The Life History and Biology of *Platynosomum Fastosum* Kossak, 1910 (*Trematoda: Dicrocoeliidae*)[1]

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VERY LITTLE is known of the biology of trematodes belonging to the family *Dicrocoeliidae* Odhner, 1910. This is due, partly, to a lack of interest on the part of helminthologists and also to the fact, well illustrated by the studies on *Dicrocoelium lanceatum*, that the life cycles of this group are somewhat complicated.

Platynosomum fastosum is a member of this group. In Puerto Rico, it infects the majority of domestic cats and inhabits the bile ducts, causing changes similar to those in other hepatic distomiases. The possible value of this parasite in studying various aspects of distomiases of medical importance and the need to enlarge upon its present knowledge demand the elucidation of its life history.

REVIEW OF THE LITERATURE

The genus *Platynosomum* was created by Looss[2] for dicrocoeliids of birds and mammals that differ from the related genera *Dicrocoelium*, *Eurytrema*, and *Lyperosomum* in the marked width of the adults with relation to length, the maximum width being at the level of the ovary with testes placed side by side and very near to, or touching, the acetabulum. Up to the present there are 21 species in the genus, 15 of which infect the liver of birds, and the rest a wide range of mammals.[3] Of these last, only two species have been found in cats. The first, described by Braun[4] in a civet cat, was named *Dicrocoelium concinnum*. Looss[5] considered it a link between *Platynosomum* and *Eurytrema* because of certain characteristics he found common to both genera. In this author's opinion, its fine, compact,

1. Received for publication December 6, 1944. Abridgment of a thesis submitted to the Graduate Faculty of the University of Minnesota, August 1944, in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

2. A. Looss, Ueber einzige zum Teil neue Distomen der europanischen Fauna. Centralbl.f. Bakt.u.Parasit., I (Abt. Orig.), **43**:604, 1906–7.

3. E. Heidebegger and H. Mendheim, Beitrage zur Kenntniss der Gattung *Platynosomum*, Part I, Ztschr.f.Parasitenk., **10**:94, 1938–39.

A. O. Foster, Some helminths of the woolly opposum in Panama. Tr.Am.Micr.Soc., **58**:185, 1939.

4. M. Braun, Ein neues *Dicrocoelium* aus der Gallenblase der Zibeth Katze. Centralbl.f. Bakt.u.Parasit., **30**:700, 1901.

5. A. Looss, *op. cit.*

scaly body-covering distinguished it from any of these genera, but the parasite has been considered a member of *Platynosomum*.

The second species, *Platynosomum fastosum*, was found by Kossak[6] in *Felis minuta*. It has been recorded in the common house cat (*Felis catus domestica*) from distant tropical regions and in the ferret (*Grisson vittatus*) of Brazil by Pinto and Almeida,[7] who renamed the parasite, *Eurytrema fastosum*.

Platynosomum fastosum was first recorded in Puerto Rico by Dikmans[8] and later by Hoffman.[9] van Volkenberg,[10] however, claims the parasite is *Platynosomum concinnum*. A comparison of the original descriptions given by Braun and Kossak with the local form confirms it as *Platynosomum fastosum*, provided adult morphology is accepted as a firm criterion of differentiation, but evidently further studies are needed to determine the exact taxonomy of these parasites. It is possible that the two species will be considered synonymous when their life histories are known. We know that adult characteristics and host specificity do not constitute a safe basis for classification, particularly since Lent and Texeiras[11] observe that individual variations are pronounced in dicrocoeliids.

Very little has been published on the genus *Platynosomum* with the exception of certain studies on its taxonomy and pathogenicity. Magarinos and Pinto[12] studied the pathological alterations in light infections in Brazilian cats. This same subject has been discussed by Ware[13] for various members of *Dicrocoeliidae* affecting domestic animals. van Volkenberg[14] tried to elucidate the life cycle of *Platynosomum fastosum;* he found the molluscan intermediate host in Puerto Rico but failed to complete the cycle by feeding infected snails to kittens.

The works of Henkel,[15] Mattes,[16] and Neuhaus[17] on *Dicrocoelium*

6. W. Kossak, Neue Distomen. Centralbl.f.Bakt.u.Parasit., **56**:114, 1910.
7. C. Pinto and J. L. Almeida, Sinopse des helmintos dos animaes domesticos do Brasil. O Campo (Rio de Janeiro), **6**:60, 1935.
8. G. Dikmans, Report of the Parasitologist. An.Rep.Agric.Exp.Sta., Mayagüez, Puerto Rico, 1927.
9. W. A. Hoffman, Occurrence of *P. fastosum* in Porto Rico. J.Parasitol., **19**:91, 1932.
10. H. L. van Volkenberg, An.Rep.Agric.Exp.Sta., Mayagüez, Puerto Rico, 1936.
11. H. Lent and J. F. Texeiras, Pesquisas helmintologicas realisadas no Estado de Para. Mem.Inst.Oswaldo Cruz, **32**:449, 1937.
12. C. Magarinos and C. Pinto, Processos pathogenicos determinados pelos trematoides *E. fastosum* e *E. coelomaticum*. Mem.Inst.Oswaldo Cruz, **31**:731, 1936.
13. F. Ware, Some members of the family *Dicrocoeliidae* affecting domestic animals. J. Comp.Path. and Therap. (Edinburgh), **36**:33, 1923.
14. H. L. van Volkenberg, An.Rep.Agric.Exp.Sta., Mayagüez, Puerto Rico, 1937.
15. H. Henkel, Untersuchungen zur Ermittelung des Zwischenwirtes von *D. lanceatum*. Ztschr.f.Parasitenk., **3**:664, 1931.
16. O. Mattes, Die Entwicklungsgang der Lanzettegels *D. lanceatum*. Ztschr.f.Parasitenk., **8**:371, 1936.

lanceatum, a sheep liver fluke, have served as a basis for the present study. Up to now, this is the only member of the family whose biology has been fully described. Throughout the present study the author will refer to the above-mentioned articles in order to establish a comparison between both parasites which, it is hoped, may serve as a basis for similar studies on related species.

