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AN EXPERIMENTAL *SCHISTOSOMA MATTHEEI* INFECTION IN MAN

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ABSTRACT

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Certain aspects of the immune response of a male experimentally infected with 3-day old cercariae of a pure field strain of *Schistosoma mattheei* were investigated. Among others, aspects such as the reaction of eosinophils, neutrophils and blood platelets after infection, were included in the study. The involvement of IgG and the cross reaction between these antibodies and *S. haematobium* and *S. mansoni* were also investigated. The phenomenon that the cercariae were, 3 days after shedding, still capable of penetrating the skin causing an inflammatory response was studied. The results lend some support to the surmise that a pure *S. mattheei* infection in humans is incapable of any egg production.

INTRODUCTION

In situations in the tropics where man shares common water resources with cattle, humans are frequently infected simultaneously with both *Schistosoma haematobium* and *S. mattheei*, respectively the cause of human urinary and cattle schistosomiasis in South Africa. This can largely be attributed to the fact that the two parasites share a common intermediate host *Bulinus africanus*. Furthermore, man seldom, if ever, passes cattle schistosome eggs in the absence of eggs of one or both of the local human schistosomes.

This apparent absence of eggs of pure infections of *S. mattheei* in man gave rise to the supposition that pure *S. mattheei* infections in humans are incapable of producing eggs (Pitchford, 1959; Wright & Ross, 1980). However, it was found that in case of an infection in man with both *S. mattheei* and *S. haematobium* the eggs passed often resemble in shape not only pure *S. mattheei* and *S. haematobium* eggs but also a variety of intermediate forms (Pitchford, 1959; Taylor, 1970). The hypothesis was advanced that *S. mattheei*-type eggs are passed in human urine only if female *S. mattheei* mate with and are carried to the bladder by *S. haematobium* males in which case the eggs would be hybrids. This hypothesis was confirmed by Pitchford (1961) by passaging the offspring of eggs judged on appearance to be hybrids, through rodents. He obtained eggs characteristic in shape for both *S. mattheei* and *S. haematobium* (Pitchford, 1961). Subsequently these observations were confirmed electrophoretically by Wright & Ross (1980) who showed unequivocally that hybrids between *S. mattheei* and *S. haematobium* occur naturally in man.

Little information exists in respect of the immunological response of humans after pure *S. mattheei* infections. Such cases probably occur rarely, and those that do occur are extremely difficult to detect. The nearly complete overlapping in the distribution of the 2 species in South Africa makes it almost impossible to exclude the possibility of exposure to either species in the field, and furthermore, cross reactions between antibodies raised against one schistosome species with antigens of another complicate diagnosis of pure *S. mattheei* infections in humans even more (Simpson & Crioli, 1987). These circumstances motivated the investigation of the eosinophil, neutrophil and blood platelet response as well as the involvement of immunoglobulin after accidental infection followed by experimental infection of a researcher (C. T. Wolmarans) with a pure strain of *S. mattheei*. At the same time the immunological response of this person to skin penetration by this parasite was also studied.

MATERIAL AND METHODS

The researcher involved was accidentally exposed to schistosome cercariae while collecting wild specimens of *B. africanus* in one of the fountains of the Mooi River on the farm "Bovenste Oog van die Mooirivier 88" in the Western Transvaal. The well-known pruritis of exposed areas experienced by this person after contact with the cercaria-infested water followed by focal dermatitis three days later, suggested that infection had taken place.

The more or less 120 snails that were collected, were transported to the laboratory in a well insulated flask before being removed and fixed for other studies. When it was realised 3 days after collection of the snails that the researcher could have been accidentally infected with schistosome cercariae, he was exposed for 15 min to 30 live cercariae recovered from the insulated flask that had been left standing in an air-conditioned laboratory at about 20 °C. This was done by placing the cercarial suspension for 15 min in a small cylinder positioned with the one opening on the person's calf. Five C57B mice (a strain also compatible with *S. haematobium*) were infected for 2 h in separate honey jars each containing 250 individually counted live cercariae of the same batch in 100 mℓ of water.

