STUDIES ON LARVAL HELMINTHS

with

OBSERVATIONS ON THE IN VITRO BEHAVIOUR OF ACANTHOCEPHALA.

by

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GENERAL INTRODUCTION

Our knowledge of adult stage of helminths is undoubtedly far more comprehensive than that of the larval stages. This is due not merely to the fact that a larger number of workers has studied adult helminths but rather to the possession by the latter of more easily distinguishable characters. In comparison, larval helminths are difficult to study and identify owing to their small size and to the consequent obscurity of their structural details. It is, therefore, not surprising to find in the literature that the descriptions of larval helminths are sometimes very brief and incomplete and this adds greatly to the difficulties of their proper identification.

Although much useful work has been done on larval helminths in Great Britain, our knowledge concerning them is still very inadequate. A majority of the investigators in Great Britain have concentrated on larval Trematoda, a few have studied larval Cestoda and larval Nematoda, but none, so far as the writer is aware, appears to have described larval Acanthocephala.

During a preliminary examination by the writer of the helminth parasites of brown trout (Salmo trutta)
from certain lochs and rivers of Scotland, the following parasites were obtained:

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<td>Echinorhynchus truttae</td>
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<tr>
<td>Neoechinorhynchus rutili</td>
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<tr>
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<tr>
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<td>(Plerocercoid cysts)</td>
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<td>Metacercaria</td>
<td>Eye</td>
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<tr>
<td>(Diplostomulum)</td>
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<tr>
<td>Discocotyle sagittata</td>
<td>Gills</td>
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These have already been reported from trout but the list may be of interest as a record of helminths from brown trout in Scotland.

Of the above parasites, the only larval helminth which was common enough to provide an abundance of material for further studies was the metacercaria from the eye.

Extensive work on the morphology and the development of the metacercariae from the eyes of fish has been carried out on the Continent and in America. In Great Britain, however, Taylor and Baylis (1930) gave a brief account of the morphology of a metacercaria...
from the eyes of sticklebacks but mentioned that owing to lack of time and suitable material it was not possible to give a fuller description. Rushton (1937, 1938) in his letters to "Nature" stated that the presence of metacercariae in the eyes of rainbow trout caused blindness in the fish. Baylis (1939) found similar parasites in one lens of the eye of a rainbow trout and these he assumed to be identical with Diplostomum volv ens of Nordmann (1832). It was clear, therefore, that there was scope for further work on these parasites.

Again, with a view to obtaining possible clues as to the life-history of some of the helminth parasites of trout, the writer was led to the examination of the stomach contents of the fish and it was found that some of them had been feeding voraciously on the crustacean, Gammarus pulex and on the snail, Valvata piscinalis. Attention was thus directed to the possibility of these animals serving as vectors of larval helminths. This proved very successful and provided a good deal of material for study. Reference to relevant literature revealed that, on the Continent, various workers had obtained from Gammarus several larval Cestoda and larval Acanthocephala. In Great Britain, neither larval Cestoda nor larval Acanthocephala were found by Baylis (1931) who examined over a hundred specimens of Gammarus. As for the snail, Valvata piscinalis a few larval Trematoda had
been described. It was thought, therefore, that perhaps a more intensive investigation might yield fruitful results.

During a search of the literature on larval helminths of Great Britain, it was found that as early as 1785, Prof. Alexander Monro II described some cysts as "Spheroidal bodies" from the spinal nerves of haddock. These were later regarded as encysted metacercariae of Gasterostomum gracilescens (now known as Bucephalopsis gracilescens). Very little work (except Tennent 1906, Woodhead, 1929, 1930) has been done so far on the development of the group of Trematoda to which Bucephalopsis belongs. Furthermore, there seems to be some confusion with regard to the structure of the anterior adhesive organ of these trematodes in the accounts given by various workers. This confusion exists not only in the description of the larva but even in that of the adult. It was, therefore, thought that a detailed study of the morphology and the development of this metacercaria from haddock might clear up some of this confusion.

The present thesis, therefore, contains an account of the morphology and the development of a varied collection of larval helminths from Salmo trutta, Valvata piscinalis, Gammarus pulex and Gadus oeglinus.

Six of these larval helminths appear to be new to Science. In addition, a re-study of the forms previously recorded from the above hosts has made it
possible to fill some of the existing gaps in our knowledge of these parasites.

The large amount of material of *Hchinorhynchus truttae*, which was obtained from brown trout, gave an opportunity of carrying out a preliminary study on the \textit{in vitro} behaviour of this Acanthocephalan to anthelmintics. So far as the writer is aware, no work has been done along these lines in this group of helminth parasites. It was thought that such a study might prove useful in future investigations on the treatment and control of Acanthocephala.

The thesis is divided into two sections: the first, consisting of four parts, deals with larval helminths and the second with the \textit{in vitro} behaviour of an Acanthocephalan.
ACKNOWLEDGEMENTS

The thesis embodies the results of investigations carried out in the Department of Zoology, University of Edinburgh. It is with great pleasure that I express my thanks to Prof. James Ritchie for kindly granting me permission to work in his laboratory, for giving me every encouragement and for taking a keen interest in my work. I also wish to express my thanks to Dr. R. A. R. Gresson for extending to me these facilities during the absence of Prof. James Ritchie on leave.

I wish to express my indebtedness to Dr. D. O. Morgan for helping me in various ways in my studies. He not only made suitable arrangements for obtaining material and literature but also took great personal interest during the progress of my work. I take this opportunity of expressing my thanks to him.

Thanks are also due to the Director of the Imperial Bureau of Agricultural Parasitology for his kindness in sending me from his library certain papers which were not easily available.

Finally I wish to express my thanks to Dr. D. M. Steven of the Department of Zoology, Edinburgh University and to Mr. J. G. Speed of the Royal (Dick) Veterinary College for helping me with specimens of trout from several localities in Scotland; and to Mr. R. J. Fant, chief technician, Zoology Department, Edinburgh University for the preparation of the photomicrographs illustrating this thesis.
STUDIES ON LARVAL HELMINTHS
PART I

ON THE MORPHOLOGY, DEVELOPMENT AND PATHOGENICITY OF
DIPLOSTOMULUM TRUTTAE N.Sp. FROM THE EYE OF THE
BROWN TROUT, SALMO TRUTTA.

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INTRODUCTION

Nordmann (1832) described two metacercariae under the name Diplostomum from the eyes of fish. A decade later, Steenstrup (1842) also found larval trematodes in the eyes of pike and perch. Brandes (1892) introduced the name "Diplostomulum" to designate the larva from the eye of Abramis brama and this name has been frequently used since. Lühe (1909) mentioned three species of Diplostomum and one of Tylodelphys as inhabitants of the eyes of fish. Szidat (1924) recorded frequent occurrence of Diplostomum volvens in the eyes of sticklebacks. Timmernann (1936) gave an account of the biology of cercaria of D. volvens.

In America Cooper (1915) found trematode larvae in the lens of Micropterus dolomieu, and La Rue, Butler and Berkhout (1926) described strigeid metacercariae from the eyes of various fishes. Hughes (1929) gave a review of all the known species of Diplostomulum from the eyes of fish.

In Great Britain attention was drawn to the strigeid metacercariae from the eyes of fish by Taylor and Baylis (1930) who obtained the metacercariae in the eyes of Cottus gobio, Phoxinus phoxinus, and Gasterosteus aculeatus. Rushton (1937, 1938) in two brief notes, stated that these parasites cause blindness in fish. Later Baylis (1939) demonstrated
before the Linnean Society, a specimen of the lens of a rainbow trout containing similar parasites, and assumed these to be the young stages of Diplostomum spathaceum.

During a preliminary investigation of the helminth parasites of brown trout from Scottish lochs and rivers, I obtained a large number of Diplostomulum, in different stages of development, in the eyes of this fish. In certain cases the infection was very heavy; the parasites being present in both the eyes, largely in the vitreous humour and also within the lens. Taylor and Baylis (1930) in their study of the metacercarie in the sticklebacks do not give a detailed account of the parasites owing to lack of material and time and they do not appear to have studied the extent of the damage done to the eye by these parasites. I have, therefore, endeavoured to elucidate some of these points by studying the morphology, developmental stages and pathogenecity of this Diplostomulum from the eye of the trout.
MATERIAL AND METHODS

The eyes of trout were dissected out and the cornea around the pupil cut away. The lens could then be lifted out with a forceps and along with it usually came away the jelly-like vitreous humour. The parasites could be seen as minute greyish-white specks in the vitreous humour from which they were liberated and examined both in the living and in the fixed and stained condition.

For whole mounts the usual staining procedure was adopted. Sections of the lens of the eye, about 15 μ thick were also cut by the freezing method and stained with Haemotoxylin and Eosin. Sketches were drawn with the help of the Camera lucida from fresh living material but fixed unstained and stained preparations were equally utilized for photo-micrographs and diagrams.
Table 1 - showing the dates, localities in Scotland and the number of trout infected with Diplostomulum.

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Text Fig. 1. - A fully developed DIPLOSTOMULUM TRUTTAE n.sp. showing morphological details. (Excretory system not shown in this figure.)

Text Fig. 2. - Anterior ends of D. TRUTTAE n.sp. showing retracted and protruded lateral adhesive organ.

e.b. - excretory bladder; e.p. - excretory pore; h.o. - holdfast organ; l.a.o. - lateral adhesive organ; o.su. - oral sucker; ph. - pharynx; sph.b. - spheroidal bodies; v.su. - ventral sucker.
STRUCTURE OF DIPLOSTOMULUM LARVA

The larva which is whitish-grey in colour lies free mostly in the vitreous humour or within the lens, and when liberated it exhibits active contraction and expansion of the body. The body of the larva (Text Fig.1) is oval to cylindrical in shape and is divided into two parts - a large anterior part and a very small posterior part. The larva shows a good deal of variation in shape, particularly in its anterior end. When the larva is contracted the anterior end is more or less rounded with a faint projection in the middle but when fully extended it becomes trilobed; a large middle lobe formed by the oral sucker and two lateral ones formed by the lateral adhesive organs (Text Fig.2).

There are no spines on the cuticle but its entire surface is covered with very minute papillae which are best seen either in the living condition or in fixed unstained toto mounts. They appear as dark bodies in the photomicrograph (Plate 1, Fig.1).

The larva (Text Fig.1) measures 0.85 mm. to 1.5 mm. in length and 0.45 mm. to 0.5 mm. in breadth. In a specimen of about 1.1 mm. length, the ratio of the anterior to the posterior part of the body is as 0.95 mm. : 0.15 mm. The oral sucker is more or less circular and measures 0.11 mm. in diameter. It leads into a short prepharynx which may become almost
invisible when the larva is contracted. This is followed by a pharynx, oval to spherical in shape, measuring about 0.05 mm. in diameter. A short oesophagus runs behind the pharynx to divide into two intestinal caeca which extend into the smaller posterior part of the body. The ventral sucker is smaller than the oral and measures 0.075 mm. in diameter. The hold-fast organ measures 0.19 mm. X 0.16 mm. The lateral adhesive organs are situated on each side of the oral sucker and may protrude in the form of ear-like flaps or retract into the body as deep cup-shaped cavities (Text Fig. 2). The walls of these organs show fine muscular strands. No lateral adhesive organs were seen in the metacercaria described by Taylor and Baylis (1930) from sticklebacks.

The body of Diplostomulum shows a large number of translucent so-called "spheroidal bodies." These show a characteristic distribution; in the present case they lie on the lateral sides of the body and extend inwards towards the median line in front of the ventral sucker (Text Fig. 1.). They also extend along the lateral sides of the oral sucker. They are sparingly present in the region of the holdfast organ and posterior to it. The "spheroidal bodies" have been associated with the excretory system of various species of Diplostomulum. I have always found them in greater number in the fully grown Diplostomulum than in its earlier stages.
Text Fig. 3 - *DIPLOSTOMULUM TRUTTAE* n.sp. drawn from a compressed living specimen, showing the distribution of the excretory vessels and the flame-cells.

Text fig. 4 - A portion of the excretory branches of *D. TRUTTAE* showing spheroidal bodies.

a.e.c. - anterior excretory canal; c.e.c. - chief excretory canal; e.b. - excretory bladder; e.p. - excretory pore; e.v. - excretory vessel; f.c. - flame-cell; h.o. - holdfast organ; l.a.o. - lateral adhesive organ; o.su. - oral sucker; ph. - pharynx; p.e.c. - posterior excretory canal; sph.b. - spheroidal bodies; v.su. - ventral sucker.
Taylor and Baylis (1930) gave no details of the excretory system of the metacercaria from the eye of sticklebacks. In the present form from the trout, the minutiae of the excretory pattern could be clearly seen in fresh living specimens under considerable pressure of the cover slip (Text Fig. 3).

The chief excretory canal of each side is formed by the union of an anterior and a posterior excretory canal near the equatorial plane of the body. From this point the chief excretory canal runs obliquely in a postero-medial direction and receives a transverse vessel, after which it runs backwards nearly parallel to the lateral margin of the body until it reaches the posterior margin of the holdfast organ. Here it curves medially and opens into the antero-lateral angle of the bladder. The right and left anterior excretory canals receive a branch each from the lateral sides of the oral sucker and just posterior to the pharynx they join up and at their point of union they receive a median dorsal canal which can be traced backwards, dorsal to the ventral sucker and holdfast organ, to a point terminating in the posterior part of the holdfast organ. There are small lateral branches meeting the anterior, posterior and median dorsal canals. The finer terminations of these lateral branches could not be clearly traced owing to the presence of a large number of "spheroidal bodies", nor were the exact number and position of the flame-
cells determined. However, I was able to count as many as 22 flame-cells and I do not think the total would greatly exceed this figure. It was also seen that each of the finer excretory branches terminated near a "spheroidal body" (Text Fig. 4.). The excretory bladder is a bilobed shield-like vesicle and when fully expanded it may have a bicornuate shape with a deep cleft dividing the vesicle. The vesicle opens out by an excretory pore at the postero-dorsal end of the animal.

No other structures such as gonads or vitelline glands were seen at this stage.

The larva now probably feeds since granules of food material were found within the intestinal caeca. Moreover, I have seen fluid being sucked in by the pharynx and also flowing out through the mouth opening by contraction of the pharynx which probably acts as a pump.
SYSTEMATIC POSITION

The larva (Diplostomulum) described in the present paper has the following distinguishing features:

1) Body smooth devoid of spines, divided into two parts - anterior much larger than the posterior and more than twice longer than broad in well developed specimens.

2) The oral sucker larger than the ventral.

3) The lateral adhesive organs capable of either great protrusion into ear-like processes or retraction into deep cup-shaped sucking organs.

As already stated, a number of species of Diplostomulum have been described from the eyes of fish in different parts of the world. Various characters have been taken into account in distinguishing species but the main criteria have been the size of the body and its two parts, the relative size and position of the suckers and the nature of the lateral adhesive organs.

Baylis (1939) obtained a metacercaria in the lens of a rainbow trout and assumed it to be Diplostomulum volvens which is the larval stage of Diplostomum spathaceum. Rushton (1938) apparently on a suggestion from Rothschild, also regarded his specimens from the yearlings of rainbow trout and roach as identical with the same species. The present writer was also tempted to regard the larvae from the eyes of brown
trout as identical with *Diplostomulum volvens* but a detailed study of the material revealed certain fundamental differences.

Commenting on the lateral adhesive organs and the suckers in *Diplostomum volvens*, Ashworth and Bannermann (1927, p.167) say, "The lateral processes at the anterior end, though partly retractile, are not capable of being converted into sucking organs.... In *D. volvens* the transverse diameter of the ventral sucker is about twice that of the oral sucker..... The ventral sucker of *D. volvens* is slightly anterior to the middle of the body."

On referring to the original paper by Nordmann (1832), the present writer found that although Nordmann does not specifically mention the comparative sizes of the oral and ventral sucker it is clear from his diagrams that the ventral sucker is larger than the oral and is situated in the anterior half of the body. The lateral adhesive organs are, however, described as retractile in the same way as the tentacles of molluscs and in none of his numerous diagrams are the lateral organs shown retracted within the body into cup-shaped cavities.

As will appear from the preceding description, the larva from brown trout, on the contrary, has an oral sucker which is larger than the ventral (seen clearly in a photomicrograph vide Plate I.Fig.1.), and the latter is either equatorial or post-equatorial
in position. Furthermore, the lateral adhesive organs are capable of retraction into deep cup-shaped sucking organs. In view of these two fundamental differences I am unable to regard my specimen as identical with *Diplostomulum volvens* (Nordmann 1832) and consequently with either that of Baylis (1939) or that of Rushton (1937, 1938).

If we assume that Braun (1894) and Szidat (1924) were correct in associating the adult strigeid *Diplostomum spathaceum* with *Diplostomulum volvens*, then I have further reason to believe that my specimen is different since the oral sucker of *Diplostomum spathaceum* is also smaller than the ventral.

Again assuming that Baylis (1939) was correct in calling his specimens *D. volvens*, it follows that there is more than one species of *Diplostomulum* parasitizing the eyes of trout in Great Britain. It may be interesting to quote in this connection Wesenberglund (1934, p.155) who says, "That we have to do with different *Diplostomum* species in the eyes of fishes is a fact beyond doubt which was already observed by Nordmann, and here is an untilled field for future investigators."

Of the various other strigeid metacercariae described from the eyes of fish, I find that the present form from brown trout, on a general comparison of structures, comes nearest to the American forms *D. gigas*, *D. huronensis* and *D. scheurini* but it is not identical with any of them.
D. rigos according to authors (Hughes and Berkhout 1928, p. 483) occurred exclusively within the lens of the eye in all the fishes examined by them. Furthermore the lateral adhesive organs of D. rigos are not capable of retraction into deep and broad cup-shaped cavities. D. huronensis has oral and ventral suckers of practically the same size (vide Tables I and II p. 492, Hughes and Hall 1928). D. scheuringi is very much elongated, its lateral organs poorly differentiated, its body showing conspicuous longitudinal muscle strands and its oral and ventral suckers practically of the same size.

I have, therefore, no doubt in my mind that the present form from brown trout is a distinct species. I name it Diplostomulum truttæa n.sp.
Text Figs. 5, 6, and 7. - METACERCARIAL ECDYSIS stage showing three phases in the ecdysis.

a.o. - anterior oral organ; cu. - cuticle; e.b. - excretory bladder; ph. - pharynx; v.su. - ventral sucker.
EARLY DEVELOPMENTAL STAGES AND ECDYSIS

The parasites obtained from the eyes of brown trout were usually fully developed Diplostomulum truttae n.sp., but in two trout from Crosswood Reservoir, Edinburgh, I noticed in the vitreous humour a few very small elongated tail-less but cercaria-like bodies among the larger Diplostomulum larvae. There was a conspicuous difference between the creeping Diplostomulum, constantly altering its shape, and these tail-less immobile stages which otherwise showed features associated with a furcocercous type of cercaria. I regard these immobile forms as early stages in the transformation of the parasite from a Cercarial to a Diplostomulum stage.

The earliest stage obtained is shown in Text Fig. 5. It measures approximately 0.56 mm. X 0.2 mm. and has a cylindrical body with a tapering anterior end and a rectangular posterior end. The anterior oral organ is muscular and trapezoid and measures 0.14 mm. X 0.1 mm. The surface of the anterior oral organ is beset with very fine spines as is sometimes seen in that of a furcocercous cercaria. There is a small pharynx about 0.028 mm. in diameter preceded by a short prepharynx and followed by a short esophagus. The intestinal caeca are not clearly defined. The ventral sucker, smaller than the anterior oral organ, lies about the middle of the
body and measures 0.085 mm. in diameter. A small bilobed excretory bladder is present at the posterior end of the body.

In some other specimens which probably represent the next step in the transformation to the metacercaria, the body is seen to be drawn away from the marginal thin hyaline cuticle and this contraction is most conspicuous in the anterior part of the body (Text Fig. 6.). In the region of the anterior oral organ the cuticle shows minute spines. Also there is a ring of slightly thicker spines round the base of the ventral sucker (Plate I, Fig. 2). The internal structures of the body are not seen clearly.

In the specimen shown in Text Fig. 7, the original cuticle seems to be peeling off the body and this feature is particularly prominent in the region of the ventral sucker. Here the cuticle projects in the form of a cone-like structure carrying with it the ring of spines. A small ventral sucker devoid of spines is left just below this projection. I have not been able to notice the exact process by which the entire cuticle is lost and the active larval stage released.

The released larva (Text Fig. 8) has an oval body and shows almost all the structures of Diplospomulum except for the division of its body into two parts and the extension of the intestinal caeca more posteriorly. This stage which occurs very commonly
Text Fig. 8- PRE-DIPLOSTOMULUM stage showing the body undivided into two parts.

e.b. - excretory bladder; h.o. - holdfast organ; l.a.o. - lateral adhesive organ; o.su. - oral sucker; v.su. - ventral sucker.
in the eyes of fish seems to be the precursor of the Diplostomulum larva which is probably derived from it merely by a further growth of its structures and a division of its body into two parts.

The above observations suggest very clearly that within the substance of the vitreous humour of the eye, a gradual transformation from the Cercarial stage to the Diplostomulum stage is brought about. This is accompanied by what appears to be a process of ecdysis during which the cuticular wall of the cercaria is cast off leaving behind the smooth body of the Diplostomulum larva.

Furthermore, it is clear that there are at least three distinct stages in this transformation. These stages in the development of Diplostomulum have not hitherto been recognized although it seems likely that they do occur in other species of Diplostomulum. I have thought it proper, therefore, to give them the following names:

1. **Metacercarial Ecdysis stage:**
   Various phases of this are shown in Text Figs. 5, 6 and 7. Cuticle of the original Cercarial stage is cast off.

2. **Pre-Diplostomulum stage:** (Text Fig. 8)
   Body like Diplostomulum but without the division into a larger anterior and a smaller posterior part. Other structures such as the oral and ventral
suckers, lateral adhesive organs, holdfast organ etc. are present.

3. **Diplostomulum stage**: (Text Fig. 1.)

   Body divided into an anterior large and a posterior smaller part. Intestinal caeca extend into the posterior part of the body. "Spheroidal bodies" may be present in large numbers.