METHODS OF EXPERIMENTATION

The first step in studying the life cycle of *Platynosomum fastosum* consisted in confirming or refuting van Volkenberg's assertion that *Subulina octona* serves as the intermediate host. A survey was made of the various species of snails in house-yards frequented by cats and those collected were brought to the laboratory for dissection. Larval stages of *Platynosomum* were identified by comparison with those of *Dicrocoelium lanceatum*, according to the published description. At the same time parasite-free snails, particularly *Subulina octona*, reared in the laboratory, were exposed to cat excreta-bearing eggs, sedimented. The best results were obtained by previously starving the snails.

Some of the experimentally infected *Subulina* were used to study larval development. A number were dissected five minutes and up to three and a half hours after they were observed ingesting the egg. Their digestive tracts were examined directly under the microscope or sectioned to study hatching and the free-swimming miracidium.

Early larval stages were studied in sections. The snail was carefully removed from the shell, fixed in Bouin or Zenker fluids, sectioned at 6 to 10 microns, and stained in Heidenhain iron-hematoxylin or in Bullard hematoxylineosin. Later stages were studied in sections or alive, sometimes stained *in toto*. Neutral red was employed as a vital stain in some cases.

The factors affecting the viability of the egg were studied by spreading sedimented excreta on filter papers. Some of these papers were then exposed to direct sunlight for one to six hours; others were allowed to dry in the shade for one to eight days. After each interval, they were moistened, and young snails allowed to feed on the excreta. Viability was determined by microscopical study of the eggs and of their ability to hatch in the snails or to infect them.

The effect of drying was also studied by burying excrement in dry sand and examining it after two and four weeks. The behaviour

(*C. vitrina*). Ztschr.f.Parasitenk., **8**:431, 1936; Der Invasionsweg der Lanzettegel—Cercariae bei der Infektion der Endwirtes. Ztschr.f.Parasitenk., **10**:436, 1938.

of the eggs under optimum conditions was studied by burying excrement in the shade during the wet season, a portion being removed weekly for the first month and afterwards, every two weeks, and brought to the laboratory for study by the usual procedures. Effect of temperature was determined by exposing excreta at 40° C and at 44° to 47° C, examining it at intervals.

The relation of light to emergence of daughter sporocysts was determined by irradiation of groups of snails with a bright lamp for various lengths of time. Each trial lasted for 14 days, at the end of which the groups were given other exposures. As controls, one group was kept constantly under room light conditions and another in the dark. The output of sporocysts was recorded daily. The effect of continuous irradiation was studied by comparing the output of four *Subulina* during alternate seven-day periods of illumination and room light. The role of moisture was determined by comparing the output under alternate dry and humid conditions.

An idea as to the mode of arrival of the parasite in the definitive host was obtained by preliminary attempts at direct infection of cats through feeding them daughter sporocysts that had emerged from the snail. A small cat was exposed to a variety of conditions: by caging him several times with infected snails, by spraying sporocysts on his fur, and by feeding him liberated cercariae. Eight more cats were used in these experiments and all, except one, were killed after several weeks and thoroughly examined.

Failure to infect these animals suggested the existence of a second intermediate host. Lizards (*Anolis* spp.) were considered the most probable vectors. A young cat was fed 108 of these lizards collected from several localities in San Juan. Three weeks after the last feeding the animal was autopsied and a large number of *Platynosomum* obtained from the liver. Subsequently, 80 lizards were autopsied. Many of the larvae obtained were fed to 3 cats and 4 mice to determine the mode of arrival and development in the liver.

OBSERVATIONS AND RESULTS

The Egg (*Fig. 1*). As observed in the excreta, the egg is thick-shelled, symmetrical, operculated, and fully embryonated, averaging 30 microns long by 24.5 at its widest part. The shell is dark and smooth, sometimes with a small posterior knob. The operculum is at times difficult to outline due to inner incrustations of the shell. The miracidium appears as a pyriform structure, its anterior extremity projecting into the zone of the operculum. It is surrounded by granules exhibiting Brownian movement and remnants of yolk cells. These granules increase in amount, if the egg is stored, and may constitute excretory products of the miracidium.

The egg has proved to be very resistant under abnormal situations, particularly sunlight. Even 6 hours of continuous radiation failed to kill many of them although some, which appeared normal, failed to hatch in the snail. They may stand drying for over three days; in our experiment none was able to hatch after 8 days. At 40° C they were still alive after 21 hours and at 44° to 47° C, after 4 hours. Under optimum conditions, they lived for several months. Putrefactive products in unchanged water or charcoal mixtures caused their death in a few days.

These observations demonstrate that the parasite is able to live under a wide range of conditions. Our assertion is supported by its occurrence in every locality of Puerto Rico, in spite of pronounced climatic variations.

The Molluscan Intermediate Host. Subulina octona was proved to act as the intermediate host of *Platynosomum fastosum* both by survey of the molluscan fauna and by experimental infections. The snail, 9 to 20 mm. long, is sharply conical with a transparent shell. Adults carry 3 to 6 white, spherical, hard-shelled eggs a millimeter in diameter. Young hatch within the marsupium although at times the eggs are laid and then hatch within two days. The snail may acquire aquatic habits.

In Puerto Rico *Subulina octona* also acts as host for other parasites; at least three other trematodes have been found in it during this study.[18] According to van Volkenberg[19] it may also harbour the larva of the cat lungworm, *Aelurostrongylus abstrusus*, and the poultry tapeworm, *Davainea proglottina*. Alicata[20] reports it as a host of the adolescaria of *Postharmostomum gallinum*, a cecal fluke of poultry.

The Miracidium (*Fig. 2*). Within 15 minutes after the egg arrives in the crop of the snail, the operculum suddenly opens and the miracidium becomes partially extruded within its embryophore. The process is identical to that described by Henkel[21] for *Dicrocoelium lanceatum*. The force that causes the release of the operculum is not well understood. Digestive enzymes apparently play no part in it, for *in vitro* experiments with artificial gastric juice and the

18. W. A. Hoffman, An. Rep. School of Tropical Medicine, San Juan, Puerto Rico, 1943. J. F. Maldonado, A note on the life cycle of *Tamerlanea bragai*. J.Parasitol., 29(6): 424, 1943.

