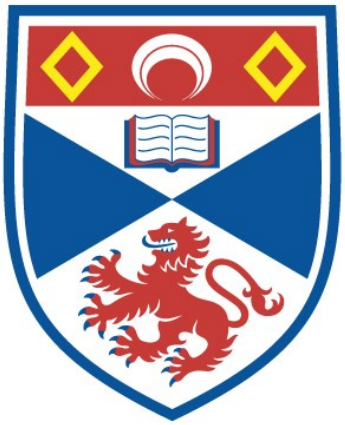
PARASITOLOGICAL STUDIES: POST-EMBRYONIC DEVELOPMENT IN THE POLYCERCUS OF PARICTEROTAENIA PARADOXA (RUDOLPHI, 1802) IN ALLOLOBOPHORA TERRESTRIS (SAVIGNY, 1826)

James Stuart Scott

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1963

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being a Thesis presented

by

JAMES STUART SCOTT

to the University of St. Andrews,

in application for the Degree of

Doctor of Philosophy

St. Andrews, 1963

POST-EMBRYONIC DEVELOPMENT IN THE POLYCERCUS

of *Paricterotaenia paradoxa* (Rudolphi, 1802) in

Allolobophora terrestris (Savigny 1826)

RESEARCH TRAINING

I commenced my parasitological studies on 1st October, 1960 as a Research Student under University Court's Ordinance 350 (General No. 12) and was enrolled as a candidate for the degree of Ph.D. under Ordinance 16 on 10th October, 1961. The results of my research are now submitted as a Ph.D. Thesis.

I wish to take this opportunity of expressing my gratitude to Professor H.G. Callan for making it possible for me to carry out my research and to Mr. D.R.R. Burt, my supervisor, for placing at my disposal his time, knowledge and experience.

I also wish to express my thanks to Madras College, St. Andrews and to the Department of Scientific and Industrial Research for awarding me Research Grants to enable me to complete my studies.

DECLARATION

I hereby declare that the following thesis is based on the results of observations and experiments carried out by me, that, apart from Appendix V, the thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The Research was carried out in the Department of Natural History, United College, St. Andrews.

(J.S. Scott)

CERTIFICATE

I hereby certify that James Stuart Scott was admitted as a Research Student in the Department of Natural History, University of St. Andrews on 1st October, 1960. He has spent nine terms on research on Parasitology, has fulfilled the conditions of Ordinance No. 16 (St. Andrews) and is qualified to submit the following thesis in application for the degree of Doctor of Philosophy.

David R.R. Burt, Supervisor.

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INTRODUCTION

The following thesis describes the occurrence, development and morphology of Polycercus lumbrici Villot, 1883, the so-called larval* form of the dilepid tapeworm Paricterotaenia paradoxa (Rudolphi, 1802). This larva has not been recorded in Britain and has been described on only one previous occasion, by I. Metchnikov who found it in an Earthworm in Russia in 1867. Most points of Metchnikov's account are confirmed, for the first time, but his conclusions have been modified in some respects and his work has been considerably extended. Experiments on infestation of Earthworms with the larvae of P. paradoxa and on the culture and evagination of the cestode in vitro are described. In addition, the discovery of the polycercal larva of P. burti Sandeman, 1959 is recorded for the first time and other cysticercoid larvae of tapeworms which infest charadriiform birds are identified.

My interest in the larval cestodes arose from the fact that, while making a general study of the cestodes found in the wading birds of the St. Andrews area in 1960-1961, I was struck by the apparent lack of host specificity in the parasites of these birds. The distribution of the cestodes appeared to be determined by the feeding habits of the host rather than by phylogenetic relationships.

* The term 'larva' is used in cestodology to include the post-onchospheral forms in the intermediate host or hosts. There is no true metamorphosis in the change from the cysticercoid to the adult worm.

This may seem a very obvious conclusion and not an original one, but, as the principle of host specificity has gained much of its strongest supporting evidence from observations on avian parasites, it was surprising to find so many examples of single groups of cestodes distributed among widely different groups of birds. My study of the feeding habits of the birds led naturally to a consideration of the intermediate hosts and life-cycles of their parasites.

It was with great interest, therefore, that I learned that in November, 1956 a number of cysts containing larval cestodes had been found in the coelom of an Earthworm while being dissected by a member of the First Year Class in Zoology at St. Andrews University. On examination, each cyst was seen to contain a number of 'cysticercoids' in which the hooks were readily distinguishable. The cysts were examined, photographed and then fixed and preserved by Mr. D.R.R. Burt, Senior Lecturer and Mr. I.M. Sandeman, then an Honours student, in the Department of Natural History. This material was provisionally labelled 'Cysticercus' and received no further attention until it was handed to me for examination.

Closer inspection of the hooks, virtually the only diagnostic features of a cysticercoid, showed that they corresponded in number, size and form to those of the dilepid tapeworm Paricterotaenia paradoxa Rud., a parasite of the Woodcock (Scolopax rusticola L.) and a few other wading birds. Unfortunately, the Earthworm host was so badly mutilated in the course of dissection that the species could not be positively determined although it was submitted to experts in the British Museum.

Acting on the assumption that the larvae were indeed those of P. paradoxa and with the knowledge that this tapeworm is a common parasite of the Woodcock, I collected large numbers of Earthworms from likely feeding-places in areas where Woodcock were known to occur, in the hope that I might find the larvae. This hope was immediately realised as I obtained a number of Earthworms infested with larval cestodes which appeared identical with the original material and this led to the detailed study of the development, structure and classification of Polycercus lumbrici which follows.

MATERIAL AND METHODS

OBSERVATIONS

THE INTERMEDIATE HOST.

Collection of Earthworms

The Earthworms were collected from areas where Woodcock were definitely known to occur. In such areas (Kippo Wood, Tayfield, Kilmarron Castle) soft, damp ground in drainage channels, on the banks of streams or in hollows was dug up and the worms picked out by hand. This was not so easy as it sounds as suitable spots were usually near trees whose matted roots made digging and extraction of the worms hard and often unrewarding work.

I tried the technique of soaking the ground with a solution of potassium permanganate, which is advocated as a means of driving Earthworms to the surface of the soil in large numbers. They can then be picked up without the labour of digging. I found this method quite useless as it appeared to have little effect on the larger worms, although it brought up small worms, and the solution burned the skin of the worms and caused portions of the cuticle to slough off.

The Earthworms were examined alive as soon as possible after collection, usually within the hour. If it was not possible to examine them immediately, they were kept in closed polythene boxes or bags, in damp moss or soil.

Infested worms, which were to be used in experiments or which were to be dissected or sectioned, were kept in moist blotting-paper or moss to clear themselves of particles of organic matter or grit in their intestines.

Technique

The examination of the samples of Earthworms to obtain infested specimens was carried out under low-power, binocular microscope by reflected light and against a black background. I have not been able to separate infested worms from the uninfested without the aid of a lens. Even with the microscope it is not always easy to determine whether or not a worm is infested. This is partly due to the movements of the worm and partly to the fact that the cysts are translucent.

The most difficult cases to detect are those in which infestation is light and the cysts are in the early stages of development. In order to check that I was not missing any infested worms in the samples, I collected a large sample (150 specimens) from an area with a 'good' rate of infestation (about 10% normally) and examined them alive in the usual manner. All infested specimens which I could detect were removed and the remaining worms were then killed and dissected so that they could be closely examined for the presence of parasites. Not one of the dissected worms showed any sign of infestation with Polycercus lumbrici, i.e. in this instance examination of the living material was 100% effective and for reckoning the rates of infestation I accepted the figures so obtained in all examinations.

Site of Infestation

The earliest indications of infestation were seen in the region of the host just posterior to the gizzard. The very early cysts

appear in reflected light as a number of small, dark globules on the outer surface of the intestine, while a few cysts are found posterior to this along the line of the intestine.

In general, the cysts in early stages of development are more commonly associated with the anterior part of the intestine than with the posterior part where there tends to be a higher proportion of fully developed larvae. However, the very earliest stages may be found together with the very latest in any part of the worm.

Identity of Host

In the samples of Earthworms which I collected from various places, I found Polycercus lumbrici in Allolobophora terrestris only. It is remarkably absent from Lumbricus terrestris and L. rubellus, the other Earthworms most commonly found with A. terrestris, nor was it found in the relatively small numbers of Bimastus spp., A. foetida and other species which were collected together with the more common species.

The numbers of the different species of Earthworms were found in the areas concerned to be approximately in the percentages:

Allolobophora terrestris	45%
Lumbricus rubellus	25%
Lubricus terrestris	20%
Other species	10%

Incidence of Infested Earthworms

The rate of infestation varies from place to place but in the 'best' areas it attained 10% of all the worms examined which gives the infestation rate for A. terrestris alone, on the basis of the above percentages, as approximately 22%.

The incidence also varies from time to time in the same place. For instance, in Spring, 1961 rates of incidence of over 10% were found in two widely separated localities, but by the early Summer of 1962 the rates had fallen to 0% in one place and 2% in the other. There are numerous factors which may explain the variation: season, weather with movement of intermediate host and consequent movement of final host, removal of the 'reservoir' by collection, and so on. I have not collected the Earthworms regularly over a sufficiently long period to be able to discuss the effects of these factors and the figures I have given are put forward as indications of incidence of infestation rather than as absolute data.

Degree of Infestation

There is great variation, also, in the number of cysts found in each infested Earthworm. In some, comparatively few cysts - a few dozen - were found, while in others there were many hundreds, or even thousands (Figs. 30, 31). A rough calculation of the number of individual larvae in a single, heavily-infested Earthworm gave the approximate 'load' as 215,000. It is not surprising, therefore, that the number of the adult cestodes in a single Woodcock may run into hundreds of thousands.

The degree of host specificity exhibited by the larvae is notable in itself, but even more remarkable is the fact that I found the larvae in immature specimens only of A. terrestris; that is, in specimens in which the reproductive organs and the clitellum had not developed.

Where the body cavity of the host is filled with larvae to such an extent that it assumes the appearance of a sac filled to bursting point with the parasites, it is obvious that, besides possible glandular changes, there will be changes in the structure of the Earthworm due to the simple mechanical effects of pressure on the organs of the host. This is exemplified in the heavily infested Earthworms where the intestine, blood vessels and the musculature of the body wall, particularly in the posterior part of the worm, are distorted and these structures have deteriorated to such an extent that they are barely recognizable, or even missing (Fig. 30).

Earthworms in this condition die under adverse conditions more quickly than healthy, uninfested worms. On one occasion, a heavily infested Earthworm which I had put into a petri-dish became moribund and cast off sections of its body from the posterior end. A constriction formed a few segments from the end of the worm, became deeper and then broke off while another constriction appeared a few segments further along towards the head of the worm and the whole process was repeated.

Sample	No. of Earthworms	No. of infested Earthworms	% infested	Period (days)
1.	7	1	14	59
2.	6	1	17	59
3.	43	3	7	93
4.	36	4	11	109
5.	16	5	31	259
6.	10	6	60	273

Table 1 : Infestation of Earthworms with Polycercus lumbrici by feeding with eggs of Paricterotaenia paradoxa.

Experimental Infestation of Earthworms

In the hope of confirming that Polycercus lumbrici is indeed the larval form of Paricterotaenia paradoxa and that the Earthworm is infested from the Woodcock, I attempted to feed a number of Earthworms with ripe proglottides of P. paradoxa taken from the intestine of the Woodcock. My first attempts were made by forcing the material into the oesophagus of the Earthworm through a blunt-nosed syringe. This was completely unsuccessful as the worms everted their pharynges and ejected the cestodes.

I then resorted to the more effective and simple, but less satisfactory method of mixing large quantities of cestodes with earth in flower pots and then placing a number of uninfested Earthworms in the pots. This method is less satisfactory than the former as it gives no indication of when the worms ingest the eggs and, therefore, it is not possible to establish, with any degree of accuracy, the time required for development of the larvae in the Earthworm. It also means that the Earthworms could not be killed at intervals in order to follow the migration of the oncosphere from the intestine to the body cavity.

The results of the experiment are listed in Table 1. Although the rates of infestation were not high in all cases, they do establish that the Earthworm can be infested from material passed out by the Woodcock and that Polycercus lumbrici is the larval form of Paricterotaenia paradoxa, as had been already deduced from morphological and ecological data.

The minimum period before infestation was detected was three months, while the maximum rate of infestation in the experiment was obtained in a pot in which the worms had been kept for ten months. In the latter case, however, most of the infested worms were very young and I am fairly certain that they were offspring of the worms originally introduced. If this is the case, it indicates that the eggs remain viable in the soil for a considerable length of time, as one would expect in infective stages which are passed out in the excreta of a bird and are subjected to the vagaries of British weather.

A check on the viability was attempted by placing quantities of cestodes from a Woodcock in soil and leaving them for three months in alternately dry and wet conditions. The soil was then mixed with other soil and Earthworms introduced. None of the Earthworms became infested and the eggs of the cestode do not appear to be able to survive for as long as three months after being expelled from the final host.

As a matter of interest, gravid segments of P. paradoxa were placed in aquaria in which Tubifex was kept and, later, with a mixture of fresh-water invertebrates including Tubifex, Daphnia, Cypris, Planorbis and Gammarus. These were examined to see if they became infested with the cysts of the cestode but no case of infestation was found.

THE FINAL HOST

Identity of Host

Paricterotaenia paradoxa has been recorded from the following birds: Woodcock (Scolopax rusticola L.), Great Shipe (Gallinago media Latham), Common Snipe (Gallinago gallinago gallinago L.), Jack Snipe (Lymnocryptes minimus Brünnich), Oyster Catcher (Haematopus ostralegus L.), Lapwing (Vanellus vanellus (L.)), Phalarope (Phalaropus lobatus (L.)), Golden Plover (Charadrius apricarius L.).

To this list I add the Curlew (Numenius arquatus (L.)) as I found a few specimens of P. paradoxa in the anterior part of the duodenum of one of these birds shot at the Eden Estuary, St. Andrews in November, 1960.

Although it is recorded from several hosts, it is in the Woodcock that P. paradoxa is most commonly found and attains its largest numbers. The Woodcock, then, can be presumed to be the principal host and the other birds secondary hosts.

Woodcock: Incidence and Habits

Woodcock are by no means rare in the East of Scotland, although they do not reach the numbers recorded in the West and in Ireland, probably due to the fact that most of the birds arrive in the East of Scotland in October-November as migrants from Scandinavia and pass on to the West where they remain until the Spring exodus to their breeding grounds in the North. This appears to be the general picture of migration of the Woodcock in this area, but the picture is by no

means clear and much is still to be learned about the movements of the bird. Some are known to move South and cross to the Continent, spreading out to East and West and reaching at least as far as the South of Spain. A certain number remains to nest in the St. Andrews area and, presumably, there is a number of true residents which remain year after year.

The bird is unpredictable in its movements and, therefore, it is to be expected that the incidence of infestation of Earthworms with the larvae of the parasite it carries will vary according to the density of the Woodcock population from season to season.

I have stated that the Woodcock is not rare in the East of Scotland, but it is not often seen other than by such as shooting parties and by keen observers moving about at dusk, when the bird, crepuscular in habit, moves to its feeding grounds. It has the reputation of being very timid, no doubt because of the sudden upward spring and rapid flight of the bird when flushed from its roosting-place in daytime. It belies its reputation, however, in the way in which it 'sits tight' uhtil the intruder is almost on top of it and in its lack of fear at dusk. On several occasions I have approached at dusk to within ten yards of a Woodcock in the open before it flew off, and on one occasion a Woodcock settled in front of my car as I drove along a forest road, allowed the car to stop about ten yards from it and a passenger to alight and approach to within five yards, before flying off into the trees.

A very detailed survey of the Woodcock in Great Britain was carried out by W.B. Alexander (1934-1935), adding much to our knowledge of the bird but leaving much to be explained.

Metchnikov's discovery of the larvae of P. paradoxa in Russia and my own work in the St. Andrews area are nicely tied together by the Woodcock which was ringed in Berkshire, England on 2nd February, 1959 and was recaptured or, more likely, shot at Leningrad, Russia on 2nd May, 1959. Its route probably followed river valleys across the Continent and it would be interesting to search for Earthworms infested with the larvae of P. paradoxa along the most likely routes between Britain and Odessa where Metchnikov found the larvae in 1867. I would expect to find reservoirs of infested Earthworms in most of the areas where Woodcock occur in Europe.

Degree and Site of Infestation.

Of nine Woodcock I have examined, only in one was P. paradoxa missing. The degree of infestation varies from bird to bird and in light infestations the cestodes are usually confined to the anterior part of the duodenum, while in heavy infestations they are found in all but the posterior part of the small intestine.

The extremely large numbers of the larvae of P. paradoxa found in the Earthworm have already been commented on and correspondingly large numbers of the adult are found in the Woodcock. Joyeux and Baer (1939) quote a figure of 20,520 specimens from a single bird. In a fairly heavy infestation, I obtained a conservative estimate of

120,000 cestodes in a single Woodcock after a careful count. Sandeman who has also made counts of the cestode in the Woodcock, has estimated 'loads' of double my figure (Personal communication).

The large number and small size of the cestodes, and the few proglottides of the adult suggest that the life of the parasite in the intestine of the host is comparatively short and that there is a rapid 'turnover'.

The staple diet of the Woodcock is Earthworms. I cannot give a definite figure for the number of worms a single bird would eat in one day, but, if the infestation rate of the larva of P. paradoxa in the Earthworms in an area frequented by Woodcock is 10%, which is not unusual, and the bird eats 20 worms each day, which is a low estimate, a tremendous number of cestodes could be built up in the intestine of the bird in the course of only a few days.

It was my intention to feed infested Earthworms to a Woodcock or Snipe and, I hoped and expected, to recover cestode eggs from the excreta. This would prove in the laboratory the Earthworm-Woodcock link in the life-cycle of the parasite. Lack of adequate facilities and the great difficulty in obtaining the birds prevented this, however. I did rear a young Common Snipe, but unsatisfactory safety precautions in an inadequate bird-pen permitted a cat or a rat to destroy the bird before I could carry out the experiment.

The life-cycle of the parasite in Woodcock and Earthworm is illustrated diagrammatically in Fig. 51.

THE DEVELOPMENT OF Polycercus lumbrici

Technique

When the larvae in the Earthworms were required alive for physiological experiments, I found that the most effective method of killing the worm was to immerse it for a few seconds in hot water and then it could be transferred to cold Locke's solution where it could be cleanly and easily dissected. The short immersion in hot water had no apparent effect on the larvae.

If required for sectioning, the Earthworms were killed and fixed in Bouin's fluid or in 10% formalin and then transferred to 70% alcohol until I was ready to use them. Latterly, I preferred the formalin to Bouin's fluid because of the difficulty of getting rid of the picric acid from the latter by repeated washings in alcohol or by addition of a little lithium carbonate to the alcohol. If not carefully removed, the picric acid had an adverse effect on the staining of the sections.

Dehydration, clearing and embedding were carried out in the usual manner through the alcohols, cedarwood oil and paraffin wax. I also tried dehydrating and clearing in Dioxan before embedding, and I tried embedding in Aquax but, in spite of the time and labour saved by using these materials, I did not find the end-results as satisfactory as those obtained by the older methods.

For examination in the living state I found Locke's solution to be as satisfactory as any medium for maintaining the larvae.

Fixation was carried out in Bouin's fluid and, later, in 10% formalin, after which the cysts were transferred to 70% alcohol.

On dissection of the infested Earthworm in water or physiological solutions the cysts drift out of the body cavity as soon as it is opened and fall to the bottom of the dissecting dish. They are easily seen against a dark background, even with the naked eye, as minute, whitish, translucent globules.

In the early stages of development the cyst is a very delicate, thin-walled bladder and the usual technique of dehydration through 70%, 80%, 90% alcohol to absolute alcohol and then to cedarwood oil resulted in the collapse of the spheres. This was overcome to a large extent by introducing intermediate grades of alcohol and by leaving the material for at least several hours in each grade. It was then transferred to increasing concentrations of cedarwood oil in absolute alcohol until ready for embedding.

To examine the different stages of development of the larvae by preparing sections of them, I at first removed them from the host, fixed them and transferred them to 70% alcohol, and then separated them into groups, each group representing a different stage of development. As the cysts were too easily lost to be dehydrated and cleared as a loose group, they were placed in a small container which held them together through the various processes up to the finished, stained sections. (The empty puparia of Drosophila melanogaster were readily available and were used.) Later the cysts were not put into the container until the cedarwood oil stage as, tedious and time-consuming as this

delicate operation was, it was even worse in the alcohols and the cysts often escaped from the container as the alcohols were changed. These manipulations were carried out under low-power binocular microscope in solid watch-glasses (embryo blocks) with the aid of a fine pipette.

This technique I found only fairly satisfactory. Among other things, it was difficult to select groups of cysts at roughly the same stage of development and some stages were certainly missed. Finally, I reached the rather obvious conclusion that the best container for the cysts was the earthworm in which they occurred and, even if the cysts could not be separated into selected groups, comparatively few would be lost if the whole Earthworm were killed and fixed and then the required parts of it processed and sectioned. This simple technique has its drawbacks, for instance the difficulty of impregnating thoroughly the tissues of the Earthworm but, on the whole, I found it very satisfactory. It meant, of course, that a great number of sections had to be prepared and carefully scrutinized to ensure that every possible stage in development was represented and detected. In all, some 4,000 sections of Earthworms and a somewhat larger number of sections prepared from selected cysts were examined. Since a single section of a heavily-infested Earthworm might contain a hundred or more sections of different larvae, the figure for the number of larvae examined is certainly in keeping with the figures for the estimated parasite pbpulations of the Earthworm and Woodcock.

A number of different staining techniques was used, including Heidenhain's and Ehrlich's haematoxylins, Ehrlich's and Eosin, Methylene Blue (for nervous tissues), Mallory's Triple stain, Masson's Ponceau-Acid Fuchsin counter-stained with Light-green, Heidenhain's Azan, Alcoholic Hydrochloric Acid Carmine, Gower's Aceto-carmine and Betchaku's technique for nervous systems. For differentiation of tissues, dependability and ease of use I found Mallory's triple stain very satisfactory.

Sections were cut at 7μ, 10μ and 15μ, depending on what I was looking for. The 15μ sections were most useful for examination of the musculature of the scolex in the fully-developed larva. For special purposes, e.g. examination of the complete larva in early stages of development, sections up to 100μ were cut. A hand-microtome was used for the latter in place of the rotary microtome used for thinner sections.

A very useful technique in the study of cestode hooks, for the introduction to which I am indebted to I.M. Sandeman, is to make a 'squash' preparation of the scolex in Liquide de Berlese, a powerful clearing agent which renders invisible under the microscope virtually all the tissues but leaves the hooks standing out clearly. The preparation can be pressed under a cover-slip until the hooks are lying perfectly flat on the microscope slide, so enabling accurate measurements and drawings to be made.

Description of Cysts

These occur as small, translucent bodies (Figs. 14, 16) lying free in the body cavity of the Earthworm, for the most part, but in the early stages of their development they are often embedded in, or associated with the chloragogenous cells and other material surrounding the outer wall of the intestine. They are spherical in form in the early stages, but in heavy infestations they are irregular in shape because of the pressure of the other cysts with which they occur. They vary in diameter from about 40μ in the earliest stage I have been able to detect, to about 1.5 mm. in a large cyst enclosing many larvae.*

* Measurements given throughout the description of the larvae are for material fixed in Bouin's fluid, preserved in 70% alcohol, dehydrated in the alcohols, cleared in cedarwood oil and embedded and sectioned in paraffin wax.

The size of the living cyst depends on the osmotic pressure of the medium surrounding it, retaining its natural size in 0.53% NaCl which is isotonic with the coelomic fluid of the Earthworm.

The processes of fixing, dehydrating, clearing, and embedding each shrink the material to some degree. I found, by taking measurements of a single cyst in a very early stage of development, that the diameter of the cyst altered as follows:

Diam. in 0.53% NaCl	209μ
Diam. after 20 hours in Bouin's fluid	158μ
Diam. after dehydration and clearing	134μ

This cyst, then, shrank to almost 70% of its original diameter during processing, most of the shrinkage occurring in fixation.

In a fully developed, individual larva the overall dimensions do not change to any great extent during processing, but the scolex within the surrounding membranes shrinks very considerably.

A cyst may contain a single larva (single cyst) (Fig. 24, 25), or two (double cyst) (Fig. 23) or more larvae (multiple cyst) (Fig. 18). A detailed description of the larva follows.

DEVELOPMENT OF THE LARVA

The cyst is derived from the onchosphere contained in the egg produced by the adult Paricterotaenia paradoxa. It can be assumed, from the known pattern of development of other cestodes, that the onchosphere is liberated from the egg in the alimentary canal of the Earthworm by the action of the intestinal juices of the host and then migrates through the wall of the intestine to the coelom of the worm where it undergoes the remainder of its development in the Earthworm.

I have not found the onchosphere or its hooks in any Earthworm. The earliest stage of development found by Metchnikov consisted of a solid, spherical mass of cells contained in a thick cuticle. This later becomes hollow. In the earliest stage I have observed, (Figs. 1a, 1b), a central cavity is evident. This cavity has been likened to the blastocoele of a developing embryo. There are relatively few cells, the nucleus, nucleoli and mitochondria being fairly obvious in many of them.

The hollow sphere now grows; the cuticle of the wall becomes thinner; the cells divide and move out to line the inside of the cuticle and, eventually, the almost solid sphere becomes a thin-walled

bladder, lined by a layer of apparently undifferentiated cells (Figs. 2a, 2b). At this stage the sphere is of no fixed diameter but it would appear that its size is determined by the space available in the body cavity of the host because the largest cysts are to be found in large specimens of the Earthworm with comparatively few parasites.

Whatever be the case, there now appears a number of centres of proliferation in the cell-layers (Fig. 26). The larger the sphere, the greater the number of these 'buds' or 'tubercles' but there is no obvious pattern in their distribution and their number is variable in the cysts. The number varies from one to fifteen while Metchnikov gives only thirteen as his largest count. These buds appear at first as formless condensations of cells, but grow and become almost hemispherical (Figs. 3a, 3b, 14, 15, 27).

As the bud grows, the first signs of differentiation of the cells appear: the cells assume a columnar arrangement normal to the inner surface of the sphere while some of the cells furthest from the base of the bud appear larger than those nearer the cyst wall (Figs. 4a, 4b).

At the base of the bud the cuticle of the cyst thickens and consists of an inner and an outer zone. The latter is minutely granular but the former is a clear zone on the inner surface of which numerous extremely small fibrillae are visible. The outer zone is bounded on each side by a band of cuticular material which (in Mallory's triple stain) stains slightly darker than either of the zones (Figs. 4a, 4b). The edge of this cuticle is at first parallel to the wall of the

'mother cyst' but, at a later stage, it extends, just inside the periphery, towards the free end of the bud (Figs. 5a, 5b).

The centre of the external surface of this cuticle now separates from that of the 'mother cyst' but the edges of the bud remain attached to the cellular layer lining the cyst although they are now structurally distinguishable from it (Figs. 6a, 6b, 20). This separation is followed by retroversion of the bud, on the completion of which process the larva has attained a bell-shaped or ovate form (Figs. 7a, 7b, 16,17). The future scolex of the larva is directed towards the wall of the cyst and the side-walls of the 'bell' towards the centre of the cyst, still attached to the cellular layer which becomes partly or wholly detached from the 'mother cyst'.

Contrary to Metchnikov's belief, the side-walls of the bell do not disappear but persist and become the walls of the caudal bladder of the fully-developed larva.

The convex, solid end of the larva is anterior, the site on which the scolex will eventually develop. The side-walls grow until they almost meet at the opposite, posterior end.

The larva now grows and changes to become pear-shaped, the narrower part of the body being at the posterior end (Fig. 2, 8a). The body is bounded by a very thin limiting-membrane. In the centre of the broad, anterior end a densely-staining ball of cells becomes apparent and from this a single column of long, narrow cells runs to the point of junction of the side walls of the larva at the posterior end. At this position in the larva there is a small vacuolated area

or sac. The cells of the column are orientated with their long axes at right-angles to the antero-posterior axis of the larva and from each cell fine processes run out into the parenchyma. These cells persist for some time but later disappear posteriorly and are then to be found only in the anterior end, associated with the bulb of the almost fully-developed larva.

The above developments are illustrated diagrammatically in Fig. 48 and those which follow in Fig. 49.

During further growth, the larva becomes proportionately longer and the posterior bladder outgrows the 'head', with a thinner layer of cells inside its walls (Fig. 19). The larva is no longer bounded by a thin membrane, but has a distinct cuticle. The cells contiguous with the cuticle have lengthened, their long axes normal to the inner wall of the cuticle, and are already assuming the appearance of the sub-cuticular cells of the adult cestode and, evidently, their function, as the cuticle has already formed around the larva.

At the anterior end of the larva the constituent parts of the adult scolex gradually differentiate. The mass of cells in the centre of the region grows and the cells arrange themselves in columns in an antero-posterior direction. In front of this an ellipsoidal structure is formed in the cells at the anterior extremity of the larva, its long axis at right angles to the long axis of the latter, and four densely-cellular areas on the sides of the larva at the level of the central mass of cells mark the primordia of the suckers (Figs. 9a, 9b).

The central mass of cells, the prebulb*, now moves forward in relation to the suckers and forms a double ring of cells separated by a vacuolated layer round the base of the former anterior ellipsoidal structure. This latter, the bulb*, has now become spherical with a conspicuous ring of large cells round its anterior part. The bulb appears to be drawn out at its base into processes which run posteriorly through the centre of the prebulb, joining an axial column of cells running between the suckers to the neck region of the larva (Figs. 10a, 10b, 22, 28, 29).

The constituent parts of the scolex are now all present, in a very early form, even to the hooks of the rostellum which appear as two rings of minute hooklets, one ring associated with the vacuolated tissues between the two rings of the prebulb, the other associated with the margin of the posterior ring of the prebulb. Later, the definitive hooks of the adult cestode appear as hollow cones in the anterior ring of hooklets while the minute hooklets of both rings are lost. The definitive hooks grow and assume their final form as the remainder of the scolex develops.

* The terms 'Bulb' and 'Prebulb' are used here as they were by Crusz (1947) as there is a close parallel between the relationships in these structures in the material I am describing and those at a similar stage of development in Cysticercus fasciolaris Rud., as described by him.

The Bulb

This first appears as a barely discernible differentiation of the tissues at the extreme anterior end of the larva after formation of the central mass of cells of the prebulb, but before any sign of development of the suckers is evident. Gradually, a flattened sphere with membranous walls is formed, with a conspicuous ring of large cells round its anterior surface and other cells arranged in columns round its walls. These cells are the myoblasts of the elevator* muscles of the hooks. The tissues of the bulb extend posteriorly through the centre of the prebulb and between the suckers and are associated with a column of cells which passes into the neck region of the larva.

As development continues, the prebulb invests the bulb, carrying its hooks and myoblasts with it until it fuses in front of the bulb, now completely enclosed. Behind the narrow neck of the developing rostellum the suckers have appeared as clearly marked hemispheres of densely packed cells.

* I use the term 'elevator' as it is used by other authors (Rees, 1960) to designate the muscles used to expose the blades of the hooks in some tapeworms although the movements of the hooks and the points of attachment of the muscles differ greatly from tapeworm to tapeworm. The term 'extensor' would be equally applicable in the case of P. paradoxa as it conveys the impression of an opening out of the ring of hooks similar to the movement of the ribs of an umbrella. 'Abductor' would describe the movement of the hooks away from the median line, while the similarity between the operation of the hook muscles in P. paradoxa and the operation of the 'hair-raising' muscles of the mammals and the 'feather-raising' muscles of the birds would be conveyed by coining the term 'arrectores uncinolurum'.

I use the term 'retractor' to convey the opposite meaning to 'elevator' although 'adductor' or 'depressor' might be more suitable.

The bulb, inside the prebulb, is spherical in form and, as the muscle cells of the prebulb divide and form columns round its sides, a long, narrow cone of what appears to be connective tissue develops in the sphere. The base of the cone is at the anterior margin and the apex at the centre of the bulb. Round the cone, the myoblasts of the bulb orientate themselves and a number of large, clear cells near the periphery of the sphere probably indicates the beginning of the formation of the glandular structure of the rostellum (Figs. 11a, 11b).

The main elements of the bulb, the central part of the rostellum of the adult, are now all present: the walls of the cone become bounded by bands of circular muscles; the system of muscles for extending the hooks forms; the glandular cells and their processes fill the centre of the bulb behind the cone.

The Prebulb

This is the first obvious structure to form in the larva after the early retroversion. It appears as a dense, spherical mass in the centre of the anterior part of the larva and, shortly afterwards, is connected to the posterior margin of the larva by a median column of cells. As the bulb develops, the prebulb widens and its cells, which stain very deeply and are densely packed, divide transversely with regard to the long axis of the larva to form two rings of cells separated by a clear, vacuolated zone. The anterior ring is very narrow, the posterior broad and well-defined.

