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COMPARATIVE STUDY OF THE BIOLOGICAL BEHAVIOUR IN HAMSTER OF TWO ISOLATES OF LEISHMANIA CHARACTERIZED RESPECTIVELY AS L. major-like AND L. donovani.

M. I. S. DUARTE (1), M. D. LAURENTI (1), H. F. ANDRADE JR. (1, 2) & C. E. P. CORBETT (1)

SUMMARY

Hamster inoculated intraperitoneally with 1 x 10^7 parasites of **L. donovani** and **L. major**-like of the New World were studied in groups of 15, 30, 60 and 90 days of infection. The parasite load and density showed progressive increase with the evolution of the infection and was higher in the **L. donovani** groups than in the **L. major**-like groups. The **L. major**-like groups showed parasite density higher in the spleen than in the liver and was similar in both organs in **L. donovani** groups. The histopathology showed a diffuse marked hyperplasia and hypertrophy of the reticuloendothelial system with high parasitism in the **L. donovani** groups while there was focal involvement of these organs in **L. major**-like groups, forming nodules of macrophages that were scantly parasitised.

The biological behaviour could be useful in the preliminary studies of **Leishmania** strain in regional laboratories and understanding the histopathology of lesions caused by different leishmania species.

KEY WORDS: Leishmania; **L. donovani**; **L. major**-like; Experimental infection.

INTRODUCTION

The leishmanias are intracellular parasites whose tissue tropism vary from species to species. The infection caused by **L. donovani** shows visceral involvement with predilection for organs rich in reticuloendothelial tissue such as spleen, liver and bone marrow (ADLER, 1963; BRAY, 1974; BRADLEY & KIRKLEY, 1977 and MELENEY, 1925). The cutaneous leishmaniasis, such as those caused by the **L. tropica** and **L. major** in the Old World and **L. brasiliensis** and **L. mexicana** in the New World, show a parasite preference for skin in spite of the detection of cryptic infections in other organs of experimental animals (COUTINHO & COELHO, 1972; SCHNUR, ZUCKERMAN & MONTILO, 1973).

Previous studies on **L. donovani** in hamsters showed an inverse correlation between the mean lifetime and the number of parasites in the inoculum and a direct correlation with the final parasitism of the spleen and liver (STAUBER, 1958). The inoculation route is also important in the development of the disease (BRADLEY & KIRKLEY, 1977; OTT, HANSON & STAUBER, 1967 and STAUBER, 1958). The differentiation of species of leishmanias using biological beha-

(1) Departamento de Patologia da Faculdade de Medicina da Universidade de São Paulo.
(2) Instituto de Medicina Tropical de São Paulo — Laboratório de Protozoologia.
Address for correspondence: Dra. Maria Irma Seixas Duarte. Faculdade de Medicina da Universidade de São Paulo, Departamento de Patologia — Av. Dr. Arnaldo, 455 — 1º andar — sala 26 — CEP 01246 São Paulo, SP, Brasil.

viour in hamsters has revealed many problems. We do not know of any quantitative studies comparing visceral involvement of different species, but we think, that this could be useful for initial studies using the analysis of the biological behaviour of new isolates for differention of the lesh mania species.

The histopathological study of experimental visceral leishmaniasis has been carried out by many authors (GELLHORN, et al., 1946; GUTIERREZ, MAKSEN & REINER, 1984; MELENEY, 1925; RITTERSON, 1955; ZUCKERMAN & LAINSON, 1977). We have been studying the histopathology of various organs from experimental visceral leishmaniasis in hamster and have previously described the visceral changes which occur (DUARTE & CORBERTT, 1984; DUARTE, SESSO & BRITO, 1978).