19. H. L. van Volkenberg, *op. cit.* (14).

20. J. E. Alicata, The life cycle of *P. gallinum*, the cecal fluke of poultry. J.Parasitol., 26:135, 1940.

21. H. Henkel, *op. cit.*

intestinal contents of the snail failed to affect the egg. Faust and Khaw[22] and Henkel[23] suggest osmotic forces are principally concerned with the process, although the mode by which they act is not clearly explained.

Extrusion of the egg contents is followed by activation of the miracidium, which shortly thereafter frees itself through a vent made with the help of its stylet. The free-swimming miracidium is pear-like, about 30 microns long. Its body is partially covered with long cilia that produce a rotatory movement. On the anterior extremity, the organism possesses a slender stylet observed best in dead or crushed specimens. Just back of this stylet there is a histolytic gland whose secretion facilitates penetration of the host; the space back of the gland is occupied by germ cells that can be seen only in stained forms. They are four in number and exactly similar in nuclear characteristics, differing from Henkel's report for *Dicrocoelium lanceatum*. The excretory system is composed of two flame cells placed in the midbody. The miracidium is characterized by the presence of two groups of 6 to 8 refractile bodies at its back end.

The life of the miracidium is very short unless it is able to reach the tissues of the host. It may move actively in 0.85 percent salt solution for 10 or 15 minutes but thereafter, loses its energy rapidly. Death ensues within 30 minutes. It was not possible to observe actual migration of the miracidium into the tissues of the snail. Sections of recently infected *Subulina* demonstrated that the organism may move into the intestine and from here into the surrounding connective tissue by piercing the wall (Fig. 3). When larval stages appear among the follicles of the digestive gland, a route by the bile duct is suggested; no evidence of penetration through the crop or the oesophagus was obtained.

The migratory ability of this organism, once it reaches the tissues, depends on its reserve of energy. It always restricts itself to the connective tissues of the snail, showing a predilection for the zone of the respiratory cavity.

The Mother Sporocyst (*Figs. 3, 4*). During the ensuing five days, the miracidium becomes adapted to its new environment. The locomotive organs, its histolytic gland, and stylet disappear. The organism transforms into a mother sporocyst, appearing at this time like a simple bag with the sole function of sheltering the four germ cells.

After the adaptation stage passes, the nuclei of these cells enlarge

22. E. C. Faust and Oo-Keh Khaw, Studies on *Clonorchis sinensis* (Cobbold). Am.J.Hyg., Monographic Series 8, 1927.

23. H. Henkel, *op. cit.*

(Fig. 3) previous to mitosis. At this time there is no apparent difference among these cells, contrary to what has been reported by Mattes[24] for *Dicrocoelium lanceatum*. On the tenth day the mother sporocyst is already conspicuous. The numerous germ cells that it carries vary as to size, chromatin content, and presence or absence of a large, eccentric karyosome. These variations may indicate the metabolic activity of the cell, the larger open nuclei being noted in those ready to divide.

On the thirteenth day the mother sporocyst has lost its original oval shape and becomes irregular in form by extending through the weaker spots in the tissues. At this stage some germ cells have already started to aggregate into groups by failing to separate upon mitosis and thus constitute the primordia of second generation sporocysts. Beyond this period and up to the fourth week, development is characterized by marked enlargement of the entire structure and by the increasing numbers and size of germ masses.

By the twenty-eighth day, the mother sporocyst has attained maturity, that is, it has reached the stage at which the first daughter sporocysts become able to leave it. Its size and form cannot be determined at this time since the parasite cannot be separated intact; however, portions that protrude through the tunica propria (Fig. 4) give a good idea of its structure.

The parasite is very delicate and consists of a simple membrane enclosing a large number of germ masses of various sizes and islands of germ cells. The organism is motionless, although motile germ masses inside give the false idea that it can move slightly. The membrane forms a complex with the tissues of the host and thus appears to be absent. In old infections the load of daughter sporocysts obscures the outlines of the mother, which lives and functions for a long time, however, whilst large numbers of young forms are continuously being produced.

The Daughter Sporocyst (*Figs. 5, 6*). The daughter sporocysts are formed in the same general pattern of other trematodes. Mitosis of individual germ cells may go on indefinitely in the mother sporocyst. At times, the resulting units fail to separate and continue to divide forming a germ mass. After several divisions, a peripheral cell elongates and surrounds the rest, forming the primordial body covering. By the second week of age clefts appear in the center of the ball, and these soon enlarge and coalesce to form a body cavity.

The cells in the sporocyst segregate in this manner into two types. Some remain free in the body cavity and constitute the germinal

24. O. Mattes, *op. cit.*

tissue from which cercariae will arise. The rest become arranged at the periphery to form the soma of the daughter sporocyst (Fig. 5). The germinal tissue consists, from the start, of small aggregates of cells and free elements; the former represent the primordia of the cercariae, the latter degenerate and disappear. Figure 5 shows four of these cells in a state of disintegration with pycnotic nuclei.

The somatic tissue gives rise to the muscular and excretory systems. The outermost cells secrete a relatively thick, eosinophilic cuticle that replaces the embryonic one. The inner elements lining the body cavity form a thin membrane of great importance in later life.

The daughter sporocyst leaves the mother as soon as it becomes completely organized, the first free sporocysts being obtained some times in month-old infections. The migrating sporocyst (Fig. 6) has a well-developed musculature, which enables it to move to distant parts of the snail. Its body cavity is considerably enlarged by elongation and thinning of the wall. All individual germ cells have disappeared and the parasite bears the final share of cercarial germ masses.

The Mature Daughter Sporocyst (*Figs. 7, 8, 9*). The mature daughter sporocyst may be described as a highly organized structure, perfectly blind, containing an average of 17.6 fully developed cercariae. These characteristics distinguish it from *Dicrocoelium lanceatum*. Maturity is attained about a month after exit from the mother, during which time the former oval organism has enlarged considerably to provide space for the growing cercariae.

Its shape varies with the state of contraction, practically spherical when fully contracted and banana-shaped when relaxed. The parasite moves vigorously by worm-like movements. Motility, however, decreases with advancing age, older forms maintaining a constant spherical shape.

Mature daughter sporocysts (Fig. 7) measure from 0.6 to 1.1 mm. long, when relaxed, according to the number of cercariae contained. The cuticle is transversely striated and uniformly thick. The cells underneath are parenchymatous, with longitudinal and circular muscle fibers. The thin internal lining mentioned before has separated from the body and may be seen twisted or folded inside. It has a leathery consistency in the older stages, acting as a bag for the cercariae and suddenly unfolding if the body wall is broken.