In the clear zone between the rings, and just in front of the posterior ring, the definitive hooks of the adult form as simple, horn-shaped processes with thin walls. They are part of a band of minute hooklets and another band of hooklets is associated with the posterior margin of the cell ring.

The double-ring structure of the prebulb now proceeds to invest the bulb (Fig. 46). In this process the bulb sinks within the rings and the anterior margin of the prebulb closes in front of the bulb, leaving nothing except, perhaps, a minute pore to indicate that the bulb was previously an 'external' structure. The anterior ring of the prebulb is carried forwards and inwards to form the retractor muscles of the hooks; the hooks thereby being moved forwards into a ring in the front margin of the larva; the posterior ring of the prebulb encircles and encloses the sides of the bulb, which is now a sphere in which the hook-extensor muscles, the glandular system and the median cone are taking shape.

In the case of the anterior ring of the prebulb and of the hooks, their further development is fairly straightforward. The ring of cells, the myoblasts of the hook-retractor muscles, form circular bands of muscles in the connective tissue in which the bases of the hooks, the future guards and handles, become embedded. The hooks change from a simple horn-shape to a more advanced form with a hook-shaped blade extending from a flat, leaf-shaped base. As the blade elongates, the base lengthens and narrows, extending posteriorly, in relation to the blade, to form the handle, and anteriorly to form the guard. The

handles and guards of the hooks are embedded in connective tissue and cuticle, forming a cone lined by the series of parallel, transverse rings of the retractor muscles.

The development of the posterior ring of the prebulb is more complex than that of the anterior ring. The cells of this broad ring become arranged into a girdle of columns of cells each parallel to the median axis of the rostellum. During and after the investment of the bulb these columns split longitudinally to form an inner and an outer layer of cells which remain joined at the posterior end of the columns but diverge anteriorly. The inner column is closely associated with the bulb and develops into the basketwork of longitudinal and circular muscles of the hook-bearing part of the rostellum. The outer layer of cells develops into the hollow cone of broad bands of circular muscles forming the outer sac of the rostellum (Figs. 3, 12a, 12b).

At the completion of the developments described above the larva has attained a form easily recognisable as that of a young cestode. The rostellum is formed and fully-extended, except for the hooks which lie flush with its sides. Behind the rostellum the four suckers swell the scolex, followed by the narrower neck to which is attached the wide, bladder-like caudal vesicle.

* Invagination

The invagination of the scolex, which follows, has been described by numerous authors and requires no elaboration. The rostellum withdraws into the scolex to between the suckers, its hooks and inner sac sheathed in the outer sac. The scolex itself, by a process of retraction, becomes enveloped by the wall of the caudal vesicle which thus forms a double-walled sac round it, leaving only a narrow pore in front of the rostellum to show where the walls of the bladder meet. The larva becomes an ellipsoidal cyst, almost fully developed; the parenchymal muscles, excretory system and nervous system become evident and the hooks, up to now sinuous and malleable, harden and take on their final form (Figs. 13, 32, 33).

The Rostellar Hooks

The general scheme of the development of the hooks has been outlined before under the section dealing with the development of the prebulb. As stated there, the hooks appear first as two bands of minute hooklets round the prebulb, one band in front of the posterior ring of cells, the other close to the posterior margin of the ring. In the anterior band a certain number of large hooklets develops

* Although the term 'invagination' is commonly used to describe the process of withdrawal of the scolex into the caudal vesicle, the scolex itself is not invaginated in the case of P. paradoxa but is retracted into the vesicle by the invagination of the anterior part of the vesicle into its cavity. The relationship between the different parts of the scolex does not alter.

while the remaining hooklets in this band and all the hooklets in the posterior band disappear. The mode of disappearance has not been determined: it may be that the hooklets are resorbed, or are cast off and lost in the contents of the cyst in which the larvae lie; or they may be incorporated in the remaining hooklets which grow to become the hooks of the adult.

I have not followed the changes in hook-form during development in P. paradoxa as I have hot been fortunate enough to find the different stages in material cleared in Berlese's fluid. However, in the closely related polycercal larvae of P. burti I have obtained a number of cysts at different stages of development in which the progress of development from the earliest hooklet to the final hook can be followed. I have studied a sufficient number of the hooks of P. paradoxa at different stages to be certain that the mode of development of the hooks in P. paradoxa is the same as that in P. burti, the only significant difference lying in their sizes. The hooks of the adult P. paradoxa are 75-108μ in length, those of P. burti 44-52μ, while the range in the number of hooks in P. paradoxa is 14-18, that in P. burti 14-16. (Metchnikov found 14-17 hooks in his material, I found 16-18 in mine.) In both cases the form of the hooks is characteristic of the Genus Paricterotaenia (Fig. 59).

The hooks are not everted or retroverted when they are exposed to pierce the mucosa in the intestine of the final host, but are carried clear of the scolex when the inner sac of the rostellum is

forced out of the outer sac and are then rotated, by the contraction of the hook elevator muscles, so that the blades move clear of the rostellum (Fig. 47).

An account of the development of the hooks in P. burti is given in Appendix 1 .

THE FUNCTIONAL MORPHOLOGY OF THE FULLY-DEVELOPED SCOLEX

THE MUSCULATURE

It is convenient, in examining the muscles of the scolex and their functions, to consider them in two separate groups: (i) the muscles of the rostellum and (ii) the muscles of the remainder of the scolex.

The Intrinsic Muscles of the Rostellum

The rostellum of the fully-developed larva consists, basically, of two muscular, doliiform sacs, a hollow outer sac which houses an inner sac on which is mounted the proboscis with its ring of hooks (Figs. 13, 32). The outer sac is a succession of parallel, broad rings of muscle, gradually diminishing in diameter to the pointed, posteriorly-directed apex (Fig. 43). Typically, the sac is about 200μ in length and 80-100μ in diameter at its widest part but the dimensions vary considerably. The muscle-rings are 60-70 in number and each ring is 2-4μ broad. A few strands of longitudinal muscle occur in the wall of the sac, but it is, in essence, a sheath of circular muscles which, by contraction, drives the inner sac forwards and partly out of the scolex to enable the proboscis to penetrate the mucosa and force the hooks into the villi of the intestine of the host.

Bands of muscle run between the suckers and the outer sac of the rostellum (Fig. 37) and four strong bundles of muscle fibres run from the parenchymal musculature to the sides of the sac. These are discussed later.

The inner and outer rostellar sacs are connected, at the apices only, by a strong process and at the apex of the outer sac there is a small, hollow, spherical structure which is obvious in some specimens but not in others. I have not been able to relate it definitely to any of the systems in the larva but, from its position, it appears to be associated in some way with the connection between the two sacs. As there is no other obvious connection between the inner sac and the rest of the scolex, a branch of the nervous system and of the excretory system apparently runs through the junction into the inner sac.

The most complex part of the scolex is the inner sac of the rostellum with the associated proboscis (Fig. 13). The posterior part of this structure appears, in median section, to have an elongated, heart-shaped outline with the apex directed posteriorly. The side-walls of the 'heart' have the appearance of a basketwork of circular and longitudinal muscles (Fig. 42, 44). The anterior end, with the invagination, is of fine circular muscles only. These muscles are not so thick, individually, as the circular muscles of the outer sac, but they are more numerous. The inner sac is about 120μ long and 75-80μ in diameter at its widest part. There are some 75 bands of longitudinal muscle and 60-70 bands of circular muscle in the part where they occur together, the bands being about 1-2μ in width. There are about 80 bands of the fine circular muscle in the anterior part, these bands being about 0.5-1μ in width. Although the muscles of the posterior part appear to be a basketwork, they are not interlaced: the layer of circular muscles lies inside the layer of longitudinal muscles, the layers being closely adjacent.

Contraction of the longitudinal muscles shortens and widens the sac. This is co-ordinated with relaxation of the circular muscles of the outer sac and contraction of the longitudinal muscles of the parenchyma. The final result is a rapid withdrawal of the rostellum into the scolex, the hooks, of course, being retracted. During this process the fine circular muscles of the anterior part of the 'heart' contract, elongating the invagination and carrying the inner ends of the hook-elevator muscles down into the interior of the sac. This contraction of circular muscles in step with the contraction of longitudinal muscles in another part of the rostellum, may seem unacdeptable at first sight, but it must be remembered that the invagination in the sac is the cone of the bulb and, therefore, its muscles and those of the anterior part of the 'heart' are derived from the bulb, whereas those of the side-walls and of the outer sac are derived from the prebulb.

In the parenchymatous interior of the sac are two layers of muscles, an anterior and a posterior layer, and a glandular structure (Figs. 34, 35, 36, 21), which is discussed later. The posterior layer of muscles consists of eight pairs of strong bands radiating from the apex of the invagination and with the outer ends terminating in the connective tissue of the wall of the sac at the level of the tips of the hooks when they are in the retracted position (Fig. 35). The anterior layer of muscles lies parallel to this but consists of thirty-two bands of muscle, radiating from near the base of the invagination in the retracted position, and terminating in the connective tissue

of the wall of the sac just posterior to the level of the guards of the hooks (Fig. 34). In longitudinal sections these rings of muscle strands appear as two layers inside the sac (Figs. 36, 50); in transverse sections they appear as the radii of circles with the invagination as centre and the wall of the sac as circumference (Figs. 34, 35). The muscles are completely relaxed, broad and curved, when the hooks are in the fully retracted position and at this time they lie in two planes almost normal to the axis of the invagination to which they are attached. These muscles, very conspicuous in stained thin sections of the scolex, are the hook-elevator muscles. They are not attached directly to the hooks but to the connective tissue in which the handles and guards of the hooks are embedded. They do not act directly on the hooks, but raise the blades of the hooks by modifying the shape of the rostellum (Fig. 47).

When the rostellum is in the extended position the circular muscles round the posterior part are contracted, those in the anterior part relaxed. The pressure of the contraction pushes out the invagination and stretches the connective tissue between the bases of the hooks, forcing the anterior part of the rostellum into a bulbous shape. The hooks at this point, were there no further changes in shape, would be almost flush with the front of the rostellum, their blades only slightly exposed (Fig. 47C).

The complete exposure of the blades of the hooks is brought about by contraction of the double ring of hook-extensor muscles. The origins

of these muscles, i.e. their inner ends, are carried forwards when the invagination to which they are attached is forced out. The origins of the anterior layer are subsequently carried outwards towards the equator of the bulging anterior end of the rostellum while the origins of the posterior layer reach the centre of the anterior inner surface of the rostellum (Fig. 47D). The muscles then contract, flattening and widening the sphere to the form of an oblate spheroid, in which the hooks lie, pulling down the handles of the hooks and drawing the walls of the rostellum away from the blades of the hooks. Each hook is thus brought to a position almost at right angles to the long axis of the rostellum, its blade fully exposed (Fig. 47E). The action of the hooks is comparable to the action of the moveable barbs of a harpoon: they lie flush during penetration but expand in the tissues and so resist withdrawal of the 'head'. This system does not lend itself to deep penetration of the walls of the intestine of the host. Examination of sections of adult P. paradoxa in situ in the intestine of the Woodcock shows that the cestode does not penetrate the wall of the intestine but that the scolex usually lies in the mucosa or, less commonly, embedded in the villi (Fig. 36). The nature of the 'matrix' is such that hooks with small blades would gain no purchase and hooks with large blades prevent the parasite from being swept along and out of the intestine.

Retraction of the hooks is a reversal of the process of elevation. The elevator muscles and the circular muscles of the greater part of the rostellum and the outer cone relax; the circular muscles of the

anterior part of the inner rostellar cone contract, re-forming the invagination which carries the ends of the elevator muscles back into the central part of the rostellum; the longitudinal muscles of the rostellum and the circular muscles of the proboscis contract, the latter drawing the handles of the hooks together.

The proboscis is the anterior, hook-bearing part of the rostellum. It contains a mass of connective tissue, the inner part of which is very elastic and appears fibrous under the microscope, the fibrous structure running parallel to the longitudinal axis of the rostellum. The handles and guards of the hooks are embedded in the non-fibrous outer part in a circle, the handles lying just outside the circumference of a cylindrical series of rings of circular muscle, the hook-retractor muscles (Figs 13, 41). By contraction, these muscles draw the guards and handles of the hooks inwards into the retracted position. The hook-retractor muscles and the tissues in which they lie originate in the anterior ring of the prebulb.

The Extrinsic Muscles of the Rostellum

The inner sac of the rostellum is quite separate from the rest of the scolex, except for the connection at its apex with the outer sac. The latter, however, is intimately related to the remainder of the scolex and to the rest of the cestode. This is well illustrated by the muscles which connect the rostellum to (a) the suckers and (b) the parenchymal muscles of the strobila.

Apart from the rostellum, the most conspicuous muscles in the scolex are bands which run from the outer sac of the rostellum to the suckers. They may be divided into two groups: those which run from the lower half of the rostellum to each sucker and those which run from the upper half of the rostellum to each sucker (Figs. 37, 52). They are inserted on the median face of each sucker well inside its rim. There are approximately twelve bands of muscle from the lower half of the rostellum to each sucker but only three or four from the upper part.

Contraction of these muscles will pull the suckers in towards the rostellum, lengthening and narrowing the scolex, and will also draw the rostellum forwards in relation to the remainder of the scolex. If this is done at the same time as the outer sac of the rostellum contracts, the whole scolex will become long and narrow with the proboscis protruded, i.e. the scolex will be in its best form for insertion into the mucosa or between the intestinal villi of the host.

The parenchymal muscles of P. paradoxa are very strong. They occur as a dorsal and ventral layer of longitudinal bundles of muscle fibres which separate the parenchyma into cortex and medulla. There are approximately eighteen bundles of muscle fibres in each layer and each bundle is, at its widest, about 6μ in diameter. It is impossible to give definite numbers of measurements for these muscles as each bundle consists, for most of its length, of three or four smaller bundles, each usually about 1.5μ diameter, which branch off and merge with other main bundles. The parenchymal muscles, therefore, form a very loose

network or mesh between cortex and medulla (Fig. 40). As these muscles pass into the neck region from the strobila four of the central bundles, two dorsal and two ventral, are inserted into the sides of the rostellum (Figs. 38, 39). The remainder continue forwards and terminate in the suckers.

The parenchymal muscles, by contraction, shorten the cestode along its length and withdraw the rostellum into the scolex.

The Inter-Acetabular Muscles

The remainder of the intrinsic muscles of the scolex conform to what appears to be a standard arrangement in the Cyclophyllidea: adjacent suckers are connected by a number of fine muscle fibres and diagonally-opposite suckers are similarly linked. In addition, the anterior rim of each sucker is linked to the posterior rim of the adjacent suckers by a few fibres. All these muscles pass outside the walls of the rostellum.

The Acetabula

Each of the four suckers consists of the usual arrangement of radial muscles bounded on the inner surface by the basement membrane and on the other surface by cuticle. Within the body of the sucker and closely applied to the basement membrane is a layer of fine strands of concentric, circular muscle fibres. The circular and radial muscles of the sucker are simple modifications of the circular and longitudinal peripheral musculature.

As has been already noted, the suckers are intimately linked with the rostellum, the longitudinal muscles of the parenchyma, and with each other.

It is obvious, from the complicated pattern of muscles, that the scolex is an extremely mobile structure. Its shape is very variable, from the broad, flat form of the contracted cestode to the long, narrow, pointed form of the cestode in the fully-extended position.

The suckers themselves are large (about 120μ in diameter when fixed in the extended state), strong and mobile. When the parasite lies in the intestine of the host they grasp the mucosa and (with the aid of the hooks) hold the tapeworm in position (Fig. 39). It is probable that they are also used in penetrating the mucosa and inserting the scolex in between the villi. Evidence in favour of this lies in the actions of living specimens of newly-evaginated P. paradoxa in vitro. The active parasite, the proboscis probing to and fro, travels rapidly over the bottom of the glass dish by moving foward one side of the scolex then the other, the suckers adhering strongly to the glass.

THE EXCRETORY OR OSMO-REGULATORY SYSTEM

Posteriorly to the scolex the longitudinal excretory vessels of P. paradoxa consist of two lateral canals, one dorsal and one ventral on each side of the cestode. The ventral vessels, relatively large and thin-walled are connected posteriorly in each proglottis by a transverse vessel. The dorsal vessels, on the other hand, are of small diameter and are relatively thick walled.

As the vessels pass into the scolex, however, they divide to form an intricate labyrinth of vessels which ramifies through the whole of the anterior part of the scolex outside the rostellum. The paired canals of the strobila and the major vessels in the scolex stain readily and are easily traced, but, as they sub-divide, they become more and more difficult to follow until they are indistinguishable from the parenchyma of the interior of the cestode (Figs 54,.61).

THE NERVOUS SYSTEM

The nervous system in the Cestoda is notoriously difficult to stain for examination and I found P. paradoxa no exception. Mallory's Triple stain was as effective as any of the other general techniques used, and it proved more satisfactory than Methylene Blue or Bechaku's method for staining the nervous system. Even in the best stained sections, however, the main parts of the system could only be partly distinguished, and this with great difficulty, while any minor structures that might exist went unobserved. Comparison of many sections produced a picture

of the general scheme of the nervous system which conforms, as far as can be determined, to the basic plan found in other cyclophyllidean tapeworms.

Two lateral nerve trunks run the length of the strobila, one on each side just lateral to the excretory vessels. These arise from a pair of ganglia in the central part of the scolex. From the ganglia, nerves run out to the suckers, to the remainder of the scolex and forward to a nerve-ring round the anterior part of the rostellum (Fig. 60).

The pair of ganglia lies close to the wall of the rostellum, one ganglion on each side. They are joined, presumably, by a ring-commissure encircling the rostellum, which, however, I have not been able to detect. Anteriorly, each ganglion gives rise to a nerve-trunk which runs forward along the side of the rostellum to join a nerve ring, the anterior commissure, from which four strong nerve trunks run out, one to each sucker, and smaller nerves branch off to various parts of the scolex. Presumably, a branch runs posteriorly from each ganglion to the apex of the rostellum where they join to pass into the inner rostellar sac (Fig. 52).

It is certain that the inner cone of the rostellum must be well-supplied with nerves to its intricate musculature, but I have not been able to make out any part of the nervous system in this region. There is a conspicuous group of cells in the centre of the rostellum with branching processes running out to the muscular walls. My first impression was that this was a central nerve ganglion with nerves to the muscles, but further examination revealed an absence of the

characteristic nervous tissues in the structure and it did not stain as did the remainder of the nervous system. In fact, it appears to be a glandular structure and is dealt with below.

THE GLANDULAR STRUCTURE OF THE ROSTELLUM

Within the rostellum of the dilepid tapeworms a structure has been noted which Joyeux and Baer (1960) describe as glandular in nature. In P. paradoxa, when the rostellum is fully extended, it appears as a group of large cells near the apex of the inner sac from which a pair of processes runs forwards and then branches into finer processes which eventually terminate in the wall of the sac. A few processes also run to the wall of the sac near the apex. Several large cells are to be found in the main anterior processes as well as in the central group. The actual arrangement of the parts varies in different specimens and appears to depend on the degree of contraction of the rostellum, the cells being distributed throughout the interior of the sac when the rostellum is withdrawn into the scolex and the musculature of the walls of the inner sac is relaxed.

The cells in the structure stain readily, in contrast to their processes and the tissues in which they lie. The former do not stain as muscle, nor as connective tissue, nor as nerves. They stain well with gold in Bechaku's method for the nervous system, but known nervous structures in the same sections do not take up the stain and the central structure cannot be assumed to be associated with the nervous system. Bechaku's method gave an excellent rendering of the whole of the main structure (Fig. 45). The probable nature of this body is discussed later.

THE INTERMEDIATE HOST

DISCUSSION

Incidence

Although some authors have stated that Polycercus was found in the common Earthworm Lumbricus terrestris L., Metchnikov did not name the species of Earthworm in which he found his cysts. He simply mentioned it in Russian as the 'Rainworm'.

According to my observations the larvae occur in Allolobophora terrestris only, and moreover, were found only in immature specimens of this Earthworm. This suggests three interesting possibilities.

(1) The infestation with P. paradox induces parasitic castration in the Earthworm, suppressing development of the reproductive organs. Some of the infested Earthworms were of a length such that one normally would have expected the presence of a prominent clitellum and well-developed gonads, but these were absent. If parasitic castration is indeed the case, the suppression of the development of the sexual characteristics is presumably due to interference in the normal system of endocrine control of hormone production in the life-cycle of the Earthworm.

(2) The Earthworms develop a high degree of immunity to infestation by the larvae as they mature. This immunity could not be inherent as, if it were, it would presumably be effective in the young Earthworms as well as in the older. It is difficult to see how it can be acquired as this would infer previous infestation and subsequent suppression or discharge of the parasites. In some cases the larvae were found

concentrated in the posterior end of the host, but in others they were distributed throughout the Earthworm where shedding of the posterior segments, as has been noted, would not rid the Earthworm of them.

(3) The feeding habits of the adult Earthworms are so different from those of the immature worms that only the latter eat the eggs of the parasite and are, in turn, eaten by the final host, or die before they reach maturity because of the deleterious effect of the parasites.

One other alternative, of course, is that, by chance, I have missed infested adult worms in collection and/or examination. This appears, to me, less probable than the first alternative.

It is generally accepted that the oncosphere enters the coelom of the intermediate host from the intestine by a simple process of penetrating the wall of the intestine and breaking free into the body cavity. Although I have little evidence in support of this and have found no oncopheres in the blood vessels of the Earthworm, I am of the opinion that the oncospheres of Polycercus lumbrici are distributed throughout the Earthworm by penetrating the wall of the intestine and then entering a blood-vessel. They may then be carried by the blood until they lodge in a capillary where they start to develop, if they have not already started while in the blood stream, by shedding their hooks and secreting a thick cuticle round the solid mass of cells.

This suggestion explains a number of facts:

(1) I have not been able to find the hexacanth hooks in any of the post-embryonic stages of Polycercus lumbrici*.

* Other workers also remark on their inability to find hexacanth hooks in proliferating cestode larvae.

(2) The larvae are found in the anterior segments of the Earthworm, in front of the level of the gizzard. On the assumption that the oncosphere must be subjected to the action of the digestive juices of the host before it hatches and penetrates the wall of the intestine, it is difficult to see any explanation for the presence of the larvae anterior to the intestine other than migration either in the blood system or in the coelomic fluid through the pores in the septa.

(3) The larva of the closely related cestode Paricterotaenia stellifera Krabbe, 1869 is always associated with the main bloodvessel of its host, Tubifex rivulata*, and is surrounded by an adventitious cyst, filled with blood or a bloody fluid, which protrudes into the coelom of the host.

* I found many specimens of Tubifex infested with the cysticercoid larva of P. stellifera while I was examining various freshwater invertebrates in search of cestode larvae.

The occurrence of a cysticercoid in Tubifex has alaready been noted by Mrazek (1907) and one of the species I have found corresponds to his description. He did not offer any suggestion as to what might be the final host of the larva, but the hooks correspond to those of Paricterotaenia stellifera and I have no doubt that this cysticercoid parasite of Tubifex is indeed the larva of P. stellifera. Supporting evidence for this lies in the facts that P. stellifera is a common parasite of Snipe, Tubifex is a favourite food of these birds and large numbers of the Common Snipe (Gallinago gallinago gallinago, L.) and the Jacksnipe (Lymnocryptes minimus Brünnich) frequent the areas where I found the infested Tubifex.

Durihg the Autumn of 1961, I found about 10% of the Tubifex in two different areas (Mount Melville and Boarhills) infested with the cystericercoids of P. stellifera. There was usually only a single cyst in each infested worm but a few had two cysts and exceptionally there were more than three.

In approximately 1% of the Tubifex collected at Mount Melville at this time I also found minute cysticercoids whose hooks are similar to those of Fimbriariodes falciformis Linton, 1927. In one case, both species of larva were in the same specimen of the host.

THE DEVELOPMENT OF Polycercus lumbrici

As Metchnikov has been mistranslated, misrepresented and doubt has been cast on the accuracy of his observations, the salient points of his paper should be re-examined. Translated as literally as possible, they are:

(1) The cysts occur in the Russian 'rain worm'. The scientific name of the host is not given.

(2) The scolices have a 'crown of long hooks from 14 to 17 in number'.

(3) The stages of development:

1st Stage - A solid mass of cells within a thick cuticle.

2nd " - Central cavity forms.

3rd " - Thin-skinned bladder lined with a layer of cells.

4th " - Tubercles, the embryos of the scolex, appear on the inside of the wall of the bladder.

5th " - Central part of the base of the tubercle separated from the wall of the sphere.

6th " - Larva assumes a bell-form with thin side-walls.

7th " - Atrophy of the side-walls of the bell. Principal features of the scolex defined.

8th " - Waist forms in scolex: short head; long, broad bladder. Epithelial layer and inner layer with longitudinal muscles and central cavity formed.

9th " - Further definition of above parts. Anterior part lengthens and assumes typical cysticercoid form. Posterior part takes on appearance of bladder. Whole surface covered with cuticle.

Metchnikov's observations	Personal observations
*1. Cyst from Earthworm	From Allolobophora terrestris
2. 14 - 17 long hooks	16 - 18 hooks as in P. paradoxa
3. 1st stage : solid mass of cells	As Metchnikov
2nd " small central cavity	ditto
3rd " bladder with layer of cells surrounding cavity	ditto
4th " tubercles, embryos of scolex, appear	ditto
4. Middle of surface attached to sphere separates	ditto
*5. -	Retroversion of larva
6. Larva assumes bell-form with thin side-walls	As Metchnikov
*7. Atrophy of side-walls	Side-walls grow and almost meet at posterior end
8. Waist forms in scolex	As Metchnikov (scolex=larva)
Epithelial layer forms	ditto
Inner layer with longitudinal muscles forms	Not observed
*Central axial cavity forms	Axial line of cells forms
9. Further definition of above	As Metchnikov
Anterior part lengthens and assumes cysticercoid form	Anterior part lengthens, forms bulb and prebulb
Posterior part assumes form of bladder	As Metchnikov
Whole surface of scolex with cuticle	ditto
Two rows of hook rudiments on proboscis	ditto
Blade of hook appears, then handle and guard	Hook horn-shaped at first, gradually differentiates to form blade, handle and guard
*Calcareous corpuscles appear	Appear later
10. Final development of hooks	As Metchnikov
11. Invagination of head into caudal vesicle	ditto

Table 2 Comparison of Metchnikov's observations with the corresponding observations in the present work. (The asterisk indicates where the observations differ.)

10th Stage - Proboscis with local swellings: rudiments of hooks. Two rows of hooks of which only upper row survives. Blade of hook appears first, then handle and guard. Calcareous corpuscles appear.

11th " - Final development of hooks.

12th " - Invagination of head into caudal bladder and of proboscis into head.

(4) Infested worms fed to duck with no effect.

(5) Scolex similar to that of Echinococcus but unique in:

(a) Relationship to bladder produced by it.

(b) Brief existence of brood-capsule (= side-walls of bell-shaped larva = amnion).

(c) Position of head within bladder: no inversion of hooks or suckers.

(d) Presence of caudal bladder: no analogy with Echinococcus.

(e) Brood capsule of Echinococcus corresponds to the "amnion" of the larva.

A comparison of Metchnikov's observations and my own is given in Table 2. I have confirmed nearly all of Metchnikov's observations (for the first time), modified some and added some of my own.

It is obvious that the material provides a unique opportunity for a detailed study of the development of the larval stages in a cyclophyllidean cestode: it is readily obtainable; various stages of development are to be found in a single host; infestation is so heavy that detection of the larvae is not difficult and dissection to separate the cysts, if necessary, is very simple. On the debit side, the cysts.

are very small, but this is countered by the fact that the hooks and the associated musculature are strong. It is surprising that it has not been reported frequently and has not been more fully examined.

It should be pointed out that, although Metchnikov obviously made a thorough study of his material, noted the main points of development and realised the importance of his discovery, he did not carry out or, at least, did not publish the results of a detailed examination of the morphology of his larva. He appeared to be satisfied, as were most of his contemporaries, with following the main changes in the general form of a developing cestode and in fitting them into the picture of cestode development which was emerging at that time. This is not to detract from his work: even today the number of publications dealing with the development and detailed morphology of larval cestodes are probably fewer than those concerned with any other aspect of parasitology.

Metchnikov followed the general course of development as I have described it to this point. Here, however, our interpretations differ. The Russian author goes on to describe how the bud grows and assumes the shape of a bell, with which I agree. Then, however, he states that the sides of the bell "form a sort of amnion, or what in Echinococcus is referred to as a brood-capsule", grow thinner, change to a short stalk and finally atrophy, freeing the larva in the cyst.

According to my observations, the separation of the central part of the bud from the wall of the cyst is followed by changes illustrated diagrammatically in Fig. 48. These changes involve an increase in the size of the cavity of the bud and a retroversion of the side-walls so

that what was previously the internal surface of the bud is now the outer surface of the larva.

This is, apparently, a rapid process and although I have found one intermediate stage it was not one showing the walls of the bell turning back and future scolex emerging. The end-result in a multiple cyst should be a number of larvae attached by the bases of their thin-walled sides to a common, delicate membrane about the centre of the containing cyst, and with their densely-cellular ends pointing outwards towards the wall of the cyst. This form is, indeed, typical of the intermediate stages of development of the larvae (Fig. 6a). The pattern of development outlined by Metchnikov does not offer an explanation of how the multiple cyst with the larvae projecting outwards from a common membrane in its interior is attained.

While following the general course of development of the larva, I became more and more interested in the development of the structure of the scolex, particularly in its complex musculature. The description given (pp. 32-40) includes observations which are a repetition of some of Metchnikov's comments but the structure of the larva at different stages is discussed in greater detail than is the case in the Russian author's description.

I have not been able to confirm Metchnikov's observation of the earliest stage in the development of the larva in the coelom of the Earthworm as a 'sphere with a thick cuticle and filled with a solid mass of cells'. This stage may well occur in the body cavity of the Earthworm, but, even in the earliest stages of development that I have

observed, a central cavity is evident (Fig. 1a), very small at first but growing with the cyst to produce the hollow, thin-walled bladder which precedes the appearance of the 'buds' (Fig. 2a). The thickening and zoning of the cuticle which accompanies the growth of the bud is not mentioned by Metchnikov.

It would appear that the formation of the two zones is the first indication of the separation of the two layers of cuticle which precedes the parting of the larva from the wall of the cyst. The new cuticle of the inner zone is formed by secretory cells in the bud and the fibrillae at the base of the bud are processes from these cells (Young 1908).

The central column of cells which appears within the larva in the stage following retroversion was not noted by Metchnikov, nor has it been mentioned, to my knowledge, in descriptions of other Platyhelminth larvae. There is a number of possibilities which can be considered, on the basis of its appearance and position rather than on its function at this and future stages about which nothing is known. It may be:

(i) the primordia of the alimentary canal which probably existed in the primitive ancestors of the modern cestode.

This view is borne out by the association of the column with the bulb of the rostellum which contains a glandular structure used, it is thought, for secreting digestive enzymes to aid penetration of the intestine of the host (Joyeau and Baer, 1961), or assimilation of

nutrients. At its other extremity, the column appears to join the small bladder into which, by analogy with descriptions of other larval cestodes, the excretory system of the larva discharges.

(ii) the myoblasts of the parenchymal musculature.

This would explain the association of the column with the cell-mass which later differentiates to form the muscles of the rostellum. Some of the parenchymal muscles of the adult cestode join the rostellum.

If this were the case, however, it conflicts with Young's (1908) view that the muscles are derived originally from the parenchyma. It is possible that the myoblasts form originally as an axial column and then migrate into the parenchyma before muscle-formation commences.

(iii) the primordia of the reproductive system.

Each cell might be thought to represent the anlage of the reproductive system in a single proglottis of the adult cestode.

The third alternative appears to be the least likely; I have come to no firm decision on which of the other possible explanations is the most probable as I have not been able to follow the migration of the cells to see if they are associated with the excretory system or the musculature but, while it is perhaps easier to accept the cells as myoblasts, I favour the third alternative on the weight of the available evidence and my interpretation of it.

To my knowledge, there is no account in the literature on the Cyclophyllidea, or even on the Cestoda, which describes the development

of the larval tapeworm in all the stages following those just discussed. There is nothing, therefore, with which to compare my observations on the formation of the bulb, prebulb and the associated musculature up to the stage where the hooklets of the rostellum make their appearance. I have drawn an analogy between the structure of the rostellum in P. paradoxa and the rostellum in Cysticercus fasciolaris (pp. 24) but Crusz's description is solely concerned with the hooks and throws little light on related structures.

