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STUDIES ON THE LIFE HISTORY OF *DIPLODISCUS TEMPERATUS* STAFFORD FROM THE FROG*

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INTRODUCTION

Diplodiscus temperatus, an amphistome trematode parasitic in the rectum of frogs, was first described by Stafford (1905) from *Rana catesbeiana* and *R. pipiens* (Syn. *R. virescens*). This parasite belongs to the family Paramphistomidae Fischoeder 1901 and to the subfamily Diplodiscinae Cohn 1904. Fukui (1929), in a paper on Japanese amphistomatous parasites, with a revision of the group, includes six species in this subfamily, namely, *Diplodiscus subclavatus* (Goeze 1782) Diesing 1836 (Syn. *D. megalochrus* Johnston 1912 and *D. microchrus* Johnston 1912), *D. americanus* (Chandler 1923), *D. cornu* (Diesing 1840) Daday 1907, *D. temperatus* Stafford 1905, *Opisthodiscus diplodiscoides* Cohn 1904, and *Catadiscus dolichocotyle* Cohn 1904.

Hunter (1930) has emended the genera *Diplodiscus* Diesing 1836 and *Opisthodiscus* Cohn 1904. In the genus *Diplodiscus,* as emended, he includes *D. subclavatus* (Goeze 1782) type, *D. temperatus* Stafford 1905 (Syn. *D. ranophilus* [Millzner 1924]), *D. americanus* (Chandler 1923), and *D.*

* Contribution from the Biological Station, Zoological Laboratory, and Museum of Zoology of the University of Michigan.

intermedius Hunter 1930. It will be noted that Hunter has followed Chapin (1926) in reducing Millzner's species to synonymy and followed the view of Cort (1926) in designating the genus *Megalodiscus* Chandler 1923 as synonymous with the genus *Diplodiscus* Diesing 1836. Hunter (1930) has not included *Diplodiscus cornu* (Diesing 1840) Daday 1907 as given by Fukui (1929) in the list of species in the genus *Diplodiscus*. Fukui was probably correct in reducing *D. megalochrus* Johnston 1912 and *D. microchrus* Johnston 1912 to synonyms of *D. subclavatus* (Goeze 1782).

Experimentally determined life histories in the subfamily Diplodiscinae are limited in number. Looss (1892) published the life history of *Diplodiscus subclavatus* (Syn. *Amphistomum subclavatum*), in which he included some experimental work. Cary (1909) published a life history of *Diplodiscus temperatus* which, as definitely pointed out by Cort (1915), must be considered erroneous. Cort, in his criticism of the life history in question, proved:

"1. That Cary described two entirely different species of cercariae as belonging to *Diplodiscus temperatus;*

a. Those with stylets, which develop in sporocysts,

b. Larger forms without stylets which develop in rediae.

"2. That since the second type only were used in the infection experiments, no connection between the first type and *Diplodiscus temporatus* can have been shown.

"3. That the infection experiments were not sufficiently controlled to be conclusive.

"4. That the cercariae used could not have possibly developed into *Diplodiscus temporatus,* since the two forms differ so fundamentally in structure."

While the life history as published by Cary has often been referred to in the literature, apparently without knowledge as to its validity or the criticisms of Cort, it seems to us that Cort has so obviously proven that the life history of *Diplodiscus temperatus* as published by Cary cannot possibly be correct, that it may be entirely disregarded in the present paper.

Beaver (1929) claimed that he was able to complete the life history of the amphistome, *Allassostoma parvum,* in *Rana*

catesbeiana and *Rana pipiens*. This parasite was originally described by Stunkard (1916) from the snapping turtle, *Chelydra serpentina*. While the history worked out by Beaver may be valid, his experimental methods may be questioned. He stated: "Several *R. catesbeiana* and *C. serpentina* were periodically fed crayfish, *Cambarus* (*Faxonius*) *propinquus*, and small *R. pipiens* on which large numbers of the cercariae had encysted and the adult *A. parva* taken from these experimental hosts were easily separated into distinct groups according to their degree of maturity, and these groups were in each case easily correlated with the number of feedings and periods at which they were given."

The fact that Beaver was able to correlate the number of feedings and periods at which they were given with the size of the flukes is interesting, since with the species presented in this paper it could not be done. While the frog amphistomes do not show any marked specificity, the finding of *Allassostoma parvum* by Beaver in the bullfrog and snapping turtle is very striking.

Since the publication of Cary's work, a number of American investigators, including Cort (1915), Faust (1919), McCoy (1929), and O'Roke (1917) have described amphistome cercariae, and some of these workers have suggested the possibility that certain of these larval forms belong in the life history of previously described adult amphistomes, but no one has actually proved by experimentation that his supposition was correct.

The diversity of hosts and the wide distribution of the Diplodiscinae are interesting. Both phases are well illustrated by *Diplodiscus subclavatus* which Lühe (1909) reported from middle Europe in eight host species including frogs, toads, and salamanders. Fukui (1929) reported it from five species in Japan, including frogs, a salamander, and a snake. Johnston (1912) found it in frogs of Australia.

The life history of the present species, *Diplodiscus temperatus*, has been completed experimentally in three hosts, namely: *Rana clamitans, R. pipiens,* and *R. cantabrigensis*.

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Egg

The egg (Fig. 2) of *Diplodiscus temperatus* under normal conditions contains a mature embryo when deposited. Eggs swell slightly in water, and ten eggs after being in water a short while measured 0.120–0.128 mm. (average 0.122 mm.) in length and 0.06–0.07 mm. (average 0.062 mm.) in width. The egg is operculate (Fig. 1), oval in shape, and slightly asymmetrical. The eggshell is comparatively thin, membranous, transparent, smooth, and has no external markings. Many refractile granules are to be seen between the shell and the enclosed miracidium. The larva in the egg moves back and forth sluggishly, contracting and elongating while the cilia are lashing. Two large functional flame cells are very conspicuous even when the eggs are observed under the low power of the compound microscope.

Miracidium

The miracidium of this species is pyriform (Fig. 7), and has a rounded anterior end, and an apical papilla. The body, with the exception of the anterior papilla, is almost entirely covered with flattened ciliated epidermal cells, twenty in number, arranged in four rows (Fig. 6). The anterior row contains six cells, the second eight, the third four, and the fourth two. The six cells in the first row cover the anterior one-fifth of the body as far posteriorly as the lateral processes. Each of these cells is wide along its posterior border and narrows gradually toward the anterior end which is irregular and lies at the base of the anterior papilla. The eight cells in the second row are rectangular and extend from the lateral processes posteriorly past the middle of the body. The cells in the third row are rectangular, about the length of those in the second row and almost twice as wide. The two cells of the last row cover the posterior end of the miracidium.

Each of the epidermal cells contains an elongated and irregular nucleus, although the nuclei in the cells of the second row are usually more irregular than those in the cells of the first, third, and fourth rows. The nuclei in the cells of the first row are situated near the posterior end of the cell, those in the cells of the second and third rows about one-third of the distance from the posterior end of the cell, and those in the cells of the fourth row near the middle or anterior part of the cell.

The number of ciliated epidermal cells in species of miracidia was discussed in detail by one of us (Price, 1931) and will not be considered here in detail. Looss (1892) described such cells for the miracidium of *Diplodiscus subclavatus,* but gave no information on their number. The number present in the miracidia of other amphistomes has not been described.

