Rev. Inst. Med. trop. São Paulo
29(3):131-136, *maio-junho*, 1987

HISTOPATHOLOGICAL PATTERNS OF THE LIVER INVOLVEMENT IN VISCERAL LEISHMANIASIS

M. I. S. DUARTE & C. E. P. CORBETT

SUMMARY

The hepatic changes observed in liver specimen from either biopsy or necropsy of 47 patients with visceral leishmaniasis permited us to define three different histopathological patterns of involvement: typical, nodular, and fibrogenic.

These patterns seem to be representative of different evolutive stages of the hepatic involvement in the disease either towards a more benign evolution or to more chronic stage with fibrosis and "cirrhosis".

These histopathological evolutive stages are related to the prognosis of the disease.

KEY WORDS: Visceral leishmaniasis; liver pathology; **L. donovani.**

INTRODUCTION

Leishmanial parasitism of Kupffer cells, hyperthrophy and hyperplasia of the reticuloendothelial system are fundamental findings used to characterize visceral leishmaniasis (LAVERAN, 1917; MELENEY, 1925; GIRAUD, 1956; ANDRADE & ANDRADE, 1966). The first histopathological report was presented by CHRISTOPHER (1904). Scattered fatty changes of the liver cells and portal area infiltrated with lymphocytes and plasma cells are also commonly described (CHAPTAL, CAZAL & JEAN, 1954; WILCOCKS & MANSON-BAHR, 1972; HUTT, KOBERLE & SALFELDER, 1973; NEAFIE & CONNOR, 1976).

The typical histopathological hepatic changes usually described in visceral leishmaniasis of the Old and the New World have been similar. However the involvement varied in intensity and no systematic characterization has been made so far.

ROGERS, 1908, described in long standing Kala-azar patients a peculiar type of hepatic "cirrhosis" due to visceral leishmaniasis. There are other descriptions of more cases of "cirrhosis" reported in Kala-azar patients mainly based on clinical and laboratory dates. Few histopathological observations were carried out in these cases (NATTAN-LARRIER, 1918; WU.CHU & WU, 1949; CAZAL, 1949; CHAPTAL, CAZAL & JEAN, 1954; SEN-GUPTA et al., 1956; RODRIGUES DA SILVA, 1957; DANESHBOD, 1972; VERESS et al., 1974). GOSWAMI, 1970, studying Indian patients with long standing Kala-azar, found high incidence of "Rogers'cirrhosis" with no other disease which could be related to hepatic cirrhosis. Such a high incidence of "Rogers'cirrhosis" found in Indian patients has not been described in other parts of the world. In Brazil, the first histopathological description of a case of "cirrhosis" due to visceral leishmaniasis, — "Rogers'cirrhosis", was made by BOGLIOLO, 1956. RODRIGUES DA SILVA & De PAOLA, 1958 and COUTINHO, 1982, described the other two cases. References about other types of intralobular fibrosis of the liver in visceral leishmaniasis are rare (CHAPTAL, CA-

Department of Pathology, University of São Paulo Medical School, Brazil.
Address for correspondence: Dra. Maria Irma Seixas Duarte. Faculdade de Medicina da Universidade de São Paulo, Departamento de Patologia. Caixa Postal 8100. CEP 01246 — São Paulo, SP, Brasil.

ZAL & JEAN, 1954; RODRIGUES DA SILVA & De PAOLA, 1958).

We believe that the study of intermediate liver changes between the typical and the "Rogers' cirrhosis" in visceral leishmaniasis is important. The characterization of these changes, would be useful to explain the chronic evolution of the lesions.

The purpose of this work is to analyse the hepatic changes either through biopsy or necropsy specimens from patients with visceral leishmaniasis, trying to define different morphological patterns of the liver involvement and their possible role in the evolution of the lesions.

