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EXPERIMENTAL S. MANSONI INFECTION IN MICE. BLOOD WHITE CELLS, SERUM PROTEINS, GLYCEMIA AND BLOOD UREA IN VARIOUS STAGES OF THE INFECTION

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SUMMARY

White mice (Swiss) were individually infected with 200 cercariae from a **Schistosoma mansoni** strain (Belo Horizonte, Brasil) and examined 1, 4, 6, 8 and 10 weeks after infection. Peripheral white cells, serum proteins, blood glucose and urea were determined and serologic tests for anticercarial antibodies and adult worm counts were made. Higher eosinophil counts were observed after four weeks as well as alterations in blood glucose and albumin levels. Later on there was a marked tendency towards neutrophilia and positive serologic tests. After the tenth week there was an increase in blood urea and alpha 2, beta and gamma globulins, suggesting circulating immune-complexes with consequent renal damage. The relationship between **S. mansoni** strains and some of the parameters studied are discussed.

INTRODUCTION

Mice have been extensively used in the study of schistosomiasis mansoni, the course of the disease being very similar to its human counterpart [2,23]. Some aspects as portal hypertension and esophageal varices frequently found in the hepatosplenic form of the disease are equally present in the experimental model using white mice [21]. Different aspects of the infection such as pathogenesis, immunology, blood picture, parasite findings, biochemistry and pharmacodynamics have been studied by several authors [7,8,14,22].

The present work aims at an overall view of the complexity of this disease in infected white mice, following-up several parameters as, white blood cell counts (WBC), blood biochemical components, humoral immunity and worm burden at different stages of the infection. The chosen strain of the parasite is well adapted to both its vertebrate and invertebrate hosts and its epidemiology is well known in Brasil [11].

MATERIAL AND METHODS

Sixty white mice (Swiss) divided in six lots of ten animals each (one lot was spared for control purposes) were individually infected with 200 **Schistosoma mansoni** cercariae, mineira strain (BH) from state of Minas Gerais, Brasil, mantained through albino mice and **Biomphalaria glabrata** [10,12]. After 1, 4, 6, 8 and 10 weeks, blood was collected (retro-orbital plexus) from each lot for total and differential white cell counts (during first 8 weeks) and for determination of blood glucose [4], blood urea [25] and serum protein levels [1], using zone electrophoresis (fourth to tenth week). Antibodies to cercariae were investigated by the pe-

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ricercarial reaction [20] and indirect immunofluorescence [15]. The animals were killed, the portal system perfused with physiologic saline [26] and the adult worms collected and counted. All sera belonging to the same lot were pooled together and rabbit globulin antibodies were investigated by agglutination tests on latex particles coated with rabbit globulins.

RESULTS

Obtained data are registered in Tables I, II and III. Normal values were considered in non-infected mice (control lot). For statistical purposes the standard errors of the means were utilized.

BLOOD LEUKOCYTE PATTERN (Table I).

TABLE I

Mice[b] peripheral leukocytes in several stages of infection with **S. mansoni**

Leukocytes[a]	Controls	Stage of infection (Weeks)			
		1	4	6	8
Neutrophils	1521 (240.3)*	1764 (408.3)	1997 (142.3)	3014 (494.3)	4981 (485.0)
Eosinophils	169 (37.8)	86 (25.6)	844 (238.6)	298 (33.0)	275 (44.7)
Lymphocytes	6122 (507.1)	3513 (463.0)	7586 (986.3)	7249 (683.7)	4893 (673.7)
Monocytes	349 (60.3)	71 (20.3)	334 (62.0)	262 (22.7)	259 (42.0)
Totals	8162 (669.0)	5435 (757.0)	10762 (788.0)	10825 (941.0)	10410 (862.0)

a) per cubic millimetre
*) standard error of the mean in parenthesis
b) 10 animals per lot

WBC counts decreased 33% ($p < 0.01$) at the first week due to significant lymphocyte and monocyte drops, but in overall counting there was a significant elevation after four weeks that remained constant throughout the rest of the experiment. The changes were essentially brought about by lymphocytes and eosinophils which increased greatly after four weeks. Beyond this stage, the observed tendency was a constant drop of peripheral lymphocytes and concomitant increase of the neutrophils ($p < 0.01$).

SERUM PROTEINS (Table II)

TABLE II

Serum proteins of mice[b] in several stages of **S. mansoni** infection

Proteins[a]	Controls	Stage of infection (Weeks)			
		4	6	8	10
Albumin	2.5 (0.3)*	2.7 (0.4)	2.0 (0.15)	2.0 (0.15)	2.3 (0.3)
Alpha 1 globulin	0.3 (0.05)	0.2 (0.02)	0.2 (0.01)	0.2 (0.01)	0.1 (0.01)
Alpha 2 globulin	0.7 (0.12)	0.6 (0.08)	0.5 (0.05)	0.6 (0.05)	1.0 (0.11)
Beta globulin	0.8 (0.11)	1.0 (0.09)	1.0 (0.08)	0.8 (0.12)	1.2 (0.1)
Gamma globulin	0.4 (0.03)	0.2 (0.01)	0.5 (0.07)	0.4 (0.02)	0.6 (0.04)
Totals	4.6 (0.4)	4.6 (0.3)	4.1 (0.3)	3.9 (0.3)	5.0 (0.6)

a) grams per 100 millilitres of serum
*) standard error of the mean in parenthesis
b) 10 animals per lot

The important alterations in protein concentration began after the fourth week, mainly at tenth week when a steady raise in the gamma, beta and alpha 2 fractions ($p < 0.05$) occurred. However, alpha 1 globulin showed a significant drop in all stages ($p < 0.05$). After four weeks, the pericercarial reaction and indirect immunofluorescence were all positive

and remained so until the end of experiment. Rabbit antiglobulins were detected after the 8th week.

