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Histopathology of lymphoid organs in experimental leishmaniasis

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Summary. Hamsters *(Mesocricetus auratus)* were inoculated with *L. (L.) chagasi* and killed on days 7, 15, 30, 45 and 60 after infection.

The lymphoid organs developed initial proliferation of the B lymphocyte zone with recovery by the 60th day group when pyroninophilic cells were prominent. The T lymphocyte area showed a progressive selective decrease of lymphocytes and cellular density with cellular pleomorphism including macrophages, plasma cells and reticular cells. The mean volume of the white pulp increased with the lymphoid follicle hyperplasia but returned to its initial level by day 60.

The main red pulp change was marked hyperplasia of the phagocytic mononuclear cells containing parasites from the 30th day of infection onward.

These changes are compatible with the humoral and cellular immunoresponse found in patients with visceral leishmaniasis (VL).

Keywords: *L. (L.) chagasi*, experimental visceral leishmaniasis, pathology, lymphoid organs

The histopathology of the lymphoid organs in visceral leishmaniasis in hamster and man has been reported as due to reticuloendothelial system hyperplasia and hypertrophy with parasitism of the cells (Meleney 1925). Andrade and Andrade (1966) describing 13 cases of human visceral leishmaniasis considered three main changes to be characteristic of the visceral leishmaniasis: (1) reticuloendothelial system cell proliferation with high parasitism, lymphocyte infiltrate and plasmocytosis; (2) interstitial deposits of hyalin substance similar to secondary amyloidosis, and (3) proliferation and swelling of endothelial cells. Veress *et al.* (1974, 1977) also noted the occurrence of extracellular eosinophilic material deposits in the spleen, lymphoid atrophy of the white pulp of the spleen and a decrease in the numbers of small lymphocytes in the paracortical zone of the lymph nodes. There was also necrosis and fibrosis of the T lymphocyte dependent zone together with histiocytic hyperplasia with parasites and plasmocytosis. These changes were considered to be due to immunosuppression associated with excess antigen. Gutierrez *et al.* (1984) reported that in liver, spleen and bone marrow of mice infected with *L. donovani*, the development of granulomas was the main histopathological

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change. They also reported progressive involution in the splenic white pulp.

The hamster inoculated with 10^7 amastigotes of *L. (L.) chagasi* presents a uniform evolution of the disease which kills the animals from the 60th day onward. It has proved to be a good model for histopathological studies (Duarte *et al.* 1978, 1988; Duarte & Corbett 1984).

In this study we analyse the sequential histopathological changes in hamsters experimentally infected with *L. (L.) chagasi* in order to evaluate the involvement of lymphoid organs in this experimental model of visceral leishmaniasis.

Material and methods

Hamsters *(Mesocricetus auratus)*, 2 months old and weighing 80–90g, were inoculated with *L. (L.) chagasi* (MHOM/BR/72/LD46) isolated from a human case in Mantena, Minas Gerais, Brazil, by Dr W. Mayrink of the Federal University of Minas Gerais, Brazil. An intraperitoneal inoculum of previously infected spleen homogenate with 10^7 amastigotes was administered to groups of seven animals which were killed on days 7, 15, 30, 45 and 60. Control groups of three animals were inoculated with normal spleen homogenate and saline solution (NaCl 0.09%).

The parasite load of the spleen was calculated by determining the number of amastigotes found per 1000 nuclei of the cells × organ's weight (mg) $\times 2 \times 10^5$ in spleen smears fixed in methanol and stained by Giemsa's method (Stauber 1958).

Fragments from thymus, spleen and lymph nodes were processed for histopathology and stained by haematoxylin–eosin, Giemsa, Wilder's silver reticulin stain and by methyl green pyronin solution in order to identify plasma cells. A morphometric method using Weibel's graticule with 100 points was used for sequential quantitative analyses. The Kruskall–Wallis non-parametric test was used for statistical analyses.

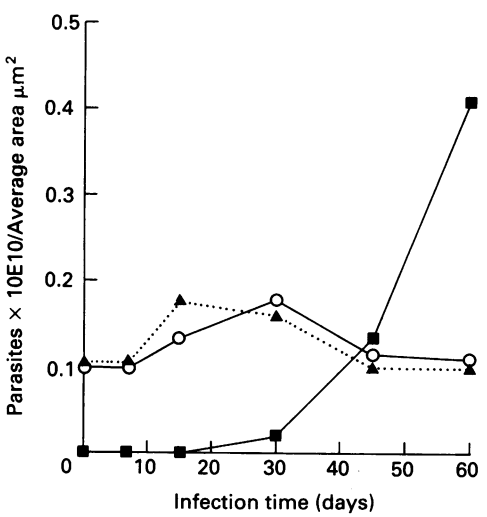


Fig. 1. Comparison of the evolution of events during experimental leishmaniasis. ■, Parasites; ○, white pulp; ▲, follicles.

Results

From the 30th day of the infection, leishmania amastigotes were detected in smears of spleen. The progression of parasite load is shown in Fig. 1 together with the variation in volume of the spleen white pulp and lymphoid follicles.

Sequential histopathological changes

Group 1 (7th day). Spleen white pulp (Fig. 2a) with normal periarteriolar lymph sheath (Fig. 4a) was separated from the lymphoid follicles by a well defined marginal sinus as usually seen in rodents (Fig. 2b). The red pulp showed congestion only. The lymph nodes were normal (Fig. 3).

Group 2 (15th day). The main change seen in the white pulp of the spleen was reactivity of the marginal centres of the lymphoid follicles, where reticular cells and mitosis were conspicuous.

The periarteriolar lymph sheath and marginal sinus were preserved. The red pulp showed a mild increase in the numbers of

plasma cells and macrophages. The lymph nodes showed activity of the germinal centres of the lymphoid follicles only.

Group 3 (30th day). There were numerous active follicular germinal centres with many mitoses in the spleen. The marginal sinus was only partially preserved. By comparison with controls, the periarteriolar sheath had a lower density and cellular pleomorphism and macrophages and plasma cells were present among the lymphoctyes (Fig. 4b). The red pulp was enlarged with increased numbers of macrophages and plasma cells. Macrophages containing parasites were identified in some, but not all, animals. The lymph nodes showed hyperplasia of the follicular germinal centres. The lymphoid follicle marginal zone showed increased cell density, mostly pyroninophilic cells representing active lymphoctyes and plasma cells. In the paracortical zone, macrophages and plasma cells were seen together with the lymphocytes.

Group 4 (45th day). The splenic white pulp did not change in volume, but there was a decrease in cell density and lymphocytes were present as well as macrophages, plasma cells and reticular cells (Fig. 4c). The follicular germinal centres, which were still hyperplastic, showed an increase in the perifollicular zone where plasma cells were also seen. The marginal sinus was partly interrupted (Fig. 5). There was an intense proliferation of macrophages mostly containing parasites which formed nodules within the red pulp.

The lymph nodes were still somewhat hyperplastic (Fig. 6) but the marginal zones also contained pyroninophilic cells and macrophages. The paracortical zone contained a few lymphoctyes and macrophages, some of them with parasites. The parasitism varied from low to high in animals within the same group. In the highly parasitized animals, there were groups of macrophages full of parasites.

