Structural and ultrastructural hepatic changes in experimental canine leishmaniasis

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Summary. Six 4 month-old beagles were inoculated with *Leishmania donovani infantum*, three of them intraperitoneally (Group A) and the other three intravenously (Group B). The animals from Group A were killed 109, 433 and 592 days after inoculation and animals from Group B 109, 171 and 334 after inoculation. The liver of each of them was examined by means of light and electron microscopy. The lesions observed in both groups were very similar, but developed more rapidly in Group B. A chronic hepatitis appeared due to infection, characterized by the presence of multiple intralobular granulomas and portal inflammatory infiltrates consisting of lymphocytes, plasmocytes and macrophages with a variable number of amastigotes. The Kupffer cells were hyperplastic and contained parasites in their cytoplasm. Gradually the hepatocytes developed a progressive cellular swelling, which during the end-stages of the process showed itself with severe nuclear degeneration, disintegration of cytoplasmic organelles, enlargement of the cytoplasmic matrix and disruption of the plasma membranes, leading to cytolysis.

Key words: Leishmaniasis, Liver, Dog

Introduction

Visceral leishmaniasis is a protozoal disease which affects man, dogs and various wild animals. The disease has been described in four continents (Europe, Asia, Africa and America) where both endemic zones and isolated cases can occur (OMS, 1984). In the Mediterranean endemic zone, in which Spain is included, the aethiologic agent is *Leishmania donovani infantum*. The disease is characterized by weight loss, fever, anaemia, oedema,

emaciation, hepatosplenomegaly and proteinuria, together with hypoalbuminaemia and hypergammaglobulinaemia.

Hepatic lesions in man (Chadli and Philippe, 1961; Cazal and Pages, 1962; Andrade and Andrade, 1966; Winslow, 1971; Veress et al., 1974; Naik et al., 1976; Quenum and Destombes, 1976) and in dogs both naturally (Giles et al., 1975; Millin et al., 1975; Corbeil et al., 1976; Tryphonas et al., 1977; Rodríguez et al., 1981; Bindseil et al., 1985) and experimentally (Bungener and Mehlitz, 1977; Keenan et al., 1984; Pospischil et al., 1987) infected, have been reported to occur during visceral leishmaniasis. However, previous reports have presented only special morphological aspects of the lesions such as the degree of hepatic parasitism (Veress et al., 1974; Corbeil et al., 1976; Tryphonas et al., 1977; Rodríguez et al., 1981; Keenan et al., 1984; Pospischil et al., 1987) or the nature of the inflammatory phenomena in the liver (Winslow, 1971; Veress et al., 1974; Giles et al., 1975; Millin et al., 1975; Naik et al., 1976; Quenum and Destombes, 1976; Bungener and Mehlitz, 1977; Keenan et al., 1984; Pospischil et al., 1987).

The present study described experimentally induced histopathological changes occurring in the liver of dogs following inoculation of *Leishmania donovani infantum*.

Materials and methods

Eight Beagle dogs, 4 males and 4 females, about 4 months old, were used in this study. The dogs were housed in individual cages and were provided with water and a standard commercial dog food (Panlab [R] Lab canine Diet, Panlab S.L., Barcelona) *ad libitum*. The eight dogs were assigned at random to three groups (A, B and C) (Table 1). The 3 animals from group A were inoculated intraperitoneally with 5.0 ml of infective material containing 2×10^8 amastigotes. The 3 animals from group B were inoculated intravenously with 5.0 ml of infective material containing 5×10^8 amastigotes.

The remaining two dogs (group C) were maintained as uninfected controls. The inoculum was prepared from two spleens macerated in physiologic saline obtained from two hamsters infected with *Leishmania donovani infantum* (isolate FVM 1001 JL). The total parasite count was determined using the same method as described in a previous study (González et al., 1983). One of the two control dogs was inoculated intraperitoneally and the other intravenously with 5.0 ml of healthy hamster spleen homogenate. The dogs from group A were killed at 109, 433 y 592 days after inoculation, the dogs from group B at 109, 171 and 334 days after inoculation and dogs from group C at 210 and 600 days after inoculation.

