

STUDIES ON THE LIFE-HISTORY AND ECOLOGY
OF TREMATODES OF THE GENUS RENICOLA COHN, 1904.

Thesis submitted for the Degree of
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by

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Abstract

Trematodes of the genus Renicola from British birds are described and evidence to show the relationship between these flukes and the "Rhodometopa" group of cercariae is presented.

The feeding habits of all of the species of birds recorded as hosts for Renicola in Western Europe are analysed to give an indication of the probable second intermediate host in the cycle.

The ecology of Turritella communis is discussed and reasons for the relation between infection with sporocysts of the "Rhodometopa" group and the size of the snails are suggested.

Attempts to infect Turritella communis with eggs from Renicola sp. are described and the results discussed.

The development of the sporocysts of the "Rhodometopa" group of cercariae within the interlobular spaces of the gonad of Turritella communis is described. A new interpretation of the structure of the sporocyst body-wall is presented.

The appearance of a second daughter generation of sporocysts is described.

The Sporocysts

Historical

An examination of some of the literature on this stage in the development of trematodes reveals a considerable variance of opinion on the structure of sporocysts and the function of various organs and cells found in them. This is possibly due to the fact that most general works refer only to the mother sporocyst and go into considerably more detail on the structure of rediae than of daughter sporocysts. Baer (1951) states that sporocysts have no mouth or intestine and that some rediae which have a poorly developed pharynx and no intestine closely resemble sporocysts. On the other hand several sporocysts are described as having a pharynx, e.g., Cercaria pekinensis Faust, 1921 which has a "pharyngeal sphincter" but no gut, and the sporocysts of the "Rhodometopa" group which, according to Rothschild (1935), have a pharynx "similar to that of C. pekinensis Faust, but surrounded by secretory cells resembling the "salivary" glands of rediae".

The histology of the sporocyst wall is an even more controversial subject. Stunkard (1932) states that the wall of the sporocyst of Cercaria rhodometopa consists of two layers, an outer one packed with deeply

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Subsidiary Papers.

1. Probable relationship between the Rhodometopa group of cercariae and the Trematode genus Renicola Cohn.
2. Trematodes of the genus Renicola from the kidneys of birds in Brazil.
3. Trematodes of the genus Renicola from birds in British zoos with descriptions of two new species.

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Introduction

Describing the excretory system of Cercaria rhodometopa Perez, 1924, Stunkard (1932) wrote - "The system is so peculiar and characteristic that it will afford a quick and certain criterion for the identification of later stages in the life-history, and may, indeed, lead to the correlation of this larva with an adult trematode having the same excretory pattern." Such an adult trematode has now been found and the following is an account of some observations on the ecology and development of both the cercariae and the adult flukes and of attempts to obtain experimental evidence of the relations between them.

In an earlier paper (Wright 1953) brief mention was made of the facts which led to the undertaking of this work. A number of infections of Renicola in the kidneys of various birds in the London and other zoos were studied (Wright 1954b). Of particular interest were the worms removed from three penguins. Campbell and Sloane (1943) also reported finding flukes of the genus Renicola in penguins at the Edinburgh zoo. These authors stated that some of the penguins found to be infected had

been hatched and raised in the menagerie. This fact and the finding of immature flukes together with very gravid specimens in the same bird in London led to the belief that the parasite was acquired from the food on which the birds were fed. Enquiries showed that in London the principal food of the penguins is herring and whiting and in Edinburgh herring and haddock. This at once seemed to implicate herring as the likely vector and since most of the fresh herring sold in this country are caught fairly close to Britain it seemed probable that the parasite would occur in British sea-birds. A further point in favour of herring acting as the vector is that the other two species of fish, both Gadoids, are rather deep-water forms and not so readily caught by diving birds as the surface-feeding, shoaling Clupeoid.

With the discovery of about ten Renicola sloanei in a Common guillemot from Pagham in Sussex the presence of the parasite in British birds was confirmed. Further work has since shown eleven further infections in three additional species^c of hosts, all British.

Anatomical studies on the material from the penguins showed that the excretory vesicle in these flukes is of a peculiar type, Y-shaped with numerous lateral branches and a cross-connection between the two arms of the Y

behind the ventral sucker. This seemed to be a suitable character by which any possibly related cercariae might be judged. Reference to the literature on cercariae from British marine gastropods soon showed that such a vesicle was to be found in the "Rhodometopa" group (Rothschild 1935). The host of these cercariae is Turritella communis Risso, a fairly deep-water, filter-feeding, prosobranch gastropod, locally common on mud bottoms.

A study of the behaviour of these cercariae in tanks in the laboratory confirmed the idea that a shoaling, plankton-feeding fish was the probable second intermediate host, but Rothschild and Sproston (1941) reported having found metacercariae of Cercaria dorchia Rothschild in two Gadus luscus, the pout whiting, from Plymouth. This species, like most other Gadoids, is a deep water form and is unlikely to be taken by birds.

A study of the food records of the species of birds acting as hosts to the parasite suggested that young herring, sprat, young gadoids and sand-eels are all frequently taken. Searches of adult herrings for metacercariae had not proved productive and when sprats became available in the London market an immediate search was started which at once proved successful. Almost simultaneously Timon -David (1953) reported metacercariae,

possibly of C. rhodometopa, from sardines in the Mediterranean near Marseilles.

This brief account is intended to give some continuity to the main body of the work which is divided into separate sections, dealing with each stage in the life cycle, for convenience of presentation.

The Adult Flukes

Historical

In 1904 Cohn published a redescription of the trematode described as Monostomum pingue Mehlis (in Creplin), 1846. By sectioning Cohn showed the presence of a ventral sucker and erected the new genus Renicola with the type species R. pinguis.

In 1914 Odhner erected the family Troglotrematidae in which Renicola was included with the other cyst living genera, Troglotrema, Paragonimus, Pholeter and Collyriclum. Baer (1931) added a sixth genus, Nephrotrema. Dollfus (1939) showed the heterogeneous nature of this family (on morphological grounds) and erected four new families, amongst them the family Renicolidae which he suggests

has some affinities with the Gymnophallidae. Dollfus (1946) included the genus Stamparia Nezlobinsky, 1926 with the Renicolidae.

In a previous publication (Wright 1954b) a list of the known species of Renicola was given. That list is now known to be incomplete and several further species have been described since the preparation of that paper. As far as can be ascertained the following list is now complete,

<u>Species</u>	<u>Host</u>	<u>Locality</u>	<u>Author</u>
1. <u>R. pinguis</u>	<u>Podiceps</u> <u>cristatus</u>	Germany	(Mehlis 1846) Cohn 1904
2. <u>R. secunda</u>	<u>Pelecanus</u> <u>onocrotalus</u>	Turkestan	Skrjabin 1924
3. <u>R. tertia</u>	<u>Sterna</u> <u>fluviatilis</u>	Turkestan	Skrjabin 1924
4. <u>R. glandoloba</u>	<u>Puffinus</u> <u>kuhli</u>	Suez	Witenberg 1929
5. <u>R. lari</u>	<u>Larus</u> <u>argentatus</u>	Marseilles	Timon-David 1933
	<u>Hydrochelidon</u> <u>nigra</u>	Siberia	Bykhovskaya- Pavlovskaya 1950
	<u>Sterna</u>	"	"
	<u>hirundo</u>	"	"
	<u>Larus</u>	"	"
	<u>taimyrensis</u>	"	"
	<u>Larus</u> <u>cachinans</u>	"	"
6. <u>R. quinta</u>	<u>Uria carbo</u>	Vladivostock	Sokolova- Andronova 1937

<u>Species</u>	<u>Host</u>	<u>Locality</u>	<u>Author</u>
7. <u>R. paraquinta</u>	<u>Larus</u> <u>ridibundus</u>	Tobolsk (Urals)	Raevski 1937
8. <u>R. umigarasu</u>	<u>Uria</u> <u>aalge</u>	Japan	Yamaguti 1939
9. <u>R. keimahuri</u>	<u>Uria</u> <u>carbo</u>	"	"
10. <u>R. pandioni</u>	<u>Pandion</u> <u>haliaetus</u>	Gorkovsk (Russia)	Sudarikov 1947
11. <u>R. undecima</u>	<u>Pandion</u> <u>haliaetus</u>	"	"
12. <u>R. magnicaudata</u>	<u>Hirundo</u> <u>rustica</u>	Siberia	Bykhovskaya- Pavlovskaya 1950
13. <u>R. medio-</u> <u>vitellata</u>	<u>Anas</u>	"	"
	<u>strepera</u>	"	"
	<u>Spatula</u> <u>clypeata</u>	"	"
	<u>Nyroca</u> <u>ferina</u>	"	"
14. <u>R. brantae</u>	<u>Branta</u> <u>canadensis</u>	Canada	McIntosh and Farr 1952
15. <u>R. bretensis</u>	<u>Pica</u> <u>pica</u>	Provence (France)	Timon-David 1952
16. <u>R. thapari</u>	<u>Pelecanus</u> <u>occidentalis</u>	Panama	Caballero 1953
17. <u>R. nana</u>	<u>Tringa</u> <u>totanus</u>	Siberia	Bykhovskaya- Pavlovskaya 1953
	<u>Vanellus</u>	"	"
	<u>vanellus</u>	"	"
18. <u>R. cruzi</u>	<u>Sterna</u> <u>maxima</u>	Brazil	Wright 1954a
	<u>Sterna</u>	"	"
	<u>hirundinacea</u>	"	"

<u>Species</u>	<u>Host</u>	<u>Locality</u>	<u>Author</u>
19. <u>R. sloanei</u>	<u>Pygoscelis</u> <u>antartica</u>	London Zoo	Wright 1954b
	<u>Eudyptes</u>	"	"
	<u>chrysolophus</u>		
	<u>Uria aalge</u>	Sussex, England	"
20. <u>R. pelecani</u>	<u>Pelecanus</u>	London Zoo	"
	<u>phillipensis</u>	(Ceylon)	
	<u>Pelecanus</u>	Chessington Zoo	"
	<u>onocrotalus</u>	(Calcutta)	
21. <u>Renicola sp.</u>	<u>Bubulcus</u> <u>coromandus</u>	London Zoo	Hammerton 1934
22. <u>Renicola sp.</u>	<u>Phaleris</u> <u>psittacula</u>	Vladivostock	Sokolova- Andronova 1937
23. <u>Renicola sp.</u>	<u>Melanitta</u> <u>fusca</u>	"	"
24. <u>Renicola sp.</u>	<u>Aptenodytes</u> <u>longirostris</u>	Edinburgh Zoo	Campbell and Sloane 1943
	<u>Pygoscelis</u>	" "	"
	<u>papua</u>		
	<u>Spheniscus</u> <u>demersus</u>	" "	"
25. <u>Renicola sp.</u>	<u>Mergulus alle</u>	Pas de Calais	Dollfus 1946
26. <u>Renicola sp.</u>	<u>Sterna</u> <u>cantiaca</u>	English Channel	Callot 1946
27. <u>Renicola sp.</u>	<u>Larus</u> <u>dominicanus</u>	Brazil	Wright ~~XXXX~~ 1954a
28. <u>Renicola sp.</u>	<u>Paecilonitta</u> <u>bahamensis</u>	Brazil	Wright 1954a
29. <u>Renicola sp.</u>	<u>Sula</u> <u>leucogaster</u>	"	"
30. <u>Renicola sp.</u>	<u>Sterna sp.</u>	"	"
31. <u>Renicola sp.</u>	<u>Threskiornis</u> <u>melanocephala</u>	London Zoo (Ceylon)	Wright 1954b
32. ~~XXXXXX~~ <u>Renicola sp.</u>	<u>Pelecanus</u> <u>onocrotalus</u>	Chessington Zoo (Calcutta)	"

The validity of some of the species listed is rather doubtful. Many of the descriptions are very inadequate and the type material is inaccessible.

Renicola sp. of Campbell and Sloane is synonymous with R. sloanei Wright, perhaps also Renicola sp. of Dollfus. McIntosh and Farr suggest the possibility that R. brantae is synonymous with R. mediovitellata Bykhovskaya-Pavlovskaya and there is a strong possibility that R. cruzi Wright may be merely a race of R. lari Timon-David. The position of R. magnicaudata Bykhovskaya-Pavlovskaya is difficult to determine. The description is very incomplete and the accompanying illustration does very little to clarify the text. The host, Hirundo rustica, the barn swallows, is almost exclusively insectivorous while the majority of the other hosts from which flukes of this genus have been recorded are either fish-eaters, shore-line feeders or omnivores which could easily scavenge along beaches. Until further material of this parasite becomes available it is perhaps best not to consider it to be a member of the genus Renicola. R. nana Bykhovskaya-Pavlovskaya is another species whose affinities are somewhat doubtful.

The only diagnostic specific character given by the author for this species, which was obtained from two

Charadriiform birds, is the uniformly small size of the mature flukes. The description indicates that the longitudinal extent of the vitelline glands is variable, the posterior limit being either the end of the second third of the body length or, alternatively, almost the posterior end of the body. No mention of the intestinal caeca is made in the text, but the illustration shows these to be short, ending just behind the level of the ventral sucker. The most peculiar feature of all in this species is that the transverse vitelline ducts and the vitelline reservoir lie well behind the ventral sucker. Dollfus (1939) mentions as one of the characters distinguishing the Renicolidae from the other families formerly grouped in the Troglotrematidae that the transverse vitelline ducts lie in front of the ventral sucker and this has been the arrangement found in all material examined by the present author. Although the superficial appearance of this species and its location in cyst-like dilations of the kidney tubules leave little doubt that it is a member of the genus Renicola, a more detailed description is required before the proper relationships of R.nana can be established.

Caballero (1953) declared R.lari Timon-David to be synonymous with R.glandoloba Witenberg. Although there

is an undoubted superficial resemblance between the two species, the characters differentiating them are at least as great as those on which Caballero differentiates his own species, R.thapari, from all others in the genus. Co-type material of R.glandoloba has been examined during the course of this work but unfortunately material of R.lari which has been promised is not yet available for comparison. Caballero also suggests that the genus Stamparia Neelobinsky, 1926 is synonymous with Renicola. This seems to be very probable.

The key for the identification of the species of Renicola given by Dollfus (1946) is excellent for the separation of the species known at that time according to their published descriptions. During the course of the present work it has been found that variation between individual specimens from any one batch of worms is so great that it must be accepted either that multiple infections are common or that most previous workers have not taken into account the degree of variation within species.

From the list of species of Renicola given above, together with their hosts, it is easy to see that host specificity in the sense of a group relationship does not apply in this genus. Apart from the Magpie, host of R.bretensis, the largely herbivorous Anseriforms harbouring

R.mediovitellata and R.brantae and the two Charadriiform hosts of R.nana, all of the other host-species are almost exclusively fish-eaters. The magpie is quite omnivorous and may easily eat fish when scavenging along the tide line, the redshank and lapwing both feed on all kinds of animal life on the shore and are likely to take fish found washed up and the Anseriforms are known to eat a considerable amount of animal food particularly during the breeding season, but the exact nature of this food is not known. The only feature in common, then, between most of the host species recorded is their fish-eating habit and for this reason the main search for Renicola in this country was concentrated on fish-eating sea birds.

Materials and methods

Some difficulty in obtaining dead sea-birds was experienced. Shooting such species as guillemots, puffins and kittiwakes is not easy except in the breeding season when they spend a considerable time on or near land. Due, however, to the rather inaccessible places chosen by these birds as breeding grounds and due to the fact that many are protected areas this method was not used much. The most useful source of material was found in the large numbers of oiled sea-birds washed up

around the coast during winter time. With the co-operation of several local ornithologists at various places a reasonable number of birds was obtained in a fresh condition. Those birds that were found to be still alive but too heavily oiled to be saved were selected, killed and at once dispatched by post, being received in London on the following day. The kidneys of all sea-birds sent to the Prosectorium of the London Zoo were preserved for examination.

The following is a list of the British birds examined.

<u>Common name</u>	<u>Species</u>	<u>Number examined</u>	<u>NUmber infected</u>
Common guillemot	<u>Uria aalge</u>	21	2
Razorbill	<u>Alca torda</u>	7	0
Puffin	<u>Fratercula arctica</u>	4	1
Greater Black Backed Gull	<u>Larus marinus</u>	5	0
Herring gull	<u>Larus argentatus</u>	2	0
Kittiwake gull	<u>Rissa tridactylus</u>	1	0
Leaches Fork-tailed Petrel	<u>Oceanodroma leucorrhoa</u>	12	0
Manx shearwater	<u>Puffinus puffinus</u>	10	8
Shag	<u>Phalacrocorax aristotelis</u>	1	0
Black-throated Diver	<u>Colymbus arcticus</u>	1	1

<u>Common name</u>	<u>Species</u>	<u>Number examined</u>	<u>Number infected</u>
Oyster catcher	<u>Haematopus ostralegus</u>	1	0
Dunlin	<u>Tringa alpina</u>	1	0
Eider duck	<u>Somateria mollissima</u>	1	0
Common Scoter	<u>Melanitta nigra</u>	7	0

All flukes found were fixed in formal-acetic and whole mounts were stained in Paracarmine, Delafield's haematoxylin or aceto-carmin. In each case a few specimens were fixed under light coverslip pressure, except of course those worms that were recovered from preserved kidneys. Serial sections of some worms were cut, Peterfi's celloidin impregnation technique being used to hold the loose mass of eggs in the uterine sac in place. Sections were stained in either Heidenhain's iron-alum haematoxylin or Mallory's triple stain.

Of the two infections in the Common Guillemot one has already been described (Wright 1954b) and the worms assigned to the species R.sloanei. The other infected bird of the same species came from Bude in Cornwall and harboured only two very gravid worms, apparently similar to R.sloanei.

Descriptions

Before presenting the descriptions of the flukes found during this work a brief summary of the generalized anatomy of the genus Renicola will be given together with a critical examination of the criteria on which the identity of the species is based.

The body size is usually small and the shape is roughly oval with or without a posterior prolongation giving a club-shaped appearance. The cuticle is usually spinous, some species are described as having no spines and in others this feature is not mentioned, but as shown by Wright (1954b) the spines in mature flukes may be mostly worn off and difficult to observe. The ventral sucker is usually roughly equatorial in position and is much reduced in some species. The oral sucker is sub-terminal and no pre-pharynx has been described. An oesophagus is usually absent and the two simple caeca extend back either to about the level of the ventral sucker or nearer to the posterior end.

The excretory pore is terminal or sub-terminal and the vesicle is usually Y-shaped. Lateral branching of the stem of the vesicle was illustrated by Yamaguti 1939 but the complex lateral branching of the arms and the cross-connection between these arms running behind the ventral sucker has only been described for R. sloanei Wright (1953 and 1954b). Lateral branching of the stem

and arms is described for R.cruzi Wright (1954a).

The genital system is very simple. Paired testes, either lobed or entire usually lie roughly in the region of the ventral sucker, either side-by-side, tandem or oblique in position. The vasa efferentia unite to form a short vas deferens which dilates to give a thin walled vesicula seminalis before joining the metraterm very near to the genital pore. The ovary is usually large, anterior to the testes and most commonly on the right hand side of the body. It is usually lobed most strongly on the side away from the mid-line. The vitelline glands form lateral extra-caecal groups of varying longitudinal distribution. The transverse vitelline ducts unite to form a vitelline reservoir immediately in front of the ventral sucker (except R.nana) and in the same area lie Mehlis' gland and the receptaculum seminis. There is no Laurer's canal.

The uterus is always very long and much coiled, usually a large uterine sac is formed in the centre of the body and this communicates by the short metraterm with the genital pore which is usually median in position and a short distance in front of the ventral sucker. The eggs are always numerous and small.

With such a simple basic structure there is little room for variations of a specific nature. The characters

most frequently used are the linear distribution of the vitelline glands, length of the intestinal caeca, relative size and shape of the testes and ovary, sizes of the suckers and the ratio between these sizes, and egg-size.

Of these criteria the size and shape of the gonads can be dismissed at once since it is so dependent on the state of maturity of the worms. Even the position of the testes with relation to one another and to the ventral sucker will be shown to be variable. The sizes and size-ratio of the suckers is a reasonable character provided all the worms are fixed by the same technique. Pressure alters the sizes slightly, and has more effect on the oral than the ventral sucker, thereby rendering the size ratio of no value.

The length of the intestinal caeca is useful, but since in most cases there are only two alternatives, either to the approximate level of the ventral sucker or more or less to the posterior end of the body, it is a character of only limited application.

The linear distribution of the vitelline glands is the principal feature on which Dollfus (1946) based his key to the genus. This is a useful criterion, but it can be distorted by pressure and is undoubtedly more variable than most authors have indicated. Wright (1954b)

showed that in R. pelecani the vitelline glands on one side or the other are sometimes completely absent.

The size of the eggs is useful in some cases but in many species the size ranges overlap within a very narrow field. In this connection it is worthwhile mentioning that the size ranges quoted by some authors seem to be unduly large. In this genus as with most other trematodes the mature eggs at the distal end of the uterus are smaller than those recently formed at the proximal end. The variation in length of mature eggs is seldom greater than ± 0.002 mm. in any of the material examined during this work, but the difference between mature and newly formed eggs may be as great as 0.006 mm. For instance, the range given by Witenberg for the egg-length of R. glandoloba is 0.031 - 0.037 mm. but in the cotype material of this ~~xxxxx~~ species examined the length of mature eggs has been found to vary between 0.032 mm. and 0.034 mm. Timon-David (1933) specifies that the measurements of eggs given for R. lari are taken from specimens in the uterine sac and he shows only a variation of 0.002 mm. in their length and 0.001 mm. in width.

Renicola sp. from Colymbus arcticus.

The bird from which these worms were obtained was an oiled one washed up at Lowestoft. It had been dead

for about forty-eight hours when it was examined but in spite of this all of the worms were alive and quite active when dissected out in warm Ringer's solution. Both very gravid mature specimens and young forms in which the gonads had scarcely begun to differentiate were found. Some of these young worms were found in the uterus but the majority were lying in pairs in the cyst-like swellings of the urinary tubules.

In mature specimens the shape is rather club-like, with the anterior portion ovoid and the characteristic "caudal extremity" (Fig.1). The uterine sac when full causes a slight blister-like swelling on the dorsal surface. Less mature forms are variable in shape but are usually roughly ovoid, wider anteriorly (Figs.5 and 6).

The following size ranges were recorded.

Body length: 0.94 - 2.10 mm.

Body width: 0.414 - 0.89 mm.

Oral sucker: 0.132 x 0.128 mm. - 0.26 x 0.25 mm.

Pharynx: 0.048 x 0.092 mm. - 0.087 x 0.087 mm.

Ventral sucker: 0.092 - 0.140 mm.

Eggs: 0.032 - 0.035 mm. x 0.015 - 0.017 mm.

The cuticle is covered with spines in a quincuncial arrangement and cuticular tubercles at irregular intervals

have been observed on some specimens extending from the oral sucker to the posteriorend of the body. The ventral sucker lies at about the middle of the body length but is forced further back in those specimens in which the uterine sac is highly developed.

There is no prepharynx, the pharynx opens immediately from the lumen of the oral sucker. In one very immature worm a short oesophagus was observed (Fig.4). The intestinal caeca extend back almost to the caudal extremity of the body.

The testes are slightly variable in position, either lying side by side behind the ventral sucker and overlapping it dorsally or being slightly separated and lying more on either side of the sucker as in Fig.3. The vesicula seminalis lies a little to the right of the genital pore which in turn lies in the mid-line in front of the ventral sucker, the distance being about equal to the diameter of the sucker.

The ovary lies to the right of the sucker slightly in front of it. As with the testes, its shape and ~~xxxxxxx~~ configuration are variable according to the state of development of the worm. When at the height of its activity it is deeply lobed on the side away from the mid-line. The vitelline glands lie outside the

caeca. and in most cases extend from just behind the level of the pharynx to about the junction of the middle and posterior thirds of the body length. In some specimens they appear to end more nearly at the junction of the third and last quarters of the body length (Fig.1).

Mehlis' gland, the receptaculum seminis and vitelline reservoir all lie immediately in front of the ventral sucker.

The excretory vesicle is of the usual Y-shape with lateral branching of the stem and arms and with the cross-connection between the arms behind the ventral sucker. In the specimen in which the vesicle is shown (Fig.4) there appears to have been some occlusion of the anterior lateral branching although there is not yet any great differentiation of the genital rudiments or any formation of the uterus.

The position of the genital rudiments in Fig.4 is of some interest as it corresponds exactly with the position of the genital anlage found in the "Rhodometopa" group of cercariae to be described later (see also Figs. 41 and 63). It appears that some form of migration of gonadial tissue must take place in order that the ovary and testes should be found in their final positions.

These worms are obviously very close to R.sloanei Wright, some of the specimens fitting exactly the

description of that species but many showing slight dissimilarities. The main difference is in the rather greater anterior extent of the vitelline glands.

Renicola sp. from Fratercula arctica.

This bird was picked up exhausted in London Docks and died the day after presentation to the London Zoo. The kidneys had been preserved in 5% formalin when examined and the cause of death determined in the Prosectorium of the Zoo was renal insufficiency. The infection is an interesting one for two reasons. Firstly, this is the first record of a natural infection of a trematode in this host species, the only other being an experimental infection with Spelotrema arenaria by Belopolskaya and Uspenskaya (1953) in Russia. Secondly, the bird was only in its first year and died on 16th October. It could not have been more than six months old and the flukes were all well developed, no very immature specimens being found. The bird was probably hatched in either the Farne Islands, Flamborough Head or one of the colonies on the east coast of Scotland and was on a southerly autumnal migration when it entered the Thames Estuary and became lost in the docks.

About forty flukes were found in the kidneys, many

were damaged during removal due to being fixed "in situ". Figs. ~~7-12~~ illustrate six specimens from this infection. The shape of the specimens is not characteristic due to the method of fixation. Fig. 10 shows a very exceptional form. Due to the more uniform state of maturity of this batch of worms the average size is a little larger than in the other groups.

Body length:	1.16 - 2.24 mm.
Body width:	0.59 - 0.83 mm.
Oral sucker:	0.16 x 0.16 mm. - 0.28 x 0.22 mm.
Pharynx:	0.08 x 0.08 mm. - 0.12 x 0.10 mm.
Ventral sucker:	0.12 - 0.15 mm.
Eggs:	0.032 - 0.034 mm. x 0.018 - 0.020 mm.

The cuticle has the usual quincuncial arrangement of spines and cuticular tubercles extending from the anterior end to the region of the excretory pore were found. Some specimens showed small groups of these tubercles at the posterior end of the body. The ventral sucker lies a little behind the middle of the body length even in the less mature specimens of this batch of worms.

The oral sucker is sub-terminal and the pharynx and gut caeca are similar to those described for the preceding group from Colymbus, but no oesophagus has been seen in any of these.

The arrangement of the testes, ovary, genital pore and other accessory reproductive organs is the same as in the material from Colymbus, but the extent of the vitelline glands is rather less. These start anteriorly at about the junction of the first and second fifths of the body length and extend back to about the junction of the third and last quarters, a distribution very similar to that found in R.sloanei.

Here again, the affinities of these worms are very close to R.sloanei but for reasons to be given below no attempt to include them in that species will be made here.

Renicola sp. from Puffinus puffinus

These infections are also extremely interesting, firstly because, as in the case of the material from Fratercula just described, this is the first record of a trematode in this host-species and secondly, the very high frequency of infection. Not only is the infection rate in the sample of birds examined from the Skokholm population very high, but in most cases the individual birds were very heavily infested with worms in both kidneys.

It seems likely that the specimens figured represent two species, Fig. 15 being a mature adult of one and

Fig.18 of the other. The only criteria by which they can possibly be distinguished ^{at}being their appearance and the greater distribution of the vitelline glands shown in Fig.18. In all other characters the two forms grade into one another and no satisfactory method for separating non-gravid specimens has been found. These differences might well have been overlooked had not one bird been found to harbour forms corresponding to Fig.15 only whereas all the others had both.

The general description of these worms is similar to that given for the preceding two batches. The shape is roughly ovate or club-shaped, the "caudal" process is variable according to the state of contraction at the time of fixation.

Body length:	0.94 - 1.65 mm.
Body width:	0.36 - 0.98 mm.
Oral suckerM.	0.136 x 0.12 mm. - 0.28 x 0.18 mm.
Pharynx:	0.06 x 0.06 mm. - 0.096 x 0.080 mm.
Ventral sucker:	0.088 - 0.144 mm.
Eggs:	0.034 - 0.038 mm. x 0.019 - 0.022 mm.

The cuticle bears spines in the same quincuncial pattern as before, no cuticular tubercles have been observed. The ventral sucker lies a little behind the middle of the body length, possibly a little further back in the form

shown in Fig.15 than in the other.

The oral sucker is sub-terminal and the pharynx is in direct contact with it posteriorly. In some of the young forms a trace of an oesophagus is present but this disappears in the gravid specimens. The intestinal caeca reach to the posterior end of the body, almost entering the "caudal" extremity.

The arrangement of the gonads and their associated structures is identical in these flukes with that found in the forms already described. The distribution of the vitelline glands in one group is from the junction of the first and second fifths of the body length to about the level of the ventral sucker (Fig.15) while in the other group the glands extend from the level of the pharynx to about the junction of the third and last quarters of the body length, well behind the ventral sucker.

Two observations of considerable significance to the life-history aspect of this work were made on very young living flukes from the shearwaters. Firstly, specimens were found bearing traces of the penetration glands of the cercariae with their ducts still plainly visible (Fig.62). Only the remains of a median group of these glands were found, the significance of this fact will be seen in the section dealing with cercariae. The other

important feature observed was that the distribution of the collecting tubes of the excretory system resembled very closely that found in the cercariae. An attempt to work out the complete flame-cell pattern was unsuccessful but the posterior three groups on one side were observed, five flame-cells per group, and the arrangement of main collecting tubes and other flame-cells seen left no doubt whatever that the systems are the same in these flukes and certain of the "Rhodometopa" group of cercariae.

Once more there is a marked similarity between these flukes and R.sloanei, but with minor variations. The original description of R.sloanei was based on type material found in Ringed penguins at the London Zoo. Specimens from the King penguins at Edinburgh Zoo and from a British Common guillemot, both of which showed minor variations from the type, were included in the species, the variations being considered insignificant and possibly due to the different hosts. Recent studies on the cercariae in which the species are very similar and yet distinct have led to the belief that R.sloanei is in fact a composite species containing several very closely related forms. These will only be distinguishable by very carefully controlled life-history experiments which are not yet possible. Rather than confuse the

systematic position any further none of the worms described above has been included with R.sloanei.

Pathology of the infected kidney

Mention is made by Campbell and Sloane (1943) of the pathological effects of Renicola sp. in the kidneys of Aptenodytes longirostris. Timon-David gives a much more detailed account of the effect on Larus argentatus of R.lari (1933) and on Pica pica of R.bretensis (1952). No other authors have discussed the histopathological details of the infections they have observed apart from the cyst-like dilation of the urinary tubules caused by the presence of the worms. Hill (1952) mentions the possibility of infestation with Renicola sp. causing debility in the host and hence making the host more susceptible to other lethal infections. Hill (1954) records Renicola sp. as the definite cause of nephrosis in Fratercula arctica (the same infection as that described above).

In the present work only Renicola sp. from Puffinus puffinus has been studied from the pathological aspect, none of the other infections having been available in sufficiently large quantities to make the observations of any great value. Comparisons have been made, however, with observations on R.sloanei in penguins and R.pelecani in pelicans.

The usual location of the worms is in the ducts of Bellini, shortly before they join the main collecting tubules. The most outstanding feature of the pathology of the infected shearwater kidney is the apparent lack of any host tissue reaction. The dilation of the duct due to the presence of the worms causes an increase of several times its original diameter and this naturally causes the occlusion of neighbouring tubules due to pressure. There is also some erosion of the epithelial lining of the duct in the immediate vicinity of the worms, but apart from these effects very little other damage is visible. In very heavy infections there is no sign of phagocytic invasion around the tubules but in light, early infections some slight eosinophilia in the neighbourhood of the cysts can be seen. Timon-David (1952) states that phagocytic activity is only visible around dead or degenerated parasites but this was not seen in this case. Scarcely any trace whatever of a fibrous connective-tissue wall around the cysts could be found (Fig.19), but in R. pelecani infections this is definitely present and in R. sloanei in penguins it is pronounced (Fig.20). In these infections in penguins strong phagocytic action around infected tubules can also be seen.

There are apparently no effects due to the blockage of the infected tubules; the parts of the tubules not containing worms appear normal and unmodified and no casts

are present in the lumen of the tubules. Casts have been observed in R.sloanei infections in penguins and Campbell and Sloane report finding a muco-purulent exudate present with the worms in King penguins.

No toxic effects were shown by the cells of the kidney, even those in close proximity to the worms, the nuclei were found to be vesicular and showed no pyknotic changes.

In sections of one of the less heavily infected kidneys a few foci in the tissues similar to those formed by the passage of larval flukes through the liver of sheep were observed. These are of probably no significance whatever, several possible causes being known. It seems most likely that the young flukes would reach the tubules by ascent of the ureters rather than passage through the tissues. No lesions in the wall of any tubule to indicate that entry of the worms was other than by the ureters have yet been found. It may be that the backwardly directed spines which are so numerous on the cuticle of young flukes and which tend to disappear in the older specimens are of use in helping the worms to maintain their position in the ureter against the downward passage of excretory material during their ascent.

Campbell and Sloane consider that gross infestations of Renicola sp. might be fatal in penguins. As already

mentioned, Hill (1952) considers that the flukes may be a contributory cause of death. In the White pelican (Pelecanus onocrotalus) in which two specimens ascribed to Renicola secunda Skrjabin were found (Wright 1954b), the cause of death was nephritis, but it was a nephritis of bacterial origin (possibly Staphylococcus aureus). The possibility of bacteria being carried up into the kidneys by young flukes can not be overlooked and may be the most serious consequence of infection with these trematodes.

As far as can be ascertained the flukes live on cell debris and the excretory material of the birds, both being frequently present in the caeca of the worms. ~~Worms~~ dissected from the kidney in Ringer's solution and allowed to remain in this together with the teased-out kidney tissue will rapidly take up blood cells until their caeca appear bright red but no evidence of this happening in the living birds has been obtained. The main excretory product of birds is uric acid and it is unlikely that the flukes are able to utilize this as a food material.

The lack of any pronounced reaction to Renicola sp. by Puffinus puffinus appears to indicate a well balanced and possibly long-established host-parasite relationship. This absence of antagonistic reaction would also account

for the build-up of large infections and the high infection rate in this host species that has been observed. The more marked reaction by penguins to R.sloanei, a parasite obviously alien to these hosts is also significant.

These observations are very similar to those by Timon-David (1933) for R.lari in Larus argentatus. In a private communication Prof. Timon-David informs me that the infection rate in Herring gulls on the Isle de Riou near Marseilles is high and it seems that here is a parallel to the host-parasite relationship between Renicola sp. and the Manx shearwaters on Skokholm Island.

Smith (1951) states that no birds are known in which the function of the kidneys is entirely aglomerular, although even in the chicken there is very little glomerular activity, a result of the necessity for water conservation associated with the excretion of uric acid. He suggests that in some marine birds which remain at sea for as much as nine months of the year and are therefore away from supplies of fresh water for that period, glomerular function may be nearly nil. Since the Manx shearwater is a bird of this type it may be that this is an additional factor in providing an environment favourable to kidney flukes.

The Ecology and feeding habits of some host birds

In this section only those birds which have been recorded as hosts for Renicola sps. in Western Europe are considered since they are most likely to give indications as to the second intermediate host in the area around the British Isles.

The only birds whose diet is known with any certainty are those penguins which were hatched in the menagerie at Edinburgh Zoo and were later found to be harbouring Renicola sp. They were fed (officially) on marine fish only, principally herring and haddock. Although none of the infected penguins from the London Zoo had been hatched in the menagerie the presence of young flukes in birds which had been in this country for about eighteen months left little doubt that these too had been acquired from the food, principally herring and whiting.

For the remainder of the host birds the knowledge of their feeding habits is based on the examination of stomach contents of shot specimens as recorded in the Handbook of British Birds except for the Manx shearwater where further evidence is available.

Both the Great crested grebe (Podiceps cristatus) and the Black-throated diver (Colymbus arcticus) feed on fresh water as well as the sea, but since the other

birds under consideration are almost exclusively marine feeders only this part of their diet will be listed.

There is little advantage to be gained from discussing the feeding habits of the Magpie, host of R.bretensis, since this bird is quite omnivorous. As with many of the Corvidae, the feeding habits of the same species may vary considerably in different areas according to the availability of food. From the evidence at present available the metacercarial stage of the genus Renicola occurs in fishes. The two Magpies found to be infected were shot near Aix-en-Provence about 20-30 miles from the sea and easy flying distance for these birds. As has been already suggested they could easily have become infected by eating fish found washed up on the beach.

The first birds to be listed are the hosts of previously recorded species, the last four are the new British records reported in this work.

(1) Podiceps cristatus host of R.pinguis

Fish taken are mainly small herrings, blennies and young gadoids. Crustacea are also eaten, mainly Pandalus.

(2) Larus argentatus host of R.lari.

92.75% of food is animal, and is very varied, including fish, molluscs, echinoderms, crustacea

of all kinds, annelids and insects, also other birds, small mammals and carrion. Fish recorded are herrings, haddock, eels and cod.

- (3) Mergulus alle host of Renicola sp. of Dollfus.

97.84% animal food, mostly crustacea, also annelids, larval molluscs and small fish, no species of which are named.

- (4) Sterna cantiaca host of Renicola sp. of Collot.

Nine stomachs only examined, food in these entirely animal. 34.79% sand eels (Ammodytes), 31.46% other fish (young herring, sprat, young whiting, weever and garfish), marine annelids 32.5% and mollusca 1.25%.

- (5) Uria aalge.

97.37% animal food, 51.6% fish, 35.52% crustacea and annelids, 10.19% mollusca. Fish recorded in Britain are mainly sand-eels (Ammodytes).

- (6) Colymbus arcticus.

Only seven stomachs of this species were examined, the food found was exclusively animal. Fish constituted 59.45%, Crustacea and Annelida 23.4% and mollusca 15.46%. Definite records of sea fish were scarce, but those identified were goby, sand-smelt, small herring and sprat.

- (7) Fratercula arctica.

98.22% animal food, 32.75% fish, 11.72% mollusca

and other marine animals 53.75%. Species of fish recorded include sand eels and fry of herring and pollack.

(8) Puffinus puffinus.

The Handbook of British Birds states "Most British records refer to smaller fish (Clupea), especially sprat, young herring, pilchard and fish offal; but also mollusca (jaws of small Cephalopoda). Winter records also chiefly of fish (pilchard, Engraulis sp. etc.). Adults feeding young contained white, semi-fluid mass of partly digested fish remains, probably chiefly young herring." Recently Lockley (1953) has shown that during the breeding season the Manx shearwaters ringed on Skokholm are frequently shot by sardine fishermen in Biscay, particularly to the south in Basque waters. They are also caught in the nets and these fishermen use the presence of the birds to locate the shoals of sardines (Sardina pilchardus). Ringing recoveries indicate that the Mediterranean race of the Manx shearwater (Puffinus p. mauretanicus) follow these fish on their northward breeding migrations, feeding almost exclusively on them. Lockley suggests that during the early part of the breeding season the Skokholm and Skomer shearwaters (also those from Annet and one or two other breeding grounds comprising a "South-Western group") feed almost entirely

on the Biscayan sardines. This continues during the incubation period, each parent spending several consecutive days away on feeding trips, but when the chick hatches and requires frequent feeding then the parents feed rather nearer home. During this period it is likely that sprats and young herrings supplement the sardines which by this time have moved to the northern part of Biscay. Personal observations on Manx shearwaters feeding near the British Isles have shown that they are usually associated with fulmar petrels and kittiwake gulls, but seldom with many auks such as guillemots, razorbills and puffins. Observations on these last three birds during the breeding season indicate that their principal food consists of sand-eels.

For reasons to be presented under the section dealing with metacercariae it will be shown that the sand-eels are not likely to be satisfactory intermediate hosts for Renicola and this adds yet another explanation to the higher rate of infection in Manx shearwaters, which live mainly on young Clupeoids, than in those birds which use Ammodytes as their principal article of diet.

Fig. 1 Renicola sp. from Colymbus arcticus. Gravid
 specimen.

Fig. 2 Renicola sp. from Colymbus arcticus. Immature
 specimen.

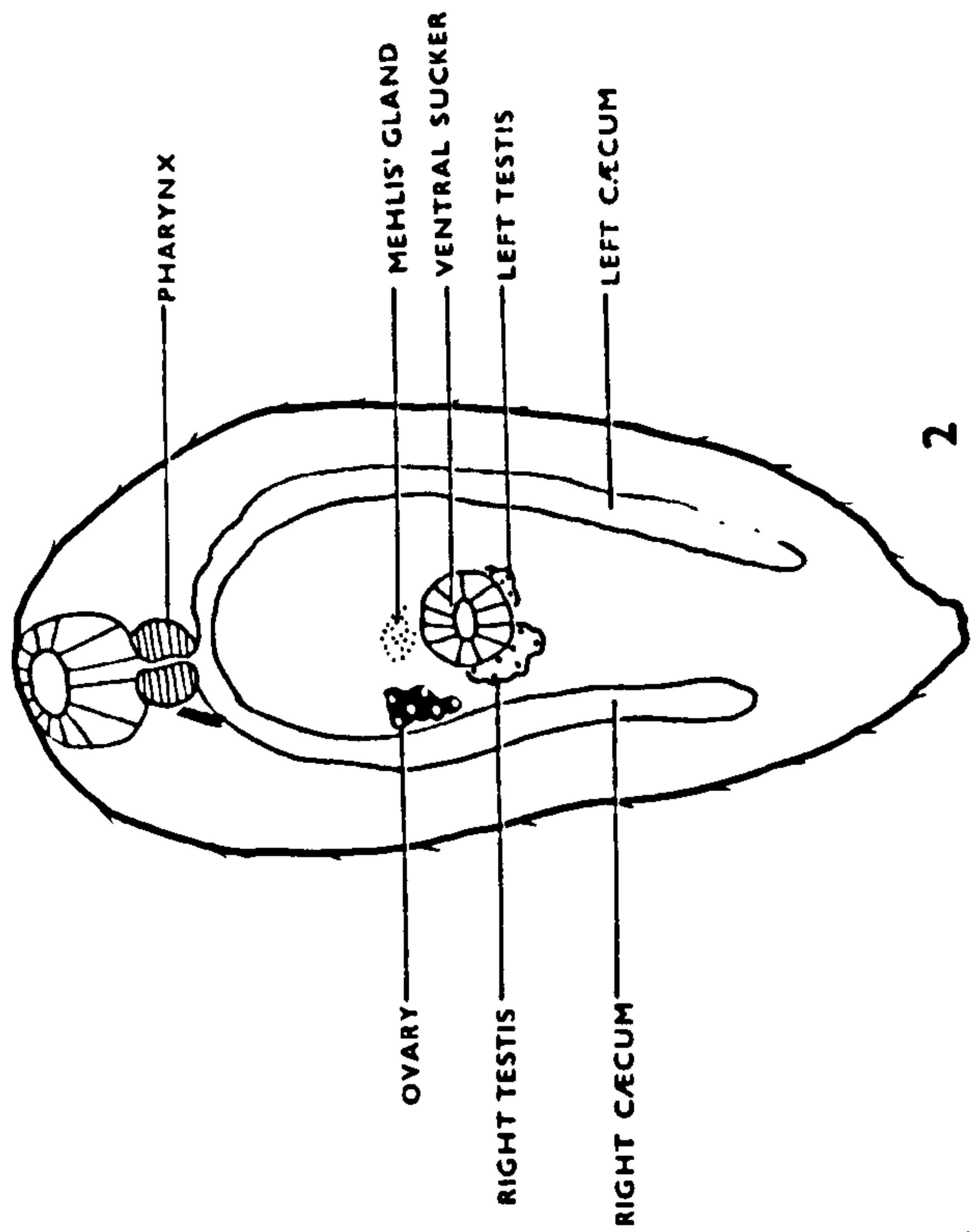
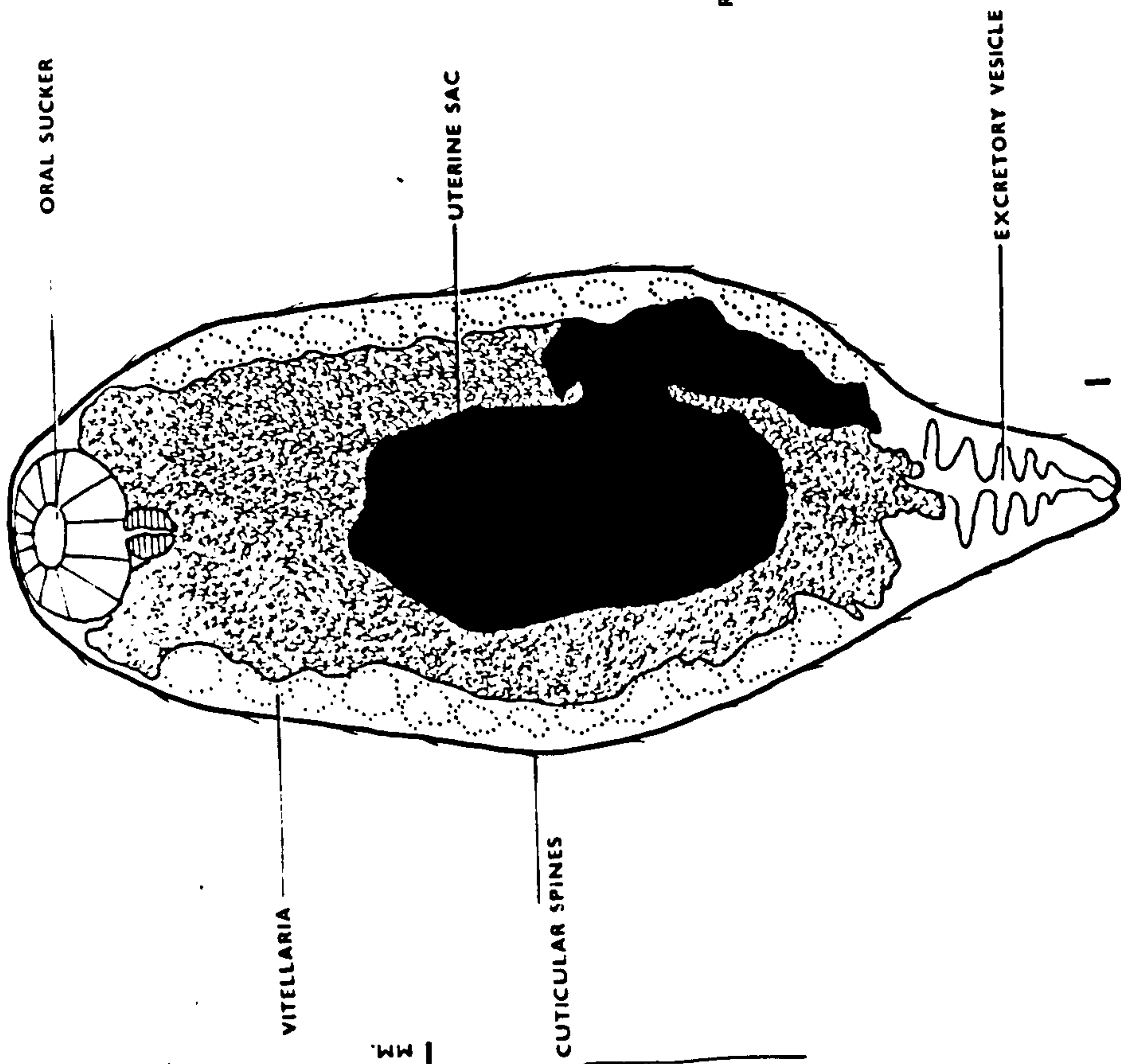


Fig. 3 Renicola sp. from Colymbus arcticus. Mature
 specimen.

Fig. 4 Renicola sp. from Colymbus arcticus. Young
 fluke.