   These observations are supported by some evidence which was obtained by Wesenberglund (1934), while describing the leaf-like Diplostomulum he says, (p. 156), "It is as if part of the process by which the furcocercaria body is altered into a Diplostomulum takes place during a short resting stage; if the old cuticula of the furcocercaria is left by an out-creeping Diplostomum I will not venture to say, but it seems to me that this really is the case."

   The phenomenon of ecdysis occurring in the development of Trematoda is rather interesting and offers some comparison to the ecdysis taking place in the development of Nematoda.

   The developmental stages of Diplostomulum in the present case were obtained in the vitreous humour and this appears to me the normal site for development. The jellylike consistency of the vitreous humour probably offers a better place for this purpose than the hard fibrous substance of the lens. Timmermann (1936 p. 59), however, states that the development into
Diplostomum volvens in both groups of his experimental fish was brought about exclusively in the lens of the eye. His exact words are, "In beiden ist eine Entwicklung der Cercarie zu Diplostomum volvens grundsätzlich möglich. Diese erfolgt bei beiden ausschließlich in den Augenlinsen."

FEEDING EXPERIMENTS ON BIRDS.

In order to study the development of this Diplostomum into an adult trematode, feeding experiments were performed on two chickens but these gave negative results.

In the first experiment a chicken about 6 weeks old was fed with 57 larvae. The bird was killed after 3 weeks but post-mortem examination did not reveal any adult trematodes.

In the second experiment a chicken about 11 weeks old was selected and kept on a low diet for a few days and then fed with 34 larvae. The bird was kept on the reduced ration during the experiment and faecal examinations were carried out from the 2nd week after feeding but no trematode eggs were recovered. On post-mortem examination after 16 days no trematodes were found either in the alimentary canal or in the liver.
INCIDENCE OF INFECTION

Previous investigators have recorded the metacercariae of the eyes of fish as generally occurring in the lens. In the present investigation, however, as already stated larvae were found mostly in the vitreous humour and only a few were actually within the capsule of the lens. Out of 105 trout examined for these parasites (vide Table 1.), it was found that only 41 were free from these parasites. Of the 64 fish infected, the parasites were present in both eyes in 59 and in only one eye in 5 cases. The maximum number of parasites recovered from one trout was 156; in this the infection was much heavier in the right eye (107 specimens) than in the left (49 specimens.).

Furthermore it is interesting to note that the infection was more common and much heavier in trout from lochs and reservoirs than in those from the rivers. In fact most of the river specimens examined in the present investigation were found to be free from infection. It is difficult to explain this difference in infection except on the assumption that there are greater chances for the concentration of the cercariae and consequent mass attack on the fish in closed waters than in the swift-flowing streams.
PATHOGENICITY

Since the time of Nordmann's discovery of the metacercaria in the eyes of fish it has been suggested that the presence of these parasites may not only impair the vision of the fish but sometimes may injure the eyes to such an extent as to make them blind. During the present investigation I noticed a high percentage infection (61%) of trout from certain lochs and reservoirs in Scotland, but even when the vitreous humour proved on examination to be teeming with metacercariae I found no external evidence of either infection or damage. Neither did I find in any fish the so-called "pop-eye" condition - (bulging out of the eye-balls of the fish) as reported by Ward and Mueller (1926) in America. There were only 4 cases of abnormal eyes which attracted my attention and in these cases two distinct types of injury could be distinguished:

1) the reduction in the size of the lens without disintegration of its capsular wall.

2) the disintegration of the outer wall of the capsule of the lens without any appreciable reduction in its size.

The first condition was beautifully represented in a fish from Gladhouse Reservoir which showed 107 parasites in the vitreous humour of the right eye and 49 in the other. The lens of the right eye was very small while that of the left was normal (Plate I, Fig.
No disintegration of the lens could be noticed although the vitreous humour was thin in texture and appeared to be partly liquefied. Another case of slight reduction of the size of the lens was seen in the right eye of one fish (with 67 parasites) from Crosswood Reservoir.

In two other cases, also from Crosswood Reservoir, the second type of injury was seen. Here the capsular wall of the lens was slightly uneven and disintegrated although the general size of the lens was not different from that of some other fish of the same length and weight from the same locality.

It is rather difficult to account for these different types of injury in the eyes of trout. However, it is possible that a heavy infection of the vitreous humour brought about at an early age of the fish may not only block the blood-vessel supplying the capsule of the lens but may also create necrotic conditions of the vitreous humour which may not, thus, be able to supply nourishment to the growing lens. The lens would thus retain its original small size. A disintegration of the capsular wall of the lens may, however, occur more commonly as a result of the irritation and necrosis produced in the vitreous humour adjacent to the lens.

Salzer (1907) studied the effect of similar parasites on the vision in trout and concluded that the parasites caused cataract of the lens. It should
be pointed out, however, that Salzer usually obtained cases of infection by the parasites of the lens epithelium or of the horny internal substance of the lens. He did not notice infection of the vitreous humour. The turbidity or opacity of the lens observed by him was not obvious in my specimens, probably due to the occurrence of very few parasites actually within the lens itself. (Plate I. Figs. 3 and 4.). Salzer also records the finding of an enlarged lens in certain cases but I have not noticed any cases of hypertrophy of the lens in my specimens. I have also not come across even a single aphakous (lens-less) condition of the eye although both Salzer (1907, p. 24) and Wesenberglund (1934, p. 151) reported the absence of lens from the eye of fish in certain cases.

It is, however, almost certain that the infection in the eyes of fish by these parasites would, in course of time, produce impairment of vision. If that be so the hooking of trout may depend more on the poor vision of the fish than on the dexterity of our anglers.
EXPLANATION OF PLATE I

LETTERING

cu. - cuticle; o. su. - oral sucker; v. su. - ventral sucker.

Fig. 1. - Photomicrograph of *D. truttae* n. sp. from an unstained balsam preparation, showing the minute papillae all over the body and the relative sizes of oral and ventral suckers. (X85)

Fig. 2. - Photomicrograph of a Metacercarial Ecdysis stage of *D. truttae* (balsam preparation) (X365)

Fig. 3. - Photograph of the lenses and vitreous humour of the eyes of a trout. The right lens is smaller than the left. A number of parasites (*D. truttae*) are shown in situ in the vitreous humour of the right eye. (X 5)

Fig. 4. - Photomicrograph of a horizontal section of the lens showing *D. truttae* in situ. (X 60)
PART II

ON THE MORPHOLOGY AND DEVELOPMENT OF THREE NEW CERCARIAE FROM THE SNAIL, VALVATA PISCINALIS.

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INTRODUCTION

*Valvata piscinalis* is a fairly common snail of the fresh-water lochs in Great Britain and on the Continent but has not often been reported as a vector of larval helminths. So far as I am aware only five cercariae have been reported from this snail, two by Harper (1929) in Great Britain and three by Wesenberglund (1934) from Denmark.

During the course of my investigation, I obtained from this snail three different types of cercariae as well as their developmental stages. These cercariae appear to be new to Science. I, therefore, propose to give here an account of their morphology and some of the experiments which were carried out to elucidate their life-history. In the case of one species the development to the adult stage was obtained and in two of them the cercariae were successfully transmitted into the second intermediate host and the metacercarial development observed.
MATERIAL AND METHODS.

The snails for the present study were collected from Duddingston Loch, Edinburgh, brought to the laboratory and kept in groups of 6 in small beakers with about 50 c.c. of water. The beakers were examined both morning and afternoon under a binocular microscope to see if any cercariae were emerging from the snails. An infected snail throwing out cercariae could then be isolated and kept singly in a beaker. In certain cases, cercariae emerged in a week or 10 days after the collection of the snails; in one case this did not take place for 22 days.

The snails, throwing out cercariae, were observed for 4 to 5 days, after which some were dissected to obtain the parthenitae, sporocyst, redia etc. The different stages of the larvae - sporocyst, redia, cercaria - were studied alive both with and without intravitam staining with neutral red and methyl blue, and Camera lucida drawings were made mostly from living specimens. Permanent balsam mounts of stained and unstained specimens were also studied. Following dehydration and embedding in paraffin by standard technique, sections 6 to 10 \( \mu \) thick, both transverse and longitudinal, were cut to study details of the internal anatomy not otherwise clearly visible in whole mounts. Infection experiments were performed on laboratory reared animals.
Table 2 - showing the number of snails examined and infected with dates of collection and types of cercariae obtained.

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>No. of snails examined</th>
<th>No. of snails infected</th>
<th>Types of cercariae</th>
</tr>
</thead>
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<tr>
<td>23.5.46.</td>
<td>34</td>
<td>4</td>
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<td></td>
<td></td>
<td>3 Xiphidiocercaria</td>
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<tr>
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<td>3</td>
<td>2 Furcocercaria</td>
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<td></td>
<td></td>
<td></td>
<td>1 Echinostome</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>2 Xiphidiocercaria</td>
</tr>
<tr>
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<tr>
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<tr>
<td></td>
<td></td>
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<td>2 Echinostome</td>
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<tr>
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</tr>
<tr>
<td>Date of collection</td>
<td>No. of snails examined</td>
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<td>Types of cercariae</td>
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<tr>
<td>7.6.47.</td>
<td>64</td>
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<td>1 Furcocercaria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Echinostome</td>
</tr>
</tbody>
</table>

Total number of snails examined - 758
Total number of snails infected - 56
Types of infection
- **Furcocercaria** - 19
- **Xiphidiocercaria** - 26
- **Echinostome** - 11

Percentage infected with different types of Cercariae
- **Furcocercaria** - 2.5%
- **Xiphidiocercaria** - 3.4%
- **Echinostome** - 1.4%
Text Fig. 9 - A portion of the SPOROCYST of CERCARIA VALVATAE N.Sp. from the hepatopancreas of Valvata piscinalis, showing sausage-shaped swellings.

Text Fig. 10 - CERCARIA VALVATAE N.Sp. showing various types of contraction of the body and of the tail.
CERCARIA VALVATAE N. SP.

SPOROCYST

The cercaria develops in long thread-like tubular sporocysts which form a tangled mass and extend deep into the tissues of the hepatopancreas of *Valvata piscinalis*. Although it is difficult to isolate one complete sporocyst, pieces varying in length from 4 to 6 mm. could be easily obtained. Such a piece may contain a dozen or more cercariae, either fully formed or incompletely developed with a number of spherical germ balls. As the sporocyst matures and proliferation of the germ cells takes place, the simple tubular sporocyst becomes marked out into several ovoid characteristically sausage-shaped swellings (Text. Fig. 9.). No birth-pore was noticed in the sporocyst but a rupture of the wall with the protrusion of the cercariae is probably brought about, by the action of the anterior spines of the cercaria.

No account is available in the literature of the exact method of the formation of the caudal furci in either a Strigeid or a Schistosome cercaria. In the cercaria described in this paper the caudal furci are developed in the following way: (Plate II, Figs. 1, 2, 3, 4.).

The spherical germ ball elongates, giving out posteriorly a cylindrical projection (tail) which
increases in length and shows a rudimentary forking at its tip (Plate II, Fig. 1). After the cylindrical projection or the tail, as we may now call it, has elongated further, the terminal forking extends deeper (Plate II, Fig. 2) giving rise to two caudal furci. These become partially constricted from the main stem or tail in much the same way as the latter is constricted off from the body (Plate II, Fig. 3). The constriction between the caudal furci and the stem disappears during further development into a cercaria (Plate II, Fig. 4).

STRUCTURE OF THE CERCARIA

The cercaria is whitish-grey in colour and is exceedingly contractile. It is divided into three main parts: 1) body 2) stem or tail and 3) caudal furci.

The body is oval to cylindrical in shape and varies from 0.18 to 0.22 mm. in length and 0.084 to 0.1 mm. in breadth, depending upon the age of the cercaria and its degree of contraction (Text Fig. 10). The cercaria is covered with a thin delicate cuticle, the anterior part of which, in the region of the oral organ, is beset with transverse rows of very minute spines (Text Fig. 11). A sub-ventral protrusible pyriform oral organ is situated at the conical anterior end of the body. This oral organ is formed of two parts, its anterior half or so is thin but the
Text Fig. 11—CERCARIA VALVATAE N. Sp., enlarged drawing showing morphological details.

- a.e.c.—anterior excretory canal
- a.o.—anterior oral organ
- c.c.—caudal cell
- c.e.c.—chief excretory canal
- c.f.—caudal furca
- e.b.—excretory bladder
- e.v.—excretory vessel
- f.c.—flame-cell
- p.gl.—penetration gland
- p.gl.d.—penetration gland duct
- ph.—pharynx
- p.e.c.—posterior excretory canal
- v.su.—ventral sucker

0.1 mm.
posterior half is thick and muscular. The anterior oral organ measures from 0.036 mm. X 0.025 mm. to 0.045 mm. X 0.03 mm. and during contraction presents a series of excentric rings starting from its anterior end, and the last one is folded in such a way as to give a false appearance of a collar. The ventral sucker is equatorial or post-equatorial and measures from 0.027 mm. X 0.025 mm. to 0.03 mm. X 0.028 mm. It is highly muscular and protrusible. A small prepharynx leads from the oral organ to open into a globular muscular pharynx about 0.014 mm. in diameter. A tubular oesophagus about 0.03 mm. long runs behind the pharynx and bifurcates immediately in front of the ventral sucker into the two intestinal caeca which extend to a point a little posterior to the sucker.

There are three pairs of penetration glands, situated posterior to the ventral sucker, but when the body of the cercaria contracts one or two pairs of these glands appear to lie antero-laterally to the ventral sucker. A wide common duct arising from the glands on each side runs forwards and is dilated near the level of the pharynx and opens at the base of the oral organ. The glands are slightly bilobed and have a granular protoplasm with a large nucleus.

There is an ovoid bicornuate excretory vesicle. The chief excretory canal of each side is formed by the union, at the level of the ventral sucker, of an anterior and a posterior excretory canal. The chief canals run posteriorly and curve medially to open into
the excretory vesicle. Each of the anterior and posterior canals of the two sides gives off lateral branches which terminate in a flame-cell. Six pairs of flame-cells have been observed, a set of five in the body and the sixth in the proximal part of the stem.

The stem or tail is cylindrical, and responsible for most of the swimming movements. It is not easily shed except when the cercaria has partially bored into the second intermediate host. The stem measures from 0.16 mm. to 0.23 mm. in length. There are six pairs of large vesicular rectangular caudal cells arranged laterally in the stem. The caudal furci are laminated structures and measure from 0.19 mm. to 0.21 mm. in length. No bristles or hairs have been observed either on the stem or on the caudal furci.

The cercariae emerge during the day although they do not have any special predilection for light when swimming about after emergence. In swimming the furcal end is kept forwards and the body is simply dragged by a jerking movement of the stem or tail. Generally the cercariae swim slowly but the speed increases on coming in contact with some object. At times the cercariae simply remain suspended in water with their caudal furci at right angles to the stem and the body which hangs head downwards. This type of temporary suspension of movement is also seen after they have been swimming briskly for some time.
Experiments were devised to find out a suitable intermediate host of this cercaria. Various animals were tried e.g. *Gammarus pulex*; *Corethra larva*; a fresh-water Oligochaete, *Lumbriculus variegatus*; fresh-water leeches, *Helobdella stagnalis*, *Glossosiphonia complanata*, and *Herpobdella octoculata*; the snails *Valvata piscinalis* and *Limnaea pereger*, and also the brown trout, *Salmo trutta*. A large number of cercariae were put in small deep petri dishes with a little pond water and the various invertebrate animals were introduced individually into these dishes. Periodic examination of the contents of the dishes was done to watch the behaviour of the cercariae. In the attempts to infect a trout, a few *Valvata piscinalis* which were throwing out cercariae were put in a small aquarium with the fish. The fish, however, did not show any signs of discomfort nor were any signs of infection noticed either in the eyes or in other viscera when the fish was killed and examined, although it had been exposed to Cercariae daily for six days. All attempts to infect the various invertebrate animals also proved negative except in the case of the leech, *Helobdella stagnalis*. 
OBSERVATIONS ON THE INFECTION OF HELOBDELLA STAGNALIS

The first clue was obtained when a leech under experiment was seen surrounded by a large number of cercariae and a few were seen actually entangled in the mucus around the skin of the leech. Having got this clue, about a dozen specimens of very young *Helobdella stagnalis* were obtained. This is easy as the leech carries the developing embryos on its ventral surface. These leeches with developing embryos were collected and put in small beakers with a little water till the young ones actually hatched out. The young leeches were put, one specimen to each, in small petri dishes and a large number of freshly emerged cercariae, collected by pipetting, were introduced into these dishes. Soon after this was done the leeches started moving vigorously owing to the irritation caused by the attacking cercariae. The cercariae were not specially attracted towards the leech but whenever their caudal furci came in contact with the body of the leech quicker movements were set up in the cercariae, the forked end turned away and the anterior ends of the cercariae were brought in contact with the body of the leech (Plate II, Fig. 5.). This sudden reversal in the mode of approach by the cercariae is very peculiar and needs further attention for any physico-chemical or physiological explanation.

As soon as this had happened, the swimming
movement of the cercaria stopped but a piston-like movement of the protruding anterior oral organ started. The minute spines on the anterior organ of the cercaria caused friction against the body of the leech; further the penetration glands poured their secretion copiously and thus helped to dissolve the tissue of the leech and make it softer for the penetrating cercaria. During this act the ducts of the penetration glands were seen to become greatly distended with the secretion. The cercaria kept a hold on the body of the leech by protruding the cup-shaped ventral sucker. If the cercaria proved successful in penetrating the leech, more than half of its anterior spiny end would penetrate the skin within a minute. Then the hold of the ventral sucker was loosened; this was facilitated by the secretion of mucus by the leech. The cercaria became slightly bent ventrally in the middle of its body and the stem and caudal furci, no longer necessary, were cast off (Plate II, Fig. 6). The cercaria, then, performed peculiar wriggling and creeping movements. In about 2-3 minutes the entire body of the cercaria entered into the tissues of the leech. In several cases, especially in very young leeches, penetration took place within one minute.

It was interesting to note that in their attempts to penetrate, a large number of cercariae perished by getting entangled in a mass of the copious mucus discharged by the leech to ward off the attacks of
the cercariae. The leech may become exhausted by its wriggling movements and by rolling in and bending out in order to escape the attack and in the experiments several leeches died as a result of the reaction to this mass attack of the cercariae. Probably in nature this will seldom happen, especially in moving or flowing water where the concentration of the cercariae is not likely to be very large at any one time. I have observed in the laboratory that when only one or two cercariae are allowed to contact the leech, the latter shows only one or two quick sudden movements but no wriggling or otherwise wild movements as noticed in a mass attack of the cercariae. The secretion of the mucus is also much less.

In one experiment, a leech was exposed to the attacking cercariae for six hours. Out of several hundred cercariae used in the experiment, about a hundred entered the body of the leech and were seen wriggling inside (Plate III, Fig.1.). The leech, however, died during this process.
Text Fig. 12. - Metacercarial stages (about 8 to 10 days old) of *Cercaria Valvatae* N.Sp.

Text Fig. 13. - Metacercaria within the cyst from the body of a leech, showing structures of the future tetracotyle (about two weeks old).

o.su. - oral sucker; v.su. - ventral sucker.
FURTHER DEVELOPMENT OF THE CERCARIA IN THE LEECH

Soon after the cercaria bores through the body wall of the leech it enters into a sinus or blood channel (Plate III, Fig. 2.). Sometimes large numbers are found in the net-like haemocoele. The tail-less cercaria, which may now be called a metacercaria, shows wriggling and creeping movements for a while when within the blood channel, but very soon these movements cease. In about 3 to 4 days the original cercarial structures have disintegrated; the penetration glands having disappeared earlier. After about 7 to 8 days new structures begin to develop in the metacercaria and then encystment begins (Text Fig. 12, a.b.c.). The metacercaria becomes rounded in shape and a cyst is secreted around it. The rudiments of several structures which are formed afresh viz. oral sucker, ventral sucker, etc. begin to appear distinctly (Text Fig. 13.). Thus the metacercaria is transformed into a tetracotyle. Some of the stages of formation of the tetracotyle are shown in Plate III, Figs. 3, 4 and 5. About 28 days are required between the cercarial penetration and the development of a fully formed tetracotyle. At this stage (Plate IV, Fig. 1.) the cysts are clearly visible in the leech as rounded bodies, each containing a fully grown tetracotyle. When removed from the cyst, the tetracotyle is broadly oval in shape with a thick body, thick muscular suckers and a holdfast organ (Plate IV, Fig. 2.; Text
Text Fig. 14 - Fully formed tetracotyle removed from the cyst showing division of the body into two parts.

h.o. - holdfast organ; o.su. - oral sucker; v.su. - ventral sucker.
Fig. 14. The details of the anatomy and the relative position of the suckers can be best seen in section (Plate IV, Fig. 3.). The tetracotyle is constricted into a larger anterior part, containing the suckers, and a small narrow posterior part which in the natural position within the cyst is compressed against the anterior part.

The leech, Helobdella stagnalis appears to be a natural second intermediate host for this cercaria as the encysted tetracotyles are seen even in some of those leeches which are collected from the lochs.

FEEDING EXPERIMENT WITH TETRACOTYLE ON A BIRD

In order to study the further development of tetracotyle into an adult trematode, a feeding experiment was performed on a chicken.

A chicken about 10 weeks old was fed with 28 cysts from the leech. It was again fed after a lapse of 12 days with 32 cysts. The bird was kept on half rations during the experiment and faecal examinations were carried out twice but no trematode eggs were recovered. On post-mortem examination, 17 days after the last feeding, no trematodes were found either in the intestine or in the liver. It is more likely that the tetracotyle would develop further in an aquatic bird but as no aquatic bird was available, the experiment could not be repeated.
The cercaria described above has the following diagnostic features:

1. Cercariae develop in long tubular sporocysts; caudal furci of about the same size as the stem and not provided with a fin or membrane.

2. Anterior end of the cercaria beset with spines; oral organ, pyriform, anterior part thin and posterior part thick and muscular; oral organ larger than the ventral sucker, both strongly protrusible.