Nucleated epidermal cells in the miracidium of *Fasciola hepatica* have been figured by Thomas (1883), Leuckart (1886), Ortmann (1908), and Coe (1896). Thomas figured these nuclei as round and in the posterior part of the cell. Leuckart represented them also as round but centrally located. Ortmann figured them as round in cross section and located toward the posterior border of the cell. Coe figured the same nuclei as elongated and branched in surface view and near the posterior end of each cell. Looss (1892) figured large, round, centrally located nuclei in the epidermal cells of the miracidium of *Diplodiscus subclavatus.* The form and position of the nuclei in the epidermal cells of the miracidium of the present species resemble those described by Coe (1896) for *Fasciola hepatica* more closely than those figured by other investigators for that or other species. It is evident from the literature that these nuclei have seldom been observed, although in the present species they were easily stained intravitally with neutral red, brilliant cresyl blue, and certain other vital dyes.

SUBEPITHELIUM

The subepithelium (Fig. 7) is a thin, transparent layer beneath the ciliated epidermal cells and is continuous around the

entire body of the miracidium, pushing slightly outward between the ciliated epidermal cells. Cell outlines were not observed in this layer although the nuclei stain well with neutral red or brilliant cresyl blue. These nuclei (Fig. 7) are round and are arranged in two rows around the miracidium with a group of two or three at the posterior end of the body. The first row of nuclei is situated about one-third of the distance from the anterior end of the miracidium just posterior to the lateral processes. The number of nuclei in this row varies from eight to eleven, but usually ten are present. The second row of nine nuclei is situated about two-thirds the distance from the anterior end of the miracidium.

GERM CELLS

The posterior half of the body is filled with large germ cells (Fig. 7), each containing a relatively large nucleus. These cells form a single large mass which was not observed to be attached to the body wall as are the individual germ cells in certain other species.

Surrounding the mass of germ cells and immediately beneath the subepithelium is a thin granular layer (Fig. 7) in which nuclei can be observed. There is a group of several nuclei near the posterior end of the body as well as a group of four or five on each side of the body, anterior to the mass of germ cells. This layer could be traced anteriorly only a short distance in front of the two lateral groups of nuclei. The function of this layer has not been determined.

INTESTINE

The primitive gut (Fig. 8) is a long sac-like structure extending posteriorly from the anterior papilla about two-thirds of the body length. It is filled with coarse granules and contains four nuclei, three of which are usually in a group at the posterior end, while the fourth is situated more anteriorly. The presence of four nuclei in the gut has been noted in different species of miracidia by several investigators. When stained intravitally with brilliant cresyl blue, the chromatin

in these nuclei appears as two masses, one a little larger than the other.

Two pairs of unicellular penetration glands (Fig. 7) are present, a pair being situated on each side of the anterior part of the gut; each discharges exteriorly through a duct which opens at the base of the anterior papilla. Each gland has a nucleus and is filled with a clear, non-granular substance which is very difficult to stain. In no other miracidium have two pairs of penetration glands been described. On each side of the body a round knoblike structure, the lateral process (Fig. 7), is situated between the first and second rows of ciliated epidermal cells. No internal connections with these structures were observed.

At the level of the lateral processes, in a slight indentation on the posterior border of each of the cells making up the first row of ciliated epidermal plates, there is a small conical papilla. Coe (1896) described similar structures for the miracidium of *Fasciola hepatica* and similar, except paired, structures, were described by Van Haitsma (1931) and Price (1931) for the miracidia of certain species of strigeids and schistosomes. Reisinger (1923) observed in the miracidium of *Schistosoma haematobium* two rows of conical papillae which he thought were connected with nerve fibers extending around the miracidium. He was also of the opinion that the circular nerves were connected with the brain. No such connections were observed in the miracidium of the present species.

The nervous system (Fig. 7) consists of a round fibrous mass situated between the penetration glands and the germ cells, and of three pairs of nerve cells. No nuclei or cells were observed around the brain, although they undoubtedly are present. Anterior to the brain are three pairs of nerve cells which have surface terminations at the base of the anterior papilla and internal terminations within the brain mass. These cells are centrally located and lie between the penetration glands.

The excretory system (Fig. 8) consists of two large flame cells and two long excretory ducts, each provided with an

excretory bladder. The two flame cells are situated in the anterior half of the body; extending posteriorly from each is a tubule which makes a loop near the posterior end of the gut and then extends anteriorly, making first an anterior and then a small lateral loop, after which it again passes posteriorly and enters an excretory bladder. In the posterior and lateral loops formed by each excretory duct is a duct nucleus. Each bladder opens exteriorly through an excretory pore situated between the third and fourth rows of ciliated epidermal cells.

Redia

The redia (Figs. 3, 4) of *Diplodiscus temperatus* is similar to the redia of all the closely related forms in the possession of two pairs of appendages. In a medium state of contraction the first pair is situated about two-thirds of the distance from the anterior end of the body, and the second pair midway between the first pair and the posterior end of the body.

Rediae elongate and contract rather sluggishly when freed from the tissue of the snail host and when fully extended may double their ordinary length. They are occasionally able to right themselves but make no progress by means of their appendages. The posterior end of the redia is smaller, more attenuated and pointed than the anterior end. Both ends are capable of considerable extension.

The rediae from several snails were all about the same size although there was a great difference in the extent of development of the contained cercariae. Living rediae in a moderate state of contraction averaged 500 microns in length and 168 microns in width. Stained and mounted specimens which had been killed in sublimate-acetic and which varied considerably in their states of contraction ranged from 400 to 800 microns in length.

The surface of the redia is thrown into fine transverse, annular bands owing to circular muscle development. The annulations are also found on the appendages, the tip of each appendage being slightly indented.

The pharynx is large and almost round, the average length and width in a measured series of living specimens being 49 by 50 microns and in preserved specimens 41 by 41 microns. The narrow lumen of the short esophagus opens into a large and sac-like gut which usually extends posteriorly to the level of the anterior pair of appendages, and is always filled with material from the host's liver. A ring of eight large unicellular glands (Fig. 4) can be seen around the esophagus immediately posterior to the pharynx. The nuclei of these cells vary in diameter from 8 to 9 microns. An additional elongated, and compact glandular mass (Fig. 4) is located just posterior to the pharynx between the gut and body wall. The cells making up the mass are about as large as the eight gland cells surrounding the pharynx. In a stained and mounted well developed redia this mass measured 62 by 25 microns.

The birth pore is situated dorsally about midway between the oral sucker and the anterior appendages.

The position of the flame cells of the excretory system is shown in figure 3. The tubules were not observed.

The developing cercariae in a single redia may number as high as twenty, the most anterior ones being more fully developed. The largest show well developed tail buds and a concentration of cells in the places of development of the future suckers and eyespots.

Cercaria

The cercaria (Figs. 5, 9) of *Diplodiscus temperatus* is a pharyngeate, monocerous amphistome with pigmented eyespots (Fig. 5). The body is wedge-shaped from a dorsal or ventral view, the tail being attached to the dorsal side immediately anterior to the posterior sucker (Fig. 5). Measurements of twenty-five cercariae killed in ten per cent hot formalin, dehydrated and mounted on slides, are given in the following table.

The measurements were all made on well extended larvae. Amphistome cercariae may contract until perfectly round, but

MEASUREMENTS IN MILLIMETERS OF THE CERCARIA OF *D. temperatus*

	Length of body	Width of body at greatest width	Length of tail	Width of tail at base
Minimum	0.382	0.160	0.685	0.062
Maximum	0.489	0.222	0.738	0.080
Average	0.423	0.187	0.713	0.070

MEASUREMENTS IN MILLIMETERS OF THE CERCARIA OF *D. temperatus* (cont.)