I have been unable to determine the exact relationship between the hooks, hooklets and the posterior ring of cells of the prebulb. The definitive hooks lie in the clear zone in front of the ring and may have some connection with a group of large, spherical cells lying inside the anterior ring of the prebulb and at the base of the bulb (Fig. 10a). It appears, however, that these cells are the primordia of the glandular system of the rostellum and that the hooks and hooklets are more closely associated with the posterior ring of cells of the prebulb and are secreted by specialised cells in this region.

I have not detected any unquestionable evidence (pp. 51,52) of the formation of the parenchymal musculature, excretory vessels nor nervous system in any of the stages prior to invagination of the scolex, in spite of careful examination. Metchnikov does not mention any of these systems except in the cysticercoid and, presumably, did not observe them in his patently careful examination of his material. They are present in the fully-developed larva and it appears, therefore, that they develop in the period following invagination of the scolex into the caudal bladder.

The Development of the Rostellar Hooks

In the living state, the hooks of the rostellum are among the more obvious features of those cestodes with armed scolices, and they are among the principal diagnostic features of the adult tapeworm. In the cysticercoid larva they are almost the sole diagnostic feature and are certainly the most evident of the structures contained in the cyst. When fully developed, the hooks are easily seen and their form can be readily studied and drawn. In the early stages of development, however, they are extremely difficult to distinguish and, being soft and thin-walled, they are readily distorted so that it is not always possible to determine their 'typical' size or form.

The use of liquide de Berlese enables more accurate measurements and interpretations of the form of the hooks to be made than were given in the original descriptions of the material. In a careful 'squash' the hooks may retain their positions relative to each other and so reveal any pattern in their distribution which might not be otherwise evident. Such refinements of technique have their drawbacks, of course. For instance, a convenient scheme of classification might be completely disrupted or complicated to an unmanageable extent by the discovery that the hooks of some members of a single group occur in a double crown instead of in a single crown, and that the dorsal and ventral hooks are slightly longer than the remainder, differences which could not be observed under less effective techniques.

The hooks first appear at a fairly late stage in development, after the bulb and the prebulb have formed but before the bulb is enveloped by the prebulb. This agrees with Crusz's (1946) account of the development of Cysticercus fasciolaris but I have not been able to confirm his and Young's (1908) observations on the stages which precede this. They found that the hooklets were formed by a cementing together of cuticular processes and that they were widespread over the prebulbar region. In spite of careful examination of my material I have not observed the appearance or the cementing together of cuticular 'hairs' and, as stated above, the hooklets in P. paradoxa lie mainly in two bands round the prebulb.

It is difficult to see any significance in the formation of the double band of hooklets and the subsequent loss of the posterior band. It may be that this is an intermediate stage between a primitive type of rostellum completely covered with small hooks, such as one finds in the protocephalidean form P. shipleyi (von Linstow 1903), and a more highly evolved type with its single ring of hooks and complex musculature.

The presence of the two bands of hooks also recalls the fact that several authors have noted that the hooks of the 'taeniid' cestodes appear to develop from two centres of chitinization, the blade and guard arising from one centre, the handle from the other. These centres appear as wedges of chitinous material in the early stages of development and unite at a fairly late stage to form the fully developed hook.

Clapham (1942) noted that in many coenuri in which the fully developed rostellum contains alternately large and small hooks, all the hooks are of the same form and size during the early stages of development but a separate wedge of chitinous material is associated with each alternate hook. Those hooks without the associated wedge become the smaller hooks of the rostellum, while the others develop into the blade and guard of the larger hooks and the associated wedges of 'chitin' grow forwards, developing into the handles of the large hooks and finally uniting with the respective blades and guards. The point of union is a weak point in some coenuri even in the fully developed rostellum.

In P. paradoxa, however, the hooklets bear no resemblance to 'wedges of chitin'. The stages of development from the leaf-like earlier stage to the fully-formed hook are simple and do not require an additional centre of chitinization to add any part to the structure.

The development of the definitive hooks in P. paradoxa agrees with Crusz's description of the process in C. fasciolaris: "Each developing hooklet of a slightly later stage is a hollow, claw-like structure formed round a conical extension of the cuticle." "The hollow, claw-like definitive hooks gradually grow out into large, thin-walled hooks. The walls then begin to thicken after the blade has attained a certain size." In P. burti (and, presumably, in P. paradoxa), however, the handle and guard (or base) of the hook do not form after the blade has thickened. Certainly, if the claw-like hook is regarded as the blade only, then the handle and guard are not present at this stage, but my interpretation of the development of the hooks in P. paradoxa

is that the development of the blade and of the base go hand-in-hand. The handle and guard are quite distinct and well-developed before there is any significant thickening of any part of the hook (Fig. 57).

There is a considerable range in the length of the hooks in the adult cestode, from about 75μ to 108μ. The lengths at the upper and lower ends of the range are sufficient to justify subdivision of the group into different species, as has been done in other genera, but, in fact, the range in P. paradoxa is continuous between the extremes. The same holds for the shape of the hooks: there is a considerable variation in the proportions of the constituent parts but, again, there is complete gradation from, say, the slender hook with rather slender blade and handle to the broad type with gently curved blade and straight handle. Even in a single specimen the hooks may show slight variations in shape.

Minor differences are to be expected in structures such as cestode hooks and it would be interesting to see if hook size, for instance, is a racial characteristic and if specimens from, say, Russia lie within a different size-range from those in Britain, although the ranges might overlap. Another factor which may affect the length of the hook is the growth of the larva. In a heavy infestation in the Earthworm the larvae may not attain the same size as they would in a light infestation where there is more room for development and proportionately more food available for each larva. I have no evidence in my material to support this suggestion but it merits consideration.

Certainly, the size of the hooks is apparently determined in the larval stage as Clapham and Peters (1941) showed by statistical analysis that in certain species of Multiceps no further growth of the hooks takes place once the larva reaches the final host.

The mode of growth of the hooks appears to be at first by internal deposition of soft material until the blade, handle and guard are formed inside a thin cuticle, and then by external deposition of the keratin-like outer shell which gives it its final form (Crusz, 1946). In the cysticercoid, the hook develops in a clear sac bounded by membranous walls. Secretion of hook material presumably takes place in the sac. On evagination of the proboscis in the final host the hooks open out and rupture the membrane, freeing their blades.

THE FUNCTIONAL MORPHOLOGY OF THE FULLY-DEVELOPED SCOLEX

THE MUSCULATURE

The gross morphology of the Cestoda has received a considerable amount of attention from that of the earliest workers. Most have been concerned with general morphology rather than with detailed structure, and many of the accounts of the musculature, excretory system and nervous system which were given up to the end of the 19th century were incidental to wider studies and often lacked detail.

This is understandable enough. As in all branches of Natural History, once a certain amount of data on numbers of differing specimens in an apparently related group has been accumulated, a few obvious features of the group are chosen, their details compared and, on the basis of rather arbitrarily selected details, the group is split up into sub-groups and the process then repeated.

In the Cestoda, the obvious features are the organs of attachment in the 'head': the bothria, bothridia and acetabula. These give a first means of division, and the study of the reproductive systems and the hooks leads to further subdivision of the group. The attention that these parts of the anatomy received, and are still receiving, as a means of 'pigeon-holing' the growing number of known cestodes far outweighs the attention which has been paid to the detailed structure and the life-histories of all but a few which are mainly those which directly affect mankind.

In the relevant works of last century and before it is difficult to find clear descriptions of the <u>whole</u> of the muscular systems. The

circular and longitudinal muscles of the body wall are noted, often in minute detail, the parenchymal muscles are mentioned, the muscles of the scolex are sketched, but, apart from a few exceptions such as are repeatedly used to illustrate such collective works as those of Braun (1894-1900), Fuhrmann (Kükenthal and Krumbach, 1928-1933), Wardle and McLeod (1952), Hyman (1951), Joyeux and Baer (1961) and so on, it is only in recent years that attempts have been made to elucidate the systems as a whole, to correlate the arrangement and function of muscles and to build up a complete picture of the anatomy of the cestode. This is, in effect, filling in the middle of the picture; the gross anatomy is known and much work has been done on structure and relationships at the level of the cell, but in between lie structures which have been inadequately described.

In the case of the muscular system, the situation has been partially rectified in recent years, and later papers, particularly those of Zschokke at the turn of the century and Rees (1960, 1961), illustrate the marvellous complexity and intricate detail to be found even in such minute structures as the scolex of a cestode.

The position is better in the cases of the excretory and reproductive systems, particularly the latter, which have been worked out in detail for many cestodes. The interest in the reproductive system is partly intrinsic but also partly for its value in classification. The excretory system has long puzzled parasitologists and, even now, there is doubt about its functions although the finest details of its structure have been examined.

The Tetraphyllidea and Trypanorhyncha have received a large share of the attention given to the structure of the scolex. In these groups there is a wider range of forms of the scolex than in the Cyclophyllidea which has had less attention, and, in considering the Dilepididae, to which the larva of Paricterotaenia paradoxa belongs, I have been unable to trace a single account of the detailed structure of the scolex. Fuhrmann (1928-1933) has outlined obvious structures; Joyeux and Baer (1961) make passing mention of the double sac of the rostellum and the central glandular structure, but go no further. I have, therefore, examined the scolex of the larval and adult stages of P. paradoxa in detail. My main interest has been in the muscular system, but I have also attempted to trace the excretory canals and nervous system, with limited success.

In my description of the musculature of the scolex I considered first the muscles of the rostellum and then the muscles of the remainder of the scolex. It must be emphasised, however, that these two groups are by no means distinct anatomical units, but are intimately connected with each other. The following description illustrates this. It is unfortunate that there are no descriptions of the detailed morphology of the scolex in other dilepid cestodes available to provide a basis for comparison and discussion. The mode of operation of the hooks and related musculature and the organisation of the rostellum in such cestodes as have been described in detail are quite different to those of P. paradoxa.

As already shown the rostellum is a development of two structures, the bulb and the prebulb, the former supplying the hook-elevator muscles, the latter the hook-retractor muscles. It is obvious that these two muscle systems cannot work independently of each other. They must be co-ordinated and, indeed, if the earlier development of the larva were not known, they would appear to be formed by differentiation in a single structure.

In the 'usual' system for exposing the blades of the hooks in the cestode scolex the hook-elevator muscles are attached to the ends of the handles of the hooks and their contraction swings the blades of the hooks into the exposed position, the guard either acting simply as a fulcrum in the system or having additional muscles attached to it to aid in the process.

When I had eventually built up a picture of the arrangement of the muscles in the rostellum of P. paradoxa, it was evident that, if the 'usual' system were operative in this case, it was an extremely inefficient one, the muscles being inserted in such positions that only a small fraction of the effort produced by their contraction would be applied to leverage of the hooks (Fig. 47).

The method of elevating the hooks which I have described is radically different from those described by Rees (1960), for instance, the blades being exposed by a change in the shape of the rostellum rather than by the straightforward contraction of muscles attached to their handles and guards. The effect is reminiscent of the erection

of the spines of the porcupine fishes (Diodontidae) by inflation of the body.

It is significant that each hook has associated with it one of the muscles of the anterior layer of hook-elevator muscles and one pair of the posterior hook-elevator muscles, indicating that the system now in use may be derived from a former system in which each hook was moved by the application of the fulcrum-and-lever principle which is retained by many of the cestodes.

THE EXCRETORY OR OSMO-REGULATORY SYSTEM

The arrangement of the excretory vessels of P. paradoxa is typical of many of the cestodes whose excretory systems have been studied in detail. It is generally assumed that the function of the system is the excretion of the waste products of metabolism, but, according to Wardle and McLeod (1952), there is no experimental evidence of this while there is strong presumptive evidence that the system is in fact osmo-regulatory.

If the system is solely concerned with the excretion of the waste products of metabolism, it is difficult to explain the presence of the plexus of canals in the scolex, but it is obvious from the concentration of these vessels that the scolex must play an important part in whatever function the system serves. If this function is metabolism only then, presumably, the presence of the plexus of canals indicates that the greater part of the metabolic processes takes place in the scolex.

Following this line of reasoning, the dorsal excretory vessel, in which the contents move anteriorly towards the scolex, may carry nutrients, absorbed through the body-wall, to the scolex where they pass into the parenchyma, the waste products being collected and passed posteriorly via the ventral excretory vessels. The scolex is the most mobile and active part of the cestode and it is in the neck region that proliferation of young proglottides takes place, so it is not unreasonable to expect that the scolex should occupy an important place in the metabolism of the cestode. There are objections, however. For instance, the plexus of vessels in the scolex is not common to all cestodes, or even to most. Many, according to descriptions given, have only a single loop or very simple ring vessel in the scolex joining the dorsal and ventral vessels.

The structure of the 'excretory' vessels is more in keeping with that of an osmo-regulatory system than with that of a purely excretory system. Wardle and McLeod (1952) describe the main function of the canals as serving "to maintain in the worm a degree of hydrostatic pressure that helps the tapeworm in extensory movements of the body and holdfast" and "to regulate the water balance of the animal". According to these authors the complexity of the system in the scolex is correlated with the degree of mobility of the scolex, the cestodes with more mobile scolices (Tetraphyllidea, Protocephala) having a more complex system than those with relatively rigid scolices (Cyclophyllidea). P. paradoxa is evidently not a typical Cyclophyllidean as it has a complex system but Hyman (1961), considers the plexus of

of canals in the scolex to be typical of the Cyclophyllidea.

Wardle and McLeod's (1952) illustrations of the canals in various cestodes (after Rees, Wagner and Gough) offer poor support for their contention that the Tetraphyllidea and Protocephala, in accordance with the more complex systems of canals in their scolices, have more mobile scolices than the Cyclophyllidea. If the complexity of the system is in fact related to the mobility of the scolex, it appears that the mode of fixation in the intestine of the host determines whether the complex system is required or not. In those tapeworms in which the scolex is fixed to the wall of the intestine by hooks a mobile scolex may not be required and a simple canal system will suffice. In those cestodes which adhere only by means of suckers or which, like P. paradoxa, adhere to the mucosa or the villi, a mobile scolex is developed, with a complex canal system and powerful suckers enabling them to maintain their positions against movement of the contents of the intestine.

It follows that all of the unarmed cestodes and certain armed cestodes may be expected to have the more complex canal systems in their scolices. The possession of the complex system is, therefore, of little taxonomic value inasmuch as it cannot be regarded as an attribute of any particular order of cestodes. It is obvious, however, that the whole system requires thorough investigation of both its structure and its function.

Although the term 'excretory' may be held to include osmoregulation, as the excretory systems of all animals serve also in

the process of water balance, it does imply the passing-out of the waste products of metabolism rather than any other function and it is misleading to apply it to a system which may be predominantly or solely osmo-regulatory in function.

THE NERVOUS SYSTEM

A study of the nervous systems of cestodes which have been illustrated by various authors (Braun (1894-1900); Fuhrmann (Kükenthal and Krumbach, (1926-1933); Rees (1960)) shows that there is a certain basic pattern common to all the systems, but the variations on this pattern are very wide. The system which I have outlined for P. paradoxa is based as much on personal observation as possible but I have had to assume the presence of certain elements by analogy with the nervous systems of other cestodes.

THE GLANDULAR STRUCTURE OF THE ROSTELLUM

In Metchnikov's description of the fully-developed larva the following passage occurs: "Beneath the proboscis is an oval gland. The cuticle, longitudinal and transverse muscles, the water vessels, calcareous bodies and parenchyma of the head also pass directly into the sac, which is provided at the top with an opening adjacent to the proboscis."

The 'oval gland' is obviously the inner sac of the rostellum and, although Metchnikov was not correct in describing it as an

integral and inseparable part of the scolex, and the 'opening adjacent to the proboscis' is, in fact, the muscular invagination to which the hook-extensory muscles are attached, the fact remains that, apart from these muscles, almost the whole of the interior of the sac is occupied by a structure which appears to be of a glandular nature.

Although there is no direct evidence, I agree with Metchnikov (1867) and Joyeux and Baer (1961) that the structure is glandular. Joyeux and Baer (1961) state that the secretion of the glands "is poured out at the base of the rostellum and its function is without doubt associated with the penetration of the organ into the intestinal mucosa of the host". This is the obvious explanation, but there are other possibilities - the expression 'without doubt' does in fact imply that the true function of the glandular secretion has not been ascertained. It may be, for instance, a lubricant which facilitates the expulsion of the inner sac of the rostellum; it may be a secretion of digestive enzymes used to break down the nutrients in the host's intestine on which the parasite feeds, or, again, it may be an antidigestive enzyme, protecting the cestode against the action of the digestive juices of the host.

There is strong circumstantial evidence in favour of the second alternative, secretion of digestive enzymes, evidence which leads to a consideration of the origin of the whole of the rostellum. If we accept that the Cestoda (and Trematoda) are derived from a turbellarian ancestor (Wardle and McLeod, 1952), the similarity between the pharynx of the turbellarian and rostellum of <u>P. paradoxa</u> must lead one to

consider the possibility of the rostellum originating in the pharynx of a remote ancestor. The scolex of P. paradoxa and the mechanism for expanding and retracting the hooks are simple when compared to the scolices in the Tetraphyllidea and the muscle systems connected with the hooks of, say, Echinobothrium brachysoma Pinter (Rees, 1960) or many of the Hymenolepididae, for instance. The relative simplicity of the system may be interpreted as being retention of a primitive form.

Associated with the pharynx of the Turbellaria are pharyngeal glands, the secretions from which aid in digestion of the food on which the animal lives. It is not impossible, then, that the glandular structure of the rostellum of P. paradoxa is, in fact, homologous with the pharyngeal glands of the Turbellaria.

Further to this argument, the development of the rostellum of P. paradoxa from the bulb and prebulb of the larva, and the association of the bulb and prebulb with the median column of cells which appears to be the primordia of the excretory system, gives additional weight to the possibility that the glandular rostellum and the excretory vessels of P. paradoxa are derived from the pharynx, pharyngeal glands and digestive system of the Turbellaria.

However, interesting as this speculation is, I am inclined to agree with Joyeux and Baer (1961) that the secretion of the glands in P. paradoxa probably aids in the penetration of the intestinal mucosa of the host. The glandular structure will then be similar in function to the glandular structures in the larvae and the scolices of many cestodes, particularly of the Tetraphyllidea.

An explanation for the presence of these 'penetration glands' in the Dilepididae and their apparent absence in many other cestodes may be found in the method of fixation of the scolices in the mucosa, villi or intestinal wall of the host. My own opinion is that, in the case of cestodes with the same type of proboscis as P. paradoxa and in the case of cestodes without hooks, the scolex cannot pierce the mucosa or insert itself in the villi without some means of breaking down these tissues in the path of the advancing tapeworm. In the case of cestodes with the type of rostellum in which the points of the hooks are directed forwards and are then retroverted, the mucosa and tissues are torn apart and there is no need for chemical or enzymatic breakdown.

I have not been able to detect any ducts through the wall of the inner sac of the rostellum to show where the secretion passes out.

It is to be expected that, if the secretion breaks down the mucosa near the scolex, there will be a reaction zone formed round the scolex. I have examined many sections of adult P. paradoxa in situ in the intestine of Woodcock. In many instances there is a clear zone round the proturded rostellum, and the mucosa or villi adjacent to the scolex and strobila stain slightly more intensely than they do elsewhere. This evidence is not conclusive: the clear zone may be due to shrinkage during processing while the more intense staining may be unconnected with the secretion from the rostellum - it may be due to digestive action by secretions from other parts of the tapeworm;

it may be due to a reaction of the host, induced by the presence of the parasite; or it may be due to the action of anti-digestive enzymes, which may be secreted by the glands, as mentioned above.

CLASSIFICATION

A study of the literature on larval cestodes reveals that I. Metchnikov discovered the multiple larval cysts of a cestode in Earthworms at Odessa in 1867. He described the cysts and gave some details of the different stages of development of the larvae in the cysts (Metchnikov, 1867). He fed the infested Earthworms to ducks in an attempt to determine whether or not the duck was a possible final host of the parasite and to obtain the adult form of the larvae, but without success. The cysts were digested by the ducks and Metchnikov could offer no suggestion as to what might be the final host nor the species of the cestode.

Metchnikov outlined the pattern of development of his larvae and described how the earliest stage of the larva, a solid sphere of cells, became a hollow sphere on the inner surface of which a number of 'buds' formed. Each bud developed into a larva with an anterior 'head' and a posterior bladder. The final stage of development, after formation of the suckers, hooks, etc. on the head, was the invagination of the head into the bladder to form the fully developed 'cysticercoid', the final stage in the intermediate host (Fig. 49). He discussed the relationship between the various parts

of the larva and compared them with the corresponding parts of Echinococcus which was receiving a great deal of attention in the latter half of the 19th century.

Metchnikov stated specifically that he could find no analogy between the caudal bladder of his larva and the brood capsule of Echinococcus. In spite of this, Moniez (1880) assumed that Metchnikov equated the caudal bladder of his larva with the brood-capsule of Echinococcus. He joined Leuckart (1886), who made the same assumption, in regarding the mother-cyst of Metchnikov's larva, i.e. the hollow sphere in which the 'buds' of the future larvae appear, as being the true 'caudal vesicle'. The posterior part of the body of the larva, which falls off after evagination in the final host, plays the role of caudal vesicle but does not fill the requirement that the true caudal vesicle is the direct derivative of the 'proscolex' and the preceding onchosphere.

Moniez proposed a system of Classification of the larval stages of the Cyclophyllidea in which Metchnikov's larva was equated with Echinococcus and he regarded the cysts as a form of Echinococcus. Villot (1883) reviewed the position of the 'cystic cestodes', grouping them in the following categories:

Group 1. Cysts in which the caudal vesicle is derived from the proscolex by simple growth and modification of structure without, strictly speaking, production of any new part:

e.g. Cysticercus
Coenurus
Echinococcus

Group 2. Cysts in which the caudal vesicle is formed by budding from the proscolex, i.e. by addition of a new part:

Section 1. Cysts in which the caudal vesicle is formed by endogeneous budding:

e.g. Polycercus
Monocercus

Section 2. Cysts in which the caudal vesicle is formed by exogenous budding:

e.g. Cercocystis
Staphylocystis
Urocystis
Cryptocystis

Villot did not agree with Moniez' view that Metchnikov's cysts were a form of Echinococcus and he discussed the position of the cysts at length. He concluded that, since Echinococcus has but three parts: head, body, caudal vesicle = proscolex, and Metchnikov's cyst has four: head, body, caudal bladder, proscolex, the two cestodes are quite distinct. Echinococcus he described as 'polycephalic, polysomatic and monocercal' while Metchnikov's cyst he described as 'monocephalic, monosomatic and polycercal'. He named Metchnikov's cyst Polycercus and included it in the first subdivision of his second group above.

Villot's views are worthy of full consideration as they have not been superseded and they were accepted by Haswell and Hill (1893)

who are the last workers to examine critically the structure and relationships of Polycercus. In a paper describing Polycercus from an Earthworm of New South Wales (Didymogaster sylvatica Fletcher), Haswell and Hill supported Villot's conclusions on the need to separate Polycercus from Echinococcus and they found additional evidence in favour of this viewpoint in the proliferating cysts which they describe in detail.

However, while Moniez' classification of cestode larvae was justifiably and, in my opinion, correctly contradicted by Villot, Haswell and Hill, while supporting Villot, threw strong doubts on Metchnikov's observations on the pattern of development of Polycercus. They found that, in their material, there was no invagination of the 'head' of the larva into the caudal bladder but that the scolex was actually formed in the centre of the growing cyst. The membranes of the cyst later fuse anteriorly and then form a pore while elsewhere they divide to form a double-walled cyst round the scolex.*

Their observations were carefully made and are, for the most part, presumably, accurate but they then made the mistaken assumptions that their material was the same as that which Metchnikov had found, that Metchnikov's observations were quite inaccurate and that the

* A re-examination of Haswell and Hill's material would probably show that the scolex is not formed in the centre of the cyst but that the invagination of the anterior part of the larva into the posterior part takes place before formation of the scolex. The later development of the scolex in the invagination would explain the mode of development which they outline.

larvae from Russia must undergo the same pattern of development as the larvae from Australia. Haswell and Hill's description of their material shows, as they themselves state, that their 'Polycercus' is a type of Staphylocystis and is quite distinct from the Polycercus of Metchnikov.

Villot's simple scheme of classification of the 'cystic cestodes' was followed by Grassi and Rovelli (1896) in their detailed account of cestode development and was also commended by Skrjabin and Mathevossian (1942) who, however, added three types of 'cysticercoids' but omitted Polycercus and Cryptocystis without giving any explanation. Their list of Cysticercoids, according to the key in their paper is:

Staphylocystis

Urocystis

Cercocystis

Ramicercus

Microcercus

Monocercus

Diplocystis

As these authors are considering only the cysticercoid group of larvae it is possible that they do not consider Polycercus or Cryptocystis to be cysticercoids. One or other of the two may have been omitted by mistake as the authors mention five types already recorded in the literature but name only four and in their key they write of a table for eight types but give only seven.

Joyeux and Baer (1961) suggest that Staphylocystis and Urocystis are the same forms at different stages of development. Ramicercus may also be included in this type: there are no embryonic hooks shown in Mrazek's illustration of Ramicercus to show where the tail is - this fits Villot's description of Staphylocystis as he found no embryonic hooks in that form either.

Joyeux and Baer (1961) follow a modified scheme in considering cyclophyllidean larvae:

Group A. Monocephalic (non-proliferating cysts)

I. Plerocercus type
II. Cysticercoid type
III. Cysticercus type

Group B. Polycephalic (proliferating cysts)

I. Cysticercoid type
II. Cysticercus type

Polycercus is included in Group B, Section I.

Another factor which would appear to be worthy of consideration in trying to define the relationships between the various types of larval cestodes is the invagination of (a) the scolex and (b) the rostellum.

In the development of the scolex there are two known patterns:

(1) the scolex develops in the non-inverted position i.e. the suckers are on the outside of the scolex which forms in the position it will hold in the adult cestode.

(2) theyscolex develops in an invagination with the suckers on the inside walls. The invagination must be everted in order to assume the position it will hold in the adult cestode.

The rostellum exhibits similar variations in development, and, in the adult cestode, the hooks may be so arranged that:

(1) they are everted as they are brought forward into the striking position;

(2) they do not evert but are formed in the striking position and are simply pushed forward with the rostellum and then open out and fix the blades in the tissues of the host.

These differences may be of no real significance and certainly should not be taken as a basis for further subdivision of a group which is hardly sufficiently well known to justify the present degree of subdivision. However, it would seem that the inverted type of rostellum, as found in the Hymenolepididae, is a more refined and effective means of penetrating and adhering to tissues than is the more straightforward type as developed in the Dilepididae. It may also be that the inverted type of scolex is a more highly evolved form than the non-inverted. Grassi and Rovelli (1896) showed that in the development of Dipylidium caninum the scolex formed as the non-inverted type and then inverted during invagination in the final stages of larval development.

A study of hook development in the various Genera of cestodes

might indicate whether or not, for instance, the typical hymenolepid hook is a more advanced type than the typical dilepid hook. Again we are brought back to the shortcomings in our present system of classification of the Cyclophyllidea. What is a typical hymenolepid hook?

Skrjabin and Mathevossian (1942) have considered hook forms in the Hymenolepididae and have proposed a distinctive term for each type of hook. They list eleven type-forms which include most of the forms to be found in the whole of the Cyclophyllidea.

Metchnikov did not attempt to name the larva he discovered and it was Villot who named it Polycercus lumbrici in 1883, apparently assuming that it had been found in a species of Lumbricus, although Metchnikov did not specifically identify the Earthworm host. Prior to this Leuckart had noted the similarity between the form of the hooks of Metchnikov's cestode and the hooks of Taenia nilotica (= Paricterotaenia nilotica) and gave as his opinion that the larva was that of Taenia nilotica, a parasite of Cursorius isabellus (C. gallicus, Gm.), found in Egypt. (In Leuckart's account of Metchnikov's discovery he describes the larva as a 'twelve-hooked embryo' whereas Metchnikov states in his description that the hooks are 'from 14 to 17 in number').

The matter rested here until 1939 when Joyeux and Baer, in describing a tapeworm found in the Woodcock, drew attention to the fact that the hooks of Polycercus lumbrici were much nearer in size and form to those of the cestode from the Woodcock than they were to

those of Taenia nilotica. They suggested, therefore, that Polycercus lumbrici was the larval form of the cestode from the Woodcock. This cestode was identified as a new species of Amoebotaenia and, in accordance with Villot's choice of name for the larva, the adult was provisionally named Amoebotaenia lumbrici (Villot, 1883), Joyeux and Baer, 1939.

Amoebotaenia lumbrici is, in fact, a synonym of Paricterotaenia paradoxa (Rudolphi, 1802). The fact that its principal host is the Woodcock, although it is a secondary parasite of other birds such as the Curlew, Golden Plover, and Snipe, affords an explanation for Metchnikov's lack of success in his attempts to infest ducks by feeding them the larval cestodes.

Paricterotaenia paradoxa.

First described by Rudolphi in 1802 and named Taenia paradoxa, it was included by Fuhrmann in his Genus Choanotaenia in 1908 as Choanotaenia intermedia. On reconsideration of this Genus in 1932, Fuhrmann included the tapeworm in his new Genus Paricterotaenia as Paricterotaenia paradoxa.

The name Amoebotaenia lumbrici was equated with P. paradoxa (as stated above) by Sandeman in 1958 and by Joyeux and Baer in 1961.

The only other synonym is Acanthocirrus multicanalis, Baczynska, 1914. This author based the new species on fragmentary material which Sandeman (1958) found on re-examination to be P. paradoxa.

EXPERIMENTS in vivo on Polycercus lumbrici

An obvious prerequisite in the study of living cysticercoids is to find a medium in which the larvae can be kept alive. Fortunately, in the case of Polycercus lumbrici this proved to be a minor problem as the larvae showed themselves capable of surviving, for hours at least, in cold, simple physiological solutions (Locke's solution, 0.53% NaCl, Ringer's) and even in tapwater.

The living cysticercoid

The fully-developed cysticercoids, inside the containing cyst, are translucent, almost transparent bodies, ellipsoidal in shape with a slight indentation at each end, that at the anterior end being the more obvious. The 'indentation' at the anterior end is, in fact, the pore formed by the meeting of the walls of the caudal bladder in front of the scolex. The latter is forced out through the pore during evagination in the final host. At the posterior end the slight invagination indicates the site of attchement of the larva to the cellular layer of the mother-cyst.

In a cyst with a single cysticercoid the latter is enclosed in a close-fitting sheath, the wall of the mother-cyst, while in the multiple cyst, i.e. that containing two or more cysticercoids, the larvae lie fairly closely-packed inside the spherical bladder from the wall of which they originated. There are few features visible in the cysticercoid: the crown of hooks appears as a truncated cone in front of the muscular rostellum, lying between the suckers.

Surrounding these can be seen the cuticle of the scolex and the double wall of the caudal bladder. (Fig. 23). Throughout the tissues of the cysticercoid there are large numbers of conspicuous calcareous corpuscles, usually spherical in shape, the larger measuring about 12μ in diameter. The cysticercoid usually measures 300μ to 400μ in length by 250μ to 350μ in diameter.

The scolex can be seen to contract periodically and to move about within the caudal bladder. The rostellum makes slight prodding motions as though preparing to force itself out of the scolex.

Movements in the newly-evaginated Cestodes

During the course of my experiments in inducing evagination and in culture of the cysticercoids I took advantage of the opportunity to study living specimens of newly-evaginated P. paradoxa in vitro under the microscope. Little more of the structure is to be seen in the evaginated cestode than is visible in the cysticercoid; the hooks, suckers and calcareous corpuscles are readily distinguishable, but little else. Numerous flame cells can be seen but there is no apparent pattern in their distribution. No longitudinal excretory vessels appear except, possibly, a few vessels mentioned below, but there does appear to be an ill-defined tract along the axis of the body of the tapeworm which is more vacuolated than the surrounding parenchyma and free of the calcareous corpuscles which are plentiful throughout the remainder of the worm except in the rostellum, suckers and peripheral layer. This tract appears to terminate in a small

bladder at the posterior extremity of the worm into which a number of fine vessels disburse. These are probably the excretory vessels.

The small tapeworms move with surprising speed over a microscope slide or the flat bottom of a petri dish. They draw themselves on by moving forward the suckers on one side of the rostellum then the other, the suckers adhering to the glass so strongly that the worms are not dislodged by fairly strong jets of water but may have to be prised off with a needle. The suckers are, therefore, well-controlled, their movements co-ordinated and they are efficient organs of attachment. They will assist the hooks in holding the cestode in place in the mucosa of the host's gut and, obviously, play a large part in penetrating the mucosa. The part they play in keeping the cestode in place in the gut against the movement of the contents of the intestine is well-demonstrated in sections of the adult cestodes in situ in the intestine of the Woodcock (Fig. 39). The suckers can be seen almost to engulf parts of the mucosa by withdrawing their central parts to form a deep hemispherical cup into which is pulled the material in which the worm fixes itself.