3. Penetration glands - three pairs, situated immediately behind the ventral sucker.

4. No eye-spots present.

5. Excretory vesicle, ovoid; six pairs of flame cells present, of which one pair is in the proximal part of the tail.

About a dozen furcocercous cercariae have been described in Great Britain from snails other than Valvata piscinalis. The present form comes nearest to Cercaria F, Harper 1931, Cercaria Y, Rees 1932, Cercaria micromorpha, Brown 1926, and Cercaria pygocytophora, Brown 1931. Of these forms Cercaria F, Harper 1931, Cercaria Y, Rees 1932, and Cercaria micromorpha, Brown 1926 have only two pairs of penetration glands while Cercaria pygocytophora, Brown 1931, has seven pairs of flame-cells. Therefore, the present form stands out clearly from all these species.
in having three pairs of glands and six pairs of flame-cells. Furthermore, in Great Britain no furcocercous cercaria has been described from *Valvata piscinalis* and none known to penetrate a leech for encystment and further development.

On the Continent, however, Wesenberglund (1934) recorded two furcocercous cercariae from *Valvata piscinalis* viz. *C. cristata* and *C. longiremis*. There is no comparison between *C. cristata* and the present form owing to very great differences in structure and shape. For example, the body of *C. cristata* bears a membranous crest, has no ventral sucker, has a sac-like gut without diverticula, has its penetration glands in the form of two fine inconspicuous threads and has rather unique double excretory vessels in the tail. *C. longiremis* has 4 pairs of penetration glands and no flame-cells in the tail. It is, therefore, obvious that none of these two continental species can be identified with the present form.

It may be pointed out here that Szidat (1929, 1931) found two tetracotyle larvae encysted in leeches and was able to show that these were produced by the cercariae of *Aptemon gracilis* and *Cotylurus cornutus*. These, however, are different from the present form, the former having bristles on the stem, four pairs of penetration glands and seven pairs of flame-cells; and the latter having two pairs of pre-acetabular penetration glands, with 10 pairs of flame-cells of
which two pairs are in the tail. There can, therefore, be no doubt that both of these are different from the present form.

As regards the American furcocercous forms I have not been able to relate even one of them to the present form although *C. burti* seems to be probably a close relative but different in having sparingly spined stem and furci, four pairs of penetration glands and seven pairs of flame-cells. It is interesting, however, to note that Castle (1900, p. 60) mentioned the occurrence of an unidentified metacercarial cyst in the leech, *Helobdella stagnalis* in America. As nothing further has been reported to throw any light on the method of infection of this leech in America, it is difficult to identify the metacercaria reported by Castle.

My present study, however, demonstrates very clearly that the same species of leech, *Helobdella stagnalis* is infected by the furcocercous cercaria from *Valvata piscinalis* in Great Britain and this may afford some clue to American workers regarding the identity of Castle's metacercaria.

From all that has been said above, it is clear that the cercaria described above from *Valvata piscinalis* is a new species. I name it *Cercaria valvatae* n. sp.

In the present state of our knowledge and according to the prevalent classification, *Cercaria*
valvatae n. sp. comes under the group of furcocercous cercariae known as the Pharyngeal longifurcate distome type. I shall, however, now proceed to examine the systematic position and classification of the furcocercous cercariae in the light of the knowledge gained by a study of Cercaria valvatae n. sp. as well as by collating the observations of previous investigators.

Since the account of the first furcocercous cercaria given by Müller (1773), this group of cercariae engaged the attention of various workers. Lühe (1909), however, attempted to give a classification of the European forms by dividing them into two groups mainly on the basis of the presence or absence of eye-spots.

As a result of Leiper’s work (1915), the Schistosome cercariae were separated from other furcocercous forms by their lack of a pharynx, absence of pigmented eye-spots and absence of a cuticular keel on the caudal furci. Further, Leiper (1915) emphasized the value of "larval" and "adult" characters in the classification of the cercariae. Later workers, however, concentrated almost entirely on certain "larval" characters in dividing the furcocercous cercariae into groups.

Cort (1917) in basing his classification of the furcocercous cercariae emphasized, besides the presence or absence of a pharynx, the characters of the tail viz. the ratio of the length of the caudal furci to
the stem and whether the caudal furci are delimited or not - delimited from the stem.

Sewell (1922), however, thought that the presence or absence of a ventral sucker was of primary importance in classifying the furcocercous forms. But a cercaria with only one sucker may sometimes develop into an adult with two suckers (Stunkard 1946, p.152.). In that case such a character as the presence or absence of a ventral sucker in the classification of the cercariae will have only secondary importance and not much significance for establishing relationships.

Miller (1926) in his comprehensive monograph on the furcocercous cercariae, while recognizing the presence or absence of a pharynx as of greater significance than the presence or absence of a ventral sucker, laid still more emphasis on the character of the tail of these cercariae in maintaining a division into a longifurcate and a brevifurcate group both for the pharyngeal and the aphanryngeal forms. He further enumerated several distinctive characters for the longifurcate and the brevifurcate groups (Miller 1926, p.63.). Later workers seem to concur with Miller (1926) in this scheme of classification. The scheme has much to commend it although as a result of more recent work on these forms the distinction between longifurcate and brevifurcate groups has grown slender. Indeed the characters used by Miller are, in many
instances, quite unsatisfactory as the following facts will show:

Miller pointed out that the anterior oral organ is modified into an anterior non-muscular part and a posterior muscular part in brevifurcate forms, while it is usually non-muscular in longifurcate forms. It can be stated, however, that the cercaria described in the present paper which is distinctly longifurcate has an oral organ showing the modification associated with the brevifurcate type. Furthermore, another longifurcate form, *Cercaria chrysenterica* Miller, 1923 also shows the oral organ of a brevifurcate type. Lastly it may be said that a brevifurcate form, *Cercaria wardi* Miller, 1923 does not show a distinct muscular and non-muscular division of the oral organ. This character, therefore, is far from satisfactory for defining the two groups.

Secondly, Miller regarded the relative sizes of the ventral and oral suckers as another distinguishing character between the longifurcate and the brevifurcate forms. The ventral sucker was thought to be as large, or even in some cases much larger than the oral organ in longifurcate forms. Moreover the ventral sucker was considered to be very protrusible in brevifurcate forms. Both in *Cercaria valvatae* n. sp. which is longifurcate as well as in two other longifurcate forms viz., *Cercaria burti* Miller, 1923 and *Cercaria*
 Miller, 1923, the ventral sucker is definitely smaller than the oral organ. Furthermore, the ventral sucker is strongly protrusible in the cercaria described by the present writer. It is, therefore, clear that this distinction between the two groups as stated by Miller has little value.

Thirdly, it was stated by Miller that the presence of a single pair of caudal flame-cells in the proximal part of the stem was usually met with in brevifurcate forms, while two pairs of caudal flame-cells seldom confined to the proximal part was a character of longifurcate forms. Both in the cercaria described by the present writer as well as in two other longifurcate forms viz., Cercaria burti Miller, 1923 and Cercaria Indicae I Sewell, 1922 there is only one pair of caudal flame-cells and these are confined to the proximal part of the stem. Evidently, therefore, the position and number of caudal flame-cells is not a sound basis for the grouping into longifurcate and brevifurcate forms.

Another point emphasized by Miller (1926) was that the caudal furci are delimited from the main stem frequently in brevifurcate forms although here too the evidence is that certain brevifurcate forms like Cercariae Indicae XXX may not have the caudal furci delimited and other longifurcate forms like Cercariae Indicae LVIII have their caudal furci delimited from the tail stem. Moreover, no account has been given.
by any investigator as to when in the development of a furcocercous cercaria, this delimitation takes place and whether this is present during the development and lost in the fully developed cercariae or vice-versa. My observations on *Cercaria valvatae* n. sp. show that there is a tendency towards delimiting the caudal furci in the same way as that of the stem from the body (Plate II, Figs. 3 and 4). While the constriction in the former case does not deepen and is lost later, that of the latter becomes increasingly prominent.

Apart from these points mentioned above, even the fundamental definition of the brevifurcate and longifurcate form is not very clear. Miller (1926) agreed with Cort (1917) in stating that a longifurcate cercaria has the caudal furci longer than one-half of the tail stem, sometimes exceeding it, and a brevifurcate form has usually caudal furci less than one-half of the stem.

This distinction also seems to be slender. Moreover, due to the contractile nature of the stem and the furci it is doubtful if the relative lengths of the two structures could be adequately appraised in all cases. Miller (1926) himself put *Cercaria elvae* [synonymous with *C. ocellata* according to Dubois (1929) and Taylor and Baylis (1930)] in the brevifurcate group although the measurements of the caudal furci and tail stem are 328 μ and 501 μ (living material).
and 290 μ and 382 μ (balsam specimen). Furthermore, Cercaria gigas Faust, 1918 was described as having a tail stem 0.32 mm. long and the caudal furci 0.18 mm. long which is more than half of the tail stem. Yet this form is put in brevifurcate group by Miller (1926).

The presence or absence of a pharynx was taken as a genuine character for dividing the furcocercous cercariae into two groups. The present writer (Lal 1937, p.37) drew attention to the observations of Oiso (1927) regarding the cercaria of Bilharziella yokogawai which possesses a muscular pharynx (clearly shown by Oiso in his diagram) but which is lost during its transformation into the adult. It may be that Oiso (1927) mistook some glandular cells for the pharynx, but if we assume that all Bilharzia worms originally had a pharynx which was lost later, it is possible to conceive that a pharyngeal cercaria may give rise to an apharyngeal adult fluke. Furthermore quite a number of cercariae possess a degenerate pharynx represented only by glandular cells: as in Cercaria gracillima and Cercaria minor.

There is no doubt, therefore, that the pharynx in furcocercariae, shows a transitory nature but a classification based on its presence or absence is certainly much more reliable than that based on the nature of the caudal furci.

Apart from this, Cercaria multicellulata is placed in the pharyngeal group both by Miller, H.M. (1926)
and, later again, by Miller, E. L. (1936) because
this cercaria resembles other pharyngeal cercariae in
general characters although it does not possess a
pharynx.

It may be interesting to note in this connection
the remarks of Johnston (1941) regarding the observa-
tions of Bradley (1926, 1933). Johnston (1941,
p.282) states that, "In a later paper (1933), Bradley
referred again to his C. pellucida and C. greeri,
calling the latter a schistosome larva. His figures
(1926) indicate C. greeri to be a longifurcate cercaria
with well developed sub-equal suckers and apparently
with small gland cells in preacetabular position. All
known schistosome cercariae belong to the brevifurcate
group. The presence of a pharynx is sometimes
detected only with difficulty and may have been over-
looked by Bradley. C. greeri seems to be a strigeid
larva."

Although the assumption, that all schistosome
cercariae are brevifurcate and strigeid cercariae
longifurcate, may generally be true, exceptions are
not wanting. For example Porter (1938, p.142) gives
the length of the stem and caudal furci in cercaria of
Bilharziella polonica as 0.26 mm. and 0.16 mm.
respectively. Again she mentions (p.145) that in
Cercaria ocellata (which Brumpt (1931) had shown to be
the cercaria of Trichobilharzia ocellata) the lengths
of the stem and caudal furci are 0.37 to 0.43 mm.
(stem) and 0.23 to 0.31 mm. (furci). Both these schistosome cercariae are, therefore, longifurcate. Furthermore, Talbot (1936, p. 379) has given a table showing the lengths of caudal furci and the stem for four schistosome cercariae and from his table it is clear that the furci are much too long to bring these cercariae into the brevifurcate group. Macfarlane and Macy (1946, p. 281) describe long caudal furci in the schistosome cercaria, Cercaria oregonensis. To my mind, it appears that the overemphasis on the character of caudal furci has introduced a good deal of confusion in understanding the true affinities of the furcocercariae.

From all that has been said above it is clear that the classification of furcocercous cercariae is still far from satisfactory. The sharply restricted groupings based on the character of the tail as suggested by various workers are rather arbitrary and may have to be given up in course of time. There is an inherent risk of misleading workers in the elucidation of the life-cycle of two cercariae which may belong to the same family of trematodes though differing in the character of their tail. In order to have a better understanding of the group of furcocercous forms as a whole and for judging closer affinities, a general resemblance in both the so-called "larval" and "adult" characters may perhaps prove more useful than that in the characters of the caudal structures alone.
Text Fig. 15. - Sporocysts of *CERCARIA DUDDINGSTONI* n.sp. from the hepatopancreas of *Valvata piscinalis* showing developing cercariae.

Text Fig. 16. - *CERCARIA DUDDINGSTONI* n.sp. enlarged drawing showing morphological details. (Body spines not shown.)

cer. - cercaria; e.b. - excretory bladder; e.v. - excretory vessel; f.c. - flame cell; o.su. - oral sucker; p.gl. - penetration gland; p.gl.d. - penetration gland duct; ph. - pharynx; st. - stylet; t. - tail; v.su. - ventral sucker.
**Cercaria Duddingstoni** n. sp

**Sporocyst**

The cercaria develops in elongated oval sporocysts which are often present in very large numbers and arranged usually in bunches in the hepatopancreas of the snail. The walls of the sporocysts adhere to one another and in many cases it is not easy to isolate complete individuals. When liberated from the tissues of the snail, the sporocysts are light grey in colour, extremely thinwalled and showing the contained cercariae. They measure from 0.35 to 0.45 mm. in length and 0.15 to 0.2 mm. in breadth (Text Fig. 15). A sporocyst may contain 3 to 12 fully developed cercariae besides a few oval germ balls or incompletely developed cercariae. No aperture or birth-pore has been noticed on the surface of the wall of the sporocyst through which the ripe cercaria might escape. In certain cases I noticed the stylet at the oral sucker of the cercaria piercing through the wall of the sporocyst and making it possible for the cercaria to emerge at this point.

**Structure of the Cercaria**

The cercaria is greenish-white in colour and is exceedingly contractile with the result that the proper shape of the body is difficult to observe. Generally the body and the tail of cercaria are flexed in a
characteristic manner when in movement. The body (Text Fig. 16) is ovoid to globular in shape and the tail which is stumpy has wrinkled margins. The cuticle is sparingly covered with minute spines and these are more prominent towards the anterior end. The body varies from 0.09 to 0.13 mm. in length and 0.075 to 0.095 mm. in breadth, depending upon the degree of contraction. The oral sucker which is sub-ventral in position measures 0.025 mm. X 0.022 mm. to 0.03 mm. X 0.025 mm. It has a feeble muscular wall but shows a conspicuously projecting stylet measuring 0.02 to 0.022 mm. in length. The stylet is shaped like a pen-nib with very slight thickenings in the anterior part. The ventral sucker which is situated in the posterior half of the body measures about 0.015 mm. in diameter.

The oral sucker leads into a minute pharynx measuring 0.003 mm.; a prepharynx can not be made out in the cercaria, and there is no trace of the remainder of the digestive system usually seen in cercariae. Even the pharynx is difficult to see in some cases.

Three pairs of large glands, the so-called penetration glands, lie antero-laterally to the ventral sucker. They have a granular cytoplasm and large nuclei and are the most prominent structures seen in the body of the cercaria. Their outline is difficult to make out in living specimens due to constant
contraction of the body of the cercaria while in preserved specimens the granules disintegrate and do not show up clearly. The glands are best seen in specimens just before they emerge from the snail. If a snail containing mature sporocysts and cercariae is crushed on a glass slide, most of the cercariae which come out on the slide show the three pairs of glands very distinctly. The glands show a lobed or crenated outline. Three greatly convoluted ducts run from these glands along the lateral sides of the body to open separately into the cavity of the oral sucker.

Due to the minute size of the cercaria the excretory system is very difficult to observe. It was only after an examination of over a hundred cercariae extending over several days that the exact pattern of the system was worked out. The chief excretory canal of each side is formed by the union of an anterior and a posterior canal at a level just in front of the ventral sucker. From this point the chief canal runs along the side of the ventral sucker parallel to the lateral margin of the body and curves medially to open into the antero-lateral angle of the excretory vesicle. The latter is a reniform chamber and is situated at the base of the body of cercaria.

Each of the right and left anterior and posterior canals divide into three branches, which in turn terminate in flame-cells. Thus we find six flame-cells on each side of the body. A small median excretory
vessel arises from the posterior wall of the excretory bladder and runs into the tail but its ultimate course could not be traced out.

The tail varies from 0.07 mm. to 0.15 mm. in length, depending upon the state of contraction. There are no processes or spines on the tail.

The cercariae usually emerge in the afternoon and exhibit slow movement of their body but very soon they start a characteristic jerky movement and swim about. During this process the body of the cercaria is kept bent inwards on its ventral surface and the tail is bent to one side. Sometimes the cercaria shows a creeping movement. The suckers seem to help in this movement and a cercaria has often been observed to turn over along its longitudinal axis if lying on its dorsal surface (under a cover slip on a glass slide) before commencing the creeping movement.

ENCYSTMENT

Encystment of this cercaria could be observed by placing it in water and allowing the water to evaporate on a slide or when methyl blue or neutral red solution was added to the drop of water containing the cercariae. When contained in a large volume of water the cercariae encyst after 3 to 4 hours after emergence but sometimes they do not encyst but die after 10 to 14 hours. When some of the snails which were throwing out the cercariae were opened and
Text Fig. 17 - CERCARIA DUCKETT N.Sp. showing encystment. The tail has been shed from the rounded cyst.

e.b. - excretory bladder; p.gl. - penetration gland; p.gl.d. - penetration gland duct; t. - tail; v.su. - ventral sucker.
examined under a binocular microscope a few large encysted bodies were found in the hepatopancreas along with the large number of sporocysts and developing cercariae.

The process of encystment was carefully watched under the microscope and may be described as follows:-

The creeping movement of the cercaria slows down almost to a standstill, although the movements of the cell contents of the body continue, aided perhaps by the rapid expansion and contraction of the three pairs of glands. During these movements an increased flow of secretion swells up the ducts of these glands. The body of the cercaria, has, in the meantime, become almost rounded and the stumpy tail projects at one end. After a while, the glands shrink in size and their ducts get merged into a thick convoluted tubular structure but the openings of the ducts could be seen separately in the oral sucker. The stylet shows a slow piston-like movement and each motion brings about a flow of large quantities of the secretion which spreads over the body of cercaria and soon hardens. The tail hangs on to the cyst for a short while but later on it is shed as the cyst-wall becomes hardened at the point of its attachment (Text Fig. 17.). The cyst later becomes completely spherical, the whole process taking about half-an-hour to complete. All the structures of the cercaria are still clearly seen through the cyst-wall. The excretory bladder assumes
a typical reniform appearance and the excretory canals are also seen.

SYSTEMATIC POSITION OF THE CERCARIA

The cercaria described above possesses the following characteristic features:

1. Cercaria very small, 0.09 mm. to 0.13 mm. in body-length, produced in oval sporocysts, more than twice as long as they are broad.

2. Oral sucker about double the size of the ventral; no trace of oesophagus and intestinal caeca.

3. Stylet, 0.02 to 0.022 mm. long, with very slight lateral thickenings.

4. Three pairs of so-called "Penetration glands."

5. Excretory bladder, reniform when full; six pairs of flame-cells in the body.

Apart from Cercaria X₃ Harper 1929, no other xiphidiocercaria has been described from Valvata piscinalis either in Great Britain or on the Continent. Harper mentioned four pairs of penetration glands in his cercaria and, therefore, my specimen from Valvata piscinalis is different as it has only three pairs of glands. Furthermore, the oesophagus and intestinal caeca are not present in my specimens but Harper (1929) describes and figures these structures.

My specimens also show some resemblance to Cercaria helvetica XII (Dubois 1929) and to Cercaria
cordiformis (Wesenberglund 1934), both from the snail Bithynia tentaculata; but Cercaria helvetica XII has twice the number of flame-cells found in my specimen and Cercaria cordiformis has no pharynx and neither has it an excretory vessel in the tail.

I therefore consider the present form from Valvata piscinalis to be a new species. I name it Cercaria duddingstoni n. sp.

From the characters enumerated above Cercaria duddingstoni n. sp. comes under the group of Cercaria microcotylae (Lühe 1909). Sewell (1922) divided this group into several sub-groups and according to his scheme the present cercaria would come under the "Pusilla" group because of its possessing three pairs of glands. Sewell included Cercaria pusilla Looss, 1896 in his "Pusilla" group but it is doubtful if it really comes under this group; as, although Looss (1896) does not mention the number of penetration glands in his description, his diagram (Plate XVI) very distinctly shows only two pairs of glands.

Furthermore, Sewell's Cellulosa group of Cercariae microcotylae has two pairs of glands and Sewell included Cercaria cellulosa Looss (1896) under this group. But according to the description given by Looss (p.228) his Cercaria cellulosa may more often possess three glands on each side.

Sewell (1922) also included Cercaria vesiculosa with a body length of more than 0.2 mm. in his
"Vesiculosa" group of Cercariae microcotylae Lühe 1909. But Lühe (1909) apparently separated the microcotylous cercariae from the rest of xiphidiocercariae mainly on the ground that these forms were very minute with a body-length less than 0.2 mm. and he had, therefore, excluded Cercaria vesiculosa from this group. I also think that if we stick to the definition of Cercariae microcotylae Lühe 1909, Cercaria vesiculosa cannot be included in it. If, however, we amend Lühe's definition it will be difficult to distinguish these microcotylous cercariae from the other xiphidiocercariae.

Wesenberglund (1934) in his description of Cercaria pusilla mentioned only 2 pairs of glands. Dawes (1946, p.449) adopted Wesenberglund's account and included it under the "Pusilla" group of Sewell but this, to my mind, is not correct as Sewell defined his "Pusilla" group as possessing 3 to 4 pairs of penetration glands. In fact there is a good deal of confusion in the allocation of various xiphidiocercariae in Sewell's sub-groups of Cercariae microcotylae.

I do not, therefore, propose to put the present form described from Valvata piscinalis viz., Cercaria duddingstoni n. sp. in any of the sub-groups of Sewell (1922) as his groupings do not appear to be very satisfactory.
ENCYSTMENT IN XIPHIDIOCERCARIE

The phenomenon of encystment in xiphidiocercarie has been observed by various investigators but there appears to be some divergence of opinion among them. Dawes (1946, p.446) states that, "Encystment in the open does not occur, the cercaria entering the body of an invertebrate such as an insect or more rarely a fish."

A somewhat similar view was expressed by Wesenberglund (1934, p.89) when he said that, "Encystment in the open has never been observed."

It is generally assumed that because the xiphidiocercariae possess a stylet they pierce through the tissues of some intermediate host where they undergo encystment. Faust (1918, p.32), however, doubted this assumption and regarded the stylet as being too delicate for the purpose.