Group 5 (60th day). The spleen changes were more marked, with the white pulp containing few lymphocytes but increased number of macrophages and plasma cells (Fig. 4d). The lymphoid follicles showed no signs of activity, no marginal sinus could be seen and the cells were mainly pyroninophilic. The periarteriolar sheaths had markedly fewer cells with few lymphocytes and macrophages mostly containing parasites and plasma cells (Fig. 7). The red pulp showed a marked increase in the number of highly parasitized macrophages which formed nodules (Fig. 8).

The lymph nodes contained follicles showing no activity and with pyroninophilic cells in the outer limits (Fig. 9). In the paracortical zone there were a few macrophages and plasma cells (Fig. 10). The medullary lymphoid sinus contained groups of plasma cells.

The white pulp became progressively hyperplastic until the 30th day with a later decrease.

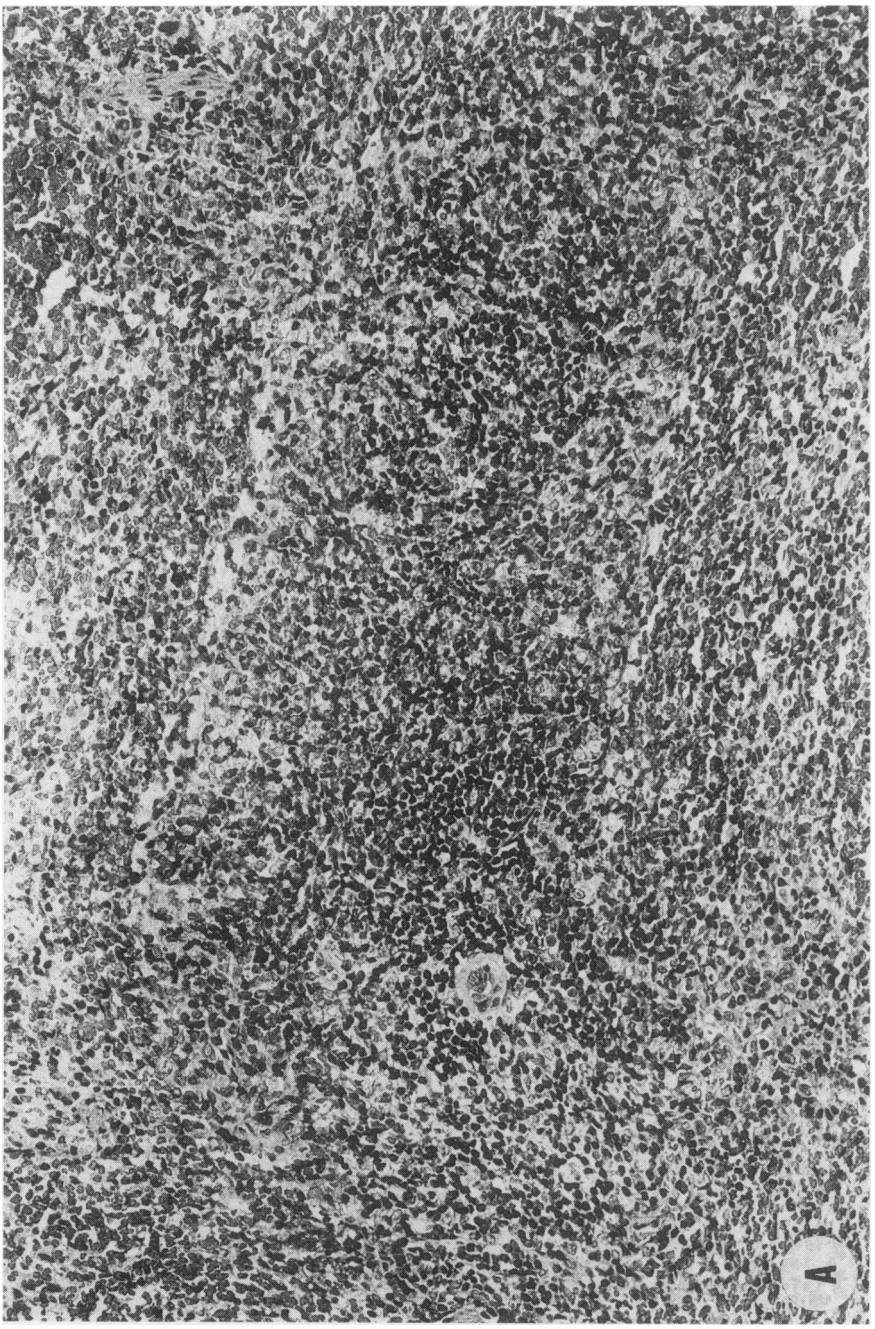
The lymphoid follicles were significantly increased at the 15th and 30th days but decreased to normal volume in the later stage of the disease.

The thymus showed only mild atrophy of the cortex with some hyalin Hassal bodies.

Discussion

Visceral leishmaniasis is known as a disease with immunodeficiency to specific and non-specific agents. Hypergammaglobulinaemia is also a regular finding. The relationship between the morphological and immunological changes has been described. Structures within lymphoid organs have been related to specific immune response activity. Since the sixties, the B and T lymphocytic zones have been characterized in spleen and lymph nodes (Parrot *et al.* 1966; Turk 1967; Silveira 1972; Nieuwenhuis & Kenning 1974; Niewnhuis & Ford 1976; Van Krieken 1989).

