STUDIES ON LARVAL TREMATODA OF BURNABY LAKE, B.C.

by

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Mr. L.W. Dunlap,
Librarian,
University of B.C.

Dear Sir:

This is to certify that Mr. Murrey Sager has submitted an acceptable thesis for the Master's degree together with an approved abstract. He has successfully passed an oral examination on the thesis.

Yours sincerely,

Head, Department of
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ABSTRACT.

Six species of larval trematoda were discovered in the mollusca of Burnaby Lake, B.C. These included two species each of echinostome cercariae, furcocercous cercariae and xiphidiocercariae. Life cycle studies were carried out with each of these larval species. A series of infection experiments proved one of the echinostome cercariae to be the larva of Echinoparyphium recurvatum, which was shown to utilize several species of snails at Burnaby Lake as first and second intermediate hosts. Morphological and experimental evidence indicated the other echinostome cercaria to be the larval stage of Echinostoma revolutum, an adult trematode parasitic in muskrat of Burnaby Lake. Both natural and experimental first and second intermediate hosts of E. revolutum at Burnaby Lake were established. The two xiphidiocercariae were found to be very similar morphologically, but were classed as separate species on the character of the stylet organ. One xiphidiocercaria bears much resemblance to Cercaria albui, Brooks, 1943, the other appears to be an undescribed species. The first intermediate snail hosts for these xiphidiocercariae have been found at Burnaby Lake and a Gammarus species has been demonstrated as being an experimental second intermediate host. Both the furcocercous cercariae discovered appear to be new species. One of these forms bears some resemblance to Cercaria oregonensis, Macfarlane and Macy 1946, and has been found capable of producing a schistosome dermatitis in humans. A high incidence of larval trematode infection exists in the snails

of Burnaby Lake. Area differences have been noted in snail populations and their trematode fauna. Additions have been made to larval trematode distribution and host records.

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Appreciation is gratefully acknowledged to Game Warden R.S. King of the Provincial Game Commission for arranging to have muskrat secured from Burnaby Lake and to Mr. P. Bettles for the bountiful supply of muskrat carcasses. A special tribute is given to fellow students Miss M.H. Thorn, who gallantly served in the schistosome dermatitis experiments, and Miss A. Redlich, who uncomplainingly cared for the many host animals used in the life cycle experiments.

To my parents I owe my greatest debt and thanks, for without their forbearance and financial assistance, this work could not have been completed.

CONTENTS.

STUDIES ON LARVAL TREMATODA OF BURNABY LAKE, B.C.

INTRODUCTION.

Three aspects of the study of the larval trematode fauna of Burnaby Lake, B.C., have been of particular concern to the author; they are, 1.) to discover what species of larval trematodes, parasitic as adult worms or "flukes" in vertebrates, are harboured by the snails of the area, 2.) to determine the incidence of infection of these larvae in the snails, and, 3.) to trace as far as possible their life cycles in an effort to determine what animals of Burnaby Lake figure in the complicated life history of these worms.

The investigation of these problems arose initially from a suggestion by Dr. James R. Adams, who indicated the value in knowing the local intermediate hosts of the Burnaby Lake muskrat (Ondatra zibethica). These trematodes must be regarded as potentially epidemic and had already shown by Musfeldt (1945) to be in large numbers in the Burnaby Lake muskrat. Her report mentions three trematodes -- Echinostomum coalitum Barker and C.A. Beaver, 1915 (Echinostomum revolutum Froelich, 1802), Quinquiserialis quinquiserialis and Notocotylus urbanensis as being parasitic in this important fur bearer. A subsequent comprehensive survey of disease and parasitism in British Columbia muskrat by the same worker (1947) showed that these same three trematodes are normal

parasites of the gut of muskrat indigenous to several other areas of the province. The latter report also noted two additional trematoda "normal" to B.C. muskrat -- Echinoparyphium contiguum and Plagiorchis proximus.

In view of this high incidence of trematode infection in B.C. muskrat, a survey of the Mollusca of Burnaby Lake, with respect to their role as intermediate hosts of trematodes, appeared warranted. The vulnerable part of trematode life histories is the intramolluscan stage and the determination of the snail species concerned with this stage is requisite to any type of control measure.

However, a preliminary survey of the Mollusca of Burnaby Lake showed that they contained several species of trematodes found in hosts other than muskrat. The problem then appeared wider in scope than the originally proposed survey of snail hosts for muskrat trematodes alone. A broader investigation was felt worthwhile to gain life history data on as many local trematodes as possible.

Burnaby Lake is a game reserve and as such harbours many animals which can and do act as hosts to several adult trematodes. The report, then, was redesigned to include the three particular aspects mentioned earlier, in the attempt of a comprehensive study of the larval trematode fauna of a selected area in the Province.

Little previous work has been done locally on larval trematodes and their hosts. Musfeldt (1945) conducted a preliminary investigation into the host cycle of Echinostoma revolutum at Burnaby Lake, incidental to her survey of parasitism in the muskrat of the area. She found that Physa occidentalis was infected with a "stylet" or xiphidiocercariae as well as a " possible non-stylet cercaria". No echinostome cercariae were found. She also discovered what she regarded as metacercariae of an echinostome, which were fed to albino rats in an effort to obtain adult worms. No adult worms were produced. Eggs from Echinostoma revolutum, and the subsequent miracidial stage were also described in detail by Musfeldt. Although she did not find echinostome cercariae to emerge from the 65 P. occidentalis under examination, she did find rediae containing cysts and active cercariae, " resembling those of echinostomes". The " non-stylet" cercaria was not classed as an echinostome because it lacked the collar spines and digestive system characteristic of the Family Echinostomidae. Musfelt concluded that "the alternate hosts of Echinostoma has as yet eluded discovery".

Going further afield, it may be noted that there has been little work done on the larval trematodes of fresh water Mollusca of the Pacific Northwest. What appears to be the earliest work recorded in this area of the continent is that of Miller (1925) on San Juan Island, Puget Sound, where he made an investigation of the larval trematode infestation of the fresh water Mollusca. In add-

ition to the fact that San Juan Island offered particularly favourable conditions for the study of trematode life histories, Miller's work was prompted by the " desireability of a study of the larval trematodes in a section of this continent from which no records have been made." As a result of the survey he found seven new species of cercariae and a high incidence of infection by these larvae in the snails of the island. Miller (1927) later made a more specialized survey, that of the forked-tailed or furcocercous cercariae in fresh water snails of the same area. This investigation added several more new species to the class and additions to cercarial distribution records.

Some recent work on larval trematodes in the Pacific Northwest has been made in connection with schistosome dermatitis or "swimmer's itch." Macfarlane and Macy (1946) investigating a case of swimmer's itch in Multnomah County, Oregon, discovered a new species of furcocercous cercaria which is capable of producing dermatitis in humans. As far as can be determined, Hunter et al (1949) have done the most recently recorded work in this area. They described a new species of dermatitis-producing furcocercous cercariae from Green Lake, within the city limits of Seattle, Washington. In their report, they state, " is the first record based on experiments to show the presence of schistosome dermatitis on the mainland in Washington, and within the city limits of

Seattle." It may be added here that, as far as can be determined, the present investigation describes the first experiments made in this province to prove the presence of a dermatitis-producing cercaria.

Generally speaking, it appears that work done on larval trematodes in Canada has been little and scattered. McLeod (1934) and Swales (1936) have both investigated cercarial dermatitis in Manitoba. A few other Canadian workers have described cercariae in papers connected with adult stages. No special reports on cercariae have been made from British Columbia.

Actually, the study of larval trematodes appears to have had a relatively short history, with much the greatest progress being made in the last few years. Siebold in Europe did a great part of the early work on trematodes during the early part of the eighteenth century. As late as 1909 Lühe is reported to have observed that up to that date, no cercariae had been identified as belonging to the adult echinostomes. Lebour and Nicoll conducted the first studies on larval trematodes to be made in England, in the early nineteen hundreds. However, it was not until Brown's work (1926) that the first life history studies of these worms were undertaken in that country.

Cort (1914) made the first comprehensive survey of

cercariae in North America. In the introduction to this work, Cort states that " practically nothing is known of the life histories of trematodes of North America. Even in Europe, where many new adults are being described each year, only a few developmental stages are completely known. One reason for this is to be found in the difficulties involved." Many of the difficulties of which Cort speaks still remain for the worker in this field, but the ever increasing knowledge of these forms is lessening the apparent, and indeed the actual, complexity of the study of trematode life cycles; it is in no way lessening the interest and fascination which this complexity holds for the investigator.

Although this present investigation is largely an extension of Musfeldt's (1945) work on the life cycle of E. revolutum, illuminating more of the life cycle of this species, it has developed into a broader study of the larval trematoda of the Burnaby Lake area.

METHODS AND TECHNIQUES.

The investigation was divided into three parts; first the collection of snails at Burnaby Lake, secondly, the dissection and examination of snails in the laboratory to detect cercariae and other intramolluscan stages, and, thirdly, the carrying out of infective feeding and exposure experiments to determine life cycles.

Details of methods and techniques employed in each of these divisions are given here while brief introductions and discussions are added to each section in the text where they are most pertinent. This is done to avoid too much cross referencing since the presentation of the data falls naturally into three somewhat distinct sections. A certain amount of repetition has been necessary, but only where it will be of some aid to the reader.

Collection of Snails: Snails were collected from three areas of Burnaby Lake. These areas have been designated as 1.) Laut Park Area, 2.) Still Creek Area and 3.) Deer Creek Area. (See Plate X). A description of each area is given in the section titled " Ecology of the Molluscan Hosts."

Snails were collected over a period of one year, from March 1949 to April 1950, with principal collections confined to the summer and fall of 1949. All collections were made personally.

No attempt was made to separate the Mollusca as to species at the time of collection. All snails found in an area were placed in common pint-sized jars in which they were carried to the laboratory.

Segregation and Maintenance of Snails.

In the laboratory the snails were washed and cleaned in tap water, segregated as to species and then placed in pint-sized glass jars which served as aquaria throughout the period of observation. Only those snails from the same collection and of the same species were placed together, with no more than twelve specimens, or six large ones, in each aquarium. These aquaria were labelled with a code number which gave the date and area of collection.

Lettuce, in both fresh and dehydrated states was the principal food supplied the snails. Most species thrived well on this diet which was chosen because of its availability and freedom from infection by larval trematodes. Water in the jars was changed once a week or oftener if it became clouded or so stained by the solution of the dried lettuce that observation for cercariae in the water was made difficult. The snails were kept at room temperature, which varied from 15°C. to 20°C. Small quantities of calcium sulphate as suggested by Swales (1935) and calcium carbonate (Stiles and Goldberger 1910) were occasionally added to the aquaria to supply the calcium required by the snails. Only

fresh unchlorinated water was used.

Detection of Cercariae:

Once segregated and labelled, the snails in aquaria were observed every two hours for the first week for the emergence of cercariae, and at least once in every twelve hours after this first week. Observation was best done by looking through the illuminated water when the aquarium was held up to a beam of light. Echinostome and stylet cercariae appeared in this manner as small white opaque objects about 0.5 mm. in diameter moving rapidly in the water. Furcocercous cercariae look like fine hair or dust particles in rapid motion.

Isolation of Infected Snails:

Snails shed larval trematodes anywhere from twelve hours to two months after collection. When cercariae were detected in the water, all snails were removed, washed and dried and then isolated singly into small jars containing fresh water. The snails which were subsequently found to be shedding the cercariae were then given a specific number, appended to the code number of the parent aquarium colony. Each snail shedding cercariae was labelled in this manner and separate records kept for each of the times of emergence, behaviour data and so forth.

Detection of Intramolluscan Stages:

If no cercariae emerged from a group of snails within one week after collection, some of the specimens were dissected and examined for intramolluscan stages. The snail was removed from its shell by means of a probe, teased on a microscope slide and examined under low-power. When the body could not be removed in this manner the shell was removed piecemeal. All whole shells were preserved in small shell vials and labelled with a code number.

Snails parasitized by larval trematodes could be readily detected as soon as the snail was removed from its shell. In some cases the sporocysts or rediae " spilled out " when the body was removed. The large digestive gland or " liver " of infected specimens was noticeably swollen and pigmented with a brown or yellow-brown colour. In others the larvae appear as small worm-like objects under the thin outer tissue of the gland.

Drawings and notes were made on all intramolluscan stages found.

Most of the snails, however, were kept alive as long as possible in the aquaria and the examination of the intramolluscan stages delayed until natural death of the animal. In this way ~~way~~ data on such matters as natural emergence periods, duration of cercarial emergence and length of development of intramolluscan

stages were gathered.

Technique of Cercarial Study:

No great departure from the standard techniques used for the study of cercariae were made. Most morphological data and all drawings were taken from living unstained specimens.

In obtaining cercariae for microscopic observation, the specimens were first located by placing the aquarium before a beam of light and removing one or more of the specimens by means of an eyedropper. When using a cover slip a small amount of water to contain the cercaria is advisable as it lessens the tendency for the cercaria to be swept to the edge of the glass.

This water containing the active larva was then placed on a microscope slide and the cercaria singled out by placing the slide on a black background. Most of the water was then drawn off with absorbent paper, leaving the cercaria in a small shallow drop which restricted the compass of its movements. This allows for greater ease in locating and following the animal under the objective of the microscope. A Number 1 cover slip placed on this drop was also useful in slowing movements and making it possible to use the oil immersion objective effectively. When using a cover slip a small amount of water to contain the cercaria is advisable as it lessens the tendency for the cercaria to be swept to the edge of the glass.

A suggestion on technique from Dr. J.R. Adams proved to be admirably suited to the study of larval trematodes. This is the use of prepared 10% methyl callulose for slowing the rapid movement of cercariae. Methyl cellulose is a highly viscous colourless medium which has been used to inhibit the activity of ciliates. The actual technique employed is to make a small ring of methyl cellulose on the microscope slide into the centre of which water containing cercariae is placed. The two media are then mixed with a probe, forming a thick syrup greatly inhibiting the violent cercarial movements but which causes no distortion or breakage of the animal. An additionally attractive feature of this medium is that the cercariae remain alive in it for a considerable length of time -- at least two hours with most species. As far as can be determined, methyl cellulose has not been used previously in the study of cercariae.

Neutral red has been found to be the most valuable intravitam dye with all species of cercariae. A solution of 5 drops of saturated neutral red in 250 cc. water is ideal for both anaesthetizing and staining. This concentration gives but a faint pink colour yet has the effect of slowing the movement of cercariae within ten minutes, and, in the same period of time, giving good differentiation of body parts. Unless neutral red is used in this weak concentration, separation of tail from body and often complete disintegration results. The stain brings out the digestive system well in both living and dead specimens. Neutral red becomes

noticeably concentrated in the genital rudiments.

Cercariae were fixed according to a method suggested by Talbot (1936). The procedure is to add to the water containing stained or unstained cercariae an equal quantity of boiling 10% formalin. The other intramolluscan stages were also successfully fixed by this method.

Drawings were made in composite from live unstained specimens only, using both high power and oil immersion lenses. The camera lucida was used in drawing quiescent and recently dead cercariae, while free hand sketches were made from the active forms. Much focusing is required as the anatomical features are found at all levels of the organisms. For drawing purposes, the flame cell patterns were best traced in the methyl cellulose medium. However, lack of time and experience hindered a complete and thorough appraisal of the excretory structures in these larvae.

Unless otherwise specified, measurements given for cercariae have been made on naturally shed living cercariae in a normally extended and quiescent state. No measurements were made on specimens under the pressure of a cover slip; a few were recorded for cercariae uniformly fixed by the hot formalin method. Although some distortion resulted from fixation it gave much less variability than did the pressure from a cover slip.

Life Cycle Studies:

The life cycle studies were of two main types

1.) infective exposure and feeding experiments, and

2.) examinations of naturally infected hosts.

Both were designed to determine the first and second intermediate hosts and the definitive hosts of the larval trematodes at Burnaby Lake. The exposure and feeding experiments included Miracidial Infection Experiments, Metacercarial Feeding Experiments and Cercarial Infection Experiments.

Miracidial Infection Experiments:

Eggs teased from adult trematodes or washed from the faeces of adult vertebrate hosts were hatched. Just prior to the hatching of the miracidia from these eggs, laboratory-raised snails were exposed to attack by these larvae.

Cercarial Infection Experiments.

Ducklings, goldfish, snails and a gammarid species were exposed to attack by all species of cercariae. The goldfish and snails were laboratory-raised uninfected specimens. The ducklings were 48 hours old when used for infection and were taken directly from the incubator to the laboratory without the possibility of becoming infected by trematodes. The gammarids were obtained from

an ornamental pond on the university campus which, as far as could be determined, was free of trematode infection. Ducklings were infected orally by putting the active cercariae in drinking water. All the other experimental hosts were exposed by being placed in large numbers of active cercariae for varying lengths of time.

Metacercarial Feeding Experiments:

The cystic or metacercarial stage of the larval trematodes, excepting furcocercous forms, taken from both naturally and experimentally infected intermediate hosts were fed to uninfected animals chosen to act as possible adult hosts for the worms. Pigeons, albino rats, guinea pigs, Pekin ducklings and goldfish were employed in these experiments.

Cysts were obtained by teasing the intermediate snail hosts in a syracuse dish or an a microscope slide. An experienced eye can locate these cysts without the aid of a microscope. They are then taken up in a clean medicine dropper and forced down the throat of the experimental host. Some of the rats and guinea pigs were fed metacercariae in small quantities of milk. Several hours prior to this feeding, the animal was kept without food or water so that the cysts supplied in the liquid stood a good chance of being eaten.

The methods and techniques used in the schistosome dermatitis experiments are given in a following section.

Life cycle data was also gained from autopsy of muskrats, and the examination of naturally infected snails, tadpoles and fish taken from Burnaby Lake.

Cultivation of Snails:

Snail egg masses collected at Burnaby Lake or from snails kept in the aquaria were hatched and colonies of uninfected mollusca raised for infection experiments. In their natural environment these egg masses are found as long sausage-shaped gelatinous masses attached to the substrata, floating articles, blades of shore grasses and on the underside of lily pads. In aquaria, the snails lay egg masses on the glass walls, or as is very often the case, on the shells of their fellows.

The egg masses were removed to fresh tap water and kept at room temperatures. As the hatching period approached, large quantities of fresh and pulverized lettuce were added to the water. Uninfected colonies of Physa occidentalis, Physa cf. traskii, Pseudocolumella, Menetus cooperi and Gyraulus vermicularis were cultivated in this manner. These molluscs were used in infection experiments when three months and over in age.

MORPHOLOGY ANDD IDENTIFICATION OF CERCARIAE AND OTHER LARVAL FORMS.

CLASSIFICATION OF CERCARIAE.

Most cercariae have been discovered and described separately from their adult forms and have, consequently, been named separately. As a result, many species of trematodes have been given two names -- one for the adult worm and one for the cercaria. For example, Echinosta revolutum is actually the adult stage of Cercaria echinata Siebold. Used in this sense, the term " cercaria" is not a genetic name but rather a group name. This lack of correlation between larval and adult forms is due largely to the complexity of trematode life cycles and to the difficulty of ascertaining exactly these life histories.

All the specimens met with in this investigation were at the outset given a tentative numerical designation based on a classification suggested by Luhe in 1909. Luhe's scheme is still used by most workers in the field. He divided the cercariae into two main groups on the basis of tail structure -- those which are separate individuals and thosw which are joined together by their tails into a kind of colony. The latter forms are all marine and are called " Rat-king " cercariae. The following is a key given by Baylis (1929) and based on Luhe's classification.

A. Cercariae separate

I. Tail well developed.

(a) Body retractile within a chamber formed in the basal part -- CYSTOCERCOUS.

(b) Body not retractile into the tail

1. Tail not forked.

(a) Tail without bristles.

(i) Tail when contracted may be as wide as or wider than body -- RHOPALOCERCOUS

(ii) Tail always considerably narrower than body -- LEPTOCERCOUS.

(b) Tail with bristles (marine forms -- TRICHOCERCOUS.

2. Tail forked at the tip -- FURCOCERCOUS.

II. Tail stumpy or absent.

(a) Stumpy -- MICROCERCOUS.

(b) Tail not developed -- "CERCARIAEUM".

B. Cercariae joined by their tails into a kind of colony -- RAT-KING CERCARIAE.

The classification of cercariae is still very much in a state of flux. Several schemes have been suggested in recent years

but data on cercariae is still too meagre to allow for the adoption of any one system. Lebour (1911) quoted by Dawes (1946) presented a classification which lays emphasis not on stmucture but upon the mode of origin. The main divisions in this classification are based on whether the cercariae develop in sporocysts or rediae. Thus it is a scheme based on life history, since subdivisions are made on type of final host.

