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# "IN VIVO" KINETICS OF EOSINOPHILS AND MAST CELLS IN EXPERIMENTAL MURINE SCHISTOSOMIASIS

H. L. LENZI, A. C. L. SOBRAL[+] & J. A. LENZI

Instituto Oswaldo Cruz, Departamento de Patologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil

*During the schistosomiasis infection there is a "dance of the cells", varying from site to site and related to the time of infection. 1 – Eosinophil levels exhibit a bimodal pattern, with the first peak related to the egg deposition and maturation and increased Kupfferian hyperplasia; the second peak precedes the death of some adult worms; 2 – The peritoneal eosinophilic levels are inversely proportional to the blood eosinophilic levels; 3 – Eosinopoiesis in the bone marrow begins at day 40, reaching the highest levels at day 50 and coincides with hepatic eosinophilic and neutrophilic metaplasia; 4 – Peritoneal mast cell levels present a bimodal pattern similar to the blood eosinophils, and inverse to the peritoneal eosinophils. They also show a cyclic behaviour within the hepatic and intestinal granulomas. Integral analysis of the events related to the eosinophils in the blood, bone marrow, peritoneal cavity and hepatic and intestinal granulomas allows the detection of two important eosinophilic phases: the first is due to mobilization and redistribution of the marginal pool and the second originates from eosinophilic production in the bone marrow and liver. The productive phase is characterized by an increase in the number of eosinophils and monocyte/macrophages, and a decrease in neutrophils and stabilization of megakariocytes and erithroid lineages.*

During *Schistosoma mansoni* infection there is an increase in the number of eosinophils both in the circulation, in the tissues, and in the peritoneal cavity (Colley et al., 1973; Mahmoud et al., 1975; Borojevic et al., 1985). This eosinophilia is not an isolated phenomenon, and can in human and experimental schistosomiasis be associated with neutropenia (Borojevic et al., 1983; Borojevic et al., 1984). Although many mechanisms were described to explain the stimulation of eosinophil production (Phillips & Colley, 1978; Bass, 1982), and considerable progress has been achieved in characterizing cytokine soups that act on eosinophils (Sanderson et al., 1985) relatively little is known about the kinetics of these cells, their balance with other cellular lineages, and its haematopoietic inductive microenvironment. On the other hand, the classical concept that mast cells and basophils may be involved in resistance to schistosomes only by recruitment and local accumulation of effector cells, such as eosinophils, and by activation of these effector leucocytes (Askenase, 1980) is changing due to the observation that mast cells are heterogeneous and show a diversity of important functions (Lee et al., 1986). At least in humans, there is also direct evidence for the clonal derivation of basophils/mast cells from multipotent progenitors (Leary & Ogawa, 1984; Denburg et al., 1985) and for a common basophil-eosinophil progenitor in peripheral blood and bone marrow (Denburg et al., 1985), turning the study of these cells more interesting and complex.

While there are several isolated reports describing the eosinophilia seen in experimental and human schistosomiasis, there are no detailed data on the kinetics of eosinophils in relation to the changes in the tissues, on the "dance" of these cells in different compartments of the organism, and on their relation to the dynamic participation of the mast cells during the infection.

For these reasons, we employed the murine schistosomal model during the acute phase of the infection, to study the following subjects: a) the comparative kinetics of the eosinophils in different tissues compartments; b) the kinetics of the mast cells in the tissues and peritoneal cavity; c) the inter-relationships of the

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Reprint requests to H. L. Lenzi.

eosinophil and mast cell responses with the pre and post-postural changes in the tissues.

## MATERIAL AND METHODS

Four sets of experiments were performed in outbred albino Swiss mice of both sexes, which were killed on day 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 70 after percutaneous exposure to 70 cercariae of the Belo Horizonte strain of *Schistosoma mansoni*. The cercariae were obtained from laboratory raised and infected *Biomphalaria glabrata*. The animals were infected when they were 5 days old, and in all experiments, 6 animals were killed every day. The same number of matched control animals were used. Blood was obtained from the axial plexus, after anesthesia by ether. Absolute leucocyte counts were done using Turck's fluid as diluent in Newbauer chamber, while absolute eosinophil counts were done using Dıscomb's fluid in a Fuchs-Rosenthal chamber. The total nucleated cell and absolute eosinophil counts in the bone marrow were done through a modification of the method described by van Furth & Cohn (1968). The right femurs of the mice were cut at both ends in the region of the metaphyses, and the bone marrow was flushed out with PBS – pH 7.2. The cell suspension was dispersed by repeated gentle aspiration in a pipette. The cells from the left femur were collected for electron microscopy (data not shown).

The peritoneal cells were collected according to conventional procedures, and were washed like the bone marrow cells by centrifugation at 4 °C, suspended in a defined volume with PBS – pH 7.2, counted and cytocentrifuged.

During necropsy, tissue samples were taken from the liver, intestines, spleen, lungs, kidneys, heart, thymus, lymph nodes and bone marrow. The specimens were fixed in formalin-Millonig (Carson et al., 1973), and embedded in paraffin. Sections were stained by the following methods: haematoxylin and eosin; PAS-Alcian blue (PAS-AB), pH 1.0 and 2.5; Lennert's Giemsa; picrosirius (for polarization microscopy) (Junqueira et al., 1979) and Gomori's silver reticulin. The bone marrow sections were decalcified with buffered EDTA.

The mast cells were counted in 10 granulomas with a central egg from hepatic and intestinal rolled sections (Lenzi & Lenzi, 1986), and stained with PAS-AB pH 1.0 and Lennert's Giemsa. Mast cells in the intestinal muscular layers and hepatic mitosis were determined in ten randomly selected microscopic fields (Leitz, 400X). The eosinophilic metaplasia was evaluated in the periphery of 10 hepatic granulomas, stained by Lennert's Giemsa.

## RESULTS

Absolute numbers of peripheral blood leucocytes and eosinophils displayed a distinct bimodal pattern with the first transient eosinophilia seen at 25 or 30 days and the second at 50 or 55 days after infection (Figs 1 and 2).