The T lymphocytes are mainly located in sheaths around arterioles and the B lymphocytes in the mantle and marginal zone of the lymphoid follicles in the spleen. In the lymph nodes the T lymphocytes are mainly in the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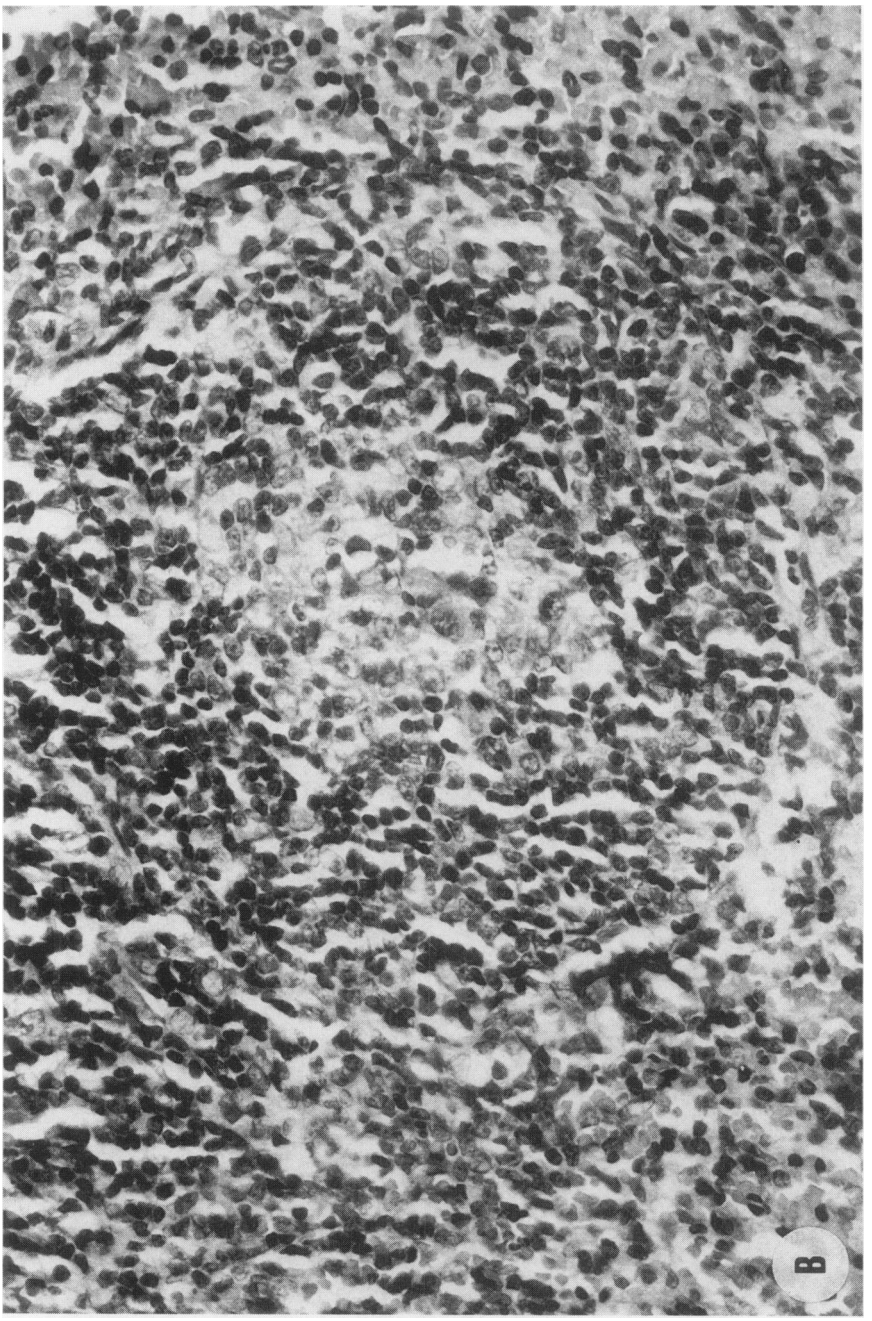


Fig. 2. Spleen white pulp. a, Normal appearance. HE. ×250; b, A well defined marginal sinus. HE. ×500.

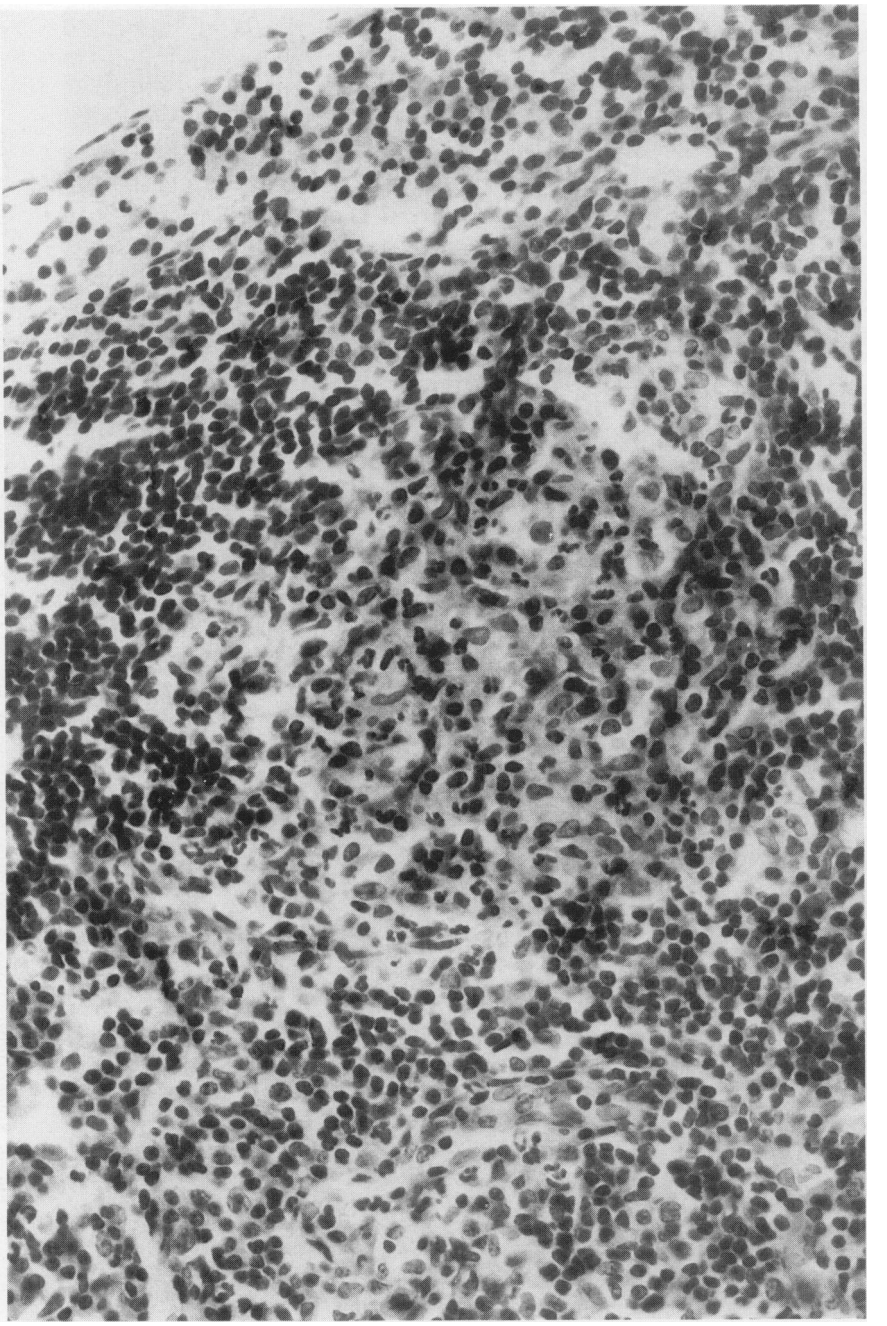


Fig. 3. Lymph node with normal lymphoid follicle. HE. × 500.

paracortical zone and the B lymphocytes in cortical areas mainly in the lymphoid follicles. In this experiment the evaluation of the white pulp of the spleen showed increase in activity of the lymphoid follicle from the 15th to the 45th day of the infection, which is compatible with the already known hypergammaglobulinaemia. However, even with decrease of the germinal centre of the lymphoid follicles at the 45th and 60th day, there was an increase in B lymphocytes and even transformation to plasma cells as indicated by the pyroninophilia of these cells. The marginal sinus, clearly visible at the 7th and 15th days, partially disappeared in the 30th day group and could not be detected at the 45th and 60th days. The T lymphocyte zones at the 30th day showed a lower cellular density and cellular pleomorphism with macrophages and plasma cells present among lymphocytes. This last group showed few lymphocytes and a lower cellular density and most of the cells were macrophages with parasites, plasma cells and reticular cells.

