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TREATMENT OF ACUTE EXPERIMENTAL SCHISTOSOMIASIS

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A model of acute schistosomiasis of the mouse was used to observe whether curative treatment would be followed by an enhancement of the hepatic and splenic lesions, as a consequence of the massive destruction of worms and eggs within the portal system. Mice infected with 50 cercariae of Schistosoma mansoni *were treated with both oxamniquine and praziquantel on the 50th day of infection and submitted to a sequential histologic examination from the 2nd to the 45th day after treatment.*

Although severe focal lesions due to dead and disintegrating worms were present in the livers of the treated animals, no aggravation of the general changes (reative hepatitis and splenitis, or periovular granulomas) was seen in comparison with a control non-treated group. Of 50 animals treated during the acute phase of schistosomiasis only one died espontaneously, while 16 out of 30 infected controls died before the end of the experiment.

The present investigation indicates that curative treatment during the acute phase of schistosomiasis does not enhance previous lesions at first and results in progressive disappearance of the lesions starting six days following chemotherapy.

Key words: acute schistosomiasis – chemotherapy – hepatic and splenic lesions – *Schistosoma mansoni*

The experimental model of acute schistosomiasis was recently described (Andrade & Azevedo, 1987). Following the infection of mice by means of a sequential histopathological examination performed every other day, from the cercarial exposure up to the moment of the differentiation of mature eggs in the tissues it was possible to identify a phase during which the lesions in the liver and spleen suddenly became acute and severe. This phase coincided with the deposition of the first mature eggs in the liver and was considered as the experimental counterpart of the so-called acute toxemic schistosomiasis seen in man.

Facing the dramatic clinical picture presented by the patients with acute toxemic schistosomiasis, clinicians have sometimes been afraid to administer drugs that kill the worms, assuming that massive destruction of parasites, with the sudden liberation of more and diversified antigens could enhance the severity of the lesions already present (Neves & Raso, 1963). Acute schistosomiasis has been compared to an immediate-type hypersensitivity disease (Diaz Rivera et al., 1956) or to serum-sickness (Hiatt et al., 1979; Bogliolo & Neves, 1965), and capable of simulating various infectious diseases (Neves et al., 1972).

Therefore, some prefer to wait until the acute manifestations subside, while others recommend the concomitant use of corticoids for the treatment of acute toxemic schistosomiasis (reviewed by Neves & Raso, 1963 and Neves et al., 1972).

In the present investigation the murine model is utilized in an attempt to find out whether curative treatment of schistosomiasis during the acute phase of the infection can indeed provoke a phase of exacerbation of the lesions.

MATERIAL AND METHODS

Young adult outbred albino mice of both sexes, weighing 18-22 grams, were individually submitted to infection with 50 cercariae of

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Schistosoma mansoni, by the transcutaneous route. Fifty animals which were eliminating viable eggs in the stools were treated on the 50th day of infection with oxamniquine and praziquantel administered simultaneously. Both drugs were given by gastric intubation. Oxamniquine was given as a single dose of 100 mg/kg b.w. and praziquantel was administered on the following day, the dose of 100 mg/kg b.w. being given four times during the day.

Thirty infected animals were not treated and served as controls.

Following treatment, two treated mice and two infected controls were killed on the days 2, 3, 6, 8, 13, 15, 17, 25, 27, 39 and 45. By day 25 all the remaining 16 infected controls, from an initial group of 30 animals, had died and only treated animals were examined from then on. As for the 50 animals in the treated group, only one died espontaneously.

At the time of sacrifice the animals were anesthesized with ether and exanguinated. The weight of the spleen was recorded. Recovering of worms were made after perfusion of the portal system with buffered saline. Pieces of liver were meshed between two glass slides for the evaluation of the types of eggs present (immature, mature and dead eggs).

Fragments of the liver and spleen were fixed in Bouin's fluid, dehydrated in ethanol and embedded in parafin. The 5 micron thick-sections were stained with hematoxylin and eosin. In some cases, special stains such as Schiff-PAS, Picro-Sirius Red and Gomori's reticulum stain were utilized. The microscopic studies of the slides were made at first "blindly", the examiner not being aware of which group the material belonged to.

RESULTS

Figure 1 shows the worm recovering rate in treated and non-treated animals. Most of the worms recovered from the treated animals on the 6th and 8th days were dead, but thereafter the numbers are representative of unmated male worms found still alive. Spleen weights for both groups of animals appear on Fig. 2. As can be noted, there was a gradual decrease in splenic weight following treatment of schistosomiasis.

The oogram in a few treated animals revealed mature and immature eggs, indicating that the treatment did not result in parasitological cure. These cases were discarded.