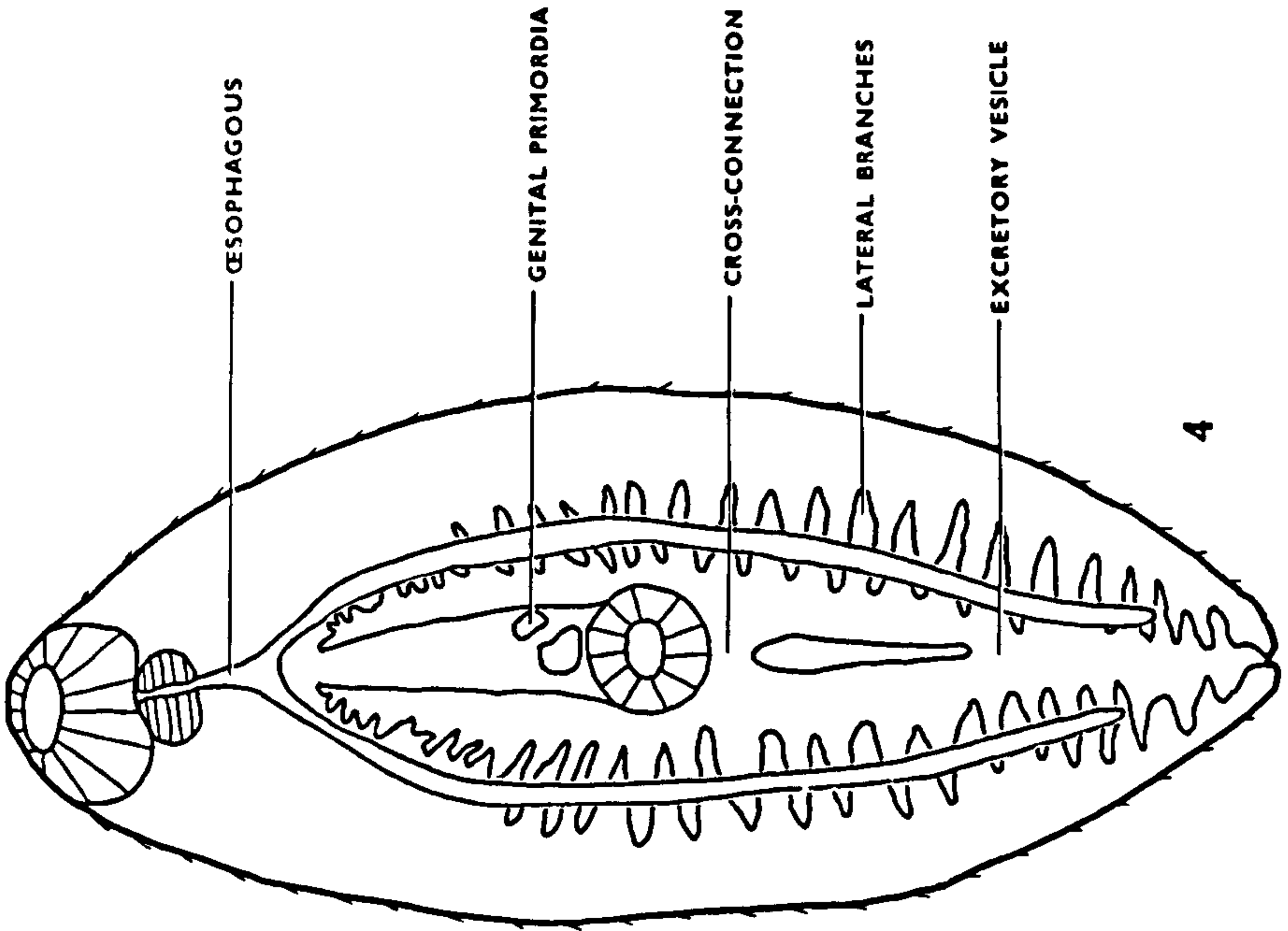
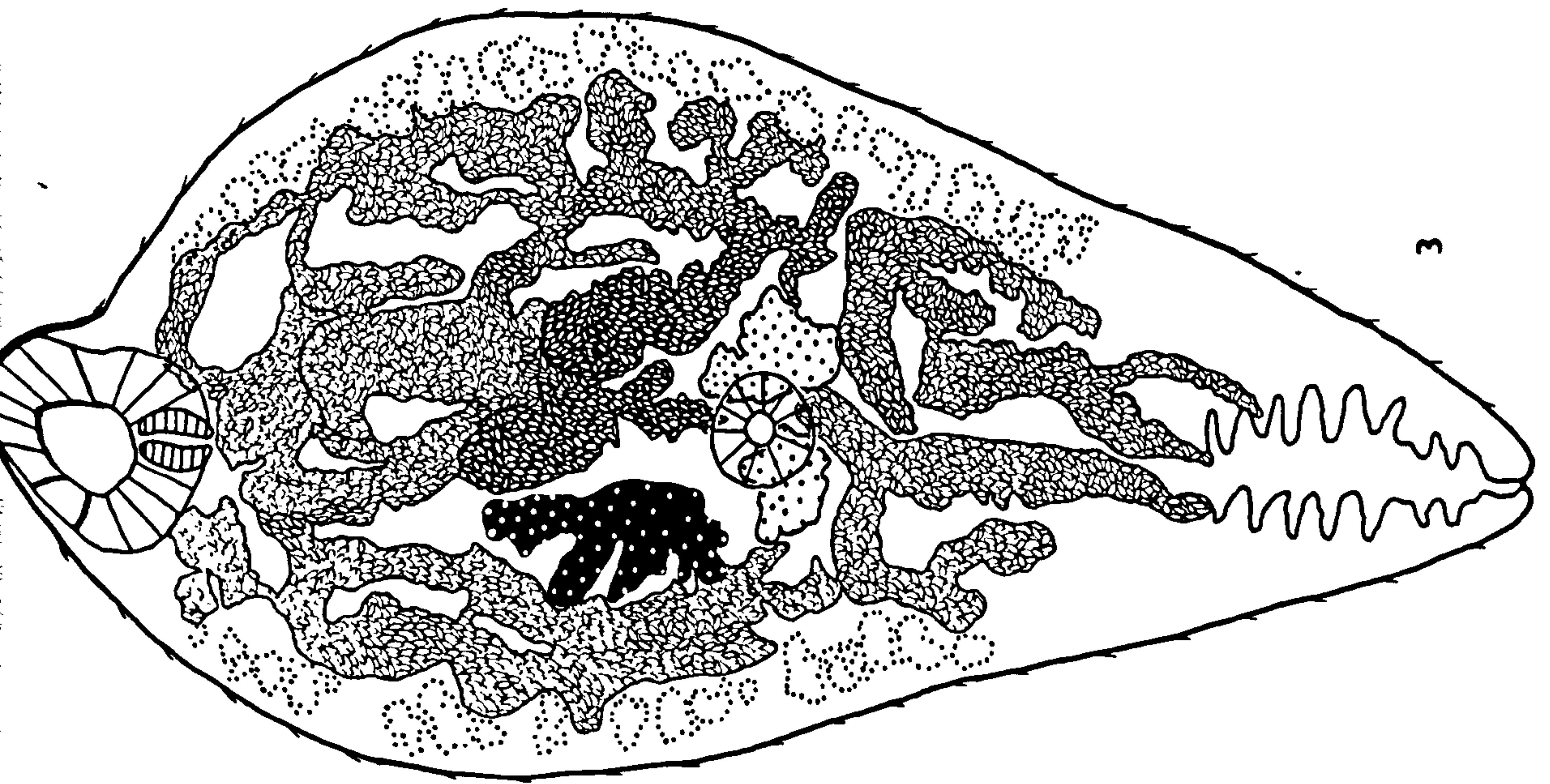
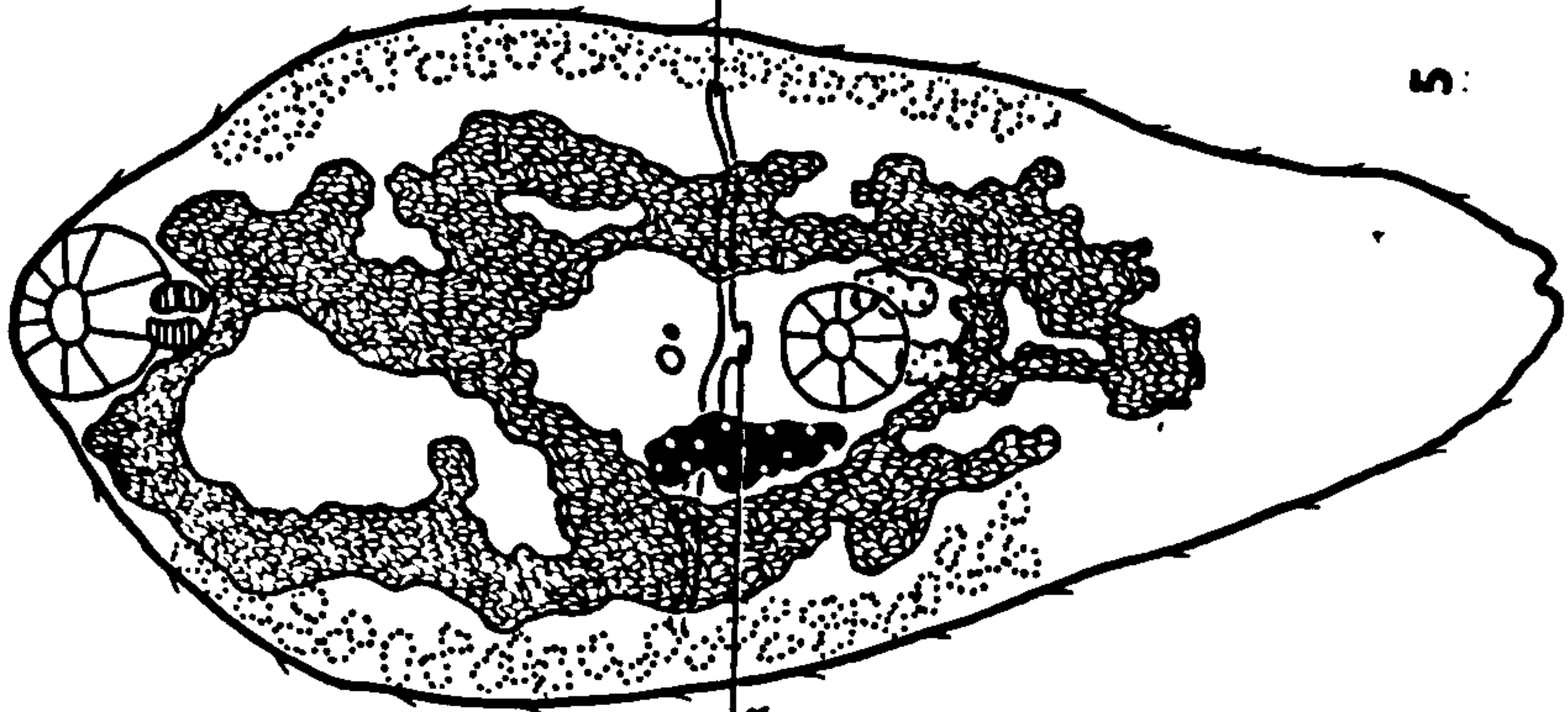


Fig. 5 Renicola sp. from Colymbus arcticus. Mature
 specimen.

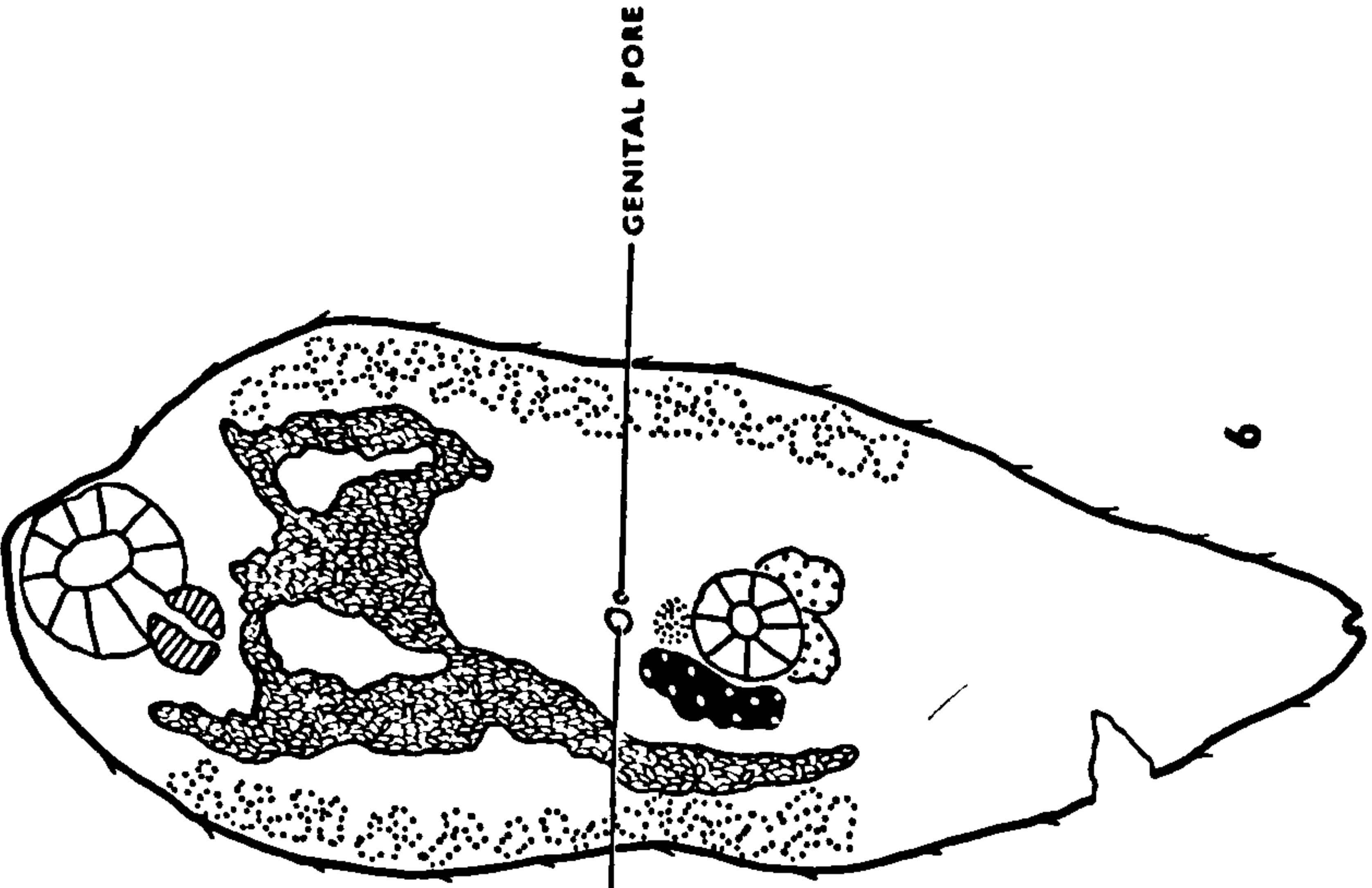
Fig. 6 Renicola sp. from Colymbus arcticus. Mature
 specimen, fixed under light pressure.



VITELLINE RESEVOIR

VITELLINE DUCT

1 MM.



VESICULA SEMINALIS

GENITAL PORE

1 MM.

- Fig. 7 Renicola sp. from Fratercula arctica. Immature
 fluke, subjected to pressure while in creosote.
- Fig. 8 Renicola sp. from Fratercula arctica. Mature
 specimen, uterus not shown.

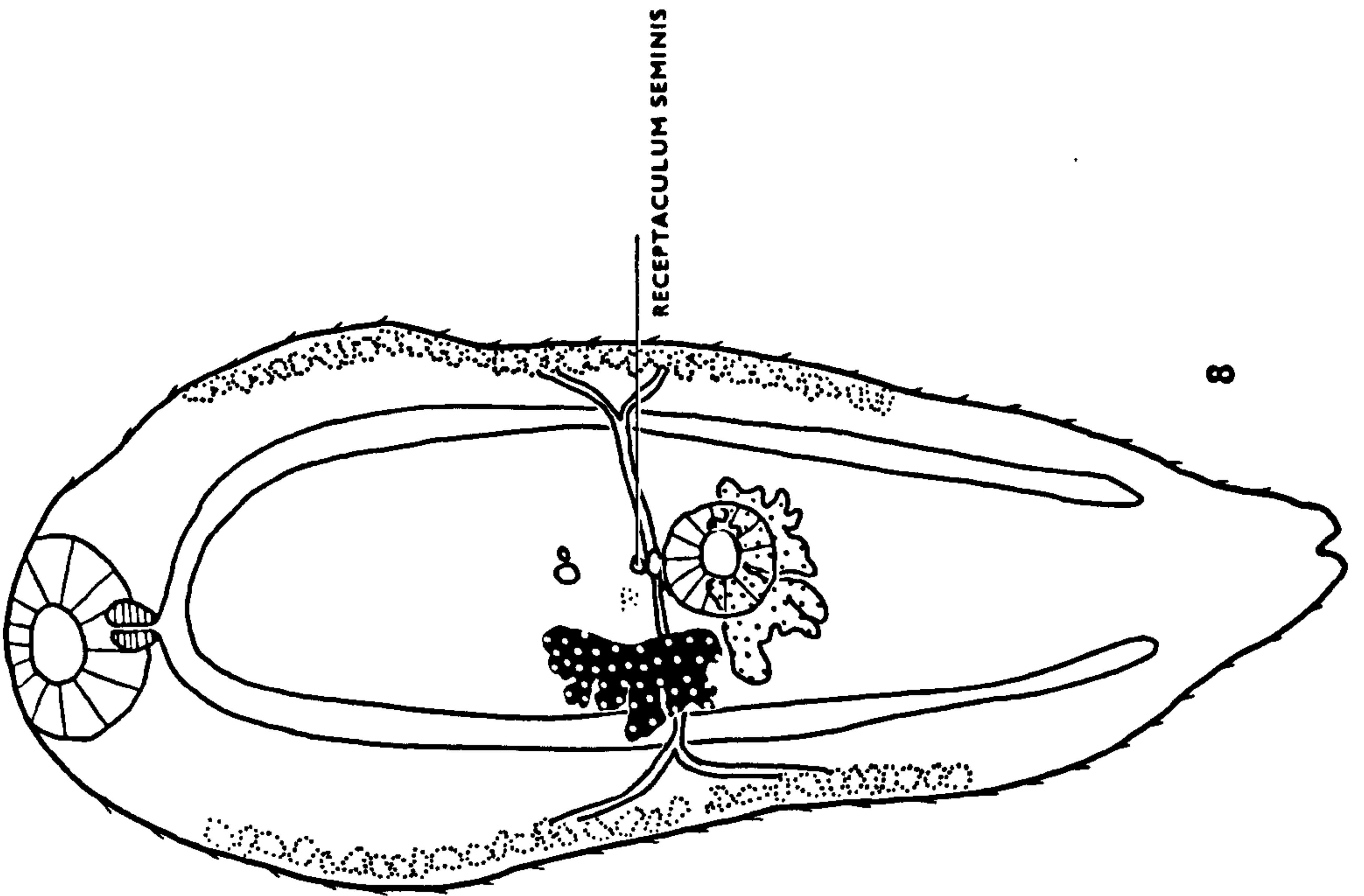
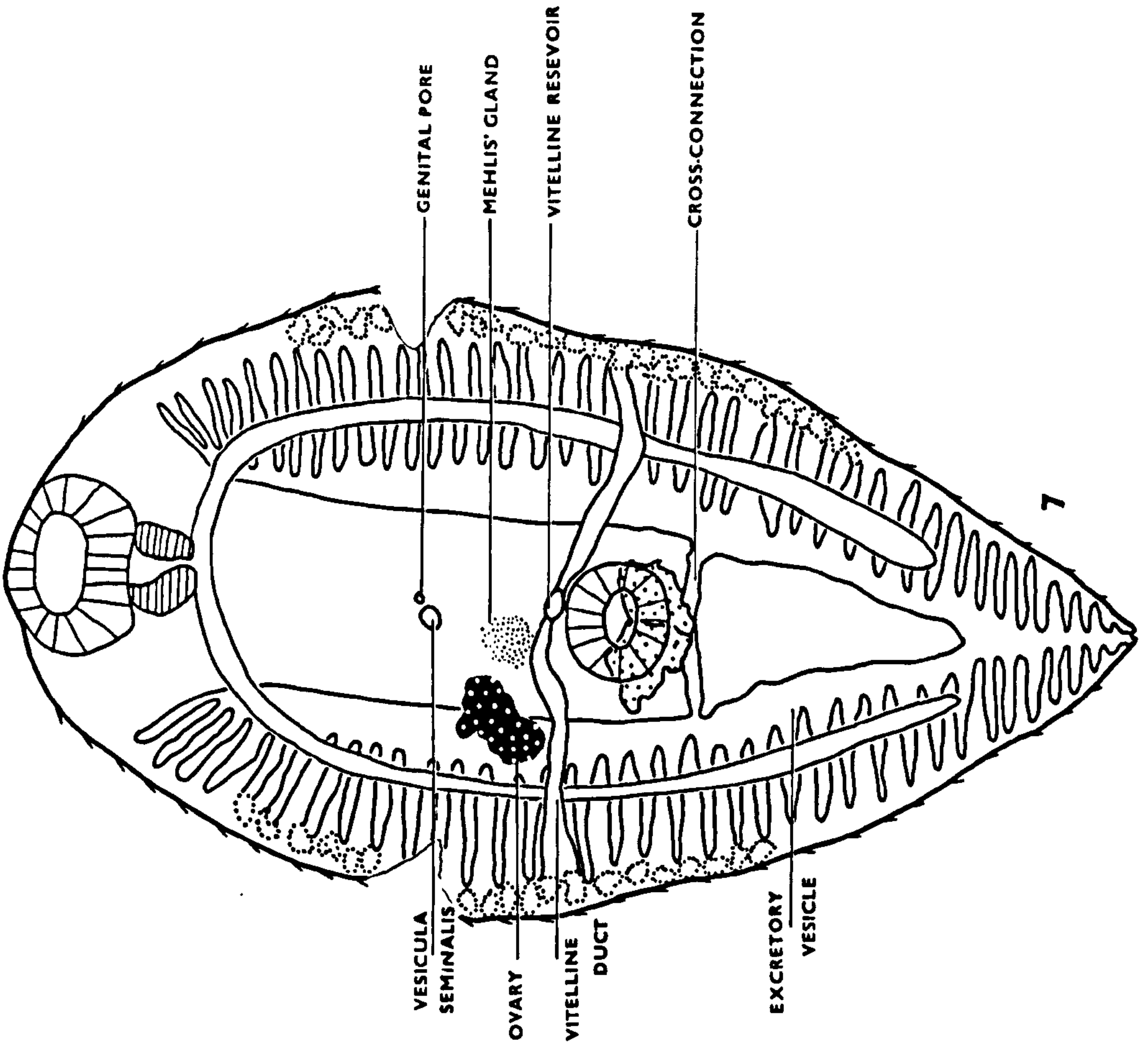
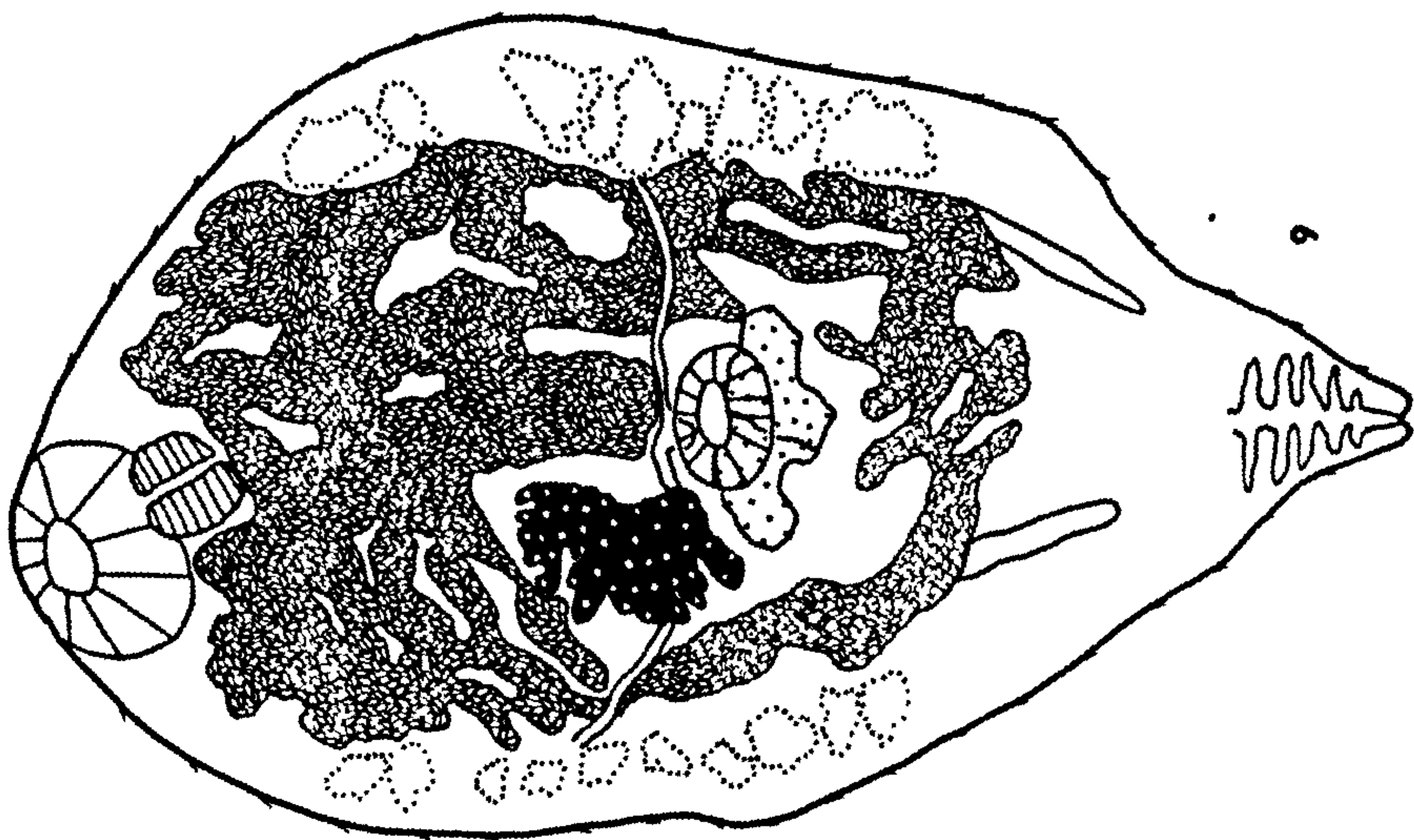
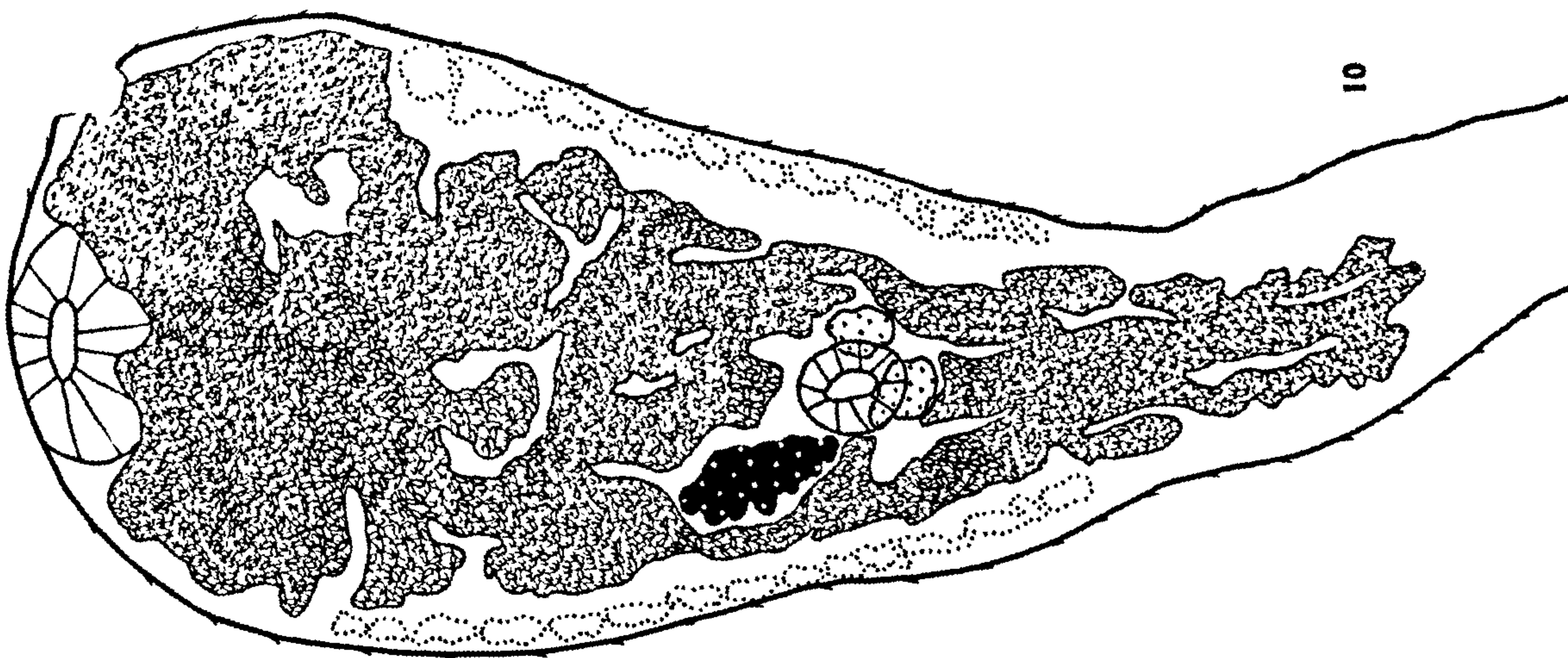


Fig. 9 Renicola sp. from Fratercula arctica. Mature
 fluke.

Fig. 10 Renicola sp. from Fratercula arctica. Mature
 fluke, posterior extremity not shown.

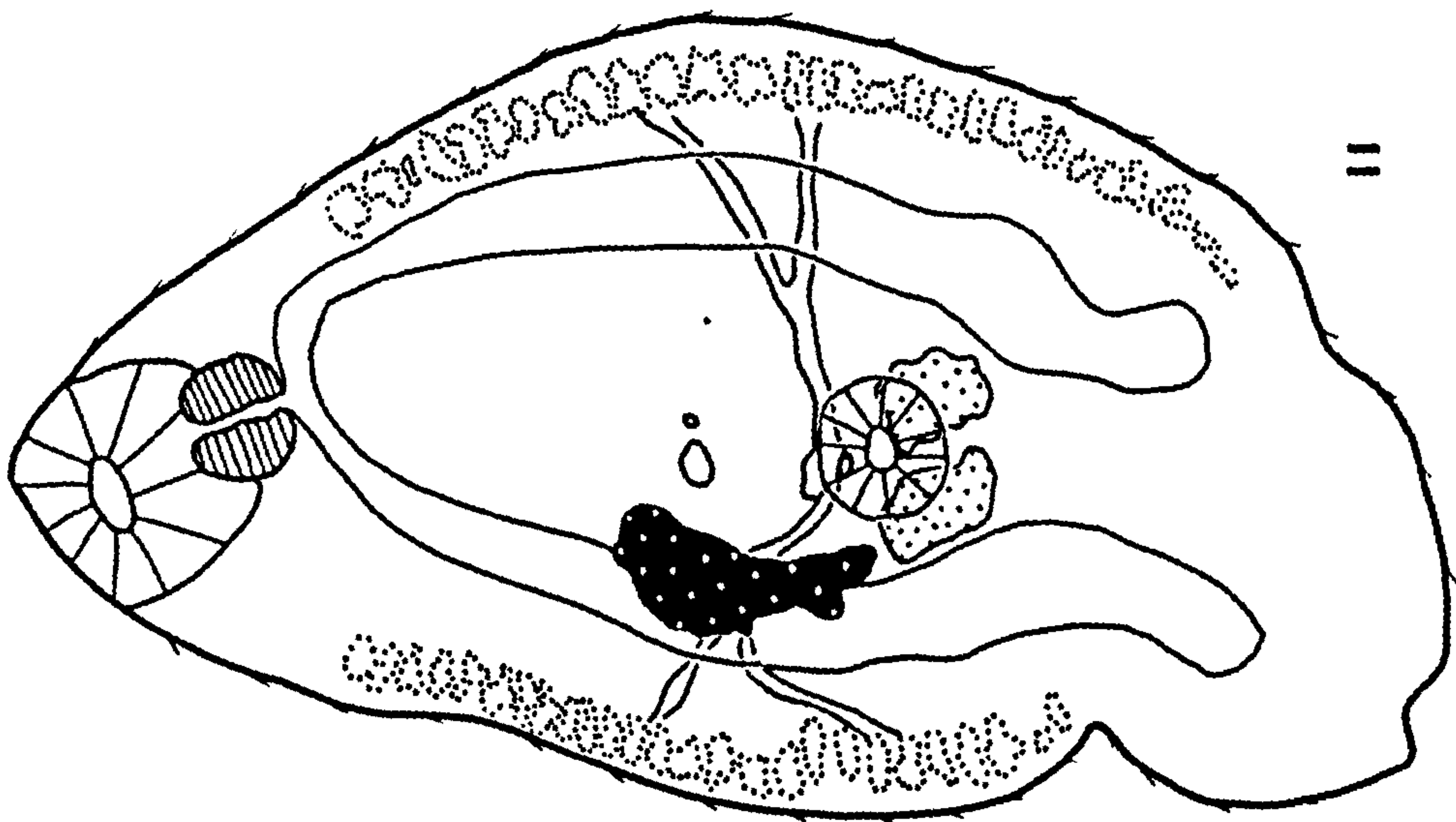
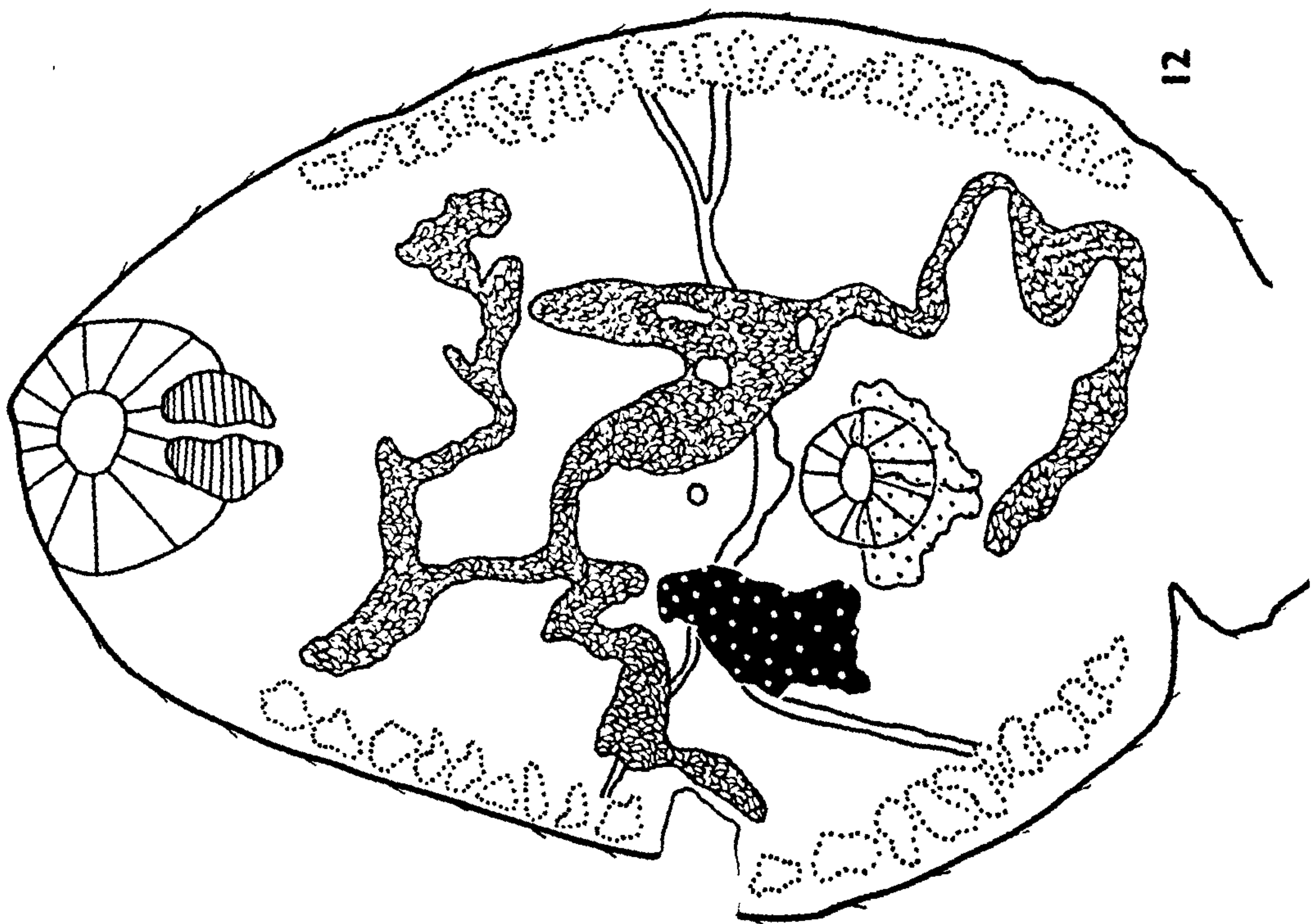
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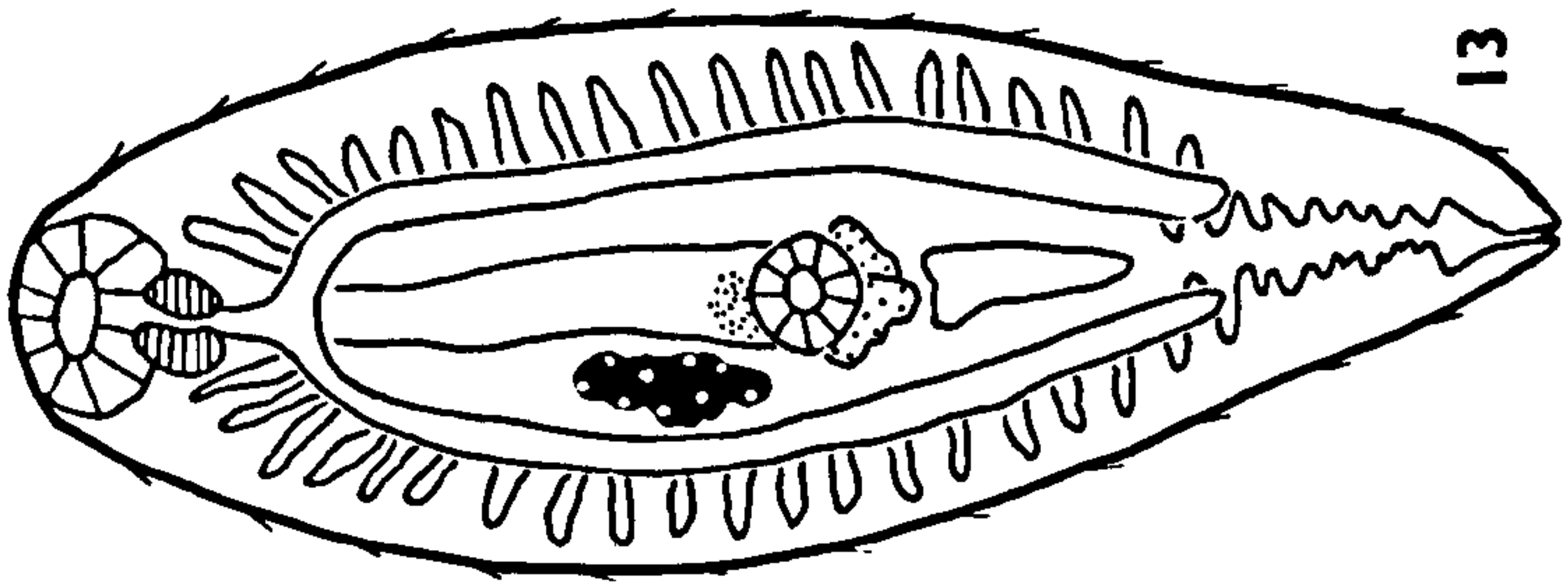
1mm

Fig.11 Renicola sp. from Fratercula arctica. Immature
 fluke.

Fig.12 Renicola sp. from Fratercula arctica. Specimen subjected to pressure in creosote.

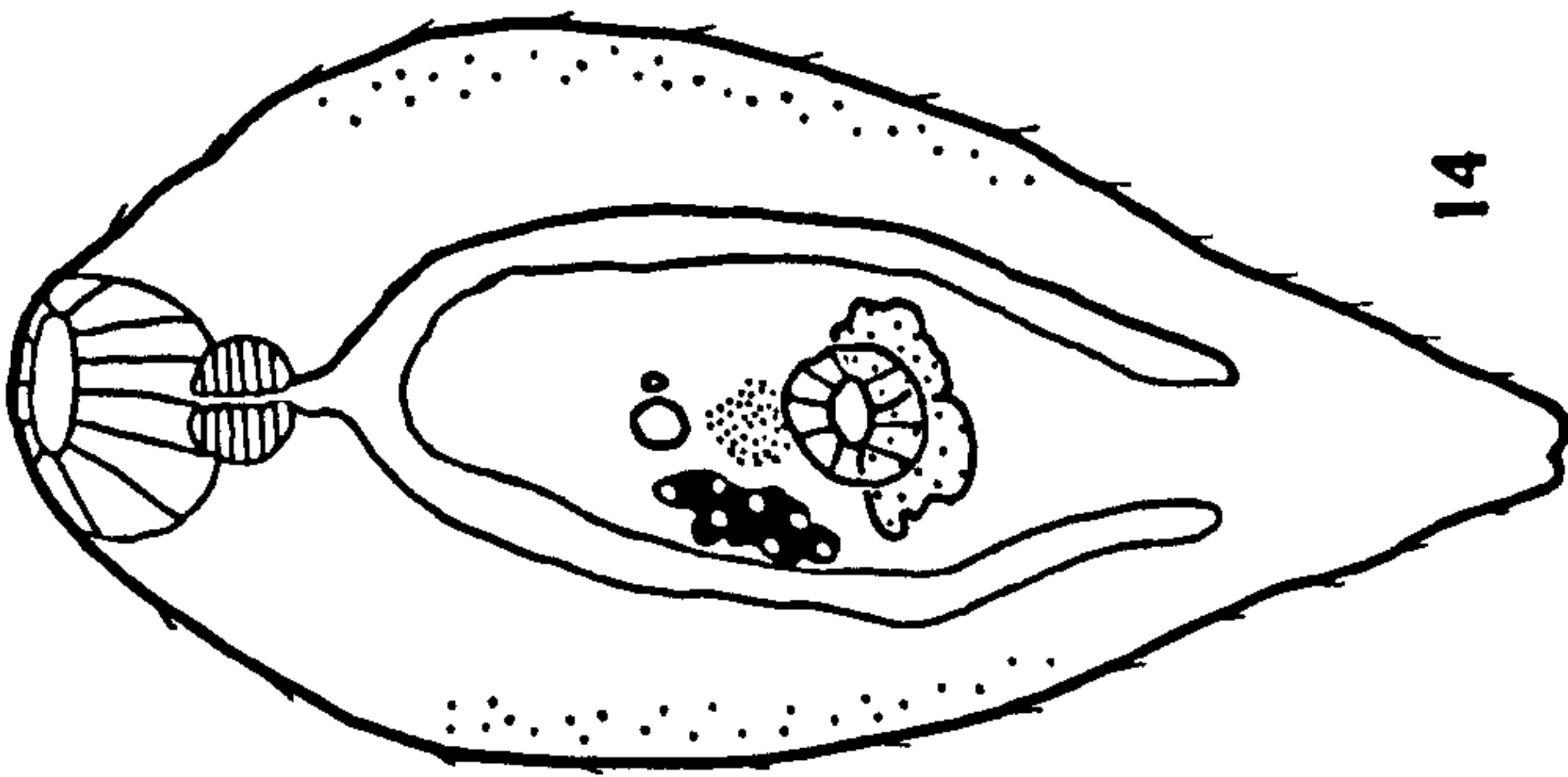


- Fig.13 Renicola sp. from Puffinus puffinus. Immature
 specimen.
- Fig.14 Renicola sp. from Puffinus puffinus. Immature
 specimen.
- Fig.15 Renicola sp. from Puffinus puffinus. Gravid
 specimen.



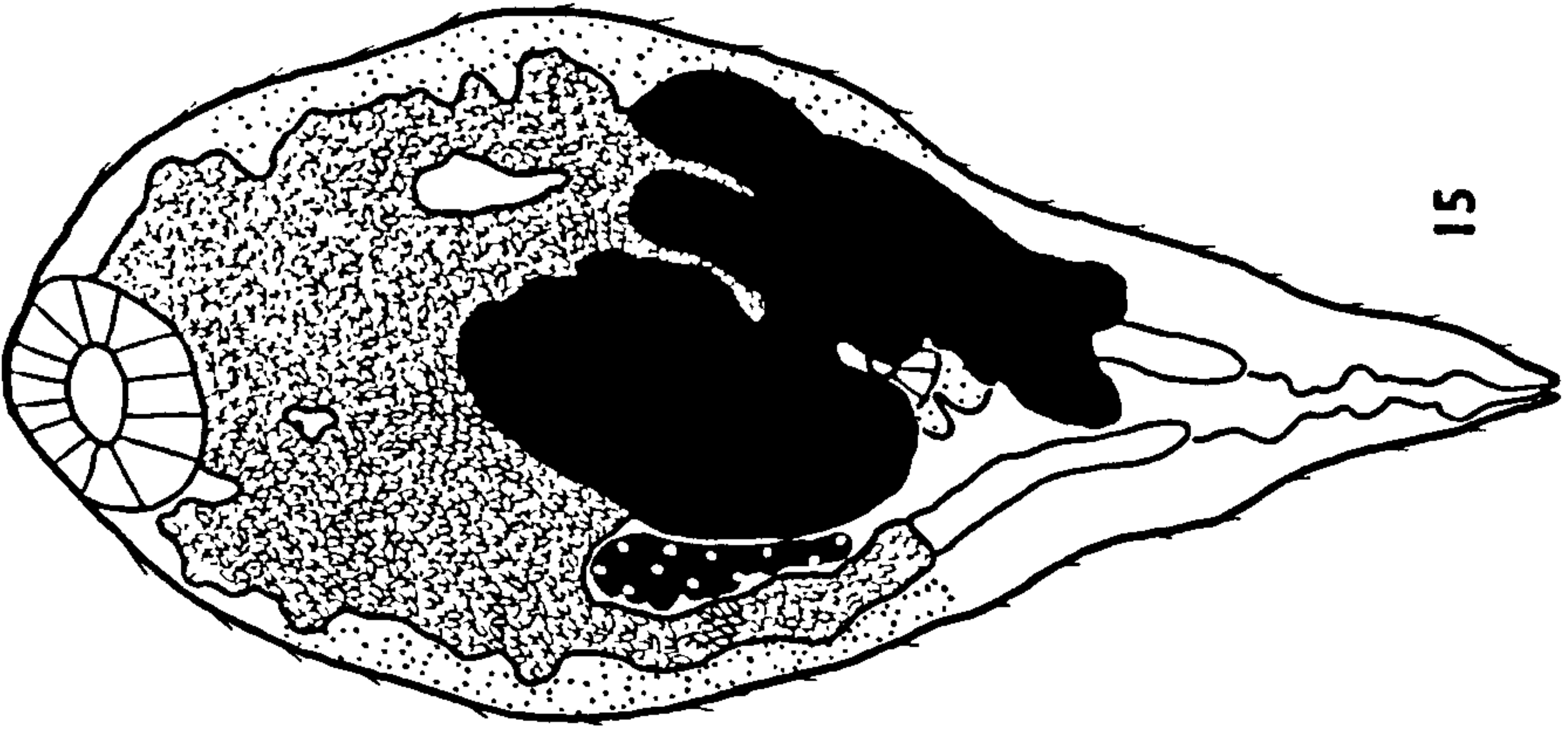
13

1 mm



14

1 mm



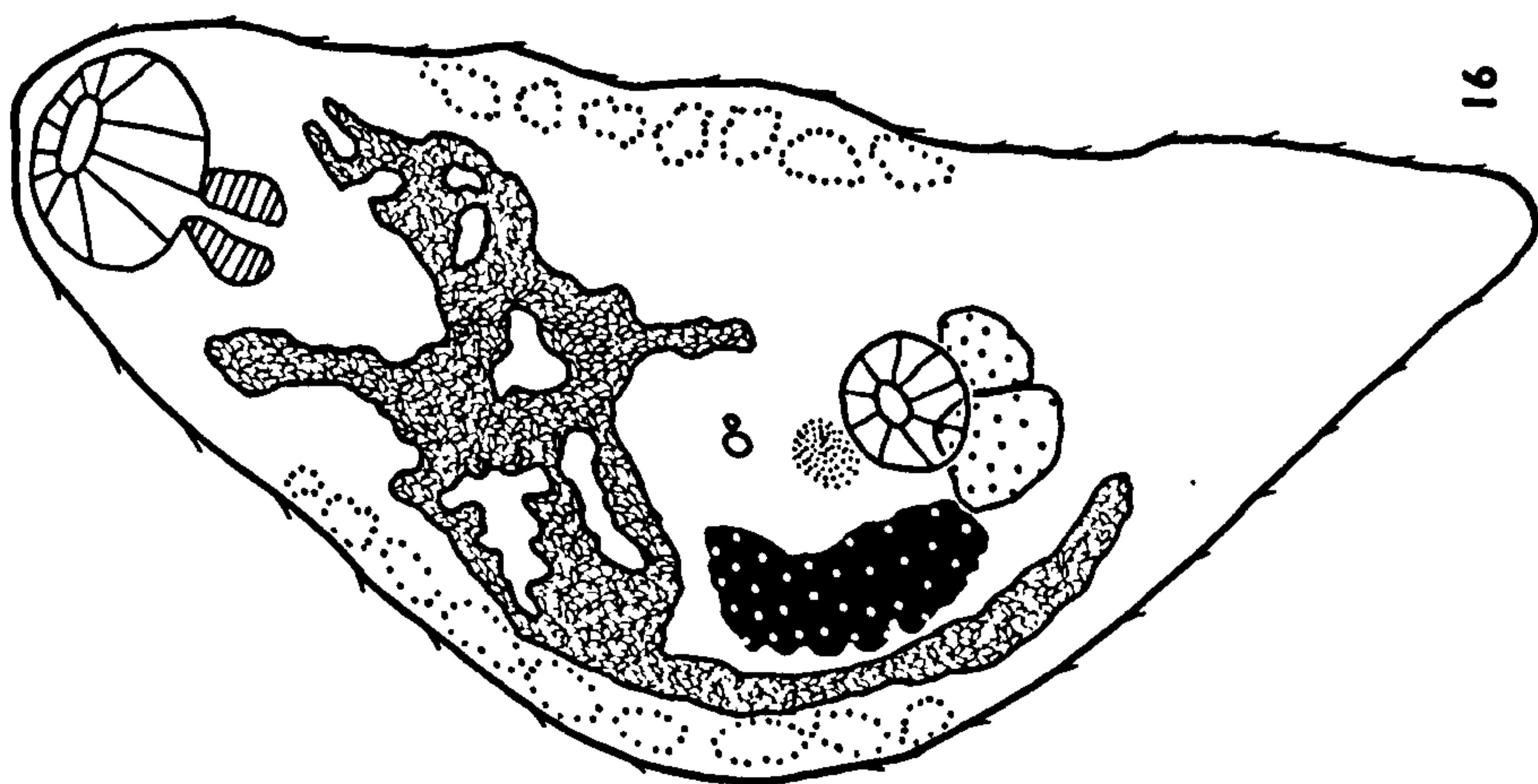
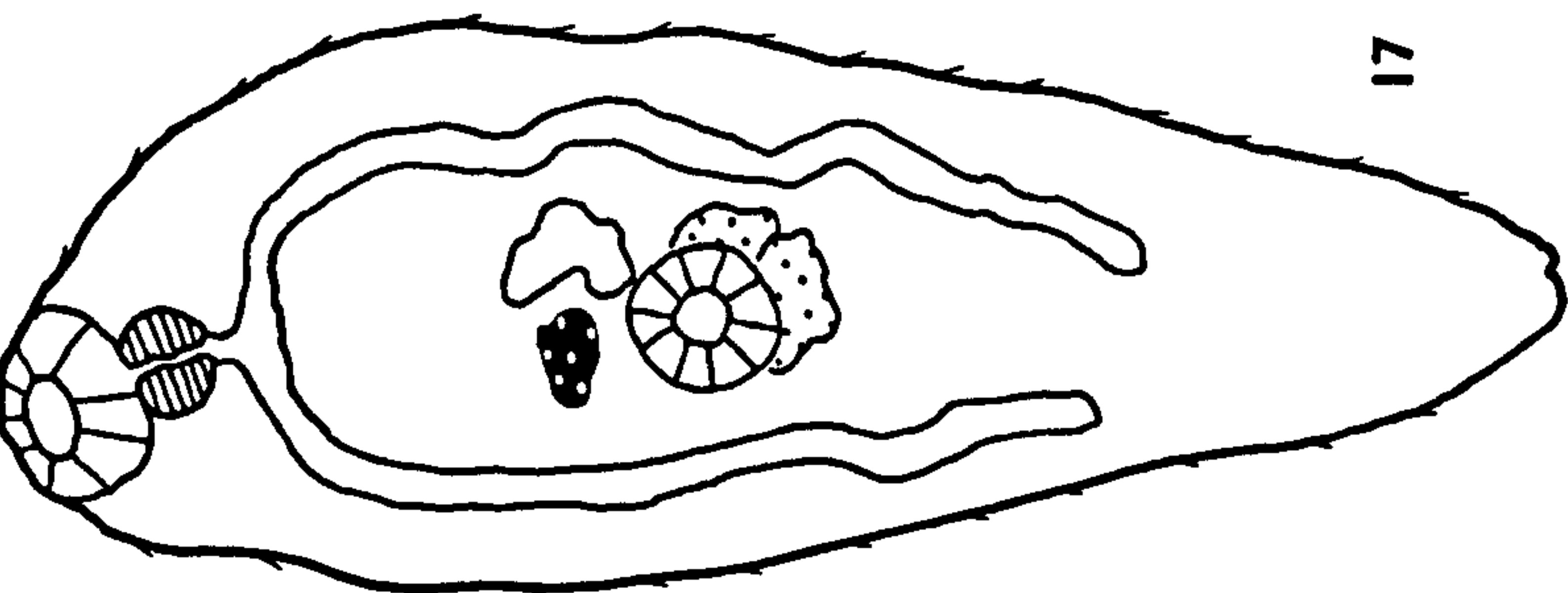
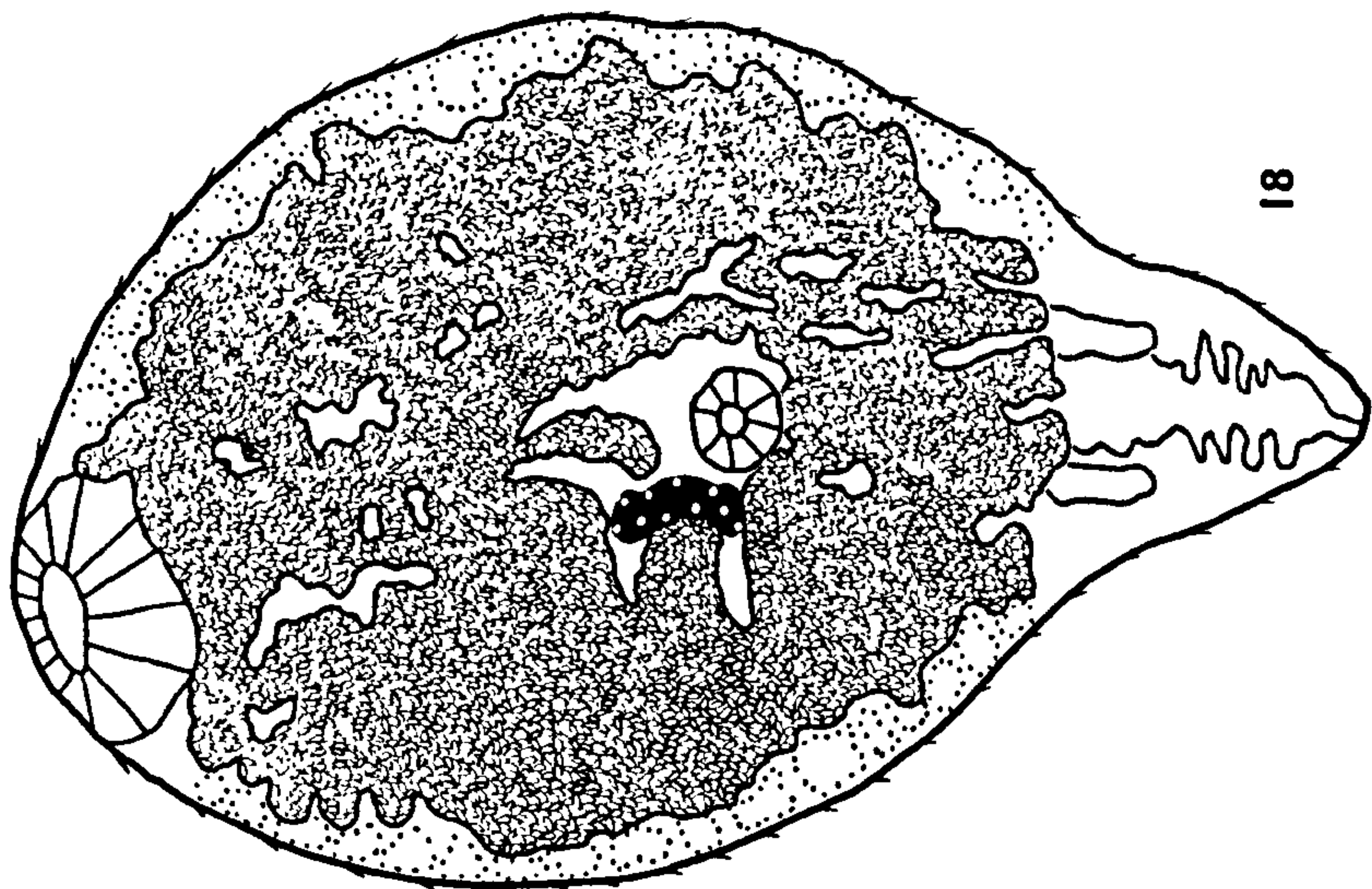
15

1 mm

Fig.16 Renicola sp. from Puffinus puffinus. Mature
 specimen fixed under light pressure.