The excretory system of the mature daughter sporocyst is fairly elaborate (Fig. 8). It consists of two opposite longitudinal trunks from whose ends primary, secondary, and tertiary tubes arise. The

latter connect to the flame cells. The entire musculature is thoroughly permeated with excretory units although these are more numerous at the extremities. A definite connection with the outside could not be observed.

When removed from the snail, the mature daughter sporocyst may remain active for 16 hours in 0.85 percent salt solution. Immature forms die very soon. In rain water some mature forms lose motility within an hour but the cercariae contained within remain active for a longer time, dying soon in others, however.

These observations indicate that the resistance of the sporocyst to extraneous conditions increases with age. The experiments with rain water suggest a difference among mature daughter sporocysts, which to all signs appear equally developed. The parasite, therefore, undergoes physiological maturation after having achieved full morphological differentiation.

As an introduction to the study of the mode of infection of the final host, mature sporocysts obtained by dissecting the snail were treated with the digestive juices from a cat. The cercariae were activated for about 10 minutes and then died; the sporocysts were immobilized in a shorter time. A young cat was also fed mature forms. Post mortem findings were entirely negative. These tests indicate that at this stage the cercariae cannot stand the conditions found in the digestive tract of the cat.

Continued observation of infected snails demonstrated later that the mature daughter sporocyst is able to emerge from its host. This process was first observed in a snail that had been exposed to sunlight in imitation of Neuhaus' procedure[25] with *D. lanceatum*. The phenomenon has been constantly observed in all infected *Subulina*. The sporocyst may reach the outside world either by way of the respiratory opening or by the tunica propria. Through the first route, the parasite must tear the respiratory epithelium so as to fall into the breathing cavity, moving slowly to the breathing pore from where it is expelled. The route of the tunica propria is chosen by those sporocysts in adjacent areas, which, after piercing it, move down between the shell and body proper to be expelled through the shell aperture.

Occasionally, sporocysts were found in the intestine, but they were always dead or devoid of musculature. Because of its rare occurrence, it was impossible to study this finding further.

Newly emerged sporocysts look like hyaline, whitish masses. Their posterior extremities remain compressed for a short time due

25. W. Neuhaus, *op. cit.* (2d part).

to the pressure of the body of the snail when expelled. The cercariae move continuously inside but are unable to free themselves. Free cercariae were never seen either in or outside of the host.

The time at which emergence commences is exactly 60 days from the ingestion of the ovum.

Motility in recently emerged forms is restricted to feeble contractions. Within 6 to 8 hours the musculature disintegrates and the leathery inner bag unfolds (Fig. 9). Under laboratory conditions, the enclosed cercariae start to die within 24 hours, and the bag slowly softens and completely disintegrates in 36 hours.

Factors Influencing Emergence. These may be considered as intrinsic and extrinsic factors, the first having to do with the sporocyst and the cercariae within. The fact that only mature sporocysts can emerge indicates that, because of some special sense, the urge to reach the outer world is an idiosyncrasy of the parasite. It is believed that the cercariae are directly related to it.

The possible role of such extrinsic factors as light and humidity was suggested by the close relationship to *Dicrocoelium lanceatum*. Temperature was not considered significant under tropical conditions.

Extensive qualitative and quantitative studies on the function of light and humidity demonstrated the latter factor as the only one of significance. The average daily output of sporocysts per snail remained practically the same irrespective of the length of exposure to bright light. These same studies showed that output is rather an individual trait of the snail, possibly determined by the degree of infection. Continuous illumination, however, resulted in an increased output of sporocysts, probably due to increased activity of the snails. It is a current experience that sporocysts emerge most often when the mollusc is active.

Moisture is entirely essential for emergence as, in its absence, the snail stays dormant and the sporocysts do not emerge. As soon as water is added to the substratum, the snail becomes active and the sporocysts come out. Moreover, moisture is needed by the parasite even after emergence for without it, it dies within several minutes.

These observations suggest that, in nature, sporocysts must come out during wet weather. In places with well-marked seasons of rainfall, the parasite may show a definite seasonal distribution.

Daily Output of Sporocysts. The number of daughter sporocysts from a single miracidium is practically impossible to determine. The daily output may give a fair answer. The studies on light which comprised several dozen snails demonstrated that the number of sporocysts per snail per day varied between 1.63 and 19.6, with an

average of 7.3. The output in nature may also lie within this range, although moisture variations undoubtedly would work a decided effect.

The Cercaria (*Figs. 6, 10*). The observations on young daughter sporocysts demonstrated that their share of cercariae is established at a very early stage in their development. The small masses remaining in the sporocyst, after the other germ elements disappear, consist of a few undifferentiated cells. One of these cells acts from the beginning as an embryonic wrapping (Fig. 6).

For a certain length of time the mass grows by repeated division of its units without exhibiting histological differentiation. Prior to organ formation the ball elongates and muscular function starts. The processes by which the various organs form are entirely identical to those so well described by Neuhaus[26] for *Dicrocoelium lanceatum* and will be described concisely hereafter.

The suckers commence to form simultaneously at the time the germ ball is about 60 microns long. The acetabulum arises as a prominent, almost terminal, protuberance, the acetabular cavity forming as a depression in the sucker at a much later stage. At first the oral sucker is very massive but can only be outlined well in sections. The oral cavity arises as a frontal indentation, gradually extending deeper until it reaches the primordium of the pharynx to continue into a very fine oesophagus.

At an early stage there are set aside in the center of the mass 8 or 10 cells that stand out by their large open nuclei and round, eccentric karyosomes. Greenish granules soon appear around these nuclei and in the neighborhood of the oral sucker (Fig. 10), suggesting that the cells have elongated in this direction. They constitute the large glands of the cercaria. Towards the end of development, two sets of small glands arise in the antero-acetabular region. The original cells from which they arise could not be distinguished, however, from the rest of the body cells by any of the techniques used.

During the first stages, the cercaria is covered with a thin cuticle of flattened cells proceeding from the original one in the very small mass. This covering is replaced towards the end of development by the definitive cuticle, a non-cellular covering secreted by the subcuticular cells.