The rostellum is usually only slightly protruded in healthy worms in vitro. Under pressure from a cover slip or in adverse conditions just before death, however, it may be fully extruded. There appears to be a muscular sphincter at the anterior end of the scolex in front of the rostellum and the latter can be seen to be forced out by withdrawal of the surrounding scolex as well as by a forward movement of the rostellum itself. This is confirmed by an examination of the

musculature of the scolex and rostellum as described earlier in the section on the rostellar musculature.

In bile broth, the cestodes are extremely active and after an initial contraction the scolex commences to make rapid spasmodic movements which pull the worm quickly over the surface of the containing vessel. The rostellum may make prodding motions as though preparing to protrude fully, and in some instances it does extend forward to its fullest extent and then withdraws, the process being repeated again and again. The hooks remain in the retracted position, however, and full expansion of the hooks appears to occur only immediately preceding or immediately following the death of the worm in vitro. In no case have I seen the hooks to be expanded and retracted again although there can be no doubt that the tapeworm can retract the hooks and withdraw the rostellum into the scolex. In a high proportion of the adult tapeworms the rostellum is fully withdrawn when the worm is taken from the final host and it is unreasonable to assume that the rostellum, in such worms, has never been protruded.

Evagination of the Cysticercoid

In attempting to induce evagination in Polycercus by artificial means I considered that two separate processes were involved. The first of these is the freeing of the larva from the cyst in which it is enclosed, the second is the actual evagination of the scolex of the freed larva.

There are four obvious means of inducing emergence from the cyst which might be met with in the alimentary tract of the bird:

(a) Mechanical, by rupture due to pressure and abrasion.

(b) Physical, by osmotic pressure, causing swelling and rupture of the cyst.

(c) Physico-chemical, (i) by rupture of the cyst by products of the reaction between an alkali and an acid;

(ii) by a combination of mechanical and chemical action.

(d) Chemical, by digesting the cyst in simple acid or alkaline solutions or by the action of enzymes.

These may also apply to inducing evagination of the scolex:

(a) by 'squeezing' the scolex out of the caudal bladder.

(b) by (i) osmotic pressure causing swelling of the caudal bladder and emergence of the scolex.

(ii) heat, increasing the activity of the cysticercoid so that the scolex forces itself out of the caudal bladder, probably assisted by contraction of the latter.

(c) by a combination of (a) and (b).

(d) by causing contraction of the caudal bladder with a resultant expulsion of the scolex.

In the following account each of the factors is considered in turn, first with regard to its effect on the cyst, then with regard to its effect on the larva within the cyst.

Mechanical Pressure and Abrasion

The method which I used to judge the effect on Polycercus of conditions similar to those in the gizzard was to insert an infested Earthworm in an ordinary rubber pipette-bulb, squeeze the air out and, pinching the neck of the bulb between the fingers of one hand, rub the walls of the bulb against each other with the fingers of the other hand for a few minutes. This ground up the Earthworm very effectively and certainly subjected the cysticercoids to pressure and abrasion, although perhaps not so extreme or prolonged as they might receive in the gizzard.

This rather crude technique proved surprisingly successful. Within about two minutes almost all of the cysts had ruptured, freeing the cysticercoids, and some 30% of the larvae had evaginated. Further rubbing could bring the level of evaginated larvae up to about 70%. It was not altogether satisfactory, however, in inducing evagination as a large proportion of the cysticercoids were only partially evaginated or were badly damaged and a very considerable proportion remained unevaginated.

Osmotic Pressure

There is no evidence that this aids in either rupture of the cyst or in evagination of the cysticercoid. In the evaginated larva the caudal bladder is contracted rather than distended, contrary to what one would expect if osmosis were responsible for forcing the scolex out of the bladder by distention of the latter. The contraction of the caudal bladder might be explained, of course, by the fact that

its contents are at a lower osmotic pressure than that of the solution into which the cyst is introduced. The reduction in volume of the containing cyst has been noted in such conditions but neither has it ruptured nor have the contained larvae evaginated.

Heat.

Although heat would not be expected to have a direct effect on rupture of the cyst, it had been noted that the larvae became more active if the medium in which they lay were warmed. The scolices contracted and expanded vigorously and the rostellum made prodding movements within the scolex.

In order to determine whether or not a rise in temperature from the cold to the body temperature of a bird would induce evagination, I transferred several cysts from Locke's solution at about 15^{o} C. to the same medium at 40^{o} C. and I also warmed Locke's solution containing a number of cysticercoids. In both cases there was an increase of activity in the larvae and, indeed, a small proportion evaginated but

(i) the cyst remained intact;

(ii) although a few cysticercoids evaginated within their cysts, the vast majority remained invaginated.

I concluded that heat may be a contributory factor in the process of evagination, but it is not a principal factor.

Physico-chemical Action

It is conceivable that the structure of the cyst or the larva might be weakened or altered in some other fashion by chemical action so that pressure or heat might bring about evagination although they are otherwise ineffective. I was unable to test this possibility but there is no evidence to suggest that it is required while there is evidence to suggest that the combination of the two are not necessary to achieve either rupture of the cyst or evagination of the scolex of the larva.

Under this heading one interesting possibility worth consideration is that, on passing through the gizzard of a bird, the wall of the cyst may become impregnated with hydrochloric acid and then be exposed to the action of alkalis in the duodenum. This might lead to the formation of minute bubbles of gas in the wall of the cyst with resultant disintegration of its substance. I tested this possibility by immersing the cyst first in hydrochloric acid then in sodium bicarbonate, and vice versa. The results show that the wall of the cyst remains intact throughout the treatment.

Chemical action

Freeing of the larvae from the cyst

In order to examine the possibility of the cyst-wall being digested or dissolved away, I exposed numbers of cysts to the actions of media containing digestive agents including those such as the cyst might encounter in the gizzard and duodenum of the bird. The following media were tried:

Dilute hydrochloric acid.

Concentrated hydrochloric acid.

" sodium bicarbonate.

Dilute hydrochloric acid + pepsin-peptone.

Gastric juice (pepsin + HCl + Ringer) (de Waele, 1933).

Pancreatic juice (pancreatin + bile + Ringer) (de Waele, 1933)

Gastric juice followed by pancreatic juice.

None of these had any effect on the cyst wall, even in high concentrations.

Evagination of the larva

In order to determine the substance or substances which induce evagination I decided to subject a number of larvae to the action of several media including synthetic gastric juice and then to transfer to synthetic pancreatic juice and other media. If any of these combinations proved successful I would then repeat the experiment with them, eliminating each of the constituents of the solutions in turn and so, I hoped, arrive at the principal cause of evagination.

The experiments were carried out, to begin with, in small petri dishes, previously sterilised by boiling. The larvae were transferred from one medium to another by means of a fine pipette. The dishes were kept in an oven at 38° C. (the body temperature of a bird) except when material was being transferred. The latter was a fairly rapid process and the results of other experiments show that it would have little or no effect on the final outcome of the experiments.

Experiment I.

To find media which will induce evagination of the cysticercoid.

A number of cysticercoids were separated from their cysts by rupturing the latter and the larvae were then divided into seven groups, each of about 20 individuals. Each group was placed in a medium (Medium I) where it remained for four hours and was then transferred to Medium II, all at 38° C.

The media used were:

Group	Medium I.	Medium II.
1	Gastric juice	Pancreatic juice
2	" "	" "
3	" "	" " without bile
4	" "	Trypsin-dextrose broth
5	Locke's soln.	Pancreatic juice
6	Ringer's + HCl	" "
7	" "	Sodium bicarbonate

The experiment was repeated three times. The results varied from experiment to experiment, but they showed that evagination may occur in:

(i) Locke's soln.
(ii) Acidified Ringer's soln.
(iii) Gastric juice.
(iv) Pancreatic juice, after treatment with gastric juice.

The proportion of the cysts in which evagination occurred in acidified Ringer's solution and gastric juice is negligible, but in Locke's solution a considerable proportion evaginated in the course of this particular experiment. However, numerous other experiments in Locke's solution showed that, although in many cases a small proportion of the larvae may evaginate, this medium is not an effective agent in inducing evagination. I am of the opinion that evagination in these media is the result of heat, not of chemical action.

The effect of the pancreatic juice on the larvae after treatment in gastric juice is immediate and remarkable. Evagination is induced almost instantaneously in a large proportion of the fully-developed larvae.

On the basis of the above findings, I accepted that treatment first with gastric juice then with pancreatic juice was the most effective means of inducing evagination.

First Medium (30 mins.)	Second Medium	No. cysts	After 1 hr. No. evaginated		After 2 hrs. No. evaginated		After 3 hrs. No. evaginated	
			Total	%	Total	%	Total	%
H_2O + HCl + Pepsin	H_2O+NaCl+Na_2CO_3+Panc.	20	7/9	35/80	7/-	35/-	5/-	25/-
	H_2O+NaCl+Panc.	15	Nil	0	-	-	-	-
	H_2O+Na_2CO_3+Panc.	34	0/20	0/60	-	-	-	-
	H_2O+NaCl+Na_2CO_3	18	1/1	5/10	1/-	5/-	-	-
	H_2O+NaCl	17	1/2	5/18	-	-	-	-
	H_2O+Na_2CO_3	35	Nil	[illegible]	-	-,	-	-
	H_2O+Panc.	22	Nil	0	-	-	-	-
	H_2O	30	Nil	0	-	-	-	-
H_2O + HCl	H_2O+NaCl+Na_2CO_3+Panc.	17	Nil	0	0/6	0/33	-	-
	H_2O+NaCl+Panc.	10	Nil	0	-	-	-	-
	H_2O+Na_2CO_3+Panc.	16	0/10	0/60	-	-	-	-
	H_2O+NaCl+Na_2CO_3	30	Nil	0	-	-	-	-
	H_2O+NaCl	40	Nil	0	-	-.	-	-
	H_2O+Na_2CO_3	11	Nil	0	0/1	0/10	-	-
	H_2O+Panc.	10	Nil	0	-	-	-	-
	H_2O	14	Nil	0	0/2	0/16	-	-
H_2O+Pepsin	H_2O+NaCl+Na_2CO_3+Panc.	24	1/7	4/33	9/-	40/-	5/-	20/-
	H_2O+NaCl+Panc.	34	21/0	63/0	21/-	63/-	21/-	63/-
	H_2O+Na_2CO_3+Panc.	25	0/24	0/95	-	-	-	-
	H_2O+NaCl+Na_2CO_3	30	0/3	0/10	-	-	-	-
	H_2O+NaCl	22	4/0	17/0	5/-	22/-	5/-	22/-
	H_2O+Na_2CO_3	38	Nil	0	-	-	-	-
	H_2O+Panc.	80	0/5	0/9	-	-	-	-
	H_2O	14	0/2	0/14	0/3	o/20	-	-

Under 'Total' x/y ; x = alive, evaginated; y = dead, evaginated.

Under '%' n/p ; n = alive, evaginated; p = alive & dead evaginated.

Table 3 **Evagination of Polycercus lumbrici in synthetic intestinal juices.**
(Determination of effective agents by process of elimination.)

Experiment II.

To determine the effective agents contained in the gastric and pancreatic juices.

A large number of larvae was collected and divided into groups which were then treated as shown in Table 13. It is immediately obvious that hydrochloric acid, by itself, has a deleterious effect on the larvae and does not contribute towards evagination.

If we postulate that an effective evaginating agent will induce evagination in at least 50% of the larvae immersed in it, then the following, in order of efficiency, are effective agents with or without survival of the larvae:

(1) Na_2CO_3 + pancreatin.

(2) Na_2CO_3 + pancreatin + NaCl

(3) Pancreatin + NaCl.

(4) Pancreatin + Na_2CO_3.

In inducing evagination with survival of the larvae for a reasonable period, only one of the media is effective:

NaCl + pancreatin.

From the above, it would appear that pancreatin is the effective agent in inducing evagination as it is the only substance common to all the effective media. It has little or no effect on its own however, as the table shows, but requires the presence of Na_2CO_3 and/or NaCl.

The most suitable technique for experimental work is that which induces evagination and also sustains the young cestodes alive. From the above experiments, immersion in H_2O + pepsin then H_2O + NaCl + pancreatin is the most suitable and this procedure was adhered to with constant success in obtaining the young tapeworms required for examination and experimental work on their development.

It should be mentioned here that, in order to verify de Waele's (1933) observations on the importance of bile in the process of evagination, several attempts were made to induce evagination by placing the larvae in bile broth. The results were mixed but, in general, evagination took place in only a small proportion of the larvae. The bile did have a decided effect on the actions of the larvae, however, producing violent spasms which soon ended in death. Diluted bile broth had no apparent effect except that the larvae did not survive for long in it. Addition of bile to the H_2O + NaCl + pancreatin medium brought about no obvious improvement in the percentage evagination but lowered the survival rate of the young tapeworms.

Medium / Time	Locke's Soln.	Serum	Changed Serum	Locke's + Serum
1 hr.	Very active, some very elongated.	-	-	-
2½ hrs.	Very active	-	-	-
20 hrs.	50% very active; most of these fully evaginated.	Most active, distended.	-	-
21 hrs.				
27 hrs.	Active; many attenuated.	Active, some have constrictions.	Active, some have constrictions.	Active.
33 hrs.	Fully evaginated active; others dead.	50% active; many distended.	40% active; all contracted.	50% active.
46 hrs.	70% evaginated alive, very active; partly evaginated all dead.	All dead.	Only one alive.	20% alive; these partly evaginated.
60 hrs.	8% alive but react only to touch.	-	All dead.	All dead.
84 hrs.	All dead	-	-	-

Table 4 **Viability of Polycercus lumbrici in vitro (at 37°C.).**

Experiment III.

To find a medium in which newly evaginated P. paradoxa could be kept alive for examination.

Several trials established that there was little to choose between Locke's solution and ¾ Locke; the young tapeworms survived in both for varying periods up to three days. The survival time varied from experiment to experiment without apparent reason: differences in the way in which they were handled, bacterial contamination and other such factors would all affect the cestodes. (Bacterial contamination is not necessarily deleterious in a culture medium; some of Smyth's experiments indicate that it might even contribute some factor essential to the development of the tapeworm). Table 4 gives the results of an experiment comparing the effectiveness of Locke's solution with horse serum and with a mixture of equal parts of horse serum and Locke's solution.

In this experiment the young cestodes were induced to evaginate by grinding them in a rubber bulb and were then washed in cold Locke's solution and placed in an oven for 2½ hours to warm up to 37° C. A number was then transferred to serum and this group was later subdivided, part remaining in the original serum, part transferred to fresh serum (to reduce bacterial contamination and to remove them from possible waste products of metabolism) and the remainder was placed in a mixture of equal parts of Locke's solution and serum.

The results show that Locke's solution was better than Locke's + serum and that the undiluted serum was the least satisfactory. There was little to choose between the unchanged serum and the fresh serum.

Although the tapeworms survived in Locke's solution they showed no signs of development. Some specimens developed constrictions posterior to the neck region which were thought to be the first indications of the formation of proglottides. Examination of sections prepared from these worms showed no evidence of the formation of the anlagen of the reproductive system and, as they occurred shortly before the death of the cestode, they were obviously pathological in nature.

Medium / Time	Undiluted Horse Serum	0.53% NaCl	¾ Locke's Soln.	Locke's Soln.	$NaHCO_3$ (sat.)
6 hrs.	Active	Inactive.	Inactive.	Active.	Inactive.
21½ hrs.	Inactive, some greatly distended.	Inactive.	Active	Active.	Inactive.
50 hrs.	Evaginated cestodes contracting spasmodically.	Possible slight activity in one cestode.	Inactive	One active.	Inactive, opaque.
74 hrs.	Inactive.	Inactive.	Inactive.	Inactive.	-
99 hrs.	Inactive, all opaque.	Inactive, all opaque.	Several active. All clear.	Inactive, opaque.	-
123 hrs.	-	-	One active, all clear.	-	-
146 hrs.	-	-	Inactive, opaque.	-	-

Table 5 Viability of Polycercus lumbrici in vitro (at 37°C.).

Experiment IV.

To determine which of a number of media would keep the unevaginated larvae alive for the longest period.

Larvae were taken from an Earthworm, cleaned by repeated washings in Locke's solution, separated into a number of groups and each group was then placed in a different medium in a container. The containers were then placed in an oven at 37° C. and examined periodically for signs of life. The media used were:

(1) Undiluted horse serum (Difco)

(2) 0.53% NaCl (Isotonic with the coelomic fluid of Lumbricus).

(3) Locke's solution.

(4) Locke's solution diluted to 75% with distilled water (¾ Locke).

(5) Saturated solution of $NaHCO_3$ in distilled water.

The results are given in Table 5 from which it may be seen that ¾ Locke appears to be the most suitable medium, several of the cysticercoids being active after 99 hours. They appeared healthy but inactive after 123 hours, except for a single worm which showed sluggish activity, and all were dead within 146 hours. Locke's solution and 0.53% NaCl sustained the larvae for 74 hours but they were all dead by 99 hours. Horse serum (undiluted) and saturated $NaHCO_3$ were least suitable, the former being slightly the better of the two. The suitability of the ¾ Locke was confirmed in other

experiments with the larvae. It was found that a good proportion could be kept alive and apparently healthy for periods of 2-3 days, which was adequate for examination. Undiluted Locke's solution was also satisfactory and was frequently used.

In this and some other experiments there were differences between the survival rates of fully-evaginated tapeworms and those which are only partly evaginated, i.e. those in which the scolex and neck were free but the body of the worm was still partly withdrawn into the caudal bladder and the posterior 'caudal appendage' had not been cast off. I saw no significance in this; sometimes the fully-evaginated worms showed the better survival rates, at other times the opposite was true.

I assume that small tapeworms with few proglottides when fully developed, such as P. paradoxa, reach maturity in a short time in the final host and would certainly show some signs of growth or formation of young proglottides within two days of entering the final host, i.e. within the period they survive in artificial media. On this assumption, Locke's solution is not a suitable medium for supporting development and I decided to try other media which might be more effective.

I encountered some difficulty in using many of the media containing enzymes as, even with reasonably careful sterilisation of the equipment, decomposition of the medium was fairly rapid. The cestodes often survived as well in badly-clouded media as in the

Medium / Time	¾ Locke's	¾ Locke's + 0.01% Merthiolate	¾ Locke's + Serum	¾ Locke's + Serum + 0.01% Merthiolate	Serum
2 hrs.	Active.	Inactive.	Active.	Inactive.	Active.
4 hrs.	Active.	Inactive.	Active.	Inactive.	Active.
7 hrs.	Active.	Inactive.	Active, distended.	Inactive.	Active.
21 hrs.	Inactive; 50% clear, 50% opaque.	Inactive, opaque.	Few active, 90% clear.	Opaque.	inactive, clear.
21½ hrs.	-	-	Fresh medium.	-	Fresh medium
24 hrs.	Inactive, opaque.	-	60% active.	-	60% active.
28 hrs.	-	-	Active.	-	Active.
45 hrs.	-	-	Active.	-	Inactive, clear.
45½ hrs.	-	-	Fresh medium.	-	Fresh medium.
69 hrs.	-	-	Two active; 14/20 react to touch. All clear.	-	Inactive, opaque.
70 hrs.	-	-	Fresh medium.	-	-
76 hrs.	-	-	Active, some constricted.	-	-
93 hrs.	-	-	Most clear but react only to touch.	-	-
100 hrs.			Active.		
117 hrs.	-	-	Inactive, clear. Fresh medium.	-	-
127 hrs.	-	-	All opaque.	-	-

Table 6 **Viability of Polycercus lumbrici in vitro (at 37°C.).**

clearer 'broths' but decomposition was certainly not desirable and, in general, had an adverse effect on the cestodes. I tried introducing 0.01% Merthiolate into the media and, although this improved the position, it also killed off the tapeworms. This is shown in Table 6 , which also shows that a medium consisting of equal parts of ½ Locke and serum can support the cestodes for at least 117 hours, but without development taking place.

Another technique for overcoming the problem of decomposition is to change the media at regular intervals. This certainly kept them clear and, although I obtained no conclusive evidence that it had any beneficial effect on the tapeworms, I adhered to this practice in most experiments. Finally, in connection with this problem, much more satisfactory results were obtained using test-tubes plugged with cottonwool as containers, rather than the petri dishes, and the former were used in all but the earliest experiments.

Experiment V.

To find a medium which supports development of P. paradoxa.

In the first attempt at inducing development of the cestode the worms were evaginated by grinding the cysts in a rubber bulb and then placing them in the following media at 37° C:

(1) Green bile broth (Gurr).

(2) Nutrient broth (Gurr).

(3) Dextrose tryptone.

(4) Glucose peptone.

(5) Trypsin.

(6) Green bile broth + serum.

(7) Nutrient broth + serum.

(8) Dextrose tryptone + serum.

(9) Glucose peptone + serum.

(10) Locke's soln. + 0.001% merthiolate + serum.

(11) Trypsin + serum.

(12) Proteose peptone + serum.

(13) Locke's soln. + serum.

The cestodes were all examined after 17 hours in the media and all were found to be dead except those in 7, 10 and 13. Those in 7 (nutrient broth + serum) were still alive after 20 hours but had died within 41 hours without showing any sign of development. Those

in the Locke's soln. + serum survived for longer, at least 42 hours in 13 (Locke's + serum) and several were alive in 10 (Locke's soln. + serum + 0.001% merthiolate) after 52 hours while one cestode was still alive after 68 hours. None of these showed any signs of development, however, and the media as used were obviously unsuitable for this purpose.

A similar experiment was carried out using cestodes which had been evaginated by placing cysts in H_2O + pepsin for 30 mins. and then transferring them to Locke's soln. + pancreatin (all at 37° C.). The evaginated worms were then freed from the containing cysts and placed in:

(1) Locke's soln. + pancreatin.

(2) Locke's soln. + pancreatin + pieces of Earthworm.

(3) Locke's soln. + pancreatin + pieces of Earthworm + dextrose-tryptone.

In 2 and 3 the worms were dead within 22 hours. A few survived for 53 hours in 1 but showed no signs of development.

My final attempt at culturing the cestodes was based on experiments on the culture of strigeid trematodes in artificial media by Williams, Hopkins and Wyllie (1960, 1961). Groups of young cestodes were placed in the following media and left for 3½ days at 40° C. to see if growth or development took place:

(1) 10 cc. egg yolk + 2 cc. albumen + 2 cc. Lock's soln. + 1.5 cc. glucose.

(2) Above diluted to 25% with Locke's soln.

(3) 2 cc. albumen + 10 cc. Locke's soln. + 1.5 cc. glucose = Basic

(4) Basic + 1 cc. yeast.

(5) 10 cc. Basic + 5 cc. horse serum.

(6) 10 cc. Basic + 1 cc. yeast + 5 cc. horse serum.

(7) 10 cc. (6) + 3 cc. Locke's soln.

(8) 10 cc. (6) + 1.5 cc. nutrient broth.

(9) 10 cc. (3) + 1.5 cc. nutrient broth.

(10) 10 cc. (4) + 1.5 cc. nutrient broth.

(11) 10 cc. (5) + 1.5 cc. nutrient broth.

(12) Nutrient broth + yeast + Locke's soln.

(13) Nutrient broth + Locke's soln.

(14) Yeast + Locke's soln.

None of the cestodes was found alive at the end of the 3½ days, nor were there any signs of development although in (2), (4) and (11) the tapeworms appeared to be larger than those in the other media and growth may have taken place before they died. This is doubtful, however, as many of the cestodes become distended in some media due to osmosis, and the size of the cestode cannot be taken as a criterion of development in culture media which are not isotonic with the body fluid of the tapeworm.

Medium	Locke's Soln.		Locke's soln. + serum.		Locke's soln. + serum + merthiolate .001%	
Time	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
	Media unchanged					
21 hrs.	Active, distended.	Inactive, opaque, distended.	Active.	Active.	Active.	Active,
41 hrs.	Inactive, opaque, distended.	-	Inactive, opaque.	Inactive, opaque.	Inactive, opaque.	Inactive, opaque, contracted.
	Media changed daily.					
21 hrs.	Inactive, opaque.	Inactive, opaque.	Active	Active, distended	Active.	Active.
41 hrs.	-	-	Inactive, clear.	Inactive, opaque.	Inactive, opaque, contracted.	Inactive, opaque, contracted.
65 hrs.	-	-	Inactive, opaque.	-	-	-

Table 7 **Comparison of the effects of aerobic and anaerobic conditions on Polycercus lumbrici in various media (at 37°C.).**

Experiment VI.

In investigating some of the factors which might influence the viability of P. paradoxa, an experiment on quite a different line of approach from the above was carried out. This was an attempt to discover if the oxygen tension in the culture medium had any effect on the cestode. Two sets of media were prepared in one of which no precautions were taken to exclude or to drive off air, but in the other the media were prepared from Locke's solution from which the air had been driven off by boiling it for several minutes. The boiled solution was then decanted into a flask and the flask stoppered and allowed to cool. Small screw-topped bottles with rubber seals were then filled to the brim with the solution after the other constituents of the media had been introduced and the young tapeworms were finally transferred to the bottles. Although these media would not be truly anaerobic, the oxygen tension in them would be greatly reduced compared with the 'normal' media.

Comparison of the cestodes in the different media (Table 7) shows that the lowering of the oxygen content has very little effect on their survival and there were no noticeable differences in size. Only in one medium (Locke's soln. + serum) was there any difference between the samples in the anaerobic and the aerobic media, those of the latter living the longer. The difference is peculiar to this medium and is so slight that I do not regard it as significant.

DISCUSSION

The availability and abundance of the polycercal larvae are great advantages in making a detailed study of their development and, of course, they are equally advantageous in a study of the living cysticercoids. The examination of the living larvae *in vitro* is not particularly informative. It is noteworthy, however, that there is no appendage or 'tail' attached to the larva, as is found in many of the non-proliferating cysticercoid larvae such as those of the *Hymenolepididae* (Lühe, 1910). In the latter the 'tail', which is shed when the larva enters the final host, carries the hooks of the onchosphere. In *Polycercus*, as in *Cysticercus pisiformis* (Young, 1908), the embryonic hooks have not been found and the exact relationship between the onchosphere and the 'mother-cyst' is not known although the latter is derived, directly or indirectly, from the former.

Evagination of the larva.

The final stage of development of the cysticercoid is the evagination of the scolex from the caudal bladder and the protrusion of the rostellum from the scolex. These are co-ordinated with the freeing of the larva from the retaining cyst.

The process itself is obvious and simple, but the factors which induce the evagination are not self-evident and a brief consideration of the changes to which the larva is subjected on being eaten by the

final host, a charadriiform bird, shows that one or more of several factors may be involved. The results of the experiments which I carried out in attempts to determine the effective factors indicate that:

(i) the larvae are freed by rupture of the cyst due to pressure and abrasion in the gizzard;

(ii) the larvae are activated by an increase in temperature;

(iii) the larvae evaginate when treated first with gastric juice and then with pancreatic juice.

The effect of pressure and abrasion

The gizzard of the Woodcock is a thick-walled, very muscular chamber between the crop and the duodenum. It is principally a crushing and grinding organ, a 'gastric mill', and material which enters it is subjected to strong pressure exerted by contractions of its walls and abrasion by grit swallowed by the bird which assists in breaking up the larger food particles.

After experiments I arrived at the conclusion that pressure and abrasion may play an important part in the process of evagination but they are not the main factors involved. Their role in the process is probably to rupture the cyst and release the cysticercoids but not to cause evagination. Admittedly, I was predisposed to make this last reservation as I was aware of the evidence of other workers which indicated that the action of digestive juices was largely responsible for inducing evagination of some species of cysticercoids other than

the one I was concerned with. Besides this, however, was the fact that full evagination in the gizzard would expose the young tapeworm to a very acid environment in which it would not survive for long, as later experiments showed.

In spite of my doubts about the true role of pressure and abrasion in the process, the method used was simple enough and efficient enough (in view of the amount of material available) to be used as a means of obtaining numbers of newly evaginated cestodes for further experiments. It has the advantage that the young tapeworms are not contaminated by chemical substances other than those from the body of the Earthworm. Certainly, this covers coelomic fluid, digestive juices, exudations from the body wall and many other imponderables, but the fact remains that, after washing in several changes of Locke's solution to get rid of extraneous matter, the young tapeworms obtained by this method showed survival rates as good as or better than those obtained by other methods and I used them for most of the simple physiological experiments which I carried out.

If the larva is freed from the containing cyst by mechanical rupture of the wall of the cyst in the gizzard of the bird it illustrates an interesting process of adaptation of the larva to the digestive processes of the bird. In Cysticercus pisiformis the cuticular cyst is attacked by 'Suc gastrique' (de Waele, 1933) but, of course, this larva is not subjected in nature to the same processes as is Polycercus, its final host being a carnivore in which there is no gizzard-like structure. Thus, the mechanical action of the gizzard of the bird is

replaced, in the mammal, by the chemical action of the digestive juices.

The effect of heat

The Earthworm is a poikilothermic animal and the cysticercoids in its body cavity will, therefore, be subjected to temperatures varying with the Earthworm's surroundings. The High Lethal Temperature for Lumbricus is 30° C. at 100% Relative Humidity (Prosser et al.) and it can be safely assumed that the High Lethal Temperature for Allolobophora terrestris will also be of this order. It is unlikely, therefore, that the cysticercoids will be subjected to temperatures even approaching anything as high as 30° C. for any length of time while they are in the living Earthworm.

The normal body temperature of a bird is approximately 38° C. rising to about 40° C. when broody. If the cysticercoids are eaten by a bird they are, then, subjected to a fairly rapid rise in temperature of 10 - 15° C. This could be quite sufficient to produce a sudden outburst of activity in the cysticercoid which would result in its evagination, as has been observed, but repeated experiments show that heat, although sometimes fairly effective as an evaginating-agent, is not a satisfactory agent and probably acts only as a contributory factor.

The effect of chemicals

(The term 'chemical' is here used to cover organic and inorganic compounds, hydrogen-ion concentration and digestive enzymes.)

In the Woodcock, the food passing down the alimentary canal enters the gizzard where it is ground up and then subjected to the action of acid secretions from the walls of the glandular stomach and proventriculum. Following this it passes into the duodenum where the acidity of the contents is reduced by the alkalinity of the bile and pancreatic juices.

In the domestic fowl the pH in the gizzard ranges from 2.7 in a 33-day chicken to 3.06 in an adult, while the respective figures for the pH of the duodenum are 4.06 and 4.24 (Prosser et al.). The figures for the Woodcock are not known but are probably of the same order; facilities to carry out these determinations were not available.

Since the highest concentrations of P. paradoxa in the Woodcock are found in the duodenum, it follows that full evagination and development take place after passage through a very acid environment into less acid surroundings in which the digestive enzymes operate. If the exact conditions could be duplicated in vitro then normal evagination and development would follow, but, of course, the conditions are dependent on a complex of factors, many of them unknown, and a rough approximation to the intestinal conditions, including the vital factors in inducing evagaination, is the best that can be hoped for.

Scott (1913) carried out experiments in inducing evagination

of Cysticercus pisiformis, the larva of Taenia pisiformis (syn. T. serrata), a common tapeworm of the dog. He immersed the cysticercoids in artificial gastric juice and then transferred them to artificial pancreatic juice. This induced evagination. He decided that the most important factors were the alkali, Sodium carbonate and the extract of pancreatin in the artificial pancreatic juice, adding that previous treatment with hydrochloric acid produced the very best results, but that the primary function of the hydrochloric acid is the destruction of bacteria and other living tissues. The Sodium carbonate appeared to be more important than pancreatin.

De Waele (1933), also working on C. pisiformis, found that if a complete cysticercoid were placed in an acid medium with pepsin the outer envelope was lost in 2-3 hours. If the larva were now transferred to a neutral or alkaline medium containing pancreatin and, most important, bile, the neck and scolex evaginate, the bile being the factor which induces immediate evagination. (Heat and the action of saliva produced much slower evagination). De Waele's results differ from mine in that I found that bile was not required to induce immediate invagination and, as I have already mentioned, the larvae are freed in the bird by the mechanical action of the gizzard, whereas de Waele suggests that the outermost envelope of Cysticercus pisiformis is destroyed in the mammal by chemical action. The time taken for the digestion of the outer envelope, the 'receptaculum' of C. pisiformis seems unduly long when we consider that the whole process of digestion in a carnivore may be completed in about four hours.

As the process of evagination does not involve the immediate disappearance of any part of the larva, the agent which induces it acts only as a stimulant and, therefore, is just as likely to be a simple salt as a complex proteolitic enzyme. The action of the stimulant is probably to induce a sudden, strong contraction of the muscles of the walls of the caudal bladder which would force the scolex of the tapeworm out through the anterior pore.