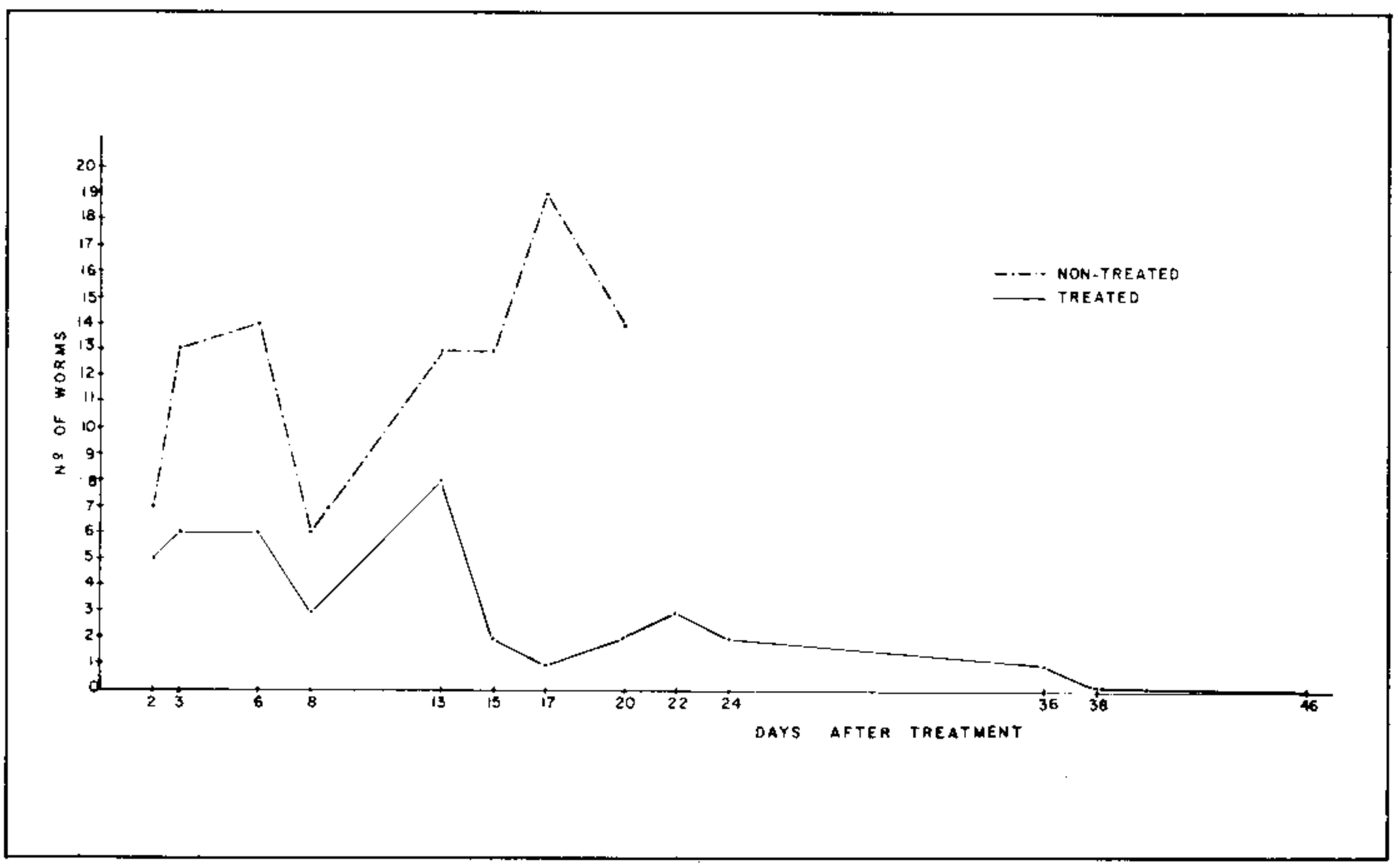

Fig. 1 : recovering of adult worms after perfusion of the portal vein system.