Faust (1924) suggested a cercarial classification based on those structural systems which are least modified in the course of development. Of all classifications, this one appears to have had the largest following. However, there has been much doubt expressed by recent workers as to the reliability of using the excretory system as a basis of cercarial classification. This is due mainly to the apparent inconsistencies in the flame cell " formulae" and to the fact that " accurate tracing of the excretory systems of cercariae ---------is exceedingly difficult, even with suitable apparatus and abundance of living material" (Harper, 1929.) Stunkard (1929) is also of the opinion that the excretory system is not an infallible guide to the diagnosis of trematodes. In some forms, he points out, there are differences occurring within one family and many show excretory systems which have additions to them in passing from the cercaria to the adult worm. Dawes (1946) suggests that the cercariae be arranged in natural groups on the basis of this change in the number of excretory ducts and flame cells. However, Brown (1926) is of the opinion that such an increase in

number may be " an expression of the phylogenetic needs of the organism, and similarity in the number of excretory units in a group or of groups within the system, the result of convergence in evolution and not necessarily an indication of the phylogenetic relationship."

Dawes (1946) concludes that the flame cell formula or pattern does not necessarily denote phylogenetic relationships and may actually give a false impression of relationship. Errors may arise from too implicit a reliance on one set of organs. Porter (1928) mentions further complications arising in the attempt to classify cercariae. " An additional difficulty has accrued, as various workers on adult flukes differ among themselves as to the exact status of certain groups, with the result that groups considered as sub families by one worker, are given family rank by other workers. In such circumstances no classification of larval flukes at present can be really satisfactory."

Naming and Identifying the Cercariae Found:

As mentioned previously, the cercariae met with in this study could not at the outset be attributed with any certainty on solely morphological grounds to known adult trematodes. Until positive identification could be made they were given the tentative designations " E.C. 1" and " E.C. 2 " for the echinostome forms, " X.C. 1 " and " X.C. 2 " for the xiphidiocercariae and " F.C. 1 " and " F.C. 2 " for the two furcocercous specimens. These names

have been followed throughout the text.

In the case of " E.C. 1 " positive identification has been possible by means of direct feeding experiments. On the basis of some gross morphological features, the other larval trematodes have been compared with forms described in the literature and given tentative identifications. These comparisons were made on the basis of body size, presence or absence of spines and fin-folds, number of penetration glands and methods of swimming and attachment.

The larval trematodes described all fall within the distome grouping, those which, like adult digenetic flukes have two body suckers with the ventral one always distant from the posterior extremity. More specifically, the cercariae belong to the leptocercous and furcocercous subdivisions of Lühe's classification. Leptocercous cercariae have tails which are straight, slender and narrower than the body. The echinostomes belong to this grouping and are characteristically provided with a head collar and a coronet of stout spines. The xiphidiocercariae are also in this group, and have the anterior end provided with a stylet or boring organ. The furcocercous forms have a narrow tail which is forked distally.

Echinostome Cercariae.

Two larval trematodes shed by the Burnaby Lake Mollusca belong to the echinostome group of cercariae. They have been so identified by the fact that they have a head collar armed with

spines. Specific identification has been based on the number and arrangement of collar spines, body size and the character of the tail, viz. the presence or absence of a tail fin. The flame cell pattern, or formula, was studied in some detail in each case but inexperience and lack of time prevented the gathering of full data on the excretory systems.

The difference between E.C. 1 and E.C. 2 in body size, number of collar spines and character of the tail are sufficient to regard them as separate species. In this respect, Beaver (1937) states that cercariae of the genus Echinostomata " exhibit few specific characters by which they may be distinguished." However, the morphological data taken on these two echinostomes would appear to indicate that they are separate species.

Echinostome Cercaria No. 1. (Plate I).

Morphology

Dimensions: (Average of 30 specimens).

Body Length	0.420 mm.
Body Width	0.095 mm.
Tail Length	0.410 mm.
Tail Width	0.038 mm.
Diameter ventral sucker	0.049 mm.

This is a relatively short cercaria, with a spadellike

EXPLANATION OF PLATE I.

E.C. 1

Fig. 1 -- General morphology of E.C. 1. x 200.

Fig. 2 -- Head region showing arrangement of collar spines.

Fig. 3 -- Freehand sketch of cercaria swimming, showing position of tail dorsal to curled body.

Fig. 4 -- Metacercaria, with collar spines visible.

Fig. 5 -- Metacercaria, with collar spines visible. x 200.

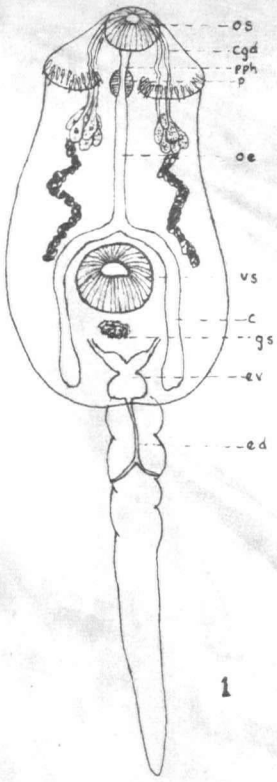
Fig. 6 -- Body in lateral view, showing ventral position of oral sucker and extreme protrusion of ventral sucker. x 100.

Fig. 7 -- Cercaria contracted in swimming, with anterior end curled ventrally. x 100.

Fig. 8 -- Redia containing cercariae. x 100.

Fig. 9 -- Redia. x 100.

Fig. 10 - Extended cercaria.



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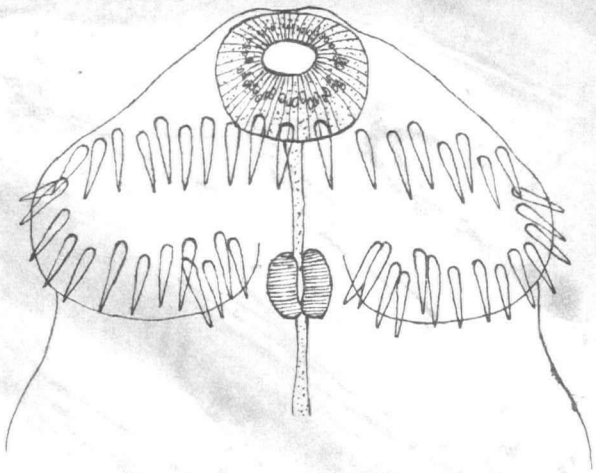

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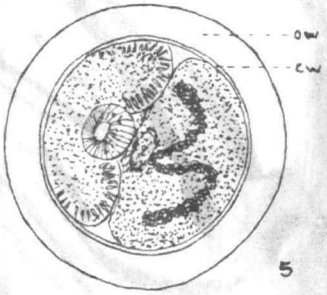

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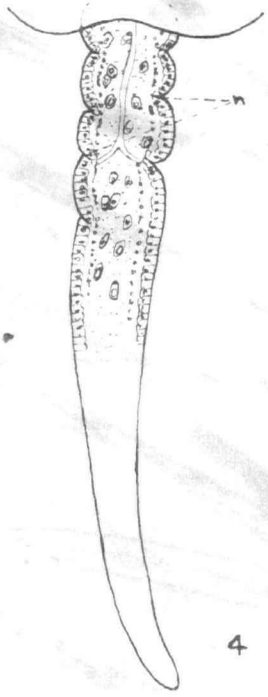

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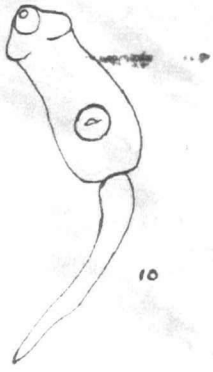

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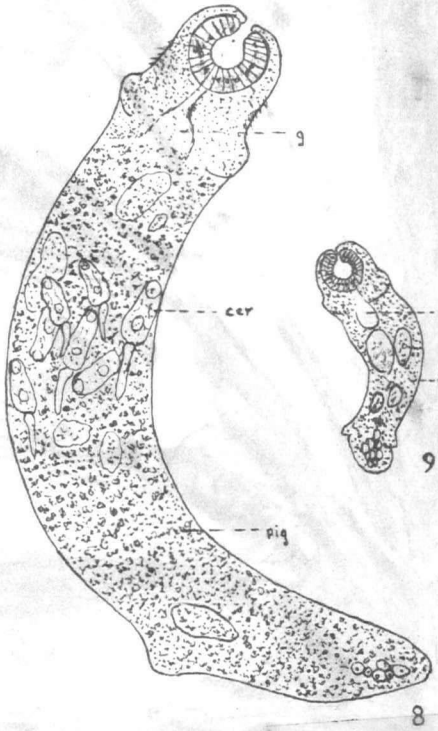

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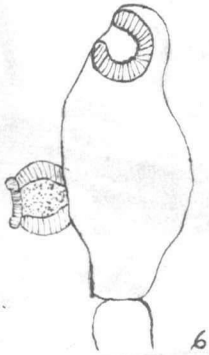

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PLATE I.

body and a stout tapering tail. When contracted the body varies from 0.140 mm. to an extended 0.525 mm. The tail also varies much in size with contraction and extension, from 0.020 mm. to 0.070 mm. at the basal section. Generally, the head and tail can be regarded as equal in length. The body is most usually rectangular in shape, becoming somewhat leaf-like when contracted and long and slender when extended (Figs. 7 and 10 respectively.)

The most prominent features of the body are the two large suckers (os and vs) situated anteriorly and mid-ventrally. The ventral sucker lies in the posterior one third of the body. It is larger than the oral sucker and is exceedingly protrusile, projecting at times the thickness of the body out from the ventral surface (Fig. 6). In lateral view the oral sucker is seen to open ventrally (Fig. 6) with the oral opening extending in a postero-dorsal direction.

The digestive system begins with an opening in the oral sucker and extends to the ventral sucker where it bifurcates and continues to the posterior extremity of the body. A pharynx is obvious at the level of the lappets.

The anterior collar is prominent and bears 43 or 45 spines (Fig. 2). Due to the overlapping of the lateral group of spines, the exact number of collar spines remains in doubt but most counts gave the total number as 45. These collar spines have the following arrangement -- a group of 4 spines in two pairs on each

lappet which are bordered by 7 or 8 unpaired lateral spines. The intervening section is filled by a group of 21 dorsal spines. The dorsal set are arranged in two alternating rows and give these spines the appearance of being grouped in staggered pairs, i.e. an oral spine placed anterior to an aboral spine. The orals appear slightly larger than the aborals.

Collar spination shows up to best advantage after the death of the cercariae and in weak neutral red stain, when the spines appear as clear unstained areas, with sharp black borders. Small cuticular spines are visible under high power on the anterior dorsal surface. These body spines extend to at least the midway point between the two suckers. No spines were observed on the ventral surface.

The excretory system can be only indefinitely described. It appears to be made up of two lateral ducts running from the posterior extremity to at least the level of the oral sucker. From these ducts many small branches arise. An extension of the system can be seen running into the basal portion of the tail (Fig. 1). Several scattered flame cells were seen but the presence of large cystogenous cells makes the examination for these cells very difficult, particularly in the region between the two suckers

The tail can very readily vary from a short stubby structure to one long and slender, tapering to a point distally. No fin membrane is apparent on the tail. A distinctive feature of the tail

is the presence of " notches " or indentations along the edge (Fig. 4). The notches are most prominent in the basal portion and expecially when the tail is contracted. They disappear when the cercaria is in neutral red or at times when the tail is extended.

The Intramolluscan Stages:

Redia (Figs. 8 and 9).

The rediae vary greatly in length. Those with birth pores range from 0.29 mm. to 1.05 mm. long, and 0.10 to 0.19 mm. wide. The diameter of the pharynx averages about 0.114 mm. The birth pore (Fig. 8), is in the posterior quarter and is best seen when the redia is in profile. No such pores are evident in the small, immature specimens. Most of these larvae are pigmented with rust coloured pigment granules. Others are filled with an evenly distributed light brown pigment. The anterior end is more transparent than the rest of the body and appears to be marked off from the remainder by indentations. In this region the muscular pharynx is very conspicuous, both in small and large rediae. In some, a short saccular gut is visible, extending only a short distance posteriorly. These rediae appear to be covered, in the anterior half at least, with small cuticular spines (Fig. 8). Many of the larger forms were observed to contain mature cercariae and a few possessed encysted metacercariae.

Metacercaria (Fig. 5).

The cyst of E.C. 1 is small, circular and transparent. It is bright yellow in colour. The cyst wall is thick, clear and obvious forming concentric circles. The outside diameter averages 0.167 mm., showing a variation of from 0.155 mm. to 0.171 mm. The wall is 0.016 mm. thick. Collar spination can be clearly seen especially under a cover slip using high power or oil immersion lenses. Portions of the excretory system can also be seen, but since the larva folds over on itself within the cyst, a thorough tracing of ducts is exceedingly difficult. Cysts are found singly or in clumps of up to two hundred in number, located most often in the liver of the the infected snail.

Behaviour Data:

Swimming

These cercariae are very active swimmers. They appear in the water to be small, white objects with a nebulous lashing motion of the tail surrounding them. Actually, the body is coiled ventrally (Fig. 3) and propelled by the vigourous lashing of the tail - which action can be observed without the aid of the microscope. They appear to favour the deeper water in the aquaria. On occasions when the water appears to be free of them some can often be seen swimming very close to the bottom. When not swimming actively, as for example when under the pressure of a cover slip, these cercariae

resort to creeping along the substratum by means of alternate attachment of anterior and posterior suckers and elongation and contraction of the body. In aged or injured specimens when the tail becomes detached from the head, creeping becomes the sole means of locomotion. This creeping activity also seems to be characteristic of moribund cercariae.

Longevity:

E.C. 1 is a relatively short lived larva, but has on one occasion been shown to be alive and active thirty hours after emergence from the snail. In this experiment a large Physa occidentalis shedding E.C. 1 was removed from its shell and placed in tap water. The cercariae free in the teased liver tissue were found to be active thirty hours later. Cercariae within the rediae also remain active for this length of time although their movements are slowed considerably.

Identification:

The most accurate spine counts on E.C. 1 give the number as being 45. This, along with relative body proportions and other morphological features gives the cercaria much resemblance to Cercaria echinoparyphium recurvatum as described by Harper (1929). There is no great discrepency in the body lengths of the two larvae, however it must be noted that the measurements given for C. echinoparyphium recurvatum are from fixed specimens while those for E.C. 1 are from living forms. The comparison then, must be but a general

one. It was found, however, that carefully fixed specimens of E.C. 1 were consistently smaller in size than the living forms so that the difference in body sizes mentioned above not as significant as it would first appear.

Harper's description of his cercaria as having a prominent collar around which there is an incomplete circle of 43-45 collar spines agrees with E.C. 1. Both also have the oral spines very slightly shorter than the aboral spines. Cuticular spines posterior to the collar and continuing to about the level of the ventral sucker is also found in both forms. The position and relative size of the suckers, a prominent muscular pharynx, tail size and structure, and structure of oesophagous and gut are also identical in E.C. 1 and C. Echinoparyphium recurvatum. Incomplete data on the excretory system for E.C. 1 made a comparison on that score impossible, however, the conspicious elements of this system in E.C. 1 agree with Harper's description in that the excretory vesicle is a single lobe and spherical, connected with two lateral collecting tubules and a single tubule from the tail (Fig. 1).

The resemblance between E.C. 1 and C. Echinoparyphium recurvatum is found also in the structure of the rediae, except in regard to the small, and apparently, younger specimens. Harper describes the latter as having a pair of mobile lateral ambulatory processes not observed in E.C. 1. The failure to note these for E.C. 1 may have been due to a lack of detailed study of the smaller

forms. Both rediae have brown pigment, a large pharynx, thick cuticle and lack of movement in mature specimens.

The metacercaria of E.C. 1 is larger than those of C. Echinoparyphium recurvatum, which has a diameter of 0.11 mm. and a wall 0.015 mm. thick. Encystment occurs not only in the same species as with C. Echinoparyphium recurvatum but also within the same host in which the cercariae develops.

Echinostome Cercaria No. 2. (Plate II.)

Morphology

Dimensions: (average of 30 specimens).

Body Length	0.385 mm.
Body Width	0.098 mm.
Tail Length	0.420 mm.
Tail Width	0.035 mm.
Diameter of ventral sucker	0.480 mm.

Unstained specimens of these cercariae are yellow in colour. When normally extended, the body is long and narrow. It is extremely contractile presenting a great variety of shapes and sizes (Figs. 8, 9, 10). Contraction takes place separately in the portions anterior and posterior to the ventral sucker. The body varies in length from a contracted 0.190 mm. to an extended 0.570 mm. The tail is also very contractile, elongating from

EXPLANATION OF PLATE II.

E.C. 2

Fig. 1 -- General morphology of E.C. 2. x 200.

Fig. 2 -- Lateral view of body, showing protrusion of ventral sucker and ventral folding of edges.

Fig. 3 -- Head region, showing arrangement of collar spines.

Fig. 4 -- Cercaria in contraction, with pronounced collar region.

Fig. 5 -- Structure of tail and fin fold.

Fig. 6 -- Metacercaria, showing curled worm and distinct cyst walls.

Fig. 7 -- Camera lucida drawing of two attached cysts. Low power.

Fig. 8, 9, 10 -- Various shapes of active cercaria.

Fig. 11 -- Freehand sketch of Redia containing cercariae.

Fig. 12 -- Redia with cyst.

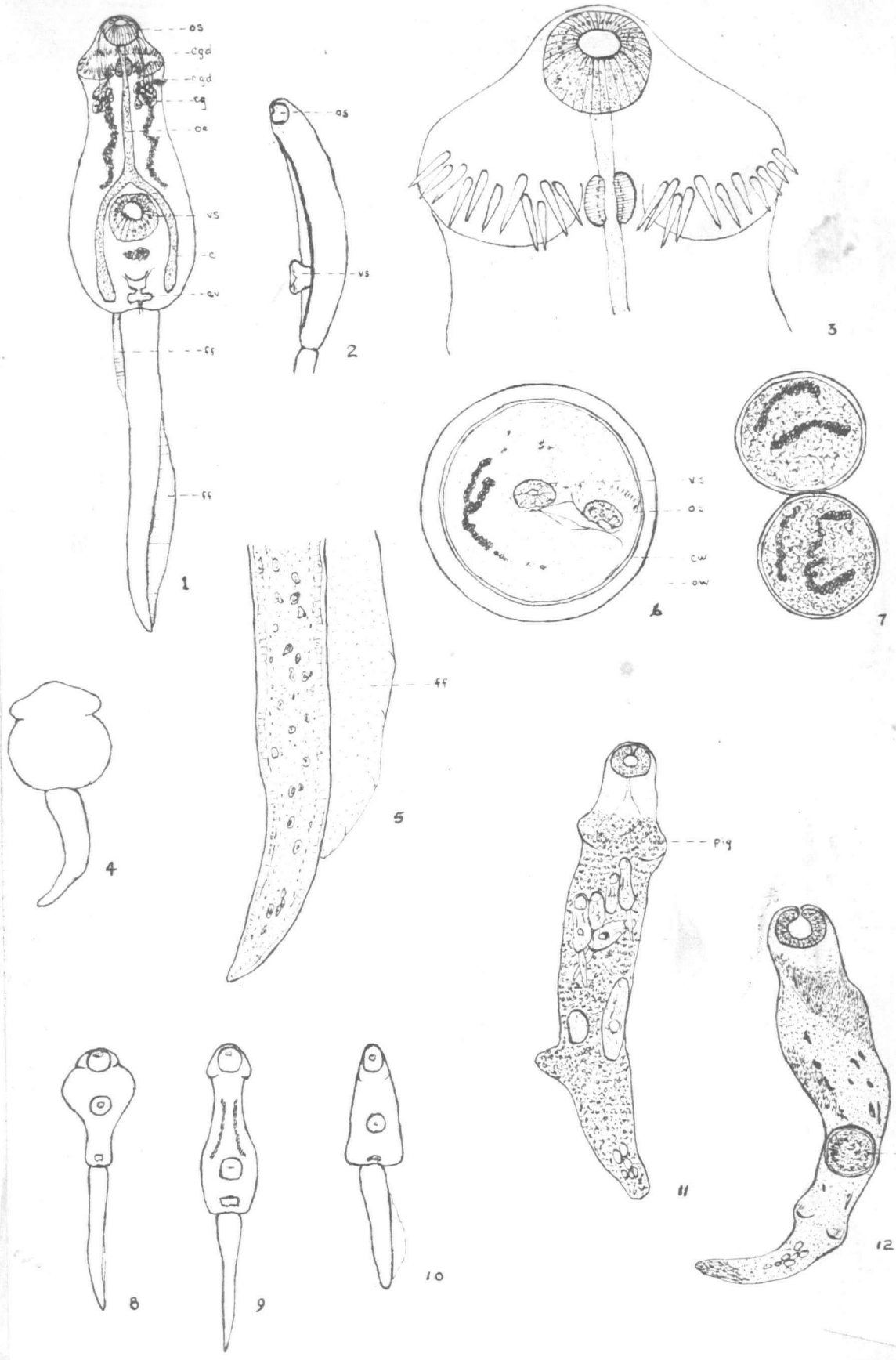
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PLATE II.

