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KINETICS OF THE CERCARIA-SCHISTOSOMULUM TRANSFORMATION *IN VIVO*

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Since most studies on the cercaria-schistosomulum transformation have been carried out in vitro, *the authors used the inoculation of cercariae into the peritoneal cavity of mice to follow the steps involved in this progressive adaptation of cercarie to the vertebrate host. The main conclusions were: 1. Most cercariae reach the schistosomular stage between 90-120 min after intraperitoneal inoculation. 2. Changes usually start with detachment of the tail followed by loss, rupture or changes of the glycocalix. 3. After 120 min most larvae loss their tails and present water sensitivity. 4. Acetabular grands depletion usually does not occur in cercaria-shistosomulum changes in the peritoneal cavity of mice. These steps differ in some way from those described in the kinetics of the* in vitro *observations performed by other investigators, and is more like those described in the penetration in the skin of living vertebrates.*

Key words: *Schistosoma mansoni.* Cercariae. Schistosomula. *In vivo* transformation.

Recently, the transformation of *Schistosoma mansoni* cercaria into schistosomulum have been investigated by several workers, mainly in relation to the immunological and biochemical aspects. At first glance a logical conclusion from the facts available is that various modification steps occur during the transformation of a cercaria (a free living larva adapted to a freshwater environment) to a schistosomulum (an organism newly adapted to the host conditions).

Various methods for collecting living schistosomula have been described and the larvae have been recovered both *in vitro* and *in vivo*. The basic differences between cercaria and schistosomulum were established by Stirewalt[18 20]. Additional differences were subsequently reported[2 3 9 11 12 22 23 24]. These differences are the standard criteria by which the organisms recovered by *in vitro* methods can be considered to be schistosomula.

It seems that, particular techniques used for cercaria-schistosomulum transformation present differences in the kinetics, so the sequences of the several steps, and the times required for them, must be characterized in each case. Pereira et al[16] described a method of collecting living schistosomula after cercariae inoculations into the peritoneal cavity of mice, which provides a rapid and easy recovery of larvae at any time. Understanding the kinetics of this model is the purpose of the present report.

MATERIALS AND METHODS

The method described by Pereira et al[16] to study the chemoprophylactic action of compounds on schistosomula recovered from the peritoneal cavity of mice was used in all experiments. Albino mice (male, weighing about 20 g) were inoculated intraperitoneally with *S. mansoni* cercariae (LE strain) shed by laboratory-reared and infected *Biomphalaria glabrata* (Belo Horizonte strain). The organisms were concentrated by the method of Pellegrino & Macedo[15], and 0.5 ml of well water containing about 500 larvae were injected using a Cornwall syringe with a 20 x 10 gauge needle.

At different time intervals ranging from 30 minutes to 3 hours after the inoculation the mice were killed by cervical fracture, and the parasites recovered from the peritoneal cavity with saline. They were then concentrated by centrifugation and counted under a dissecting microscope. Five animals were sacrificed at each time interval (30 mice for each experiment). The

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experiment was repeated four times, so data were collected from 120 animals.

The larvae were classified initially as cercariae and tailless organisms. To separate cercarial bodies (which remain alive in freshwater) from schistosomula (which die in such conditions), 5 ml of distilled water was dropped into a Petri dish containing larvae and after 10 min, alive and dead larvae were recounted. In addition to the absence of motility, dead larvae showed herniation of acetabulum. Two vital stains (neutral red and alizarin red) were added to the suspension of organisms to characterize the acetabular glands.

RESULTS

Data are presented in Fig. 1. Each column (mean of five mice) shows the percentual recovery of different larvae. About 30 percent of inoculated cercariae were recovered as larvae in different steps of transformation.

