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Liver parenchymal cell parasitism in human visceral leishmaniasis

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Summary. Liver parenchymal cell parasitism with the amastigotes form of *L. donovani* was detected by electron microscopy in human visceral leishmaniasis. Endocytosis was considered to be the mechanism by which the leishmania entered the cell. Evidence of well preserved parasites within hepatocytes suggest this parasitism as a possible reservoir for recrudescence.

Key words: Visceral leishmaniasis – *L. donovani* – Parasitism – Liver pathology – Electron microscopy

Introduction

There are rare reports on liver cell parasitism by amastigotes forms in visceral leishmaniasis. These relate to the identification of parasites using light microscopy (Christina 1910; Meleney 1925; Silva 1957; Daneshbod 1972; Coutinho 1982; Bryceson 1986). However, precise distinction of the limits of hepatocytes and the hypertrophic and hyperplastic Kupffer cells found in visceral leishmaniasis is not always possible by light microscopy. The objective of this work is to demonstrate the parasitism of liver parenchymal cells in human visceral leishmaniasis, found by electron microscopy after clinically successful treatment.

Material and methods

Liver biopsies from 14 cases of human visceral leishmaniasis were studied. The liver fragments were fixed for 2 h (4° C.) with 2% glutaraldehyde (phosphate buffered 0.1 M, pH 7.3; 400 mOsm) and post-fixed for 2 h with 1% tetroxide of osmium (phosphate buffered 0.1 M, pH 7.3).

After ethanol dehydration starting at 70% concentration, the fragments were immersed in propylene oxide and subsequently embedded in Araldite. Semithin 0.5 μm sections were stained with methylene blue (0.5%), azure II (0.5%), sodium borate (0.5%) and acid fuchsin (2%) in ethanol 50%. The material was observed in a Philips 301 electron microscope.

All cases presented a fibrogenic pattern of liver involvement (Duarte and Corbett 1987) with persisting hepatomegaly, negative myelogram and myeloculture. These cases were selected from 47 cases used in the study to define the different patterns of liver involvement in visceral leishmaniasis (Duarte and Corbett 1987).

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Results

Light microscopy showed the typical fibrogenic pattern of liver involvement in human visceral leishmaniasis (Duarte and Corbett 1987). This pattern shows mild hyperplasia and hypertrophy of the Kupffer cells and increase in reticulin and collagen fibres in the Disse space. Electron microscopy showed few Kupffer cells with amastigotes forms of *L. donovani* within phagosomes. Amastigotes were also found in the cytoplasm of the hepatocytes (Fig. 1) in one case. Some of parasites were within phagosomes (Fig. 2) with a limiting membrane closely apposed to the entire surface of the parasite forming a parasitophorous vacuole type I (Chang and Dwyer 1978; Chulay et al. 1985). Other amastigotes were in parasitophorous vacuoles type II, in which the amastigotes are eccentrically placed in a vacuole (with vacuolar membrane adherent to part of their surface) containing a flocculent precipitate of low electron density together with rests of membrane (Fig. 3). Leishmania with no clearly identified parasitophorous membrane, apparently free in the hyaloplasm of the liver cells, were also seen. The parasites were well preserved.