The lymph nodes showed a similar time scale of changes in both the B and T lymphocytes zones, apart from the lymphoid follicle reactivity which started early.

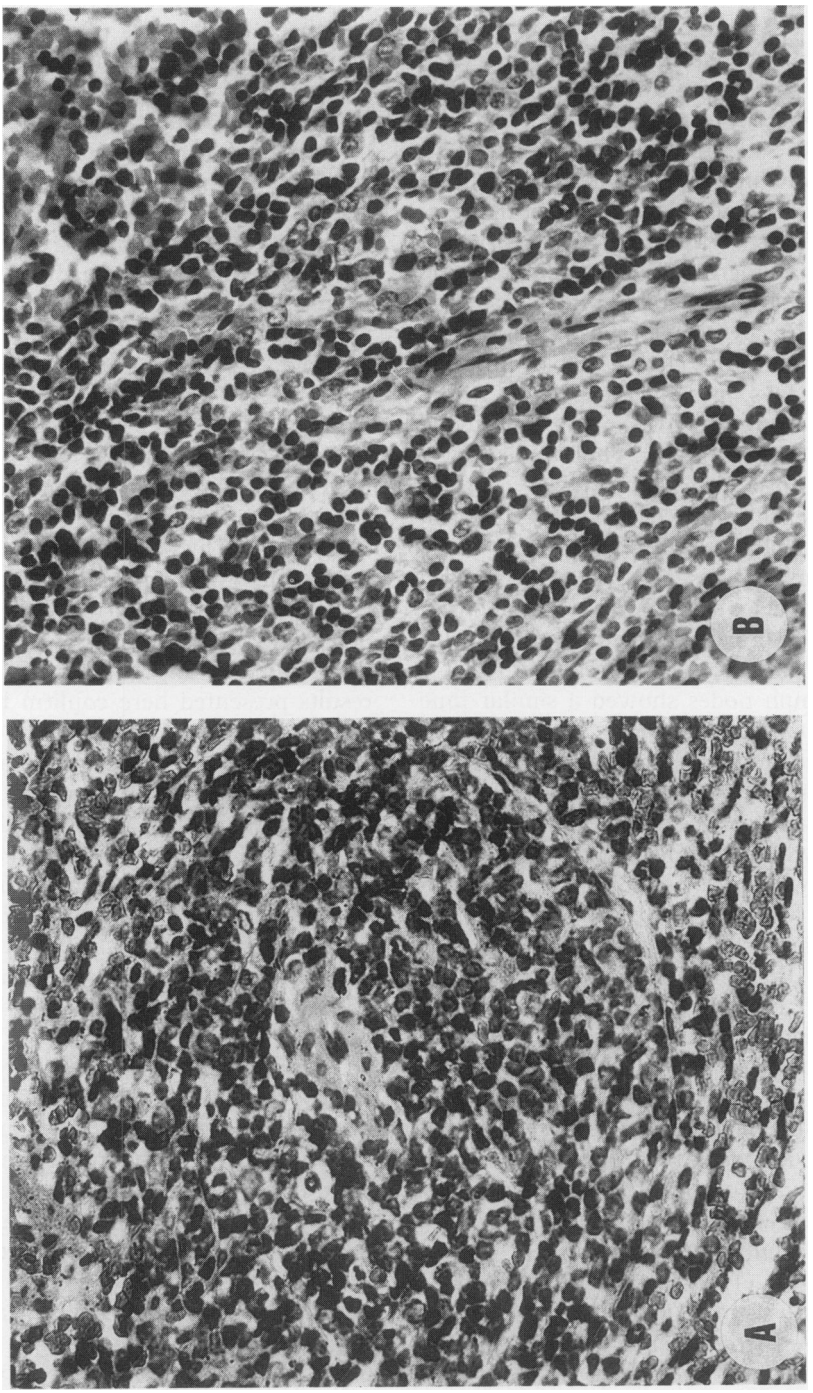
The quantitative evaluation of the white pulp showed a significant increase at the 30th day which was related to an increase in lymphoid follicles; there was a return to the same initial mean volume by the 60th day. However, there was marked selective decrease of T lymphocytes and even with low cellular density there was an increase of plasma cells, parasitized macrophages and reticular cells.

Regressive changes in the spleen and lymph nodes were reported previously as a general feature of the histopathology of visceral leishmaniasis (Meleney 1925; Bhaskara-Menon 1939; Veress *et al.* 1974). Veress *et al.* (1977) reported a morphometric analysis of human visceral leishmaniasis spleen which demonstrated a significant decrease of the white pulp and no germinal centres. They also described lower cellular density with few lymphocytes and the presence of parasitized macrophages and plasma cells in the white pulp. Similar T lymphocytic changes were also seen in lymph nodes. These authors also described necrosis, fibrosis, lymphoid follicles and extracellular hyalin deposits which they interpreted as precipitated antigen–antibody complexes due to excess of antigens. They also noted the decrease of T lymphocytes zones and related this to a reduction in the immunocellular response. In this work no necrosis, fibrosis or evident hyalin deposits were observed. However, in our experience, amyloid is present in the kidneys and in blood vessel walls in these phases of the disease.

The relationship between lymphoid follicle activity and the evolution of the disease was described in Brazilian human visceral leishmaniasis (VL) by Carvalho *et al.* (1985) who divided the VL patients into two groups, one with hyperplasia or normal follicles and the other with atrophic lymphoid follicles which was related to the duration of disease. The results presented here confirm his hypotheses.

The depressed immunocellular response reported in the active phase of the disease (Rees *et al.* 1981) and the decrease in number of T lymphocytes (Rezai *et al.* 1978; Aikat *et al.* 1979; Musumeci *et al.* 1981; Carvalho *et al.* 1985; Koech *et al.* 1987) is also compatible with the lower cellular density and pleomorphism found in the T lymphocyte zones in the spleen and lymph nodes.