Fig.17 Renicola sp. from Puffinus puffinus. Young
 fluke.

Fig.18 Renicola sp. from Puffinus puffinus. Gravid
 specimen.

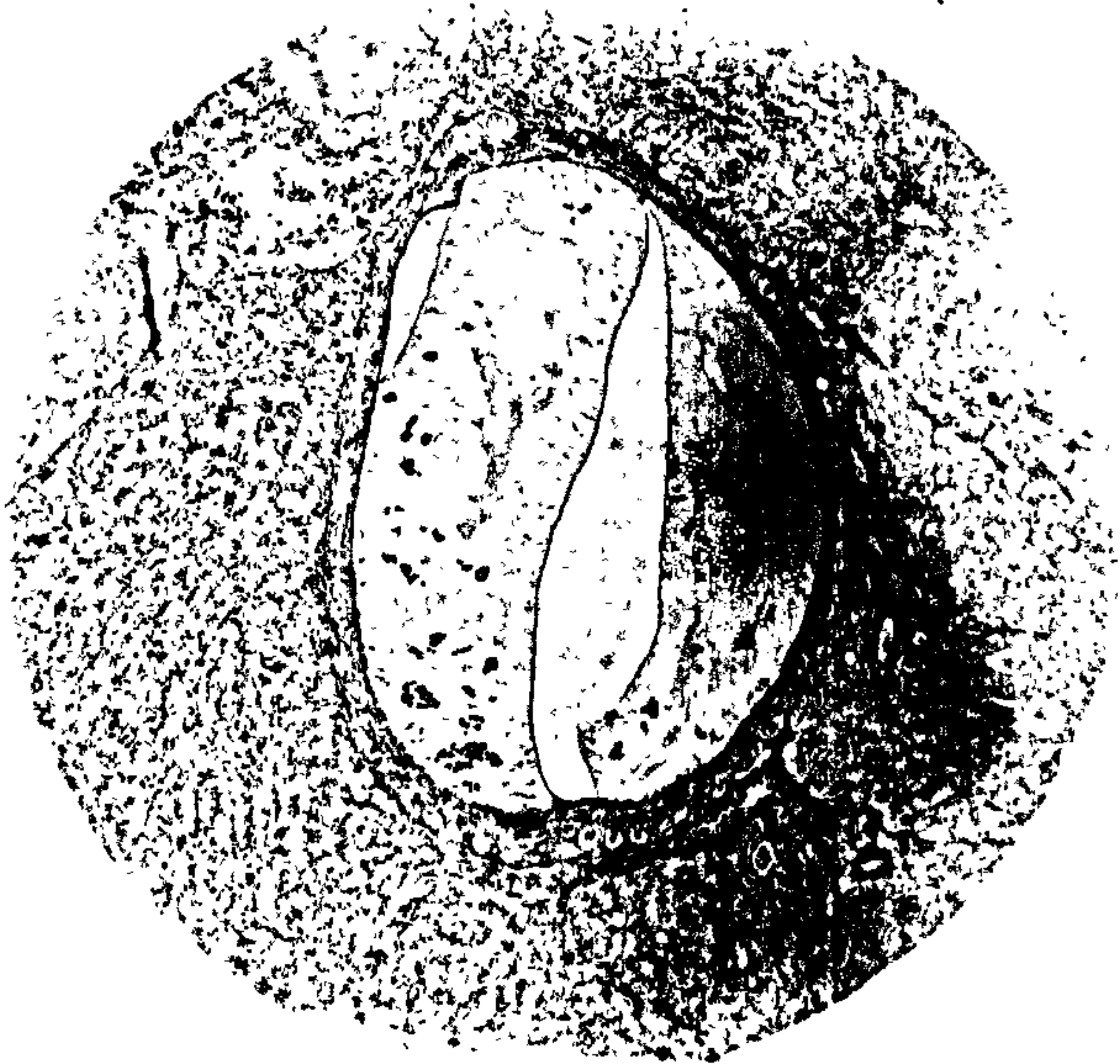
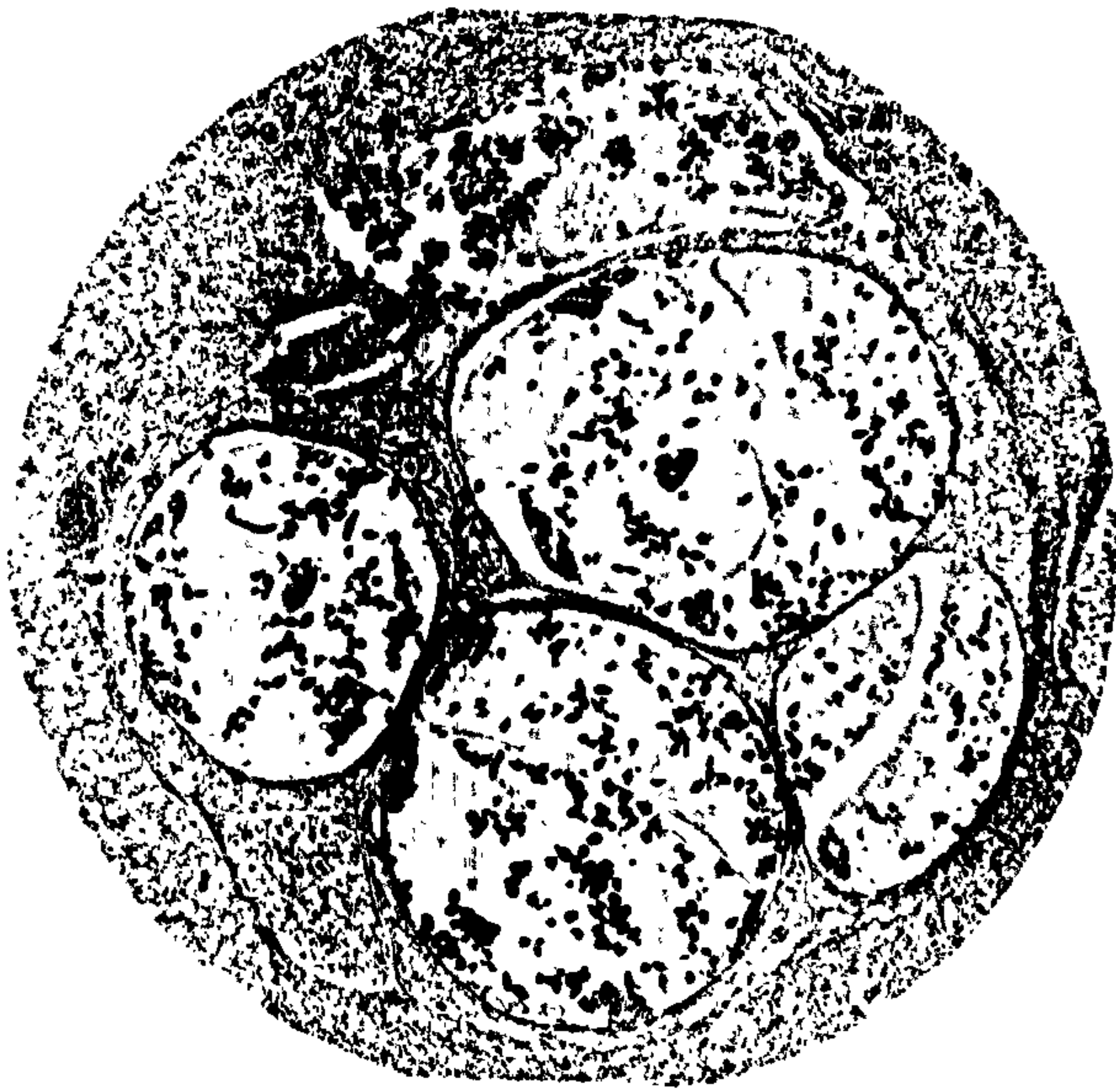


1mm.

1mm.

1mm.

- Fig.19 Photomicrograph of a section through a kidney
 of Puffinus puffinus heavily infected with
 Renicola sp.
- Fig.20 Photomicrograph of a section through a kidney
 of a penguin infected with Renicola sloanei.



The Molluscan Intermediate Host

All of the species of the "Rhodometopa" group of cercariae so far described have been found in Turritella communis Risso, a prosobranch gastropod. The genus Turritella is well known geologically and the species T. communis has been reported from Pleistocene deposits in Britain. Fleming (1828) mentions ten species of Turritella as being found in British waters, differentiating the species on the characters of the shell. The present view is that these are no more than local varieties of T. communis. These local variations are very marked, specimens from Millport and Plymouth being easily distinguished from one another, while a number of specimens kindly obtained for me from Naples by Miss Rothschild were recognized with ease when mixed with either of the foregoing groups. The variability is mostly in the ridging and roundness of the whorls, the depth of the suture between them and the colour and average length of the spire. Specimens from Millport have rather short, rough shells with a somewhat chalky appearance and the whorls slightly rectangular in cross-section while those from Plymouth are larger, smoother, a deeper shade of pink and the whorls are more rounded in section. These differences are probably governed to a considerable extent by the nature of the bottom on

which the snails live, the mud at Plymouth being much more sandy than that from Millport. Specimens collected from St. Bride's Bay, off the Pembrokeshire coast, tended to be more like those from Millport and a similarity in the type of mud was also noticed. The albino variety, T. communis var. nivea was found both at Millport and in St. Bride's Bay, but not in samples from Plymouth.

The snails live only on mud bottoms, almost entirely buried, with the shell roughly horizontal a centimetre or two below the surface. Only parts of the head, foot and mantle are exposed. They are filter feeders, drawing in fine particles by means of a ciliary current. Yonge (1946) considers them to be exclusively filter feeding while Graham (1938) suggests that the radula may also be used. Lebour (1933) states that T. communis kept in plunger jars, for observations on breeding, could be seen browsing on the algae growing on the sides of the jars. Observations during the present work seem to indicate that mucus-containing food particles is passed from the food groove to the foot where it is formed into masses by the cilia and these are then "licked" off by means of the radula.

In natural infections of Turritella with cercariae of the "Rhodometopa" group, only one snail less than 30 mm. in shell-length was found to be infected.

Rothschild (1935) states that no snails less than 30 mm. long were found to carry the parasite and Perez (1936) states that only adults were found to be infected. Various explanations of this have been sought and the following considered: firstly, the sporocysts infect the gonad of the host and the possibility that the gonad is not fully developed until the spire reaches a length of about 30 mm. seemed reasonable. This was found to be not so, a well developed gonad having been found in many specimens with a shell length of 25 mm. and in one of only 23 mm. Secondly it was thought that the size of the trematode egg might be too large for the filter-feeding ciliary current of young snails but in a private communication Dr. John Morton considered this to be unlikely and experimental observations showed that the eggs were easily taken in by snails of 20 mm. shell length. Lastly, it has been observed that when dredging on mud banks for Turritella it is unusual to find small specimens with the large ones, even when a cone dredge with a canvas lining through which no snails could be washed out is used. This suggests that it is not until a certain size of spire is reached that the snails settle down to a sedentary, filter-feeding life on the mud and that the smaller specimens are more active,

possibly moving around and feeding mostly by means of the radula. In tanks in the laboratory where the snails are provided with a soft substratum in which to burrow it has been observed that the smaller ones move around a good deal and are frequently found climbing the glass sides of the tanks. In a private communication these observations are confirmed by Prof. Graham who also considers this explanation to be the most probable one.

Another feature of natural infections of this trematode in Turritella is that female snails are rarely infected. Rothschild (1935) made a similar observation but Perez (1936) stated that the proportions of the sexes in infected specimens from the bay of Morlaise were roughly equal. The explanation given by Rothschild that the male gonad is active throughout a greater part of the year than the female and is therefore a more congenial host tissue for the parasite, providing a greater supply of food material, seems very reasonable. A complication of the facts in this connection is that in all of the ^msamples of Turritella examined during this work the females were always outnumbered by the males, but the females have a larger average size than the males. Fig. 21 shows the distribution of the male and female snails in a sample of 719 specimens from St. Bride's Bay,

also the distribution of the infected snails within the two sex groups. This sample has an unusually low infection rate, about 4%, and the fact that 14% of the infected specimens are females is also unusual. However, the diagram does show the distinctly larger trend in size in the females and also illustrates that scarcely any small specimens have been obtained with the large ones even although in this case both cone and mesh dredges were used. The few specimens of less than 30 mm. length which were found did in fact come from the mesh dredge and not the cone, in spite of extremely careful washing and sifting of the mud in the latter. This sample represents the results of two hauls only in about the centre of St. Bride's Bay (area A in Fig.21a) at a depth of about sixteen fathoms. Other hauls immediately to the north of Skomer Island and between Skokholm and Skomer islands (areas B and C respectively in Fig.21a) were entirely unproductive of snails. A small haul obtained at a depth of between five and seven fathoms in South Haven, Skomer island (area D, Fig.21a) showed an infection rate of 17% in forty-nine snails. All the infected ones were males. Unfortunately no samples were obtained from North Haven, Skomer island

where T. communis is reported to be abundant (Bassindale 1945). The low infection rate in the sample from the middle of St. Bride's Bay, several miles to the north of Skomer island may be accounted for by the fact that the principal tidal current in this area flows southward, thus carrying the majority of the eggs shed into the water by the Manx shearwaters nesting on Skomer and Skokholm away from the area where the snails were collected. The rather higher infection rate in the small sample from South Haven is interesting as this bay lies immediately below the area on which the shearwaters nest.

Fig.22 represents the size distribution in a group of snails from Millport and another group from Plymouth. Each group represents two samples from the same general area. Both of those from Millport were taken from the same locality and, although there is no definite evidence to this effect, it seems that the two samples from Plymouth were probably from different localities, one sample containing almost entirely small specimens and the other only large forms. Unfortunately no definite information as to the exact source of the small-sized sample can be obtained as it might provide useful evidence to support the theory put forward above concerning

ecological differences connected with size.

It can be seen from Fig.22 that the one infected specimen of less than 30 mm. shell length (27 mm.) came from Millport and that the size ranges of the infected specimens from this source are lower than those from Plymouth. The figure also illustrates that a high proportion of snails from Plymouth reach a considerably larger size than the maximum recorded from Millport. In view of this it does not seem that the presence of this small, infected specimen from Millport has any material effect upon the habitat-size theory. The rather arbitrary length of 30 mm., derived from observations on material from Plymouth and Naples, must be reduced to some extent when applied to snail communities in which the average size is considerably smaller.

The percentage infection rates in samples of one or two hundred snails are obviously of no great value as Turritella is very common in the localities where it is found and such samples bear very little relation to the total population. It is interesting to note however, that in a sample from Millport obtained in 1953 an infection rate of 2.62% was recorded while in a slightly smaller sample in 1954 12.82% infected snails were found. Similarly, samples from Plymouth have

shown infection rates of 3.1%, 7.9%, 5.8% and 11.25%, the average rate being about 6.9% in 360 snails, comparing with 8% in 541 snails recorded by Rothschild at Plymouth in 1935.

In spite of its specialized habitat and feeding requirements, Turritella communis is a relatively easy mollusc to maintain in the laboratory. Individuals have been kept in a finger-bowl with two or three inches of sea-water for several months, but they usually die in two or three weeks under these conditions. In the early stages of this work whenever snails were ordered from one of the marine biological stations a quantity of their mud was included in the order and was used as a substratum in the plunger jars in which they were kept. Only "outside" sea-water, collected some miles from the coast and supplied by the marine laboratory at Plymouth, was used. The use of mud in the jars was found to have several disadvantages in that it "went off" after about a month and also that the snails became quite deeply buried in it so that when they were removed for examination the mud was disturbed a great deal. It then had to be allowed to settle for about two days before snails could be returned to the same jar as when they are placed in water with a heavy

suspension of mud particles their inhalant passages become choked and they soon die. Another and more obvious disadvantage is that when mud in the tanks is renewed there is a very slight chance of the introduction of trematode eggs which would upset controlled infection experiments. The mud was replaced by carefully washed fine sand from inland sources where the chances of trematode eggs being present were very small. Although not able to burrow so rapidly in this medium as in the fine mud the snails appear to experience little difficulty in assuming their normal positions. A quantity of granular animal charcoal is mixed with the sand to reduce the effects of accumulated nitrogenous waste. This medium although very satisfactory from most points of view is sterile and contains no food material for the snails. The difficulty of obtaining a finely divided food with a reasonably balanced proportion of constituents has for some time been a major obstacle to the keeping of filter-feeding organisms in sterile cultures. The usual solution to the problem is the separate culture of diatoms which can be added to the tanks from time to time. This was not practicable due

to the uncertainty of results and the large amount of additional labour involved. It is essential that whatever the food material that is used it should be as insoluble in sea-water as possible, otherwise bacterial action will rapidly foul the water. For this reason, no protein extracts (Marmite, Bovril, etc.) are of any use. After several not very successful attempts with such materials as finely ground Bemax or porridge oats a successful substance was found in finely ground, dried yeast to which no other substances had been added. The particle size of a yeast cell is easily dealt with by the filter-feeding mechanism of the snail. Any yeast not immediately taken up by the snails does not appear to multiply, presumably due to the osmotic pressure of the sea-water and lack of carbohydrates in solution and it does not appear to decay rapidly.

Plunger jars are no longer used as they seem to be of little advantage and can go wrong periodically, causing considerable wastage of time. Instead, glass tanks of eighteen inches to two feet depth are used with a perspex cover and a gentle air bubble stream from a porous bubbler. The bubbler is kept about two to three inches below the water surface, just sufficient to maintain—

the aeration of the water and a gentle circulatory current. Polythene tubing is used on the bubblers as rubber tubing disintegrates rapidly in sea-water. Overcrowding can cause an increase in the death rate but if about one square foot of substratum is allowed for each fifty snails this is not noticeable. An additional refinement is to add a small amount of finely ground marble chips to the sand in order to maintain the calcium concentration of the water. If this is not done, after several months in the same water, the most recent additions to the final whorl of the shell are extremely thin and easily damaged. The density of the sea-water (1.0264) is maintained by the addition of glass-distilled water at about fortnightly intervals to replace the loss due to evaporation caused by the bubblers.

Provided that these conditions are maintained and the water temperature is kept as low as possible by the continual circulation of cold, fresh water around the outside of the tanks, Turritella can be kept alive more or less indefinitely in the laboratory.

Fig.21 Histogram of the size and sex distribution of Turritella communis samples from St. Bride's Bay.
Numbers of infected snails are indicated by the blocked-in areas.



Fig.21A. Map of St. Bride's Bay with the areas from which Turritella communis were collected marked.

Reproduced from Admiralty Chart No.1410 with permission of the Controller, H.M. Stationery Office, and of the Hydrographer of the Navy.

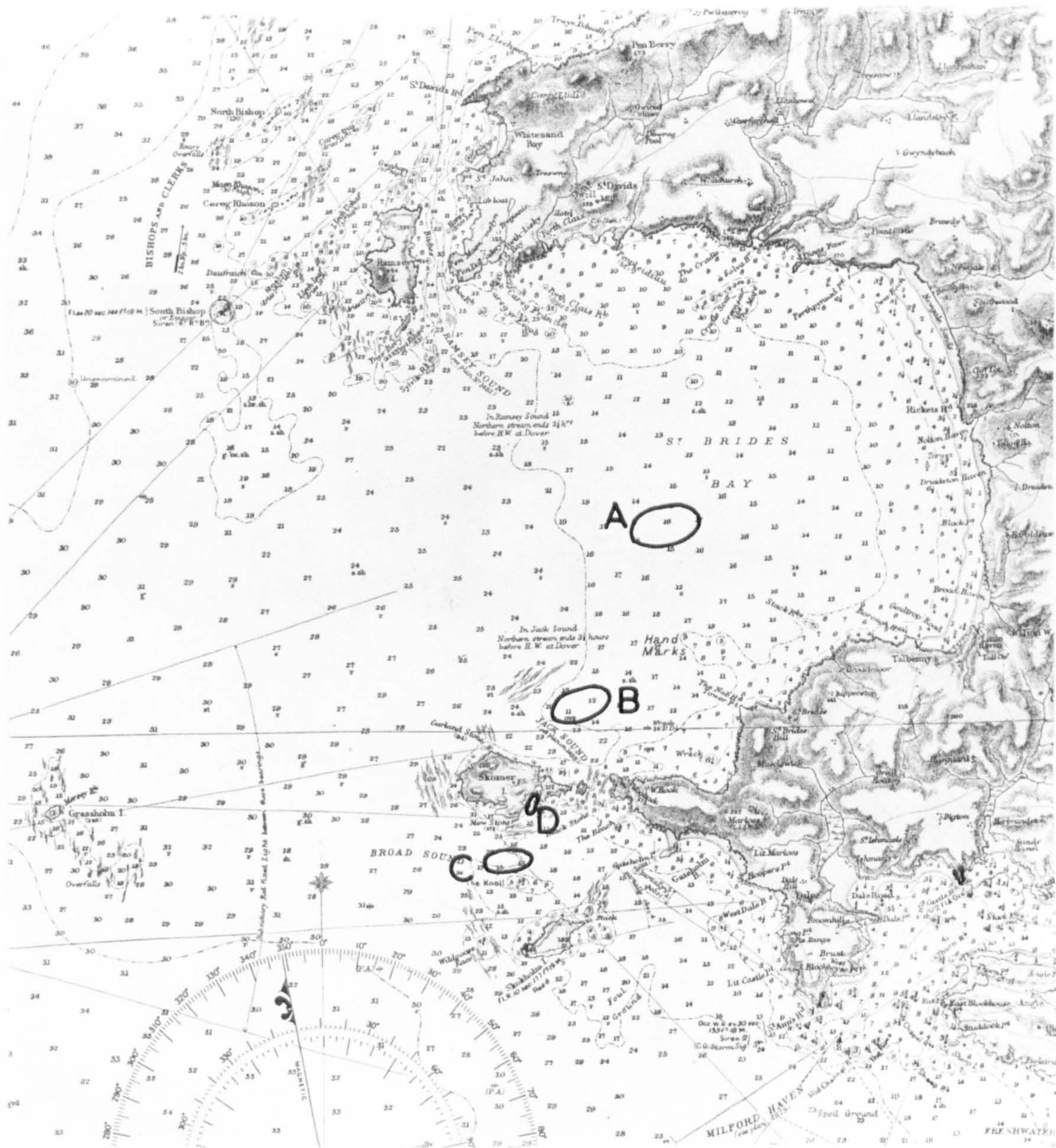


Fig.22 Histograms showing the size distribution of
 Turritella communis from Millport and
 Plymouth. Numbers of infected snails are
 indicated by the blocked-in areas.



The Egg, Miracidium and Infection experiments

The formation of the egg-shell in Fasciola hepatica has been very fully described by Stephenson (1947) and from observations on Renicola sps. during the present work there is no reason to believe that the process is in any way very different. No conclusions as to the validity of Stephenson's theory on the function of Mehlis' gland were reached. The similarity between the changes described by Stephenson for the eggs of Fasciola in their passage from the ootype through the uterus and the changes observed in the eggs of Renicola indicate that the process of the maturation of the egg-shell is probably the same. The same shrinking and darkening in colour of the egg-shell which indicates the change from the ortho-dihydroxy-phenol and protein complex to the quinone tanned leather is very apparent. In Renicola, however, the uterus is a good deal longer than in Fasciola and its convolutions are extremely complex. Stephenson suggests that the oxidation of the polyphenol to a quinone (the main cause of the darkening in colour of the egg-shell) occurs in the anterior part of the uterus, the coils of which are surrounded by a special concentration of a generally

distributed tissue haemoglobin. In Renicola the coiling of the uterus appears to be rather erratic as in different specimens the beginning of the oxidation process can be observed to take effect in different parts of the body.

As mentioned by several authors who have described various species of Renicola, the eggs are fully embryonated by the time they reach the genital pore. No estimate can be made of the time taken for an egg to traverse the whole length of the uterus, but presumably the convolution of the uterine tube is to give the embryo time to develop before reaching the uterine sac. As mentioned in an earlier paper (Wright 1954b) it appears that the contents of the uterine sac can be discharged all at once rather than as a steady stream. The exact reason for this is not easy to see unless there is some advantage to be gained from a mass of eggs descending the ureters together rather than singly.

Attempts to hatch eggs isolated from the droppings of birds or dissected from the uterine sac of a mature worm by placing them in fresh, brackish or sea-water with or without some of the excretory material of the bird have all proved unsuccessful. When placed in

sea-water the eggs sink. The size of the eggs of most species of Renicola is small, the largest on record being those of R. tertia Skrjabin which are described as 0.057 mm. long by 0.024 mm. wide. In all of the infections recorded in Britain the egg lengths are between 0.028 mm. and 0.038 mm. Particles of this size in the sea would be sorted by the currents and tides into the zones where mud banks are formed and hence into the vicinity of communities of Turritella communis. The significance of the theory postulated in the preceding section concerning the differences in the habitat of young and older snails can now be seen. It will be shown later that small snails are not immune to infection by eggs of Renicola sps. but due to their different ecological requirements they are unlikely to become infected until they reach the size at which they take up a sedentary life in the mud.

As has been stated above, attempts to hatch eggs in water failed. This suggested that they would only hatch after ingestion by the molluscan intermediate host, an idea supported by the small size of the eggs and the observed lack of eye-spots on the contained miracidia. Experiments to show this have been attempted but due to the rarity of material they are not as

complete as might be desired. Single specimens of Turritella were placed in small glass capsules with sea-water and fine sand on the bottom and left for 15-20 minutes to settle down and assume their normal feeding position. A capsule was then placed on the stage of the binocular microscope and the beam from an intensity lamp focused on the extruded part of the snail's body. The immediate reaction of the snail was to withdraw, but after a few moments it usually emerged once more and large numbers of ciliates, which appear to live within the mantle cavity, come out and swim around the foot and head. The first attempts to feed eggs to snails were always preceded by the exact location of the inhalant water current by means of a carmine suspension in sea-water. When this was known accurately a heavy suspension of eggs of Renicola sp., dissected from the uterine sac of a mature worm, was pipetted onto the surface of the water immediately above the inhalant opening in the snail's mantle folds. As the eggs sink past this opening they are drawn into the mantle cavity and reappear a short while later moving forward in the food groove on the right side of the head. After the snail had actually been observed to ingest the eggs it was removed from its shell and

dissected. In the stomach and intestine numbers of eggs have been observed, the mature ones with their operculum open and miracidia emerging or swimming nearby. Unfortunately accurate observations on the miracidia under these conditions have been impossible due to the rather dense and viscous gut contents of the snail and the presence of large numbers of ciliates which are either parasitic or symbiotic in the snail. These ciliates are so similar in size and movement to the miracidia that once the latter have left the vicinity of their open egg-shells they are extremely difficult to identify. Attempts to make eggs hatch by placing them in the fluid from a stomach or intestine freshly removed from a snail were unsuccessful. It seems that a sequence of events, possibly involving several digestive enzymes and physical movements of the living gut of the snail, are necessary to induce hatching of the eggs.

Such observations as have been made on the miracidia have been achieved by subjecting eggs on a slide to pressure beneath a coverslip. Considerable pressure is needed and fresh eggs most frequently split at the anopercular end rather than round the suture of the operculum. Eggs which have been preserved in formalin

for some time open more readily at the operculum.

The miracidia (Fig.23) are roughly pear-shaped, the tapering anterior end being very mobile, capable of considerable retraction. They are not uniformly ciliated, but the cilia, which are long, appear to be restricted to a "collar" about mid-way along the tapering anterior end. No eye-spots are present but two large cells of somewhat irregular outline just behind the anterior end may represent some form of penetration glands. No other details of the internal anatomy have been worked out.

Experimental infections of groups of Turritella communis have been carried out and details of these experiments will now be given.

Because of the impossibility (at present) of rearing Turritella from the egg beyond the late veliger stage (Lebour 1933), it is necessary to use for infection experiments snails which have been collected and kept in aquaria. To ensure as nearly as possible that the snails under experiment are free from natural trematode infections they must be kept for some time in the laboratory before they are used. During this time they are tested for shedding of cercariae at intervals of at least a week. For reasons to be shown in the

dealing with sporocysts and cercariae this testing is only of use at certain times of the year and must be repeated at frequent intervals. For testing for infection the snails are isolated in sea-water in 3" x 1" glass tubes and left overnight. Any cercariae in the tubes are easily seen with the naked eye and the snails which shed them are segregated. When, after repeated tests, no further "shedders" are found and no cercariae are seen in the tank in which the snails are kept then that batch is considered not to be carrying natural infections. The method is of course not infallible and any results of such experiments must be treated with considerable caution.

The method of infection used in all the experiments was as follows: a group of snails was isolated in 3" x 1" glass tubes and to each tube a drop of sea-water containing a suspension of eggs of Renicola sp. was added. The suspension was made by removing the uterine sac from a gravid worm and teasing it out in sea-water. Unavoidably, a considerable number of immature eggs are also taken in this way. Attempts to estimate the numbers of eggs per drop by means of a McMaster egg-counting slide were not satisfactory as the eggs sink to the bottom of the chamber and there is a tendency to

clumping so that uniform concentration cannot be guaranteed. The suspension was kept continually agitated so that every drop contained at least a few eggs. The disadvantages of this system are obvious, but in practice it was found to be the only method that was easily workable. After adding the egg suspension to each tube the snails were allowed to remain in the tube for varying periods and were then returned to one of the tanks.

Infection experiment 1.

Forty-two snails were exposed to infection in the manner described above, on 10 April 1953, with eggs of Renicola sp. from Colymbus arcticus. The snails were left in contact with the eggs overnight before being returned to the tank. They had been tested for shedding over a period of four and a half months before being used, even so cercariae were found free in the tank only ten days after the infection date. The batch was isolated and two shedders heavily infected with sporocysts were found and removed.

Rothschild (1935) reported keeping a Turritella in a plunger jar for six months and then, on dissection, finding a young infection of daughter sporocysts, indicating a very slow rate of development. In view of this

it was decided not to sacrifice many of the snails in the early stages of infection due to the rarity of material. One snail was dissected each week during the first month but no trace of infection could be found in any, even by sectioning of the gonad. From then on one snail was dissected each fortnight and no signs of infection were found until three and a half months after exposure to the eggs. One female, 35 mm. in shell length, was found to have very immature sporocysts present in the gonad (Fig.34). From that time onward no further snails were dissected, in the hope that the infections might mature and cercariae would be produced enabling a correlation between the adults and larvae to be obtained. Five months after infection only fourteen snails were left alive and by nine months from the date of infection all of these were dead with no cercariae having been produced.

This experiment is very inconclusive; the young sporocysts found in the one female snail were much earlier than those reported by Rothschild after six months. Miss Rothschild was kind enough to lend me some sections of this individual for comparison. In this experiment the snail had been in laboratory tanks for just over eight months when the infection was found.

The absence of any traces of sporocysts in the others which were dissected is difficult to explain. If any further development occurred in the remaining snails it was not found when they were examined after death because most were too decayed even for the sex to be recognizable.

Infection experiment 2.

Sixty nine snails were exposed to infection on 15 July 1953 with eggs of Renicola sp. from Puffinus puffinus and were left in contact with the eggs in the tubes overnight before being returned to the tank. This batch was composed of selected snails, all over thirty millimetres in length and had been kept in the laboratory and tested for four months before use.

As in the previous experiment sample snails were selected and examined, but only at fortnightly intervals. No signs of infection were found and after three months the whole batch was dead.

In this experiment the suspension of eggs used was very heavy and each snail must have received a considerable number. It is possible that this was the cause of the rather rapid death of all the snails. Subsequent work on the development of the sporocysts although not conclusive does lead to the idea that the early stages

may have been overlooked during dissection and that the part reserved for sectioning in each case, the apex of the gonad, may not become infected until a later stage in the development of the sporocysts.

Infection experiment 3.

Sixty snails of varying sizes, ranging from 18-36 mm. shell-length were exposed to infection on 23 July 1953 with eggs of Renicola sp. from Puffinus puffinus. The egg suspension used in this experiment was lighter than that used in the preceding one and the snails were only exposed for six hours instead of overnight.

As before, sample snails were examined at two-weekly intervals. After four weeks, three were examined, two were found to be dead and the third, a 36 mm. shell-length female showed considerable white spotting of the gonad. Sectioning, however, showed no pathological changes and no signs of the presence of any parasites.

Three and a half months after infection the batch was isolated in 3" x 1" tubes and only twenty four snails were found to be still alive. In view of this it was decided to stop fortnightly examinations, once again in the hope that cercariae would eventually be produced.

On 18 January 1954, six months after the date of

infection, the group was isolated once again and only eight snails were found alive. These were dissected and the following observations made: one male of the variety T. communis var. livea of length 24 mm., another normal specimen of the same length with no gonad developed and two females of 26 mm. and 28 mm. shell-length were all uninfected but the other four, all females with shell-lengths of 25 mm., 28 mm., 30 mm. and 30 mm. were found to have young sporocysts in their gonads. These sporocysts were in a more advanced state and were more numerous than those found in the first experiment (Fig. 36).

At the time when the infections were found the snails had been in the laboratory for just over eight months. It is strange if these were natural infections that all should be in small snails and all of them females, a combination of circumstances very unusual in nature, as was shown in the preceding section. If, in fact, they are natural infections then they provide even further proof of the incredibly long time taken for the maturation of the infection in the molluscan host. If the infections are the result of exposure to eggs in the laboratory then it is possible to account for them by the fact that female snails are, for some

reason, partially resistant to infection. Obviously also the suspension of eggs was^hnot uniform otherwise the other survivors might have been expected to be infected as well. The probable explanation is that these partially resistant snails only received a light dose of eggs compared to the others which may have had a lethal dose.

The experiment does show that small snails are not immune to infection, further evidence in support of the theory of ecological immunity given earlier.

Infection experiment 4.

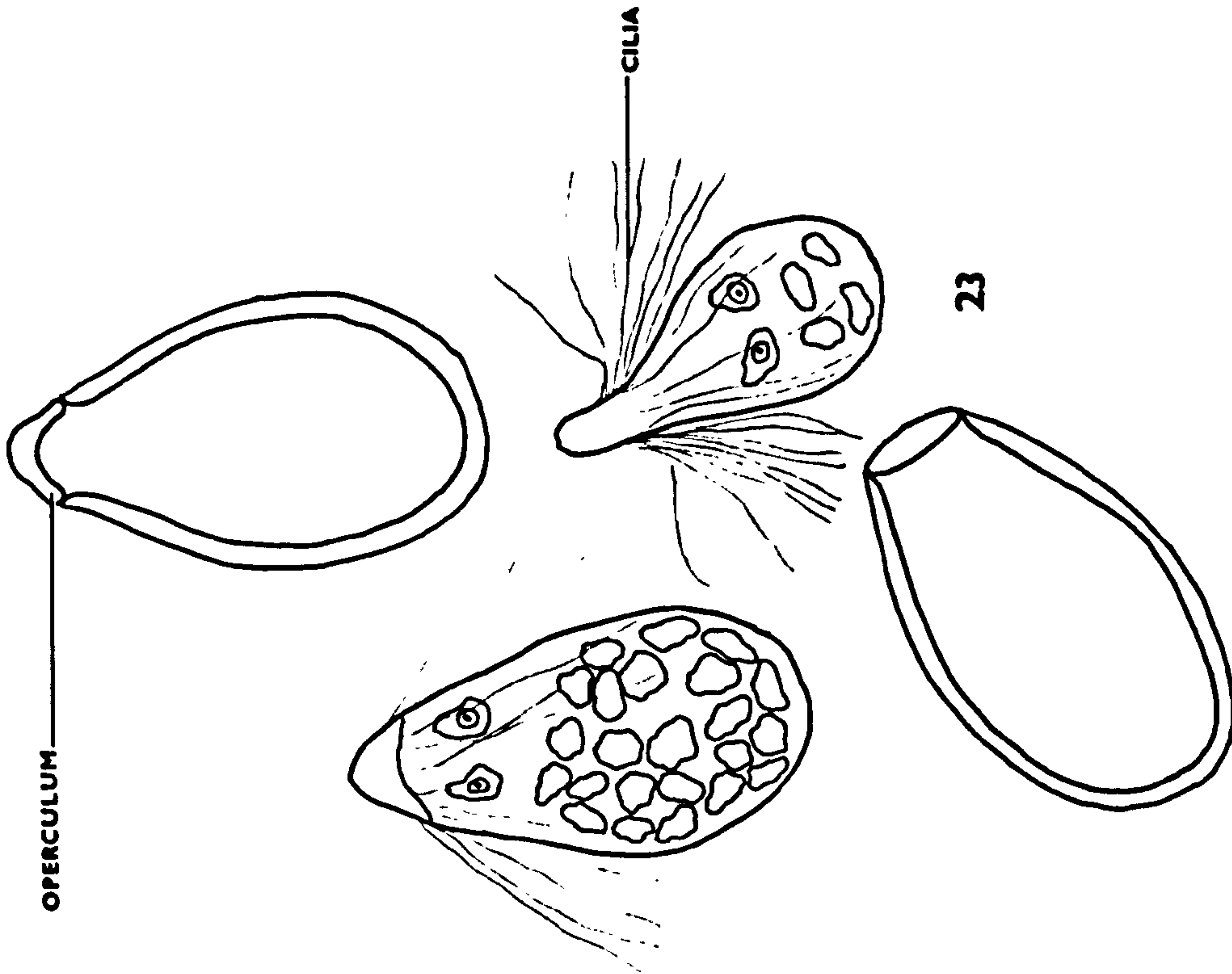
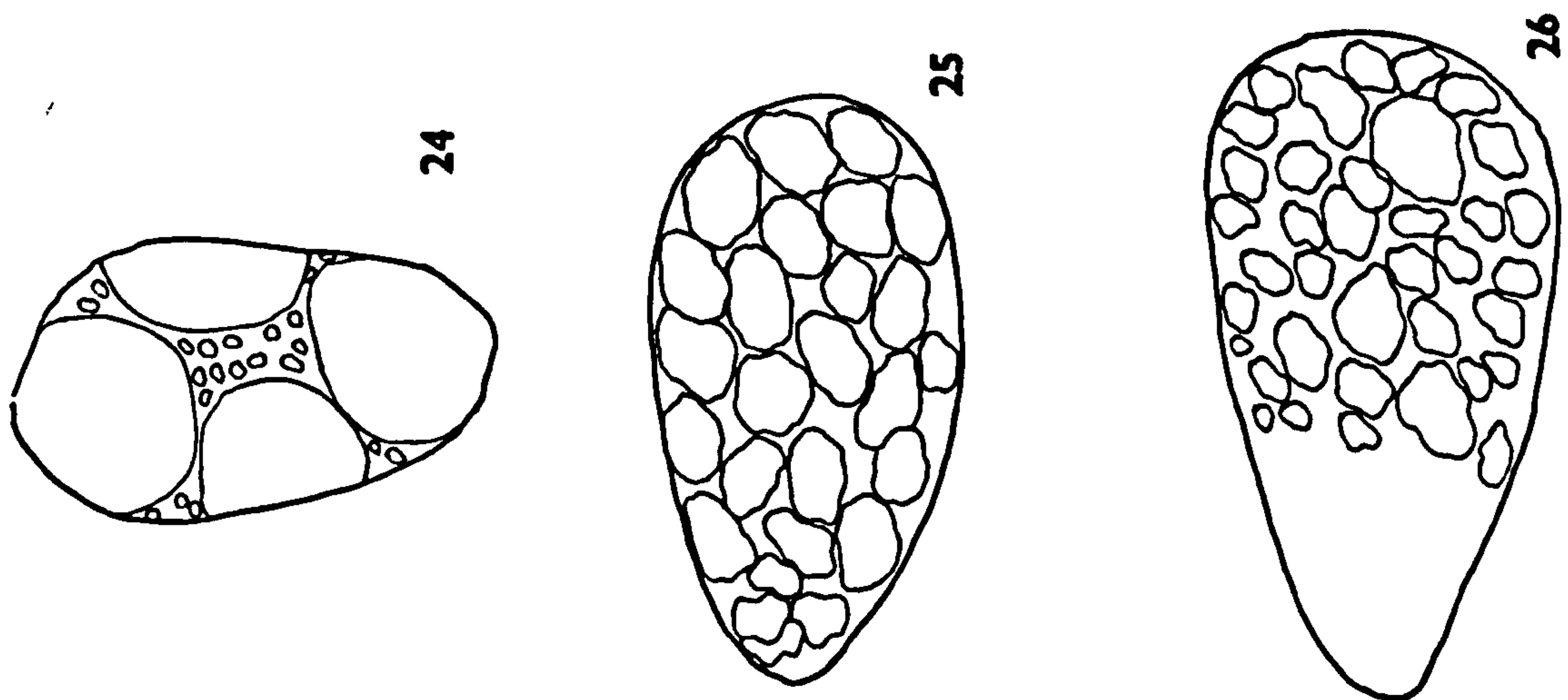
Thirty snails were exposed to infection with eggs of Renicola sp. from Puffinus puffinus on 30 June 1954. These snails had only been kept in the laboratory for a month and a half before being used but frequent testing during that time had revealed a large number of shedders which were segregated and no cercariae had been seen in the tank for two weeks before the date of infection. As a result of experience gained in the three previous experiments only a light suspension of eggs was used and the snails were returned to the tank after only four hours in contact with the eggs. In order to test whether any increased death rate was due to infection a control batch of a similar number of

snails from the same source as the experimental group were kept under as nearly identical conditions as possible in a neighbouring tank. After seven days five of the experimental group and five of the controls were dead, presumably as a result of the interference and handling necessary during the preparation of the experiment. Exactly four weeks after the infection date one of the experimental group was dead but no further casualties had occurred in the control batch. One of the experimental group was dissected but nothing indicative of infection was seen.

This experiment is still not completed. It is hoped ~~XXXX~~ that with the improved conditions in the tanks and light dose of eggs cercariae may eventually be produced.

- Fig.23 Eggs and miracidia of Renicola sp. from
Puffinus puffinus.
- Fig.24 Mother sporocyst (?) from intestine of
Turritella communis 24 hours after exposure
to eggs of Renicola sp.
- Fig.25 Mother sporocyst (?) from intestine of
Turritella communis 48 hours after exposure
to eggs of Renicola sp.
- Fig.26 Mother sporocyst (?) from intestine of
Turritella communis 3 days after exposure
to eggs of Renicola sp.

0.05 mm



0.05 mm

Fig.27 Photomicrograph of eggs of Renicola sp. from
 Puffinus puffinus.



The Sporocysts

Historical

An examination of some of the literature on this stage in the development of trematodes reveals a considerable variance of opinion on the structure of sporocysts and the function of various organs and cells found in them. This is possibly due to the fact that most general works refer only to the mother sporocyst and go into considerably more detail on the structure of rediae than of daughter sporocysts. Baer (1951) states that sporocysts have no mouth or intestine and that some rediae which have a poorly developed pharynx and no intestine closely resemble sporocysts. On the other hand several sporocysts are described as having a pharynx, e.g., Cercaria pekinensis Faust, 1921 which has a "pharyngeal sphincter" but no gut, and the sporocysts of the "Rhodometopa" group which, according to Rothschild (1935), have a pharynx "similar to that of C. pekinensis Faust, but surrounded by secretory cells resembling the "salivary" glands of rediae".

The histology of the sporocyst wall is an even more controversial subject. Stunkard (1932) states that the wall of the sporocyst of Cercaria rhodometopa consists of two layers, an outer one packed with deeply

staining nuclei and an inner layer of cells with a few faintly staining nuclei. The cell boundaries in both layers are indistinct. Rothschild (1935), describing the body wall of the sporocyst of C.pythionike, which, she says, is typical of the other members of the "Rhodometopa" group which she examined, refers to five layers. These are: an outer layer of primitive epithelium consisting of ill-defined, flattened cells with discoidal nuclei; a second layer of large, non-vacuolated cells with deeply staining cytoplasm and nuclei; a third layer of muscle fibres; a fourth layer of large, vacuolated eosinophil cells, most prominent in the anterior region but with reduced cytoplasm elsewhere and finally a fifth and innermost layer of epithelium, again of flattened cells with poorly staining lenticular nuclei. This seemed to indicate such a wide divergence from Stunkard's view that a brief check was made on some other descriptions of the histology of sporocysts in other groups. Harper (1929) describes the sporocyst of Cercaria X. 1 as having a body wall composed of two layers, an outer one of flattened cells and an internal epithelium which is most easily seen in young specimens. For Cercaria X.3 Harper describes a delicate outer epithelium

and states^{that} the cercariae arise from a germinal epithelium lining the whole sporocyst cavity. Faust (1921) gives no details of the histology of the sporocyst wall of C. pekinensis. Hyman (1951) describing a generalized sporocyst states that it is composed of an outer cuticle below which the miracidial muscles and mesenchyme persist. She illustrates a section through such a sporocyst and shows the outer cuticle, then a layer of circular muscle, followed by longitudinal muscles and then a thick, innermost layer of mesenchyme with some germinal cells embedded in it and others free in the lumen. This obviously refers to a mother sporocyst. Cort, Ameel, Van der Woude and others in a series of recent publications have shown that generalizations concerning the structure of larval trematodes cannot be made and that differences of the nature discussed above are of importance in the determination of a natural classification of the Trematoda. In this connection it is interesting to note that these workers (1954) refer to Paragonimus kellicotti as a member of the Troglotrematidae. The second larval generation in the molluscan intermediate host of this fluke is a redia. The present work shows that the corresponding stage for Renicola is a daughter sporocyst, a fact that lends

weight to the splitting of the family Troglotrematidae on morphological grounds by Dollfus (1939).