After a period of 12 weeks the mice were dissected, the schistosome eggs recovered from their livers by homogenization and filtration through a helminth filter, isolated and fixed in Todd's solution (Todd, 1986) for identification. Stool and 250 mℓ urine samples from the infected person were scrutinized for the presence of eggs. Skin biopsies were taken 2 and 4 days after the experimental exposure at the sites where the skin appeared to have been penetrated by the cercariae and fixed in Todd's solution (Todd, 1986). After fixation the tissue was cut in 2 mm × 3 mm pieces and prepared for light microscopy according to the method of Ekly-Natey, Wuest, Swiderski, Striebel & Huggel (1985) except that they were not critically point dried. The tissue was then bedded into 100 % Spurr resin. Sections of 0,5 µm were cut with an Ultracut ultra microtome, stained with 1 % toluene blue and permanent mounts prepared with Entellan mounting glue.

Differential white blood cell and platelet counts were made thrice at two-weekly intervals after infection and a fourth nine months later. Antiserum was prepared from a blood sample collected two weeks

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after infection and antigen-antibody interactions were performed in the following way. Adult *S. haematobium, S. mansoni* and *S. mattheei* worms were isolated by perfusion from the livers of infected mice and homogenised at pH 7,2 in phosfate buffered saline (PBS). Nitrocellulose paper (0,45 μm, Schleicher & Schuell) was cut into squares of 2 cm × 2 cm and the antigen solutions each added dropwise to a separate square until it was thoroughly wetted and thereafter left to couple for 30 min. Each paper square was then rinsed separately in a buffer consisting of 50 mM Tris HCl, 0,9 % NaCl and 5 mM EDTA (pH 7,0) to remove excessive antigen. Thereafter the squares were transferred to a blocking buffer (50 mM Tris HCl plus 3 g bovine serum albumen/100 mℓ buffer) for 5 h before being rinsed. They were then placed separately into 10 mℓ rinsing buffer plus 50 μℓ of the prepared antiserum (primary antibody), left overnight at 4 °C, rinsed again and placed into the secondary antibody (rabbit-antihuman IgG) at a concentration of 50 μℓ/100 mℓ rinsing buffer for 2 h. After similar coupling of the tertiary antibody (goat-antirabbit), the papers were incubated for 1 h in 100 μℓ PAP (Peroxidase anti-Peroxidase)/100 mℓ 50 mM Tris (pH 7,4) plus 0,89 % NaCl and rinsed. DAB (3,3 Diaminobenzidine) staining was then performed for 5 min in a solution of 70 mg DAB/100 mℓ ice-cold 0,5 tris HCl (pH 7,6), before being inhibited by replacing the solution with distilled water. Samples from a presumed non-infected volunteer served as controls as regards the presence of anti-bodies and white cell response.

RESULTS

During the experimental infection a pruritis reaction accompanied the penetration of the parasites. Subsequently dermatitis was observed three days after infection at the site of infection. No eggs were detected at any time in either the stool or urine samples of the infected person. On the other hand all the mice infected with cercariae were positive for only *S. mattheei* eggs.

