

IMMUNOPATHOLOGY OF *SCHISTOSOMA* *MANSONI* INFECTION IN RABBITS

(A preliminary report)

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Five rabbits infected with Schistosoma mansoni showed marked resistance, which resulted in low worm recovery and low egg production. Pathological changes appeared in liver and intestines as scattered foci of eosinophilic infiltration around immature eggs, with only occasional granulomatous formation. Antibodies to ovular and adult worm structures were demonstrated by immunofluorescence in the sera of rabbits prior to infection (natural antibodies) and specially following infection by S. mansoni. These findings point out to the peculiarities of the immunopathology of schistosomiasis in rabbits.

Rabbits are usually good models for immunological studies. A recent report claims that these rodents developed good resistance to re-infection with *S. mansoni* when immunized with whole worm extract (Scarpin et al, 1980). However, the immunopathology of Schistosomiasis mansoni in the rabbits has been little investigated. The rabbits are considered as poor experimental hosts for *S. mansoni* because infection is drastically reduced with time (Warren & Peters, 1967); the eggs in the tissues do not seem to reach maturity (Cunha et al, 1962) and because of that they do not appear in the faeces (Stewart, 1963). In an attempt to investigate the usefulness of the rabbit model for immunopathologic studies in *S. mansoni* infection and to obtain baseline information about inoculum size and histopathological lesions we performed an immunofluorescent and histological investigation on rabbits experimentally infected with *S. mansoni*.

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This investigation received support from the UNDP/World Bank/WHO Special Programme for research and training in tropical diseases.

Received for publication December 4th and accepted December 28th, 1981.

MATERIAL AND METHODS

Five healthy young male rabbits weighing 1.000 to 1.500 grams were injected subcutaneously with recently shed *S. mansoni* cercariae obtained from experimentally infected *Biomphalaria glabrata* snails. The animals were infected with 500, 1.000, 1.500, 2.000 and 3.000 cercariae respectively. When the amount of fluid to be injected exceeded one milliliter, injections were made into different parts of the subcutaneous tissue. Stool examination for detection of *S. mansoni* eggs was performed regularly up to the tenth week post-infection. One rabbit was sacrificed eight weeks after infection and the remaining four were sacrificed ten weeks after infection. The portal venous system of the rabbits was perfused with saline for the recovery of worms. Furthermore the search for worms was completed with gross and microscopic dissection of the liver and mesenteric region. Fragments of the liver, spleen, intestines, lungs and kidneys were fixed in neutral 10% formalin, embedded in paraffin and the sections were stained with Hematoxylin and Eosin.

The rabbits were bled once before infection (normal control sera) and also at the end of the experiment. The sera were used for immunofluorescent studies. Bouin fixed, paraffin sections of mouse liver containing eggs and adult worms of *S. mansoni* were tested by the undiluted sera and also by the same sera diluted to 1:2, 1:5, 1:20, 1:40 and 1:80 with phosphate buffered saline. The indirect immunofluorescence technique was performed with fluoresceinated goat anti-rabbit IgG diluted to 1:60 and with appropriate control sections. Sections mounted in phosphate buffer pH 9 were examined under ultraviolet light in an A0 microscope.

RESULTS

Relatively few worms were recovered from infected rabbits. These data appear on Table I, which also shows the results of immunofluorescent examination.

TABLE I

General parasitological and immunopathological data on schistosomiasis mansoni in rabbits

Rabbit N ^o	N ^o Cercariae	Worms Recovered		Localization and titer of positive immunofluorescence			
		N ^o	%	Before infection		After infection	
				Worm	Eggs	Worm	Eggs
1	500	27 14♂; 13♀	5.4	undiluted	1:2	1:80	1:80
2	1,000	5 3♂; 2♀	0.5	undiluted	1:5	1:40	1:40
3	1,500	2 1♂; 1♀	0.1	undiluted	1:5	1:40	1:40
4	2,000	0	0	undiluted	1:5	1:80	1:80
5	3,000	0	0	1:2	1:5	1:10	1:40