Further controversy is encountered with respect to larval reproduction at the sporocyst stage. Baer (1951) states that a mother sporocyst can only produce a single generation of daughters. This is supported by the recent papers by Cort and his co-workers, but they suggest that the mother sporocyst may produce daughters over a short period of time, not all at once as is suggested by Baer's statement. Faust (1921) described the sporocysts of C. pekinensis as some containing only sporocysts, others with sporocysts and cercariae while others contained cercariae alone. Rothschild (1935) described and figured budding and division of sporocysts of the "Rhodometopa" group as occurring both in young, immature sporocysts and again in mature forms after a large emission of cercariae. No other reference to this kind of reproduction of sporocysts has been found, but Harper (1929) refers to the fusion of sporocyst walls in heavy infections of Cercaria X.3, a phenomenon which might give the appearance of division in sections.

The area of the sporocyst wall concerned with the production of cercariae is another important factor in

the natural classification of the Trematoda. Cort et al. (1954) describe this in some detail for various groups. Rothschild (1935) shows large pedunculated cells with very large nuclei embedded in the fourth layer of the body wall and apparently distributed at random within the sporocyst but most numerous anteriorly. These are described as germ cells but in a private communication Miss Rothschild informs me that she is no longer convinced that this is their true function.

On the macroscopic appearance of the sporocysts of C. rhodometopa Stunkard (1932) states that they are bright orange and curved or coiled while Rothschild (1935) describes those of other species in the same group as varying from yellowish grey to bright orange, brightest at both ends where the wall is thickest, sausage-shaped, tapering slightly at the anterior end and closely packed and entangled but not coiled.

Materials and Methods

Sporocysts examined during the present work were found in infected Turritella communis from Plymouth, Millport, St. Bride's Bay and South Haven, Skomer Island. A number of species are involved but the specific differences at this stage are so slight that they do not affect the general description in any way.

Young sporocysts found in snails exposed to the eggs of Renicola sps. were studied, also a slide of sections of an infected snail gonad lent by Miss Rothschild.

Living sporocysts were examined by dissection with fine tungsten wire needles and neutral red intravital staining as recommended by Cort et al. (1953) for the location of germ cells was attempted. Fixed whole snail gonads with sporocysts were used for sectioning, individual sporocysts dissected from the snails were found to be too delicate for this purpose. Bonin's fixative gave the most satisfactory histological results. Mallory's triple stain was found to be the most useful for these sections as even early sporocysts were easily picked out by their different staining reaction. For fine detail Heidenhain's iron alum haematoxylin was used, also Delafield's haematoxylin with eosin counter-stain.

Development of the sporocysts.

Unfortunately very little of the early development of the sporocysts has been worked out. Snails to which a very large dose of Renicola sp. eggs had been fed were dissected twenty-four hours later and small patches were found on the wall of the intestine. These consisted of a surrounding membrane and contained usually four large cells, two of which stained fairly

easily with neutral red while the other two did not (Fig.24). In some of these five or six of these large cells were found, the additional ones usually being of the type that did not stain with neutral red. Similar objects were not found on the intestinal walls of control snails not exposed to eggs. Further snails which had received large doses of eggs were dissected after forty-eight and seventy-two hours. Similar patches were found but they were larger and contained large numbers of smaller cells, again some staining with neutral red and others not (Figs. 25 & 26). In snails which had been fed large numbers of eggs and not examined until more than three days from the time of infection no trace of these patches was found.

From evidence obtained later it is suggested that the site of the mother sporocyst is in the tissue surrounding the stomach, intestine and rectum of the snail, just below the point in the spire where the digestive gland and gonad begin. It seems likely that the patches on the wall of the intestine, described above, may be the early mother sporocysts which after about three days leave the gut wall and migrate into the surrounding tissue. Due to the shortage of material no confirmatory evidence for this supposition has been obtained.

One reason for suspecting the mother sporocyst to be in the position already described is that young infections of daughter sporocysts appear to spread up the spire. In early infections there are always more young sporocysts near the base of the gonad and the apical part does not appear to be infected until much later. This probably explains the total lack of success in finding young infections after exposure to eggs when only the top of the gonad was reserved for sectioning.

The earliest stages identified with certainty were those found in the female snail three and a half months after exposure to eggs of Renicola sp. from Colymbus arcticus. The largest sporocysts present measured about 0.25 mm. x 0.2 mm., but several small ones of about 0.05 mm. diameter were also seen. These small ones showed scarcely any lumen, it being mainly occluded by parenchymatous cells corresponding to the fourth layer of Rothschild. Numbers of large cells with large vesicula nuclei were present but none with the pedunculate structure described by Rothschild were seen. The wall of the sporocyst was thin, consisting apparently of one layer of cells only, again corresponding to the fourth layer of Rothschild. Outside this and separated

from it by a considerable space was a layer of cells corresponding to Rothschild's second layer. A more advanced sporocyst measuring about 0.18 mm. x 0.1 mm. was found to have the pharynx well developed and the lumen very pronounced. Surrounding the pharynx were several layers of vacuolated cells of the fourth layer type and behind the pharynx a cluster of the poorly-staining cells similar to the "salivary glands" of rediae was present. At the opposite end of the body a thickening of the fourth layer cells containing the large nucleated cells mentioned above formed a knob of tissue projecting into the lumen. The lumen contained about a dozen germ balls of varying size, the largest ones being at the anterior end. Two medium sized germ balls were found adhering to the knob of tissue at the posterior end and just in front of these was a group of rather smaller balls, some attached to one another and appearing to result from the sub-division of one of the medium sized balls. The wall of the body again, as in the small forms, appeared to consist of fourth layer cells which were more than one cell thick in places. As before, outside this and separated from it by some little distance was the second layer.

The largest sporocysts present have a similar

appearance in section to the medium sized ones just described but the germ balls are more numerous and in one a fairly well developed cercaria is present (Fig.32). The cercaria showed an oral sucker, pharynx, main nerve ganglion and ventral sucker all fairly well advanced and the main channels of the excretory vesicle were also formed. The body wall of the sporocyst has the second and fourth layers applied together, no trace of the intervening third, muscular layer could be found. Inside the fourth layer and lining the lumen of the sporocyst a few flattened cells similar to those described by Rothschild as the fifth layer can be made out. At the anterior (pharyngeal) end the second and fourth layers of the body wall separate and the second layer comes to lie some distance away so that the pharynx opens into the space between these layers (Fig. 32). Also, in this region, the second layer becomes confluent with that of a small specimen lying close by. In this small specimen the pharynx can just be seen differentiating from the thickened fourth layer at the anterior end. Immediately opposite to the opening of the pharynx on the larger sporocyst the second layer cells give the appearance of being drawn towards the pharyngoal opening but they do not in fact come into contact with any

of the fourth layer tissue in that area.

The next youngest stages studied were those from the four small female snails which had been exposed to eggs of Renicola ap. from Puffinus puffinus six months earlier. The sporocysts in these are considerably larger, the biggest measuring about 0.83 mm. x 0.26 mm. When alive they were a light greyish-yellow colour. Although not closely packed in any of the four snails they are more numerous than those described in the previous case and several have reached the apex of the gonad. None of the sporocysts is straight, all are at least slightly bent some almost into a V-shape.

In sections of the gonad the sporocysts can be seen to contain numbers of advanced cercariae in addition to germ balls (Fig.35). Except for their larger size and the more advanced state of the contained cercariae the sporocysts in these infections resemble the most advanced forms described in the preceding case. One series of sections was obtained longitudinally through the pharynx of one sporocyst, showing a feature of some interest. As described above, the second and fourth layers of the body wall (no muscle layer has been seen) are separated in the region of the pharynx and this organ opens into the space between the

the two layers. The sections are stained in Mallory's triple stain and the fourth layer cells are of an orange colour. On their outer surface they are covered by an exceedingly thin layer that appears to be slightly corrugated and stains a deep blue colour. This layer is thickest immediately around the opening of the pharynx. It passes down to line the pharyngeal tube and spreads to form a very thin layer on the inner surface of the fourth layer cells surrounding the pharynx. No detailed structure whatever can be made out in this layer, but it gives the appearance of being some sort of a cuticle, possibly secreted by the "salivary gland" cells lying in a cluster immediately behind the pharynx. A further point of interest in these sections is that behind the "salivary gland" cells in the lumen of the sporocyst are some granules exactly similar in size, shape and staining reaction to the yolk granules in the ovary. If these are yolk granules which have been taken in by the sporocyst it is difficult to see how they arrived there. The second layer cells appear to form a continuous sheath enclosing the sporocyst and no granules have been observed in the space between the second and fourth layers in the pharyngeal region. It will be shown in the section

dealing with cercariae that this observation may have some significance. Fig.39 is a photomicrograph of the pharyngeal region of this sporocyst.

These sections also show a possible additional reason for the low natural infection rate in female snails. The lobules of the ovary are rather compact and the sporocysts are forced to lie either near to the surface or encroaching on the less resistant digestive gland. It has been noticed that in naturally infected females the gonad does not appear to be so distorted as it is in males where the testicular lobes are softer and more easily compressed by the growing sporocysts.

The slide of the sectioned gonad of a male Turritella lent by Miss Rothschild is very interesting. This snail had been kept in filtered sea-water for six months before it was dissected. The appearance of the sporocysts is very abnormal, in the sections examined neither a pharynx nor the ingrowth of tissue at the opposite end has been observed. The lumen of the sporocysts contains only a few germ balls (one poorly developed cercarial embryo was seen) and yet the size of the sporocysts is roughly equal to that of the more advanced specimens described above. The body wall shows all five of the layers described by Rothschild.

The sporocysts appear in groups and when lying close together the large-nucleated, darkly staining second layer cells appear to run into^{one} another (Fig.36). Several of the sporocysts appear to show division forms with ingrowths of the body wall exactly similar to those figured by Rothschild, but on the limited number of sections available it seems that these are not in fact dividing sporocysts but are folds in the body wall caused by sharp bending of the body. The tissues of the snail in this case have not got a healthy appearance. The testis is inactive and the digestive gland has a rather "thin" appearance. In a private communication Miss Rothschild informs me that during the six months in which this snail had been kept in the laboratory in filtered sea-water it had had no mud or any other form of food, hence the symptoms of starvation. Under these conditions it is not uncommon for a mollusc to resorb parts of the body, in particular the gonad, and it seems reasonable to suppose that the sporocysts have suffered a retardation of their development as a result.

From the observations made so far several points concerning the probable early course of development of the mother and daughter sporocysts can be suggested. The first of these, concerning the probable location of

the mother sporocyst has already been made. The second point concerns the second layer of the body wall. Rothschild says that it is easily detachable, as a single sheet, from the rest of the sporocyst. As already described, in very young sporocysts the second layer may be completely detached from the rest of the body and merely seems to form a cover in which the sporocyst floats. In later stages as the sporocyst grows it tends to fill out the rest of the cavity and the body wall becomes closely applied to the second layer except in the pharyngeal region where it usually remains slightly detached. Rothschild illustrates a longitudinal section through "a sporocyst of the second daughter generation" in which the external opening of the pharynx is covered by the second layer.

All of these facts lead to the belief that the so-called second-layer (and presumably the primitive epithelium outside it) is not an integral part of the sporocyst body wall. As soon as this is realized the similarity between this layer and the paletot described by Cort, Ameel and Van der Woude (1952) for the Plagiorchid, Lechriorchis primus is easily seen. This structure, the paletot, is a layer of mother sporocyst cells which persists as a sheath enclosing the daughter

sporocysts. It arises as a result of a rapid growth of the mother which branches out while the embryos of the daughters develop within its lumen. The branches of the mother ramify through the organs of the snail, then constrictions in the walls of the branches appear, separating the embryo daughters from one another and giving rise to chains of daughters. Rothschild noted the presence of short chains of sporocysts but interpreted them as resulting from rapid division of a single sporocyst in which the severance was incomplete. The fact that sporocysts occur in groups in the gonad may also be accounted for by this theory, the group marking the path of a branch of the mother through the host tissue. The fact that in young infections some of the sporocysts appear to share the "second layer" between two is probably due to a recent constriction of the wall of the mother separating two daughters from one another.

If this "second layer" really is a palotot, and the evidence definitely seems to indicate that it is, then the third or muscle layer of the body wall is in fact the outer covering of the sporocyst itself, a very unlikely condition. Close examination of this layer indicates that it may well be an epithelium of a "stratified" type in which the nuclei are very difficult

to make out, giving the appearance of circular bands of muscle. This idea is strengthened by the fact that the appearance of this layer does not alter, regardless of the angle at which the section has cut the sporocyst, whereas bands of circular muscle would have a different appearance if cut across. Furthermore, living sporocysts dissected from an infected snail show no muscular movement whatever, only twitching caused by the sudden movements of the contained cercariae. In the specimens where this layer has not been observed it is probably because it has become stretched and exceedingly thin. It is significant that the only sporocysts which show this layer clearly are those retarded ones in the starved snail.

No definite cases of division in young daughter sporocysts have been observed. The fact that the pharynx has been seen in the process of formation in young specimens, differentiating from a patch of the thickened fourth layer is in opposition to the suggestion by Rothschild that the pharynx arises at the point of division. Rothschild's figure of the pharynx developing as a result of division does show clearly a double row of "second layer" cells between the two sporocysts and can be explained as two neighbouring

(78)

individuals with the pharyngeal ends apposed but with the layers of paletot cells between them.

This theory of the early development of the daughter sporocysts reduces the number of layers of the body wall to three, the "stratified" outer epithelium, the vacuolated layer with poorly staining nuclei (fourth of Rothschild and inner layer of Stunkard) and an inner epithelium. Since under most circumstances the inner and outer layers are very difficult to find the usual appearance is of a body wall made up of one type of cell only which may be more than one cell thick in places. In the mature sporocyst the paletot becomes closely applied to the body wall and gives the two layered appearance described by Stunkard.

The mature daughter sporocysts.

Nothing is known of the time taken for daughter sporocysts to mature to the point of shedding cercariae, but it can definitely be stated that the period is a very long one, probably six to seven months as a minimum. As the shedding of cercariae is seasonal (to be shown in the next section) the time may vary considerably according to the time of year when infection occurs.

When ready to produce cercariae the sporocysts are

usually 1.5 - 2.0 mm. long and about 0.5 mm. wide. The shape is sausage-like with sometimes a slight protuberance around the pharynx at the anterior end (Fig.28). The colour is variable, from greyish to a brilliant orange and is due to the presence of pigment granules in the vacuolated layer of the body wall. As stated by Rothschild, the colour is brightest at the ends of the body where the wall is thickest. It is unusual to find even more or less straight sporocysts in a heavy infection, and U and S shapes are common. According to Rothschild the pharynx acts as a birth-pore and although no evidence of this has been found, no alternative exit for the cercariae has been seen.

At the height of the shedding period sporocysts are commonly found which have a protuberance projecting from either the posterior end or one side of the posterior region of the body (Figs.30 & 32). In section these projections show a rather thick wall and small lumen. They arise from the thickened part of the body wall which appears to proliferate the germ balls into the lumen of the sporocyst itself. The lumen of the projection does not appear to be continuous with that of the projection. A section of the gonad of a snail with the sporocysts at the height of the shedding period

is shown in Fig.37.

When the season during which the cercariae are emitted is at an end the appearance of the sporocysts is rather different. The ends become even more brilliantly pigmented than before and the middle becomes distended, the wall in that region being quite translucent. The sporocysts tend to look rather attenuated and ragged when compared to those at the peak of the shedding period (Fig.33). Sections show that in some sporocysts the body wall tends to shrink away from the paletot, presumably the two are only brought into contact by the pressure of the contained cercariae on the walls of the sporocyst. Immediately the shedding period is over there are only one or two cercarial embryos to be seen within the sporocyst lumen (Fig.38), but some three weeks after the last cercariae have been shed quite large numbers of germ balls and early cercarial embryos are present.

The most interesting feature at this period of the infection is the appearance in the gonad of numbers of small sporocysts. These tend to be ovoid or almost spherical and are practically colourless when compared with the larger forms present. In section they show a very thick body wall and contain a few germ balls.

At this stage there appears to be no pharynx. They are scattered throughout the gonad but are most numerous near the base of the spire, particularly in the tissues surrounding the stomach and intestine of the snail.

Rothschild describes a vigorous division or budding of sporocysts following a large emission of cercariae and refers to a generation of smaller daughter sporocysts being formed. In the present work no division of sporocysts has been observed, but it was at first thought that the protuberances seen on shedding sporocysts were the buds from which this new daughter generation would be formed. It seems now that the most likely solution is that the mother sporocyst lies quiescent after the first generation of daughters has been produced until they have shed their cercariae. The mother then produces a new generation of daughters which migrate up into the gonad. The most likely explanation of the "buds" seen on shedding sporocysts is that they are a proliferation of the growing zone of the body wall from which the next lot of germ balls is produced once the first batch of cercariae have been released.

This most unusual production of a second daughter generation of sporocysts has not at present been recorded for any other trematodes. Unfortunately observations

over a period of more than two years have not yet been possible to determine whether further generations of daughters are produced.

The effect of the parasite on *Turritella communis*

Perez (1936) describes the main effect on the gonads of *Turritella* due to the presence of trematode parasites. He uses the term atrophy in this connection but this is only true of very gross infections. It is unusual to find snails in which all trace of the gonad has been obliterated, but this does occur. Usual infections in males result in the reduction of the tubules of the testis to mere threads but these are easily recognizable from the ovary which is seldom so seriously reduced. Many infections in males have been found in which sufficient of the testis has remained active for the vas-deferens to be packed with spermatozoa.

Nothing is known of the duration of infection in the mollusc or whether the infection will subsequently die off and regeneration of the gonad occur. Two snails of over 50 mm. shell length have been found in which the gonad contained both testicular and ovarian tissue. It is possible that if a sex change of this nature is not normal in *Turritella* these specimens were males which had been infected and, on losing the infection, the gonad

was regenerating as an ovary. No evidence to support this idea has been obtained but such sex reversals due to trematode infection of gastropods have been reported.

The concept of parasitic castration is a somewhat controversial one. Baer (1951) summarizes much of the evidence concerning the classical examples of castration by rhizocephalan and isopod parasites. He points out that the effect of the parasite is to modify the secondary sexual characters of the male host towards femaleness, but that removal of the testis has no such effect on an unparasitized male. Baer makes no comment on the so-called parasitic castration of molluscs by larval trematodes and in this field Rothschild (1936 & 1941) and more recently Boettger (1952 & 1953) have written a good deal. Both of these authors have shown how trematode infections in the gonad and or digestive gland can cause modifications in the external appearance of the snail host. These modifications usually take the form of gigantism and sometimes distortion of the whorls of the shell acquired after infection occurs. The variations in most of the cases quoted by these authors are very obvious and not easily overlooked. In Turritella communis no such abnormalities as a result of infection with sporocysts were observed. It was at first thought

that the finding of infections only in large snails might be due to a castration effect causing rapid growth of the shell but the diagrammatic analysis of the sample from St. Bride's Bay (Fig.21) shows that this is most unlikely. Infected snails kept in captivity have not grown any more rapidly than uninfected ones from the same batch.

- Fig.28 First generation daughter sporocyst of Cercaria
doricha from Turritella communis, just before
the beginning of the shedding period.
- Fig.29 Sporocyst of Cercaria doricha during shedding
period.
- Fig.30 Sporocyst of Cercaria doricha late in the shed-
ding period.
- Fig.31 Sporocyst of Cercaria doricha of second daughter
generation about two months after the end of
the shedding period.

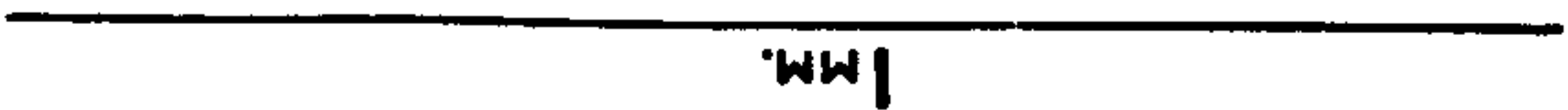
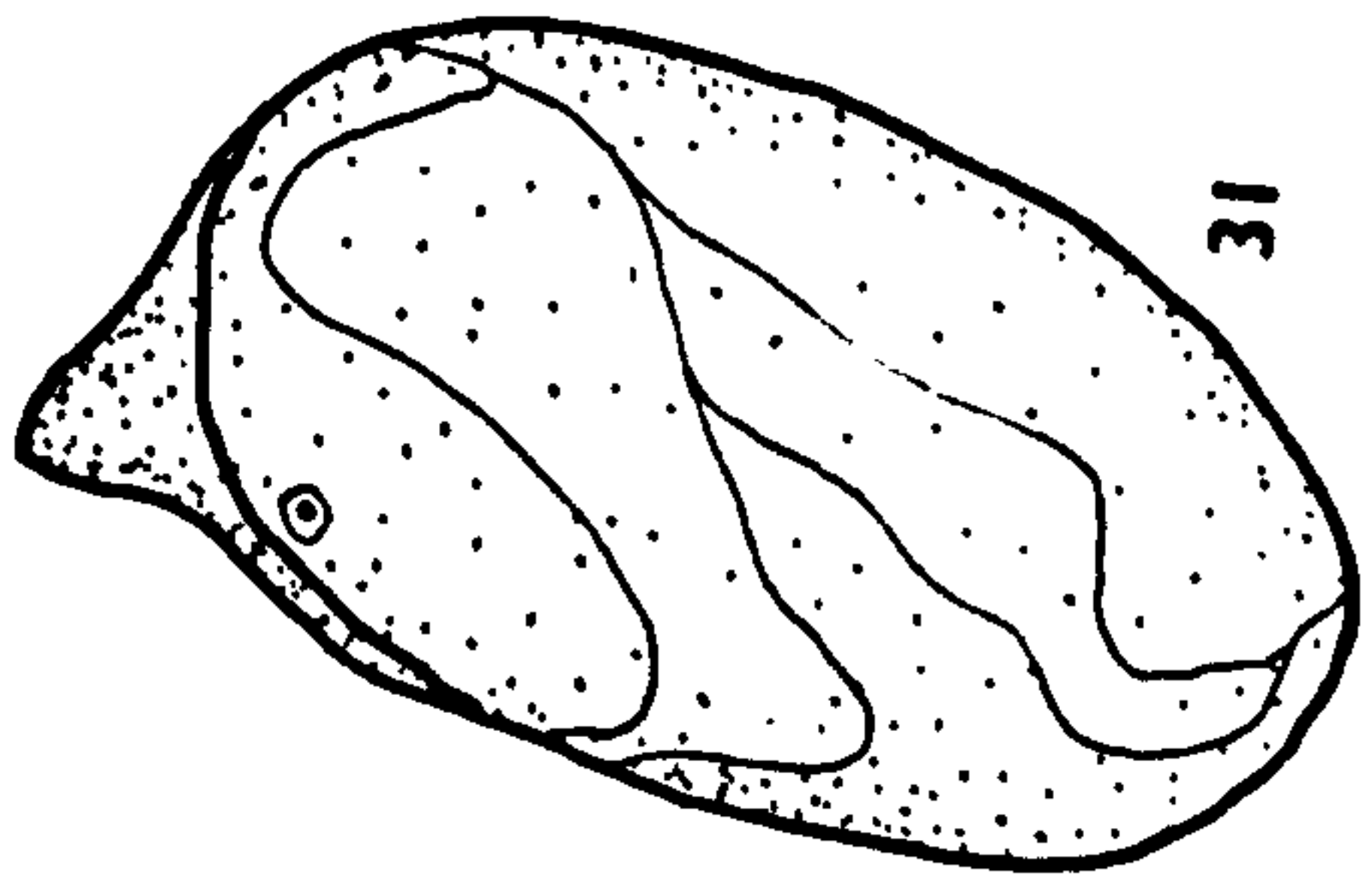
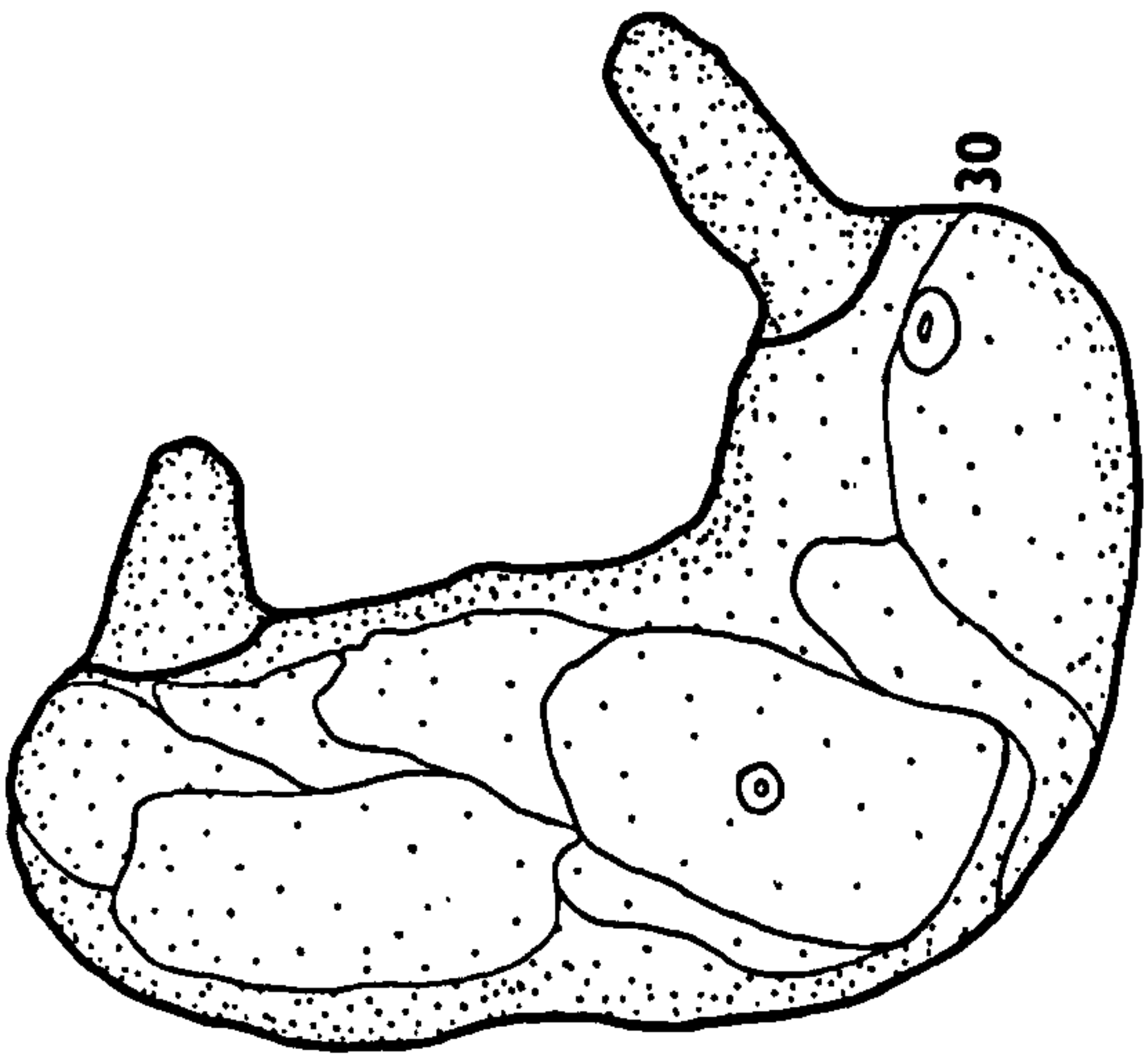
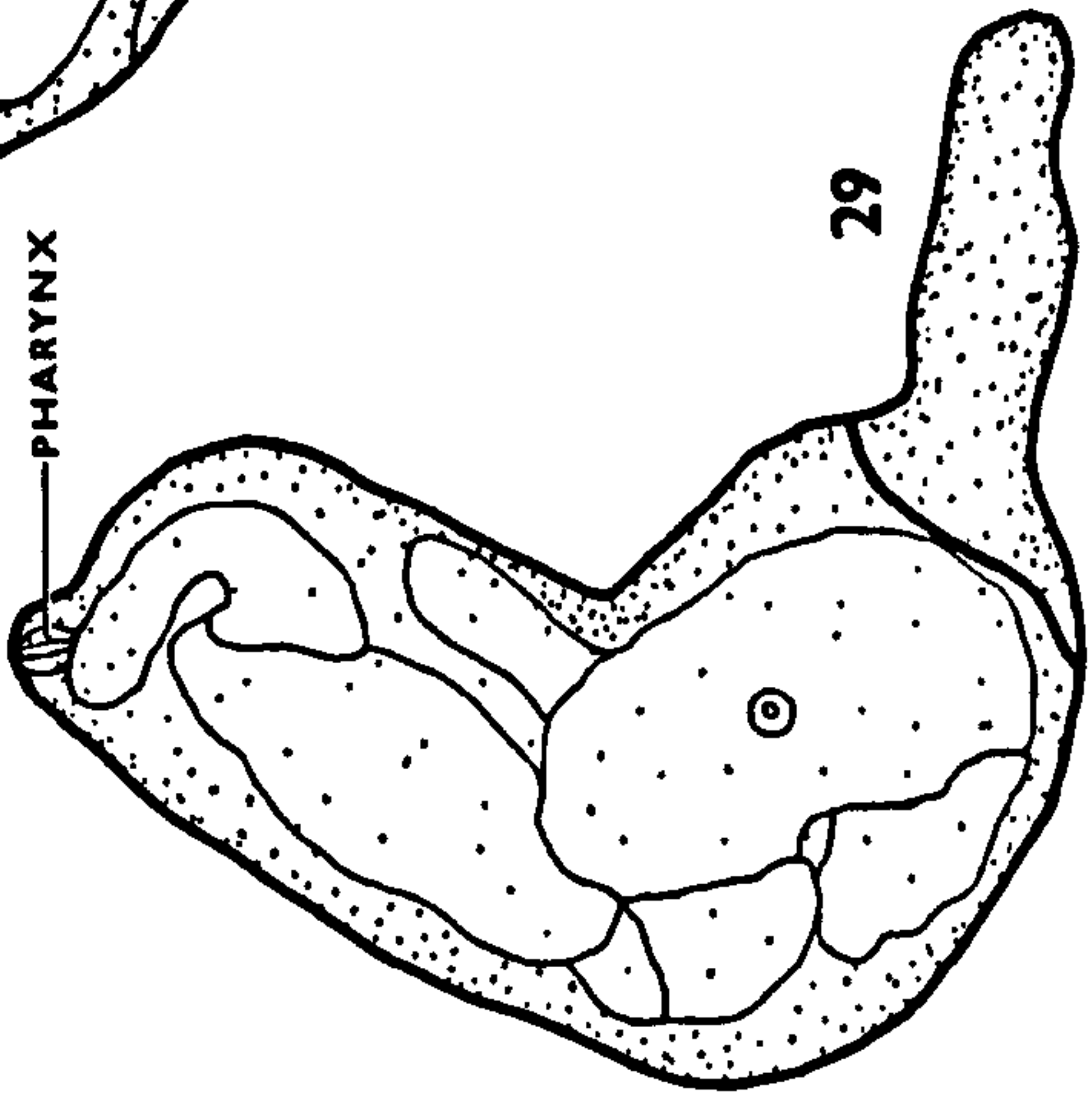
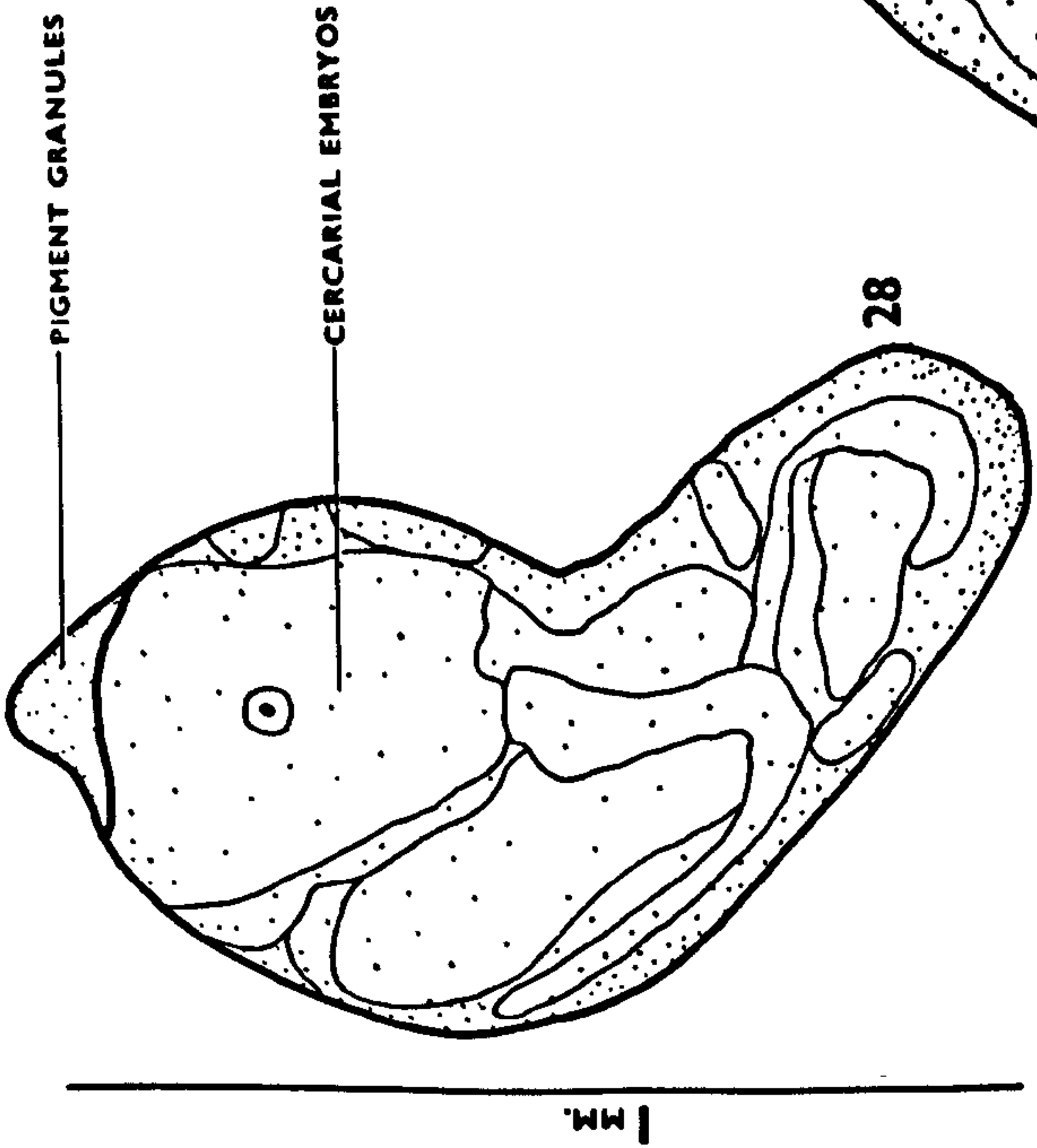


Fig.32 Photomicrograph of sporocysts of Cercaria
doricha during the shedding period.

Fig.33 Photomicrograph of sporocysts of Cercaria
doricha after the shedding period.

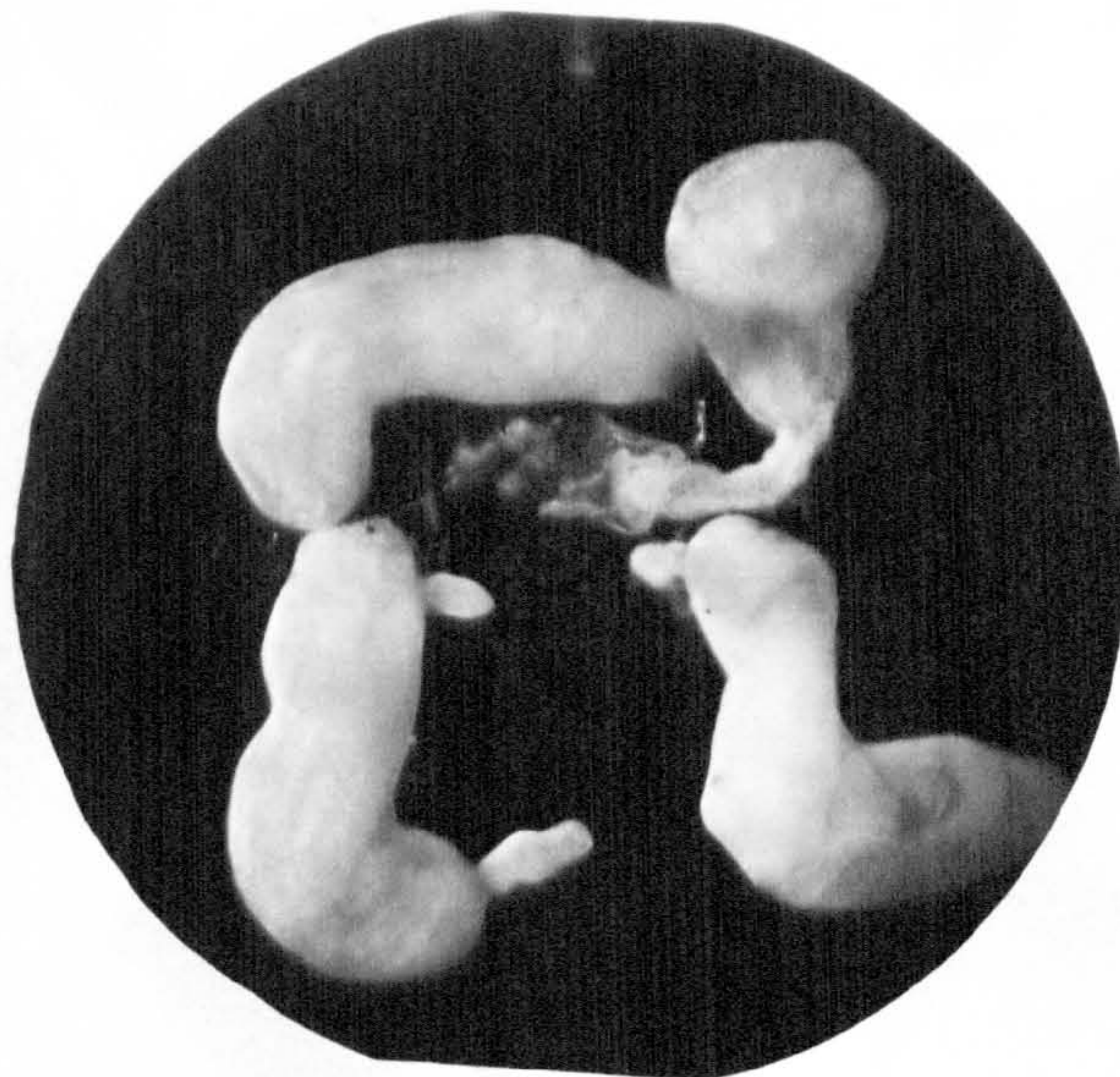


Fig.34 Photomicrograph of a section through the ovary of Turritella communis showing a sporocyst $3\frac{1}{2}$ months after exposure to eggs of Renicola sp.

Fig.35 Photomicrograph of a section through the ovary of Turritella communis showing sporocysts six months after exposure to eggs of Renicola sp.

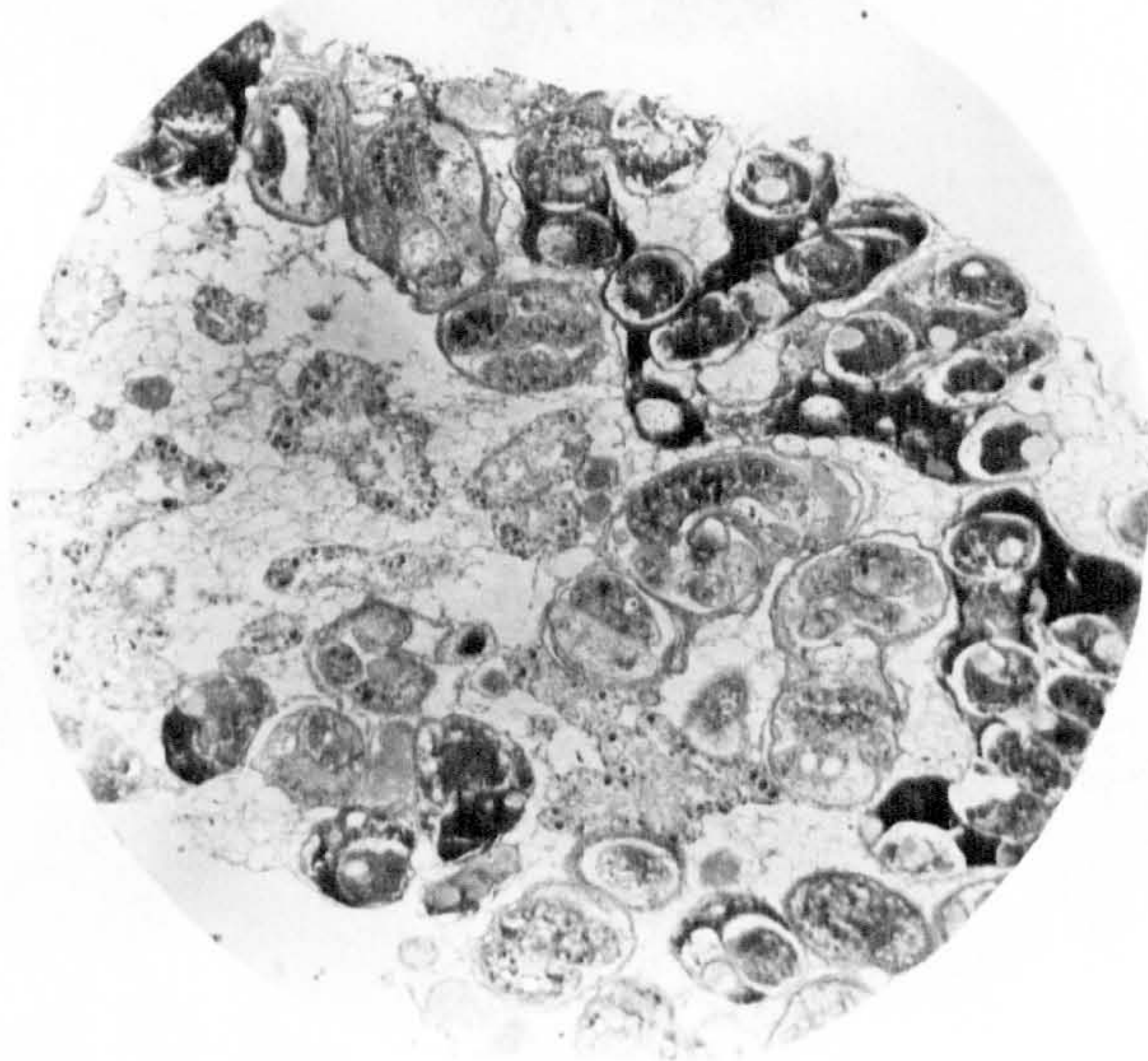
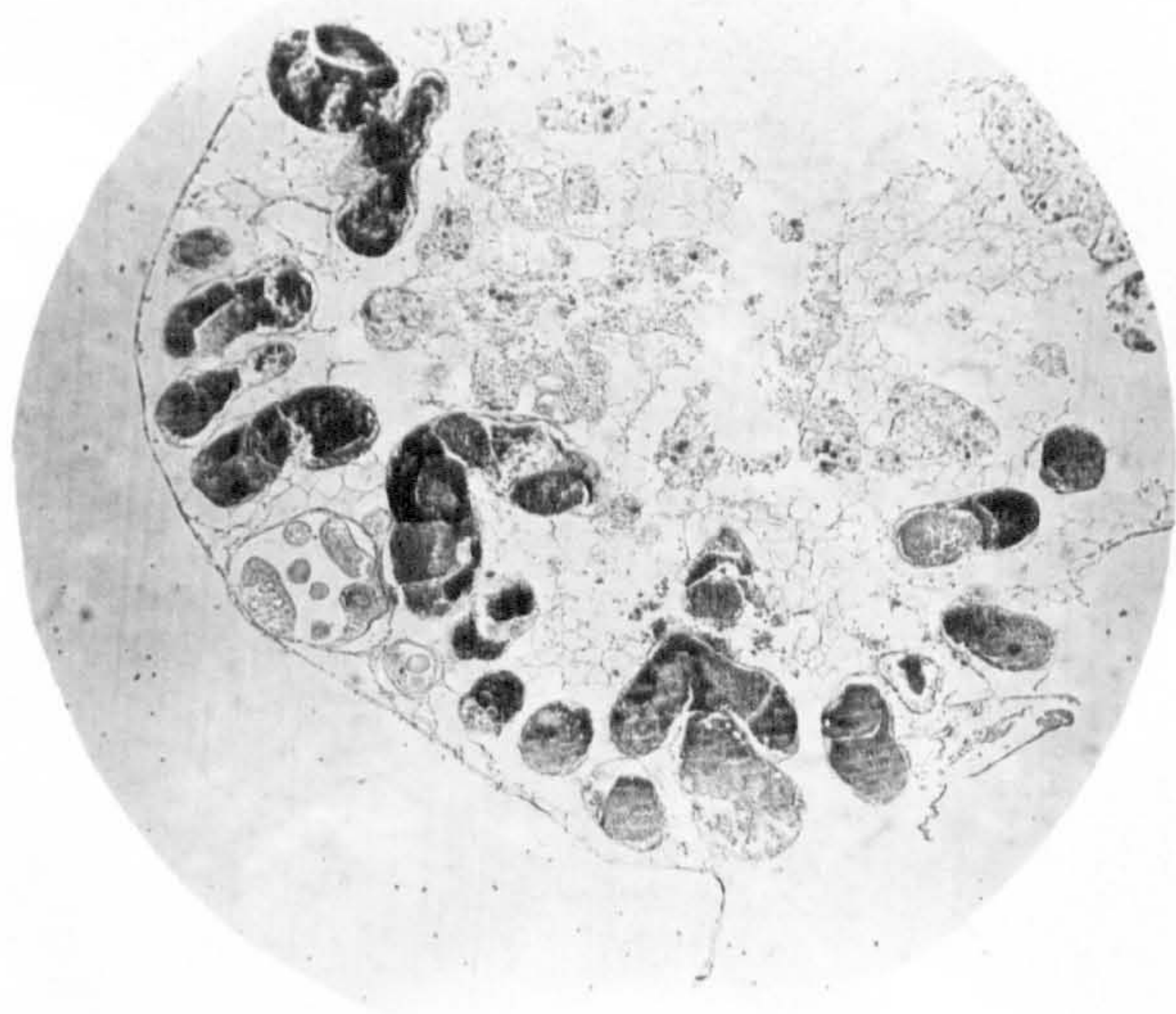


Fig.36 Photomicrograph of a section through the gonad
of Turritella communis with a natural infection of sporocysts, but the host had been
starved for six months before fixation.
(Specimen lent by Miss M. Rothschild)

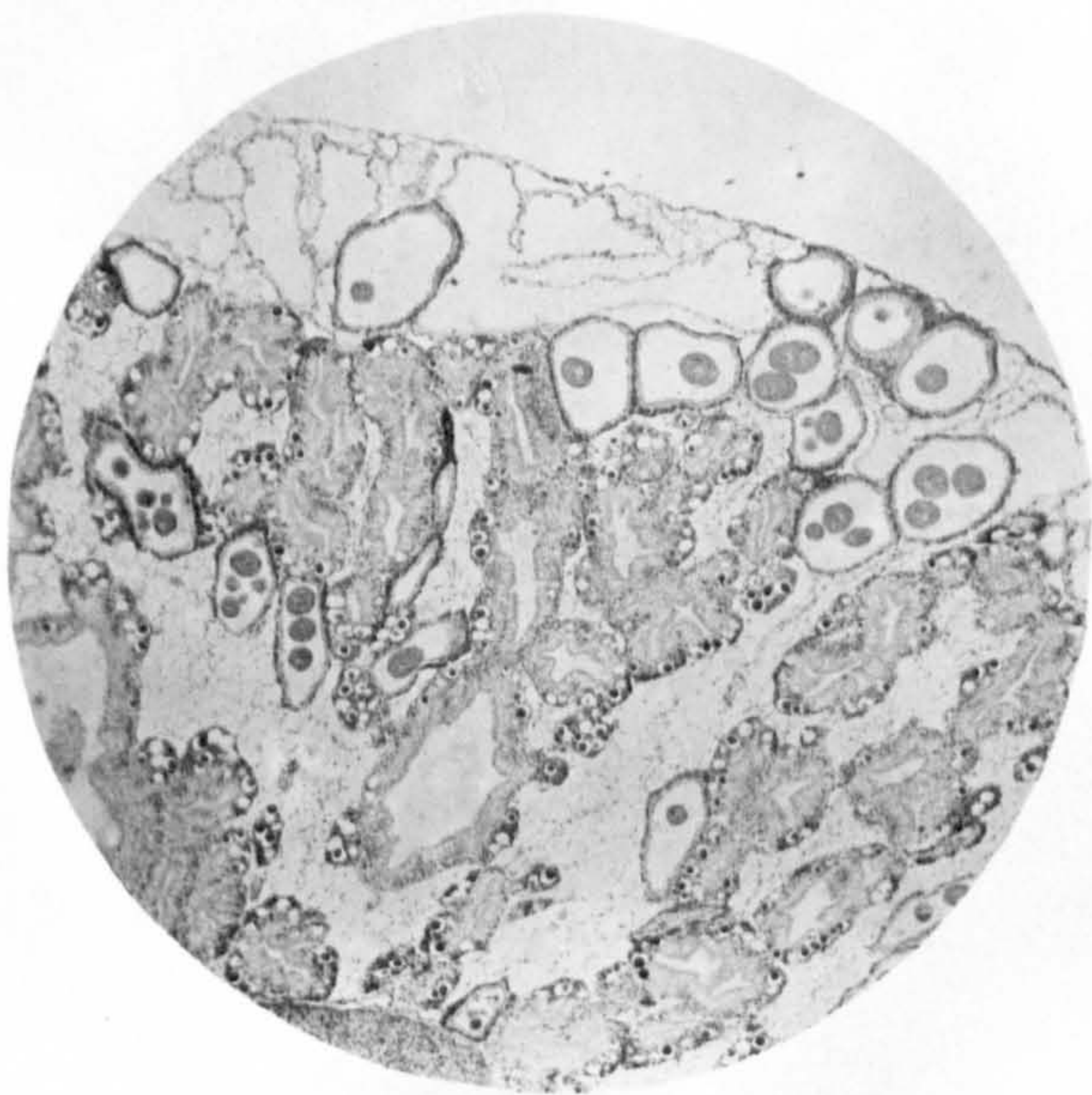


Fig.37 Photomicrograph of a section through the gonad of Turritella communis with a natural infection of sporocysts at the height of the shedding period.

Fig.38 Photomicrograph of a section through the gonad of Turritella communis with a natural infection of sporocysts after the shedding period.

