THE MATURATION OF CLINOSTOMUM COMPLANATUM (Rudolphi 1814) IN SOME MAMMALIAN HOSTS

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ABSTRACT

1. Cats could be infected with C. complanatum by feeding them encysted metacercariae. Though the worms did not produce eggs, they showed signs of maturation. Less than two percent of the metacercariae fed were recovered from cats sacrificed one to four days after feeding.

2. Completely negative results were obtained in feeding excysted metacercariae to rats and further experiments of this kind seem unwarranted.

3. Metacercariae, introduced into the abdominal cavity of LAF, C57L, and A/Jax mice, mature and produce eggs within three to four days.

4. The hosts react to this infection by encapsulating the worms. In C57L and LAF, mice this process was well advanced five to seven days after infection. In A/Jax mice the capsule developed in eight to ten days. The life span of the trematodes in these mice was therefore longer.

5. Metacercariae placed in the peritoneal cavity of male mice had a definite predilection for the scrotum, where they were found attached to both the testes and the scrotal wall. In unilaterally castrated males the worms showed no preference for either the castrated or the un-

castrated side of the scrotum.

6. Several trematodes whose intestines were damaged during mechanical extraction from cysts matured in mice and produced eggs.

7. Rudolphi described Distoma complanatum in 1814 and the morphologically identical D. marginatum in 1819. This last name is therefore regarded herein as a junior synonym of D. complanatum Rud. 1814.

I.

INTRODUCTION

Clinostomum complanatum (Rudolphi, 1814) or the "yellow grub", a trematode parasite of birds, has fresh water fish as its second intermediate host and is therefore a nuisance to fishermen (see Table I.). The presence of the yellowish metacercariae, 3 to 6 mm. long, in the flesh of the fishes is unsightly and is a matter of concern to fisheries management officials.

C. complanatum utilizes planorbid snails of the genus Helisoma as first intermediate host, and fish-eating aquatic birds as definitive hosts (see Table I.).

C. complanatum may be to some extent pathogenic to the definitive hosts. Its oral sucker is surrounded by a group of glands which produce a substance causing a strong local hyperemia (4). The parasite feeds on blood, and causes a deep lesion which bleeds profusely. Representatives of the genus Clinostomum are basically bird parasites. However, Tubangui (60) described C. abdoni from the mouth of a cat in Mindanao, Philippines, Bhalerao (7) found C. kalappahi in the mouth of a cat in India, and Ortlepp (43) recovered C. falcatum from the mouth of a cat in South Africa. The validity of these three species is doubtful and probably they are junior synonyms of C. complanatum. Yamashita, (67) gave a good drawing of C. complanatum from the pharynx of a woman

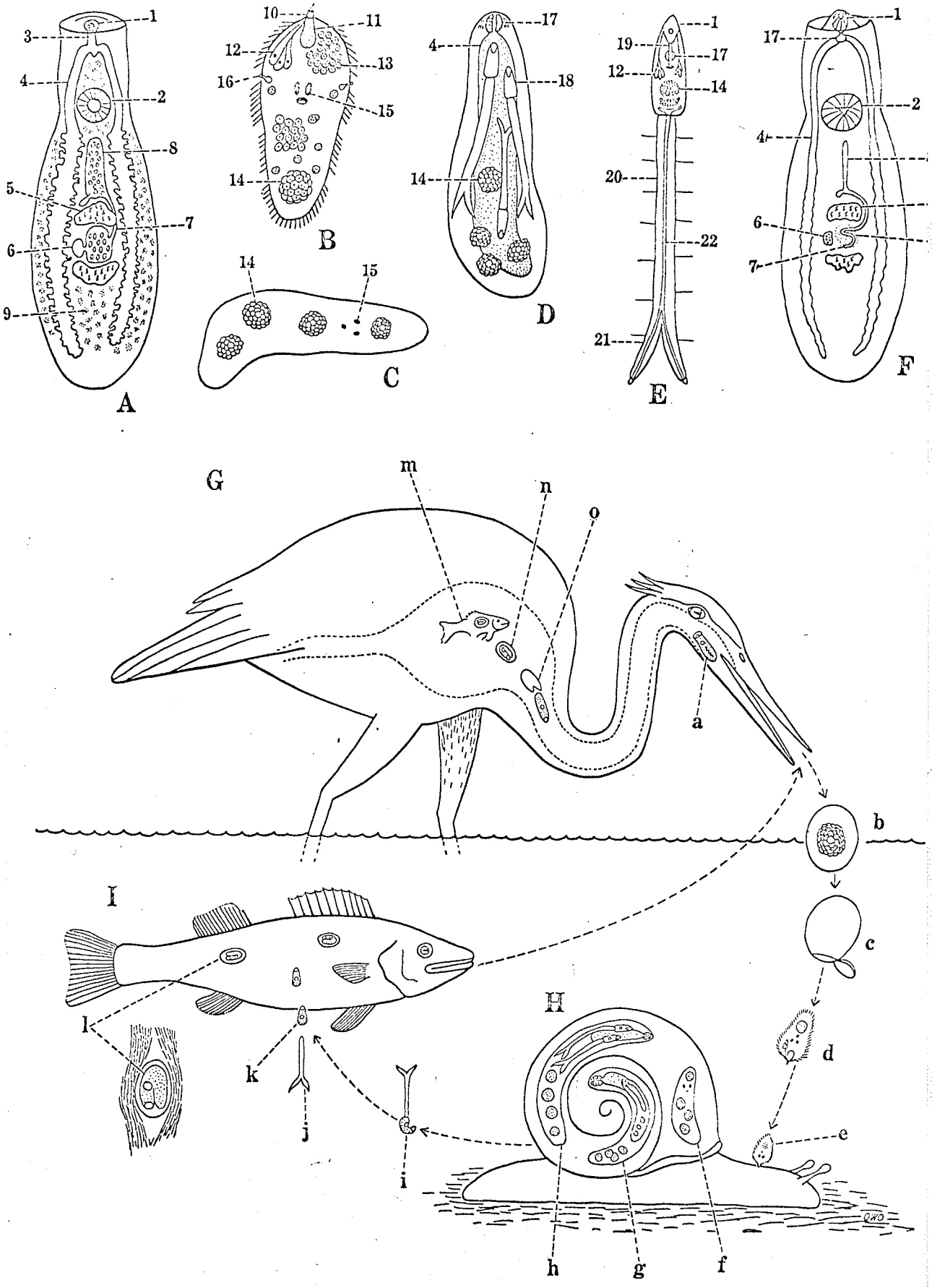
EXPLANATION OF PLATE I.

A. Adult fluke. B. Miracidium. C. Mother sporocyst. D. Daughter redia. E. Cercaria. F. Metacercaria. G. Great blue heron (*Ardea herodias*). H. Snail (*Helisoma companulatum*, *H. antrosa*) first intermediate host. I. Fish (*Perca flavescens*) second intermediate host.

1, oral sucker; 2, ventral sucker; 3, oesophagus; 4, intestine; 5, testes; 6, ovary; 7, oviduct; 8, uterus; 9, vitelline gland; 10, apical papilla; 11, apical gland; 12, penetration glands; 13, large nucleated cells; 14, germinal mass; 15, eyespot; 16, lateral papilla; 17, pharynx; 18, cercaria; 19, prepharynx; 20, tail; 21, short furca; 22, excretory tubule of tail: 23, Mehlis' gland.

a, adult fluke in mouth of heron; b, unembryonated eggs laid in mouth are washed into water as heron strikes at fish and some are swallowed and expelled in faeces; c, eggs develop in water and hatch; d, free-swimming miracidium with single germ ball inside; e, miracidium penetrating snail intermediate host; f, mother sporocyst with developing mother rediae; g, mature mother redia with developing daughter rediae; h, daughter redia containing cercariae in various stages of development; i, cercaria resting in characteristic position in water (note fin over dorsal side of body); j, cercaria penetrating fish, leaving tail behind; k, cercaria migrating in subcutaneous tissues; l, metacercaria encysted in muscles of fish; m, heron becomes infected upon swallowing fish harboring metacercariae which are released in stomach by digestive juices; n, metacercaria freed from tissues: o, young fluke escaping from cyst migrates anteriorly through oesophagus and pharynx into oral cavity where it develops to maturity in three days and remains for about two weeks when it is lost.

This explanation and Plate I have been taken from Olsen (42) who referred to *Clinostomum complanatum* as *Clinostomum campanulatum* (Rud.1819). This is obvious *lapsus calami* and should read *Clinostomum complanatum* (Rud.1814).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in Osaka, Japan. Witenberg (62) identified as C. complanatum a worm found by Gorshiov and Marshak in the throat of a patient from Tiberias who expectorated blood. Witenberg pointed out that "Halzoun", a laryngopharyngitis of mammals, including man, is caused in the Near East by C. complanatum as well as by the leech Limnatis nilotica (Savigny, 1820) but not by Fasciola hepatica (L.1758) as suggested by Khuri (vide Witenberg 1944). In addition Kamo, Ogino and Hatsushika in Japan (31) described "A unique infection of man with Clinostomum sp., a small trematode causing acute laryngitis." This trematode is almost certainly C. complanatum.

Mammalian infections with Clinostomum are summarized in the following table:

Host	Parasite	
man	Clinostomum complanatum	Yamashita (67) Witenberg (62)
	C. sp.	Kamo, Ogino and Hatsushika (31)
cat	C. abdoni	Tubangui (60)
	C. kalappahi	Bhalerao (7)
	C. falsatum	Ortlepp (43)

Only one clinostomid genus, Nephrocephalus Odhner, 1902, has been reported exclusively from mammals. However, many trematodes of birds are incidental parasites of mammals. The family Heterophyidae (Appendix I) is an example

of such parasites. Twenty-four species of this family were reported from both birds and mammals. Other families of trematodes as e.g. Strigeidae, Microphallidae, Cyathocotylidae, Echinostomidae and Psilostomidae also have species that parasitize both birds and mammals.

The above evidence led to the suggestion that C. complanatum might infect mammals and even mature in them. The present study was made to test this hypothesis. Furthermore, fishermen in northern Canada often feed fish to their dogs; it is possible that Clinostomum metacercariae may cause pharyngitis and even asphyxiation in these animals. Evans (18) a graduate student in this department fed perch infected with Clinostomum metacercariae to a cat. Two days later the cat was dead. Upon dissection Evans found 9 metacercariae in the larynx of the cat and he believed that the cat had died of asphyxiation. This led to the present study of the behaviour of the metacercariae of C. complanatum in some mammals and an attempt to bring them to maturity in these abnormal hosts.

The systematic position of Clinostomum is as follows:

Kingdom -- Animalia
Subkingdom -- Metazoa Haeckel, 1874
Grade -- Bilateria Hatschek, 1888
Superphylum -- Acoelomata Schimkevitch, 1891

Phylum -- Platyhelminthes Minot, 1876

Class -- Trematoda Rudolphi, 1808

Order -- Digenea Steenstrup, 1842

Family -- Clinostomidae Luhe, 1901

Subfamily -- Clinostominae Pratt, 1902

Genus -- Clinostomum Leidy, 1856

species -- complanatum Rudolphi, 1814

The proper scientific name is Clinostomum complanatum (Rud.1814) Braun, 1899.

Description of C. complanatum by Baer (4)(translated from French)

Body length 3-8 mm.; maximum width 3.6 mm.; ventral sucker 0.8 mm. in diameter; spinous cuticle; sex glands medial. Testes are in tandem, lobed, wider than long. Ovary and ootype situated between the testes, ovary smaller than testes; vitelline vesicle present; sex opening to the right from the mid-line, on the level of the anterior portion of the testes. Uteroduct joins uterus near its posterior portion. Uterus extends to the level of the ventral sucker. Vitellariae peripheral and extend anteriorly to the posterior margin of the ventral sucker and posteriorly past the sex glands.

TABLE I

List of North American 1st intermediate, 2nd intermediate and definitive hosts of *Clinostomum complanatum*.

1st intermediate hosts (37)	2nd intermediate hosts (1)	Definitive hosts (59)
Helisoma antrosa (Menke)	*Salmo clarki* Richardson	*Casmerodius albus* (Linnaeus)
Helisoma companulata (Say)	*Salmo gairdneri* Richardson	*Ardea herodias* Linnaeus
Helisoma trivolvis (Say)	*Semotilus atromaculatus* (Mitchill)	*Ardea cinerea* Linnaeus
	Ictalurus nebulosus (Le Sueur)	*Mycteria americana* Linnaeus
	Aphredoderus sayanus (Gilliams)	*Nycticorax nycticorax* (Linnaeus)
	Lepomis auritus (Linnaeus)	*Nyctanassa violacea* (Linnaeus)
	Lepomis gibbosus (Linnaeus)	
	Lepomis macrochirus Rafinesque	
	Chaenobryttus gulosus (Cuvier)	
	Ambloplites rupestris (Rafinesque)	
	Micropterus dolomieui Lacepede	
	Perca flavescens (Mitchill)	
	Stizostedion vitreum vitreum (Mitchill)	

II.

HISTORICAL REVIEW

Clinostomum complanatum was originally described by Rudolphi in 1814 as Distoma complanatum (51). In 1856 (34) Leidy found a trematode near Philadelphia, Pa. in cysts on the gills of the sun-fish, Eupomotis vulgaris (L. 1758) and later in the intestine of a pike. Leidy (34) established a new genus, Clinostomum, for it, and named this trematode C. gracile Leidy, 1856. Looss (36) subdivided the genus Distoma Retzius, 1786 into several genera and recognized the validity of Leidy's Clinostomum. Braun (8) placed eight species of the genus Distoma, including D. complanatum, into this genus. The name D. complanatum Rud. 1814 thus became Clinostomum complanatum (Rud.1814) Braun 1899 (8). Braun gave the date of description of D. complanatum by Rudolphi as 1819. The correct date of Rudolphi's description is 1814, as will be shown later in this chapter.

Wright (63) found encysted trematodes on the gills, branchiostegal membranes and pectoral fins of the yellow perch, P. flavescens, at Toronto, Ontario. He recognized these worms as C. gracile Leidy and referred to them as Distoma gracile. He also found mature "D. gracile" in the bittern Botaurus minor, a fish-eating bird.