Gross lesions were limited to a few and scattered whitish dots seen on the external surface as well as on the cut sections of the liver. Microscopically there were few accumulations of eosinophils and macrophages around immature eggs in the liver and intestines (Figs. 1 and 2). In the intestines the eggs were located in the mucosal and submucosal layers. In the liver, they were usually seen in the portal tract and a few within the lobular parenchyma. The liver architecture was well-preserved. Portal infiltration with predominance of eosinophils was sometimes seen, with or without the presence of eggs. Focal hyaline necrosis was rarely seen near the portal tracts together with diffuse eosinophilic infiltration (Fig. 3). Probably this was due to reaction around dead adult worms, but could not be proven in the sections examined. Most of the times the eggs were immature, surrounded by giant cell, and located near the portal tracts (Fig. 4). A well preserved, completely matured egg was never seen in the present material. Stool examination was negative for all animals up to the period they were sacrificed.

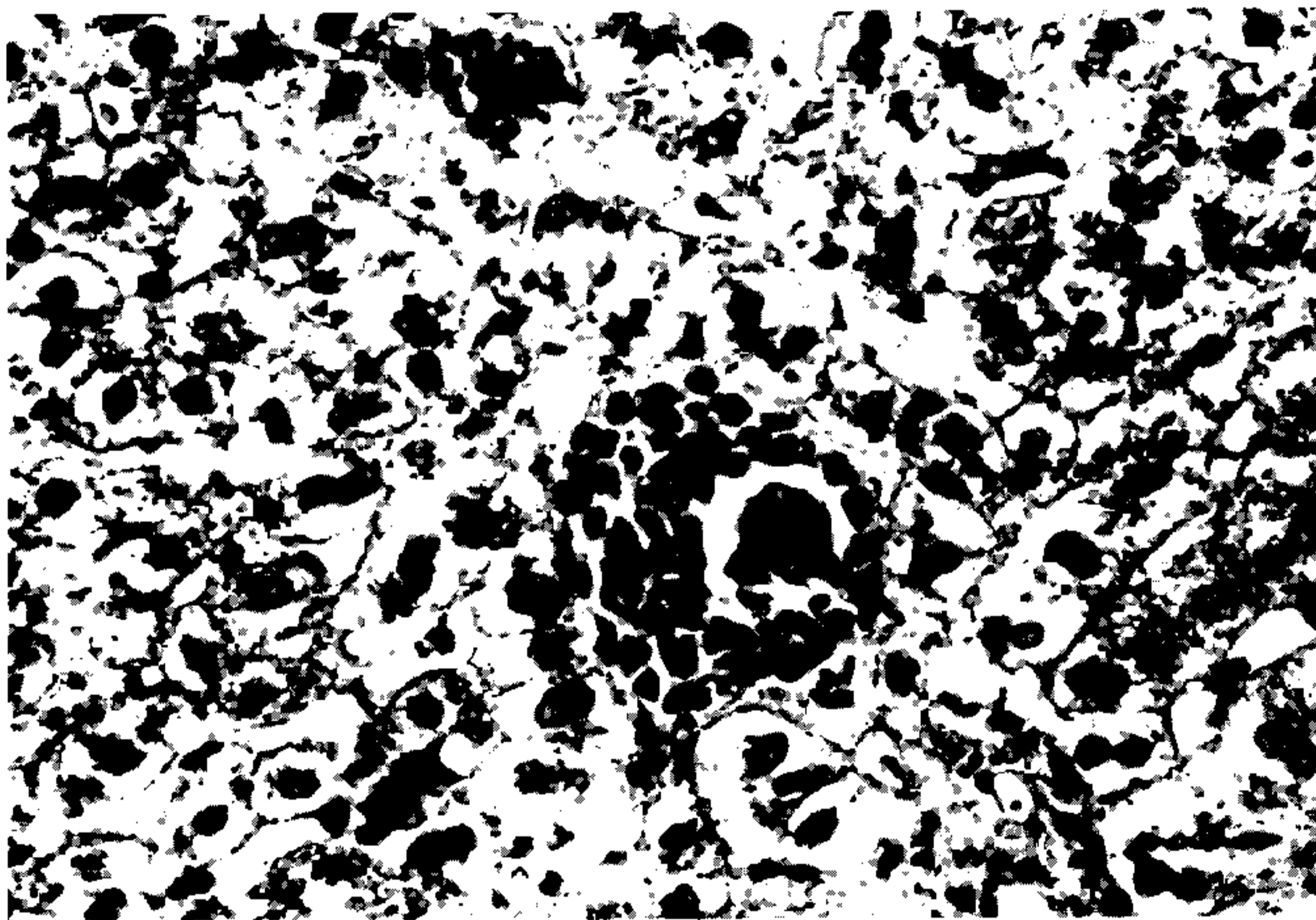


Fig. 1 – A small collection of inflammatory cells, mostly eosinophils, around a disintegrating immature *S. mansoni* egg within the hepatic parenchyma. 120xHE.

The lungs, spleen, and kidney sections were within normal limits.

Specific immunofluorescent staining localized within the eggs (Fig. 5) and in the intestinal lining of the gut of the adult worms (Fig. 6) was found positive even with normal sera collected prior to the infection of the animals (see Table I). This staining became much more intense eight to ten weeks after infection. Besides, the sera from infected rabbits bound to the tegument of the worms and also promoted a diffuse staining of all the structures of the adult worm, especially strong in the sections from female worms.

COMMENTS

Rabbits are said to be naturally resistant to infection with *S. mansoni* (Faust, Jones & Hoffman, 1934; Warren & Peters, 1967; Cunha et al, 1962) although they seem