MATERIAL AND METHODS

We studied 47 cases of visceral leishmaniasis diagnosed by isolation of leishmania from bone marrow and the histopathological study of the liver specimens. The liver biopsy was performed in 16 cases due to persistent hepatomegaly after the treatment with clinical cure. All these cases were HBsAg (—) and Schistossomin (—). The other 31 cases were obtained from necropsy of patients who died during the disease treated or not.

The age of the patients varied from 7 months to 42 years old and most of them were young children.

The liver fragment were fixed in phosphate buffered (pH 7,0) 10% formalin solution and prepared by the usual technic for histopathological section. Sections were stained by Heamatoxylin-eosin, Wilder's silver reticulum stain, Masson's trichrome, Giemsa, Gallego and Picrosirium methods (JUNQUEIRA, BIGNOLAS & BRENTANI, 1979). Giemsa and Gallego stain were performed for a better demonstration of the parasite.

There was no other previous or associated disease in our patients that could be related to hepatic fibrosis.

RESULTS

The histopathological liver changes presented different patterns that could be classified as:

1. Typical or classical patterns: 7 cases (Fig. 1) — All these cases obtained from autopsy show marked hyperthrophy and hyperplasia of the Kupffer cells, many of them with high parasitism by amastigotes. Enlarged Kupffer cells were pratically filling up all the sinusoidal lumen. The portal spaces were enlarged and infiltrated with macrophages, some of them with parasites, lymphocytes and plasma cells in a varyied amount. We also found small intralobular foci of mononuclear inflammatory cells. The hepatocytes usually showed fatty change varying in intensity, and mild degenerative changes with either focal or individual cell necrosis. Regeneration of the parenchymal cells were rare.

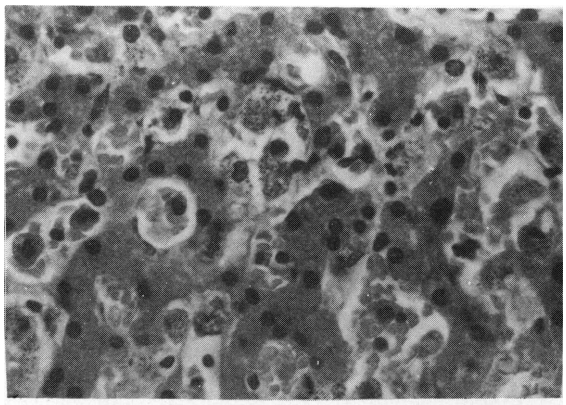

Fig. 1 — Typical pattern: hyperthrophy and hyperplasia of the Kupffer cells most of them parasited. (HE stain; x 620).

2. Nodular pattern: 5 cases (FIG. 2) — It is characterized by scattered nodules irregularly distributed in the intralobular and portal area. These nodules were made up by macrophages, lymphocytes and plasma cells infiltration with no epithelioid differentiation. The Kupffer celss showed mild hyperthrophy and hiperplasia, usually with no parasitism. Few parasites within macrophages when present were mainly in the central part of the nodules. The portal space presented also diffuse mononuclear cell infiltration. Reticulin stain showed small foci with dendritic aspect of the fibrils. The hepatocytes showed no obvious changes.
3. Fibrogenic pattern: 35 cases. It is characterized by intralobular proliferation of reticulin fibers and/or collagen band deposits. Multifocal proliferation with thickening of

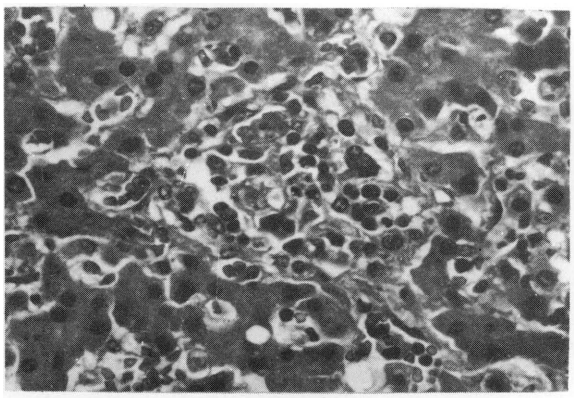

Fig. 2 — Nodular pattern: intralobular nodular infiltration by macrophages, lymphocytes and plasma cells. (HE stain; x 620.

