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THE IMPORTANCE OF SKIN AND PULMONARY PHASES TO THE DEVELOPMENT OF SCHISTOSOMA MANSONI IN ALBINO MICE (*)

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SUMMARY

Schistosomula **(Schistosoma mansoni)** prepared **in vitro** or obtained from lungs or liver of mice previously infected were inoculated into the portal vein of normal albino mice. A percentage of larvae (about 50%) develops in the portal system without skin phase, and probably without pulmonary phase. Under such conditions, the parasite presents a higher degree of development compared with transcutaneous infection, as demonstrated by schistogram pattern and quantitative oogram.

INTRODUCTION

A point of great biological interest in the study of different worms is the influence of the journey through the lungs of the host by the parasite.

In **Ancylostoma caninum**, the usual mode of infection is through larvae skin penetration and subsequent passage through the lungs of the host. However, after oral infection, the development of this hookworm can take place in the alimentary tract without passage through the lungs (SCOTT [14]; NAGAHANA & YOSHIDA [7]).

In natural infection of **Ascaris lumbricoides**, on the other hand, the second-stage larva leaves the small intestine (habitat of adult worms) and migrates to the lungs, where the larva suffers two moults before coming back to the small intestine to develop into adult worm. SYLK [15] correlated the need of pulmonary phase with the aerobic metabolism of **Ascaris** lung larvae.

The **Schistosoma mansoni** life-cycle in vertebrate host starts with cercariae skin penetration. In a few days, the schistosomula reach the lungs, and after that they migrate to the portal system.

Some authors have observed that while in the lungs schistosomula generally do not feed on blood, and only after reaching the portal system they become adult worms (FAUST, JONES & HOFFMAN [5]; KOPPISCH [6]; YOLLES, MOORE & MELLENEY [19]; WILKS [17]; BARBOSA et al. [1]). On the contrary, BRUCE et al. [3] have suggested that an absorptive function was active in the lung schistosomula integument, as well as pinocytosis and phagocytosis.

Based on these conflicting opinions about nutrition of **S. mansoni** in lungs, the present work has been conducted with the purpose of establishing whether the skin and pulmonary phases are essentials or not to the complete development of worm in the vertebrate host.

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MATERIALS AND METHODS

Cercariae of **S. mansoni,** LE strain (Belo Horizonte, Brasil), shed by laboratory-reared and infected **Biomphalaria glabrata,** were used in the present study. The cercariae were concentrated in sintered-glass crucibles as described by PELLEGRINO & MACEDO [9].

Schistosomula of **S. mansoni** were obtained by "in vitro" transformation (RAMALHO-PINTO et al. [13]), or from the lungs (BARBOSA et al. [1]), and from the liver (PELLEGRINO & SIQUEIRA [11]) of experimentally infected mice. The schistomula were inoculated into the portal vein of outbred white Swiss mice (Schistosomiasis Research Unit) weighing 20 to 30 g. Before inoculation, the lower abdomen was shaved and mice were anesthetized with Nembutal (66.5 mg/kg). The skin and the peritoneum of the animals were opened with a horizontal incision of 1.5 cm in length with the aseptic precautions used in surgical processes. The visceral contents were drawn out and protected with a gauze drenched in Hanks' balanced salt solution (HBSS).

With an sterilized disposable hypodermic syringe, 0.2 ml of the schistosomula suspension was inoculated into the portal vein. Immediately after the removal of the syringe, the portal vein was compressed with a gauze pad drenched in HBSS, to avoid hemorrhagic accidents. The guts were carefully introduced into the peritoneal cavity, and the peritoneum and skin were sutured separately with categut. The sutured area was treated with merthiolate solution.

The control group was infected with cercariae by transcutaneous route, according to BARBOSA et al. [1].

After the schistosomula inoculation into the portal vein or after the penetration of cercariae through the skin, the mice were sacrificed by cervical fracture at predetermined times. The following parasitological proceedings were used according to the purpose of each experiment:

a) Schistosomula recovery from the lungs (BARBOSA et al. [1]);

b) Schistosomula recovery from the liver (PELLEGRINO & SIQUEIRA [11]);

c) Classification of portal system worms in relation to their stage (schistogram). The gastric caecum shape was chosen for classifying the parasites,as described by BARBOSA et al. [1]:

STAGE 1 — schistosomula presenting only a light stain which stands for the beginning of the caecum;

STAGE 2 — a darker stain now bifurcating but not bypassing the acetabulum;

STAGE 3 — the dark stain bypasses the acetabulum and its branches link themselves later on;

STAGE 4 — the dark bifurcated stain after reconnection grows to the parasite end, but not longer than the bifurcated caecum;

STAGE 5 — the final linked caecum grows longer than its bifurcated section, but shorter than three times of its length;

STAGE 6 — their linked caecum grows three times longer than the bifurcated caecum (mature adults).

d) Qualitative and quantitative oograms (PELLEGRINO et al. [10]).

