

MIGRATION OF *SCHISTOSOMA MANSONI* LARVAE IN THE ALBINO MOUSE

Leógenes Horácio PEREIRA, Paulo Marcos Zech COELHO, José Júlio Andrade FONSECA,
Angelika BREDT and J. PELLEGRINO

SUMMARY

Schistosomula (*) of different ages were obtained from albino mice experimentally infected with cercariae of *Schistosoma mansoni*.

The larvae were collected from lungs (4-day-old schistosomula) and liver (10 and 20-day-old), and were inoculated subcutaneously and intraperitoneally in uninfected mice. The capacity of migration was evaluated by the finding of adult worms within the portal system.

The migration was observed using 4 and 10-day-old larvae, with both routes of inoculation. Twenty-day-old larvae were recovered as adult worms within the portal system in small numbers when injected subcutaneously. No schistosomes could be found in blood vessels after intraperitoneal inoculation of 20-day-old schistosomula.

INTRODUCTION

In human infections produced by species of the genus *Schistosoma*, the larvae, after their penetration into host skin, are carried to the lungs is well known, but the migration route followed by the larvae from the skin to the lungs is well known, but the migration from lungs to the portal system is a point of controversy.

Different lung-to-liver routes of migration have been suggested. NARABAYASHI⁴ and WILKS⁵ claimed that larvae migrate mainly through host tissues outside the blood stream. MIYAGAWA & TAKEMOTO³, FAUST & MELENEY¹, SUDAN et al.⁶ concluded that larvae reach the portal system through blood vessels.

In this paper the capacity of migration of schistosomula (larvae found in the vertebrate

host after cercarial penetration), was studied by subcutaneous and intraperitoneal inoculations in mice.

MATERIAL AND METHODS

Infection of mice — Adult albino mice were placed individually into flasks and exposed for 30 minutes to 1,500 *S. mansoni* cercariae (L. E. strain), shed by infected *Biomphalaria glabrata*.

Collection of schistosomula — The infected mice were killed by cervical fracture. Some of them had the lungs removed (after perfusion with saline injected into the right ventricle), sectioned in small pieces and incubated in a Petri dish with saline at 37°C during 30

(*) According to HSU, HYBAKKEN & HSU⁽²⁾ the correct nomenclature for developing larvae of *Schistosoma* in the vertebrate host is "schistosomulum" and "schistosomula".

This work has been supported by the "Conselho de Pesquisas da UFMG", "Conselho Nacional de Pesquisas", Brazil, and the U.S. Army (Grant number DAHC-19-71-G-0018). Contribution number 22 from the Schistosomiasis Research Unit.

"Departamento de Zoologia e Parasitologia do Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais", Belo Horizonte, Brazil

TABLE I
Distribution of schistosomes and eggs in mice inoculated intraperitoneally with schistosomula (50 larvae per animal)

Mouse number	Age of schistosomula (days)	<i>S. mansoni</i> eggs			Worms found within the portal system			Worms found in the peritoneal cavity		
		Liver	Intestine	Feces	males	females	pairs	males	females	pairs
1	4	-	-	-	—	—	—	23	—	5
2	4	+	+	+	—	1	1	2	1	—
3	4	+	+	+	9	—	4	1	—	1
4	4	+	+	+	—	—	1	21	—	2
5	4	-	-	-	—	—	1	13	—	2
6	4	+	+	+	4	—	1	19	—	10
7	4	+	+	+	—	—	5	14	—	3
8	4	+	+	+	1	—	1	12	—	—
9	4	+	+	-	2	1	1	24	—	4
10	4	-	-	-	—	—	—	11	—	8
11	10	+	+	+	2	2	1	9	—	2
12	10	-	-	-	—	—	—	1	1	—
13	10	-	-	-	—	—	—	35	—	1
14	10	-	-	-	—	—	—	39	—	—
15	10	-	-	-	—	—	—	—	1	—
16	10	-	-	-	—	—	—	10	1	3
17	10	-	-	-	—	—	—	9	—	3
18	10	-	-	-	—	—	—	14	2	—
19	10	-	-	-	—	—	—	23	3	3
20(*)										
21	20	-	-	-	—	—	—	—	—	1
22	20	-	-	-	—	—	—	—	—	—
23	20	-	-	-	—	—	—	1	—	—
24	20	-	-	-	—	—	—	—	—	—
25	20	-	-	-	—	—	—	15	—	2
26	20	-	-	-	—	—	—	—	—	—
27	20	-	-	-	—	—	—	2	1	—
28	20	-	-	-	—	—	—	—	—	—
29	20	-	-	-	—	—	—	—	—	—
30	20	-	-	-	—	—	—	—	—	—

