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MORPHOLOGICAL STUDIES OF THE SCHISTOSOMULUM OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA MARGREBOWIEI* IN LUNGS OF MICE

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Morphological studies on the *Schistosoma mansoni* and *S. margrebowiei* schistosomulum development in the lung of definitive host mouse have been reported after 2 to 21 days post-infection. The greatest proportion of schistosomula were observed within the capillaries attached to the alveoli and few in the branches of the pulmonary arteries and veins. The length and shape of schistosomulum sections were highly variable due to the randomness of the plane of sections through the worm. The differences in the diameter of the body and cuticle of the *S. mansoni* and *S. margrebowiei* schistosomulum were non-significant ($P > 0.05$).

Key words: *Schistosoma mansoni, S. margrebowiei,* Schistosomulum, Lungs, Mice.

Introduction

The larval stage of *Schistosoma* (parasite) is called cercaria. These cercariae deposit a mucoid (PAS-positive) secretion from the postacetabular secretory glands as they loop over the skin of their mammal host during exploration at the site of penetration, enter into the horny and keratogenous zones during their passage through the skin and across the cellular epidermis. The mucoid post-acetabular secretion is adhesive, lubricative and serves in protective functions. The pre-acetabular secretion is primarily enzymatic (Stirewalt and Kruidenier 1961). Histolytic enzymes are secreted from the penetration glands and the cercariae burrow through the tissues. The tail of the cercaria is shed on penetration and the tail-less cercaria is then known as a schistosomulum. The schistosomulum is now no longer able to survive in fresh water (McLaren and Hockley 1977). The process of transformation takes less than one hour to complete *in vivo* (Cousin *et al* 1981).

Schistosomula leave the skin via the blood or lymphatic vessels and ultimately pass through the right side of the heart via the venous system to be distributed to the lungs via the pulmonary arterial system. The increase in numbers of schistosomula within the pulmonary capillaries from day 2 to day 7 post infection (p i) indicates their effectiveness as a physical barrier for further migration (Wheater and Wilson 1979). The arrival of schistosomula in the lungs via the pulmonary artery is a prelude to a sequence of developmental changes which are presumably necessary for further migration. Following arrival in the lungs of mice, schistosomula undergo a phase of elongation up to four times the length observed in the skin, with a concomitant reduction in diameter and no increase in mass (Wilson *et al* 1978). This process of elongation may be a necessary prelude to the passage of schistosomulum through the narrow lumina of capillaries in the lungs (Carbtree and Wilson 1986a). Schistosomula retain this capacity for elongation until they reach the hepatic portal system where it is believed to facilitate migration through capillary beds in the lungs and systemic organs (Miller and Wilson 1980). The aim of the present research paper is to describe the morphological changes in the development of the schistosomula in the lung of the mouse during schistosome infections.

Materials and Methods

Age-matched female mice of the Bantim and Kingman Tylers Original (BKTO) strain, weighed approximately 20 - 35g, each were infected with 200 cerariae of either *S. mansoni* [Puerto Rican strain maintained in albino *Biomphalaria glabrata* snails and random-bred TO mice following the methods of Taylor *et al* (1969) or *S. margrebowiei* (originally obtained from Lochinvar National Park, Zambia] and maintained in *Bulinus natalensis* intermediate host snails (the original stock was obtained from the Experimental Taxonomy Unit of the British Museum of Natural History, London, UK). Before administering the cercariae, the experimental animals were anaesthetized with sodium pentobarbitone (Nembutal) and the abdominal hair was clipped. The cercariae were applied to the abdominal skin by using ring. All mice were killed at day 2, 3, 4, 6, 8, 10, 16 and 21 and autopsies were performed immediately after the animals were killed by dislocation of neck region. The lungs from each animal were fixed in Heidenhain's Susa fixative, washed, dehydrated with ethanol, infiltrated and

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embedded in historesin. Selected 4 µm thick sections were stained in haematoxylin and eosin method. The sections were interpreted on Emst Leitz Wetzlar light microscope (Model No. 786554).

Results and Discussion

Morphological characteristics of *S. mansoni* and *S. margrebowiei* were studied in the lungs of mice.

Normal schistosomula were observed in both groups from day 2-16. During lung migration, the greatest proportion of schistosomula of both species were observed within the capillaries attached to alveoli and few were present in the branches of the pulmonary arteries (Fig 1-3). Some degenerated *S.mansoni* schistosomula were observed on day 21 in the branches of the veins. In *S.margrebowiei*, these were observed in the capillaries on day 8 and 21 pi. Few of the schistosomula were also observed in the pleural surface on days 2, 3, 6, 16 and 21 after infection.

