THE JOURNAL OF PARASITOLOGY
Vol. 50, No. 3, June 1964, p. 441–444

The Protective Action of *Chaetogaster limnaei* on Snails Exposed to *Schistosoma mansoni**

Edward H. Michelson
Department of Tropical Public Health,
Harvard School of Public Health, Boston, Massachusetts

ABSTRACT: Experiments demonstrated that infestation with the oligochaete worm, *Chaetogaster limnaei*, afforded a degree of protection to the snail *Australorbis glabratus* when the snail was exposed to *Schistosoma mansoni* miracidia. To a lesser extent, the oligochaetes protected snails exposed to echinostome cercariae. The presence of *C. limnaei* within the kidney of the snail *Physa heterostropha* is reported.

An intimate association between the oligochaete worm *Chaetogaster limnaei* von Baer and various species of aquatic snails has long been recognized (von Baer, 1827), yet the nature of this relationship has not been adequately defined. Early naturalists considered *C. limnaei* to be a true parasite which was thought to feed upon the "slime" produced by the host. However, Wagin (1931) and subsequent investigators demonstrated that the oligochaete fed principally on microorganisms, and thus the worm has been considered a commensal. Wagin also observed that the oligochaete ingested cercariae and suggested that *C. limnaei* might be of value in controlling trematode transmission. While Krasnodebski (1936) confirmed Wagin's field observations and experimentally demonstrated that the oligochaete would ingest various types of cercariae, he did not believe that *Chaetogaster* could play a significant role in the control of trematodes. Backlund (1949) observed that cercariae of *Fasciola hepatica* were ingested by *Chaetogaster*, and Ruiz (1951) later reported a similar observation with respect to *Schistosoma mansoni*. Coelho (1957) noted that *Chaetogaster* frequently ingested *S. mansoni* miracidia, but commented that the worms did not protect snails from infection. However, Khalil (1961) presented circumstantial evidence suggesting that snails infested with *C. limnaei* were refractory to infection with *Fasciola hepatica* under laboratory conditions.

The present study was initiated to determine quantitatively whether *Australorbis glabratus* infested with *C. limnaei* were protected against invasion by miracidia of *S. mansoni* and by cercariae of an echinostome.

MATERIALS AND METHODS

Specimens of *C. limnaei* were obtained from *Physa heterostropha* collected in Boston. The oligochaetes were freed by immersing the snails in 1% Urethan (ethyl carbamate) for 3 to 5 min. Anesthetized worms were collected by pipette, allowed to recover in charcoal-filtered tap water, and used immediately or colonized for later use (Brandwein, 1937). A Puerto Rican strain (PR-1) of *Australorbis glabratus* was used in all experiments. This snail strain has been maintained in our laboratory for several years and has been free from infestation with *C. limnaei*. Miracidia were hatched from eggs obtained from the livers of mice infected with a Puerto Rican strain of *S. mansoni* and were used within 30 min of emergence (Michelson, 1964). Cercariae of an unidentified echinostome were obtained from *Physa heterostropha* collected from local ponds.

Five *C. limnaei* were added to individually isolated *A. glabratus* in 5-ml beakers containing 3 ml of charcoal-filtered tap water. The snail was confined with the oligochaetes for 1 hr or until all worms became attached. At this time and depending upon the experiment, one to five miracidia or ten cercariae were added to each beaker. After an exposure of approximately 16 hr, the snails from any one experiment were maintained together in a battery jar containing 2.5 liters of water. The battery jars were continually aerated, maintained at 25 ± 1 C, and the snails fed Romaine lettuce. Con-

Received for publication 3 March 1964.

* These studies were supported in part by Grants AI-00513 and 5 TI AI 46 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, Bethesda, Maryland.