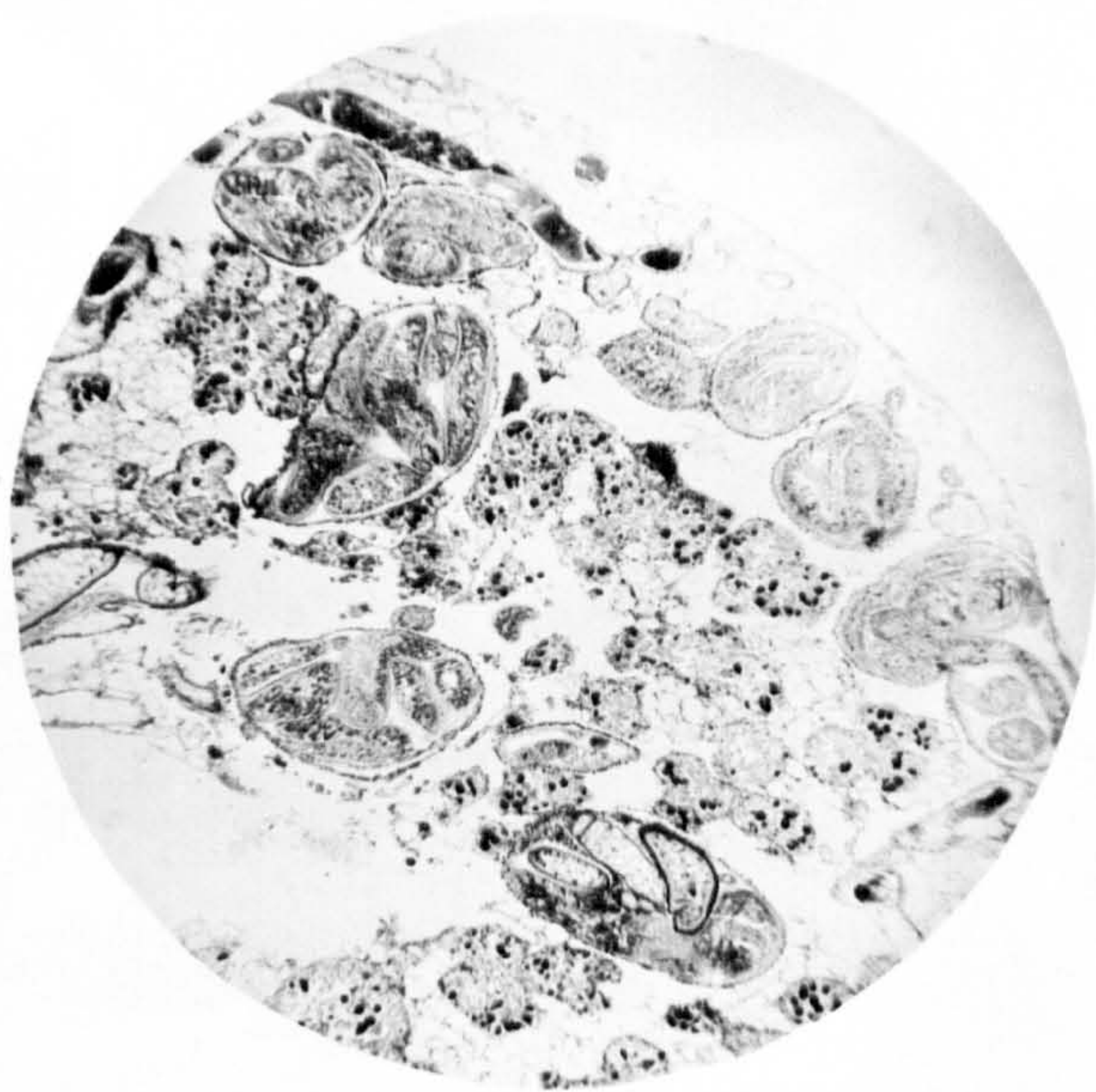


Fig. 1. The anterior end of the sporozoite and lining of the intestine.

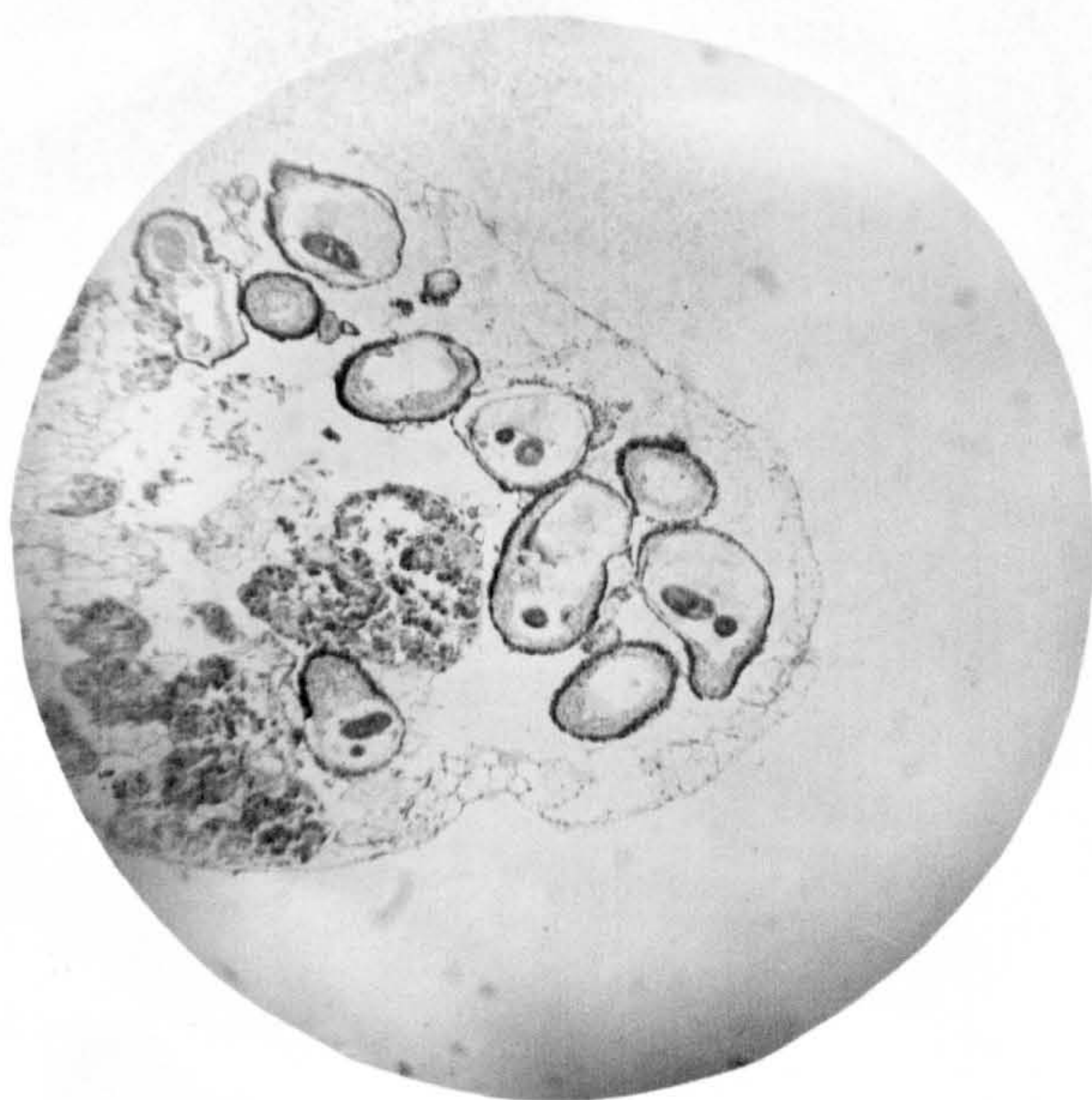


Fig.39 Photomicrograph of a section of the pharyngeal region of a sporocyst showing the relationship of the paleotot to the rest of the body wall and the darkly staining layer covering the anterior end of the sporocyst and lining the pharynx.

Fig. 10. 100x

Fig. 10. 100x

Fig. 10. 100x

Fig. 10. 100x

Fig. 10. 100x

Fig. 10. 100x

Fig. 10. 100x

Fig. 10. 100x

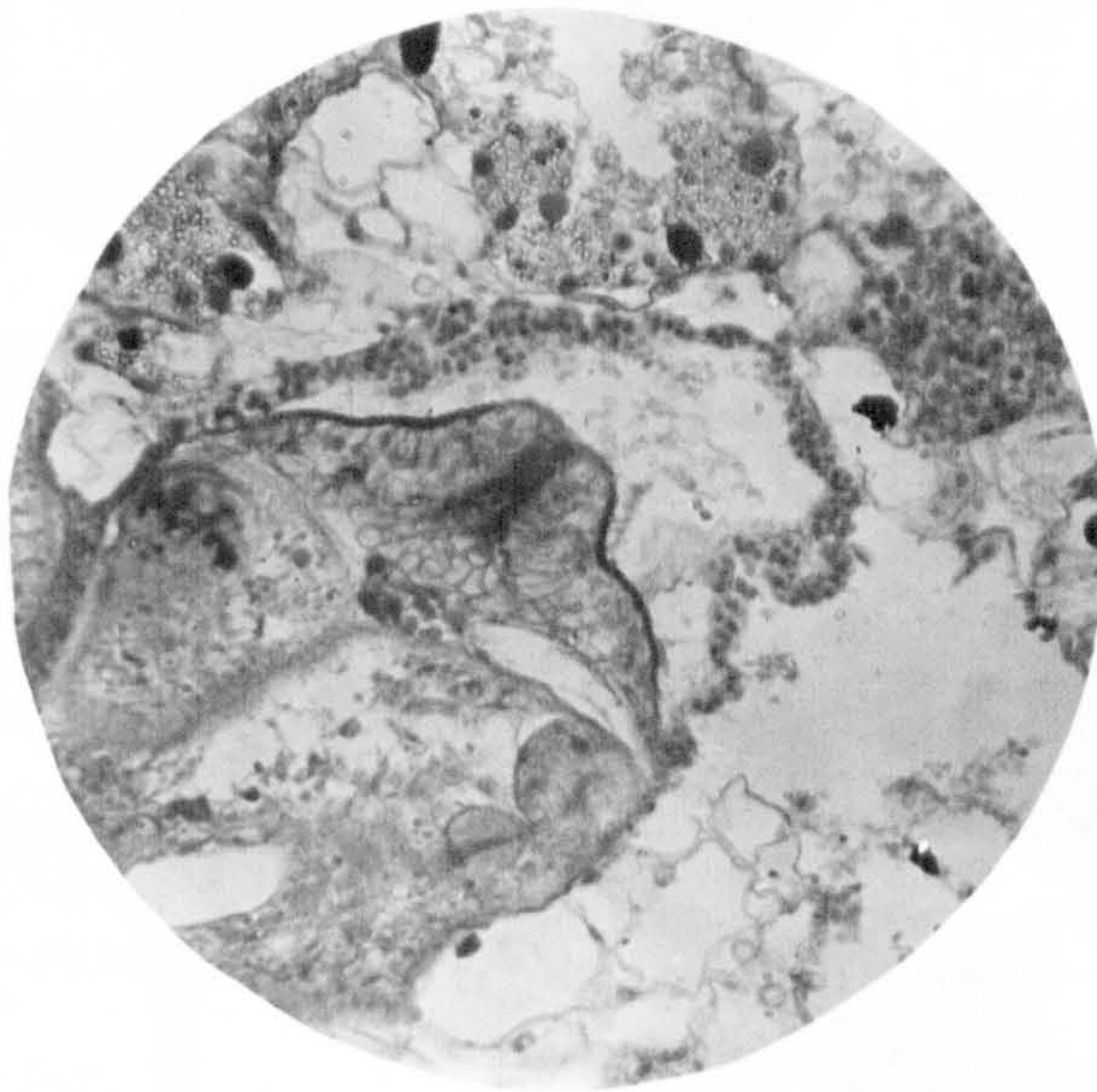


Fig. 10. 100x

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Fig. 10. 100x

The Cercariae

Historical

Cercaria rhodometopa, the type species of the group was named and very briefly characterized by Perez (1924) from Roscoff and the bay of Morlaise. Stunkard (1932) found an infection of the same species in a Turritella from Roscoff and published a more complete description. Rothschild (1935) described six new species of the same group, four from Plymouth and two from Naples.

It is unfortunate that the material on which Stunkard based his redescription of the type species was all obtained from one dissected snail, consequently no definite estimate as to the maturity of the cercariae he described can be made. His prophecy that the peculiarity of the excretory vesicle with its laterally branched Y-shape would lead to the finding of later stages in the life history is in no way impaired by the fact that he appears to have overlooked the most unusual feature of all in that vesicle, the cross-connection between the arms of the Y behind the ventral sucker. The work of Rothschild was based on larger quantities of material and on cercariae which had actually escaped from the snail host.

The most useful characters used by Rothschild for the separation of the species she described are unfortunately not mentioned by Stunkard for C. rhodometopa, however Stunkard does mention the presence of a well developed prepharynx, a character only occasionally found in one of Rothschild's species, and he was unable to find any trace of an intestine while the later author describes the presence of intestinal caeca in all of her species.

The work of Rothschild shows clearly that there are two distinct subdivisions of the "Rhodometopa" group of cercariae. The first of these includes the forms with only a median cluster of penetration gland cells and five flame-cells in each group. Most of

them have a brightly pigmented anterior end and their bodies are usually rather translucent. This subdivision is characterized by Cercaria pythionike Rothschild 1935, being the most fully described, and to it belong C. rhodometopa Perez, C. nicarete Rothschild, C. horpsyllis Rothschild, C. ampelis Rothschild and C. ranzii Rothschild. To the other subdivision belongs C. doricha Rothschild, a species considerably larger than the others with two lateral clusters of penetration glands, lying outside the main arms of the vesicle, in

addition to the median cluster. There are six flame-cells per group in this species. Due to the smaller size of the granules in the excretory vesicle of this species and their far greater profusion than in most species of the other group the body of C.doricha appears opaque. Further differences between these two subdivisions will be shown later.

Life history experiments by Rothschild were all unsuccessful. Two specimens of C.doricha made an abortive attempt to penetrate the skin on the fins of Pleuronectes platesaa, but as Rothschild suggested, her observations on the behaviour of the cercariae made it unlikely that the second intermediate host would be a bottom-living form.

Species found during the present work.

Not all of the species described by Rothschild have been found during the present work. Of 360 T.communis from Plymouth twenty five have been found to be infected, twenty four with C.doricha (Fig.40) and one with C.pythionike (Fig.42). This shows an infection rate of 6.6% for C.doricha and of 0.27% for C.pythionike, compared to a rate of 5% and 2% respectively recorded by Rothschild (1935). From Millport 269 snails were examined, twenty one were infected, five with C.doricha,

four with C.pythionike, one with C.nicarete (Fig.43) and eleven with a new species to be described below under the name C.doricha pigmentata n.sp. Of the 719 snails from St. Bride's Bay twenty nine were infected also eight of the forty nine from South Haven, Skomer Island. In these last two cases all of the infections were discovered by dissection and only one or two of the snails contained cercariae in an advanced state of development. These, however, did not resemble any of the previously described forms and will be described below under the name of C.cooki n.sp.

Of the British species so far described only C.herpsyllis Rothschild has not been seen and two new species have been found.

Cercaria doricha-pigmentata n.sp. (Fig.45 & 49)
4.2% infection in Turritella communis from Millport in the Firth of Clyde. This is the most common of the species found in snails from this region, but has not been found in material examined from any of the other localities.

As the name suggest, this cercaria belongs to the C.doricha sub-group and is the only other species recorded with lateral clusters of penetration glands and six flame-cells per group. It is exceedingly like

C.doricha but differs from it in having pink pigment around the oral sucker, also down the whole length of the tail. Rothschild (1935 & 1936) mentions two infections of C.doricha "in which 99% of the cercariae over 1 mm. in body length had pink pigment present in the tail". It is quite possible that these infections belonged to this species as occasionally the anterior pigment granules are so scattered as to be difficult to observe. The pigment is usually well marked but not so extensive as in C.pythionike. The colour is not quite the same, having a somewhat duller or more brownish quality than that in the other pigmented species, while the colour in the tail is definitely pink.

The measurements of this species are rather smaller than those of C.doricha, the body length in the largest specimens found being 1.08 mm. with a width at the level of the ventral sucker of 0.44 mm. The cuticle is uniformly covered with spines in a quincuncial arrangement and cuticular tubercles are present with a distribution extending from the anterior end to about the level of the ventral sucker. The ventral sucker is situated slightly above the middle of the body length and measures 0.085 mm. in diameter.

The oral sucker is sub-terminal and measures about 0.10 mm. in diameter. The pharynx usually lies immediately behind the oral sucker, but in some of the specimens examined there is a short prepharynx. Beyond this no trace of any intestine has been found. The excretory vesicle is of the usual Y-shape with lateral branches and the cross-connection behind the ventral sucker. The lateral diverticula appear to be rather more numerous than in C.doricha, but, as will be shown later, this has no great significance. The granules in the excretory vesicle are small, 0.004 mm. in diameter, and very numerous, particularly in the anterior region. The lateral branches behind the level of the ventral sucker contain relatively few granules and usually not many are present in the main stem of the vesicle. As far as can be made out the flame-cell arrangement, based on six to a group, is the same as that in C.doricha, $2[(6+6+6+6+6+6) + (6+6+6+6+6+6)]$. The complete pattern could not be worked out because, as in C.doricha, the lateral groups of penetration glands obscure the more anterior flame cells.

The penetration glands are exactly similar to those in C.doricha, but the two lateral groups do not stain intra-vitam with neutral red quite so easily as do the median group. This median cluster of gland cells contains

two kinds of cells, the anterior ones which stain easily with neutral red and a more posterior group which do not stain so easily and have a less well defined outline.

The genital primordia are located in a crescent-shaped patch of cells with deeply staining (in haematoxylin) nuclei a very short distance in front of the ventral sucker and slightly to the left of the mid-line.

The species is extremely close to C.doricha and may perhaps be only a race of that species but it is certainly more than a mere variation as Rothschild described the forms she found with pigmented tails. It is distinguished from C.doricha by its smaller size, slightly larger excretory granules and the presence of pigment at both the anterior end and in the tail. It replaces C.doricha as the most common form in the Firth of Clyde from the Millport area.

Cercaria cooki n.sp. (Fig.44)

The exact percentage infection with this species cannot be recorded with any certainty as there is no guarantee that all of the infected snails from St.Bride's Bay and South Haven, Skomer Island were carrying the same species. However, all of those in which cercariae

were sufficiently developed to be recognized with accuracy were of this species. The species is named after H.M.S. "Cook", the ship from which the St. Brides Bay samples were obtained.

This cercaria belongs to the pythionike sub-division, having only a median cluster of penetration glands and five flame cells per group.

The dimensions of the body are small, the largest specimens examined measured only 0.69 mm. in length by 0.38 mm. in width. These measurements fall within the limits given by Rothschild for C. herpsyllis, the smallest of the British species. The fact that the material on which this description is based was all obtained by dissection and not from fully mature, shed cercariae means that any body sizes given are not necessarily the maximum which can be reached by the species.

The cuticle is covered with a quincuncial arrangement of spines and cuticular tubercles are well developed. These have a distribution similar to those in C. nicareto, from the anterior end right down to the point of junction between the body and tail. Bright red pigment is present at the anterior end but its distribution seems to be slightly more peripheral than in C. pythionike.

The ventral sucker is almost exactly central in position and measures about 0.06 mm. in diameter.

The oral sucker is sub-terminal and measures 0.064-0.072mm. The pharynx lies immediately behind it. No prepharynx has been seen in any of the specimens examined and no trace of any other part of an intestine, neither oesophagus nor caeca has been seen.

The excretory vesicle, of the usual laterally branched Y-shaped type with a cross-connection behind the ventral sucker, contains a few large granules, mainly in the anterior branches. The largest of these granules measure 0.008 mm. in diameter. The flame cell pattern was observed to be the same as that for the other species in this sub-group, $2[(5+5+5+5+5+5) + (5+5+5+5+5+5)]$.

The penetration glands comprise only the median cluster, with the anterior mass of irregularly lobed, granular cells, staining easily with neutral red and the more posterior group, surrounding the base of the ventral sucker, which have a more regular outline, fewer cytoplasmic granules, and do not stain so readily with neutral red.

The genital primordia, as in the previous species, form a crescent shaped mass of cells in front of the

ventral sucker and slightly to the left of the mid-line.

The affinities of this species are more difficult to analyse than those of the one described before. The size is similar to that of C.herpsyllis, but the size of the excretory granules differentiate it from that species, and, for the reasons already given, the body measurements are not altogether reliable. The distribution of the cuticular tubercles is the same as that for C.nicarete, but the new species is pigmented at the anterior end and has smaller excretory granules. The excretory granule size is most near to that of C.pythionike, but the greater extent of the cuticular tubercles in the new species provide a distinguishing feature. In one further respect does this new species differ from the descriptions of all other British members of the sub-group, it has no oesophagus or intestinal caeca. In this respect it is similar to the type species, C.rhodomotopa, except that a propharynx has been described in that form. Overlooking this as a possible artefact caused by coverslip pressure, it is difficult to assess the relationship between the new species and the type, as none of the criteria on which a separation from the British species has been made have been described for the type. A possible reason

for suspecting the identity of C.cooki and the type is based on ecological ideas. As shown by Lockley (1953), the main feeding grounds for the Skokholm and Skomor Manx shearwaters during the breeding season is in Biscay and up the North-West coast of France. Their principal food is the young Sardina pichardus which could easily become infected with Cercaria rhodomotopa from the Bay of Morlaix. It seems fairly certain that the cercaria will be found in other regions along the Biscayan coast where the nature of the bottom favours the molluscan intermediate host. It is very probable, then, that the shearwaters which have already been shown to harbour adult Renicola in considerable numbers, are carrying the adults of the type species and transferring the infection to the Turritella living near the birds' breeding grounds. This hypothesis will not be capable of proof until a redescription of Cercaria rhodomotopa from Roscoff is available, or even better, when living cercariae from this locality and St. Bride's Bay can be compared together. Until then, Cercaria cooki n.sp. must stand with the suggestion of its possible synonymity with the type species.

Some observations on the anatomy of the "Rhodomotopa" group.

In both of the new species just described as well

as in the type species no gut has been found beyond the pharynx. Rothschild describes the presence of an oesophagus and intestinal caeca which are more or less easy to trace in all six of the species she described. In none of the three of her species found during the present work, and examined with great care, has any sign of a digestive system behind the pharynx been seen. In a private communication Miss Rothschild informs me that she observed the oesophagus and caeca only in very young cercariae in which the penetration glands and cystogenous glands were not yet formed. In specimens of this nature the alimentary system was seen by neutral red intra-vitam staining which showed numbers of very fine granules in both the oesophagus and caeca. The same procedure has been repeated here, but in these very immature forms it has been found that the main trunks of the nervous system stain slightly with neutral red and follow roughly the course where the caeca might be expected to lie. Other attempts have been made to locate the system both in living and fixed material. Cercariae observed under phase-contrast, dark ground and polarizing microscopes have shown nothing, probably because they are too large and thick to give satisfactory results with any of these methods.

Whole mounts stained with haematoxylin and eosin or aceto-carmin with or without indigo-carmin counter stain have similarly shown no signs of a digestive system. Finally, serial sections, both frontal and longitudinal, of mature cercariae have shown that although the pharynx has a well developed lumen, in connection with that of the oral sucker, no trace of it can be found behind the pharynx.

The excretory system has also been examined in some detail and although the validity of the flame-cell formula is undoubted, the exact distribution of the flame-cells themselves within the groups is so variable that it is of no systematic value whatever. The nature of the granules in the excretory vesicle has not been determined, but they are definitely granules and not oil-droplets as found in Fasciola hepatica by Stephenson (1947). The larger granules can be crushed by pressure on a coverslip and can be seen to fracture, indicating their solid nature. They are soluble in ~~XXXXXX~~ dilute acetic acid and formalin but if they are soluble in alcohol it is only very slowly. In C.doricha, where the granule size is small, concretions of several granules are sometimes found but they seem to be expelled fairly rapidly through the excretory pore.

At first sight the size of these granules in the excretory system seems to be a not very reliable specific criterion, but repeated measurements in numbers of specimens of the same species have shown a remarkable constancy. The size variation of the granules in different species of the cercariae is usually fairly large, but in the sporocyst stages where the granules are very small and the size differences only slight they cease to be a ^{useful} very specific character.

It has been found that the number of lateral branches of the excretory vesicle is variable in different specimens of the same species of cercaria. Longitudinal sections show this to be due to the fact that all of these branches, particularly in the central third of the body length, do not lie in the same plane but overlap one another (Fig.61). If coverslip pressure is applied to flatten a cercaria then more of these diverticula become visible as a result.

The other very useful character for recognition of different species within the "Rhodometopa" group is the distribution of the lateral cuticular tubercles. These are of particular interest since exactly similar tubercles have been found on adult flukes. They are not always easy to find and appear to be distributed

only around the edges of the cercaria, not on the dorsal or ventral surfaces. Interest in the function of these tubercles led to an attempt to discover if they had any special nerve supply. Two techniques were attempted, the first, Unna's intra-vitam method (Pantin 1948) using a reduced methylene blue solution. This gave a good picture of the gross structure of the nervous system but no detail. The other method was that of Holmes (1947) involving the use of silver solutions and toning with gold chloride on specially fixed (hot formal-sublimate) whole cercariae. This method is very inconsistent, out of several hundred cercariae used and subjected to identical treatment only a few specimens which showed much detail were obtained. Many of the others had parts of the system well stained and all were examined for some sign of fibres leading to the tubercles but none was found. The two nerves running almost horizontally from either side of the nerve collar and then dividing to give an anterior and posterior branch near the lateral edge of the body are the most likely to be involved in this connection, but no evidence of this was found (Fig.41). The function of these tubercles, if any, remains unknown. In the absence of any other obvious organs of special sense or general

receptors it is perhaps these tubercles which are in some way connected with the behaviour of the cercariae to be discussed later.

The development of the cercariae.

The origin of the germ-balls within the sporocyst has already been shown to be the thickened part of the vacuolated layer of the body wall in the posterior region. Some sections have shown what appears to be a break-down of some of these balls after formation to give a number of smaller masses which develop independently. There is no evidence to show that this is a general rule, but, if it is, it may account for the occasional monsters which are produced. Such a monster was observed in an infection of C.doricha in which one specimen had the anterior end divided, each part bearing a separate oral sucker. Such specimens could possibly be the results of incomplete separation of two of the smaller germ-balls.

The earliest recognizable cercarial embryos are germ-balls which have become flattened discs of cells, rather ovoid in shape, with a small projection at one end which represents the future tail. As these embryos grow the first organs to be formed, at a very early stage, are the suckers. The ventral sucker arises as

a papilla which stands well out from the ventral surface of the body and later invaginates at the tip to form the typical sucker-like structure.

After the appearance of the suckers the excretory vesicle begins to differentiate in the manner shown by Rothschild. First, two roughly parallel channels appear in the posterior region of the body. These then extend both forward and backward, the posterior extensions approximating more closely to one another, until they fuse to form the stem of the vesicle. Next, slight bends in the two branches appear, forming a pair of projections facing one another just behind the ventral sucker. These grow towards one another, forming the cross-connection between the two arms. At about the same time the lateral evaginations of the stem and branches begin to appear.

The tail, by this stage, has become separated from the main mass of the body and is attached to it only by muscle fibres. The fins begin to develop, the two main ones being dorsal and ventral as described by Rothschild and not lateral according to Stunkard.

The last organs to appear are the penetration and cystogenous glands, the latter scattered over the body just below the cuticle, but most numerous in the middle

region, and the cuticular spines and tubercles. Even after the appearance of all of these structures there is still some time before the giant cells of the tail have degenerated, as described by Rothschild, and the cercariae are ready to emerge.

Nothing is known of the time taken for this "embryonic" development of the cercariae, from the formation of the germ ball to the emergence of the mature larva, but evidence indicates that even when the cercariae are fully developed they may not emerge at once, but wait until conditions are correct.

The shedding or emergence of the cercariae.

Rothschild (1935) mentions that cercariae rarely emerge when the temperature of the water in which the snails are kept is below 15°C . She further states that two infected snails were kept at a temperature of less than 8°C . for a period of nine weeks and that on dissection the sporocysts were longer than usual and contained large numbers of moribund cercariae in an advanced state of development. It is unfortunate that the time of year at which these observations were made is not recorded.

When this work was started a number of Turritella communis were obtained from Plymouth in November 1952.

When these were isolated in 3" x 1" glass tubes no cercariae were shed until the water in the tubes was allowed to warm up slowly in the laboratory. About ten minutes after reaching 15°C. a number of cercariae were found in one of the tubes. This occurred on the 28th November. On subsequent occasions, once or twice a week, this snail was warmed up slowly and on almost every occasion when the temperature reached 15°C. a few cercariae were shed. This was repeated until the snail died during the Christmas holiday, sometime between the 23rd and 27th December. The temperature of the water in the plunger jars, in which the snails were kept at that time, was never above 12°C. and usually varied between 10°C. and 10.5°C., but occasionally fell to just below 9°C.

Information was sought concerning the temperature of the sea throughout the year at the depth at which Turritella most commonly occurs off Plymouth (15-30 fathoms). Although no really concise and definite answer could be obtained it was found to be very unlikely that a temperature of 15°C. would be reached even during the summer and that for the majority of the year it was quite likely to be below 8°C. This was rather what had been expected and consequently the real reason for the

shedding at 15°C. in the laboratory had to be found.

Various alternatives were considered, amongst them the possibility that bacterial activity in the mud in which the snails live was responsible for a slight increase in the temperature. This was discounted when it was considered that the snails are seldom buried more than half an inch below the surface of the mud and that to maintain that layer at about 15°C. when the surrounding water was at about 8°C. was almost impossible. The most reasonable solution to the problem ~~XXXXXXXX~~ seemed to be that the temperature increase itself was not responsible for the shedding but that it activated the gonad in some way and that this in turn stimulated the sporocysts, resulting in the shedding of cercariae.

The stimulation of the gonads of molluscs by temperature changes is a fairly well known phenomenon, for example the sex reversal in oysters which is closely associated with temperature change. The idea was supported by a private communication from Dr. Annie Porter in which she stated that she had observed a similar emission of cercariae following an increase of water temperature when she was working on the larval trematodes parasitizing fresh water molluscs in South Africa. She further stated that sections of snails fixed after such

an emission showed the intake of yolk granules by the sporocysts, suggesting a sudden increase in the activity of both the gonad and the parasite. This possibly accounts for the yolk-granules seen inside a sporocyst, mentioned in the section dealing with sporocyst development. This snail was dissected in January when the water temperature in the tanks was low and had probably been kept in the warm laboratory for some hours before dissection and fixation.

If the theory that the temperature stimulated the gonad and thus brought about shedding was correct, it seemed probable that when the gonads were naturally active during the breeding period of the snails that cercariae would emerge at temperatures of less than 15°C. Lebour (1933) found that the egg-laying period for Turritella at Plymouth begins in early April and continues until June.

On 26th March, 1953, when the temperature of the water in the plunger jars was just below 13°C., a number of C.doricha were found free in the water. From then on cercariae were shed fairly regularly, in spite of the water temperature occasionally falling below 12°C., until the early part of July.

It was found that individual infected snails were

usually rather difficult to keep isolated for more than about a month, consequently shedding records for groups of snails infected with the same species of cercariae were kept. Each day the cercariae were removed from the tank by means of a pipette and the numbers recorded. Since during the shedding period occasionally more snails were added to a group under observation or a snail was removed for dissection the numbers of snails in a group were not constant. In view of this the figures have been reduced to an average shed per snail per week. The histograms in Figs.57-60 therefore represent the cercarial shedding activity of a colony of snails rather than the production of an individual, except that for C.nicarote where only one shedding snail was found.

Records for individual, isolated, infected snails showed a considerable difference from one another, some never shed more than about thirty cercariae a day, while others produce as many as a hundred and eighty during a twenty-four hour period. The shed of an individual snail shows no uniformity, one specimen kept under observation for a two week period by itself shed 86 on the first day, none on the next, 119 on the next, then 2, then 8, then 151, none on the next day, 180 on

the following, then 6, then 18, followed by two days with no cercariae at all being produced. In a colony this individual irregularity is smoothed out, giving the histograms shown. Not all of the snails begin to shed at once when the breeding season commences, and the peak reached in late May and early June (Fig.57) probably represents the period during which the greatest number of snails are shedding at the same time as well as the greatest peak of individual production by each snail. In the first batch of snails examined (those obtained in November 1952), two snails were found which did not begin to shed until late April 1953, some considerable time after the others had begun. The possible explanation for this may be that those snails which start to shed right at the beginning of the season are those with a residual infection from the preceding year, while the late starters are those which only became infected during the previous year. This is borne out by the fact that the early starters usually shed more cercariae than those which begin later in the season and on dissection they show a more massive infection with sporocysts, a result of the added second daughter generation after the previous year's shedding.

For about four months after the end of the shedding

period no amount of warming of the snails will induce the production of cercariae, simply because of the absence of any sufficiently mature larvae. By late November or early December, however, a few will be emitted as has already been shown. This seems to indicate that a minimum period of four to four and a half months is required for a cercaria to mature from the germ-ball stage.

The breeding period for snails from Millport starts nearly a month later than that at Plymouth and consequently the shedding period is similarly delayed. The snails obtained from St. Bride's Bay in April were not yet breeding and only a few of the infected ones contained mature cercariae.

If the two infected snails kept at a temperature of less than 8°C. for nine weeks were subjected to these conditions just before the breeding season or early during that period then this would account for their condition and the absence of shedding during that period. Had they been allowed to warm up a little during the spring it is quite possible that the well developed but moribund cercariae would have emerged normally.

The behaviour of the cercariae.

The behaviour of C.pythionike was observed and comparison with that of C.doricha made by Rothschild (1935). During the course of the present work independent observations on the behaviour of C.doricha have been made by K. N. Brown, whose work is at present in preparation for publication and who has kindly allowed me to see the manuscript.

Rothschild points out that the typical resting and sinking positions of C.pythionike and C.doricha are different. This has now been established as a difference between the sub-groups of which these two species are characteristic. C.doricha pigmentata n.sp. is very similar to C.doricha in that when sinking passively the body is held straight with the tail in line with the body and very contracted, the cercaria sinking more or less head first or at a very steep angle (Figs. 51 & 52). C.nicarete behaves very like C.pythionike, sinking with the body slightly bent but more or less horizontal in position with the contracted tail held at right angles to the ventral surface of the body (Fig. 55). This species sinks with the ventral side uppermost. In both groups the initiation of ^{the} swimming movement is basically the same. The body is suddenly

bent ventrally with the oral sucker reaching back to just beyond the base of the tail (Figs. 53 & 56). The tail is extended to between three and four times its resting length and it begins to lash with a rapid S-shaped action, driving the body through the water with the middle of the dorsal surface to the front (Fig. 54).

Rothschild placed freshly emerged specimens of C.pythionike in the bottom of a 30 ft. long vertical tube of sea-water and found that they reached the surface in about five hours. C.doricha, on the other hand, seldom rose much above ten feet from the bottom. Brown also found that C.doricha did not rise to a height of more than a few feet in a vertical tube.

After emergence from the snail in an ordinary tank not more than two feet in depth all of the cercariae studied during this work swam at once to the surface. On reaching the surface the resting attitude is adopted and the cercariae slowly sink for a short distance then start to swim upward toward the surface once more, repeating the action. This behaviour is mentioned by Rothschild and was studied in great detail by Brown, particularly the relation between periods of activity and the age of the cercariae.

This type of small vertical movement is typical of many planktonic animals, particularly some of the copepods such as Calanus sps. which form a very large part of the food of plankton-feeding fishes. The effort to reach the surface and hence the richest plankton layers of the sea is well marked in C.pythionike, but is apparently not so great in C.doricha. As will be shown in the section dealing with metacercariae, there is reason to believe that C.doricha is more active in nature than it appears to be in the laboratory. A consideration of the possible stimuli which may initiate this attempt to reach the surface is useful. The most likely physical stimuli are light, temperature and water pressure. The last is the least likely as, if a certain water pressure is required to initiate the upward swim it is hardly likely that the behaviour pattern in the thirty foot vertical tube and in a tank of only two feet depth would be substantially the same. Light may well be important, but only in the later stages of the vertical movement as, on the authority of a naval diver, at a depth at which Turritella lives, in muddy water around the British coast, light would not penetrate. Rothschild states that C.pythionike is phototactic, swimming gradually away from a light source. Brown

found a similar reaction for C.doricha. In the present work observations have indicated that all species studied are negatively phototactic to a light source of bright intensity, but they do not seek total darkness. They appear to choose a position where the light is moderate but not too shaded. This again is a similar reaction to that of many planktonic organisms which achieve the same result by adjusting the depth at which they swim to the intensity of illumination. Thus, on a bright, sunny day plankton hauls will probably be found to be best at a depth of three or four fathoms while in dull weather they will be better nearer to the surface.

This leaves temperature as the third alternative for the stimulus to reach the surface. There is no doubt that the water at a depth of fifteen or more fathoms is much colder during the spring than the upper layers which are beginning to warm up and it is possible that it is this thermal gradient which influences the upward movement of the cercariae. This is difficult to check in the laboratory, firstly because the exact temperatures involved are not known and secondly because of the practical difficulties in reproducing these conditions. Even if temperature does play a part in the action it cannot be the whole solution, but it may

account for the difference in observed behaviour between C.pythionike and C.doricha if one of the two is more sensitive to the stimulus than the other.

The possibility of vertical water currents about which little is known at present and even tidal action acting as agents in the raising of cercariae from the bottom, cannot be overlooked.

So far only the individual behaviour of cercariae has been considered, but what seems to be more important and has been largely overlooked by the two previous workers is the group behaviour of large numbers of cercariae together. The manifestation of this is the tendency of individuals to gather together and form large clumps, all remaining independent of one another within the clump. They follow their own individual behaviour patterns, swimming up and sinking down again, but in such a way that the centre of the clump remains stationary. The stimulus to this reaction has not been discovered but the following experiment is interesting. A glass tank measuring eighteen inches in all directions was filled with sea water and placed where, as far as could be judged, the illumination was uniform. The water was stirred well to produce a uniform temperature and five hundred Cercaria doricha were added. The water

was again stirred well to distribute the cercariae as evenly as possible. The tank was then allowed to stand and within one and half hours nearly all of the cercariae were concentrated within a few cubic inches of water in a fairly dense group. This was repeated on several occasions with the same result, but the group did not form in exactly the same part of the tank each time. To test whether this was due to eddying in the water, as a result of the stirring, the whole experiment was repeated when the water was quite still and the cercariae were scattered on the surface, by means of a pipette, with as little disturbance to the water as possible. Under these conditions the group was formed within an hour, even more quickly than before, and again, when this was repeated the group did not form in the same place each time. Once the group was formed it did not disperse even when the tank was rocked gently to produce a slight "wave" motion. Only vigorous stirring was found to disperse the cluster and after such a dispersal the group was reformed once more, not necessarily in the same place. In all of these experiments the group was formed near the surface, with its centre some three or four inches down.

The form of the group was never compact, due to the

continual movement of the individuals forming it, but it resembled the mating swarms of some dipterous insects with a fairly dense centre, thinning out near the edges. This experiment seems to indicate that neither temperature nor light intensity are of any significance in initiating this grouping behaviour. It appears that the presence of some cercariae in a given locality attracts to others to the same area. To test this the previous experiment was repeated, but when the water was still two groups of cercariae of equal numbers (fifty in each) were introduced to different parts of the tank by carefully pipetting them in so that they were not separated by turbulence. After just over an hour when the tank was examined two separate clumps of cercariae had formed in approximately the positions where the two small groups had been introduced. The two formations were not quite equal in size and the tank was left undisturbed overnight. By the following morning the two groups had coalesced but were slightly deeper in the water than before. During the night considerable differences in light intensity had occurred, probably also minor variations of temperature. This test left no doubt, however, that the motive for this grouping is the presence of other cercariae rather than external physical stimuli.

Such "social" behaviour in invertebrates is known from a number of phyla. Allee (1941) summarizes many of these instances and in each case the reason for grouping activities has been found to be connected with reproduction, mutual protection or some physiological function as in the case of echinoderm embryos which develop more rapidly when crowded than in small groups. In these cercariae the advantage to be gained from grouping is that the chances of completing the life-cycle will be improved. As will be shown in the section dealing with metacercariae, the cercariae do not penetrate the exterior of the fish host but must be swallowed by it. This accounts for the characteristic up and down movement of the cercariae which is so similar to the behaviour of many of the planktonic animals on which fish feed. It is also the most useful way for a group of individuals to keep together as a group but remain out of physical contact with one another. A large group of cercariae is much more likely to attract the attention of feeding fishes rather than single individuals scattered at random through the plankton.

It has been shown that these groups are not broken up by the rather choppy artificial wave action produced in a laboratory tank and it seems equally unlikely that

they would be disturbed by the ordinary wave action of the sea, with its much longer periodicity, provided that the group was not too close inshore.

The probable sequence of events following on the shedding of cercariae in the sea is as follows: Firstly, an upward swim, the initiation of which is not known, then, when either the surface or some optimum depth is reached (the optimum may possibly be influenced by conditions of temperature and light intensity) the cercariae will tend to form into aggregations, the principle influence in this case being the presence of other cercariae.

An attempt to prove this in St. Bride's Bay in April 1954 by taking numbers of plankton tow-net hauls at various depths failed. When the Turritella which were being dredged at the same time were examined it was found that the sporocysts in the infected ones were not yet shedding cercariae.

Due to the lack of numbers of cercariae of other species these grouping experiments were tried only with C.doricha. It would be interesting to find out whether there is any difference in the rapidity with which groups are formed in species with different distributions of cuticular tubercles.

It has been stated that the grouping behaviour will assist in the completion of the life-cycle as it will attract the fish intermediate hosts to swallow the cercariae. It may also attract predators which can eat the cercariae without themselves becoming infected. In this connection it was interesting to discover a predator on these cercariae which at one time was a serious nuisance in the tanks in which cercarial shedding counts were made. Growing on the operculum of many Turritella communis is to be found a hydroid of the genus Leuckartiara. The species is probably L. octona (= Perigonimus repens Hincks) and it is most common on snails from Millport. When the Turritella is lying in its normal feeding position these hydroids are placed so as to be able to gather food particles from the inhalant current of the snail. It is quite easy for a cercaria in leaving an infected snail which is also carrying this hydroid to brush past their tentacles. To find out if there was an immunity to the nematocysts of this species, cercariae were induced to sink towards a clump of the hydroids. Immediately they touched the tentacles they were caught and engulfed. Digestion appears to be slow in these hydroids and the distended white appearance given by the prey lasts for some time.

An infected snail carrying this hydroid was frequently examined and every time several of the individuals were found to contain the remains of cercariae. The medusae had to be removed from the tanks as soon as they were budded off as, if allowed to mature, they were found with numbers of cercariae entangled in their tentacles.

Fig.40 Cercaria doricha Rothschild.

Fig.41 Cercaria doricha, nervous system.

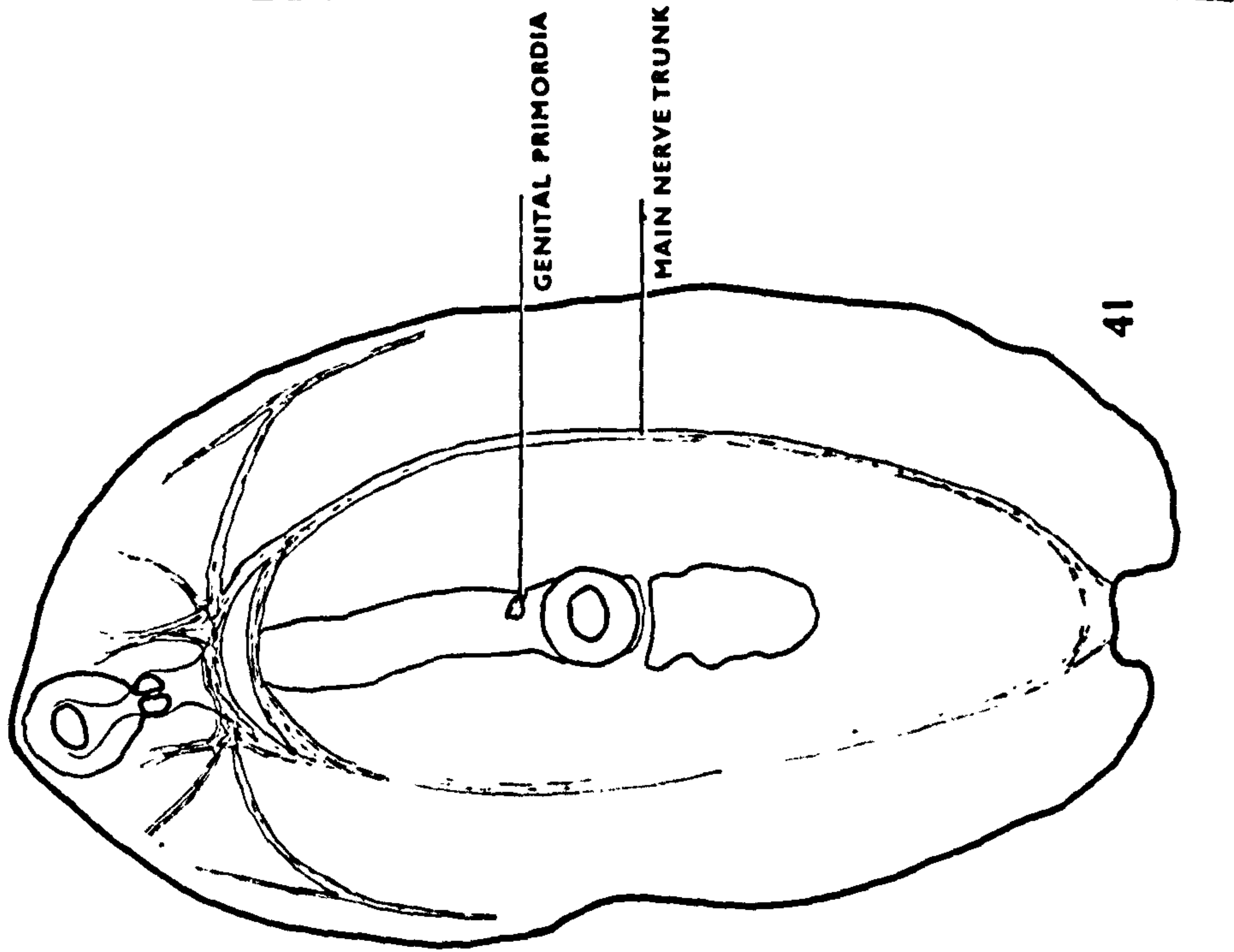
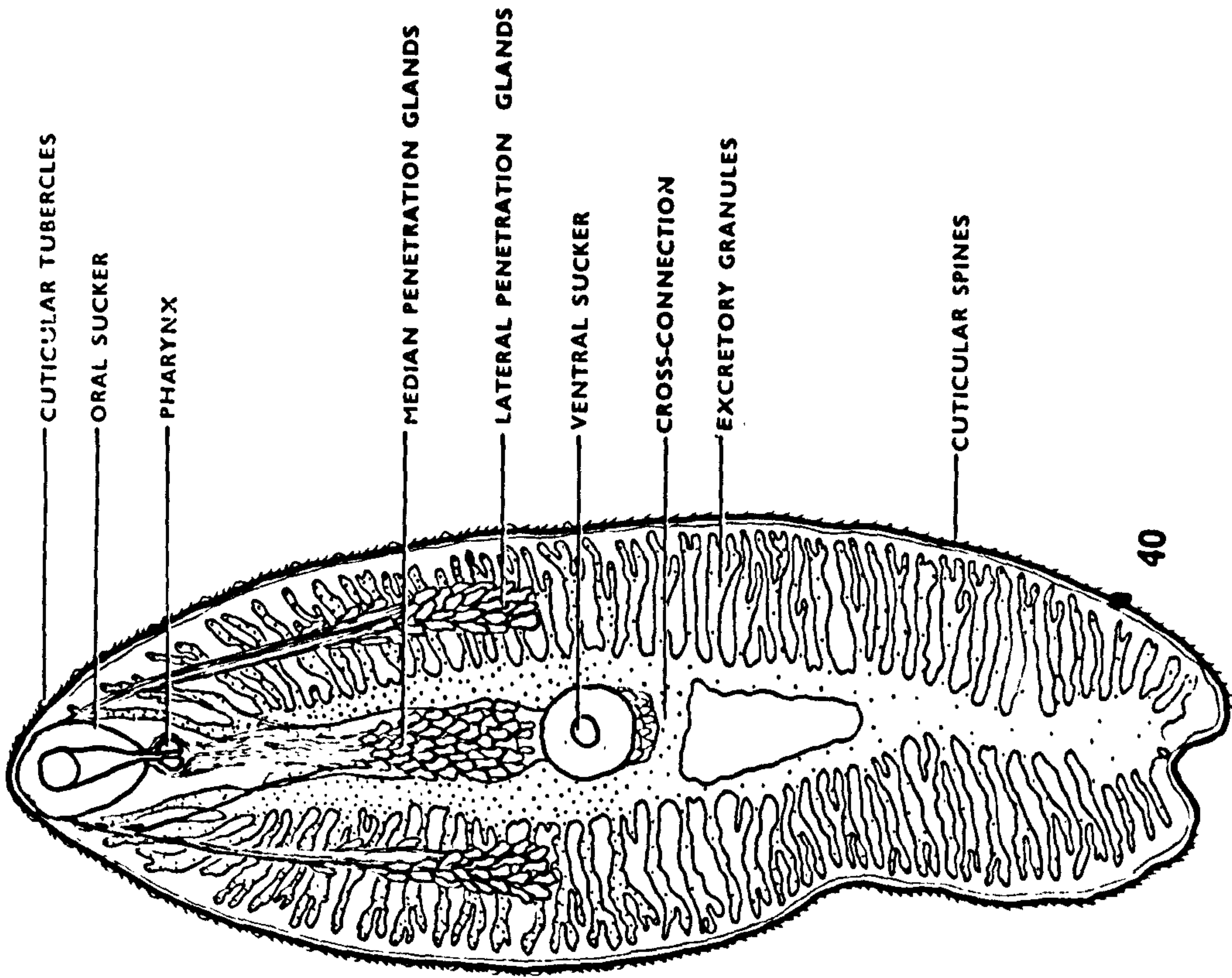


Fig.42 Cercaria pythionike Rothschild.

Fig.43 Cercaria nicarete Rothschild.