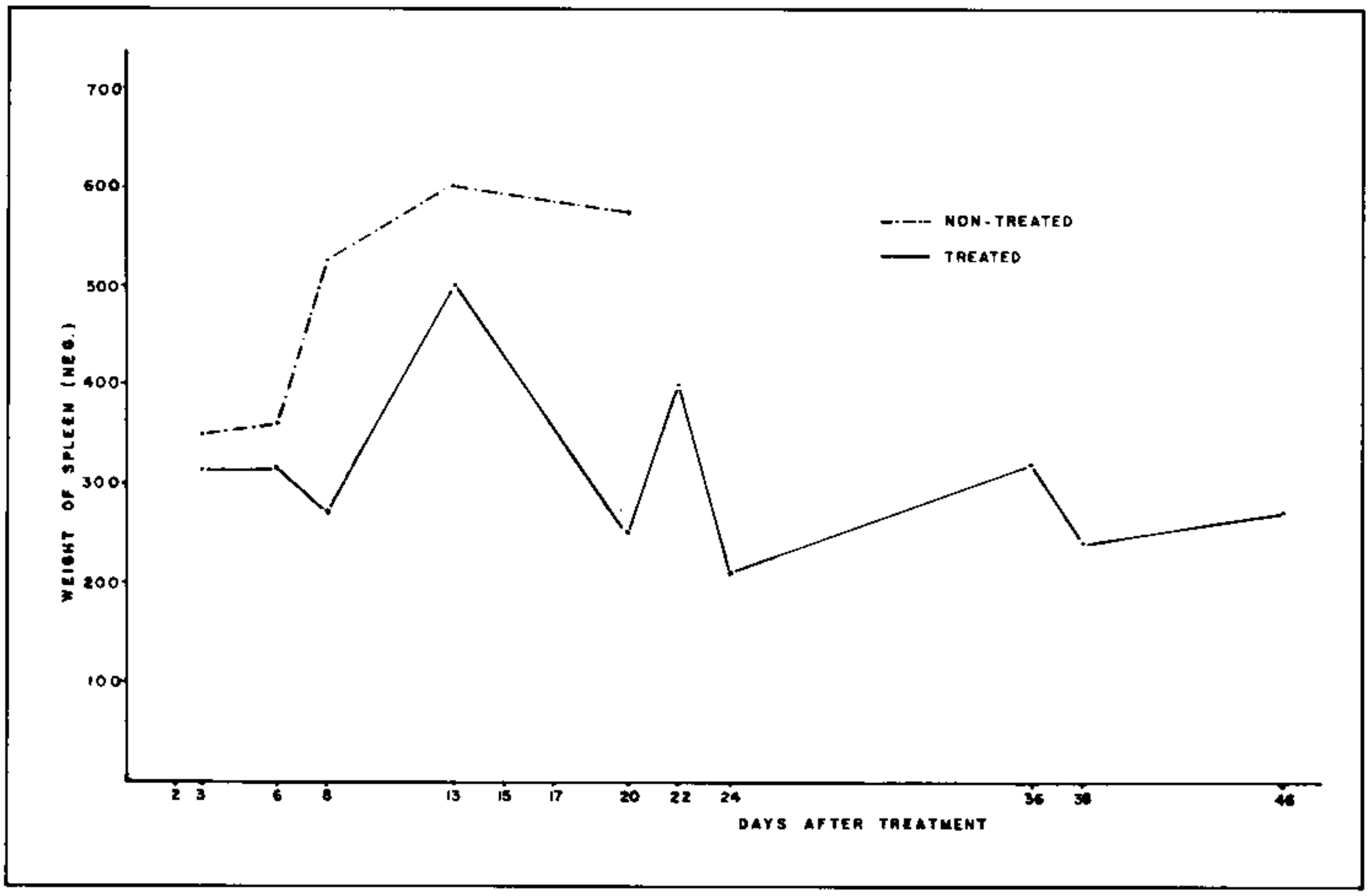

Fig. 2: curves showing the weights of the spleen in mice infected with *Schistosoma mansoni*, treated and non-treated.