CERCARIAE AND ADULT WORMS RECOVERY

The infection technique utilized allowed control the number of cercariae that effectively penetrated the animal tail. In fourteen animals tested, an average of 94.8% (189 ± 1.4) penetration was obtained. Adult worms recovery was about 25% with clear male predominance (29.3 ± 4.4 per mouse) as compared to the female worms (18.9 ± 3.4 per mouse) in forty animals.

BLOOD UREA AND GLUCOSE (Table III)

TABLE III

Mice[b] serum glucose and urea in several stages of **S. mansoni** infection

Stage of infection (Weeks)	Glucose[a]	Urea[a]
Non infected	162 (10.7)*	46 (4.3)
4	182 (13.4)	43 (2.2)
6	58 (6.7)	45 (5.8)
8	78 (8.5)	42 (4.4)
10	78 (9.6)	61 (6.7)

a) milligrams per 100 millilitres of serum
*) standard error of the mean in parenthesis
b) 10 animals per lot

There was a blood urea increase in the tenth week ($p < 0.01$) and a marked drop in the blood glucose level by the sixth week followed by slight elevation towards the end of the observation period, but still definitely lower than control levels ($p < 0.01$).

DISCUSSION

Many workers have strongly emphasized the importance of technical standardization in experimental studies on schistosomiasis [3], since investigators can easily and inadvertently introduce irrelevant variables. For instance, in repetitive bleedings, even when the animals are kept 5 days apart, have showed a tendency to increase neutrophils. Although the cause of this remains unclear, a possible explanation could be the stress caused by frequent manipulation of the animals [18]. Also blood counts vary depending on the bleeding technique, and heart blood is cited to have fewer cells than blood from the tail vein [17]. These facts led us to use a single bleeding in each lot, using exactly the same procedure. The control lot was examined at the beginning of the experiment.

Eosinophil patterns have been previously studied in schistosomiasis [3,5]. Highest values were found after the fourth week, which is in agreement with DE WITT [5], though this author worked with moderate infections (100 cercariae from a Puerto Rico strain). COLLEY [3] found maximum count at the 8th week in mice infected by 75 cercariae, possibly from a Puerto Rico strain.

The introduction of bias in the present study might be represented by extraneous preexisting intestinal helmintiasis, and in fact in all our lots we were able to detect two different nematode species: **Syphacia obvelata** and **Aspiculuris tetraptera.** A similar situation was observed by DE WITT [5].

Apparently, after four weeks of infection, the worm maturation process was reasonably developed in the liver and mesenteric veins, enabling the initiation of adult sexual activity and eggs production. From then onwards it looks as though the total worm immunogenic surface area and its metabolic activity approached the maximum level, causing more prominent alterations in the parameters under study. The maximum eosinophil count was paralleled by a sharp drop in blood glucose, possibly due to the high metabolic activity of the worms [16,19]. Rheumatoid factor-like antibodies could be detected after the 8th week, and these remained detectable until the end of the experiment [24]. The same held true for increases in globulin fractions alpha 2, beta and gamma. These data show an important stage greatly influencing the host immunological mechanisms. So, hepatic granulomas and circulating antigen-antibody complexes might represent an important etiopathogenic mechanism that directly contributes to the renal damage inferable from high urea levels in the 10th week. DE WITT & WARREN [6] observed similar blood globulin patterns in moderate infections with 125 cercariae from a Puerto Rico strain.

In the appraisal of experimental models of schistosomiasis, attention should be directed to the choice of parasite strains, because the pathogenicity seems to be intimately relat-

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ed to the degree of adaptability of the worm to both invertebrate and vertebrate hosts. PARAENSE & CORREA [13] have shown the coexistence in the same zoogeographic area of two **S. mansoni** strains with dissimilar behaviour in molluscs and in vertebrate hosts [9]. The present paper presents data on one of these strains (Belo Horizonte, Brasil) which shows good adaptability to the two hosts (**Biomphalaria glabrata** and mammals), which would in theory explain the high pathogenicity observed by MAGALHÃES et al. [11]. Recovery of adult worms in this study was superior ($p < 0.05$) to the 19.1% obtained by DE WITT & WARREN [6].

Profound alterations in the vital functions of the mice under study resulted in death to some after the 10th week. This caused on one hand, great limitations on the follow up, and on the other, restricted the inclusion of additional parameters. By using a smaller number of cercariae, we would possibly have had better survival.

The existence in nature of many **S. mansoni** strains seems to be a reality, and they possibly differ in their degree of adaptation to their respective hosts. Standardized comparative studies of these populations could provide an important key for the better understanding of the ecology and epidemiology of mansoni schistosomiasis throughout the world.

RESUMO

Infecção experimental de camundongos por S. mansoni. Leucócitos periféricos, proteínas séricas, glicemia e uremia em vários estágios da infecção.

Camundongos albinos foram individualmente infectados com 200 cercárias de **Schistosoma mansoni** (linhagem de Belo Horizonte) e examinados após 1, 4, 6, 8 e 10 semanas de infecção. Leucócitos periféricos, proteínas séricas, glicemia e uremia foram determinados e testes sorológicos para detecção de anticorpos anti-cercária foram feitos nos períodos assinalados. Foram observadas altas contagens de eosinófilos após 4 semanas, assim como alterações nos níveis de albumina e glicose. Em etapa posterior, houve tendência ao aparecimento de neutrofilia e positividade nos testes sorológicos. Após a 10.ª semana houve aumento na uremia e globulinas alfa 2, beta e gama, sugerindo a presença de imune-complexos circulantes com conseqüente injúria renal. Discute-se a relação entre a patogenicidade de algumas linhagens do parasita e alguns dos parâmetros avaliados.

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