Two names, Clinostomum complanatum and C. marginatum, are in current use for the designation of the most common species of the yellow grub and it is not obvious which is

the correct name. American authors prefer the name C. marginatum, European and other authors, C. complanatum. Moreover the years of description of these two species cited by various authors vary greatly, as the following list shows:

C. complanatum (Rud.1809)....Baer (4), Dawes (16).

C. complanatum (Rud.1819)....Braun (8), Feizullaev (19), Skrjabin (54), and Yamaguti (66).

C. marginatum (Rud.1819).....Braun (8), Osborn (44), Richard (48), Uzmann and Douglas (61).

Cort (13), Hunter and Hunter (27, 28, 29), Cort, Ameel and Van der Woude (15) and Klass (32) are authors who use the name C. marginatum (Rud.) but give no date. Fischthal (24), and Elliott and Russert (17) use the name C. marginatum but give no authority or date. Yamashita (67) and Witenberg (62) use the name C. complanatum but give no authority or date.

The year of description of C. complanatum has been given by various authors as 1809 and 1819 and of C. marginatum as 1819 (see previous list). These discrepancies in the date of description cause confusion as to the priority of the name. To find out the correct date of description of these two species it was necessary to study the original descriptions by Rudolphi as well as the subsequent literature. A thorough perusal of

Rudolphi's publication "Entozoorum sive vermium intestinalium historia naturalis" of 1809 (50) showed that the names Distoma complanatum and D. marginatum did not appear among the 82 species listed (see Appendix I). However, both names are present in Rudolphi's "Entozoorum synopsis" of 1819 (52), D. complanatum on page 376 and D. marginatum in the appendix on page 680. Both species are designated as "R. n. sp." and could be thus regarded as first descriptions. However, in the early portion of the 19th Century many authors used the designation "n. sp." repeatedly in several publications; thus it became necessary to check Rudolphi's papers published between 1809 and 1819.

In 1814 Rudolphi's (51) "Erster Nachtrag zu meiner Naturgeschichte der Eingeweidewurmer" appeared, in which D. complanatum was described (p.103 l.c.). This description reads as follows:

> "58. Distoma complanatum R. Nova species Entoz. 11.1.p.373. pone n.15. inserenda.
> Distoma: depressum, oblongum, antice subattenuatum, pori antici apertura orbiculari, ventralis majoris longitudinali.
> Hab. In Ardeae cinereae oesophago am. Rosenthal Aprili mense me suadente in vermes inquisivit et quatuor reperit Distomata.
> Descr. Vermes unam cum dimidia ad duas cum dimidia lineas longi, vix dimidiam lati, albi punctis nigris varii.

Corpus oblongum, complanatum, antice perum attenuatum, postice obtusum, marginibus obtusiusculis. Poruanticus terminalis ad inferiora paulumum vergens, margine tumido, apertura orbiculari, magna; ventralis lineae quartam partem distans, major, margine tumido, apertura mox triangulari (apice infero), mox oblonga. Ovaria utrinue decurrentia, vase fusco medio ante corporis apicem posticum coeuntia. Cirrus non visus.

Obs. Species tam Distomati hianti quam D. heterostomati affinis, ad hoc tamen et corporis forma et pororum ratione diversa, ab illo vero corpore tenui, albo, poris aliter comparatis abunde differt."

A translation of this description is as follows:

"58. Distoma complanatum R. Nova species. To be inserted into Entozoorum Historia Naturalis volume 2, part i, page 373 after species number 15.

Distoma: flattened, elongated, slightly attenuated anteriorly, the opening of the anterior pore circular, that of the ventral pore mostly longitudinal.

Habitat: On my urging Rosenthal studied parasitic worms and found four Distoma in the oesophagus of Ardea cinereae, in the month of April

Description: Worms from 1½ to 2½ lines long and about ½ line wide, white, with black dots.

Body elongate, compressed, with slightly attenuated anterior end; posterior end blunted, margins slightly rounded. The anterior pore terminal directed slightly ventrad with a swollen margin and a large round aperture. Ovaries running on both sides and joined by a dark medial vessel just before the posterior end of the body. Cirrus not seen.

Observations: Species related to both D. hians and D. heterostomum, but differing from both in the shape of the body which is narrow and white, and in the relative sizes of the pores."

As D. complanatum was not described by Rudolphi in the 1809 paper (50) we must regard the above description as the first of the species; thus the proper name of D. complanatum is Clinostomum complanatum (Rud.1814) Braun 1899.

D. marginatum, however, is not mentioned in Rudolphi papers of 1809 (50) and 1814 (51) and Rudolphi's description of this species published in 1819 (52) is the first. The proper name of D. marginatum is thus Clinostomum marginatum (Rud.1819), Braun 1899.

Braun (9) regarded C. complanatum and C. marginatum as two distinct species and discussed the morphological differences in his paper of 1900. He said that C. complanatum was short and white but not as wide or as flat as C. marginatum. He used these criteria to separate the species in the type material of Rudolphi. He pointed out other morphological differences: in C. complanatum the neck is short and the ventral sucker close to the anterior end, the genital pore considerably displaced to the right from the mid-line, and the anterior ends of the vitellariae much farther from the anterior end of the body and their posterior ends farther from the posterior end of the body, than in C. marginatum.

Baer (4), however, wrote (translated from French):

> "We regard C. marginatum as being a synonym of C. complanatum as it is impossible to

> distinguish them morphologically one from the other; the metacercarial stage of both are found in related fishes and the adult stages in the same genus of birds, Ardea. We do not think that the geographical distribution is sufficient to separate these two species. The morphological differences indicated by Braun (1901)[1] are subject to large individual variation. The specific name marginatum must fall before complanatum as this last name has the priority of pages in the work of Rudolphi."

I agree basically with the view of Baer who regards C. marginatum as a synonym of C. complanatum. It must be pointed out, however, that this is not a priority of pages, as Baer thought, but a priority of years, as C. complanatum was described by Rudolphi in 1814 (51) and C. marginatum in 1819 (52). There certainly are no reliable morphological characteristics which would serve to differentiate these two species. It must be stressed, however, that the type locality of C. complanatum is Germany, whereas the type locality of C. marginatum is Brazil. This may explain why American authors prefer the name C. marginatum, whereas European authors prefer C. complanatum.

Krull (33) described the cercarial and redial

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1. Braun's paper "Die Arten der Gattung Clinostomum Leidy," appeared in Vol.14 of Zoologische Jahrbucher whose title page was printed in 1901. However the first issue of this volume in which this paper was published appeared in 1900.

stages of Clinostomum complanatum (Rud.1814) from a naturally infected planorbid snail, Helisoma antrosa (Menke). The cercariae from snails were used to infect the pumpkin-seed fish, Eupomotis gibbosus (L. 1758) Centrarchidae and the resulting metacercariae fed to a young black-crowned night heron, Nycticorax nycticorax naevius (Bodd.). Eggs appeared in the faeces three days later. Krull thus demonstrated for the first time the complete life history of a member of this genus. Hunter and Hunter (27) reported in detail their experiments on the life history of C. complanatum (their C. marginatum) confirming the findings of Krull.

In 1911 Osborn (44) published a paper on the distribution and "mode of occurrence" of "C. marginatum" and in 1912 another paper on its morphology (45). He called this trematode "a parasite of the frog, bass and heron". It seems, however, that he confused two species. Indeed, Cort (12) showed that specimens from frogs differ from those from fish sufficiently to be considered distinct species. He thought that the bittern served as a definitive host for the trematodes from frogs which he described as C. attenuatum. Hunter and Hunter (28) carried out feeding experiments with metacercariae both from frogs and fish using bitterns and herons and confirmed Cort's contention that the worm from frogs is

a separate species.

Price (46) tentatively identified a trematode from the tracheal mucosa of a fowl as C. attenuatum, and thus showed that this parasite could develop to maturity in chickens. Manter in 1937 (38) found an immature specimen of C. attenuatum in the small intestine of a pigeon and suggested that the pigeon had eaten what to him looked like seeds in the body of a dissected frog. These two cases are examples of accidental parasitism.

Encysted metacercariae of "C. marginatum" survive in their fish host for a long time. Fischthal (24) described the overwintering of "yellow grub" in perch, bass, bluegills and pumpkin-seed fishes and stated that the loss of "grubs" in winter was negligible. Uzmann and Douglas (61) reported that trout kept for ninteen months in captivity yielded many vigorous metacercariae upon dissection. These authors also were the first to report C. complanatum from Pacific slope waters.

Klass (32) carried out an ecological investigation of C. complanatum near a heron nesting colony in Kansas. This investigation dealt with the foraging movements of the herons, the incidence of infected fish and snails and the distribution and location of "yellow grubs" in the fish. Klass found that there was no apparent relation-

ships between "yellow grub" infections and size and abundance of fish. He suggested a reduction of the submergent vegetation in ponds as a natural means of partial control rather than the impractical elimination of herons or snails.

Although the morphology, life cycle, ecology and possible economic importance of C. complanatum have been studied, no attempts have been made to infect mammals with this trematode experimentally. Thus this present study on the maturation of C. complanatum in some mammals.

III.

REVIEW OF THE FAMILY CLINOSTOMIDAE

The family Clinostomidae is divided into two sub-families, Clinostominae, parasitizing birds and Harmotreminae parasitizing reptiles. A review of the first sub-family, especially of the type genus Clinostomum, based on that given by Skrjabin (54) follows:

Clinostomidae Lühe 1901

Clinostominae Pratt, 1902. Diagnosis-Oral zone provided with accessory musculature. Pharynx usually rudimentary, but may be well developed. Caeca often with numerous diverticula. Ventral sucker always well developed. Parenchyma with numerous glandular cells. Excretory bladder V- or Y-shaped. Collecting tubules much branched, especially in anterior portions of the body. Sex organs mostly in the posterior half of the body. Ovary situated between the testes. Sexual opening mostly near the mid-line of ventral surface, usually in the posterior quarter of the body.

Cirrus sac pear-shaped, without prostatic portion, or long and with a prostatic portion. Laurer's canal present. Receptaculum seminis absent. Vitelline reservoir mostly present.

Uterus is usually sac-shaped and situated between the sexual glands and the ventral sucker. It may be

provided with diverticula. As a rule the uteroduct opens into the uterus laterally and at a distance from the ventral sucker. The uterus cylindrical or hose-shaped and always has an ascending and descending portion. Terminal portion of the uterus opens into the common genital pore. Vitellariae consist of small follicles situated peripherally to the sex organs. Eggs are large and often fully embryonated when deposited. Adults are found in the oral cavity, oesophagus and intestine of birds and reptiles. Metacercariae in fishes and amphibians.

Type sub-family Clinostominae Pratt, 1902.

KEY TO SUB-FAMILIES OF FAMILY CLINOSTOMIDAE

1. (2) Cirrus sac without prostatic portion.

   Uterus sac-shaped.

   Parasites of birds -------- Clinostominae Pratt, 1902.

2. (1) Cirrus sac with prostatic portion.

   Uterus cylindrical.

   Parasites of reptiles.

3. (4) Cirrus sac large, elongated with a conspicuous coil.

   Eggs embryonated when laid.

   Parasites of oesophagus of crocodiles -------- Opisthophallinae Travassos, 1926.

4. (3) Cirrus sac small, slightly curved.

Eggs not embryonated when laid.

Parasites of the intestines of snakes and crocodiles -------- Harmotreminae Yamaguti, 1933.

Sub-family Clinostominae Pratt, 1902.

Diagnosis - Clinostomidae with a Y-shaped excretory bladder. Cirrus-sac small, pear-shaped, without prostatic portion. Uterus sac-shaped. Eggs unembryonated when laid. Type genus Clinostomum Leidy, 1856.

KEY FOR IDENTIFICATION OF GENERA OF SUB-FAMILY CLINOSTOMINAE

(after Baer, 1933).

1. (2) Intestine with long lateral diverticula which may be branched -------- Euclinostomum Travassos, 1928.

2. (1) Intestine with short lateral diverticula, never branching.

3. (4) Body up to several cms. long. Vitellaria not penetrating into the anterior half of the body -------- Ithyoclinostomum Witenberg, 1926.

4. (3) Body never several cms. long. Vitellaria penetrating into anterior half of body -------- Clinostomum, Leidy, 1856.

(Syn. Clinostomatopsis Dollfus, 1932).

Diagnosis - Clinostomidae of medium size. Pharynx absent or rudimentary. Sex organs near the middle of the body or in its posterior half. Uteroduct opens into the posterior portion of the sac-shaped uterus. Adults parasitize Ardeiformes, Pelicaniformes, Lariformes.

Metacercariae in fishes and amphibians. Type species C. complanatum (Rudolphi, 1814).

KEY FOR IDENTIFICATION OF THE SPECIES OF THE GENUS CLINOSTOMUM

(after Baer, 1933).

1. (2) Vitellaria extend anteriorly to the ventral sucker -------- C. sorbens M. Braun, 1899.

2. (1) Vitellariae not extending anterior to the ventral sucker.

3. (6) Sex organs median, near middle of the body.

4. (5) Vitellariae radial -------- C. foliiforme M. Braun, 1899.

5. (4) Vitellariae not radial -------- C. complanatum (Rud.1819). (obvious lapsus calami. Must be (Rud.1814) Auct.).

6. (3) Sex organs in the posterior half or third of the body.

7. (8) Uterus with lateral evaginations -------- C. detruncatum M. Braun, 1899.

8. (7) Uterus without lateral evaginations.

9. (12) Distance from the bottom of the mature uterus to the ventral sucker exceeds the distance to the oral sucker.

10. (11) Sex organs not near the middle of the body. C. heluans M. Braun, 1899.

11. (10) Sex glands near the middle of the body -------- C. attenuatum Cort, 1913.

12. (9) Ventral sucker closer to the bottom of the uterus than to the oral sucker.

13. (16) Body not over 5 mm. long.

14. (15) Oral sucker 0.16 mm. in diameter -------- C. lambitans M. Braun, 1899.