In our laboratory working with a strain of **Leishmania**, isolated from the liver of a dog from an endemic area of human visceral leishmaniasis, we found, in hamster, parasite proliferation in liver and spleen with no evident involvement of the skin. This strain was earlier characterized as **L. donovani** but a new identification using biochemical and immunological methods (MOMEN et al., 1984; PACHECO, 1985 and SHAW, 1985 — personal communication) established as being a **L. major**-like. However, the visceral involvement was much more benign than that found in visceral leishmaniasis caused by a well characterized strain of **L. donovani chagasi.**

In this work we have carried out a comparative study of the parasitism and the histopathology of the spleen and liver of infected hamsters looking for a possible differentiation between the **L. donovani** and **L. major**-like isolated in the New World.

MATERIAL AND METHODS

Animals: male, outbread, hamsters, age 45-60 days old, from the University of São Paulo Medical School General Colony and kept in our laboratory were used (VAN JOOST & SLUTERS, 1972).

Parasites: L. donovani (MHOM/BR/72/LD 46) was isolated by Dr. W. Mayrink, Federal University of Minas Gerais, Brazil, in October, 1972, from an human case of visceral leishmaniasis comming from Mantena (MG). The parasite was isolated using bone marrow aspirate and inoculated into hamsters. We have maintained this parasite by inoculation every three months. It was characterized at the Wellcome Parasitology Unit, Instituto Evandro Chagas, Belém, PA, using monoclonal antibodies and by isoenzyme methods, in 1985 (SHAW — personal communication).

L. major-like (MCAN/BR/73/LD 70) isolated by Dr. Magno Dias, Federal University of Minas Gerais, from liver of a dog from Conselheiro Pena (MG) in 1973. It was maintained in the laboratory by sub-inoculations every three months (PACHECO, 1985; SHAW, 1985 — personal communication).

Eight experimental groups of hamsters infected with either **L. donovani** or with **L. major**-like with at least four survining animals. Each animal was inoculated intraperitoneally with 1 x 10^7 amastigotes, determined by the Stauber method (STAUBER, 1958) at intervals of 15, 30, 60 and 90 days after inoculation. Fragments from the liver and spleen were processed for light microscopy and for parasite load determination. Fragments from spleen and liver were fixed in buffered 10% formalin solution (pH 7.2) and processed by usual histopathological techniques and stained with haematoxylin-eosin.

The spleen and liver smears were fixed in methanol and stained by Giemsa's method. The parasite load of the spleen and liver were calculate by determining the number of amastigotes found per 1000 nuclei of the organs cells x 2 x 10^5 (STAUBER, 1958). The parasite density was obtained by dividing the parasite load of the organ by its weight in milligrams (STAUBER, 1958). Statistical analysis was performed using Students "t" test, with 0.05 significance levels.

RESULTS

The distribution of the spleen and liver parasite load can be seen in table 1. There was a progressive increase of parasite in both species. The increase was bigger in animals infected with **L. donovani** than in those inoculated with **L.**

TABLE 1

Parasite load and density of **L. donovani** and **L. major**-like in the spleen and liver of hamster.

Experimental group	Number of animals	Time of infection (days)	Strain	Spleen			Liver		
				Weight (mg) ± standard error (S.E.)	Parasite load § (10^6) ± S.E.	Parasite density & (10^3) ± S.E.	Weight (mg) ± S.E.	Parasite load (10^6) ± S.E.	Parasite density (10^3) ± S.E.
I	9	15	L. donovani	143.33 ± (31.89)	2.39 ± (1.59)	10.60 ± (5.03)	4413.33 ± (387.67)	54.63 ± (24.24)	12.73 ± (5.92)
II	10	30	L. donovani	244.00 ± (20.23)	13.02 ± (4.14)	46.60 ± (12.95)	4118.00 ± (205.54)	177.66 ± (66.67)	47.99 ± (16.90)
III	6	60	L. donovani	366.67 ± (37.21)	496.66 ± (150.50)	1422.91 ± (521.00)	5175.00 ± (198.42)	4383.00 ± (1198.17)	842.60 ± (226.00)
IV	4*	90	L. donovani	530.00 ± (82.86)	700.70 ± (438.46)	2017.00 ± (1541.00)	4757.00 ± (650.00)	1897.47 ± (1260.70)	498.00 ± (373.00)
V	6	15	L. major-like	253.33 ± (92.00)	0.42 ± (0.11)	2.10 ± (0.60)	3783.00 ± (152.00)	8.43 ± (2.76)	2.21 ± (0.75)
VI	7	30	L. major-like	324.33 ± (31.00)	1.73 ± (0.69)	6.10 ± (2.67)	5184.00 ± (213.00)	10.58 ± (3.00)	2.14 ± (0.64)
VII	5	60	L. major-like	220.00 ± (20.98)	3.97 ± (1.51)	18.90 ± (8.37)	3606.00 ± (477.05)	22.48 ± (13.05)	7.75 ± (5.60)
VIII	5	90	L. major-like	284.00 ± (15.03)	9.90 ± (5.34)	37.20 ± (21.80)	4578.00 ± (156.76)	57.40 ± (30.66)	16.70 ± (6.69)