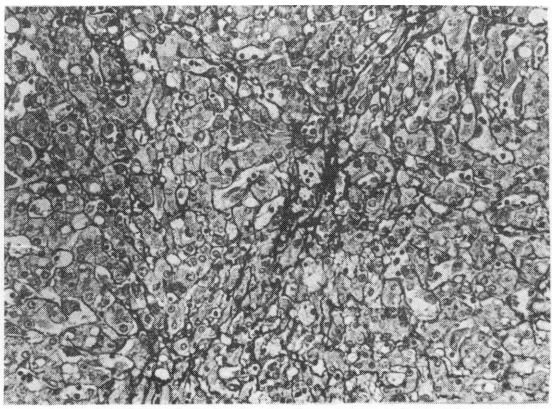

Fig. 4 — Fibrogenic pattern; multifocal persinusoidal collagen fibers irregulary distributed (Picro-sirius stain; x 240).

the reticular framework with preserved architecture was found in 15 of these cases. There was no preference for any particular area of the lobule. The Kupffer cells showed mild hyperplasia and hyperthrophy with rare parasitism. Slight intralobular and portal space mononuclear cells infiltrate was seen and the Ito's cells were prominent. The hepatocytes were usually normal. In 19 cases collagen deposition feading to multifocal perisinusoidal fibrosis was seen, (Fig. 3 and 4), with preference to the vicinity of the portal area. The reticulin fibers were also proliferated.

One case (Fig. 5), showed diffuse intralobular fibrosis compatible with the description made by ROGERS (1908) (so called "Rogers' cirrhosis"). In this case the organ architecture was alterated by diffuse intralobular fibrosis isolating either individual or small groups of liver cells with intralobular collagen bands in continuity with the portal fibrosis, and portal spaces enlarged by striking fibrosis. There was also some grade of hyperthrophy and hyperplasis of Kupffer cells, few of them parasitized. Portal and intralobular infiltration by macrophages, lymphocytes and plasma cells were also found. The reticulin fibres were diffusely proliferated. The hepatic cells showed degenerative changes. Necrosis and bile pigment within hepatocytes and bile thrombi were also found. Liver cells regenerating plates appearing as two cells layers thick or acinar like regenerative process were present.

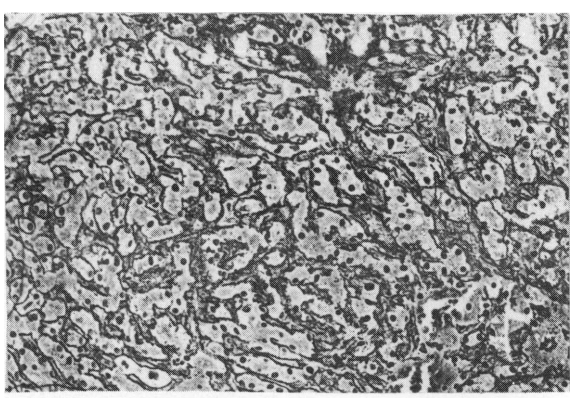

Fig. 3 — Fibrogenic pattern: perisinusoidal proliferation of reticulin fibers. (Wilder's reticulin stain; x 240)

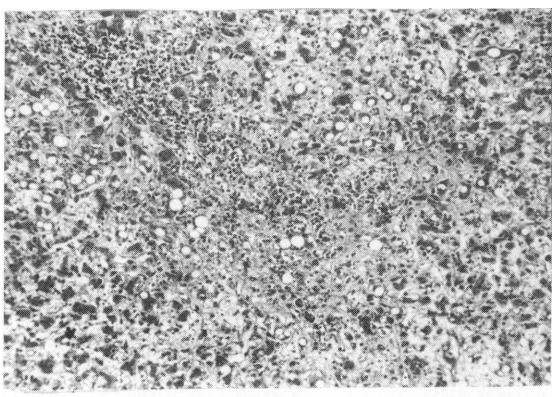

Fig. 5 — Diffuse intralobular fibrosis: "Rogers's cirrhoss" with bands of collagen tissue isolating small groups of liver cells. (Masson's stain; x 125).