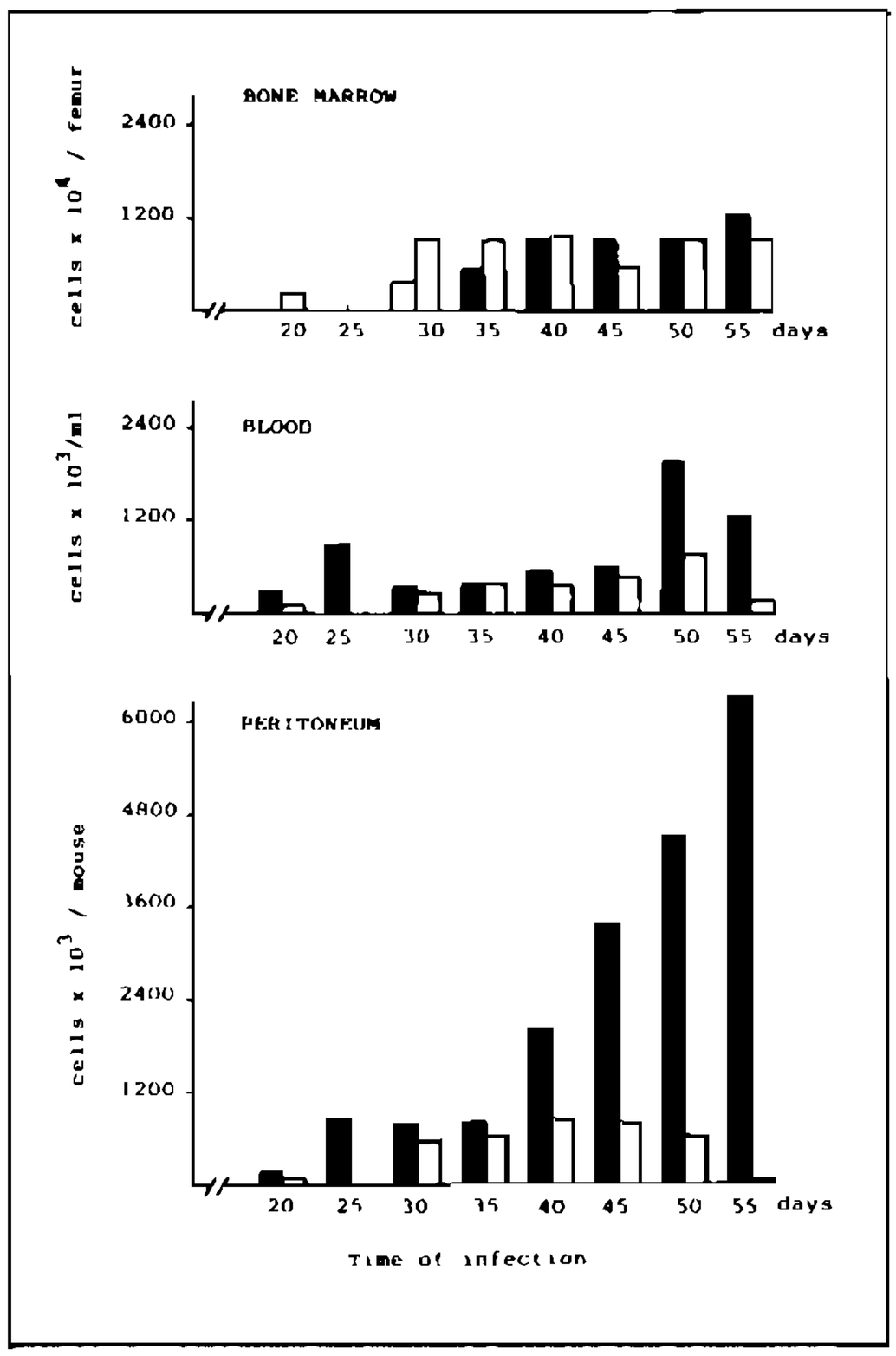



Fig. 1: mean absolute peripheral blood leucocytes and total peritoneal and bone marrow nucleated cell counts in normal (□) and in mice infected with *Schistosoma mansoni* (■) (Exp. II).

The total number of leucocytes in the peritoneal cavity showed a considerable increase from the 25th until the 60th day of infection, with posterior reduction (Figs 1 and 3) while the number of eosinophils presented a cyclic

pattern, with the first peak antedating the first peak seen in the peripheral blood (Fig. 2). The number of mast cells also exhibited a bimodal pattern and an inverse relationship existed between mast cell and eosinophil numbers in peritoneal fluid (Fig. 4). The total nucleated cell counts in the bone marrow did not shown a significant change during the infection until the 55th day, when they increased for the first time in number (Fig. 1). The eosinophils, on the other hand presented an absolute and relative increase from 22 x $10^4$ (day 35) to 650 x $10^4$ cells/femur (day 55), and at the 55th day as much as 70 percent of the bone marrow cells were eosinophils (Fig. 2). Extramedullary eosinophilic granulocytopoiesis occurred in the periphery of hepatic schistosomal granulomas and in the hepatic parenchyma from the 40th day, becoming more intense on the 50th and the 60th days, following the same pattern observed in the bone marrow (Fig. 5). The myeloid foci were composed by myeloblasts, metamyeloblasts, ring cells and mature eosinophils.

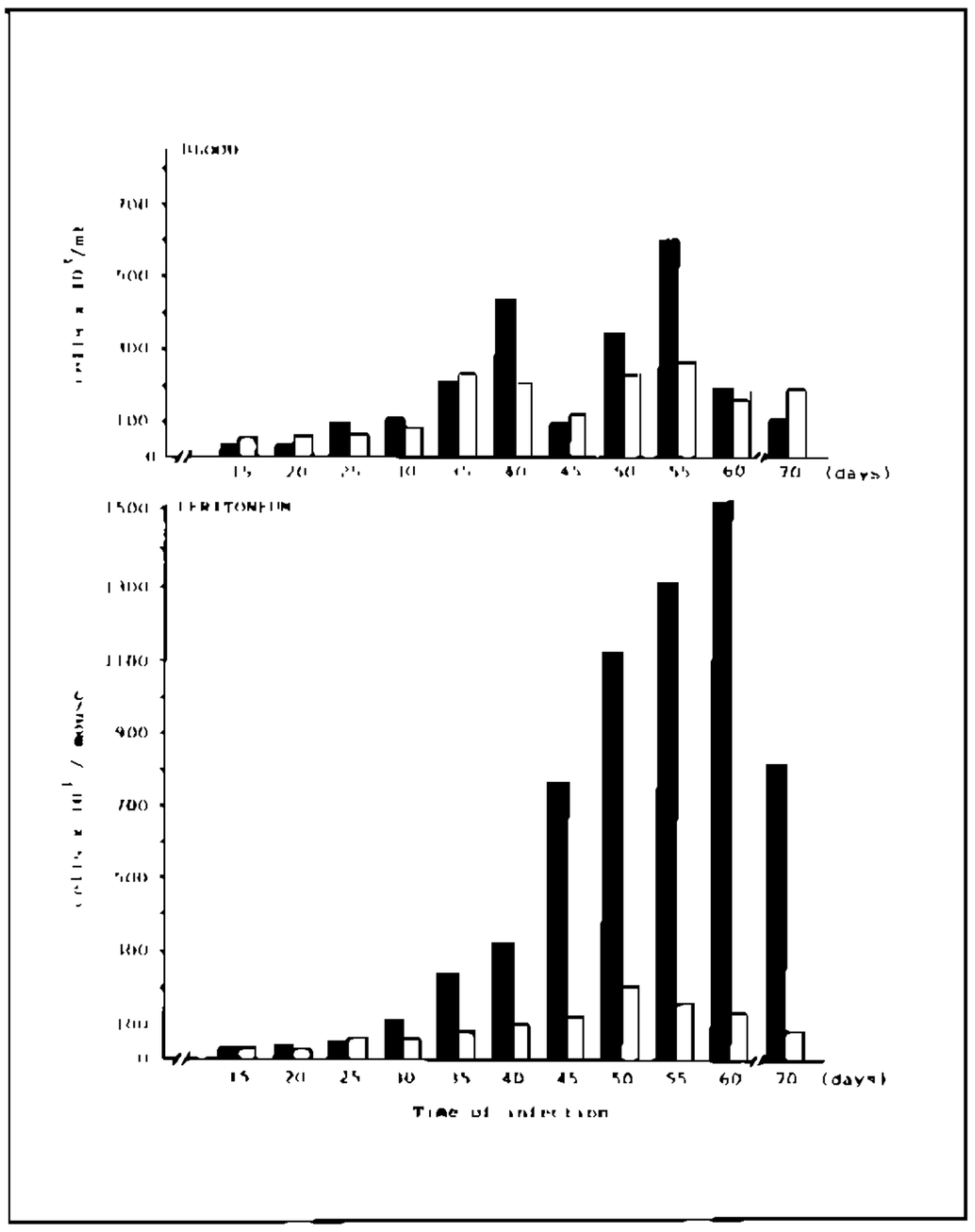



Fig. 3: mean absolute peripheral blood leucocyte and total peritoneal cell counts in normal (□) and in mice infected with *Schistosoma mansoni* (■) (Exp. I).