* 6 died before the 90th day. — § Number of amastigotes per 1000 nuclei of tissue cells x $2x10^5$
& Number of amastigotes per milligram of the organ.

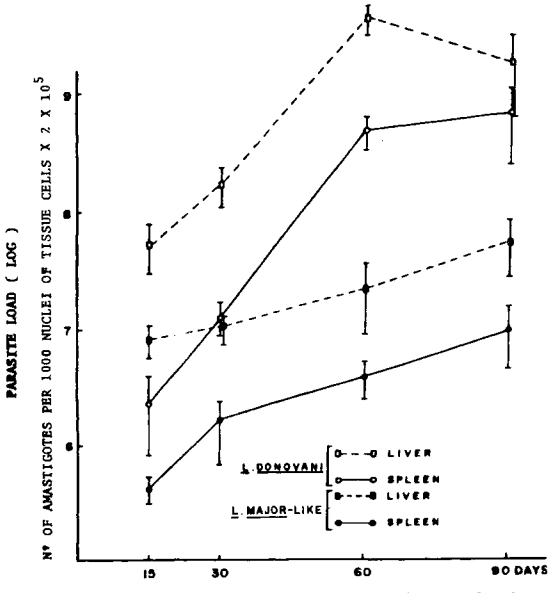

Fig. 1 — Evolution of the parasite load in liver and spleen of hamster inoculated with **L. donovani** and **L. major**-like.

major-like and the difference was significant ($p < 0,05$) from the 15th day of infection onwards (Fig. 1).

The parasite density, i. e., the number of parasites per milligram of the organ (Fig. 2) showed clear differences in the behaviour of these two strains.

The **L. donovani** groups showed a faster proliferation of parasites than the **L. major**-like groups ($p < 0,05$). The parasite density in the spleen and liver in all **L. donovani** groups was similar. Nevertheless in the **L. major**-like group this density was significantly higher in the spleen than in the liver with a ($p < 0.10$ and $p > 0.05$, respectively). The 90 day **L. donovani** group had only 4 animals because the other 6 had died before this time. These animals showed high parasitism of the spleen and liver and marked typical histopathological lesions of the disease.

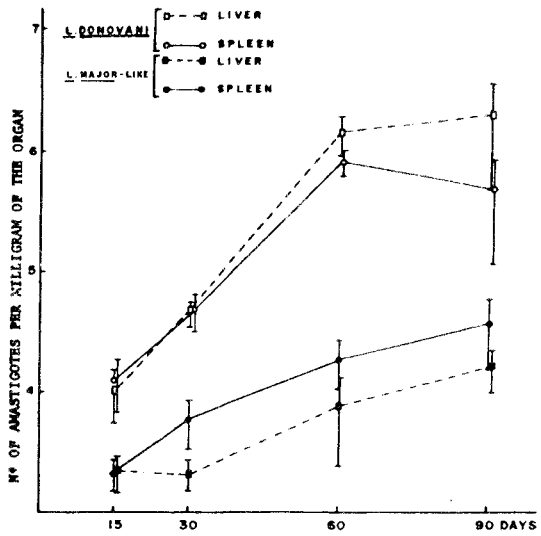

Fig. 2 — Evolution of the parasite density in liver and spleen of hamsters inoculated with **L. donovani** and **L. major** like.

