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Histology and Histopathology

Amyloidosis in adrenal glands of hamsters experimentally infected with *Leishmania infantum*

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Summary. Thirty 10-week-old hamsters were inoculated intraperitoneally with *Leishmania infantum* amastigotes and were serially killed after 15, 30, 45, 60, 75 and 89-90 days. The adrenal glands of each of them were examined by means of light and electron microscopy. The cortex and medulla of the adrenal gland showed a progressive deposition of amyloid, selectively identified by both optical and ultrastructural techniques. It has been suggested that adrenal amyloidosis during visceral leishmaniasis is directly related to a stimulation of the phagocitary mononuclear system due to the persistence of the antigenic stimulation of the parasite. In addition to these deposits, the presence of inflammatory infiltrates containing lymphocytes, plasmocytes and macrophages with leishmanias confirmed the establishment of leishmaniasis. The deposition of the amyloid fibrils finally leads to the partial destruction of the adrenal parenchyma.

Key words: Leishmaniasis, Amyloid, Adrenal gland, Hamster

Introduction

Visceral leishmaniasis is a protozoal disease which affects man, dogs and other mammals. 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 infantum*.

Adrenal lesions both in man (Chadli and Philippe, 1961; Cazal and Pages, 1962) and in animals (Gellhorn et al., 1946; Stauber, 1955; Rioux et al., 1971; Lennox et al., 1972; Anderson et al., 1980; Longstaffe et al., 1983) have been reported to occur during visceral *leishamaniasis*. However, these reports have contained few details and have only concentrated on the degree of adrenal parasitism (Stauber, 1955; Chadli and Philippe, 1961; Cazal and Pages, 1962; Rioux et al., 1971; Lennox et al., 1972), on the nature of the inflammatory phenomena (Cazal and Pages, 1962; Rioux et al., 1971; Lennox et al., 1972; Longstaffe et al., 1983) and on the presence of amyloid deposits in the adrenal glands (Gellhorn et al., 1946).

The present study describes experimentally-induced histopathological changes occurring in the adrenal glands of hamsters following inoculation of *Leishmania infantum*.

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Materials and methods

Thirty-six 10-week-old Syrian golden hamsters *(Mesocricetus auratus)*, 18 males and 18 females, with a mean weight of 100 g, were fed a commercial diet supplemented with lettuce, carrots and sunflower seeds. Drinking water was supplied *ad libitum*.

They were divided into two groups: group A, 30 animals, were inoculated i.p. with 1.0 ml of infective material containing 1.7×10^7 amastigotes. The inoculum was prepared from a spleen homogenate obtained from a hamster infected with *Leishmania infantum*. The total parasite count was determined using the same method as described in a previous study (González et al., 1983). The hamsters were serially killed at 15, 30, 45, 60, 75 and 88-90 days after inoculation. Group B, 6 animals, were inoculated i.p. with 1.0 ml of healthy hamster spleen homogenate.

Tissue samples for light microscopy study were fixed in 10% formalin, dehydrated, embedded in Histosec (Merck) and cut at 5 μm. The sections obtained were stained with hematoxylin and eosin, Masson's trichrome, Congo red and Wright's method modified for differentiating amyloid AA (Van Rijswijk and Van Heusden, 1979).

The Congo red-stained sections were viewed with polarized and fluorescent light. Samples for transmission

