

THE PENETRATION OF MAMMALIAN SKIN BY CERCARIAE OF *TRICHOBILHARZIA* SP. (TREMATODA: SCHISTOSOMATIDAE) FROM SOUTH AFRICA

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ABSTRACT

APPLETON, C. C. & BROCK, KRISTEN, 1986. The penetration of mammalian skin by cercariae of *Trichobilharzia* sp. (Trematoda: Schistosomatidae) from South Africa. *Onderstepoort Journal of Veterinary Research*, 53, 209–211 (1986)

The ability of the cercariae of *Trichobilharzia* sp., the commonest avian schistosome in South Africa, to cause dermatitis in man is confirmed, and their progress through and resultant histopathology in rodent skin are documented. These cercariae reach the subcutaneous tissue within one hour after exposure, and some migrate to the lungs. Between 46 and 97 h later, those stranded in the skin have died, while those in the epidermis are extruded by 97 h. There were haematological changes in the peripheral blood and a rapid rise and fall in the mitotic rate of the basal epithelium of the epidermis.

INTRODUCTION

The most widespread avian schistosome in South Africa is an undescribed species of *Trichobilharzia* (Appleton, 1982; 1986). It is a common parasite of the spurwing goose, *Plectropterus gambensis*, and its intermediate host is the freshwater snail, *Lymnaea natalensis*. Anecdotal reports suggest that the cercariae of this blood-fluke are a cause of dermatitis in man (Appleton, 1984). This paper presents experimental evidence confirming this and describes the histological responses of mouse skin to penetration by these cercariae.

MATERIALS AND METHODS

Twenty unsensitized BALB/c mice were anaesthetized with a 1:4 ml dilution of pentobarbitone sodium (Sagatal) at a dosage of 0,1 ml/mouse. Each mouse was then exposed for one hour to 29–77 (mean 44) freshly emerged cercariae of *Trichobilharzia* sp., using the ring method of Smithers & Terry (1965). The diameter of the rings used was 11,6 mm and the area of skin exposed was therefore 105,7 mm². Biopsies of the exposed skin of 2 mice were then taken after each of the following intervals: 1, 3, 20, 28, 46, 73, 97, 120 and 216 h. The skin was then serial-sectioned at 5 µm and stained in haematoxylin and eosin. A control series of sections was made of skin from an unexposed animal. Smears of venous blood were taken from the orbital sinuses of 2 additional exposed mice, using microhaematocrit tubes, for white blood cell differential counts of the peripheral blood of exposed mice to be recorded parallel to the series of skin biopsies. The lungs of 3 animals were examined for evidence of cercarial migration by teasing them apart in 0,9 % saline (Bacha, Roush & Icardi, 1982).

A mitotic index, $M/(I+M) \times 100$, where M = total mitotic and I = total interphase cells (Hsu, 1973), was calculated for the basal epithelium across the width of sectioned lesions in the epidermis. Between 34 and 883 (mean 333) cells were counted for each lesion, with 5–10 replicates per time interval.

Unless otherwise stated all measurements are given \pm standard deviation.

RESULTS

Laboratory confirmation of the ability of the cercariae of *Trichobilharzia* sp. to cause dermatitis in man was

demonstrated by exposing the forearms of 3 volunteers to these larvae. In 2 out of 3 cases this resulted in the development of itchy, erythematous papules similar to those of avian schistosome dermatitis, as described by Appleton & Lethbridge (1979) and Shimuzu, Matsuoka & Ando (1981). A short-lived, prickling sensation was felt 5–10 min after the cercariae were pipetted onto the skin. These "pinpricks" itched for a further 30–60 min. Small, hard papules with a diameter of approximately 4,5 mm, surrounded by an area of erythema, appeared 5–30 h later and itched for the subsequent 2–3 days. These papules persisted for 8–9 days before fading gradually.

Penetration of mouse skin

From the number of tails recovered from the rings after the exposures it was estimated that 85,2 % of cercariae attempted to penetrate. Of these, approximately 41 % were found adhering by means of suckers to the outer surface of the epidermis, while the remaining 59 % had entered the skin (Table 1). The mean depth of penetration, viz., outside of corneous layer to the centre of the inflamed area, was $0,57 \pm 0,09$ mm (n=9), with a maximum recorded depth of 0,72 mm. It is noteworthy that, in sections taken after 97 h, there was no evidence of cercariae in the epidermis, though they could still be found in the dermis. Except in 2 instances where they had not completely penetrated, all cercariae seen in the sections were in cross-section. This indicates that they were lying at right angles to the presumed axis of penetration.

While in most cases cercariae could be seen to have penetrated through the corneous layer of the epidermis, there was evidence that some cercariae used hair follicles as an entry route, causing a folliculitis. Up to and including 46 h, morphological features of the cercariae, such as the acetabulum and the pigment granules of the eyespots, could be recognized in the sections. At 73 h and later, only the pigment granules could be recognized, and these were usually scattered and no longer in aggregations as shown by Van der Roemer & Haas (1984) for fresh cercariae. Cercariae were therefore considered to have died between 46 and 73 h post-exposure. This compares favourably with the LT50 of 60 h for cercariae in water (Fig. 1). Only one schistosomulum, measuring 529 µm, was recovered after 97 h from lung tissue from a mouse exposed to 300–350 cercariae. Some of these schistosomula do therefore migrate from the dermis to the lungs.