The histopathological analysis of the lesions in all groups gradually increased with time and a difference between the **L. donovani** and **L. major**-like involvement was noticed. In spite of all groups showing histopathological lesions increasing with the time there was a different pattern of involvement in the **L. donovani** group and in **L. major**-like group. The changes were more diffuse in the **L. donovani** group and nodular in the **L. major**-like group. In each parasite species group the type of histopathological involvement was similar varying only in intensity with the time of infection.

The spleen and liver reticuloendothelial system was highly parasitized from the begining in **L. donovani** infected animals. The early liver changes (15 days infection) were moderate diffuse hyperplasia and hypertrophy of the Küpffer cells with discreet parasitism. Intralobular aggregates of the phagocytic cells with high parasitism were irregulary distributed within the hepatic lobules, with no preference for any particular zone. The portal spaces showed mild infiltration by lymphocytes, plasma cells and macrophages, some of them containing leishmanias. After 30 days of infection there was an increase in the hypertrophy and hyperplasia of the Küpffer cells and also higher parasitism. At the same time there was a decrease of the intralobular macrophage aggregates and portal mononuclear cells began to infiltrate the lesions (Fig. 3).

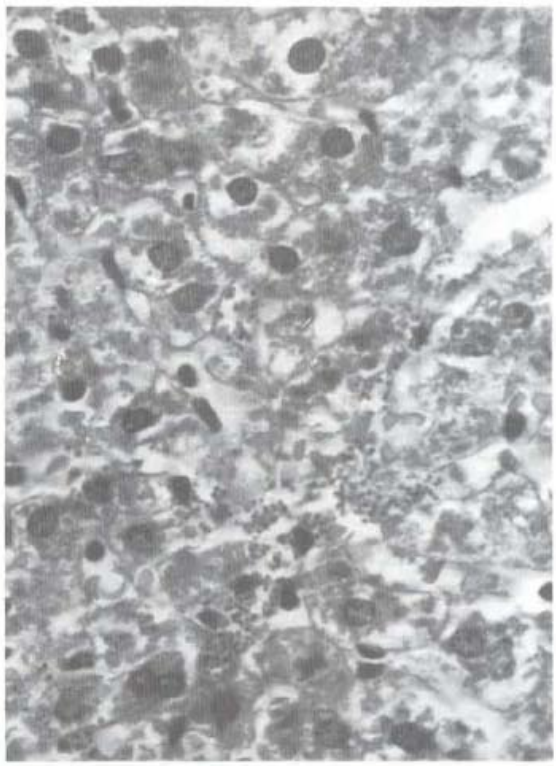

Fig. 3 — Liver from **L. donovani** group 60 days after infection: diffuse hypertrophy and hyperplasia of the Küpffer cells with severe parasitism (x 506,9).

The parasite density in the liver was lower with **L. major**-like than with **L. donovani**. The histopathology of the **L. major**-like group showed multi-focal lesions with no diffuse reticuloendothelial system hyperplasia as seen in **L. donovani** group. The most frequent histopathological changes found were infralobular inflammatory nodules scattered throughout the parenchyma. These nodules were made up of macrophages together with lymphocytes forming small aggregates where the leishmanias were either few or absent. There was also mild hyperplasia of the Küpffer cells which did not show any parasitism. The portal spaces presented discreet infiltration by lymphocytes and macrophages up to 30 days of infection groups and moderate in the others two which showed also an increase of plasma cells (Fig. 4).