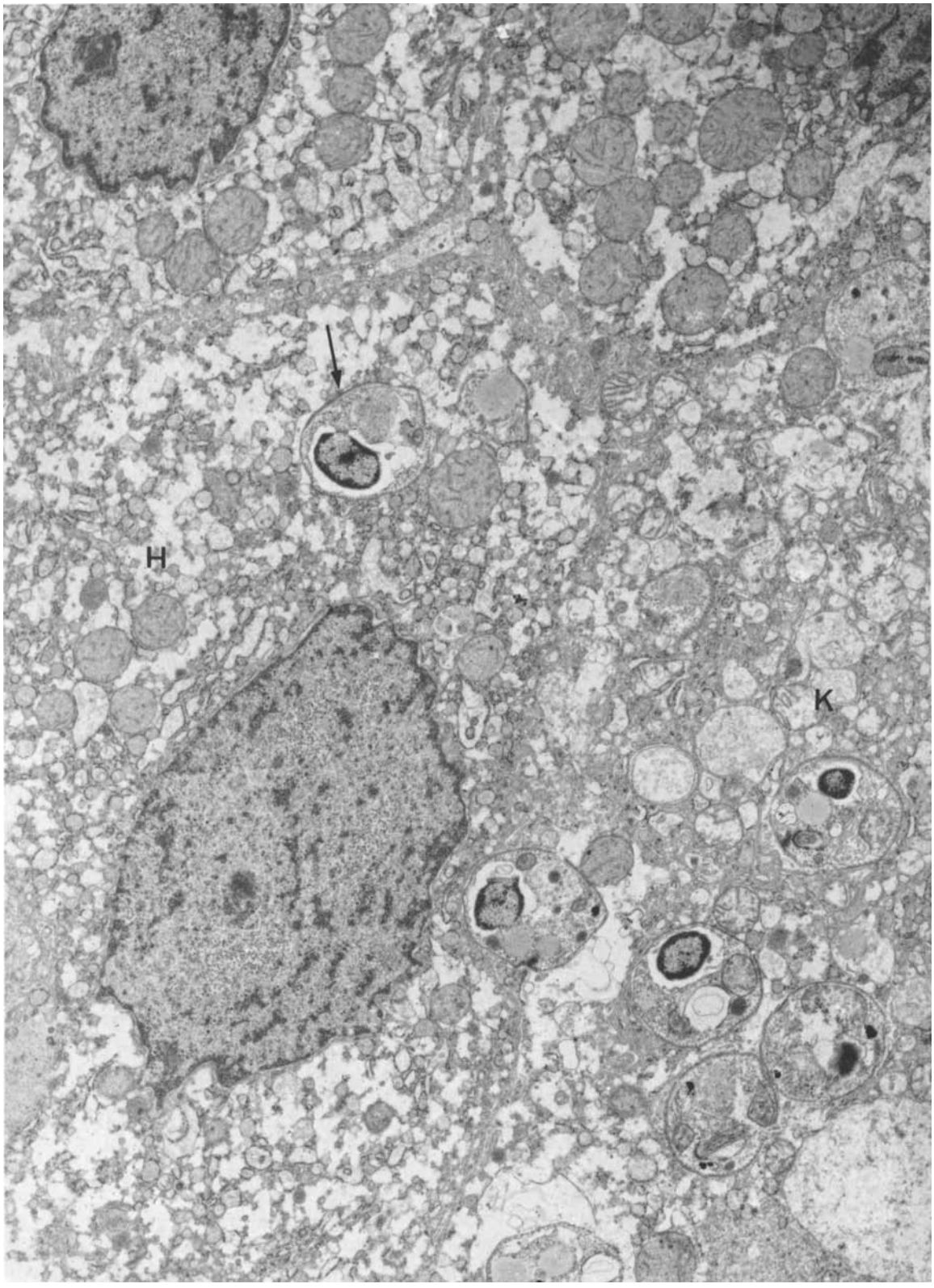

Fig. 1. Amastigotes form of *L. donovani* within hepatocyte (*H*) and Kupffer cell (*K*) cytoplasm and apparently entering the hepatocyte. (×10000)