	Length of oral organ and pouches	Length of posterior sucker	Width of posterior sucker	Width of eyespots
Minimum	0.115	0.044	0.115	0.026
Maximum	0.142	0.089	0.151	0.040
Average	0.128	0.060	0.135	0.030

these did not. The average body length of ten cercariae killed by the heat method on a slide without a cover glass was 0.439 mm., and the tail length 0.772 mm. The length of the body naturally varies considerably owing to different states of contraction, but the length of the tail shows much less variation.

The body and tail are entirely spineless, being covered only by a smooth cuticula of more or less uniform thickness, measuring 0.006 mm. in living specimens and 0.004 mm. in preserved specimens. On the dorsal side of the anterior three-fourths of the body is a scattered, blackish pigment which is more abundant toward the anterior end with the exception of the oral sucker region which is free from pigment. The pigment diminishes gradually toward the anterior end of the acetabulum.

The extent and intensity of the pigmentation always varies with the individual. The pigment granules are sometimes

massed together and give the cercaria a patchy appearance. An occasional specimen may show some pigment on the ventral side.

A pair of large pigmented eyespots are situated on the dorsal side of the body between the buccal pouches and the intestinal ceca. Each eyespot is provided with a large lens. The lenses are turned laterally almost at right angles to the longitudinal axis of the cercaria.

Faint longitudinal and transverse striae can be seen on the surface of the cercaria and since the longitudinal ones are more pronounced when the larva is extended and the transverse ones more pronounced when the larva is contracted, they are probably slight folds in the cuticle which follow the contour of the deeper musculature.

The cystogenous gland cells produce groups of small rod-shaped structures which are destined to form the cyst. These structures stain a bright red with Mallory's triple stain and sections show that they are not confined to the cells just underneath the cuticle but are found in undifferentiated cells throughout the body.

The oral sucker (Fig. 9) has a muscular wall with two buccal pouches extending from its posterior border. These retrodorsal pouches are very muscular, capable of much contraction, and each is somewhat shorter than the oral sucker. A relatively long esophagus extends posteriorly between and ventral to the buccal pouches and before opening into the two intestinal ceca is surrounded by a large muscular pharynx, the longitudinal axis of which is usually turned dorso-ventrally.

The intestinal ceca are thick-walled, about five times as wide as the esophagus, and extend backward to near the acetabulum. Sometimes the ceca are filled with a light brown watery material, possibly liver from the snail host.

The genital field is midway between the two ends of the cercaria. Three relatively large oval bodies, the primordia of the reproductive organs, are situated between the intestinal ceca. The two largest of these bodies, one of which is situated

to the left and a little anterior to the other, are the primordia of the testes. The smallest of the three bodies, the primordium of the ovary, is situated in an intermediate position between the primordia of the two testes but always on the side of the most anterior testis and nearly median in position. The testes are slightly unequal in size and normally are lobed, but after a cercaria has been on a slide for some time the lobing is lost and the testes become broadly oval in shape. The ovary has an irregular margin, is more rounded than the testes and much smaller. The rather conspicuous median genital pore is situated on the ventral side just posterior to the level of the eyespots, and is closed by a plug of tissue.

The large posterior sucker is terminal and directed slightly ventrally. Its measurements are included in the table.

The excretory system (Fig. 10) is complicated. There is a rather small muscular bladder located immediately anterior to the acetabulum and just beneath the dorsal body wall. Its contents are discharged through a dorsal median pore. Three main excretory tubules discharge into the bladder. The posterior median one enters the tail, passes posteriorly, and bifurcates near the end of the tail, the two branches passing to the surface laterally, but having no external openings. A pair of tubules originating close together at the anterior end of the bladder proceed laterally, then anteriorly by a winding course to the level of the eyespots where they loop back, the lumen becoming somewhat smaller, and continue posteriorly about one-half the length of the cercaria where they bifurcate. Several other branching longitudinal tubules are observed, but their connections could not be found. Smaller tubules ramify to all parts of the body, and a reticulum of fine tubules which are thought to originate from a pair of tubes directly connected with the bladder can be observed in the posterior sucker. Some flame cells were seen but their connections could not be determined. The tubules are free from excretory granules except at the anterior ends of the main trunks just before they turn posteriorly at the level of the eyespots.

Here, the tubes are distended by a few globular concretions of variable size (Fig. 10). The number on each side in a series of twenty-five specimens ranged from none to fifteen, the average being seven. They are relatively stationary in the tubes and sometimes fused together. Some of the concretions appear to be structureless while in others concentric markings can be seen. When the water evaporates from under the cover-glass in fresh preparations the granules break up along their radii into four or five major pieces.

The cercaria of *Diplodiscus temperatus* belongs to a group for which Sewell (1922: 80) proposed the name Diplocotylea. These larval forms, as pointed out by Cort (1915) belong to the subfamily Diplodiscinae Cohn. Sewell (1922) placed in the group Diplocotylea six forms, *Cercaria diplocotylea* Pag., the larval form of *Diplodiscus subclavatus* (Gze.), *Cercaria gastrodisci aegyptiaci* Looss, *C. inhabilis* Cort, *C. diastropha* Cort, *C. cortii* O'Roke, and *C. indicae* XXI Sewell. As pointed out by McCoy (1929: 200), who added *C. missouriensis* to the group, *C. convoluta* Faust must also be placed here. Beaver (1929) added the cercaria of *Allassostoma parvum* Stunkard to the Diplocotylea group, and now the cercaria of *Diplodiscus temperatus* is to be added making a total of ten forms included in the group. These cercariae are all characterized by the presence of pharyngeal pockets with the exception of *C. cortii* in which they are absent. All of the American forms are parasitic in *Helisoma trivolvis*.

The cercaria of *Diplodiscus temperatus* differs from all the other individuals in the group with the exception of *C. missouriensis* and *C. convoluta* either in size and pigmentation or in the number and distribution of the concretions in the excretory tubules. With the exceptions noted, all of the other cercariae in the group have been described or figured with numerous concretions filling the main excretory tubules. As previously pointed out, the number of concretions in the excretory ducts of the cercaria of *Diplodiscus temperatus* may vary from none to fifteen, but they are never numerous and are

always confined to a definite area, never occupying the entire length of the main excretory tubules. In this respect, the present cercaria also differs from *C. missouriensis* for which McCoy (1929: 200) states that, "The number of these large concretions is remarkably constant, six to eight, and since no exceptions were noticed, their presence may even be considered a specific character." *C. missouriensis* likewise differs from the cercaria of *D. temperatus* in body and tail measurements.

The description of *Cercaria convoluta* by Faust (1919) is too inadequate either to identify it with or to differentiate it from the cercaria of *Diplodiscus temperatus* although the measurements are somewhat different. During the present investigation, three series of measurements were made on cercariae. One series, seven in number, killed and measured in ten per cent hot formalin, measured 0.44–0.56 mm. in length (average 0.50 mm.). A second series, consisting of twenty-five cercariae killed in the same way but measured after staining and mounting, measured 0.38–0.49 mm. (average 0.42 mm.). A third series of ten specimens killed with heat measured 0.35–0.50 mm. (average 0.44 mm.). The whole series varied in length from 0.35 to 0.56 mm. Faust (1919) gives the body length of *C. convoluta* as 0.4–0.76 mm. The length of the tail in all three of the measured series of the cercaria of *D. temperatus* measured 0.68–0.82 mm. (average 0.75 mm.). Faust gave the tail length of *C. convoluta* as twice as long as the body. Thus it will be seen that if measurements are of value in the differentiation of species, *C. temperatus* may be considered as distinct from *C. convoluta.*

Adult

The amphistome, *Diplodiscus temperatus* (Fig. 11), whether juvenile or adult, has a deflected cone shape, the deflection indicating the ventral side. The largest parasites when preserved are 6 mm. in length, 2.5 mm. in width, and 2 mm. thick. The greatest width of the body is two-thirds of the distance from the anterior end. The diameter of the posterior sucker in these large ones is about the same as the thickness of the

worm and may have a cavity 1 mm. in depth. The posterior sucker may have a small papilla in the center. The organ is very muscular and mobile, and capable of great variations in its apparent shape, size, and thickness.