(*) Died before being examined

minutes. The tissues were then removed from the Petri dish and the larvae present in the saline concentrated by centrifugation. This method was used to obtain larvae 4 days after exposure. Other mice were killed as described above and the larvae collected in liver perfusates, by severing the portal vein and injecting heparinized saline into the hepatic venous sinus. This method was used to collect larvae 10 and 20 days after the initial exposure.

Inoculation of schistosomula — Larvae were inoculated subcutaneously through a syringe in 10 mice and intraperitoneally in another group of 10 animals. Each animal received about 50 larvae. Schistosomula used were 4, 10, and 20-day old.

Examination of mice — Animals were killed with ether. The abdominal cavity was opened and carefully inspected for collection of worms. Thereafter the liver and the mesenteric vessels were perfused as recommended by PELLEGRINO & SIQUEIRA⁵. Worms collected from the peritoneal cavity and from the portal system were counted under a dissecting microscope and fixed in 5% formalin for later studies. Pieces of liver and intestine as well as samples of feces were microscopically examined for *S. mansoni* eggs.

RESULTS

Data are shown on Tables I and II. Larvae of different ages that reached the portal

TABLE II

Distribution of schistosomes and eggs in mice inoculated subcutaneously with schistosomula (50 larvae per animal)

Mouse number	Age of schistosomula (days)	<i>S. mansoni</i> eggs			Worms found within the portal system		
		Liver	Intestine	Feces	males	females	pairs
31	4	+	+	+	13	—	11
32	4	+	+	+	2	—	5
33	4	+	+	+	11	—	7
34	4	+	+	+	—	—	11
35	4	+	+	+	6	—	6
36	4	+	+	+	5	1	—
37	4	+	+	+	4	2	1
38	4	+	+	+	—	—	5
39	4	+	+	+	—	—	5
40	4	+	+	+	7	—	7
41	10	+	+	+	1	—	—
42	10	+	+	+	—	—	5
43	10	+	+	+	2	1	2
44	10	—	—	—	1	—	—
45	10	+	+	+	2	—	1
46	10	—	—	—	—	—	—
47	10	+	+	+	—	—	1
48	10	—	—	—	—	—	—
49	10	—	—	—	—	—	—
50	10	—	—	—	—	—	—
51	20	—	—	—	—	—	—
52	20	—	—	—	—	—	—
53	20	—	—	—	—	—	—
54	20	—	—	—	—	—	—
55	20	—	—	—	—	—	—
56	20	—	—	—	—	—	—
57	20	+	+	+	—	—	1
58	20	—	—	—	—	—	—
59	20	—	—	—	—	—	—
60	20	—	—	—	—	—	—

system and developed into adult worms were recovered by perfusion in the following percentages:

Intraperitoneal inoculation: 4-day-old: 9.6%; 10-day-old: 1.2%; 20-day-old: none

Subcutaneous inoculation: 4-day-old: 33.4%; 10-day-old: 5.0%; 20-day-old: 0.4%.