0.09 mm. to 0.65 mm.

The anterior collar and spines are fairly prominent (Fig. 3). The number of spines counted was not consistent, the average count being 37. This variation in collar spine number is due to the difficulty in determining exactly the number of overlapping lateral spines. The arrangement of collar spines appear to be as follows -- 5 on each lappet or ventral termination of the collar, 6 alternating lateral or corner spines and 15 in two alternating rows on the dorsal surface. As in the case of E.C. 1, there is at least an apparent difference in the size of these alternating spines, the orals being slightly shorter than the aborals, as with E.C. 1. The difference observed here may be the result of different angles of insertion.

Small cuticular spines appear to cover the entire body surface. They are especially obvious in the shoulder region, just posterior to the collar. These cuticular spines are small and inconspicuous and can usually be seen under high power magnification only, and in lateral view.

The body suckers are large and prominent, and of approximately the same size. The ventral sucker is in the posterior one third of the body.

The digestive system begins with an oral opening in the anterior sucker. The prepharynx is difficult to trace and leads to

a fairly prominent pharynx at the level of the lappets (Figs. 1 and 3). The oesophagus is long, bifurcating just in front of the ventral sucker (Fig. 1), with the two intestinal caeca extending to the posterior of the body.

Penetration glands are best seen in stained specimens . They are in two groups, lateral to the oesophagus, and are 10 or possibly 12 in number. Although the glands are large and prominent in stained specimens, the actual number present is somewhat obscured by overlapping cystogenous cells.

The contractile excretory vesicle is in two parts, one in front of the other (Fig. 1), in the posterior of the body. From this organ two ducts extend anteriorly and a single duct enters the basal portion of the tail.

The flame cell pattern is difficult to trace in this cercariae, principally because small vibratile portions of the excretory ducts obscure the flame cells. Flame cells were seen extending from the region of the vesicle, lateral to the ventral sucker and anteriorly as far as the oral sucker.

The tail is long and slender, usually longer than the body. A fin membrane or fold is present on this structure. Since the fin membrane arises vertically from the dorsal surface of the tail, it is not always clearly visible(Figs. 1 and 5).

The Intramolluscan Stages:

The Intramolluscan Stages:

Redia and Daughter Redia (Plate II, Figs. 11 and 12).

Almost invariably the liver of the snails infected with rediae and daughter rediae of E.C. 2 are orange or brown in colour. These rediae are of variable shapes and sizes but are generally elongate.

Mother rediae measure on the average 2.3 mm. by 0.42 mm. In some forms a short saccular gut is visible, as well as a birth pore near the posterior extremity. Ambulatory processes are especially prominent in smaller forms. The pharynx measures about 0.120 mm. in large specimens. No flame cells were seen. In many instances these rediae contained encysted cercariae and active cercariae.

The rediae are capable of slight independent movement. It is probable that this movement, combined with the appetite of the larvae, cause the damage to the liver of the snail host. The digestive gland of many snails were found to be completely replaced by rediae.

Metacercaria (Plate II. Fig. 11 and 12).

The cyst is comparatively large and perfectly round, with the outer and cyst walls forming concentric circles. Under high power the young trematode can be seen curled up with the ventral anterior surface in contact with the tip of the posterior end.

Groups of up to 40 - 50 can be found in the infected tissue of the snail.

Behaviour Data:

Swimming:

The single tail in rapid movement creates the appearance of several flagellae in motion around the cercaria. Under the microscope the body is seen to be strongly contracted when swimming. The tail is also somewhat contracted and lashes about close to and dorsally to the body. This cercariae would appear to be swimming on its back. Periodically swimming ceases and the body is stretched or pushed out. At this time a creeping movement may be commenced along the substratum by means of the two body suckers. The body is alternately stretched and contracted in the regions anterior and posterior to the ventral sucker. There appears to be no definite attraction to snail or tadpole hosts. Contact with the host appears to be random, but once contact is made swimming usually ceases and creeping begins. Like E.C. 1 they appear to favour the bottom of the aquarium, where they can be readily detected as opaque, pearl-like objects.

Longevity:

The life os these cercariae was found to be ralatively short, being about 18 or 19 hours.

Escape process from Snail Host (Pseudosuccinea columellae).

The emergence of mature E.C. 2 from the tentacles of a Molluscan host was observed. A description of the phenomenon is thought of value since, as far as can be determined, no such account has been reported in the literature on larval trematodes.

Cercariae were seen emerging from a large Pseudosuccinea columella. They appeared in a canal or duct in the parenchymatous tissue of the anterior or leading edge of the broad tentacle. At one time five cercariae were observed along the length of this canal, wiggling in quick movements and gradually progressing toward the distal opening. The contractions and extensions of body and tail characteristic of creeping movements were also observed. Within half an hour two cercariae had partially emerged from the canal opening body first while a third made violent motions to free itself, proceeding tail first. At the moment the first two escaped, the snail reacted by a sharp contraction of its tentacle.

Most cercariae did not become free of the host tissue in a single unhindered movement but exhibited difficulty in progressing within the canal and wiggling clear at the opening. The tail of the cercaria remained attached to the host tissue for some time, with the body lashing about freely in the water. Whenever cercariae did escape quickly from the canal the tentacle of the snail contracted sharply.

Forty-four cercariae were seen to emerge from the tentacle in a period of 1 1/2 hours.

Identification:

In gross morphology at least, E.C. 2 closely resembles Cercariae echinostoma revolutum as described by Beaver (1937). Obvious features of collar spination and general body size exhibit particular similarity. The description of C. echinostoma revolutum as seen by Beaver has 37 collar spines in an arrangement identical to that of E.C. 2. A comparison of excretory systems is impossible since complete data was not obtained for E.C. 2, but the information that was gathered for this system indicates that the general arrangement of the system in both cercariae is similar. Beaver (1937) in discussing the difficulty of obtaining complete data on the excretory systems has this to say, " After long and tedious study on the excretory systemsand so called " flame cell formula" or " pattern " of this and other species, I am inclined to aggee with Wernberg-Lund (1934, p. 81) that while it is an admirable ambition it is also a practical failure, and I must confess with him and Tubanqui (1932 b) that I cannot with absolute certainty determine the number of flame cells in the species."

The rediae and daughter rediae of E.C. 2 agree in detail with the description given by Beaver (1937) for C. Echinostoma revolutum. However, Beaver (1937) does not mention finding cercariae or metacercariae in the rediae of C. revolutum. This is a

point on which several workers have diaagreed. Faust (1917) for instance reported that cercariae did not encyst while still in the rediae.

FURCOCERCOUS CERCARIAE.

The furcocercous cercariae have long forked tails into which the body cannot be contracted. They usually develop in sporocysts. Lühe separated 9 forms on the basis of presence or absence of eyespots, character of tail and furcae, nature of sporocysts and upon hosts. H.M. Miller (1926) devised a classification of these cercariae which has become widely used. He subdivided these cercariae into " pharyngeal " and " apharyngeal". Each of these groups were subdivided into " brevifurcate", those with furcae less than half as long as the tail stem, and the " longifurcate", those cercariae with the furcal rami as long as or longer than the tail stem.

FURCOCERCOUS CERCARIAE NO. 1 (Plate III).

Morphology

Measurements (average of 30 unfixed specimens).

Body Length	0.228 mm.
Body Width	0.030 mm.
Tail stem Length	0.247 mm.
Tail stem Width	0.038 mm.
Furcae length	0.228 mm.

This cercaria is short and relatively stout in general appearance. Unstained specimens are deep yellow in colour. The elongated body is about equal in length, but narrower than, the tail stem.

EXPLANATION OF PLATE III.

F.C. 1

Fig. 1 -- General morphology of F.C. 1. x 400.

Fig. 2 -- Diagram of body region of cercaria, showing some of the flame cells and ducts of the excretory system.

Fig. 3 -- Anterior region, showing protrusile suctorial apparatus.

Fig. 4 -- Diagram of cercaria attached to the wall of aquarium.

Fig. 5, 6, 7 -- Cercaria in various typical positions. Fig. 6 shows position of cercaria when floating.

Fig. 8 -- Body region in lateral view.

Fig. 9 -- Sporocyst containing cercariae.

Fig. 10 - Immature cercaria teased from sporocyst.

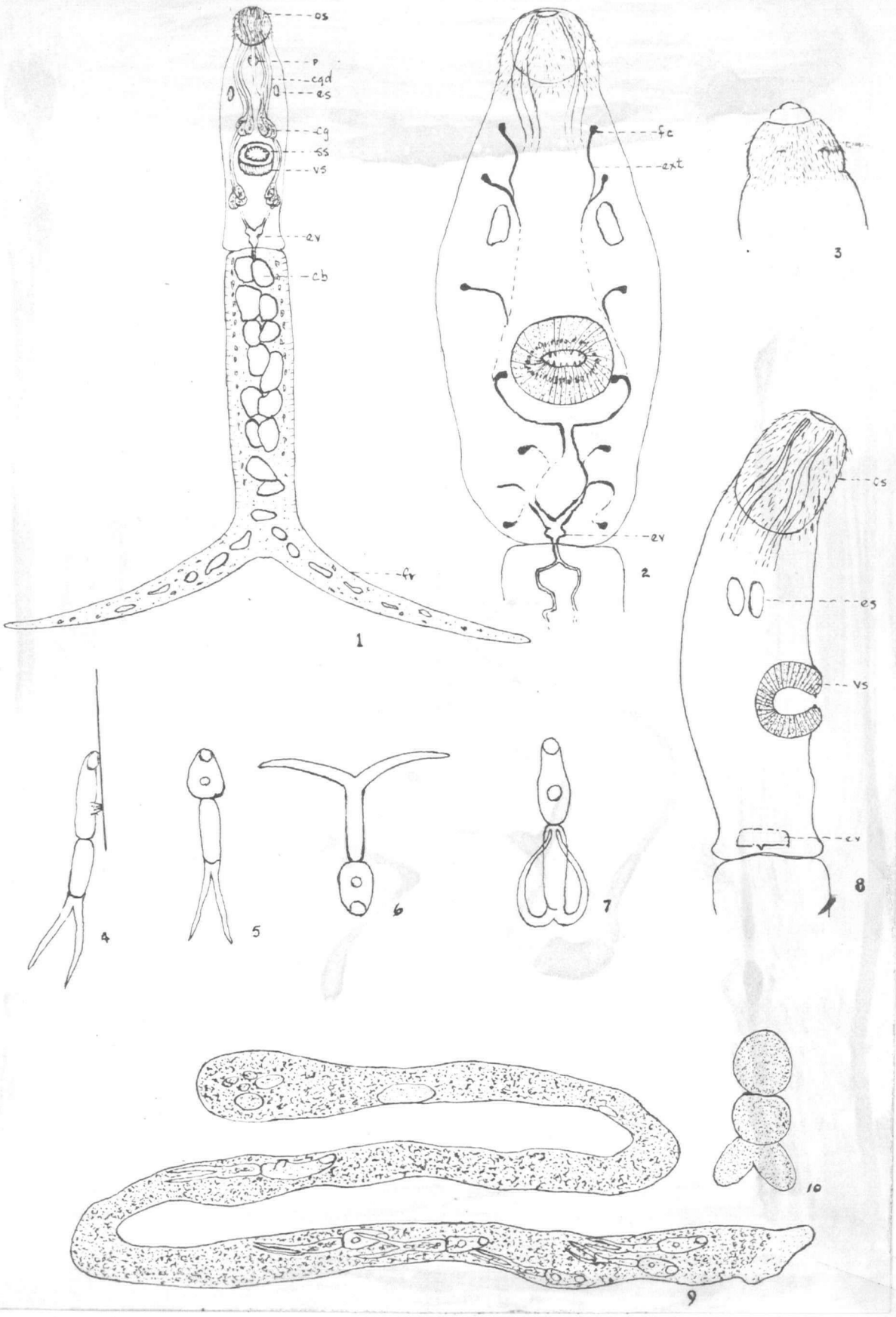

PLATE III.

The body is very contractile, becoming at times a rounded ball (Fig. 5) anterior to the non-contractile tail stem. The anterior end is provided with an oral suctoral pouch which can be inverted. The oral sucker is terminal anteriorly; the ventral sucker located just posterior to the centre of the body. One or two rows of short stout spines surround the concavity of the ventral sucker. As far as could be determined, there are six pairs of penetration glands, three (in 2 groups) in the anterior region between the two suckers and posterior to the ventral sucker. They appear to open at the anterior tip by at least four ducts which pass through the anterior organ. The digestive system begins with a mouth that opens on the anterior ventral surface, surrounded by the oral sucker and connected with a long oesophagus which bifurcates at the level of the ventral sucker and terminates a short distance posteriorly. Two vacuole-like structures in the body region between the two suckers are prominent and are considered to be unpigmented eyespots.

The excretory system has been only partially traced. Seven flame cells can be counted on each side running laterally to the ventral sucker. A prominent bladder or excretory vesicle is present in the posterior end of the body. Two ducts pass from the vesicle into the tail stem and ramify between the caudal bodies. This ramification extends the length of the tail stem and for at least a short distance into the furcae.

Spines are confined to the anterior cap region. These small spines extend posteriorly from the anterior tip to almost

the end of the anterior organ. There is a sharp demarcating line where this spined portion ceases. Neither spines nor flagellets are found in tail stem or furcae.

The tail stem is stout, with strongly notched or indented edges, due to slight and persistent contraction. This portion is apparently non-contractile. This structure contains very obvious and distinctive caudal blocks or bodies which are large cell-like objects stained deeply yellow. There are 14 caudal bodies running the length of the tail stem. In freshly emerged cercariae the bodies are somewhat paired but this arrangement is lost in older specimens. The posterior pairs are smaller than the anterior few and are continuous with several smaller bodies extending into each furca. During contraction and extension, the caudal bodies move up and down within the tail stem.

Behaviour Data.

Swimming:

These cercariae swim by means of a vigourous movement in which the furcae pull and the body pushes. At rest the cercariae appear as small hair-like dust particles about 1/32 of an inch long. They are often inactive for long periods of time and sink slowly in the water, with the furcae uppermost and at a wide angle to each other.

Staining Reaction:

Neutral red becomes more concentrated in the body than in either the tail stem or furcae. When the cercaria is placed in weak neutral red, the body at first contracts into a sphere. The caudal bodies disintegrate after a short time, followed by the disintegration of the tail stem and furcae. The body very often becomes detached from the tail stem in neutral red but keeps alive for as long a time as those which remain attached.

Effect of Temperature:

Experiments were undertaken to illustrate the effect of temperature upon F.C. 1. It was observed that cercariae subjected to 25°C. for 15 minutes suffered no effects other than a slowing of body movements. At 39°C., the cercariae ceased swimming; as soon as the temperature was lowered to 35°C., swimming recommenced. Activity was renewed also when the temperature was lowered to 35°C. after being at 40°C. for approximately 10 minutes. The cercariae died in water at temperature 54°C.

Effect of Light:

These cercariae are positively phototactic. On several occasions the cercariae were observed concentrated in the top 2 inches of the water after the aquarium had been under a lighted lamp for 10 minutes. When subsequently kept for a few minutes in diffuse light the cercariae became evenly distributed throughout the water.

The Intramolluscan Stage (Plate III).

The Sporocyst:

The sporocysts of F.C. 1 are very long and slender, with little modification. Cercariae in all stages of development are found in the sporocysts, from the rudimentary bulbous beginnings to the mature active cercariae. The sporocyst averages 10 mm. in length, varying greatly from 5 to 12 mm. It averages 0.20 mm. in width. Most have light brown pigment concentrated in the peripheral regions as a dark narrow border. This pigment is found in a lesser degree throughout the larva. Macroscopically, and as seen through the thin wall of the digestive gland, the sporocysts resemble small cestodes, being long slender and cream coloured. The liver or digestive gland of snails infected with this larva are usually in a gorged condition but are not as strongly pigmented as in the case of snails infected with the Echinostome and Xiphidiocercariae. The genital pore is relatively large, located about 0.133 mm. from the posterior end. It averages 0.098 mm. in diameter.

There appears to be no localization of cercarial development in these sporocysts. Cercariae of equal maturity were observed at both ends as well as in the central portion of the sporocyst. These sporocysts are capable of independent movement.

Identification:

The identification of F.C. 1 must remain, as previously

stated, largely in doubt. It possesses features in common with several strigeid cercariae as described in the literature and yet has others that do not permit it to be specifically likened to any particular one.

The body dimensions place it in the group of longifurcate pharyngeal cercariae under Faust's classifications. F.C. 1 resembles Cercariae dohema, Cort and Brackett (1937) in general appearance, having small spines at the anterior extremity only and with a large number of caudal bodies in the tail stem. Furthermore, C. dohema possesses two very large unpigmented eyespots in the anterior of the body which are not present in F.C. 1. Another strigeid with caudal bodies almost identical with those of F.C. 1 in shape and number is C. okobojensis, Brooks (1943 a) a species taken from the Iowa Lakes region. The "notched " or indented appearance of the tail stem is apparent in both these cercariae.

But, the resemblance fails since the body and furcae of C. okobojensis are almost entirely covered with spines, whereas F.C. 1 possess spines at the anterior tip only. The tail stem of the former cercaria also has flagellets projecting from it, structures which were not observed in F.C. 1. A similarity to C. ramae Dort and Brackett (1938) ceases because of the lack of spination in F.C. 1. C. pseudoburti, Rankin (1939) appears to resemble very closely F.C. 1 in body size but again the lack of spination and character of the caudal bodies of F.C. 1 would appear to give it a

status of its own.

It seems highly probable that this cercaria, like the forms mentioned above, is the larval stage of one of the holostome group of trematodes, of the Family Strigeidae, Railliet, 1919 (Syn. Holostomidae Brandes, 1890). The adult worms are found in the gut of many birds. The cercariae commonly encyst as tetracotyles or diplostoma in the flesh of fish. Several attempts were made to infect some experimental hosts with this cercaria, and will be discussed in a following section. Of the cercariae discussed above which bear a resemblance to F.C. 1, C. ranae, Cort and Brackett (1938) has been reported to be able to produce diplostoma in tadpoles, causing a fatal bloat disease. This feature of forming encysted larval holostomes in vertebrate hosts is apparently not common to all Strigeidae similar in appearance, since none of the experiments performed with F.C. 1 were successful. Rankin (1939) was also unsuccessful in attempts to infect may fly and dragon fly nymphs, gammarids, tadpoles, fishes and mice with C. pseudoburti Rankin.

FURCOCERCOUS CERCARIAE No. 2 (Plate IV).

Morphology:

Dimensions (average of 30 unfixed specimens).

Body Length	0.385 mm.
Body Width	0.059 mm.

EXPLANATION OF PLATE IV.

F.C. 2

Fig. 1 -- General morphology of cercaria. x about 400.

Figs. 2 and 3 -- Various shapes of anterior suctorial apparatus.

Fig. 4 -- Diagram showing protrusibility of anterior end and terminations of gland ducts.

Fig. 5 -- Diagram of tail furcae, showing flagellets of the fin membrane and terminal excretory papillae.

Fig. 6 -- Position of cercaria at surface film of water.

Fig. 7 -- Lateral view of body region showing protrusion of ventral sucker.

Fig. 8 -- Sporocyst.

Fig. 9 -- Distal portion of caudal furcus of Cercaria mibleri.