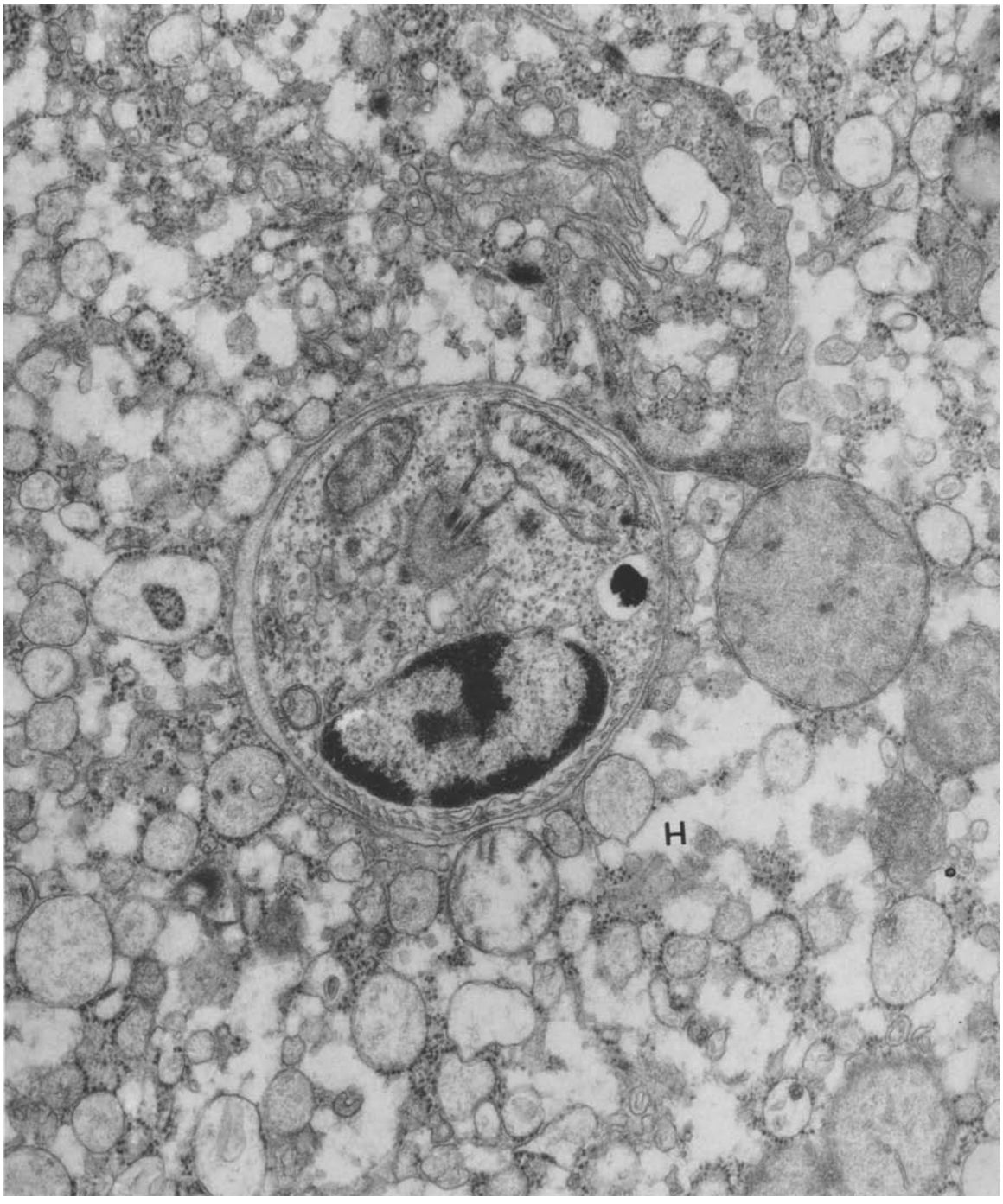

Fig. 2. Amastigote form of *L. donovani* within hepatocyte cytoplasm in parasitophorous vacuole type I. (×16400)

Amastigotes have also been identified in the Disse space in close relation to the hepatocyte. At this point the liver cells showed concavity or the cell surface with loss of microvilli, suggesting endocytosis (Fig. 4). The amastigote seen had well preserved sub-pelicular microtubules, megasome (juxtanuclear vacuole), flagellum and endoplasmic reticulin.

Discussion

Hepatocyte parasitism with amastigotes in visceral leishmaniasis has been demonstrated by electron microscopy. Light microscopy is not adequate in evaluating the sinusoidal and perisinusoidal elements of this disease. The close apposition of the cytoplasmic process of the sinusoidal lining cells