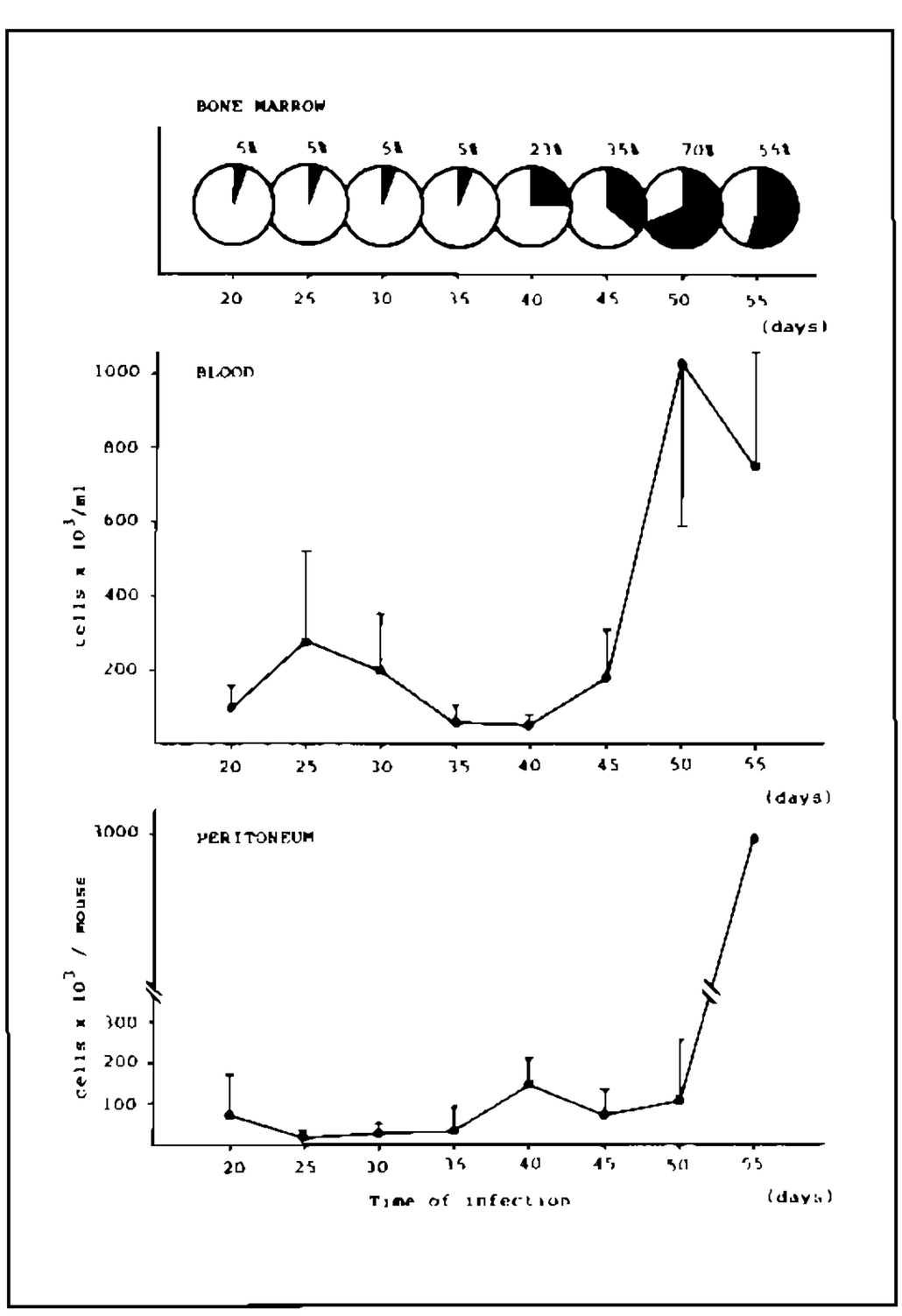



Fig. 2: eosinophil levels during murine infection with *Schistosoma mansoni* in three different compartments. Blood and peritoneum values are mean ± SD (n = 6). (Exp. II)

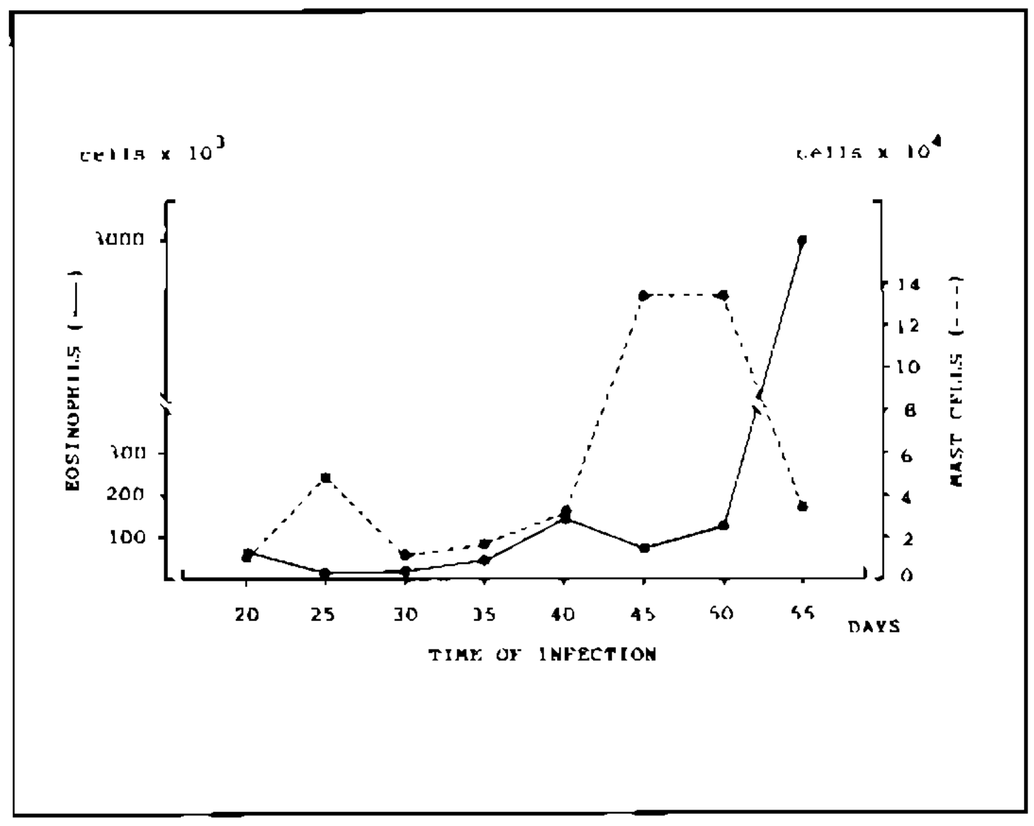



Fig. 4: mean of total peritoneal mast cells and eosinophils during murine schistosomiasis.