There is also ample evidence of encystment of xiphidiocercariae occurring in the soft tissues of the snail originally producing these cercariae and it is considered that in such cases although the cercariae may emerge out of the snail, they re-enter it for encystment. In other cases, however, the cercariae may encyst without emerging and in unfavourable circumstances encystment may even occur within the sporocyst.
Sewell (1922) seems to be contradictory on this point. For example, in his general remarks on the xiphidiocercariae he mentions that, "Encystment takes place in the intermediate host" and yet when describing Cercariae Indicae XIX (p.184) he states that, "the cercariae readily encyst when liberated from the parent sporocyst and set free in a watch glass."

This implies encystment in the open.

My observations on the present xiphidiocercaria show, as already pointed out, that encystment can occur in the open. Both Porter (1938) and Miller, E.L. (1936) also, on many occasions observed the encystment of the xiphidiocercariae in the open. Porter (p.331) states that, "As already mentioned, cystogenous cells are present and encystment occurs on vegetation." Further she states (p.333) that, "On the microscope slide the cercariae have encysted."

The observations of Miller are very interesting. He states (1936, p.58) that, "After removal of these forms from containers each formed a cyst wall. Tapping on the cover-glass caused this wall to break and the worm to crawl out. However, I have never observed these forms to encyst a second time, for they always died soon after leaving the broken cyst wall."

From these observations it appears that encystment in xiphidiocercariae is far from being a rigid
phenomenon. Both from my own observations and from those of the other workers, there is absolutely no question that encystment can take place in the open even in those species which encyst in an intermediate host such as the same or other snails. Miller's observation on the re-emergence of the metacercariae from a cyst are extremely interesting and might be worthy of much further study, particularly on the physiology of the so-called "penetration glands" and on the nature of the chemical composition of the secretion. It would seem that, since the cercariae of the same species can encyst, either in the open or after penetration into an intermediate host, there are either two types of secretion produced by these glands or that the secretion is capable of both lysis and cyst-formation.

At this stage, however, all that one can say is that the statements of Dawes (1946) and Wesenberglund (1934) that xiphidiocercariae do not encyst in the open are unwarranted.
Text Fig. 18- Three REDIAE of CERCARIA ECHINOPARYPHIUM Sp. from hepatopancreas of Valvata piscinalis showing length of gut in relation to length of body.

b.p.- birth-pore; g.- gut.
CERCARIA OF ECHINOPARYPHIUM SP.

REDIA

The cercariae develop in long cylindrical rediae which are light yellow or whitish-grey in colour and fill up the entire tissue of the hepatopancreas of Valvata piscinalis. The rediae exhibit very slight contractile movements, more prominent in younger stages. The length of the rediae varies from 0.7 mm. in younger forms to about 1.75 mm. in fully grown specimens. A birth-pore is seen on the lateral wall of the redia towards its anterior end. The body of redia is covered with smooth cuticle, except in the anterior fifth where it shows very minute spines. The pharynx is muscular and leads into a fairly long gut whose length varies in different individuals (Text Fig. 18). In young rediae it extends beyond the middle of the body and sometimes approaches the level of the locomotory processes or procruscula. In fully-grown rediae it is, on the other hand, relatively much shorter. With the development of a large number of cercariae within the body of a redia, the gut may be pushed to one side or may be folded so that its full length can not be made out except by a study of serial sections (Plate V, Figs. 1 and 2.). There appears to be no annular collar in these rediae but
Text Fig. 19 - CERCARIA ECHINOPARYPHIUM sp. drawn from live specimen showing the excretory system, spines on the collar and the fin on the tail. (Body spines not shown.)

a.e.c. - anterior excretory canal; c.e.c. - chief excretory canal; co.sp. - collar spines; e.b. - excretory bladder; o.su. - oral sucker; p.e.c. - posterior excretory canal; t. - tail; t.f. - tail fin; v.su. - ventral sucker.
the shape of the anterior end strongly suggests the presence of such a structure.

STRUCTURE OF THE CERCARIA

The cercaria (Text fig. 19) has a large white opaque body with a broadly flattened tail. Generally during movement, the body is flexed ventrally at the level of the ventral sucker. The body of the cercaria is covered over with a thin cuticle which is beset with rows of very minute spines extending from the anterior end to the level of the ventral sucker. Immediately behind the conical anterior end there are minute lateral projections which appear to be the precursors of the "collar" of the Echinostomes. This is beset with 45 spines, arranged in alternate rows and not interrupted on the dorsal side. The collar spines, in very young cercariae obtained by dissecting rediae, are not properly developed and even in some of the recently discharged cercariae their shape, size and arrangement is not very definite. The best specimens for the study of collar spines are those which have lived a free-swimming life for a couple of hours. In these the proper arrangement of the collar spines in two rows can be seen after treating them with a weak solution of methyl blue and the arrangement appears to be as follows:-
Body of

Text Fig. 20 - *Cercaria Echinoparyphium* sp. - greatly enlarged drawing showing the arrangement of the collar and body spines and other structures. (Excretory system not shown.)

co.sp. - collar spines; c.s.rud. - cirrus sac rudiments; cy.gl. - cystogenous gland; e.b. - excretory bladder; go.rud. - gonad rudiments; o.su. - oral sucker; ph. - pharynx; sp. - spines; v.su. - ventral sucker.
\[(3 \times 2) + 1 + (7 \times 2) + 3 + (7 \times 2) + 1 + (3 \times 2) = 45.\]

The spines in the anterior row are slightly shorter than those in the posterior row.

The body of the cercaria (Text Fig. 20) measures from 0.3 to 0.4 mm. in length and 0.175 to 0.225 mm. in breadth, depending upon the degree of its contraction. The oral sucker measures approximately 0.06 mm. in diameter and a much larger and muscular ventral sucker measures from 0.08 mm. X 0.07 mm. to 0.1 mm. X 0.05 mm. The latter is situated in the post-equatorial part of the body. The oral sucker leads into a small prepharynx, approximately 0.04 mm. in length followed by a globular muscular pharynx measuring about 0.03 mm. X 0.025 mm. The oesophagus measures approximately 0.13 mm. and divides in front of the ventral sucker into the two intestinal caeca which extend almost up to the posterior end of the body.

Large cells, the cystogenous glands containing dark granules, fill up the body of the cercaria and mask the rest of the structures so that the excretory system is very difficult to see completely. In some cases rudiments of gonads and a cirrus sac are also visible.

However, it is possible to recognize an excretory bladder which is somewhat squarish in shape and into this open the two chief excretory canals (Text Fig. 19). These extend lateral to the ventral sucker and forwards
as far as the level of the pharynx. Dark granules fill up these two chief excretory canals which are slightly dilated in the region of the oesophagus. At the level of the pharynx, these canals form a loop and run backwards, practically along the same course as before till they reach the posterior level of the oesophagus where it is possible to see in some specimens, the anterior and the posterior canals uniting together to form these two chief excretory canals.

The tail of the cercaria measures from 0.25 mm. to 0.42 mm. in length and is provided with an extremely small, hyaline and thin membranous fin which runs along one side of the posterior half of the tail. In the contracted state it appears as an undulating membrane (Plate V, Figs. 3 and 4). This fin was difficult to see in freshly emerged cercariae and was never seen in the young cercariae obtained by dissection of the rediae. Indeed it only appears clearly in the cercaria which have been swimming freely in water for a couple of hours. This structure, probably, attains its development sometime after the cercariae have emerged from the body of the snail.

The cercaria swims about with a powerful movement of the tail but the strokes are slow and the progress consequently not very rapid. The cercaria also exhibits creeping movements but generally only when the tail is shed.
ENCYSTMENT AND METACERCARIA

During the course of this investigation the writer found, on removing the shells of some *Valvata piscinalis*, certain tail-less cercariae, along with a few others with tails still intact, showing slow creeping movements over the mantle of the snail. At the same time they discharged large quantities of a viscid fluid which restricted their movement and very soon glued them to a spot over the mantle. The body of the tail-less cercariae folded on itself and became more globular in shape while the viscid secretion formed a layer or coating over its surface. The metacercaria, as it may now be termed, thus became encysted. In the beginning, the cyst has an irregular oval shape but after some time it assumes an almost spherical shape with a smooth surface.

The creeping tail-less cercariae were seen both in snails which had shown emerging cercariae previously and in other snails which did not show any infection of their hepatopancreas when dissected out and their viscera examined. Apparently the cercariae which had become tail-less had reached these latter snails from outside.

It was also possible to infect young *Valvata piscinalis* snails, reared in the laboratory, with emerging cercariae. In these the spherical metacercarial cysts showed full development in about
6 to 10 days.

The metacercarial cysts usually occur on the mantle in groups of 50 to 60 or more, depending upon the degree of infestation. They are of variable sizes, from 0.11 mm. × 0.12 mm. to 0.17 mm. × 0.19 mm. In one cluster of 50, some were observed to be of smaller size than the others. The cysts are more or less transparent and the contained metacercaria with its collar of spines and suckers can be clearly seen through the cyst-wall. In a few cases the metacercaria was pressed out of its cyst wall and on examination it showed 45 collar spines very distinctly.

FEEDING EXPERIMENTS ON BIRDS

In order to study the further development of the metacercaria into an adult trematode, two feeding experiments were performed on chickens.

In the first experiment, a chicken about 11 weeks old was fed with 23 metacercarial cysts along with the tissues of the snail, Valvata piscinalis. After a fortnight the bird was killed and dissected. Only one adult Echinostome was recovered from the intestine of the bird. The bird had been kept on a diet which precluded the possibility of natural infection.

In the second experiment, a chicken about 12 weeks old was kept without food for two days and then
fed with 60 metacercarial cysts. The cysts were carefully scraped by a sharp scalpel from the tissues of the snail and squirted into the oesophagus of the bird with a little water by means of a pipette. After a lapse of three days during which the bird had been kept on a low diet, a second feeding with 37 cysts was done in the same way. The bird was kept on low diet during the next fortnight and was given no food for 24 hours before being killed. During this period faecal examination of the bird was done twice but no eggs of trematodes were recovered.

On post-mortem examination, 21 days after the last feeding, with cysts, eleven specimens of an Echinostome were recovered from the anterior part of the intestine. The trematodes were active and well developed; eight of them contained yellowish-brown eggs in the uterus.

An examination of the trematode showed it to be a member of the genus *Echinoparyphium*. There were 45 collar spines, and their arrangement was exactly the same as that in the cercarial stage. It resembled in most of the features the species, *Echinoparyphium recurvatum* and *Echinoparyphium baculus*, but differed from the former in having smaller eggs and from the latter in possessing prominent spines on the body-cuticle.
SYSTEMATIC POSITION OF THE CERCARIA

The cercaria described above has the following diagnostic features:

1. Cercaria is produced in long cylindrical rediae whose anterior fifth body is covered with minute spines.

2. Body of cercaria (excluding tail) has a length from 0.3 to 0.4 mm. and a breadth from 0.175 to 0.225 mm. Body covered with minute spines, arranged in transverse rows, as far back as the level of the ventral sucker.

3. Collar with 45 spines, arranged in alternate rows and not interrupted on the dorsal side; spines in the two rows differing slightly in size.

4. Large cystogenous cells fill up the entire lateral field.

5. Tail provided with a thin membranous fin on the posterior half of its length.

From the characters enumerated above, this cercaria belongs to the group of Echinostome cercariae. So far only two Echinostome cercariae have been described from *Valvata piscinalis*, one viz. Cercaria of *Echinoparyphium recurvatum* by Harper (1929) and the other viz. Cercaria abyssicola by Wesenberglund (1934).

Harper's specimen is narrower in shape and has 43 to 45 collar spines. The oral and ventral suckers
and the pharynx are smaller than in my specimens from *Valvata piscinalis*. Furthermore no tail-fin is present in Harper's specimen.

Wesenberglund's specimen has only 30 collar spines and it is not possible to imagine Wesenberglund missing 15 collar spines.

It is, therefore, quite clear that both Harper's and Wesenberglund's cercariae are different from my species.

Besides these cercariae there are a few others (from snails other than *Valvata piscinalis*) viz., *C. helvetica* XXVI, *C. helvetica* II, *C. spinifera*, and *C. echinatoides* which appear to show some resemblance to my species.

Of these, *C. helvetica* XXVI (Dubois 1929) has been mentioned as possessing 45 (4+18+1+18+4) collar spines but unfortunately this cannot be compared with my species because of its incomplete description. Dubois (1929, p.51) own description is: "Malheureusement, nos notes très incomplètes ne permettent pas d'en faire une description suffisante."

_Cercaria helvetica_ II has no membrane on the tail, it has oral and ventral suckers of almost equal size and the grouping of the collar spines is 5+17+1+17+5. It is, therefore, quite different from my specimen.

Of the other two cercariae viz., *Cercaria spinifera* and *Cercaria echinatoides*, the former has 40 to 45 collar spines, oral and ventral suckers of equal
size, cystogenous cells arranged in two clusters and six small projecting processes just in front of the oral sucker. The latter species viz. C. echinatoides is described as possessing more than 40 collar spines (which may mean any number beyond it). Furthermore, in this species the spines on the collar in both the oral and aboral row have the same size and according to Wesenbergglund (1934, Plate VII, fig. 1) there are four ventrolateral spines on each side which are very large from the rest.

Moreover both C. spinifera and C. echinatoides have a fin running on both sides of their tail and are, therefore, different from my specimen from Valvata piscinalis.

As already stated, it was possible to trace the development of my species through to the adult stage. The adult obtained undoubtedly belongs to the genus Echinoparyphium. There are only two species of Echinoparyphium with a collar of 45 spines which have been recorded from Great Britain. These are E. recurvatum and E. baculus.

Since the cercaria of E. recurvatum has been shown repeatedly by Mathias (1927), Harper (1929) and Rasin (1933) to be devoid of a fin on the tail, it is not possible to regard my specimen which possesses a tail fin as identical with that cercaria.

As already stated, the adult fluke obtained by me experimentally resembles E. baculus in almost all its
features, but the latter is according to Dietz (1910) devoid of body spines and these are very prominent in my specimen. Further, since the cercaria of E. baculus has not been described and can, therefore, not be compared with the cercaria obtained by me, it is difficult to relate my specimen to E. baculus with any certainty.

It has, therefore, been thought advisable to regard the cercaria obtained by the writer from Valvata piscinalis as a new species, belonging to the genus Echinoparyphium. It is named Cercaria Echinoparyphium sp. n. sp.

AGE OF REDIA AND LENGTH OF ITS GUT IN ECHINOSTOMES

Lühe (1909) thought that the length of the gut in the rediae of Echinostomes varied with the different species and he used this character as a basis for classifying them into sub-groups. Recently, Rasin (1933) also thought that the length of the digestive tube probably formed a generic feature in the rediae.

Sewell (1922, p. 127) and Wesenberglund (1934, p. 59) thought that the length of the gut of the rediae of some Echinostomes depended upon their producing either daughter rediae or cercariae. Unfortunately these authors do not seem to be clear on this point since both their descriptions and their figures are contradictory. They describe and show in the figures
(Sewell 1922, p.133 and Wesenberglund 1934, Plate IX, Figs.2 and 5) an almost equally proportionate length of the gut both in the rediae-producing rediae and cercaria-producing rediae.

The writer's observations, as based on the present investigation, show that the length of the gut both in the mother and daughter-rediae increases gradually when the rediae are young and feeding. Later on when the rediae start propagating, either daughter-rediae or cercariae, they stop feeding but continue to increase in length, especially towards their posterior end, probably as a result of the pressure produced by the growing germballs. During this increase in length, the gut, which by this time has finished its function of feeding, remains almost constant in size. In matured rediae the gut gets folded and pressed against the side due to the pressure of the contained cercariae; it is therefore not properly seen and its actual length can only be revealed in many cases by examination of serial sections.

The early investigators on larval Trematoda do not appear to have emphasized the age-gut relationship in the rediae. But on reading their descriptions it is found that this relation exists not only for Echinostomes but in Amphistomes as well. For example the relative lengths of the gut given for young and old rediae of some Amphistomes both by Sewell (1922, p.72) and Bennett (1936, p.61) suggest strongly that
there is a diminishing ratio between the length of the gut and that of the body as the redia becomes older. Whether a similar relation exists in other groups of redia-producing trematodes may be well worth investigation.

It may, however, be stated that it would be a mistake to attach any value to the length of the gut in distinguishing the rediae in Echinostomes as has been done by some workers.
EXPLANATION OF PLATE II.

LETTERING

c.f. - caudal furca; s. - stem.

Fig. 1. - Photomicrograph of an early stage in the development of *Cercaria valvatae* n. sp., showing rudiments of caudal furci. (X280)

Fig. 2. - Photomicrograph of a later stage in the development of *C. valvatae* showing lengthening of the caudal stem and formation of stump like caudal furci. (X280)

Fig. 3. - Photomicrograph of a young *C. valvatae*, showing division into body, stem and caudal furci. There is a constriction at the base of the caudal furci. (X280)

Fig. 4. - Photomicrograph of a fully formed immature *Cercaria valvatae* n. sp. with well developed body, stem and caudal furci. Constriction at the base of furci disappearing. (X280)

Fig. 5. - Photomicrograph showing *Cercaria valvatae* n. sp. attacking the body of a leech (*Helobdella stagnalis*). (X 90)

Fig. 6. - Photomicrograph of a stained balsam preparation of a leech, showing partial penetration of *C. valvatae*. (The stem and furci have been shed.) Note the prominent ventral sucker. (X365)
EXPLANATION OF PLATE III.

LETTERING

cy. - cyst; o. su. - oral sucker; v. su. - ventral sucker.

Fig. 1. - Photomicrograph of a part of the body of a leech, Helobdella stagnalis after six hours exposure to Cercariae. Body is full of dark metacercariae. (X60)

Fig. 2. - Photomicrograph of the blood sinus of an infected leech, showing a metacercaria in situ. (X365)

Fig. 3. - Photomicrograph of a part of the body of a leech, showing cyst formation around the metacercaria. (X60)

Fig. 4. - A very early tetracotyle (about 2 weeks old) stage taken out of a cyst. (X165)

Fig. 5. - A later stage in the development of tetracotyle (about 18 days old.) The oral and ventral suckers have become muscular (body folded). (X125)
EXPLANATION OF PLATE IV.

LETTERING

cy. w. - cyst wall;
cy. - cyst;  o. su. - oral sucker;  n. p. - posterior part;
v. su. - ventral sucker.

Fig. 1. - Photomicrograph from a balsam preparation of a part of the body of a leech 28 days after infection. The cysts are prominent rounded bodies. (X60)

Fig. 2. - Photomicrograph of a fully formed tetracotyle (about 4 weeks old) removed from the cyst. (balsam preparation.) (X280)

Fig. 3. - Photomicrograph of a transverse section through the body of an infected leech, showing the tetracotyle in sagittal section within the cyst. (X280)
EXPLANATION OF PLATE V.

LETTERING

g.-gut; ph.-pharynx; t.f.-tail fin.

Figs. 1 and 2.- Photomicrographs of two of the serial sagittal sections through the anterior end of a redia of Cercaria Echinonaryphium sp., showing the extension of the gut. (X125)

Fig. 3.- Photomicrograph of the tail of Cercaria Echinonaryphium sp. showing the membranous tail fin. (X280)

Fig. 4.- Photomicrograph of the tail of Cercaria Echinonaryphium sp. showing the fin resembling an undulating membrane near the posterior end of the tail. (X280)
PART III

ON THE MORPHOLOGY AND DEVELOPMENT OF FIVE LARVAL
HELMINTHS FROM THE AMPHIPOD, GAMMARUS PULEX.

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INTRODUCTION

Gammarus pulex as a carrier of larval helminths has been known for a long time but, so far as I am aware, only a few larval stages of helminths have, hitherto, been described from this crustacean in Great Britain. Brown (1926) gave an account of a metacercaria and Harper (1929) described the encysted stage of his Cercaria X₁ from Gammarus pulex. Baylis (1931) obtained three larval helminths, viz., two metacercariae and one nematode larva, from the same host.

During my investigations I obtained from Gammarus pulex five different larval helminths, of which two cestode larvae are new to science and two Acanthocephalan larvae are described for the first time in Great Britain. The metacercaria which had already been recorded by Brown (1926) and Harper (1929) is redescribed in order to elucidate its morphological details and throw light on its proper systematic position. I have also succeeded in tracing the development of the two larval Acanthocephala to their respective adults (viz. Polymorphus minutus and Echinorhynchus truttae.)
MATERIAL AND METHODS.

Gammarus pulex is fairly common in the Braid Burn, Edinburgh, throughout the year and specimens were obtained at frequent intervals and brought alive to the laboratory in glass tubes. They were then transferred to small aquaria in which they thrived well if kept in a small amount of water so as to ensure direct aeration. A little sterile organic debris was added to this water.

Larval stages were obtained by dissection of the crustacean in small flat glass dishes in fresh-water. The different stages of the larvae were studied alive, under slight pressure of the cover slip, with and without intra-vitam staining and sketches made, with the help of the Camera lucida, both from living and preserved and stained specimens. In some cases the larval stages were kept in vitro at room temperature and some at 104° F and observations made on their further development.
Table III showing the dates of collection and the number of *Gammarus pulex* examined and found infected with different types of helminth larvae.

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<th>Number examined</th>
<th>Number infected</th>
<th>Type of larva</th>
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</table>

Total number of *Gammarus pulex* examined = 1225
Number of *G. pulex* infected = 229
Percentage of *G. pulex* infected = 18.7%

Percentage of infection with Polymorphus minutus = 7.3%
Percentage of infection with Echinorhynchus truttae = 0.98%
Percentage of infection with Cysticercoid braidburni = 0.57%
Percentage of infection with Cysticercoid gammari = 0.33%
Percentage of infection with Metacercaria Plagiorchis sp. = 9.7%

* (There were three cases with double infections of Metacercariae and Acanthocephalan larvae.
This explains the discrepancy in the percentages given above.)
Text Fig. 21 - Metacercaria of *PLAGIORCHIS* Sp. within the cyst, showing morphological details.

o.su. - oral sucker; ov. - ovary; ph. - pharynx; st. - stylet; tes. - testes; v.su. - ventral sucker.
METACERCARIA OF *PLAGIORCHIS* SP.