The hyperplasia and parasitism of the phagocytic mononuclear system was the most striking change in the red pulp of spleen at the 45th and 60th day. Before that, there were great variations between animals in the same group. These changes were reported in the earliest histopathological observations of VL either in hamsters (Hu 1933) or in man (Hu 1936). These data demonstrate a highly proliferated and parasitized phagocytic mononuclear system and confirm the accepted characteristic histopathological changes.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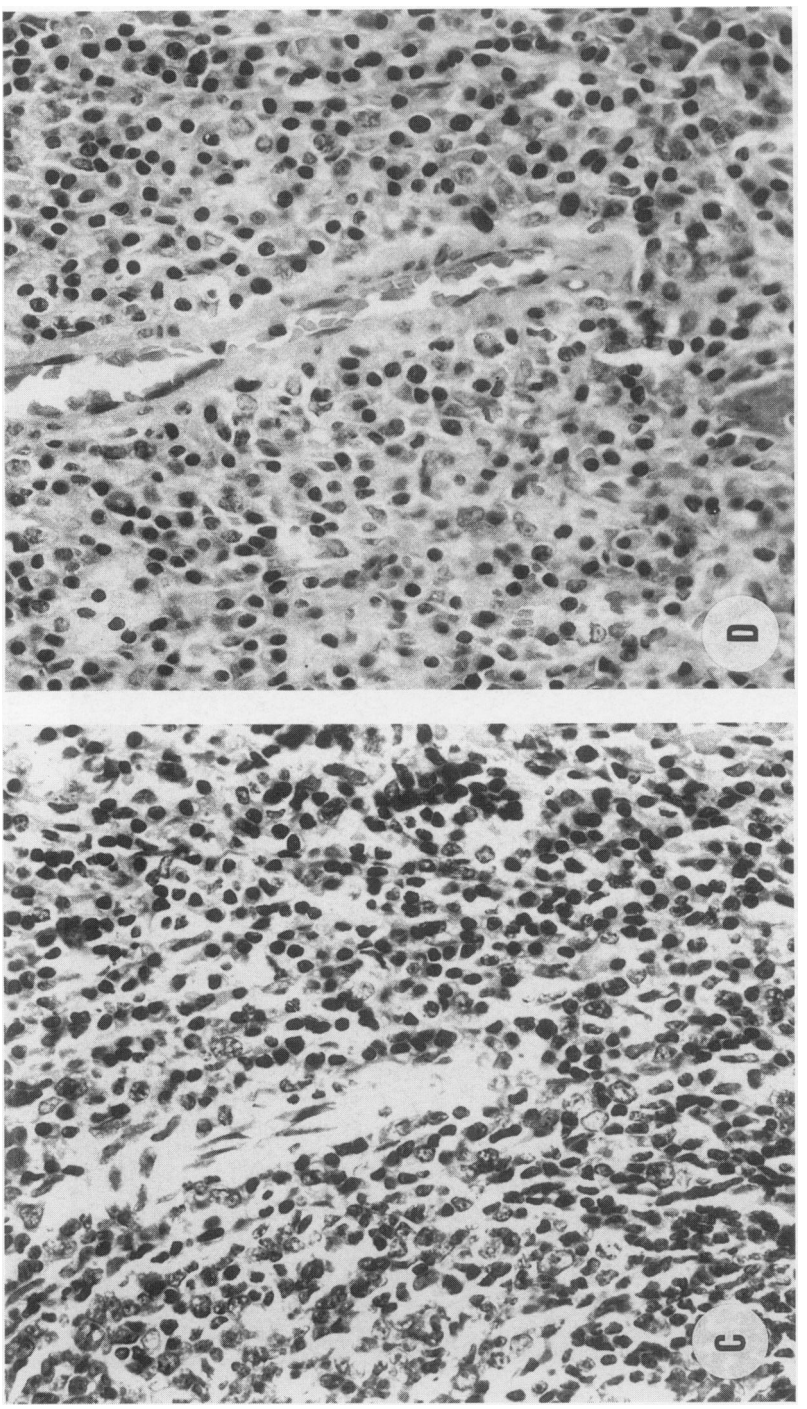

Fig. 4. Periarteriolar sheath. a, Normal cellularity (7th day). HE. × 500; b, Mild decrease of cellularity and cellular pleomorphism (30th day). HE. × 500; c, Low cellular density and cellular pleomorphism (45th day). HE. × 500; d, Lymphocyte depletion with low cellular density and cellular pleomorphism (60th day). HE. × 500.

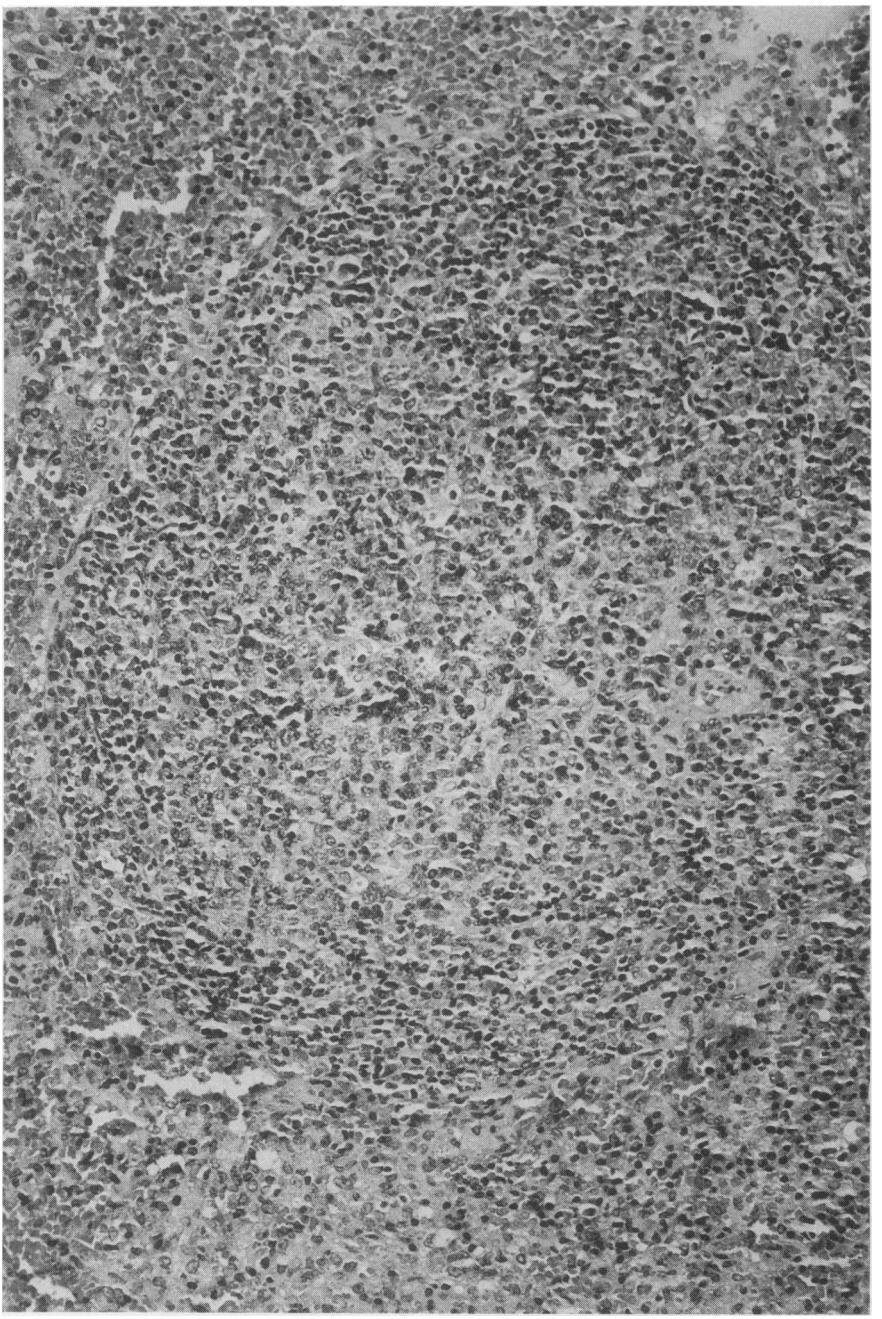

Fig. 5. Spleen white pulp with hyperplasia of the lymphoid follicle and a partially interrupted marginal sinus (45th day). HE. × 250.