TABLE I. *Influence of* Chaetogaster limnaei *infestation on the rate of infection of snails exposed to* S. mansoni *miracidia and to echinostome cercariae.*

Group no.	Snails with *C. limnaei*[1] No. positive/ no. exposed	Control snails No. positive/ no. exposed
	5 miracidia of *S. mansoni* per snail	
1[2]	2/7	4/8
2[2]	7/18	14/20
3[2]	1/5	5/6
4	5/10	8/10
5	2/10	10/10
Total	17/50 (34%)	41/54 (76%)
	1 miracidium of *S. mansoni* per snail	
6	1/10	5/10
7	2/15	7/15
Total	3/25 (12%)	12/25 (48%)
	10 cercariae of echinostome sp. per snail	
8	7/10	10/10
9	7/10	10/10
Total	14/20 (70%)	20/20 (100%)

[1] All snails infested with 5 worms each.
[2] Test groups 1, 2, and 3 had one, two, and one snails die before they could be examined for the presence of infection; these have not been included with the data.

trol snails, free of *C. limnaei*, were exposed to miracidia or cercariae in the same manner. Two weeks postexposure, control and *Chaetogaster*-infested snails were examined for the presence of *S. mansoni* sporocysts by the technique of Chernin and Dunavan (1962). Snails exposed to echinostome cercariae were examined 7 days postexposure for the presence of metacercariae. For this purpose the snail was removed from its shell, the mantle cut along its left margin and reflected to the right, and the pallial cavity examined under a 13 × dissecting microscope.

Experimental results

In five experiments a total of 54 snails, each infested with five *Chaetogaster limnaei*, were exposed individually to five *S. mansoni* miracidia and 17 (34%) of 50 snails which survived were found infected (Table I). By comparison, in the control groups of snails 41 of 54 snails (76%) were infected. Thus, a mean reduction in the rate of infection of 42% was associated with the presence of *C. limnaei*. In two additional experiments (Table I), snails harboring five *C. limnaei* were exposed individually to a single miracidium of *S. mansoni*. Of a total of 25 snails so exposed, three (12%) became infected. An infection rate of 48% (12/25 snails) occurred, however, in the two groups of control snails. A mean reduction of 36% in the infection rate was again observed to be associated with the presence of *C. limnaei*.

Snails were exposed to echinostome cercariae in two experiments (Table I), and all the control snails were later found infected. However, only 70% (14/20) of the snails infested with *C. limnaei* had metacercarial cysts. In the first experiment 38 and 58 metacercarial cysts were recovered from the *Chaetogaster*-infested and control snails respectively, and in the second experiment 22 and 69 cysts respectively. Infected snails were found to have from three to eight cysts each.

Miscellaneous observations

The intensity of *Chaetogaster* infestation in snails under field conditions would appear to vary considerably. Backlund (1949) observed that no more than ten *Chaetogaster* were found on specimens of *Lymnaea stagnalis* and *L. ovata*; however, Krasnodebski (1936) noted as many as 300 worms on a single specimen of *L. stagnalis*, up to 60 worms on *Physa fontinalis*, and a mean of 1.3 worms on specimens of *Ancylus lacustris*. In our own experience, as many as 25 *C. limnaei* have been recovered from a single *Physa heterostropha*. In one collection of 105 *P. heterostropha* there was a mean of nine worms per snail. The heaviest infestations were found on older and larger snails, particularly those which had overwintered. Quantitative data concerning natural infestations of *Chaetogaster* on the snail *Australorbis glabratus* are not available, although infestations of field populations have been reported (Coelho, 1957). Natural infestations of South African populations of *Bulinus tropicus*, *Bulinus africanus*, and *Biomphalaria pfeifferi* have also been noted by Bayer (1955). Likewise, laboratory colonies of *A. glabratus*, as well as *Bulinus truncatus* and *B. tropicus*, have been observed to be infested with *Chaetogaster* (Khalil, 1961).