RESULTS

In Table I, it can be observed that the age of lung schistosomula do not promote differences in the recovery rates of larvae from the portal system and lungs, when inoculated into the portal vein of mice. Twelve-day-old schistosomula obtained from the liver of mice, and inoculated into the portal vein were recovered mainly in the portal system.

Yet, in Table I, it can be seen that the "in vitro" prepared schistosomula inoculated into the portal vein of mice can be recovered from the portal system and lungs, at different time intervals. In spite of parasite migration to the lungs, the relative percentage of larvae recovered in the portal system, suggests that about 50% of parasite remain in the inoculation site. Reinforcing this evidence, the schistogram pattern (Figs. 1 and 2), and the quantitative oogram (Table II and Fig. 3) indicated that the degree of worm development in the group inoculated into the portal vein, using "in vitro" prepared schistosomula, is higher than that observed in the group infected transcutaneously. Finally, in Fig. 2 the rates of recovery of 6th stage worms obtained in the group inoculated into the portal

vein using "in vitro" prepared schistosomula are compared with those obtained in the group infected by cercarial skin penetration. As can be seen, between the 23rd and 33rd days after infection, the group submitted to portal vein inoculation presented statistically significant higher rates of 6th stage worm recovery (Student test, $P < 0.05$).

TABLE I

Recovery of **S. mansoni** schistosomula from lungs and liver of mice, after inoculation into the portal vein of schistosomula "in vitro" prepared or obtained from lungs or liver of mice.

Schistosomula Source	Mean of inoculated schistosomula	Number of animals	Days after inoculation	Mean of schistosomula recovery (%)	
				Lungs	Liver
"In vitro"	560	4	3	24.37	75.63
"In vitro"	560	4	5	51.22	48.78
"In vitro"	560	4	7	26.93	73.07
Lungs (4 days)	40	6	3	18.57	81.43
Lungs (6 days)	57	6	3	21.33	78.67
Lungs (8 days)	40	5	3	10.00	90.00
Lungs (10 days)	40	5	3	18.41	81.59
Liver (12 days)	128	4	3	0.00	100.00
Liver (12 days)	128	4	5	1.70	98.30
Liver (12 days)	128	3	7	0.00	100.00

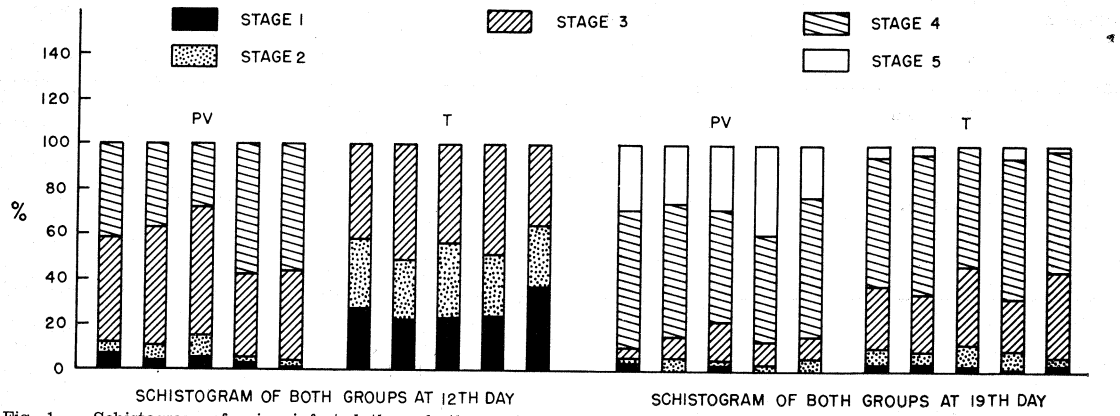

Fig. 1 — Schistogram of mice infected through the portal vein with 335 "in vitro" prepared schistosomula (PV) or transcutaneously with 282 **S. mansoni** cercariae (T)

DISCUSSION

BARBOSA et al.[1] showed that after transcutaneous infection the increase of larvae in the liver is correlated with a decrease of pulmonary larvae. After schistosomula migration to the liver the number of larvae drops to zero. The results (Table I) showed that a significant percentage of "in vitro" prepared schistosomula inoculated into the portal vein reaches the lungs. Nevertheless, about 50% of the larvae remain in the portal system, since the number of schis-

