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# AUTORADIOGRAPHIC ANALYSIS OF *SCHISTOSOMA MANSONI* MIGRATION IN THE NZ RABBIT

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*The migration of larval* Schistosoma mansoni *was tracked by means of autoradiographic analysis in naive rabbits percutaneously exposed to L-($^{75}$Se) selenomethionine-labeled cercariae on serial intervals of 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40 and 50 days post-infection.*

*Autoradiographic foci were detected from the 1st day in the skin, up to the 15th day in the liver. Adult and mature worms were recovered either paired or not 60 days after infection, by perfusion of hepatic and mesenteric veins. Morphometric analysis under optical microscopy, showed that worms were within regular dimention limits as compared to adult worms harboured by other host species.*

*These observations extend previous informations on the* S. mansoni*-rabbit association and clearly demonstrate the post-liver phase of* S. mansoni *life-cycle in this host.*

Key words: *Schistosoma mansoni* – migration – autoradiographic analysis – rabbit model

Most of the available information on the *Schistosoma mansoni* migration route, was obtained in a wide range of mammals. Those experiments were mainly based on mean rates of parasites recovered, by means of perfusion of organs (Perez et al., 1974; Sher et al., 1974; Miller & Wilson, 1978). Nevertheless, interpretation of results is complicated by the failure of some parasites in crawling out of the chopped tissues, leading to the underestimation of the number of penetrating cercariae (Chandiwana, 1988; Fusco et al., 1988).

In this context, autoradiographic tracking of radiolabeled schistosomula through host tissue (Georgi et al., 1982), has highly contributed towards a better understanding of migration patterns (Knopf et al., 1986; Karnija & McLaren, 1987). Moreover, successful labeling of cercariae with radioselenium is being applied to locate and quantify schistosomulum-bound label within the host and determine the fate of challenge infections in immune hosts (Dean & Mangold, 1984; Wilson et al., 1986).

Aiming to extend our knowledge on the *S. mansoni*-rabbit interaction, we performed the autoradiographic analysis of *S. mansoni* migration in naive NZ rabbits infected with L-($^{75}$Se) seleniomethionine labeled cercariae.

Results herein reported, clearly demonstrate the post liver phase of the life cycle of this parasite in the rabbits and provide new informations on the dynamics of schistosome infection.

## MATERIAL AND METHODS

*Parasite and animals* – LE strain of *S. mansoni* is currently maintained in our laboratories in Swiss mice and *Biomphalaria glabrata.*

Male Swiss mice (aging 6-8 weeks) and male New Zealand rabbits (weighing 2 kg) were obtained from Oswaldo Cruz Institute's animal house and Granja Cantopi (Rio de Janeiro) respectively.

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*Cercarial labeling* – Forty *B. glabrata* adult snails were infected with 5-7 miracidia/each. After seven weeks, infected snails were simultaneously exposed to 500 $\mu$ Ci of ($^{75}$Se) L-seleniomethionine (specific activity of 0.6-4 Ci/mmol, Amershamp Corp.) in 1 ml destilled water/snail, for 5 h. Labeled cercariae were obtained 4 days later.

*Infection protocol* – A total of 28 unprimed rabbits were infected simultaneously with 500 labeled cercariae/animal, percutaneously through the abdominal skin by an adaptation of the ring method (Smithers & Terry, 1965).

Briefly, rabbits were restrained in dorsal recumbency. The labeled cercariae were applied to the shaved skin of the abdomen, using inverted funnels of 6 cm diameter. After a 45 min exposure period, funnels were removed and the remaining fluid from each animal was examined under a dissecting microscope to ascertain the number of penetrating cercariae. Usually, no remaining intact larvae were observed (Almeida et al., 1989).

*Autoradiography* – For autoradiography tracking of migrating larvae, infected rabbits were killed with a rapid intravenous (marginal vein of the ear) injection of 20 ml of air, and tissue specimens (skin, lung and liver) removed on days 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50 from two animals/day were processed as described by Georgi (1982). Results are expressed as mean count of foci for each site/day.