The blast cells were in direct contact with hepatocytes and intermixed with reticular and collagen type III fibers. At day 70, the metaplasic foci exhibited a predominance of mature cells over blast cells. Similar foci of neutrophilic metaplasia were also observed at days 50 and 60 post infection. Antedating and concomitant with the begining of the extramedullary granulocytopoiesis a large number of hepatic cells in mitosis were counted (Fig. 5).

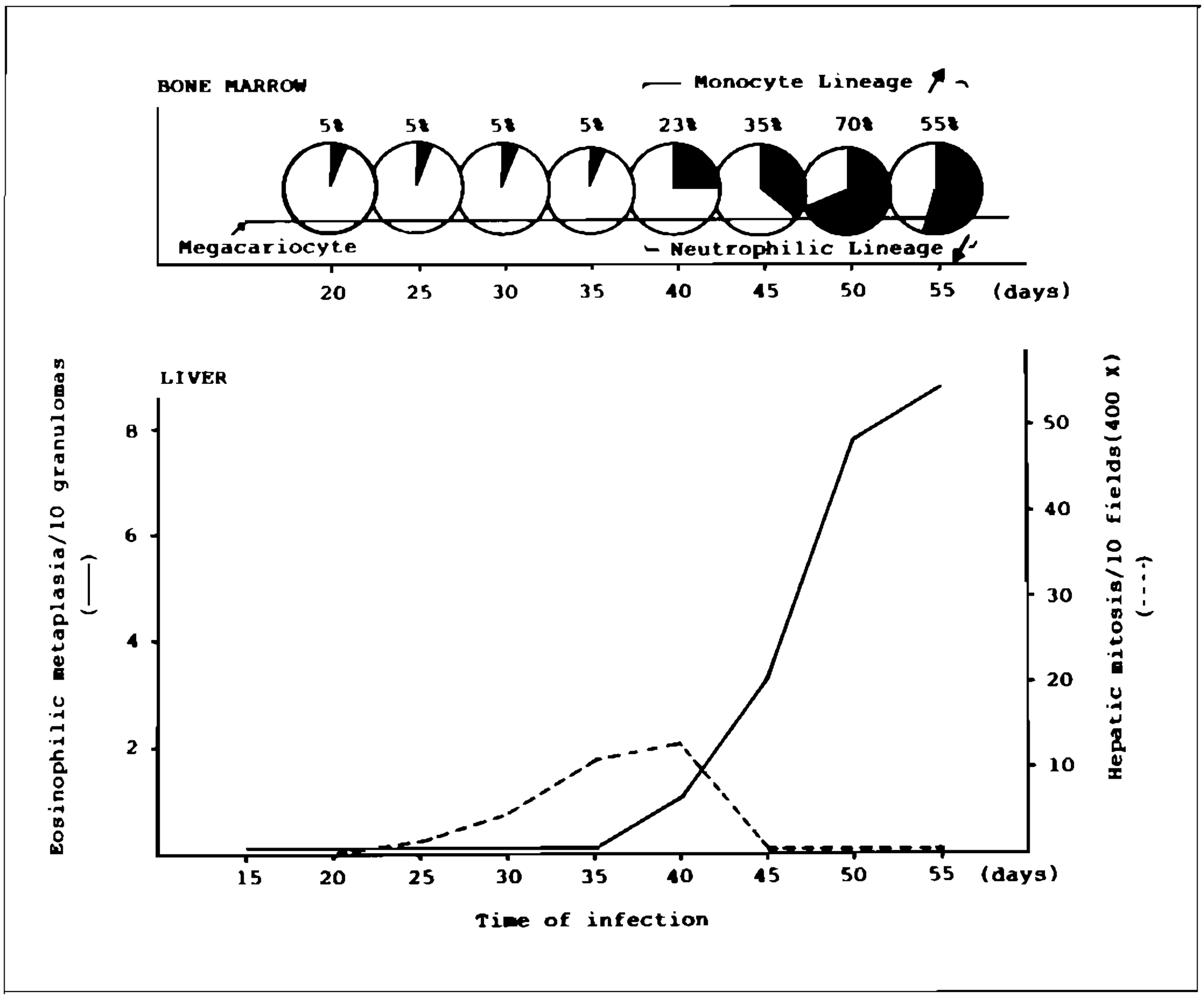



Fig. 5: parallelism between eosinophil metaplasia in the liver and eosinopoiesis in the bone marrow, and alterations in other cellular lineages. Period of hepatic mitosis.

Mast cell numbers also presented a bimodal pattern inside the hepatic and intestinal granulomas while they showed an exponential increase in the muscular layers next to the granulomas (Fig. 6). At day 70 of infection eosinophils were observed in the subserosa layer of the intestines causing sometimes focal eosinophilic serositis, with dropping of eosinophils to the peritoneal cavity (Figs 7 and 8).

The principal parasitic occurrences and host tissue reaction in the liver and intestines, and their relationship with the blood and peritoneal eosinophils are shown in the Figs 11, 12 and 13.

## DISCUSSION

In our experiments with acute schistosomiasis, the peripheral blood eosinophilia displayed a bimodal pattern, as described before (Colley et al., 1973; Mahmoud et al., 1975) showing two distinct waves.