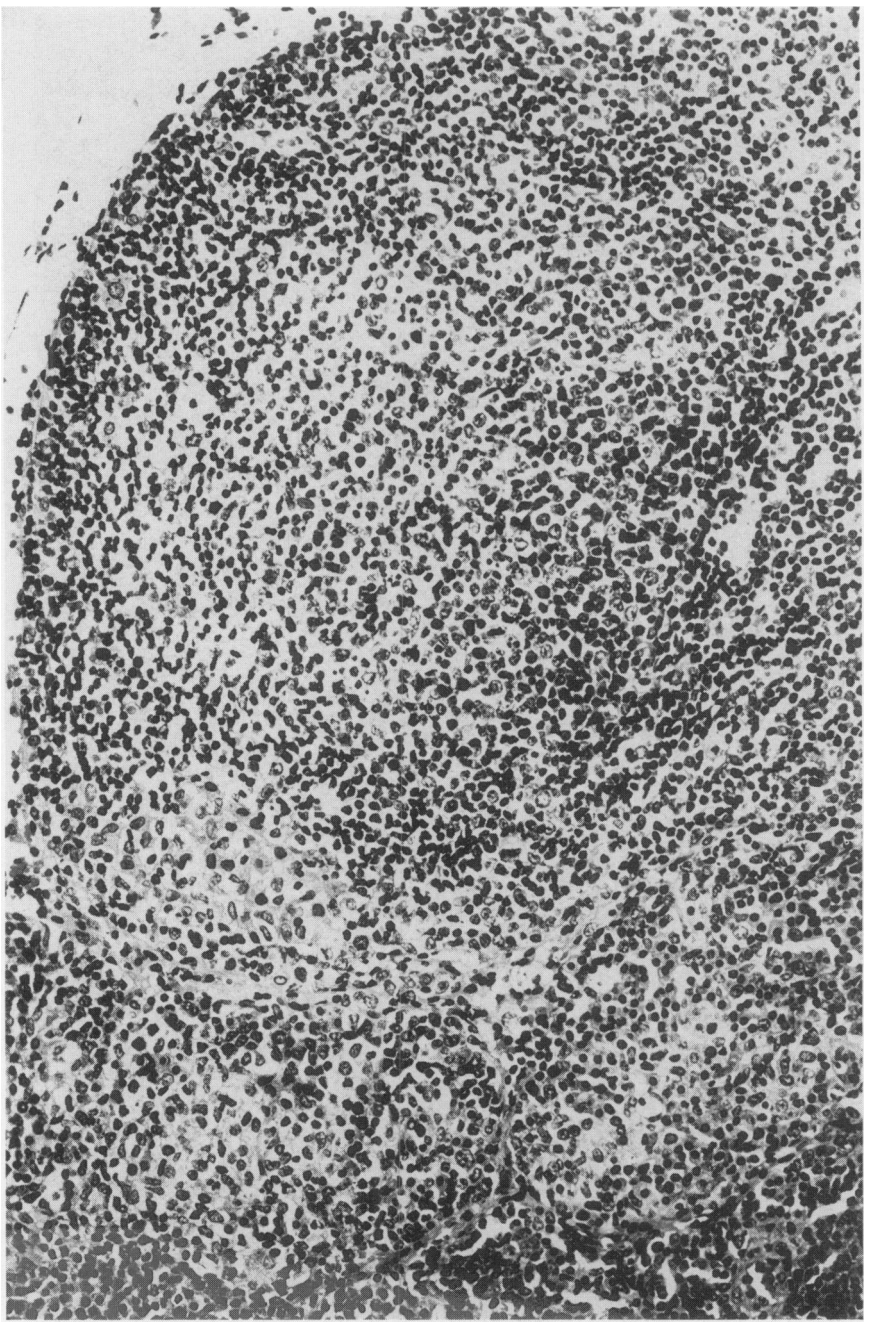

Fig. 6. Lymph node with lymphoid follicle hyperplasia (45th day). HE. ×250.