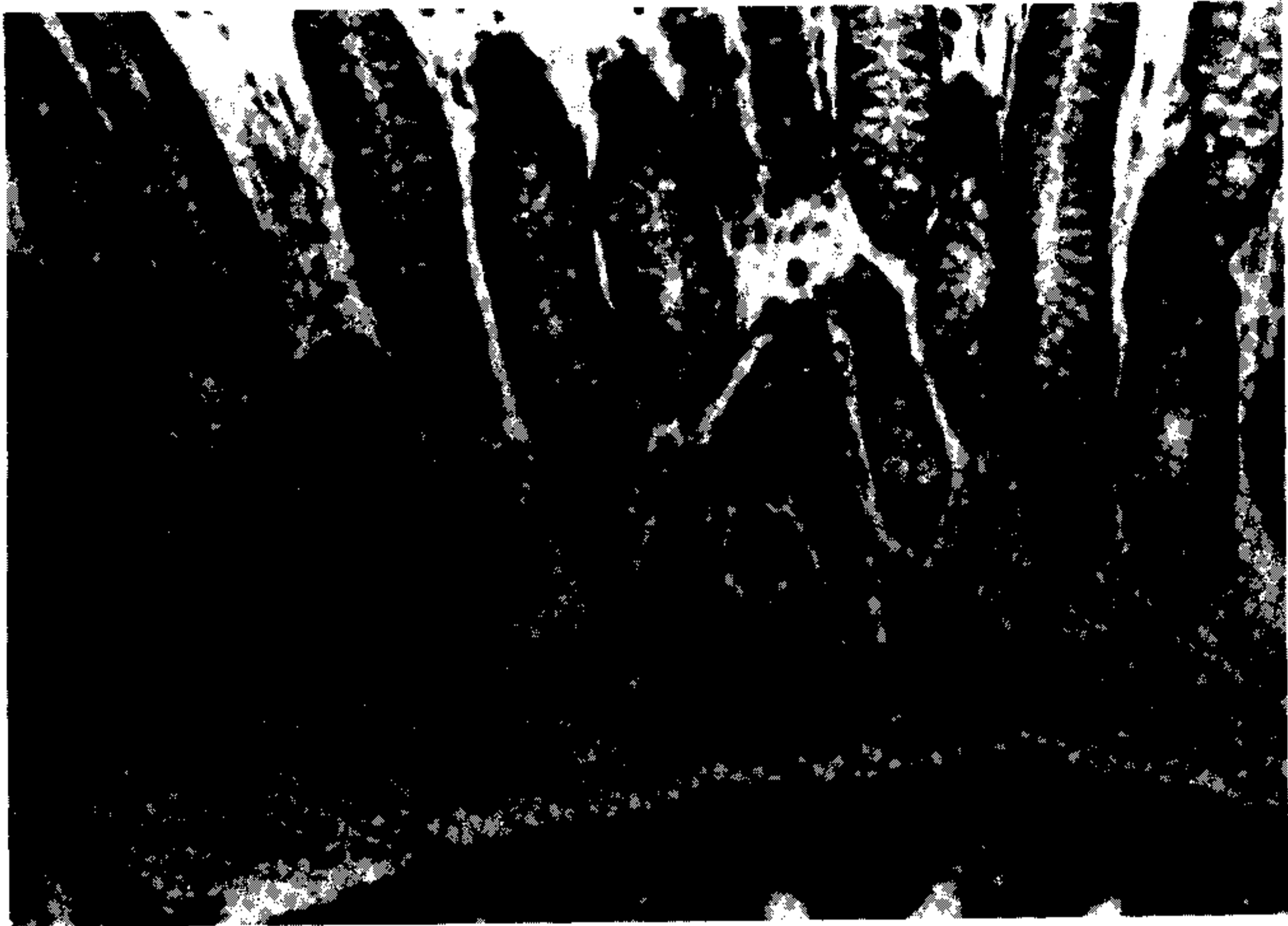


Fig. 2 Accumulations of eosinophils around immature eggs of *S. mansoni* in the mucosa of the colon. 120 x HE.