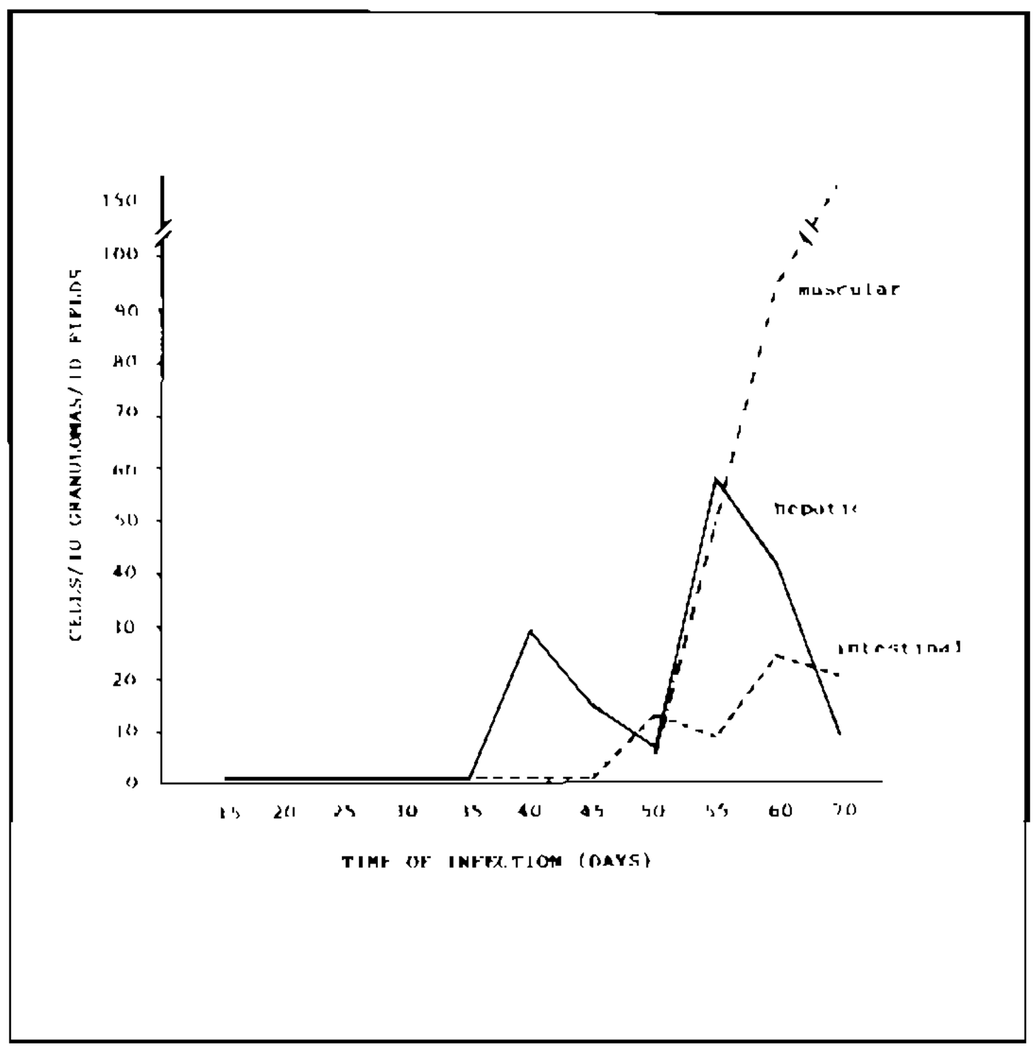
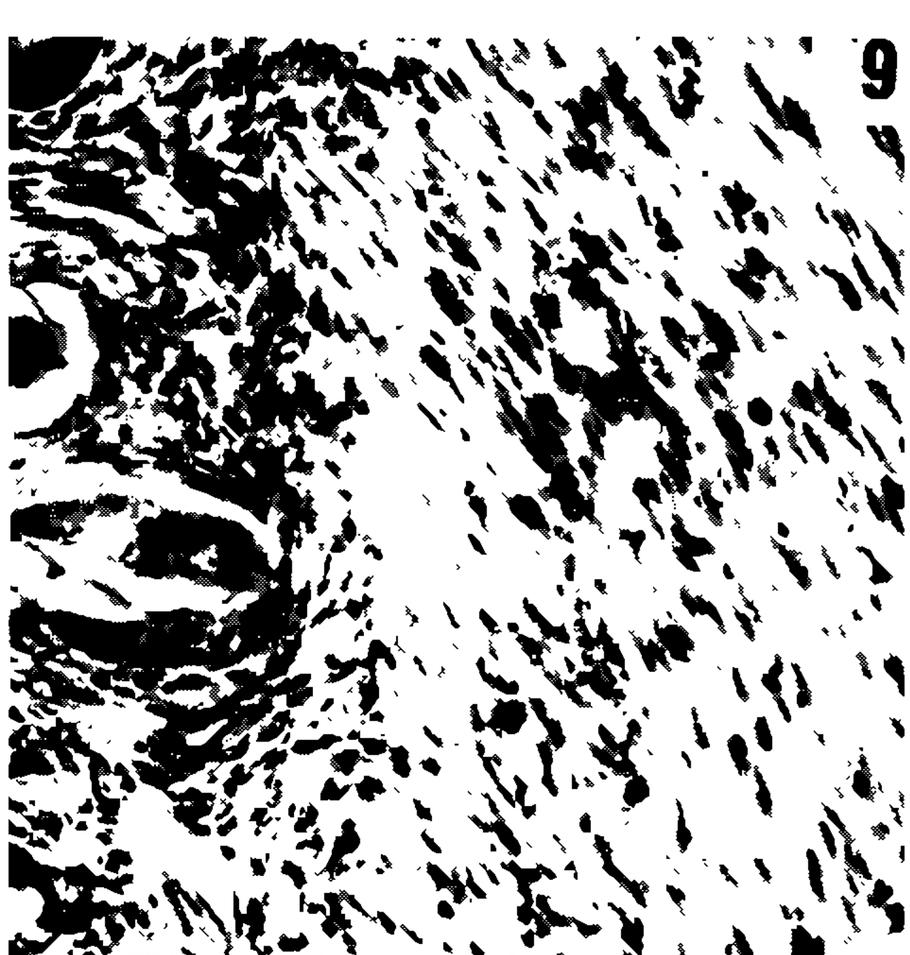



Fig. 6: number of mast cells in hepatic and intestinal granulomas and in the muscular layer of the intestines of mice infected with *Schistosoma mansoni*.