Briefly, skin specimens were mounted on a cardboard and flattened with doubly adhesive tape. Lungs and livers were also placed on cardboard. All organs were similarly covered with celophane film and flattened under the weight of a metal cylinder (soft tissues). For skin preparations, a typographic press was used to flatten properly the removed rabbit abdominal skin specimens. The cards with flattened tissues were dried at 37-50 $^{o}$C, during 48 h. Under the safe light illumination, in the darkroom a X-ray film (Kodak, 30 x 40 cm) was apposed to the side of each card bearing the labeled and flattened organs and placed in a plywood screw-press. The loaded screw-press was kept at room temperature.

After 75 days, films were developed in an automatic processor and silver foci were counted under 6X magnification of a stereoscopic microscope.

*Adult worm recovery* – Two rabbits infected with labeled cercariae were subjected to perfusion of hepatic and mesenteric veins for adult worm burden evaluation, 60 days after infection (Pellegrino & Siqueira, 1956).

Specimens were collected in a 0.85% NaCl solution, fixed uncompressed in cold AFA, dehydrated in alcohol, stained by Meyer's carmine and cleared in beechwood creososote for morphological analysis.

## RESULTS

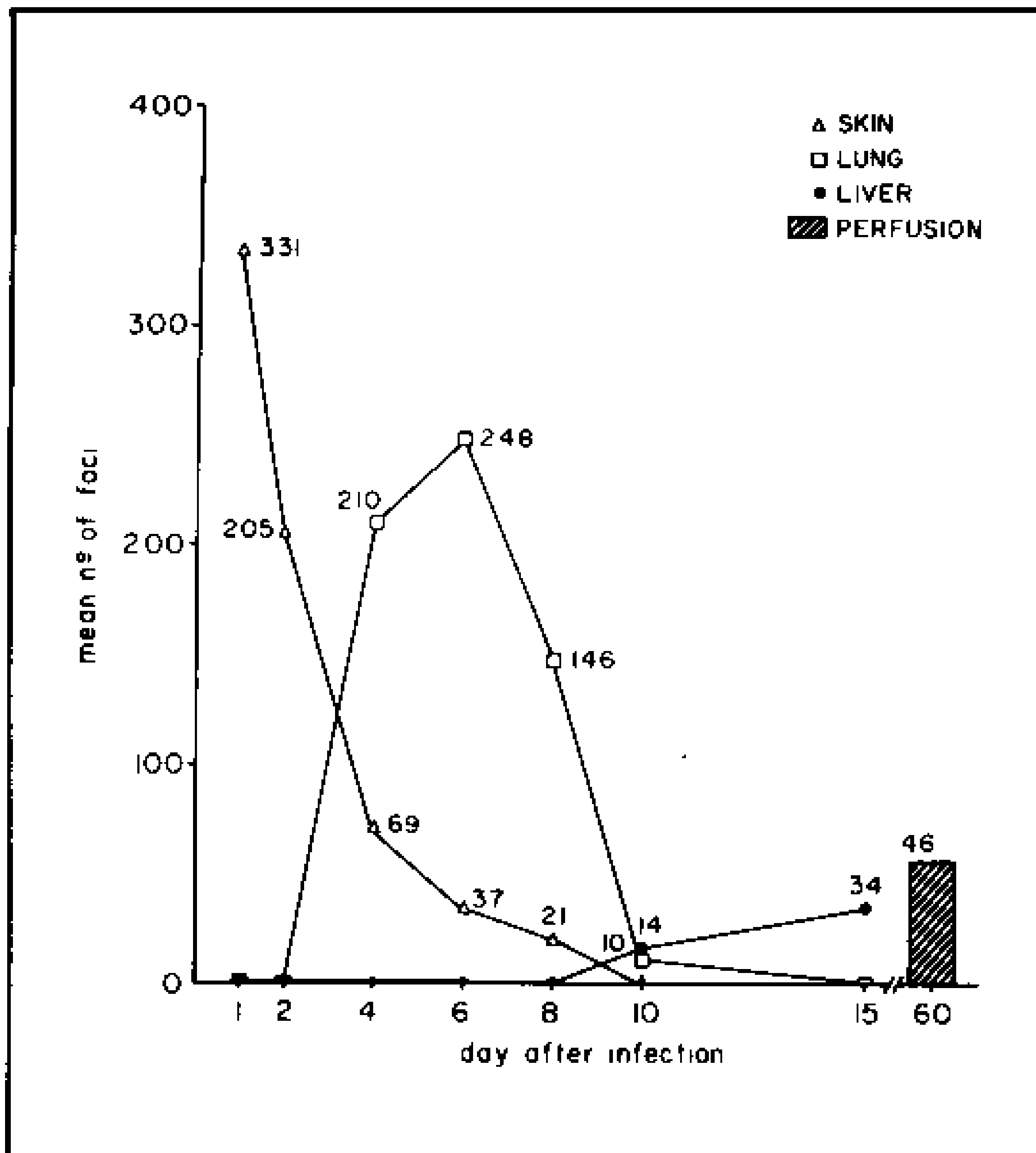
Results of migration tracking (Fig.) show that by day 1, 331 larvae (66.2% of infecting cercariae) could be counted as reduced silver foci in the skin. By day 2, 205 foci were counted in this site and none could be detected yet in the lungs. At the 4th day, 69 foci were counted in the skin, while 210 foci had shifted to the lungs. By day 6, 248 schistosomula were detected in the lungs, while 37 persisted in the skin.

