*Rev. Inst. Med. trop. São Paulo*
*16(3):127-131, maio-junho, 1974*

*UDC 616.995.122.22*

# INFECTIVITY OF SCHISTOSOMULA *(SCHISTOSOMA MANSONI)* RECOVERED FROM THE SKIN AND THE LUNG OF INFECTED MICE

J. C. HOLANDA (1) and J. PELLEGRINO (2)

## SUMMARY

Schistosomula (*Schistosoma mansoni*) were obtained by exposing the abdominal skin of mice to about 3,000 cercariae and by removing the exposed area (after 30 min) and mincing the tissue in saline. Three groups of 10 mice were then injected (50 larvae per animal) by the subcutaneous, intraperitoneal and intravenous routes. The percentages of worms recovered as adult schistosomes were 25.4, 29.2 and 19.6, respectively. Schistosomula, recovered from the lung of mice (4 days after exposure to 3,000 cercariae) were injected intravenously (50 larvae per animal) into 10 mice. The percentage of worm recovery was 64.8.

## INTRODUCTION

Relatively, little is known about the infective capacity of schistosomula (*Schistosoma mansoni*) obtained *in vitro* and *in vivo*. STIREWALT & UY [11] mention that schistosomula obtained *in vitro* produce infections in mice following intravenous, but not intracutaneous or intraperitoneal inoculations. PEREIRA [9] and PEREIRA et al. [10] demonstrated that schistosomula collected from lungs of infected mice (4-day-old larvae) and liver (10-day-old larvae) are infective to mice when injected by subcutaneous and intraperitoneal routes. HOLANDA, PELLEGRINO & GAZZINELLI [4] showed that a high percentage of recovery (about 40%) is reached when schistosomula obtained *in vitro* are injected in mice by the subcutaneous and intravenous routes. MOREIRA et al. [6] were not able to infect adult hamsters (cheek pouch) and newly-born hamsters (skin) using schistosomula obtained from the lung of mice (3 to 5-day-old larvae).

The present paper deals with the infective capacity of schistosomula, obtained *in vivo*, when injected by the subcutaneous, intraperitoneal and intravenous routes to mice.

## MATERIAL AND METHODS

*Cercariae of S. mansoni* — In all experiments cercariae (L.E. strain, Belo Horizonte) of *S. mansoni* were shed by infected *Biomphalaria glabrata*, maintained and reared in the laboratory.

*Recovery of schistosomula from skin* — The method used was similar to those described by STIREWALT [11], CLEGG [2], and CLEGG & SMITHERS [3]. A standard glass ring with

This paper was supported, in part, by the "Conselho Nacional de Pesquisas" (MINIPLAN), Brazil, and the U.S. Army (Grant DADA 17-73-G-9378)

(1) Department of Zoology and Parasitology, Federal University of Minas Gerais. Fellow from the "Conselho Nacional de Pesquisas"

(2) Schistosomiasis Research Unit, Federal University of Minas Gerais, Belo Horizonte, Brazil. Fellow from the "Conselho Nacional de Pesquisas"

Contribution no. 50 from the Schistosomiasis Research Unit. Address for reprints: Caixa Postal 1404, 30000 — Belo Horizonte, Brazil

an inside diameter of 2.0 cm was placed on the shaved abdominal skin of 5 anesthetized mice. The ring was filled with 2 ml of a heavy cercarial suspension (about 3,000 cercariae) which was left in contact with the skin for 30 min. The animals were killed and the area of the skin covered by the glass ring was washed with saline and rapidly excised. The schistosomula were recovered from the pieces of the skin by chopping the skin into small pieces which were incubated in saline for 1 hour at 37°C.

*Recovery of schistosomula from lungs* — Mice were killed 4 days after exposure to about 3,000 cercariae per animal. The animals were opened and 10 ml of saline containing 0.1 mg heparin per ml was injected into the right venticle to perfuse the lungs. The left ventricle was punctured to allow the perfusate to flow away. The perfused lungs were chopped into small fragment (1 $mm^3$). The lung fragments were incubated in saline for 1 hour at 37°C. During this time schistosomula crowled out from the lung capillaries. Finally, lung fragments were separated from the schistosomula by passing the suspension through a stainless steel wire (mesh 60).

*Infection of mice* — The animals were injected with 50 schistosomula. According to each experiment the larvae were injected by the subcutaneous, intraperitoneal and intravenous routes. In the last case the larvae were injected via the dorso cervical veins of the tail.