Worms found within the portal system, independently of the route of inoculation, were of normal morphology. Worms were found in the peritoneal cavity only if the inoculation of larvae was intraperitoneal. However, they were much smaller than those found within the portal vein and no pigment

was seen in the worms as well as eggs in the females.

DISCUSSION

STIREWALT⁷ inoculated schistosomula of *S. mansoni* into mice using the intravenous, intraperitoneal and intracutaneous routes. Larvae were collected *in vitro* (schistosomula-collecting apparatus) and *in vivo* (from skin). Only the larvae inoculated intravenously could be recovered as adult worms within mesenteric vessels. The Author claimed that the enzymic armentarium of acellular glands is necessary for migration through host tissues. However, WILKS⁸, exposing mice to *S. mansoni* cercariae by the percutaneous route, demonstrated that

migration actually occurs, since schistosomula were found in lung parenchyma, pleural cavity, diaphragm muscle, peritoneal cavity and liver parenchyma.

The present data show that schistosomula from lungs and portal system were able to migrate reaching maturity within blood vessels.

The following conclusions can be drawn:

1) It is possible to infect albino mice with schistosomula of *S. mansoni* by subcutaneous and intraperitoneal inoculations.

2) Lung larvae (4-day-old) and liver larvae (10-day-old) of *S. mansoni* are able to migrate from the site of inoculation reaching the portal system, where they produce fertile eggs. The migratory capacity of 20-day-old larvae is extremely reduced.

RESUMO

Migração das larvas de Schistosoma mansoni no camundongo albino

Camundongos albinos adultos foram expostos a cercárias de *S. mansoni*. Os esquistossômulos foram retirados do pulmão (4 dias após a exposição) ou do fígado (10 e 20 dias após a exposição) dos animais e inoculados pelas vias subcutânea e intraperitoneal em camundongos não infetados. A capacidade de migração das larvas foi avaliada, após 2 a 3 meses, pelo achado de vermes adultos dentro do sistema porta.

A migração ocorreu com larvas de 4 e de 10 dias, utilizando-se as duas vias de inoculação. Larvas de 20 dias, injetadas subcutaneamente, foram recuperadas como vermes adultos, no sistema porta, em pequeno

número. Nenhum esquistossomo foi encontrado nos vasos sanguíneos após inoculação intraperitoneal de larvas de 20 dias.

REFERENCES

1. FAUST, E. C. & MELENEY, H. E. — The life history of *Schistosoma japonicum* KATSURADA. The causative organism of oriental schistosomiasis in China. *China Med. J.* 37: 726-734, 1923.
2. HSÜ, S. Y. L.; HYBAKKEN, O. E. & HSÜ, H. F. — "Schistosomulum", "schistosomula", or "schistosomule"? *J. Parasitol.* 55:876, 1969.
3. MIYAGAWA, Y. & TAKEMOTO, S. — The mode of infection of *Schistosomum japonicum* and the principal route of its journey from the skin to the portal vein in the host. *J. Path. Bact.* 24:168-174, 1921.
4. NARABAYASHI, H. — *China Med. J.* 22:1, 1917. In MIYAGAWA & TAKEMOTO, 1921.
5. PELLEGRINO, J. & SIQUEIRA, A. F. — Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infectadas. *Rev. Brasil. Malar.* 8:589-597, 1956.
6. SADUN, E. H.; LIN, S. S. & WILLIAMS, J. E. — Studies on the host parasite relationships to *Schistosoma japonicum*. I — The effect of single graded infections and the route of migration of schistosomula. *Amer. J. Trop. Med. Hyg.* 7:494-499, 1958.
7. STIREWALT, M. A. — *Schistosoma mansoni*: Cercarial penetration and schistosomule collection in an *in vitro* system. *Exp. Parasitol.* 26:17-28, 1969.
8. WILKS, N. E. — Lung-to-liver migration of schistosomes in the laboratory mouse. *Amer. J. Trop. Med. Hyg.* 16:599-605, 1967.

Recebido para publicação em 23/2/1972.