**STRUCTURE OF THE CYST**

Cysts containing metacercariae were found singly or in greater numbers in the body-cavity of *Gammarus pulex*. These (Text Fig. 21) are oval or spherical bodies, more or less transparent and measure from 0.35 mm. X 0.29 mm. to 0.4 mm. X 0.265 mm. The metacercaria lies doubled up inside the cyst and measures approximately 0.56 mm. in length and has a body-wall covered with very minute spines. With slight pressure of the cover slip, most of the internal structures of the metacercaria are visible through the transparent cyst-wall. The oral sucker, which is large and muscular, measures 0.13 mm. X 0.12 mm. to 0.14 mm. X 0.12 mm. The ventral sucker, situated about the middle of the body, is much smaller, measuring 0.06 mm. to 0.08 mm. in diameter. Immediately behind the oral sucker is seen a small muscular pharynx measuring approximately 0.06 mm. X 0.04 mm. In most cysts, the reproductive organs of the future trematoda have already made their appearance in the posterior third of the body of metacercaria. Usually the testes are visible at an earlier stage in the development of the metacercaria than the ovary. The two oval testes lie close together, the anterior measuring approximately 0.035 mm. X 0.05 mm., the
posterior approximately 0.08 mm. x 0.07 mm. The ovary which is almost spherical, lies between the anterior testis and the ventral sucker and measures approximately 0.05 mm. in diameter. The stylet of the cercarial stage is retained, very often lying as a free, detached structure within the cyst. In shape it looks like a pen-nib and measures 0.025 mm. long.

Quite a number of free metacercariae which may be termed agamodistomes or immature flukes were recovered from *Gammarus pulex*. This shows that, after a certain stage in development has been attained, the thin cyst-wall ruptures and liberates the young trematode. The actual process of emergence was observed in the laboratory by placing some of these cysts in fresh-water or in 0.5% sodium chloride solution at room temperature for a couple of hours.

**STRUCTURE OF THE AGAMODISTOME**

The emerged metacercaria or agamodistome shows almost all the organs of the adult except the uterus and the eggs, and the relative positions of various organs within the body can be made out with greater accuracy. Text Figure 22 shows one of these agamodistomes with a broad anterior end which narrows slightly towards the posterior end. It can be seen that the oral sucker is large and conspicuous and measures approximately 0.14 x 0.11 mm. This leads into
Text Fig. 22 - An excysted agamodistome of *Plagiorchis* Sp. showing morphological details (Dorsal View)
c.s. - cirrus sac; e.b. - excretory bladder;
o.su. - oral sucker; ov. - ovary; ph. - pharynx;
tes. - testis; v.gl. - vitelline glands;
v.su. - ventral sucker.
a distinct prepharynx followed by a globular muscular pharynx and an extremely short oesophagus which bifurcates into the two broad intestinal caeca. The ventral sucker lies at a distance of 0.28 mm. from the anterior end and measures 0.06 mm. in diameter. Closely applied to the right side of the ventral sucker is a very conspicuous crescent-shaped cirrus sac which opens at the genital pore 0.025 mm. in front of the ventral sucker in the median line. The testes lie slightly obliquely in the posterior third of the body. The anterior testis measures 0.075 mm. X 0.06 mm., the posterior 0.09 mm. X 0.07 mm. The ovary measures 0.045 mm. in diameter. The vitelline glands are faintly developed in the lateral field and appear as small granular bodies extending from about the level of the pharynx to the posterior end. The main excretory stem is Y-shaped and extends between the testes and bifurcates immediately in front of these organs. There is no distinct excretory vesicle although the posterior part of the main stem shows a slight swelling. The main stem is very long and the two lateral limbs very short. The vesicula seminalis and other associated glands are not clearly differentiated at this stage. No eye-pigment granules or spots are present.
The metacercaria described overleaf has the following diagnostic features:

1. Oval or rounded cysts, 0.35 mm. to 0.4 mm. long and 0.265 mm. to 0.29 mm. broad.

2. Body wall of the metacercaria slightly spiny.

3. Oral sucker almost twice as large as the ventral; oesophagus extremely short.

4. Agamodistome shows almost fully formed gonads; testes, two, oblique, behind the ovary in the posterior third of the body.

5. Cirrus sac, crescent-shaped, closely applied to the ventral sucker and terminates in a median genital pore.

6. Vitelline glands, small, granular, distributed in the lateral field between the pharynx and the posterior end.

7. Excretory system Y-shaped.

From the characters enumerated above, especially the Y-shaped excretory system with its sinuous main canal passing obliquely between the testes; the much greater size of the oral sucker as compared with the ventral, and the general disposition of the gonads, cirrus sac and the vitelline glands, I consider this metacercaria to be an encysted stage of a Plagiorchis sp.

Several metacercariae from Gammarus pulex have
been described in Great Britain. Baylis (1931) described two metacercariae, one of which he regarded as a larval stage of *Crepidostomum farionis*. He did not give any detailed account of this metacercaria but from his diagram the oral sucker appears to have lobes and thus it differs entirely from the other metacercariae recorded from *Gammarus*. Furthermore, the oral and ventral suckers of his metacercaria are equal in diameter and it also shows eye-pigment granules which were not observed in my specimen.

The second metacercaria obtained by Baylis was regarded by him as being identical with *Distomum agamos* of von Linstow (1872). In this form the ventral sucker is much larger than the oral and the body-cuticle is devoid of spines.

Hence it is not possible to relate the species described in this paper to any of those described by Baylis (1931) from *Gammarus*.

Brown (1926) and Harper (1929) have also described metacercaria from *Gammarus* and both regarded their species as identical with *Distomum pulicis* of von Linstow (1892).

Harper (1929), however, doubted the identity of his metacercaria with that of Brown (1926) but the differences pointed out by Harper, viz., in the size and colour of the sporocysts and the length of the oesophagus in the cercaria, seem to be rather insignificant. It may be pointed out that in a
diagram given by Harper (1929), the excretory system is V-shaped but in his description it is mentioned as Y-shaped. I have studied the excretory system in my specimens and find it definitely Y-shaped. In spite of this discrepancy I consider the metacercaria described in this paper as being the same as that described by Brown and that by Harper; although there are a number of minor differences in the measurements given for the various organs and in the position of cirrus sac. Whether the present form can be related to Distomum pulicis, von Linstow (1892) as mentioned by Brown and by Harper or to Distomum gammari, von Linstow (1877) is far from clear as von Linstow gave only a very brief description of his metacercariae and neither the position of the cirrus sac nor that of the ovary is clear from his account.
LARVAL CESTODA

A number of larval Cestoda have been described from invertebrate hosts in Great Britain [Rosseter (1891, 1893, 1896, 1897); Nicoll & Minchin (1911); Harper (1930); Oldham (1931); Rayski (1945)] but strangely enough none so far from the Amphipoda (Malacostraca) which have been reported as carriers of larval Cestoda on the continent, (Hamann 1890, 1891; von Linstow 1892; Joyeux 1926).

Rosseter (1897) who described several larval Cestoda from Copepods and Ostracods did not find any of them in Gammarus (Amphipoda). Baylis (1931) also examined about 100 specimens of Gammarus but did not obtain larval Cestoda from this host. Leiper (1936) stated that a number of hymenolepid cestodes e.g. H. collaris, H. fasciata, H. gracilis, H. microsoma, H. setigera, and H. tenuirostris of aquatic birds all reached the infective stage in Cyclops (Copepoda). It is, therefore, obvious that Copepods as vectors of larval Cestoda among the crustaceans have attracted most attention.

During the course of the investigations two different Cysticercoids were obtained from Gammarus pulex (Amphipoda) and these appear to be new to Science and are described in the following pages.
Text Fig. 23 - **CYSTICERCOID GAMMARI** n.sp. drawn from a fresh specimen, showing the layers of the cyst-wall and a part of the tail.

Text Fig. 24 - A part of **CYSTICERCOID GAMMARI** n. sp. drawn from a fresh specimen, showing the arrangement of suckers and hooks (greatly enlarged.)

c.b. - calcareous bodies; hk. - hooks; ros. - rostellum; su. - sucker; t. - tail.
These cysticercoids (Plate VI, Fig. 1.) are not of common occurrence in *Gammarus pulex* and when present they are about 15 to 20 in number. Each cysticercoid is provided with a long tail which is several times longer than the body and serves as a process for attachment to the tissues of the host either to the external surface of the intestinal wall or to the neighbouring connective tissue. The cuticle of the tail is not continuous with the outer covering of the cyst in this case but is seen to pierce the cyst wall to join the contained cysticercoid. (Text Fig. 23.)

The body of the cyst is oval or sub-spherical in shape and appears dark due to the interior being filled with dark refractile granules. A large number of oval calcareous bodies are also present inside the body of the cysticercoid. In the fresh condition the cyst measures approximately 0.31 mm. X 0.33 mm. and has a double wall, the outer being gelatinous, more or less hyaline, and formed of fibrous tissue; and the inner granular and forming a close fitting envelope round the cysticercoid. The rostellum (Text Fig. 24), which is an elongated elliptical structure lying obliquely between the suckers, measures 0.096 mm. in length and 0.027 mm. in breadth at the level of the hooks. There are 10 hooks, slightly curved and measuring about 0.025 to 0.027 mm. in length and approximately
0.0038 mm. in width. There are four suckers which are muscular, and provided with thick walls. They are ovoid in shape and measure approximately 0.07 mm. x 0.065 mm. in diameter. There are no spines on the suckers.

**SYSTEMATIC POSITION**

The cysticercoid described above has the following diagnostic features:

1. Almost spherical cysts, provided with a very long tail, and measuring 0.33 mm. x 0.31 mm.
2. Suckers, four, about 0.07 mm. x 0.065 mm. in diameter and not provided with spines.
3. Rostellar hooks, slightly curved, 10 in number and measuring 0.025 to 0.027 mm. in length and 0.0038 mm. in width.

Several cysticercoids with 10 rostellar hooks have been described so far and some of these have been reported from *Gammarus pulex* on the Continent. Most of these cysticercoids, however, differ from the cysticercoid described above in the size of the rostellar hooks, size of the suckers and shape and size of the cysts. The only forms which approach nearest to my specimens, in general characters, are *Cysticercus tenuirostris*, *Cysticercus echinocotyle*, *Cysticercus fimbriariae*, *Cysticercus mirabilis*, and
Cysticercus 'B' Harper.

Of these Cysticercus echinocotyle and Cysticercus 'B' Harper, apart from other differences, have spines on the suckers and can, therefore, be ruled out. The cyst of Cysticercus tenuirostris is much smaller (practically half) in size than that of the cysticercoid described in the preceding pages. Moreover, the rostellar hooks of C. tenuirostris are also much smaller and even in its corresponding adult viz. Hymenolepis tenuirostris, they have never been found to attain a length of more than 0.023 mm. while in the cysticercoid described in the preceding pages the hooks are 0.025 to 0.027 mm. in length. It is inconceivable to refer a cysticercoid to an adult which shows smaller rostellar hooks than those of its larva. Cysticercus mirabilis has rostellar hooks of an entirely different shape and of a smaller size than those of my specimens. Its body is also smaller than that of the latter. Lastly my specimens differ from Cysticercus fimbriariae in having a larger body and larger rostellar hooks.

It seems clear, therefore, that the present cysticercoid cannot be related to any of the cysticercoids previously described and there appears to be every justification for the creation of a new species. It is named Cysticercoid gammaris n. sp.
FEEDING EXPERIMENT ON A BIRD

An attempt was made to obtain the adult of *Cysticercoid gammari* n. sp. by feeding this cysticercoid to a chicken about 10 weeks old. In the experiment 25 cysticercoids were fed to the bird but on post-mortem examination after 3 weeks no trace of the cysticercoids was obtained. Owing to the relatively small numbers of cysticercoids available and due to the difficulty of obtaining suitable birds this feeding experiment could not be repeated.
Text Fig. 25 - An early stage of **CYSTICERCOID BRAINTURNI** n.sp. showing the short tail.

Text Fig. 26 - **CYSTICERCOID BRAINTURNI** n.sp. showing the ring of hooks and muscular suckers.

Text Fig. 27 - A few rostellar hooks of **CYSTICERCOID BRAINTURNI** n.sp. showing the characteristic shape.

hk. - hooks; ros. - rostellum; su. - sucker; t. - tail.
CYSTICERC OID BRAID BURNI N. SP.

This cysticercoid was also of infrequent occurrence but when present there were usually 12 to 36 individuals found attached by their small tail to the external surface of the intestine of *Gammarus pulex*. (Plate VI, Fig. 2; Text Fig. 25). The cysts are oval to elliptical in shape and measure from 0.23 to 0.28 mm by 0.16 to 0.175 mm. The tail breaks off easily leaving the free tail-less cysts floating in the body cavity of the intermediate host (Plate VI, Fig. 3). The body of the cysticercoid (Text Fig. 26) is enclosed within a closely fitting membrane which is continuous with the cuticle of the tail. The tail is about 1 to 2 times the length of the body, and usually contains granular protoplasm. There are four suckers with muscular walls, devoid of spines and measuring 0.07 to 0.08 mm by 0.03 to 0.047 mm. The hooks on the rostellarium enclose an area of about 0.06 mm x 0.045 mm. The number of hooks varies between 55 to 59. Each rostellar hook (Text Fig. 27) is a minute bifid structure of characteristic shape. The "blade" of the hook is curved and tapers to a sharp point. It is longer than the "guard" and the latter is longer than the handle. The length of the hook from its tip to the base of the "handle" is approximately 0.01 mm and the length from the tip...
of the 'guard' to the base of the 'handle' is 0.0075 to 0.0085 mm.

SYSTEMATIC POSITION

The cysticercoid described above has the following diagnostic features:

1. Oval to elliptical cysts, provided with a short tail 1 to 2 times the length of the body.

2. Cysts measure 0.23 to 0.28 mm. by 0.16 to 0.175 mm.

3. Suckers, four, measure 0.07 to 0.08 mm. by 0.03 to 0.047 mm., and not provided with spines.

4. Rostellar hooks, bifid, 55 to 59 in number and measure 0.01 mm. in length.

Both in its habitat in *Gammarus*, as well as in its general structure, this cysticercoid appears to be closely allied to *Cysticercus integrus* Hamann, 1890.

The cyst of *Cysticercus integrus* measures 0.45 to 0.5 mm., its rostellar hooks from 0.017 to 0.018 mm. and the number of hooks varies according to Joyeux (1926) between 64 to 68. The present form, therefore, differs from *Cysticercus integrus* in having a body of about half the size of the latter and in having much smaller and fewer hooks.

Furthermore, as has been shown by Joyeux (1926), *Cysticercus integrus* with 64 to 68 rostellar hooks
develops into the adult *Haploparaksis dujardinii*. This cestode has, according to Krabbe, only 46 rostellar hooks; and it is said that the reduction in the number of hooks may perhaps be brought about by the falling off of the rostellar hooks during development. Even if *Cysticercus integrus* does develop to *Haploparaksis dujardinii*, the shape of the rostellar hooks of the latter is very different from that of the hooks of the present cysticercoid.

The cysticercoid described in the preceding pages is, therefore, regarded as a new species. It is named *Cysticercoid braidburni* n. sp.

No experiments on the further development of this cysticercoid could be carried out for want of a suitable bird for the purpose.

SOME GENERAL REMARKS ON LARVAL CESTODA FROM GAMMARUS

Although work on *Gammarus pulex* was based on specimens obtained from one stream viz., the Braid Burn, Edinburgh, certain points of interest may be mentioned here.
INCIDENCE OF INFECTION

Gammarus pulex as a vector of larval Cestoda in Great Britain is being recorded for the first time, but the incidence of infection is very low (vide supra: Table III). The maximum number of larvae obtained from a single specimen of this crustacean was 36. The two species of cysticercoids were never found in the same crustacean.

As regards seasonal variation of the cestode infection in Gammarus pulex, my observations do not point to any restricted or periodic infection. As will appear from the list of specimens examined (Table III), I obtained cestode larvae practically throughout the year, even during the very cold weather in the early part of February 1947. This shows that the extreme rigours of winter probably do not have any adverse effect on these crustaceans, much less on their internally lodged cysts (cysticercoids). However, this may not always be true. Baylis (1931) while looking for larvae of the cestoda Gyathocephalus in Gammarus examined more than 100 specimens and although he obtained larval Trematoda and larval Nematoda, no larval Cestoda were found to be present in those specimens. Baylis (1931) accounts for the absence of larval Cestoda from Gammarus by saying, "These individuals were collected in December, and it seems probable that this was not a suitable
time of the year, and that *Gammarus* taken in the spring or summer might prove more productive."

I have, however, recovered larval Cestoda from *Gammarus pulex* in Edinburgh during both December 1946 and February 1947.

**EMERGENCE OF THE SCOLEX OF CYSTICERCOID BRAIDBURNI N.SP.**

Another point of interest which may be considered here deals with the development of a cysticercoid into the adult cestode. It is generally believed that the cysticercoid must reach the intestine of the definitive host before it can hatch into a juvenile cestode. Southwell (1930, p. 24) stated that, "We have already noted that, except in *Hymenolepis nana*, the final host can only become infected with the adult by swallowing the larval form. The latter consists essentially of the head, or scolex, of the future worm, usually enclosed in one or more membranes. When this is swallowed, the membranes are digested and the larva is set free in the lumen of the digestive tract."

My observations on *Cysticercoid braidburni* showed that the emergence of the scolex may occur in the body cavity of the intermediate host or even *in vitro*. This takes place merely by a split in the wall of the cysticercoid and digestion of the membranes covering...
Text Fig. 28 - Emergence of the scolex of CYSTICERCOID BRAIDBURNI N.Sp. (in vitro).

Text Fig. 29 - Emerged scolex of CYSTICERCOID BRAIDBURNI N.Sp. from the bodycavity of Gammarus pulex (greatly enlarged - balsam preparation).

hk.- hooks; sco.- scolex; su.- sucker; t.- tail.
the cysticercoid does not seem to be essential.

When specimens of *Gammarus pulex* were dissected in order to obtain larval Cestoda, a number of small bodies (everted scolices) were found floating in the body-fluid of this crustacean. At first I took them for cestode larvae of another species as they looked so different from the tailed oval encysted cysticercoids. As, however, they were found along with the tailed forms in the same crustacean I thought they might be a modified form of the same. Closer and continued examination in vitro of the tailed cysticercoids, one day solved the mystery as I actually noticed the emergence of this vase-shaped body out of the tailed oval cyst by a split in its wall. (Plate VI, Fig. 4. Text Fig. 28.) The emerged scolex (Text Fig. 29) is larger in size than that usually contained within the cyst. It appears, therefore, that this emergence may be a normal process which can take place when the cysticercoid has grown to a certain size and is ripe for further development into the adult. Joyeux (1920, pp. 108-09) arrived at a similar result with *Hymenolepis diminuta* where the scolex emerged in vitro through a split in the cyst-wall.

Jouyeux (op. cit.) mentioned that for bringing about the emergence of the scolex in vitro it was necessary to treat the cysticercoid with a weak solution of caustic soda. In my in vitro experiments showing this phenomenon, only a 0.5% sodium chloride
solution was used. This phenomenon was also noticed while dissecting *Gammarus pulex* in fresh-water. It is, therefore, clear that treatment with caustic alkali, as suggested by Joyeux, is not a pre-requisite for the evagination of the scolex and that this phenomenon can take place naturally within the intermediate host after the scolex has attained a certain stage in its development.
LARVAL ACANTHOCEPHALA

INTRODUCTION

Our knowledge of the life-history of Acanthocephala is still meagre as compared to that of the other groups of helminths. The most comprehensive account of the life-cycle of an Acanthocephalan was published by Meyer (1933, 1938). Later workers, Helen Ward (1940), Kates (1943), and Moore (1946) have also contributed to our knowledge of the life-cycles of various Acanthocephala.

Besides this, observations have been made on the morphology and detailed histology of the various organs of the larval stages of Acanthocephala, notably by Hamann (1891) and Kaiser (1893).

Greeff (1864), who described some of the larval stages in the development of Polymorphus minutus (regarded by him as Echinorhynchus polymorphus) from Hammarus pulex, gave very sketchy diagrams, so much so that it is difficult to follow from his account the proper sequence of development. Hamann (1891) pointed out the limitations of Greeff's work and stated that the complex organization of these larvae could be better studied from their microtome sections. He, therefore, described the histology of the various embryonic structures in great detail but did not figure the
entire larvae in the various stages of development. Meyer (1933) in his monograph on Acanthocephala reproduced Greeff's figure to represent the juvenile stage of Polymorphus minutus but this diagram hardly shows any of the diagnostic features present in the larva at this stage.

Similarly Scheer (1935) and Steinsträsser (1936) mentioned that the larval stages found by them in Gammarus pulex were those of Echinorhynchus truttae but they gave no detailed description of those stages.

During the present investigation I obtained from Gammarus pulex two species of larval Acanthocephala, one developing into an adult in a bird, the other in a fish. The account which follows is a detailed description of the morphology of both these larval Acanthocephala, together with diagrams and photomicrographs of their various stages, and also of their development into the respective adults viz., Polymorphus minutus and Echinorhynchus truttae.
There has been some confusion regarding the use of a proper terminology for the various developmental stages of the Acanthocephala and this calls for some comment.

The development in the intermediate host from the time of infection up to the attainment of the final stage is very complicated and not marked by sharp stages of ecdysis or metamorphosis. Van Cleave (1937), however, distinguished two main stages in the development of an Acanthocephalan, basing his observations mainly on the larval stages of Macracanthorhynchus hirudinaceus. He proposed two names: Acanthor and Acanthella. He states, (p. 741). "the name Acanthor is here proposed for the larval Acanthocephalan which emerges from the embryonic membranes. This stage varies considerably in external appearance and in degree of internal organization among the various genera."

Further he states that, "the term Acanthella is here proposed to designate the series of immature stages of an Acanthocephalan which develop progressively within the body of the invertebrate host."

There is no doubt that the term "Acanthor" has been rightly used by later workers to denote the larval form which emerges from the embryonic membranes. The term "Acanthella", however, has been variously
used in a modified sense by different workers and some new terms have been introduced.