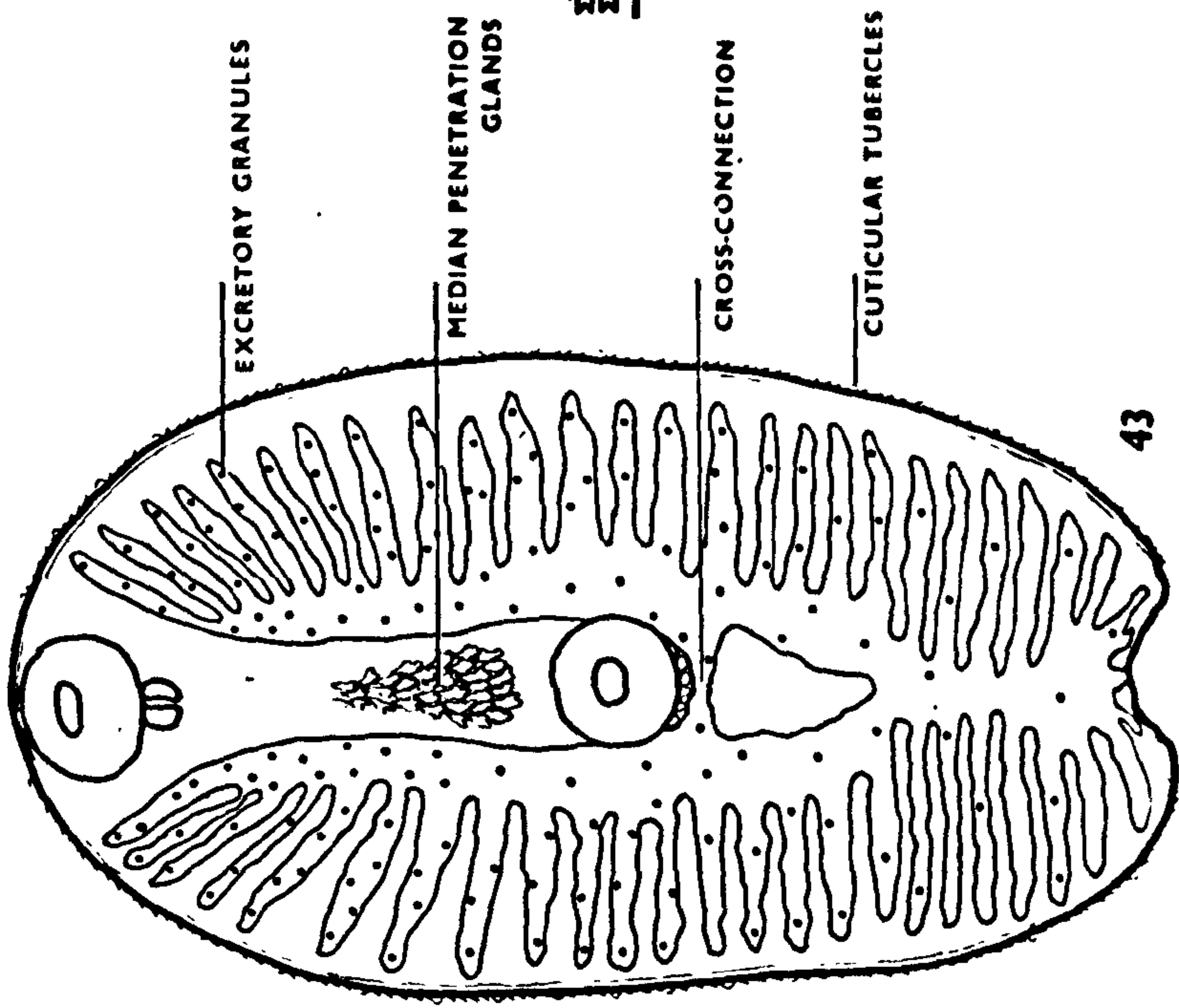
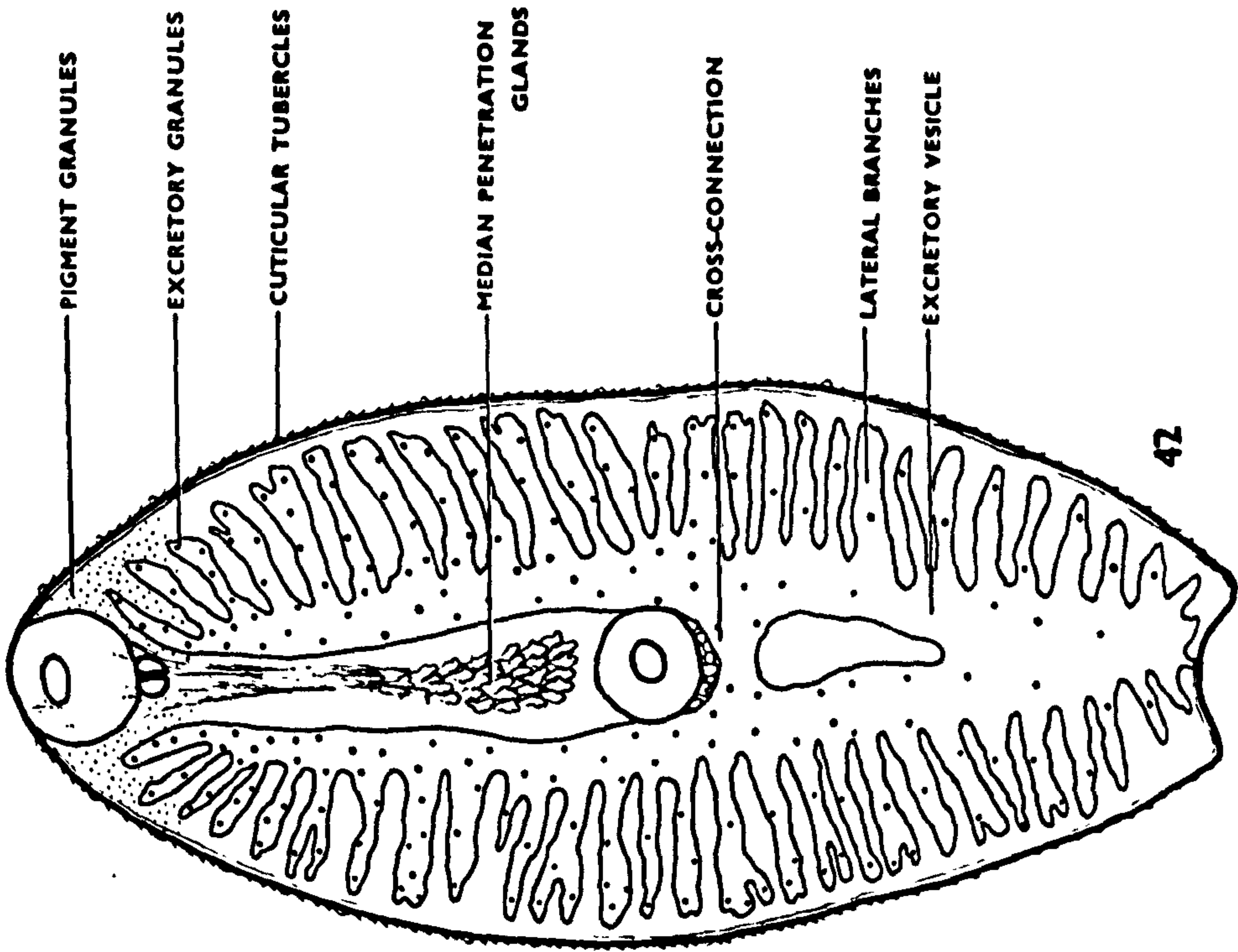
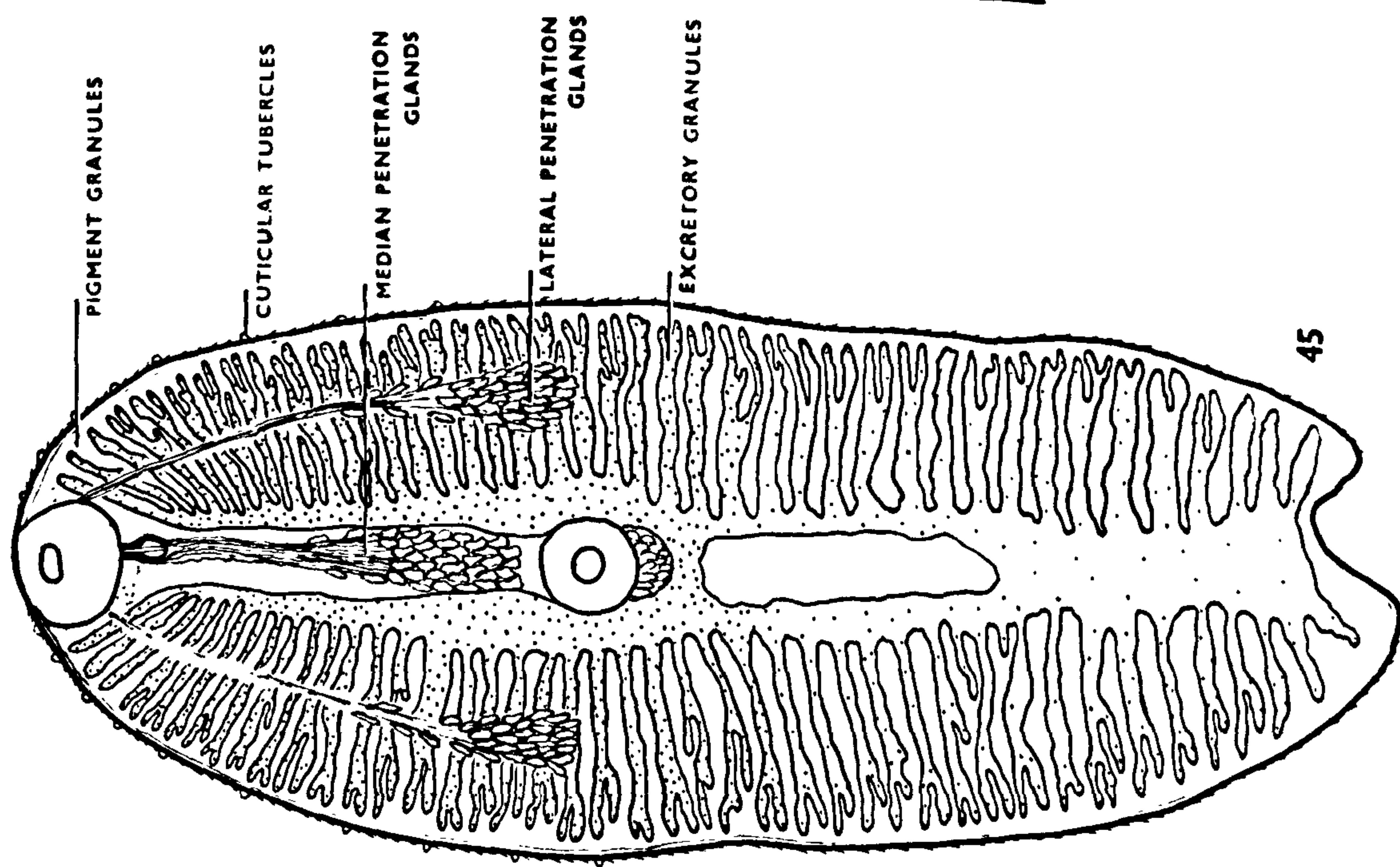


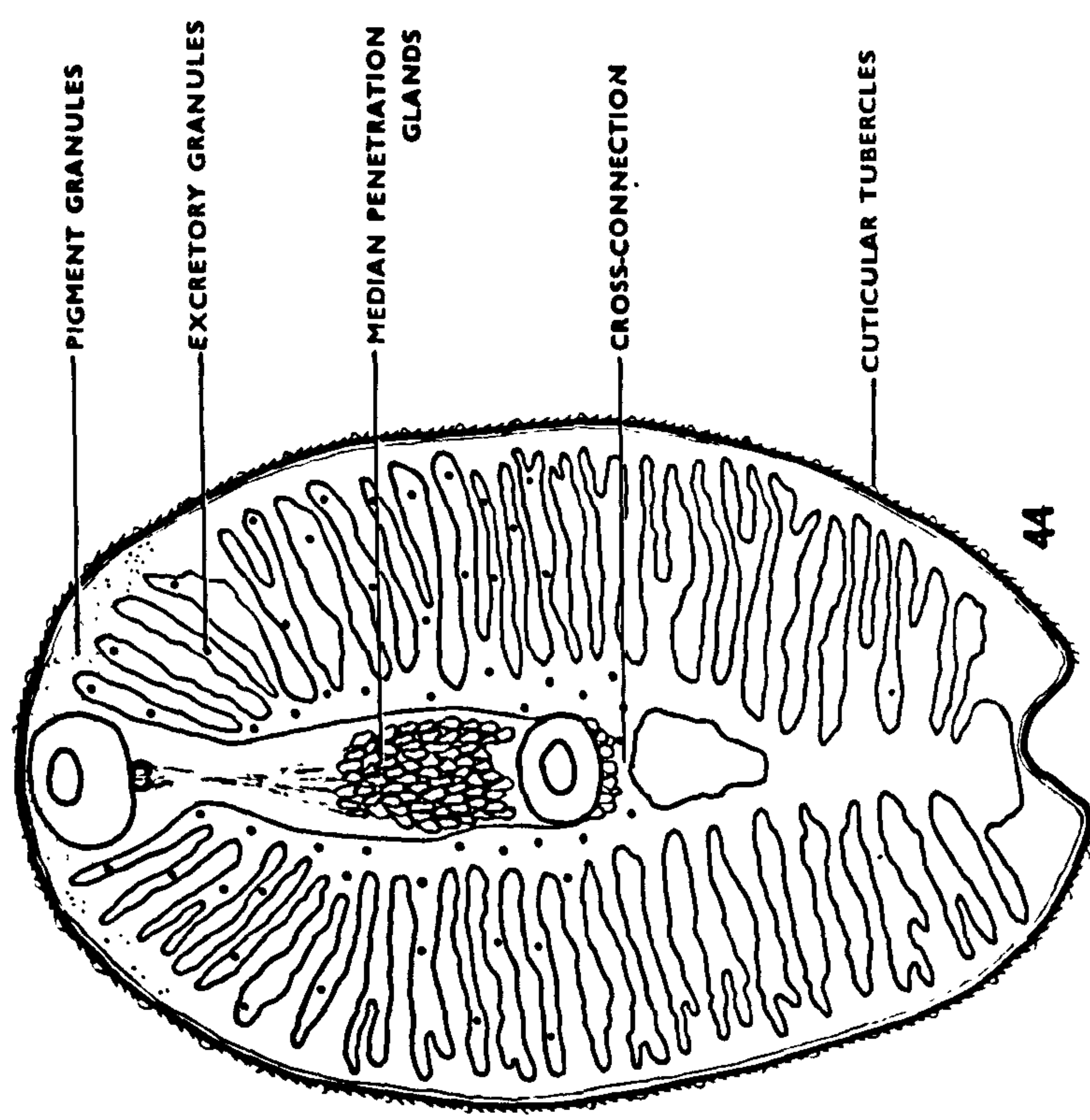
Fig.44 Cercaria cooki n.sp.

Fig.45 Cercaria doricha pigmentata n.sp.

0.5 mm



45



44

0.5 mm

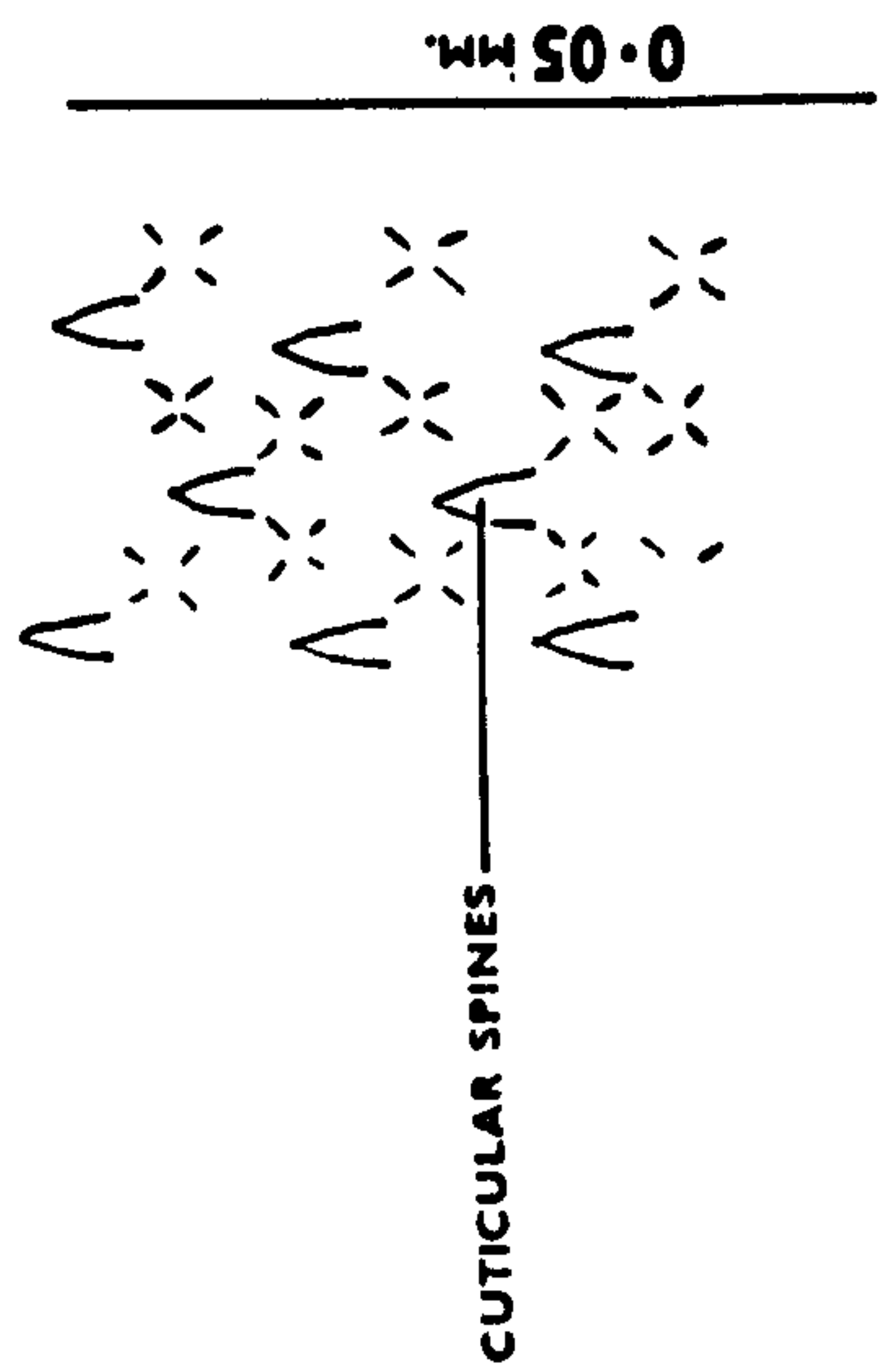
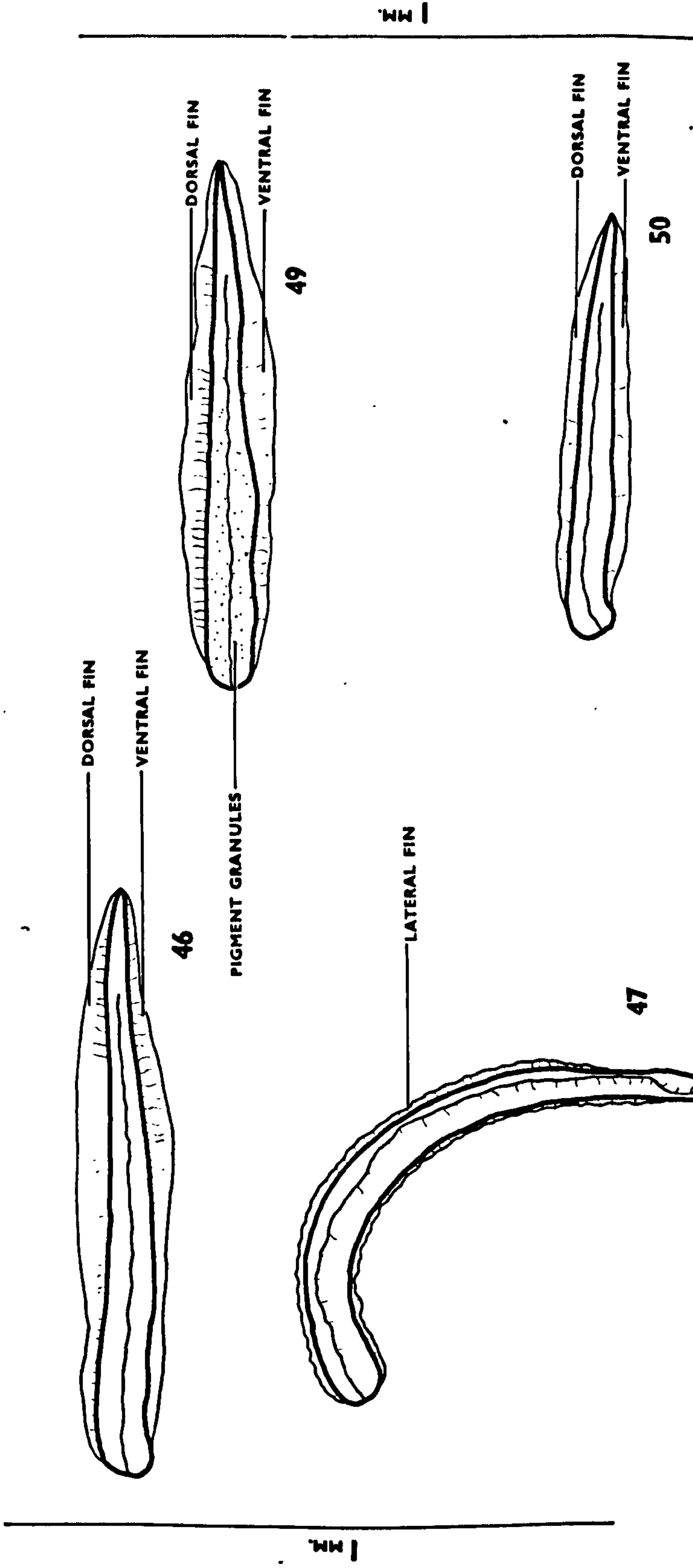
Fig.46 Tail of C.doricha lateral view.

Fig.47 Tail of C.doricha ventral view.

Fig.48 High power surface view of cuticle of
C.doricha.

Fig.49 Tail of C.doricha pigmentata n.sp. lateral
view.

Fig.50 Tail of C.nicarete lateral view.



- Fig.51 C.doricha, characteristic resting position.
- Fig.52 C.doricha pigmentata, characteristic resting position.
- Fig.53 C.doricha pigmentata about to swim.
- Fig.54 C.doricha pigmentata swimming.
- Fig.55 C.nicarete, resting position.
- Fig.56 C.nicarete about to swim.

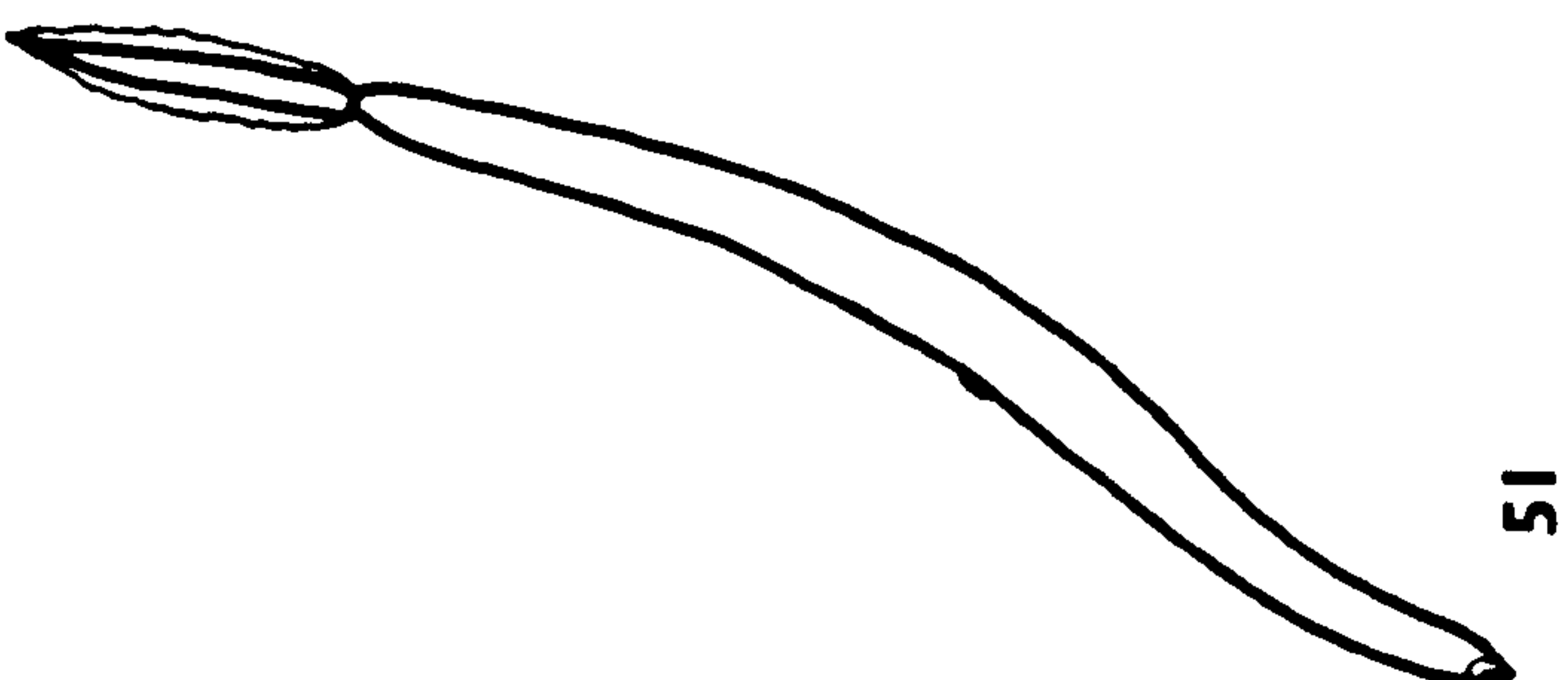
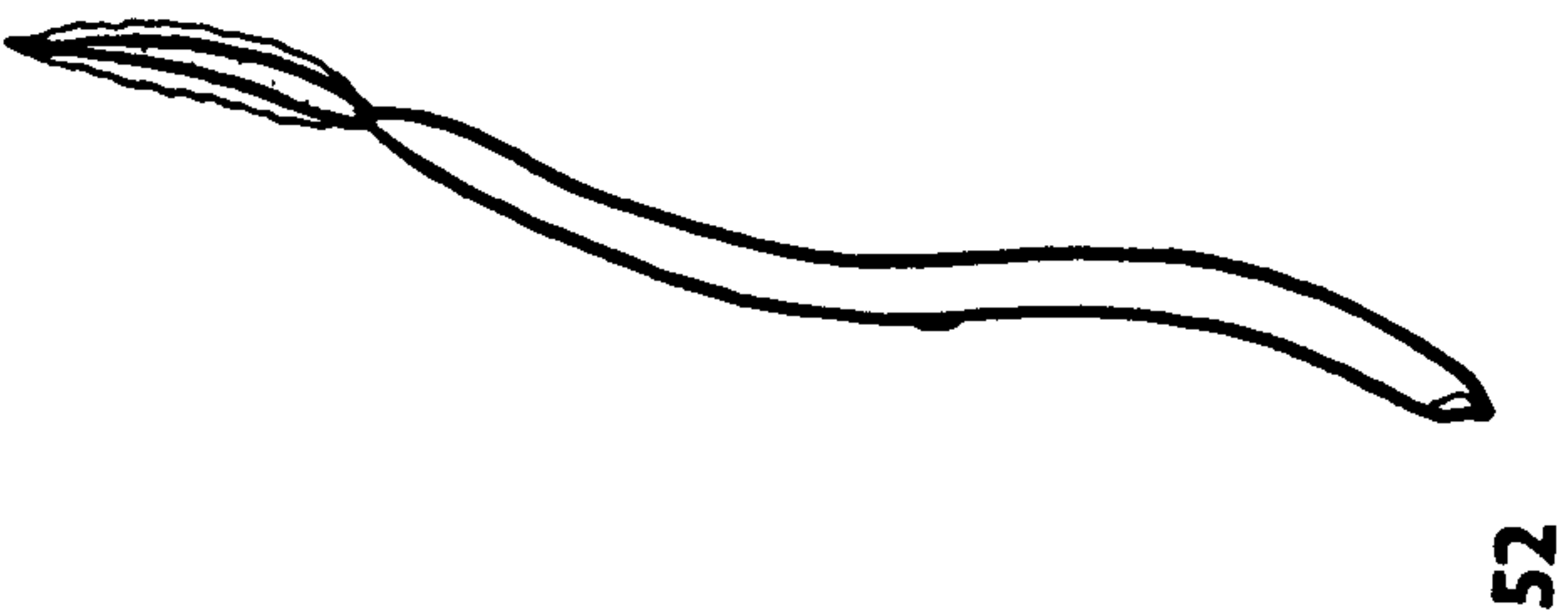
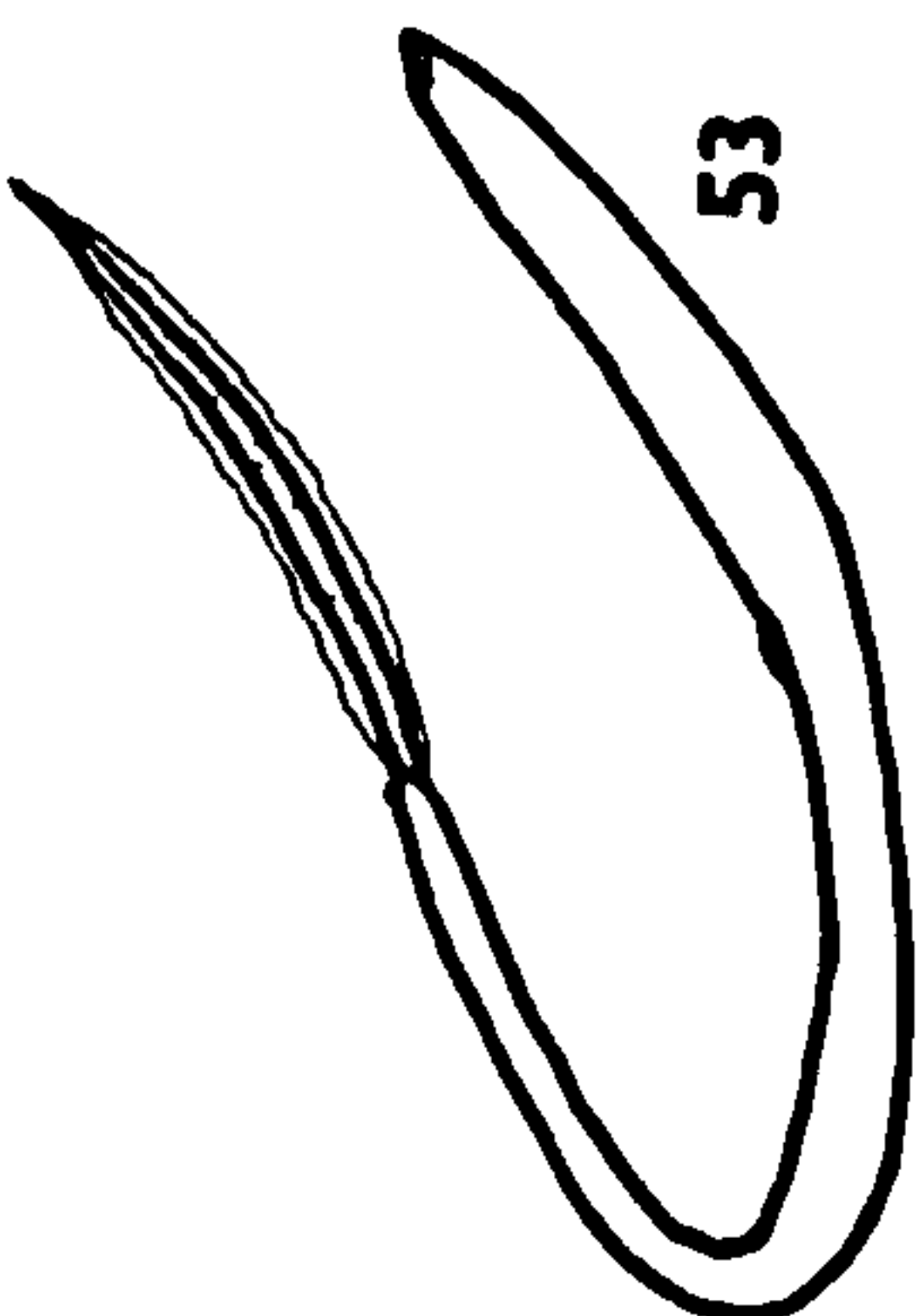
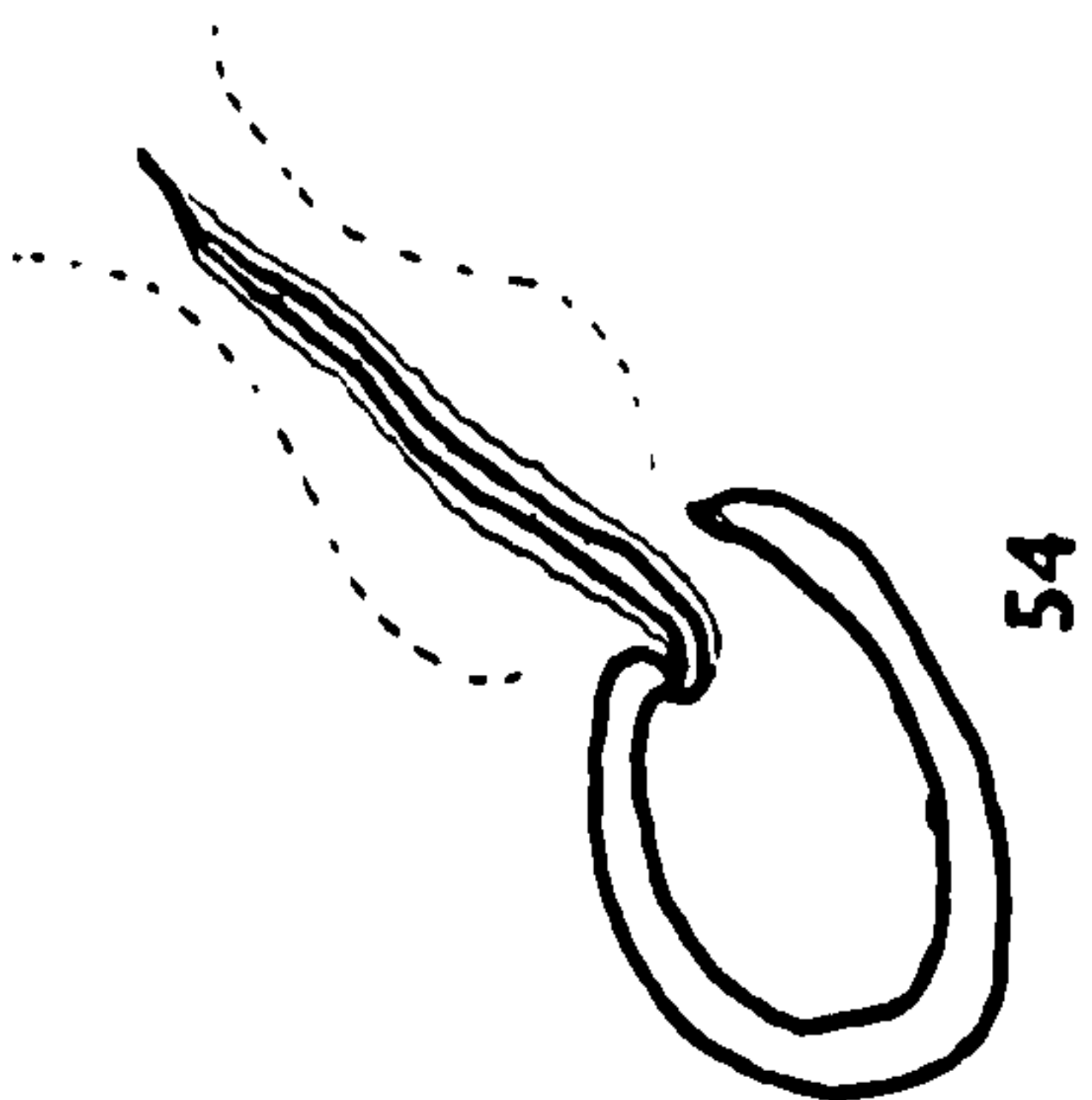
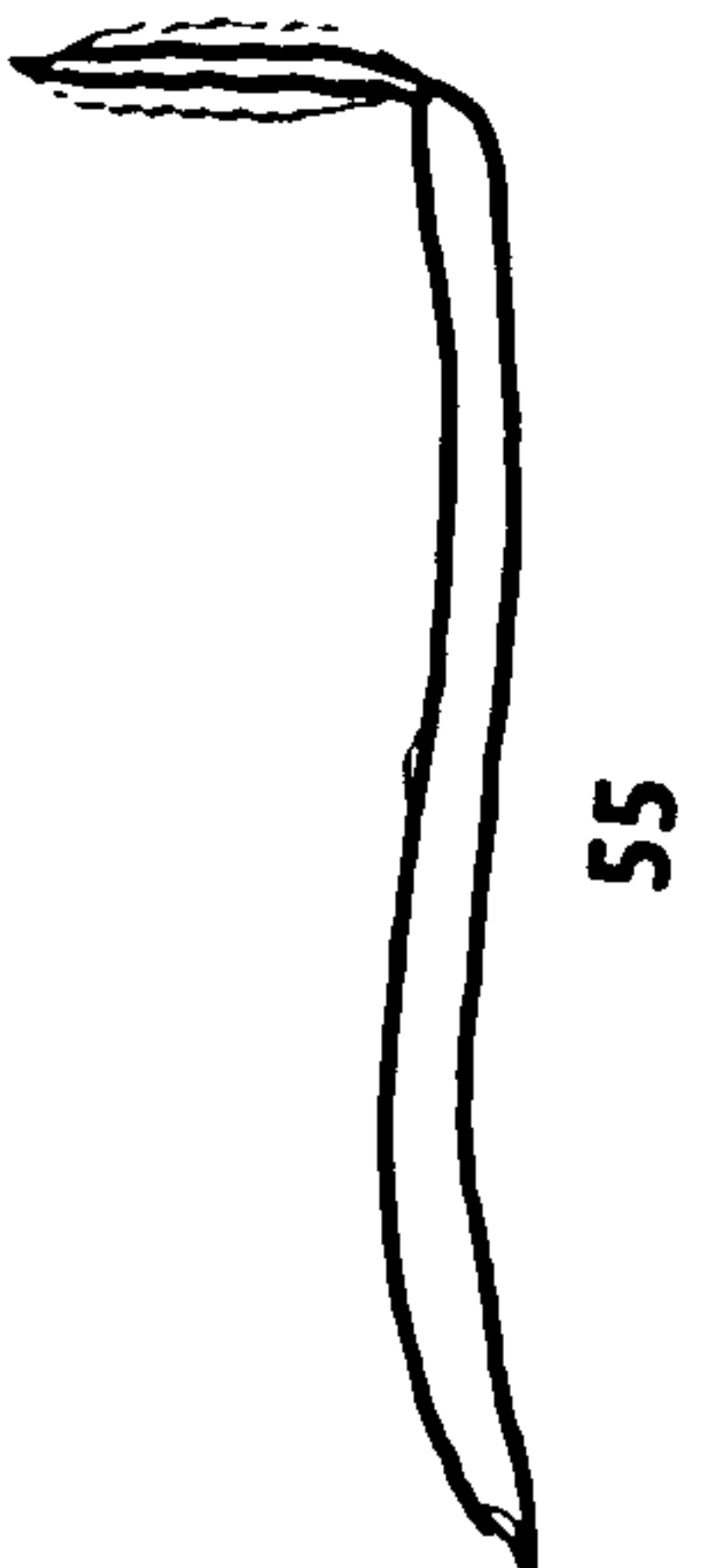
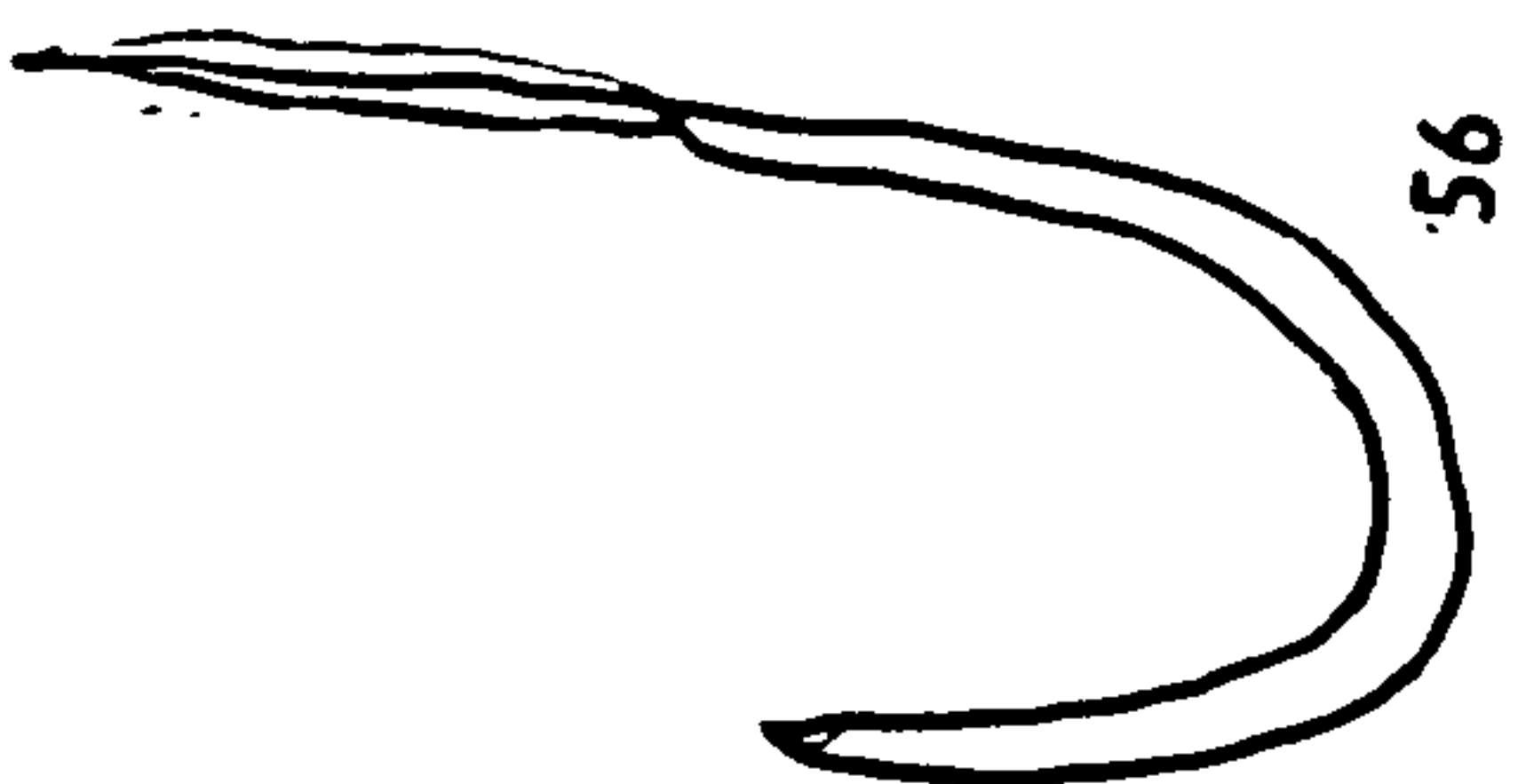


Fig.57 Histogram of shedding period of Corcaria doricha.

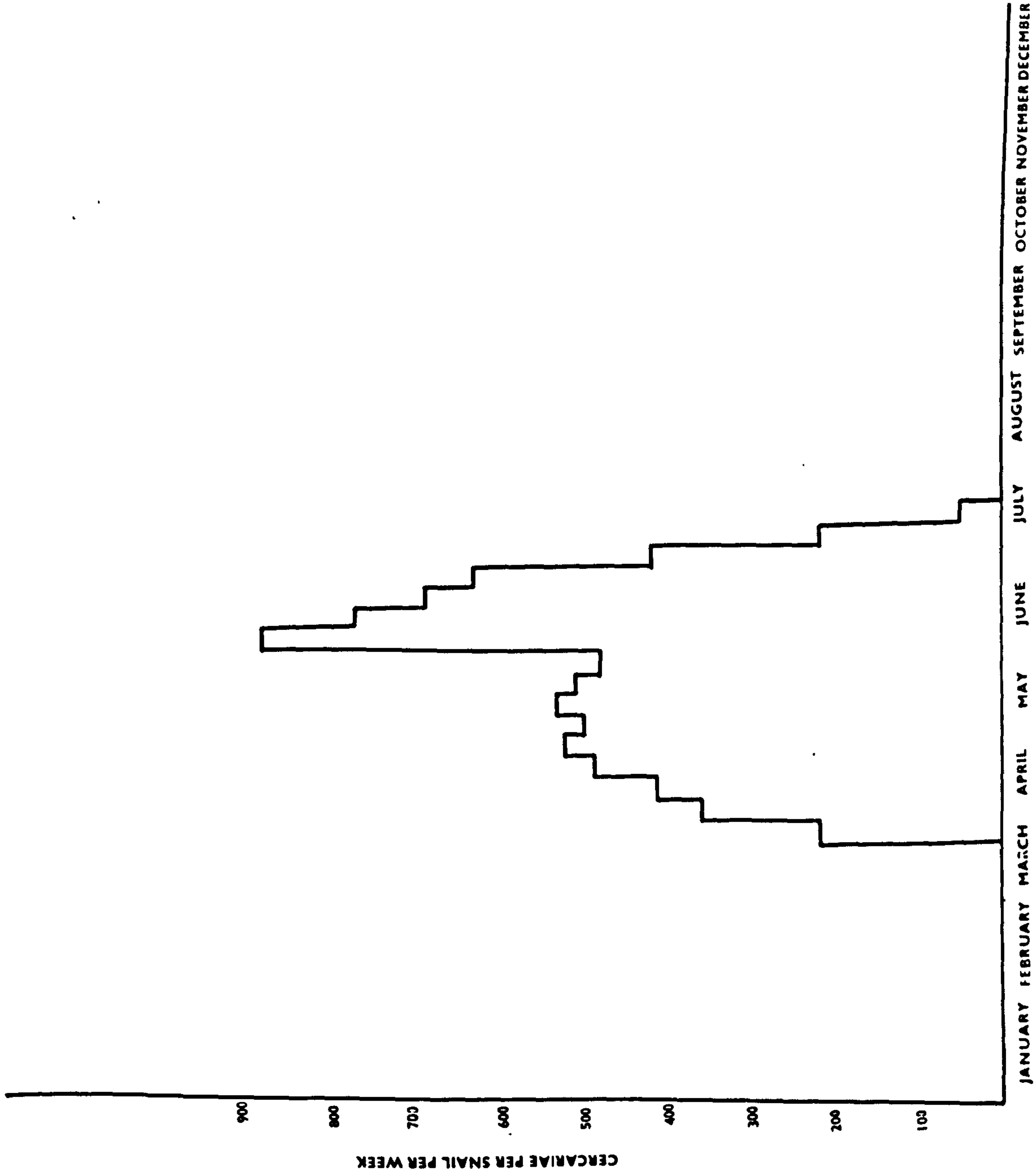


Fig.58 Histogram of shedding period of Cercaria
pythionike.

Fig.59 Histogram of shedding period of Cercaria nicarete

Fig.60 Histogram of shedding period of Cercaria
doricha pigmentata.

Note: In all three of these graphs the beginning is not shown as observations were not started until after the shedding period had begun.