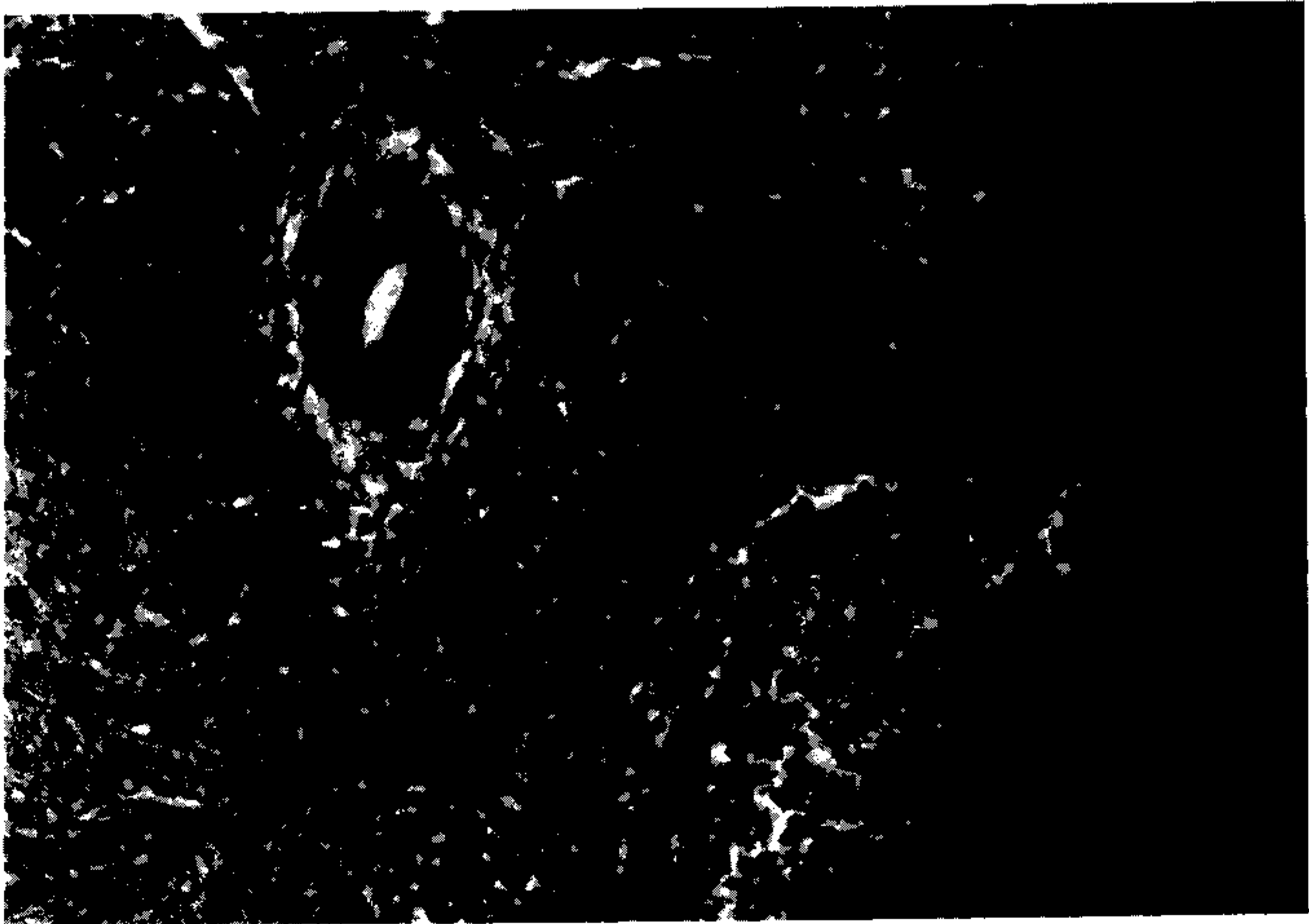


Fig. 3 - Diffuse infiltration of a portal area by polymorphonuclear eosinophils in the vicinity of a focus of hyaline necrosis, which is surrounded by a palisade of macrophages. 100 x HE.



Fig. 4 - Granuloma with a foreign body giant cell encircling a disintegrating immature egg in a portal space which is densely infiltrated with eosinophils, lymphocytes and plasma cells. 120 x HE.