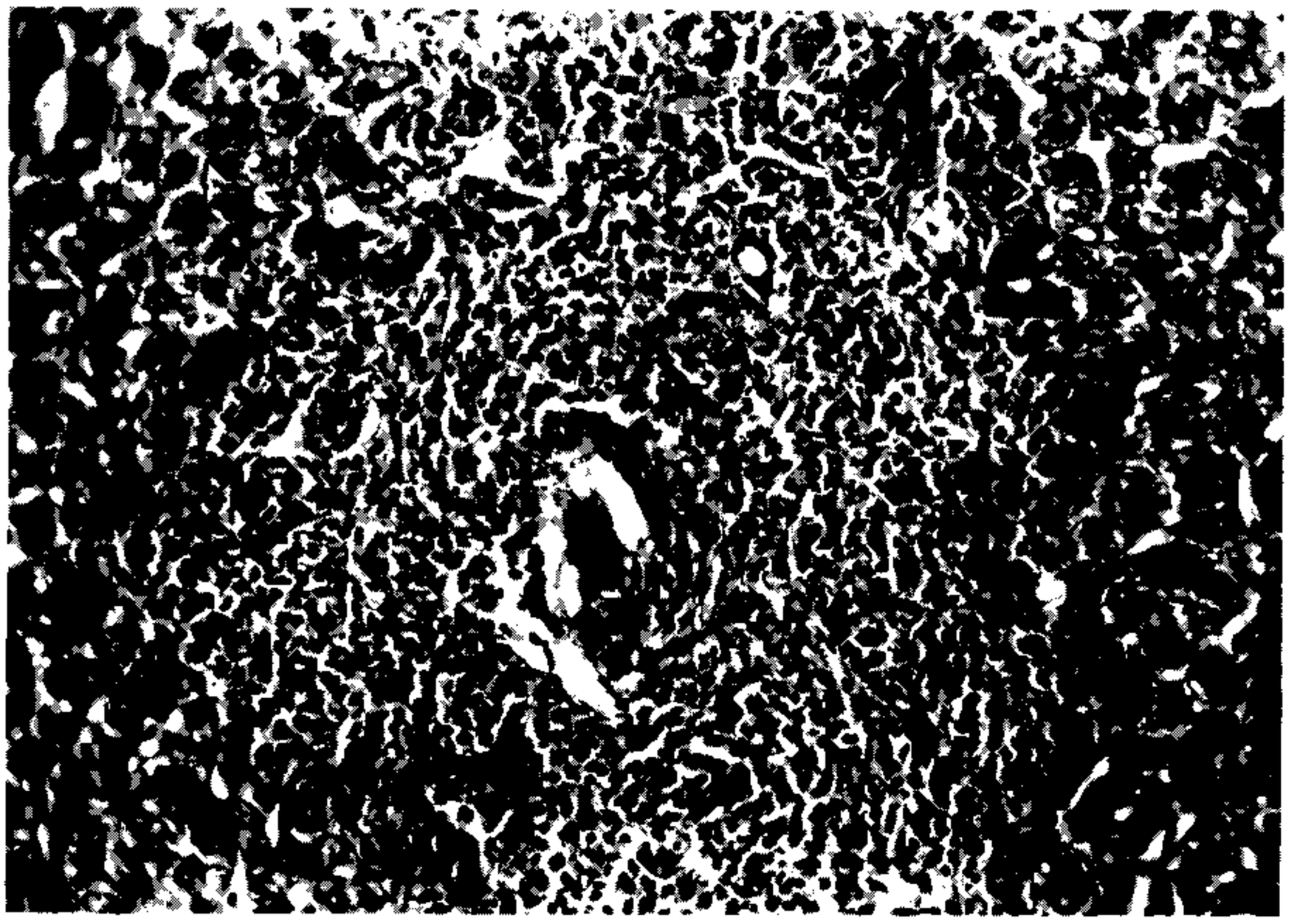

Fig. 3: a large granulomatous lesions around a mature *Schistosoma mansoni* egg in the liver of a mouse at the 53rd day of infection. There is a central area of purulent necrosis, a ragged periphery and many eosinophils interspersed with macrophages, a typical aspect for the acute phase of schistosomiasis. H. & E., X 150.

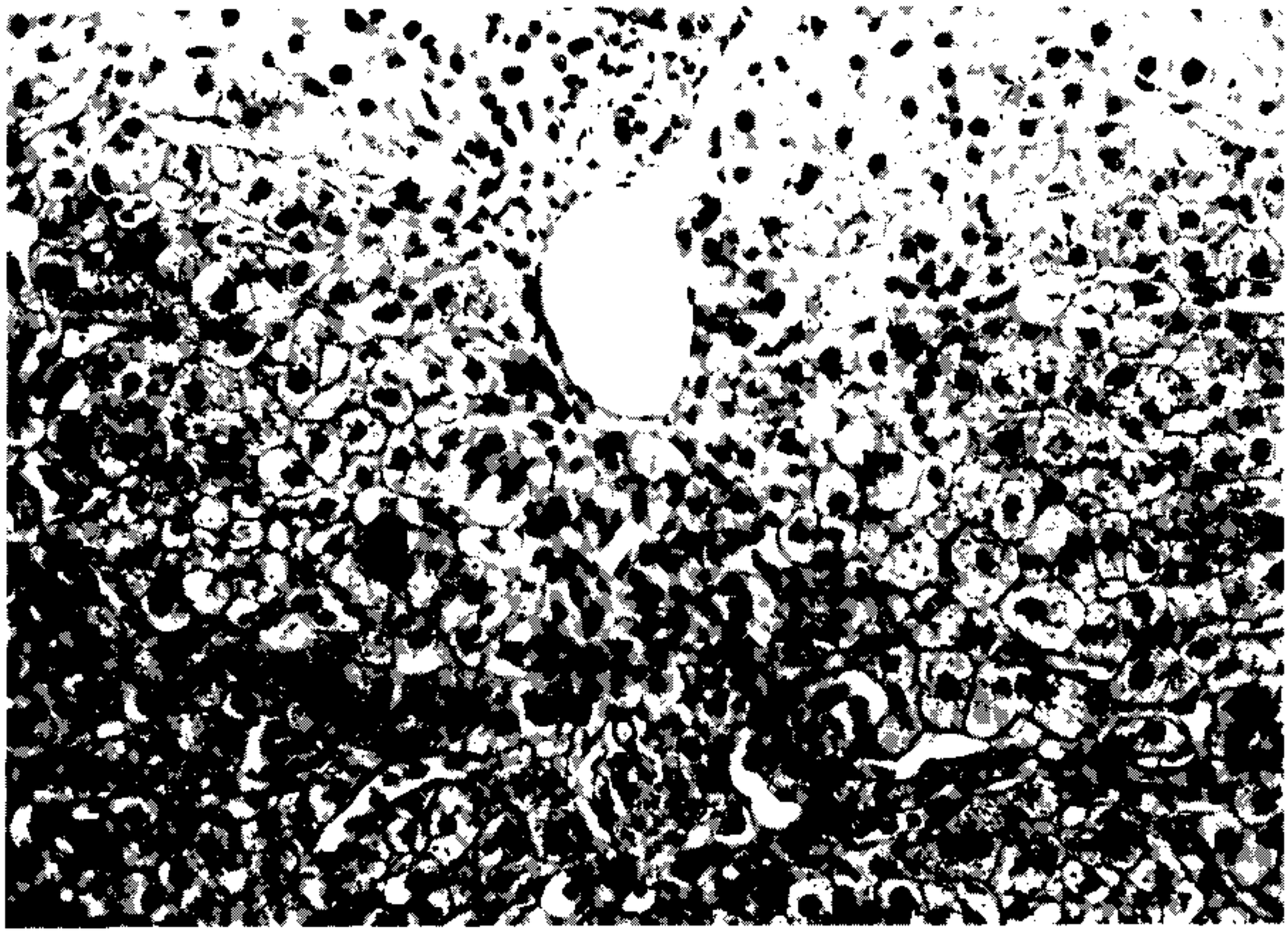

Fig. 4: reactive hepatitis in acute schistosomiasis of the mouse. Central portion of the hepatic lobule showing mobilization of Kupffer cells and focal parenchymal accumulation of mononuclear cells (single cell necrosis). H. & E., X 150.