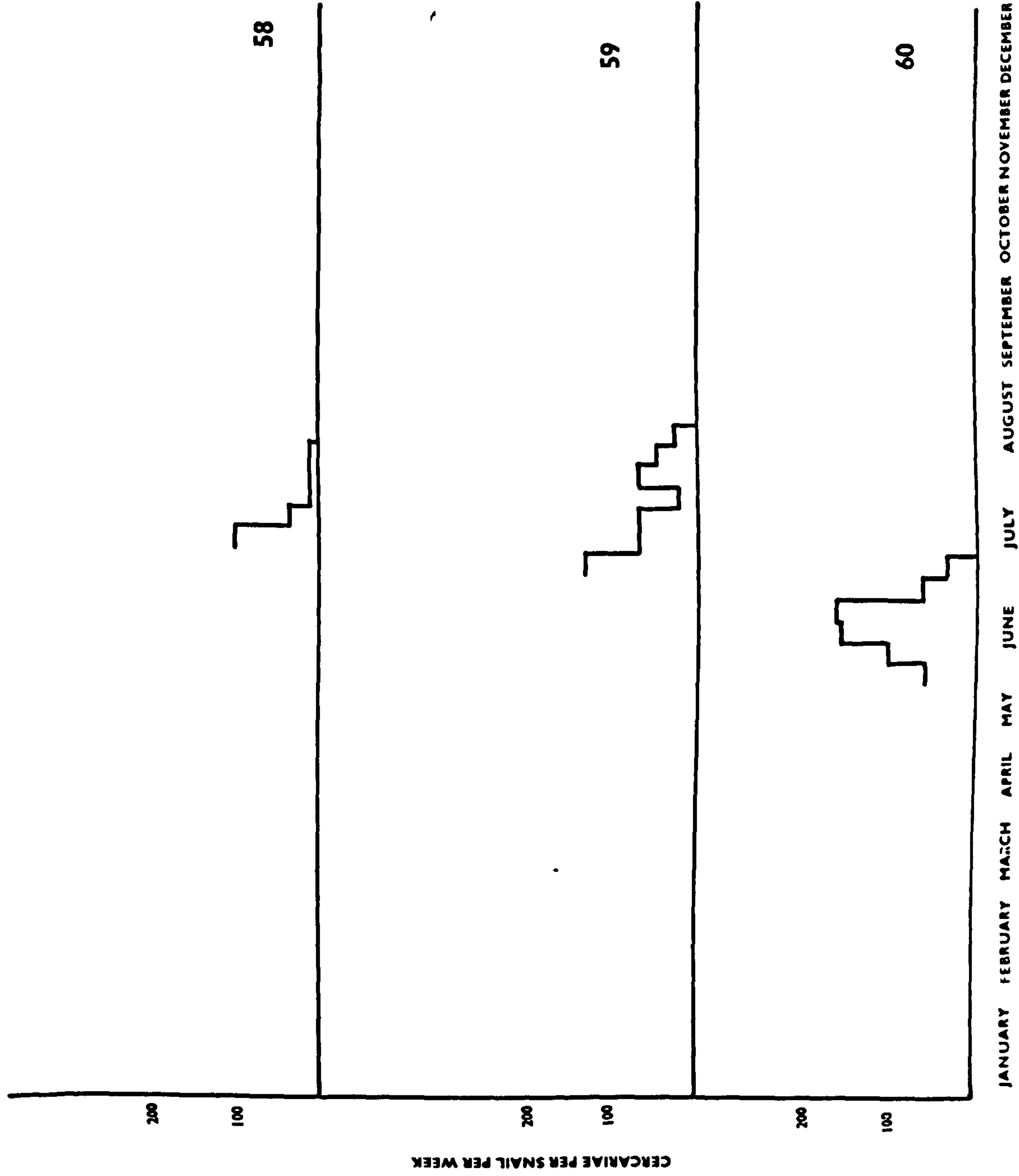
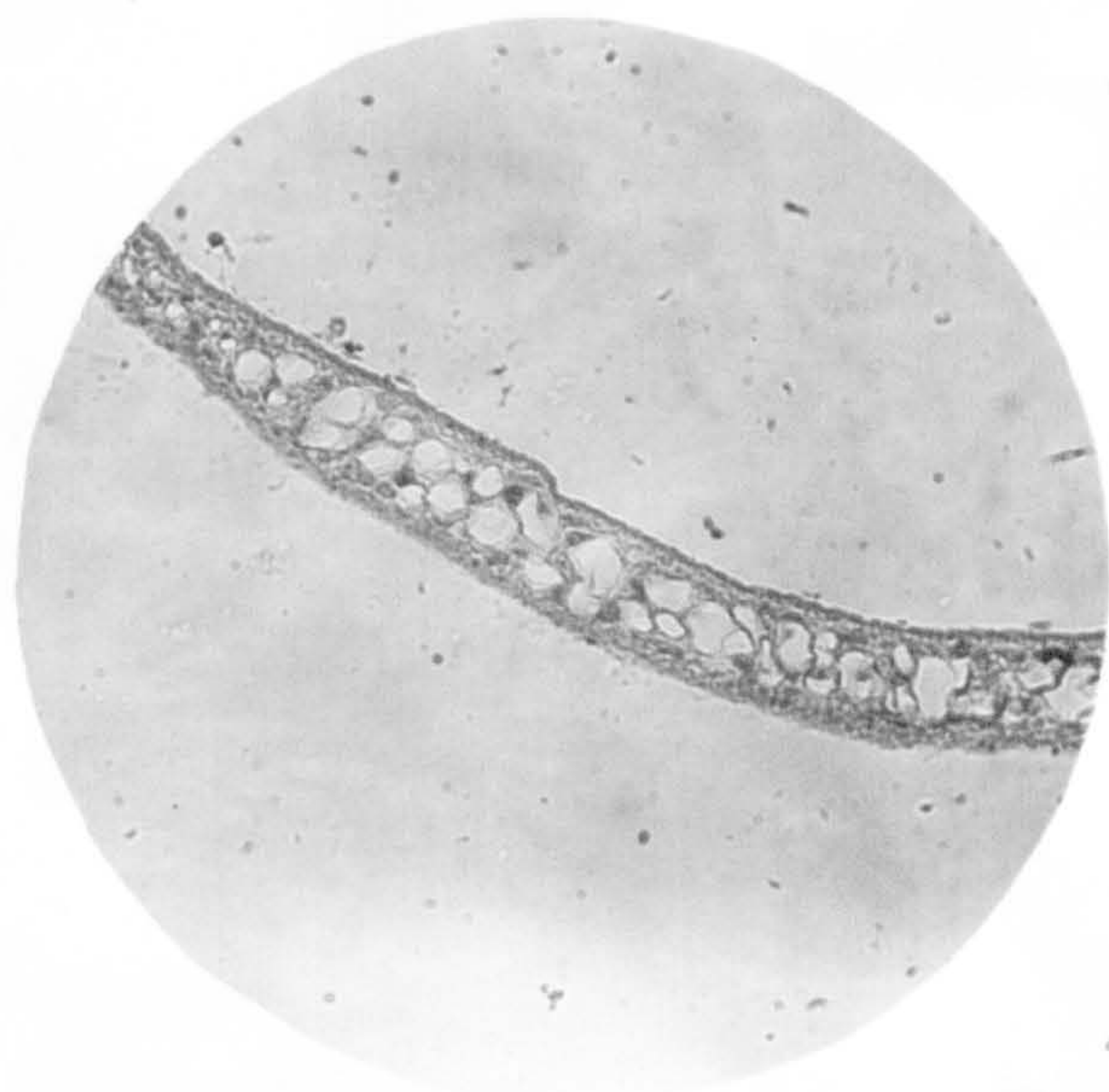


Fig.61 Photomicrograph of a longitudinal section of
 Cercaria doricha showing the arrangement
 of the lateral diverticula of the excretory
 vesicle.



The Metacercariae

Historical

Rothschild and Sproston (1941) published a description of the metacercaria of Corcaria doricha found in Gadus luscus from the Ramo mud (the principal locality for Turritella communis) at Plymouth. Two out of six of these fish which were examined were found to harbour cysts, one had about a hundred and the other, two. Fifty Gadus merlangus from various localities were also examined and two of these were found to contain three cysts each.

The location of the cysts was in the mesenteries connecting the pyloric caeca of the fish. The suggestion is made that the cercariae are swallowed and do not make their way through the skin of the fish. Rothschild (1935) had already shown that the cercariae were unable to penetrate the skin of a plaice.

The last part of this paper is concerned with speculations as to the life-history and final host of these metacercariae. The fact that C.doricha was observed (Rothschild 1935) never to rise far off the bottom when placed in a vertical tube was taken to indicate that this species is a bottom-living form. Because Gadus luscus is a bottom feeding fish it was

considered to be the normal metacercarial host. Gadus merlangus was thought to be a less likely natural host as it does not feed so consistently on the bottom. Because the supposed normal fish host is usually found at a depth of twenty fathoms or more it is stated that the final host is unlikely to be a bird. Various speculations as to the predators on both G. merlangus and G. luscus being the final host are then made. Those mentioned are: Lophius piscatorius, Rhombus maximus, Rhina squatina, Gadus pollachius, large Raja spp. and Trigla spp.

More recently Timon-David (1953) has reported that the sardines caught in the Mediterranean sea near the Provençal coast are very frequently infected with a metacercaria of the "Rhodometopa" group. Unfortunately the species of sardine involved is not named. The location of the cysts is given as the pyloric caeca, also the wall of the intestine and the mesentery. The author gives a detailed description of the metacercariae and draws the conclusion that these are the encysted stages of the type species, C. rhodometopa. From the size of the excretory granules and the anatomical details mentioned it seems much more likely that they belong to the species C. ampelis Rothschild, a Mediterranean species.

Timon-David differentiates his specimens from C.ampolis by the smaller size of the pharynx in his material, not a very reliable character. It is interesting to note that he was unable to find any trace of intestinal caeca or oesophagus in these metacercariae.

Whatever the species of these metacercariae from Marseilles there is not doubt that they belong to the pythionike sub-group mentioned in the previous section, also that their host, most probably the young of Sardina pilchardus, is a surface-living, shoaling, plankton feeding fish that can be easily caught by birds.

Metacercariae found during the present work

When the idea of a connection between the "Rhodometopa" group of cercariae and the genus Renicola was first developed the deep-water hosts recorded by Rothschild and Sproston for the metacercariae were considered to be a major obstacle to the theory.

Attempts to obtain Gadus luscus were made and were eventually successful. The fish is only of local and seasonal occurrence around the British coast. The material was obtained from a Newhaven trawler captain who gave the information that while Pout (the local name for G.luscus) is caught in fairly deep water during the day it is frequently taken at night in the nets of

drifters, fishing at or near to the surface. The fish when examined proved to be thirty-seven G.luscus and three G.merlangus. The examination for cysts was limited to the pyloric caeca, intestinal wall and associated connective tissues. No cysts were found.

When, later, the behaviour of the cercariae had been observed personally and the observations of Rothschild had been confirmed and extended, no doubt remained that the correct host must be a small, surface-feeding fish easily caught by birds. Reference back to the food lists of the birds recorded as hosts for various species of Renicola in Western Europe showed the recurrence of such fish as Ammodytes sps., the sand eels, small herring (Clupea harengus), sprats (Clupea sprattus), young whiting (Gadus merlangus), young pollack (Gadus pollachius) and other small gadoids, in the diet of most of these birds.

Adult herrings were examined but no success was obtained and the examination was rendered extremely difficult by the presence of large quantities of fat laid down in the mesenteries of the pyloric caeca.

Ammodytes sps. were largely discounted as possible hosts when it was found that these fish have only a single caecal appendage (Day 1884) and not the circlet of caeca

in which cysts were found by Rothschild and Sproston. Subsequent examination of a number of sand-eels has not shown any of them to harbour metacercariae. This left young herrings, young gadoids and sprats as possible sources of material. Young fish are difficult to obtain in this country due to the laws regulating the minimum size of various species which may be landed. Sprats are only of seasonal occurrence. Immediately sprats became available examinations were started and in the first batch dissected twenty-four out of twenty-seven fish were found to be infected with metacercarial cysts of "Rhodometopa" group cercariae. Shortly after this discovery came Timon-David's report of metacercariae in Mediterranean sardines.

Subsequent batches of sprats contained variable numbers of infected fishes and these will be discussed later. Two substantially different kinds of cysts were found, one type was larger and more rounded than the other which had an elongate ovoid form. The first kind were found to contain metacercariae of the doricha type and the second enclosed forms of the pythionike sub-group. This, then, is yet another difference between the two sub-groups, in addition to the anatomical and behaviouristic dissimilarities already mentioned.

The doricha type of cysts were by far the most numerous and were concentrated mainly in the mesenteries of the pyloric caeca. Not many of the cysts were reserved for anatomical study, the majority were used in infection experiments. Those that were examined bore a very close resemblance to the description given by Rothschild and Sproston. Most of those cysts which were measured fell within the size limits given by the previous authors, 0.53 mm.-0.395 mm. x 0.40 mm.-0.26 mm. The largest found measured 0.55 mm. x 0.41 mm. The cyst wall is hyaline and fairly thick, but very pliable. The worm within the cyst is bent so that the posterior end does not quite meet the oral sucker and there is ample room for movement. When removed from the fish the worm within the cyst can be seen to be moving almost continuously. When dissected out of the cysts the metacercariae are seen to be practically indistinguishable from young flukes recovered from the kidneys of birds (Figs. 62 & 63). Similarly they show scarcely any change from the cercarial form except for the loss of the tail and most of the penetration glands, also a slight reduction in size as noted by Rothschild and Sproston. The only way in which this material differed from the description given by the previous authors was

in that no prepharynx was seen in any specimen. When the material was available time did not allow of a detailed anatomical study of living specimens so no observations on the flame-cell pattern were made. The size of the excretory granules, 0.002-0.003 mm. leaves no doubt that these cysts were those of Cercaria doricha.

The cysts of the pythionike type were much less common than the preceding forms and were frequently found associated with them in the same fish. Although most commonly located in the mesenteries of the pyloric caeca these cysts were also found embedded in the fatty tissue down either side of the intestine. Only a few of these cysts were opened and due to a variety of circumstances the contained larvae were not examined in a living condition. Fig.63 illustrates a fixed and stained specimen. The cysts are hyaline and thick walled (up to 0.020 mm.) and measure usually about 0.38 mm. long by about 0.22 mm. in width. These measurements are very close to those given by Timon-David for the metacercariae from sardines. The contained larva is much more closely folded than is that of C.doricha, the anterior and posterior ends being bent sharply in a ventral direction and there is little spare space within the cyst. The larvae do not appear to be so

active as are those of the larger species. The pink pigment in the anterior end shows clearly through the cyst wall and can be seen at once when the cysts are examined under the binocular microscope.

The excretory vesicle of the contained metacercaria is visible through the cyst wall and it was noted that the excretory granules in the vesicle were large and few in number. This eliminates C. herpsyllis as a possible species, while the presence of pink pigment at the anterior end rules out C. nicarete. It is probable, therefore, that these cysts were either of C. pythionike or the new species C. cooki.

The origins and infection rates of *Clupea sprattus*.

Nearly all of the fish used in these examinations were obtained either through the Billingsgate fish market or local retailers in London who had in turn bought the fish from the main market. In most cases the dealers either could not or would not give the origin of the fish or even the port at which it was landed. Enquiries as to how long the fish had been dead were equally unsuccessful. Through the kindness of W. Young and Son two batches of fish were obtained during the off-season together with information as to their origin and the time dead. Some material was

kindly supplied by the Ministry of Agriculture and Fisheries laboratory at Lowestoft together with accurate collecting data but this ^{material} was all preserved in formalin.

The following table shows the findings of a number of examinations. In each case only the pyloric caeca, intestine and associated mesenteries were examined.

<u>Date</u>	<u>Number</u> <u>Examined</u>	<u>Number</u> <u>Infected</u>	<u>Origin</u>	<u>Maximum no. of</u> <u>cysts in any</u> <u>one fish (approx.)</u>
7.1.54.	27	24	East Coast (?)	120
12.1.54.	30	23	Unknown	80
13.1.54.	36	27	"	300
14.1.54.	34	26	"	80
15.1.54.	26	21	"	70
19.1.54.	35	5	Landed at Brixham (?)	15
20.1.54.	33	24	Unknown	280
24.1.54.	12	2	"	3
31.1.54.	25	1	"	1
9.2.54.	86	20	"	190
10.2.54.	83	14	"	240
11.2.54.	84	21	"	170

The percentage infection rate of the sprats examined as shown in the above table is 40.6% in five hundred and eleven fish. It will be seen that the last date on

which fish were examined was 11th February, 1954. Enquiries amongst wholesale fish dealers gave the information that the best period for sprat-fishing was in March, consequently the majority of the material obtained up until the date shown was used for infection experiments on birds. Unfortunately the sprat season for 1954 appeared to terminate abruptly and no further fish were obtained for many months.

The preserved material from the Fisheries Laboratory at Lowestoft consisted of batches caught off Cromer, Mablethorpe, Bridlington and just north of Spurn Head. Most of the fish in these samples were very young "white-bait" and no cysts were found in any of them.

At the end of July the two off-season batches supplied by Young's were caught by the whitebait fishermen in the Thames estuary. The majority of the fish examined measured between 5 and $7\frac{1}{2}$ cms. long and in the opinion of Mr. N.B. Marshall of the British Museum (Natural History) they were probably little over six months old. Of two hundred and twenty seven fish examined only five were found to be infected and those contained only one cyst each. The cysts were of the pythionike type but only one contained an active larva, the other four were apparently moribund. The fish had

been dead for just over twenty-four hours when examined. The fishermen who caught these fish were surprised at the presence of specimens of such large size in the Thames estuary during July.

The results given above are all unfortunately rather vague. The sources of the heavily infected fish are in no case known with certainty and of the batches whose origins are known none were found to carry any significant numbers of cysts.

Since the breeding season of sprats in the North Sea is said to be from March until June and growth is rapid in the early stages there is little doubt that the fish caught off the east coast in the samples from Lowestoft were little more than two or three months old. The breeding period of sprats in other areas is not well known. In the English Channel it is said to begin in January, but no information for the west coast could be obtained. The movements of the adult sprats during the off-season fishing period from the end of March to November are not well known and are at present under investigation by various workers. The most recent theory is that they move off-shore into deeper water. This may have some considerable significance when considered in the light of the known shedding period (March-July)

and behaviour of Cercaria doricha with which so many of these fish were found to be infected.

The actual degree of infection in sprats carrying cysts is worth some consideration. In the tabulated results given above the maximum number of cysts found in a single fish in each batch is listed in the right-hand column. The greatest number ever found was about three hundred and in this batch several fish were found to have infections of this size. In each of the heavily infected batches there were usually one or two fish carrying a hundred or more cysts and several with about fifty. The majority usually had an average of 6-12 cysts each and a few had less. This wide divergence in the degree of infection in sprats which were always obtained ~~XXXXXXXXXXXXXXX~~ from the same approximate locality (batches were always obtained direct from the boxes which are packed on board the boats) is perhaps unexpected when the fish are all of about the same age group.

If the cercariae were distributed randomly throughout the plankton then a more uniform infection rate in the fishes would be expected. If, however, the distribution in the plankton is not uniform and the cercariae tend to form groups, as described in the previous section,

then the infection rate expected in fishes would be similar to that which has actually been found. The sprat is a shoaling species and the individuals within a shoal may be very close together. If such a shoal encountered a group of cercariae then those fish passing through the centre of the group would be most heavily infected while those passing through the less dense edges of the cercarial cluster would obtain a proportionately lower infection.

The finding of large numbers of Cercaria doricha cysts in Clupea sprattus raises once more the problem of whether this species of cercaria does behave the same way in nature as it does in the laboratory. If the sprat is exclusively a surface feeding fish then the cercariae must rise to the surface layers of the sea. If, however, the theory concerning a deeper water phase in the life of the sprat is correct then it is probable that the cercariae do not rise to much greater heights from the bottom than the experiments of Rothschild showed. Whatever the correct answer to this problem may be it seems to indicate that Gadus luscus is not the natural intermediate host that it was assumed to be by Rothschild and Sproston. The diurnal vertical migration of this fish described by the trawler captain suggests that it

might easily be caught by birds fishing at night but there is no evidence to show that birds ever do this. Although G.luscus undoubtedly has plenty of opportunity to become infected with cercariae, particularly in its surface-living post-larval stage through which all gadoids pass, it seems probable that the two infections recorded by the earlier authors are "cul-de-sacs" from the life-history point of view.

The finding of both doricha and pythionike types of cysts in the same fish confirms the view expressed in the section on adult flukes that mixed infections of different species in birds may occur.

The work of Timon-David shows that sardines, probably of the species Sardina pilchardus, are capable of acting as hosts for the metacercariae of the "Rhodometopa" group. Although his records are from the Mediterranean there is no reason to believe that the sardines from the Biscayan coast may not equally well serve as vectors, as has been suggested in the preceding section, accounting for the large infections in the Manx shearwaters which live on these fish.

Excystation.

For observations on the anatomy of the metacercariae the cysts were teased open with fine tungsten wire

needles, a relatively easy operation. An attempt to bring about excystation by means of an *in vitro* digest failed. The digest used was of the usual peptic type employed in the liberation of larval Trichinella spiralis from their cysts. The solution was buffered to a pH value of between 2.0 and 2.2 and incubated at 40°C. Within the first thirty minutes the cysts had swollen to nearly twice their original size and over half of the contained larvae were dead. By the end of an hour the remainder of the worms were dead and disintegrating. A papaine digest was tried with similar results but it was later found that this had not been correctly prepared. In a private communication Miss Rothschild informs me that she also attempted excystation experiments but that all were unsuccessful except for a "bacteri^ological ferment" the nature of which was not very clear and which appears to have arisen as something of an accident in one of the other digests.

The damaging effect of a peptic digest buffered to normal mammalian conditions led to a search for information on digestion in birds. The only paper forthcoming was that by Vonk et al. (1946) on gastric digestion in chickens. A dissertation by Mennoga (1938) was quoted in which a pH value of 3.5-5.89 had been found in the

stomach of herons, the only fish-eating bird investigated. Protein digestion in these birds is rapid but the pH values given are far too high for any known peptic digestion to be effective. The inference is that either proteolytic digestion in herons is not by means of pepsin or, if it is, then the pepsin involved is not similar to that found in mammals. This may well account for the failure to excyst these metacercariae by means of a mammalian type of digest.

It is also possible that no one enzyme acting alone will be sufficient to break down the cyst wall. It has already been suggested that the young flukes reach the kidneys by ascending the ureters in the manner described for Tamerlania bragai in pigeons by Maldonado (1943 and 1945). If this is so then there is no need for the larvae to leave the cysts until their passage through the intestine is nearly completed. Under these circumstances the outer wall of the cyst will be subjected to a series of digestive enzymes like any other food material.

Infection experiments on birds.

Three attempts to feed encysted metacercariae of both the doricha and pythionike types to different species of birds have been made. The birds used were a domestic duckling about six weeks old, a domestic chicken also

about six weeks old and an adult greater black-backed gull.

For these experiments cysts were removed from sprats by dissection and placed in Ringer's solution. When in Ringer's solution they were examined and only those containing actively moving metacercariae were selected. These were counted out into gelatine capsules which were then fed to the birds. In the cases of the duckling and chicken the beak was opened and the capsule pushed down into the oesophagus until it could be felt from the outside. It was then pushed gently down by running a finger and thumb down either side of the oesophagus from the outside until it was nearly in the stomach. This technique is of no use with gulls which can regurgitate the whole of their stomach contents when excited or frightened. The capsules were fed to the gull concealed inside pieces of whale-meat.

The duckling received 300 cysts and was killed after one week. A very careful examination of the intestine, ureters and kidneys revealed no flukes.

The chicken also received 300 cysts but was not killed for four months, during which time weekly examinations of its excretory material were made for the presence of eggs. None were found and no flukes were found at the post-mortem examination.

The greater black-backed gull received 1700 cysts in doses of 300-600 over a period of two weeks. Daily examinations of the excretory material were started one week after the first dose of cysts. These were continued for two weeks and were then reduced to twice weekly examinations for the remainder of the time until the bird was killed, four months after receiving the first cysts. No flukes were recovered during the post-mortem examination and no eggs were passed during the period after infection.

None of these three experiments was successful. The reasons may be various. Firstly, the three species of birds used have not been recorded as hosts for natural infections of Renicola and may have some immunity to the flukes. This is not very likely in view of the great variety of species that has been found to harbour the flukes in nature. Secondly, it is possible that the young chicken and duckling had some age immunity. In a private communication Dr. Annie Porter informs me that she has frequently been unable to infect young birds with species of flukes that normally occur in adult birds of the same kind. She suggests that this is due to the rapid passage of food through the intestine in young birds, not giving the cysts time for the walls to be

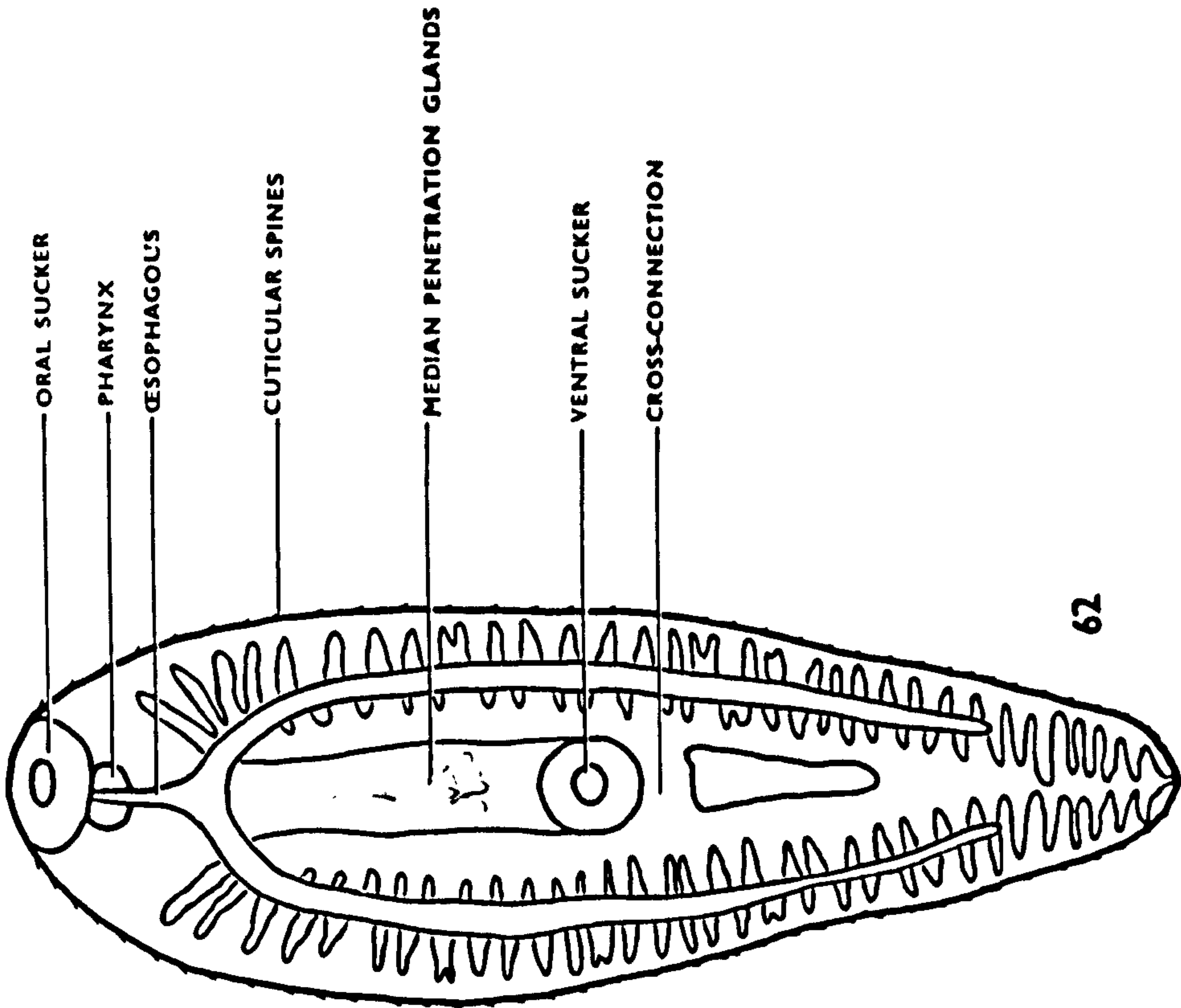
digested, or alternatively, sweeping the young flukes out before they have time to become established.

Lastly, the most likely reason for the lack of success in these experiments is that the cysts, although containing living worms, were no longer infective. No information could be obtained from the suppliers of the heavily infected sprats as to how long they had been dead. Sprats decay fairly rapidly, particularly around the intestine, site of the encysted metacercariae. It may be that although the contained worms were still actively moving they were in fact moribund and if they did emerge from the cysts they were no longer capable of establishing an infection.

Due to the lack of material none of these very unsatisfactory experiments has been repeated under different conditions.

Fig.62 Young Renicola sp. from Puffinus puffinus.
Living specimen stained with neutral red and
drawn under coverslip pressure.

Fig.63 Metacercaria of the pythionike type from Clupea
sprattus, removed from cysts, fixed and
stained.



ORAL SUCKER

PHARYNX

ESOPHAGOUS

CUTICULAR SPINES

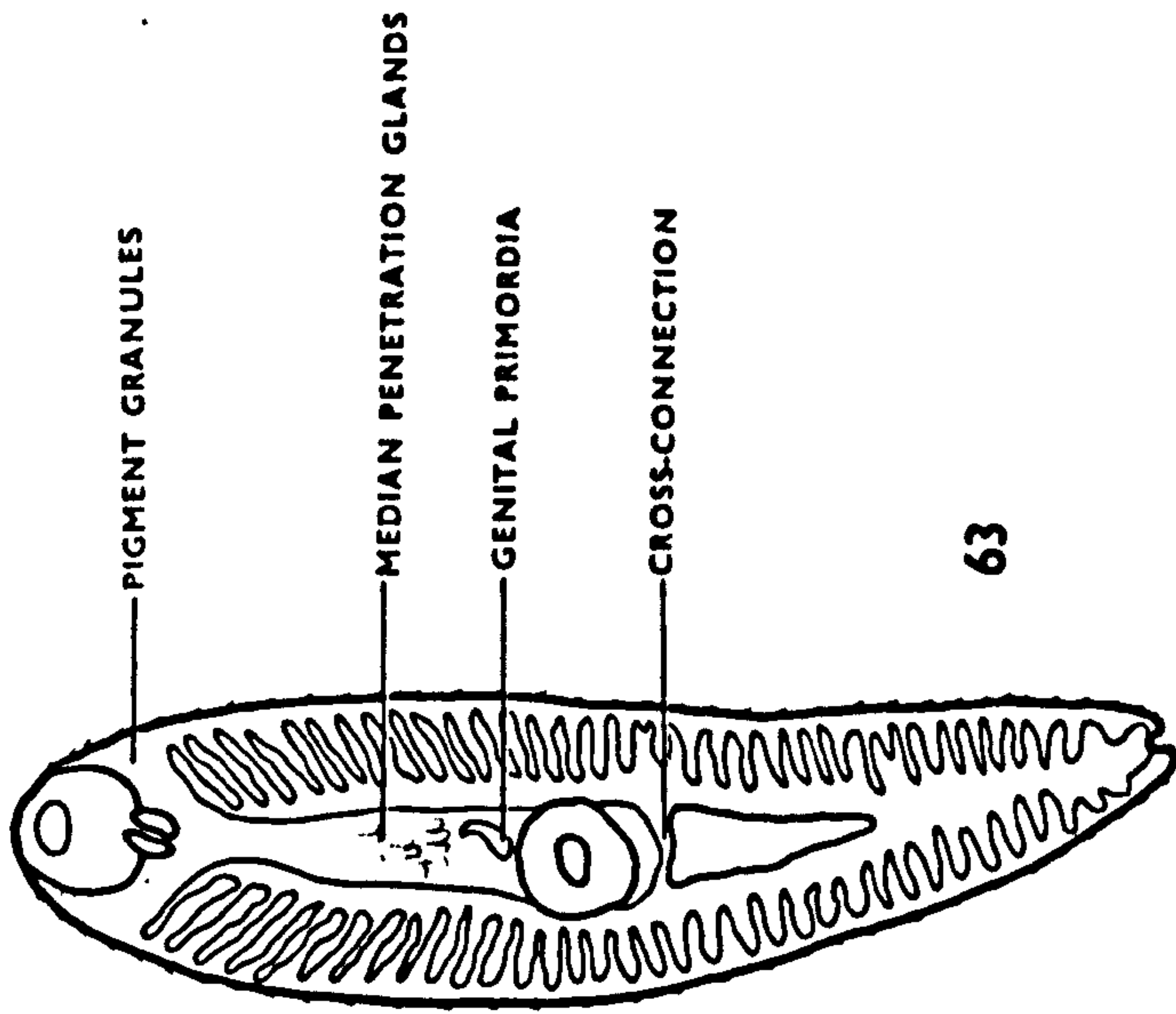
MEDIAN PENETRATION GLANDS

VENTRAL SUCKER

CROSS-CONNECTION

62

1 mm.



PIGMENT GRANULES

MEDIAN PENETRATION GLANDS

GENITAL PRIMORDIA

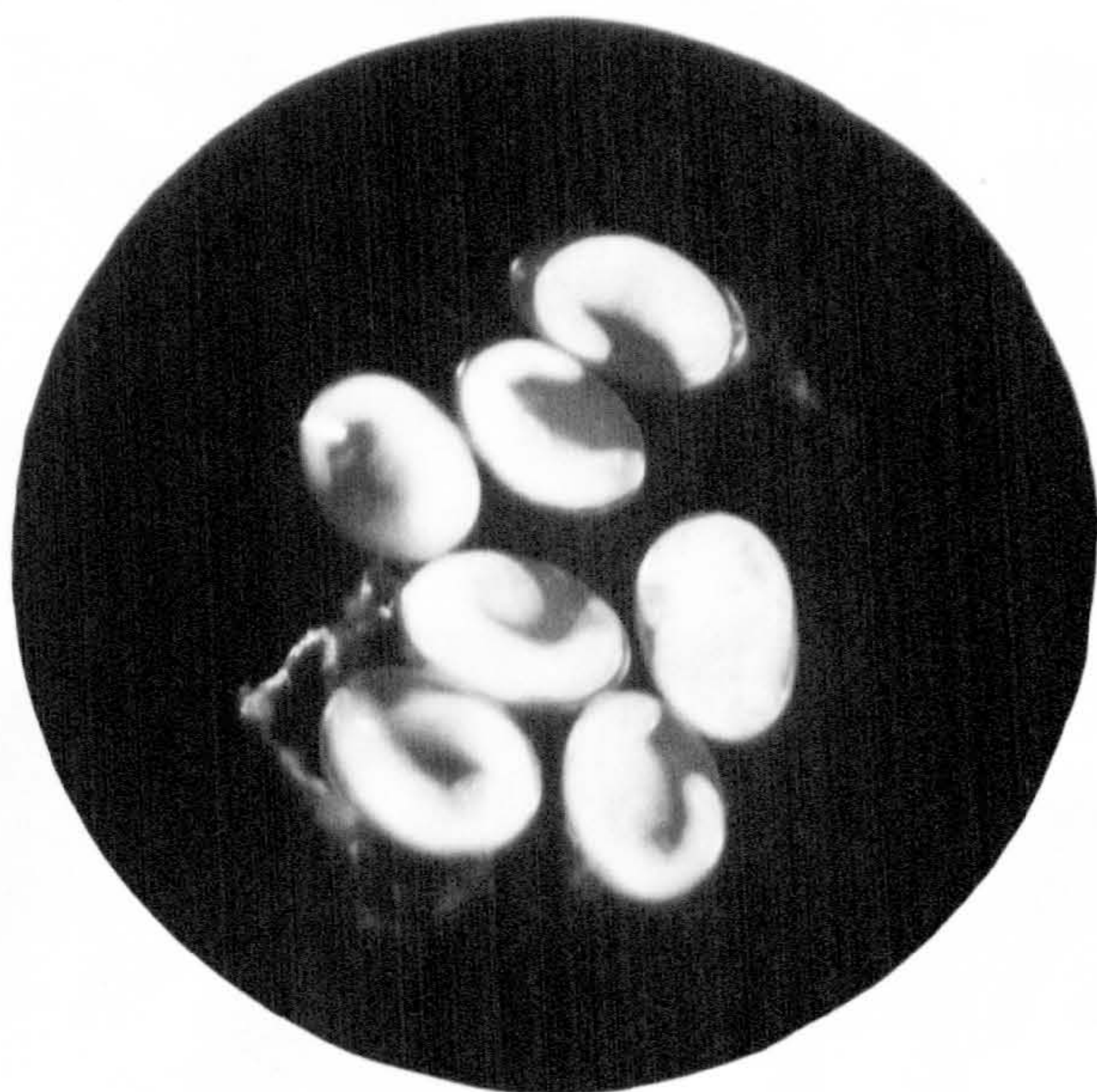
CROSS-CONNECTION

63

1 mm.

Fig.64 Cysts of metacercariae of the doricha type.
 Photomicrograph by reflected light.

Fig.65 Cysts of metacercariae of the doricha type.
 Photomicrograph by transmitted light.



Discussion

The morphological evidence presented in this work leaves no doubt as to the definite connection between the "Rhodometopa" group of cercariae and the trematode genus Renicola. Experimental evidence in support of this is poor, but the ecological aspects presented lend a good deal of weight to the theory.

Since the morphological and anatomical evidence is the most strong and the most straightforward of the three it is best to discuss this before the others.

Firstly, the cuticle and its structures. In all of ~~of~~ the adult specimens of Renicola examined the same quincuncial arrangement of spines has been observed. All of the cercariae of the "Rhodometopa" group have an exactly similar arrangement. Cuticular tubercles whose longitudinal distribution is of importance in the identification of the cercarial species have also been found on the adult flukes. In some of the flukes these tubercles have only been traced as far back as the level of the ventral sucker while in others they extend right to the posterior end of the worm. The position of the ventral sucker in young flukes may prove of value in identifying them with larval stages when more is known of the metacercariae and the modifications in structure

which may arise in this stage.

Secondly, the excretory system, on the close similarity of which between the larvae and adults this work was largely started. The basic form of the Y-shaped vesicle with its lateral branches and cross-connection between the arms behind the ventral sucker has been found to be constant in all adults and cercariae that have been examined. No other vesicle of this nature appears to have been recorded in any fluke or larval stage. The number and character of the lateral branches of the vesicle may vary slightly from one species to another but the possible variation within a species has been shown to be so great that this character is of little value in identification. The complex flame-cell pattern of the larvae has not been related completely to the adult flukes. The posterior three groups of flame-cells and the main collecting tub^uercles of the other groups have been observed in young flukes removed from the kidneys of Manx shearwaters. The arrangement was that characteristic of a cercaria~~ae~~ of the pythionike sub-group. In the same young flukes traces of a median cluster of penetration gland cells, also characteristic of the same sub-group of cercariae were found. So far the excretory granules, so important in determining the

species of cercariae, have not been observed to persist in the adult stage.

Thirdly, and of as much significance as the excretory vesicle, is the position and shape of the genital primordium. This crescentic group of deeply staining cells is present in the cercariae, metacercariae and very young flukes. The position is always the same, slightly in front of the ventral sucker and just to the left of the mid-line. It appears that the gonads do not differentiate until some time after the young flukes have reached the kidneys of the final host.

The last of the anatomical characters about which something must be said is the digestive system. This is present in all adult flukes but has not been seen in some very immature specimens. Of the workers who have studied either the cercariae or the metacercariae only Rothschild has reported the presence of a gut, and this only in late cercarial embryos in which neither the cystogenous nor penetration glands were developed. Neither Stunkard, Timon-David nor the present author has been able to find any trace of even an oesophagus.

The anatomical similarities described above are greater than those to be found between many other cercariae and their adults and they do establish beyond any reasonable

doubt that the genus of flukes and the group of cercariae under discussion are related.

The experimental evidence produced to support the anatomical studies is small but is significant. The difficulty of attempting to induce the hatching of eggs has been described, also the fact that the intestines of Turritella communis to which large numbers of eggs had been fed were found to contain eggs with open opercula and free miracidia near by. Existing evidence indicates that in those flukes where the egg has to be swallowed by the molluscan intermediate host hatching will not occur in the wrong species of snail. The development of lesions on the intestine of snails within twenty-four hours of the ingestion of eggs of Renicola sps has been noted. These lesions appear similar to mother sporocysts. The rapid death-rate of batches of snails to which large doses of eggs have been given and the finding, in one case, of immature daughter sporocysts in half of the survivors has been described. Alternative explanations for these occurrences have been sought but the only satisfactory solution to account for them is that they are the results of artificial infections produced in the laboratory.

No such confirmatory evidence has been obtained

from the very inadequate attempts which have been made to infect birds by the administration of encysted metacercariae.

Ecological studies have provided several useful links. The most notable of these was the determination of the probable second intermediate host in the cycle. A study was made of the food records of birds known to act as hosts to Renicola sps., also the behaviour patterns of the "Rhodomotopa" cercariae. The combination of the two demanded that the host should be a small, surface-feeding fish easily caught by birds and likely to be attracted by a behaviour pattern similar to that of many small planktonic crustacea. The various alternatives were considered and the sprat was selected as a probable vector. It was in this fish that the metacercariae were found in profusion. When it became realized how specialised in their choice of site within the fish the metacercariae are it was seen that Ammodytes was anatomically unsuited to act as intermediate host. A further point was thus established to account for the higher rate of infection in Manx shearwaters, feeding primarily on young clupeoids, than in the auks whose food consists mainly of sand eels.

A small contribution in the ecological field was

the finding of a very high rate of infection (17%) in Turritella communis dredged from South Haven, Skomer Island, almost immediately below one of the nesting areas of the Manx shearwater. Not too much importance should be attached to this observation as the sample was a small one and possibly not truly representative of the population in South Haven.

This is the evidence in favour of the relationship between the flukes and cercariae. In the succeeding pages discussion of some of the observations made during the course of the work will be made.

An analysis of the observations on the development of the sporocyst sheds some light on the natural affinities of the genus Renicola. The eggs are small and must be ingested by the snail before hatching occurs. The miracidia appear to penetrate the gut of the snail and develop for a short time on the outer wall of the intestine. They then seem to leave the intestine and probably move into the body tissues at the base of the spire. The finding of a structure very similar to a paletot surrounding the daughter sporocysts in the gonad of the snail suggests a course of development very closely analogous to that described by Cort, Ameel and Van der Woude for the Plagiorchid Lechrionchis primus. In this species

the daughters form within the mother which then branches out carrying the daughters into the interlobular spaces of the snail's gonad. The development of the cercarial embryos from germ balls produced by a thickened area of the sporocyst body wall in the posterior region is again similar to the system described for the Plagiorchids by Cort and his co-workers. The cercariae, however, possess no stylet and are therefore dissimilar from the Plagiorchoidea in this respect. The production of a second generation of daughter sporocysts, whether it be by budding or from a persistent mother, is not known as yet for any other group of trematodes and constitutes a profound difference from the other groups.

No support can be found for the suggestion by Dollfus that the Ronicolidae are parallel to the Gymnophallidae. In that family the first intermediate host is a Pelecypod Mollusc and the second is also a bivalve.

New information regarding the behaviour and ~~shedding~~ shedding of the "Rhodometopa" group of cercariae has been obtained and may well give a lead to similar problems in trematodes of economic importance.

The individual behaviour of the cercariae described by both Rothschild and Brown has been shown to be closely

connected with the more important grouping behaviour of large numbers of individuals. It is unfortunate that Cercaria dorchia was the only species available in sufficient numbers for studies on the communal behaviour. It would be interesting to discover whether the action is so marked in other species and also what the effect of large batches of cercariae of mixed species would be. Would each species cluster separately or would large, mixed groups be formed? The survival value of this communal behaviour is obvious and the connection between this activity and the infection rate in the fish intermediate host is striking.

The close connection between the breeding period of Turritella communis and the shedding of cercariae is another feature of this life-history with very wide possible implications. Unfortunately not a lot is known as yet of the breeding behaviour of the molluscan intermediate hosts of many of the trematodes of economic importance. It is probable that such a connection will only be found in those species where the sporocyst or redia stages parasitize the gonad of the intermediate host. In the genus Renicola it is quite possible that when more is known of the biology of the sprat that a close ecological connection between this limited shedding

period and the behaviour of the sprat at that time of year will be found.

In conclusion it should be mentioned that the records of natural infections of Renicola sps. made during this work are all new to Britain, also that with the exception of Uria aalge all of the hosts recorded are new ones for the genus Renicola. It is also the first time that a natural infection of a trematode from either Puffinus puffinus or Fratercula arctica has been found. In addition, two new species of cercariae of the "Rhodometopa" group have been described and Clupea sprattus has been found to be a common host to the metacercarial stages of this group.

Appendix

An attempt was made to hatch and raise a few seabirds in captivity for use in infection experiments. Rothschild 1936 and 1940 published accounts of attempts to raise Larus ridibundus and Larus argentatus in captivity. Success was obtained in the first species, but none at all with the other. In the account of the unsuccessful attempt with the Herring gull eggs several suggestions for the benefit of future workers were made and these proved useful in the present work.

In order to avoid transporting the eggs over considerable distances a visit was made to Skokholm bird observatory during the week 4th to 12th July, 1953. An incubator was improvised from an old paraffin stove oven heated by a chimney-type of paraffin lamp supported a suitable distance below the oven. A tray of water was fixed in the open bottom of the oven to distribute the heat as evenly as possible and to maintain a high humidity. This incubator maintained a temperature level which fluctuated between 37°C. and 41°C.

Due to the rather primitive nature of the apparatus it was considered desirable that the eggs should remain in it for as short a time as possible. For this reason only eggs which were beginning to show signs of chipping

were collected.

The following is a list of the eggs collected and the results obtained:

<u>Species</u>	<u>Number collected</u>	<u>Number hatched</u>	<u>Number addled</u>
<u>Larus fuscus</u>	17	6	8
<u>Larus marinus</u>	2	1	1
<u>Alca torda</u>	9	1	5
<u>Uria aalge</u>	4	0	3

The rather large number of addled eggs is probably due to the fact that the collecting was done late in the season. The greatest number of casualties occurred actually at hatching. Three Lesser black-backed gulls, two razorbills and one guillemot were lost at this stage, after the chick had broken through the shell but before it had emerged completely. The time lapse in some of the successful hatchings between the first break-through of the egg-shell to the final emergence of the chick was as much as forty-eight hours. The only egg which was not addled and which failed to develop further in the incubator was one of the Razorbills.

The chicks were allowed to remain in the incubator after hatching until the down was fully dry and they were then transferred to a box with dry grass litter and

kept out of draughts. For the first two or three days the chicks were fed on chopped fresh rabbit liver, a source of food which it was considered was not likely to carry metacercariae of Renicola. After three days chopped rabbit meat was used and by this time the gulls had learned to feed themselves from a dish, little help with forceps being needed. Feeding took place at four hourly intervals four times each day.

After returning to London it was decided to substitute whale meat for rabbit as it was more easily obtainable and equally likely to be free from infective stages of trematodes. When the chicks were about ten days old several showed signs of weakness in the legs and all had soft beaks. These symptoms appeared to indicate a deficiency of calcium in their diet, probably due to the absence of bone in their unnatural food. Daily doses of finely powdered egg-shell were given, sprinkled over the whale-meat and to this was added brown bread, cod-liver oil and a powdered yeast product. Within two days the chicks were all up and walking about and their beaks were hardening.

One of the Lesser black-backed gull chicks was lost at the age of about two weeks as a result of falling off a bench just after it had eaten heavily. It died two hours later.

The remaining birds progressed well until the age of about four to five weeks when the down had been almost completely replaced by feathers. Within one and ^a/₂ weeks all but one of the Lesser black-backed chicks were dead, also both the Greater black-backed and Razorbill chicks. In each case the symptoms were the same, a sudden refusal of food, laboured breathing and slight conjunctivitis followed by death within about twelve hours. Some peeling of the skin of the web of the foot was observed in two of the cases. Post-mortem examination of the dead chicks showed them all to be in an apparently healthy state. No conclusions as to the cause of death have been made but Dane et al. 1953 suggest that puffinosis, the virus epizootic disease of shearwaters on Skomer and Skokholm islands, is actually a disease of gulls. The symptoms observed in the chicks are similar to those described by these authors except that definite blistering of the web of the foot was not observed.

The one remaining Lesser black-backed gull chick lived to the age of about six months and then died suddenly without any previous signs of sickness. Post-mortem examination revealed no signs of disease but a dent in the base of the skull which had in turn damaged

the brain was found. How this occurred is not known but it gave the appearance of being an old injury, possibly inflicted by one of the other chicks some months before.

Although this attempt was not entirely successful it seems fairly certain that the raising of some of these sea-birds may prove more easy than has been thought in the past, provided that only chipping eggs are collected and incubated more or less on the spot.

Summary

1. All of the known records of the genus Renicola are listed and the validity of some species is discussed.
2. A summary of the morphological criteria on which the species of adult flukes of the genus Renicola are separated is given and an analysis of the value of these criteria is made.
3. Three new host records for the genus Renicola are given and the worms found are described.
4. The pathology of the infected kidney is discussed.
5. The food records of the birds known to act as hosts to the genus Renicola in Western Europe are listed.
6. The ecology of Turritella communis is discussed and possible explanations of the relationship between the size of the snail and infection with sporocysts of the "Rhodometopa" group of cercariae are given.
7. Methods for keeping Turritella communis in the laboratory are described.
8. The eggs and miracidia of Renicola sp. from Puffinus puffinus are described.

9. Experimental infection of Turritella communis with eggs of Ronicola sp. is described and the results are discussed.
10. The differing views of previous workers on the structure of the sporocysts of the "Rhodometopa" group and those of some other cercariae are mentioned.
11. Suggestions as to the early course of development of the sporocysts of the "Rhodometopa" group are made and later stages are described. A new interpretation of the sporocyst body wall is made.
12. The effects of the sporocysts on Turritella communis are discussed.
13. A summary of previous work on the cercariae of the "Rhodometopa" group is given.
14. The species of cercariae found in this work are given and descriptions of two new species are made.
15. Some general observations on the anatomy of cercariae of the "Rhodometopa" group are made.
16. The development of cercariae is described.
17. Observations on the shedding or emergence of the cercariae from infected snails are described and related to the breeding season of the host.

18. The behaviour of the cercariae, both as individuals and as groups is described, also experiments to determine the stimuli which initiate this behaviour.
19. A case of a predator on these cercariae living in close ecological conjunction with them is described.
20. Previous records of the metacercariae of the "Rhodometopa" group are discussed.
21. A new metacercarial host is recorded and the rates of infection from a number of samples are given.
22. A relationship between the distribution of the cysts in the fish and the behaviour of the cercariae is shown.
23. Experimental attempts to induce excystation of the metacercariae "in vitro" are described.
24. Attempted infection experiments by feeding metacercariae to birds are described.
25. An account of an attempt to raise some seabirds in captivity for infection experiments is given in an appendix.

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Probable Relationship between the *Rhodomotopa* Group of Cercariae and the Trematode Genus *Renicola* Cohn

DURING the past year, a study of the trematodes of the genus *Renicola* from birds in British zoos has been carried out and certain interesting points concerning the genus have come to light.

Among other features to which particular attention was given is the excretory vesicle in a species which has been found on several occasions in the kidneys of various penguins. This species was first reported by Campbell and Sloane¹ from king penguins in the Edinburgh Zoo. The fact that they had found specimens in birds hatched in the menagerie, and certain other features of infections observed in London, seemed to indicate that this is not a normal parasite of penguins, but is more likely to be found in British sea-birds. With this in view, a search was started and, after several unsuccessful examinations, about ten specimens, similar to those from the penguins, were found in the kidneys of a common guillemot (*Uria aalge*).

An examination of the literature on larval trematodes from British marine gastropods showed almost at once the very marked similarity between the excretory vesicle of young adults of this species of *Renicola* and that of the *Rhodomotopa* group of cercariae, particularly *C. pythionike* Rothschild². In both cases the vesicle is Y-shaped with numerous lateral branches and with a connecting channel joining the two arms of the Y immediately behind the ventral sucker.

Evidence of this kind seems fairly strong, and further work is being carried out in the hope that a knowledge of the larval stages may help to establish more definitely the true systematic position of this somewhat disputed genus.

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¹ Campbell and Sloane, *Vet. J.*, 99, 291 (1943).

² Rothschild, M., *Parasitology*, 27, 152 (1935).

TEXT BOUND INTO THE SPINE

TREMATODES OF THE GENUS "RENICOLA" FROM THE KIDNEYS OF BIRDS IN BRAZIL¹

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(With 4 text-figures)

The specimens to be described in this paper were seen in the collection of the Instituto Oswaldo Cruz in Rio de Janeiro (I.O.C.Col.) during the month of August 1952. A considerable amount of material from several species of birds was examined, but only the specimens from two species of terns proved to be in a sufficiently good condition to allow of a detailed examination. Other species of birds from which unidentified specimens of *Renicola* were obtained are: — *Larus dominicanus*, *Poecilonetta bahamensis*, *Sula leucogaster* and *Sterna* sp.

My very great thanks are due to Dr. LAURO TRAVASSOS for his permission to examine this material and for placing laboratory facilities at my disposal. To Dr. TEIXEIRA DE FREITAS and the other members of the Helminthology Laboratory I am indebted for all their kindness and help.

Renicola cruzi sp.n.