The formation of the excretory system may be followed best in the living state. Before any organs have appeared, it consists of a pair of flame cells near the equator of the mass, each cell connecting to the outside by a fine, winding tube that opens at the postero-

26. W. Neuhaus, *op. cit.* (1st part).

lateral margins. The number of flame cells increases by mitosis of the original ones. The distal portion of the excretory tubes bend toward each other until they meet at the center, forming the primordial excretory bladder, which elongates slowly but is not yet fully developed by the time the cercaria attains its final form.

The process by which the stylet develops could not be followed accurately. It is definitely the last organ to develop, appearing at first transparent and delicate but shortly after becoming chitinized and stout.

The genital primordium arises from a small group of cells lying dorsal to the acetabulum, which gradually increase in numbers and extend forward and back from the original site until they surround the inner face of the sucker. These cells are distinguished by their dark, compact, and relatively smaller nuclei. In the final stages of development, a broad protuberance forms at the distal end of the body. By constriction at the base a globular tail arises that fails to grow beyond this shape.

The Mature Cercaria (*Figs. 11, 12; 13*). At rest, this organism is ellipsoidal, 209 to 235 microns long and 81 to 88 microns at its widest level. Its cuticle is smooth, 4 microns thick, with short, thick-set spines at the extremities. The lips of both suckers are provided with sensory hairs.

The oral sucker lies ventrad, 47 microns long by 36 wide; the oral cavity opens directly ventrad with a pharynx 14 microns wide, oval and non-muscular. The oesophagus is thin, almost imperceptible, reaching to the small glands; its bifurcation could not be observed. The stylet, 21 microns long, is located dorso-ventrally above the front end of the oral sucker; the acetabulum is muscular, oval, 36 microns long by 50 wide, and centrally located. The acetabular cavity is large with lobed lips.

Large glands appear in two sets, the fundi over the acetabulum giving the cercaria a hunched form. Their ducts consist of the prolongation of each cell into a narrow neck extending over the latero-dorsal part of the oral sucker and opening on both sides of the stylet. The secretory granules are greenish; the consistency of the glandular contents varies, becoming more fluid with increasing age.

Eight or 10 small glands are placed in two sets just in front of the large ones. In vital staining with neutral red, their secretion appears dust-like and homogenous. The ducts of these glands extend forward and bend to merge with those of the large glands. The excretory system is identical in pattern to that of *Dicrocoelium lanceatum*, with the excretory bladder Y-shaped and extending two-

thirds the distance between the posterior tip and the acetabulum, opening distally. Each arm of the bladder continues into a tortuous collecting tube which reaches to the oral sucker and turns back to end near the tip of the body. The flame pattern is 2 [(2 + 2 + 2) + (2 + 2 + 2)].

The bulk of the genital anlage (Fig. 13) occupies the area between the excretory bladder and the acetabulum. It projects forward over the sucker as a double cordon of cells to end just anterior to it. These details, however, may be seen in sections only. The tail is rudimentary, globular, and firmly anchored to the body.

During the emergence of the sporocyst, the cercaria releases the contents of the large glands; this usually occurs as soon as the sporocyst comes in contact with the air. The secretion comes out in a constant stream and forms a mass that floats back and forth within the sporocyst, changing into large floccules within an hour and followed by disintegration. Some cercariae may release their secretion by pressing them under a cover glass, but this is merely a mechanical force that cannot explain the nature of the process in unhandled sporocysts.

All the activities of the cercaria are restricted to the enclosure of the sporocyst; it dies within 24 hours in a moist environment.

Extra-molluscan Phase of the Life Cycle

(a) *Studies on direct transmission.* In order to determine whether the cat acquires *Platynosomum* by ingesting the emerged sporocysts, or the cercariae freed from them, 8 young cats were treated as described in the section on methods. Briefly, it may be said that at no time was an infection obtained by feeding large numbers of sporocysts passed by the snails over 24-hour periods, by feeding freed cercariae, or by exposing the cats to the parasite under various conditions. All possible ways by which infection could occur were tried in the laboratory, but the larva was unable to establish itself in the cat by direct contact. The already mentioned *in vitro* observations with digestive juices represent a simple chemotactic effect and suggested the existence of a second intermediate host.

(b) *Studies on the second intermediate host* (*Figs. 14, 15*). The high incidence of *Platynosomum* in Puerto Rico indicates that the cat acquires this parasite easily. Inasmuch as the infection must be acquired *per os*, a study of the feeding habits of the cat furnished the clue to the mode of transmission.

Among the various animals on which this feline is known to prey, the only possible vector considered was the common lizard, *Anolis*

cristatellus. Practically every cat in Puerto Rico uses this reptile as a source of food, and the experiments undertaken proved conclusively that it acts as the second intermediate host. It has been impossible, however, to determine by which mode the lizard becomes infected, hence this aspect of our investigations is still under study.

All the data on *Anolis cristatellus* as intermediate host of *Platynosomum fastosum* was obtained by the study of naturally infected specimens; in all, 80 lizards were collected from several places in the City of San Juan. The incidence of the parasite varied from 30 to 75 percent in different groups, with a load of one to well over 300, the average being 16. In every case all the forms encountered had attained the infective stage and no intermediate stages in development were obtained.

The infective stage of *Platynosomum* inhabits the common bile duct of the lizard (Fig. 14). This organism measures 450 microns long by 200 wide in the resting state, that is, about twice the size of the cercaria (Fig. 15). Its oral sucker is muscular, 87 microns in diameter, and located subventrad; the acetabulum is powerful, 120 microns wide, and located near the center of the body. Its body is covered with a thick cuticle bearing blunt protuberances, which are more numerous around the oral sucker and suggest their sensory nature. The digestive tract is already clearly outlined, though not very conspicuous. The oesophagus extends half-way the distance between the pharynx and the acetabulum. The small glands described in the cercaria are preserved, but the most prominent structure is the excretory bladder. It is saccular, filling practically all of the post-acetabular region. The collecting tubes are prominent. The number of flame cells has remained constant.

The changes undergone by the cercaria are characterized by an increase in muscularity. The stylet and tail have been lost; its suckers are powerful, and resistance to extraneous conditions and power of locomotion have improved markedly. It may live for various hours in 0.85 percent saline solution, moving vigorously with the help of its suckers. It is evident that the parasite is now prepared to stand conditions in the digestive tract of the final host and to move rapidly into its final habitat.