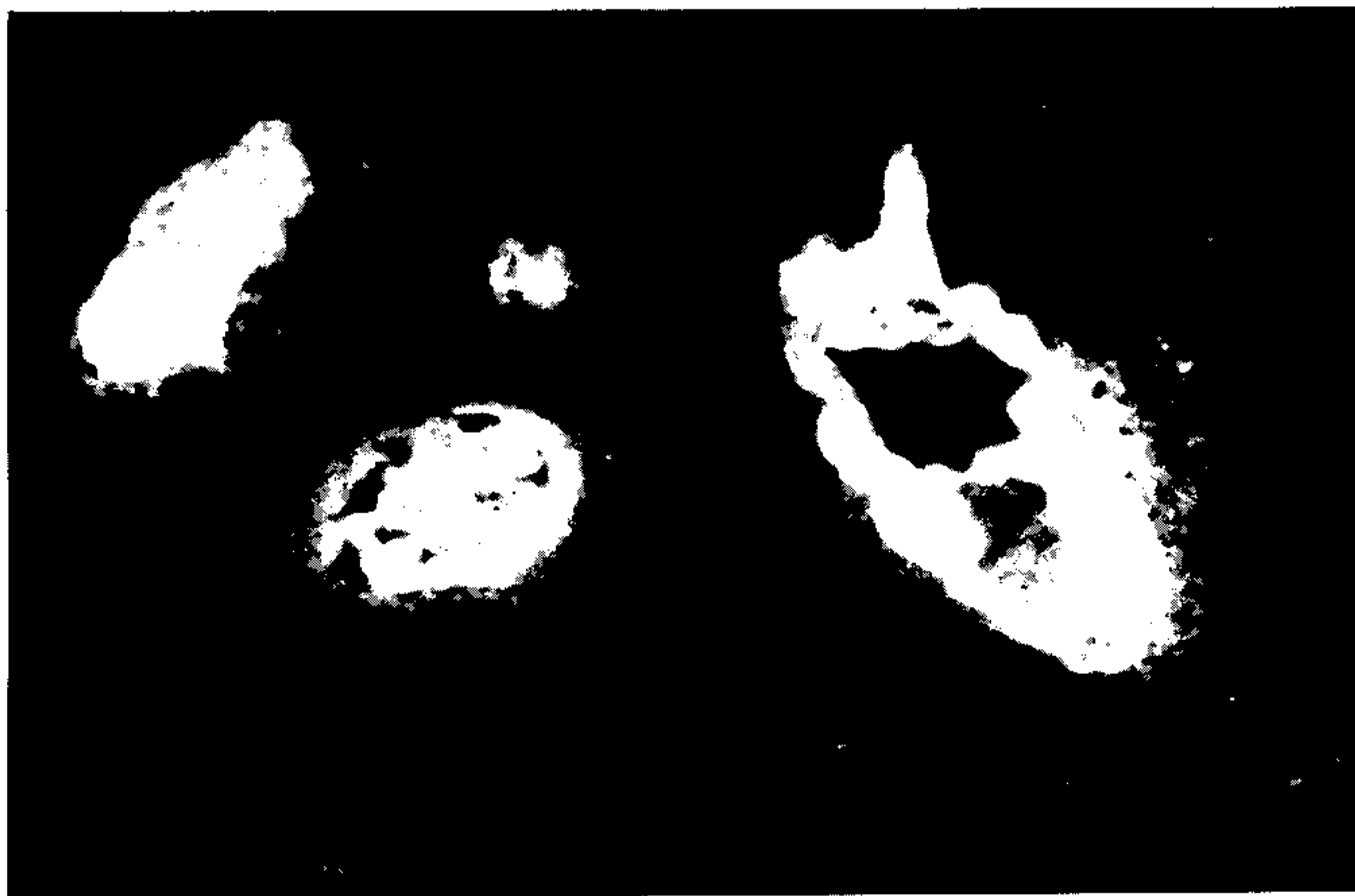


Fig. 5 - Eggs of *S. mansoni* showing specific fluorescence after being treated with normal (right) and infected (left) rabbit sera followed by fluoresceinated anti-rabbit globulin. 250 x.