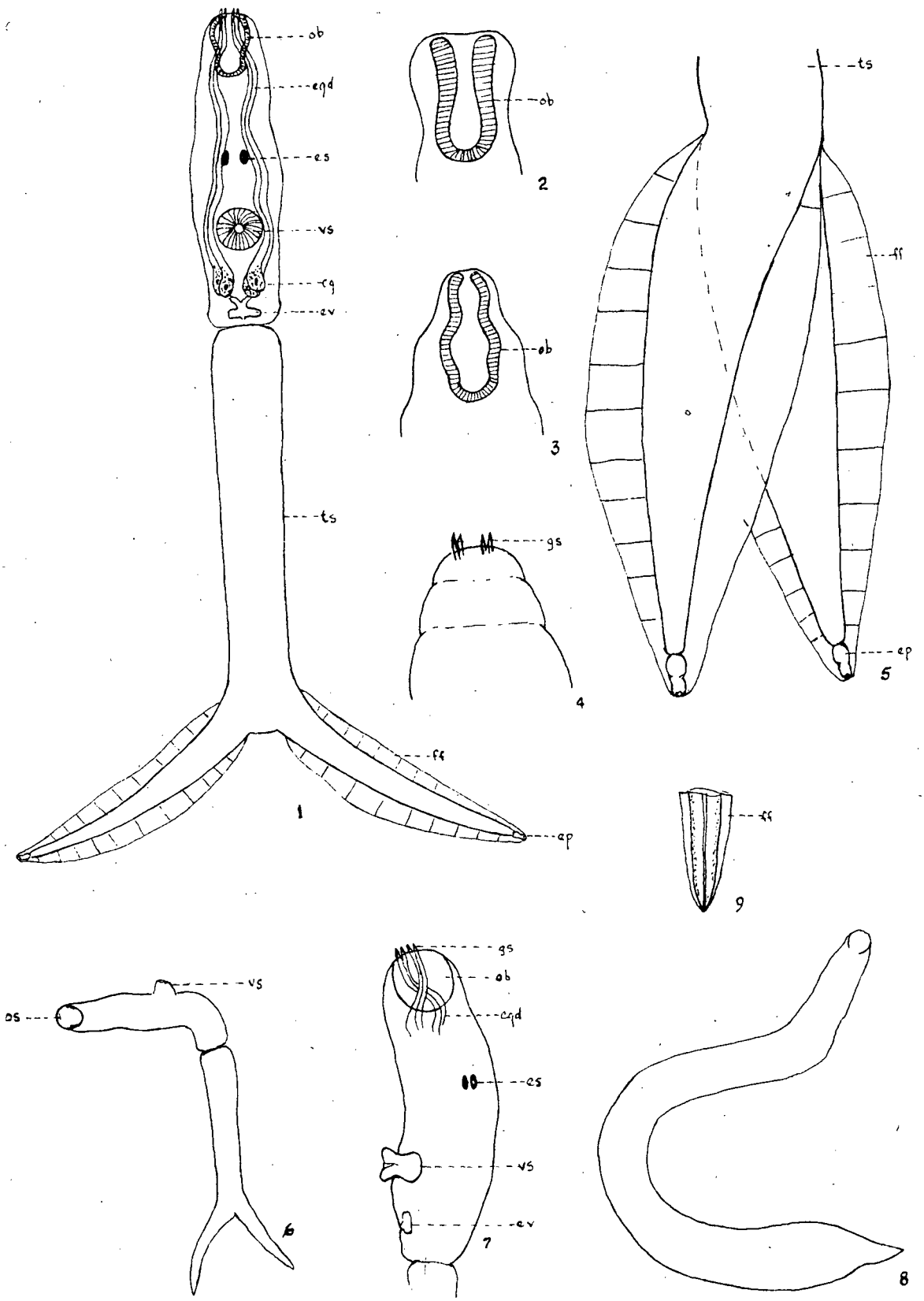
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PLATE IV.

Tail Stem Length	0.425 mm.
Tail Stem Width	0.052 mm.
Furcae Length	0.285 mm.

F.C. 2 is a long apharyngeal brevifurcate distomate cercaria closely resembling the members of the Cercaria elvae, Miller, 1923 group. The body and tail stem are narrow, the furcal rami stout and tapering to a sharp point. In a normally extended condition the body is slightly narrower than the tail stem.

The most prominent features of the anatomy are two dark pigmented eyespots located approximately 0.020 mm. apart and situated one third of the body distance behind the anterior sucker.

Another characteristic and obvious feature of F.C. 2 is the large "oral bulbous" (Faust, 1926), in which is found the anterior suctorial apparatus. This head organ extends about half way between the oral and ventral suckers. The oral bulbous replaces the suctorial disc of other distomes and is actually a suctorial proboscis (Figs. 2, 3 and 4). The large tortuous cephalic gland ducts open as four spine-like projections from the anterior tip of the cercaria (Figs. 4 and 7). The glands themselves are situated posterior to the ventral sucker.

The digestive system consists of an inconspicuous tube which appears to bifurcate and terminate just anterior to the ventral sucker. There is no pharynx.

The ventral sucker is small and extremely protrusile, often projecting out from the subintegumentous tissue (Fig. 7).

The excretory system has been incompletely traced. At least three main excretory ducts can be seen, two run laterally to the ventral sucker from the region of the anterior organ to the excretory vesicle. The third appears to run down the mid-line of the body. Excretory ducts extend through the furcal rami and apparently terminate at the excretory papillae (Fig. 2). In unstained cercariae the vibratile portions of the ducts, and the flame cells themselves, are pink to mauve in colour. The body is enclosed in a relatively thick, obvious body wall.

The tail stem possesses few distinguishing features such as were present in the tail stem of F.C. 1. No caudal blocks are present in E.C. 2. The tail stem is non-contractile.

The furcal rami are short, stout and strongly pointed. They are highly contractile. Transparent furcal folds are seen under high power. An obvious excretory papilla is found at the tip of each ramus. The furcal fold can best be seen under high power or oil immersion only when the furcae are in contraction. In this condition, flagellets, appearing as long dark lines, can be seen projecting from the furcae. These would appear to be creases in the relaxed furcal fold. The flagellets are 0,010 mm. long. Under low power magnification the terminal excretory papillae appear as curved spines on hooks. The papillae are flexible terminations.

Morphology of the Other Intramolluscan Stages: Plate IV. Fig. 8.

Sporocysts:

The sporocysts are long and stringy in appearance, and can be readily detected within the infected portion of the snail. In detail they are similar to the sporocysts of F.C. 1 and characteristic of the schistosome group as a whole. They are capable of independent movement.

Behaviour Data:

Swimming:

This cercaria is a powerful swimmer. In swimming the tail is forwardly directed. In a diffuse light the cercariae tend to concentrate at the surface. When crawling, these cercaria proceed in measuring worm fashion.

On many occasions these cercariae can be found attached to the walls of the aquarium by means of the ventral sucker. This is a strong attachment, many of the cercariae being unable to be loosened with a probe without causing damage to them. However, it is not a permanent attachment for they can free themselves and recommence swimming or creeping. The ventral sucker only is used in adhering to substrata. When floating at rest in the water, F.C. 2 hang vertically with furcae uppermost and spread wide apart -- at approximately 180 from each other.

Longevity:

Several experiments to ascertain the longevity of F.C. 2 were undertaken. After a large number of these forms were shed from a Physa occidentalis, the snail was removed from the aquarium and the cercariae kept in the water at similar temperature and light conditions. At least half of the cercariae were alive and active at 60 hours. All the larvae were dead at 72 hours.

Effect of Temperature:

In several instances it was observed that infected snails kept at 13 - 14°C., shed but a few F.C. 2. When these snails were placed in aquaria with water at 20 - 21°C., the cercariae were again shed in great numbers within the period of an hour.

Effect of Light:

Some experiments were carried out to show the effect of light on this cercaria. A strong light was placed under an aquarium in a darkened room. At the outset, all F.C. 2 were swimming in the topmost 1/3 of the water. After a period of 15 minutes in this lighted condition, approximately 90% of the cercariae were observed swimming in the bottom 1/3 of the aquarium water.

Identification:

Primarily the identification of this cercaria has been made on the presence of body size, number of penetration glands,

spination, and the presence or absence of eyespots and furcal folds. On this basis F.C. 2 appears to bear resemblance to Cercariae oregonensis, Macfarlane and Macy (1946), C. milleri, Faust (1926) which is a member of the C. elvae group (Miller, 1926). Like C. oregonensis, F.C. 1 has been found to be experimentally capable of producing a dermatitis in man. Also similar to C. oregonensis are the body dimensions, the comparison of which is given here:

	F.C. 2.	C. oregonensis M. & M. 1946.
Body Length	0.385 mm.	0.315 mm.
Body Width	0.059 mm.	0.690 mm.
Tail Stem Length	0.425 mm.	0.426 mm.
Tail Stem Width	0.052 mm.	0.047 mm.
Furcae Length	0.285 mm.	0.222 mm.

In addition both possess eyespots. However, there is no similarity in the matter of spination. In C. oregonensis the body, tail stem and furcae are all uniformly spined. Spines in F.C. 2 are present only at the anterior tip. F.C. 2 most certainly is a member of the C. elvae group as described by Miller, but its specific identification can not be made from the present observations.

It must be pointed out that these comparisons are made on superficial grounds. Faust (1926) mentions that the essential differentiating features of the apharyngeal fork-tailed cercariae consist in the michrochemical reaction, as well as the number of the excretory cells or penetration glands.

An interesting comparison can be made here with C. milleri, Faust 1926. C. milleri is an apharyngeal fork-tailed cercaria which Faust described as a new species from Durban, Natal. It is a form closely resembling Cercaria elvae, Miller, 1923. The fact that such an exotic form should bear such resemblance to a local species is worthy of note. The body measurements of these two forms agree closely, as do the character of eyespots and oral bulbous.

XIPHIDIOCERCARIAE:

The xiphidiocercariae are characterized by having an anterior end provided with a stylet or boring organ. They are not, however, alone in this respect for the Microcercariae, Rhapalocercous and Cystocercous groups also possess stylets. According to Porter (1928) it was Diesing in 1855 who first used the term xiphidiocercariae to describe these forms. In 1919, Lühe further defined the group and noted that these cercariae have characteristically slender tails, penetration glands and no eyes. Xiphidiocercariae develop in sporocysts and encystment takes place in second intermediate hosts.

Lühe's four main groups of xiphidiocercariae are Cercaria microtyle, Cercaria vergulae, Cercaria ornatae and Cercaria armatae. Cort (1915) further suggested a Polyadena group. Sewell (1922) reclassified the xiphidiocercariae, subdividing Lühe's four main groups and placing the Polyadena group as a subdivision of the Armatae cercariae.

The Xiphidiocercariae found at Burnaby Lake fall within the Polyadena grouping.

Xiphidiocercariae No. 1 (Plate V).

Morphology:

Measurements (average of 30 unstained normally extended specimens.)

Body Length	0.216 mm.
Body Width	0.094 mm.
Tail Length	0.133 mm.
Tail Width	
Diameter oral sucker	0.534 mm.
Diameter ventral sucker	0.35 mm.

This cercariae is small and extremely contractile. When contracted state the head is either broad and leaf-like (Fig. 13) or almost a perfect shpere (Fig. 16) while the tail is stubby and sharply pointed. The body varies from a contracted 0.114 mm. in diameter to an extended 0.285 mm. in length. Two prominent body suckers are present. The oral or anterior sucker is slightly smaller than the ventral sucker which is located just posterior to the mid-line of the body. When the body elongated the anterior sucker is especially obvious, as a result of the greatly narrowed mid-portion of the body between the two suckers. The anterior sucker is usually directed ventrally (Figs. 6 and 8) and surrounds an

EXPLANATION OF PLATE V.

X.C. 1

Fig. 1 -- Sketch of entire cercaria showing some structural details. About x 400.

Fig. 2 -- Cyst, showing position of curled worm.

Fig. 3 -- Diagram of contracted tail.

Fig. 4 -- Stylet, dorsal view.

Fig. 5 -- Stylet, lateral view, showing the straight ventral surface.

Fig. 6 and 8 -- Diagram of anterior end of cercaria showing various positions of stylet and shapes of oral opening.

Fig. 7 -- Position of cercaria while swimming.

Fig. 9 -- Diagram showing lobed structure of tail.

Fig. 10 - Metacercaria, showing the extreme curling of larva.

Fig. 11, 12 and 13.- Various shapes of cercaria.

Fig. 14 -- Outline sketch of entire sporocyst.

Fig. 15 -- Sketch of portion of sporocyst containing cercariae.

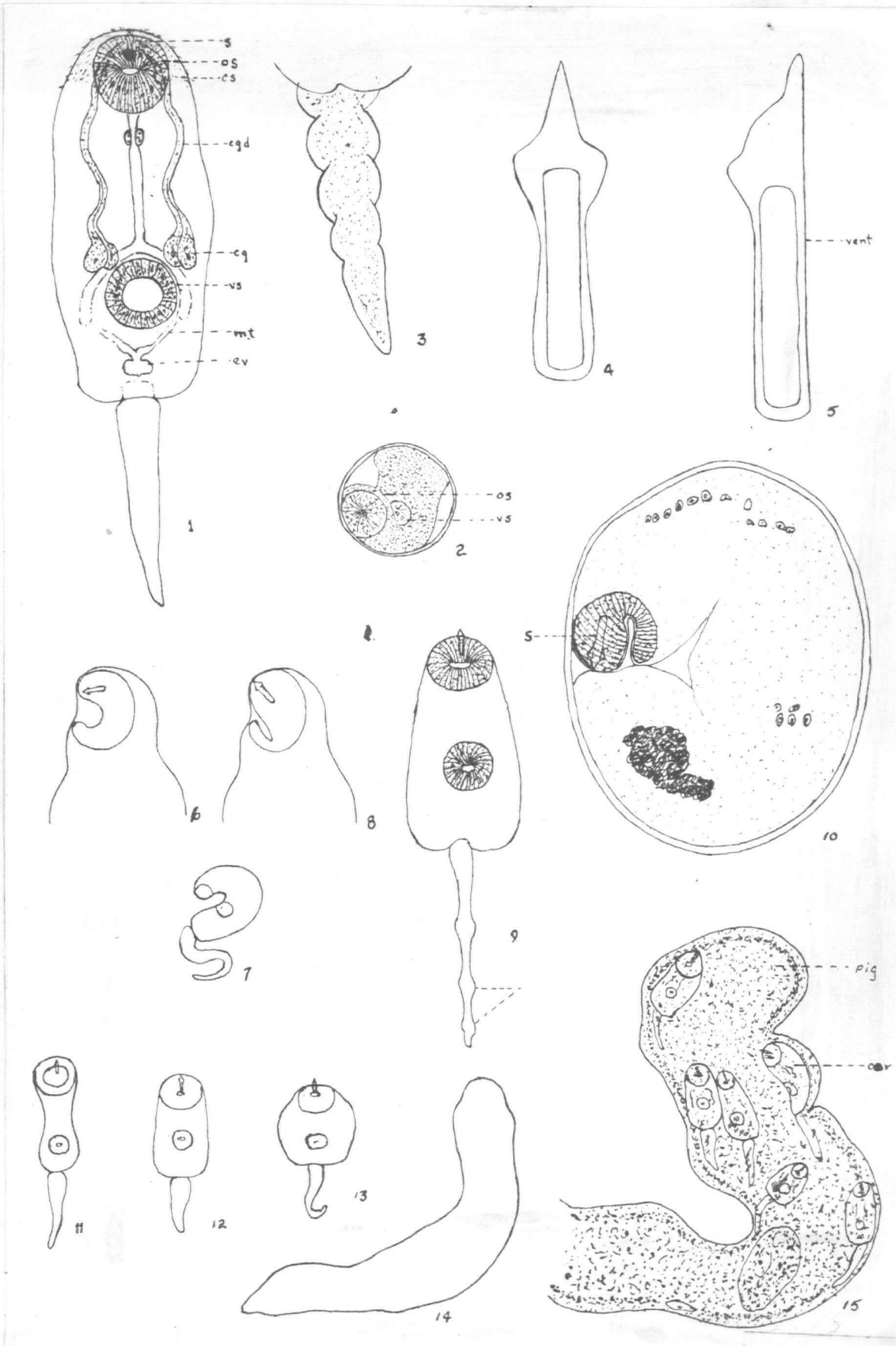
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PLATE V.

oral opening. Depending upon the state of contraction this opening appears as either a posteriorly directed slit or as a wide concavity surrounded by the narrow muscular border of the sucker.

The stylet (Figs. 4 and 5) is embedded in the anterior sucker, dorsal and anterior to the oral opening. It is 0.0196 mm. in length, with a point of 0.005 mm. long. The structure of the stylet is seen best in unstained specimens under the pressure of a cover slip. The pressure of a cover slip causes the mass of the cercaria to change from a clouded appearnce to extreme clarity making the sharp outline of the stylet stand out in contrast. In appearance the stylet is a sturdy, nail-like structure, with three bulbous areas on the dorsal and two lateral surfaces near the point. The base is the same size as the rest of the stalk and the has rounded corners. It is noteworthy that the stylet remains intact sometimes long after the total disintegration of the cercaria.

Two pair of large penetration glands open by one, or possibly two ducts on the anterior tip of the cercaria near the point of the stylet. These ducts extend in corkscrew fashion almost to the glands near the level of the ventral sucker.

Five spines or hairs are found from the anterior tip of the cercaria to a point 1/3 the length of the oral sucker. No spines are present on any other region of the body surface.

The excretory system has been incompletely traced.

When the cercaria is not swimming the tail of X.C. 1 assumes a short and stubby shape (Fig. 3). In this contracted shape, the " notched " or crenated appearance of the borders of the tail becomes prominent. In swimming the tail is greatly extended, becoming a long slender pointed structure, losing its notches. Tail crenation disappears also in stained specimens. A characteristic feature of the tail, when it is somewhat extended is the appearance of four or five bumps or " knots " at regular intervals along its length, (See Fig. 9). These notches appear to be a characteristic or at least a consistent feature of this cercaria. They are arranged alternately on each side, with several successively smaller ones in the distal half.

Morphology of the Intramolluscan Stages: Plate V., Figs. 14 and 15 15).

The Sporocyst:

The sporocysts X.C. 1 are of a great variety of shapes and sizes. Generally, the sporocysts are undifferentiated, slender, terminally club-shaped structures. They range in length from 0.209 mm. to 0.760 mm. and in width from 0.078 to 0.133 mm. Many contain the developing as well as mature active cercariae, the latter being found in small numbers only. Stained in weak neutral red, the active cercariae within the sporocysts concentrate much more of the dye than does the surrounding material.

Brown pigment is present in most of the sporocysts and is

concentrated in a narrow area bordering the wall. It seems probable that the sporocysts without these granules are the more mature forms in which the pigmentation has been lost. Many of the smaller, presumably younger larvae have a pink colouration. A brown pigmentation colouring the whole of the digestive gland of the snail host almost invariably indicates infection by these larvae.

None of the sporocysts examined contained metacercariae. In this respect, care must be taken by the observer to assure that a cyst which appears to be within the sporocysts is actually contained and not, as often occurs, free and merely superimposed or underneath the sporocyst cercaria.

The Metacercaria:

The cysts are colourless, oval shaped bodies. There is an apparent variation in the size of the cysts, which as far as can be determined, is due to the cysts lying at various angles to the horizontal. In an end view the cyst is almost a perfect circle, measuring 0.122 mm. in diameter. The oval shaped cysts are 0.124 x 0.133 mm.

The wall is narrow but clearly defined. Within the cyst wall the metacercaria is doubled upon itself so that the stylet in some instances is in contact with the posterior end.

In Lymnaea palustris and L. proxima at least, the cysts were found in small quantities in the mantle cavity and digestive gland.

As stated previously no positive evidence of cysts within the sporocyst was observed. However, in one Helisoma trivoluis, in which sporocysts were found to be in an advanced stage of decomposition objects looking much like decomposing cysts were seen within the larval walls. No definite cyst wall or stylet was observed but the dimensions (0.133 x 0.130) agree favourably with those found for the cyst of X.C. 1.

Behaviour Data:

Swimming:

Swimming is accomplished by the propelling action of the tail aided by a sympathetic rhythmic movement in the curled body. Just prior to swimming the tail is stretched greatly. The body in swimming is bent ventrally and is strongly contracted, the tail lashes about dorsally to the body. Although the swimming activity is vigorous, the cercaria gains little distance -- virtually no more than if the animal had spent the time in creeping over the substratum. X.C. 1 has been observed to maintain a rapid swimming activity for about two minutes, then to suddenly commence a creeping movement of sucker traction and body elongation. After a minute or so of creeping, the ineffective swimming is resumed. Older and moribund specimens were found to creep almost exclusively. This cercaria creeps in a fashion similar to that as described for the other cercariae.

Action of the Stylet:

The action of the stylet organ, presumably used in the penetration of the 2nd intermediate host tissue, has been observed. Embedded as it is in the muscular anterior sucker, the stylet has great manoeuverability. While the animal creeps along the substratum the stylet is in constant probing movement. A close observation of this movement reveals that it is a lever type, with the fulcrum located at or near the bulbous portion, causing the posterior tip of the stylet to transcribe large arcs. That is, the stylet scoops down towards the oral opening and then up and out towards the substratum.

Longevity:

Experiments showed X.C. 1 to be short lived.

Penetration and Encystment of Cercaria:

On two occasions, X.C. 1 was observed in the process of piercing the surface meniscus of water on a glass surface. The cercaria was only successful however, in pushing a channel of water before it. In a short period of time, the creeping movement slowed and the tail ceased lashing about. Later, the tail became detached from the body and disintegrated. An exudate then appeared on the anterior tip of the body, preparatory, it is assumed, to the formation of an encircling cyst wall. This was an abortive attempt for the secretion failed to encircle the body but instead mixed with the

surrounding water. It would thus appear that satisfactory conditions for cyst formation were not present. The resistance afforded by the miniscus was possibly a sufficient stimulus for the process of cyst formation but the character of the surrounding medium prohibited completion.

Identification:

Due to insufficient morphological data gathered from this species, a positive identification has been impossible. Other cercariae which show similarities to X.C. 1 are Cercaria conneae, Brooks 1943, C. dorotti, Brooks 1943, and C. albui, Brooks 1943, C. leptosoma , Brown 1929, with most resemblance towards the C. albui.