Although it has generally been thought that *C. limnaei* acts as a commensal of snails, feeding principally on aquatic microorganisms, there is some evidence to suggest that this oligochaete may also be a parasite. The observation of Lankester (1870) of *C. limnaei* in the kidney of a single *L. stagnalis* has been overlooked. Our examination of both field and laboratory populations of *P. heterostropha*

TABLE II. *Distribution of* Chaetogaster limnaei *in a collection of* Physa heterostropha.

Snail no.[1]	Number *C. limnaei* present	
	On body surface and in mantle cavity	Inside kidney
1	1	0
2	0	7
3	0	8
4	0	5
5	2	1
6	6	5
7	1	3
8	0	7
9	0	5
10	1	5
11	2	5
12	0	2
13	3	1
14	1	5
15	5	18

[1] Five snails in this collection had no *Chaetogaster* and are not included in the table.

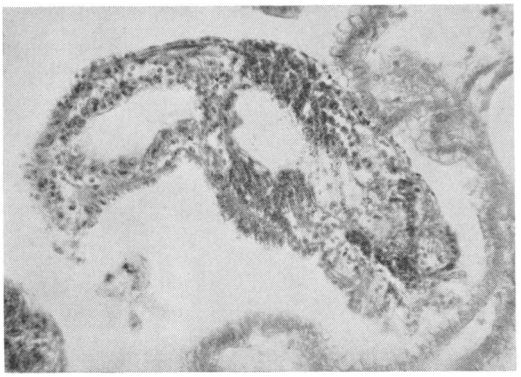

FIGURE 1. Longitudinal section through the kidney of *Physa heterostropha* showing a specimen of *Chaetogaster limnaei* attached to the epithelium. Hemalum and eosin. × 245.

revealed that under some circumstances large numbers of *C. limnaei* may be found within the kidney of the snail. In one group of *P. heterostropha,* examined 3 days after collection, 15 of 20 snails were infested. Fourteen of the 15 infested snails harbored 1 to 18 worms within the kidney; whereas only nine of these 15 snails had external infestations (Table II). Of further interest is the fact that snails could be free of external infestation while harboring numerous worms in their kidney. Histologic studies demonstrated that most of the oligochaetes were in the anterior portion of the kidney, but any part of the organ might harbor the worms (Fig. 1). No tissue reactions were observed in sections of snails so infected, although some oligochaetes appeared attached to the renal epithelium and renal concretions were observed in the intestinal tract of a few worms. Oligochaetes were not found in the kidney of experimentally infested *A. glabratus.*

DISCUSSION

It is evident that under laboratory conditions, *A. glabratus* infested with *C. limnaei* are afforded a degree of protection against infection by *S. mansoni* miracidia. To a lesser extent, protection against a cercaria of an echinostome was also demonstrated. The role of *Chaetogaster* in nature is a matter of conjecture and, at this time, attempts to transpose laboratory data to field conditions are neither possible nor desirable. It is important, however, to recognize that *Chaetogaster*-infested snails may be refractory to experimental trematode infections and that laboratory snail colonies should be free from such infestations. Snails can be freed from external infestation of *C. limnaei* by immersion in 1% Urethan for periods of 10 to 20 min. The effect of Urethan on a renal infection has not been determined.

The nature of the association between *C. limnaei* and aquatic snails requires further investigation. Factors which predispose the oligochaetes to enter the kidney of a snail are not understood, nor can we be sure that the renal phase of this oligochaete is, in fact, parasitic.

ACKNOWLEDGMENTS

The author is indebted to Drs. Eli Chernin and Thomas E. Frothingham for their generous advice in preparing this manuscript and to Mrs. Clark B. Adams for technical assistance.

LITERATURE CITED

BACKLUND, H. O. 1949. En kommensal som äter sitt värddjurs parasiter. Fauna o. flora, Pop. Tidskr. Biol. **44**: 38–41.

BAYER, F. A. H. 1955. Notes on a carnivorous oligochaete commensal on certain fresh-water snails in South Africa. Proc. Zool. Soc. London **125**: 407–409.

BRANDWEIN, P. 1937. *In* Culture methods for invertebrate animals. Comstock Publ. Co. Inc., Ithaca, N.Y., p. 142–143.