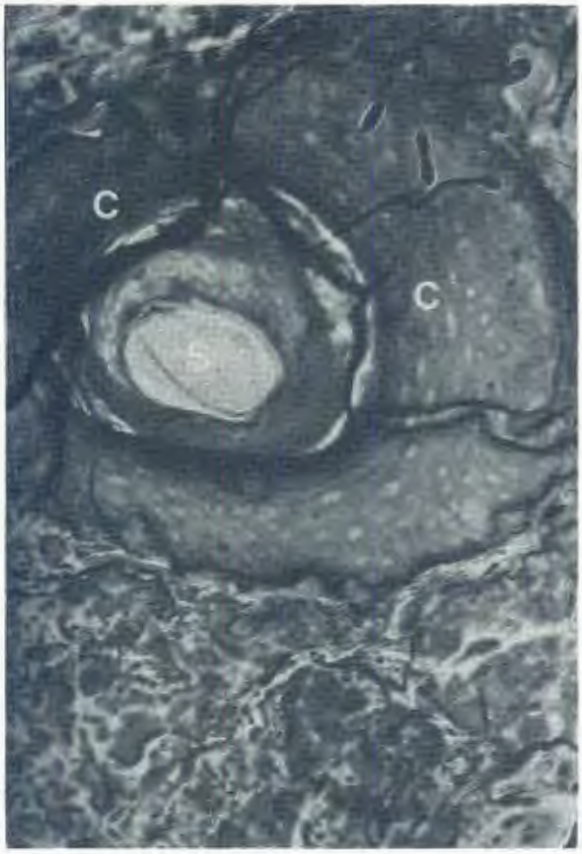

FIG. 1 A skin biopsy to show a two day old schistosomulum (S) and the surrounding cellular (C) reaction

FIG. 2 A skin biopsy four days after cercarial penetration to show the penetration site and a shed glycocalyx (G). No schistosomulum was present

In Fig. 1, of a biopsy 2 days after penetration, cell infiltration, most propably including polymorphonuclear neutrophils, lymphocytes, mononuclear cells,

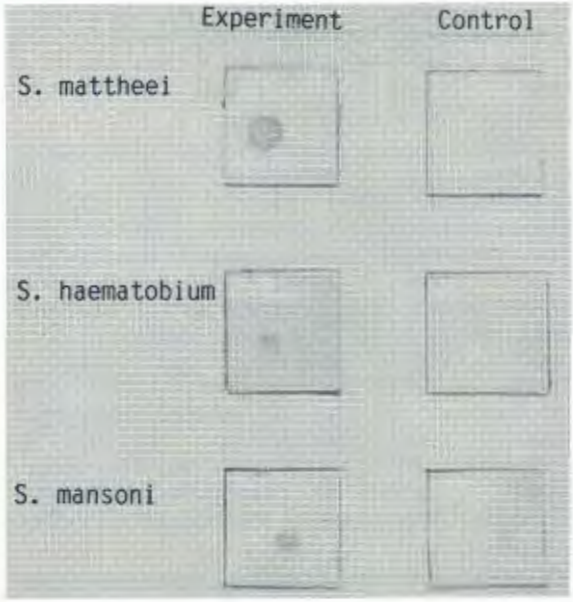

FIG. 3 The interaction between antibodies raised against *S. mattheei* and antigens of *S. mattheei, S. haematobium* and *S. mansoni*

plasma cells and eosinophils, is visible around a schistosomulum (S). Fig. 2 depicts the biopsy taken 4 days after infection and shows the perforation in the skin and parts of the cercarial glycocalyx (G) shed during penetration. Muscle necrosis was observed directly beneath the penetration site of the cercariae.

From Fig. 3 it is obvious that antibodies (IgG) raised against a pure *S. mattheei* infection cross-react with antigens of both *S. haematobium* and *S. mansoni*. The control serum was negative for all the parasite antigens used. As far as the differential white cell counts are concerned it is clear from Table 1 that the eosinophil counts were zero 2 weeks after penetration and thereafter increased to 1,3 and 0,7 respectively 4 and 6 weeks after penetration. The platelet and neutrophil counts fluctuated within the limits of the normal range. The counts of the control blood sample did not deviate from the normal range in any of the cases.

TABLE 1 The absolute eosinophil (E), neutrophil (N) and blood platelet (P) counts (number of cells/10^{-9} ℓ) at different time invervals after a pure *S. mattheei* infection

	Analysis time after infection				
Responses	2 weeks	4 weeks	7 weeks	9 months	Normal range
Experimental data					
E	0,0	1,3	0,7	0,1	0,04–0,4
N	4,9	3,7	2,1	3,5	2,00–7,5
P	209	190	209	217	150–450
Control					
E	0,0	0,2	0,0	0,2	0,04–0,4
N	4,8	5,3	3,4	3,2	2,00–7,5
P	232	240	276	258	150–450

DISCUSSION

The fact that no *S. mattheei* eggs could be detected in urine or stool samples of the experimentally infected person, lends some support to the supposition made in previous studies that *S. mattheei* infections in man are not capable of egg production unless accompanied by other schistosome species.