15. (14) Oral sucker 0.40 mm. in diameter ---------- C. hornum Nicoll, 1914.

16. (13) Body up to 11 mm. long.

17. (18) Vitelline vesicles present ---------- C. lophophallum Baer, 1932.

18. (17) Vitelline vesicle absent.

19. (20) Ventral sucker on the boundary between the anterior and middle thirds of the body ---------- C. intermedialis Lamont, 1920.

20. (19) Ventral sucker on the boundary between the first and second fourth of the body ---------- C. phalacrocoracis Dubois, 1930.

Baer has not included in this key C. australiense Johnstone, 1916, because its description is based on sub-adults only.

IV.

MATERIALS AND METHODS

1. Procurement of metacercariae

Fish infected with metacercariae of *C. complanatum* were taken from Barren and Jessica Lakes in Manitoba and Lawrenson Lake in Ontario. They were caught either by angling or the use of a gill net. The fish were kept in a retaining tub and transported in plastic bags containing a solution of Tricaine Methane Sulphonate (MS222). One gram of MS222 was dissolved in five gallons of water. One gallon of this mixture was placed in a five pound plastic bag with two pounds of ice cubes. Three fish were placed in each bag and the bags sealed with copper wire tabs, leaving as much air as possible in the upper portion of the bag.

This concentration of MS222 anesthetized the fish to about stage 2 of the McFarland classification (39); i.e. there was total loss of reactivity to stimuli, except strong manual pressure, and a slight decrease in opercular rate. On arrival at the laboratory the fish were transferred into 45-50 gallon tanks filled with dechlorinated, filtered, well aerated water and kept in a constant temperature room at 45° F.

The fish ranged in weight from 12 to 450 grams.

Mortality of fish was low; of 120 specimens collected only 3 died in transit. The perch recovered in about 15-20 minutes after being placed in the fresh water, the smaller fish returning to normal most quickly.

2. Method for digestive excysting of metacercariae

Whole fish heads and slices of fish flesh with metacercariae were placed in glass jars containing one per cent pepsin solution in half per cent HCl and incubated at 37° C. for 20-30 minutes. Excysted metacercariae were removed with an eye-dropper and placed in sterile 0.85% NaCl.

3. Infection procedure

1. Excysted trematodes were washed in three changes of sterile 0.85% NaCl and placed in 0.85% NaCl with 6,000 units of penicillin per ml.

2. Mice were anaesthetized with ether, and 0.1 cc. of Avertine solution per 10 gms. of body weight injected intraperitoneally. (Avertine solution was prepared in diluting 0.1 cc. of Avertine with 5 cc. of distilled water and kept at 40° in a water bath).

3. A longitudinal incision 10 mm. in length ventral to the kidney and 12 mm. from the spine was made through the skin and musculature of the abdominal wall. The trematodes were placed into the peritoneal cavity with an eye dropper. The muscles and the skin

were each sutured separately with two interrupted stitches.

4. Infected mice were placed one to a cage in clean plastic cages.

4. Fixing and staining procedures

Trematodes removed from the experimental animals were left in cold water for relaxation, then compressed between two slides and immersed in 4 per cent formaldehyde for 6 hours. The slides were sufficiently heavy to flatten the trematodes, but not heavy enough to crush them. After washing in running water for 6 hours the trematodes were stained in Gower's Carmine for 12-18 hours, and differentiated in acidified alcohol (70% ethyl alcohol and 0.5% HCl) for 3-6 hours.

The specimens were washed 1-2 hours to remove HCl and dehydrated in a series of alcohols: 50%-75%-85%-95%-95% two changes of absolute, 50-50 mixture of absolute and xylene, two changes of xylene-, and mounted in Permount. Timing was as follows: sixty minutes in each alcohol including 95%, then 6 hours in each of the two changes of absolute alcohol. Thirty minutes in a 50-50 mixture of alcohol and xylene and 30 minutes in each of the two changes of xylene.

5. Technique of castration

1. The mice were anaesthetized with ether and 0.1

cc of Avertine solution per 10 gms. of body weight injected intraperitoneally. (Avertine solution was prepared as before).

2. A 10 mm. longitudinal incision slightly to the left of the mid-ventral line approximately 20 mm. anterior to the anus was made through the skin and musculature of the abdominal wall.

3. The left testis was extracted with tweezers through the incision and the spermatic cord ligatured and cut. The stump was pushed back into the abdominal cavity. The muscles and the skin were each sutured separately, with two interrupted stitches.

V.

OBSERVATIONS

1. Comparison of the morphology of C. complanatum metacercaria with that of the adult.

To evaluate the development of C. complanatum in experimental animals, it is necessary to describe the larval form as it is when excysted from the fish. The late immature stages from the fish and mature worms differ little in form and body proportions. However, a great deal of change takes place in the internal morphology.

The testes of the immature worm are well developed but slender and show extensive lateral branching. As the worm matures the testes increase in size and the lateral branches become wider and gradually round out. The immature testes stain deeply with Gower's carmine but as they mature they stain lighter. The ovary of the metacercaria is inconspicious, and appears as a small dark mass to the left of the oötype. With maturation the ovary increases in size and becomes a darker sphere. The uterus of the immature worm is a long, slender tube; in the maturing worm it becomes wider and develops many lateral outpouchings. When full of eggs, it extends laterally to the inner margins of the intestinal caeca. The caeca of the metacercariae stain light red, but in the maturing and feeding

individual, they become distended and filled with brown masses of food. There is no evidence of vitellariae in the metacercariae. With maturation the vitellariae first appear in the lateral fields and posterior to the testes as small spots which later become larger and more distinct (Figs.1-6).

2. Occurrence.

Ninety seven perch from Barren Lake and 6 perch from Jessica Lake in the Whiteshell region of Manitoba and 75 perch and 4 walleyes from Laurenson Lake at Kenora, Ontario provided the metacercariae for my experiments.

Approximately 1200 perch from Crawford Lake and Sandy Lake in the prairie region of Manitoba were examined but no "yellow grubs" were found. Forty bass, 20 perch, 30 carp, 30 bullheads and 1 pike from Nettley marsh were examined with negative results.

The heron is present both in the prairie region, including Nettley marsh, and in the Whiteshell area. Thus the absence of Clinostomum in the prairies may indicate that the snail hosts are absent from this area.

PLATE II. Structure of a metacercaria of *C. complanatum* from perch. All taken at 60X.

Fig. 1. Uterus.

Fig. 2. Testes, ovary, and oötype.

Fig. 3. Posterior end.

FIG. 1

FIG. 2

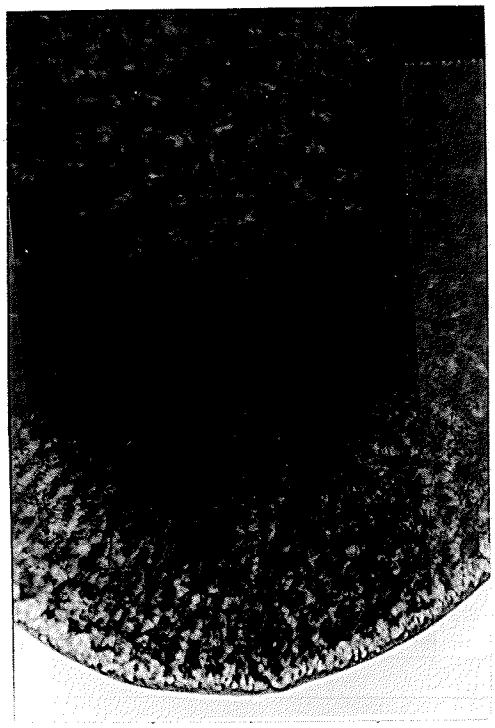

FIG. 3

PLATE III. Structure of an adult C. complanatum from abdominal cavity of A/Jax male mouse. All taken at 60X.

Fig. 4. Uterus.

Fig. 5. Testes, ovary, and oötype.

Fig. 6. Posterior end.

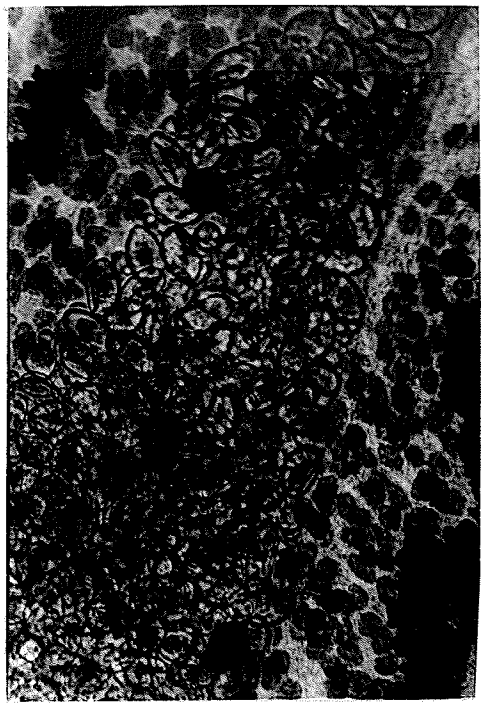

FIG. 4

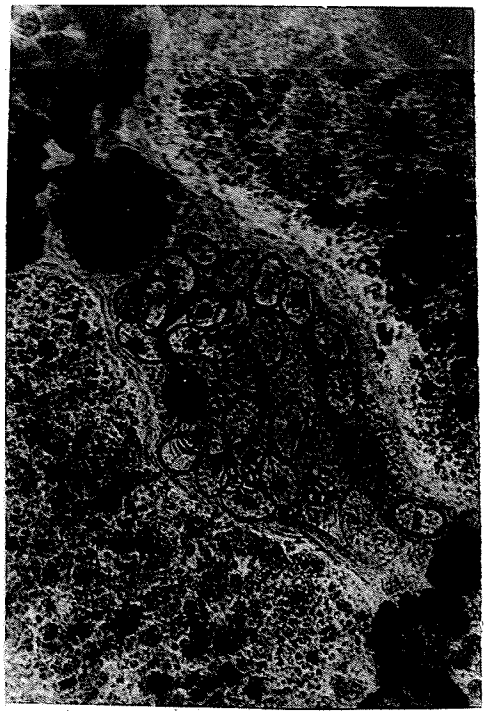

FIG. 5

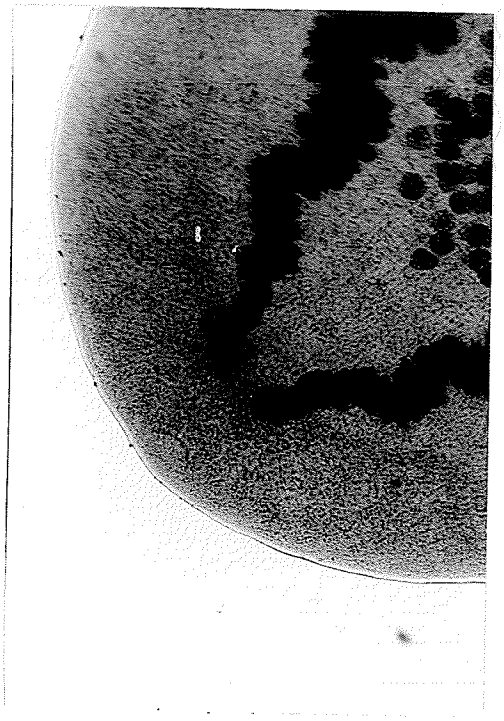

FIG. 6

VI.

RESULTS OF EXPERIMENTS

As already stated in the Introduction, Clinostomum has been found in the mouth of cats by Evans (18) and by several other authors (see page 2). This led to my experimental infection of cats. At the same time an attempt was made to infect white rats by force feeding them metacercariae.

1. Experiments with cats

On May 14, 1965 three adult cats were fed fresh perch fillets containing metacercariae of C. complanatum. The first cat was given ten encysted metacercariae, the second cat twenty, and the third cat, forty. All cats were sacrificed four days after infection. The oesophagus, larynx, pharynx and oral cavity were examined for the presence of trematodes. None were found in the first or second cat, but in the third cat, one trematode was found near the middle of the oesophagus. It was firmly attached to the mucous membrane and obviously feeding, as the intestinal caeca contained ingested material. Its testes, ovary, ootype, oviduct and uterus were well developed, and the vitellariae were in the process of development. There were, however, no eggs in the oötype or the uterus (Fig.7).

On May 19, 1965 two more adult cats were fed fifty encysted metacercariae each. These cats were sacrificed two days later. Three trematodes were removed from the oesophagus of the first cat. None were found in the second cat. The trematodes showed some maturation. Though there was no sign of food in the intestinal caeca, their testes were rounding out and the ovaries were starting to form.

Eleven hundred and fifty grams of fresh perch fillets containing approximately five hundred encysted metacercariae were fed to four kittens over a period of three days, August 5-8, 1965. Two of these kittens were sacrificed on August 9, 1965, and one trematode was found in the oesophagus of eachkitten. Again there was little evidence of maturation, the caeca did not contain food, but the testes showed some development as evidenced by a slight change in their shape and structure. The ovary in each trematode was starting to form (Fig.8).

On August 10, 1965, kittens number three and four were sacrificed. Kitten number three had two living trematodes, one in the larynx and one in the oesophagus. Kitten number four had four living trematodes, three in its larynx and one in its oesophagus. The signs of maturation were again slight however. There was no

indication that the trematodes had been feeding, the testes had developed slightly, and again, the ovary was beginning to form (Fig.9).

These experiments prove that cats can definitely be infected with C. complanatum. Although no fully mature trematodes were recovered, it is possible that they will mature in cats. From the nine cats fed a total of six hundred and seventy larvae, only twelve worms were recovered, an extremely low yield. None of these cats showed any signs of asphyxiation. Table III gives a summary of experiments with cats.

2. Experiments with white rats

On May 14, 1965, five excysted metacercariae of C. complanatum were forced fed to each of two white rats. The rats were dissected on May 18, 1965, and their oral cavity, pharynx, larynx and oesophagus examined. No trematodes were found. The experiment was repeated on May 19, 1965, by feeding ten excysted metacercariae to each of two white rats. The first rat was dissected one day later and the second two days after infection, both with negative results. Two more rats were force fed ten metacercariae on June 21, 1965, and dissected four days later; again the results were negative.