The remainder of this description is based on small mature specimens, 2.5 mm. to 3 mm. in length, developed in experimental animals. Both sectioned material and whole mounts were used. The cercariae giving rise to these adults were all obtained from the same snail.

The oral sucker is large, well developed, and globular in a worm moderately contracted. Buccal pouches are present. The openings to the pouches are slightly dorsal to the esophageal opening. The pouches are very contractile, about two-thirds the thickness of the wall of the oral sucker and are somewhat less muscular. The ratio of the length of the oral sucker to that of the buccal pouches in preserved specimens is 3 : 2. The walls of the two pouches fuse for about half their length, but the fusion is not readily discernible except in sectioned material. The rather narrow prepharynx is quite long and rarely straight even in extended specimens, and is surrounded by small, unicellular esophageal glands. It is terminated by a pharyngeal swelling which is more or less oval in shape and only slightly smaller than one of the buccal pouches. The pharynx consists of a mass of circular muscle from 35 to 40 microns in thickness. The wide thick-walled ceca arise immediately behind the pharynx and extend along the sides of the body to near the acetabulum.

The testes, subequal in size, are intercecal and situated in the anterior half of the body, the posterior one extending back to the middle of the body. They may be contiguous and overlapping for about half their length in contracted specimens. They are relatively large in small mature specimens and small in large specimens, and are always as large as the oral sucker or larger. Usually they are somewhat irregular and may appear to be lobed close to the ventral body wall. The anterior end of the vas deferens is muscular, form-

ing the pars prostatica, and opens with the uterus through a common genital pore.

The ovary, situated dorsally in the posterior half of the body, is submedian in position and one-half to two-thirds as large as a testis. The oviduct, which may arise from the side or anterior end of the ovary, extends posteriorly where it is somewhat dilated for holding spermatozoa and narrows again before opening into the oötype. The vitellaria consist of about fifteen follicles on each side of the body, and these extend from the level of the posterior testis to near the acetabulum and are arranged in two groups. The two anterior groups are ventro-lateral while the posterior ones are dorso-lateral. The lateral vitelline ducts open by a common duct into the narrowed portion of the oviduct a short distance posteriorly from the opening of Laurer's canal. The oötype is surrounded by small cells, the Mehlis gland, and continues as the much coiled uterus to the genital pore. In large, mature worms, the uterus occupies all of the available body space between the intestinal ceca. Laurer's canal extends to the dorsal surface of the body above the ovary. The median genital pore opens ventrally at about the level of the pharynx.

The large excretory vesicle which is just anterior to the acetabulum is median and dorsal in position and opens to the dorsal body surface. Deeply staining cells surround the short excretory duct. Large excretory trunks are found laterally which receive branches that form a very complicated system of tubules all through the body.

The lymph system of this parasite has recently been described by Willey (1930).

Experimentation and Life History Determination

Definitive Host.—Mature specimens of *Diplodiscus temperatus* have been taken from four species of naturally infected frogs, *Rana pipiens* (leopard frog), *R. clamitans* (green frog), *R. cantabrigensis* (wood frog), and *R. catesbeiana* (bullfrog), and also from experimentally infected frogs of the first three

species. Stafford (1905) collected this amphistome from *R. pipiens* (Syn. *R. virescens*) and *R. catesbeiana.*

The mature and immature flukes occur in the tadpole as well as in the adult frog, but mature parasites are not numerous in either as a rule, and usually do not grow as large in the tadpole as in the frog. This parasite, which lives in the rectum, is never found in the lower end of the large gut except in extreme instances of crowding as experiments described later indicate. Under crowded conditions it prefers, however, the small intestine to the lower part of the large gut.

Intermediate Host.—*Helisoma trivolvis* serves as the intermediate host. The snail has not been found in abundance in the Ann Arbor or the Douglas Lake region, but a few scattered ones are always found along lake shores, ponds, drainage ditches, and rivers. No other species of *Helisoma* has been found to harbor this parasite.

All attempts to infect *H. trivolvis* experimentally have been negative. Numerous experiments to infect snails ranging in size from newly hatched to mature ones were attempted without success. Such experiments have been tried with miracidia of different ages and by using water of different temperatures, but only negative results have been obtained. In some of the experiments a number of snails were put in a large container and free miracidia or eggs containing miracidia were added to the container. Other snails were kept individually in large shell vials in which were put two or three miracidia or several eggs containing mature miracidia. In another experiment several frogs carrying amphistomes were put into an aquarium with adult *H. trivolvis* and kept together for months, but in all instances the results were negative. According to Looss (1892) snails are easy to infect and begin to shed cercariae in eight weeks if summer heat is maintained, development being slowed down by winter temperatures.

Miracidium.—If large mature flukes are removed from a frog to water, the thin-shelled eggs usually begin to appear in the dish after ten or fifteen minutes. The first eggs deposited contain fully developed miracidia, but if the parasites are

allowed to remain in the water for some time eggs are deposited which contain developing miracidia. Such eggs which were kept in a refrigerator at a temperature of 45° F. continued their development, a few hatching daily over a period of more than a month. This made possible the study of the miracidium without the frequent sacrifice of a host. Looss (1892) stated that such immature eggs continue their development and finally hatch. When small mature parasites are removed from a frog to water, eggs containing immature miracidia are deposited.

Redia.—Since snails were not infected, the mother sporocyst was not observed. The rediae are found in the liver. Several snails harboring an infection were dissected and examined. The extent of the infection varied with the specimens. In hosts with a slight infection one invariably finds that the very tip end of the liver is affected. It seems that this is the seat of the initial infection and that it spreads from this point. In specimens in which at least half the liver is destroyed, one finds by carefully breaking and removing the shell without tearing the mantle that the cercariae in various advanced stages of development greatly outnumber the rediae.

There is also a difference in the distribution of the two larval forms. The cercariae seem to be at the center of the cone of host tissue, while the rediae are more abundant around the periphery just underneath the mantle. Cercariae less than half grown are discharged from the rediae through a birth pore and complete the rest of their development outside of the parent, free in the tissues of the host. At the time of discharge from the rediae, the cercaria have no pigmented eyespots. In snails where infections are not so far advanced, that is, when a third or less of the liver is destroyed, one finds that the tip end is nearly a solid mass of advanced developing cercariae intermingled with a very few rediae. Furthermore, the rediae become more numerous as one proceeds toward the unaffected liver, near which the rediae are very abundant and the cercariae comparatively few in number.

When rediae and cercariae are placed in water or normal saline, activity is seen in all rediae but only in cercariae which are mature or nearly mature. The developing cercariae are not active except for occasional spasmodic contractions in various parts of the body and tail. Mature cercariae move in the usual way. The rediae elongate and contract more or less sluggishly and try to move around by means of their appendages and oral sucker. They have never been observed to make any progress, however, except by elongating and contracting the body. The rediae live on liver tissue with which their gut is usually distended.