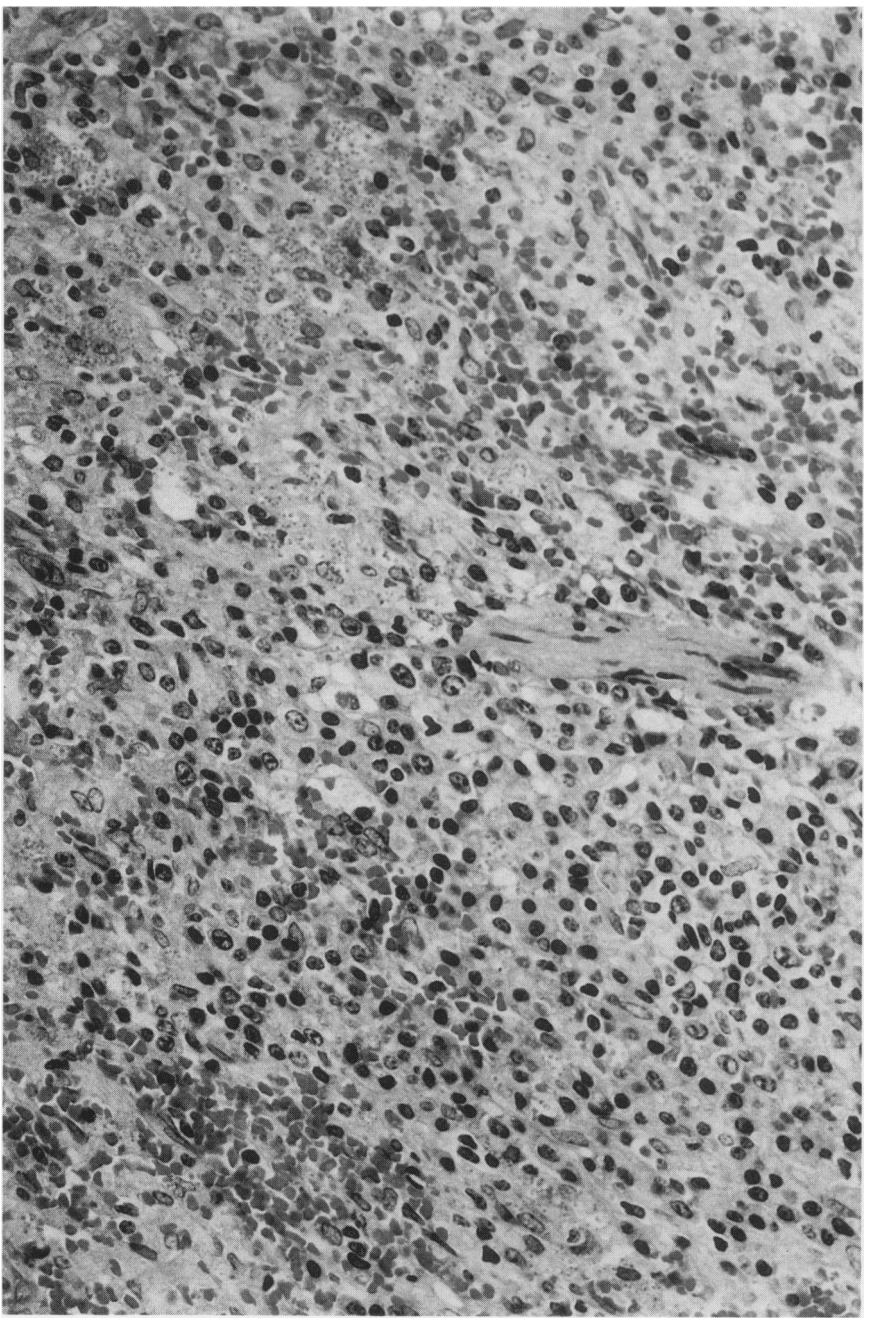

Fig. 7. Spleen white pulp showing marked lymphocytic depletion and macrophages with parasites (60th day). HE. × 500.

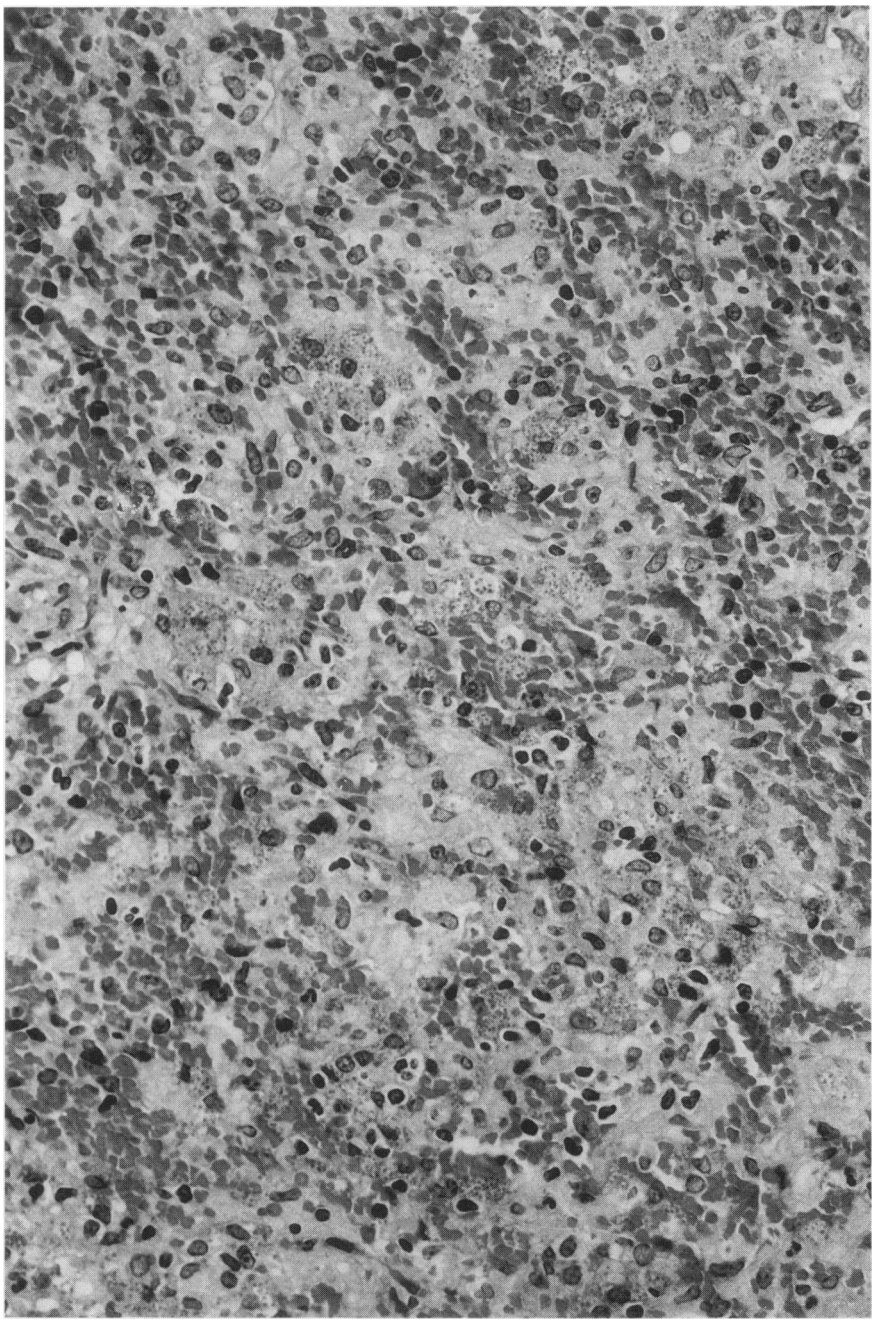

Fig. 8. Spleen red pulp with marked hyperplasia of the phagocytic mononuclear system and a high degree of parasitism (60th day). HE. × 500.