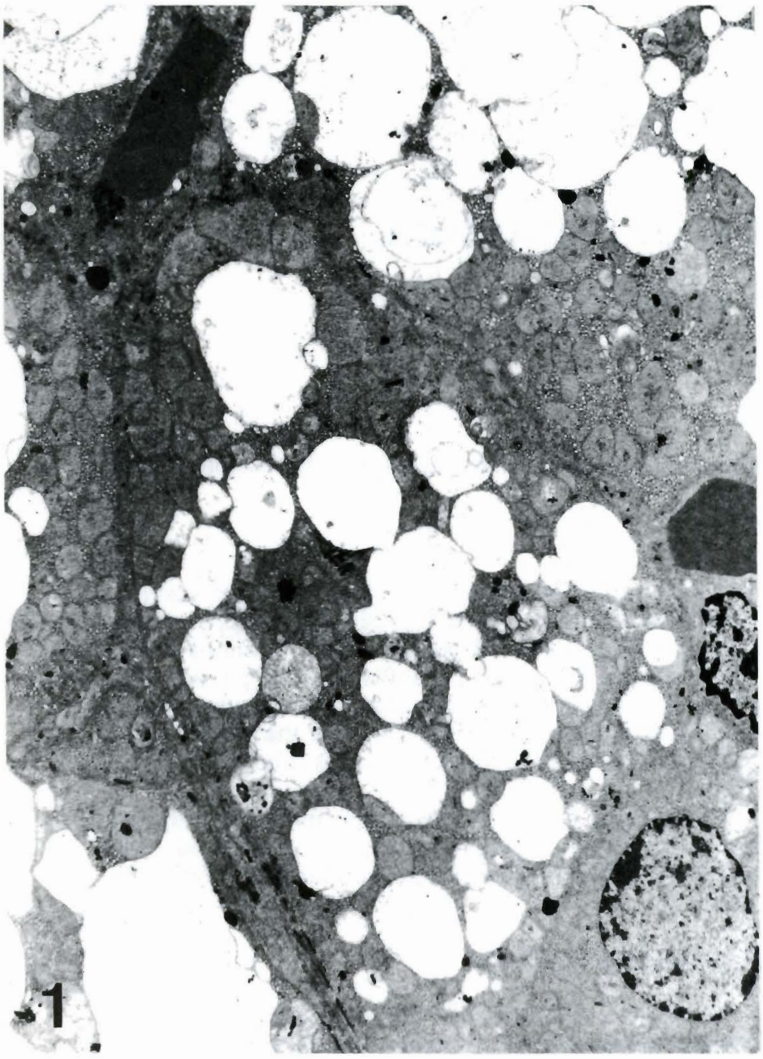

Fig. 1. Zona fasciculata, 60 days after inoculation. Hydropic degeneration of fascicular cells. × 5,500

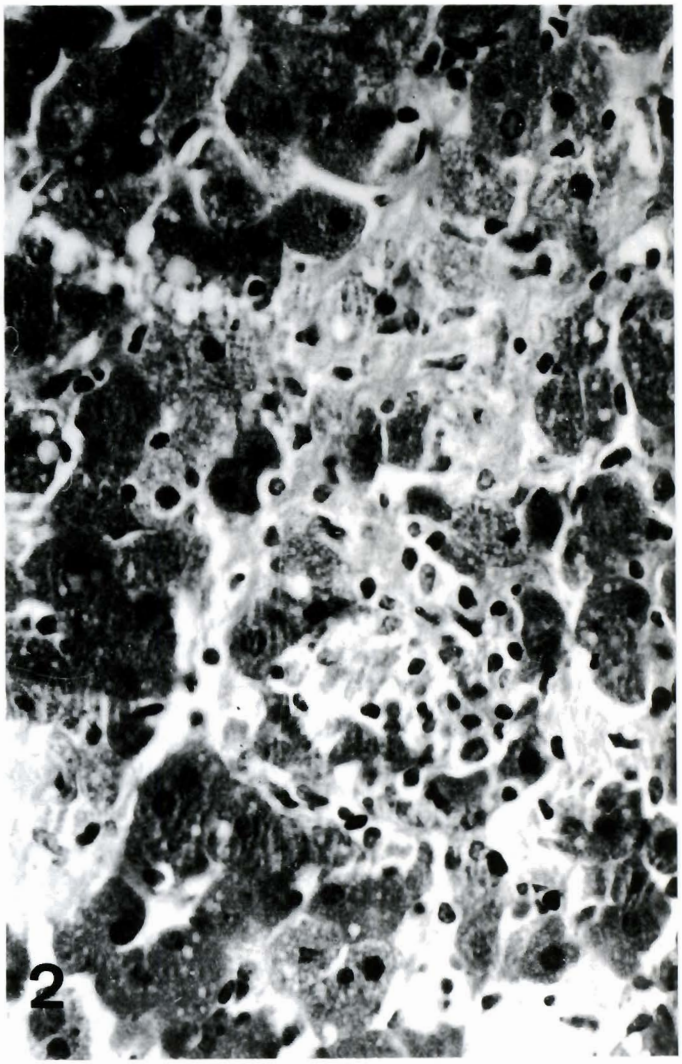

Fig. 2. Zona fasciculata, 60 days after inoculation. Interstitial lympho-histio-plasmacytic inflammatory infiltration. Intercellular amyloid deposit. Congo-red × 400

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 and ultratome, stained with 2% aqueous uranyl acetate and lead citrate and examined in a JEOL 100B electron microscope at 80 kV.