Thus the experiments on force feeding of excysted

TABLE II

Summary of experiments with cats.

Cat number	number of metacercariae fed	number of metacercariae recovered	location	
			oesophagus	larynx
1	10	0	0	0
2	20	0	0	0
3	40	1	1	0
4	50	3	3	0
5	50	0	0	0
6	125	1	1	0
7	125	1	1	0
8	125	2	1	1
9	125	4	1	3

PLATE IV. *Clinostomum complanatum* from cats, all taken at 10X.

Fig. 7. Trematode from oesophagus of cat four days after feeding.

Fig. 8. Trematode from oesophagus of kitten four days after feeding.

Fig. 9. Trematode from larynx of kitten five days after feeding.

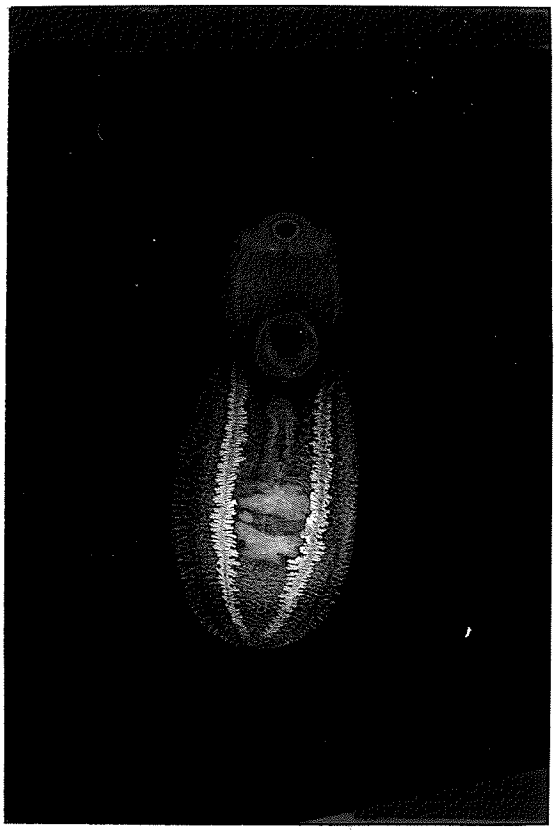

FIG. 7

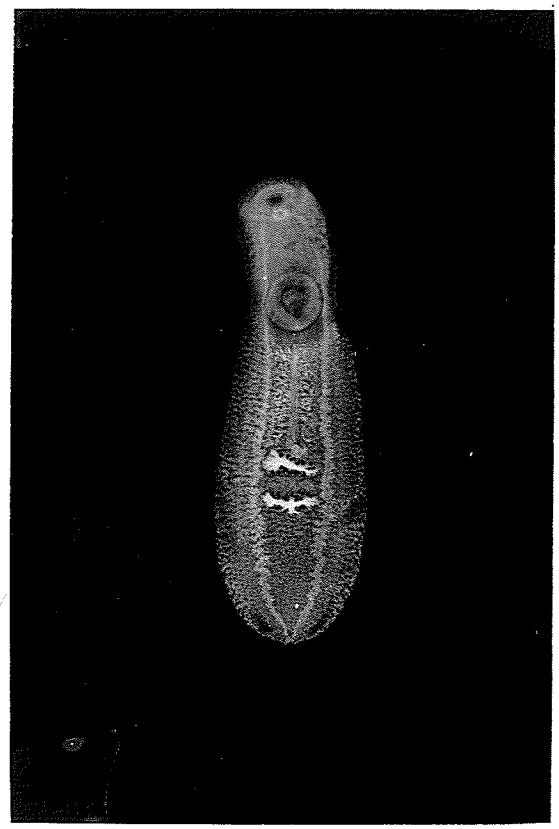

FIG. 8

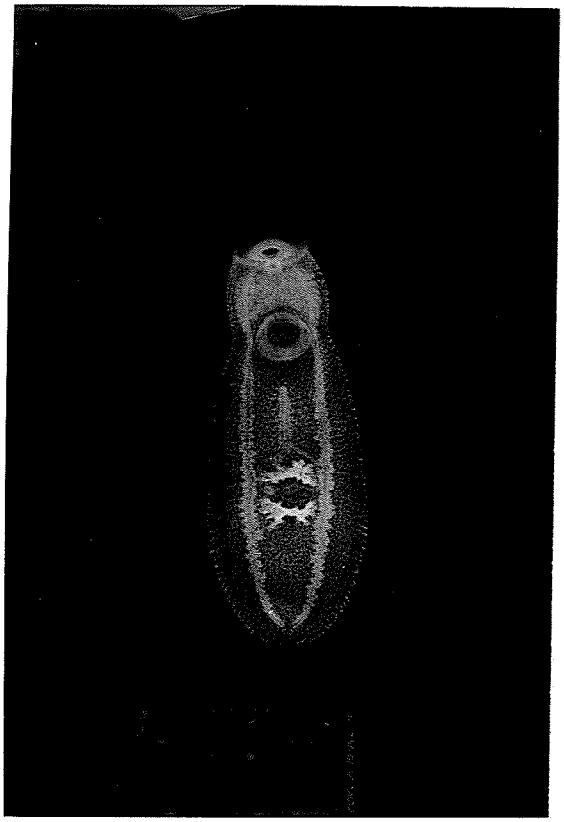

FIG. 9

metacercariae to all six white rats gave negative results.

3. Experiments with mice

The escape of the worms from the mouth with or without subsequent digestion by the experimental host probably accounted for the low yield and negative results from cats and rats respectively. This led to an attempt to bring Clinostomum to maturity in the abdominal cavity of laboratory mice from which the trematodes could not escape.

On June 23, 1965, six A/Jax male mice were infected intraperitoneally with five excysted metacercariae each. One mouse was dissected each day, starting on June 25, through June 30. The trematodes recovered were mounted in toto. (Fig.10-17).

Fig.10 shows a metacercaria from the fish, Fig.11 a worm after two days in the mouse. In this specimen the intestinal caeca are partially filled with food, the testes somewhat larger, the ovary more conspicuous and the uterus wider. Fig.12 shows a worm after three days in the mouse. Its intestinal caeca are well filled with food, the testes rounding out, the ovary still larger, the oötype has started to differentiate, the uterus has become wider and shows lateral outpouchings, the vitellariae are now discernable both in the lateral fields and posterior to the testes. Fig.13,

that of a trematode after four days in the mouse, shows the caeca to contain food, the testes still more rounded, the ovary well developed and spherical, the oötype, oviduct and uterus containing eggs, and the vitellariae more pronounced.

Figs. 14 and 15 represent worms after five and six days in the mouse respectively. They show little change as compared to the worm in Fig.13, though the testes are still more rounded, the oötypes filled with eggs, the vitellariae well developed and filling the greater portion of the body.

Fig.16 represents C. complanatum which was in the peritoneal cavity for seven days. Its cacea were filled with food, its testes have become quite large, lost their lateral branches and are probably degenerating. The uterus has enlarged and is full of eggs.

Fig.17 is that of an encapsulated trematode which was ten days in the peritoneal cavity of an A/Jax mouse. The wall of the capsule is approximately 0.25 mm thick. In A/Jax mice the capsule formation required eight to ten days, in C57L and LAF, mice, only five to seven days. Although encapsulated trematodes always succumbed, many of them matured first, as indicated by the presence of eggs in the capsule. In the specimens which died in the capsule the acetabulum assumed a distinctive

triangular shape (Fig.18) different from that of specimens which were fixed (Fig.19).

Though the trematodes could be found anywhere in the peritoneal cavity of mice, they had a pronounced predilection for the scrotum, where they attached themselves to either the testes or the scrotal wall. These observations led to a study of the distribution of Clinostomum in unilaterally castrated male mice. On July 27, 1965, left testes were removed from four A/Jax mice and five trematodes placed in the peritoneal cavity of each animal. Six days later the mice were dissected and the distribution of the trematodes in their peritoneal cavities studied. The results are summarized in the following table.

Mouse number	Abdominal cavity	Scrotum Lt.	Scrotum Rt.
1	4	0	1
2	0	3	2
3	1	2	2
4	3	1	1

Of the twenty trematodes removed from these four mice, twelve were in the scrota, six on either side. Thus, unilateral castration did not effect the distribution of C. complanatum in the peritoneal cavity, the trematodes being found as often in the castrated side as in the

uncastrated.

On September 17, 1965, experiments on the condition and distribution of the trematodes in the peritoneal cavities of mice were repeated with larger numbers of animals. Ten C57L and thirteen A/Jax male mice were infected intraperitoneally with 5 C. complanatum metacercariae each. Three C57L and three A/Jax mice were dissected five days later, on September 22. Five unencapsulated and twelve encapsulated worms were recovered from the three C57L mice. All unencapsulated worms were found in the scrotum; the majority of encapsulated ones were also in the scrotum, though some were scattered throughout the abdominal cavity. From the three A/Jax mice 16 unencapsulated worms were recovered, 15 from the scrotum and 1 attached to the liver.

One day later, six days after infection, the remaining seven C57L and ten A/Jax mice were dissected. All 31 trematodes removed from C57L mice were encapsulated. Eighteen of them were in the scrotum, 9 on the liver, and 4 scattered in the abdominal cavity. Of the 49 trematodes from the ten A/Jax mice only 13 were encapsulated. Forty worms were in the scrotum, 33 unencapsulated and 7 encapsulated. The remaining 9 worms were distributed throughout the abdominal cavity, 6 of them encapsulated and the others free. Thus the results of the second

series of experiments with a larger number of animals closely parallelled those of the first series.

Mice were infected with 5 metacercariae each in all preceding experiments. On November 11, 1965, ten A/Jax mice were infected intraperitoneally with 7-11 trematodes each. Five mice died on the day of infection and the 5 remaining were dissected seven days later, on November 18. Forty-seven trematodes were recovered, all but one living. The number of trematodes used in infection and their distribution is summarized in the following table.

Mouse	Number of metacercariae placed in peritoneal cavity	Location	
		Abdominal cavity	Scrotum
1	7	4	3
2	9	1	8
3	9	1	8
4	10	1	9
5	11	1	10

All trematodes recovered, but one, were living, some of them, however, were in the process of encapsulation. Mice 2, 3, 4 and 5 had in their scrota a large number of trematodes clumped in writhing masses. All contained great numbers of eggs which they released promptly on being placed into cold water.

PLATE V. Maturation of *Clinostomum* in laboratory mice, all taken at 10X.

Fig. 10. Metacercaria from fish.

Fig. 11. Trematode after 2 days in the peritoneal cavity.

Fig. 12. Trematode after 3 days in the peritoneal cavity.

Fig. 13. Trematode after 4 days in the peritoneal cavity. (note: first evidence of eggs).