Cercaria.—A single infected snail, No. 264, forms the basis for almost all of the infection experiments with cercariae and observations on them. It was collected in Delhi Pool, near Ann Arbor, on October 26, 1929, and was kept in the laboratory until it died on August 2, 1930. While in the laboratory it lived in a large moist chamber 12 inches in diameter, filled with tap water to which was added a handful of pebbles. It was fed on fresh lettuce, and dead leaves. It was very active until two or three days before death. A post-mortem examination showed a complete destruction of liver tissue which was, no doubt, the cause of death.

Cercariae were shed every day during the time the snail was kept in the laboratory. There was a considerable variation from day to day in the number escaping, but there was no marked reduction in the number of cercariae until the last two weeks prior to death. A few cercariae escaped at all hours of the day but in larger numbers in the afternoon. In order to count the cercariae which escaped in the afternoon hours the snail was put in a small shell vial containing a little lettuce and water from the moist chamber in which the snail was kept. Since the cercariae could be easily seen, they were removed with a pipette from the container each half hour and counted. With this procedure it was found that the snail was only slightly disturbed each time. The following table gives the result of the count on the four days that it was made.

TABLE I

SHOWING NUMBER OF CERCARIAE WHICH ESCAPED DURING REGULAR TIME INTERVALS

Time of day	No. cercariae Jan. 18, 1930	No. cercariae Feb. 8, 1930	No. cercariae Feb. 10, 1930	No. cercariae Feb. 12, 1930	Total
11:00 A. M. to 11:30 A. M. ..	0	0	0	2	2
11:30 A. M. to 12:00 noon ..	1	0	0	23	24
12:00 noon to 12:30 P. M. ..	12	0	4	43	59
12:30 P. M. to 1:00 P. M. ..	18	0	14	46	78
1:00 P. M. to 1:30 P. M. ..	38	2	17	32	89
1:30 P. M. to 2:00 P. M. ..	42	9	18	28	97
2:00 P. M. to 2:30 P. M. ..	21	17	9	21	68
2:30 P. M. to 3:00 P. M. ..	12	11	8	37	68
3:00 P. M. to 3:30 P. M. ..	17	3	5	21	46
3:30 P. M. to 4:00 P. M. ..	2	6	5	12	25
4:00 P. M. to 4:30 P. M. ..	0	1	4	7	12
4:30 P. M. to 5:00 P. M. ..	0	0	0	1	1
5:00 P. M. to 5:30 P. M. ..	0	0	0	1	1
Total	163	49	84	274	570

While nothing particularly significant is shown in the table, the apparent regularity of the number of cercariae escaping with the duration of time during which they were shed and the constancy in the time of maximum degree of escape is interesting.

Cercariae were shed in small numbers on dull, cloudy days in spite of the fact that room temperatures were the same. To compensate for lack of light on dull days, a lighted incandescent lamp was placed over the dish in which the snail was kept, but it did not seem to influence the number of cercariae

which escaped. Whether there is actually a correlation between cercaria production and weather conditions is problematic, nevertheless, there is a suggestion of this relationship. These observations are not extensive enough to be conclusive, although additional support of these facts is given by Cort (1922) who made some observations on the escape of cercariae from snails and also noticed a considerable variation from day to day. He observed that there were given times in each day when cercariae escaped and that these times remained the same for a specific kind of cercaria, but differed for different species. He was impressed with the relative constancy of this cycle of escape which is illustrated in the amphistome under discussion. The intervals of time used by Cort are too large and too indefinite to permit an analysis of the hourly production of cercariae.

There is a very definite heliotropic response in these cercariae which can be easily demonstrated with the electric light in the following way. If a number of cercariae are drawn into a pipette which is then held perpendicular to the edge of a lamp shade covering a lighted electric lamp with the full length of the pipette exposed to the light, the cercariae will be seen going back and forth in the tube. If then, the pipette is drawn past the edge of the shade so that the upper end is darkened, the cercariae rapidly move into the lighted part, and never leave the lighted area for any length of time. By continuing to raise the pipette all the cercariae can be concentrated in the very small tip and by squeezing the bulb slightly and quickly, all can be dropped into a watch glass in a single drop of water. If the dish is lighted from above the cercariae swim near the top; if lighted more strongly from below, most of them remain near the bottom.

At first they appear to swim continuously or to stop only long enough to settle momentarily to the bottom of the dish. Cercariae that have been in water for five hours are only slightly slower than those recently emerged, but after this time they begin to show greater effects of fatigue and their move-

ments become progressively slower. They rise shorter distances from the bottom and rest more frequently; their movements become so sluggish that they are unable to rise from the bottom, but they continue to lash their tails while their bodies elongate and contract. The tail is often detached but the body continues to squirm. Finally, movements of the tail cease and the body elongates and contracts until death. After death, the cercariae swell to two or three times their original size.

Several authors have described amphistome cercariae which are very closely related to the one discussed and have noted the sluggishness of the cercariae. While the present form is relatively slow-moving as compared with most fork-tailed cercariae, its movements are not sluggish. When the cercariae first escape from the snail they swim actively but are easily caught with a pipette. They are very awkward looking creatures as they swim by lashing their tails. They have a greenish gray color and are somewhat transparent when examined with the naked eye.

Cercariae are readily entangled in any débris in the water. Certain materials may collect numbers of them. Curiously enough, in spite of the fact that the tails can apparently be detached easily, they are not lost when the cercariae are caught in débris. The cercariae when entangled by foreign material continue their usual movements for some time during which the tails are mutilated, collapsed, and some times twisted to form a threadlike structure.

The conditions under which the cercariae encyst are very interesting. If they are put into a small covered dish of water, only a small percentage, as a rule, encysts. Many poorly shaped cysts are formed under these circumstances by the discharge of the cystogenous material which fixes the cercaria in a certain position that varies greatly with the individual. When this happens the cercariae die in about the same time as those which do not encyst. Those which encyst properly do not usually remain in the cyst very long but crawl out of it and soon die. If cercariae are put into a dish of

shallow water and it is left open so that the water evaporates, the cercariae all encyst perfectly, encystment beginning as soon as the water becomes so shallow that the cercariae are exposed to the atmosphere and cannot swim. Thinking that absence of suitable material on which to encyst was responsible for failure of the cercariae to encyst when plenty of water was present, we placed rough and smooth pebbles, lettuce, and dry leaves in the container in which there were cercariae, but the number which encysted was not noticeably changed. Finally, a small wood frog was placed in a watch glass of water containing twenty-four cercariae and covered with a second watch glass. The cercariae began to encyst on the surface of the frog with great rapidity, and within three minutes all except three had encysted. *R. catesbeiana, R. pipiens,* and *R. clamitans* were tried and served equally well.

The cercariae do not ordinarily encyst on the non-pigmented parts until forced to do so because of lack of room. The cysts do not collect in a mass on the dark spots, but when these are filled more of the cercariae encyst on the lesser pigmented regions and occasionally on the non-pigmented parts of the body. They prefer the dorsal side of the legs, particularly the hind legs, the pigmented region around the forelimbs, and the parts posterior to the eyes. A frog is apparently not inconvenienced by the encystment of cercariae, but one day a cercaria was observed to encyst at the external canthus of the eye, and the frog appeared very much concerned and made unsuccessful attempts to remove the encysting cercaria. Tadpoles are somewhat disturbed by the presence of large numbers of cercariae swimming around them, but there is no indication that the encystment of the cercariae on the body has any effect on the tadpole.