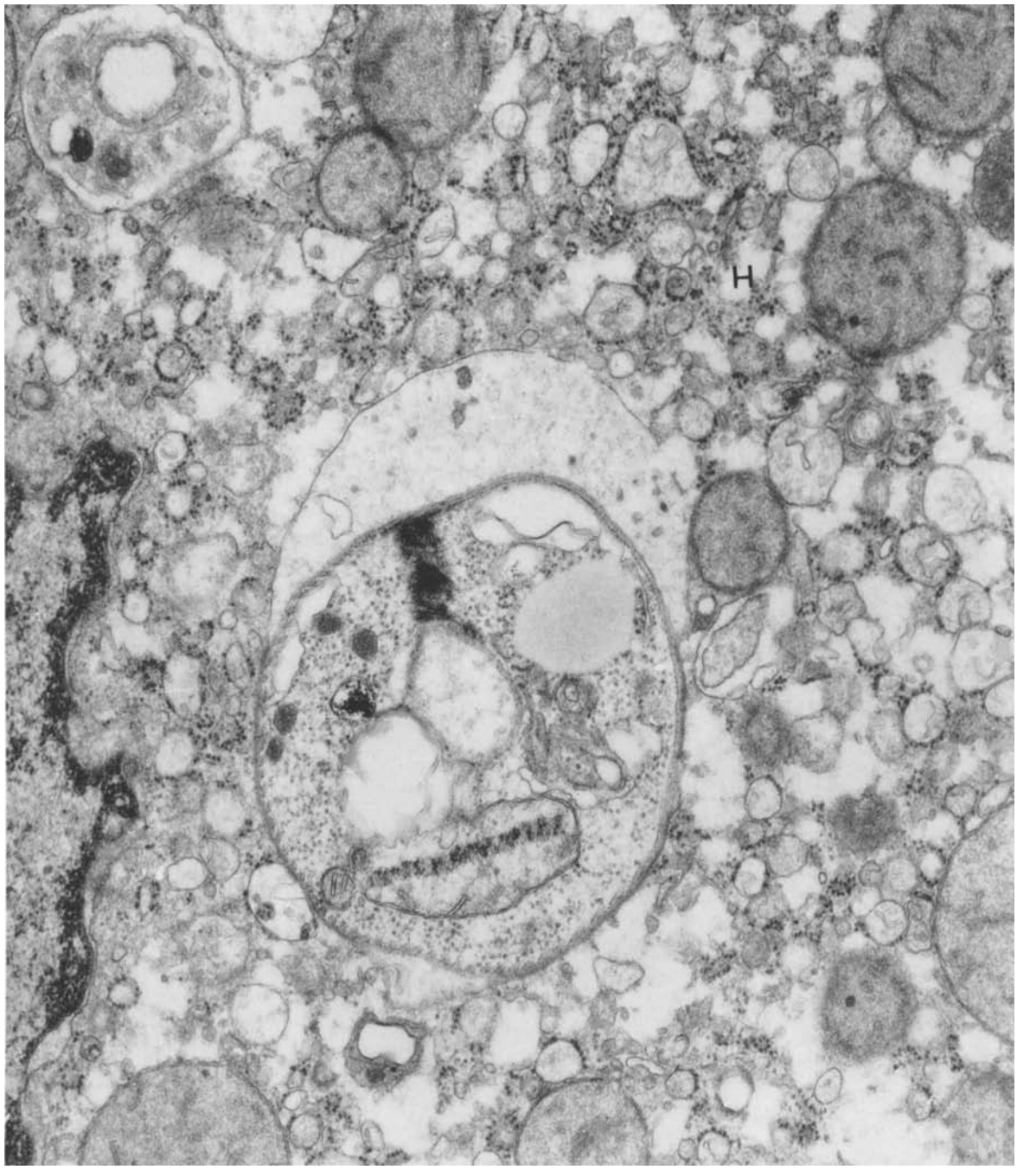

Fig. 3. Amastigote form of *L. donovani* within hepatocyte cytoplasm in parasitophorous vacuole type II. (×16600)

mixed with flocculent material in the Disse space make the membrane limits of regional cells poorly defined. In the normal liver these elements are more easily defined in histological section because there is much less overlapping of the cytoplasmic processes. All cases studied showed enlargement of the Disse space either by flocculent material or by fibrosis accompaining the persistent hypertrophy and hyperplasia of Kupffer cells and perisinusoidal cells. These alterations may suggest the "endothelial massage" mechanism as proposed by Wisse et al. (1983, 1985, 1986) which may promote changes in the metabolic exchange and transport mechanisms leading to stasis and fibrogenesis. We have detected hepatocyte concavity with loss of microvilii close to a leishmania in the Disse space,