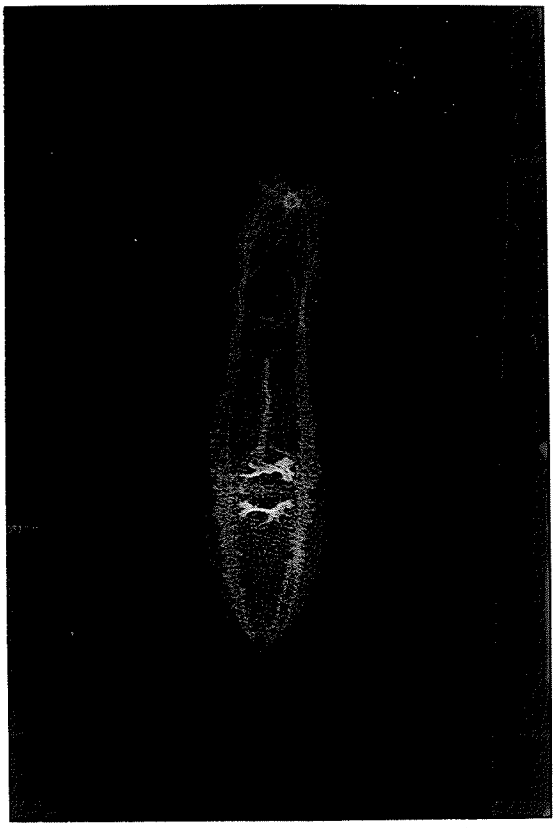

FIG. 10

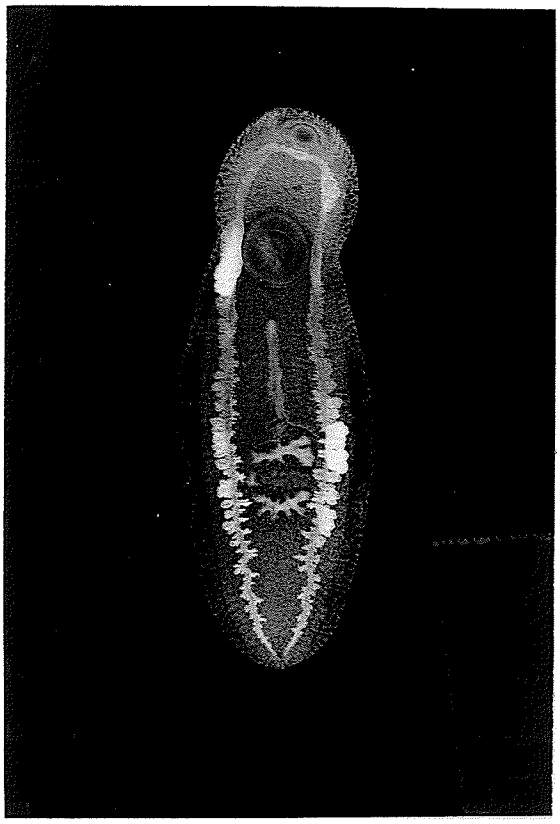

FIG. 11

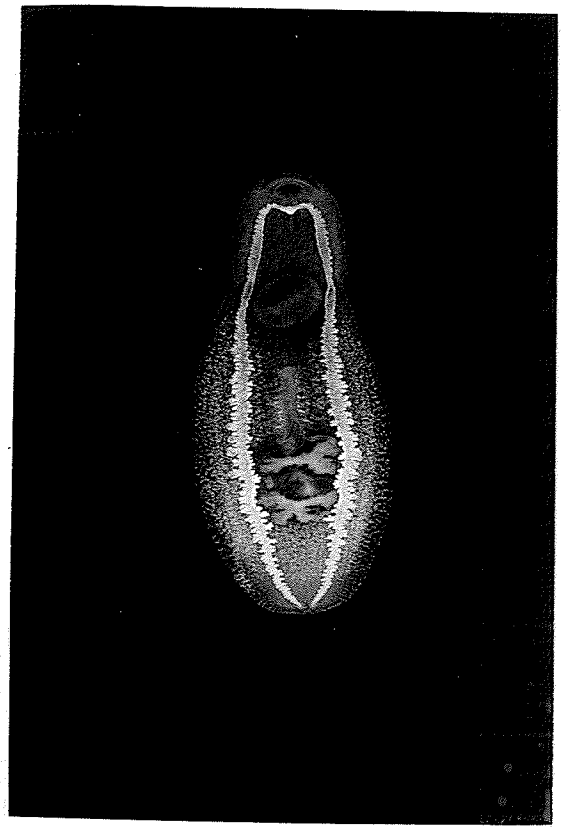

FIG. 12

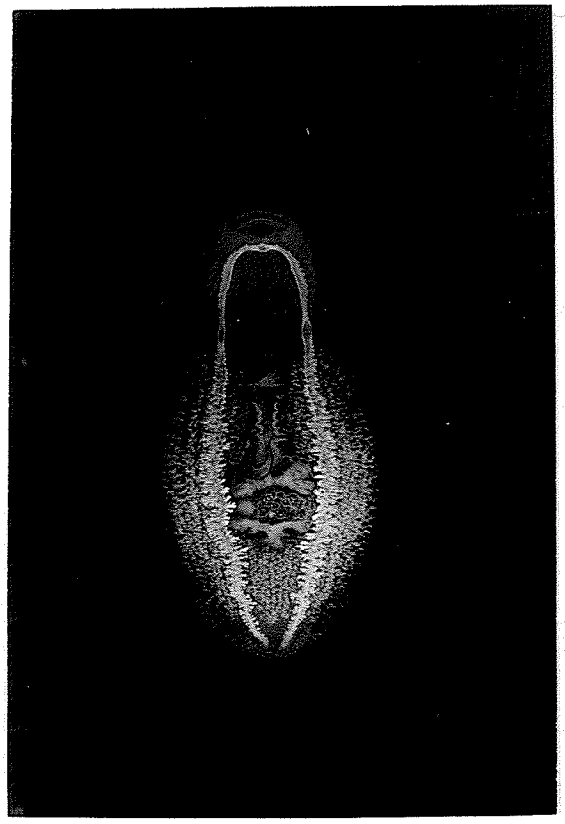

FIG. 13

PLATE VI. Maturation of *Clinostomum* in laboratory mice, all taken at 10X.

Fig. 14. Trematode after 5 days in the peritoneal cavity.

Fig. 15. Trematode after 6 days in the peritoneal cavity.

Fig. 16. Trematode after 7 days in the peritoneal cavity.

Fig. 17. Trematode after 10 days in the peritoneal cavity.

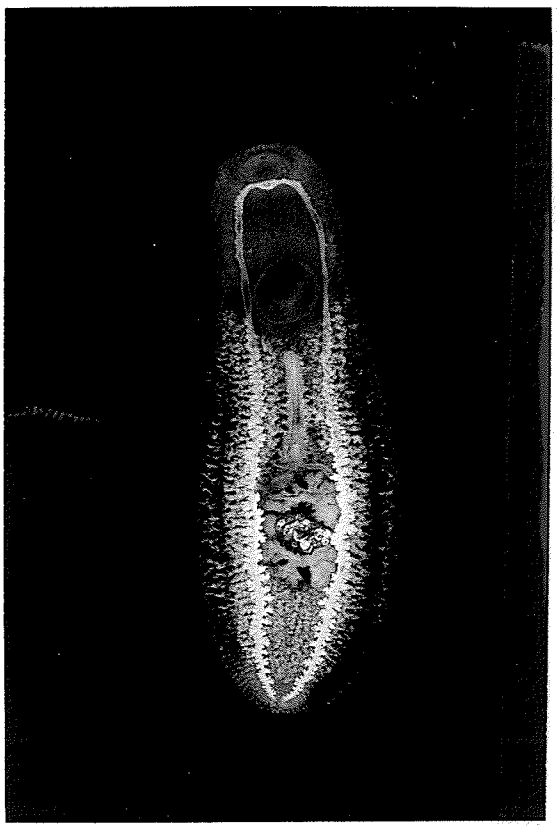

FIG. 14

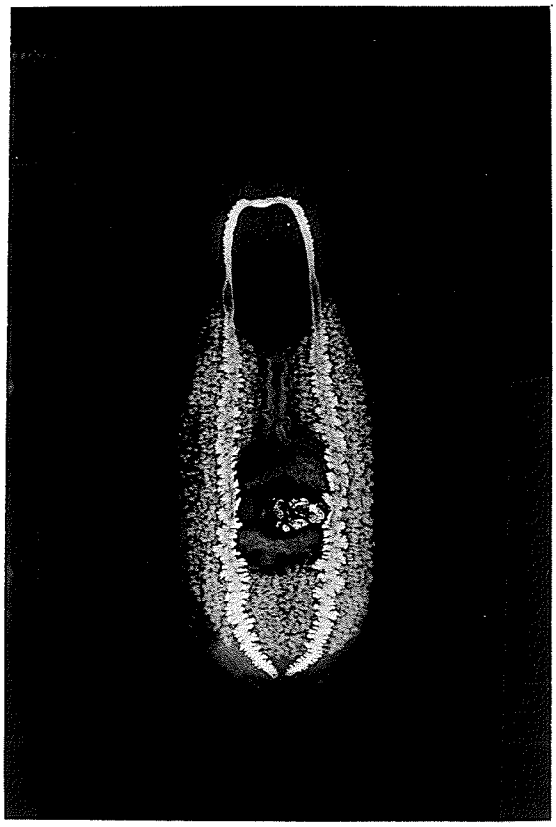

FIG. 15

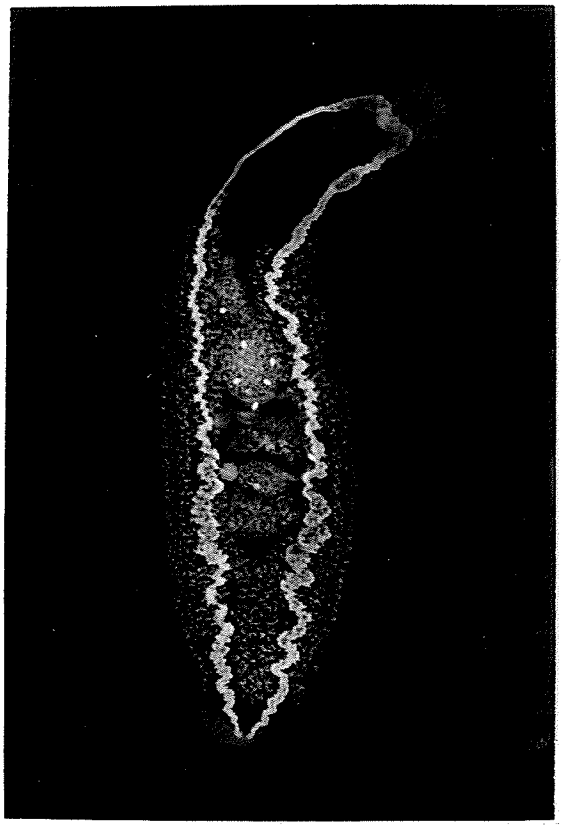

FIG. 16

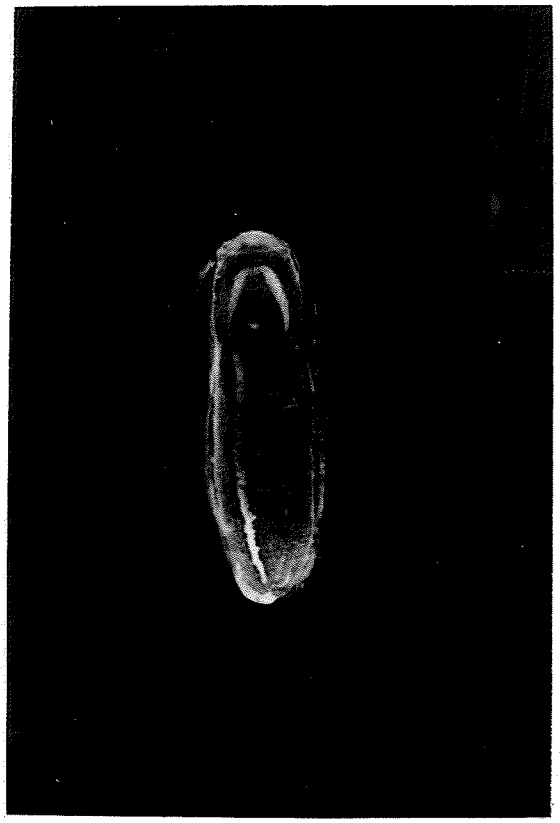

FIG. 17

PLATE VII. Shape of the acetabulum, all taken at 10X.

Fig. 18. Trematode which died due to encapsulation.

Fig. 19. Trematode which died in fixative.

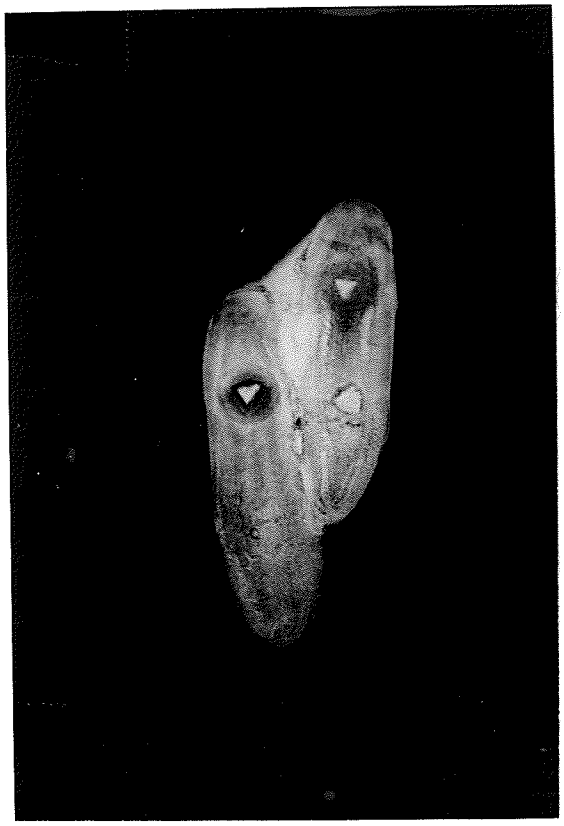

FIG. 18

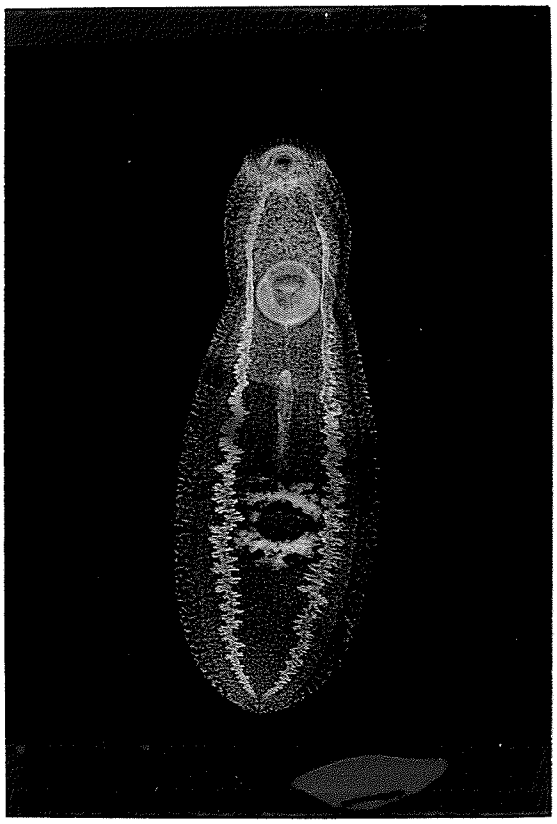

FIG. 19

4. Maturation of damaged Clinostomum in mice

Several trematodes used to infect mice were inadvertently damaged; the injuries often resulted in the severance of one or both intestinal caeca (Figs. 20-23). The effect of this damage was studied on four mounted specimens. Fig.20 presents a trematode after seven days in the peritoneal cavity of an A/Jax mouse. The right caecum and the oviduct of this worm were severed anteriorly to the testes. The ovary and testes are well developed. The vitellariae on the left side are much better developed than those on the damaged right side. The ovary is pushed to the left by the oötype which is distended with eggs because of the occlusion of the severed oviduct.

The worm depicted in Fig.21 was also seven days in the peritoneal cavity of an A/Jax mouse. Again the testes and ovary are well developed, and the vitellariae on the undamaged side are larger than those on the damaged side. The uterus is packed with eggs probably because of the occlusion of the damaged genital pore and displaces to the left, the functional left caecum. Fig.22 is that of a worm after five days in an A/Jax mouse. The changes in its reproductive organs closely resemble those of specimens of Figs.20 and 21.

Fig.23 shows a worm whose entire anterior end

including the acetabulum was severed. It was three days in the peritoneal cavity of an A/Jax mouse. Its intestinal caeca were empty, and yet this worm was definitely maturing. The testes and the ovary were well developed, the uterus wide, and the vitellariae, though small, were conspicuous.

There was no regeneration of damaged structures in these worms, possibly due to the short duration of the experiment (seven days at most). There was no difference in the rate and manner of maturation between the damaged and undamaged trematodes.