If a cercaria strikes a frog but does not encyst it is slowed down very decidedly or ceases to swim altogether for a moment, acting as if paralyzed or shocked. When one strikes for encystment it apparently strikes "head on" and attaches itself by the oral sucker. Whether or not it hangs on by suction, for which its oral sucker is well fitted, is not known. At the

moment of contact the cercarial body is perpendicular to the host and motionless for a second or two. In some instances it has been noticed that instead of being motionless, the whole cercaria trembles during this time as if injured, then the cercaria flattens out and bends so that the ventral side is very closely applied to the surface of the skin of the host. After a brief pause there is a moderate cupping of the cercaria as a result of a slight pulling away from the host, but at this time the metacercaria is found to be actively moving around in the cyst, while the tail outside of the cyst begins to vibrate rapidly.

The tail may remain attached to the cyst until it disintegrates or may free itself by its great activity. If freed, it moves rapidly in the water and is easily mistaken for a cercaria of another kind. The tail becomes progressively weaker and at the end of twelve minutes is unable to swim. It settles to the bottom of the container, continues to undergo contractions for a while, and in twenty minutes from the time of encystment of the cercaria the tail is inactive and apparently disintegrating as indicated by a change in color.

A metacercaria immediately after encystment moves actively around in the cyst as if in an attempt to make it smooth. This apparently is the only explanation since no appreciable increase in the thickness of the cyst has been noted as a result of this activity. The movements of the metacercariae within the cysts become progressively slower and at the end of twenty minutes most metacercariae are inactive, except that a few make occasional slight adjusting movements. After this almost the only visible evidences of life are the pulsations of the bladder and occasional movements of the buccal pouches.

An experiment to determine the length of life of the metacercaria indicates that this period may be rather short. In this experiment, twenty-four cercariae were forced to encyst in a watch glass by allowing the water to dry up. They were then washed, covered, and set away in water. The water was changed after thirty-six hours and at that time one parasite had crawled half way out of the cyst and was dead. Seven

days later the rest of the parasites had become opaque in the cyst indicating that they were dead.

The cysts hold to the skin of the frog tenaciously. They can hardly be dislodged with a pair of needles. By pinching the skin in a pair of forceps it was possible to force off the cysts. Stained sections of the skin with attached cysts revealed no unusual structures to explain this apparent tenacity. Pieces of frog with skin and with skin removed were tried and were found to serve equally well for encystment. In one experiment, the front legs were cut from a frog, one was skinned and this, with the intact appendage, was put into a watch glass of water containing 50 cercariae. They began to encyst immediately on the black spots, then on the lesser pigmented areas, and as these parts became crowded they encysted on the white parts. In an hour all were encysted, but none were found encysted on the dish, on the cut ends of the appendages, or on the skinned leg. As previously mentioned encysting cercariae prefer the black spots and these areas are usually entirely covered before any number of cercariae encyst on the less pigmented areas.

The cysts vary somewhat in shape and in the tenacity with which they hold to the host. Those which are formed on anything but the skin of a frog are relatively loosely attached. Cysts formed on either rough or smooth glass are easily detached. Those formed on tadpoles are easily rubbed off when the tadpole comes in contact with solids. In fact, they become detached by the mere swimming of the tadpole through the water. The cysts along the back and at the base of the tail are not so easily dislodged and if any are to be found on the tadpole they can be seen in these regions.

An attempt was made to induce the cercariae to encyst on an artificial background having spots. A white piece of paper the size and shape of the bottom of the watch glass was divided in the middle and one-half of it was spotted with black ink to resemble frog skin. A watch glass containing 50 cercariae was placed over the piece of paper. No encystment took place and no response of the cercariae to the black spots

was observed. In another instance two small bluish gray pebbles were washed and after one of them had been rubbed on the back of a frog both were placed in a watch glass containing a large number of cercariae. The cercariae made some response to the pebble rubbed on the frog's back as shown by their striking it and acting for the moment as if they were going to encyst. They made no response to the other pebble, however, and during the three hours the experiment was in progress no encystment took place on either. While other factors may be involved, these experiments seem to show that encystment is prompted by a heliotropic-chemotactic complex.

Encystment can be almost an instantaneous process. One evening a number of cercariae were taken from the water containing the infected snail and transferred to a watch glass. A pipette was then filled with the water from the watch glass containing the cercariae which were concentrated by the method already described and then dropped into a clean, dry watch glass. The cercariae ceased all activity on striking the dish and it was thought for the moment that they had been overheated. Microscopical examination, however, revealed that they had all encysted. An experiment was begun to find out what condition or complex of conditions prompted the encystment. After trying varying distances for the fall of the cercariae from the pipette, temperature changes, weak acid, and cercariae of various ages, this response was not duplicated. It is possible that the rapid spreading of the drop of water on the clean dish prompted the encystment.

The time which elapses between the escape of cercariae from the snail and encystment is a variable one. According to Cawston (1920) the survival time of cercariae after escaping from the snail is short. He says cercariae do not usually live longer than the day on which they commence their aquatic life. Lang (1892) found that the cercariae of *Diplodiscus subclavatus* were active only fifteen hours. Looss (1892) found that these cercariae were able to sustain themselves in the water for twenty-eight hours.

The cercariae of *D. temperatus* seem to be no exception to the rule, but a small percentage live somewhat longer. When kept in watch glasses and fingerbowls at room temperature some of them remain active enough to rise from the bottom of the dish for at least twenty hours, but at the end of that time they are sluggish. Several cercariae have been kept for thirty hours, and at the end of this period they were still lashing their tails but were making no progress and after forty hours all those which had not encysted were dead. The free-living existence of the cercariae may be prolonged for a period of days by reducing the temperature, which does not prevent them from encysting.

Cercariae are able to encyst as soon as they escape from the snail, in fact, it has been found, that when released by dissection of the snail, they are potentially able to undergo encystment as long as they are active. Cercariae will encyst on a microscope slide, covered or uncovered. The metacercariae cannot withstand dessication, and are dead as soon as the drying distorts the cyst. In timed experiments, the metacercariae were dead in 30 minutes after the water evaporated. The cysts are resistant to reagents. Some loose, newly formed cysts were placed in formol-acetic-alcohol and the metacercariae remained active for two and one-half minutes.

As stated on an earlier page, encystment on vegetation, either fresh or decaying, seems to be the exception rather than the rule. No cysts have been found on lettuce, leaves, or stones, but some were occasionally found on the bottom of the dish. The cysts are sticky and readily adhere to teasing needles. Loosened cysts often cohere, collect débris, and are easily entangled in vegetation. They are thin, hyaline, and very tough, the contact side being more flattened than the exposed side. The average diameter of sixteen cysts formed on tadpoles was 0.247 mm., height 0.195 mm. Cysts on the tadpole are decidedly higher than those on the frog.

As pointed out earlier, the tadpole as well as the frog becomes infected. Fuhrmann (1928) states that the frog amphistome is the only trematode in which the cercaria encysts

on the host that it infects. Lang (1892) described the method by which frogs become infected and recovered the parasites from the rectum of the frogs to which he had given pieces of skin on which the cercariae were encysted. When encystment takes place on the frog, infection follows the eating of the skin with the attached cysts. Looss (1892) on the basis of his observations concluded that this was not the only method by which the frog became infected. He stated that frogs do not consume their skin so regularly and so completely as to account for all the infections taking place in this manner. He apparently placed little faith in the phenomenon discovered by Lang. Looss stated that cysts accumulate throughout the summer on the sediment in the water and are taken up chiefly during the winter by their hosts. He claimed that frogs eat the accumulated sediment on the bottom of ponds and obtain the encysted parasites in this way. In support of this conclusion he found that the frogs which he obtained in the winter and early spring had a considerable amount of mud in the digestive system along with thirty young amphistomes of cercarial proportions. In addition he usually found cyst fragments near these parasites. The fact that so many amphistomes were found in the early spring was used by him as an argument against the idea that encystment on the host was the usual means of transfer. Looss stated that on the basis of the explicit data which he presented he omitted feeding experiments. Had Looss carried out some experiments he, no doubt, would have been convinced that the method described by Lang was as important as his own.