Faust (1939, p. 559) restricted the term "Acanthella" to cover only the infective stage of Acanthocephala but Kates (1943) used the term "Infective Acanthella" for the same stage.

Moore (June 1946, p. 259), however, mentioned that the term "Acanthella", as used by Van Cleave (1937), is a blanket term, covering a long period of development and at least two distinct stages. He, therefore, like Faust (op. cit.) restricted the term "Acanthella" to cover only the infective stage; and for the stage immediately preceding it he introduced a new term "Pre-Acanthella."

In defining the restricted term "Acanthella", Moore (op. cit.) mentioned that at this stage the proboscis reaches its full development and is invaginated within the proboscis receptacle (basing his observations on the larval stages of Moniliformis dubius.). In this case, as well as in the genus Macracanthorhynchus, the proboscis develops in an extroverted position and is later on introverted at the "Acanthella" stage.

Van Cleave (Dec. 1946, p. 516) pointed out that the term "Acanthella" should not be applied to the larval stage in the intermediate host after the proboscis is fully formed and functional. He stated that the metamorphosis is completed when the proboscis
becomes functional and this stage should, therefore, be designated "post-Acanthella" or Juvenile Acanthocephala. He defined this stage by saying that, "The terms Juvenile Acanthocephala or "post-Acanthella" may be used for the final stage in the arthropod host after the Acanthella has completed its metamorphosis and has attained rudiments of organs of the mature worm and tissues of the body-wall have lost their embryonic character."

It may be pointed out here that the terminology suggested by various workers has been based entirely on the developmental stages as seen in the species of the order Archiacanthocephala Meyer 1931. During the present investigations on the development of two forms viz., Polymorphus minutus and Echinorhynchus truttae, both belonging to the order Palaeacanthocephala Meyer 1931, the writer found certain characteristic differences which add to the difficulty of adopting a terminology on the above lines.

For example, in the species described in this paper, the proboscis and its hooks are developed originally in the introverted position. Furthermore, even at a stage when the proboscis is fully developed and functional and the reproductive organs almost completely formed, the embryonic dendritic branches which give rise to the integumentary vessels of the adult and the scattered large rounded nuclei are still present (Plate VIII, Fig. 2).
It becomes, therefore, impossible to apply the nomenclature suggested by previous investigators - in the sense in which it was originally used - to the larval stages of *Polymorphus minutus* and *Echinorhynchus truttae*.

Finally it may be pointed out that the term "Acanthella" is already preoccupied since it has been used twice in a generic sense (probably by mistake), once for a genus of *Sponges* (Schmidt, 1862) and a second time for a *Coelenterate* (Allman, 1883). It may, therefore, be expedient to give up the use of the term "Acanthella" for a larval stage of Acanthocephala, but perhaps it is better to wait until some more Acanthocephalan life-histories have been worked out, before adopting a new nomenclature. Until then the terms in current use are retained.

**PHASES IN THE DEVELOPMENT OF ACANTHELLA**

In the present investigation it was observed, both in the larvae of *Polymorphus minutus* and *Echinorhynchus truttae*, that there are three distinct phases of "Acanthella" through which the transformation from the "Acanthor" to the "juvenile Acanthocephala" takes place.

**Acanthella phase 1** - At this phase there is no differentiation of structures; the larva simply shows a large number of scattered rounded nuclei.
It appears to be a **Primordial** phase in the development of the Acanthella.

**Acanthella** phase 2 - Most of the organs of the Acanthella develop during this stage. It, therefore, appears to be a **Growth** phase.

**Acanthella** phase 3 - At this phase, the Acanthella has a fully formed proboscis with a full number of hooks and the gonads are clearly defined. It appears to be a resting stage awaiting the opportunity of infecting the definitive host. It is, therefore, considered as an **Infective** phase.

These three phases viz., the Primordial, the Growth and the Infective, are seen in the development of Acanthella of all the known forms, even in those of the order Archiacanthocephala Meyer, 1931. I have, therefore, no hesitation in suggesting the use of these "phase names" in order to specify particular stages and to enable a proper and connected description of their development to be given.
Text Fig. 30 - ACANTHELLA (Primordial phase) of Polymorpha minutus showing arrangement of nuclei.

ev. - envelope; nu. - nucleus.
LARVAL STAGES OF POLYMORPHUS MINUTUS (GOEZE)

ACANTHELLA PHASE 1 (PRIMORDIAL PHASE) -

(Plate VII, Fig.1.; Text Fig.30.)

This is the earliest stage which was obtained in the body cavity of *Gammarus pusillus*. The larva has a light yellow colour and is contained within a very thin membranous envelope which fits very closely around the embryo. At this stage the larva appears to grow very rapidly - as it was noticed that not only was there a slight increase in its size but also a change in the appearance of certain structures on keeping the larva in vitro for 24 hours. The larva measures approximately 0.47 mm. in length and 0.17 mm. in width. Several large rounded nuclei are present within the larva which also shows a faint demarcation of the body into an anterior and a posterior part. The nuclei are spherical bodies, arranged more or less in transverse rows in the body of the larva. Later on there appears another faint constriction in the anterior part and this gives rise to an anterior, a middle and a posterior part of the body, seen clearly at the next stage.
Text Fig. 31 - ACANTHELLA (Growth phase) of POLYMORPHUS MINUTUS, showing morphological details.

ev. - envelope; f.z. - fibrous zone; hk. - hooks;
lem. - lemnisci; lig. - ligament; m. - muscles;
pr. - proboscis; rep. rud. - rudiments of reproductive system; sp. rud. - rudiments of spines.
ACANTHELLA PHASE 2 (GROWTH PHASE)

(Plate VII, Figs. 2 and 3; Text Fig. 31.)

The larva, which is still within the membranous envelope, shows a well marked division of the body into an anterior, a middle, and a posterior part. The length of the larva is approximately 1.7 mm., of which approximately, the anterior part is 0.75 mm., the middle 0.76 mm. and the posterior 0.19 mm.

The proboscis and proboscis sheath have developed and remain in an invaginated position, and there are visible signs of the development of proboscis hooks. The neck of the larva does not show any spines on its external surface, although their rudiments are seen distinctly. There are two elongated, more or less flask-shaped, sacs known as the lemnisci and these are placed laterally in the anterior region of the larva. They show a large number of spherical nuclei in their wall.

The middle part of the larva is the widest and shows a division into a thickened outer fibrous zone and a granular, thin, inner zone. The latter is filled with a proliferating mass of cells, but does not show further differentiation of structures.

In the posterior narrowest part of the larva, rudiments of the reproductive organs are discernible in the form of a cluster of several large rounded nuclei. A thin strand of tissue, viz., the ligament
Text Fig. 32 - Anterior end of an ACANTHELLA (Growth phase) of P. MINUTUS, showing further growth and the disposition of various organs (greatly enlarged).

hk. - hook; lem. - lemnisci; m. - muscles; n.m. - nerve mass; nu. - nucleus; pr. - proboscis.
runs longitudinally within the middle part of the larva and this connects the anlage of the reproductive organs with the proboscis sheath. The muscles of the proboscis are only slightly developed at this stage.

As the development proceeds further, the rudiments of different structures take the shape of fully formed organs. There is formation of pigment, giving the larva a deep orange colour. The proboscis, though still remaining in the invaginated position, shows greater development of the hooks and the proboscis muscles also become thicker (Text Fig. 32.). A nerve mass measuring 0.1 mm. X 0.06 mm. becomes clearly marked out in the form of a group of large rounded cells at the base of the proboscis and in between the lemnisci. The lemnisci are now large vesicles and measure approximately 0.22 mm. X 0.7 mm. The neck of the larva shows minute spines on its external wall. The rudiments of the reproductive organs do not yet show development into definite gonads.

This larva after a further growth starts retracting its anterior and posterior part into the wider middle part which has by this time attained a thickly fibrous wall and is barrel shaped with marked constrictions at each end. I have noticed this retraction taking place in a specimen removed from Gammarus pulex and kept in 0.5% sodium chloride solution. The posterior part of the body gets folded
Text Fig. 33 - A fresh ACANTHELLEA (Infected phase) of P. MINUTUS showing the introverted capsule within the envelope.

Text Fig. 34 - ACANTHELLEA (Infected phase) of P. MINUTUS showing the internal structure of the capsule. (Balsam preparation)

cp. - capsule; ev. - envelope; hk. - hook; ne. - neck; pr. - proboscis; pr. s. - proboscis sheath.
or rolled inwards as it is withdrawn rather quickly inside the barrel shaped middle body (Plate VII, Fig. 3). The anterior part of the larva, viz., the spiny neck containing the proboscis, lemnisci and other structures, is withdrawn inside the middle part by the action of the muscles of the proboscis. Each end of the barrel-shaped middle part becomes greatly constricted.

ACANTHELLA PHASE 3 (INFECTIVE PHASE) -

(Plate VII, Fig. 4.; Text Figs. 33, 34.)

The larva at this stage assumes a typical barrel shape, deep orange in colour, lying within the elliptical membranous envelope (Text Fig. 33.). The barrel or capsule, as it may now be termed, has a smooth thick wall and it floats in the fluid inside the surrounding envelope. During this introverted stage, complete development of the reproductive organs takes place. The brain and its nerves, the muscles of the proboscis and the full number of hooks are all formed and the larva is now said to be at its " Infective" phase. Owing to the thickness of the wall of the capsule, no structures can be seen within it; but if the larva is removed from its membranous envelope and dehydrated and cleared in cedar wood oil by standard technique, certain internal structures become clearly visible (Plate VII, Fig. 4.; Text Fig. 34.).
The capsule measures from 0.9 mm. to 1.0 mm. in length and about 0.57 mm. in breadth. The anterior retracted part of the body, viz., the neck containing the proboscis and hooks lies bent in the form of a loop by the side of the body. At this stage the larva shows sex differentiation. In the male, the oval or rounded testes can be made out in the central part of the capsule. The thick covering is extremely hard and slippery; and the larva is well protected and can remain in this condition for a long time before it is finally transferred to the definitive bird host.

EMERGENCE OF THE PROBOSCIS (IN VITRO.)

In order to study how exactly the "Inf ective" stage is changed into the "Juvenile" form or the adult Acanthocephalan, the following experiment in vitro was devised for the purpose:

A large number of these infective barrel-shaped capsules with their membranous envelopes intact were removed from Gammarus and put in 0.5% sodium chloride solution, some at room temperature and others at 104°F. After remaining in this solution for 3 to 4 hours, the capsule showed a slight shrinkage of its body. After 24 hours at room temperature, or about 6 to 8 hours at 104°F, the capsule, while still
Text Fig. 35 - A juvenile male *P. MINUTUS* (obtained in vitro), showing extroversion of the spiny neck with the contained proboscis.

bu. - bursa; ev. - envelope; lem. - lemnisci;
pr. - proboscis; pr.s. - proboscis sheath; tes. - testis.
within its membranous envelope, began to show slight lateral zigzag movement. At the same time, towards its anterior apex, a small area became prominent by a thickening of the rim around it. Within this enclosed area part of the still introverted neck of the larva protruded out. Soon after this the entire neck containing the introverted proboscis of the juvenile Acanthocephala protruded out. The rest of the body remained within the capsule, and there was no emergence of the body of the larva at the posterior end of the capsule (Plate VII, Fig. 5.; Text Fig. 35.).

At this stage the evaginated juvenile Acanthocephala, still enclosed within the membranous envelope, shows a smooth barrel measuring about 0.74 mm. long, and the neck about 0.6 mm. long and covered with minute spines. The lemnisci lie in the neck at the sides of the introverted proboscis and measure 0.50 to 0.52 mm. in length; a part of the lemnisci still lies invaginated along with the proboscis. The reproductive organs can be clearly seen in the middle of the vesicle. In the male, there are two testes measuring from 0.1 to 0.15 mm. in diameter. The future cement glands lie between the testes and the posterior part of the body or bursal region but are not very distinct. In the female, an ovisac becomes prominent, and lies along the ligament.

Soon after the emergence of the neck, the proboscis also extroverts, but rather incompletely, and shows
Text Fig. 36. — A juvenile female *P. MINUTUS* (obtained *in vitro*), removed from the envelope, showing extroverted proboscis.

hk. — hook; lig. — ligament; pr. — proboscis; sp. — spines.
large proboscis hooks at its tip. This is the first time when the proboscis with its hook evaginates.

Usually the membranous envelop ruptures at this stage by the protrusion of the hooked proboscis.

This stage (Plate VII, Fig. 6.; Text Fig. 36.) may now be termed a "Juvenile" Polymorphus minutus. It measures approximately 1.9 mm. long, of which the oval vesicle measures 0.9 mm., and the neck and proboscis 1. mm. The spiny neck measures 0.47 mm. long. A small part of the body measuring about 0.3 mm., between the hooked proboscis and the spiny neck, has a smooth cuticle. The number and arrangement of hooks is similar to that of Polymorphus minutus.

Development in vitro did not proceed beyond this stage.

FEEDING EXPERIMENT ON A BIRD

In order to obtain the adult Acanthocephala corresponding to these larval forms, a duckling about two weeks old, which had been reared in the laboratory and had been previously kept on a diet which precluded helminthic infection was fed with 23 larvae (orange coloured capsules) removed from Gammarus pulex. The bird was kept on half-rations during the period of the experiment. Thirty days after feeding with larvae, the bird was killed and examined. Three well developed specimens of Acanthocephala were obtained.
with the proboscis deeply embedded into the intestinal wall of the host. They conformed in all respects to *Polymorphus minutus* (Goeze). I, therefore, consider these larval stages in *Gammarus pulex* as larvae of *P. minutus*. 
Text Fig. 37 - ACANTHELLE (Primordial phase) of Echinorhynchus truttae showing rudiments of structures.

ev. - envelope; pr. rud. - proboscis rudiments.
ACANTHELLA PHASE 1 (PRIMORDIAL PHASE) - (Text Fig. 37.)

This is the earliest stage which was obtained in the body cavity of *Gammarus pulex*. The larva has a pale yellow colour and is elliptical in shape and is contained within a closely fitting membranous envelope. The larva measures about 1.15 mm. X 0.35 mm. At its anterior end the larva shows a proliferation of small cells which form the rudiments of the proboscis. Similarly in the posterior part of the body is another group of rounded cells. The middle part of the larva shows a few large round cells and a greater number of small ones. No distinct structures can be seen at this stage, although it is possible to distinguish this larval stage from that of *Polymorphus minutus* owing to the smaller size of the latter and the better differentiations of its structures.
Text Fig. 38 - ACANTHELLA (Growth phase) of *Echinorhynchus truttae* showing morphological details.

ev. - envelope; hk. - hook; lem. - lemnisci; pr. - proboscis.
ACANTHELLA PHASE 2 (GROWTH PHASE) - (Text Fig. 38.)

During further development the larva becomes an elongated cylindrical structure and still lies within the membranous envelope. Generally the larva lies doubled up in the body cavity of *Gammarus pulex* (Text Fig. 38.). The body of the larva is light yellow in colour or whitish with orange spots or stripes; it measures approximately 3 mm. x 0.45 mm. The covering membranous envelope is a transparent structure and fits closely along most of the length of the larva except for a slight space at the anterior end and a larger space at the posterior end. The envelope contains a fluid in which the larva is bathed. The development of the proboscis has taken place in its introverted position at the anterior part of the larva and the proboscis shows the beginning of the formation of the hooks. A small pair of lemnisci are also present towards the anterior end of the proboscis.
Text Fig. 39 - ACANTHELLA (Infected phase) of a female *E. TRUTTAE* showing an early stage in the formation of the ovary.

hk. - hook; lem. - lemnisci; lig. - ligament; pr. - proboscis; ov. - ovisac.
At a later stage, the lemnisci become flask-shaped and extend half-way along the sides of the introverted proboscis. The hooks of the proboscis have become more prominent. A larva removed from its envelope at this stage is shown in Text Fig. 39. It measures approximately 4.8 mm. in length and 0.5 mm. in width. The proboscis sheath is 1.11 mm. long and the lemnisci about 0.53 mm. in length. There is a ligament running from the base of the proboscis sheath to the posterior end of the larva and rudiments of the reproductive organs are now distinctly seen. At this stage the larva shows sex differentiation. In the female, the ovisac is developed towards the posterior end of the larva and shows proliferating cells. A group of large cells posterior to the ovary mark the position of the uterine bell. In the male, there are two faint oval masses, the testes, in the middle of the body of the larva and rudiments of the cement glands may also be distinguished towards the posterior part of the body of the larva. The larva has attained the "Inf ective" phase and can remain within its envelope in this condition for some time before it is finally transferred to the definitive fish host.
EMERGENCE OF THE PROBOSCIS (IN VITRO)

At this stage of development, the larva, with its envelope intact, can be removed from the body of *Gammarus pulex* and kept in vitro in 0.5% sodium chloride solution at room temperature. In this medium it is usually possible to see the emergence of the proboscis in about 24 hours, but the larvae do not seem to survive much beyond this time.

It is seen that the anterior end of the larva shifts gradually towards the role of the envelope by a slight twisting movement followed by a few jerky movements which bring about a partial extroversion of the proboscis. The extroversion is slow and gradual and brought about by alternate movements of partial extroversion and introversion. Ultimately the proboscis extroverts completely by rupturing the covering envelope.

This stage of development when the proboscis is extroverted and shows its full number of hooks (Plate VIII, Fig.1.) and when the gonads are clearly defined marks the "Juvenile stage" of *Echinorhynchus truttae*. 
Text Fig. 40- A juvenile male *E. TRUITEAE* (obtained in *vitro*) showing extroverted proboscis and other structures.

- **d.i.v.** - dendritic integumentary vessels;
- **bu.** - bursa;
- **c.gl.** - cement glands;
- **fem.** - lemnisci;
- **pr.** - proboscis;
- **pr.s.** - proboscis sheath;
- **tes.** - testes.
A male "juvenile" Echinorhynchus truttae is shown in Text Fig. 40. The body is cylindrical and measures (including the proboscis) about 4.7 mm. in length. The proboscis measures approximately 0.65 mm. X 0.22 mm. and the proboscis sheath is 0.93 mm. long. The flask-shaped lemnisci measure 0.76 to 0.83 mm. in length. The testes are oval bodies, the anterior measures 0.33 mm. X 0.2 mm.; the posterior 0.3 mm. X 0.17 mm. The cement glands are six in number and there is a prominent bursa bearing small papillae present at the posterior end. Two longitudinal vessels run along the lateral margins of the animal. Each one sends out a number of dendritic branches towards the interior of the body. This system of vessels is the rudiment of the integumentary vessels of the adult. Besides these structures, the body contains large, rounded, scattered nuclei which are more clearly observed in the posterior part of the body (Plate VIII, Fig. 2.).

A female "juvenile" Echinorhynchus truttae is shown in Text Fig. 41. The body is cylindrical and measures about 6.7 mm. (including the proboscis), in length. The proboscis is cylindrical and measures approximately 0.64 mm. X 0.34 mm. and the proboscis sheath measures about 1.3 mm. in length. The lemnisci extend about two-thirds of the distance along the proboscis sheath and the muscles of the proboscis
Text Fig 41 - A juvenile female *E. TRUTTAE* (obtained in vitro) showing extroverted proboscis and other structures. (Proboscis compressed; dendritic vessels not shown).

hk. - hook; lem. - lemnisci; m. - muscles; ov. - ovisac; pr. - proboscis; pr. s. - proboscis sheath.
are also very distinct. The entire body of the "juvenile" Acanthocephala (female) is filled with ovarian balls which have arisen as a proliferation of the ovisac which lies along the ligament in the posterior part of the body. The uterine bell is also clearly marked by the presence of a mass of large rounded cells at the posterior end of the body.

The number and arrangement of hooks in both the male and the female "juvenile" Acanthocephala correspond to those in the adult *Echinorhynchus truttae* Schrank, 1788. There are 20 to 22 longitudinal rows of hooks with 13 to 15 hooks in each row.

**FEEDING EXPERIMENT ON A BROWN TROUT**

One of the trout yearlings obtained from the Stirling hatchery and kept in the laboratory on a diet which precluded any natural infection by helminths was selected for the experiment.

Two larvae of the supposed *Echinorhynchus truttae* were obtained from *Gammarus pulex* by dissection and immediately squirted down the oesophagus of the fish by means of a pipette. The fish was taken out of water, held in a piece of cloth a little behind the operculum and kept under a slow stream of water running from a tap. Within a moment the fish started gasping and thus afforded the opportunity to squirt water containing the larvae into the opening of the oesophagus. On releasing the pressure of the hand
on the post-opercular region of the fish it immediately swallowed the contents.

Next day two more larvae were fed to the fish; this time the fish was induced to swallow the partly dissected bodies of *Gammarus pulex* containing the larvae.

Fourteen days after the second feeding, the fish was killed and on post-mortem examination revealed one Acanthocephalan in its intestine. The Acanthocephalan was still alive as it showed extroversion and introversion of its proboscis. On examination of its detailed morphology, it appeared to agree in all respects to a male *Echinorhynchus truttae* Schrank, 1788.

In view of the fact that no Acanthocephalan was found when six other trout from the same aquarium were dissected, it is fairly safe to assume that this Acanthocephalan, obtained alive from the experimental trout, had developed as a result of feeding the larval forms from *Gammarus pulex*.

Furthermore, the juvenile Acanthocephala obtained by keeping the larval stages from *G. pulex* in vitro also agreed in all essential respects with *Echinorhynchus truttae* Schrank, 1788.
EXPLANATION OF PLATE VI.

LETTERING

hk. - hooks.

Fig. 1. - Photomicrograph of a number of Cysticercoid gammarid n. sp. from the connective tissue of Gammarus pulex. (X45)

Fig. 2. - Photomicrograph of a part of the intestine of Gammarus pulex showing the attached Cysticercoid brandburni n. sp. (X 90)

Fig. 3. - Photomicrograph of Cysticercoid brandburni, showing the crown of hooks. (X165)

Fig. 4. - Photomicrograph of an emerging scolex of Cysticercoid brandburni n. sp. (in vitro.) (X125)
EXPLANATION OF PLATE VII.

LETTERING

ev.-envelope; lem.-lemnisci; ne.-neck; ne.sp.-neck spines; nu.-nucleus; pr.-proboscis; pr.hk.-proboscis hooks.