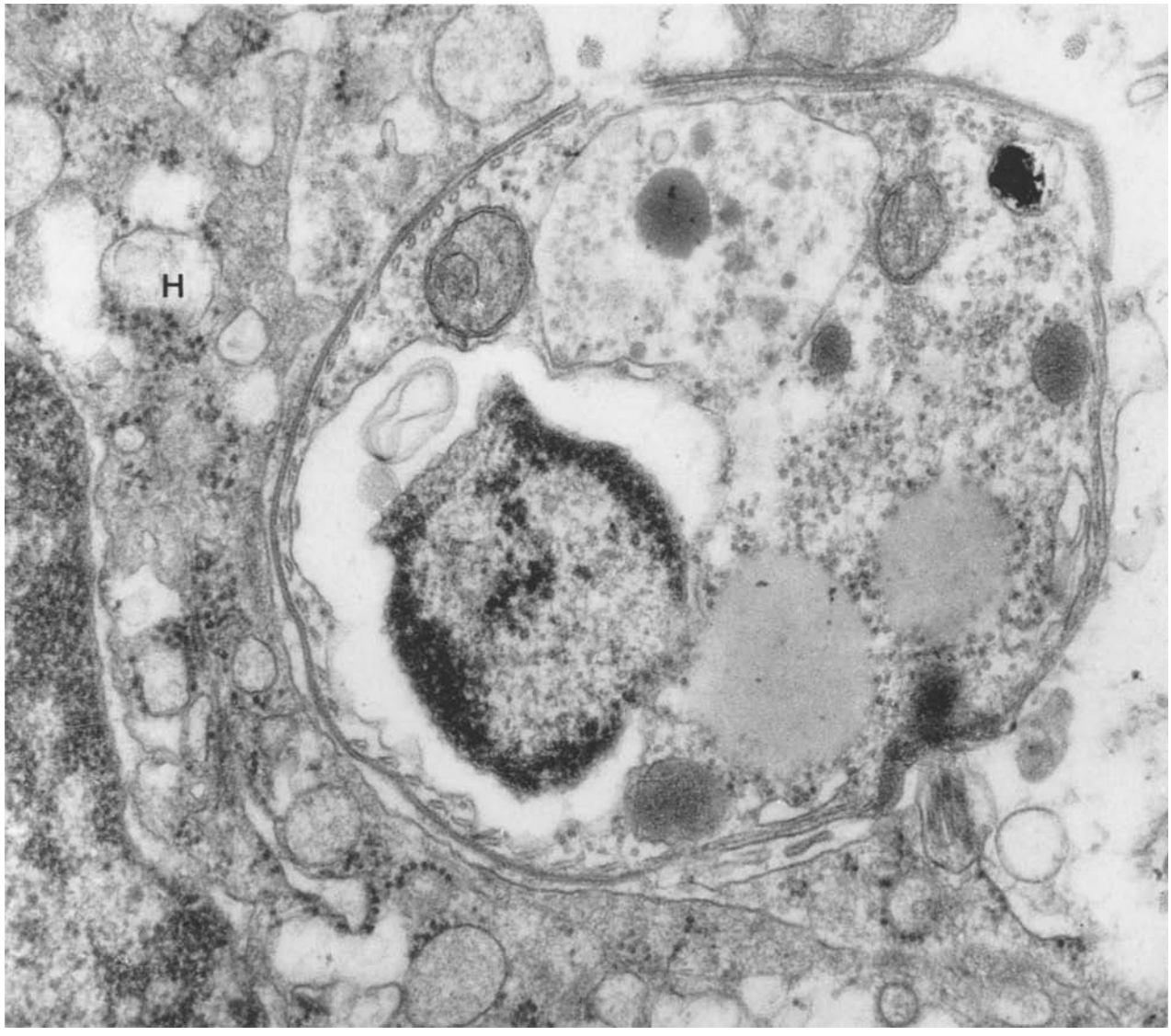

Fig. 4. Well preserved amastigotes of *L. donovani* apparently entering the hepatocyte. (× 73 500)

which suggest endocytosis as the mechanism for entry into the cell. Parasites within the parenchymal cells were either in parasitophorous vacuoles type I or II as described by Chang and Dwyer 1978 and Chulay et al. 1985, or were apparently free in the cytoplasm.

Hepatocyte and Kupffer cell parasitism may be considered as a possible reservoir for recrudescence of the disease, since the parasites were apparently well preserved within these cells.

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