Irrespective of the load, the lizard stands the infection successfully. Its only pathological reaction is a hypertrophy and thickening of the main bile ducts; in heavy infections its gall bladder occasionally appears shrunken.

(c) *The infection in the final host* (*Fig. 16*). Experiments with white mice have shown that the metacercaria reaches the liver via the common bile duct, the first ones arriving at the finer ducts two hours after feeding. During the first three days, the only visible change in the parasite is a clearer demarkation of the digestive tract. Following this period of adaptation, the post-acetabular region elongates, and the entire worm flattens markedly. In adults the acetabulum will consequently lie in the anterior body fourth.

Both suckers grow at about the same rate but, due to a faster development of the body, these organs attain in the adult a relatively smaller size than in the larva. The pharynx becomes muscular and functional. The oesophagus shortens as the intestinal tract increases in caliber and undulations.

The excretory bladder elongates simultaneously with the posterior body and becomes claviform, extending along the posterior two-thirds of the parasite. The distal end develops a sphincter that regulates its function. The collecting vessels and flame cells increase in caliber, but their pattern remains unchanged.

The small glands of the cercaria, still present in the stage in the lizard, are also preserved in three-day-old forms obtained from mice. These glands evidently represent the cephalic glands of the adult parasite. Neuhaus[27] described similar organs in *Dicrocoelium lanceatum*, although his ideas about their mode of development differ from the present observations. In contrast with his opinion, it is suggested that the cephalic glands are organs that may pass directly from one stage to another without appreciable modification.

The development of the reproductive system occupies the limelight in the final stages. A week after arriving in the liver, the genital primordium has enlarged without differentiation (Fig. 16a). By the second week the testes have segregated as antero-lateral round masses (Fig. 16b). The rest of the germinal tissue gives rise to other male and female organs, as described graphically in Fig. 16c and 16d. Adult development is remarkably similar to that of the lancet fluke. The reader is therefore referred to Neuhaus' work (1938) for detailed information.

Eggs begin to form four to five weeks after arriving in the liver and may start to pass in the excrement no less than eight weeks after.

The old adult *Platynosomum fastosum* (Fig. 17) measures about 6.0 to 8.0 mm. long, which is a greater size limit than that reported by Kossak.[28] Its other characteristics closely resemble the original description. The infection in laboratory mice runs the usual course as in the cat. A detailed study with this new host is now under way.

27. W. Neuhaus, *op. cit.* (2d part).
28. W. Kossak, *op. cit.*

DISCUSSION

The observations presented above are doubtlessly of interest to parasitologists, both from the standpoint of enlarging upon the knowledge about the group of trematodes, to which *Platynosomum* belongs, but also for the addition of new facts on the very variable biological characteristics of trematodes in general.

The peculiarity of emergence of daughter sporocysts from the molluscan host is a rare phenomenon. As far as is known, there are two other examples of this kind. Thomas[29] has referred to the ability of sporocysts of *Cercaria limacis* to emerge through the integument of the intermediate hosts, the slugs, *Arion ater* and *Limax cinereus*. *Leucochloridium*, a genus of harmostomes infecting birds, produces large, colored, active sporocysts that may come out of the tentacles of the snail.[30]

Neuhaus[31] has observed that some daughter sporocysts of *Dicrocoelium lanceatum* may become activated upon irradiation of the snail and accompany the cercaria in its migration through the vena magna. The sporocysts are unable, however, to pass into the respiratory cavity to be expelled eventually. This peculiarity probably constitutes a link in the evolution of the migratory instinct of sporocysts of *Platynosomum fastosum;* the parasite has become completely transformed in order to successfully accomplish its mission. In contrast to the lancet fluke, the sporocyst has restricted germinative properties, giving rise to a fixed number of cercariae that originate and develop uniformly. These are entirely unable to leave their enclosure since the sporocyst is a blind sac.

It is practically impossible to determine the nature of the migratory instinct. Although with *Dicrocoelium lanceatum* a phototropism is suggested, in *Platynosomum fastosum*, emergence of sporocysts does not depend on any special extrinsic stimulus. The parasite has no definite sensory organs to which this function could be attributed.

The similarities between both trematodes indicate that they have followed a parallel evolutionary course during part of their life cycles. It is possible that the study of other members in the family may establish the link between those points on which they differ.

The use of a second intermediate host by *Platynosomum fastosum* constitutes the widest divergence between both parasites. It is of interest since this is the first instance in which *Anolis* have been

29. A. P. Thomas, The life history of the liver fluke (*Fasciola hepatica*). Quart.J.Micr.Soc., No. 1, 1883.
30. A. E. Woodhead, The mother sporocysts of *Leucochloridium*. J.Parasitol., 21:337, 1935.
31. W. Neuhaus, *op. cit.* (2d part).

implicated in the transmission of helminths. By the adoption of a second intermediate host, the parasite has taken a progressive step over its close relative in order to overcome the other handicaps it possesses. This same factor, however, delimits its distribution to places where the reptile lives.

The susceptibility of white mice to *Platynosomum fastosum* raises a question on the degree of specificity of this trematode. Pinto and Almeida[32] have found the ferret (*Grisson vittatus*) naturally infected. It is supposed that the parasite occurs mainly in cats because of their predilection for lizards, but it is also possible that other animals may serve as natural or accidental hosts. Kossak[33] observes that *Platynosomum semifuscum* and *Platynosomum illiciens*, two bird parasites, are morphologically similar to *Platynosomum fastosum;* Travassos[34] found a very similar one, *Platynosomum arietis*, in sheep.

SUMMARY

The biology of *Platynosomum fastosum* Kossak, 1910, the Puerto Rican cat liver fluke, has been described briefly. The molluscan intermediate host, *Subulina octona*, becomes infected by feeding on the egg. The miracidium emerges in the digestive tract of the snail, enters the tissues, and transforms into a mother sporocyst. This gives rise to numberless daughter forms. The daughter sporocyst is a blind, muscular sac containing a small number of cercariae (ave. 17.5), which originate and develop simultaneously. This daughter sporocyst comes out of the snail after maturing. The lizard *Anolis cristatellus* acts as a second intermediate host. The cercaria becomes established in the common bile duct where it changes considerably and becomes infective for the final host. The cat acquires the infection by preying on lizards.