Results

In adrenal glands of animals killed at 15 days after inoculation no significant lesions were found when compared to controls.

Animals killed 30 days after inoculation showed slight signs of cellular swelling both in the adrenal *cortex* and *medulla*. In the cortico-medullary junction some lymphocytes and histiocytes were observed.

45 days after inoculation cellular swelling was still discrete in both the *medulla* and *zona glomerulosa* and intense in *zona reticularis* and *zona fasciculata*. Thus, with the electron microscope, the cells showed mitochondrial swelling and enlarged cisternae in the Golgi complex and the endoplasmic reticulum.

A notable finding at this stage was the accumulation of an amorphous eosinophilic and acellular material in the *zona reticularis* interstitium which was positive to Congo red and had yellow-green birefringence under polarized light, typical of amyloid. Alterations in affinity of amyloid for Congo red after incubation of tissue sections with potassium permanganate, demonstrated that it was amyloid AA. Ultrastructurally it appeared fibrillar with characteristic thin transversally striated fibrils arranged randomly.

Discrete lympho-histio-plasmacytic inflammatory infiltration in the *medulla* and in the cortico-medullary junction was observed.

The adrenal glands of animals killed 60 days after inoculation showed a greater degree of cellular swelling

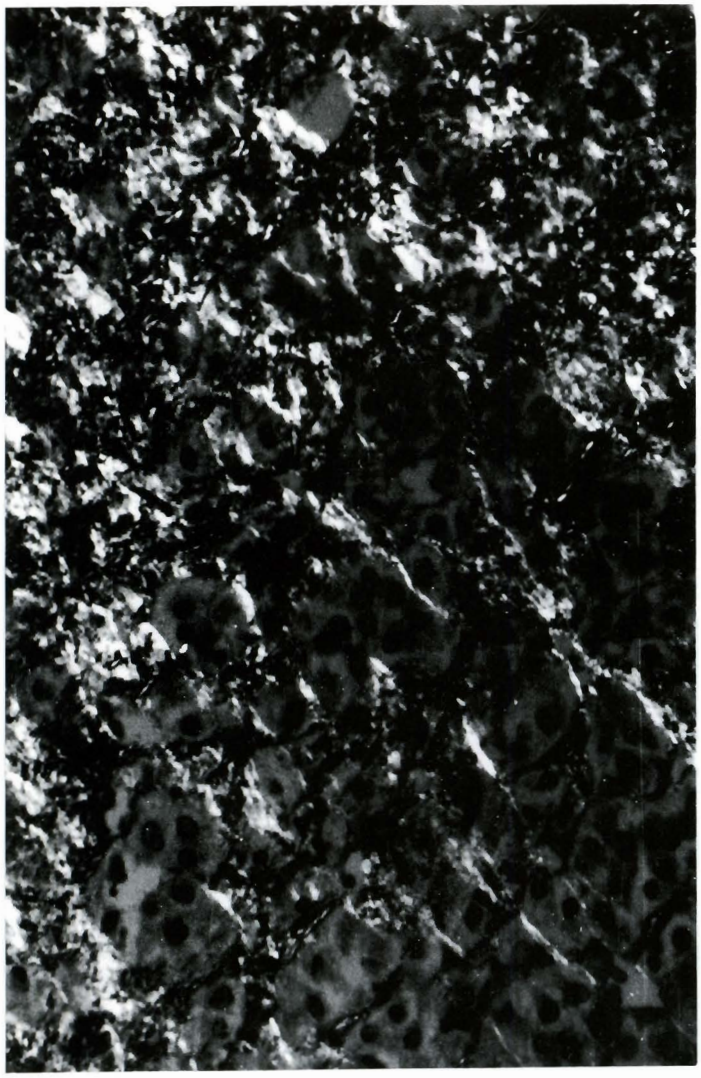

Fig. 3. Medulla, 75 days after inoculation. Birefringent amyloid deposits in the interstitium. Congo-red with polarized light. × 312,5