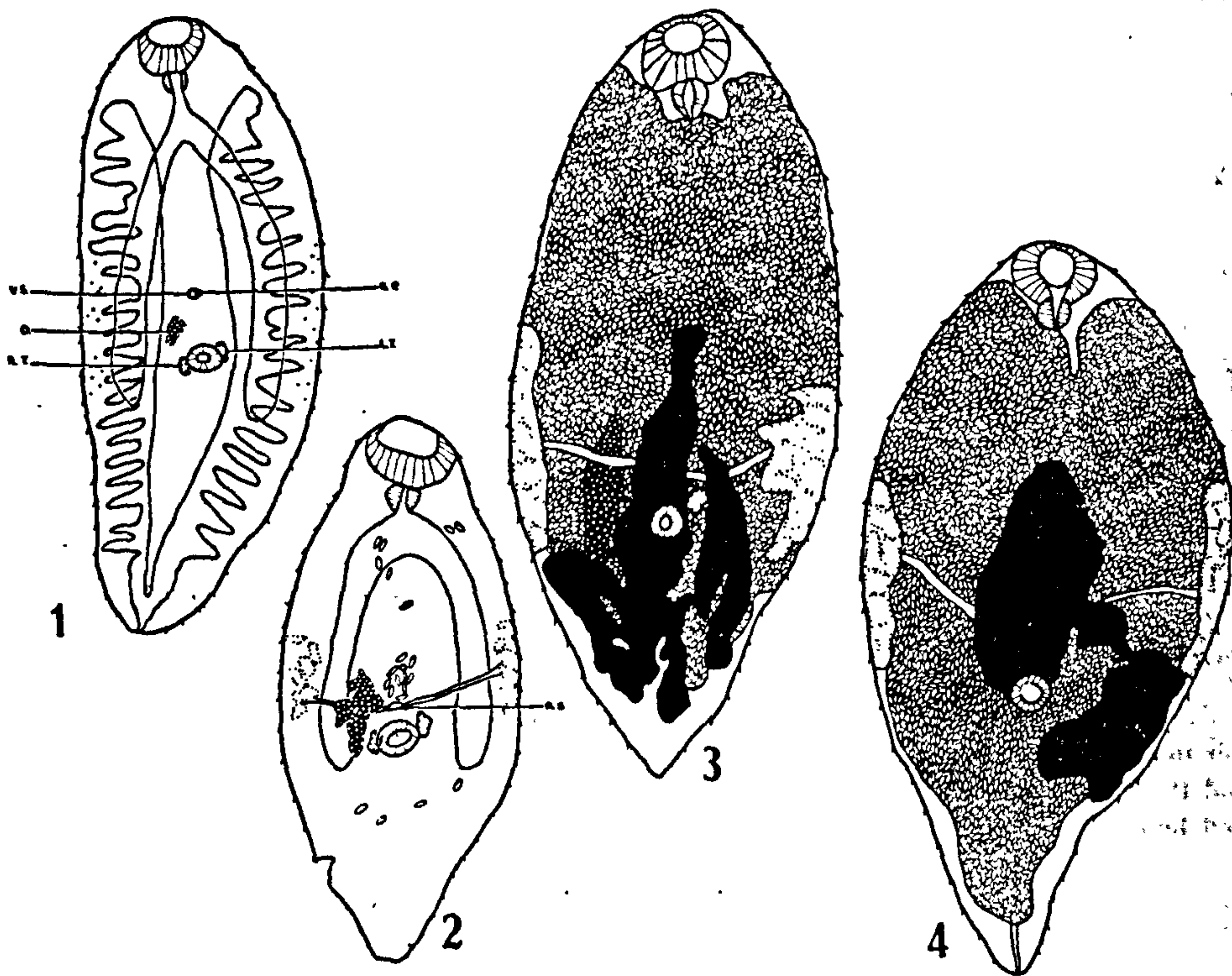
Hosts — *Sterna maxima* (I.O.C.Col. Nos. 19831 and 19832a-f) and *Sterna hirundinacea* (I.O.C.Col. No. 19833a-d) from Manguinhos, Distrito Federal. *Sterna maxima* (I.O.C.Col. No. 19834a-g) from Itanhaem, S. Paulo.

Location — In the kidney tubules.

Description — Body roughly ovoid, tapering slightly posteriorly, 0.87 — 1.69 mm. long and 0.29 — 0.73 mm. in maximum width. The cuticle is uniformly covered with spines in a quincuncial arrangement. The ventral sucker in the fully mature adult worm lies at the junction of the middle and posterior thirds of the body length, but in the immature stages it is more nearly central, being pushed back later by the developing uterus. It measures 0.75 — 0.10 mm. in diameter.

¹ Received for publication December 22, 1953.

The oral sucker is subterminal and large, 0.117 – 0.193 mm. x 0.105 – 0.155 mm. in mature specimens but in one of the rather immature specimens from *S. hirundinacea* it measured only 0.084 mm. x 0.088 mm. The lumen of the oral sucker opens directly into the pharynx which is rather barrel-shaped and measures 0.042 – 0.080 mm. x 0.058 – 0.080 mm. In one of the specimens examined there was a very short oesophagus but in all of the others the intestinal caeca divide immediately behind the pharynx. The caeca are short, reaching back only to the level of the posterior edge of the ventral sucker.



Renicola cruzi sp. n. — Fig. 1: Young specimen from *Sterna maxima*, showing the laterally branched excretory vesicle, also a short oesophagus (I.O.C.Col.No. 19832b, paratype.); fig. 2: young specimen from *Sterna maxima*, reproductive organs well developed (I.O.C.Col.No. 19831 holotype); fig. 3: mature gravid specimen from *Sterna maxima*, the black areas indicate the coils of the uterus bearing mature eggs and the uterine sac (I.O.C.Col. No. 19834a, paratype); fig. 4: mature gravid specimen from *Sterna maxima* (I.O.C.Col. No. 19834b, paratype). (G.P. — Genital Pore; L.T. — Left Testis; O. — Ovary; R.S. — Receptaculum Seminis; R.T. — Right Testis; V.S. — Vesicula Seminalis).

The excretory vesicle is Y-shaped, the stem being very short and the arms extending forward to the level of the posterior edge of the pharynx. Both the stem and the arms have a considerable number of lateral branches throughout their length. No cross-connection between the arms of the vesicle was observed, as is the case in some species of this genus.

The testes lie on either side of the ventral sucker, that on the left being set slightly further forward than that on the right. The testes are not greatly

lobed, in the very immature specimens they are quite smooth in outline but this later becomes somewhat irregular. The vesicula seminalis lies slightly to the right of the mid-line, on a level with the genital pore which is about 0.14 mm. in front of the centre of the ventral sucker.

The ovary is large and lobed on the side away from the mid-line. It lies on the right, extending forward to about the level of the genital pore and back to almost the posterior edge of the ventral sucker. The vitelline glands lie laterally to the caeca and consist of either a single or double row of large follicles. Due to the fact that all of the specimens had been fixed under pressure the exact distribution of the glands is not constant, but they extend posteriorly to about the level of the ventral sucker and anteriorly for a distance equivalent to a little less than one third of the body length of the worm. The transverse vitelline ducts run across a short way in front of the ventral sucker and in the mid-line at this level the receptaculum seminis and Mehlis' gland occur. In whole mounts of mature worms neither of these last two organs is visible, being obscured by the uterine sac. As is characteristic of members of this genus, the uterus is very strongly developed, filling almost the whole body. The eggs when mature are brown-shelled and contain a miracidium. They measure 0.034 — 0.042 mm. x 0.017 — 0.021 mm.

This species is very similar to *R. lari* Timon-David, 1933 and is only to be distinguished from it by its uniformly smaller size and the smaller size of the eggs. No mention is made of any lateral branching of the excretory vesicle in *R. lari*, but this is frequently a difficult feature to observe and appears, in most of the species examined by the author, to be suppressed in the gravid adults. It may be that this species is no more than a race of *R. lari*, but this will only be shown by detailed life-history studies.

Renicola sp.

Host — *Larus dominicanus* (I.O.C.Col. No. 19835a-b) from Manguinhos, Distrito Federal.

Location — In the kidney tubules.

Description — The material examined was very gravid and fairly badly damaged. The following measurements were made from pressed specimens: Body about 2.0 mm. long x 1.0 mm. broad. Oral sucker 0.24 mm. x 0.15 — 0.18 mm. Pharynx 0.084 mm. x 0.080 mm. Ventral sucker 0.105 mm. in diameter. Eggs 0.038 — 0.042 mm. x 0.017 — 0.021 mm.

No further details could be made out.

Renicola sp.

Host — *Poecilonetta bahamensis* from Manguinhos, Distrito Federal. (I.O.C.Col. Nos. 7430, 7512, 8355).

Location — In the kidney tubules.

Description — This material consisted of three pieces of kidney each of which was heavily infected with worms. Attempts to remove any worms by dissection from the fixed kidney were all unsuccessful, consequently the observations made were all from serial sections of pieces of infected kidney. These have proved extremely difficult to orientate and the reconstructions made have indicated some rather unusual features. In view of this it has been decided to refrain from publishing a description until an opportunity for examining whole specimens has occurred.

Renicola sp.

Host — *Sula leucogaster* from the Ilha de Paquetá, Distrito Federal.

Location — In the kidney tubules.

This material was collected by the author and is not in a very good state. Dr. TEIXEIRA DE FREITAS informs me that a description of a *Renicola* from this species of bird is to be published shortly by him and no attempt therefore will be made here.

SUMMARY

A number of species of *Renicola* in the collection of the Instituto Oswaldo Cruz have been examined and a new species, *R. cruzi*, has been described. These are the first records of any trematodes of this genus from the New World.

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TREMATODES OF THE GENUS *RENICOLA* FROM
BIRDS IN BRITISH ZOOS, WITH DESCRIPTIONS OF
TWO NEW SPECIES

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Trematodes of the genus *Renicola* from birds in British zoos, with descriptions of two new species.

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(With 23 figures in the text.)

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INTRODUCTION.

The genus *Renicola* Cohn, 1904 contains a number of species all of which inhabit the tubules and ureters of birds' kidneys. The members of the genus are for the most part known from only a few specimens and there is, therefore, ample scope for both anatomical and ecological study of the group. It is intended in the present work to describe the anatomy of two new species.

HISTORICAL.

In 1846 Creplin published a report of a trematode found in the kidneys of a Great Crested Grebe (*Podiceps cristatus*) by Mehlis in Germany. No adequate description of the worm was given but it was named *Monostomum pingue* and quoted as such by several authors until in 1904 Cohn obtained some of the original material and, by sectioning, showed the presence of a ventral sucker. He proposed the name *Renicola* for the genus which at that time still contained only one species. The systematic position of the genus was doubtful and, largely because the worms cause cyst-like swellings in the urinary tubules of the host, it was placed with the other cyst-living genera—*Paragonimus*, *Pholeter*, *Troglotrema*, *Collyriclum*—in the family Troglotrematidae. Dollfus (1939), however, separated these genera owing to the lack of anatomical relationship between them, and erected the family Renicollidae in which the rather doubtful genus *Stamparia* Nezlobinsky, 1926 was included with *Renicola*.

Since the work of Cohn many more species of the genus have been described, but most of them appear never to have been recorded more than once, and some of the descriptions, based on inadequate material, are not as complete as

desirable. The following table gives the species described, together with their hosts and localities.

Date	Species	Author	Host	Locality
1846	<i>R. pinguis</i>	(Mehlis) Cohn 1904	<i>Podiceps cristatus</i>	N.W. Germany
1924	<i>R. secunda</i>	Skrjabin	<i>Pelecanus onocrotalus</i>	Russian Turkestan
1924	<i>R. tertia</i>	Skrjabin	<i>Sterna fluviatilis</i>	Russian Turkestan
1929	<i>R. glandoloba</i>	Witenberg	<i>Puffinus kuhlii</i>	Suez
1933	<i>R. lari</i>	Timon-David	<i>Larus argentatus</i>	Marseilles
1937	<i>R. quinta</i>	Sokolova Andronova	<i>Uria carbo</i>	Vladivostock
1937	<i>R. paraquinta</i>	Raevski	<i>Larus ridibundus</i>	Tobolsk, Urals
1939	<i>R. umigarasu</i>	Yamaguti	<i>Uria aalge</i>	Japan
1939	<i>R. keimahuri</i>	Yamaguti	<i>Uria carbo</i>	Japan
1947	<i>R. pandioni</i>	Sudarikov	<i>Pandion haliaetus</i>	Gorkovsk Prov. U.S.S.R.
1947	<i>R. undecima</i>	Sudarikov	<i>Pandion haliaetus</i>	Gorkovsk Prov. U.S.S.R.
1912	<i>Renicola</i> sp.	Dollfus	<i>Mergulus alle</i>	Pas de Calais
1933	<i>Renicola</i> sp.	Hammerton	<i>Bubulcus coromandus</i>	London Zoo (India)
1937	<i>Renicola</i> sp.	Solokova Andronova	<i>Phaleris psittacula</i>	Vladivostock
1937	<i>Renicola</i> sp.	Sokolova Andronova	<i>Melanitta fusca</i>	Vladivostock

Campbell & Sloane (1943) reported a possible new species of *Renicola* from a number of King Penguins, *Aptenodytes longirostris*, a Black-footed Penguin, *Spheniscus demersus* and a Gentoo Penguin, *Pygoscelis papua* which had died in the Edinburgh zoo.

MATERIAL AND METHODS.

Most of the material on which the present work is based was obtained in the Prosectorium of the Zoological Society of London, in Regent's Park, but some was obtained from the Veterinary Laboratories Poultry Advice Service Department at Weybridge. In several instances the birds from which worms were obtained had been dead for over twenty-four hours and the worms were either dead or moribund when fixed.

The following is a list of the birds from which material was obtained, together with the place of origin of the bird, the time it had been in the Zoo and the state of the worms when fixed.

Ringed Penguin (1)	<i>Pygoscelis antarctica</i>	South Georgia	18 months	live
Macaroni Penguin	<i>Eudyptes chrysolophus</i>	Edinburgh Zoo		live
Ringed Penguin (2)	<i>Pygoscelis antarctica</i>	South Georgia	19 months	live
Spot-billed Pelican	<i>Pelecanus phillipensis</i>	Ceylon	1 day	live
Black-headed Ibis	<i>Threskiornis melanocephala</i>	Ceylon	1 day	live
White Pelican (1)	<i>Pelecanus onocrotalus</i>	Calcutta	7 months	dead
White Pelican (2)	<i>Pelecanus onocrotalus</i>	Calcutta	21 days	dead
White Pelican (3)	<i>Pelecanus onocrotalus</i>	Calcutta	28 days	dead
Common Guillemot	<i>Uria aalge</i>	Sussex	14 days	live

Many other birds have been examined, but no other infections were found.

The material collected was dissected out of the kidney tubules, usually by teasing the tubule open with fine needles. This method frequently damages the specimens but a sufficient number of undamaged ones was obtained in nearly every case. The flukes were fixed in 5 per cent formal-saline; when sufficient numbers were available a few were fixed under light coverslip pressure and some observations on distortion of the specimens were made.

Examination of the material in whole mounts was extremely difficult as the uterus in mature worms is enormously developed, a feature characteristic

of the genus, which obscures nearly all of the anatomy. Useful information on the genital organs was obtained from whole mounts of immature specimens, but sectioning was necessary to show detailed structure. Peterfi's celloidin impregnation technique was used with good results for both sagittal and frontal series at 0.008 mm, but the loose mass of eggs rendered thinner sections unsatisfactory. Sections were stained in Delafield's haematoxylin with eosin counter-stain, Heidenhain's iron-alum haematoxylin, and borax carmine with picro-indigo-carmin. Of these the iron-alum-haematoxylin proved most useful. Whole mounts were stained in Delafield's haematoxylin or Gurr's paracarmine.

The material from the three penguins and the Common Guillemot proved to be the same species as that from Edinburgh. It was checked against a description kindly lent by Mr. Sloane, and against material sent by Dr. Campbell. A description of this species is given below.

The material from the Black-headed Ibis consisted of four specimens only; they were not in sufficiently good condition for any detailed observations to be made, but they do not appear to correspond with any known species.

The specimens from the first White Pelican consisted of two worms only, bearing a resemblance to *R. secunda* Skrjabin. Unfortunately one was badly damaged and the genital organs and ventral sucker of the other were completely obscured by the dense mass of eggs in the uterine sac.

The worms from the spot-billed and from the other two White Pelicans all proved to belong to the same hitherto undescribed species. In all cases the infection was heavy, particularly in the White Pelicans, in the second of which there was a mixed infection of this species together with a few examples of an unidentified form.

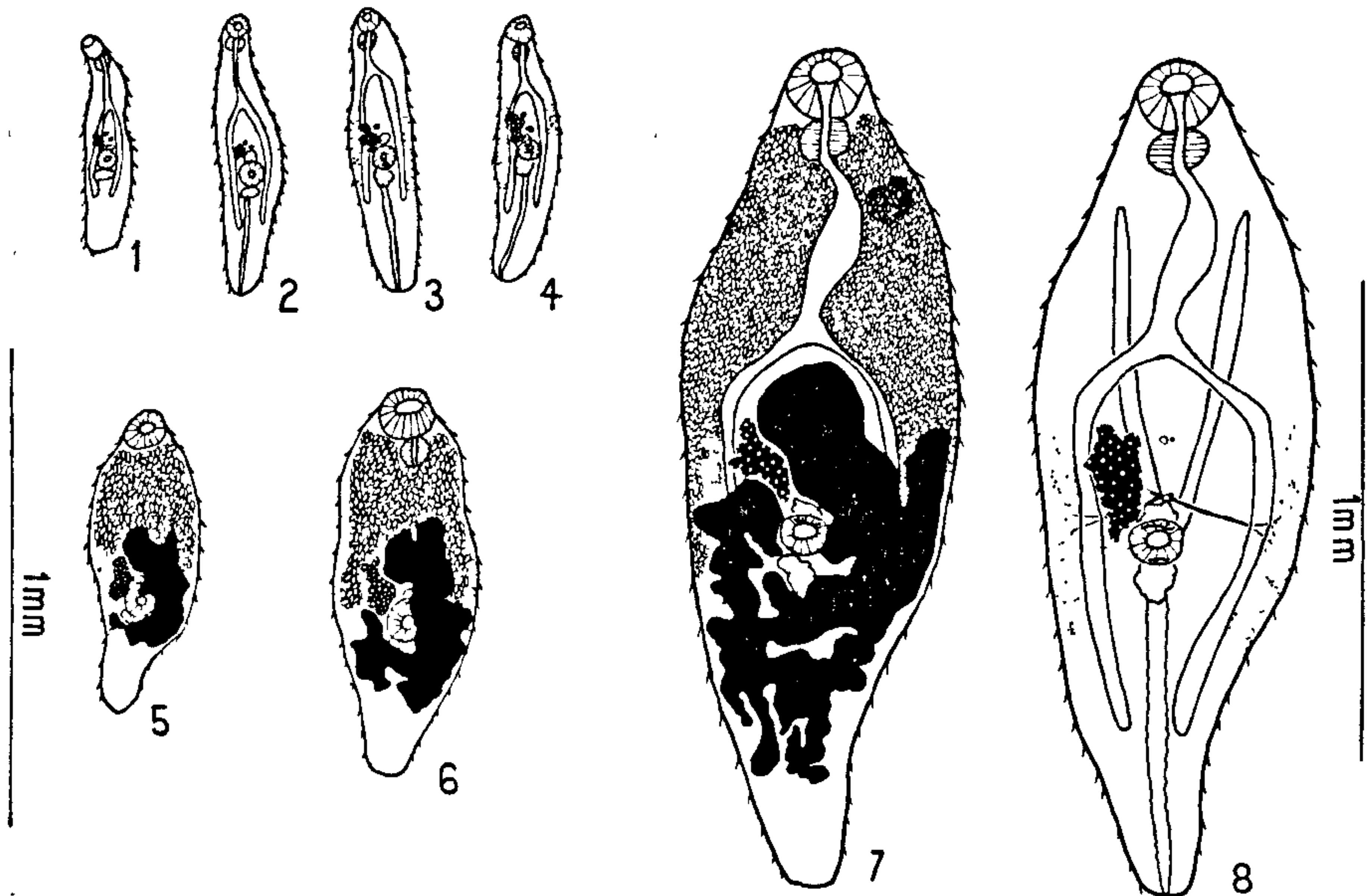
The first infection with these trematodes was found in the kidneys of a Spot-billed Pelican which died very soon after its arrival by sea from Ceylon. Ceylon must, therefore, be regarded as the type locality of the species, although the two cases from Calcutta seem to indicate a fairly wide distribution.

As is characteristic of members of this genus the worms were found lying in pairs in the urinary tubules. A few specimens were also found in the ureters, but this possibly represented merely a movement after the death of the host.

RENICOLA PELECANI SP. N.

The body of the parasite is somewhat spindle shaped and slightly dorso-ventrally flattened, the ventral surface being slightly convex and the dorsal surface rather flatter except where the uterine sac causes a swelling. The following size ranges are based on a series of mature specimens, with the minimum sizes at which immature specimens were recognizable placed in brackets afterwards. The body measures 0.93–1.82 mm. (0.51 mm.) in length and 0.23–0.62 mm. (0.114 mm.) in maximum width normally in the middle region. In some specimens the posterior end is rounded but in others it is distinctly truncated; in few cases is this extremity so apparent as in other species of the same genus. The cuticle is uniformly covered with scale-like spines which show a quincuncial arrangement. In many of the older specimens, however, numerous spines appear to have fallen off, particularly in the middle region of the body. The ventral sucker lies, in the mature specimens, at about the junction of the middle and posterior thirds of the body length. In the immature stages this sucker is more nearly equatorial, giving the impression that subsequent increase in size, whether by growth or merely by stretching due to the increase in size of the gravid uterus, takes place principally in the anterior region of the animal. The diameter of the ventral sucker ranges from 0.057–0.086 mm. in the specimens examined; in the majority of the mature specimens however, it is uniformly 0.086 mm. in diameter. In sagittal sections it can be seen that this sucker opens in a somewhat posterior direction.

The oral sucker is almost terminal and very thick-walled and powerful. In the specimens examined, where it is in different stages of contraction, it measures 0.114–0.143 mm. $\times$ 0.100–0.143 mm. (0.043 mm. $\times$ 0.057 mm.). Immediately behind the oral sucker lies the pharynx, which may be either barrel-shaped or elongate, slightly wider at the anterior end. In contrast to previously known species of this genus, the oesophagus is long, being equal to about one-third of the body-length. The intestinal caeca extend well behind the ventral sucker, terminating about midway between it and the posterior end of the body.



Figs. 1–4.—*R. pelecani* sp. n., immature stages. Figs. 5 and 6.—*R. pelecani* sp. n., young stages to show development of the uterus. Fig. 7.—*R. pelecani* sp. n., mature worm very slightly flattened. Fig. 8.—*R. pelecani* sp. n., diagram reconstructed from serial sections of mature worms, uterus not shown.

Ovary, white dots on black ground.

Testes, black dots on white ground.

Vitelline follicles, dotted outline.

Uterine sac and coils of uterus containing mature eggs completely black.

The excretory vesicle is Y-shaped, the stem dividing just behind or above the ventral sucker. The excretory pore is on the posterior extremity and may be either terminal or slightly ventral. The anterior extent of the arms of the vesicle appears to be slightly variable, but in the majority of specimens the arms terminate almost on a level with the pharynx.

The male genitalia consist of two tandem testes, slightly lobed and with their long axes in the transverse plane. The posterior testis which is the larger of the two lies immediately behind and slightly dorsal to the ventral sucker. In the immature stages this appears to be the active testis, the anterior one often being much smaller; in more mature worms, however, the anterior testis appears to develop and take over the function of producing spermatozoa. The anterior testis is situated dorsally above the ventral sucker and a little in front of it. The vasa efferentia unite just in front of the anterior testis and the vas deferens runs forward to the genital pore which lies in the mid-line about 0.18 mm. in front of the centre of the ventral sucker. Before reaching the genital pore the vas deferens expands to form a thin-walled seminal vesicle

which lies a little to the right of the mid-line and on a level with the genital pore. This organ is usually visible in whole mounts. It is connected to the terminal part of the metraterm by a short ductus ejaculatorius. There is no cirrus pouch as stated by Dawes (1946) in the key to the genera of Troglotrematidae occurring in birds.

The ovary is much larger than either of the testes. It lies in the ventral parenchyma to the right of the mid-line, its posterior edge being more or less level with the centre of the ventral sucker; it extends forward to about the level of the genital pore. It is an elongate organ, with its main axis in the longitudinal plane, deeply lobed, mainly on the side away from the mid-line. The vitelline glands lie lateral to the intestinal caeca but overlap them slightly dorsally. The follicles are fairly large and are arranged in a single line reaching anteriorly to about the level of the anterior margin of the ovary (this is roughly the junction of the anterior and posterior halves of the body), and passing behind the level of the ventral sucker for approximately an equal distance posteriorly. In several of the specimens examined the vitellaria on one side or the other were completely absent. The transverse vitelline ducts run just in front of the ventral sucker, dorsal to the anterior testis. The vitelline reservoir lies just to the right or slightly in front of this testis, and closely associated with it is the small receptaculum seminis and Mehlis' gland, surrounding the transverse oviduct. None of these structures is easily visible in whole mounts, being obscured either by the inner margin of the ovary or by a posterior extension of the dorsal uterine sac. The uterus is very long and much coiled, reaching posteriorly into the hinder region of the body; anteriorly there is a loop running forwards on either side of the pharynx. The remainder of the body is occupied by the uterus and uterine sac; the latter, although not so well developed as in other species of the genus, extends from the intestinal bifurcation to well behind the ventral sucker, mainly on the left side of the body. The eggs when mature are brown shelled, but in the most proximal coils of the uterus they are colourless. They are operculate, measure 0.024–0.027 mm. $\times$ 0.012–0.015 mm., and contain a miracidium while in the uterine sac.

This species does not appear to be closely related to any of those hitherto described. It is easily separated from all other known members of the genus by the well developed oesophagus and by its spindle-shaped body. For it I propose the name *Renicola pelecani* sp. n. The type specimens have been deposited in the British Museum, Natural History. B.M. (N.H.) Regd. No. 1954.1.15.8–15.

RENICOLA SLOANEI SP. N.

To this species I have referred specimens from two Ringed Penguins (*Pygoscelis antarctica*), a Macaroni Penguin (*Eudyptes chrysolophus*) and a Common Guillemot (*Uria aalge*). In spite of a number of small size-differences between the worms from the penguins and the guillemot there is no significant anatomical variation between them, and the egg-sizes fall within the same range. In the following description the measurements for the specimens from the guillemot are given in brackets.

Body ovate, blunt anteriorly, tapering posteriorly, 1.47–2.71 mm. (0.93–1.82 mm.) long and 0.69–1.26 mm. (0.42–0.71 mm.) in maximum breadth. The body is either rather pear-shaped or roughly oval. The cuticle is covered with spines arranged quincuncially. The ventral sucker lies at about the junction of the middle and posterior thirds of the body, and measures 0.114–0.129 mm. (0.073–0.10 mm.) in diameter. The oral sucker is terminal or sub-terminal and measures 0.257–0.329 mm. (0.129–0.143 mm.) $\times$ 0.229–0.286 mm. (0.186–0.228 mm.). It leads directly into the pharynx, and may frequently overlie this organ which is barrel-shaped and measures

0.114 × 0.114 mm. (0.047 × 0.073 mm.—0.085 × 0.085 mm.). There is no oesophagus. The intestinal caeca divide immediately behind the pharynx and pass right back into the posterior extremity of the worm. The excretory vesicle is Y-shaped, the division of the arms being about mid-way between the ventral sucker and the posterior end. A feature of particular interest is the presence of a connecting channel between the two arms of the vesicle, running transversely just behind the ventral sucker. There is a very pronounced lateral branching of both the stem and arms of the vesicle.

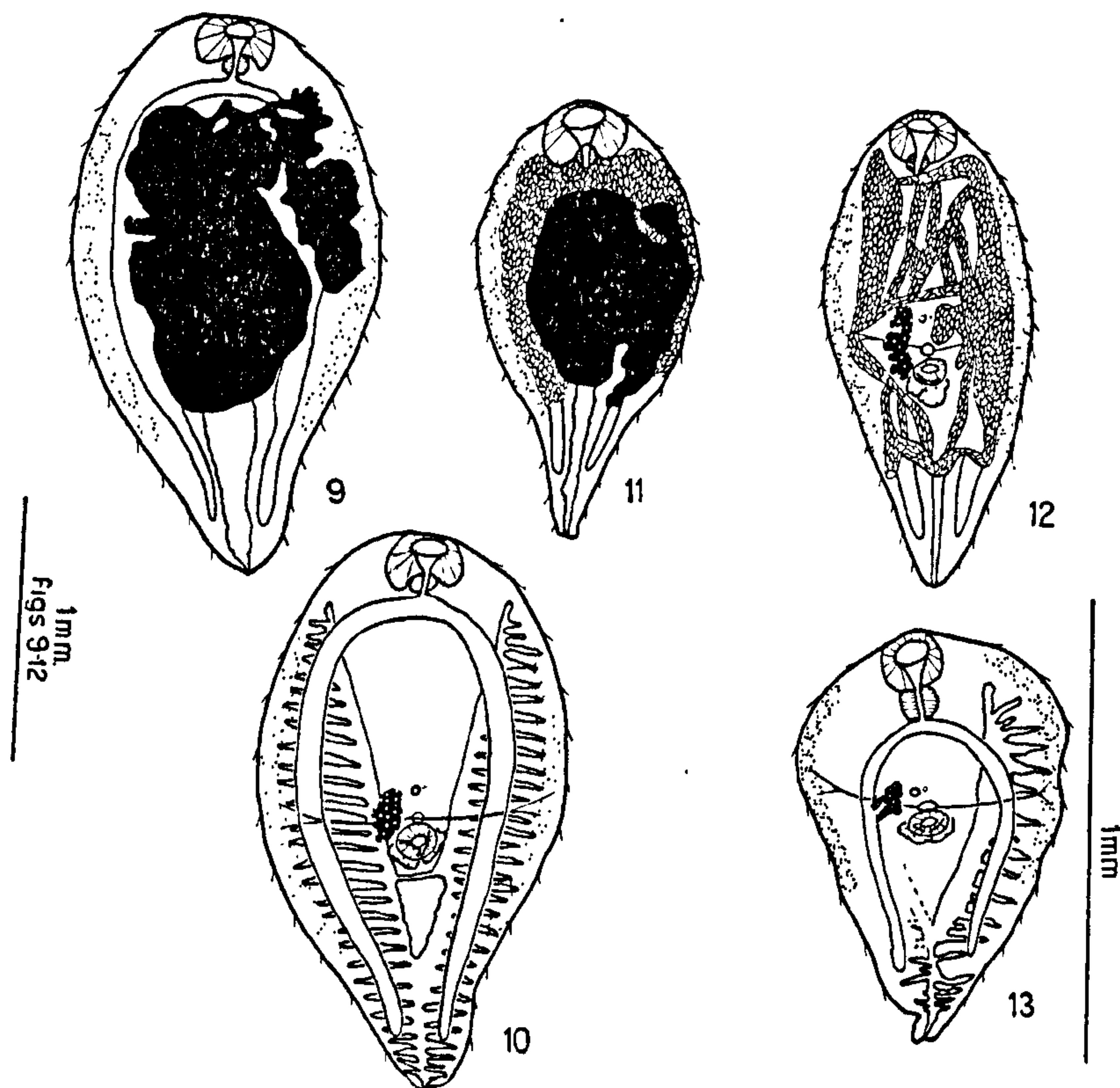


Fig. 9.—*R. sloanei* sp. n., gravid specimen from Ringed Penguin (*Pygoscelis antarctica*).
 Fig. 10.—*R. sloanei* sp. n., diagram reconstructed from serial sections, uterus not shown. Fig. 11.—*R. secunda* (?) Skrjabin, mature specimen from *Pelecanus onocrotalus*. Fig. 12.—*R. sloanei* sp. n., specimen from Common Guillemot (*Uria aalge*). Fig. 13.—*R. sloanei* sp. n., less mature specimen from *Uria aalge* uterus not shown.

Ovary, white dots on black ground.

Testes, black dots on white ground.

Vitelline follicles, dotted outline.

Uterine sac and coils of uterus containing mature eggs completely black.

The testes lie on each side but slightly posteriorly to the ventral sucker, both overlapping the sucker dorsally. The left testis extends slightly anteriorly as well as posteriorly. Both are considerably lobed; the genital pore lies in the median line about 0.22 mm. in front of the centre of the ventral sucker; the cuticular spines in the opening of the pore are particularly well developed. Immediately to the right of the pore lies the vesicula seminalis which is connected

to the terminal part of the metraterm by a short ductus ejaculatorius. The ovary lies to the right of the mid-line and extends forward from the ventral sucker almost to the level of the genital pore. It is a large, lobed organ with its major axis in the longitudinal plane and most of the indentation on the outer side. The vitelline glands lie in a single row of extra-caecal follicles extending from the beginning of the second fifth of the body length to the beginning of the last quarter. The transverse vitelline ducts run across about mid-way between the ventral sucker and the genital pore; in the mid-line at this point lie the vitelline reservoir, receptaculum seminis and Mehlis' gland. The uterus is extremely long and much coiled, extending anteriorly in two loops on each side of the pharynx and posteriorly almost into the caudal extremity of the body. There is a very large uterine sac which in mature specimens obscures all of the genitalia except the vitelline glands. In one of the specimens from the guillemot the whole of the contents of the uterine sac was discharged when the worm was placed in physiological saline solution, showing that the presence or absence of a uterine sac is not a diagnostic feature of any significance. The eggs are operculate and dark brown when mature; they measure 0.028–0.034 mm. (0.032–0.038 mm.) $\times$ 0.016–0.018 mm. (0.0165–0.0195 mm.).

This species can be distinguished from other described species by the different distribution of the vitelline glands. It is most closely related to *Renicola umigarasu* Yamaguti, but is distinguished from that species by the size of the eggs and the greater extent of the vitellaria. It may perhaps be the *Renicola* sp. of Dollfus from *Mergulus alle*. The type material has been deposited in the British Museum (Natural History). B.M. (N.H.) Regd. No. 1954.1.15.1–4.

RENICOLA SPP.

Brief descriptions are now given of various other members of the genus *Renicola* which were found during the course of this work but which cannot, for various reasons, be identified with any certainty.

Renicola sp. from *Threskiornis melanocephala*.

The body is roughly oval, and the tapered posterior end is somewhat rounded. Length 0.78–0.90 mm.; breadth 0.49–0.51 mm. Oral sucker large, subterminal, 0.128 $\times$ 0.118 mm.; pharynx oval, slightly broader at the anterior end, 0.057 $\times$ 0.043 mm.

The uterus is very strongly developed and obscures the remainder of the reproductive organs. There appears to be no development of a uterine sac. The eggs are light brown when mature and measure 0.036 $\times$ 0.018 mm.

Renicola sp. from *Pelecanus onocrotalus*.

Body clavate, the posterior end very strongly tapered, length 1.73 mm., width 0.79 mm. The cuticle is spiny, but no regular arrangement of spines can be made out. The ventral sucker lies post-equatorially and measures (in frontal section) 0.143 mm. in diameter. The oral sucker is slightly sub-terminal, very powerful and thick walled, with a funnel-shaped lumen. Its external measurements are 0.33 $\times$ 0.243 mm. It leads directly into the elongate barrel-shaped pharynx which measures 0.126 $\times$ 0.072 mm. It appears from the very poor sections obtained from the damaged specimen that there is a short oesophagus proximal to the division of the intestinal caeca. The caeca are very long, extending just into the caudal end of the body.

Owing to the very gravid state of the specimen the only parts of the reproductive organs that can be seen are the vitelline glands. They extend in a linear series of lateral follicles from the pharynx to the junction of the third and fourth quarters of the body length, and possibly extend into the last quarter. The uterus is very strongly developed and a large uterine sac occludes

the whole of the central part of the specimen. No details of the reproductive organs could be made out from the sectioned specimen. The eggs are operculate and brown in colour, measuring $0.028-0.034 \times 0.013-0.016$ mm.

These specimens were dead on removal from the bird and the measurements and proportions of the body length given may not be those that would have been obtained had the specimens been fixed alive under light pressure. It seems, therefore, on the basis of egg-size, distribution of vitellaria and the host species, reasonable to refer these specimens tentatively to *R. secunda* Skrjabin.

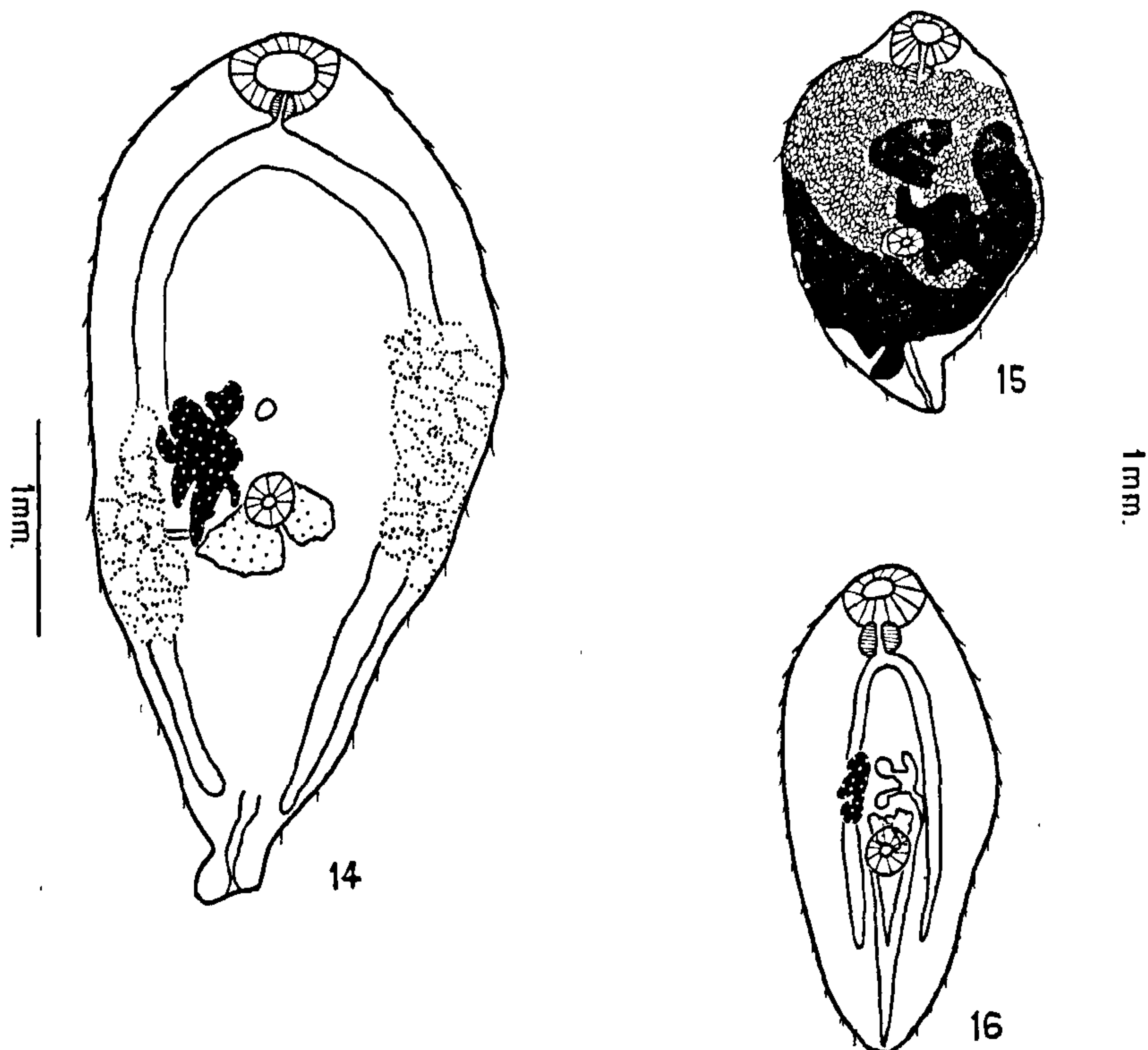


Fig. 14.—*Renicola* sp. Hammerton, specimen from Indian Cattle Egret (*Bubulcus coromandus*), very much flattened. Fig. 15.—*Renicola* sp., from Black-headed Ibis (*Threskiornis melanocephala*). Fig. 16.—*Renicola* sp. from *Pelecanus onocrotalus* composite drawing from three immature specimens.

Ovary, white dots on black ground.

Testes, black dots on white ground.

Vitelline follicles, dotted outline.

Uterine sac and coils of uterus containing mature eggs completely black.

Renicola sp. from *Pelecanus onocrotalus*.

One of the white pelicans which bore a heavy infection of *R. pelecani* harboured also a number of specimens of a different species. The adults of the other species were all in very poor condition, being little more than bags of eggs. None was removed intact, and all that can be said is that they were rather pear-shaped with a fairly pronounced narrow posterior extremity, and an enormous uterine sac covering nearly the whole body which was about 2.0 mm. long and 1.5 mm. wide. One of these egg masses was sectioned and showed signs of a considerable posterior and anterior extent of vitellaria, but

the gonads were only to be made out as degenerate traces amongst the eggs. The ventral sucker was observed, however, and its diameter in frontal section was 0.14 mm. The size of the eggs was 0.032–0.034 mm. $\times$ 0.015–0.018 mm.

In the same kidneys a number of immature flukes was found. They did not correspond with the immature stages of *R. pelecani*, and they may possibly belong to the unidentified species of which the adults were found. The following notes were made on these specimens.

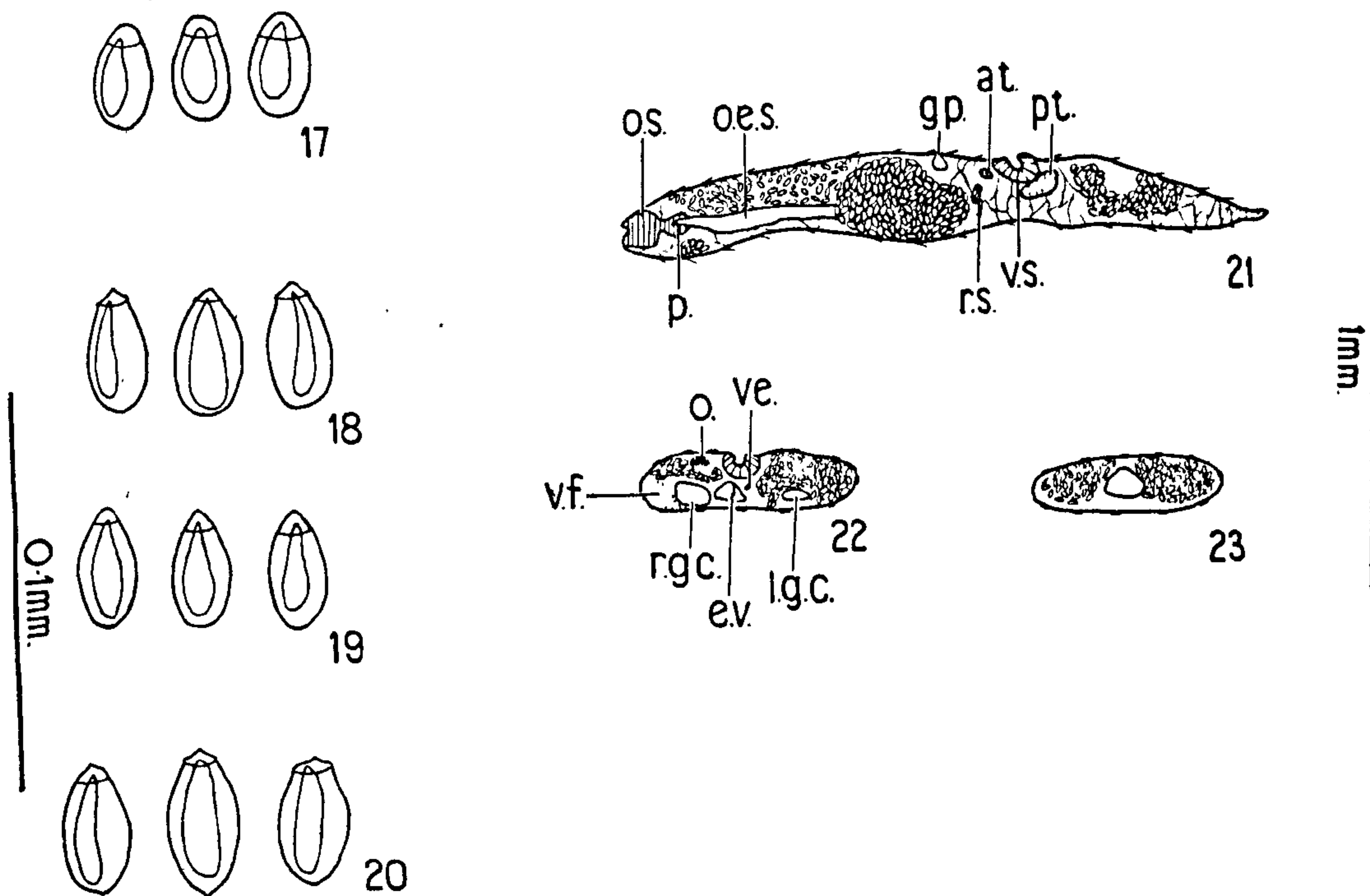


Fig. 17.—Eggs of *R. pelecani* sp. n. (Drawn in formal-saline.) Fig. 18.—Eggs of *R. sloanei* sp. n. from Ringed Penguin (*Pygoscelis antarctica*). (In balsam.) Fig. 19.—Eggs of *R. secunda* (?) Skrjabin. (In formal-saline.) Fig. 20.—Eggs of *R. sloanei* sp. n. from Common Guillemot (*Uria aalge*). (In sea-water.) Fig. 21.—Sagittal section (slightly oblique) through ventral sucker and genital pore of *R. pelecani*. Fig. 22.—Transverse section through ventral sucker of *R. pelecani* sp. n. Fig. 23.—Transverse section through oesophageal region of *R. pelecani* sp. n.

a.t. anterior testis. e.v. excretory vesicle. g.p. genital pore. l.g.c. left gut caecum. o. ovary. o.s. oral sucker. oes. oesophagus. p. pharynx. p.t. posterior testis. r.g.c. right gut caecum. r.s. receptaculum seminis. v.f. vitelline follicle. v.e. vas efferens (from posterior testis). v.s. ventral sucker.

Ovary, white dots on black ground.

Testes, black dots on white ground.

Vitelline follicles, dotted outline.

Uterine sac and coils of uterus containing mature eggs completely black.

Body roughly elliptical, tapered posteriorly, length 1.0–1.34 mm., breadth 0.29–0.56 mm. The cuticle is covered with fine spines. The ventral sucker lies almost in the centre of the body and measures 0.1–0.14 mm. in diameter. The oral sucker is terminal, 0.14–0.18 $\times$ 0.095–0.165 mm.; its lumen is funnel shaped and leads directly into the pharynx which measures 0.05–0.062 mm. $\times$ 0.074–0.082 mm. There is no oesophagus, the two intestinal caeca appearing to divide directly behind the pharynx and reach almost to the posterior end of the body.

In only one specimen were the genital rudiments sufficiently well organized for their relationships to be determined. A pair of lobed testes lie immediately in front of the ventral sucker but overlapping it slightly, and a trilobed ovary on the right and slightly anterior to the testes. There is a complex structure on the left, apparently connected to two deeply staining vesicles in the mid-line (possibly vesicula seminalis and receptaculum seminis), but the details cannot be made out.

The excretory vesicle is Y-shaped with the division of the two arms occurring about mid-way between the ventral sucker and the posterior end of the worm. The anterior limit of the branches is not clear. There are no eggs in any of the specimens. No conclusions on the probable relationships of these worms can be made in view of the fact that the observations are based on a small amount of indifferently preserved material.

Renicola sp. Hammerton from *Bubulcus coromandus*.

Although this form was not actually found during the present work the following description is included because, although these worms have been mentioned in other works, there does not appear to be any description of them in existence. The specimens were obtained from the kidneys of an Indian Cattle Egret at the London Zoo in 1933. They were mentioned in the Prosector's report for that year by Hammerton (1934) and were mentioned by Dollfus in 1946. Of the specimens examined, one was unpressed and no details of the anatomy could be made out, but the other two were pressed so that the structure is visible although the degree of distortion appears rather great.

Body pear-shaped, dorso-ventrally flattened, wider anteriorly 2.6–3.9 mm. long and 1.8–2.3 mm. in maximum width. The cuticle bears a few spines but too many have been worn off to be able to make out their arrangement. The narrow caudal end of the body forms a small bilobed protuberance on which the excretory pore opens. The ventral sucker lies just behind the centre of the body and is 0.14 mm. in diameter. The oral sucker is sub terminal, measuring 0.37×0.44 mm. It opens immediately into the barrel-shaped pharynx which measures 0.128×0.114 mm. The oesophagus is virtually absent, and the intestinal caeca are long, extending almost to the posterior end of the body. The testes lie more or less side by side posterior to the ventral sucker and overlapping it slightly dorsally. The right testis is somewhat lobed and lies more or less in the mid-line; in the pressed specimen on which all the observations on the genitalia were made, it measures 0.42×0.29 mm.; the left testis is more rounded and measures 0.28×0.26 mm. The vesicula seminalis lies in the mid-line about 0.36 mm. in front of the centre of the ventral sucker. The ovary is very strongly lobed, it lies to the right of the median line and measures 0.68×0.28 mm. The vitelline glands are arranged laterally and overlap the intestinal caeca dorsally, extending for about a quarter of the body length. In the pressed specimens their position has been greatly distorted, but in the unpressed specimen they have their anterior limit just in front of the ventral sucker and extend back to a point about halfway between this sucker and the posterior end of the body. The vitellaria are disposed in large, closely set follicles. The transverse vitelline ducts run across immediately in front of the ventral sucker. A loop of the uterus with mature eggs obscures further details of the genitalia. The position of the genital pore was not determined. The uterus is very long and much coiled, extending almost into the caudal extremity of the body, and anteriorly on either side of the oral sucker. There is no great development of the uterine sac in any of the specimens examined. The shells of the eggs in the first coils of the uterus are completely colourless, but as they mature they change through yellow to dark brown. They measure $0.028\text{--}0.034 \times 0.015\text{--}0.017$ mm.

This species appears to be most closely related to *R. lari* Timon-David, but differs from it in several respects, notably the size of the eggs. The complete details of the anatomy have not, however, been made out and the measurements given are liable to considerable inaccuracy. The species must therefore continue to stand simply as *Renicola* sp. Hammerton.

SUMMARY.

A number of cases of infection of the kidneys in birds by trematodes of the genus *Renicola* Cohn, 1904 is reported and two new species of the genus are described. One of them, *Renicola pelecani* sp. n., has been found in both the White Pelican (*Pelecanus onocrotalus*) and the Spot-billed Pelican (*Pelecanus phillipensis*). The other, *Renicola sloanei* sp. n., has been found in a number of species of penguin. It has also been found in a Common Guillemot (*Uria aalge*) from Sussex, this being the first record of a *Renicola* from a wild bird in Great Britain.

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