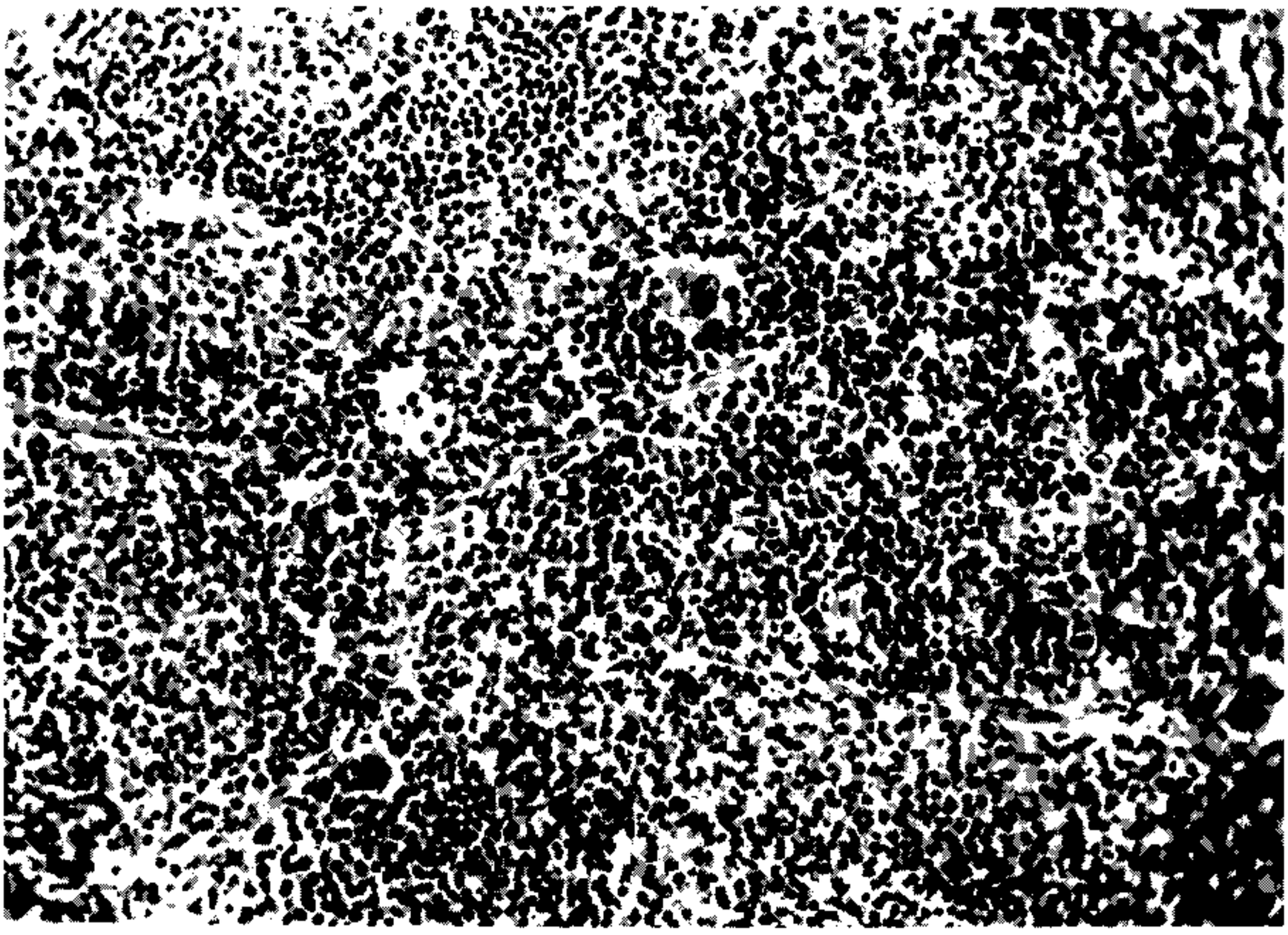

Fig. 5: marked proliferation of basophilic cells in the red pulp of the spleen during acute schistosomiasis. A portion of the hyperplastic white pulp is seen at the superior right angle of the picture. H. & E., X 100.