5. Splenectomy

On September 6, 1965, fifteen LAF_1 male hybrid mice were splenectomized and simultaneously, 3 to 7 metacercariae were placed into the abdominal cavity of each mouse. At the same time eighteen intact (non-splenectomized) LAF_1 hybrid control mice were infected, also intraperitoneally with 3 to 7 metacercariae each. Twelve mice from each group were sacrificed seven days later. The number of trematodes recovered, their condition (encapsulated or unencapsulated) and their distribution in the peritoneal cavity are summarized in Table III.

All 47 trematodes recovered from the splenectomized mice were encapsulated. Twenty-five of them were in the abdominal cavity and 22 in the scrotum. Of the 59 worms recovered from the control mice, 55 were encapsulated and

PLATE VIII. Maturation of damaged trematodes, all taken at 10X.

Fig. 20. After 7 days in peritoneal cavity of A/Jax ♂ mouse.

Fig. 21. Also 7 days in peritoneal cavity of A/Jax ♂ mouse.

Fig. 22. After 5 days in peritoneal cavity of A/Jax ♂ mouse.

Fig. 23. After 3 days in peritoneal cavity of A/Jax ♂ mouse.

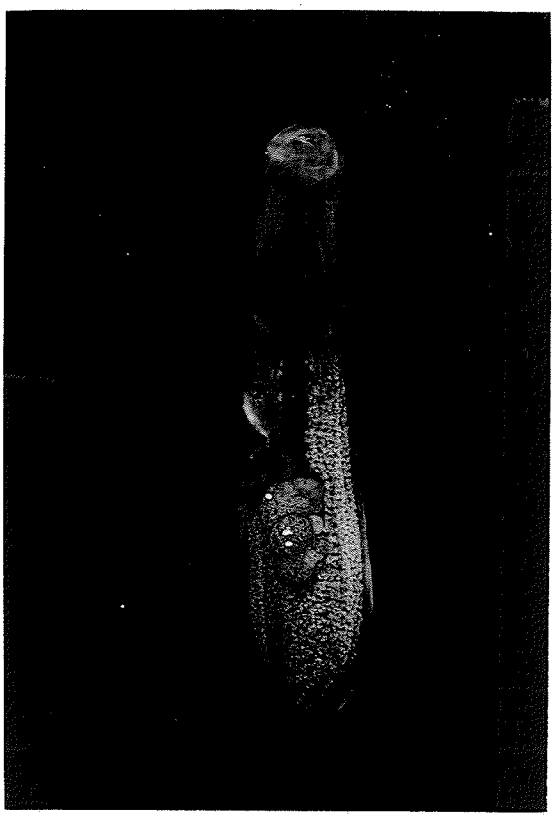

FIG. 20

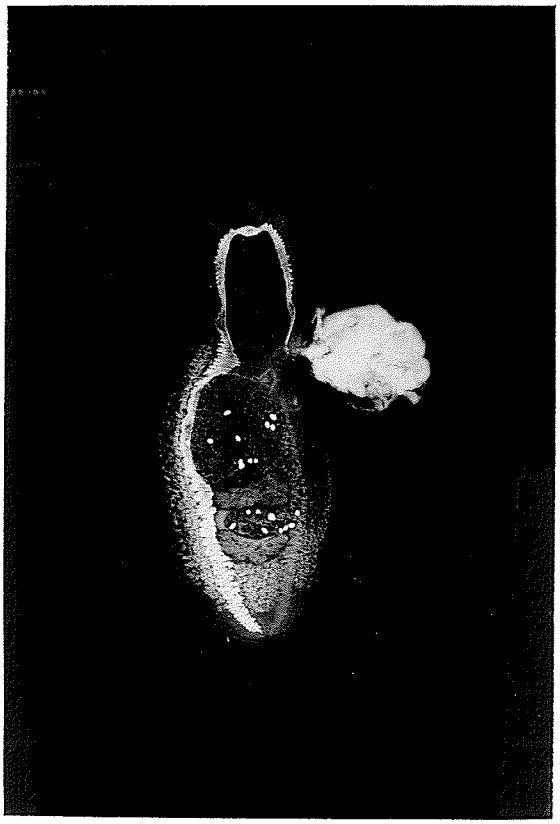

FIG. 21

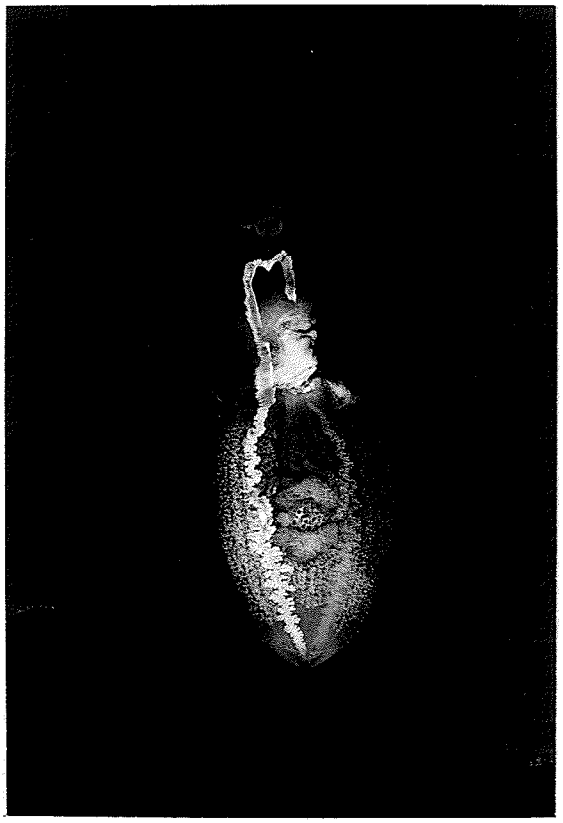

FIG. 22

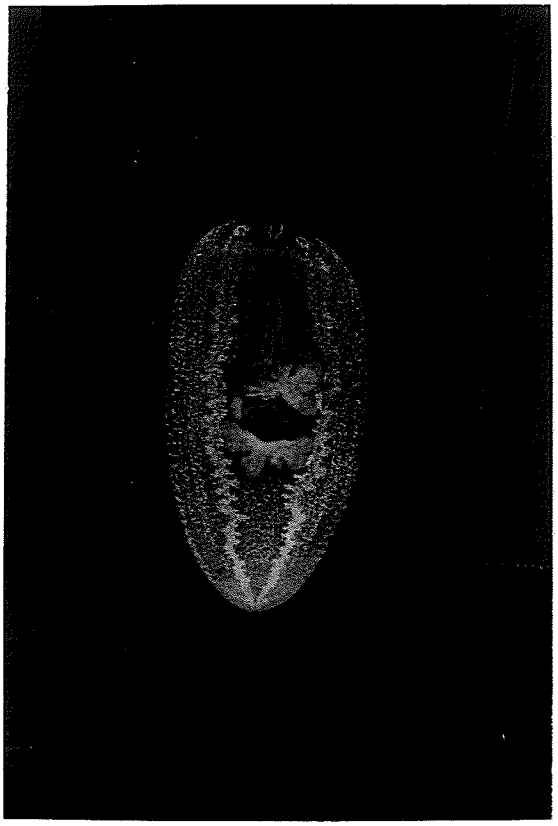

FIG. 23

4 unencapsulated. Nineteen were in the abdominal cavity and 40 in the scrotum.

All trematodes recovered from splenectomized mice were encapsulated, whereas 4 out of 59 worms (7%) from control mice were not encapsulated. It is obvious that splenectomy did not suppress the process of encapsulation.

6. Egg measurements

Dawes (16) gave measurements for eggs of Clinostomum complanatum from "birds" as: length 104-140 μ, width 66-73 μ. One hundred and eleven eggs deposited by trematodes which had matured in the peritoneal cavity of A/Jax male mice were measured. The average length of the eggs was 103±1 μ with the observed limits of variation 69.5 to 124.5 μ, the average width 66±1 μ with limits of 39.5 to 79.5 μ. The frequency distribution of lengths and widths are shown on Figs. 24 and 25.

TABLE III

Summary of experiments with splenectomized and control LAF_1 male hybrid mice

Splenectomized

Mouse	Number of trematodes recovered	Encapsulated	Unencapsulated	Location	
				Abdomen	Scrotum
1	4	4	0	0	4
2	4	4	0	2	2
3	3	3	0	3	0
4	3	3	0	0	3
5	4	4	0	2	2
6	3	3	0	3	0
7	4	4	0	0	4
8	6	6	0	4	2
9	3	3	0	3	0
10	6	6	0	3	3
11	3	3	0	3	0
12	4	4	0	2	2
Total	47	47	0	25	22

Control

1	4	4	0	1	3
2	7	6	1	2	5
3	4	4	0	2	2
4	4	3	1	1	3
5	1	1	0	1	0
6	5	4	1	1	4
7	2	1	1	1	1
8	7	7	0	3	4
9	7	7	0	3	4
10	6	6	0	1	5
11	6	6	0	2	4
12	6	6	0	1	5
Total	59	55	4	19	40

Fig. 24. Variation of the length of Clinostomum eggs produced in the peritoneal cavity of A/Jax ♂ mice.

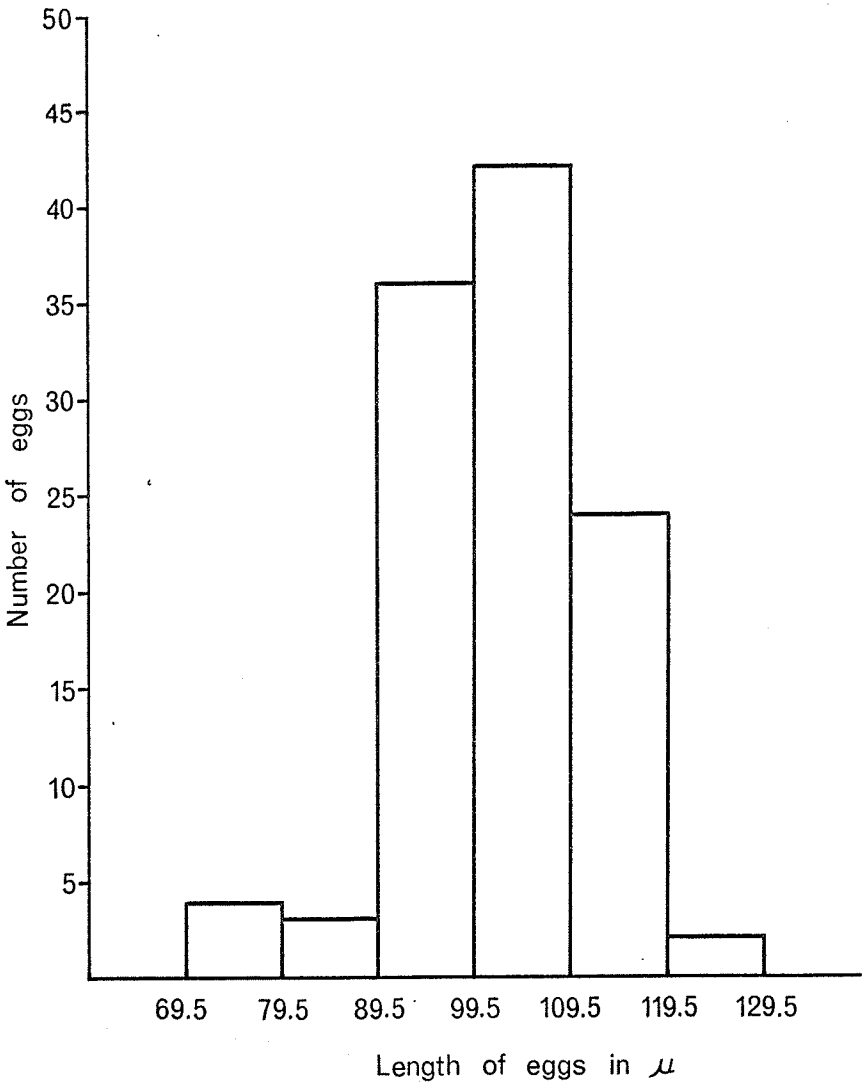
50
45
40
35
30
25
20
15
10
5
Number of eggs
69.5
79.5
89.5
99.5
109.5
119.5
129.5
Length of eggs in μ

FIG. 24

Fig. 25. Variation of the width of Clinostomum eggs produced in the peritoneal cavity of A/Jax ♂ mice.

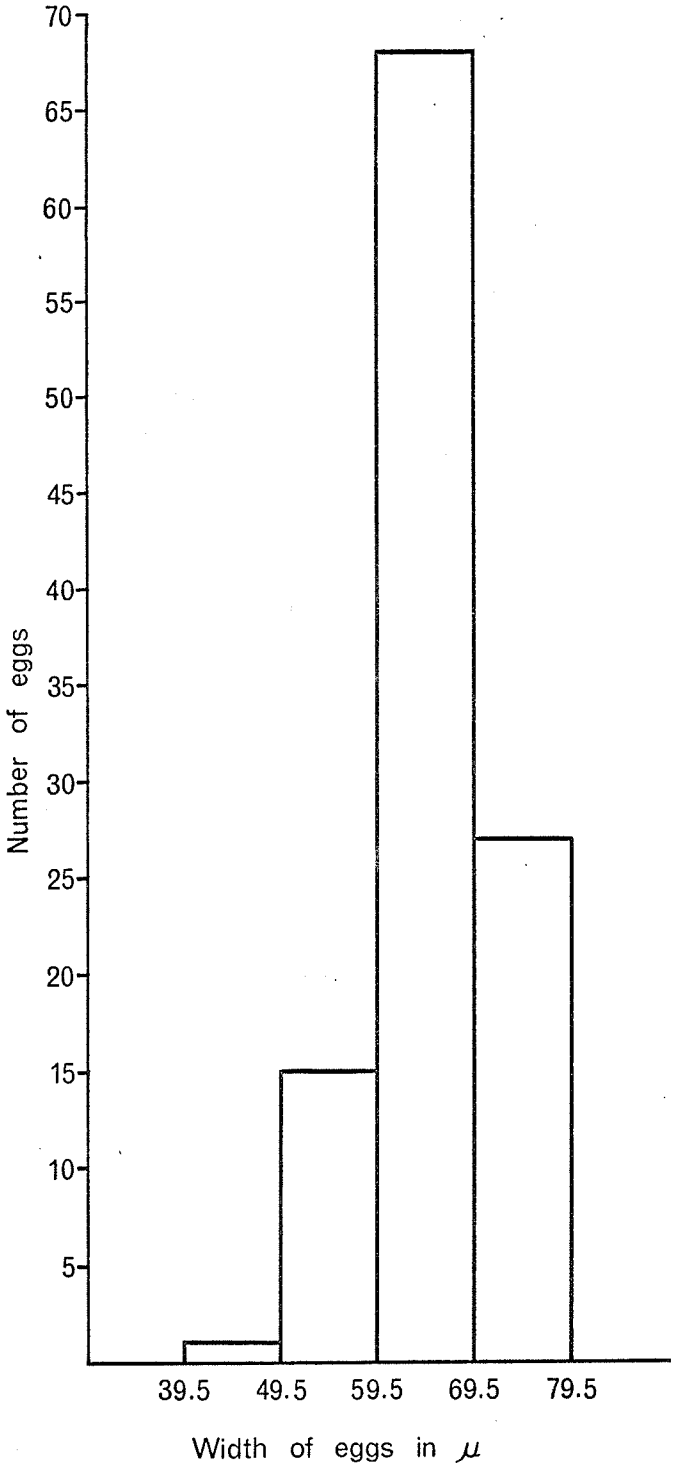
70
65
60
55
50
45
40
35
30
25
20
15
10
5
Number of eggs
39.5
49.5
59.5
69.5
79.5
Width of eggs in μ

FIG. 25

VII.