The tapeworm after evagination

On completion of evagination in vitro, the young tapeworm has assumed a form commonly found in the intestine of the Woodcock: a well-formed scolex with the harrower neck joining it to the sac-like body. In the vessel in which the evagination is carried out, each young cestode is accompanied by a large number of calcareous corpuscles and a caudal vesicle lying free on the bottom of the container. The caudal vesicle is cast off when the cestode is fully evaginated. It is the outermost wall of the cysticercoid and is 'pinched off' at the level which formed the anterior pore of the cysticercoid through which the scolex and remainder of the cestode are forced out during evagination. The cuticular wall of the sac is lined with longitudinal and circular muscles, as is the rest of the body wall of the tapeworm, and the sac can be seen to expand and contract spasmodically sometime after being separated off. Such contractions are probably responsible for forcing the scolex out of the caudal vesicle during evagination.

The sac is then lost as is the caudal appendage or 'tail' of many of the cysticercoids of the non-proliferating cysts, the part of the larva which bears the hooks of the oncosphere and which is cast off when the larva is ingested by the final host.

The fact that numerous calcareous corpuscles are freed during evagination is interesting and possibly significant. It is generally assumed that these are cellular secretions, whether intra-cellular or extra-cellular, and, although it is not certain what functions they serve, they are thought to be either excretory products, antacid, or contain stores of glycogen or other nutrients. As many are freed from the larva during evagination, they must have formed outside the larva of have passed out of its body either into the space between the scolex and the enveloping wall of the cysticercoid or through the region where the caudal bladder separates from the young worm. This suggests that they are excretory products or, as suggested by von Brand et al. (1960), they are a protective device against the acidity of the gastric juice of the host or the acid products of the metabolism of the cestode itself. However, histochemical and X-ray analysis of the calcareous corpuscles of Taenia taeniaeformis with the aid of the electron-microscope reveal the presence of two polysaccharides in the interior of the corpuscles and unidentified proteins and lipids on the outside. This certainly suggests that they play a part in the 'economy' of the tapeworm. (Joyeux and Baer, 1961).

Culture of P. paradoxa in vitro.

A great deal of attention has been concentrated on the physiology of the Platyhelminthes in recent years, particularly on the problems of development and maturation of their larvae in vitro. A considerable degree of success has been achieved with the Trematoda but, although some notable advances have been made, attempts to culture Cestoda in artificial environments have met with little reward except in the cases of procercoid and plerocercoid larvae of certain tapeworms. Such successes as have been achieved, while the results of meticulous and patient experiments, are still largely inconclusive. Repeated experiments give varying results; development proceeds in conditions previously assumed to be adverse; the simplest of media, with no obvious nutritional value, appear to be more effective at times in supporting development than the most complex physiological broths and at other times the reverse is true.

The most valuable contributions to this field have been made by Smyth (1946 et seq.), working principally with the plerocercoids of Ligula intestinalis and Schistocephalus solidus. His papers give an excellent review of the work done by himself and others, but his work is confined to larvae very different from and much easier to work with than the minute cyclophyllidean larvae of P. paradoxa. Except for a little work on the larvae of those parasites which infest man or are of economic importance to man, (Dévé, 1926; Coutelen, 1926, 1929) cyclophyllidean larvae have not been used in

physiological experiments. Principles which apply to one species or genus do not necessarily apply to another, and it is not to be expected that techniques which succeed with a plerocercoid will apply equally well to a cysticercoid although they may well indicate the general lines along which experiments on the latter may be carried out. The position is very well summed up by Wardle and McLeod (1952):

> "The inescapable conclusion from the data presented is that the saline media usually employed in physiological studies are useless for the study of tapeworm physiology, and conclusions based upon experiments carried out in such media cannot be accepted as giving an accurate picture of the physiological processes taking place in the worm that is living in the animal gut. That is to say, the bulk of the information already accumulated upon tapeworm physiology, scanty as it is, is practically worthless, and workers in the field of physiology will have to adopt a technique more akin to that of the bateriologist than that of the physiologist ..... The nutritional requirements of such a tapeworm when <u>in vitro</u> may prove to be less exacting than has been supposed, and it may not be necessary to duplicate the nutrient complex that surrounds the tapeworm <u>in situ</u>. Wardle (1937).
>
> "Tapeworm cultivation is thus essentially a problem of establishing an equilibrium between the tapeworm's internal environment and a laboratory imitation of its external environment. Relatively little information is available, however, about the chemical and physical conditions of the two environments or about the inter-environmental exchanges."

The work of Smyth confirms the above observations, his techniques being those of the bacteriologist rather than those of the physiologist.

My first ventures into the field of physiology involving <u>P. paradoxa</u> were simple efforts to keep the cysticercoids and newly-evaginated tapeworms alive and healthy for the time required to examine them in the living state. This subsequently led to experiments

to determine the media in which they lived for the longest periods and try to find media in which the post-larval stages would grow and finally mature. Some success was achieved in the first objective, little in the second and none in the third.

For the purpose of my experiments, a cestode was not presumed dead until it was both inactive and opaque. Many of the young cestodes remained apparently motionless for long periods. The healthy young tapeworm, in vitro, is translucent with an almost octahedral scolex and the rostellum protruding only slightly in front of the suckers. After examining large numbers of living and dead cestodes it is fairly easy to distinguish between the two.

The experiments were based mainly on Smyth's findings that standard physiological solutions formed a good basis for maintaining and culturing the plerocercoid larvae of Ligula intestinalis and Schistocephalus solidus, with or without the addition of other agents. Horse serum appeared to have a beneficial effect in some instances and was employed in conjunction with the physiological solutions in the hope that it supplied a vital factor in promoting development.

Having established that the cysticercoids could be kept alive in simple solutions, I turned my attention to the problem of keeping the evaginated tapeworms alive in artificial media.

The foregoing experiments show that:

(1) A considerable proportion of the cysticercoids and newly-evaginated young of P. paradoxa can be kept alive, healthy and active in simple

artificial culture media for two days, and life may be sustained in these media for 5½ days of more (Tables 5, 6).

(2) None of the media used has promoted development of the cestodes or furnished any indication of conditions in which they might mature.

So far as I can ascertain, there have been no previous attempts to culture cysticercoids or young cyclophyllidean tapeworms immediately after evagination, and there are no data, therefore, with which I can fairly compare my results. Smyth's (1946) summary of previous attempts to culture cestodes in vitro gives figures for maximum viability ranging from 0.5 days for a plerocercoid, Triaenophorus tricuspidatus, to 35 days for the cysticercus of Taenia teniaeformis, but he himself has kept the plerocercoid larva of Schistocephalus solidus alive in peptone-broth for 300 days. He has also brought the plerocercoid to sexual maturity in a few days, producing eggs which developed into normal coracidia. He attained a considerable degree of success using simple methods such as I followed, but his ultimate achievements were the result of a careful and refined technique involving the use of sterile media at high temperature with provision for constant removal of the waste products of metabolism and for a suitable substratum to which the plerocercoids could adhere.

The facilities for applying Smyth's techniques to P. paradoxa were not available to me but, even if they were, it must be borne in mind that there is a considerable difference in dealing with a fairly large plerocercoid and a minute cysticercoid. As has been stated

previously, techniques which have been reasonably successful in the culture of one species of cestode have failed with other species.

APPENDIX I.

Paricterotaenia burti Sandeman (1958).

Sandeman (1958), while examining material from the Jacksnipe (Lymnocryptes minimus) and the Curlew (Numenius arquata) in the area of the River Eden near St. Andrews, found numbers of a very small cestode in both of these birds. The hooks of the worms corresponded in size and shape to those included in Krabbe's (1869) description of Paricterotaenia stellifera. In this description, two sets of hook characteristics are given, one accepted by later workers as those typical of P. stellifera while the other is apparently ignored. The latter corresponds to that of the hooks of the minute tapeworm found in the Jacksnipe and Curlew and, as this tapeworm is anatomically quite distinct from P. stellifera, Sandeman named it Paricterotaenia burti.

Later P. burti was found in large numbers, together with P. paradoxa, in the Woodcock (Scolopax rusticola). These two tapeworms are so similar in size and form that the fact that there are the two species in many Woodcock has been overlooked by other workers who may have examined the birds in which they occur. The most obvious difference between the two cestodes is in the size of the hooks: P. paradoxa has 14-18 hooks of 72-108μ in length; P. burti has 14-16 hooks of 44-52μ in length. Another fairly obvious difference lies in the number of testes: P. paradoxa has 5-9 testes, P. burti 16-20.

In examining Earthworms from Kippo Wood for the polycercal larvae of P. paradoxa, I discovered that, in a large proportion (about 30%) of the Earthworms infested with Polycercus, the fully developed dysticercoids were smaller than the typical cysticercoids of P. paradoxa and their hooks were only about half the size of the hooks of the latter. From the size, shape and number of the hooks in each cysticercoid it was obvious that I had found a second polycercal cestode, the larval form of P. burti. This has not been previously recorded. The fact that P. burti is found in Woodcock in this area is supporting evidence in favour of my identification of the Polycercus.

A careful study of the anatomy of the larva of P. burti at different stages of development shows that it is very similar, except for size, to that of P. paradoxa, both as regards the process of development and structural detail.

There are two very interesting points about the occurrence of the two polycerci. Firstly, they both occur in the same host, Allolobophora terrestris, and have not been found in other species of Earthworm. Secondly, although the adult tapeworms of both species are found side by side in the Woodcock, the polycerci have not been found together in a single specimen of the Earthworm. This suggests that, when the Earthworm is infested by one species of the polycercus it may develop an immunity to the other species. It is quite possible, also, that the Earthworm, once infested, may develop an immunity to subsequent infestation by the same species and that a heavy infestation

is the consequence of a single heavy initial intake of the cestode eggs, not of a series of successive, smaller intakes.

Hook development in P. burti.

In the course of examining the polycerci of P. burti I treated several groups of the larvae with Liquide de Berlese and I was fortunate enough to find that, on one occasion, I had treated material which included a number of cysts showing the hooks at different stages of development. This was particularly valuable as I had been unable to obtain similar material for P. paradoxa. Since the process of development in P. burti appears to be identical with that of P. paradoxa, the pattern of hook development in the former should indicate the pattern in the latter. This is confirmed by the fact that such stages of hook development in P. paradoxa as had been observed were exactly similar to the corresponding stages in P. burti.

The stages found are illustrated in Figs 52-59 . The earliest (Fig. 52) is that in which the double band of hooklets has formed and the definitive hooks have started to develop in the anterior band. The hooklets are 1-5μ in length and even the smallest that are distinguishable appear rod-like or conical rather than as cuticular 'hairs'. They increase in size, become conical and then curve to become claw-like. Among the larger hooklets there are innumerable small hooklets and many of these appear to run in a few definite tracts between the two main bands. The largest of the hooklets, about 5μ in length, are leaf-shaped in outline, rather than claw-like, swelling from the base then tapering to a fine point.

The definitive hooks are easily distinguished. They are twice as large as the larger hooklets, being approximately 10μ in length and consist of a rudimentary, curved blade rising from a relatively large leaf-shaped base (Fig. 52). They lie on the anterior edge of the band of hooklets with which they are associated.

The arrangement of the hooks and the hooklets, as a whole, is more complicated than appears at first sight. Basically, it consists of two bands of hooklets, the anterior band including the larger definitive hooks. These bands are not separate, however, but are connected by tracts of minute hooklets. The anterior band contains comparatively few of the smaller hooklets and none of the larger although the distinction between the larger hooklets and the smaller definitive hooks is not great. The arrangement suggests two possibilities: (a) the whole of the prebulb was originally covered by hooklets which later became restricted to the two main bands;

(b) the posterior band of hooklets forms first and then there is a migration of this material to the site where the definitive hooks form, the material contributing to the formation of the larger hooks. The pattern of distribution of the hooklets does suggest a flow of material ffom the posterior band to the anterior along well-defined tracts, and this mode of development would explain the disappearance of most of the hooklets, but there is insufficient evidence to postulate that such a migration does take place.

In the next stage found almost all the hooklets have disappeared

except for a scattering of granules among the definitive hooks (Figs 53, 54). These latter appear as simple cones with straight walls, or are curved and claw-like. They are 12-15μ in length. This stage is followed by a lengthening of the base of the cone (Fig. 55) so that the hook becomes thorn-shaped and its parts can be readily related to the parts of the hook of the adult: the long, oval base becomes the handle and guard, and the sharp point of the 'thorn' develops into the long blade.

The succeeding stages are a continuation of this process: the base lengthens and flattens, extending forwards to form the handle and backwards to form the guard; the blade grows backwards as a delicate, often sinuous process along the side of the rostellum and then broadens (Figs 56, 57). At this point, before the hooks begin to harden by deposition of chitinous material over the thin walls, they have attained a length of about 30μ and are easily recognizable as 'paricterotaeniid' hooks. The final stage of development is the deposition of the keratin-like outer shell of the hook which builds it up to its final size and form (Figs 58, 59).

APPENDIX II.

Identification of Cysticercoids

In the course of my investigations, the life cycles of three species of the Genus Paricterotaenia have been elucidated: P. paradoxa and P. burti whose principal definitive host is the Woodcock and whose intermediate host is the Earthworm Allolobophora terrestris, and P. stellifera whose definitive host is the Snipe and whose intermediate host is Tubifex rivulata.

An interesting discovery was the occurrence of two specimens of Cysticercus pachycanthus v. Linst. in the gizzard of a single specimen of the Common Snipe, Gallinago gallinago L. which was shot near Lathones, Fife. This cysticercoid had been previously recorded by von Listow as occurring in Gammarus pulex. Its hooks correspond to the description of those of Diagonaliporus skrjabini given by Krotov (1952), who found the cestode in the Snipe Gallinago solitaria japonica Br. in the Sakhaline Islands. However, as I.M. Sandeman has pointed out (Unpublished communication), Krotov's description of the crossing over of the genital pore, which is the diagnostic charactertistic of his Genus Diagonaliporus, is difficult to understand in the context of our present interpretation of the process of cestode growth, i.e. the budding off of proglottides from the neck-region of the tapeworm, and it is not accepted as a characteristic on which a new genus can be founded. Diagonaliporus has been classed as synonymous

with Valipora Linton (1927) by Yamaguti (1959) and Diagonaliporus skrjabini renamed Valipora skrjabini (Krotov, 1951).

In the intestine of the same Snipe as the cysticercoids of Cysticercus pachycanthus were found I also obtained a number of mature cestodes which agree with Krotov's description of D. skrjabini and whose hooks are identical with those of the cysticercoids. The evidence is fairly conclusive, therefore, that Cysticercus pachycanthus is the larval form of Valipora skrjabini, its final host the Snipe and its intermediate host the Crustacean Gammarus pulex.

In the above cases the hooks of the cysticercoids have been the main features by which the larval forms have been correlated with the adults. In addition to this, however, attention has been paid to the nature of the intermediate host and its relationship to the final host. When these requirements, similarity of hooks and association of final and intermediate hosts, are met I accept them as sufficiently good grounds for naming the cysticercoids as the larval forms of the corresponding adult cestodes. Thus, the larval forms of P. paradoxa and P. burti are found in the Earthworm, staple diet of the Woodcock; P. stellifera is found in its larval form in Tubifex, which is eaten in large numbers by Snipe; Gammarus pulex is also a favourite food of the Snipe and it is not surprising, therefore, that the cysticercoid Cysticercus pachycanthus, parasitic in Gammarus, is the larval form of Valipora skrjabini, a tapeworm parasitic in the Snipe.

It has been the practice of parasitologists from as far back as the mid-18th century to try to determine the adult form of a larval

cestode by feeding the larva to what is thought to be a likely final host and then trying to recover the adult from this host. Because of the apparent high degree of host specificity among many cestodes and the difficulty of finding a single, possibly small, cestode in the intestine of an animal, the chances of finding the right host and the adult cestode are remote. Such techniques served a very useful purpose when comparatively few cestodes had been described and there was not sufficient evidence to show that the size and form of the hooks of the worms were characters of high diagnostic value.

Our present, fairly comprehensive knowledge of, at least, the more common tapeworm parasites of the vertebrates makes it possible to correlate a larval armed cestode with its adult form from ecological and anatomical data. There is no need to carry out experimental infestations which, although desirable as confirmatory evidence, should not be stipulated as a necessary condition for attributing to the larval form the name of the adult. To insist on this condition is to retreat towards the position in which parasitologists worked before it was realised that the 'cystic cestodes' found in invertebrates were in fact the larval stages of the tapeworms found in vertebrates. The taxonomics and systematics of the Cestoda are complicated enough without the larval forms being given names which bear no relationship to the names of the adults, as has been done in the past. This practice has been adhered to even up to the present day and no real attempt has been made to name the larval cestodes in

such a way that their connection with the adult form is made clear. A trinomial system of nomenclature for the 'cystic cestodes' would be a simple matter, e.g. the polycercal larva of Paricterotaenia paradoxa could be known simply as Polycercus paricterotaenia paradoxa, or, if the Latin grammar is to be invoked, Polycercus paricterotaeniae paradoxae, but it would be even simpler to give the larva the name of the adult as is the practice in dealing with other classes of the animal kingdom.

APPENDIX III.

The Genus Paricterotaenia Fuhrmann

Phylum Platyhelminthes; Class Cestoda; Order Cyclophyllidea; Family Dilepididae; Sub-family Dilepidinae, the uterus sac-like, persistent, more or less lobed or branched or, rarely, ring-shaped or reticulate; Genus Paricterotaenia, single crown of hooks, genital apertures alternating irregularly, genital ducts between excretory canals, testes numerous, lying behind the female organs; Genotype P. porosa Rudolphi 1810.

Paricterotaenia paradoxa (Rudolphi.

Joyeux and Baer (1939) describe P. paradoxa (Amoebotaenia lumbrici) as follows (extract):

"Length about 1 mm. Greatest breadth 120μ.

Scolex measures 200-25μ in diameter, the rostellum being half-invaginated in its interior. The suckers are 90-135μ (in diameter). The rostellum is 250-340μ long, with a maximum diameter of 70-100μ. There is a simple crown of 16 hooks of length 87-92μ.

The genital pores alternate. There are 7-9 testes, rarely 10, in the posterior part of the proglottis. The cirrus sac is fairly large, 62-67μ long by 25-30μ in diameter. It passes the ventral excretory vessel and contains a seminal vesicle which is difficult to see. The cirrus is armed.

The ovary is bilobed, the vitelline gland large. The seminal

receptacle, oval in form, measures 50μ by 25μ in the third proglottis. The inner shell of the ripe egg measures 27μ in diameter; the outer shell is imperfectly formed. The embryo is 20μ in diameter."

This description is in accord with my observations but it differs from that of Fuhrmann (1936) who, among other things, gives the length of the cestode as 1-12 mm. and the number of testes as 20. As stated by himself, Fuhrmann was obviously dealing with a 'composite species' probably including P. paradoxa, P. burti and P. stellifera.

According to Fuhrmann, P. paradoxa is "adult in the Woodcock, Scolopax rusticola L.; the Snipe, Gallinago media (Lath.), Gallinago gallinago L. and Lymnocryptes gallinula (L.); the Oystercatcher, Haematopus ostralegus L.; the Plover, Charadrius apricarius L.; the Lapwing, Vanellus vanellus L. and the Phalarope, Phalaropus lobatus (L.) - Development unknown."

Paricterotaenia burti Sandeman.

Sandeman (1958) describes P. burti as follows:

"Syn. Paricterotaenia stellifera (Krabbe, 1968) ex parte.

Host: Lymnocryptes minimus and Numenius arquatus.

The strobila is exceedingly small, it has a length of up to 0.6 mm. and a maximum breadth of 0.17 mm. There are only two or three segments, the last of which is often gravid. The scolex has a diameter of 130-260μ. The rostellum, of length 65μ and breadth

about 50μ, bears a single crown of 14-16 hooks of length 44-52μ.

The genital poree are alternating and open on the lateral margin of the proglottis, slightly anterior to the centre. The longitudinal canals were not observed. There are 5-8 testes of diameter 40μ situated in the posterior part of the proglottis. The cirrus sac runs obliquely forwards from the genital pore, it has a length of 54-70μ by 10-13μ. The ovary has two rounded lobes and is situated centrally in the anterior centre of the proglottis where it is expanded to form a receptaculum seminis of size 33μ by 16μ. The uterus is sac-like and occupies the whole ventral part of the proglottis. The eggs have a diameter of 15-17μ."

P. paradoxa is normally associated with other cestodes in the intestine of the Woodcock. Almost without exception, the Woodcock which I have examined have harboured large numbers of Haploparaxis parafilum and several members of an unidentified species of the Genus Amoebotaenia, described below. H. parafilum is usually confined to the middle part of the intestine and I have never found it in the duodenum. The Amoebotaenia also occurs in the mid-gut but extends forwards into the posterior part of the duodenum and backwards into the third quarter of the gut. In addition, as has been mentioned previously, it is not unusual to find P. burti with P. paradoxa.

None of these tapeworms have any obvious effect on the occurrence of P. paradoxa. Although the latter occurs in its highest concentrations in the anterior part of the duodenum, it is widespread throughout

the anterior two-thirds of the intestine. It is found in the immediate vicinity of H. parafilum and Amoebotaenia sp. so they, apparently, do not induce any reaction in the host nor produce any secretions which prevent the occurrence and development of P. paradoxa in the same host, or vice versa. Such 'acquired immunity' has been suggested as an explanation for the fact that certain tapeworms occur in surprisingly small numbers in some hosts and, also, it would explain the occupation of certain regions of the host's gut by single species while other species may be found only in other well-defined regions characteristic of these species.

APPENDIX IV.

Amoebotaenia sp.

Host: Scolopax rusticola L.

Locality: Easter Balrymont, St. Andrews.

Description: Length up to 3 mm.; breadth up to 0.27 mm.

There are a moderate number of segments, 23 in a specimen in which the gravid segments were missing. The scolex measures 200μ in diameter by 160μ in length. The four suckers are circular with a diameter of 80-90μ. The rostellum is extremely long and slender, 400μ by 30μ and has a bulbous tip 80μ in diameter on which is mounted the single crown of 23-26 hooks of length 32μ. The neck is very short, about 40μ long by 140μ wide.

The genital pores are regularly alternating and open on the lateral margin of the proglottis at about its mid-point. The excretory vessels are not well-enough defined to established the relationship between them and the genital ducts. The cirrus sac runs diagonally forward from the genital atrium and measures approximately 90μ in length by 12μ in diameter. The cirrus is unarmed. There are 16-18 testes in a compact group behind the female organs in the posterior part of the proglottis. The ovary is about 140μ wide by about 25μ deep lying ventrally across the proglottis between the testes and the moderately convoluted vas deferens. The uterus is sac-like, almost filling the gravid segments. No receptaculum seminis nor seminal vesicle was observed in the specimens examined.

The cestode has the characteristics of the Genus Amoebotaenia, common in the Charadriiformes, but the hooks do not correspond in number, shape and size to any of the species of Amoebotaenia described. I class it therefore, as Amoebotaenia sp. incog. pending further elucidation of its anatomy. In view of its common occurrence I am reluctant to assume that it is a new species.

APPENDIX V.

A FORM OF COLONIAL SCOLEX

by Ilya Mechnikov

(pp. 263-266 of the Proceedings of the Zoological Series of the St. Petersburg Academy of Sciences.)

In the body-cavity of many rain worms studied by me in Odessa in the autumn of 1867 I discovered lenticular white corpuscles, which on closer examination proved to be capsules containing the heads of a type of Taenia unknown to me. The number of heads lying completely freely inside the capsule, without being in any way attached to it, varied in the extreme: some capsules contained only one head each, while others contained as many as 13. The capsules themselves were also free, not being surrounded with a cyst or other forms so often associated with parasite-infested animals. The capsule is in the form of a bladder of a fairly thick amorphous chitinous cuticle, beneath which is a layer of cells with nuclei and nucleoli. The scolices contained in the capsules look more or less like round bodies consisting of a sac enclosing the head: the latter is not turned inside out, as in the case of the Cysticerci, etc., but is in a completely normal position (i.e. with the suckers outwards), like the scolices found by Meissner in the body of Limax. The head has approximately a conical form with the wider side outwards. In front it has four suckers, and in the middle a proboscis drawn up inside the head and provided with a crown of long hooks, from 14 (?) to 17 in number.

These hooks are three-pointed: the upper straight point, like a long thorn, is joined to the lower curved arm of about the same length, and with a lateral arm, also curved and sharp. Beneath the proboscis is an oval gland. The cuticle, longitudinal and transverse muscles, the water vessels, calcium bodies (?) and parenchyma of the head also pass directly to the sac, which is provided at the top with an opening adjacent to the proboscis.

Apart from the forms just described, I discovered in the body-cavity of the rain worms a whole series of other younger growths. The earliest stage is in the form of a sphere provided with a thick cuticle and filled with a solid mass of cells, containing a round aqueous (?) nucleus with small nucleoles. The next stage differs from that just described not only by its greater size, but by a thinner cuticle and the presence of a central cavity. Further development continues in this direction, and the result is a bladder contained in a skin and consisting of a layer of cells surrounding a large cavity filled with aqueous liquid. On the inside surface of this bladder appear, in varying numbers, embryos of the scolex. At first they have the form of tubercles, broader than they are tall. As they develop these tubercles grow taller while the middle of the surface attached to the cellular layer of the bladder separates from it. As a result the embryo assumes the shape of a bell, joined at the edges to the cellular envelope of the bladder and containing within its cavity the gradually increasing body of the developing embryo. This bud, with its free edge facing the surface of the bladder, is itself

a future scolex, since the side walls of the bell, as they grow gradually thinner, form a sort of amnion, or what with Echinococcus is referred to as a 'brood-capsule'. However, whereas the capsule of Echinococcus is a phenomenon of long duration, that of the complex scolex of the rain-worm exists for a very short period. In addition to atrophy of the brood-capsule (which changes meanwhile into a short stalk), the connection between the embryo and the wall of the bladder is broken. This, however, occurs at a fairly late stage when the principal features of almost all the parts of the scolex have become defined. As it grows in length, the embryo of the scolex appears divided into two parts with a waist in the middle: a shorter, conical, round-ended fore part, which is the future head; and a broader, longer rear part, which is the embryo of the bladder. At about this time the young scolex clearly reveals at its periphery an epithelial layer, somewhat thicker at the fore end of the embryo, and an inner, thicker layer in which longitudinal muscles can be distinguished. Within the axis of the embryo there is a cyclindrical cavity (caused, evidently, by separation of the cells), which becomes wider in the rear portion of the embryo. It is also possible to observe in the young scolex the rudiments of four suckers in the region of the inner layer, and also the rudiments of the glandular organ of the proboscis.

Further development consists in the main of a shapper definition of those parts which have been already observed in the early stages. The attenuation of the portion between the bud of the head and the

bud of the bladder becomes even more striking, and in addition a change takes place in the shape of both parts: the anterior part which had been short now considerably lengthens, taking on the shape characteristic of the heads of all types of Cysticercus; the posterior part assumes meanwhile more of the appearance of a bladder, the walls becoming thinner and the interior filling with a quantity of aqueous liquid. During the period we have described, the proboscis takes shape at the front of the head, and suckers protrude behind the proboscis. At this time the whole surface of the young scolex is covered with a thin cuticle which forms on the proboscis local swellings which are the rudiments of hooks. It is remarkable that these buds appear in several rows, as Naunin discovered in the case of Echinococcus; only one upper row out of two survives, the other atrophying. First appear the lower, curved extremities of hooks and then the straight upper extremities are formed. We must also mention the calcium bodies which appear at about this time. These bodies are contained within the bladders from the very start of their existence, but I was unable to establish the process of their development.

During all the changes I have described the young scolex within the bladder which it had produced (acephalocyst) was so disposed that the head lay free, without being drawn into the caudal bladder, (i.e. the bladder forming the posterior portion of the scolex itself). Once the hooks have ceased development the position of the parts of the scolex changes, its anterior portion, i.e. the head, drawing back into the caudal bladder, while the cavity of the bladder becomes

smaller and smaller and finally is almost undetectable. At the same time the proboscis withdraws within the head and the scolex assumes its final position inside the acephalocyst in the same way as was described at the beginning of this account.

In order to solve the problem of the type of Taenia to which our scolex belongs, I fed a duck every day for three days with rain-worms containing it. At the end of this period I killed the duck, but not only found no further development of the scolices, but established that they had been completely digested. As a result of this, and having moreover a shortage of material, I postponed a solution of this question until a more favourable period, such as the spring, when one might count on there being moles and other insectivores, containing, perhaps, Taenia of our scolex. My researches on parasites encountered in the internal organs of domestic animals in Odessa have given me no guidance towards solving the problem of the further development of our scolex.

Although the scolex of the rain-worm is extremely like Echinococcus, it is a somewhat unique organism. One of its characteristics is its relationship to the bladder produced by it, another is the brief existence of the brood-capsule. Another peculiarity is the position of the head within the bladder, whereby it differs from Echinococcus and is similar to single scolices found in the body of many invertebrates. Of greatest importance, of course, is the presence in our scolex of a caudal bladder, for which we find no analogy in Echinococcus, the only cystiform parasite with an acephalocyst (the brood-capsule of

Echinococcus no doubt corresponds to the amnion of our scolex, and cannot therefore be compared with a caudal bladder).

SUMMARY AND CONCLUSIONS

1. Metchnikov's discovery of *Polycercus lumbrici* in the Earthworm, made in 1867, is confirmed for the first time.

2. The conclusion of Joyeux and Baer that *Polycercus lumbrici* is the larval form of *Paricterotaenia paradoxa* (= *Amoebotaenia lumbrici*) is confirmed.

3. The intermediate host is identified as *Allolobophora terrestris* and the principal final host as the Woodcock (*Scolopax rusticola*).

4. The polycercus has been found only in immature specimens of *A. terrestris*. The implications of this are discussed.

5. Earthworms were experimentally infested with *Polycercus lumbrici* by feeding them with mature proglottides of *Paricterotaenia paradoxa* from the Woodcock.

6. The incidence and habits of the Woodcock are discussed.

7. The degree and site of infestation of *Paricterotaenia paradoxa* in the Woodcock are discussed.

8. The development of *Polycercus lumbrici* in the Earthworm, from the earliest form of the larva to the cysticercoid, is described in detail for the first time.

9. Metchnikov's outline of the development of Polycercus lumbrici is amended to include a retroversion of the larva prior to the differentiation of the primordia of the scolex, retention of the side-walls of the larva and the appearance of an axial column of cells. His account is also extended to give, for the first time, a complete description of the development of the scolex. The rostellum is formed, as in Cysticercus fasciolaris, from a bulb and prebulb, the former supplying the hook-elevator muscles and the glandular elements, the latter the muscles of the walls of the rostellum and the hook-retractor muscles. The remainder of the muscles of the scolex are modifications of the parenchymal and peripheral musculature.

10. The muscular, excretory, nervous and glandular systems are described and discussed.

11. A description is given of experiments to determine:

(i) suitable media for keeping the larvae alive in vitro;

(ii) agents for freeing the cysticercoids from the cyst;

(iii) agents which induce evagination of the scolex;

(iv) suitable media for culture and development of the evaginated tapeworms.

The results of the experiments indicate that:

(i) the larvae can be kept alive for periods of up to five days in simple physiological solutions such as Locke's;

(ii) the larvae are freed from the cyst in the gizzard of the final host by a purely mechanical process;

(iii) the larvae are induced to evaginate in the final host by the action of gastric juice followed by pancreatic juice, the effective agents being pepsin in the first and pancreatin in the second.

(iv) the tapeworm will not develop to maturity in any of the media used.

12. The discovery of a second polycercus, the larval form of Paricterotaenia burti Sandeman, 1959 is recorded from the Earthworm Allolobophora terrestris.

13. Hook development in Paricterotaenia burti is described: it follows the same general pattern as that of P. paradoxa.

14. An unidentified species of Amoebotaenia is described.

15. Evidence is offered which elucidates the life-cycles of the following cestodes:

(i) Paricterotaenia paradoxa: intermediate host, the Earthworm Allolobophora terrestris; final host, the Woodcock (Scolopax rusticola).

(ii) Paricterotaenia burti: intermediate host, the Earthworm Allolobophora terrestris; final host, the Woodcock (Scolopax rusticola).

(iii) Paricterotaenia stellifera: final host, various charadriiform birds; intermediate host, Tubifex.

Cysticercus pachycanthus is identified as the larval form of Valipora skrjabini a parasite of the Common Snipe (Gallinago gallinago).

An unrecorded cysticercoid from Tubifex is noted.

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POSTSCRIPT

Since completing this thesis I have become acquainted with the work of A.K. Berntzen (The In-vitro Cultivation of Tapeworms. I. Growth of Hymenolepis diminuta (Cestoda: Cyclophyllidea). J. Parasit. 47, 3, 1961). This worker has successfully cultured H. diminuta in vitro from the cysticercoid stage to the adult with proglottides containing developing oncospheres. The culture-medium used was a very complex one which included the plasma of human blood, but the success of the experiments is attributed mainly to the use of an ingenious apparatus which ensured 'a continuous flow of medium over the worms at a controlled rate'. It allowed for the removal of medium which might have been altered by the metabolic processes of the tapeworm.