*Recovery of schistosomes* — The method of PELLEGRINO & SIQUEIRA [8], adapted to mice, was used to perfuse the liver and mesenteric vessels, 7 weeks after infection with schistosomula. In case the animals had been infected intraperitoneally, after the perfusion the peritoneum and intestinal loops were washed carefully, as mentioned by BRENER [1], with saline.

*Oogram* — The oogram was performed in all animals following the technique of PELLEGRINO & FARIA [7].

## RESULTS AND COMMENTS

The results obtained after injecting schistosomula recovered from skin, in mice, are summarized in Table I. As can be seen. the mean worm recoveries were $12.7 \pm 4.7$, $14.6 \pm 6.1$ and $9.8 \pm 3.8$ for the subcutaneous, intraperitoneal and intravenous routes. The statistical analysis showed that only the comparison between the mean worm recovery from the animals injected by intraperitoneal and intravenous routes was significant ($p < 0.05$). It is important to note that the great majority of the larvae injected into the peritoneal cavity remained there developing to adult but stunted schistosomes. The worms recovered from the peritoneal cavity were considerably smaller than those found in the hepatic-portal system and have no hematin in the intestinal ceca, indicating that they had not fed on blood (MOORE & MELENEY [5]). The oogram was normal in all mice with exception of 3 animals (no eggs in intestinal fragments) injected with schistosomula by the intraperitoneal route. With this route, the ratio male: female schistosomes was very high. Our data show that schistosomula recovered from the skin are able to infect mice through the conventional routes of infection except the skin. This agree with the data reported by PEREIRA [9], PEREIRA et al. [10], and, in part, with those of STIREWALT & UY [12].

As can be seen in Table II, a high mean of worm recovery was observed ($32.4 \pm 10.5$) in mice injected intravenously with schistosomula (4-day-old larvae) recovered from the lung. The oogram was normal in all animals. The percentage of recovery was 64.8.

## RESUMO

*Infectividade, para o camundongo, de esquistossômulos* (Schistosoma mansoni) *obtidos da pele e pulmão de camundongos infectados*

Esquistossômulos (*Schistosoma mansoni*) foram obtidos expondo-se a pele do abdomen de camundongos a cerca de 3.000 cercárias. Após 30 minutos a pele exposta foi removida e cortada em pequenos fragmentos, em salina. Três grupos de 10 camundongos foram injetados com esquistossômulos (50 larvas

TABLE I

Infective capacity of schistosomula (% of worm recovery as adult worms) collected from the skin and injected into mice. The animals were sacrificed 7 weeks after infection

| Routes of infection | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subcutaneous | | | | | Intraperitoneal | | | | | Intravenous | | | | |
| Mice | P | ♂ | ♀ | T | Mice | P | ♂ | ♀ | T | Mice | P | ♂ | ♀ | T |
| 1 | 4 | 7 | 3 | 18 | 11 | — | 3 | 2 | 5+ | 21 | 2 | 4 | — | 8 |
| 2 | 2 | 2 | 1 | 7 | 12 | 2 | 6 | 3 | 13 | 22 | 4 | 1 | — | 9 |
| 3 | 1 | 7 | 2 | 11 | 13 | 4 | 11 | 2 | 21 | 23 | 2 | 1 | 1 | 6 |
| 4 | 5 | 3 | 4 | 17 | 14 | 2 | 6 | — | 10 | 24 | 2 | 2 | 1 | 7 |
| 5 | 3 | 6 | 5 | 17 | 15 | 2 | 12 | 7 | 23+ | 25 | 2 | 6 | — | 10 |
| 6 | 1 | 2 | 1 | 5 | 16 | 1 | 6 | 2 | 10+ | 26 | 5 | 5 | 4 | 19 |
| 7 | 2 | 6 | 2 | 12 | 17 | 4 | 12 | 5 | 25 | 27 | 2 | 3 | 1 | 8 |
| 8 | 3 | 8 | 5 | 19 | 18 | 2 | 6 | 2 | 12 | 28 | 2 | 5 | 6 | 15 |
| 9 | 2 | 3 | — | 7 | 19 | 1 | 8 | 3 | 13 | 29 | 1 | 5 | 3 | 10 |
| 10 | 3 | 3 | 5 | 14 | 20 | 4 | 4 | 2 | 14 | 30 | 2 | 1 | 1 | 6 |
| Total | 26 | 47 | 38 | 127 | | 22 | 74 | 28 | 146 | | 24 | 33 | 17 | 98 |
| Mean & s.e. | 2.6 | 4.7 | 3.8 | 12.7±4.7 | | 2.2 | 7.4 | 2.8 | 14.6±6.1 | | 2.4 | 3.3 | 1.7 | 9.8±3.8 |
| Recovery (%) | | | | 25.4 | | | | | 29.2 | | | | | 19.6 |