Fig. 1.- Photomicrograph of an Acanthella (Primordial phase) of Polymorphus minutus. The envelope is ruptured at the anterior end and shows the protruding body of the Acanthella. (X 90)

Fig. 2.- Photomicrograph of an Acanthella (Growth phase) of P. minutus within the envelope showing the division of the body into 3 parts. The lemnisci are clearly seen in the anterior part of the body. (X40)

Fig. 3.- Photomicrograph of an Acanthella (Growth phase) of P. minutus removed from its envelope, showing further growth of the neck spines and the proboscis hooks. The posterior part of the Acanthella is withdrawing into the capsule. (X40)

Fig. 4.- Photomicrograph of a fully introverted Acanthella (Infective phase) of P. minutus showing the capsule-like body, (removed from its envelope.) (X40)

Fig. 5.- Photomicrograph of a juvenile P. minutus, (obtained in vitro) still within its envelope and showing extroverted neck. Note the pigment patches covering the body. (X40)

Fig. 6.- Photomicrograph of a juvenile P. minutus (obtained in vitro) showing the partly extroverted proboscis. (X40)
EXPLANATION OF PLATE VIII.

LETTERING

lem.-lemnisci;
d.i.v.-dendritic integumentary vessels;
nu.-nucleus.

Fig. 1.- Photomicrograph of the anterior end of a juvenile *Echinorhynchus truttae*, showing the proboscis with the arrangement of the hooks, and the lemnisci. (X65)

Fig. 2.- Photomicrograph of the posterior end of a juvenile *E. truttae*, showing the scattered rounded nuclei and the dendritic branches of the integumentary vessels. (X125)

Fig. 3.- Photomicrograph of the proboscis of an experimentally developed adult *E. truttae* showing the arrangement of hooks. (X65)
PART IV

ON THE MORPHOLOGY, DEVELOPMENT AND PATHOGENECITY OF A METACERCARIA FROM THE SPINAL NERVE OF THE HADDOCK, GADUS OEGLIFINUS.

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PATHOGENECITY ..................................... 193
INTRODUCTION

The remarkable discovery by Monro (1785) of oval cysts in the brain, spinal cord and spinal nerves of haddock was recorded in his classical monograph on the structure and physiology of fish. He designated these cysts, "Spheroidal bodies" and wrote (op. cit. p.106) that, "Spheroidal bodies consist of a tough transparent membrane of skin, containing a transparent viscid liquor, in the centre of which one or two white or opaque serpentine bodies are lodged." Since that time various attempts have been made to elucidate the morphological details of these encysted bodies. Sharpey (1836) regarded these bodies as "cystic entozoa." Goodsir (1844) named these cystic entozoa "Neuronaia monroii", and his brother (Goodsir, H. D., S., 1845) regarded this parasite as a distome since he associated the anterior sucker of this parasite (which is a Gasterostome) with the mouth opening and the real mouth opening (oral sucker) as the acetabulum.

Madox (1867) gave an elaborate account of these encysted bodies, designating them "Spheroidal bodies of Monro", and further regarded them as encysted young stages of Gasterostomum gracilescens (Rud.). Johnstone (1905) described cysts from the brain of Phycis, Cod and Haddock. Lebour (1907) regarded these cysts as the second stage in the development of the bucephalid, Gasterostomum gracilescens.
During the course of this investigation cysts of this bucephalid metacercaria were obtained from the spinal nerves (most commonly from the caudal but sometimes from the trunk region) of Haddock. In view of the fact that certain important morphological details of this encysted trematode seemed to have escaped the notice of previous workers, a full account is given here of its morphology and particularly of the peculiar structure associated with its anterior sucker. This structure is important since it throws light on the phylogeny of the species within this group. Furthermore, the extent of the damage caused by these encysted metacercariae to the spinal nerves of Haddock is also discussed.
MATERIAL AND METHODS.

The material for the present investigation was obtained from Gadus oeglifinus (haddock) purchased from the local fish market in Edinburgh. The metacercarial cysts were collected from the spinal nerves. In most of the cases the recovered specimens were alive within the cysts, although the fish had been dead for 12 to 18 hours. Specimens were removed from the cysts and studied both in the living and fixed and stained condition. Using standard technique, microtome sections, both of the spinal nerve containing the cysts and of the Agamodistome, were cut 6 to 8 μ thick and stained with Haemotoxylin and Eosin. Sketches were made with the help of the Camera lucida in each case.

Feeding experiments were tried on the brown trout, Salmo trutta obtained from the hatchery at Stirling. Attempts were also made to keep the Agamodistomes alive in vitro; but although the parasites survived for about 24 hours in vitro and almost certainly for several hours in the fish, very little development was observed.
Text Fig. 42 - A portion of the spinal nerve of a haddock, showing cysts in situ. One cyst contains four parasites.

a. su. - anterior sucker; cy. - cyst; sp.n. - spinal nerve.
STRUCTURE OF THE METACERCARIA

The oval cysts containing the metacercaria are present generally as swellings of the spinal nerves to which they impart a beaded appearance. They are most abundantly met with in the nerves of the caudal region but are also sparingly present in those of the trunk. The wall of the cyst is composed of two layers, an outer tough but more or less transparent layer and an inner granular layer. The cysts (Text Fig. 42.) vary in size from 0.78 mm. X 0.475 mm. to 0.85 mm. X 0.7 mm. and usually only one metacercaria is contained in each cyst. Some cysts may contain two individuals according to Maddox (1867), but I found on several occasions cysts containing as many as four individuals (Plate IX, Fig. 1.; Text Fig. 42.). This latter type of cyst is relatively larger, about 1.01 mm. X 0.65 mm. in diameter, although the individuals contained within are smaller in size than those occurring singly.

When liberated from the cyst, the metacercaria (Text Fig. 43) or the Agamodistome as it may now be called, is elongated and cylindrical in structure and is very active. The entire body is beset with transverse rows of minute spines, but these are more thickly set in the anterior two-thirds of its body. Immediately below the spiny cuticle there is a layer of muscle fibres which runs obliquely over the surface
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When liberated from the cyst, the metacercaria (Text Fig. 43) or the Agamodistome as it may now be called, is elongated and cylindrical in structure and is very active. The entire body is beset with transverse rows of minute spines, but these are more thickly set in the anterior two-thirds of its body. Immediately below the spiny cuticle there is a layer of muscle fibres which runs obliquely over the surface
Text Fig. 43 - An early stage of an agamodistome removed from the cyst. (Body spines not shown.)

Text Fig. 44 - An agamodistome, drawn from a compressed living specimen, showing the distribution of the excretory vessels and the flame-cells.

a.e.c. - anterior excretory canal; a.su. - anterior sucker; c.s. - cirrus sac; e.b. - excretory bladder; e.p. - excretory pore; f.c. - flame-cell; g. - gut; o.su. - oral sucker; p.e.c. - posterior excretory canal.
of the metacercaria (Plate X, Fig. 3.). The Agamodistome measures about 0.9 mm. X 0.27 mm. The anterior sucker measuring 0.14 mm. X 0.07 mm. is situated sub-ventrally. The oral sucker lies at a distance of about 0.2 mm. from the anterior end and appears to be thickly muscular and carries behind it the rudimentary intestine measuring approximately 0.17 mm. X 0.07 mm. The excretory vesicle, when fully extended, reaches the level of the oral sucker; the excretory pore lies a little in front of the posterior end of the animal. A faint outline of the cirrus sac is seen at this stage but no structures can be seen within it.

As far as I am aware no description has been given so far of the excretory system in the metacercaria or the Agamodistome of any of the Bucephalid forms. It has, however, been described for several Bucephalid cercariae in which, as the accounts available show, there is a good deal of variation in the arrangement and number of flame-cells. For example, Woodhead (1931) studied the excretory system in the cercariae of vasterostomes and found the number of flame-cells present on each side varying from 18 (Cercaria scioti, C. argi, C. basi) to 46 (Cercaria elegans).

Cercaria elegans is, according to Woodhead (1930) the cercarial stage of Bucephalus elegans; and this author (1930) found that the number of flame-cells was
reduced in the mature Cercaria. For example in the young Cercaria elegans, the total number of flame-cells was 92 while in the mature cercaria he found only 67.

The excretory system in the present metacercaria was observed clearly in the specimens which were removed from the cyst and kept in vitro for about 24 hours. During this period the dark contents of the excretory vesicle are excreted and the body of the Agamodistome also becomes slightly more transparent. It is, therefore, easy to locate the flame-cells and the branches of the excretory vessels (Text Fig. 44).

The sinuous excretory vesicle extends almost halfway forwards in the body and the two chief excretory canals open into the vesicle about the middle of its length. Each of these excretory canals is a much convoluted tube and is formed by the union of an anterior and a posterior canal. The anterior excretory canal of each side runs forward to the level of the anterior sucker and during its course gives off small capillary vessels each terminating in a flame-cell. There are eight flame cells associated with the anterior canal. The posterior excretory canal is short and gives off three capillary vessels each terminating in a flame-cell. There are, thus, 11 flame-cells on each side.

Unfortunately the excretory pattern and the full number of flame-cells are not known for the cercarial
stage of the present metacercaria to enable me to find what reduction in the number of flame-cells has been brought about during the transition from the cercarial to the metacercarial stage. How far this reduction in the number of flame-cells is carried further in the adult Gasterostome is also not known.

The pattern of the excretory system, with the main excretory vessels opening into the lateral sides of the excretory vesicle and not at its tip, is a characteristic feature of the Gasterostomata and probably occurs in very few other trematodes [c.f. *Paragonimus westermani* (Kobayashi 1919)].

**STRUCTURE OF PARTIALLY DEVELOPED TREMATODE**

When one of these agamodistomes is kept alive *in vitro* at room temperature, certain structures viz., the gonads, the cirrus sac, and a small button-like process at the anterior sucker become clearly visible after a lapse of about 10 to 12 hours. But these structures are still more clearly seen in specimens recovered from the intestine of a brown trout which has previously been fed with these cysts. Besides these structures the number and position of the vitelline glands are also more clearly marked in specimens from the trout. One of the trematodes recovered from the intestine of an experimental fish is sketched in Text Fig. 45.
Text Fig. 45 - A partially developed BUCEPHALID TREPATODE from the intestine of a trout, showing morphological details. (Body spines and excretory system not shown.)

a. su. - anterior sucker; c.s. - cirrus sac; g. - gut; oo. - ootype; o. su. - oral sucker; ov. - ovary; tes. - testes; ut. - uterus; v.d. - vas deferens; v. gl. - vitelline glands; v. sem. - vesicula seminalis.
The trematode measures approximately 2.9 mm. X 0.46 mm. The anterior sucker shows a small button-like process, but this is better seen during the protrusion of the anterior sucker. The muscular oral sucker measures approximately 0.13 mm. X 0.11 mm. and leads into a sac-like gut measuring about 0.43 mm. X 0.2 mm. The reproductive organs are fully laid down at this stage. The two spherical testes lie close together behind the middle of the body and measure approximately 0.2 mm. in diameter. The cirrus sac is more or less cylindrical and encloses the vesicula seminalis, prostate gland cells, pars prostatica and the cirrus. The cirrus sac leads into a bulbous structure which forms the genital atrium and into this open both the male and female genital ducts. The genital atrium ultimately opens to the exterior a little in front of the excretory pore. The ovary lies on the same side of the body as the testes, about 0.15 mm. in front of the anterior testis. The ovary is spherical in shape and measures 0.11 mm. in diameter. An ootype can be seen in some specimens situated a little posterior to the ovary. The uterus shows the beginning of the formation of its loops lying between the genital atrium and the level of the ovary. The vitelline glands appear as 15 to 18 small rounded follicles along each lateral side of the body. They extend from the posterior level of the
Text Fig. 46 - Anterior ends of three agamodistomes showing degrees of protrusion of the "process" of the anterior sucker (pr.a.su.).
ut to a point a little behind the anterior sucker where they tend to converge towards the middle.

The anterior sucker shows certain peculiar modifications. In the early stage when the metacercaria is still enclosed within the cyst in the spinal nerve the anterior sucker is simple with a shallow cavity and a fairly thick muscular margin. As the metacercaria grows in size the thickness of the muscular margin gradually diminishes and the cavity also becomes shallower. A ridge appears along the ventral wall of the sucker at this stage and this gradually thickens and develops into a small process or projection (Plate X, Fig. 2). After a brief period of free existence outside the cyst, the agamodistome shows a rapid development of this process which may show partial or complete eversion from the anterior sucker (Text Fig. 46). I observed this process for the first time in a dead partially developed trematode obtained from the intestine of an experimental brown trout fed 18 hours previously. In some cases, however, this process is not everted out of the sucker but remains retracted within it and can be seen only in a microtome section of the anterior sucker. When fully everted (Plate IX, Fig. 2) this process is a spoon-shaped structure and measures approximately 0.12 mm. in longer diameter.
SYSTEMATIC POSITION

The metacercaria described above has the following diagnostic features:

1. Skin spiny, anterior sucker feebly muscular with shallow cavity and provided with a spoon-shaped process.

2. Ovary and the two testes lie on the right side. Ovary is pretesticular.

3. Oral sucker, about the middle of the body and leads into a posteriorly directed sac-like gut.

4. Vitelline glands, 15 to 18 follicles, situated near lateral margins and extending from the level of the ovary to that of the anterior sucker where they tend to converge towards the middle.

5. Cirrus sac cylindrical and situated in the posterior quarter of the body.

6. Uterine loops at this stage do not extend anteriorly beyond the level of the ovary.

It is difficult to relate this metacercaria with certainty to any of the known adult Gasterostomes. Lebour (1907), without obtaining any experimental evidence, tentatively suggested that the metacercaria from the spinal nerves of Haddock might be the second larval stage of Bucephalopsis gracilescens. There are unmistakable resemblances between the metacercaria and B. gracilescens except for the presence of a small process at the anterior sucker in the former and its
absence in the latter. It is probable, however, that Lebour did not see this structure in the cysts which she examined. Unfortunately it was not possible for me to develop this metacercaria into the adult trematode, but if the process seen at the metacercarial stage is carried into the adult stage, this form can not, under the existing classification, be identified with B. gracilescens as the latter has no process at the anterior sucker.

The present metacercaria also shows some resemblances to Prosorhynchus gracilescens, as described by Linton (1940), which has a sucker and a process at the anterior end, and also to Rhipidocotyle baculum (Linton 1905) Eckman 1932 on similar grounds. It may, however, be better to regard this metacercaria as a species incertae sedis until its development into an adult trematode is carried out experimentally.

FEEDING EXPERIMENTS ON BROWN TROUT.

Undoubtedly a marine carnivorous fish would be the more likely definitive host to use for a feeding experiment but this could not be easily arranged under laboratory conditions. There were, however, at this time a number of yearling trout in small tanks in the laboratory of the Zoology Department, Edinburgh University, and it was decided to feed the metacercarial cysts to one or two of these in the hope that
some degree of development to the adult stage might be obtained. The trout were kept under conditions where infestation from this particular type of parasite was not likely to occur.

It was no easy matter to persuade the fish while in the aquarium to swallow the cysts of the metacercariae. When fragments of nerves with the enclosed cysts were taken by the fish they invariably threw them out immediately.

In the first experiment, a fish was taken out of water held in a piece of cloth a little behind the operculum and kept under a slow stream of water running from a tap. Within a moment, the fish started gasping and thus afforded the opportunity to squirt water containing cysts from a pipette into the opening of the oesophagus. On releasing the pressure of the hand on the post-opercular region of the fish it immediately swallowed the contents. A fish could be held in this manner out of water for 2 or 3 minutes before returning it to the aquarium. Although this method of feeding was occasionally successful, in the majority of attempts the cysts were regurgitated. The process of feeding was repeated three times within an hour and about 150 cysts in all were introduced into the oesophagus. It is probable, however, that only about half this number would be retained by the fish. The fish was killed and dissected after about 18 hours, and 20 specimens
of trematodes were found emerged from their cysts in the stomach and intestine but all were dead.

A better method of feeding was devised for the second experiment. The trout in the aquarium had been trained by this time to feed on small fragments of earthworms dropped into the water and this suggested a simpler method of experimental feeding. Very small bits of earthworm about 6 to 8 mm. in length, were taken, their viscera scooped out, and refilled with cysts. These were dropped into the aquarium, one by one, and were devoured by the trout with avidity. About 120 cysts were fed to a fish by this method. No regurgitation of the cysts took place although the fish refused to eat after swallowing three bits of earthworm.

After 20 hours, this fish was killed and examined for the developing trematodes and although 36 specimens were recovered from the stomach and intestine they were again found dead.

As was expected the trout did not prove a satisfactory fish for developing these metacercariae experimentally. Probably the metacercariae, too, were not in a perfectly viable condition as the haddocks had been dead 16 to 18 hours before the feeding experiments were performed.

Although the feeding experiments were not very successful, an important morphological fact of considerable taxonomic importance was revealed by the
examination of these dead trematodes which had emerged from their cysts. A large spoon-shaped process projecting out of the anterior sucker was clearly visible in some specimens (Plate IX, Fig. 2.). This structure was noticed for the first time in these partially developed trematodes from the trout. Later on, it was possible for me to detect this structure even in those specimens which had been kept in the laboratory, in vitro, in nutritive fluids and also, at least in a rudimentary stage, when a closer study was made of the metacercaria within the cyst (Plate X, Fig. 2.).

Another feature revealed in the dead trematode from the intestine of the trout was the development of the full number of vitelline follicles and their convergence towards the mid-line behind the anterior sucker.

IMPORTANCE OF THE ANTERIOR SUCKER IN THE STUDY OF THE PHYLOGENY OF GASTEROSTOMATA

The structure of the non-perforate anterior sucker (anterior adhesive organ) in the Gasterostomata has been greatly emphasized in the classification of these trematodes. The occurrence of a projecting process associated with the anterior sucker in the present metacercaria can not, therefore, be disposed of without comment.
It may be mentioned that Linton (1940, p. 31) while describing an adult bucephalid, Prosorhynchus gracilescens found a process associated with the anterior sucker which showed great variation in the degree of its protrusion. He says, "In some there is a distinct cap overhanging the anterior sucker. In others the cap is reduced to a button like process and in still others there is no trace of it." Linton, however, did not explain whether the apparent absence of this process in some specimens was due really to its non-development or merely to the extreme contraction of the process making it inconspicuous. It is difficult to believe that this structure can either be present or absent within the same species.

In my studies on the metacercaria described above I found varying degrees of contraction of this process and in some cases it appeared to be entirely absent. In the latter case, however, when microtome sections were cut the process was clearly revealed (Plate IX, Fig. 5).

It is probable, therefore, that in those of Linton's specimens which did not show this process, the latter was completely withdrawn within the sucker and could have been seen only in section.

Nicoll (1910, p. 351) while describing the adult Prosorhynchus aculeatus states that, "rhynchus (or rostellum) may be protruded like a small button, or
retracted. In the latter case a shallow sucker-like depression is formed."

Johnstone (1905) while describing the metacercaria from the haddock showed a button-like process of the sucker in his diagram but did not mention this in his description and similarly Lebour (1911) was apparently not clear about this structure in her specimen from haddock when she wrote (op. cit. p. 425), "At the anterior end is a sucker-like organ." (italics mine).

There is no doubt, however, that this structure does make its appearance at the metacercarial stage in my specimens and develops further in the agamodistome. Whether this process is a permanent structure and persists in the adult or atrophies, the present writer can not say as his attempt to develop this metacercaria into a mature adult trematode did not succeed.

Van Cleave & Mueller (1934), however, thought that these processes of the anterior sucker in certain Bucephalid forms appear merely as signs of senescence. But this can not be the case since this process is clearly defined even in the metacercaria.

The phylogenetic significance of this structure in the Gasterostomata can be seen more clearly when we study the variation which occurs in this organ in the various genera and species of this group of trematodes. The variations, of this structure which is termed the anterior adhesive organ, may be classified as follows:-
(a) a simple muscular sucker.
   e.g. in Bucephalopsis gracilescens.

(b) a muscular sucker with tentacular processes.
   e.g. in Bucephalus varicus.

(c) a muscular sucker with tentacular processes
and a ventral broader process (rhynchus).
   e.g. in Bucephalus introvercus.

(d) a muscular sucker with short papillae and
a rhynchus.
   e.g. in Rhipidocotyle galeatum.

(e) a shallow sucker with a rhynchus.
   e.g. in Rhipidocotyle baculum.

(f) a large rhynchus only.
   e.g. in Prosorhynchus.

From the above it is evident that there is a
great deal of variation in the anterior adhesive
organ within this small group of trematodes; but it is
not possible at this stage of our knowledge to trace
the exact derivation of the rhynchus (as the process
may be termed) from the true sucker or vice versa.
My own observations, however, on the metacercaria
from haddock throw a ray of light on this problem.

It was seen in the metacercaria from haddock
that at first only a thick-walled muscular anterior
sucker was present and that later a small process
A large process (rhynchus) only

Sucker atrophies.

A shallow sucker with a process (rhynchus).

Reduced tentacles entirely disappear

A sucker with reduced papilla-like tentacles and a large process.

Reduction of tentacles and growth of process.

A sucker with tentacles and a broad process.

Growth of process along with tentacles.

A sucker with tentacular process.

Tentacles grow out from sucker.

A muscular sucker.

Proserhynchus.

Rhipidocotyle baculum.

Rhipidocotyle galeatum.

Bucephalus introversus.

Bucephalus varicus.

Bucephalopsis gracilescens.

Text Fig. 47 - Schematic diagram of the evolution of the anterior adhesive organ.
arose from the sucker whose cavity became shallow. The process then became enlarged during further development and assumed a spoon-shaped form.

It follows, therefore, that the sucker with its muscular walls is the basic structure in this trematode and that the process or rhynchus is only a secondary development.

Proceeding on this assumption it seems very likely that the trend of evolution of the anterior adhesive organ in this group of trematodes has been from a *Bucephalopsis* type (Text Fig. 47a) where only a sucker is present to a *Prosorhynchus* type (Text Fig. 47f) where a rhynchus is present and no sucker.