The peculiarities of greater interest in the life cycle of *Platynosomum fastosum* are the ability of the daughter sporocysts to emerge from the snail and its use of the lizard as a second intermediate host. The first is a rare process and dependent only on the idiosyncrasy of the parasite and the favorable conditions of moisture, which it may encounter.

Throughout this study the author has strived to establish a comparison between this parasite and *Dicrocoelium lanceatum*, the lancet fluke, to which it is closely related.

32. C. Pinto and J. L. Almeida, *op. cit.*
33. W. Kossak, *op. cit.*
34. L. Travassos, Helmintes parasitos dos animaes domesticos. Rev.Vet.e Zootechnia (Rio de Janeiro), 8:3, 1918.

All figures, except numbers 7, 8 and 14, are camera lucida drawings and refer to *Platynosomum fastosum*

PLATE I

Fig. 1. Ovum in freshly passed excrement.

Fig. 2. The free-swimming miracidium obtained from the crop of the snail host.

Fig. 3. Miracidium lying in the peri-intestinal connective tissue along the respiratory cavity (to the right, not shown). Notice adjacent gap in the wall of the intestine.

Fig. 4. Piece of mother sporocyst projecting through the tunica propria of the snail. Larger masses are daughter sporocysts. Germ cells in islands.

Fig. 5. Section through a daughter sporocyst on the thirteenth day of infection. Starting formation of body cavity. Segregation in the center of cercarial germ cells and masses.

Fig. 6. Sagittal section of daughter sporocyst soon after leaving mother form. Germinal cells disappeared; only cercarial masses left.

Fig. 7. Mature daughter sporocyst. Musculature intact, inner bag folded. Free-hand drawing.

Todos los grabados, excepto los Núm. 7, 8 y 14, son dibujos del *P. fastosum* ejecutados en cámara clara

LÁMINA I

Grab. 1. Huevecillo tal como aparece en excremento acabado de expulsar.

Grab. 2. Miracidio moviéndose libremente en el cuerpo del caracol.

Grab. 3. Miracidio situado en el tejido conjuntivo que rodea al intestino, paralelamente a la cavidad intestinal (fuera del grabado). Nótese el hueco existente en la pared intestinal contigua.

Grab. 4. Porción de un esporoquiste primario saliendo a través de la túnica propia del caracol. Las masas grandes son esporoquistes secundarios. Las células germinales están dispuestas en islotes.

Grab. 5. Corte transversal de un esporoquiste secundario al décimo tercer día de parasitado el caracol. Comienza a formarse una cavidad, en cuyo centro se agrupan las células germinales y las masas.

Grab. 6. Corte sagital de un esporoquiste secundario inmediatamente después de separarse del esporoquiste primario. Han desaparecido las células germinales; solamente aparecen masas germinales.

Grab. 7. Esporoquiste primario en plena madurez. El sistema muscular aparece intacto; el saco interno, plegado (Dibujo a mano).

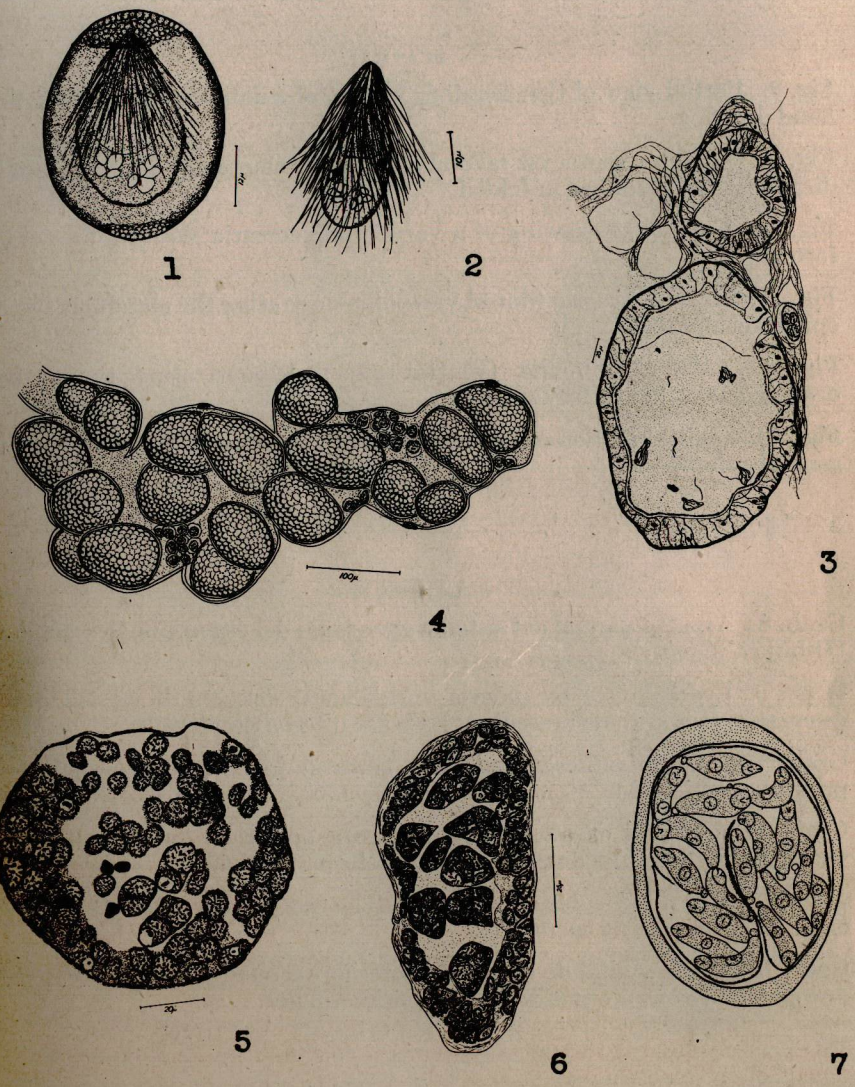


PLATE II

Fig. 8. Partial view of the excretory system of a daughter sporocyst. Free-hand drawing.

Fig. 9. Daughter sporocyst several hours after emerging. Musculature has disappeared. Inner bag unfolded.