It is noteworthy that the author 'excysted' the larvae by placing them in an oven, in Tyrode's solution, at 37° C. He found this an unsatisfactory method which produced a very low proportion of 'excysted' worms and states that a more effective method must be used. This difficulty might be resolved by using a technique for evagination similar to that described by me.

ILLUSTRATIONS

A number of the following photographs of sections showing different stages in the development of *Polycercus lumbrici* are accompanied by drawings of the same stages. The drawings were prepared to show principal structures which may not be satisfactorily revealed in the photographs. They are semi-diagrammatic and sometimes composite, being made in some cases from two successive sections of the same specimen. They are not necessarily drawn from the same sections as the accompanying photographs.

The drawings were all made with the aid of the camera lucida. The photographs were taken with an Edixamat Flex B, 35 mm. single-lens reflex camera, using Ilford Pan F and Micro Neg Pan film.

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Fig. 60. Diagram of nervous system in scolex.

Fig. 61. Diagram of osmo-regulatory system in scolex.

PLATE 1. *Polycercus lumbrici*.

Figs 1a and 1b. 2nd stage larva with small central cavity.

PLATE 2. *Polycercus lumbrici*.

Figs 2a and 2b. 3rd stage larva.

PLATE 2

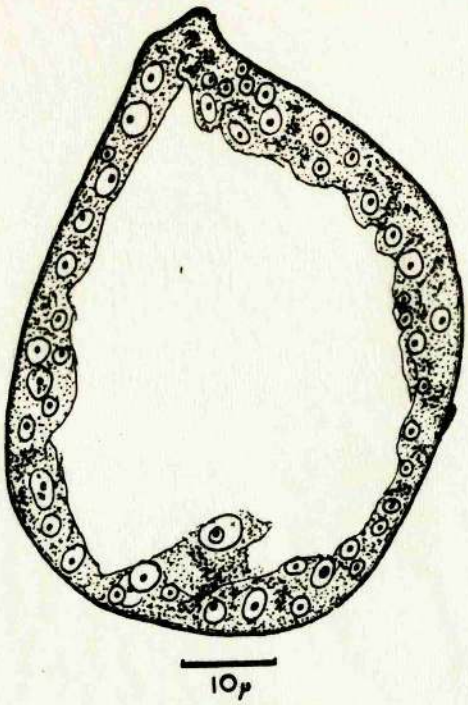

Fig. 2a

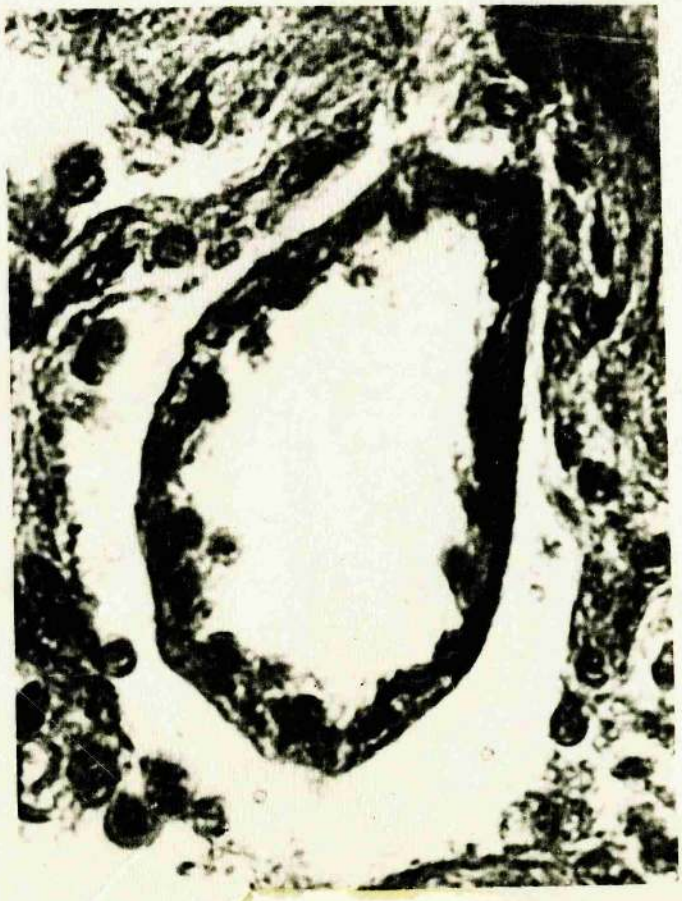

Fig. 2b

PLATE 3. Polycercus lumbrici.

Figs 3a and 3b. 4th stage larva. Cyst with single bud.

PLATE 3

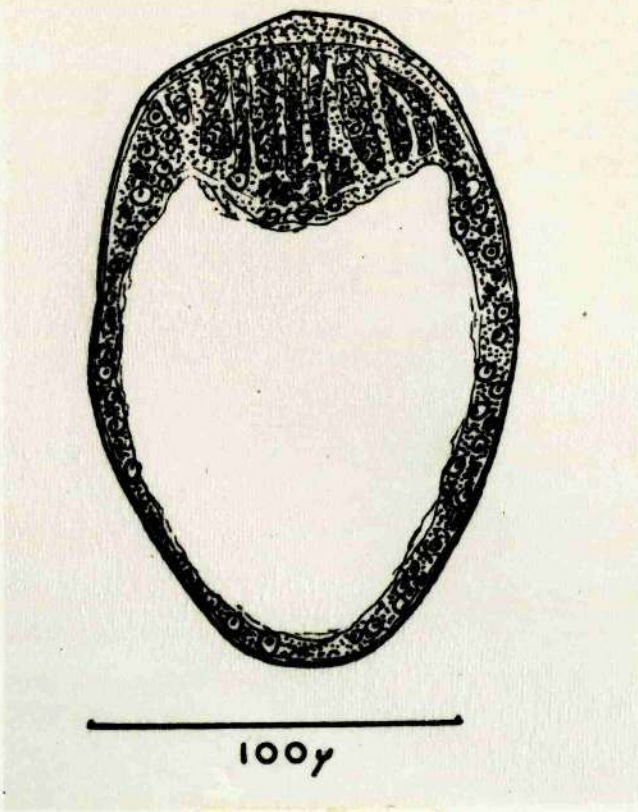

Fig. 3a

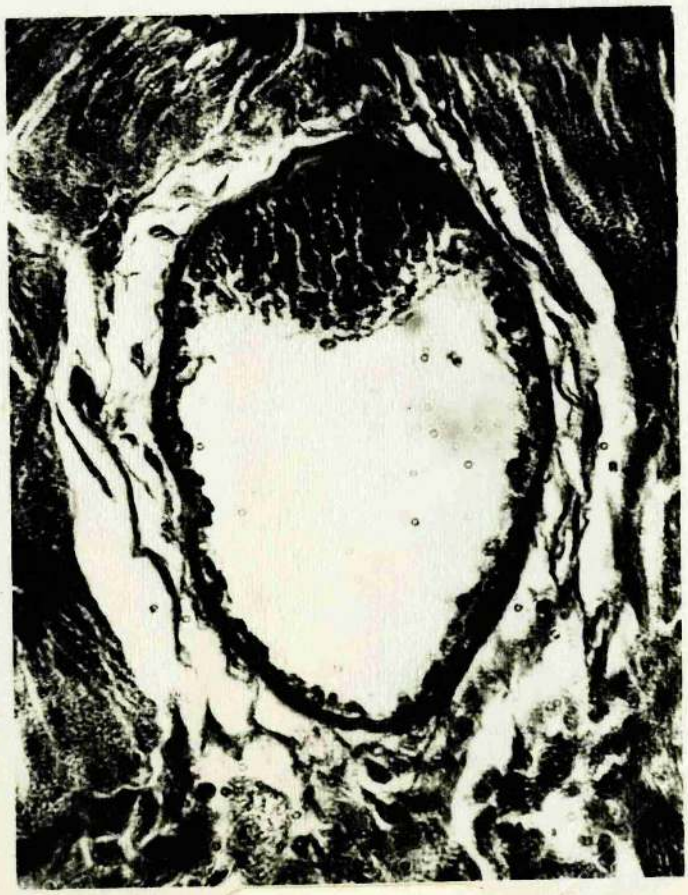

Fig. 3b

PLATE 4. Polycercus lumbrici.

Figs 4a and 4b. 4th stage larva. The bud, showing columns of cells and the thickening and zoning of the cuticle adjacent to its base.

a - granular zone.
b - separating layer.
c - clear zone.
d - fibrillae.

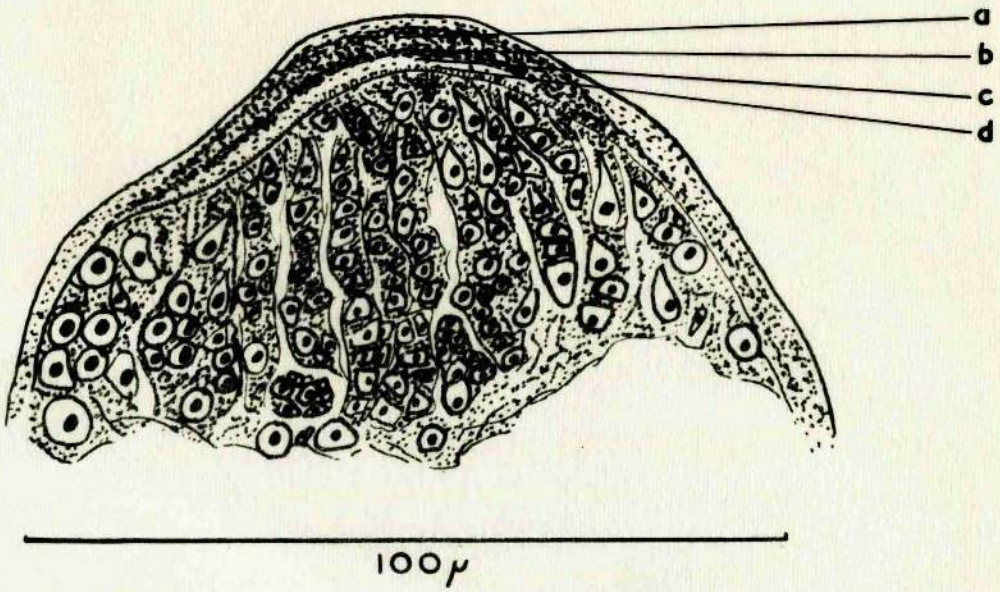

Fig. 4a

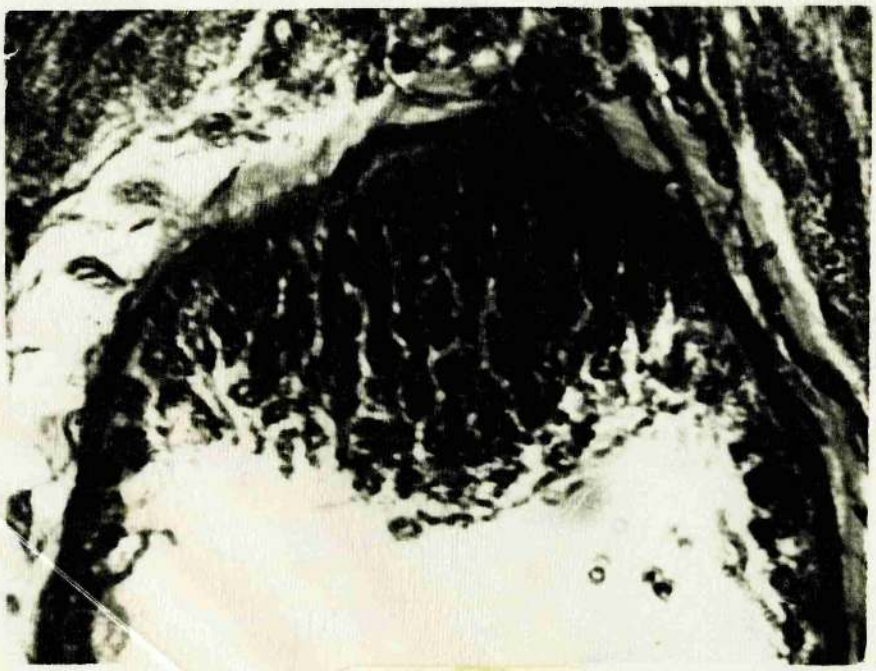

Fig. 4b

PLATE 5. Polycercus lumbrici.

Figs 5a and 5b. Differentiation in the bud prior to separation from the wall of the cyst.

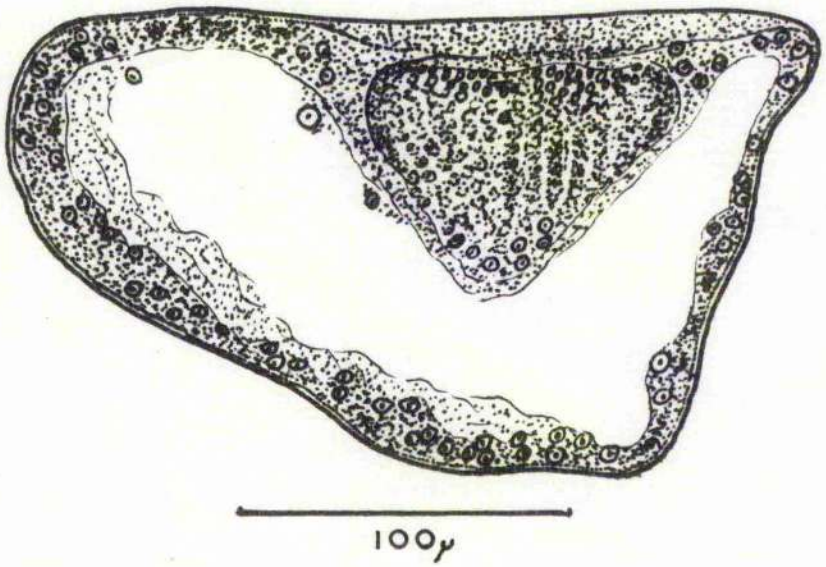

Fig. 5a

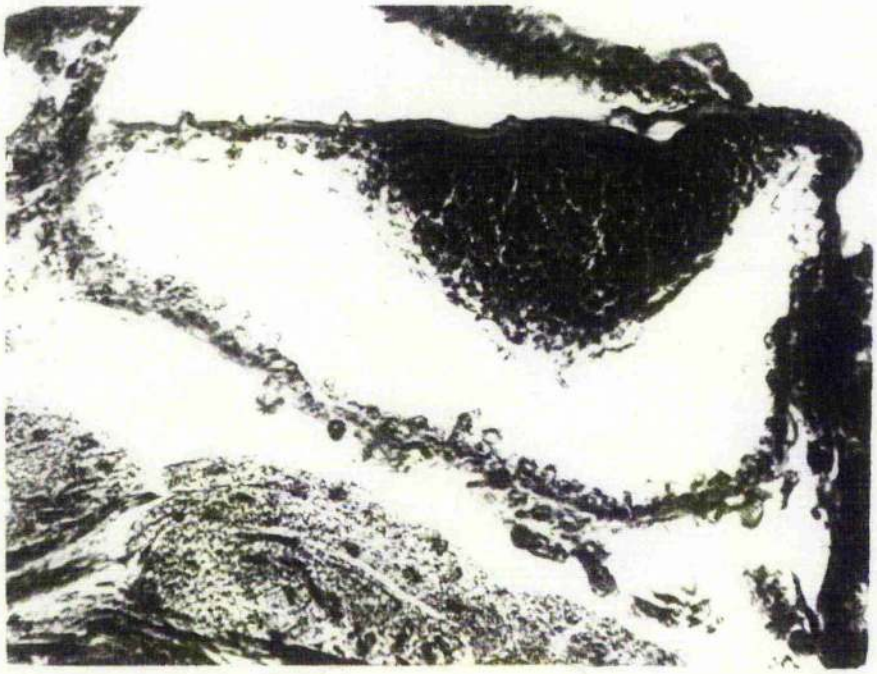

Fig. 5b

PLATE 6. *Polycercus lumbrici*.

Figs 6a and 6b. 5th stage larva. Separation of the central part of the base from the cyst wall.

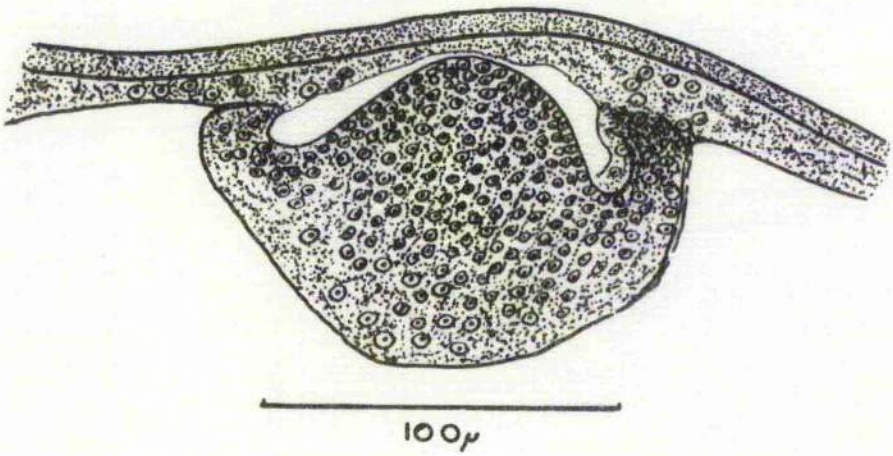

Fig. 6a

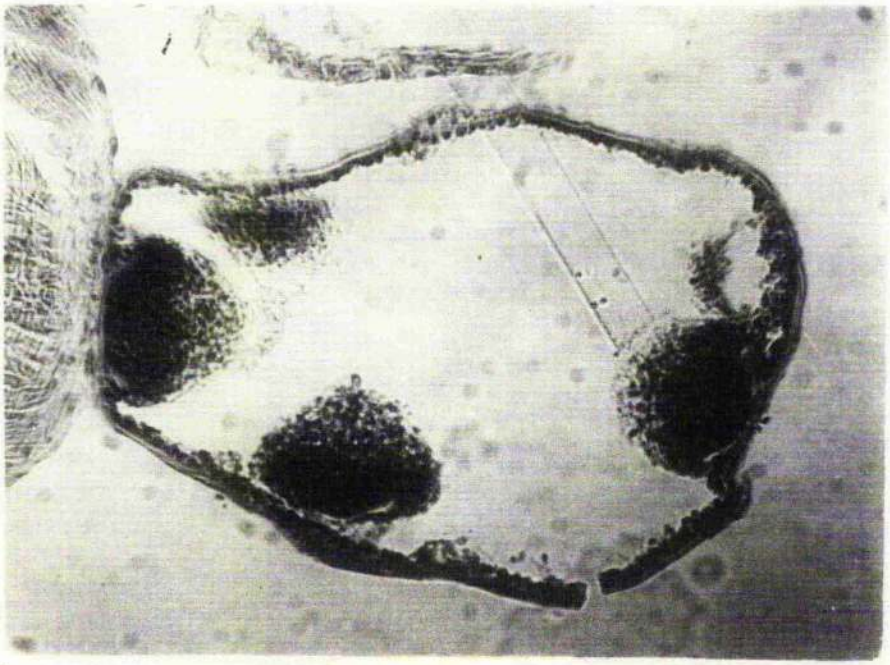

Fig. 6b

PLATE 7. Polycercus lumbrici.

Figs 7a and 7b. Multiple cyst after retroversion of the larvae.

a - membrane detached from cyst.
b - wall of 'mother cyst'.

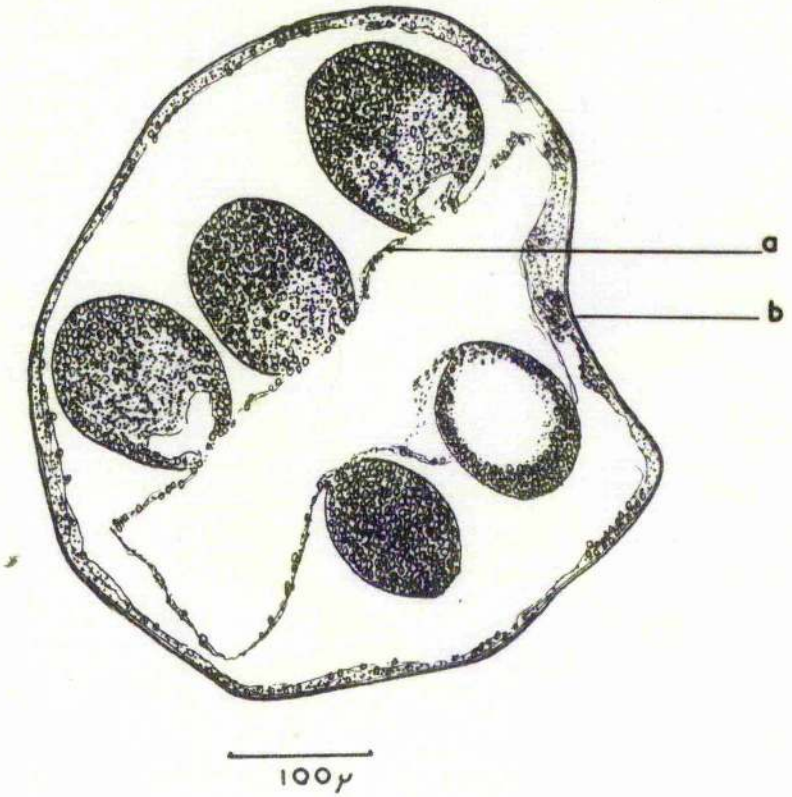

Fig. 7a

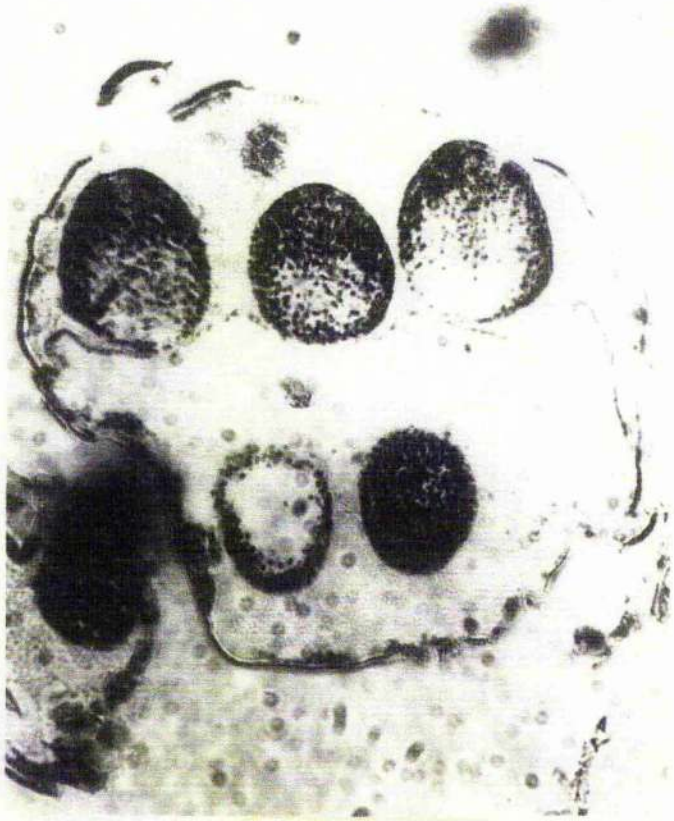

Fig. 7b

PLATE 8. Polycercus lumbrici.

Figs 8a and 8b. Differentiation of cells in larva.

a - central 'ball' of cells.

b - axial column of cells.

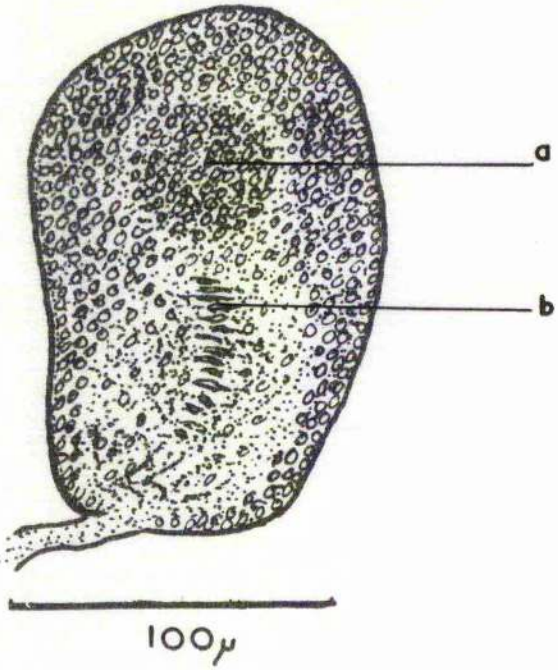

Fig. 8a

Fig. 8b

PLATE 9. Polycercus lumbrici.

Figs 9a and 9b. Early development of bulb, prebulb and suckers.

a - bulb.
b - prebulb.
c - sucker.
d - axial column of cells.
e - posterior invagination.

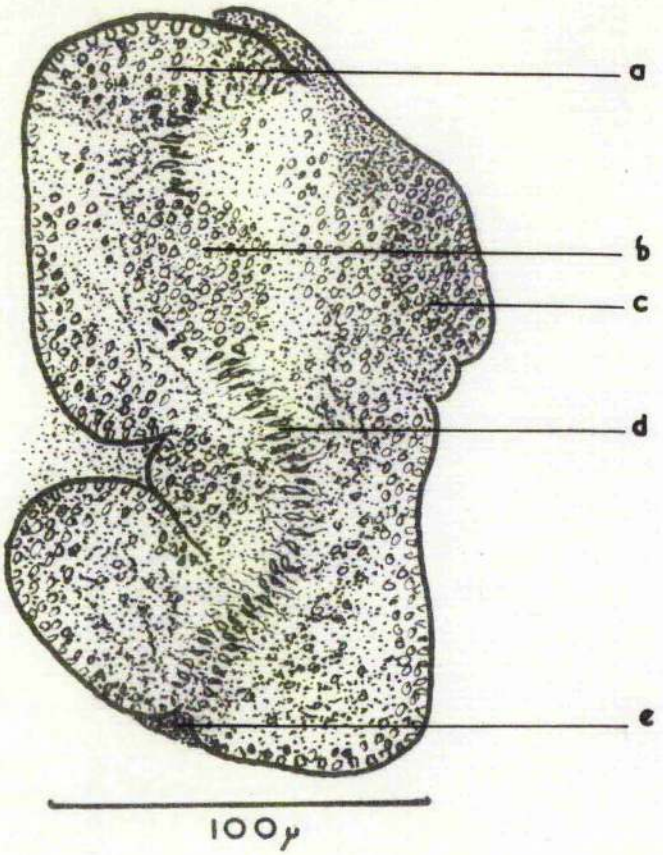

Fig. 9a

Fig. 9b

PLATE 10. Polycercus lumbrici.

Figs 10a and 10b. Scolex prior to investment of bulb by prebulb.

a - bulb.
b - anterior ring of prebulb.
c - early definitive hooks.
d - posterior ring of prebulb.
e - axial column of cells.
f - sucker.

PLATE 10

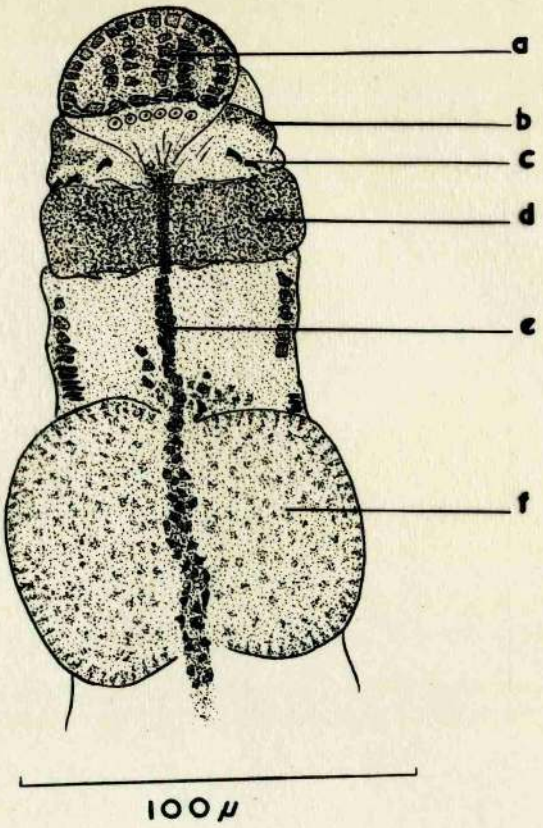

Fig. 10a

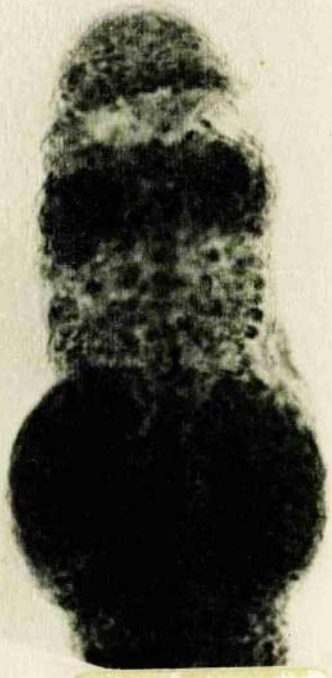

Fig. 10b

PLATE 11. Polycercus lumbrici.

Figs 11a and 11 b. Scolex after investment of bulb by prebulb.

a - hook.
b - cone of bulb.
c - myoblasts in bulb.
d - myoblasts in prebulb.
e - bulb.

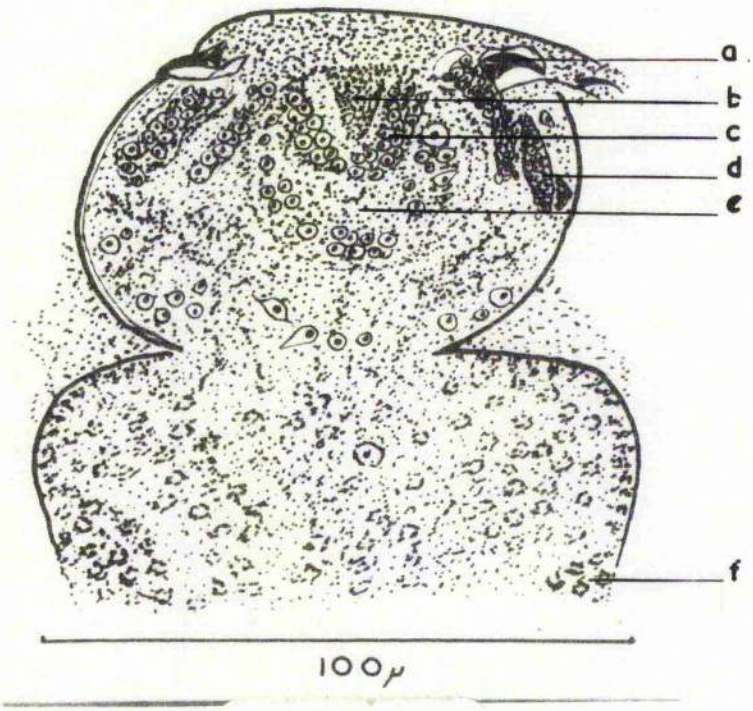

Fig. IIa

Fig. IIb

PLATE 12. Polycercus lumbrici.

Figs 12a and 12b. Larva prior to invagination.

a - retractor muscles of hooks.
b - hook.
c - cone of bulb.
d - myoblasts of prebulb.
e - sucker.

