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INFECTIVITY FOR MICE OF CERCARIAE, SCHISTOSOMULES AND INTERMEDIATE FORMS OF *SCHISTOSOMA MANSONI*, OBTAINED *IN VITRO*

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SUMMARY

Schistosomules of *Schistosoma mansoni* were obtained from cercariae by *in vitro* induction of tail loss, evacuation of acetabular glandular secretion and coat removal. Besides the initial (cercaria) and final (schistosomule) classical forms, the following ones were prepared: cercarial bodies, cercarial bodies without secretion and with coat and cercarial bodies without coat. Cercariae and schistosomules were infective by subcutaneous, intraperitoneal and intravenous routes. The percentage of worms recovered were 23.6, 30.5 and 9.4 for cercariae and 40.2, 40.3 and 44.0 for schistosomules, respectively. Cercarial bodies, cercarial bodies without secretion and with coat and cercarial bodies without coat were also infective for mice by the subcutaneous, intraperitoneal and intravenous routes. It was found that cercarial bodies and schistosomules are not able to infect mice through the skin.

INTRODUCTION

The penetration of *Schistosoma mansoni* cercariae into the skin of vertebrate hosts is associated with the metamorphosis of the larvae to schistosomules. This process is known to involve the following steps: a) change in cercarial behaviour with exploratory movements for entry site; b) secretion of adhesive mucus from the postacetabular glands; c) muscular activity for penetration; d) secretion of a proteolytic enzyme from the preacetabular glands; e) loss of tail, and f) alterations of the external layer of the tegument (STIREWALT [10]; STIREWALT, MINNICK & FREGEAU [11]; STIREWALT & UY [12]).

In 1966, STIREWALT, MINNICK & FREGEAU [11] showed that the process of cercarial penetration and transformation into schistosomules also occurs in the presence of dried, scraped and plucked skin of a potential host. A simple apparatus for *in vitro* collection of large numbers of schistosomules was then described.

More recently, GILBERT et al. [5] demonstrated that schistosomules (*S. mansoni*) can be obtained *in vitro* without the aid of biological membranes. Soybean lecithin, a product which contains many of the phospholipids present in skin fat produced, when fresh, the transformation of 50-80% of exposed cercariae into schistosomules. Creaming of egg lecithin with cold water (1:50) was as effective as skin lipid in the process of transformation.

GAZZINELLI et al. [3] and RAMALHO-PINTO et al. [9] established a defined system for stepwise metamorphosis of cercaria to schistoso-

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mule *in vitro*. This simple but effective technique opened interesting and new fields for investigation. In fact, since the steps of the process of transformation are known, intermediate or new forms could be obtained with less than 5% contamination with other larval forms: cercarial bodies, cercarial bodies without secretion but with coat and cercarial bodies without coat.

In this paper, the results of infection of mice with intermediate forms as well as cercariae and schistosomules, using different routes for inoculation, will be presented.

MATERIALS AND METHODS

Cercarial suspension — Cercariae of *S. mansoni* (L. E. strain, Belo Horizonte, Brazil) were shed by *Biomphalaria glabrata* infected and reared in the laboratory. The suspension of cercariae was concentrated as previously described (GAZZINELLI et al. [3]).

Cercarial bodies — Ten ml aliquots of the cercarial suspension (ca. 1500 organisms/ml) were pipetted into 15 ml glass conical centrifuge tubes with an Oxford pipettor (Oxford Laboratories, San Mateo, California) and cooled in an ice bath for 10 min to reduce motility of the organisms. Following a slow speed centrifugation at ambient temperature (26 to 28°C) for 1 min the supernatant was discarded. The packed cercariae from 2-4 tubes were pooled and resuspended in 2 ml of ice-cold Hanks'-BSS, pH 7.4. This heavy suspension of cercariae was whorled in a vortex mixer (Scientific Industry Inc., Queens Village, N.Y.) for 1 min in order to effect the decaudation of the organisms. The isolation of cercarial bodies from tails was achieved by increasing the volume of the suspension containing bodies and tails to 7-10 ml with Hanks'-BSS. After 8 min, by gravity sedimentation, the cercarial bodies concentrate on the bottom of the centrifuge tubes. The tail-rich supernatant was decanted and this step repeated once again. Cercarial bodies thus obtained were less than 5% contaminated with tails and more than 90% of the larvae presented normal motility.