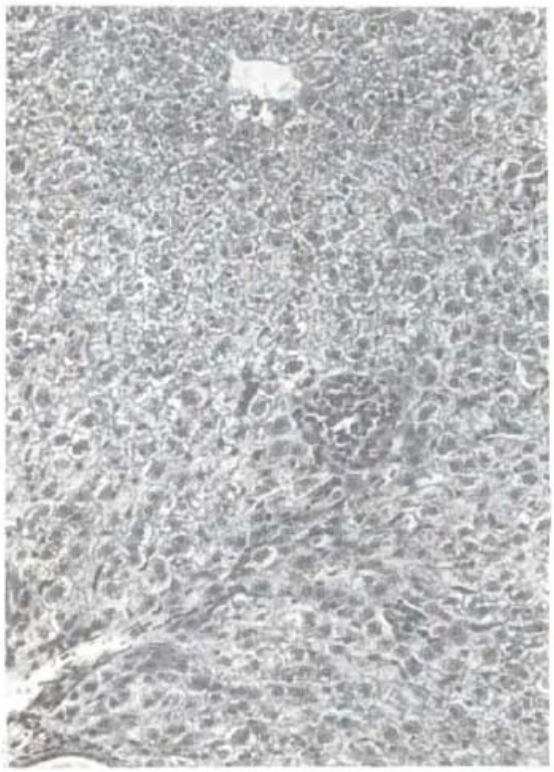

Fig. 4 — Liver from **L. major**-like group 60 days after infection: intralobular nodules of macrophages, lymphocytes and plasma cells. There was scanty parasitism. (x 126,7).

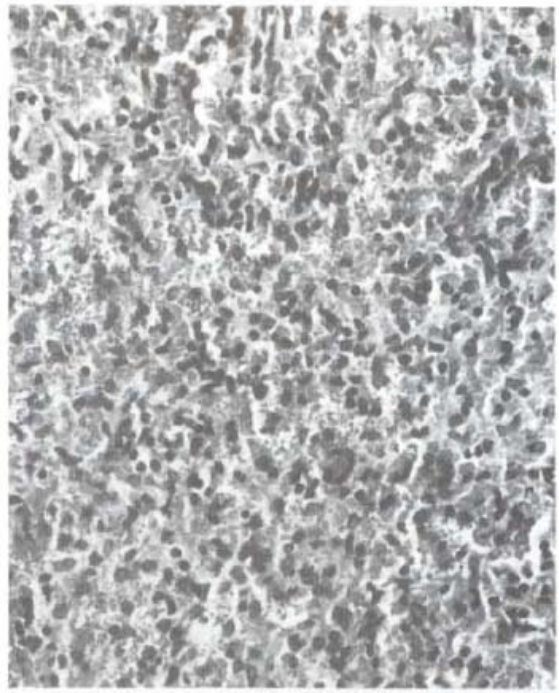

Fig. 5 — Spleen from **L. donovani** group 60 days after infection: diffuse hypertrophy and hyperplasia of the reticuloendothelial system with high parasitism. (x 253,4).

In the spleen the most prominent changes found in the **L. donovani** groups were diffuse hyperplasia and hypertrophy of the reticuloendothelial system within the sinusoids increasing in intensity in the other groups accompanied by marked parasitism (Fig. 5). On the other hand the **L. major**-like groups showed mainly nodules made up of macrophages with either mild or moderate parasitism scattered in the red pulp of the spleen (Fig. 6). In the oldest group there was also mild diffuse hyperplasia of the reticuloendothelial system. The lymphoid follicles of the white pulp moderately increased in volume with hyperplasia and parasitism of the macrophagic cells in the **L. donovani** group. In the **L. major**-like groups there were discreet increase in the germinative centers with mild hyperplasia of the macrophages and occasional parasites were seen. The T-lymphocytes density in the lymphoid follicles was decreased only in the 90 days old **L. donovani** groups.

Fig. 6 — Spleen from **L. major**-like group 60 days after infection: hypertrophy and hyperplasia of the mononuclear phagocitic cells arranged in nodules with rare parasites. (x 126,7).

There was no change in the lymphocyte density either in the B or T dependent zone, in any the **L. major**-like groups.