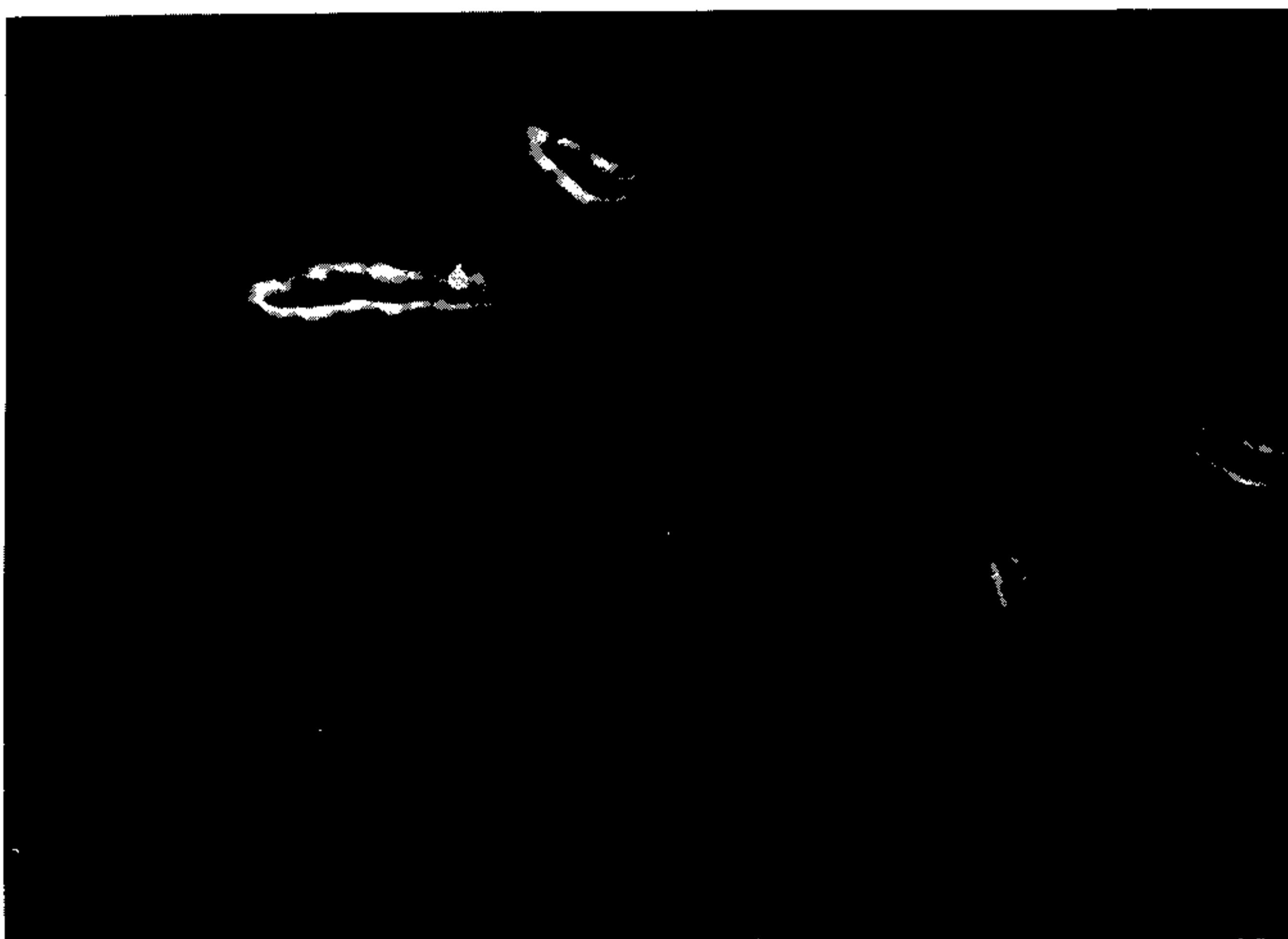


Fig. 6 – Fluorescence in the epithelial lining of the *S. mansoni* gut which appeared after treatment with normal rabbit serum followed by fluoresceinated anti-rabbit globulin. 150 x.

to be good hosts for *S. japonicum* (Tsutsumi & Nakashima, 1972; Cheever, Duvall & Minker, 1980). Studying the oogram in rabbits infected with *S. mansoni* Cunha et al (1962) observed that 58.2% of the eggs were dead and only 32.5% caused granuloma; only 9.3% were viable and no egg was found to have fully developed miracidium. It is not known whether such resistance is due to immunological or non-immunological factors. During this investigation it was disclosed the presence of a serum factor, probably natural antibodies, with affinity to *S. mansoni* structures. The level of these antibodies increased after infection, as seen now with the immunofluorescent technique. We are inclined to suggest that such natural antibodies may play a role in the resistance shown by the rabbits. Anyway, when considering experiment to test resistance to *S. mansoni* with the rabbit model, these natural antibodies have to be taken into consideration. The irregular recovery of worms as seen in the present work, which showed no correlation with inoculum size, suggests that these antibodies and or other factors may contribute to turn the results of infection with *S. mansoni* in rabbits really erratic. Although no worms have been recovered from two of the rabbits, they showed egg-related lesions in the liver and intestines. Either, there was a failure to recover a few worms or they might have died after producing eggs.