Fig. 10. Composite drawing of a very young cercaria showing first organs forming.

Fig. 11. Mature cercaria (dorsal view) demonstrating the glandular system, spination and stylet.

Fig. 12. Mature cercaria (ventral view) demonstrating the suckers, oesophagus and excretory system.

Fig. 13. Sagittal section of posterior half of the cercaria demonstrating the genital anlage.

LÁMINA II

Grab. 8. Aspecto parcial del sistema excretorio del esporoquiste secundario (Dibujo a mano).

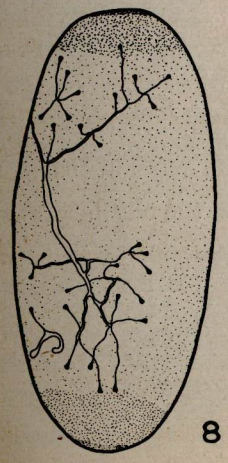
Grab. 9. Esporoquiste secundario varias horas después de su salida. Ha desaparecido el sistema muscular. La bolsa interna desplegada.

Grab. 10. Dibujo representando una cercaria juvenil y su organización primitiva.

Grab. 11. Cercaria en plena madurez (cara posterior) en que puede observarse el sistema glandular, la espina posterior y el estilete.

Grab. 12. Cercaria madura (cara ventral) en que pueden verse las ventosas el esófago y el sistema excretorio.

Grab. 13. Corte sagital de la mitad posterior del cuerpo de una cercaria en que pueden verse los órganos genitales primordiales.



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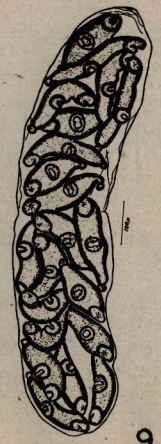

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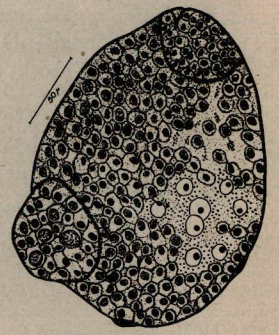

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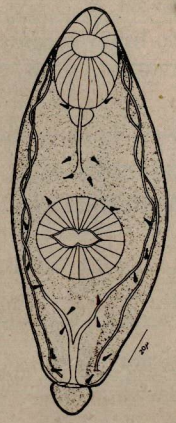

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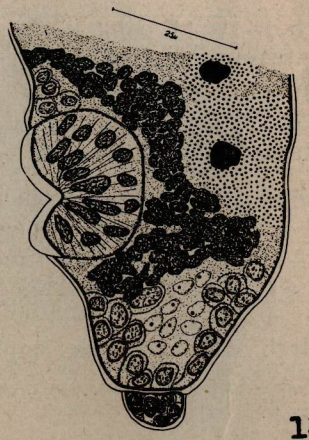

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PLATE III

Fig. 14. Photomicrograph of common bile duct of *Anolis cristatellus* heavily infected with metacercariae in the infective stage. Magnif. 100x.

Fig. 15. Ventral view of the metacercaria obtained from the bile duct of an *Anolis*. Resting position.

Fig. 16. Developmental stages of the adult:

a—One week of age.
b—Two weeks of age.
c—Third week of age.
d—Young adult—4 to 5 weeks of age.

Fig. 17. Fully developed *P. fastosum*.

LÁMINA III

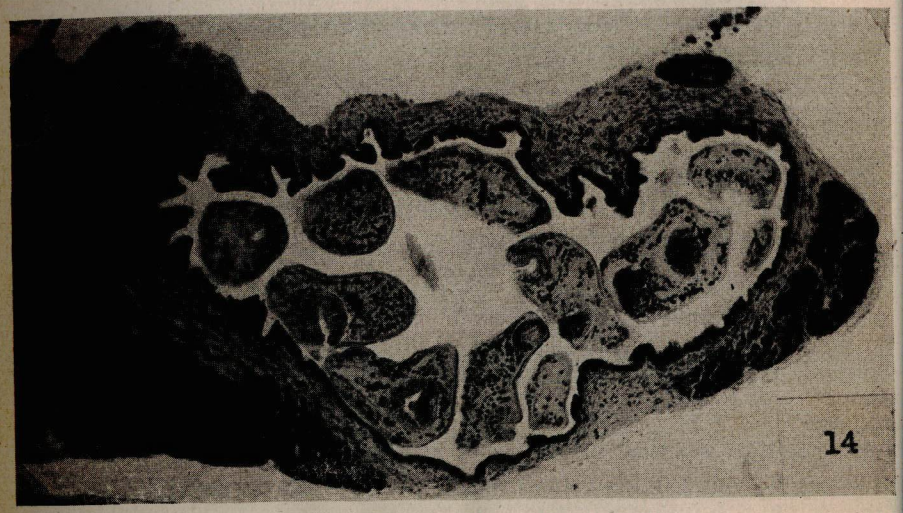
Grab. 14. Microfotografía del conducto biliar común del *Anolis cristatellus*, intensamente parasitado con metacercarias en su etapa infectiva. (Mag. 100x).

Grab. 15. Cara ventral de una metacercaria en reposo procedente del conducto biliar de un *Anolis*.

Grab. 16. Etapas de evolución del parásito adulto:

a—De una semana.
b—De dos semanas.
c—De tres semanas.
d—Adulto juvenil—de 4 á 5 semanas.

Grab. 17. *P. fastosum* completamente desarrollado.



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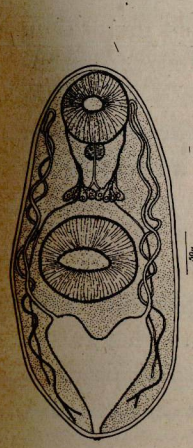

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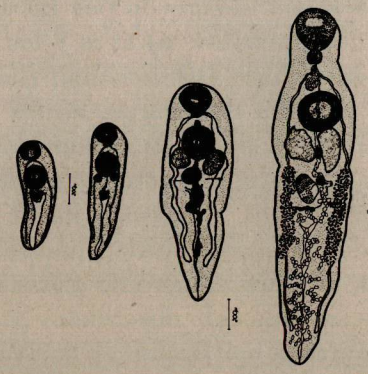

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