Cercarial bodies without pre-acetabular secretion — These intermediate forms were obtained according to the following procedure: cercarial bodies were packed by low speed centrifugation for 2 min and by incubating the larvae in a minimum volume of Hanks'-BSS (pH 7.4) at 30°C for 40 min. At this condition, 50-80% of the glandular material was eliminated as evaluated by the proteolytic activity assayed on ATEE (acetyl tyrosine ethyl ester) according to GAZZINELLI et al. [3, 4]. Cercarial bodies so obtained were washed twice in Hanks'-BSS to eliminate the secreted material.

Cercarial bodies without coat — These forms were obtained by incubating the cercarial bodies (ca. 1,000 organisms/ml) in TC-199 (Difco) medium (pH 7.4) at 37°C for 60 min with constant shaking in a Dubnoff Incubator (Precision Scientific Co., Chicago).

The loss of the coat was evaluation by the water sensitivity test with diluted methylene blue (CLEGG & SMITHERS [1]). More than 90% of the organisms thus obtained stained blue after 10 min at room temperature.

Schistosomules — The last stage of transformed organisms was prepared *in vitro* by the procedure of RAMALHO-PINTO et al. [9]. To remove the coat, heat-inactivated (56°C for 60 min) sera of different mammals were used instead of TC-199 medium at the same conditions described above. These schistosomules have been shown to be viable in culture up to 40 days (TIBA et al. [13]).

Infection of mice — Five groups of 30 Swiss mice were injected by subcutaneous, intraperitoneal and intravenous routes, with the following living material: cercariae, cercarial bodies, cercarial bodies "without secretion", cercarial bodies "without coat" and schistosomules. Four groups of 10 animals were infected by intravenous route, with schistosomules prepared in different incubation media: TC-199 and inactivated serum from mice, hamsters and rats.

The mice from both groups were infected with 100 ± 20 organisms per animal. Seven weeks after infection the animals were sacrificed and the worms collected by perfusion of the liver and mesenteric vessels. The peritoneal cavity of the mice infected through the peritoneal route was carefully washed before the perfusion. Oograms from intestinal fragments were performed according to PELLEGRINO & FARIA [7].

The differences among the means from worm recoveries were assessed by the Student test.

RESULTS

The results obtained by injecting mice with the initial (cercaria) and final (schistosomule) stages of *S. mansoni* as well as intermediate forms obtained *in vitro* are summarized in Table I. The highest worm recovery was observed in animals infected with schistosomules, with all routes of inoculation. After the infection with intermediate forms the worm burden was particularly low, the mean schistosome recovery being higher in mice infected by the intraperitoneal route. In groups 2, 3, and 4 (Table I), in which the intraperitoneal route was used for infection, most of worms were found in the peritoneal cavity. This explains the fact that no *S. mansoni* eggs were found in the liver and intestine from 30% of the mice. The oogram was normal in the remaining animals.

Table II shows the worm recovery from mice infected by intravenous injection of schistosomules obtained by incubating cercarial bodies in heat-inactivated serum of mice, rats, and hamsters, as compared with those prepared in TC-199 medium. There were no significant differences between groups.

Attempts to infect mice by the transcutaneous route using cercarial bodies and schistosomules obtained *in vitro* were not successful.

DISCUSSION

Results of subcutaneous, intraperitoneal and intravenous injections with cercariae, intermediate larvae, and schistosomules have important bearing on the mechanisms of penetration and enzymatic involvement in infection. The difficulty is to prepare and to monitore intermediate forms by the *in vivo* and *in vitro* techniques available, for the collected organisms constitute a mixture of a continuum corresponding to cercaria-schistosomule stages. Recently, a stepwise procedure developed by RAMALHO-PINTO et al.[9] enabled the preparation of intermediate forms from transforming cercariae which

TABLE I
Mean worm recovery from mice infected with cercariae, schistosomules and intermediate forms (*Schistosoma mansoni*) obtained *in vitro*

Groups of 10 mice	Living organisms	Routes of inoculation and worm recovery		
		Subcutaneous mean & s.e.	Intraperitoneal mean & s.e.	Intravenous mean & s.e.
1	Cercariae	23.6 ± 10.9	30.5 ± 7.0	9.3 ± 4.0
2	(*) Cercarial bodies	7.3 ± 3.5	15.2 ± 7.1	8.9 ± 3.1
3	Cercarial bodies without secretion and with coat	5.1 ± 1.8	22.0 ± 6.4	8.8 ± 5.9
4	Cercarial bodies without coat	6.0 ± 4.5	23.3 ± 5.9	6.1 ± 3.2
5	(*) Schistosomules obtained in inactivated guinea pig serum	40.1 ± 9.8	40.3 ± 7.3	44.0 ± 10.1