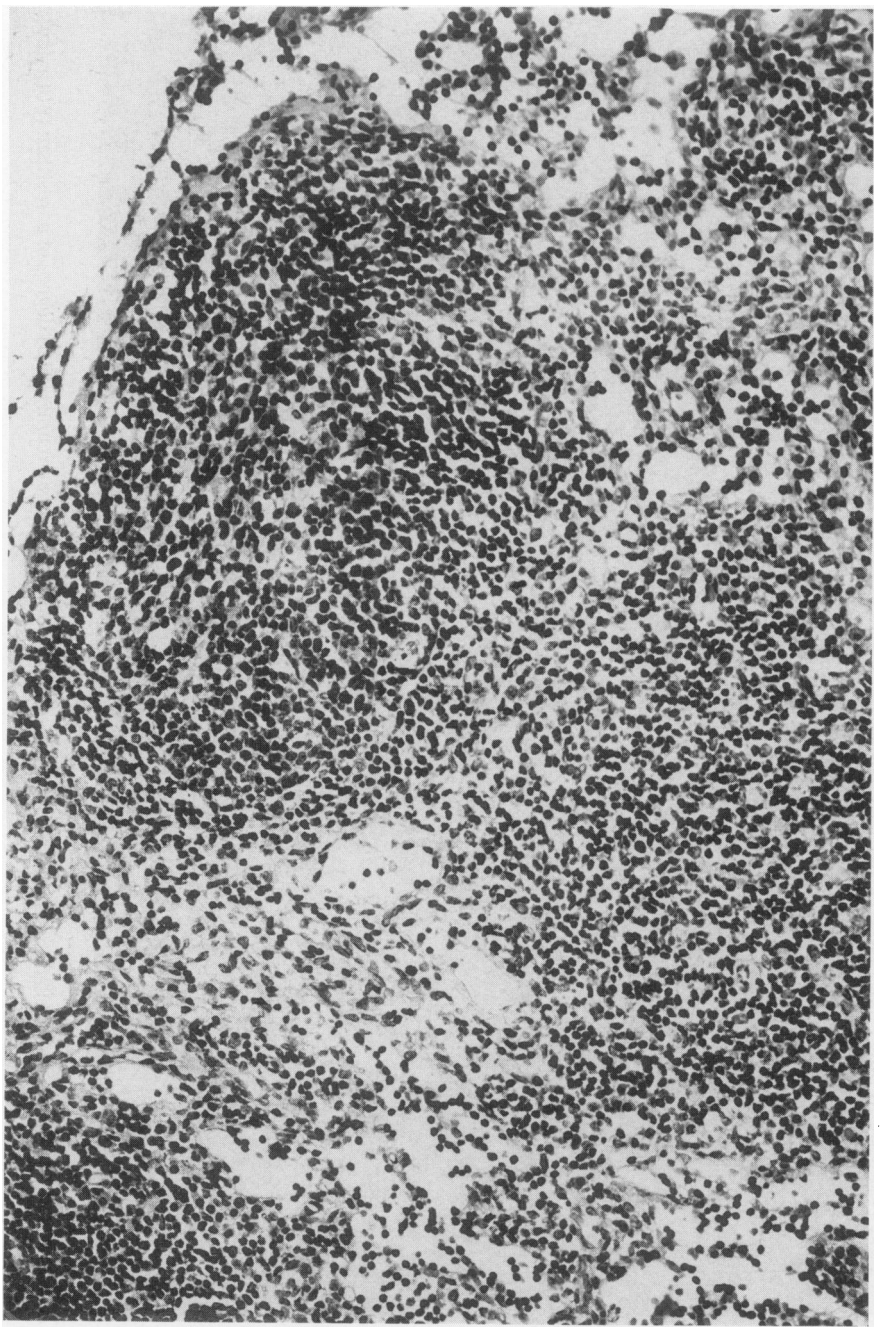

Fig. 9. Lymph node with pyroninophilic cells in the outer limit of the lymphoid follicle (60th day). Methyl green pyronin. ×250.

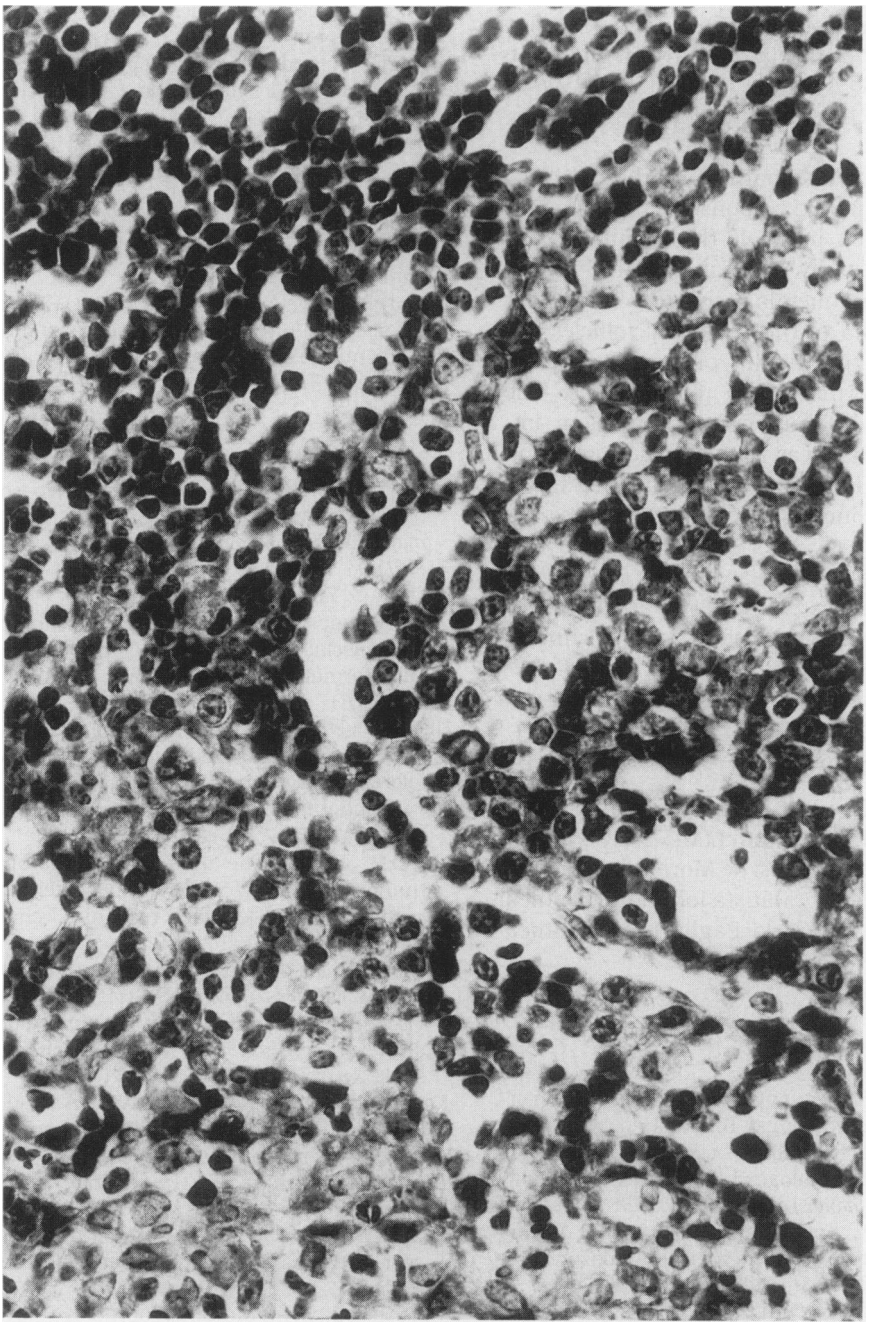

Fig. 10. Lymph node paracortical zone with lymphocyte depletion (60th day). HE. ×787.5.

Epithelioid granulomas which were occasionally noted in previous studies (Bell *et al.* 1958; Jehan *et al.* 1982; Gutierrez *et al.* 1984) were not seen in this experiment. Similarly, extracellular hyalin deposits (Andrade & Andrade 1966; Veress *et al.* 1974, 1977) were not evident in this material.

Sequential histopathology thus demonstrates a dynamic response of the lymphoid organs which parallels the immunological changes usually described. The hypergammaglobulinaemia seems to be the result of hyperplasia of the lymphoid follicles followed by hyperactivity of lymphocytes or their transformation to plasma cells. The compromised T lymphocyte-dependent immune response was related to selective and progressive lymphocyte depletion in the T dependent zones in 60th spleen and lymph nodes.

A progressive increase in the phagocytic mononuclear system activity was also visible and is characteristic of visceral leishmaniasis.

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