Size of lung schistosomula. In the histological examined material, the length and shape of worm sections was highly variable due to the randomness of the plane of sections through the worm. In an attempt to compare sizes, the minimum diameter of transverse sections through the worms was determined. Observations on schistosomula in the mouse lungs were consistent with the organisms having an elongated thin shape. From 2-21 days pi, the diameter of the body and thickness of the cuticle of at least five healthy and five abnormal schistosomula were recorded in both species. The mean and standard deviations of the diameter of the body of the schistosomula of *S. mansoni* were 2.2560 ± 1.1280 µm and in *S. margrebowiei* they were 2.5790 + 0.9036 µm. The differ-

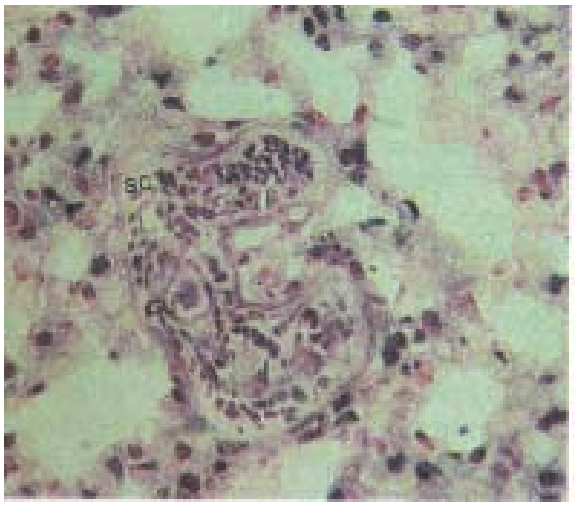

Fig 1. Mouse lung 3 days post-infection with *S.margrebowiei* cercariae. SC=Schistosomulum; BV=Blood vessel; N=Nuclei of differentiated cells. Stain; Haematoxylin and eosin.

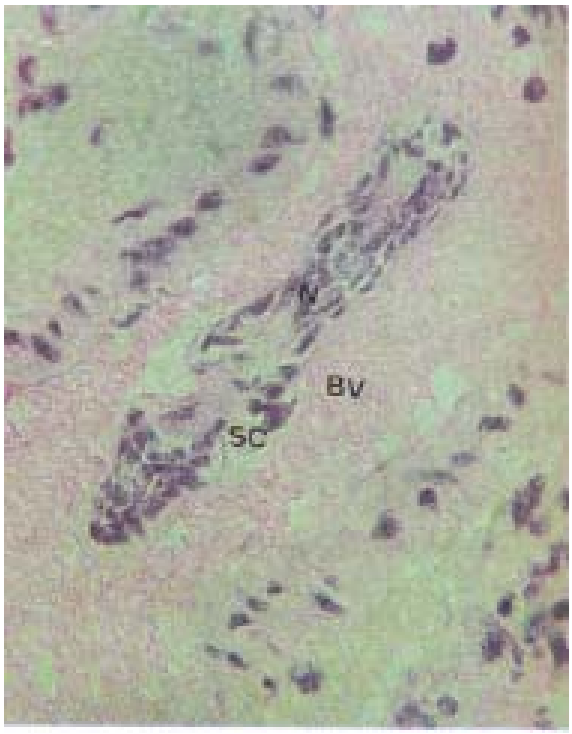

Fig 2. Mouse lung 4 days post-infection with *S.Margrebowiei* cercariae. SC=Schistosomulum; GU=Gut caecum; N=Nuclei of differentiated cells. Stain: Haematoxylin and eosin.

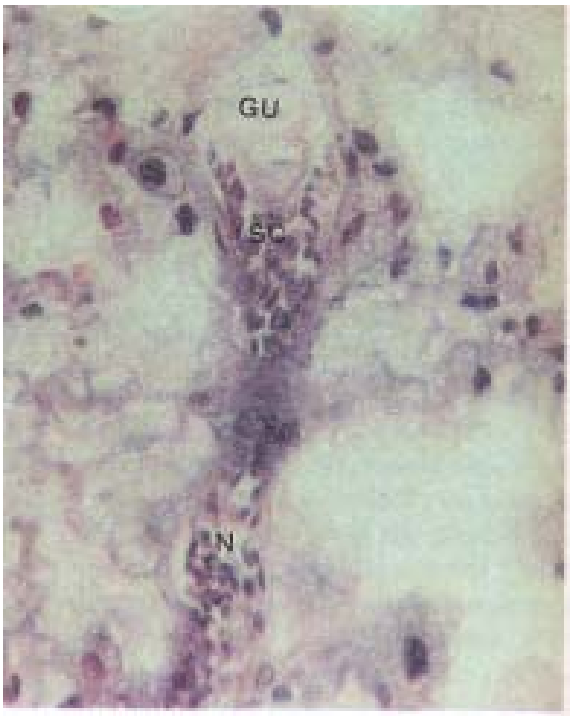

Fig 3. Mouse lung 4 days post-infection with *S.margrebowiei* cercariae. SC=Schistosomulum; GU=Gut caecum; N=Nuclei of differentiated cells. Stain: Haematoxylin and eosin.

Table 1
Mean diameter of the body and thickness of the cuticle of the *S. mansoni* and *S. margrebowiei* schistosomulum in the lungs of mice.