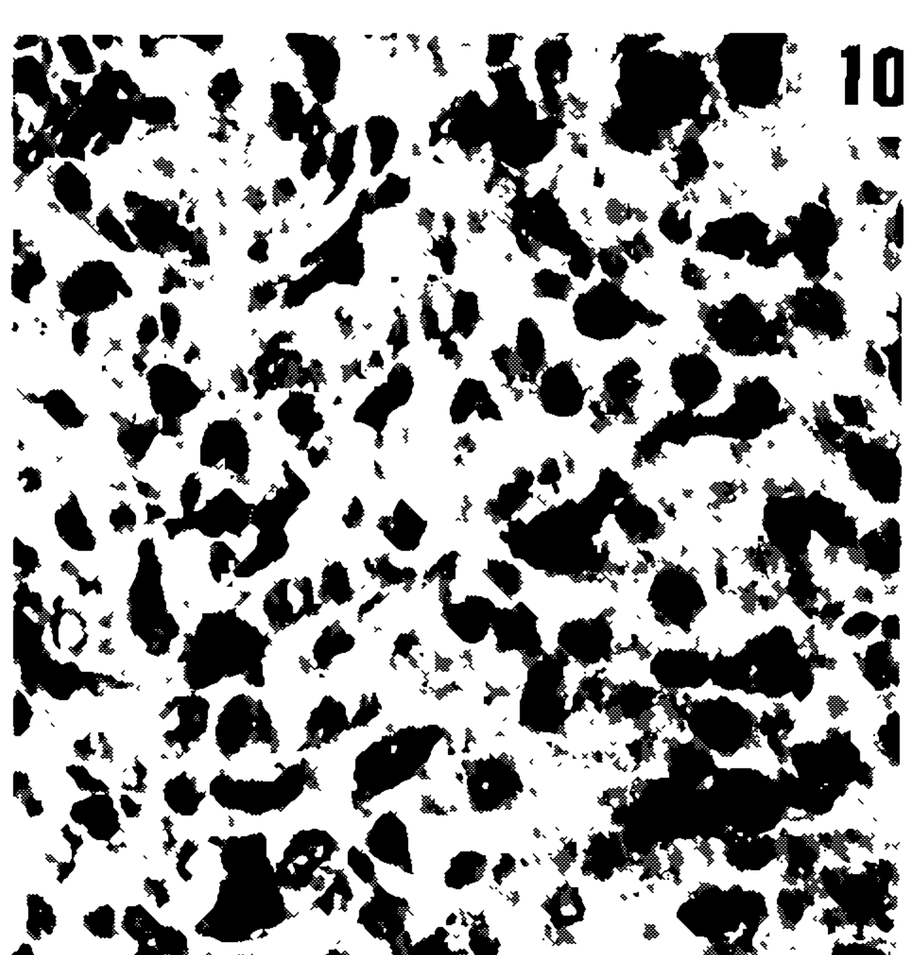




Fig. 7: focal eosinophil serositis and eosinophils permeating the muscular layers close to granulomas (Infection time: 70 days – Exp. I) Lennert's Giemsa. X260. Fig. 8: eosinophil and monocytic infiltrate in the subserosa covered by hypertrophic mesothelial cells. Rows of eosinophils in the intestinal muscular layer and dropping of eosinophils into the peritoneal cavity (→). (Infection time: 50 days – Exp. I). Lennert's Giemsa. X500. Fig. 9: mast cells in the intestinal muscular layer and granulomas in the submucosa. (Infection time: 70 days – Exp. I). Lennert's Giemsa. X200. Fig. 10: detail of the mast cell infiltrate in the intestinal muscular layer (Infection time: 60 days – Exp. I). Lennert's Giemsa. X640.

The development of the first peripheral blood eosinophil wave was coincident with egg deposition and maturation in the liver, while in the peritoneal cavity the rise in eosinophils antedated the blood eosinophilia and the detection of egg deposition in the intestinal tissue (Figs 2, 11 and 12). We do not know yet how to explain this event, which occurs at the end of the maturation process of the worms and at the begining of their migration from the portal vein to the mesenteric vessels.

The second peripheral blood eosinophilia can be related at least partially to the release of antigens from dead adult worms (Fig. 11). This hypothesis is reinforced by the occurrence of peripheral eosinophilia around 10 days after specific treatment of patients infected by *S. mansoni* (Cunha & Cançado, 1970). However, this and other subsequent peaks depend on a variety of antigenic influences and complex pathogenic mechanisms, not yet elucidated. Colley et al. (1973) showed that peripheral blood eosinophilia remained elevated during a period when lymphocyte reactivity declined, and eosinophilia followed closely the rise and fall of heat-labile reagin-type antibody levels. The T-lymphocyte depletion had little or no effect on the first eosinophil peak but either markedly impaired or completely abolished the second eosinophil rise, which indicates that at least two quite different mechanisms underlie the development of the two episodes of eosinophilia observed during the course of primary infection with *S. mansoni* (Fine et al., 1973). It appears that the first eosinophil episode is dependent on eosinophil releasing factors (Spry, 1971), whose activities are mainly directed at mobilizing the stored mature eosinophils in the bone marrow or in unknown marginal pools. This fact could not easily be detected by the observation of the numbers of the bone marrow cells because it has been estimated, for example in the guinea pig, that for each eosinophil found in the circulation, 400 more reside in the marrow and another 300 in other tissues (Weller, 1984).

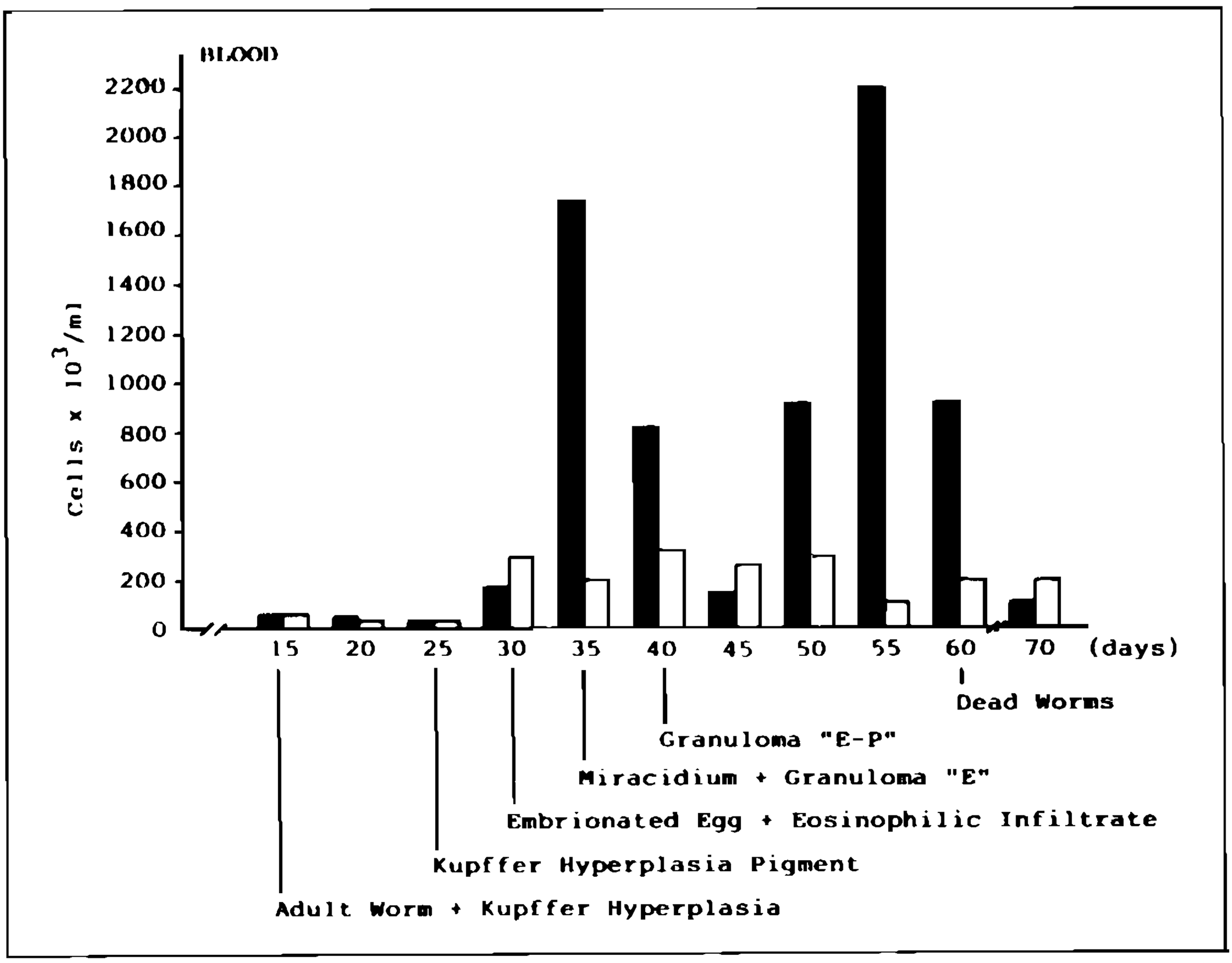



Fig. 11: relation of blood eosinophils and principal hepatic occurrences during murine schistosomiasis (Exp. I). Infected (■); control (□); E: exsudative granuloma; E-P: exsudative-productive granuloma.