Xiphidiocercaria No. 2 (Plate VI).

Morphology:

Very little morphological data and no measurements were made on X.C. 2. This stylet cercaria was seen on but one occasion, emerging from a large Helisoma trivolvis hornii (Tyron). In size it is slightly larger than X.C. 1. Unstained specimens are tinted a deep yellow colour.

The oral sucker is large and is constantly contracting and relaxing. This alternate muscular action results in the oral opening fluctuating between a large gaping orifice to a narrow slit. In the latter case the oral opening is directed antero-ventrally.

EXPLANATION OF PLATE VI.

X.C. 2

Fig. 1 -- Sketch of entire cercaria showing some structural details.

Fig. 2 -- Extended cercaria, lateral view.

Fig. 3 -- Stylet, dorsal view.

Fig. 4 -- Stylet, lateral view.

Fig. 5 -- Small sporocyst containing cercariae.

Fig. 6 -- Metacercaria.

Fig. 7 and 8 -- Diagrams of anterior end showing various shapes of oral opening.

Fig. 9 -- Sporocyst.

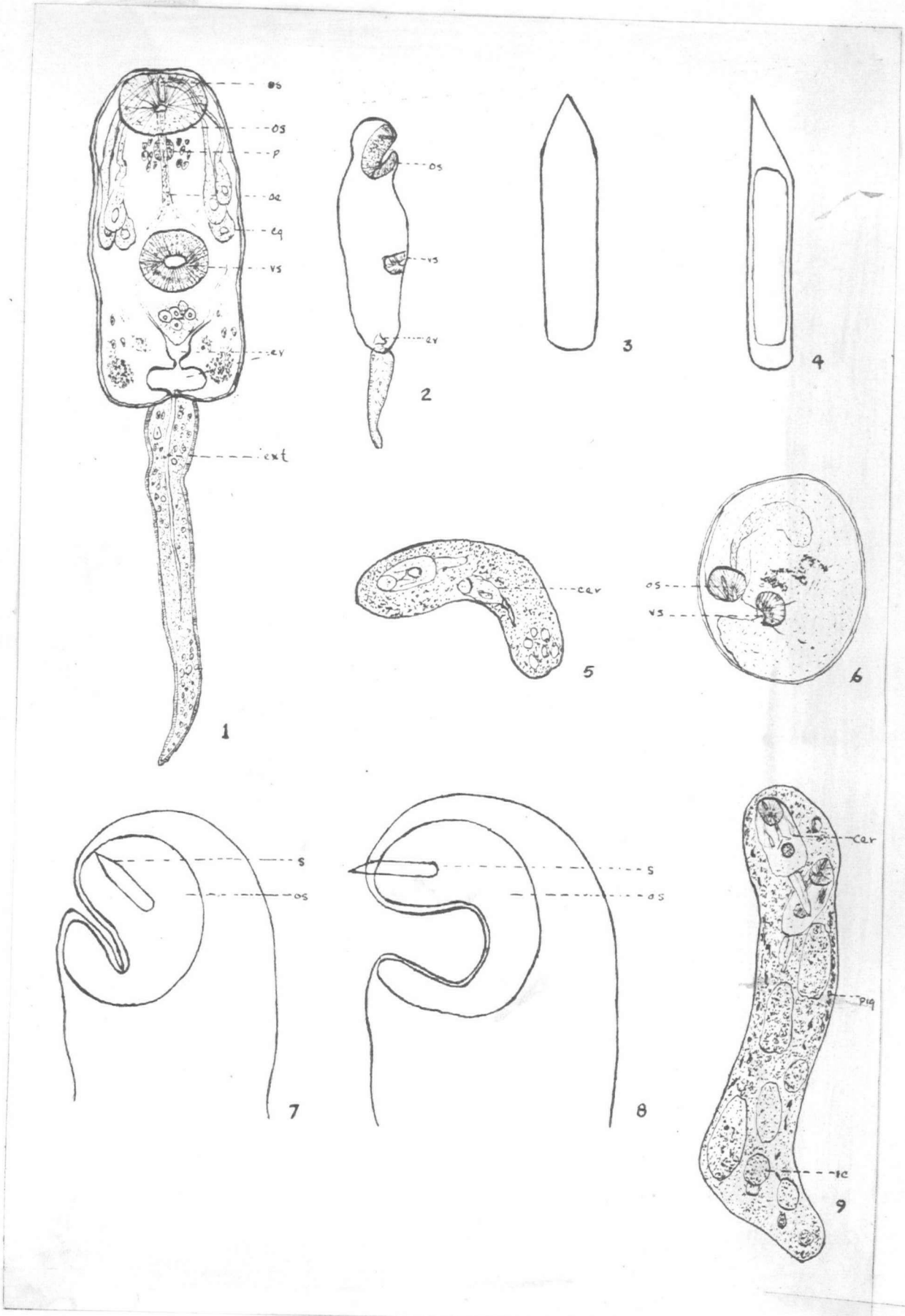
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PLATE VI.

The stylet is simple in structure and measures 0.163 mm. long. It lacks the bulbous thickenings characteristic of X.C. 1. It is located in the musculature of the oral sucker, dorsal and anterior to the oral opening. The movement of the stylet is identical to that as described for X.C. 1, with a fulcrum near the anterior tip.

The tail is long and slender and averages 0.150 mm. in length. When extended and at rest the tail has several " knots" along its length. Notches or crenations along the edge of the tail are present but not obvious as in the case of X.C. 1. In many specimens, the tail showed a slight bend toward the left on the basal portion (Fig. 1).

Morphology of the Intramolluscan Stages:

The infected digestive gland of the snail was swollen to a mass larger in size than the uninfected portions of the body. Blood red patches covered the external surface of the digestive gland. This phenomenon had not been observed in any other infected snail.

The sporocysts are short, broad and have blunt ends. They range in length from 0.150 mm. to 0.76 mm., with an average width of 0.098 mm. Most of the sporocysts have an orange pigmentation. Immature and mature cercariae are present in the sporocysts. The active cercariae creep along the wall. When the end of the sporocyst is reached the cercaria contracts and, conforming itself to the curvature of the sporocyst, proceeds to creep along the opposite

wall. The parenchymous tissue within the mother larva appears to offer no difficulty to the progress of the cercariae.

The Metacercaria:

No metacercaria were found in the snail.

Behaviour Data:

Swimming:

The method of swimming in X.C. 2 is indistinguishable from X.C. 1.

Occurrence:

The H. trivolvis from which X.C. 2 everged was collected in the Deer Creek Area, on April 3rd. The snail-was found on the surface of the moist mud of the lake-shore. It is very likely that this snail had been out of water for 6 months, for the water level of the lake had been lowered for this period of time prior to collection. The cercariae emerged from the snail within 12 hours after isolation in the laboratory. Large numbers were shed for a period of two days. The snail died on the third day and on examination was found to be highly parasitized.

Identification:

Insufficient morphological data was gathered to give a positive identification to this cercaria. It seems likely however,

that X.C. 2 is the same cercaria as that described by Musfeldt (1945) and found in Physa occidentalis from Burnaby Lake. The structure of the stylet organ as described for Musfeldt's cercaria shows much similarity as that found in X.C. 2.

EXPERIMENTS TO DETERMINE LIFE CYCLES.

Introduction:

The experiments reported in the following section describe the attempts with each cercaria to trace its development toward the adult stage of the worm. This work involved two types of infection experiments;

1.) Cercarial Infection Experiments, in which several species of vertebrate and invertebrate experimental hosts were exposed to attack by each species of cercaria.

   (a) Experiments using Echinostome and Xiphidiocercariae to determine which animals serve as second intermediate hosts.

   (b) Experiments using the Furcocercous cercariae to determine the adult hosts of these forms.

2.) Metacercarial Infection Experiments, in which cysts of Echinostome and Xiphidiocercariae were fed, in a number of controlled experiments to vertebrate hosts to determine in which the larval trematode would attain adult development.

A summary and discussion of the results with each cercaria follows a brief description of the experiments used with each.

ECHINOSTOME CERCARIAE NO. 1.

Cercarial Infection Experiments:

Experiments with Mollusca:

1.) A small uninfected Physa occidentalis was placed in a small amount of water in a syracuse dish along with approximately 20 E.C.1. After 22 hrs. the cercariae were no longer seen in the water. The snail was then kept in an aquarium for two weeks. The snail was not examined until two days following its death so that the body was found in an advanced stage of decomposition. No metacercariae were found.

2.) A large uninfected P. occidentalis was placed in a stender dish with 10 E.C. 1 for a period of twenty-four hours. The snail was then examined. No metacercariae were found.

3.) A large laboratory bred, uninfected P. traskii was placed in a small aquarium with a Lymnaea proxima rowellii shedding E.C. 1. After two days of such exposure the Physa was crushed and examined under low power of the microscope. Three cysts, identical with the 45-spined cysts of E.C. 1 were found.

4.) A small uninfected P. occidentalis and Pseudosuccinea columella were placed in an aquarium with a Lymnaea proxima shedding E.C. 1. The snails were exposed to cercarial attack for 24 hours, after which they were crushed and examined. Each was found to contain 5 - 10,

45 spined cysts.

5.) A medium sized Pseudosuccinea columella was exposed for 4 days to E.C. 1 emerging from a large P. occidentalis. Upon examination the exposed snail was found to contain approximately 150, 45-spined cysts.

Experiments with Vertebrates:

The vertebrates exposed to attach by E.C. 1 included tadpoles, cat fish and goldfish. No metacercariae were found in any of these exposed animals.

Metacercarial Infection Experiments:

Pigeon Experiments:

Two pigeons were used in these experiments. One bird was fed cysts from an experimentally infected P. occidentalis, and the other was fed cysts, on two separate occasions, from naturally infected snails.

Pigeon Experiment No. 1.

An adult pigeon was kept in captivity for a period of three weeks. During this time several faecal examinations were made to determine the presence or absence of trematodes in the alimentary canal. No eggs were detected in these examinations and the pigeon was regarded as uninfected. The bird was then fed a crushed medium-sized P. occidentalis containing at least 5 experimentally infected

45-spined metacercaria. Feeding was accomplished by means of a medicine dropper. The pigeon was kept for a period of two weeks on a diet of grain and poultry starter mash. Faecal examinations were then commenced and kept up bi-weekly for a period of 6 weeks. The pigeon was sacrificed on the 45th day following infection and its alimentary tract thoroughly examined. No trematodes were found.

Pigeon Experiment No. 2.

Part (a) An adult pigeon was kept for three weeks in captivity, as in Experiment No. 1., prior to infective feeding. During this time, repeated faecal examinations failed to disclose the presence of trematodes in the digestive tract, thus the animal was considered to be uninfected. The pigeon was then fed 10 - 15 cysts removed from a naturally infected P. cf traski. These cysts were identical, as far as could be determined, with the 45-spined metacercariae of E.C. 1. Faecal examinations were made every 2 - 3 days for two weeks but failed to reveal the presence of trematode eggs in the gut. The diet of the pigeon throughout the experiment was grain and poultry starter mash. After a period of 2 weeks had elapsed during which no trematode ova could be detected in the faeces, the infection was assumed to be unsuccessful and the pigeon again regarded as " uninfected."

Part (b) The pigeon from part (a) was fed a "clump" of 150 or more cysts removed from a naturally infected P. occidentalis. These cysts included an unknown number of 45-spined cysts and

possibly a number of 31-spined cysts. The pigeon was kept for a period of 21 days on grain and starter mash. The pigeon was sacrificed on the 32nd day and its alimentary canal thoroughly examined. Eight small mature echinostomes were found in the upper small intestine. These trematodes possessed 45 collar spines, and were tentatively identified as Echinoparyphium recurvatum. A description of this fluke is given in a following section.

Duckling Experiment:

A small number of 37 and 45-spined cysts removed from a naturally infected Pseudosuccinea columella were fed via medicine dropper to a small 3 day old duckling. The duckling was incubator raised and thus considered to be uninfected. It was fed a diet of starter mash. Nine days following the infective feeding, the duckling was inadvertently destroyed by a predator. An examination of the section of the digestive tract which remained revealed no immature trematodes.

Guinea Pig Experiment:

180 or more E.C. 1 cysts removed from a naturally infected Physa occidentalis were fed via medicine dropper to an adult guinea pig. The cysts were teased from the digestive gland of the snail, 6 hours prior to the infective feeding and kept in water.

Movement of the metacercaria within the cyst was observed at the time of feeding. The animal was sacrificed after 43 days and its digestive tract thoroughly examined. No trematodes were

found.

Rat Experiments:

Rat Experiment No. 1.

20 cysts identical with those of E.C. 1 were removed from a naturally infected Pseudosuccinea columella and fed to an adult albino rat with a medicine dropper. Faecal examinations prior to feeding of cysts showed the rat to be uninfected by trematodes. It was kept on a diet of milk and starter mash for 43 days after infective feeding. During this period, no trematodes were found in the faeces. On the 44th day the rat was sacrificed and its digestive tract thoroughly examined. No trematodes were found.

Rat Experiment No. 2.

8 or 9 forty-five spined cysts taken from a Physa sp were fed via drinking milk to a medium-sized albino rat. Faecal examinations were made two and three times a week for a period of 5 weeks after infective feeding. No trematodes were found in the intestine or viscera when the rat was killed and examined.

Rat Experiment No. 3.

15 forty-five spined cysts were fed to an adult albino rat in milk. The rat was kept for 35 days on a diet of grain pellets, during which time repeated faecal examinations failed to reveal trematode ova in the faeces. The rat was then fed a clump of

150 or more cysts, containing 37 as well as 45-spined metacercariae. The rat was sacrificed 31 days after this feeding but no trematodes were found in its intestine.

The Identification of the Adult Echinostome (Plate VII).

The echinostome produced in the pigeon from an infective feeding of cysts of E.C. 1 is, as far as can be determined, identical with Echinoparyphium recurvatum (Linstow 1873) Lühe 1909. The most obvious features in common with the adult, cercaria and metacercaria are the number and arrangement of the collar spines. (Fig. 5). The following is a description of the adult 45-spined echinostome obtained from the pigeon which was fed 45-spined metacercaria identical with E.C. 1.

The worm is short and stout and possesses an obvious head collar bearing 45 rostellar spines (Fig. 5). The anterior 1/3 of the body is bent ventrally almost at right angles to the main axis. as seen in Fig. 1. The dorsal surface at this point forms a distinct shoulder. Posterior to the collar there is a short narrow neck region. A distinctive feature of the anterior portion of the worm is the ventral folding of the edges which results in a ventral groove extending from the pharynx approximately to the ventral sucker. Both body suckers are prominent, the ventral being slightly larger than the oral, and located in the anterior one third of the worm.

The 45 collar spines appear to have a definite arrangement.

EXPLANATION OF PLATE VII.

Echinoparyphium recurvatum.

Fig. 1 -- Ventral view of adult, showing general morphology and relative body proportions. About x 40.

Fig. 2 -- Freehand sketch of a portion of the excretory duct showing the direction of activity of vibratile patches along its length.

Fig. 3-- Outline diagram of egg. x 30.

Fig. 4 -- Lateral view of anterior end, showing position of oral sucker and paired arrangement of the collar spines.

Fig. 5 -- Ventral view of anterior end, showing the arrangement of collar spines.

Fig. 6 -- Lateral collar spine. About x 1000.

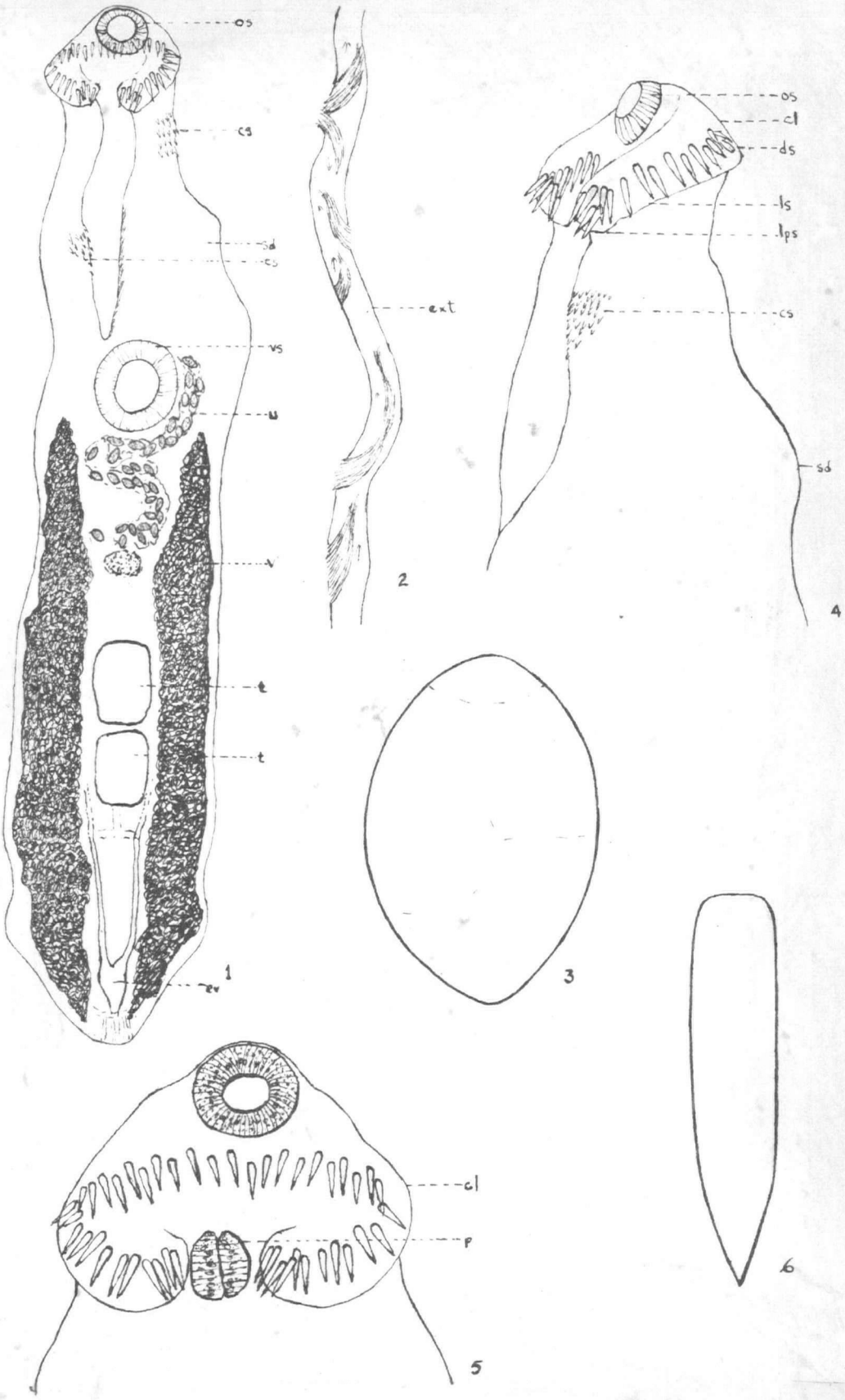


PLATE VII.

There are three pairs of spines on each lappet, each somewhat spindle-shaped. Two pair are more anterior than the third group, and all are larger than the lateral and dorsal spines. Bordering each lappet group is a single spine and then begin the paired dorsal series. The dorsals form two rows, one the oral, the other the aboral. These spines are spiked or wedge-shaped in comparison with the slightly spindle-shaped lappet spines. Their average length is .057 mm. The collar spines of this worm are brittle, and resist dislodgement to a fair degree. When the worms were shaken in saline and straightened out with a fine hair brush, some of the ends of the collar spines were found to be broken off.

Cuticular spination, partially represented in Figs. 1 and 2, extends from the anterior collar to the level of the ventral sucker at least, on both the dorsal and ventral surfaces. These spines are in rows about 0.028 mm. apart. In the neck region the row arrangement of the cuticular spines is somewhat lost, due, perhaps, to contraction of the cuticle. The body spines are 0.023 mm. long. They can be seen well in unstained worms under No. 1 coverslips.

Of the internal anatomy, the testes are most prominent. They appear as two oblong structures in the mid-line, just posterior to the centre of the worm. Anterior to the testes, the uterus coils several times before reaching the level of the ventral sucker. In the mature worm, eggs in the uterus can be readily seen as very yellow, ovate bodies within the folds of the uterus. The eggs are operculate. Vitellariae extend laterally from the ventral sucker to

almost the posterior tip of the body.

Measurements (Average).

Body Length	4.18 mm.
Body Width	0.627 mm.
Diameter Anterior sucker	0.095 mm.
Diameter Ventral sucker	0.590 mm.
Diameter of Collar	0.226 mm.