Hepatic tissue samples for light microscopy study were fixed in neutral buffered formalin, dehydrated, embedded in Histosec (Merck) and cut at 5 μm. The sections obtained were stained with hematoxylin and eosin, Gallego's trichrome, Masson's trichrome, Best's carmine, methylene blue and Congo red. Samples for transmission electron microscopy were fixed in 3% Millonig-buffered glutaraldehyde, pH 7.3 and post-fixed in 1% osmium tetroxide. The samples were dehydrated through graded alcohols and embedded in epon-araldite. Thin sections were cut with an ultratome, stained with 2% aqueous uranyl acetate and lead citrate and examined under a JEOL 100 B electron microscope at 80 Kv.

Results

GROUP A (intraperitoneal inoculation)

Throughout the infectious process, all the animals in this group presented multiple, randomly distributed liver granulomas. These consisted of macrophages containing amastigotes of *Leishmania donovani infantum*, as well as concentrically arranged lymphocytes and plasmocytes (Figs. 1, 2). The portal tracts showed progressive lympho-histio-plasmocytic inflammatory infiltration, which, in the terminal stage of the process (dog No. 3) and due to its extension, caused compression of the adjacent liver parenchyma (Fig. 3). The Kupffer cells appeared hyperplastic and with a variable number of parasites in their cytoplasm.

In all the animals of this group, the liver parenchyma showed progressive swelling of the liver cells. At the onset of infections (dog No. 1), some hepatocytes without definite location presented a cloudy and paler than normal cytoplasm. In addition, ultrastructural swelling of the mitochondria was observed (Fig. 4), which revealed rupture of their cristae and cristolysis, central osmiophilic bodies and myelin-like lamellae (Fig. 5). The cisternae of the endoplasmic reticulum were enlarged. As the infection progressed (dog No. 2), the degenerative process involved the entire hepatic lobule. The hepatocytes showed enlarged cisternae in the Golgi complex and the endoplasmic reticulum, as well as a considerable degree of mitochondrial swelling (Fig. 6).

The cytoplasmic matrix appeared spacious and electrolucent, with a marked decrease in glycogen particles. The nuclei were pycnotic and/or swollen. In the terminal phase of the process (dog No. 3) the cells appeared voluminous and rounded, with a completely vacuolized cytoplasm and peripherally displaced nuclei (ballooned cells). With the electron microscope intense cell degeneration was observed, including cytoplasmic organelle disintegration (Fig. 7), considerable matrix enlargement and focal ruptures of the plasma membrane.

GROUP B (intravenous inoculation)

The dogs in this group presented a hepatic inflammatory reaction which was similar to that observed in Group A, although it developed more rapidly. The number of parasites observed in Kupffer cells and macrophages throughout the infection was lower than that recorded for the intraperitoneally inoculated animals.

The liver parenchyma presented a progressive degenerative process, characterized by cellular swelling and a more rapid evolution than in Group A. Thus, a marked increase in cell volume was observed, together with vacuolar transformation of the endoplasmic reticulum, mitochondrial swelling, enlargement of the cytoplasmic matrix and different degrees of nuclear degeneration. Eventually (dog No. 6) this lesion gave rise to irreversible hydropic degeneration of the hepatocyte (Fig. 8) leading to its cytolysis.

With the Congo red technique, no hepatic amyloid deposits were observed in any of the experimental dogs.

Discussion

In this study the sensitivity of the Beagle to visceral leishmaniasis (Bungener and Mehlitz, 1977; Reiter et al., 1985; Pospischil et al., 1987) was confirmed reproducing this disease by means of intraperitoneal and intravenous inoculation of *Leishmania donovani infantum*. Hence, and in the light of the results obtained, this breed was considered an adequate experimental model for the study of the histo-pathological lesions ocurring in the liver in the course of leishmaniasis.

In our study a focal chronic inflammatory reaction was observed, typically developing multiple intralobular granulomas, similar to those described for leishmaniasis in man (Cazal and Pages, 1962; Andrade and Andrade, 1966; Naik et al., 1976; Quenum and Destombes, 1976) and in dogs (Bungener and Mehlitz, 1977; Tryphonas et al., 1977; Rodríguez et al., 1981; Bindseil et al., 1985). The granulomas originated in response to the presence of parasites incorporated into macrophages, in whose cytoplasm they multiply in an uncontrolled way favoured by the incapacity of the macrophages to destroy them. Due to the antigen stimulation triggered by these parasites, a progressive hyperactivation of humoral immunity was produced (Corbeil et al., 1976) and, consequently, lymphocytic and plasmocytic infiltration

Table 1. General inoculation scheme of *Leishmania donovani infantun* in dogs.