The incidence of each histopathological type found in the autopsy and biopsy material is shown in table 1.

TABLE I

The incidence of each type of liver involvement found in autopsy and biopsy specimens

		Visceral leishmaniasis Liver involvement		
	Number of cases	Typical	Fibrogenic	Nodular
Autopsy	31	7 (22,58%)	21 (67,74%)	3 (9,67%)
Biopsy	16	—*	14 (87,5%)	2 (12,5%)
Total	47	7 (14,89%)	35 (74,46%)	5 (10,63%)

* Liver biopsy was performed only in patients with persistent hepatomegaly after treatment. No typical pattern was found in this cases.

DISCUSSION

In the literature the most reported liver involvement in visceral leishmaniasis is the typical pattern. These reports usually describe: a) hyperthrophy and hyperplasia of the reticuloendothelial system; b) parasitism; c) portal area infiltrated by macrophages, lymphocytes and plasma cells; d) scanty changes of the hepatocytes.

In spite of the discussion on the possibility of the visceral leishmaniasis to cause true cirrhosis, a particular type of "cirrhosis" has been demonstrated and accepted by the authors (ROGERS, 1908; BOGLIOLO, 1956; SEN GUPTA et al. 1956; RODRIGES DA SILVA & De PAOLA, 1958; GOSWAMI, 1970; WILCOCKS & MANSON-BAHR, 1972; COUTINHO, 1982). The "Rogers' cirrhosis" as described is not nodular but presents a marked diffuse fibrosis isolating small groups of liver cells with regenerative changes. All these hepatic changes lead to portal hypertension and hepatic insufficiency. In a way there is a histopathological similarity with the congenital hepatic fibrosis due to siphilis (BOGLIOLO, 1956; EDINGTON, 1979). From the liver pathology point of view "Rogers'cirrhosis" doesn't represent a real cirrhosis as recommended by a working group sponsored by the World Health Organization in 1977 (ANTHONY et al.). However, this group does not discuss visceral leishmaniasis as cause of either cirrhosis or fibrosis. Based on this we will call the "Rogers' cirrhosis" as diffuse intralobular fibrosis. This diffuse intralobular fibrosis, — "Rogers'cirrhosis" —, appears mainly in adult with long standing disease. The Children often have a more rapidly progressive disease with no time for the fibrotic change to occur (GOSWAMI, 1970). It is important to point out that the visceral leishmaniatic "cirrhosis" has been diagnosed more often in Indian patients than in other endemic areas. In Indian Kala-azar it has been reported with higher incidence, varying from 25% (ROGERS, 1908) to 60% (GOSWAMI, 1970). In Brazil the "Rogers'cirrhosis" is far less frequent than in Indian Kala-azar. There are only four cases described with histopathological confirmation (BOGLIOLO, 1956; RODRIGUES DA SILVA & De PAOLA, 1958; COUTINHO, 1982 and our case). Our material showed only one conclusive case for intralobular diffuse fibrosis (ROGERS' cirrhosis) with portal hypertention and hepatic insufficiency. Cases with foci of intralobular fibrosis in visceral leishmaniasis with no portal hypertention and hepatic insufficiency has occasionally been reported (RODRIGUES DA SILVA & De PAOLA, 1958). We found in our material high incidence of intralobular fibrosis with 74.4% of cases showing at least scattered small foci of perisinusoidal fibrosis. The biopsy material showed a distribution of each type which could not be considered as representative of the population. The persistent hepatomegaly after clinical cure was the biopsy indication and the patients submitted to liver biopsy showed higher incidence of the fibrogenic pattern. The hepatomegaly was smaller than usually found in the active phase of the disease. We couldn't find any report considering a possible fibrogenic process secondary to glucantine treatment. LIEBER, 1985 has not considered nutritional changes as responsible for hepatic fibrogenic process. The pathogenesis of the fibrotic changes is not known yet, but the participation of specific immunological response to the parasite or some of its components could be considered as factor of activation of interstitial elements leading to the fibrogenic process. However, the dynamic of the immune response in the visceral leishmaniasis is not so far clear.