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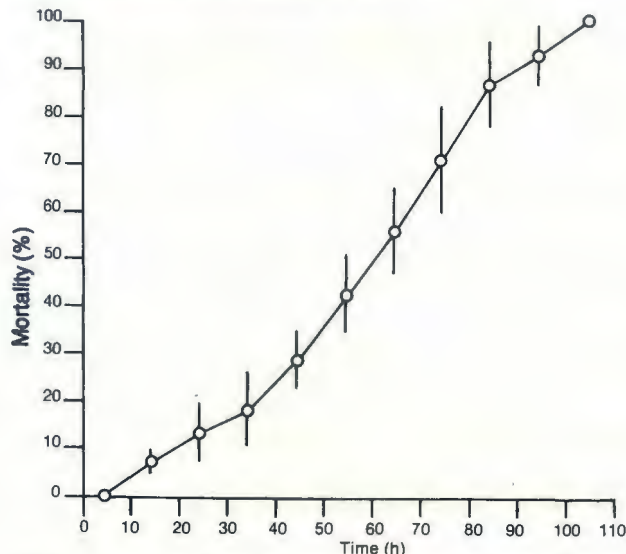


FIG. 1. Longevity of freshly-emerged cercariae of *Trichobilharzia* sp. in water. Vertical bars = standard deviations.

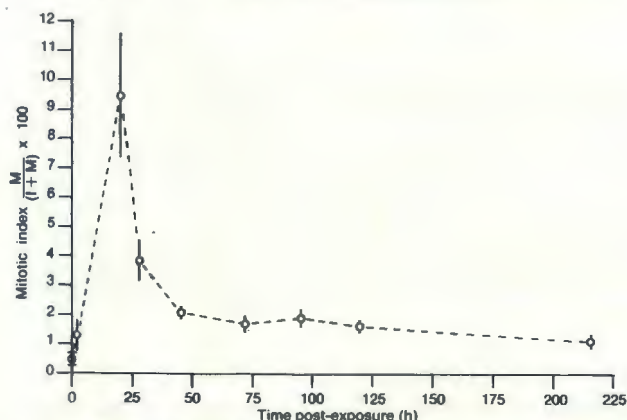


FIG. 2. Rise and fall of the mitotic index of the basal epithelium of the epidermis between 1 and 216 h after exposure to cercariae of *Trichobilharzia* sp. Vertical bars = standard errors

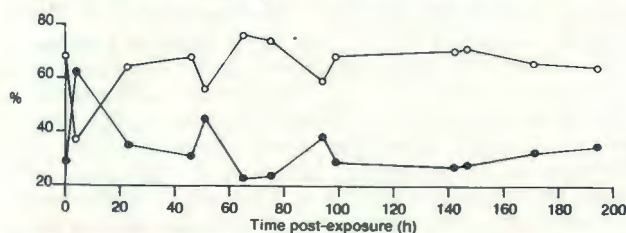


FIG. 3. Fluctuations in the differential counts of neutrophils (●) and lymphocytes (○) in the peripheral blood of BALB/c mice exposed to *Trichobilharzia* sp. cercariae.

Inflammatory response

Cercariae in the epidermis elicited very little inflammation. At 1 h post-exposure, a few lymphocytes and neutrophils were seen around the points of entry, and at 3 h, superficial ulcers had developed at these sites where the epidermis had been disrupted. A sparse population of neutrophils was visible around the cercariae themselves.

By 20 h the inflammation, consisting mostly of neutrophils, had become diffuse, and occasional macrophages and lymphocytes were present around the cercariae. Inflammatory crusts of packed neutrophils in keratin had formed at the penetration sites, and an unusually marked rate of mitotic division could be seen in the basal epithelium of the epidermis in these regions. At 28 h the cell population around the invading cercaria showed a chronic inflammatory response consisting mainly of lymphocytes and histiocytes. In contrast to earlier sections, very few neutrophils were present. By 46 h the

TABLE 1 Percentages of *Trichobilharzia* sp. cercariae seen in the epidermis and dermis of BALB/c mouse skin between 1 and 216 h post-exposure. Actual numbers are given in parentheses.

Time interval post-exposure (h)	Adhering to the outside of the epidermis	In epidermis	In dermis + subcutaneous tissue	Total
1	74,3 (26)	27,1 (6)	8,6 (3)	35
3	65,0 (13)	20,0 (4)	15,0 (3)	20
21	40,8 (31)	44,7 (34)	14,5 (11)	76
28	40,5 (17)	45,2 (19)	14,3 (6)	42
46	33,9 (19)	57,1 (32)	8,9 (5)	56
72	21,7 (10)	8,7 (4)	69,6 (32)	46
97	88,9 (16)	0	11,1 (2)	18
120	62,5 (30)	0	37,5 (18)	48
216	55,9 (19)	0	44,1 (15)	34

response has changed and was again dominated by neutrophils. One cercaria was found lodged in a layer of connective tissue between 2 layers of muscle and had clearly passed through the outer of these layers.