Histologically there were no evident differences between the lesions present in the liver and spleen of treated and untreated animals, up to the 6th day after treatment. At least it was not possible to recognize to which group the cases belonged just by looking at the microscope slides. Therefore, the lesions in both groups are described together: hepatic granulomas around mature eggs were large, with a central area of necrosis or a mixture of necrosis with many eosinophils; peripheral zone between liver parenchyma and inflammatory cells was irregular and blurred; eosinophils and lymphocytes predominated over macrophages in periovular granulomas (Fig. 3). Away from the granulomas, the liver exhibited hypertrophy and hyperplasia of Kupffer cells as well as isolated liver-cell necrosis with focal infiltration of eosinophilic and mononuclear cells (Fig. 4). Portal infiltration with numerous eosinophils and some macrophages, lymphocytes and plasma cells was an outstanding feature and did not seem to be always related to periovular granulomas.

In the spleen there was amplification of germinal centers, in which macrophages were seen engulfing nuclear debris and presenting signs of mitotic activity. The most remarkable alterations occurred in the red pulp, which was markedly congested and enlarged, with numerous foci of proliferating basophilic cells, probably immunoblasts, and some other more differentiated elements of the plasma cell line. These foci of accumulation of basophilic cells tended to become quite prominent in some cases, especially at one and two weeks following the onset of oviposition (Fig. 5).

However, on the 6th day, lesions caused by dead and disintegrating worms begun to appear within small and medium sized portal veins. Some few male worms scaped destruction and could appear preserved, side by side with others in advanced disintegration (Fig. 6). Such findings did permit the identification of slides as belonging to animals of the treated group. From then on, there was no difficulty in separating the groups by looking at the microscopic slides. Starting from the 8th day after treatment, signs of reactive hepatitis subsided almost completely, the granulomas begun to shrink and their periphery became discrete and fibrotic, while the granuloma cellularity decreased and fibroblasts and macrophages were seen to predominate over the eosinophils (Fig. 7). Lesions around dead worms also underwent fibrotic replacement and progressive shrinkage, sometimes with the addition of calcification.

After decoding the slides and assessement of the results, a second through examination of the slides was made, aiming at a close comparative study of the two groups, especially concerning the earliest stages at the 2nd, 3rd and 6th days after treatment, in order to detect any histological signs of enhancement of the inflammatory, vascular and degenerative changes. Results were essentially negative on this regard, although some sections of the liver, belonging to treated animals, could show an impressive picture, with several focal lesions around disintegration worms, vascular obstruction, reactive hepatitis, focal ischemic necrosis and portal accumulations of eosinophils. However such changes were focal and seen only occasionally with such degree of severity.

A striking change seen in the treated animals was the early disintegration or even the disappearance of the miracidium within the egg shell. As early as on the 3rd day this phenomenum could be observed and it was more evident thereafter. Inflammatory and degenerative changes around such eggs could persist for some time as acute and severe as in the granulomas of non-treated controls, which were centered with eggs containing well preserved miracidia (Fig. 8).

DISCUSSION

Curative treatment of acute schistosomiasis in mice, with a combination of two drugs (oxamniquine plus praziquantel), although resulting in intrahepatic focal vascular lesions caused by dead and disintegrating worms did not cause any apparent enhancement of the general lesions present in the liver and spleen (reactive hepatitis and splenitis), nor in periovular granulomas. Six days after treatment the lesions started to ameliorate and that tendency became quite evident subsequently.

The difference in survival rate for the two experimental groups was remarkable: while only one out of 50 animals in the treated group died espontaneously during the time of the experiment (12 weeks following cercarial exposure), 16 out of 30 non-treated infected controls had died by the end of the 9th week of infection.