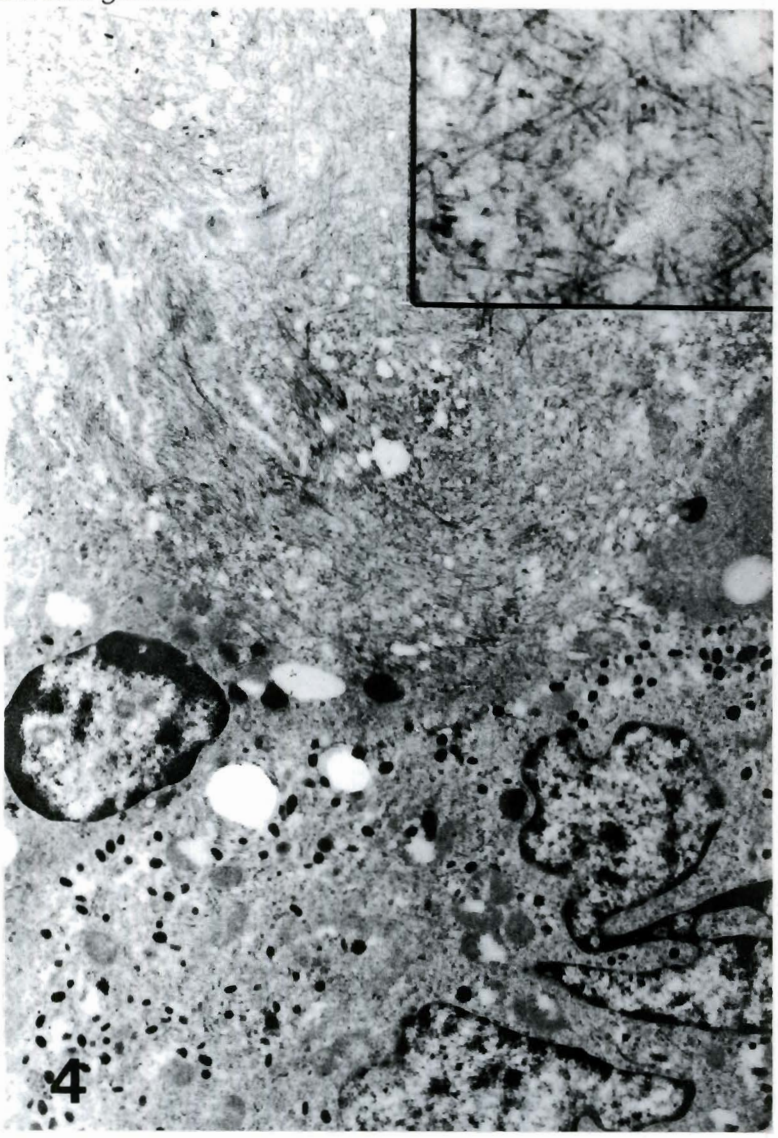

Fig. 4. Medulla, 75 days after inoculation. Amyloid fibrils compress chromaffin cells. × 10,000. Insert: magnification of amyloid fibrils.

both in the *cortex* and *medulla*. Ultrastructurally we observed a marked increase in the cellular volume with a vacuolar transformation of the endoplasmic reticulum (Fig. 1), mitochondrial swelling with cristolysis and different degrees of nuclear degeneration.

An increase of amyloid accumulation surrounding the capillaries and among the *zona reticularis* cells was observed. These deposits of amyloid also appeared in the zona fasciculata interstitium.

Discrete diffuse lympho-histio-plasmacytic infiltration was observed in the whole adrenal gland (Fig. 2).

In the animals killed 75 days after inoculation a greater amyloid deposit was observed in the whole *zona reticularis*, a great part of the *zona fasciculata* and the periphery of the *medulla* (Fig. 3). By means of electron microscopy these amyloid fibrils appeared to be extracellular, and compressed the cells (Fig. 4) which showed marked signs of hydropic degeneration.

Adjacent to these amyloid deposits an inflammatory infiltrate was seen with a predominance of histiocytes, lymphocytes and plasma cells. *Leishmania infantum* amastigotes were not present in macrophages.

In the end phase of the disease, at 88-90 days after inoculation, the amyloid occupied small parts of the *zona glomerulosa*, large parts of the *zona fasciculata*, the whole *zona reticularis* and half of the *medulla* (Fig. 5). As a consequence of this amyloidosis, a progressive parenchymatous atrophy of the *medulla* as well as of the cortical was originated. The parenchymatous cells not destroyed exhibited signs of an intense cellular degeneration, including enlarged cisternae in the Golgi complex and the endoplasmic reticulum, a considerable degree of mitochondrial swelling and pycnotic or swollen nuclei.

Lympho-histio-plasmacytic diffuse inflammatory infiltration was observed. Some macrophages containing 2 or 3 amastigotes in their cytoplasm were seen (Fig. 6).