DISCUSSION

1. Literary review

Many trematodes reach an advanced stage of morphological development in the intermediate host, whereas others remain relatively undifferentiated. Bell and Smyth (5) for purposes of reference, divided the process of maturation into the following phases:

"(a) Cell multiplication: the period during which a larva increases its number of cells without these cells undergoing differentiation.

(b) Body shaping: a stage corresponding to segmentation in cestodes, but often not clearly seen in trematodes; the body takes on its general adult features.

(c) Organogeny: the outline of the main genitalia, especially the tubular uterus, begins to appear.

(d) Early gametogeny: the appearance of the primordia of the testes and ovaries.

(e) Late gametogeny: the appearance of active gametes.

(f) Shell formation: the vitelline cells reach maturity and pass into the uterus preparatory to releasing egg-shell material.

(g) Oviposition: fully formed, shelled eggs appear in the uterus."

At one end of the scale are metacercariae such as those of Fasciola hepatica and Diplostomum phoxini, which are poorly differentiated and undergo all their sexual maturation stages in the definitive host. At the other end are the progenetic[1] metacercariae such as Coitocaecum anaspidis which become sexually mature in the intermediate crustacean host. In the crustacean, however, they produce a smaller number of eggs than in their definitive fish-host.

Between these extremes are species whose metacercariae reach varying degrees of sexual differentiation. Thus Clinostomum complanatum metacercariae show marked organogeny and possess the primordia of both male and female reproductive systems. C. complanatum produces

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1. "The terms progenesis and neoteny are almost synonymous. Maturation of the gonads in a larval animal is known as neoteny. Advanced development of genitalia in larva, without maturation, is known as progenesis. An advanced progenetic condition clearly becomes neoteny, but the terms neotenic and progenetic are rather loosely used." (56)

eggs after four days in the oesophagus of Ardea sp. their natural definitive host and in the same time in abdominal cavity of laboratory mice. The strigeid Linstowiella szidati develops to maturity in the intestine of a chick in three days (2). Miller (40) obtained adult Posthodiplostomum minimum one and a half days after feeding metacercarise to chicks. Hugghins (26) found that the strigeid Hysteromorpha triloba produced eggs two and a half days after the metacercariae were ingested by the definitive host Phalacrocorax auritus.

Although progenetic metacercariae occur in many common animals, few attempts have been made to bring them to maturity in vitro. In the 1940's Ferguson worked with cercariae of the strigeid Diplostomum flexicaudatum which develop normally into metacercariae in the lenses of many fishes, their natural hosts (20). He showed that these metacercariae will also grow normally in the lenses of living frogs, turtles, chicks, ducklings, laboratory mice, rats, guinea pigs and rabbits (21). Cercariae of D. flexicaudatum remain alive in sterile Tyrode solution, without lens material for about a week, but little or no development occurs. When lens material from cold or warm blooded vertebrates was added to the culture medium, the cercariae of D. flexicaudatum developed into metacercariae at room temperature (22).

In the 1950's Miller (40) experimented with the strigeid Posthodiplostomum minimum. In this trematode the primordia of the genitalia were present in the metacercariae which matured in one and a half days both in the natural definitive host, the heron, and in experimental chicks. Ferguson (22) found that the metacercariae of P. minimum required three days to mature in vitro and produced abnormal, unviable eggs. These adults, however, were also abnormal in that their vitellariae were less developed than those of adults in the natural host.

In the 1960's, Wyllie, Williams and Hopkins (64) used gluco-saline, albumin, yeast extracts, serum and amino acids in an attempt to bring strigeid trematodes to maturity. The cultivated worms produced large masses of sperm and developed vitellariae, but only five percent of them produced eggs and these were not viable. Senft and Senft (53) used a completely synthetic medium containing amino acids, nucleic acid deviatives, vitamins, salts and glucose for the maintenance of Schistosoma mansoni in vitro. This was not an optimum medium, but the worms survived for about twenty days, laid eggs, and otherwise displayed normal behaviour.

As has been shown above, progenesis occurs in a number of trematode families, however, this phenomenon is

best demonstrated by the family Microphallidae. In the great majority of microphallids the life cycle involved two intermediate hosts and one definitive host, although some representatives of this family use in their life cycles one intermediate and one definitive host.

The microphallid metacercariae which are produced in the first or second intermediate host, have a completely developed genital system, which insures fast production of eggs in the definitive host. Morphologically and physiologically these metacercariae correspond to adults or sub-adults of other trematode species, which attain maturity only in their definitive hosts. Thus, the life cycle of these microphallids is abbreviated because of the shorter time necessary for the maturation of the adult.

Buttner (10) who has made intensive studies of progenetic trematodes, feels that the metacercariae of Levinseniella pellucida are in a condition closely resembling progenesis because they produce eggs a few hours after the infection of their definitive hosts. Many microphallids require only one day or less for the development of eggs in their definitive hosts, (Microphallus opacus, M. pirum, Levinseniella amnicole, Maritrema obstipum, M. kitanensis are examples).

Rothschild (49) pointed out that the common gulls infected with encysted metacercariae of Maritrema oöcysta

pass eggs of this trematode on the third day after infection. The fact that the metacercariae of this family are prepared to start sexual multiplication is supported by the results of their cultivation in vitro. Microphallus opacus, M. papillorobustus, Levinseniella tridezitata, Maritrema eroliae, M. macrovestibulum, M. urayasensis and Gynaecotyla adunca are species which mature easily in vitro.

Rausch (47) cultured excysted metacercariae of Microphallus opacus in Ringer's solution with the addition of media used for the cultivation of Protozoa: His experiments were carried out at temperatures of: 12° C., room temperature and 37° C. In trematodes cultured at room temperature and 37° C., eggs appeared in the uteri in twelve hours. At 12° C. the development was slow. Hunter (30) incubated the metacercariae of Gynaecotyla adunca in 1% sea water at 40° C. Eggs with shells appeared ten to twelve hours after incubation started and were laid 80 hours later. In this medium G. adunca survived up to eight days.

Ogata (41) infected mice with metacercariae of Maritrema eroliae and simultaneously cultured other metacercariae of M. eroliae at a temperature of 40° C. Eggs appeared in both the cultured trematodes and those from the definitive hosts, mice in about ten to twelve hours. Some metacercariae of M. eroliae started producing

eggs in the cyst and their receptaculum seminis contained sperm. This indicates the possibility of self fertilization in M. eroliae.

Cable, Connor and Balling (11), found that metacercariae of Megalophallus diontis in dead crabs (Calinectes) started producing eggs within a few hours. This is probably an adaptive reaction which insures propogation of this species.

Many species of Microphallidae normally produce eggs in the second intermediate host (Microphallus minus, M. progeneticus, Maritrema eroliae, Maritreminoides caridinae and Pseudo-levinsiella cheni). Yeh and Wu (68) pointed out that the progenetic eggs of Microphallus minus differ from normal eggs both in their size and shape. They also showed that while these eggs were present in the metraterm of the encysted trematode, no eggs were present in the cyst cavity. According to Yamaguti and Nisimura (65) encysted metacercariae of Maritreminoides cardinae in the liver and sex glands of the prawn Neocaridina denticulata produced eggs and oviposit filling the cysts with eggs. These authors believe that the life cycle of this trematode may be completed without passage through the definitive vertebrate host.

Sogandares-Bernal (57), described a new species, Microphallus progeneticus, which matures in its second

intermediate host, the crayfish Cambarellus puer. This trematode does not encyst in the crayfish. In one crayfish Sogandares-Bernal found over 100 trematodes all containing eggs with mature miracidiae.

Some species of the Microphallidae, Microphallus pygmaeus, Maritrema oöcysta and Levinseniella minuta are almost mature in their first intermediate host the snail. Belopol'skaia thinks that the finding of species which reach maturity in snails is to be expected (6).

Though the phenomenon of progenesis occurs in many trematode families, the great majority of the species of only one family, the Microphallidae are actually or potentially progenetic. The development of progensis results in the possibility of trematodes maturing in a great number of different definitive hosts because maturation takes only a short time. The end result may be, however, the complete elimination of the vertebrate host from the life cycle of these trematodes. (6)

The strigeid, Cotylurus flabelliformis, an intestinal parasite of ducks, uses Lymnaea stagnalis and Stagnicola palustris is 1st intermediate hosts, the cercariae developing in the digestive glands. After immergence they penetrate into the gonads of other individuals of the same species and develop into metacercariae. The cercariae often attack physid and

planorbid snails. If these snails are uninfected by other trematodes, the cercariae of C. flabelliformis die; however, if the physid or planorbid snails harbour sporocysts and rediae of other trematodes, C. flabelliformis cercariae invade these and readily develop into metacercariae. Cort, Olivier and Brackett (13) and Cort, Brackett, Olivier and Nolf (14) demonstrated experimentally that Helisoma trivolvis and H. campanulata parasitized by Clinostomum complanatum served as 2nd intermediate hosts for Cotylurus flabelliformis and that its metacercariae developed normally in the sporocysts and rediae of Clinostomum complanatum. They suggested that in these the cercariae of Cotylurus flabelliformis are protected from the immune reaction of the abnormal host. As hyperparasites the Cotylurus cercariae utilize the food absorbed by Clinostomum sporocysts and rediae from the tissues of the snail.

The phenomenon of progenesis occurs also in cestodes, the well developed plerocercoid of the cestode Diphyllobothrium dendriticum matures in the duodenum of gulls of the genus Larus in only six days. This cestode will also develop in the duodenum of mammals; e.g. dogs, cats, rats, and mice, and also in man. Its development in these incidental hosts is often abnormal and takes a longer time because of the lower temperature of the mammalian

hosts (3).

Although the plerocercoids of most cestodes do not reach a high degree of differentiation within the intermediate host, there are some exceptions. The most remarkable of these are the genera Biacetabulum and Caryophyllaeus, who parasitize fresh water oligochaetes as intermediate hosts and fish as definitive hosts. The progenetic larvae of Biacetabulum sieboldi have been known since 1855 and were regarded as adults of 'Archigetes' sieboldi Leuckart 1878 (35). The procercoid occurs in the coelom of fresh water oligochaetes where it grows and develops into a progenetic plerocercoid with mature reproductive organs. The name 'Archigetes' was given to these progenetic larvae before the adult, Biacetabulum sieboldi (Leuckart 1878) occuring in the intestine of fish, was identified in 1937 by Szidat (58).

In Caryophyllaeus sp. procercoids, occur in the coelom of fresh water oligochaetes. The primordia of the reproductive organs are well developed but complete maturation does not occur until the plerocercoids are eaten by the definitive fish host (58).

In the plerocercoids of Schistocephalus solidus developing in teleost fishes; the genital primordia are well formed. This cestode matures in the gut of the definitive bird host in two days. Hopkins and Smyth (25)

have shown that these plerocercoids have sufficient food reserves to enable them to mature *in vitro* without any addition of exogenous food material. A temperature of 35-40° C. and suitable physiochemical conditions are sufficient to bring about their maturation.

Ligula intestinalis a diphyllidean cestode of aquatic birds, whose plerocercoids often cause castration of their second intermediate hosts, the fishes, is less progenetic than *S. solidus*. These plerocercoids take three days to mature in their definitive hosts, aquatic birds. They can be brought to maturity *in vitro*, though experiments of this type with *Ligula* were not as successful as those with *Schistocephalus* (55).

The phenomenon of progenesis is not uncommon in trematodes and cestodes. Helminthologists have realized that the progenetic species mature faster *in vitro*, than those which are less mature in the metacercarial or plerocercoid stages. *Linstowiella szidati*, *Posthodiplostomum minimum*, *Hysteromorpha triloba* and *Clinostomum complanatum* all mature quickly after being ingested by their natural definitive hosts. They also develop quickly and completely in some unnatural hosts. *In vitro*, however, the development of the first three to maturity often requires a longer time, and the adults produced are usually abnormal. *In vitro* experiments with *C. complanatum* have not been done.

2. Experimental results

The specimens of C. complanatum recovered from the body cavities of mice were in the same stage of maturity as the type specimens of Distoma complanatum Rud. as they are depicted in Braun's paper (9) (plate I figs. 6 and 7) and his type specimens of D. marginatum Rud. (plate I. figs. 4 and 8 and plate II. figs. 19 and 20). It is not possible to distinguish them morphologically one from the other. Both are found in the metacercarial stage in related fishes and inhabit as adults birds of the genus, Ardea. I do not think that the geographical distribution is sufficient to separate these two species. Morphological differences indicated by Braun (9) are subject to large individual variation. Therefore I am in full agreement with Baer (4) who regards C. marginatum as a synonym of C. complanatum and this latter name has been used herein.