The first development, therefore, would be a protrusion of folds or tentacles from the sucker giving the condition met with in *Bucephalus varicus* (b). The next stage would be the development of a small broad process along with the tentacles which would give rise to the condition seen in *Bucephalus introversus* (c). It is possible that this broad process may be more useful than the tentacles for attachment to the tubular pyloric caeca of the fish. Thus in the next stage one would find a reduction of the tentacles and a more pronounced development of the broad process as seen in *Rhipidocotyle galeatum* (d). The subsequent stage in the supposed evolution of this adhesive organ would show the complete atrophy of the tentacles and the very marked
development of the broad process as seen in *Rhinidocotyle baculum* (e.).

Finally one would see the full development, from the process, of the so-called *rhynchus* entirely replacing the sucker and giving the condition seen in *Prosrhynchus* (f.).

The case of the genus *Alcicornis*, which shows a *rhynchus* and also a number of tentacles but no sucker, has not been included in the above discussion. It is difficult to explain the origin of this condition except on the assumption that it has been derived as an offshoot from *Bucephalus introversus* by the retention of the tentacles; the gradual atrophy of the sucker and the progressive development of the process (*rhynchus*).

The present metacercaria from haddock shows a direct modification, without the intervening stages, from the type seen in *Bucephalopsis gracilescents* to that seen in *Rhinidocotyle baculum*.

As already stated the line of evolution of the anterior adhesive organ of Gasterostomata indicated in the schematic diagram (Text fig. 47.) is purely a tentative one. Only a study of the complete development from the cercaria to the adult of *Prosrhynchus* may probably give us direct proof, although it is doubtful if the entire phylogenetic picture would be displayed in the ontogeny of any living representative of the genus.
Text Fig. 48 - A transverse section of an uninfected spinal nerve of a haddock showing the arrangement of the bundles or fasciculi.

enm.- endoneurium; epm.- epineurium; pem.- perineurium.
PATHOGENECITY

The metacercarial cysts occur in the spinal nerves of the tail of haddock and a single nerve may contain as many as 20 to 30 cysts in its course. It is very likely that with such a heavy infestation with parasites, the nerves would not be able to discharge their normal function. Previous investigators on the metacercaria of haddock do not seem to have considered this aspect and no mention is made of the possible damage caused by the parasites to the nerves.

In order to have a clear idea of any nerve injury caused by the parasite under consideration, it is necessary to know the histological picture of an uninfected spinal nerve in the haddock. One of the normal nerves in transverse section shows the following structures (Text Fig. 48.):

A very large number of fibres, each composed of an axon, myelin, neurolemma and the sheath, are arranged in bundles or fasciculi, of varying size. These are bound together by loose fibro-elastic tissue. A layer of connective tissue, the epineurium encloses all the fasciculi and forms the external boundary of the nerve. This connective tissue continues in between the various fasciculi and forms a more dense layer, the perineurium around each fascicula. Inside each fascicula there is a loosely distributed delicate
connective tissue, the *endoneurium* between the adjacent component fibres. As the nerve extends out from its central origin, it branches and at each branching there is a gradual diminution both in the number of the fasciculi and the number of fibres in each *fascicula*. The connective tissue of the nerve has a special vascular supply, the *vasi nervorum*.

A series of transverse sections (Plate X, Figs. 1, 2 and 3) passing through an infected nerve show the extent of the damage caused to the nerve. When the parasite is small and lies either within or without a *fascicula* it occupies only a small part of the width of the nerve. The presence of the parasite, however, brings about a disintegration or necrosis of the neighbouring tissue and a protective sheath surrounding the parasite seems to develop within the nerve (Plate X, Fig. 1.). As the parasite increases in size, the cyst also increases and the neighbouring *fasciculi* are pressed further and further towards the *epineurium* (Plate X, Fig. 2.).

When the cyst is fully developed practically the whole width of the nerve is occupied by the cyst and the nerve tissue is reduced to an extremely thin layer at the periphery immediately underlying the epineurium (Plate X, Fig. 3.). It is quite evident that with this reduction of the nerve tissue within the nerve combined with the disintegration of its fibres, the nerve would no longer be capable of functioning.
properly. The extent of the damage caused to a single nerve may be realized when one considers that during its course a single nerve may have 20 to 30 cysts. The muscles of the caudal region in haddock which are innervated by these nerves may thus be paralyzed and bring about a restricted movement of the tail. Such a fish would naturally be unable to swim rapidly and, becoming increasingly sluggish, would fall an easy prey to its enemies.

Regarding the course of migration of the metacercaria to this unusual habitat we have little experimental evidence although I may venture to suggest, from the structure of the nerve already discussed, that it is most likely that the parasite is brought into the interior of the nerve through its vascular supply - the vasi nervorum.
EXPLANATION OF PLATE IX.

LETTERING

a. su. - anterior sucker; pr. a. su. - process of the anterior sucker (adhesive organ).

Fig. 1.- Photomicrograph of a part of an infected spinal nerve of haddock, showing cysts in situ. One cyst contains four parasites. (X20)

Fig. 2.- Photomicrograph of a part of a partially developed Bucephalid trematode from the intestine of a trout, showing the "process" of the anterior adhesive organ. (X60)

Figs. 3 and 4.- Photomicrographs of two of the serial sections of the anterior adhesive organ, showing the protruded "process." (X125)

Fig. 5.- Photomicrograph of a sagittal section of the anterior adhesive organ of an Agamodistome from trout, showing the "process" in the retracted condition. (X280)
EXPLANATION OF PLATE X.

LETTERING

c.s. - cirrus sac; cy.w. - cyst wall; e.b. - excretory bladder; M. - Metacercaria; pr.a.su. - process of anterior sucker; sp. - spines.

Fig. 1. - Photomicrograph of a transverse section of an infected spinal nerve of haddock showing the parasite in situ. The cyst wall is being formed. (X125)

Fig. 2. - Photomicrograph of a transverse section of an infected spinal nerve of haddock containing a large cyst showing the beginning of the formation of a small "process" of the anterior sucker. (X125)

Fig. 3. - Photomicrograph of a transverse section of an infected spinal nerve of haddock showing the large cyst filling up all the space within the nerve. (X125)
OBSERVATIONS ON THE IN VITRO BEHAVIOUR OF ACANTHOCEPHALA
PART V

OBSERVATIONS ON THE IN VITRO BEHAVIOUR OF THE ACANTHOCEPHALAN, ECHINORHYNCHUS TRUTTAE.

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INTRODUCTION

During investigations on the helminth parasites of brown trout it was noticed that the acanthocephalan Echinorhynchus truttae occurs in about 82% of the fish examined from a number of lochs and rivers of Scotland. As an abundant supply of material was available an opportunity was taken for studying the in vitro behaviour of this Acanthocephalan.

Very little is known concerning the value of anthelmintics in removing or killing Acanthocephala in vivo and practically nothing, as far as the writer is aware, regarding the in vitro behaviour of Acanthocephala to anthelmintics. Although the in vitro tests may possibly not reproduce exactly the response of helminth parasites in vivo, they give an opportunity of comparing directly the action of a large number of old as well as new anthelmintic drugs in a short time and results so obtained may suggest methods of combating Acanthocephala in vivo. Furthermore, in vitro observations are also useful for various physiological tests on these parasites.

A good deal of attention has been paid lately to such studies on nematodes by Baldwin (1943), Trim (1944) besides several continental workers but a general difficulty has been felt in keeping nematodes
alive in the laboratory and some of these workers have worked on fragments of the body of these parasites.

The present investigation was, therefore, carried out in order to find the viability in vitro of Echinorhynchus truttae in different concentrations of sodium chloride solution and in solution or suspension of certain anthelmintics.

The writer can, however, claim only a few tests so far (Lal, 1947) and these are fully described in the following pages.
MATERIAL AND METHODS

Specimens of **Echinorhynchus truttae** were collected from the intestine of the trout and placed in 3" diameter petri dishes in sodium chloride solutions, different concentrations ranging from 0.5% to 2% being used in different experiments. Several changes of the saline solution were made to rid the parasites of any adhering tissue or faecal matter after which they were kept in fresh solution of the same strength. After a lapse of two hours the most actively motile worms were selected and kept in small petri dishes in saline solutions. Petri dishes were kept in darkness as it was found that light had an adverse effect on the survival of these worms. Contact with metallic instruments was also avoided as this too was found to have a deleterious effect. The viability of the Acanthocephala in different concentrations of the saline solution was studied. All experiments were performed at room temperature.

The second stage of the experiments was designed to test the \textit{in vitro} behaviour of these worms to anthelmintics. The 1% saline solution, which was found to be more or less isotonic, was used for collecting, washing and selecting the most normal individuals. This done, six worms were put in small petri dishes which contained the anthelmintics in solution or suspension in 1% saline solution.
Gentle tilting of the dishes brought the whole surface of the worms in contact with the chemicals. The time required in each experiment for the apparent cessation of motility in the worms was recorded. After this three of the inert worms were transferred to another petri dish containing a 1% saline solution. The three remaining worms were left for a further 5 minutes before transfer to the saline solution. The saline solution was changed several times within the next 10 minutes to remove all traces of the drugs from the worms, which were then left overnight in 1% saline solution and examined next morning for motility or any signs of life. A control in the form of normal worms in a 1% saline solution was always maintained for comparison. For distinguishing dead from live worms, both in the experiments with saline and with the anthelmintics, a blunt glass needle was used for gently probing the worms. Absence of any movement was regarded as a fair indication of death.
VIABILITY OF *E. TRUTTAE* IN DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE SOLUTION.

1. Effect of 0.5% sodium chloride solution.

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2. Effect of 0.75% sodium chloride solution.

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3. Effect of 1% sodium chloride solution.

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4. Effect of 1.5% sodium chloride solution.
5. Effect of 2% sodium chloride solution.

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From the experiments given in the preceding pages, it is clear that a 1% sodium chloride solution is the best of these solutions for maintaining this Acanthocephalan in vitro. More than 50% of the worms not only survive in this solution for a fortnight but they exhibit a wrinkled natural appearance and rhythmic expansion and contraction of the body. There are no deaths during the first three days and only 3% perish by the end of six days. A gradual fall in survival percentage is seen from the 7th to the 15th day, after which there is a sudden drop. This solution appears to be isotonic to the worms.

Both the 1.5% and 0.75% solutions of sodium chloride have practically the same effects on this Acanthocephalan and seem to be the next best for maintaining this worm in vitro. More than 50% of the worms survive for 8 to 9 days in these solutions. The 1.5% solution is slightly the better of the two.

Both the 0.5% and the 2.0% solutions are quite unsuitable for maintaining this worm in vitro. While in the former, the worms show a marked bulging of the cuticle (seen slightly even in 0.75%), in the latter they exhibit a much shrunken appearance. The survival percentage is very low and 50% of the worms die during one and three days respectively. The 2.0% solution is slightly better than the 0.5%.
VIABILITY OF E. TRUZZAE IN DIFFERENT CONCENTRATIONS OF ANTHelmINTICS

Having obtained a fairly satisfactory medium for the maintenance in vitro of Echinorhynchus truttae, it was decided to study the effects of certain anthelmintics on the viability of these worms. The following drugs in common use as anthelmintics were used in these experiments:

1. carbon-tetrachloride
2. copper sulphate
3. oil of chenopodium
4. santonin
5. sulphathiazole (soluble)
6. thymol.

In order to ascertain which of these substances are lethal to this Acanthocephalan, a 1% solution or suspension was prepared in a 1% sodium chloride solution, and used for the test. It was found that while in certain solutions the Acanthocephala survived even a 24 hours exposure, in others they died within a few minutes. The former were regarded as non-lethal and the latter lethal for these worms; and the various substances were, therefore, grouped in two categories:

**Group A.** (lethal)
- carbon tetrachloride
- copper sulphate
- thymol

**Group B.** (non-lethal)
- santonin
- sulphathiazole

Oil of chenopodium did not give very conclusive results and is, therefore, not included in the above groups.
1. Effect of carbon tetrachloride.

A - 0.05% carbon tetrachloride in 1% sodium chloride solution.

<table>
<thead>
<tr>
<th>Number of observations</th>
<th>Number of worms used</th>
<th>Number of worms surviving after lapse of minutes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>6 6 6 6 5 5 3 1 X</td>
</tr>
<tr>
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</tr>
<tr>
<td>3</td>
<td>6</td>
<td>6 6 6 6 5 2 1 X</td>
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<td>6 6 6 4 4 2 X</td>
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<td>6</td>
<td>6 6 6 6 5 3 2 X</td>
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<td>6</td>
<td>6 6 6 6 4 2 2 X</td>
</tr>
</tbody>
</table>

Total number of worms used and surviving: 36 36 36 34 29 21 14 5 X

B - 0.1% carbon tetrachloride in 1% sodium chloride solution.

<table>
<thead>
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<th>Number of worms used</th>
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</tr>
</thead>
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<tr>
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<td>6 5 4 2 X</td>
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<td>6</td>
<td>6 6 2 X</td>
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</tbody>
</table>

Total number of worms used and surviving: 36 36 27 13 4 X
2. Effect of copper sulphate.

A - 0.05% copper sulphate in 1% sodium chloride solution.

<table>
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</tr>
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<td>6</td>
<td>6</td>
<td>6 6 5 4 4 4 1 X</td>
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<tr>
<td>Total number of worms</td>
<td>36</td>
<td>36 36 34 26 24 22 11 X</td>
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<tr>
<td>used and surviving</td>
<td></td>
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</table>

B - 0.1% copper sulphate in 1% sodium chloride solution.

<table>
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<th>Number of observations</th>
<th>Number of worms used</th>
<th>Number of worms surviving after lapse of minutes.</th>
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<td>6 6 6 5 4 2 X</td>
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<td>6</td>
<td>6 6 5 5 4 X X</td>
</tr>
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<td>Total number of worms</td>
<td>36</td>
<td>36 35 33 27 22 8 X</td>
</tr>
<tr>
<td>used and surviving</td>
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### 3. Effect of thymol.

**A - 0.05% thymol in 1% sodium chloride solution.**

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</table>

**Total number of worms used and surviving:**

36 36 36 36 32 32 31 26 22 6 X

**B - 0.1% thymol in 1% sodium chloride solution.**

<table>
<thead>
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<th>Number of observations</th>
<th>Number of worms used</th>
<th>Number of worms surviving after lapse of minutes.</th>
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<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>X</td>
</tr>
</tbody>
</table>

**Total number of worms used and surviving:**

36 36 25 17 5 3 1 X
4. Effect of Oil of Chenopodium.

The experiments with this drug did not yield conclusive results. While in certain cases a 1% concentration killed the worms, in others they became quiescent in about 17 minutes but revived on transfer to a 1% sodium chloride solution. With concentrations of 0.5% and less, the worms invariably became simply immobile after 24 to 30 minutes but revived when transferred to a 1% sodium chloride solution.

NATURE OF THE ACTION OF ANTHELMINTICS

Although it was difficult to determine the exact nature of the action of the anthelmintics on this Acanthocephalan certain observations were made on the behaviour of the worms to the drugs used. For example, it was observed that a rapid introversion and extroversion of the proboscis, accompanied by alternate contraction and expansion of the body, were brought about by carbon tetrachloride and thymol, while copper sulphate did not produce such marked effects. Oil of Chenopodium seemed to be much milder and produced only a slightly rapid expansion and contraction of the body followed by a wriggling of the body into an S-shape. In a 0.1% concentration, carbon tetrachloride and thymol both acted in more or less the same way, killing about 50% of the worms in about 3 minutes. With a similar concentration of
copper sulphate the time taken was about 6 minutes.

Of the three drugs, carbon tetrachloride, thymol and copper sulphate, therefore, the last is the least lethal when used in a 0.1% concentration. In a 0.05% concentration, on the other hand, copper sulphate behaves in almost the reverse manner and is more quickly lethal than the other two drugs.

Probably only a cytological examination of the worms treated with these drugs would explain this variation in their behaviour.

From the experiments described in the preceding pages it is clear that *Echinorhynchus truttae* might prove a useful helminth both for physiological studies and for testing anthelmintics *in vitro*, since it can be kept alive so easily in the laboratory for about two weeks.

The following tables give the results of the experiments with sodium chloride solution and with the anthelmintics, *in a condensed form*. Approximate percentages are calculated in each case.
Table 1 - Abstract of results with sodium chloride solution  
(Graph 1.)

<table>
<thead>
<tr>
<th>Concentrations of sodium chloride solution</th>
<th>Percentage of worms surviving after the lapse of days</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0.5%</td>
<td></td>
</tr>
<tr>
<td>0.75%</td>
<td>58</td>
</tr>
<tr>
<td>1.0%</td>
<td>100</td>
</tr>
<tr>
<td>1.5%</td>
<td>100</td>
</tr>
<tr>
<td>2.0%</td>
<td>100</td>
</tr>
</tbody>
</table>
Graph II - Viability of *Echinorhynchus truttae* in 0.05 and 0.1 per cent concentrations of carbon tetrachloride, thymol, and copper sulphate.

---
- 0.05 %
- 0.1 %

---
- carbon tetrachloride
- thymol
- copper sulphate.
<table>
<thead>
<tr>
<th>Name of anthelmintic used and its concentration</th>
<th>Percentage of worms surviving after the lapse of minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon tetrachloride 0.05%</td>
<td>100 100 100 95 81 58 39 14 X</td>
</tr>
<tr>
<td>Carbon tetrachloride 0.1%</td>
<td>100 75 36 11 X</td>
</tr>
<tr>
<td>Copper sulphate 0.05%</td>
<td>100 100 94 72 67 61 31 X</td>
</tr>
<tr>
<td>Copper sulphate 0.1%</td>
<td>100 97 92 75 61 22 X</td>
</tr>
<tr>
<td>Thymol 0.05%</td>
<td>100 100 100 89 89 86 72 61 17 X</td>
</tr>
<tr>
<td>Thymol 0.1%</td>
<td>100 70 47 14 8 3 X</td>
</tr>
</tbody>
</table>
GENERAL CONCLUSIONS AND SUMMARY.

The present thesis embodies the results of a study of a varied collection of larval helminths from Salmo trutta, Valvata piscinalis, Gammarus pulex, and Gadus oeglifinus. The morphology of the various larvae has been described and an attempt has been made to elucidate their life-history. Feeding experiments were performed on various laboratory reared animals and also in vitro experiments devised to obtain partial development. The last part of the thesis deals with the observations made on the in vitro behaviour of an Acanthocephalan, Echinorhynchus truttae.

PART I.

1. A new species of Diplostomulum larva viz., D. truttae n. sp. from the eye of brown trout is described.

2. An account is given of the early developmental stages of this Diplostomulum and evidence is adduced to show that a phenomenon of Ecdysis takes place in the life-history of this trematode. This tends to confirm the suggestion made by Wesenberglund (1934) on this point and it is suggested that future investigations may reveal its occurrence in certain other trematodes.
3. The development of *D. truttae* n. sp. within the eye of fish seems to be marked out into three distinct stages. These stages which had not previously been recognized in any *Diplostomulum* are, therefore, given separate names viz., Metacercarial Ecdysis stage, Pre-*Diplostomulum* stage and *Diplostomulum* stage.

4. This parasite was found chiefly in the vitreous humour, but sometimes also within the lens of the eye. Observations were made on its pathogenicity and a reduction in the size of the lens associated with a heavy infection of the vitreous humour was found.

**PART II.**

1. Three new species of Cercariae, together with their parthenitae, from the snail, *Valvata piscinalis* are described in detail.

2. The second intermediate hosts were experimentally determined for two of these cercariae and one of these cercariae was traced through to the adult trematode.

3. One of these cercariae, *Cercaria valvatae* n. sp., which is a furcocercous form, was shown to enter a leech (*Helobdella stagnalis*) and its develop-
ment into a tetracotyle was traced.

4. Attempts to infect a fowl by feeding with this tetracotyle were not successful.

5. The basis of the existing classification of the furcocercous cercariae was analysed and the value of both the so-called "Adult" and the "Larval" characters of the cercaria is emphasized.

6. Another cercaria which belongs to the genus *Echinoparyphium* develops into a metacercaria in the same snail. The adult fluke was obtained by feeding this metacercaria to fowls, but its specific identity must await further work in this group.

7. A study of the redia of this cercaria shows that there is a diminishing ratio between the length of the gut and that of the body as the redia becomes older.

8. The third cercaria described viz. *C. duddingstoni* n. sp. is a microcotylous xiphidiocercaria. Its excretory system is described in detail.

9. This cercaria frequently encysts in the open, without penetrating any host. The mode of encystment in xiphidiocercariae is discussed. Contrary to the general belief of various workers it is shown that encystment in this group can
take place in the open.

PART III.

1. Five larval helminths from *Gammarus pulex* are described.

2. Of these, two cysticercoids are new to Science; two Acanthocephalan larvae are traced through to their respective adults and the fifth, a metacercaria, is now shown to belong to the genus *Plagiorchis*.

3. *Gammarus pulex* is recorded for the first time as an intermediate host for larval Cestoda and larval Acanthocephala in Great Britain.

4. The emergence of the scolex by a split in the cyst-wall has been shown to occur in *Cysticercoid braudburni* n. sp. both naturally in the body of its intermediate host and *in vitro* at room temperature.

5. In the description of larval Acanthocephala, the nomenclature used for the various larval stages is discussed and the limitations of the existing terminology pointed out.

6. The development of "Acantholla" is shown to take place in three phases which are named "Primordial", "Growth" and "Infective" phases.
PART IV.

1. A description is given of the morphology and partial development of a Bucephalid metacercaria, found encysted in the spinal nerves of haddock.

2. As details of the excretory system of any metacercaria of the Gasterostomata are not known, a full description of this system has been given for the above metacercaria.

3. The so called "process" of the anterior adhesive organ of this Bucephalid has been shown to arise from the simple sucker. This study has thrown some light on the trend of evolution of the anterior adhesive organ in this group of trematodes.

4. The systematic position of this metacercaria is discussed and this is regarded as species incertae sedis until its development into a mature adult is experimentally determined.

5. It is shown that considerable damage is done to the spinal nerves of haddock by this parasite.

PART V.

1. An account is given of a preliminary investigation on the in vitro behaviour of the Acanthocephalan, Echinorhynchus truttae.
2. Results obtained indicate that this Acanthocephalan may be easily maintained in vitro in the laboratory for over two weeks and may thus prove useful for physiological studies.

3. The effects of a few anthelmintics on this Acanthocephalan, in vitro, have been studied. As far as the writer is aware such a study has not previously been made for any member of this group.
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mit Schwanzanhängen.

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