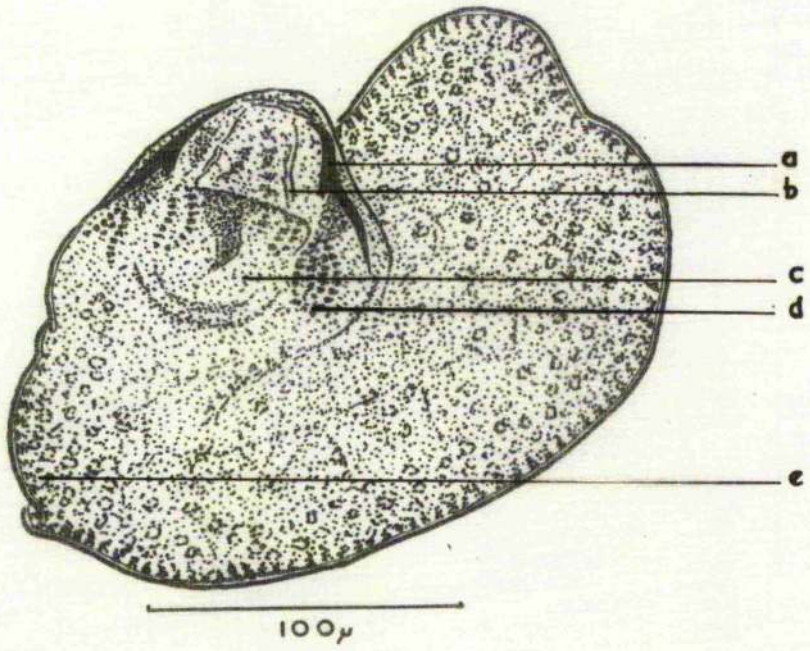

Fig. 12a

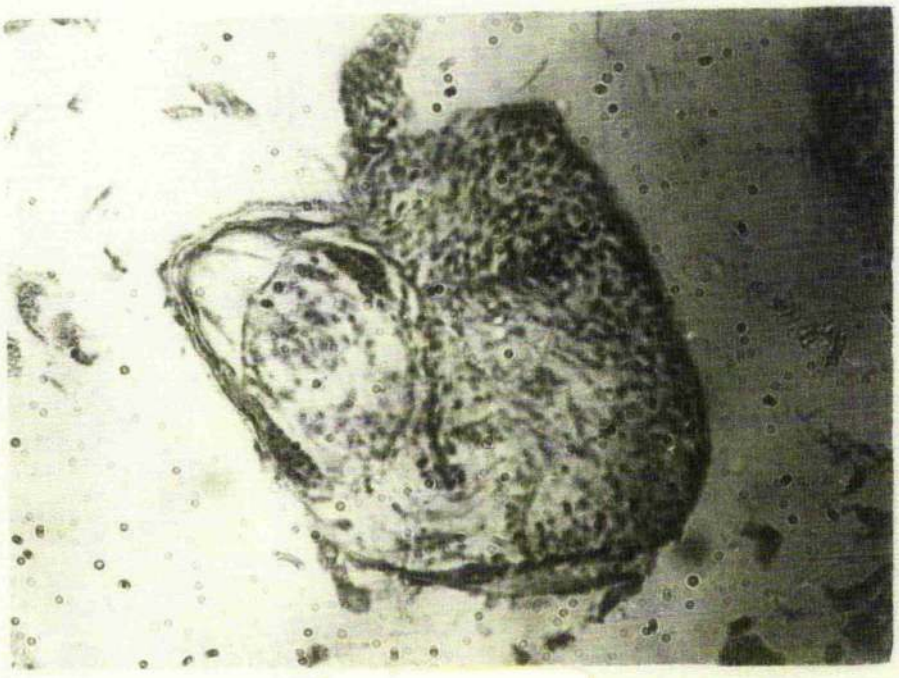

Fig. 12b

PLATE 13. Polycercus lumbrici.

Fig. 13. Fully-developed cysticercoid.

a - anterior pore.
b - parenchyma.
c - cuticle.
d - sub-cuticular cells.
e - hook-retractor muscles.
f - hook.
g - outer sac of rostellum.
h - anterior hook-extensor muscle.
i - central cone of rostellum.
j - posterior hook-extensor muscle.
k - inner sac of rostellum.
l - glandular structure of rostellum.
m - longitudinal muscle of parenchyma.

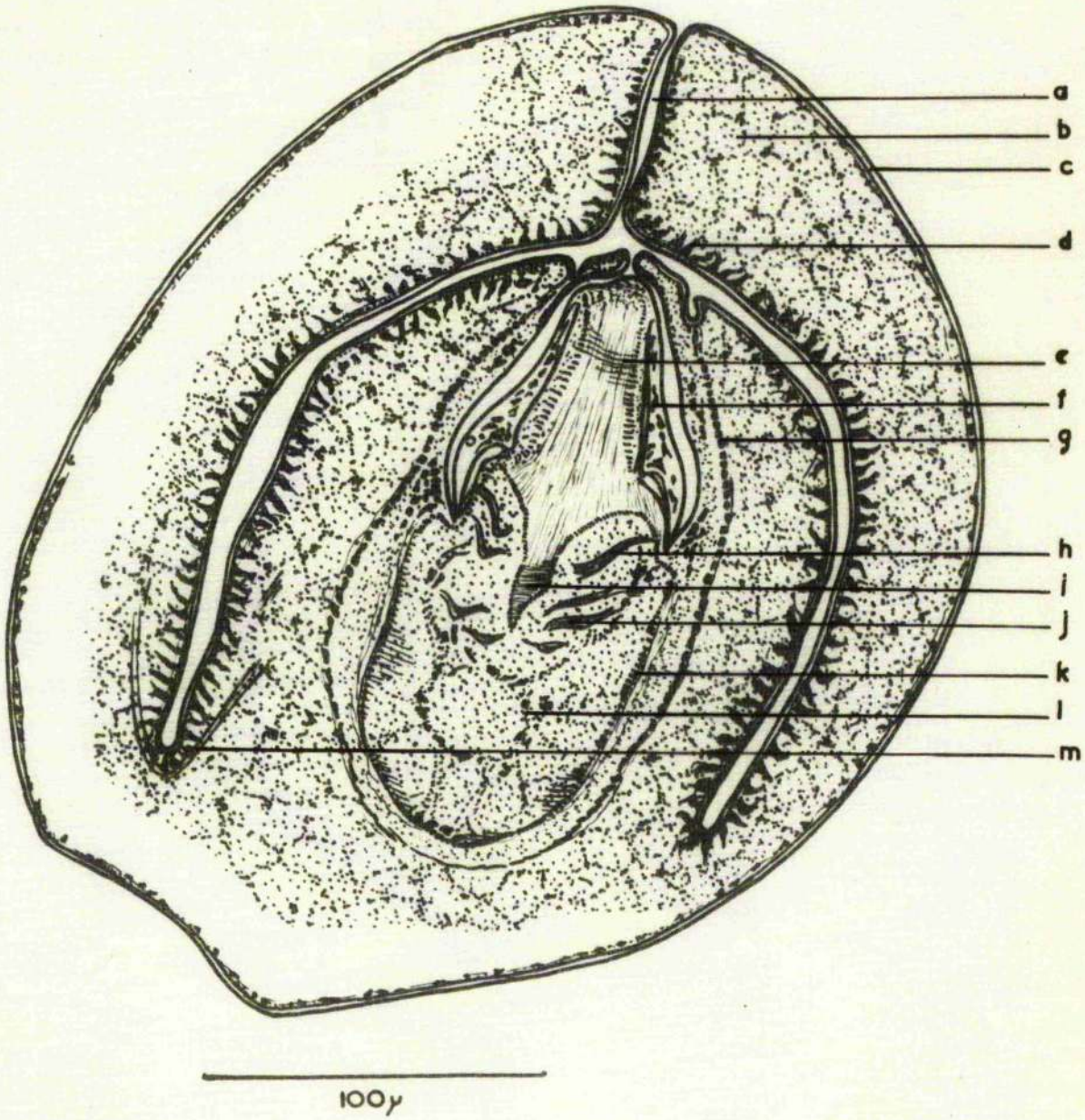

Fig. 13

PLATE 14. Polycercus lumbrici.

Fig. 14. Cyst with early, undifferentiated buds.

Fig. 15. Section through early, undifferentiated bud.

Fig. 16. Cyst with larvae shortly after retroversion.

Fig. 17. Section through cyst showing early differentiation in larvae.

Fig. 14

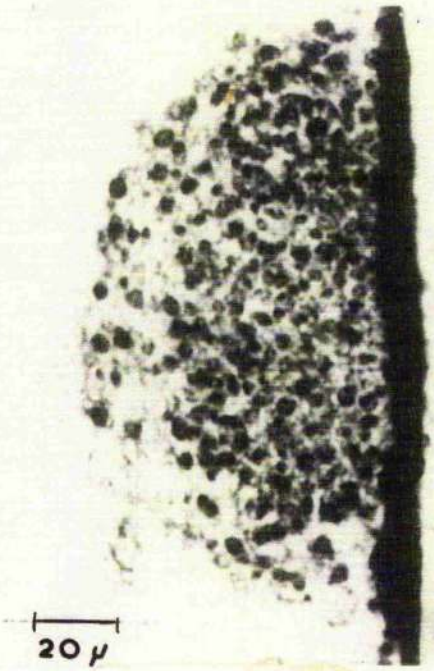

Fig. 15

Fig. 16

Fig. 17

PLATE 15. Polycercus lumbrici.

Fig. 18. Cysts with larvae showing development of scolex and caudal bladder. (Fixed material).

Fig. 19. Section through cyst with larvae showing development of scolex and caudal bladder.

Fig. 20. Cyst with buds prior to retroversion. (Live material).

Fig. 21. Cyst with larvae just prior to differentiation of scolex. (Live material).

Fig. 18

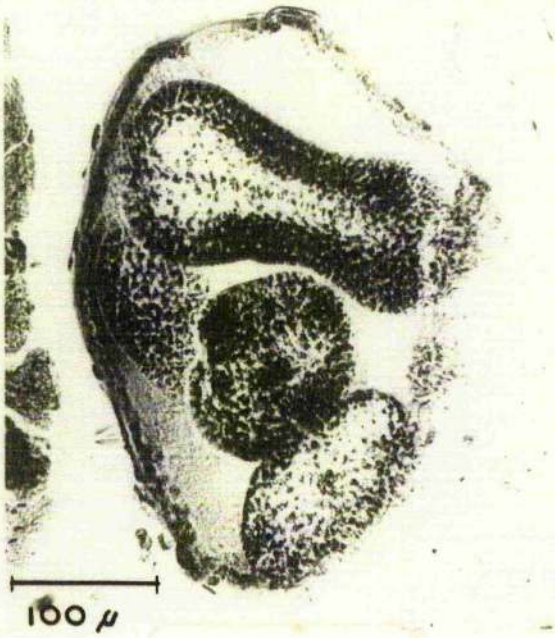

Fig. 19

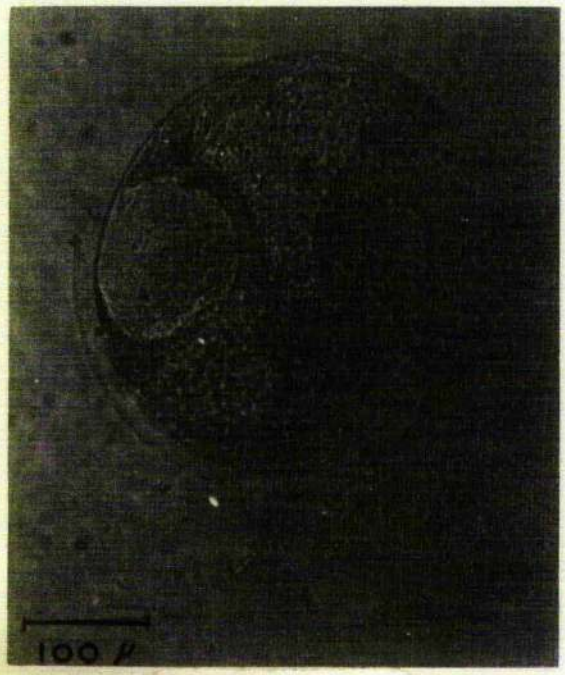

Fig. 20

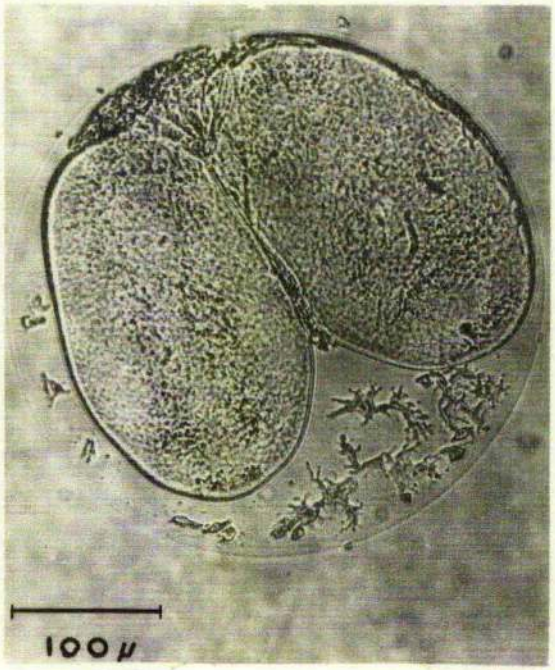

Fig. 21

PLATE 16. *Polycercus lumbrici*.

Fig. 22. Cyst with three larvae showing differentiation of bulb, prebulb, suckers and caudal bladder.

Fig. 23. 'Double' cyst with fully developed cysticercoids.

Fig. 24. Cysticercoid with partly-protruded rostellum. (Abnormal state).

Fig. 25. Cysticercoid with fully protruded rostellum but scolex still invaginated in caudal bladder. (Abnormal state).

Fig. 22

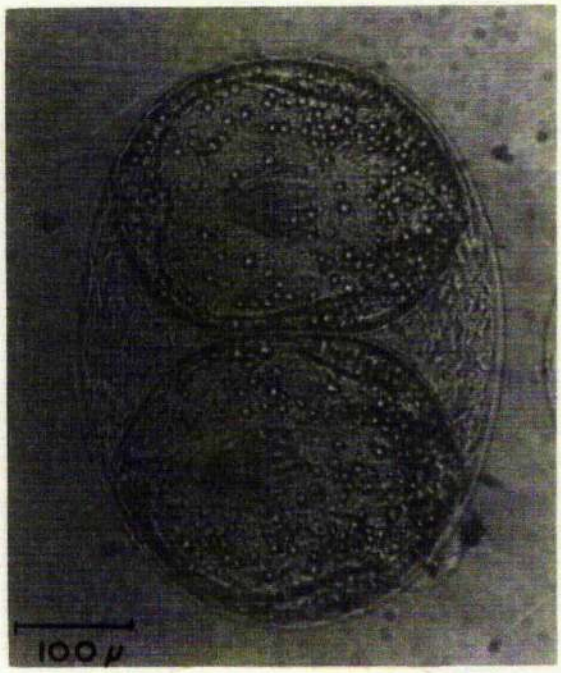

Fig. 23

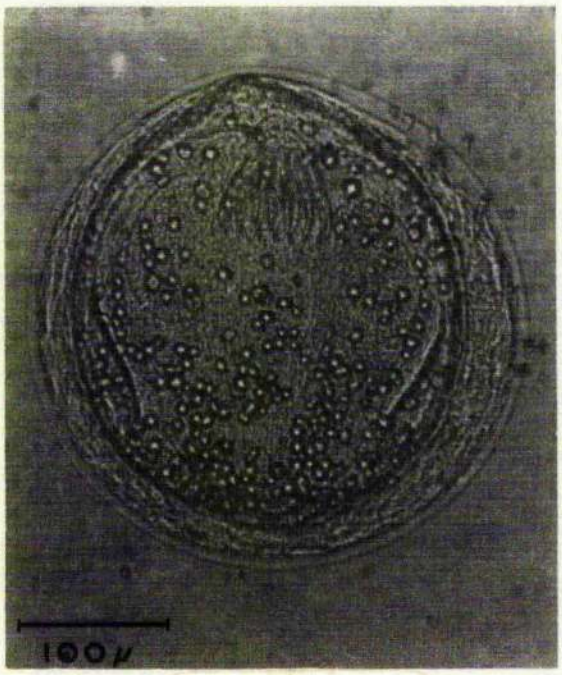

Fig. 24

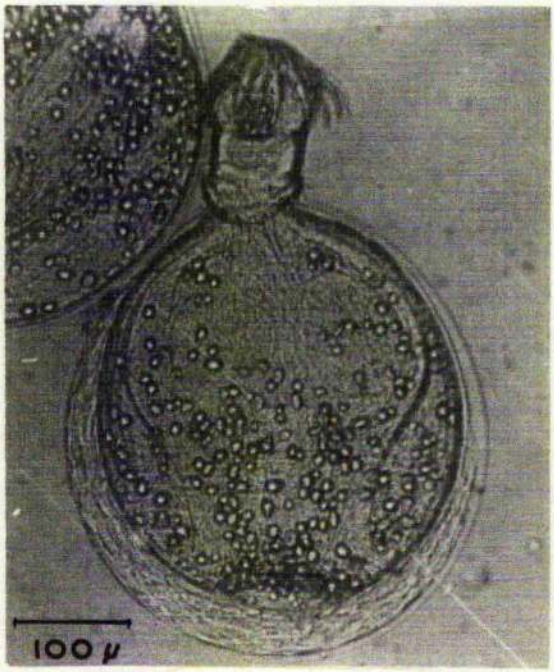

Fig. 25

PLATE 17. Polycercus lumbrici.

Fig. 26. Cyst showing 'condensations' of cells to form buds. (Live material).

Fig. 27. Cyst with well-developed buds prior to retroversion. (Live material).

Fig. 28. Well-developed larva before withdrawal of the scolex into the caudal bladder. (Live material).

Fig. 29. Enlarged view of the scolex of larva as in Fig. 28. The bulb is partly invested by the prebulb and the suckers are well-formed. (Live material).

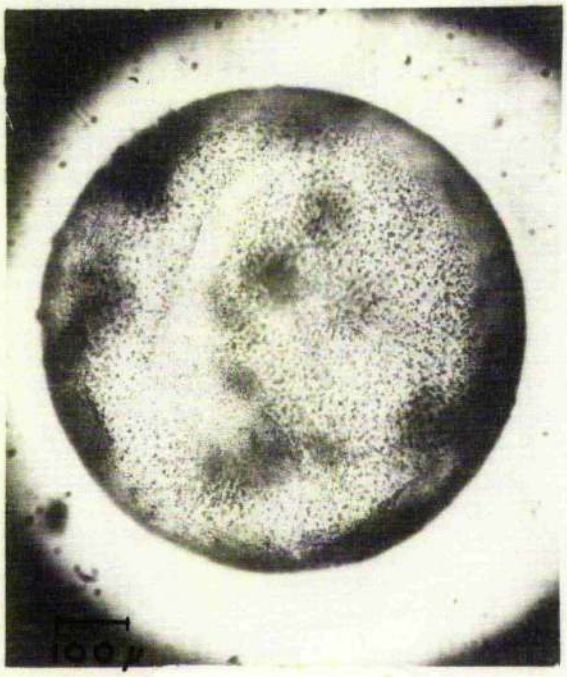

Fig. 26

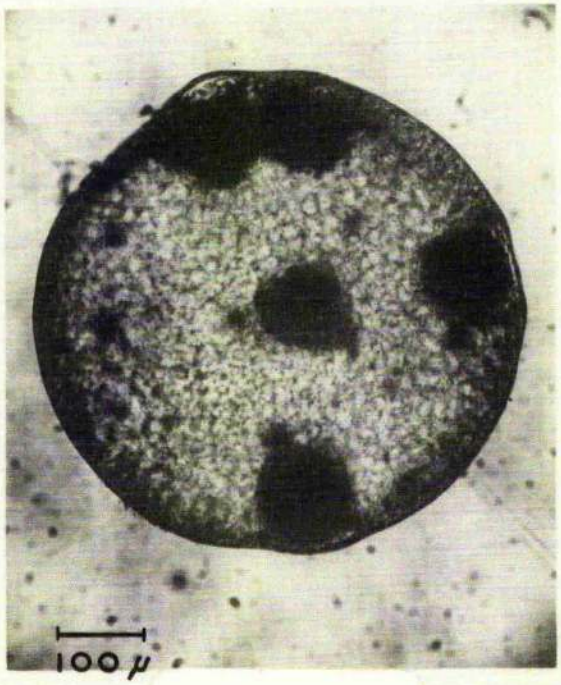

Fig. 27

Fig. 28

Fig. 29

PLATE 18. *Polycercus lumbrici*.

Fig. 30. Transverse section of heavily-infested Earthworm (*Allolobophora terrestris*).

Fig. 31. Longitudinal section of heavily-infested Earthworm (*Allolobophora terrestris*).

Fig. 32. Longitudinal section through fully-developed cysticercoid.

Fig. 33. Transverse section of fully-developed cysticercoid through the guards of the hooks.

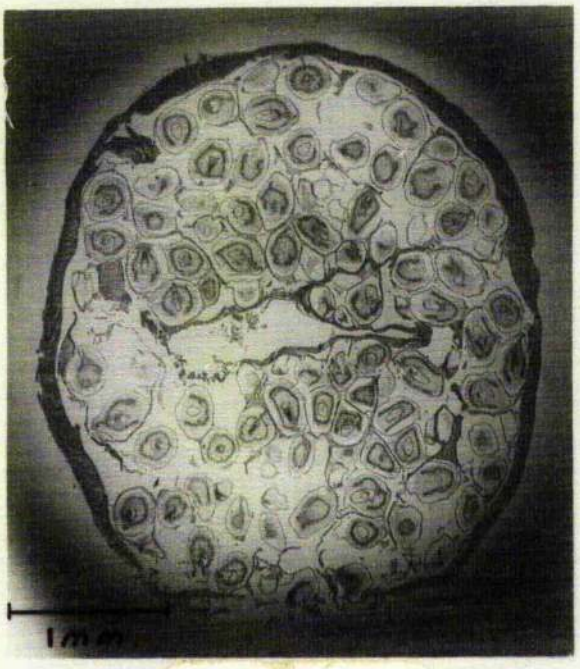

Fig. 30

Fig. 31

Fig.32

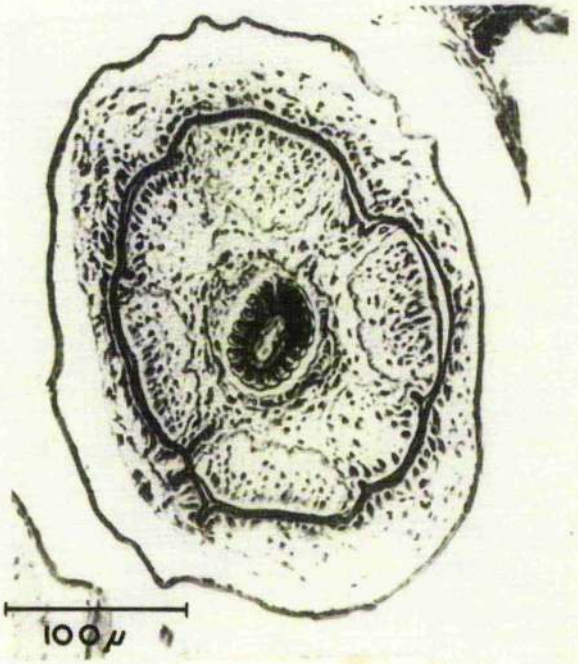

Fig. 33

PLATE 19.

Fig. 34. Polycercus lumbrici.

Transverse section of fully-developed cysticercoid showing the anterior layer of hook-extensor muscles.

Fig. 35. Polycercus lumbrici.

Transverse section of fully-developed cysticercoid showing the posterior layer of hook-extensor muscles.

Fig. 36. Paricterotaenia paradoxa. Longitudinal section of scolex in situ in villi of intestine of Scolopax rusticola.

Fig. 37. Paricterotaenia paradoxa. Longitudinal section of scolex in situ in intestine of Scolopax rusticola.

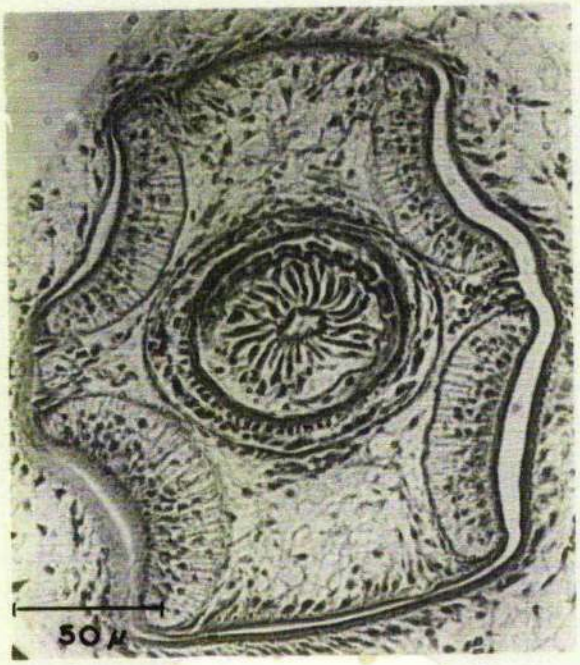

Fig. 34

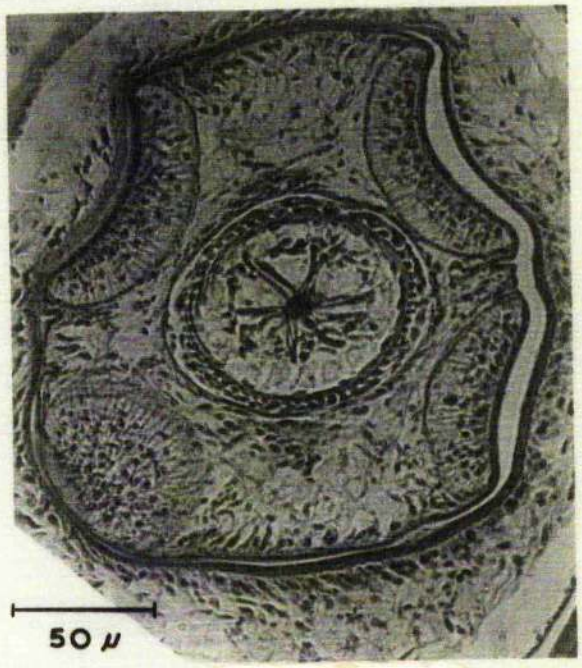

Fig. 35

Fig. 36

Fig. 37

PLATE 20.

Fig. 38. Paricterotaenia paradoxa. Longitudinal section showing branching of parenchymal muscles to either side of rostellum.

Fig. 39. Paricterotaenia paradoxa. Oblique-longitudinal section of strobila showing parenchymal muscles.

Fig. 40. Paricterotaenia paradoxa. Transverse section of proglottis showing parenchymal muscles.

Fig. 41. Polycercus lumbrici. Longitudinal section of rostellum.

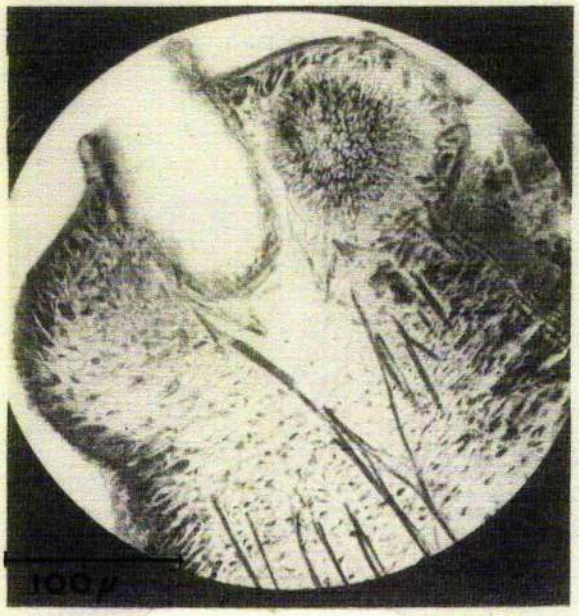

Fig. 38

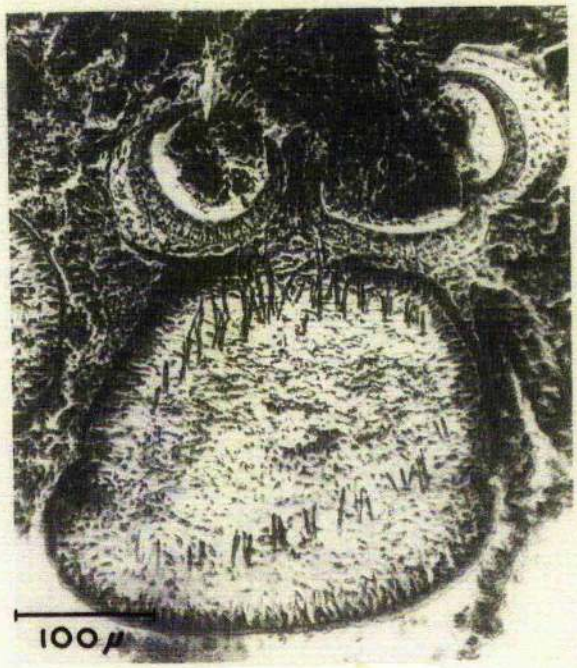

Fig. 39

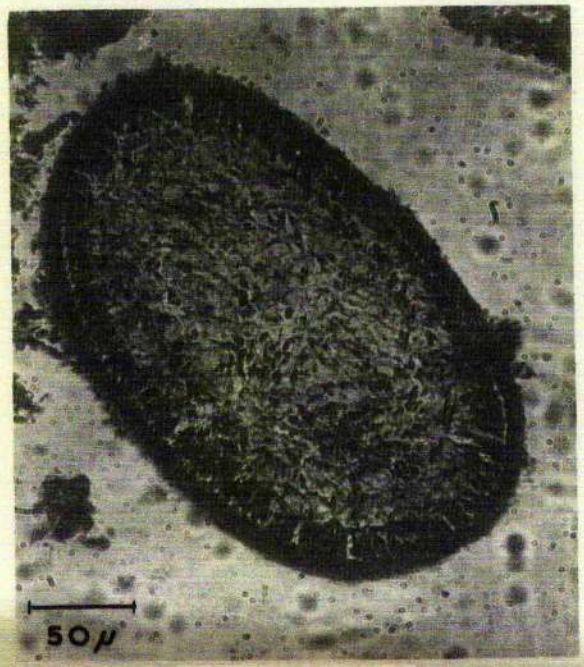

Fig. 40

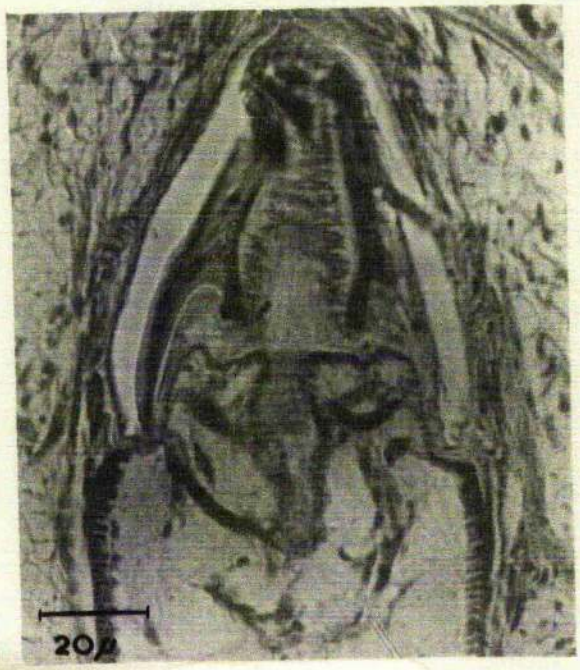

Fig. 41

PLATE 21.

Fig. 42. Polycercus lumbrici. Wall of inner-sac of rostellum showing longitudinal and circular muscles.

Fig. 43. Polycercus lumbrici. Wall of outer-sac of rostellum showing circular muscles.

Fig. 44. Polycercus lumbrici. Transverse section at posterior tip of rostellum showing longitudinal and circular muscles of inner sac.

Fig. 45. Paricterotaenia paradoxa. Longitudinal section of scolex showing glandular structure of rostellum.

Fig. 42

Fig. 43

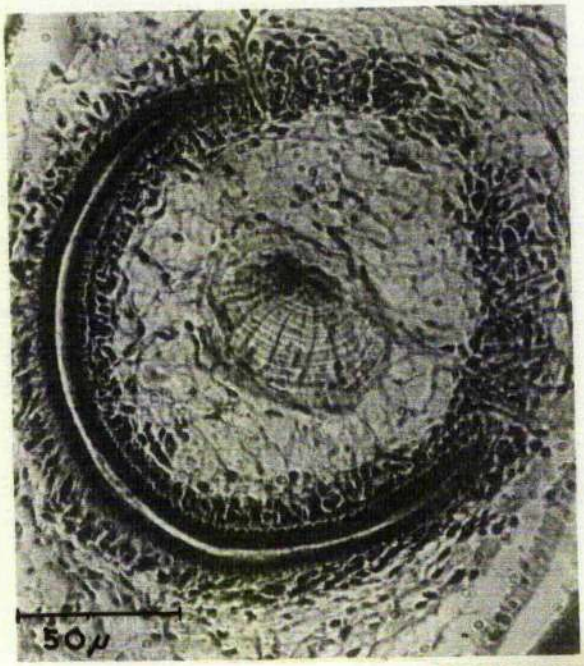

Fig. 44

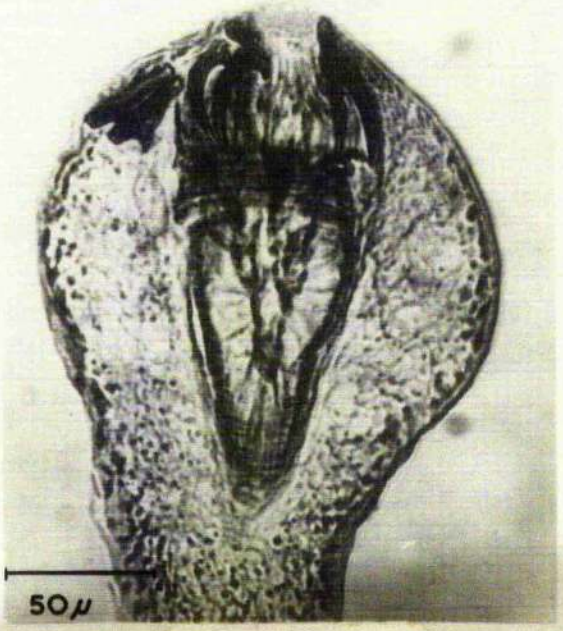

Fig. 45

PLATE 22. Polycercus lumbrici

Fig. 46. Development of rostellum; investment of bulb by prebulb.

a - before investment.

b - bulb almost completely invested by prebulb. Muscles round bulb dividing to form walls of inner and outer sacs of rostellum. Anterior coneof prebulb forming. Hooks at 'claw-like' stage of development.

c - bulb completely invested by prebulb. Hook-extensor muscles formed. Hooks well-formed and not hardened.