A large bullfrog which was kept from August until January in the laboratory at room temperature and away from possible infections was examined. Many small amphistomes, some of cercarial proportions, were found in the rectum.

The amphistomes found in naturally infected *Rana cantabrigensis* in March have usually been adults although occasionally some were very small. Throughout the summer amphistomes of all sizes may be found in the frogs and tadpoles, but in the fall, as late as October, the large adult parasites become very scarce.

Many wood frogs and leopard frogs collected and examined a day or so after they came out of hibernation had no mud in the small intestine, but the large intestine in many cases was full of black material which seemed to be mostly mud. Early in the spring frogs eat earthworms almost exclusively and much dirt is present in the intestine. Many times, however, this material also contains insect remains and almost invariably, large pieces of *stratum corneum* have been found in the posterior end of the small intestine. Whether this enters the digestive system prior to hibernation or during hibernation we are not prepared to say. If frogs are collected in the fall and kept in the laboratory during the winter, the content of the large intestine is the same as that found in frogs after they come out of hibernation in the spring. Remains of cysts under these conditions have not been found, but the exceedingly small number of parasites accounts for this condition.

Snails collected in March and brought into the laboratory immediately began to give off cercariae. Snails kept at low temperatures in a refrigerator also gave off cercariae which were able to swim and even encyst; thus it appears that cercariae are present when the frogs come out of hibernation. In a temporary pond about a hundred feet in diameter and not over three feet deep where considerable collecting was done throughout the early spring, the infected wood and leopard frogs were rather numerous. One spring efforts were made to secure *Helisoma trivolvis* from this pool and after using various methods of collecting only five specimens were obtained, two of which were infected. It is possible but not probable that the wood and leopard frogs would eat enough mud, as Looss (1892) claimed, to get their infections where such a small number of snails existed.

A series of experiments has shown that the methods described thus far are not the only ways by which the hosts may become infected. In one experiment six small wood frogs raised from eggs under trematode-free conditions were placed in a covered moist chamber with a snail which was actively shedding cercariae. The frogs were between 18 and 20 mm.

in length. Sixteen days after the experiment was begun one frog was found dead and 12 amphistomes were recovered from its rectum. These worms varied in size, some showed considerable growth while others had cercarial proportions. *Stratum corneum* was found in the rectum of the frog. Six empty cysts, each with a slitlike opening, were found in the rectum, which showed that the metacercariae did not escape as a result of the cyst being digested by the host, but freed themselves as a result of their own efforts. A second frog was found near death on the same day (16 days after the experiment was begun). A small piece of *stratum corneum,* to which three cysts were attached, was hanging from its mouth. The digestive system was carefully opened so as not to disturb its contents. The devoured skin of the frog continued in one piece to the posterior end of the stomach. Twenty-four cysts were attached and tangled up in it, the parasites alive and inactive in the cysts. The terminal part of the small intestine and the rectum were distended with immature amphistomes, only a few of which had blood in the ceca indicating that these were the only ones which had taken food. Ninety-six amphistomes were recovered from the rectum. This was the heaviest infection found in any of the many naturally or experimentally infected frogs and tadpoles examined. Some of the parasites were attached to the terminal part of the small gut, which unusual situation was attributed to the crowded condition of the rectum, but the lower part of the large gut was comparatively free from infection. A third experimental frog was then examined externally under a binocular microscope and cysts were observed on the pigmented parts of the body. These facts prove conclusively that frogs infect themselves by eating the *stratum corneum.* In our estimation this is the usual mode of infection of frogs while other methods are only secondary or accidental.

Infection in tadpoles takes place in a different way. The cysts formed by the cercariae are not firmly fastened to the tadpole, but usually drop off or are brushed off soon after they are formed. The cysts, being sticky, clump together,

become entangled in débris or may gather particles of material around them. To determine whether the encysted parasites which formed on the tadpoles and dropped off were infective, several cercariae were added to a watch glass of water, containing a wood frog tadpole raised in the laboratory. As the cercariae encysted on the tadpole and dropped off, the cysts were removed with a dropper and transferred to one of six finger bowls, each of which contained a wood frog tadpole which had been raised under controlled conditions. Since it had been previously found that the metacercariae sometimes crawled out of the cysts and died, the tadpoles in this experiment were starved for several days prior to the time they were used so that they would feed immediately after the encysted parasites were put with them, thus reducing possible mortality of the parasites. The tadpoles were examined several days later and all had amphistomes of cercarial proportions. This shows that tadpoles may become infected by picking up loose cysts.

Several laboratory-raised wood frogs were fed cercariae with a pipette and the process watched under a binocular microscope to make sure that the cercariae did not escape. The frogs were examined after several days, and very young amphistomes were recovered from the rectum. This suggested a new mode of infection in the tadpoles, and the following experiments were undertaken.

A large wood frog tadpole raised in the laboratory was placed in a watch glass containing water and after the larva had become quiet some cercariae were placed in the water. They disappeared one by one as they were drawn into the mouth of the tadpole in the respiratory currents and did not reappear. In a second experiment with another laboratory-raised tadpole, nine of eleven cercariae disappeared in ten minutes and were swallowed by the tadpole after they had been taken into the mouth by the respiratory currents. This tadpole was examined 24 hours later and all of the larval flukes were out of the cysts and attached to the wall of the rectum.

In order to determine what happened to the cercariae and encysted metacercariae which were fed to frogs and tadpoles, additional experiments were undertaken. A medium sized leopard frog was pithed, the anterior part of the digestive system quickly opened and while the frog was ventral side up in a dissecting pan a drop of water containing cercariae was placed near the anterior end of the roof of the mouth. The cercariae remained active until most of the water had been moved down the esophagus by the cilia; then they very suddenly encysted, and the cysts were carried by the ciliary currents to the stomach. A second small leopard frog was fed eight cercariae with a dropper; after five hours it was examined and two cysts were still in the mouth, five were in the stomach and duodenum, and one parasite was out of the cyst in the ileum. A third leopard frog (48 mm. long) which had been kept in the aquarium room without food for four months during the winter, was fed 75 cercariae with a dropper. Six hours later all of the parasites were found in properly formed cysts in the stomach. Finally a well fed leopard frog which had been kept in the laboratory several months was given a piece of frog's leg, the skin of which was covered with freshly formed cysts. Twelve hours later the skin was found in the ileum, the rest of the leg in the stomach. Ten encysted metacercariae were found midway in the intestine, five free metacercariae in the ileum, and the rectum was full of very young parasites and empty cysts.

These experiments show that the cercariae encyst and are infective when taken into the mouth of the host and this, no doubt, is an important mode of infection in tadpoles. They also bring out the fact that the metacercariae escape from the cyst in the terminal part of the small intestine. Frogs may be infected with cercariae experimentally, but in nature such infections would be accidental.

Six attempts at rectal infections with cercariae were negative. The cercariae in a small amount of water were inserted directly into the rectum through the anal opening. One half of the frogs were examined eighteen hours after the cercariae

had been administered and the others were examined in three days.

In the experiments detailed above, approximately 75 frogs and tadpoles most of which had been laboratory-raised, or had been collected as tadpoles from an acid lake and allowed to transform under laboratory conditions, were used in experiments. Details concerning the acid lake are given by Krull (1931). Control animals were kept for experiments even when laboratory-raised specimens were used.