GROUPS	DOG No.	SEX	ROUTE OF INOCULATION	NUMBER OF AMASTIGOTES/5ml INOCULATED	DAYS KILLED AFTER INOCULATION
	1	male	i.p.	2×10^8	109
A	2	male	i.p.	2×10^8	433
	3	female	i.p.	2×10^8	592*
	4	female	i.v.	5×10^8	109
B	5	male	i.v.	5×10^8	171
	6	female	i.v.	5×10^8	334*
	7	male	i.p.	—	210
C (control)	8	female	i.v.	—	600

* Killed due to severe clinical condition (generalized lymphadenomegaly, cachexia, hemorrhagic diarrhea, ascites and peripheral edema).
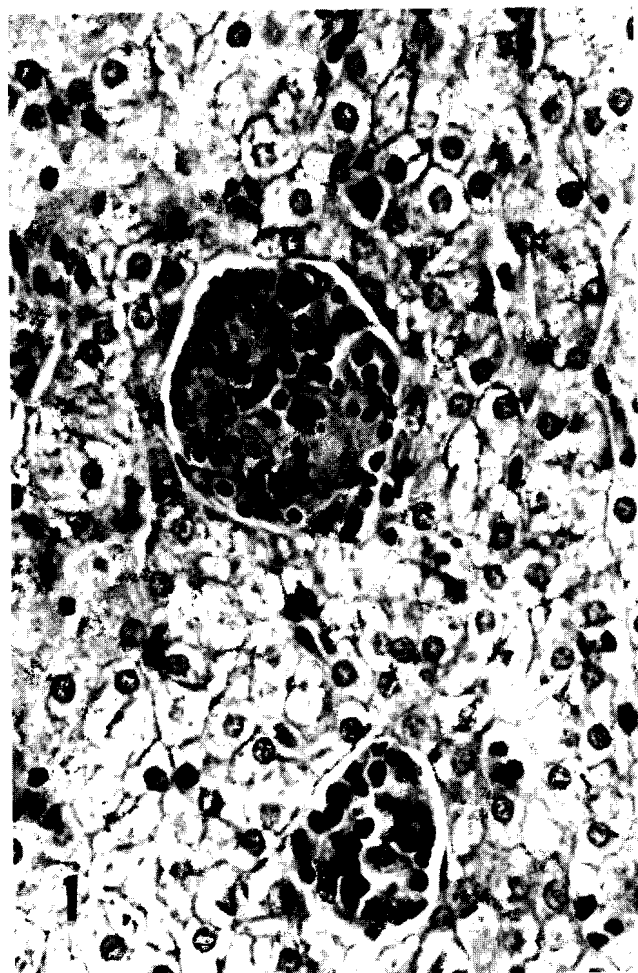
Fig. 1. Dog No. 2, 433 days after inoculation. Two inflammatory granulomas. Swollen hepatocytes. Gallego's trichrome × 400