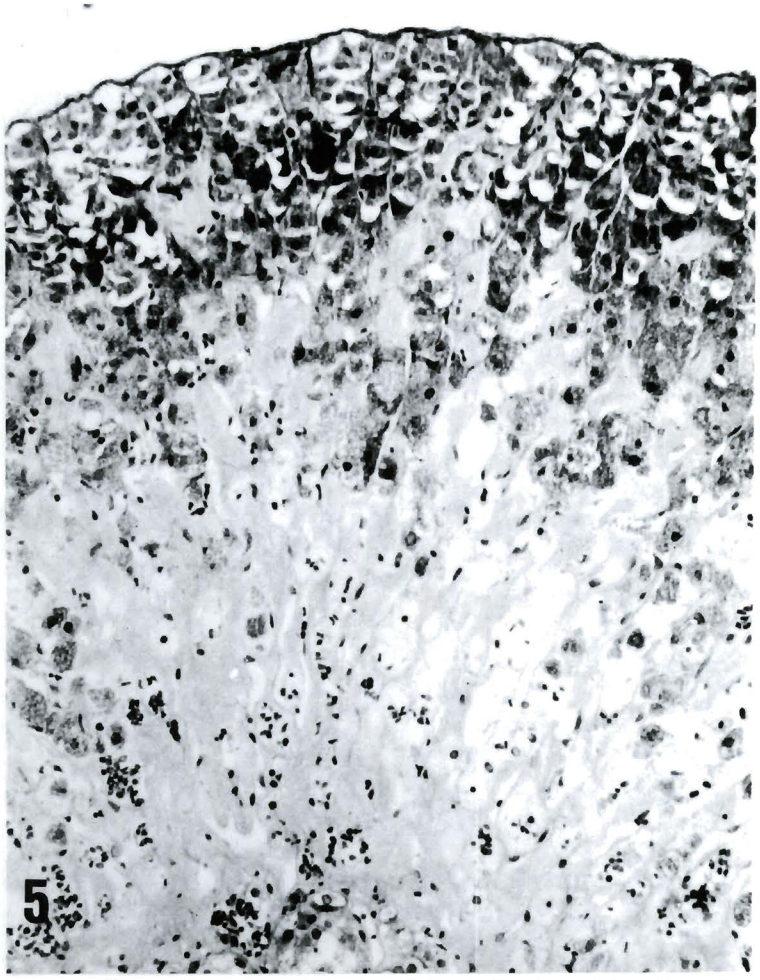

Fig. 5. Cortex and medulla, 90 days after inoculation. Extensive amyloid deposit in the zona fasciculata and the zona reticularis. Slight amyloidosis in the zona glomerulosa and periphery of the medulla. Congo-red. × 160