Rate of growth and time of maturity of the parasite in the final host are very problematic subjects. In the first place, it is very difficult to determine just when the parasites are mature because of their thickness and mobility. They mature, however, when still rather small and under certain conditions grow to be four or five times larger than they are at maturity. A group of frogs was given cercariae or encysted metacercariae and examined at irregular intervals thereafter and the parasites preserved. This procedure was repeated with other frogs, and the size of the parasites in the two series was compared. These experiments revealed no constancy in the growth rate of the parasites. It was found that in the same number of days, the parasites were small in one frog and much larger in another frog. The parasites from some frogs had ceca filled with blood while the ceca of parasites from another frog were empty. In some cases parasites appeared to be abnormal because the blood in the ceca was pinkish instead of reddish. When such specimens were placed under a cover glass, the pressure forced from the ceca great numbers of very small protozoans which were responsible for the color. In several cases, material that resembled fecal material filled the ceca of worms which otherwise looked perfectly healthy.

It appears that the presence of a large number of parasites decreases the rate of growth of the individual worms, but the rate is very difficult to measure since it seems that some frogs are better hosts than others and are able to support more parasites. In exceptional cases, if the parasites were not too numerous in the frog, a number of eggs was found in the

uterus of some of the flukes in three weeks. One frog examined 27 days after infection contained two amphistomes, each of which gave off two or three eggs containing mature miracidia. Two or three months was the usual length of time necessary for these parasites to reach maturity in experimental frogs. If the parasites were numerous, fifteen or more, three to four months was needed and then only one or two specimens were mature, while the rest showed very little growth. Looss (1892) found that the development of *Diplodiscus subclavatus* also took place slowly.

Fully grown mature amphistomes are usually not found in abundance in the rectum. From one to three specimens are usually found, but in bullfrogs and green frogs we have in exceptional cases found as many as ten. Smaller parasites, some mature but not fully grown, are found in large numbers. From these facts and experimental data it must be concluded that the rectum is generally able to support only a small number of fully grown adults, and consequently, the mortality of these parasites after entering the frogs in nature must be great. Looss (1892) found practically the same condition concerning the number of flukes; usually he found the large adults singly and only once did he find two in a large frog.

The eyespots characteristic of the cercariae and metacercariae persist a long time after the fluke is in the frog. The age of the parasite apparently has little to do with the loss of eyespots; growth seems to be the determining factor. As the parasite grows larger the eyespots become smaller and disappear at about the time when the first eggs with fully developed miracidia are present.

Experiments have been carried out to determine what happens to the parasites during the metamorphosis of the infected tadpole. By a series of comparisons of frogs which were infected while tadpoles with frogs and tadpoles infected before or after metamorphosis, it appears that the number of parasites is very materially reduced during metamorphosis; in many cases all flukes are eliminated, but in some several remain. By dissecting naturally infected frogs during the

time of metamorphosis it has been found that, when the parasites are quite numerous, they migrate to all parts of the intestine as far forward as the stomach. This dispersal continues as long as the tadpole type of gut remains, but as soon as the frog gut is formed the amphistomes which remain are confined to the rectum.

Experiments designed to determine the length of life of the parasite in the frog were molested and all the frogs set free. From examinations of hosts kept from one season to the next it seems that the length of life of the parasite is quite variable, but in general, it appears that the parasites live in the frog from one summer to the next when the frog becomes reinfected.

Summary

The life history of *Diplodiscus temperatus* Stafford has been established and the morphology of the stages described.

Laboratory-raised *R. cantabrigensis* tadpoles and frogs were used as final hosts in establishing this life history.

Trematode-free *R. pipiens* and *R. clamitans* were also infected in the laboratory.

Frogs become naturally infected by devouring their own *stratum corneum* on which the cercariae encyst. Tadpoles become naturally infected by eating encysted metacercariae which they pick up with pond ooze or by taking the active cercariae into the mouth in respiration.

When cercariae are eaten by the final host the cercariae encyst while being carried to the stomach.

Parasite cysts on frog skin are firmly attached while those on tadpoles are very loosely attached and drop off or are scraped off very readily.

Cercariae exhibit a decided positive heliotropism and are easily concentrated in a small amount of water by using light as a stimulus.

Encystment of cercariae seems to be produced by a heliotropic-chemotactic complex.

Adults with eggs containing mature miracidia can occasionally be produced in a month under laboratory conditions, but the usual time is two or three months.

Metamorphosis of the tadpole brings about a migration of the flukes into all parts of the intestine during which time many are lost. In the frog or tadpole they are usually confined to the rectum.

The cercariae prefer the pigmented areas on the frogs and tadpoles for encystment.

Helisoma trivolvis (Say) is the only snail which has been found to be infected by this species of parasite.

The escape of cercariae from the snail is cyclic, the height of escape being between one and two o'clock in the afternoon.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Egg shell.
Fig. 2. Egg containing mature miracidium.
Fig. 3. Redia, showing pharynx, gut, flame cells, appendages, and developing cercariae.
Fig. 4. Redia, showing glandular structures at the anterior end.
Fig. 5. Cercaria, side view.
Fig. 6. Miracidium, showing arrangement of ciliated epidermal cells.
Fig. 7. Miracidium, combining optical sections at various levels to show details of structure.
Fig. 8. Miracidium, showing gut and excretory system.

PLATE I

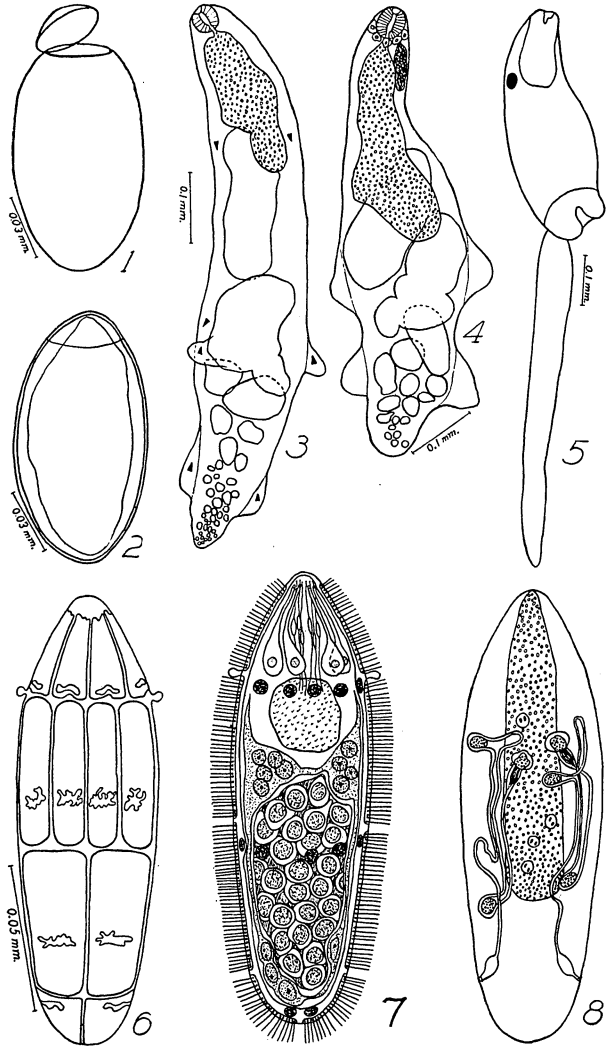

PLATE II

Fig. 9. Cercaria, ventral view, showing internal structures.

Fig. 10. Cercarial body, ventral view, showing the main excretory channels and their connections as far as could be determined.

Fig. 11. Adult, ventral view, showing the main structures, coils of the uterus having been omitted for the sake of clarity.

PLATE II