CHERNIN, E., AND C. A. DUNAVAN. 1962. Influence of host-parasite dispersion upon the capacity of *Schistosoma mansoni* miracidia to infect *Australorbis glabratus.* Am. J. Trop. Med. Hyg. **11**: 455–471.

COELHO, M. V. 1957. Aspectos do des envolvimento das formas larvais de *"Schistosoma mansoni"* em *"Australorbis nigricans."* Rev. Brasil. Biol. **17:** 325–337.

KHALIL, L. F. 1961. On the capture and destruction of miracidia by *Chaetogaster limnaei* (*Oligochaeta*). J. Helm. **35:** 269–274.

KRASNODEBSKI, F. 1936. Untersuchungen über die Nahrung des Oligochaeten *Chaetogaster limnaei* K. E. v. Baer. Zoologica Poloniae. (Lemberg) **1:** 199–208.

LANKESTER, E. R. 1870. A contribution to the knowledge of the lower annelids. Tr. Linn. Soc. London **26:** 631–646.

MICHELSON, E. H. 1964. Miracidia-immobilizing substances in extracts prepared from snails infected with *Schistosoma mansoni.* Am. J. Trop. Med. Hyg. **13:** 36–42.

RUIZ, J. M. 1951. Nota sôbre a cercariofagia de um oligochaeta do gênero *Chaetogaster* v. Baer, 1827. An. Fac. Farm. Odontol., Univ. São Paulo **9:** 51–56.

VON BAER, K. E. 1827. Beiträge zur Kenntnis der niedern Thiere. Nova Acta Caes. Acad. Leop. Car. **13:** 523–762, 881–882.

WAGIN, W. L. 1931. *Chaetogaster limnaei* K. Baer als Cercarienvertilger. Zool. Anz. **95:** 55–59.

RESEARCH NOTE . . .

First Record of Mating of *Cuterebra angustifrons* Dalmat (Diptera: Cuterebridae)

Studies with warble flies have been hampered by difficulties in obtaining mated females. Weintraub (1961, Can. Ent. **93:** 149–156) was the first to obtain regular matings of *Hypoderma* spp. (cattle warble flies) by tethering the flies to strings or by decapitating the male. Catts (1963, Ph.D. thesis, University of California) was able to induce copulation in two species of *Cuterebra* (rodent warble flies) using the tethering procedure.

Previous attempts to induce copulation in *Cuterebra angustifrons*, a parasite of the white-footed mouse *Peromyscus leucopus noveboracensis* (Fischer), have met with failure (1956, Sillman, Rept. Ent. Soc. Ont. **87:** 28–40; 1959, Sillman and Smith, Science **130:** 165–166). However, the author has recently been successful in inducing copulation between two adults of this species, reared from pupae obtained in September 1963.

Confinement of the flies in a free flight cage, tethering, and stroking the genitalia of the male for prolonged periods failed to induce any copulatory response. After decapitation of the male, its genitalia were stimulated by stroking with a camel hair brush until they became active. This took about 30 min. Although the female had begun to deposit eggs, and extruded eggs interfered with the action of the male's copulatory apparatus, repeated contacts between the genitalia of the two hand-held flies eventually resulted in establishing a union. After copulation, which lasted 3 to 4 min, the female immediately resumed ovipositing, approximately 800 eggs being laid over a period of about 36 hr after mating. Examination of the spermathecae, upon death of the female 2 days later, revealed the presence of live sperm. Larvae began to hatch from fertilized eggs in response to human breath on the 7th day after oviposition in a sample kept at 24 C and 99+% relative humidity. The infectivity of the larvae has been demonstrated by their establishment in captive white-footed mice. No development took place in 46 eggs laid by this female immediately prior to mating.

This work was supported by a grant from the Ontario Research Foundation.

Th. H. Scholten, Department of Zoology, University of Western Ontario, London, Ontario, Canada