Fig. 2. Dog No. 2, 433 days after inoculation. Inflammatory granuloma with lymphocytes, a plasmocyte and a macrophage containing an amastigote of *Leishmania donovani infantum.* × 10,000
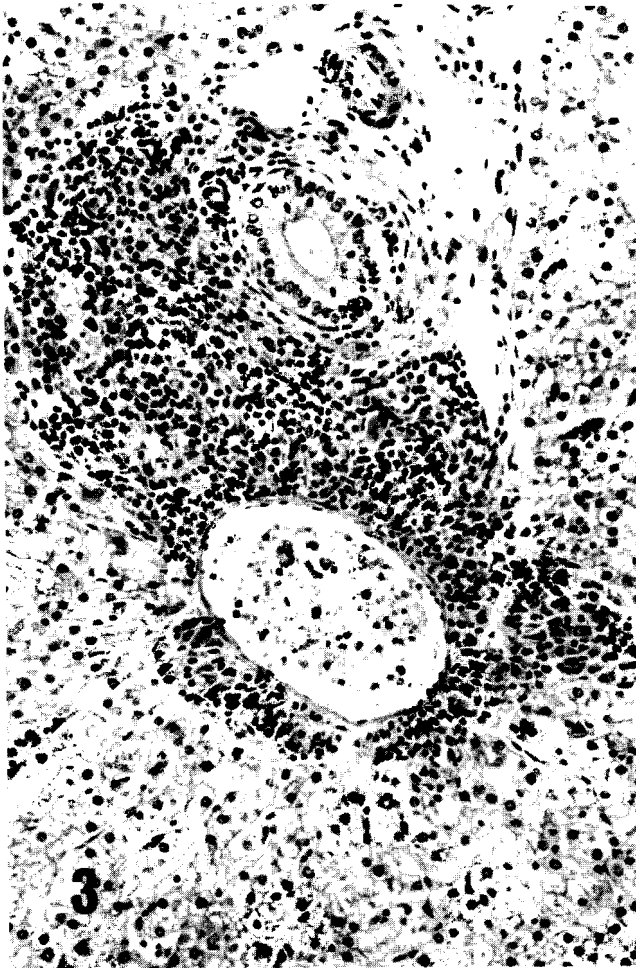
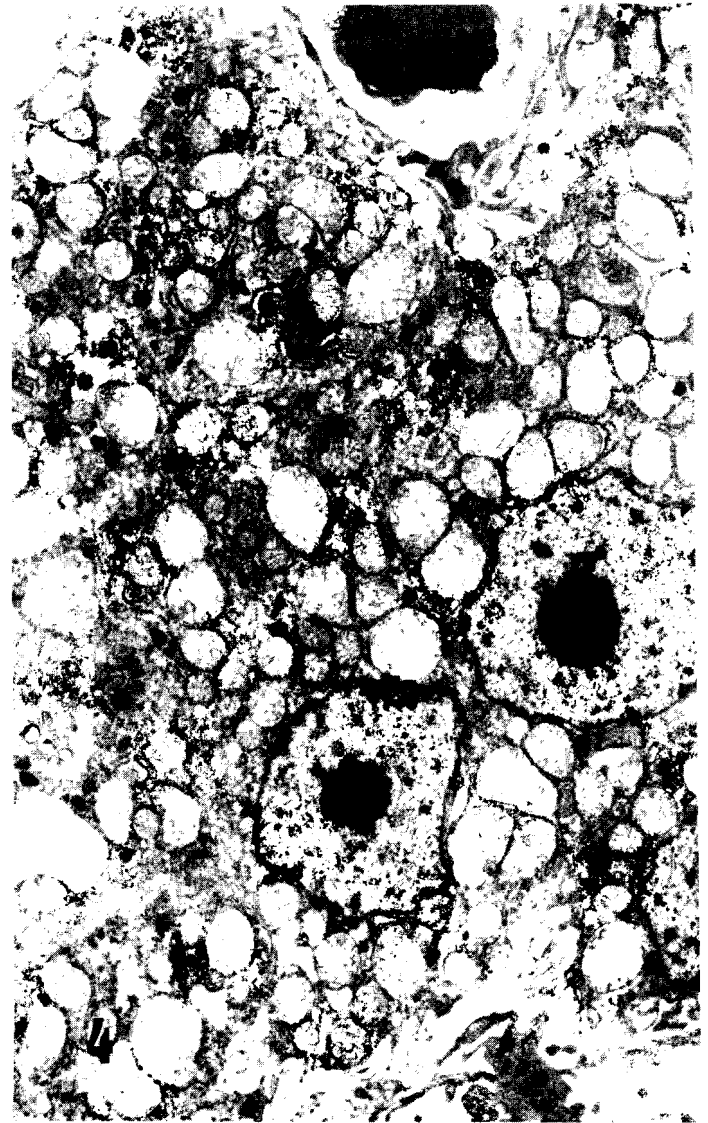**Fig. 3.** Dog. No. 3, 592 days after inoculation. Large portal inflammatory infiltration. HE × 160

Fig. 4. Dog. No. 1, 109 days after inoculation. Hepatocyte with swelling of the mitochondria. × 5,200

in the vicinity of the parasite-infected macrophages. Eventually the granulomas acquired their characteristic concentric arrangement, with the macrophages positioned centrally, and the plasma cells and lymphocytes displaced peripherally. Throughout the evolution of this process, the granulomas did not show any presence of giant cells (Rodríguez et al., 1981) or focal necrosis (Quenum and Destombes, 1976). Together with granuloma development, progressive inflammatory invasion of the portal tracts was observed, which caused compression of the adjacent liver parenchyma. These portal infiltrates described in man (Chadli and Philippe, 1961; Cazal and

Pages, 1962; Andrade and Andrade, 1966; Winslow, 1971) and in dogs (Giles et al., 1975; Millin et al., 1975; Bindseil et al., 1985), consisted of macrophages with a variable number of leishmanial organisms, plasmocytes, lymphocytes and, in the early stages of infection, some neutrophilic leucocytes. Portal fibrosis (Chadli and Philippe, 1961; Andrade and Andrade, 1966) was not found in any of the experimental animals.