Legend: 1. bulb.

2. anterior part of prebulb.

3. posterior part of prebulb.

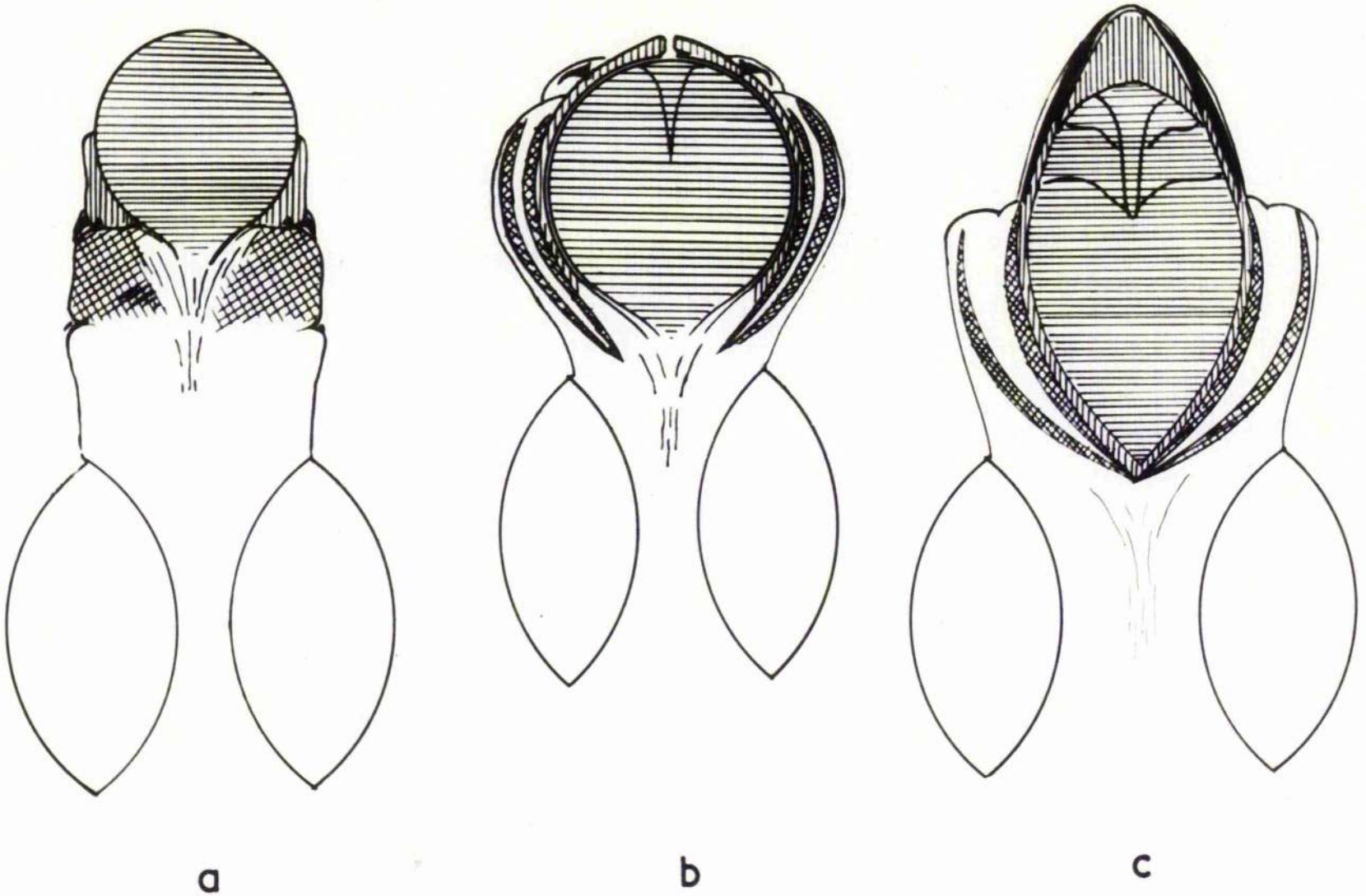

Fig. 46

PLATE 22

PLATE 23. Paricterotaenia paradoxa.

Fig. 47. Elevation of the hooks.

A - Rostellum fully relaxed; cone invaginated to fullest extent.

B - Circular muscles of rostellum partly contracted; cone being evaginated, carrying inner ends of extensor muscles forwards.

C - Circular muscles of rostellum almost fully contracted; cone almost fully-evaginated; extensory muscles relaxed.

D - Extensory muscles partly contracted; hooks partly raised.

E - Extensor muscles contracted; anterior part of rostellum flattened; hooks fully extended.

a - Hook.

b - cone.

c - anterior hook-extensor muscle.

d - posterior hook-extensory muscles.

e - inner sac of rostellum.

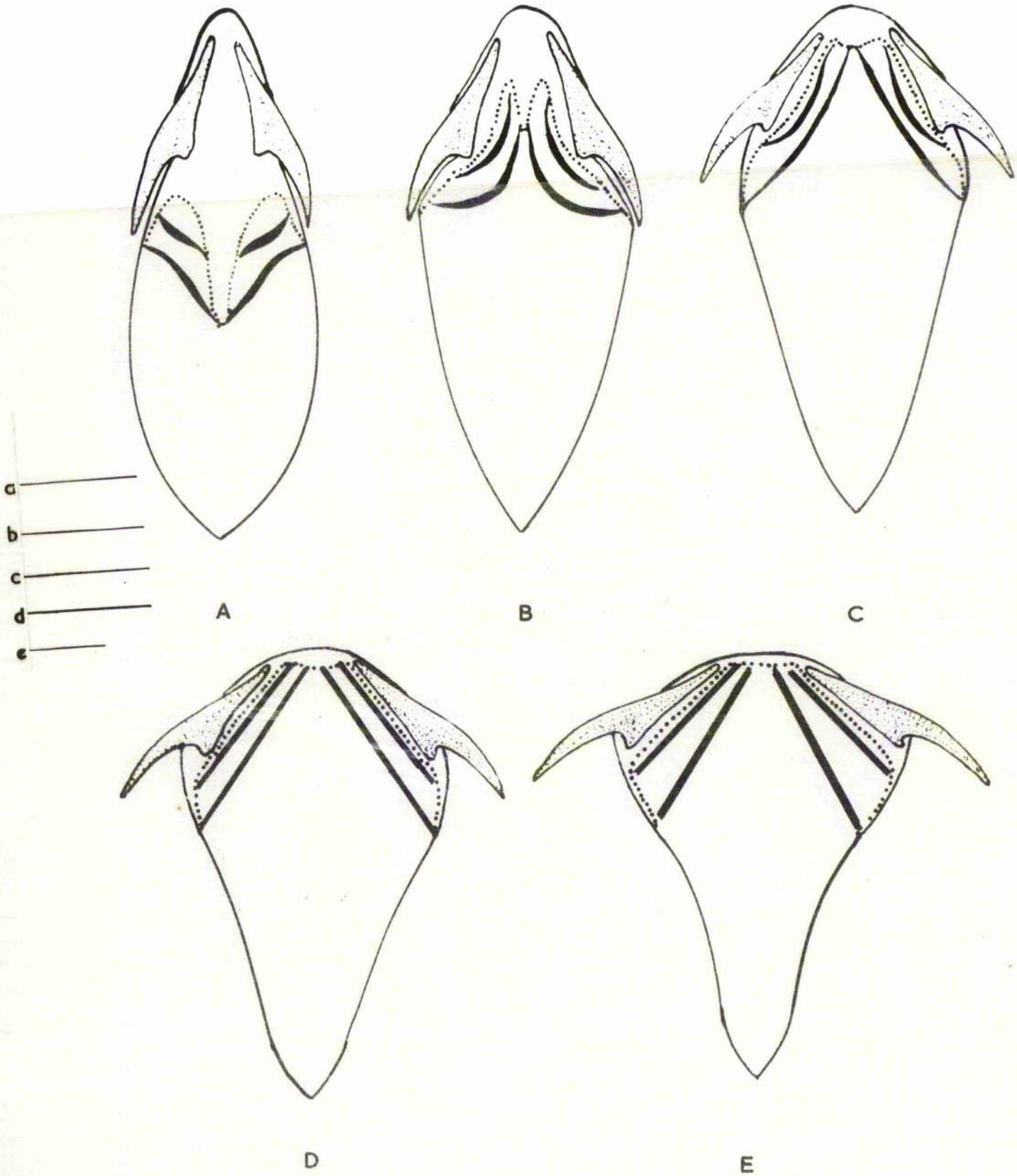

Fig. 47

PLATE 24. Polycercus lumbrici.

Fig. 48. Development of larva.

a - c. Growth of central cavity.

d - e. Growth of bud.

f - g. Separation of bud from wall of cyst.

h - j. Retroversion of larva.

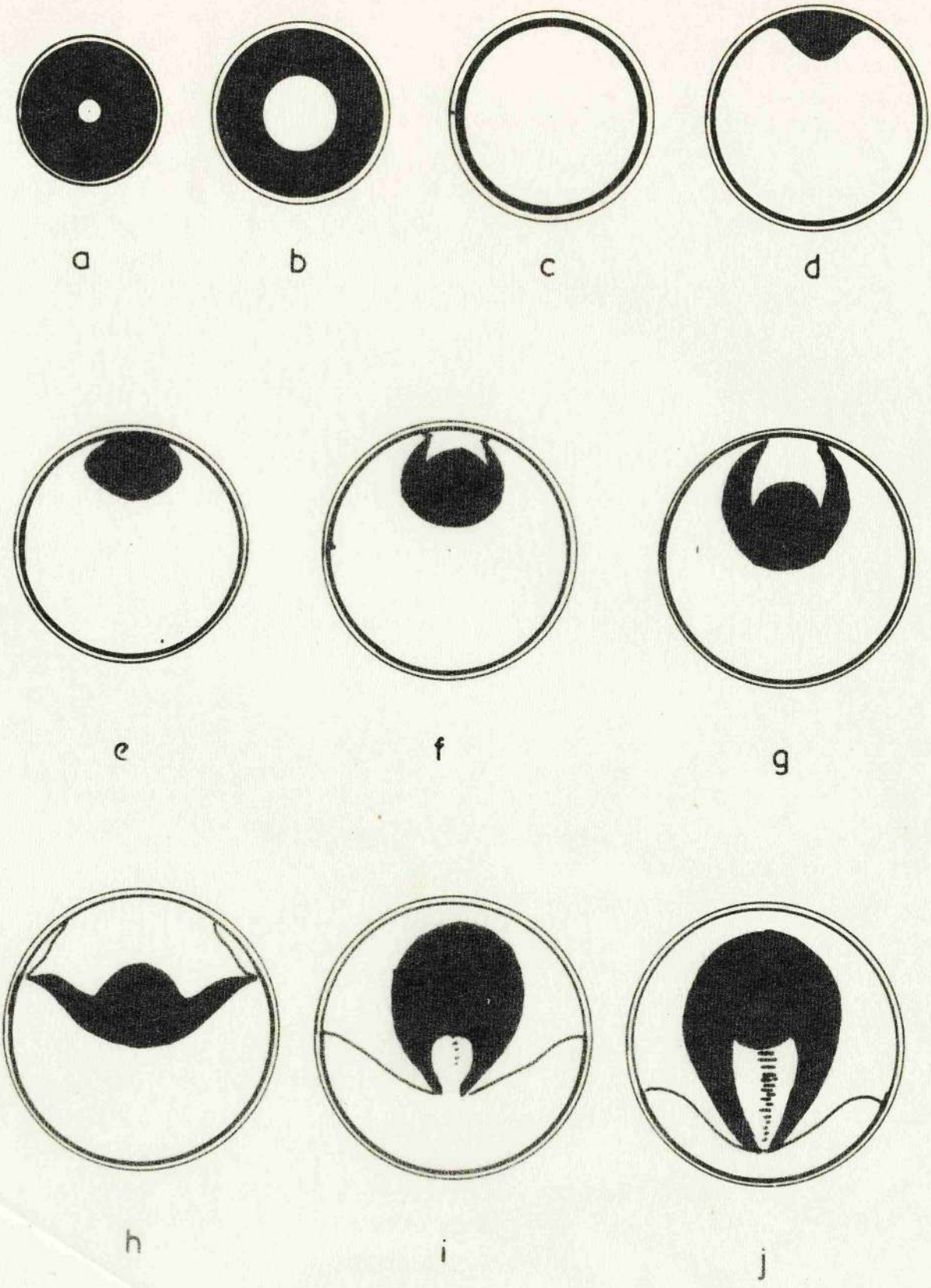

Fig. 48

PLATE 25. Polycercus lumbrici.

Fig. 49. Development of larva.

a - c. Formation of bulb and prebulb.

d. Formation of suckers.

e - f. Investment of bulb by prebulb.

g - i. Withdrawal of scolex into caudal bladder.

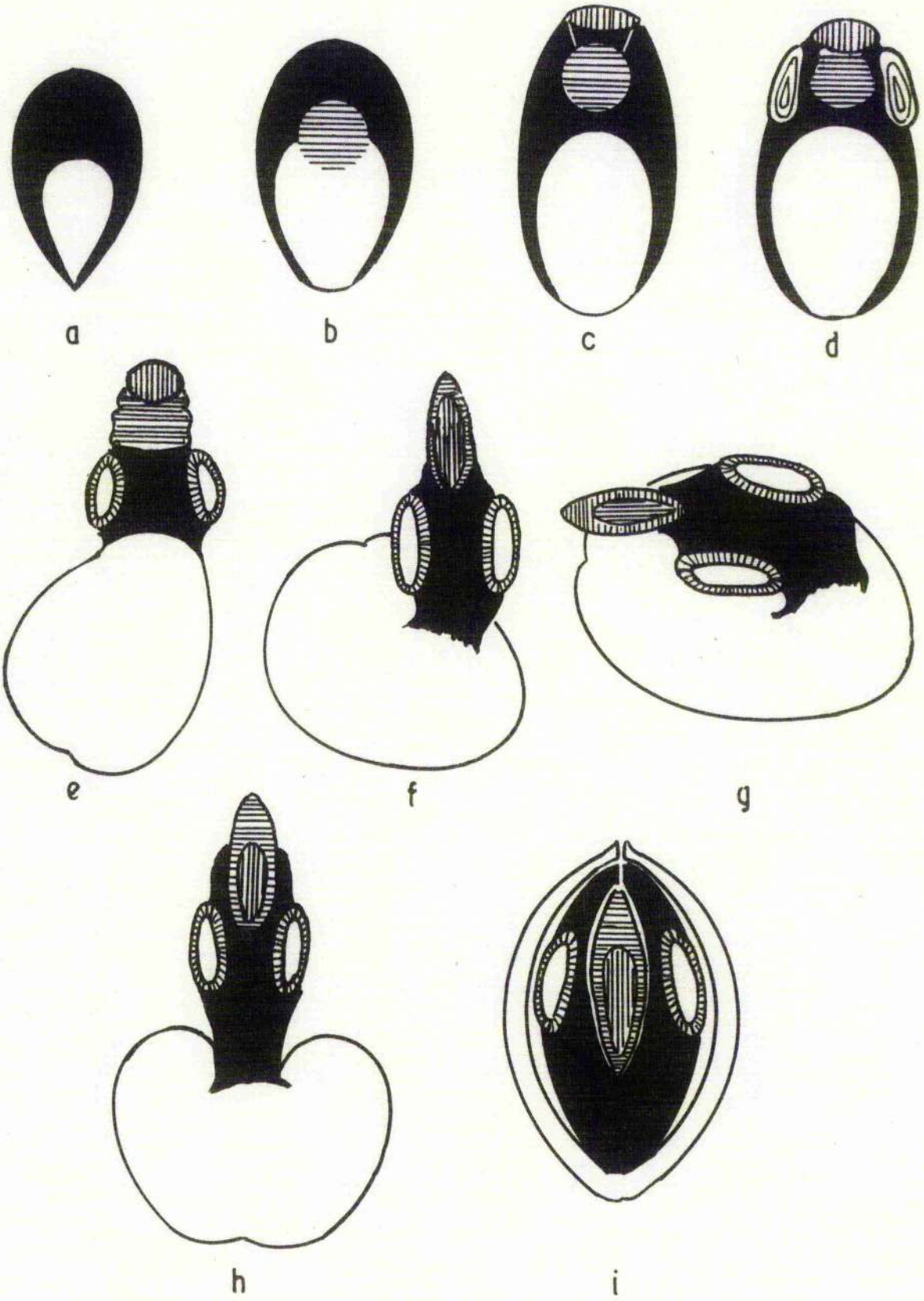

Fig. 49

PLATE 26. *Polycercus lumbrici*.

Fig. 50. Structure of rostellum.

a - anterior pore.

b - elastic connective tissue.

c - hook-retractor muscles.

d - hook.

e - cone of rostellum.

f - anterior hook-extensor muscles.

g - posterior hook-extensor muscles.

h - longitudinal muscles of inner sac of rostellum.

i - circular muscles of inner sac of rostellum.

j - glandular structure.

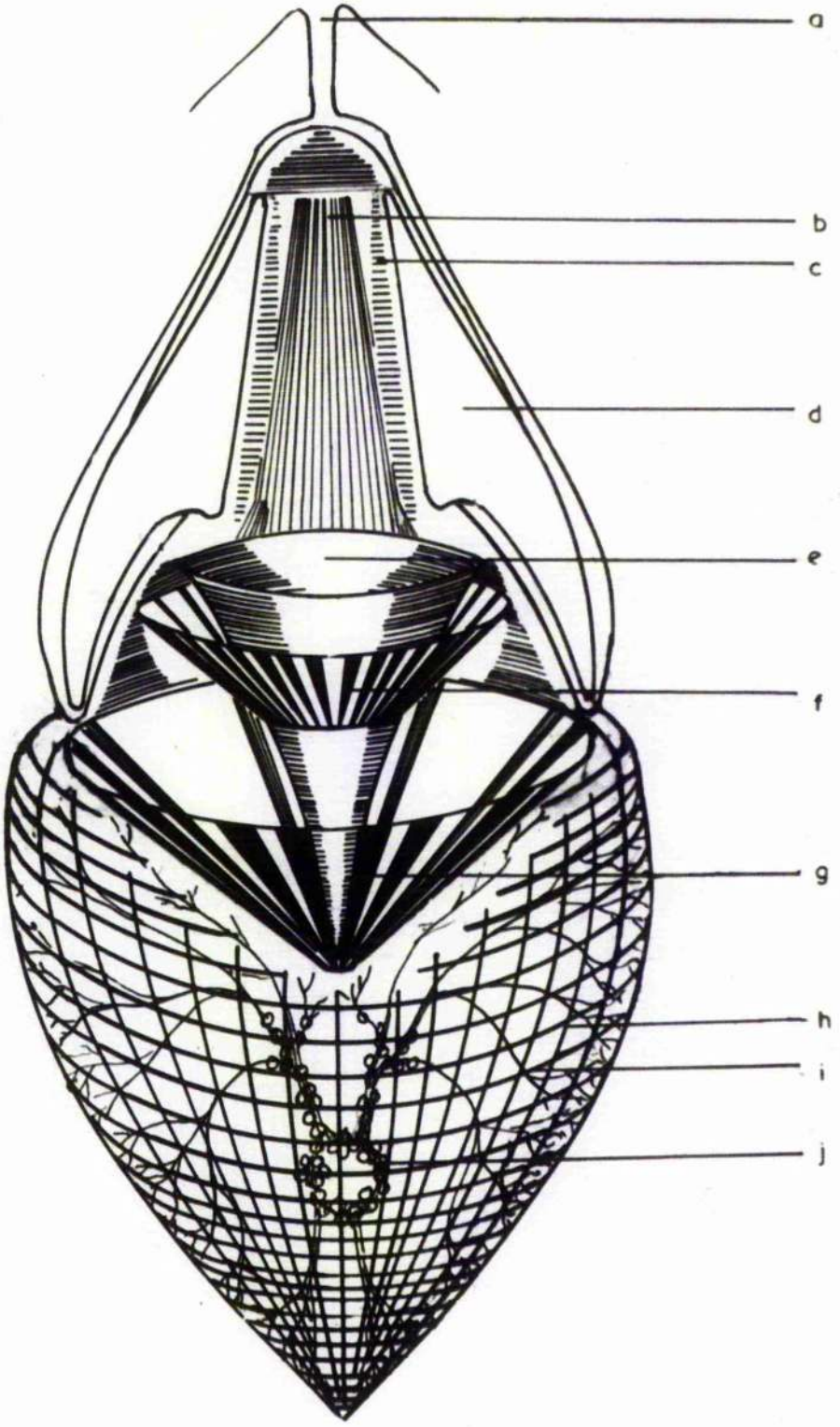

Fig. 50

PLATE 27. Paricterotaenia paradoxa.

Fig. 51. Life cycle of Paricterotaenia paradoxa.

A - Definitive host, the Woodcock. (Scolopax rusticola).

B - Ripe proglottis.

C - Intermediate host, the Earthworm (Allolobophora terrestris).

D - Oncosphere.

E - Solid sphere of cells.

F - Formation of small central cavity.

G - Enlargement of central cavity to form cyst.

H - Formation of 'buds' or 'tubercles'.

I - Retroversion of 'buds'.

J - Cysticercoids in cyst.

K - Cysticercoid swallowed by Woodcock on eating Earthworm.

L - 'Evagination' of cysticercoid in intestine of Woodcock.

M - Adult cestode.

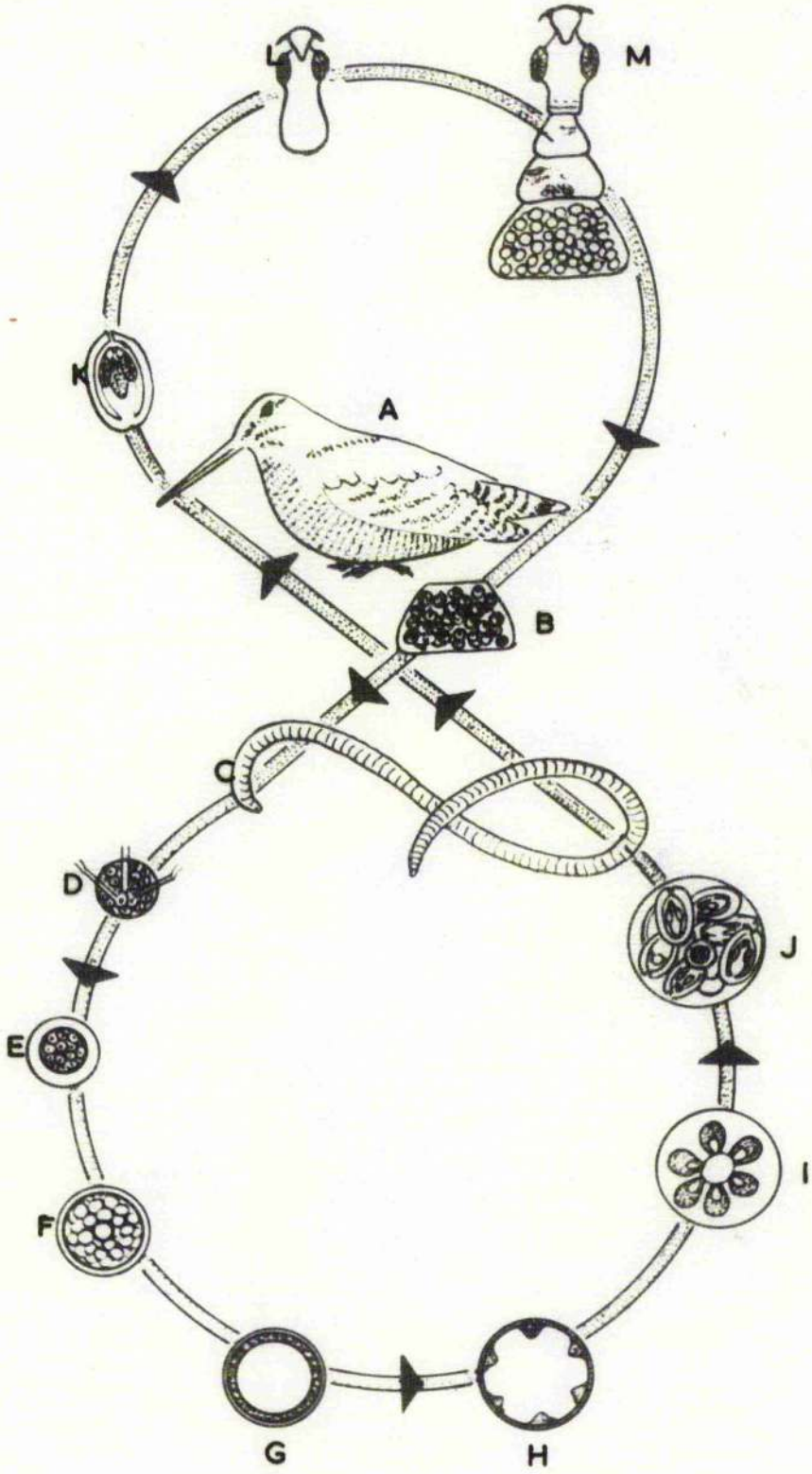

Fig. 51

PLATE 28. Paricterotaenia burti.

Fig. 52. Two rings of hooklets with larger, definitive hooks in anterior ring.

Fig. 53. Claw-like, early definitive hooks.

Fig. 54. Early hook showing differentiation of blade and base.

Fig. 55. Ring of hooks showing early differentiation of blade handle and guard.

Fig. 52

Fig. 53

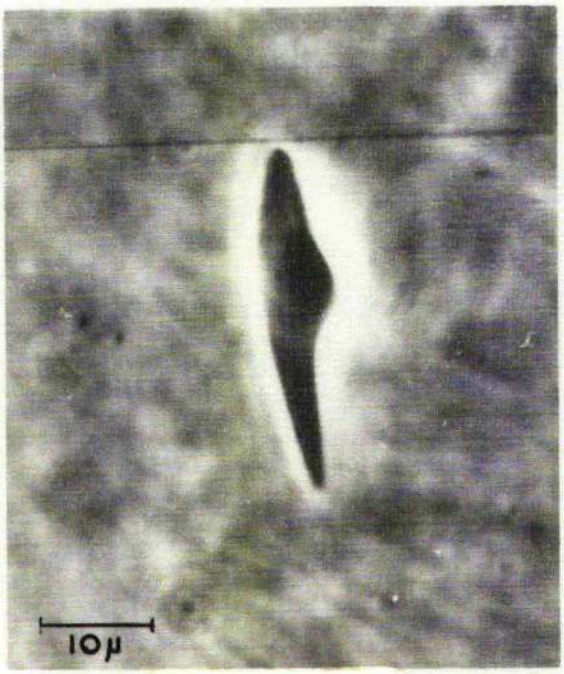

Fig. 54

Fig. 55

PLATE 29. Paricterotaenia burti.

Fig. 56. Hooks well-formed but soft.

Fig. 57. As Fig. 56.

Fig. 58. Hooks almost fully-formed before invagination of the scolex.

Fig. 59. Hook of adult.

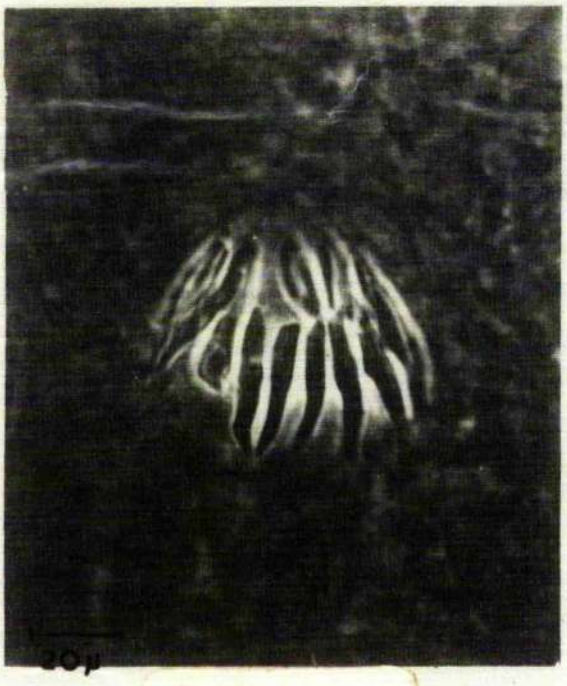

Fig. 56

Fig. 57

Fig. 58

Fig. 59

PLATE 30. Paricterotaenia paradoxa.

Fig. 60. Diagram of nervous system in scolex.

a - to anterior part of scolex.
b - to suckers.
c - anterior commissure
d - ganglion.
e - posterior commissure.
f - to posterior part of scolex.
g - outer sac of rostellum.
h - to inner sac of rostellum.

Fig. 61. Diagram of osmo-regulatory system in scolex.

a - outer sac of rostellum.
b - dorsal excretory vessel.
c - ventral excretory vessel.

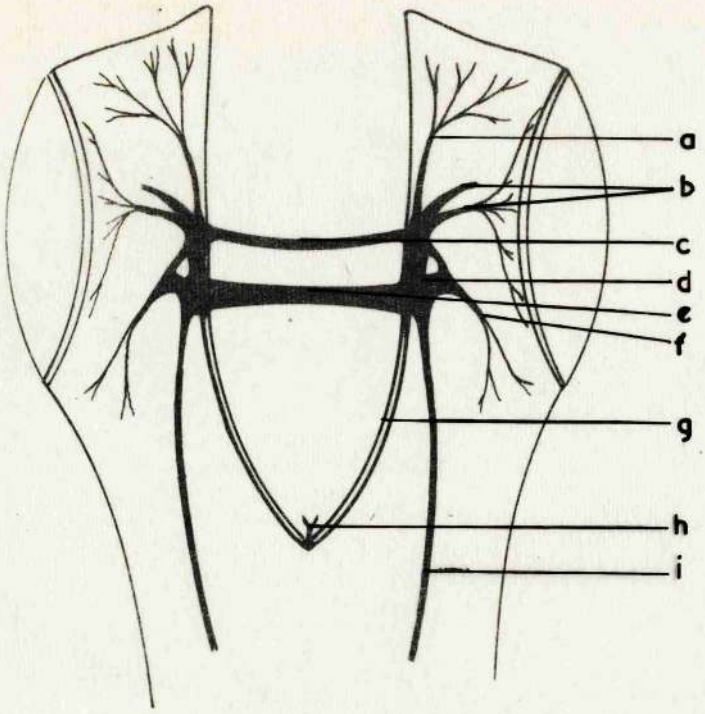

Fig. 60

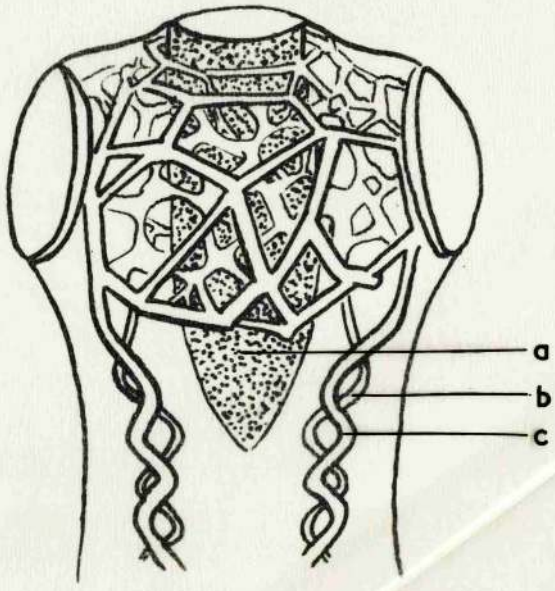

Fig. 61