At 73 h the inflammation had become reduced to localized patches of infiltration around the remains of the cercariae and measured $131,6 \pm 15,5 \times 99,3 \pm 16,2 \mu\text{m}$ (n=17). These were mixed cell populations, consisting of lymphocytes, histiocytes and neutrophils. This situation persisted through the 120 and 216 h sections. By 120 h, however, these focal responses had assumed a granulomatous character with the appearance of several multinucleate giant cells in each granuloma and an increase in size to $193,9 \pm 38,0 \times 144,1 \pm 42,2 \mu\text{m}$ (n=8). At 216 h, areas of dark pink coloration could be seen within the cytoplasm of these giant cells and are believed to be the remnants of the cercariae. No eosinophils were seen in any of the sections.

The mitotic index rose rapidly after exposure from a normal level of 0,4 % to 9,4 % after 21 h (Fig. 2). A slightly higher rate was probably reached a few hours earlier, at approximately 16 h post-exposure. This high level dropped just as rapidly to 2,0 % at 46 h and continued falling gradually to reach 1,7 % by 216 h.

Differential counts

Counts over a period of 194 h post-exposure (Fig. 3) provided evidence of 3 haematological changes of successively decreasing magnitude in the peripheral blood, viz., between 4-23 h, 46-65 h and 73-200 h. These corresponded respectively to (1) the initial neutrophil-dominated response, as recorded in the 3 and 22 h sections, the change to (2) a lymphocyte-dominated response and (3) to the death of the cercariae, coupled with the reduction of the responses to focal ones. After 100 h post-exposure, the lymphocyte and neutrophil levels showed only slight changes from normal, and these corresponded to the formation of granulomata around the remains of the stranded cercariae. Eosinophils were rare in these smears.

DISCUSSION

Like many species of avian schistosome, the cercariae of *Trichobilharzia* sp. from South Africa do cause dermatitis in man. These cercariae are able to penetrate mammalian skin rapidly and reach the vascularized dermal layers as well as the subcutaneous tissue within 1 hour of exposure. In previous studies (Brackett, 1940; Macfarlane, 1949), the cercariae of several North American and New Zealand species of *Trichobilharzia* were found not to have penetrated deeper than the Malpighian layer of the epidermis, though in both cases human skin was involved.

Yang, Tchou, T'ang, Ho & Luo, (1965) noted that *Trichobilharzia* spp. in China penetrated to the subcutaneous tissue of white mice within 1.5 h, while in human skin they failed to go beyond the papillary layer of the dermis. In Japan, Shimuzu *et al.* (1981) failed to locate cercariae of *Gigantobilharzia* in sections of exposed human skin, but noted that all evidence of cellular infiltration was confined to the epidermis and papillary dermis.

In general, however, the histopathological changes described in this paper are comparable with those recorded in both unsensitized rodent and human skin, following exposure to other species of *Trichobilharzia* (Brackett, 1940; Macfarlane, 1949; Shimuzu *et al.*, 1981; Yang *et al.*, 1965). The intensity and speed of the reactions would probably be accelerated in sensitized subjects.

Estimates of the longevity of these cercariae in unsensitized skin vary from 21 h (Yang *et al.*, 1965), 29 h (Brackett, 1940), 31 h (Shimuzu *et al.*, 1981) to <42 h (Macfarlane, 1949). The criteria used by these workers to indicate death are a general breakdown of normal cercarial structure and, more particularly, nuclear degeneration and cellular pyknosis. In the present study, as was noted earlier, identifiable cercarial structures were seen up to 46 h and most cercariae were therefore considered to have died between 46 and 73 h post-exposure.

Another feature of the present series of sections was the disappearance of cercariae from the epidermis of skin 97 h or more after exposure. This is probably due to their having been extruded as a result of an accelerated rate of skin growth in the affected areas (Macfarlane, 1949) and exemplified by the high level of mitotic activity recorded here up to approximately 28 h post-exposure (Fig. 2).

Lung migration by cercariae of avian schistosome has been demonstrated for species of *Trichobilharzia* and *Austrobilharzia* in laboratory rodents and monkeys (Bacha *et al.*, 1982; Olivier, 1953; Yang *et al.*, 1965). The finding of only a single *Trichobilharzia* schistosomulum in the lungs in the present case shows that at least a small proportion of cercariae do enter the bloodstream and reach the lungs. The important question that we, in common with other workers who have investigated this consequence of schistosome dermatitis, still have to answer is whether or not this migration also takes place in man. The fact that Olivier (1953) demonstrated its occurrence in monkeys suggests that it might also occur in man, but the observations of Brackett (1940) and

Macfarlane (1949) that congeneric cercariae failed to reach the dermis of human skin, or, as Yang *et al.* (1965) reported, penetrated only its outer layer, tend to militate against this.

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