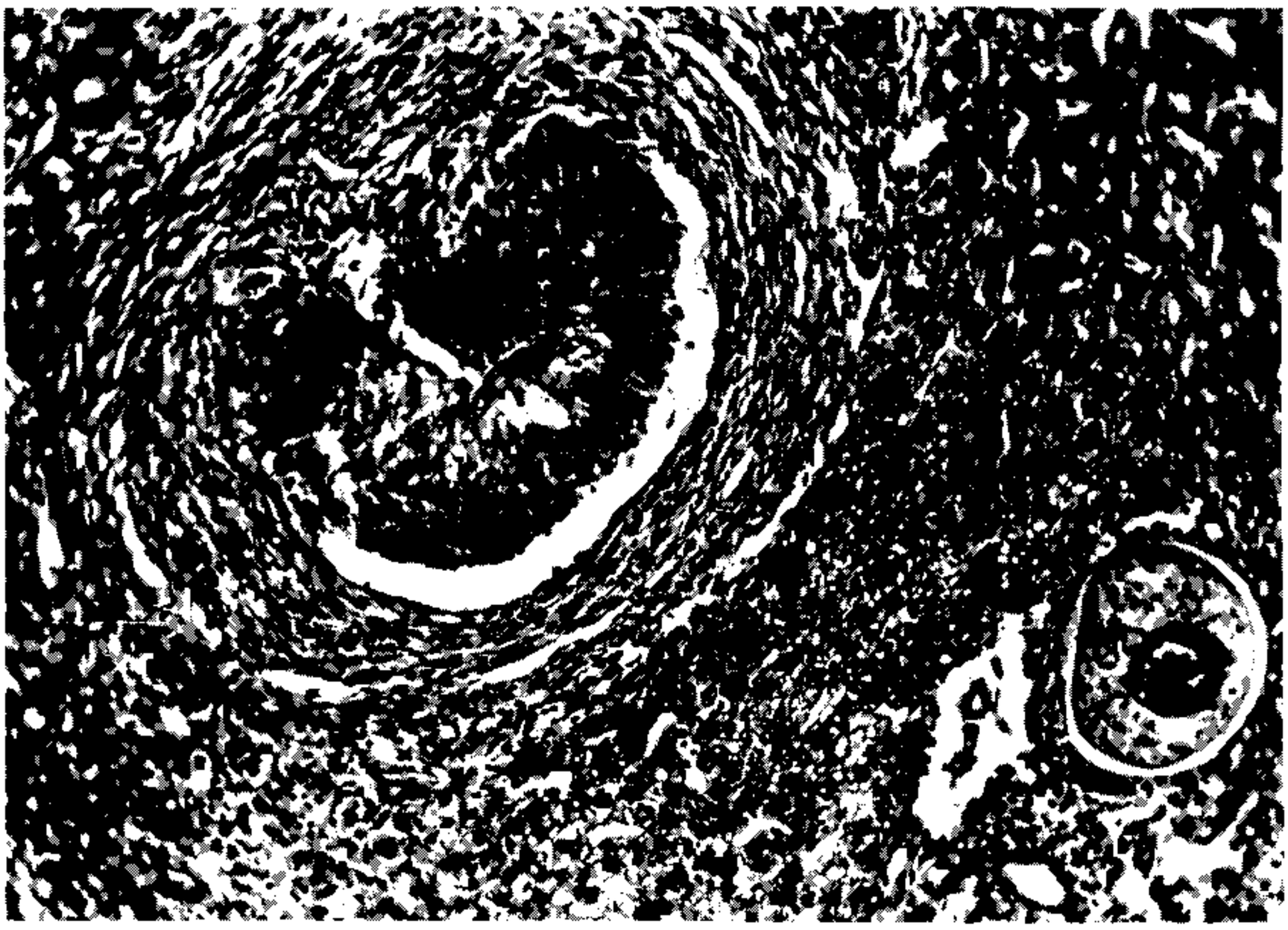

Fig. 6: liver of a mouse treated during the acute phase of schistosomiasis. There is a disintegrating worm already surrounded by a fibrotic ring and nearby a male worm which is well preserved. H. & E., X 150.

Fig. 7: an involuting periovular granuloma in the portal space of a mouse 20 days after treatment. Liver parenchyma is clean, with no signs of reactive hepatitis. H. & E., X 100.