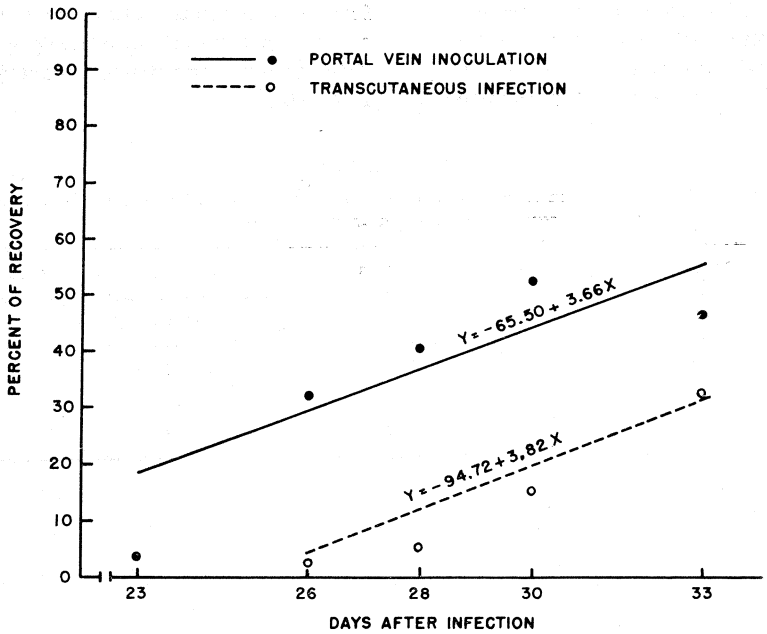

Fig. 2 — Linear relationship between sixth stage schistosomula percent and time of infection (days) in mice, after portal vein inoculation with 167 "in vitro" prepared schistosomula or after transcutaneous infection with 150 **S. mansoni** cercariae

T A B L E II

Quantitative oogram of mice after portal vein inoculation with 241 "in vitro" prepared schistosomula or after transcutaneous infection with 206 **S. mansoni** cercariae

Days after infection	Infection route	Number of mice	Number of worms	Viable eggs per gram of intestinal tissue					Total viable eggs per gram of tissue
				Stages					
				1st	2nd	3rd	4th	Mature	
27	Portal vein	1	96	0	0	0	0	0	0
		2	33	0	0	0	0	0	0
		3	75	0	0	0	0	0	0
		4	74	0	0	0	0	0	0
27	Transcutaneous	1	98	0	0	0	0	0	0
		2	121	0	0	0	0	0	0
		3	123	0	0	0	0	0	0
		4	117	0	0	0	0	0	0
28	Portal vein	1	108	171	17	35	0	0	223
		2	52	0	0	0	0	0	0
		3	107	61	0	0	0	0	61
		4	59	0	0	0	0	0	0
28	Transcutaneous	1	119	0	0	0	0	0	0
		2	83	0	0	0	0	0	0
		3	103	0	0	0	0	0	0
31	Portal vein	1	51	1202	119	44	0	0	1365
		2	15	767	329	137	0	0	1233
		3	66	2000	325	175	0	0	2500
		4	66	526	18	561	0	0	1105
31	Transcutaneous	1	114	49	0	0	0	0	49
		2	56	29	0	0	0	0	29
		3	91	0	0	0	0	0	0