P = Pairs T = Total + = No *S. mansoni* eggs in the liver and intestine s.e. = standard error

TABLE II

Worm recovery from mice, 7 weeks after infection, by the intravenous route, with 50 schistosomula obtained from the lung of infected mice

| Mice | Pairs | ♂ | ♀ | Total |
|---|---|---|---|---|
| 1 | 18 | 6 | 2 | 44 |
| 2 | 19 | 5 | 4 | 47 |
| 3 | 4 | 7 | 4 | 19 |
| 4 | 10 | 3 | 4 | 27 |
| 5 | 15 | 9 | 5 | 44 |
| 6 | 7 | 10 | 4 | 28 |
| 7 | 3 | 5 | 2 | 13 |
| 8 | 8 | 9 | 9 | 34 |
| 9 | 7 | 10 | 7 | 31 |
| 10 | 13 | 6 | 5 | 37 |
| Total | 104 | 70 | 46 | 324 |
| Mean & s.e. | 10.4 | 7.0 | 4.6 | 32.4±10.5 |
| Recovery (%) | | | | 64.8 |

s.e. = standard error

por animal), pelas vias subcutâneas, intraperitoneal e endovenosa. As percentages de vermes adultos recuperados foram de 25,4, 29,2 e 19,6, respectivamente.

Esquistossômulos obtidos do pulmão de camundongos infectados (4 dias depois da exposição a 3.000 cercárias) foram injetados por via endovenosa (50 larvas por animal) em 10 camundongos. A percentagem de recuperação de vermes adultos foi de 64,8.

## REFERENCES

1. BRENER, Z. — Esquistossomose experimental. *Rev. Brasil. Malariol. Doenças Trop.* **11**:473-506, 1959.

2. CLEGG, J. A. — "In vitro" cultivation of *Schistosoma mansoni*. *Exp. Parasitol.* **16**: 133-147, 1965.

3. CLEGG, J. A. & SMITHERS, S. R. — Death of schistosome cercariae during penetration of the skin. II — Penetration of mammalian skin by *Schistosoma mansoni*. *Parasitol.* **58**:111-128, 1968.

4. HOLANDA, J. C.; PELLEGRINO, J. & GAZZINELLI, G. — Infection of mice with cercariae and schistosomula of *Schistosoma mansoni* by intravenous and subcutaneous routes. *Rev. Inst. Med. trop. São Paulo* **16**: 132-134, 1974.

5. MOORE, D. V. & MELENEY, H. E. — Development of *Schistosoma mansoni* in the peritoneal cavity of mice. *J. Parasitol.* **41**: 235-245, 1955.

6. MOREIRA, C.; PEREIRA, L. H.; ARAÚJO, C. C. & PELLEGRINO, J. — Attempts to infect hamsters through the cheek pouch and skin using schistosomula (*Schistosoma mansoni*). *Trans. Royal Soc. Trop. Med. & Hyg.* **67**:308, 1973.

7. PELLEGRINO, J. & FARIA, J. — The oogram method for the screening of drugs in Schistosomiasis mansoni. *Amer. J. Trop. Med. & Hyg.* **14**:363-369, 1965.

8. PELLEGRINO, J. & SIQUEIRA, A. F. — Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infestadas. *Rev. Brasil. Malariol.* 8: 589-597, 1956.

9. PEREIRA, L. H. — *Capacidade de migração das larvas de* Schistosoma mansoni *Sambon, 1907, no camundongo albino.* Thesis. Institute of Biological Sciences, Federal University of Minas Gerais, 1971, 38 pp.

10. PEREIRA, L. H.; COELHO, P. M. Z.; FONSECA, J. J. A.; BREDT, A. & PELLEGRINO, J. — Migration of *Schistosoma mansoni* larvae in the albino mouse. *Rev. Inst. Med. trop. São Paulo* **14**:306-309, 1972.

11. STIREWALT, M. A. — Cercaria vs. schistosomule (*S. mansoni*): absence of the pericercarial envelope *in vivo* and the early physiological and histological metamorphosis of the parasites. *Exp. Parasitol.* **13**: 395-406, 1963.

12. STIREWALT, M. A. & UY, A. — *Schistosoma mansoni*: cercarial penetration and schistosomule collection in an *in vivo* system. *Exp. Parasitol.* **26**:17-28, 1969.

Recebido para publicação em 5/11/1973.