Days post infection	*S. mansoni* No.	Body µm	Cuticle µm	*S. margrebowiei* No.	Body µm	Cuticle µm
2	1	39.98	0.95	3	36.17	2.85
3	2	62.83	0.95	4	34.27	1.42
4	3	28.56	2.85	2	37.12	3.80
6	6	25.70	2.69	2	28.56	1.42
7	3	29.51	2.85	2	27.60	2.85
8	4	28.56	1.58	2	31.41	1.90
10	2	25.70	1.90	-	-	-
16	4	29.51	2.13	2	27.60	1.42
21	2	98.05	0.95	5	90.44	1.33

Abbrevation: dpi=Days post-infection, S=Schistosoma, µ=Micron.

ences in the diameter of the body of *S. mansoni* and *S. margrebowiei* schistosomulum were non significant, ($P > 0.05$). The mean and standard deviation of the thickness of the cuticle of *S. mansoni* larvae were 1.8720 + 0.8128 µm, whereas, in *S. margrebowiei* they were 2.1240 ± 0.9282 µm. The difference in the thickness of the cuticle of *S. mansoni* and *S. margrebowiei* schistosomulum was non significant, ($P > 0.05$). Hence there is no statistically significant difference in the diameter of the body and thickness of the cuticle of the two species of schistosomulum during their development in the lungs of mice between day 2 to 21 pi (Table 1).

Majority of the *S. mansoni* and *S. margrebowiei* schistosomula were presented in the capillaries of the lungs of mice from day 2 to 16 after infection, however a few were in the branches of arteries and veins of the lung. These observations are in close agreement with those of previously researched by Wheater and Wilson (1979) for *S.mansoni*, Ogbe (1983,1985) and Soomro (1996) for *S.margrebowiei*. Similar results were also reported by Yixun and Xinwu (1985) for *S.japonicum* schistosomula in the lungs of mice.

In the present study, the mean diameter of the body of the schistosomula of *S. mansoni* and *S. margrebowiei* was lower than reported by Carbtree and Wilson (1986a) where the minimum body diameter of the lung schistosomula was about 8 µm at day 7 pi Bloch (1980) reported that schistosomula had pigment in their caeca and the diameter varied between 40 - 175 µm. Clegg (1965) reported that average measurements of the 7 day old schistosomula (252 x 25 µm) are compatible with the view that the schistosomula elongates in the lung capillary but does not grow. Bruce *et al* (1974) reported that the mean cross section diameter of the intravascular organism was 20 µm. Wilson *et al* (1978) reported that maximum and minimum length of the 8 day schistosomulum was 365 µm and 197 µm respectively, giving maximum amplitude of 168 µm. The lung worm can constrict regions of its body to the diameter of less than 10 µm.

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References

Bloch E H 1980 *In vivo* microscopy of schistosomiasis. II. Migration of *Schistosoma mansoni* in the lungs, liver and intestine. *American Journal of Tropical Medicine and Hygiene* **29** 62-70.

Bruce J I, Pezzlo F, Yajima Y, Mc Carty, J E 1974 *Schistosoma mansoni*: pulmonary phase of schistosomula migration studied by electron microscopy. *Experimental Parasitology* **35** 150-160.

Carbtree J E, Wilson R A 1986a *Schistosoma mansoni*: an ultrastructural examination of pulmonary migration. *Parasitology* **92** 343-354.

Clegg J A 1965 *In vitro* cultivation of *Schistosoma mansoni*. *Experimental Parasitology* **16** 133-147.

Cousin C E, Stirewalt M A, Dorsey C I I 1981 *Schistosoma mansoni* ultrastructure of early transformation of skin and shear-pressure derived schistosomula. *Experimental Parasitology* **51** 341-365.

McLaren D J, Hockley D J 1977 Blood flukes have a double outer membrane. *Nature* (London) **269** 147-149.

Miller P, Wilson R A 1980 Migration of schistosomula of *Schistosoma mansoni* from lungs to the hepatic portal system. *Parasitology* **80** 267-288.

Ogbe M G 1983 *In vivo* and *vitro* development of *Schistosoma margrebowiei*. *Journal of Helminthology* **57** 31-235.

Ogbe M G 1985 Aspects of the life cycle of *Schistosoma margrebowiei* infection in laboratory mammals. *International Journal of Parasitology* **15** 141-145.

Soomro N M 1996 Pathology of schistosome infection in mice and vector snails. Ph.D thesis, University of Wales Bangor, U.K.

Stirewalt M A, Kruidenier F J 1961 Activity of the acetabular secretory apparatus of cercariae of *Schistosoma mansoni* under experimental conditions. *Experimental Parasitology* **11** 191-211.

Taylor M G, Amin M A, Nelson G S 1969 Pathogenesis in *Schistosoma mattheei*. *J Helminthology* **43** 197-206.

Wheater P R, Wilson R A 1979 *Schistosoma mansoni*: a histological study of migration in laboratory mouse. *Parasitology* **79** 49-62.

Wilson R A, Draskau T Miller P, Lawson J R 1978 *Schistosoma mansoni*: the activity and development of the schistosomulum during migration from the skin to the hepatic portal system. *Parasitology* **77** 57-73.

Yixun He, Xinwu P 1985 Scanning electron microscopy of *Schistosoma japonicum* during growth and maturation in mice. *Acta Zoologica Sinica* **31** 138-142.