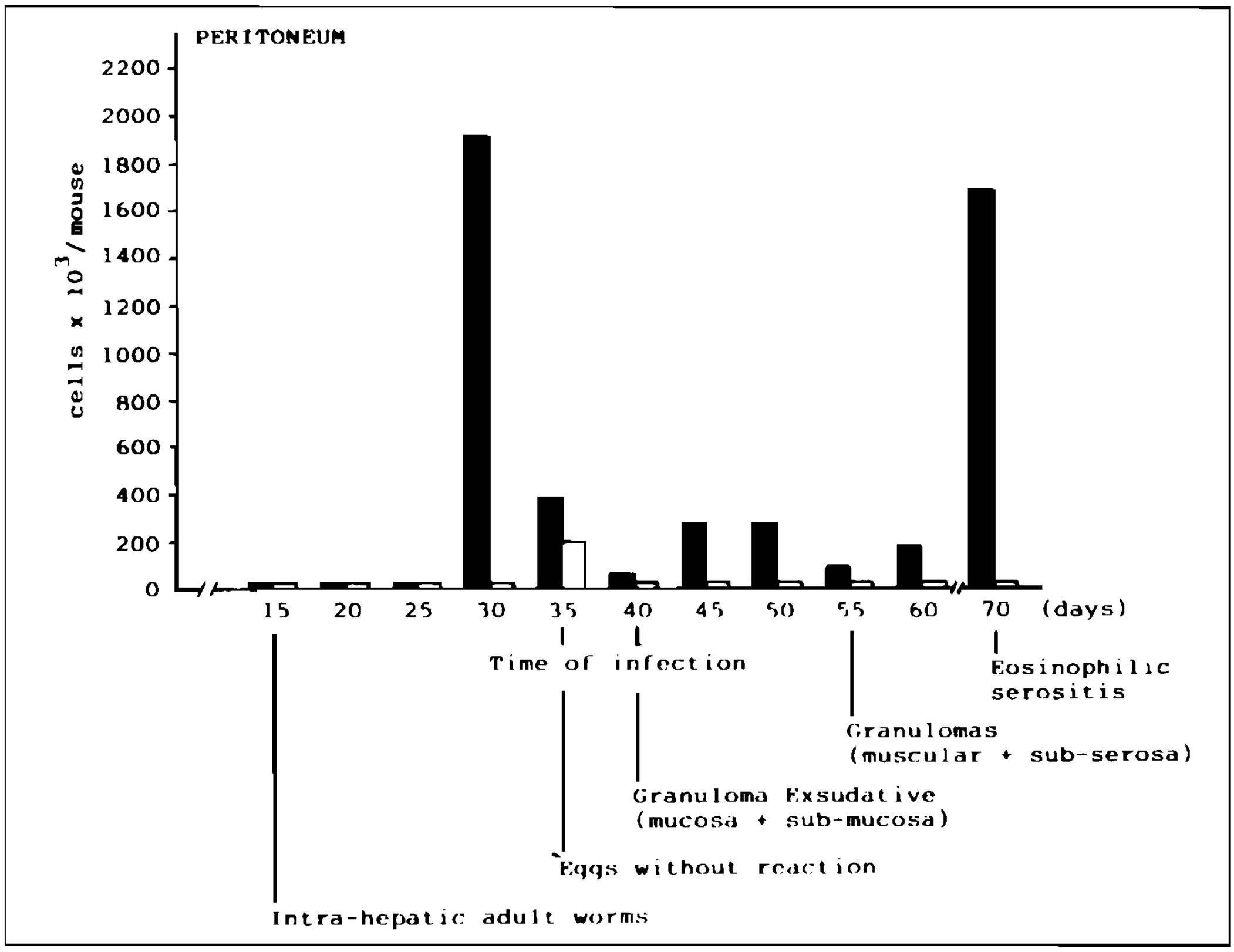



Fig. 12: relation of peritoneal eosinophils and principal intestinal occurrences during murine schistosomiasis. (Exp. I). Infected (■); control (□).

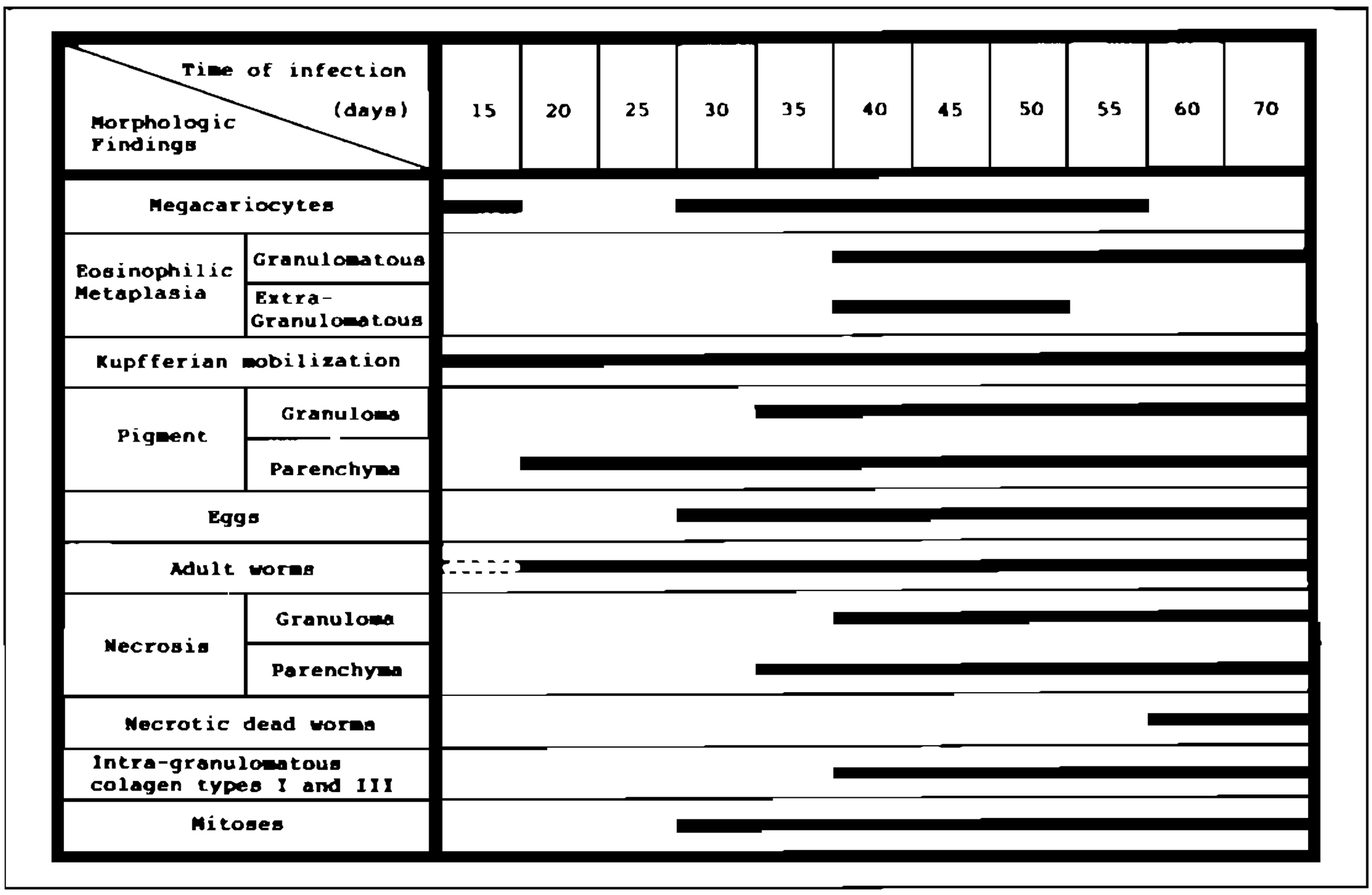



Fig. 13: appearance of the principal morphologic alterations in the liver during murine schistosomiasis.