The hepatic inflammatory reaction was evidenced in all the dogs of our series, although it varied greatly in intensity, independent of the route and dose of inoculation. This latter fact suggests that the inflammatory process
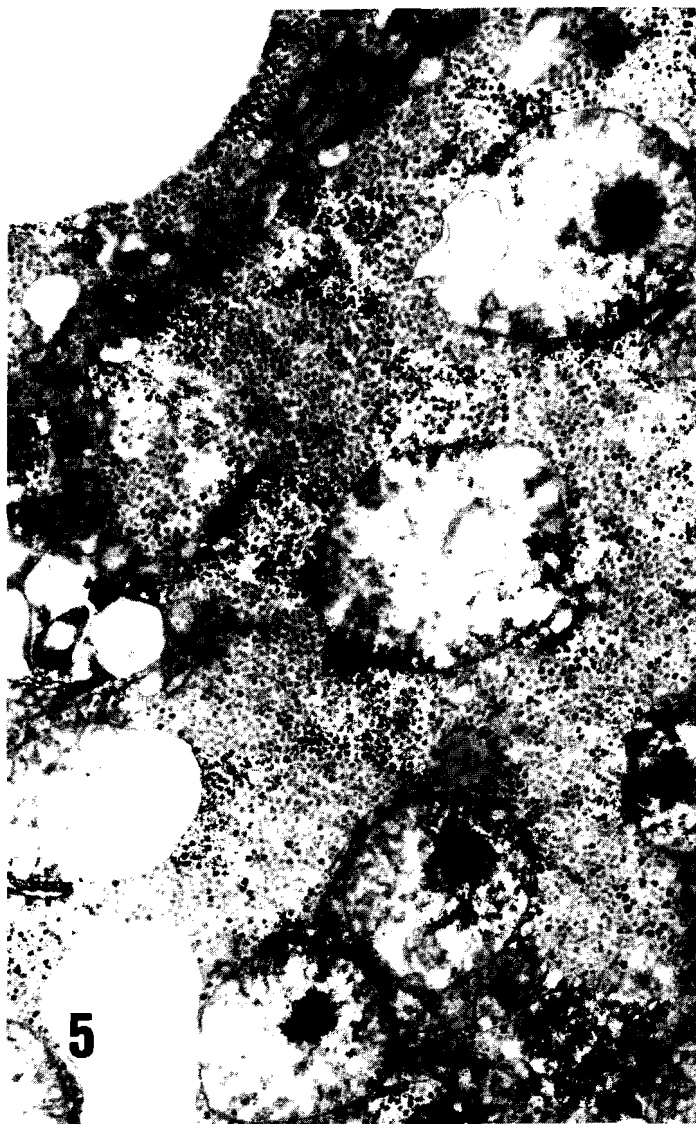
Fig. 5. Dog. No. 1, 109 days after inoculation. Mitochondria which show disintegrated cristae, osmiophilic bodies and myelin-like lamellae. Abundant glycogen particles. × 16,500
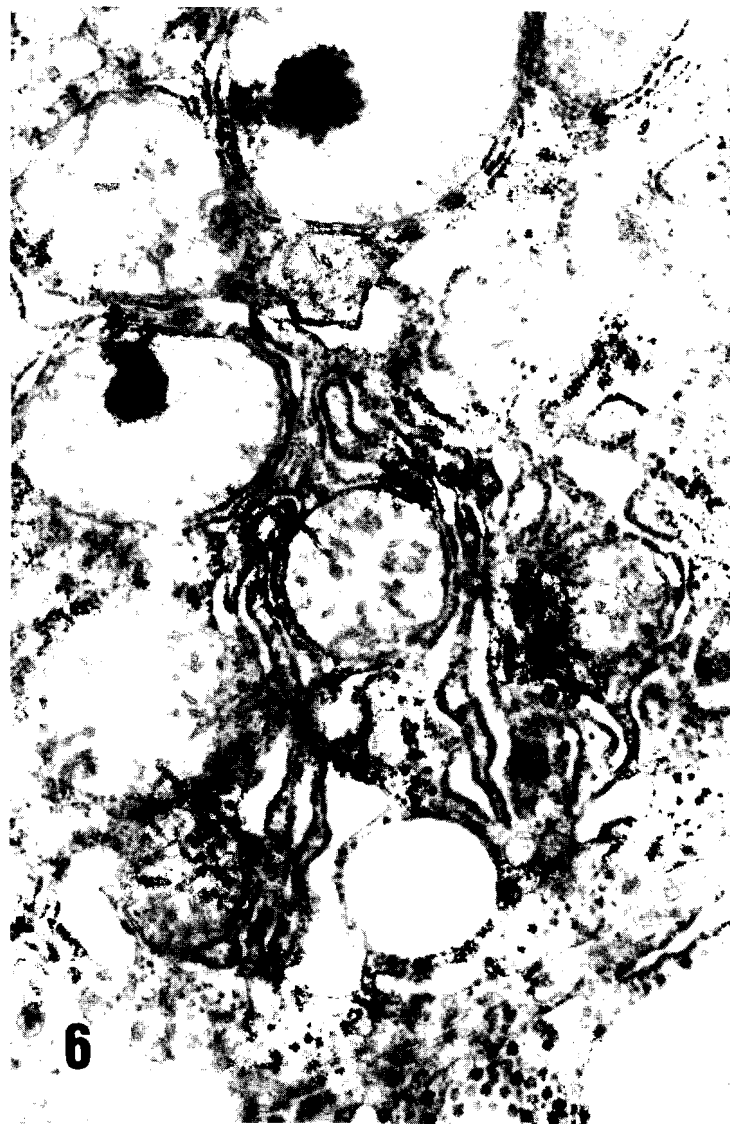
Fig. 6. Dog. No. 2, 433 days after inoculation. Hepatocyte showing mitochondrial swelling and enlargement of the cisternae of the endoplasmic reticulum. × 33,000