The lesions caused by the *S. mansoni* eggs were quite mild. This resulted not only because the eggs were few but because they did not attain full maturity. Most of the eggs were infiltrated by cells, especially polymorphonuclear eosinophils, while still immature. This is different from what one usually sees in mice (Prata, 1957) and man (Andrade, 1965) where immature eggs do not seem to evoke host reaction.

So, infection by *S. mansoni* in the rabbits shows a peculiar behavior, and it seems that anyone interested in such model should be aware of it. Some details, such as presence of natural cross-reacting antibodies against *S. mansoni* and its products, the progressive

decrease of worm load with time, and the failure of the eggs to reach maturation are some of the points that should be carefully considered when the utilization of this animal model is contemplated for the study of immunopathology of schistosomiasis.

RESUMO

Cinco coelhos infectados experimentalmente pelo *Schistosoma mansoni* exibiram acentuada resistência, a qual esteve refletida na baixa recuperação dos vermes e na escassa produção de ovos. As lesões histopatológicas se limitaram a focos esparsos de infiltração eosinófila em torno de ovos imaturos e alguns raros granulomas periovulares.

No soro dos coelhos normais (pré-infecção) foi detectada por imunofluorescência indireta a presença de anticorpos contra as estruturas ovulares e dos vermes adultos. Estes anticorpos se tornaram mais evidentes após a infecção pelo *S. mansoni*. Os achados deste estudo preliminar acentuam as peculiaridades da imunopatologia da esquistossomose no coelho.

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