Identification:

The most obvious features in common with the adult E.C. 1 echinostome and Echinoparyphium recurvatum are the number of collar spines and the body dimensions. The arrangement of the collar spines is somewhat different in the two worms, for Echinoparyphium recurvatum has four spines on each lappet, in two pairs, whereas the adult form E.C. 1 has six lappet spines obviously paired. The remainder of the collar spines in both worms are arranged in two dorsal rows, with those in the oral row smaller than those in the aboral.

The following is a comparison of dimensions for the adult E.C. 1 and Echinoparyphium recurvatum (from Dawes, 1946).

	Adult from E.C. 1.	Echinoparyphium recurvatum
Body Length	4.18 mm.	4.5 mm.
Body Width	0.065 mm.	0.05 - 0.06 mm.
No. Collar Spines	45	45
Lappet Spines	12	8

Dorsal Spines	33	37
Egg Dimensions	0.102 - 0.065 mm.	0.108 - 0.081 mm.

Summary of Life Cycle Studies for E.C. 1:

E.C. 1 was experimentally infected in three species of snails. Physa occidentalis, P. cf traski, and Pseudosuccinea columella. An examination of naturally infected snails revealed that P. cf traskii, Pseudosuccinea columella and Lymnaea proxima, contained ~~forty-five~~ 45 spined cysts identical with those from E.C. 1. Tadpoles, catfish and goldfish were unsuccessfully exposed to E.C. 1. Rats, guinea pigs and ducklings were found to be unsuccessful as experimental adult hosts for this cercaria. E.C. 1 reached the adult stage in an experimentally infected pigeon. The cysts fed to this pigeon came from naturally infected snails collected at Burnaby Lake. The cysts were morphologically identical with those of E.C. 1. The pigeon was proven to be uninfected by trematodes prior to the infective feeding experiment. On the basis of collar spine number and arrangement, body dimensions and other morphological features, the adult worm has been identified as Echinoparyphium recurvatum.

Echinostome Cercaria No. 2.

Cercarial Infection Experiments:

Experiments with Mollusca:

Several species of uninfected, laboratory-raised snails were exposed, as described in experiments with E.C. 1, to attack by

E.C. 2. Of these, Physa occidentalis and P. cf traskii were unsuccessfully infected. Pseudosuccinea columella and Gyraulus vermicularis were unsuccessfully exposed to E.C. 2, but since the number of cercariae used was small, the failure to infect can not be regarded as evidence of immunity.

Experiments with Vertebrates:

Two tadpoles taken from Burnaby Lake were exposed to attack by E.C. 2. These animals were examined three weeks later and were found to contain several echinostome cysts. The exact number of collar spines and flame cell formula in these cysts was difficult to determine, however the size agrees very favourably with the size of the cysts experimentally infected from E.C. 2. It must be noted that since the tadpoles cannot be regarded as uninfected, the success of the infection experiments is in doubt. The indication is, though, that the tadpoles of Burnaby Lake do act as second intermediate hosts to E.C. 2.

Metacercarial Infection Experiments.

As in the metacercarial infection experiments described for E.C. 1, several animals were selected as possible adult hosts for E.C. 2 and fed cysts either via medicine dropper or in drinking milk. All these experiments, using the cysts identified as the encysted stage of E.C. 2, were unsuccessful. This undoubtedly was due in some instances to an insufficient number of cysts being used, and in others, to what may have been natural immunity of the host.

All these experiments were carried out as described in the experiments for E.C. 1. For the sake of brevity it may be stated here that pigeons, ducklings, guinea pigs and albino rats were unable to be infected with the metacercariae of E.C. 2.

Summary of Life Cycle Studies with E.C. 2.

1st Intermediate Hosts:

None of the miracidial infection experiments involving E.C. 2 were successfuly However, three species of snails from Burnaby Lake have been found to be naturally infected with E.C. 2. These snails are -- Pseudosuccinea columella, Physa occidentalis and Helisoma trivolvis.

2nd Intermediate Hosts:

Physa occidentalis and P. cf traski have been found to be 2nd Intermediate hosts for E.C. 2. Pseudosuccinea columella, P cf traskii and P. occidentalis have been found to be naturally infected second Intermediate hosts for E.C. 2.

Adult Hosts:

None of the experimental adult hosts were successfully infected. The negative results are possibly due to improper diet, or more probably, to the fact that too few number of cysts were fed to to the animals.

EXPLANATION OF PLATE VIII.

Echinostoma revolutum.

Fig. 1 -- Sketch of adult worm from hand lens showing some of the structural details. x 10.

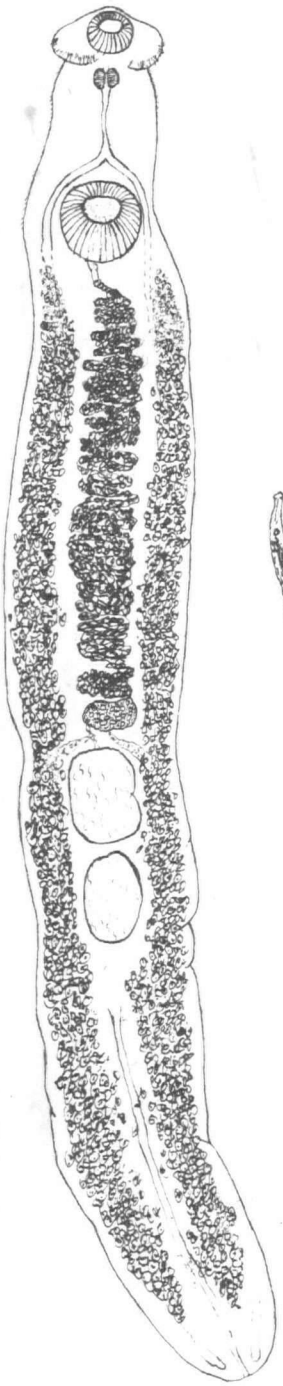
Fig. 2 -- Ventral view of anterior end showing arrangement of collar spines. x 200.

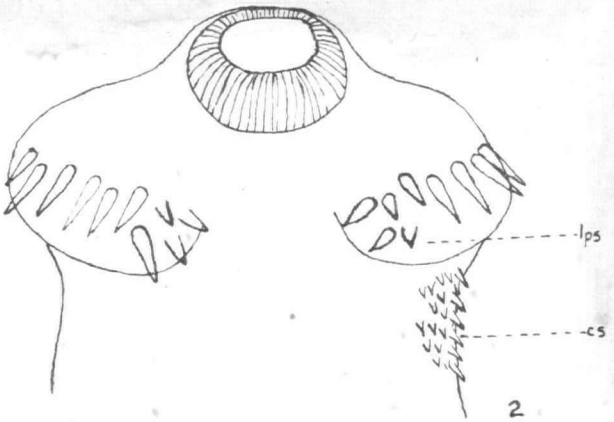
Fig. 3 -- Adult worm, actual size.

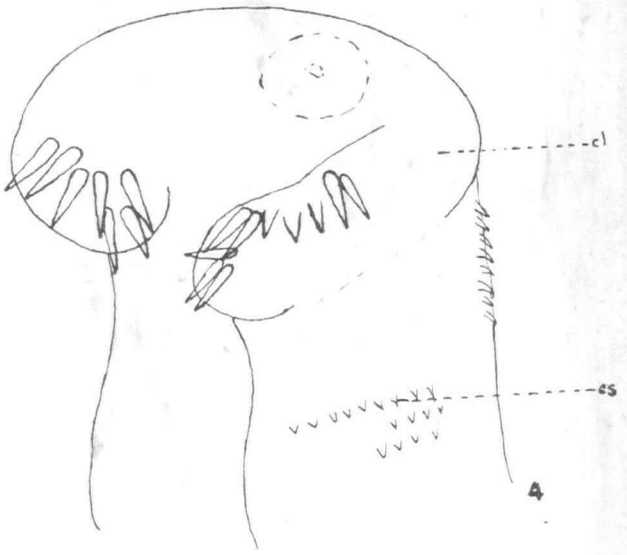
Fig. 4 -- Lateral view of anterior end showing lappet spines. x 200.

Fig. 5 -- Camera lucida drawing (low power) of developing miracidium.

Fig. 6 -- Mature miracidium within egg. Camera lucida drawing, high power.







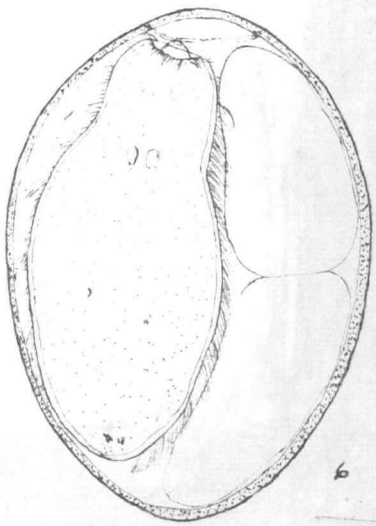


PLATE VIII.

Identification of Adult Stage of E.C. 2:

On morphological grounds at least, the adult stage of E.C.2 is undoubtedly Echinostoma revolutum found in the muskrat of Burnaby Lake. As previously stated (Page 35), E.C. 2 is decidedly similar to Cercaria echinostomum revolutum as described by Beaver (1937). On these grounds the linkage with E. revolutum appears conclusive, for as Beaver (1937) concludes in his monumental work on this trematode " the Cercaria resembles the adult so closely in cephalic spination that positive identifications can be made from this character alone."

Xiphidiocercaria No. 1.

Cercarial Infection Experiments:

Experiments with Mollusca.

1.) Two large uninfected P. occidentalis were exposed for 24 hours to approximately fifty X.C. 1., which emerged from a large Lymnaea palustris. The snails were crushed and examined 9 days later but did not contain metacercariae.

2.) A large laboratory-bred uninfected Physa cf traskii was placed in an aquarium containing a L. proxima shedding large numbers of X.C. 1. The uninfected snail remained exposed to the cercariae for 10 days, after which time it was removed from its shell and thoroughly examined under low power magnification. No encysted cercariae were found.

3.) Two uninfected P. occidentalis and one uninfected Pseudosuccinea columella were exposed to large numbers of X.C. 1 for 2 days. These snails were then kept in an aquarium for 6 weeks, following which they were teased and examined. No cysts were found in any of the snails.

4.) An uninfected Menetus cooperi was exposed to 15 X.C. 1 in a syracuse dish. After 2 days the snail was removed to an aquarium and kept for 3 weeks. No cysts were found when the snail was crushed and examined.

Experiments with Catfish--

On two instances, two small catfish taken from Burnaby Lake were placed for 48 hours in an aquarium with a large L. palustris from which large numbers of X.C. 1 were emerging daily. One month after exposure these fish were killed and thoroughly examined externally and internally. No metacercariae were found.

Experiments with Goldfish:

Approximately 300 X.C. 1 were added for three successive days to an aquarium containing a medium sized goldgish. Two weeks later the fish were killed and a thorough examination made. No cysts were found.

Experiments with Gammarus sp :

All gammarids used in these experiments were considered free of infection by trematodes after an examination of 30 specimens

taken from a lily pond showed no cysts were present.

1.) Three gammarids werepplaced in an aquarium with a large L. palustris shedding large numbers of X.C. 1. On the second day these amphipods were found dead. An examination revealed no metacercariae.

2.) Four additional gammarids were placed intthe same aquarium as No. 1 for two days. An examination of the crushed bodies of the gammarids on the third day showed that no cysts were present.

3.) Five gammarids were placed in a large number of X.C. 1, being shed from a L. proxima. Three gammarids were dead on the second day of exposure. No cysts were found after a thorough examination of these animals under low power magnification. The remaining two gammarids died the following day and in each, four stylet cysts were found. As far as could be determined, these cysts were identical to the cysts found in snails infected with X.C. 1. One cyst was found in the flesh of a basal appendage segment. All other cysts, appeared to be free in the teased remains of the gammarid.

4.) Three gammarids were placed in an aquarium for three days, with large numbers of X.C. 1 shed from the L. proxima used in No. 3. On examination two days later each gammarid was found to contain many stylet cysts (35, 40 and 80). Most of these cysts were located free in the teased gammarids, but many were observed within the appendage segments.

5.) Two gammarids were added to water containing a fair number of X.C. 1. One was examined two days later and contained

a single cyst. The second gammarid yielded nine cysts, none of which were in the flesh of the appendages. All metacercaria showed movement.

Metacercarial Infection Experiments:

Experiments with Goldfish:

1.) Eight stylet cercaria taken from an experimentally infected snail of the above Cercarial Infection Experiments were fed, via a medicine dropper, to a large goldfish. On the 14th day following infective feeding the fish was killed and examined. No trematodes were found in intestines or viscera.

2.) Fifteen to twenty stylet cysts taken from experimentally infected gammarids were fed to a small goldfish. Ten days after this feeding the goldfish was killed and examined. No trematodes were found.

Duckling Experiments:

Nine stylet cercariae were fed via medicine dropper to a duckling two days old. Thirteen days after infective feeding, the duckling was sacrificed and a thorough examination made of the viscera. No adult worms were discovered.

Experiments with Rats:

1.) Ten cysts taken from an experimentally infected Gammarus sp. were fed to an adult albino rat. Six weeks after feeding this rat was sacrificed. No trematodes were found in an

examination of the intestines.

2.) Nine stylet cysts of C.X. 1 were fed to a small albino rat. On the 34th day after feeding, the rat was killed and examined. No adult trematodes were found.

Experiments with Guinea Pigs:

Four X.C. 1 cysts taken from an experimentally infected gammarid were fed to a guinea pig, via medicine dropper. The animal was killed and examined 34 days after feeding. No trematodes were found in its gut.

Summary of Life Cycle Studies with X.C. 1.

It would appear that the 2nd Intermediate hosts of X.C. 1 are specific. Experimental encystment of X.C. 1 was unsuccessful in L. palustris, L. proxima, Menetus cooperi, goldfish and catfish.

A Gammarus species was found to be an experimental 2nd Intermediate host for X.C. 1. No successful infections were made with X.C. 1 shed from L. palustris. This may be the result of insufficient numbers of cercariae used, the premature death of the gammarids, or the fact that the cercariae emerging from the L. palustris were not X.C. 1. Encystment in gammarids appears to take place in the body as well as in the fleshy portion of the appendages. No encystment was observed on the substratum. Unsuccessful attempts were made to infect goldfish, ducklings, rats and guinea pigs with the cysts of X.C. 1.

Xiphidiocercaria No. 2:

Life cycle experiments were confined to the exposure of a number of ininfected gammarus sp to attack by X.C. 2. The experiments were performed as described for X.C. 1. On two occasions gammarids exposed to large numbers of X.C. 2 for two days contained 40 and 80 stylet cysts. These metacercaria were not studied in any detail, but appeared to show much resemblance to those of X.C.1

Furcocercous Cercaria No. 1:

Several experiments were designed to determine whether or not F.C. # develops as a tetracotyle in a second intermediate host or is directly infective to the adult host, either orally or cutaneously. All snails used as experimental hosts were laboratory-raised uninfected specimens. The ducklings used were incubator hatched. Gammarids, as in previously described experiments were taken from a lily pond and considered to be uninfected. The tadpoles and catfish were taken from Burnaby Lake, and could not be considered as uninfected.

The animals were exposed to F.C. 1 by placing them in 500 cc. of water in an aquarium containing large numbers of the cercariae.

Results of Experiments:

Experiments with Mollusca.

Physa occidentalis, P. cf traskii, Pseudosuccinea colum-

ella were unsuccessfully exposed to F.C. 1.

Experiments with Gammarus sp. -- unsuccessful.

Experiments with Ducklings -- unsuccessful.

1.) The feet and legs of a four day old duckling which had just died were immersed for three hours in water containing a great number of F.C. 1. The tissue of the foot was then examined under low power for the presence of penetrating or penetrated cercariae. No cercariae were found. The tissue of the foot was then teased and examined. No cercariae or tetracotyles were found.

2.) A duckling was fed approximately 50 F.C. 1 via drinking water for a period of one week. This animal was prematurely and inadvertently destroyed by a predator three days after oral feeding ceased. An examination could not be made before much of the blood had congealed, making perfusion experiments for the presence of blood parasites impossible. The animal was exsaginated by decapitation and as much blood as possible was thoroughly examined. No flukes were found.

Goldfish Experiments -- unsuccessful.

Summary of Life Cycle Studies with F.C. 1.

Physa occidentalis, P. cf traskii and Pseudosuccinea columella were unsuccessfully exposed to infection by X.C. 1. On one occasion, a Pseudosuccinea columella collected at Burnaby Lake was found to contain eight large cyst-like objects, which showed much resemblance to the tetracotyles of strigeid cercariae. There

is, then, evidence that the Burnaby Lake snails do act as natural hosts for tetracotyles of strigeid cercariae, to which group E.C. 1 belongs.

Tadpoles and catfish also taken from Burnaby Lake contained strigeid-like tetracotyls. Thus, although it has not been supported by experimental evidence from this investigation, there are indications that the snails, tadpoles and catfish of Burnaby Lake harbour tetracotyles of strigeid trematodes, one of which might be F.C. 1.

Gammarus sp, ducklings and goldfish apparently do not serve as hosts for F.C. 1.

Furcocercous Cercaria No. 2.

Similar experiments as those for F.C. 1 were carried out using F.C. 2. The results of these experiments are presented here in brief form.

Experiments with Molluscs:

Three Physa sp were exposed to F.C. 2 for periods of one, three, and 11 days respectively. No larval trematodes was found when the snails were crushed and examined.

Experiments with Tadpoles:

A tadpole taken from Burnaby Lake was exposed to F.C. 2 shed from L. palustris for a period of 22 hours. Two weeks later the tadpole was teased and examined. No larval trematodes were found.

Experiments with Goldfish:

A large goldfish was exposed to F.C. 2 for three days, after which it was crushed and examined under low power. No larval stages were found.

Experiments with Ducklings:

A two day old duckling was fed 30 or 40 cercariae in 200 cc. of drinking water daily for one week. On the eleventh day the animal was examined for flukes in the blood and viscera. No larval stages were found.

Summary of Life Cycle Studies with F.C. 2:

Snails, tadpoles, goldfish and ducklings do not serve as experimental hosts for F.C. 2. The number of experiments undertaken prohibit these negative results from being regarded as conclusive.

SCHISTOSOME DERMATITIS EXPERIMENTS.

INTRODUCTION

Schistosome dermatitis is a skin infection in humans caused by the penetration of the cercariae of avian trematodes. It is common in north and central United States and Canada, and is popularly called "swimmer's itch," or " swimmer's rash." In many instances a severe dermatitis results from exposure to these cercariae. However laboratory experiments have shown that the penetration is otherwise harmless, proceeding only a short distance before the cercariae die.

Cort (1928) was the first worker in America to demonstrate experimentally that non-human schistosome cercariae could penetrate human skin and cause papular eruptions. He found four furcocercous cercariae responsible for this dermatitis in Michigan, all belonging to the C. elvae group. Several years later, Cort (1936 a) (1936 b) presented a comprehensive review of the work up to date on schistosome dermatitis in America. This was followed by several pertinent papers by Cort and his associates Cort (1936 b), Talbot (1936), Cort and Talbot (1936) and Brackett (1940) (1941). The most recent reports on the subject are by MacMullen and Meaver (1945), Macfarlane and Macy (1946), Olivier (1947), and Hunter et al (1949).

Although these worms are harmless to humans, the possibil-

ity that their intermediate hosts might be the intermediate hosts to human schistosomes also has interested at least one worker. Stunkard (1946) tested the possibility of the American species of snails acting as hosts to the cercariae of human schistosome flukes commonly found in Asia and tropical latitudes. He exposed representative specimens of the more common and readily available snails from eastern United States to the miracidia of human schistosomes. Among the snails used were L. palustris, Pseudosuccinea columella, Physa spp, H. trivolvis and H. anceps, which are to be found at Burnaby Lake. No positive results were obtained in any of these experiments. Stunkard states that " the failure to obtain cercariae is not surprising since it is difficult to consummate well known trematode life cycles under laboratory conditions, even when natural intermediate hosts are employed." He suggests that these negative results are far from being conclusive and may lead to a false sense of security about local species of snails beingimmune to human schistosomes.

Until McLeod's (1934) work appeared all cercariae causing schistosome dermatitis were apparently of the apharyngeal brevifurcate distome cercariae of the C. elvae group, that is, typical members of the Schistosomatidae. McLeod discovered two species, besides that of C. elvae Miller, 1923, from Clear Lake, Manitoba, which produced swimmer's itch. These cercariae are members of the Strigeidae (pharyngeal longifurcate distome cercariae) and named C. Wardlei McLeod, 1934 and C. bajkovi McLeod, 1934.

Swales (1936) working in the same Clear Lake, Manitoba, found C. elvae Miller, 1923 and a Cercaria sp in Stagnicola emarginata Canadensis (Sowerby) causing dermatitis, along with five species of Strigeid cercariae, " none of which indicated any power of penetration onto the human skin."