A systematically description of the lesions and the correlation with possible evolution has not been made so far.

We believe that visceral leishmaniasis determine liver alteration ranging from reticulo-

endothelial system reactivity and the acompanying parasitism to a fibrogenic response, eventually evolution to intralobular diffuse fibrosis ("Rogers' cirrhosis"). Therefore, between the exuberant reactivity of the reticuloendothelial system and the "Rogers' cirrhosis" there is a fibrogenic process evoluting through proliferation of reticulin fibers and multifocal fibrosis. The chronic parasitism with long standing reactivity of the reticuloendothelial system would stimulate the fat-containing perisinusoidal cells (Ito's cell) leading to increased production of reticulin fibers. The maintenance of the stimulus would lead to collagen fibers formation, at the perisinusoidal space, and subsequent fibrosis. The multifocal fibrosis was the most common finding seen in our biopsy material, probably due to the fact that the biopsed patients have had clinical and laboratorial cure with persisting hepatomegaly.

The liver involvement here described can also be included in the group of the mesenchymal hepatitis (MARTIN et al., 1984) or the perisinusoidal fibrosis (FEROLDI & MALLET-GUY, 1979, 1984).

We are convinced that the visceral leishmaniosis causes an interstitial disease also present in other organs such as lungs and kidneys. We have already described the lung involvement in experimentally infected hamster (DUARTE, 1984) and an interstitial nephritis in man (DUARTE et al., 1983). The hepatic changes now described can be considered as part of this interstitial change.

In the nodular pattern, the hyperplasia and hyperthrophy of the reticuloendothelial system is not marked, parasitism is low and there is small foci of inflammatory mononuclear cells infiltrate. In the literature there are reports describing nodular liver infiltration in visceral leishmaniasis (CAZAL, 1949) or "Granulomas" (SEN GUPTA et al., 1956) or even groups of mononuclear cells forming sometimes "real nodules" (GIRAUD, 1956) and nodules of cells (CHAPTAL, CAZAL & JEAN, 1954). This pattern has also been found in experimental animals evoluting to cure and in "glucantine" treated patients with good clinical response, which suggest this pattern as an evolution to cure. However, we are inclined-to think that this pattern would also be found in oligosymptomatic patients living in endemic areas. The pattern could also be related to other than **L. donovani** leishmania causing visceral changes (DUARTE et al., 1984) as seen in experimentally infected animals.

All these histopathological patterns observed could be representative of different evolutive stage of the liver involvement in visceral leishmaniasis whether we consider an evolution for cure (nodular pattern) or to a more chronic response of the organ towards diffuse intralobular fibrosis (Rogers' cirrhosis).

These histopathological results leads to the necessity of more research on clinical and laboratorial aspects of the hepatic involvement in visceral leishmaniasis looking for a histopathological and clinical correlation.

RESUMO

Padrões histopatológicos do acometimento hepático na leishmaniose visceral

As alterações hepáticas observadas no fígado em material de biópsia ou necrópsia de 47 pacientes com leishmaniose visceral nos permitiu definir três padrões histopatológicos diferentes de acometimento hepático: típico, nodular e fibrogênico.

Estes padrões parecem ser representativos de diferentes formas evolutivas do acometimento hepático na doença para uma evolução mais benigna ou para uma evolução mais crônica com fibrose e "cirrose".

Estas formas histopatológicas evolutivas estão relacionadas ao prognóstico da doença.