taking place in the liver, in course of experimentally induced leishmaniasis in dogs, is determined by the individual immunological response of each single animals.

The number of parasites present in Kupffer cells (Corbeil et al., 1976; Rodríguez et al., 1981; Keenan et al., 1984) and in the macrophages (Bungener and Mehlitz, 1977; Tryphonas et al., 1977; Pospischil et al., 1987) varied remarkably in the different stages of the process, and was higher in the intraperitoneally infected animals. The degree of the parasite infection did not seem to be directly correlated to the intensity of the

inflammatory reaction and/or liver cell degeneration.

In all dogs the liver parenchyma presented an obvious degenerative process, characterized by liver cell swelling, which developed more rapidly in the intravenously inoculated animals than in the intraperitoneally infected group. This process is likely to originate from the toxic action of the parasite, which would either have a direct impact on the plasma membrane or else interfere with the metabolic activity of the cell organelles. During the early stages of infection cell swelling partially affected the hepatic lobule, yet lacked a precise location. Subsequently the degenerative process involved the
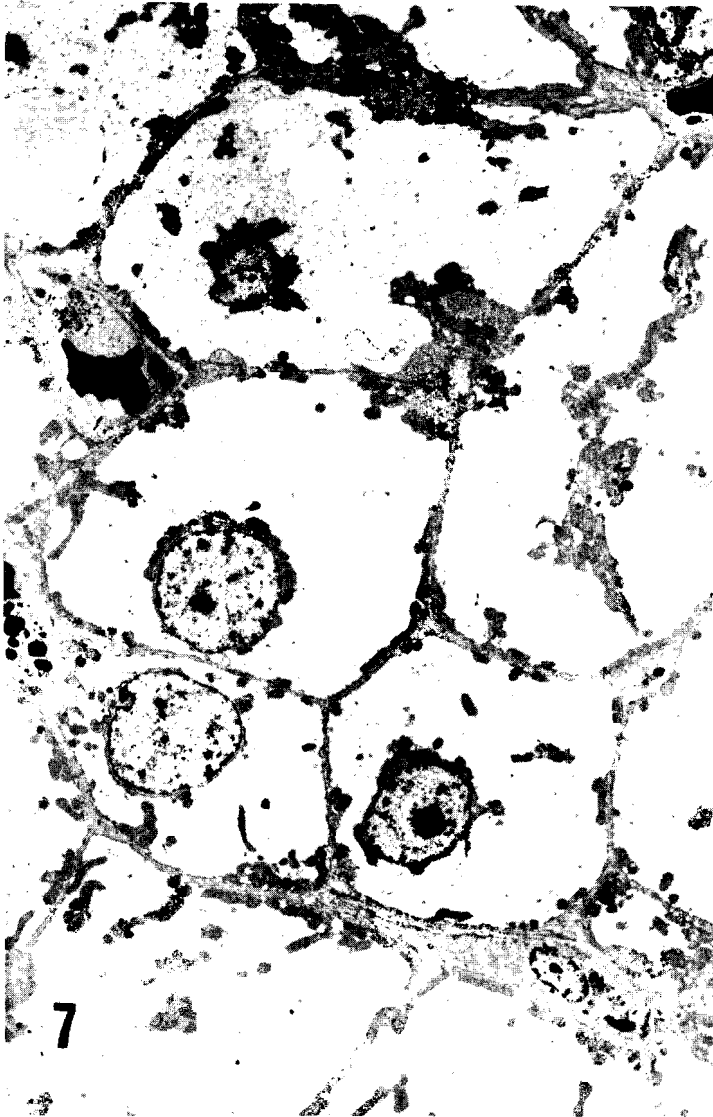
Fig. 7. Dog. No. 3, 592 days after inoculation. Ballooned cells characterized by disintegration of cytoplasmic organelles and considerable enlargement of the matrix. × 2,500
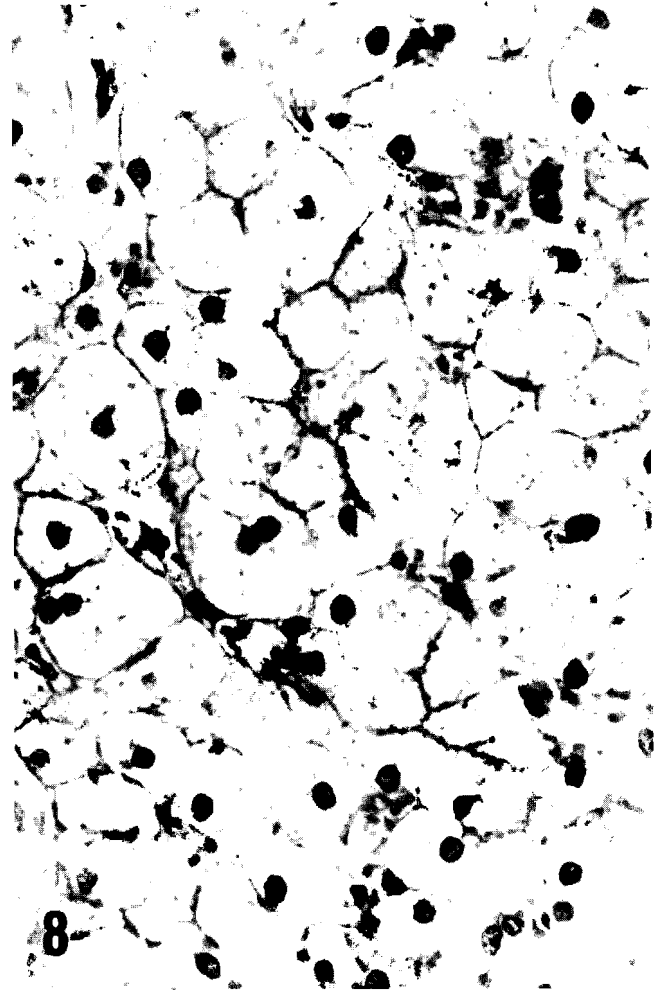

Fig. 8. Dog. No. 6, 334 days after inoculation. Hydropic degeneration of the hepatocytes with disruption of the plasma membrane. HE × 400

entire liver lobule, whose cells showed a maximum degree of hyperhydration, which eventually determined their cytolysis.

Liver amyloidosis, however, as described in the literature for leishmaniasis in man (Andrade and Andrade, 1966) and in the dog (Corbeil et al., 1976; George et al., 1976; Bungener and Mehlitz, 1977), was not observed at any stage of our study.

Experimental infection of the Beagle with *Leishmania donovani infantum* thus causes chronic hepatitis, characterized by the presence of multiple intralobular granulomas and portal inflammatory infiltrates consisting

of lymphocytes, plasmocytes and macropaghes with a variable number of amastigotes. The liver cells develop a progressively greater degree of cellular swelling, which terminates in their cytolysis.

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