Methods:

Two experimental human hosts were used in the experiments with F.C. 1 and F.C. 2, one a student-colleague (Host 1), the other the author (Host 2). Two procedures were adopted, one called the Drop Method and the other the Immersion Method.

1. Drop Method:

This method consisted of placing a drop of water containing one or more cercariae on the flexor surface of the forearm. This relatively hairless portion of the arm allows for more sensitive reactions than other skin surfaces. The drop of water was allowed to stand for a certain period and then shaken off and air-dried. Each drop of water was ringed with indelible ink.

In removing the cercariae from the aquaria, a medicine dropper was utilized. As little water as possible was taken up so that when it was expelled, the drops of water formed on the skin surface would not be so large as to easily roll off. Just prior to expelling the drops on the arm, the dropper was held before a beam

of light in order to check the number of cercariae contained and to make certain that none had become attached to the sides. Both the furcocercariae experimented with here were found to become readily and stubbornly attached to the inside of the medicine dropper. It was thus found advisable to make the time between sucking up the cercariae to placing the drop of water on the arm as short as possible.

Several of these drops of water containing active cercariae were placed along the length of the arm, each one ringed with indelible ink. The arm was held steady and the drops left undisturbed for periods up to an hour in length, then the water was shaken off and the infected areas allowed to air-dry. Notes were made on any subsequent sensations experienced and skin reactions observed.

Drops of water containing no cercariae were placed on the flexor surface of the other arm in order that a comparison could be made of cercarial and non-cercarial water.

2. Immersion Method:

The second method consisted of placing the elbow or flexor surface of the wrist in a stacking dish, or a petri dish, containing a large number of the active cercariae. The corresponding surface of the other arm was immersed in an equal quantity of water with no cercariae.

The skin surface was kept in the water for a period of five minutes, then removed and allowed to dry. This was then followed

by separate immersions of 10 and 25 minutes, each followed by a drying period. In this manner, the periodic immersion and drying of a person while swimming was simulated, representing the conditions under which actual or natural, cercarial attack is made.

Results and Discussion of Infection:

Furcocercaria No. 1.

Several attempts, using both the above methods, were made to produce dermatitis using this cercaria. None of the experiments on either host was successful. It seems very likely, to this worker at least, that F.C. 1 is not a dermatitis-producing schistosome.

Furcocercaria No. 2.

Drop Method:

Case No. 1 (Colleague).

Three drops of water containing one or more cercariae each were placed on the flexor surface of the right arm. Several drops without cercariae were placed on the left arm. A slight itching sensation and intermittent sharp pricks commenced within 15 minutes. This "prickly heat feeling " stopped within an hour and a half. One hour after exposure, three small erythematous spots were observed. By seven hours these red areas had increased to 2 mm. in diameter. No further itching was experienced. At twenty hours the erythema had become more diffuse, extending now to 3 mm. in diameter. At this stage the spots were definite papules with

minute white heads. Erythema decreased after 24 hours. By 48 hours all trace of the infection had disappeared. No such reaction was observed in the left arm.

Case No. 2. (Colleague).

Seven drops of water, each containing one or two cercariae, were placed on the flexor surface of the right forearm. Approximately 15 minutes later a prickling sensation was experienced. Itching became intermittently more intense but did not result in pruritus. After half an hour, the drops were shaken off and allowed to air-dry. At this time seven small erythematous areas were observed. These were sharply defined areas 1mm. in diameter. The slight itching ceased after about an hour and was not experienced again. Two hours after exposure, the erythema had increased to 1.5 mm. in diameter. At 22 hours, pink papules appeared, and at 28 hours these papules measured three mm. in diameter and about 1.5 mm. high. By the second day the papules had become pale. On the third day all skin reaction disappeared. No reaction was experienced in the left arm.

An interesting reaction in this case appeared three weeks later. At spots on the arm, which, as far as could be determined, were identical with the originally papulated areas, new small red papules appeared. These raised areas developed to 1.5 mm. in diameter. No itching or tingling sensations were experienced. These new areas remained obvious for seven days.

Case No. 3. (Author).

Seven drops of water, each containing one or more F.C. 2 were placed on the flexor surface of the left arm. Several control drops were placed on the right arm. The drops were left on for ten minutes and then shaken off. No reactions whatsoever were experienced.

Case No. 4. (Author).

Seven drops of water containing one or more F.C. 2 were placed on the flexor surface of the left arm and allowed to remain for thirty-five minutes. The drops were then shaken off and allowed to air-dry. A strong pruritus commenced in two or three of the exposed areas after ten minutes. In thirty minutes, four erythematous spots were observed in as many ringed areas. Intense itching in and around these areas was experienced at this time. Itching decreased after an hour but the number of erythematous areas had increased to six, each approximately 2mm. in diameter. No further pruritis developed. At 20 hours following exposure, the spots had become papular. At 24 hours the papules had become very prominent and had begun to disappear at 30 hours. On the second day, erythema was less localized, each area now extending 4 or 5 mm. in diameter. Papules were present but not obvious; a slight pain was experienced when they were touched, otherwise no sensations were felt. Erythema remained for about two weeks as small unraised areas on the skin, about 1.5 mm. in diameter.

Case No. 5 (Author).

This experiment utilized the same arm surface as in Case No. 4 and was started three days after the drops in Case No. 4 were placed on the arm. Eight additional drops of water containing one or more cercariae were placed near the previously infected areas. Within ten minutes intense itching began in one area very close to a previously papulated area. Five separate erythematous spots developed in this new area. Erythematous macules appeared in thirty minutes. At 35 minutes itching was almost unbearable. The drops were shaken off at 45 minutes, at which time three red spots were present. Itching continued for one hour. By 1 1/2 hours, fifteen small red areas could be seen. One area of five spots developed into a common raised welt about 2 cm. in diameter.

On the 3rd day, some of the papules were purposely irritated. Within twenty-four hours these had become very slightly pustular, with a small scab on the surface. These minute pustules remained obvious for a week, during which time there was a gradual disappearance of the erythema. The small scars remained evident for six weeks.

Immersion Method:

Of several attempts to produce dermatitis using this method only once was there any thing more than slight itching experienced by either host. This itching was only slightly more intense than the " tingling " sensation induced in the areas immersed in non- cercarial water.

Summary of Results of Laboratory Exposure to F.C. 2.:

Colleague: Slight itching at 15 minutes.
No intense itching or pruritus.
Itching ceased after 1 1/2 hours.
Erythematous macules at 30 min., decreased after 28 hours; maximus papulation at 24 hours.
No pustule development.
Skin reaction disappeared in 3 days.

Author: (In general reactions more acute than in colleague).
Extreme itching and pruritis at 10 min., continued for 1 hour.
Erythema macules appeared at 30 min.
Slight itching after 1 1/2 hours.
Papules developed to maximum by 24 hours, remaining for three days.
Erythema lasting two weeks.
Welts, pustules and intense erythema if irritated.

Conclusions:

1.) F.C. 2 is capable of producing Schistosome dermatitis.
2.) There appears to be a difference in host reaction to the penetration of F.C. 2.
3.) The intensity of the reaction is increased in proportion to the number of infecting cercariae per unit area.
4.) Pustulation and scar tissue result if the papules are

irritated.

5.) The strigeid cercaria F.C. 1 is apparently incapable of producing a dermatitis in humans.

Ecological Relations of Larval Trematodes of Burnaby Lake:

In this discussion of the degree of larval trematode infestation in B urnaby Lake snails it is considered to be of value to preface the findings with some remarks on the collection areas themselves, and also upon the snail populations found. Since the snails that were examined in the laboratory were taken from definite areas of Burnaby Lake, it was hoped that in addition to determining the degree and species of infection, that the investigation might disclose possible area differences which exist in the snail fauna and their larval trematodes.

Collection Areas at Burnaby Lake (Plate X).

Three shore areas of Burnaby Lake from which snail collections were made were selected on the basis of ecological differences and accessibility. Although the lake is small, and thus the collection areas not far removed from each other, it was felt that the areas chosen represented sufficient differences in habitat to warrant observations being made for the possibility of fauna differences.

In general, Burnaby Lake area is a typical marsh and bog area, with marsh and mud shores bordering the lake proper. It is a Provincial Game Reserve and as such harbours a large muskrat population, large numbers of migratory water fowl at various times of the year, and many other vertebrates, resident and migratory, which are possible adult trematode hosts.

1.) Laut Park Area.

This area is on the north side of Burnaby Lake, at the base of Burnaby Mountain. It is reached by means of the Laut Park Road which follows the course of a small clear water stream entering the lake on this north shore.

The bulk of the snails taken from this area were collected from approximately two hundred feet of shore line to the west of the mouth of the Laut Park stream. The shore here varies from a sand and gravel district near the stream mouth to a muddy shore with much decaying vegetation at the western extremity. Most of the snails were collected from the surface of the mud and shallow shore pools and offshore marshes.

2.) Still Creek Area.

Collecting in this area was confined to the region around the mouth of Still Creek, at the western-most end of Burnaby Lake, and along the creek banks for a distance of five hundred feet from the lake. The banks of this creek are of hard clay or mud and bordered by cattail (Typha latifolia), rushes (Juncus sp) and bent grass (agrostis). Most of the snails were collected from the leaves of the rushes and grasses bent over and submerged at the creek's edge. Some snails were also collected from the mud surface at the mouth of Still Creek.

3.) Deer Creek:Area:

3.) Deer Creek Area:

Collections in this area were made in the vicinity of the mouth of the stream which connects Deer Lake with Burnaby Lake. This is the largest of the shore areas from which collections were made. Snails were taken from the shore of the lake extending both east and west of Deer Creek mouth, but principally in the eastward direction for a distance of approximately 200 feet. This area is typical fresh water bag. Rushes and bent grass border the lake and the banks of Deer Creek. Most of the snails were collected from the surface of the moist mud and decaying vegetation, and in shallow bog pools of the offshore regions.

The limits of all these areas are shown in Plate X.

Snails Found at Burnaby Lake (See Plate IX).

The following is a list of the snail species collected at Burnaby Lake as identified by Dr. Henry van der Schalie, of the University of Michigan.

Pseudosuccinea columella (Say)

Lymnaea proxima rowelli (Tyron)

Lymnaea palustris (Muller)

Helisoma trivolvis hornii (Tyron)

Helisoma anceps (Menke) -- formerly called antrosum (Conrad).

Menetus cooperi (F.C. Baker) -- formerly called planulatus (Cooper).

EXPLANATION OF PLATE IX.

Snail Species collected at Burnaby Lake.

1. Pseudosuccinea columella (Say)
2. Lymnaea palustris (Muller)
3. Lymnaea proxima rowelli (Tyron)
4. Helisoma trivolvis hornii (Tyron)
5. Helisoma anceps (Monke)
6. Menetus cooperi (F.C. Baker)
7. Gyraulus vermicularis (Gould)
8. Physa occidentalis (Tyron)
9. Physa cf traskii (Lea)

1

2

3

4

5

6

7

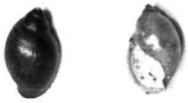


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PLATE IX.

Gyraulus vermicularis (Gould)

Physa occidentalis (Tyron)

Physa cf. traskii (Lea) -- may be only a form of Physa occidentalis (Tyron).

Ferrissia caurina (Cooper).

Collection Areas and their Snail Populations:

Tables No. I, II, and III give data on area differences at Burnaby Lake, both of the snail host populations and their larval trematode infections. From these tables the following generalizations have been taken.

1.) Laut Park Area:

In the number of snails collected and examined, this area heads the other two. However, most of the snails collected were of one species, Pseudosuccinea columella, so that the number examined gives no indication of the richness of the molluscan fauna.

Pseudosuccinea columella were mostly found on the mud shore when the water level of the lake was low, as well as in small water-filled depressions, and on moist duckweed and decaying vegetation. These snails were found throughout the year. During December, January and February, this species was the only one found in any quantity in the area. In the ice and snow free sections of the shore Pseudosuccinea columella was usually found on the under-surface of objects lying in the mud.

The species found in next greatest abundance were the two Physas -- Physa occidentalis and P. cf traskii. A total of 102 of these two species were collected and examined as compared with about 200 Pseudosuccinea columella. Lymnaea proxima and L. palustris were found in small numbers -- only three and one respectively being taken from the area. One Helisoma trivolvis was collected. Very few of the inconspicuous Gyraulus vermicularis were taken, although they appeared to be present in large numbers on the mud surface here.

No Helisoma anceps or Ferrissia caurina were collected at Laut Park. However, the collections were in no way exhaustive and there appeared no reason why this species cannot be found in this habitat.

2. Still Creek:

During the warm late spring and summer months, large quantities of snails were found in this area. The majority of specimens were collected from submerged rushes at the edte of the creek. Many were found floating at the water surface near the creek banks. Great quantities of the gelatinous egg masses are attached to the submerged vegetation. By late August, the number of snails to be found in this area fell off sharply. No snails in any appreciable quantity could be found in this area from late October to April. Physa spp. and Pseudosuccinea columella were in greatest concentration in this area.

Although very few snails from this area were examined for

larval trematodes, it was found that during the summer months it harboured more specimens than the other two areas.

3.) Deer Creek:

This area was found to have the most bountiful molluscan fauna. In contrast to Laut Park Area, Deer Creek was without Pseudosuccinea columella during winter months. Physa occidentalis was in greater abundance during the summer months. L. proxima, L. palustris and H. trivolvis were collected during all seasons at Deer Creek but in relatively small numbers. During the winter season these species could be found on the surface of the mud and amongst duckweed.

Pseudosuccinea columella, Physa spp are in large supply on the under surface of the pond lily (Nymphea polysepala) which almost completely covers the lake surface during late spring and summer. Egg masses are also plentiful on the under surface of this plant.

Summary of Area Differences in Larval Trematode Infection:

The percentage infection by each larval stage is given in Table III.

The three collection areas at Burnaby Lake are no great distance apart and yet appreciable differences in the degree of larval trematode infection of their snails has been shown to exist. A total of five hundred and sixty-two snails were examined in the laboratory for the presence of cercariae and other intramolluscan

EXPLANATION OF PLATE X.

Map of Burnaby Lake showing Collection Areas.

Shaded portions indicate regions from which collections were made.

A. Laut Park Area.

B. Still Creek Area.

C. Deer Creek Area.

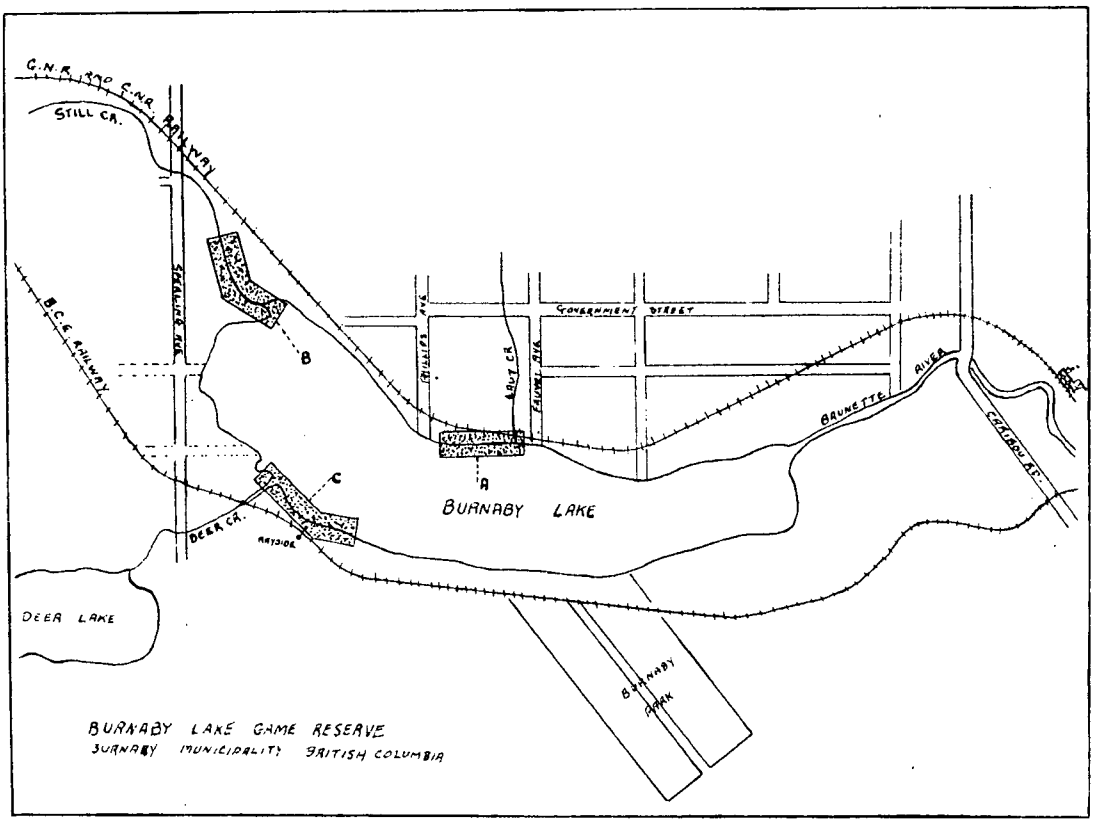
C.N.R.
C.N.R.
STILL CR.
STANLEY AVE.
B.C.E. RAILWAY
B
GOVERNMENT STREET
GILLEY'S AVE.
ROUT CR.
FADDEN AVE.
BRUNETTE RIVER
CANADA AVE.
A
BURNABY LAKE
C
DEER CR.
DEER LAKE
BURNABY PARK
BURNABY LAKE GAME RESERVE
BURNABY (MUNICIPALITY) BRITISH COLUMBIA

FIG. X.

stages.

Laut Park snails are the least parasitized by cercariae, rediae and sporocysts, but were found to be most heavily infected with the cyst stage. One percent of all snails examined containing cercariae and other intramolluscan stages were collected at Laut Park, as compared to two percent and seven percent from Still Creek and Deer Creek respectively. Deer Creek also possessed the greatest number of species of cercariae. No xiphidiocercariae were found in snails collected at Laut Park and Still Creek.

Furcocercous cercariae were found in greater percentage in snails of all three areas than were the echinostome and xiphidiocercariae.

These area differences can in no way be regarded as rigid. The number of snails examined from each area is too small, and the survey was for one year only, so that definite conclusions cannot be made from the data.

Discussion of High Degree of Infection at Deer Creek:

The Deer Creek area appears to be the most favourable for infection of snails by miracidia. This may be due to the combination of the area having a greater year round abundance of snails and a large adult host population from which the infection of these snails can arise.

No accurate data on the distribution of muskrat or other non-avian hosts within the Burnaby Lake area has been taken. Observations that were made in this regard indicate that the Deer Creek area is more heavily populated by muskrat than either the Laut Park or Still Creek areas. This was determined by the number of muskrat actually seen, the number of defecating posts found, sedge cuttings and other typical signs of a muskrat population.

During migratory seasons, waterfowl were seen in great numbers on Burnaby Lake. Although the actual distribution of the birds among the three areas was impossible to determine, it was noted that signs of birds predominated in the Deer Creek area. On most occasions the shoreline here was noticeably marked by avian faeces and scored by bird tracks. This area appears to provide an excellent feeding ground for water fowl, especially when the water level is low and much muddy shoreline is laid bare.

A large vertebrate population such as may be found in the Deer Creek area means a large quantity of trematode eggs being passed with the faeces into the lake water and moist shore. Subsequently a large number of the infective miracidia developing from these eggs are liable to cause a high percentage of infection in the snails of the area.

Degree of Larval Trematode Infection in Burnaby Lake Snails:

The following is a list of the snails collected and their percent infection by all intramolluscan stages,

Helisoma trivolvis	92%
Physa occidentalis	73%
P. cf traskii	70%
Lymnaea palustris	38%
Pseudosuccinea columella	27%
Menetus cooperi	26%
L. proxima	24%
H. anceps	0%
Gyraulus vermicularis	0%

Snail Species and their Infections

See Tables I, II.

1.) Helisoma trivolvis. Only 12 of thes species were examined. Eleven were infected by one stage or another of the larval trematodes. The majority of these snails came from Deer Creek (See Table I) and were for the most part infected by xiphidiocercariae. Echinostome and furcocercous cercariae were also found in this species of snail.

Summary of Larval Trematode Infection in 12 H. trivolvis (92% infected).

Number	Larva	Percent Infection
1	E.C. 1	9%
4	X.C. 1	33%
1	X.C. 2	9%
3	F.C. 1	25%

2	Echinostome cysts	17%
1	Large unidentified cyst (tetracotyle?)	9%

2.) Physa occidentalis. The larval trematode infection in this snail was found to be almost identical with that of P. cf. traskii. Dr. van der Schalie in his identification of the Burnaby Lake snails, suggests that these two species may be one and the same thing, which may partly explain the similarity in infection. One hundred and forty specimens were examined and found to be 73% infected by larval trematodes. The echinostome and furcocercous cercariae were present in equal degree. No Xiphidiocercariae were found infecting these forms. As regards area differences (See Table I) an equal quantity of snails from Deer Creek and Still Creek was infected. Two snails only from Laut Park harboured larval flukes, both infected with E.C. 1. This snail variably contained echinostome metacercariae (44% infected). Musfeldt (1945) found a stylet and possible non-stylet cercariae in this species at Burnaby Lake.