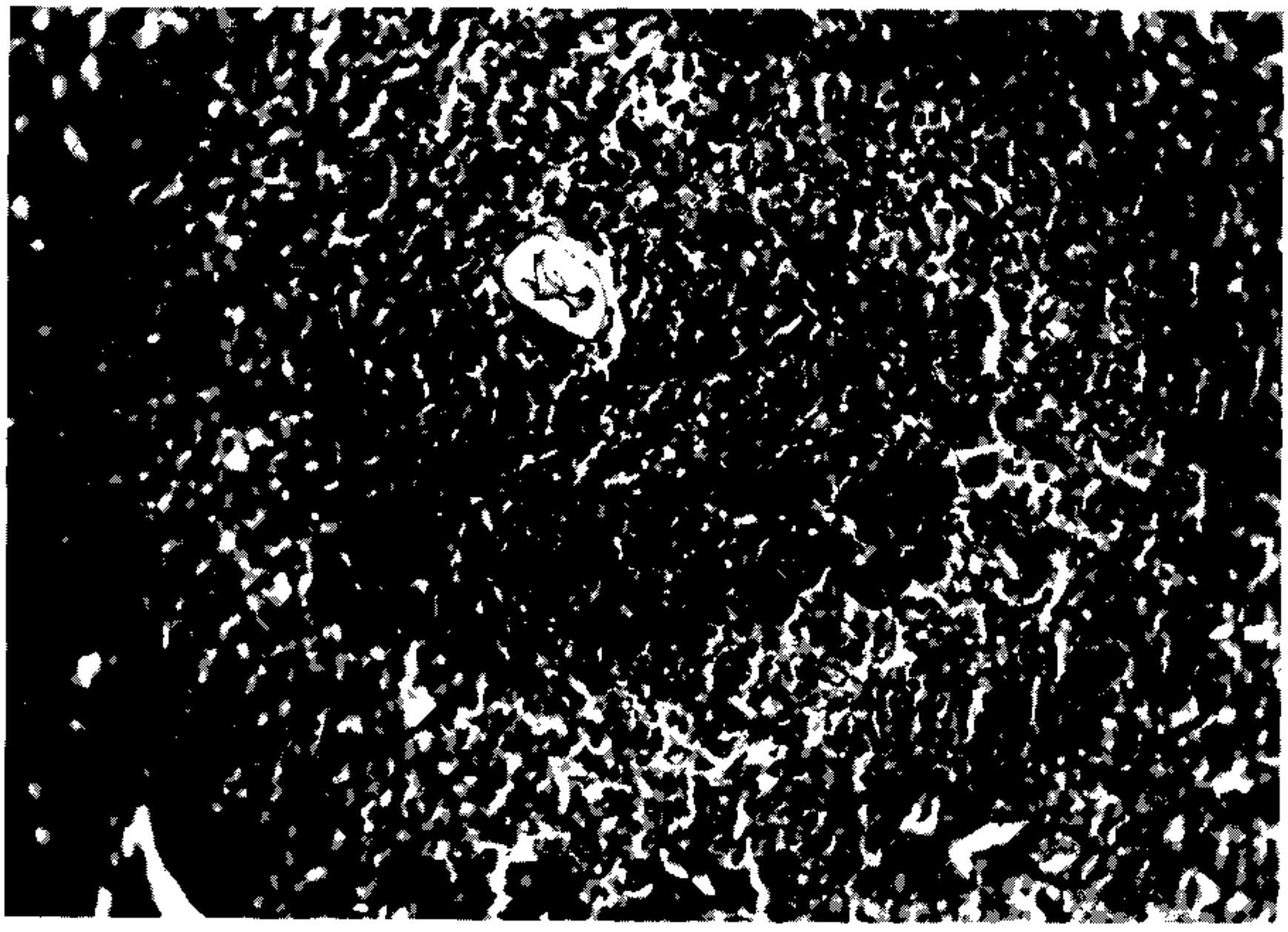

Fig. 8: three days after the beginning of treatment the egg in the center of the granuloma shows only a miracidial shadow, but the inflammatory and degenerative changes remain severe. H. & E., X 150.

Therefore, the findings here presented give no support to the assumption that treatment of acute schistosomiasis can provoke the worsening of the host lesions. As a matter of fact such worsening has never been documented in humans. On the contrary, patients with acute toxemic schistosomiasis have benefited from specific treatment (Ferreira et al., 1966), even when clinical presentation appeared extremely severe (Neves et al., 1972).

Histological changes in the spleen were more difficult to be comparatively evaluated, since the reactive changes both in the white and red pulps seemed to persist for longer time than the hepatic changes and to vary according to the area examined. However, the gradual decrease of spleen weight after treatment, as seen in Fig. 2, leaves no doubt that the splenic reaction gradually subsided in the treated animals.

There seems to be a relationship between the presence of adult worms and the size and cellular composition of periovular granulomas. This was first noted during studies with *in vitro* granulomas (Doughty & Philips, 1982). It was then seen that when the worms were present in the culture medium, the granulomas were larger and with more compact cellular arrangement. In mice treated with drugs (hycanthone and oxamniquine) that rapidly kill all worms, but do not affect directly the eggs (Reis & Andrade, 1987a), changes in size and cellularity in periovular granulomas occurred in the first few days after treatment (Andrade & Grimaud, 1986).

In the present investigation, the early granulomas did not seem to change even when the centrally located eggs presented with advanced signs of miracidial disintegration. Praziquantel is known to cause rapid destruction of miracidia (Matsuda et al., 1983; Reis & Andrade, 1987b).

Since the combination of drugs used (oxamniquine and praziquantel) killed the worms gradually and failed sometimes to erradicate all male worms, the present findings give support to the idea that the presence of live worm can influence the morphology of periovular granuloma.

During acute schistosomiasis signs of reactive hepatitis are prominent and consist of single-cell necrosis, mobilization of Kupffer cells and portal infiltration with inflammatory cells,

especially eosinophils. Such changes have been noted both in man (Diaz-Rivera et al., 1956; Bogliolo & Neves, 1965) and in experimental animals (Andrade & Azevedo, 1987). However, especially in the latter, the doubt whether such non-specific changes are really due to schistosomiasis has always remained. The gradual disappearance of the reactive hepatitis seen now in the treated animals and its persistence in the controls stands as a strong argument in favor of its relationship with the schistosomal infection.

RESUMO

Tratamento da esquistossomose aguda experimental – Foi utilizado um modelo de esquistossomose aguda do camundongo para testar se o tratamento curativo da parasitose nesta fase poderia produzir uma exacerbação das lesões hepáticas e esplênicas, em virtude da destruição maciça de vermes e ovos no interior do sistema porta.

Camundongos infectados com 50 cercárias do *Schistosoma mansoni* foram tratados no 50º dia da infecção por uma combinação de oxamniquine e praziquantel e submetidos a exames histopatológicos seqüenciados desde o 2º até o 45º dia após o tratamento.

Muito embora tenham sido encontrados lesões focais intensas causadas por vermes mortos no interior do fígado, não foi encontrada qualquer evidência de agravamento das lesões gerais (hepatite reacional e esplenite, ou nos granulomas periovulares) quando se fez comparação com um grupo controle de animais não tratados. Dos 50 animais tratados durante a fase aguda da esquistossomose apenas um morreu espontaneamente, enquanto no grupo controle 16 de 30 animais morreram antes do fim do experimento.

A presente investigação indica que o tratamento curativo durante a fase aguda da esquistossomose não agrava as lesões gerais desta fase no fígado e no baço e contribui para o desaparecimento gradual das lesões já a partir do 6º dia após a terapêutica.

Palavras-chave: esquistossomose aguda – quimioterapia – lesões hepáticas e esplênicas – *Schistosoma mansoni*

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