DISCUSSION

The **L. donovani** group presented a parasite load and density higher than the **L. major**-like group in spleen and liver. However, the **L. major**-like group showed higher concentration in the spleen than in the liver while the **L. donovani** groups showed no difference in these organs. The histopathology also showed significant differences between there two groups. The **L. donovani** groups had a more diffuse involvement with severe reactivity of the reticuloendothelial system and high parasitism. The **L. major**-like groups showed focal involvement with intralobular macrophages nodules scaterred, throughout the organs, with few or no leishmania found into these nodules. There was low reactivity of the reticuloendothelial system where no parasite was seen. These differences seem to be determined by the two species studied (HOMMEL, 1978; MAUEL & BEHIN, 1982). Even with the outstanding taxonomic problems related to the leishmanias species of the New World (GARDENER, 1977; HOMMEL, 1978) it is accepted that the **L. donovani** has a tropism for the viscera (ADLER, 1963; BRAY, 1974; BRADLEY & KIRKLEY, 1977) and the **L. major**-like for the skin (BRAY, 1974; ZUCKERMAN & LAINSON, 1977). Using biochemical methods and monoclonal antibodies (MOMEN et al., 1984; PACHECO, 1985; SHAW, 1985 — personal communication) this strains of a neotropical **leishmania** have recently been identified as **L. major**-like. However, the strain used in the present studied is different from the **L. major**, reference strain, in relation to the KDNA restriction enzymes analysis (MOMEN et al., 1985; PACHECO, 1985).

Strain MCAN/BR/73/LD70 was originally, isolated from dog liver and has not showed cutaneous tropism when inoculated intraperitonealy in hamsters.

Others strains in the New World have been identified as **L. major**-like but there have been considered in some cases as result of laboratory "mix-ups" (SHAW, personal communication). The exact nature of **L. major**-like strains from Brazil must be investigated in greater detail. Epidemiological studies and identifications of new isolates must now be performed.

In conclusion we feel that it is very important to investigate the biological behaviour of new isolates for initial studies of different leishmania species which together with clinical and epidemiological observations will be useful for the understanding of the pathological changes causes by each species. Both studied species showed significant biological behaviour differences between them indicating that, such differences can be detected using quantitative and histopathological methods.

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RESUMO

Estudo comparativo do comportamento biológico de dois isolados de Leishmania **caracterizados respectivamente como** L. major**-like e** L. donovani, **em hamster.**

Experimentos utilizando-se hamsters inoculados intraperitonealmente com 1×10^7 parasitas de 2 cepas, **L. donovani** (MHOM/BR/72/LD 46) e **L. major**-like (MCAN/BR/73/LD 70) isoladas no Novo Mundo foram realizados e estudados em grupos de 15, 30, 60 e 90 dias de infecção. A carga e a densidade parasitária mostraram progressivo aumento com a evolução da infecção e foi maior nos grupos inoculados com **L. donovani** do que nos grupos inoculados com **L. major**-like. Os grupos inoculados com **L. major**-like mostraram densidade parasitária maior no baço que no fígado e foram semelhantes em ambos os órgãos nos grupos inoculados com **L. donovani**. A histopatologia mostrou intensa e difusa hiperplasia e hipertrofia do sistema reticuloendotelial com alto parasitismo nos grupos inoculados com **L. donovani**, enquanto foi encontrado envolvimento focal nestes órgãos nos grupos inoculados

com **L. major**-like, formando nódulos de macrófagos discretamente parasitados.

O comportamento biológico seria útil em estudos preliminares de identificação de cepas de **Leishmania** em laboratórios regionais e na compreensão da histopatologia das lesões causadas por diferentes espécimes de leishmanias.

REFERENCES

1. ADLER, S. — Immunity to protozoa. Oxford, Blackwell Scientific Publication, 1963. p. 235.

2. BRAY, R. S. — Leishmania. **Ann. Rev. Microbiol., 28**: 189-217, 1974.

3. BRADLEY, D. J. & KIRKLEY, J. — Regulation of Leishmania populations within the host. I. The variable course of **L. donovani** infections in mice. **Exp. Immunol., 30**: 119-129, 1977.