(*) Not infective by transcutaneous route
s.e. = standard error

TABLE II
Mean worm recovery from mice infected with schistosomules obtained *in vitro* and injected intravenously

Groups of mice	Living organisms	Worm recovery from mice infected intravenously	
		Number of mice	Worm burden Mean & s.e.
1	Schistosomules obtained in TC 199 medium	10	32.4 ± 5.6
2	Schistosomules obtained in inactivated mice serum	10	31.9 ± 9.4
3	Schistosomules obtained in inactivated hamster serum	10	34.5 ± 11.4
4	Schistosomules obtained in inactivated rat serum	10	36.9 ± 5.4

s.e. = standard error

may be defined by morphological and biochemical parameters (OLIVEIRA et al. [6]). Advantage was taken of this procedure to prepare the intermediate forms described here (cercarial body, cercarial body without secretion and cercarial body without coat). These forms were characterized: a) by simple observation at the stereomicroscope; b) by assaying the proteolytic activity of the larval extract, and c) by testing the sensitivity of the larvae to water. The infectivity of the intermediate forms, as well as the initial (cercaria) and the final (schistosomule) was evaluated in mice by worm recoveries.

It should be stressed that the worm burden from cercariae injected subcutaneously reported here was low when compared to the data of PETERS & WARREN [8] using a Puerto Rican strain of *S. mansoni*. However, the recovery of mature worms varies widely according to the strain of mice (COLLEY [2]), and perhaps also with *S. mansoni* strains. In addition, these results have been consistently found in our laboratories.

It is interesting to note that cercariae were also found infective when injected by intravenous route and that intermediate forms were infective by all routes employed, although in a smaller degree. The low recovery of worms by subcutaneous and intravenous injections of intermediate forms indicates that in most of the larvae the process of transformation did not completed *in vivo* when initiated *in vitro*. This may be explained either as due to the manipulation of the organisms during preparation or by the lack of glycocalyx or proteolytic secretion in the intermediate forms. An indication that the larvae were not lesioned during preparation was the high recovery of the intermediate forms as adult worms in peritoneal cavity. Besides, schistosomules prepared *in vitro* were submitted to similar manipulation and showed a worm recovery as high as 40%. It can be seen (Table I) that schistosomules prepared by the procedure here employed were able to infect mice intracutaneously as well as intravenously. These data did not agree with those reported by STIREWALT & UY [12] who stressed that no infection occurred when schistosomules obtained *in vivo* and *in vitro* were inoculated intravenously. This discrepancy can not be explained presently since the schistosomule prepared by the technique here described was defined by the criteria established by STIREWALT, MINNICK & FREGEAU [11]. It is possible that in the future some differences

could be detected between the schistosomules obtained by the stepwise procedure and by biological-membrane techniques.

It was observed small differences in infectivity of schistosomules prepared in different media. However, the figures were not statistically significant.

RESUMO

Infecção do camundongo por cercárias, esquistossômulos e formas intermediárias de Schistosoma mansoni *obtidas* in vitro

Esquistossômulos de *Schistosoma mansoni* foram obtidos *in vitro* induzindo a perda da cauda, do glicocalix, e eliminação das glândulas acetabulares. Além das formas inicial (cercária) e final (esquistossômulo), as seguintes formas intermediárias foram obtidas: corpos cercarianos, corpos cercarianos sem secreção e com glicocalix e corpos cercarianos sem glicocalix. A recuperação de esquistossomos de camundongos infectados com cercárias e esquistossômulos, por via subcutânea, intraperitoneal e intravenosa foi, respectivamente, 28,6, 30,5 e 9,4% para cercárias e 40,2, 40,3 e 44,0 para esquistossômulos. Corpos cercarianos intactos, corpos cercarianos sem secreção e com glicocalix, e corpos cercarianos sem glicocalix e com secreção foram capazes de infectar camundongos pelas mesmas vias. Corpos cercarianos e esquistossômulos não foram capazes de infectar o camundongo pela pele.

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