Observations on the Effects of Fish Serum on Cercarial and Metacercarial Stages of *Posthodiplostomum minimum* (Trematoda: Diplostomidae)

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The effects of vertebrate sera on cercariae have been reported occasionally in the literature. Culbertson and Talbot (1935) observed cercaricidal activity of serum from uninfected mice, rats, snakes, frogs, and fish. True cercaricidal activity was preceded by the formation of a granular or globular precipitate which surrounded first the tail and later the body. A period of reduced, uncoordinated larval movement occurred immediately prior to death, this state being determined by an absence of motility and flame cell activity. Experimental studies indicated the responsible factor to be storage and heat labile (56°C for 30 min). Papirmeister and Bang (1948) recorded another phenomenon when cercariae were placed in either uninfected or infected *Schistosoma mansoni* mouse and rat serum. Finely granular or globular surface deposits accumulated around the larvae in what they termed the precipitin reaction. When these workers exposed cercariae to heat inactivated serum, a pericercarial envelope always formed. Liu and Bang (1950) reported agglutination of cercariae into large clumps in infected mouse and hamster serum. Studies by Stirewalt and Evans (1955) indicated that agglutination might be a stage in a weak or slowly developing *cercarienhullenreaktion* (CHR) of Vogel and Minning (1949). This reaction was produced when *S. mansoni* cercariae were placed in serum of infected mice and hamsters and in heat inactivated serum of infected rats (Stirewalt and Evans, 1955). Stirewalt (1963) demonstrated that newly recovered schistosomules did not give the CHR as did cercariae, this indicating a change in the outer surfaces of the cercarial integument. Further, cercaricidal serum was found to be ineffective against the schistosomule, suggesting a lack of correlation between in vitro cercaricidal activity and the susceptibility of individual hosts.

The studies of Culbertson and Talbot (1935), as noted earlier, dealt with cercaricidal effects of serum from uninfected *Ictalurus nebulosus* (Le Sueur) and did not include the metacercarial stage. In the present study, serum from centrarchid fishes both infected and uninfected with metacercariae of the trematode *Posthodiplostomum minimum* (MacCallum, 1921) Dubois 1936 were analyzed for their cercaricidal and metacercaricidal activities.

**Materials and Methods**

Fourteen fishes were used in the investigation including five *Lepomis macrochirus* Rafinesque, one *L. megalotus* Rafinesque, four *L. microlophus* (Gunther), and four *Chaenobryttus gulosis* (Cuvier), collected by either seine or hook from Club Lake, an area in east Texas which has a high incidence of *P. minimum* and from Lake Granite Shoals, central Texas, where the parasite is essentially absent. Blood was obtained by cardiac puncture, allowed to clot at 5°C, and serum extracted using a Lourdes refrigerated centrifuge. All fishes were given thorough post-mortem examinations to determine the extent of metacercarial infection with *P. minimum* as well as to eliminate those possessing other parasites. Cercariae used in the study were collected after their spontaneous emergence from snails, *Physa halei* Lea; whereas, metacercariae were obtained from heart and liver tissue of fishes and extracted and washed in physiological saline before being exposed to serum. *Schistosoma mansoni* were obtained from snails, *Australorbis glabratus* Say. Eight larvae were added to each unpooled serum sample in a depression slide with several samples being taken from each of the 14 fishes.

**Results**

Cercariae exposed to fresh unheated serum from infected and uninfected fish reacted similarly in being initially hyperactive and vigorous
in movement. Within three minutes globular secretions were copiously exuded from the oral end of the cercariae, and this continued until death. Detachment of tails normally occurred within 10 minutes, although some were retained for 30 min (Fig. 1). After 30 min a soft mucoid sheath enveloped the larvae causing debris to adhere to the surfaces. Most cercariae were immobilized and appeared dead after several hours. Those placed in serum heated at 56°C for 30 min on the other hand produced no oral exudate, failed to detach their tails during the first hr, and remained motile after 12 hr. A similar reaction was observed using Schistosoma mansoni cercariae indicating the nonspecific nature of the reaction (Fig. 2).