4. COUTINHO, E. & COELHO, M. V. — Leishmaniose tegumentar experimental. III. Patologia comparada da infecção de amostras de **L. braziliensis, L. mexicana** e **L. tropica**, em animais de laboratório. **Rev. Inst. Med. trop. S. Paulo, 14**: 12-29, 1972.

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

6. DUARTE, M. I. S.; SESSO, A. & BRITO, T. — Relationship between glomerular mesangial cell proliferation and amiloid deposition as seen by ultrastructural and morphometric analysis in experimental Kala-azar of the hamster. **Amer. J. Path., 92**: 157-173, 1978.

7. GARDENER, P. J. — Taxonomy of the genus Leishmania. A review of nomenclature and classification. **Trop. Dis. Bull., 74**: 1069-1088, 1977.

8. GELLHORN, A.; VAN DYKE, D. B.; PYLES, W. J. & TUPIKOVA, N. A. — Amyloidosis in hamster with leishmaniasis. **Proc. Soc. exp. Biol. (N. Y.), 61**: 254, 1946.

9. GUTIERREZ, Y.; MAKSEN, J. A. & REINER, N. E. — Pathologic changes in murine Leishmaniasis (**L. donovani**) with special reference to the dynamics of granuloma formation in the liver. **Amer. J. Path., 144**: 222-230, 1984.

10. HOMMEL, M. — The genus Leishmania, biology of the parasites and clinical aspects. **Bull. Inst. Pasteur, 76**: 5-112, 1978.

11. MAUEL, J. & BEHIN, R. — Leishmaniasis: immunity, immunopathology and immunodiagnosis. In: COHEN, S. & WARREN, K. W., ed. — **Immunology of parasitic infections**. 2nd. ed. London, Blackwell Scientific Publication, 1982. p. 300-355.

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

13. MOMEN, H.; GRIMALDI Jr., G.; PACHECO, R. S.; JAFFE, C. L.; McMAHON PRATT, D. & MARZOCHI, M. C. A. — Brazilian Leishmania stocks phenotypically similar to **L. major**. **Amer. J. trop. Med. Hyg., 34**: 1076-1084, 1985.

14. OTT, K. J.; HANSON, W. L. & STAUBER, L. A. — Course of infection of **L. donovani** in hamsters inoculated by the intraperitoneal route. **J. Parasit., 53**: 641-645, 1967.

15. PACHECO, R. S. — Caracterização de cepas e clones de Leishmania pela análise do DNA do cinetoplasto por enzimas de restrição e por hibridização molecular. Rio de Janeiro, 1985. (Tese de Mestrado — Instituto Oswaldo Cruz (FIOCRUZ).

16. RITTERSON, A. L. — Leishmaniasis in golden hamster. **J. Parasit. 41**: 603-612, 1955.

17. SCHNUR, L.; ZUCKERMAN, A. & MONTILO, B. — Dissemination of leishmanias to the organs of Syrian Hamsters following intra-splenic inoculation of promastigotes. **Exp. Parasit., 34**: 432-447, 1973.

18. SHAW, J. J. — Personal communication, 1985.

19. STAUBER, L. A. — Host resistence to the klartoun strain of **L. donovani. Rince Institut Pamphect, 45**: 80-96, 1958.

20. VAN JOOST, K. S. & SLUTERS, J. F. — Appearance of **L. donovani** (Kenya strain) in the blood of experimentaly infected golden hamsters (**Mesocricetus auratus**). **Trop. geogr. Med., 24**: 292-297, 1972.

21. ZUCKERMAN, A. & LAINSON, R. — 3. Leishmania. In: KREIER, J. P., ed. **Parasitic protozoa.** London, Academic Press, 1977. v. 1, p. 57-133.

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