Until the second episode or wave of peripheral blood eosinophilia, there was a temporal dissociation of tissue, blood, peritoneal and marrow eosinophil levels following the eosinophilogenic stimulus. This dissociation is depicted graphically in Fig. 2 in which a developing eosinophil infiltrate in the egg-granulomas is seen to induce a temporary blood eosinopenia (Fig. 11) that leads to subsequent marrow, then blood and then a more intense tissue and peritoneal eosinophilia. Therefore the eosinophilia in marrow lags behind that of blood, tissue and peritoneal cavity. Mahmoud et al. (1977) have demonstrated that specific acute depletion of eosinophils generates eosinophilopoietin, which is T-cell dependent.

The hepatic eosinophil granulocytopoiesis or metaplasia described by Goennert (1955), Grimaud & Borojevic (1972), Byran et al. (1978) and Borojevic et al. (1981) follows the same pattern observed in the bone marrow during the acute schistosomiasis (Fig. 5). The increase in hepatic mitosis before and during the begining of the eosinophil metaplasia suggests that some transformations in the hepatocytes are required to facilitate this *fetalization* of the hepatic tissues. The selective stimulation of eosinophilopoiesis and, more rare, of the neutrophilic lineage, could be achieved either by progenitor selection or through specific stimulation of less differentiated stem cells (Borojevic et al., 1981). One possible hypothesis is that Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) is bound to glycosaminoglycans in the supporting stroma of the bone marrow and hepatic granuloma and is then released in marrow and hepatic microenvironments, where it acts on nearby progenitors. Borojevic et al. (1981) considered the Kupffer-cell stimulation and hyperplasia as the responsibles for this extramedullary granulocytopoiesis. The discrepancy between a non-significant change in the total nucleated cell counts and the increase in the absolute and relative number of eosinophils in the bone marrow is indicative that eosinophils are competing with other cellular lineages (Figs 1, 2 and 5). In fact, together with the eosinophil increase in the bone marrow, we detected an increase in the monocyte lineage and decrease in the neutrophilic lineage. There were no significant changes in the erythroid and megacariocytic series (Fig. 5). Borojevic et al. (1983) have shown that sera from patients with schistosomiasis modified the proliferation of murine bone-marrow cells in soft-agar cultures. A total inhibition of neutrophilic differentiation was compensated for by a proportional increase in macrophage differentiation. According to these authors, the neutrophil-inhibitory activity of sera of patients with schistosomiasis may be responsible for the delayed *in vivo* maturation of neutrophils in the bone marrow and spleen of these patients. It has also been reported that eosinophils may suppress neutrophil production in an *in vitro* bone marrow culture system (Tebbi et al., 1980). It appears that during schistosomic infection there are multiple and complex interactions among the Colony Stimulating Factors themselves and different sequences or cascates of cytokines may occur. Interleukin-5 (eosinophil differentiation factor) appears to have the same activity as B-cell growth factor-2 (T-cell replacing factor). When added after primary stimulation of bone marrow with IL-3, IL-5 strikingly potentiates formation of eosinophilic colonies. By contrast, simultaneous exposure of bone marrow to IL-3 plus IL-5 results in mostly granulocyte-macrophage colonies. IL-1 not only stimulates production of CSFs, but may also increase the number of progenitor cells responsive to CSFs, via modulation of their CSF receptor (see meeting report on Hematopoietic Growth Factors by Groopman, 1987).

The mast cell levels also exhibited a cyclic pattern within the hepatic and intestinal granulomas (Fig. 6) and in the peritoneal cavity (Fig. 4). It appears that the first mast cell wave in the peritoneal cavity is T-cell independent, while the presence of these cells inside the granulomas are, at least partially, regulated by T lymphocytes. In fact, a relationship has been established between the thymus derived T-cells and the presence of mast cells within the schistosome egg granuloma (Epstein et al., 1979). Weinstock & Boros (1983), by passive transfer studies, have shown that the Lyt $1^+$ T-cell population may exert an inductive role on intra-lesional mast cell accumulation. This is consonant with a report that showed that cloned Lyt $1^+$ T-cells produced a factor that selectively induces the *in vitro* proliferation of mast cells (Nabel et al., 1981). The mast cells, via histamine, may participate in the regulation of the granulomatous inflammatory process (Weinstock et al., 1983). The fact that the number of eosinophils in the peritoneal fluid rises as that of the mast cells falls is an intrigant problem (Fig. 4). We do not know if this event

is centrally regulated within the bone marrow, or depends on different recruitment mechanisms of cells from the blood and tissue to the peritoneal fluid. Previous observations have shown that if, instead of the sudden changes caused by chemical histamine releasers, the mast cell damage is prolonged, as occurs when repeatedly washing out the peritoneal cavity of rats with saline or as a result of magnesium deficiency in rats (Hungerford & Karson, 1960), the number of eosinophils in the peritoneal fluid rises as that of the mast cells falls. Presumably, this is due to a continuous slow release of substances from the mast cells attracting the eosinophils (Parish, 1970). At least the last rise of the eosinophil numbers at the end of the experiment (Figs 2, 4 and 12) can be explained by the gradative deepening of the granulomas in the intestinal wall, causing an eosinophil serositis with dropping of these cells to the peritoneal fluid (Figs 7 and 8). In contrast the mast cells otherwise accumulate each time more within the muscular layers of the intestinal wall (Figs 6, 9 and 10) close to egg granulomas. Preliminary investigations show that intestinal smooth muscle is hyperresponsive after parasite-induced inflammation, but whether this relates to mast cell-neuropeptide axis remains to be studied (Shanahan et al., 1985). We do not know what is the importance of the "milk spots" in the regulation of the eosinophils-mast cells in the peritoneal cavity, because Parish (1970) observed that in mesenteric spreads from guinea-pigs dying of anaphylaxis after intravenous antigen, or killed after intraperitoneal injection of a very dilute solution of antigen, there was a tendency for eosinophils to accumulate around disrupted mast cells or around the small dense collection of macrophages and basophilic cells known as "milk spots". The present observations, together with data from the literature, point to the following conclusions: I. During the murine schistosomal infection there are two distinct phases: a – NON-PRODUCTIVE PHASE (before 35-40 days of infection); b – PRODUCTIVE PHASE (after 35-40 days of infection). II. The productive phase is dynamic and characterized by an increase in the number of eosinophils and monocyte/macrophages, and a decrease in the numbers of neutrophils and stabilization of megakaryocytes and erithroid lineages. III. The parallelism between eosinophilic metaplasia in the liver and eosinopoiesis in the bone marrow suggests that stimulating common factor(s) are acting in both sites at the same time (GM-CSF, IL-5, IL-3, acute phase proteins etc. . .?). The eosinophilic metaplasia is not compensatory but probably due to an overflow of growth and differentiation factors. IV. The "Dance" of the eosinophils and mast cells during the infection is complex and the mechanisms are still unknown.

## ACKNOWLEDGMENTS

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