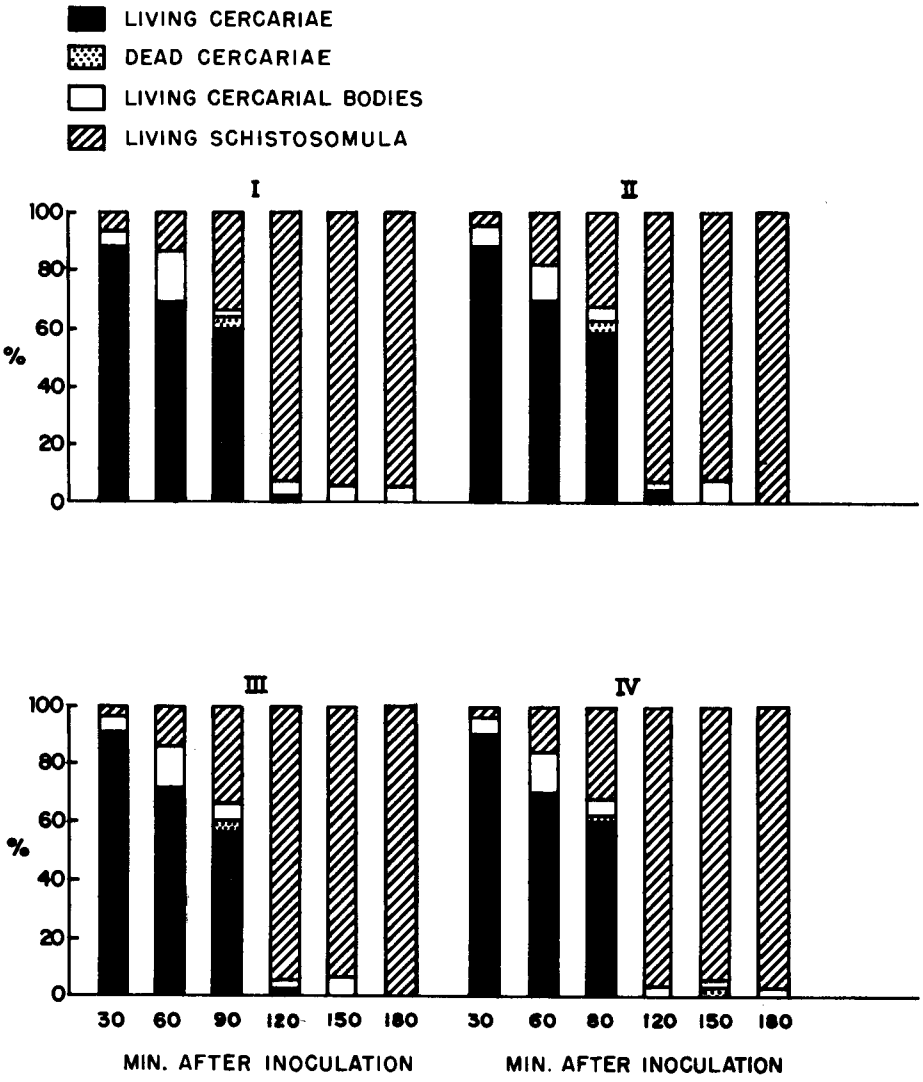

Figure 1 – Kinetics of cercaria-schistosomulum transformation in the peritoneal cavity of mice, in four separate experiments (each column represents the mean numbers of larvae recovered from five mice).

We observed that when alizarin red or neutral red were used, almost all schistosomula incorporated the vital stain, the colour being generally intense (as in the cercariae), a few schistosomula presenting a more pale staining.

As can be seen, after 30 min of inoculation, very few schistosomula are found, most the organisms being living cercarial bodies or cercariae. After 60 min the numbers of cercarial bodies decrease. At 90 min larvae are found mostly as schistosomula or cercariae, and a low percentage of dead cercariae are found. After 120 min and 150 min the majority of larvae are schistosomula. The transformation is fully achieved at 180 min, except for the persistence of gland contents in almost all larvae.

DISCUSSION

Early schistosomula, soon after cercarial transformation, are often obtained by several procedures: 1. They can be recovered from *in vivo* infections after penetration of cercariae into fresh skin *in situ* of laboratory animals[2 3 9 12 18 20 22]; 2. They have been collected in vitro after cercariae have penetrated excised fresh host skin, or dried rat or human epidermis[4 10 11 12 14 21 23 24]; 3. Schistosomula collections after unsuccessful penetration attempts by cercariae, or collection without membranes or demonstrable penetration responses[1 5 7 8 9 13 17].

The cercaria-schistosomulum changes follows defined steps in each procedure but the resulting larvae from the *in vitro* methods may differ in some way from those occurring in the natural infection. Although the inoculation of cercariae into the peritoneal cavity of mice is also artificial, it provides easy recovery of larvae at any time. The experiments are highly reproducible and have the advantage of being done *in vivo*.

Since the initial work by Cram & Bozicevich[6] and Yolles[25] who described the development of *Schistosoma mansoni* after intraperitoneal inoculation in laboratory animals, knowledge about the factors involved in the process of cercaria-schistosomulum conversion in the peritoneal cavity of mice remains deficient.

It is generally accepted that the transformation process occurs rapidly under natural conditions. Stirewalt[18], examining the recovered living organisms 15 min after infection via ear or abdominal region of rats, mice and hamster, found 100% of these organisms presenting water sensitivity. Clegg & Smithers[3] showed in the same conditions, that sensitivity to the medium is progressive, and is determined by schistosomula surface changes.

As stated by Stirewalt[19], the body-tail separation can be the first trigger for initiating the change, perhaps because tail loss results in interruption of the surface integrity of the cercaria. The body-tail separation has been also induced by several stimuli as lipids, physical treatments and, more frequently, it has resulted from a intense muscular activity of cercariae[19].

In this work, most larvae lost their tails and can be considered to be schistosomula because they presented body-tail separation, water intolerance, a wormlike flacid appearance, wormlike peristaltic locomotion with constant elongation and contraction, and free lateral body bending. One of the standard criteria for identification of the schistosomula is acetabular gland depletion[19], but some investigators did not utilize this parameter because if the cercariae penetrate more easily, with minor effort, their glands are not depleted[10]. According to Eveland[7], the complete depletion of acetabular glands in the peritoneal cavity of mice rarely occurs and seems not to be an essential requirement for the transformation process.

RESUMO

Uma vez que a maioria dos estudos da transformação cercária-esquistossômulo têm sido realizados in vitro, *os autores usaram a inoculação de cercárias na cavidade peritoneal de camundongos para seguir as etapas envolvidas nesta adaptação progressiva das larvas ao hospedeiro vertebrado. As conclusões principais foram:*

1. A maioria das cercárias atinge o estádio de esquistossômulo entre 90 – 120 minutos após a inoculação intraperitoneal.

2. As modificações usualmente são iniciadas com a perda da cauda, seguidas pela perda, ruptura ou modificações do glicocálice.

3. Após 120 minutos do inóculo, a maioria das larvas perde sua cauda e apresenta intolerância à água.

4. A depleção das glândulas acetabulares habitualmente não ocorre durante o processo de transformação cercária-esquistossômulo, na cavidade peritoneal do camundongo.

Os resultados da inoculação intraperitoneal em camundongos aproximam-se mais dos achados da penetração da cercária na pele de hospedeiros vertebrados, e diferem, em parte, das observações realizadas in vitro.

Palavras chaves: *Schistosoma mansoni.* Cercária. Esquistossômulo. Transformação *in vivo.*

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