About half of the migrating larvae, left the lungs by day 8, since 146 autoradiographic foci were detected in this site. By day 10 only 10 foci could be seen in lung tissues and 14 foci appeared in the liver. By day 15 no foci could be observed in the lungs while 34 were counted in the liver. Peak values for schistosomula counts in the skin and lungs were observed respectively on the 1st and 6th days after infection. Between, days 8 and 15 there was a progressive disappearance of foci in the lungs as depicted in the Fig.

Adult worms were recovered by venous perfusion from 2 additional remaining rabbits, infected simultaneously to animals utilized for autoradiographic tracking of migrating larvae.

Rabbits subjected to perfusion yielded 46 and 47 worms/rabbit, respectively. Adult worms were recovered either paired or not. The distribution of specimens corresponds to 61.7% found paired in the mesenteric veins and 15.3% in the liver. The remaining 23.0%, were recovered unpaired: 14.6% in the mesentery and 8.4% in the liver.

The mean ratio of adult worms obtained by venous perfusion of liver and mesentery, 60 days after infection, was 1:1.9 respectively.




Autoradiographic tracking of *Schistosoma mansoni* migration in the naive New Zealand rabbit. Expressed values correspond to mean number of foci/site/day.

Adult worms presented length values of 6.4-6.6 mm (males) and 5.3-7.0 mm (females) and width measures of 0.34-0.44 mm and 0.11-0.12 mm for males and females respectively.

Morphological analysis under optical microscopy, showed no abnormalities in the internal structures of both sexes individuals.

## DISCUSSION

The wide range of laboratory animals and the behavior they present while infected with schistosomes, has been exaustively investigated aiming the stablishment of suitable animal models for the study of infection related phenomena and mechanisms of resistance to *S. mansoni* (Moore et al., 1949; Stirewalt et al., 1951; Kuntz & Malakatz, 1955; Bruce et al., 1960; Clegg & Smithers, 1968; Pearce & McLaren, 1983; Damian, 1984). It is now generally accepted that there is no ideal animal model, for schistosomiasis, since a large body of evidence has shown great diversity mainly on immune mechanisms against schistosomes in different hosts (Capron & Capron, 1986).

Through autoradiographic tracking of radiolabeled cercariae, we assessed the migration pattern of *S. mansoni* in naive NZ rabbits.

Data shown in the Fig. clearly indicate that days 1 and 6 post infection are respectively the times of peak skin, and lung schistosomula accumulation. In addition, marked reduction in the number of parasites was detected after the 8th day. This result suggests that major attrition and elimination of schistosomes in naive rabbits, occurs only after lung passage of the worms similarly to data reported for schistosoma infection of naive mice, either outbred or inbred strains (Georgi et al., 1982; Dean & Mangold, 1984; Pinto et al., 1987).

Furthermore we clearly demonstrated that the mesenteric phase is achieved by *S. mansoni* in this host, although without faecal elimination of eggs. Most of the rabbits adult worm burden evaluated at the 60th day after percutaneous infection, was recovered either paired or not from mesenteric veins, before the liver was perfused. Our data are in accordance to Andrade et al. (1988) who recently reported on the pathology induced by *S. mansoni* infection in rabbits.

The results herein presented confirm and extend earlier observations in the context of attrition site of parasites and migration pattern of *S. mansoni* infection in the rabbit.

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