However, these results cannot be accepted as proof of this supposition. Furthermore it must be kept in mind that mice excreta was not screened for *S. mattheei* ova during the present study. Although eggs were detected in the livers of infected mice it can not be assumed that ova were passed in their excreta.

The fact that no ova were detected in the excreta of the infected researcher does therefore not exclude the possibility of the presence of eggs in his liver. It must be accepted that man is a poor host for *S. mattheei*, as, with few exceptions, the prevalence of this infection is low, even in regions where nearly all the cattle are infected, and water sources are shared by man and beast (Pitchford, 1959). Furthermore, those few humans who are infected pass but few ova daily (Pitchford, 1961) even though some are probably exposed to large numbers of viable cercariae while swimming. This implies that only small numbers of fully viable *S. mattheei* cercariae are able to mature in man, even in the presence of the human schistosomes. The researcher in this study was exposed to only 30 cercariae that were 3 days old, while previous reports indicate that, for full viability, cercariae should have access to their host within about 3–4 h (Olivier, 1966; Stirewalt & Fregeau, 1968). The cercariae were apparently able to migrate away from the penetration site, but it is quite possible that the process of penetration and intial migration could have sapped them to such an extent of their available energy, that they were not able to cross further hurdles in the form of migration through the lungs, liver, etc.

It should also be kept in mind that, judging from the reaction to the presumed unintentional exposure while collecting snails, the researcher had previously been sensitised to schistosomes, and that attrition of schistosomula in rodent models occur in either the skin, or the lungs of the host (Von Lichtenburg, Sher, Gibbons & Doughty, 1976; Von Lichtenburgh & Byram, 1980). There is insufficient information on the immunological response of humans in reaction to pure *S. mattheei* infections largely due to the extreme difficulties involved in detecting such cases. Cross reactions between antibodies raised against one schistosome species with antigens of another schistosome species complicate the diagnosis of pure *S. mattheei* infections in humans even more (Simpson & Crioli, 1987). In this study it was found that the immunological responses investigated closely resemble those in humans infected with human schistosomes (DuMondé, Humblin & Lloyd, 1982). The finding that the infected person experienced pruritis accompanied by cercarial dermatitis suggests that he had been sensitised during a previous infection. Hardly any detectable reaction is shown by individuals during a first infection and this makes detection of such cases extremely difficult (Winslow, 1967). The finding that only the glycocalyx of a cercaria was present in the skin of the person 4 days after penetration (Fig. 2) and that muscle necrosis occurred directly beneath the penetration site of the cercaria showed that the cercariae were not only able to penetrate but were also able to migrate further away from the penetration site. This together with the presence of *S. mattheei* ova in the homogenized livers of all the infected mice, indicated that the cercariae were still infective despite being 3 days old at that time.

The findings of Savage & Colley (1980) that the eosinophils lie in close proximity to the parasites, but that direct contact has yet to be established on ultrastructural level, is supported by our results as is evident in Fig. 1. Eosinophilia as observed in this study is also a feature of hepatosplenic bilharziasis (Moosa, Ata, El Rooby, El Garem, Abdel Wahab & El Raziki, 1967).

The cross reactions between antibodies raised against *S. mattheei* and antigens of *S. haematobium* and *S. mansoni* was partly to be expected, as it is a common phenomenon among schistosome species. Simpson & Crioli (1987) found cross-reactions with antigens having masses of >200 kDa, 30 kDa and 28 kDa, all of which were demonstrated in various schistosome species.

The fact that no *S. mattheei* eggs could be detected in urine or stool samples of the experimentally infected person, lends some support to the supposition made in previous studies that *S. mattheei* infections in man are not capable of egg production unless accompanied by other schistosome species.

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