ACKNOWLEDGEMENTS

Work supported by FINEP — Projeto 43/83/0006/00 and 43/86/0361/00. We thank Dr. H.F. Andrade Jr. for his valuable advices.

REFERENCES

1. ANDRADE, Z. A. & ANDRADE, S. G. — Alguns novos aspectos da patologia do Calazar (Estudo morfológico de 13 casos necropsiados). **Rev. Inst. Med. trop. S. Paulo, 8: 259-266, 1966.**

2. ANTHONY, P. P.; ISHAK, K. G.; NAYAK, N. C.; POULSEN, J. E.; SCHEUER, P. & SOBIN, L. H. — The morphology of cirrhosis: definition, nomenclature and classification. **Bull. Wld. Hlth. Org., 55: 521-540, 1977.**

3. BOGLIOLO, L. — Nova contribuição ao conhecimento da anatomia patológica da leishmaniose visceral. **A**

propósito de um caso brasileiro e com especial referência à fibrose hepática. **Hospital (Rio de J.), 1:** 393-440, 1956.

4. CAZAL, P. — L'hépatite leishmanienne démontrée par les ponctions biopsies. **Ann. Biol. Clin., 7:** 113-120, 1949.

5. CHAPTAL, J.; CAZAL, P. & JEAN, R. — Étude sur le Kala-azar. Lésion des organes hemato-poiétiques au cours du Kala-azar infantile. Leur evolution sous l'effer du traitement. **Arch. Pediat., 11:** 281-290, 1954.

6. CHRISTOPHER, S. R. — On a parasite found in persons suffering from enlargement of the spleen in India. Second Report. Scient. Mem. of the Government of India. New Serie n.º 11 — Calcutta, Government Printing, 1904. Apud: Sen Gupta et al., (1956). The liver in Kala-azar. **Ann. trop. Med. Parasit., 50:** 352-359, 1956.

7. COUTINHO, E. M. — Contribuição para o estudo anátomo-patológico da leishmaniose visceral. Recife, 1982. (Tese — Faculdade de Ciências Médicas da Universidade Federal de Pernambuco).

8. DANESHBOD, K. — Visceral leishmaniasis (Kala-azar) in Iran: a pathologic and electron microscopic study **Amer. J. clin. Path., 57:** 156, 1972.

9. DUARTE, M. I. S. & CORBETT, C. E. P. — Histopathological and ultrastructural aspects of interstitial pneumonitis of experimental visceral leishmaniasis. **Trans. roy. Soc. trop. Med. Hyg., 78:** 683-688, 1984.

10. DUARTE, M. I. S.; LAURENTI, M. D.; GOTO, H.; ANDRADE JR., H. F. & CORBETT, C. E. P. — Comportamento visceralizante da **Leishmania mexicana amazonensis** em hamsteres. In: CONGRESSO DA SOCIEDADE BRASILEIRA DE MEDICINA TROPICAL, 20., Salvador, 1984. **Programa e resumos.** Salvador, Gráfica **Econômico, 1984. p. 88-89.**

11. DUARTE, M. I. S.; SILVA, M. R. R.; GOTO, H.; NICODEMO, E. L. & AMATO NETO, V. — Interstitial nephritis in human Kala-Azar. **Trans. roy. soc. trop. Med. Hyg., 77:** 531-537, 1983.

12. EDINGTON, G. M. — Other viral and infection disease in pathology of the liver. In: MacSWEEN, R. N. M.; ANTHONY, P. P. & SCHEVER, P. J. — **Pathology of the liver.** Edinburgh, Churcill Livingtone, 1979. p. 201-202.

13. FEROLDI, J. & MALLET-GUY, Y. — Fibrillogénèse collagène intrahépatique et cellules de stockage de graisse. **Arch. Anat. Cytol. Path., 27:** 377-382, 1979.

14. FEROLDI, J. & MALLET-GUY, Y. — Les fibroses perisinusoidales. **Arch. Anat. Cytol. Path., 32:** 287-295, 1984.

15. GIRAUD, P. — Importance des lésions hépatiques au cours de la leishmaniose viscérale (Kala-azar). **Rev. int. Hépat., 6:** 769-777, 1956.