Clinostomum complanatum is a progenetic trematode, whose male and female reproductive organs are present in the metacercariae. In my experiments, eggs were present in the uteri of the worms after four days in the peritoneal cavity of mice. These findings are similar to those of Hunter and Hunter (27) who found immature worms in the mouth of the great blue heron two days after feeding, and mature worms at the end of the fourth day.

In my experiments with cats, the animals showed no signs of asphyxiation which, according to Witenberg (62), happens in heavier infections. However, in the present experiments the greatest number of worms removed from the larynx and oesophagus of any one cat was four, despite the use of large numbers of metacercariae (up to one hundred per cat). Experiments with still larger numbers of metacercariae would be necessary to determine whether the trematodes can cause suffocation of their mammalian hosts.

Clinostomum lives in the mouth, pharynx and upper portion of the oesophagus of its natural host, where the body temperature, normally about 102° F., may be lowered by currents of inhaled air. This may explain the marked predilection of these worms for the scrotum of mice where the temperature is lower than in the body proper.

Clinostomum metacercariae which had been damaged by mechanical excysting, developed as rapidly as undamaged worms in the abdominal cavity of mice. These damaged worms showed no signs of regeneration, probably because the life span of Clinostomum in the peritoneal cavity was shortened by the host reaction. If this reaction could be suppressed by corticosteroids, the blockage of the reticulo-endothelial system, or by splenectomy, the regenerative ability of Clinostomum could

probably be studied. The life span of this trematode in the peritoneal cavity of mice, about seven days, was at least three times shorter than that in the natural host, the heron.

Trematodes of birds have never before been brought to maturity in the peritoneal cavity of mice. This method may be useful in the study of life cycles, not only of Clinostomum, but also of other trematodes which may be "cultured" in this way.

Since the early 1940's a great deal of research has been done in culturing trematodes and cestodes in vitro. This work has been carried out principally with helminthes which exhibit progenetic tendencies. Since C. complanatum mature quickly (4 days) both in their natural hosts, the herons (see page 70) and in my experimental mice, and since according to my observations this trematode can live for a week in 0.85% saline in the refrigerator at 1.5° C. an attempt to culture them in vitro seems warranted.

Experiments with cats showed that the great majority of trematodes were lost when these animals were infected by direct feeding. The worms were either digested or escaped from the mouth. Placing the metacercariae in the abdominal cavity of mice prevented their escape, thus insuring a greater return. Mice are relatively inexpensive and easily maintained, and easy to work with.

In the experiments with mice, two strains, and one hybrid were used; these were: A/Jax male, C57L male, and LAF male hybrids. It was observed early in the experiments that the C57L strain and the LAF, hybrids responded with an intensive inflammatory reaction to the infection. This reaction consisted of the production of a percipitate infiltrated with leucocytes which formed around the trematodes, completely encapsulating them in 4 to 5 days. In the A/Jax mice, however, this reaction took 7 to 8 days to develop. As it takes the parasite 4 to 5 days to mature, the A/Jax strain proved to be the most suitable host.

Experiments with splenectomized mice did not show that splenectomy depresses the host reaction to the worms, as all 47 trematodes recovered from operated mice were completely encapsulated at the end of seven days. Of the 59 worms recovered from the control mice 55 were encapsulated and 4 were unencapsulated. In this group more worms were found in the scrotum (67.8%) than in the group of splenectomized mice (46.8%).

VIII.

CONCLUSIONS

1. The hypothesis that Clinostomum complanatum may infect mammals proved to be correct. The experimental cats were infected by feeding them excysted metacercariae. The worms showed signs of maturation but had not produced eggs. Even though up to 100 metacercariae were fed to each cat, only approximately 2% of them were recovered from the larynx and the cats showed no signs of asphyxiation.

2. Clinostomum complanatum metacercariae introduced into the abdominal cavity of mice mature as rapidly as they do in their natural hosts, the herons.

3. Maturation of the bird trematode, Clinostomum complanatum in the peritoneal cavity of mice is a new technique. This new method may be significant for future work on other parasites.

4. Clinostomum metacercariae placed in the abdominal cavity of male mice, showed a marked predilection for the scrotum.

5. Damaged trematodes in which at least one branch of the intestine was severed, developed as rapidly as the undamaged specimens. No sign of regeneration was seen.

6. Excysted metacercariae of C. complanatum can live for a week in 0.85% saline in the refrigerator of 1.5° C. During this time no further development was observed.

7. Clinostomum complanatum was described by Rudolphi in 1814 on the basis of specimens taken from the oesophagus of Ardea in Germany (51). Clinostomum marginatum was described by the same author in 1819 on the basis of specimens from the oesophagus of Ardea in Brazil (52). There are no significant morphological differences which permit one to regard C. marginatum as a separate species distinct from C. complanatum. Thus, C. marginatum (Rud. 1819) Braun 1899 is a junior synonym of C. complanatum (Rud. 1814) Braun 1899.

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APPENDIX I.

Avian trematodes as incidental parasites of mammals.
(based on the monograph by Yamaguti (66))

Trematode	Avian Hosts	Mammalian Hosts	Vectors	Location
I. Heterophyidae Odhner, 1914				
1) *Heterophyes heterophyes* (Siebold, 1852) Stiles and Hassall, 1900.	*Milvus migrans* *M. parasiticus* *Pelicanus onocrotalus*	Man, cat, dog wolf, fox and weasel	*Mugil cephalus*	Egypt, Japan China
2) *H. expectans* (Africa and Garcia, 1935) Tubanqui and Africa, 1939	*Fregata ariel ariel*	dog		
3) *H. continua* Onji and Nishio, 1916	*Colymbus articus* *Larus argentatus*	cat (exper) dog and man	*Mugil cephalus* *Harengula zunasi*	Japan
4) *Apophallus muehlingi* (Jagerskiold, 1899) Luhe, 1909	*Larus ridibundus* *Larus argentatus* *Larus canus* *Pelicanus onocrotalus* *Himantopus himantopus* *Cancroma cochlearia* *Phalacrocorax pigmeus* *Colymbus septentrionalis*	dog and cat	*Abramis brama* *Leuciscus rutilus* *Blicca bjorkna* *Scardinius erythrophthalmus* *Perca fluviatilis*	Europe (Rumania, Poland)

5) A. donicus (Skrjabin and Lindtrop, 1919) Price, 1931	Buteo buteo Asio otus, Ciconia ciconia, Merqus merganser, Columba livia, Nycticorax nycticorax Larus ridibundus, Sterna cantiaco, Coturnix communis Turtur communis	dog, cat, rat, rabbit (exper.) Vulpes lagopus	Perca, Lucioperca Blicca, Acerina, Abramis, Scardinius	Europe and North America
6) A. imperator Lyster, 1940	pigeon (exper.) Ardea herodias, Mergus merganser, Lophodytes	cats (exper.)	Salvelinus, Salmo, Perca, Catostmus	North America
7) Phagicola arnoldoi Travassos, 1928	Diomedea melanophys	dog, mouse		Brazil
8) P. longus Ransom, 1920	Milvus migrans	Vulpes, Lutra, cat, dog	Mugil, Lichia Bargus	North America
9) P. minutus (Looss, 1899) Stunkard and Haviland, 1924	Ardea cinerea	dog, cat		Egypt
10) Centrocestus armatus (Tanabe, 1922) Isumi, 1935	Nycticorax nycticorax Ardea cinerea Phalacrocorax carbo Milvus migrans	cat man, cat, dog, rat mouse and rabbits (exper.)	cyprinids	

11) C. caninus Leiper, 1912	Nycticorax nycticorax Egretta intermedia Platalea leucorodia	man, cat, dog, rat guinea-pig (exper.)	Channa formosana Cyprinus carpio, C. auratus, Gnathopogon elongatus Acheilognathus spp., Misgurnus anguillicaudatus, Parasilurus asotus Opsariichthys uncirostris	Formosa
12) Cryptocotyle concava (Crepl., 1825) Luhe, 1899	Colymbus rufogularis Phalacrocorax, Anas, Anser, Nyroca, Oidemia, Merqus Alga, Glaucionetta, Ardea, Larus, Meleagris, Columba, Gallus, Risso, Sterna, Somateria	dog pig and rabbit (exper.)	Atherina, Gobius Mesogobius, Mullus	
13) C. americana Ciurea, 1924	Marine birds	dog Phoca vitulina		North America
14) C. jejuna Nicoll, 1907	Totanus calidus, Larus argentatus, L. ridibundus, Sterna hirundo, Rissa, Fratercula, Uria, Cepphus Phalacrocorax, Stercorarius Somateria	dog	Gobius melanostomus Peringia ulvae Gobius minutus G. ruthensparri	Europe

15) C. lingua (Crepl., 1825) Fischoeder, 1903	Larus, Sterna, Fratercula, Uria, Cepphus, Phalacrocorax, Somateria, Stercorarius	dog, cat, Vulpes fulva white rat and guinea pigs (exper.)	Tautogolabrus adspersus, Tautogaonitis, Cottus scorpius, Syngnathus typhle	Europe, North America, Japan, Siberia
16) Haplorchis taihokui (Nishigori, 1924) Chen, 1936	Nycticorax nycticorax	dog, cat, rabbit, rat mouse, guinea-pig, man	Cyprinidae, Siluridae Cobitidae and Mullet	Formosa
17) Metagonimus minutus Katsuta, 1932	piscivoruos birds	cat, mouse (exper.)	mullet	
18) Dexiogonimus ciureanus Witenberg, 1929	Larus spp.	dog, cat		Palestine
19) Stellanchasmus falcatus Onji and Nishio, 1915	Colymbus arcticus	cat (exper.)	Mugil cephalus	
20) Pygidiopsis genata Looss, 1907	Pelicanus onocrotalus Butorides virescens Columba livia Numida meleagris Anas domestica Anser domesticus Milvus migrans	cat, dog, rat (exper.)		Egypt, Palestine, China, Europe

21) *P. marivillai* Refuerozo and Garcia, 1938	*Haliaetus leucogaster*	dog (exper.)	*Mugil dussumieri*	
22) *P. summa* Onji and Nishio, 1916	*Colymbus arcticus* *Milvus migrans*, *Nycticorax nycticorax*	cat, dog (exper.) rat	*Mugil cephalus* *Glossogobius bunneus*	Japan, China
23) *Stictodora sawakinensis* Looss, 1899	*Larus* sp., *Puffinus kuhli*, *Anas platyrhyncha*, *Sterna hirundo*	dog, cat	*Mugil* spp.	Egypt, Philippines, Palestine, W. Siberia
24) *S. guerreroi* Garcia and Refuerzo, 1936	*Larus ridibundus*	dog, rat, mouse, puppy, cat (exper.)	*Hepsetia balabacensis* *Hemiranyphus georgi* *Ambassis buruensis* *Mugil dussumieri*	Philippines

APPENDIX II

Species of the genus Distoma listed by Rudolphi in his "Entozoorum sive vermium intestinalium historia naturalis" (50).

1. D. hepaticum Abildg.
2. D. ovatum R.
3. D. cuneatum R.
4. D. hians R.
5. D. cucumerinum R.
6. D. incisum R.
7. D. transversale R.
8. D. atomon R.
9. D. polymorphum R.
10. D. globiporum R.
11. D. cygnoides Zed.
12. D. seriale R.
13. D. simplex R.
14. D. divergens R.
15. D. longicauda R.
16. D. delicatulum R.
17. D. maculosum R.
18. D. elegans R.
19. D. cirratum R.
20. D. nanum R.
21. D. involutum R.
22. D. crassicolle R.
23. D. tereticolle R.
24. D. heterostormum R.
25. D. caudale R.
26. D. soleaforme R.
27. D. purillum Zed.
28. D. macrostomum R.
29. D. mesostzmum R.
30. D. microstomum R.
31. D. hyslinum R.
32. D. flexuosum R.
33. D. clavatum R.
34. D. cylindricum R.
35. D. granulosum R.
36. D. inflexum R.
37. D. varicum Zed.
38. D. ovreatum R.

39. D. gibbosum R.
40. D. excavatum R.
41. D. appendiculatum R.
42. D. areolatum R.
43. D. alatum Zed.
44. D. crenatum R.
45. D. scabrum R.
46. D. crassiusculium R.
47. D. punctum Zed.
48. D. nodulosum Zed.
49. D. lanceatum Zed.
50. D. lineare R.
51. D. tregonocephalum R.
52. D. echinatum Zed.
53. D. uncinatum Zed.
54. D. miliare R.
55. D. cinctum R.
56. D. apiculatum R.
57. D. denticutatum R.
58. D. spinulosum R.
59. D. ferox R.
60. D. Lima R.

Species dubiae

61. D. falconis Chrgaetis
62. D. Falconis M'loi
63. D. Buteonis
64. D. Collurionis
65. D. Anatis furcae
66. D. Anatis domesticae
67. D. Gruis
68. D. Ardeae stellaris
68. (?) D. Testusinis Mydae
69. D. Crotali Durissi
70. D. colubri Natricis intest
71. D. Colubri Natricis pulmonale
72. D. Colubri
73. D. Rajae
74. D. Sturionis
75. D. Anarrhychus Lupi
76. D. Coryphaene
77. D. Clupeae Rhenanae
78. D. Cyclopteri
79. D. Esocis Lucii
80. D. binode Zed.
81. D. districhum Zed.

Note: 82 species in all, considering that 68 has been repeated.

APPENDIX III

Species of the genus Distoma listed by Rudolphi in his "Erster Nachtrag zu meiner Naturgeschichte der Eingeweidewurmer" (51).

D. hepaticum
D. hians
D. polymorphum
D. anguillae
D. maculosum
D. clavatum
D. laureatum
D. echinatum
C. uncinatum
D. ferox
D. Crotali Durissi
D. Colubri Natricis pulmonale
D. complanatum R.
D. Globulus
D. torulosum R.
D. truncatum Abildg.