Excysted metacercariae exposed to infected and uninfected serum likewise secreted copious quantities of water-insoluble exudate in amounts approaching the size of the metacercariae itself, and continued to secrete it until succumbing. Within 10 min a thin mucoid sheath began forming around the excysted metacercariae and in 15–20 min the metacercarial membranes of many larvae appeared to weaken and balloon, often in several different places on each parasite (Fig. 3). Excysted metacercariae began to lyse at the weakened surfaces 30–60 min after exposure to serum, all dying within 2 hr. Excysted larvae placed in heat inactivated serum produced small amounts of oral exudate within 30 min, but no lysis of membranes occurred and all were viable after 2 hours. Intact metacercariae in cysts were unaffected by serum. When they were exposed to serum for 2 hr, mechanically excysted, and the excysted larvae subjected to direct exposure to serum, lysis occurred within the hour. Reactions obtained were identical to metacercariae not incubated in their cysts prior to exposure. Larvae incubated in cysts, excysted, and placed in heat inactivated serum were unaffected. Controls placed in saline showed normal motility after several hours with no obvious deleterious effects.

**Discussion**

The present study is the first to report a metacercarial factor present in centrarchid fish serum taken from hosts uninfected or infected with metacercariae of *Posthodiplostomum minimum*. That this is possibly a nonspecific reaction is evidenced by the fact that cercarial effects on both *P. minimum* and *S. mansoni* were likewise displayed. Metacercariae were obviously shielded from the factor(s) while in intact cysts. Even when incubated in serum for several hours prior to excystment and exposure, no differences in survival rate were apparent. Both cercarial and metacercarial properties of the serum were destroyed by heating at 56°C for 30 min and by storage for 48 hr.

The comparative responses of *P. minimum* and *S. mansoni* larval stages when exposed to fish serum are of interest. Stirewalt (1963) reported cercariae of *S. mansoni* reacted in host serum while schistosomules were unaffected. This is supported by recent micrographs by Lichtenberg (1967) that illustrate distinct differences between the cercarial and schistosomule integuments of *S. mansoni*, a fact which suggests a possible physiological alteration of the worm as a prerequisite for survival in the host. *Posthodiplostomum minimum* apparently does not undergo such alteration upon excystment since both larval stages are serum-sensitive. In view of the nonspecificity of the reaction with respect to types of cercariae employed and the loss of both cercarial and metacercarial factors by heat inactivation, it may be suggested that identical serum factor(s) are involved. The movement of cercariae through the host circulatory system without damage from cercarial agents prior to excystment as metacercariae is unexplained.

**Summary**

Serum from fishes both infected and uninfected with metacercariae of *Posthodiplostomum minimum* were found to have cercari-
cidal and metacercaricidal activities. Metacercaiae exposed to serum in intact cysts were unaffected but displayed metacercaricidal behavior when exposed without cysts. When heated for 30 min at 56°C, serum produced no response from either larval stage.

**Literature Cited**


\textbf{Diplectanum lacustris} sp. nov. (Dactylogyroidea: Diplectanidae), a Monogenetic Trematode from the Gills of the Nile Perch

\textbf{JUNE P. THURSTON\textsuperscript{1} AND I. PAPERNA\textsuperscript{2}}

During surveys of fish parasites in Ghana and Uganda, specimens of a monogenetic trematode were obtained from the gills of two species of \textit{Lates}, the Nile Perch. The trematode was identified as a new species of \textit{Diplectanum} (Dactylogyroidea: Diplectanidae). \textit{Diplectanum} is predominantly a parasite of marine teleosts, and the present species is therefore unusual in occurring on a fresh water fish. Interestingly, however, the genus \textit{Lates} is classified by Greenwood (1966) in the Family Centropomidae, which is composed mainly of marine fish. \textit{Lates calcarifer}, which is the host of \textit{Diplectanum latesi} Tripathi, 1955 in India, is an estuarine species.

**Materials and Methods**

Five specimens of \textit{Lates albertianus} were obtained in Uganda from Lake Albert and nine from the River Nile between Lakes Victoria and Kyoga, while three specimens of \textit{Lates niloticus} were obtained from the newly formed Volta Lake in Ghana.

Methods used in collecting the monogeneids, and in their fixation, mounting and measurement were similar to those used in earlier studies (Paperna and Thurston, 1969). In addition, some specimens were stained with Semichon’s carmine and cleared in clove oil, but examination of these preparations revealed little more anatomical detail than the examination of specimens mounted in glycerin jelly.

\textbf{Diplectanum lacustris} sp. nov.

**Description**

This parasite exhibits a wide range of shapes and sizes, from typical “slender” forms in which the opisthaptor is well delineated from the body, to “gravid” forms which are proportionately wider and usually longer than the “slender” forms and in which the opisthaptor is almost completely embedded in the pos-