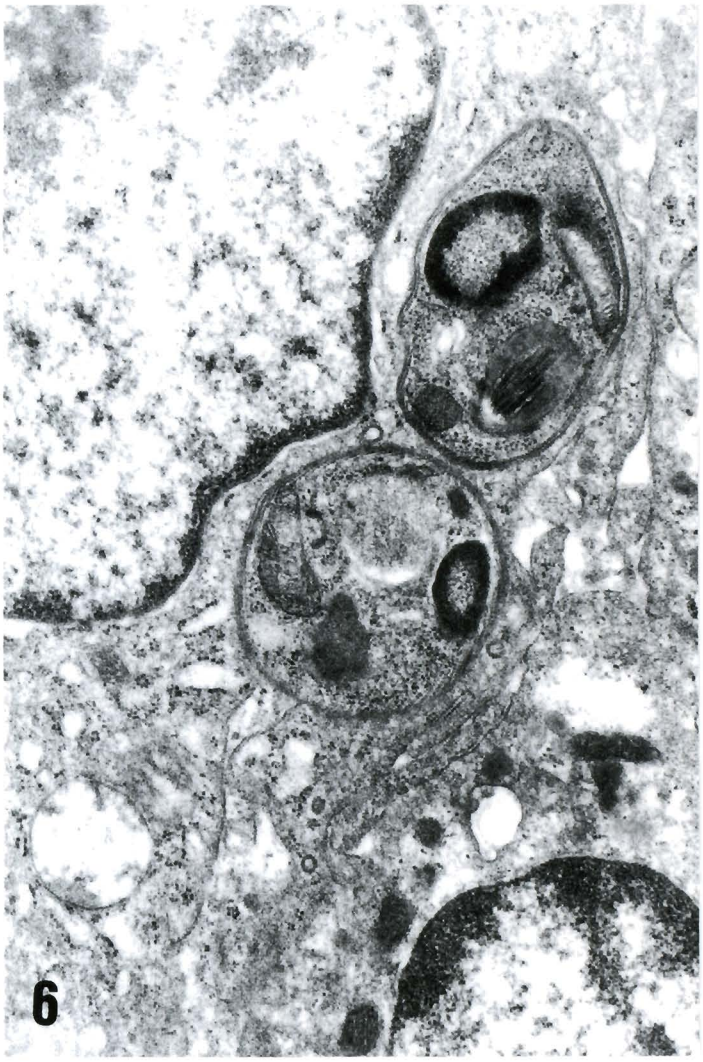

Fig. 6. Interstitium, 90 days after inoculation. Amastigotes contained in the cytoplasm of a macrophage. × 20,000

The control animal's adrenals presented no lesions.

Discussion

The present study demonstrates that experimental infection of hamsters with *Leishmania infantum* causes adrenal amyloidosis, characterized by the deposit of amyloid in cortex and medulla.

Amyloidosis as a complication of visceral leishmaniasis has been reported in man (Andrade and Andrade, 1966; Andrade and Iabuki, 1972), in dogs (Corbeil et al., 1976; George et al., 1976; Büngener and Mehlitz, 1977) and in hamsters (Hinglais et al., 1964; Abruzzo, 1971; González et al., 1983, 1986), but these deposits were described in liver, spleen, kidney, testis and intestine. However, the presence of amyloid in the adrenals of hamsters have only been reported by Gellhorn et al. (1946), which located these deposits exclusively in the cortex.

Amyloid is characterized by a mesh of rigid and non-ramified fibrillar proteins with a thickness ranging between 80 and 100 Å (Glenner and Terry, 1973). X-ray diffraction has shown these proteins to be formed by polypeptide chains arranged as ß-pleated sheets, a stable molecular conformation which makes them extremely insoluble and totally resistant to normal proteolytic enzymes (Tyzard, 1982). Consequently, accumulation of amyloid within tissue is generally irreversible and leads to pressure atrophy and progressive tissue destruction.

The pathogenesis of amyloid in leishmaniasis is still controversial. We have found that an increased amyloid deposition is directly related to an increased lympho-histio-plasmacytic infiltrate. The relationship between secondary amyloidosis and persistent antigenic stimulation triggered by these parasites is clear (Cheville, 1988).

However, absence of amyloid fibrils in the cytoplasm

of plasma cells, as has been observed in a previous study in the intestine (González et al., 1986), questions the hypothesis that amyloid synthesis during visceral leishmaniasis could be due to a disfunction of the plasma cells (Corbeil et al., 1976).

On the other hand, a progressive amyloidosis affecting the whole gland has been observed throughout this study. Amyloid begins to deposit interstitially in the *zona reticularis* 45 days after inoculation. Afterwards, it extends into the *zona fasciculata*, the *zona glomerulosa* and the *medulla*. Finally, amyloid occupies small portions of the *zona glomerulosa*, a great part of the *zona fasciculata*, the whole *zona reticularis* and a large area of the *medulla*. As a consequence of this amyloidosis a parenchymatous atrophy of the *cortex* and the *medulla* is originated.

Diffuse lympho-histio-plasmacytic infiltrates, which never constituted granulomas (Rioux et al., 1971; Longstaffe et al., 1983), were seen associated with the deposits of amyloid, both in the *cortex* and *medulla* (Chadli and Philippe, 1961; Lennox et al., 1972). The reports of macrophages carrying amastigotes in man (Chadli and Philippe, 1961; Cazal and Pages, 1962) and in animals (Stauber, 1955; Rioux et al., 1971; Lennox et al., 1972) during visceral leishmaniasis, were confirmed in this study made in hamsters. However, the number of leishmanias was small (maximum of 3 parasites per cell) and they were only observed at the end stage of the disease.

Together with amyloidosis, both cortical and medullary cells developed a progressive hydropic degeneration which finally led to their cytolysis.

The experimental infection of hamsters with *Leishmania infantum* produces a progressive amyloidosis in the *cortex* and *medulla* of the adrenal gland which at the final stages determines the partial destruction of adrenal parenchyma. Associated with amyloidosis, a lympho-histio-plasmacytic inflammatory infiltration has been observed.

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