Summary of Larval Trematode Infection in 140 P. occidentalis (73% infected).

Number	Larva	Percent Infection
6	E.C. 1	4%
2	E.C. 2	1%
6	F.C. 1	4%
2	F.C. 2	1%

67	Echinostome cysts	48%
17	Large unidentified cysts	14%

3.) Physa cf. traskii. Fifty-seven specimens were examined and found to be 70% infected by six probable infecting species. No xiphidiocercariae were found

Summary of Larval Trematodes infecting 57 P. cf. traskii (70% infected).

Number	Larva	Percent infected.
3	E.C. 1	5%
1	E.C. 2	2%
3	F.C. 1	5%
2	F.C. 2	3%
25	Echinostome cysts	44%
6	Unidentified cysts (Tetracotyles?)	15%

4.) Lymnaea palustris. Xiphidiocercariae were present in greatest percentage. The numbers of specimens examined was too small to permit generalizations to be made regarding area differences or immunity existing in these snails. Only one specimen was taken from Laut Park and Still Creek, neither of which were infected. Relatively few echinostome cysts were found.

Summary of Larval Trematodes infection in 24 L. palustris (38% infected).

Number	Larva	Percent infected.
2	E.C. 1	8%
1	X.C. 1	4%
1	F.C. 1	4%
3	Echinostome Cysts	13%
1	Unidentified cysts	8%

5.) Pseudosuccinea columella. The majority of this species were collected at Laut Park. Only one of these specimens was infected, by a F.C. 1. Only one from Still Creek was found to be infected by a E.C. 1. No xiphidiocercariae were found in these snails.

In general, larval trematode infection in Pseudosuccinea columella was relatively small; of two hundred and fifty-nine snails examined, only five were found infected by cercariae. The infection by cystic stages in this species was of a high degree. Of two hundred and fifty-two Pseudosuccinea columella examined specifically for metacercariae, fifty-five contained cysts identified as belonging to Echinostomes, while in twelve specimens large unidentified cysts were found. Of all species examined this proved to be least infected-- 2% (See Table IV) by cercariae, and 27% by both cercariae, and metacercariae.

Summary of Larval Trematode Infection in 259 Pseudosuccinea columella (27% infected).

Number	Larva	Percent Infected.
1	E.C. 1	0.4% i
1	E.C. 2	0.4%
1	F.C. 1	0.4%
1	F.C. 2	0.4%
55	Echinostome cysts	21 %
13	Unidentified cysts	5%

6.) Menetus cooperi. Of twenty-seven specimens examined, 26% were found to be infected by echinostome cysts. No cercariae of any species were observed in this snail. The infected snails were collected at Still Creek and Deer Creek.

Summary of Larval Trematode Infection in 27 Menetus cooperi (26% infected.

Number	Larva	Percent Infected
7	Echinostome cysts	26%

7.) Lymnaea proxima. Thirty-five snails were examined, the majority of which were collecte at Deer Creek. One specimen only came from Still Creek and was uninfected. Twenty-one percent of the snails were infected with four cercariae species. Nine snails were so infected, counting all types of cysts, there were six probable infecting larval trematode species in L. proxima,(See Table IV).

Summary of Larval Trematode Infection in 35 L. proxima (24% infected).

Number	Larvae	Percent Infection
4	E.C. 1	11%
2	X.C. 1	6%
1	F.C. 1	3%
1	F.C. 2	3%
4	Echinostome cysts	11%
1	Unidentified cyst	3%

8). Helisoma anceps. Only three H. anceps were examined. No specimens were found infected by larval trematodes. All specimens examined came from Deer Creek.

9.) Gyraulus vermicularis. Small numbers of this species were collected and examined from each area. No trematode infection was discovered in this species.

Comparison with Infection found at San Juan Island (Miller 1925).

Tables IV and V indicate that Burnaby Lake snails are more heavily parasitized than those of San Juan Island, as reported by Miller (1925). These tables show in particular that the percentage of infection and the number of infecting larval species are higher in Burnaby Lake snails than in those collected on San Juan Island.

The species of snail common to both areas is L. proxima

and in these the difference in the degree of infection of the snails of both areas is particularly evident. L. proxima from Burnaby Lake are 24% infected, by a total of six different larvae as compared with L. proxima from San Juan Island which are only 8% infected, by two different infecting species.

The Physa spp from Burnaby Lake also show a higher degree of infection than the Physas reported by Miller. Burnaby Lake Physae were 73% infected, while only 3% of the San Juan Island physa were infected.

In the snails of both areas much variation in infection of individual species was found. There appears to be no relation between the degree of infection of a snail species in one area with the same species in the other. The degree of infection in the snails seems to be a characteristic of the locale.

Summary of Host Records for Burnaby Lake Larval Trematodes.

The following is a list of snails found at Burnaby Lake which were found shedding each cercaria. Table VI gives the complete host records for each cercaria spp.

E.C. 1.

Pseudosuccinea columella

Lymnaea proxima

L. palustris

Physa occidentalis

P. cf. traskii

Helisoma trivolvis

E.C. 2.

Pseudosuccinea columella

P. occidentalis

H. trivolvis

X.C. 1

L. proxima

L. palustris

H. trivolvis

X.C. 2

H. trivolvis

F.C. 1

Pseudosuccinea columella

L. proxima

L. palustris

P. occidentalis

P. traskii

H. trivolvis

F.C. 2

Pseudosuccinea columella

L. proxima

P. occidentalis

Evidence of Host Specificity of Cercariae.

There appears to be little specificity shown by trematode miracidia infesting Burnaby Lake in the choice of snail hosts.

The greatest degree of host specificity has been found with X.C. 2, which was found in one snail species only, that of H. trivolvis. X.C. 2 and F.C. 1 show the next highest degree of host specificity, for each are found in three species of snail only, which fall into two separate genera. E.C. 2 was found in three species of snails, comprising three genera. E.C. 1 and F.C. 1 exhibit the least amount of specificity in choosing their intermediary hosts. Both cercariae were shed from the same host species, comprising six species of three different genera.

It may be of interest to add here that H.M. Miller Jr. (1925) observed that no one of the nine species of furcocercous cercariae he found at San Juan Island were shed from more than one genus of snail host. Cort (1915) found furcocercous forms parasitizing three generalof snails in Michigan.

Evidence of Multiple Infection ofSnail Hosts:

No case of a snail playing host to more than one species of larval trematode was found in the snails of Burnaby Lake. Although this would appear to indicate the existence of a natural immunity of Burnaby Lake snails to second infection, the relatively small number of snails examined prohibits such a conclusion. The

presence of two species of rediae or sporocysts within an individual snail may have been obscured due to the small amount of one or the other present and lack of experience in distinguishing rediae and sporocysts on specific grounds.

It can be definitely said that no snail from Burnaby Lake, from which cercariae emerged, shed more than one type during the time it was under observation.

In the case of each of the six cercariae discovered, the snails shedding the cercariae were in several instances found to be harbouring echinostomes or metacercariae of another species. Many snails were also found to contain both echinostome and large unidentified cysts.

Evidence of Harm done to Snail Hosts:

It is difficult to determine whether or not larval trematodes living within snails can be a cause of the snails' death. Several snails were proved on examination to be extremely parasitized, to the extent that the liver or digestive gland was composed almost exclusively of sporocysts and rediae. These snails it is true, may have died from old age rather than from the cumulative effect of harbouring the active larval worms. Examination, nonetheless, showed that very little of the normal tissue could be found. Rees (1931) reports much the same condition in the case of the liver fluke, Fasciola hepatica. Here, he reports, the intramolluscan stages create much disintegration of the snail host,

reducing the liver cells to a thin layer of protoplasm containing the nuclei.

In the case of infected Burnaby Lake snails, the digestive gland was invariably found to have an unhealthy appearance. Most cases were such that the infected portion of the snail was larger in mass than the uninfected portions. The infected digestive gland varied from a cream to rust in colour.

It is apparently the rediae which are agents of most of the destruction within the snail hosts. Rediae are muscular and capable of independent movement. In addition, they have a mouth surrounded by a sucker, and in some cases may be provided with an anterior collar of spines. It is conceivable, then, that as a result of their movements and feeding these larvae are capable of causing much destruction within snails.

The infected snail, it must be noted, seems to be able to present some degree of fortitude and counteraction to the presence of larval trematodes. Active and apparently healthy snails have been examined which contained nothing more than a sac of active cercariae in place of the digestive gland.

Brown (1926) is of the opinion that snails infected by echinostome and xiphidiocercariae die sooner than those infected by furcocercous forms. A similar observation has been made in the case of Burnaby Lake snails. It was found that those snails which s

shed cercariae and lived longest had the furcocercous cercariae emerging from them.

In summarizing the harmful effects of larval trematodes upon their snail hosts, Brown (1926) makes the statement regarding Echinostome cercariae, that " it is very difficult to estimate the effects of these parasites in their hosts, that they are harmful is indisputable," which in general covers the situation found with all cercariae species at Burnaby Lake.

Infestation by metacercariae appears to have no detrimental effect upon the snail. This is to be expected since metacercariae are non-motile and non-feeding. Large clusters or " clumps" of cysts were often found in snails, but apparently their presence in quantity is as innocuous as single infestations.

SUMMARY

Six species of larval trematoda have been discovered in the Mollusca of Burnaby Lake, B.C. These cercariae emerged from snails collected over a period of one year.

All snails examined came from three specific areas of Burnaby Lake. They were observed in aquaria for the natural emergence of cercariae, or crushed to detect the presence of other intra-molluscan stages. The nature and intensity of the larval trematode infection has been noted and the details for each presented in tables.

The cercariae found comprise two species each of echinostome cercariae, furcocercous cercariae and xiphidiocercariae. The tentative identifications of E.C. 1 and E.C. 2 for the echinostomes, F.C. 1 and F.C. 2 for the fork-tailed and X.C. 1 and X.C. 2 for the xiphidiocercariae were made. Comparisons and tentative identifications with cercariae reported in the literature have been carried out. The results of these comparisons is as follows:

E.C. 1 Cercaria echinoparyphium recurvatum (proved by infection experiment).

E.C. 2 C. echinostomum revolutum (indicated by close morphological resemblances).

F.C. 1 shows resemblance to C. dohema, Cort and Brackett 1937.

F.C. 2 shows resemblance to C. oregonensis, Macfarlane and Macy, 1946 ; and C. milleri, Faust 1926.

X.C. 1 shows much resemblance to C. albui, Brooks 1947.

X.C. 2 appears to be an undescribed species.

Each species of larval trematode has been described.

A series of extensive observations and experiments were conducted to discover the possible life cycle of each of these larval worms. These life cycle studies have included 1) a survey of the naturally infected snails, as well as some tadpoles and catfish, to determine which species act as intermediate hosts to larval trematodes, 2) exposure of laboratory-raised snails, and of Burnaby Lake tadpoles and catfish, to attack by various cercariae, and 3) feeding of the encysted cercariae to various vertebrates in order to discover which are the adult hosts of the trematodes.

The life cycle of E.C. 1 (= Echinoparyphium recurvatum) at Burnaby Lake has been demonstrated. The first intermediate host of this worm has been determined as being Pseudosuccinea columella, Lymnaea proxima, L. palustris, Physa occidentalis, P. cf traskii and Helisoma trivolvis. E.C. 1 utilizes the same snail species for both the first and second intermediate hosts, for metacercariae have been found in the molluscan species from which cercariae emerged. Three species of laboratory-raised snails have been

infected by E.C. 1. These experimental second intermediate hosts are Pseudosuccinea columella, P. occidentalis and P. cf traskii.

Metacercariae identical with the 45-spined experimentally produced cysts of E.C. 1 were fed to guinea pigs, rats, ducklings and pigeons. In one pigeon, eight small adult echinostomes were discovered 33 days after the experimental feeding. These worms have been identified as Echinoparyphium recurvatum. Close morphological comparisons of E.C. 1 and its cyst with the adult trematode, and the fact that, as far as could be determined, the pigeon was free of trematode infection prior to the experiment, indicates that E.C. 1 is the larval stage of Echinoparyphium recurvatum.

Several unsuccessful experiments were made to determine possible intermediate hosts of E.C. 1. This cercariae has been identified as pharyngeal longifurcate distome cercariae, and is very likely the larval stage of a strigeid trematode. It was proved to be incapable of producing schistosome dermatitis in humans.

The second furcocercous cercariae found at Burnaby Lake has been discovered capable of producing dermatitis or " swimmer's itch " in humans. The symptoms recorded agree generally with those reported in the literature for other schistosome dermatitis experiments.

Although the two xiphidiocercariae were very similar morphologically, they have been classed as separate species on the basis of stylet structure. The first intermediate hosts of X.C. 1

are L. proxima, L. palustris and H. trivolvis. No naturally infected second intermediate hosts of this cercariae were discovered. A gammarus species was proved to be at least an experimental second intermediate host. X.C. 1 was unable to be infected in ducklings, goldfish, rats and guinea pigs.

X.C. 2 was found in only one snail species, that of H. trivolvis. A gammarus species was found to be an experimental intermediate host of this cercaria.

Burnaby Lake was found to have a high degree of larval trematode infection, both in the number of species of larvae and in the number of snails infected.

There is at least an apparent difference in snail populations, and their larval trematode fauna, between snails of the three areas under study at Burnaby Lake. The Deer Creek Area has the richest molluscan fauna, and its snails have the highest incidence of infection by larval trematodes.

There appears to be little or no host specificity in the larval trematodes at Burnaby Lake. X.C. 2 exhibits the only noticeable degree of specificity, being found in H. trivolvis only. All the other cercariae are found in snail species comprising at least two genera.

F.C. 2 has been proved a causative agent of schistosome dermatitis. As far as can be determined, this is the first experi-

mental demonstration of schistosome dermatitis in British Columbia. The dermatitis is manifest by the production of erythematous papules and itching.

EXPLANATION OF LETTERING OF PLATES.

act, anterior collecting tubules.

bp, birth pore.

c, caecum.

cb, caudal bodies or blocks

cer, cercaria.

cg, cephalic or penetration gland.

cgd, cephalic gland duct.

cl, collar.

cs, cuticular spines or hairs.

cw, cyst wall.

ds, dorsal spine region.

ext, excretory collecting tubule.

ep, excretory papilla.

es, eyespot.

ev, excretory vesicle.

fc, flame cell.

ff, fin fold or membrane.

fr, furcal ramus.

g, gut

gs, spines of glands.

ic, immature cercaria.

ls, lateral spine region.

mt, main excretory tubule.

lps, lappet spine region.

n, " notches " or " crenations ".

o, ovary.

ob, oral bulbous or suctorial apparatus.

oe, oesophagus

og, oral gland.

os, oral sucker.

ow, outer cyst wall.

p, pharynx.

pig, pigment.

pph, prepharynx.

s, stylet.

sd, shoulder region.

sp, suctorial proboscis.

ss, sucker spines.

t, testis.

ts, tail stem.

u, uterus.

v, vitellaria.

vent, ventral surface.

vtd, viteline duct.

TABLE I.

Summary of Occurrence of Cercariae by Host and Area.

SNAIL SPECIES.	LAUT PARK AREA. No. Exd.	LAUT PARK AREA. No snails infected with cerc. sp. XC1	XC2	XC1	XC2	FC1	FC2	STILL CREEK AREA. No. Exd.	STILL CREEK AREA. No. snails infected with cerc. sp. XC1	XC2	XC1	XC2	FC1	FC2	DEER CREEK AREA. No. Exd.	DEER CREEK AREA. No. snails infected with cerc. sp. XC1	XC2	XC1	XC2	FC1	FC2	Total Exd.
Pseudosuccinea columella	173						1	45	1						45	1		1			1		259
Lymnaea proxima	5						1	1							31	4		2			1	1	35
L. palustris	1							1							22	1		3			1		24
Physa occidentalis	74	2						23	2	2			2	1	43	2					4	1	140
P. traskii	28	1			1	1		10						2	19	2	1				2		67
Helisoma trivolvis	1					1	1	2							9	1		4		1	2		12
H. anceps	0							0							3								3
Menetus cooperi	2							5							20								27
Gyraulus vermicularis	4							1							0								5
Ferrissia cowrina	0							0															0
TOTALS.	286	3				2	2	86	3	2			2	3	190	11	2	7		1	11	2	Total No. Snails Exd. 562

TABLE II.

Summary of Occurrence of Encysted Forms by Host and Area.

SNAIL SPECIES.	LAUT PARK AREA.			STILL CREEK AREA.			DEER CREEK AREA.			Total Exd.
	No. Exd.	No. snails infected with cysts.		No. Exd.	No. snails infected with cysts.		No. Exd.	No. snails infected with cysts.		
		Echinost cysts	Unident. cysts		Echinost. cysts	Unident. cysts		Echinost. cysts	Unident. cysts.	
Pseudosuccinea columella	170	36	9	43	14	2	39	5	1	252
Lymnaea proxima	3	1	1	1			22	3		26
L. palustris	1			0			18	3	2	19
Physa occidentalis	66	41	8	19	6	4	36	20	7	121
P. traskii	26	10		8	5	2	14	10	4	48
Helisoma trivolvis	1			2	2	1	1			4
H. anceps	0			0			3			3
Menetus cooperi	2			5	2		19	5		26
Gyraulus vermicularis	4			1			0			5
Ferrissia caurina	0			0			0			0
TOTALS.	273	88	18	79	29	9	152	36	14	504

TABLE III.

Percent of all Snails examined infected with Intramolluscan stages of Larval Trematodes.

AREA FROM WHICH SNAILS WERE COLLECTED.	LARVAL STAGES AND PERCENT INFECTION IN ALL SNAILS EXAMINED.				
	Echinostome cercariae	Xiphidio-cercariae	Furcocercous cercariea	Cercariae and other Intramolluscan	Cysts *
Laut Park	0.5%	—	0.7%	1%	20%
Still Creek	0.9%	—	0.9%	2%	8%
Deer Creek	2.0%	1.6%	2.2%	6%	12%

N.B. * Based on the examination of 504 specimens (See Table II).

TABLE IV.

Summary of Larval Trematode Infection in Burnaby lake Snails.

SNAIL SPECIES.	No. Examined	No. of infecting c cercariae sp	No. infected by cercariae spp	% infected	Probable No. of Total infecting spp	% infected by total infecting species.
Pseudosuccinea columella	259	4	5	2%	7	27%
Lymnaea proxima	35	4	9	21%	6	24%
Lymnaea palustris	24	3	3	13%	5	38%
Physa occidentalis	140	4	16	12%	7	73%
Physa cf. traskii	57	4	9	16%	6	70%
Helisoma trivolvis	12	3	9	75%	5	92%
Helisoma anceps	3	0	0		0	0
Gyraulus vermicularis	5	0	0		0	0
Ferrissia caurina	0	0	0		0	0
Menetus cooperi	27	0	0		1	26

TABLE V.

Summary of Larval Trematode Infection in Snails of San Juan Island, Puget Sound, as given by Miller (1925).

SNAIL SPECIES.	No. Examined	Percent Infected	Probable Number of Infecting Species.
L. proxima	76	8%	2
L. stagnalis	84	65%	6
P. species	75	3%	1
Planorbis (3 or more)	376	44%	12
Succinea retusa	77	0%	0
Sphaerium	32	0%	0

TABLE VI.

Summary of Life Cycle Studies

Natural and experimental hosts of larval trematodes found at Burnaby lake.

CERCARIA SPECIES.	1st INTERMEDIATE HOSTS (all naturally infected)	2nd INTERMEDIATE HOSTS (naturally and experimentally infected.	ADULT HOSTS (naturally and experimentally fected.
E.C. 1	Pseudosuccinea columella Physa occidentalis P of traskii Lymnae proxima Helisoma trivolvis	Pseudosuccinea columella(Exp) L. proxima L. palustris P. occidentalis (Exp) P. of traskii (Exp) H. trivolvis	Pigeon (experimental)
E.C. 2	Pseudosuccinea columella P. occidentalis H. trivolvis	Pseudosuccinea columella P. occidentalis (Exp) P. of traskii (Exp)	Muskrat (natural)
X.C. 1	L. proxima L. palustris H. trivolvis	Gammarus (Exp) sp	———
X.C. 2	H. trivolvis	Gammarus sp (Exp)	———
P.C. 1	Pseudosuccinea columella L. proxima L. palustris P. occidentalis P. of traskii H. trivolvis	———	———
P.C. 2	Pseudosuccinea columella L. proxima P. occidentalis	———	———

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