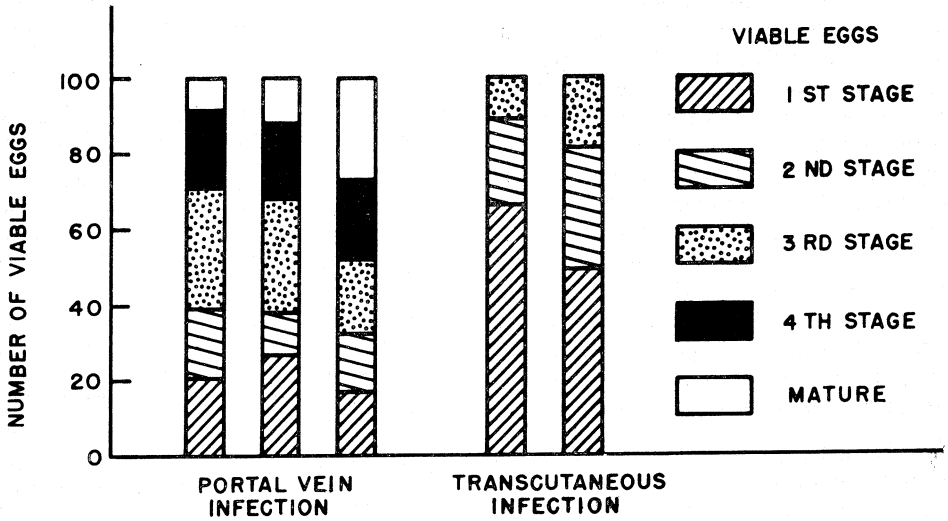

Fig. 3 — Proportion of viable eggs stages concerning counting of 100 eggs in the small intestine of mice, 34 days after portal vein inoculation with 241 "in vitro" prepared schistosomula or after transcutaneous infection with 206 **S. mansoni** cercariae.

tosomula in that site never drops below approximately 50%.

FAUST, JONES & HOFFMAN[5], KOPPISCH[6], YOLLES[19], OLIVIER[8], WILKS[17], and BARBOSA et al.[1] showed that the blood feeding of schistosomula occurs only in the portal system. So, the real development of the worm possibly starts in the liver. The results presented in Figs. 1 and 2 corroborate this hypothesis. Portal vein inoculation of "in vitro" prepared schistosomula induced to a high degree of parasite development, compared with transcutaneous infection, evaluated by schistogram pattern (BARBOSA et. al[1]). In this way, Table II and Fig. 3 show a precocious egg laying and an increase in the egg production in mice infected through the portal vein, as demonstrated by the oogram method (PELLEGRINO et al.[10]). The total number of eggs per gram of intestinal tissue presented significant statistical differences between the two groups (Table II). These differences were attributed to the higher proportion of mature females found in the group inoculated through the portal vein.

PRATA[12] demonstrated that **S. mansoni** egg, lodged in tissue, needs about three days to reach the 3rd stage. It can be seen (Table II) that mouse 1 (28th day after portal vein inoculation) presented some 3rd stage eggs. Thus, in this case, the egg laying started nearly at 25th day after inoculation. Also from the experimental data in Fig. 2, it can be concluded that after portal vein inoculation the parasite reaches the 6th stage earlier than in transcutaneous infection.

In two experiments, worm pairs were first observed in the 23th after portal vein inoculation with 156 and 167 "in vitro" prepared schistosomula. Through these data we may infer that portal vein inoculation reduces five days in the life-cycle of **S. mansoni** in the vertebrate host (BRENER[2]; CLEGG[4]; BARBOSA et al.[1]).

This is the period of time that the parasite needs to migrate from the skin to the lungs, and after that to the portal system.

The age of lung larvae seems not to be a determinant factor in the migration process when these larvae were inoculated into the portal system (Table I). The 12-day-old schistosomula practically have not migrated to the lungs after portal vein inoculation (Table I). These last results suggest that the size of inoculated larvae is the mainly factor in promoting the migration to the lungs after portal vein inoculation. WHEATHER & WILSON[16] also suggest that the portal tract end of the hepatic sinusoides functions as a filter preventing the recirculation of schistosomula through the liver.

On the other hand, it was observed that after portal vein inoculation practically the totality of the remaining worms in the liver presented some food in their guts, while in lung worms this phenomenon was rare. This was

true in experiments carried out with schistosomula obtained "in vitro" or from lungs. It is possible that the parasites that feed in portal system apparently remain there. WILSON et al.[18] suggested the hypothesis that worm nutrition in portal system induce the stimulus to parasite growth and probably this stimulus prevents migration. Other workers (FAUST, JONES & HOFFMAN[5]; OLIVIER[8]; WILKS[17]; BARBOSA et al.[1]) reported that only a reduced number of lung worms presents blood contents in their guts. This last observation in conjunction with our data allow to conclude that the recirculation of parasites between lungs and portal system seems not to be a valuable hypothesis.

In conclusion, the skin and the pulmonary phases seem to be rather a hemodynamic phenomenon than an actual necessity for further development of **S. mansoni** in mammalian host.

RESUMO

Importância da migração através da pele e do pulmão no desenvolvimento do Schistosoma mansoni em camundongos albinos

Esquistossômulos **(Schistosoma mansoni)**, preparados **in vitro** ou obtidos dos pulmões e fígado de camundongos previamente infectados, foram inoculados na veia porta de camundongos albinos normais.

Cerca de 50% das larvas desenvolvem-se no sistema porta sem passar pela pele e, provavelmente, também sem a fase pulmonar. Sob tais condições, o parasito apresenta maior grau de desenvolvimento, comparado com a infecção transcutânea, como foi demonstrado através do schistograma e do oograma quantitativo.

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