16. GOSWAMI, B. M. — A study of cirrhosis of the liver due to chronic Kala-azar. **J. Indian med. Ass., 54:** 315-320, 1970.

17. HUTT, M. S. R.; KÖBERLE, F. & SALFELDER, K. — Leishmaniasis and trypanosomiasis in tropical pathology. In: SPENCER, H.; DAYAN, A. D.; GIBSON, J. B.; HUNTSMAN, R. G.; HUTT, M. S. R.; JENKINS, G. C.; KÖBERLE, F.; MAEGRAITH, B. G. & SALFELDER, K. — **Tropical pathology.** New York, Springer-Verlag, 1973. p. 356.

18. JUNQUEIRA, L. C. U.; BIGNOLAS, G. & BRENTANI, R. R. — Picro-sirius staining plus polarization microscopy: a specific method for collagen detection in tissue sections. **Histochem. J., 11:** 447-455, 1979.

19. LAVERAN, A. — **Leishmanioses:** Kala-azar. Bouton D'Orient. Leishmaniose Américane. Paris, Masson, 1917. p. 173-175.

20. MARTIN, E.; RAIN, B.; LANGUILLE, O. & PERRIER, Ph. — Les hépatites mésenchymateuses. Les lésions non tumorales primitives ou secondaires du mésenchyme hepátique. **Arch. Anat. Cytol. Path., 32:** 277-282, 1984.

21. MELENEY, H. E. — The histopathology of Kala-azar in the hamster, monkey and man. **Amer. J. Path., 1:** 147-168, 1925.

22. NATTAN-LARRIER, L. — Les cirrhoses hépatiques du au Kala-azar. **Bull. Acad. Méd. (Paris), 79:** 402-403, 1918.

23. NEAFIE, R. C. & CONNOR, D. H. — Visceral leishmaniasis. In: BINFORD, C. H. & CONNOR, D. H., ed. — **Pathology of tropical and extraordinary diseases.** Washington, D. C., Armed Forces Institute of Pathology, 1976. p. 265-272.

24. RODRIGUES DA SILVA, J. — Leishmaniose visceral (Kala-azar). Rio de Janeiro, 1957. (Tese — Faculdade Nacional de Medicina da Universidade do Brasil).

25. RODRIGUES DA SILVA, J. & DE PAOLA, D. — O problema das fibroses hepáticas na leishmaniose visceral americana. **Rev. Ass. Méd. bras., 4:** 8-21, 1958.

26. ROGERS, L. — A peculiar intralobular cirrhosis of the liver produced by the protozoal parasite of Kala-azar. **Ann. trop. Med. Parasit., 2:** 147-152, 1908.

27. SCHNUR, L. F.; CHANCE, M. L.; EBERT, F.; THOMAS, S. C. & PETERS, W. — The biochemical and serological taxonomy of visceraling **Leishmania. Ann. trop. Med. Parasit., 75:** 131-144, 1981.

28. SEN GUPTA, P. C.; CHAKRAVARTY, N. K.; RAY, H. N. & DAS GUPTA, B. — The liver in Kala-azar. **Ann. trop. Med. Parasit., 50:** 252-259, 1956.

29. VERESS, B.; MALIK, M. O. A.; SATIR, A. A. & EL HASSAN, A. M. — Morphological observation on visceral leishmaniasis in the Sudan. **Trop. Geogr. Med. 26:** 198-203, 1974.

30. WILCOCKS, G. & MANSON-BAHR, P. E. C. — Leishmaniasis. In: Manson's Tropical Medicine. London, Baillière and Tindall, 1972.

31. WU, C. S. C.; CHU, I. T. & WU, T. T. — A case of Kala-azar with cirrhosis of the liver and jaundice **J. Path. Bact., 2:** 209-215, 1949.

Recebido para publicação em 23/4/86.