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EXPERIMENTAL CUTANEOUS LEISHMANIASIS

II — The pathology of leishmaniasis by *Leishmania mexicana*

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SUMMARY

The pathology of *Leishmania mexicana*, the causative agent of "chiclero's ulcera", has been studied on albino mice and golden hamsters.

The lesion caused by *L. mexicana* had tumor-like or nodular appearance, either ulcerated or not, and, on section, the tissues had lard-like aspect.

Metastases were produced in the ear, scrotum, snout and feet and, in six hamsters, the strain underwent visceralization. Hepato-splenomegaly was present in about 90 per cent of the animals.

Microscopically, the main lesion in the dermal and subcutaneous layers, showed: a) either the compact type of lesion, with large-sized histiocytes, a sole intracytoplasmic vacuole and leishmaniae grouped in a garland-like fashion; b) or the loose type of lesion, with variable degree of interstitial oedema, small-sized multivacuolated histiocytes (foam cells), rupture and fragmentation of the reticulum, a smaller number of leishmaniae than in the former type.

Those lesions were accompanied by an infiltrate of lymphocytes and plasma cells with a tendency to concentrate around blood vessels and nerves. Foci of purulent necrosis were very frequent and sometimes coalesced, the pus being then eliminated through the epidermis.

The occurrence of hyperplasia and parasitism of the endothelial cells of blood vessels, especially of the capillaries and venules, was frequent and, then, an histological picture of obstructive or semi-obstructive leishmaniotic phlebitis was disclosed. Intravascular micropolyps, formed by collections of parasitized macrophages on the valves of dilated lymph vessels, were also found.

Nodular foci of histiocytosis were found in both the liver and the spleen. Amyloid degeneration of the liver, spleen and kidneys was observed from the 86th to the 580th day of infection.

Atrophy of the lymphoid follicles of the spleen was detected in 76.4 per cent of hamsters and 60 per cent of mice.

Parasitism of the bone marrow was detected not only at the site of inoculation but also in metastatic foci.

In mice and hamsters, the cutaneous lesion caused by *Leishmania mexicana* proved to be histologically different from the lesion caused by *Leishmania tropica*. In the latter case the cellular arrangement was more compact and showed a predominance of groups of epithelioid cells (parasitized or not) with tuberculoid-like granulomata and, sometimes, giant cells. This picture has never been observed in animals infected with *L. mexicana* and sacrificed after the same length of infection.

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INTRODUCTION

BIAGI [6, 7, 8], in México, called attention to a form of cutaneous leishmaniasis locally known as "ulcera de los chicleros" which had already been previously described by SEIDELIN. The disease was later on found in Guatemala and British Honduras [13] and clinically characterized by ectimatoid lesions (chiefly located on the ear [6, 7, 8, 13, 20]), chronic course, tendency to ulceration and seldom infecting the mucosae.

Considered at first as a sub-species (*L. tropica mexicana* [7, 12, 30]), this leishmania was recently classified as *Leishmania mexicana* [14, 20, 21], owing to its clinical and epidemiological features. Recent observations by ADLER [1] show that *L. braziliensis*, *L. tropica* and *L. mexicana* are antigenically different, which further justifies the individualization of *L. mexicana*.

Proceeding with our studies on experimental leishmaniasis, the present paper deals with the histopathology of *L. mexicana* in mice and hamsters with an aim to find out whether pathological changes determined by *Leishmania* in laboratory animals can contribute to distinguish between *Leishmania* species.

MATERIAL AND METHODS

There have been used fifty-five albino mice (*Mus musculus*) and thirty-four hamsters (*Cricetus auratus*) of both sexes. Thirteen out of the thirty-four hamsters were reinfected with the same or different species of *Leishmania*. The mice were subcutaneously infected, at the age of two months (18-20 g), with *L. mexicana* from lesions of mice or hamsters. This strain was isolated from a human case of "ulcera de los chicleros" in British Honduras and has been kept in the laboratory for three years, either in culture or in hamsters and albino mice, through succeeding subinoculations.

The animals were inoculated in the snout, base of tail, scrotum or peritoneum and observed for 80 to 580 days.

For comparative studies, 11 hamsters were inoculated (in the snout, scrotum and peritoneum) with a strain of *L. tropica* from Israel and killed 6 months after inoculation, when the cutaneous lesions was quite visible.

Then, they were fixed in Bouin's fluid, embedded in paraffin and stained with haematoxylin-eosin, Mayer's haemalumen, Mallory-Russel's trichome, Benhold's stain, P.A.S., Giemsa, Mallory's phosphotungstic acid haematoxylin, Verhoeff-van Gieson and Perdrau's silver impregnation.

Sections (10-15 μ thick) were cut off in the freezing microtome and stained with Sudan III for fat.

Decalcification of bones was performed in alcohol at 70% with nitric acid at 5% for 7 to 10 days.

RESULTS

I — *Gross pathology*

Animals inoculated in the snout (65) showed a tumoral or nodule-tumoral lesion, ulcerated or not, covered with a crust (Fig. 1A). In 27 animals inoculated with *L. mexicana*, the skin lesion was quite inconspicuous when located at the scrotum, perineum or at the base of the tail but, at the snout, it was quite visible and gave the local tissues a lard-like appearance and consistency.

Scrotum lesions usually showed heavy oedematous interstitial infiltration and the organ attained giant size.

Skin lesions did not ulcerate until about three months after infection.

In some hamsters inoculated by either subcutaneous or intraperitoneal routes there appeared a condition of generalized oedema and the animals died from 8 to 16 weeks later.

II — *Metastases*

In 4 mice and 12 hamsters the primary lesion gave origin to metastatic foci and parasites were detected in the liver, spleen, ear (inoculation in the snout), scrotum (intraperitoneal inoculation), and feet.

Involvement of the skull bones close to the primary lesion (snout) was not evident and the viscera seemed normal except for the hepato-splenomegaly present in about 90 per cent of the animals.

III — *Microscopic pathology*

1) *Skin* — The skin covering the primary lesion at the site of inoculation showed squa-

mous epithelium slightly cornfied that was either normal or atrophic, sometimes with areas of acanthosis. In some cases there could be seen extensive ulceration (protected with a crust) extending down to the derma.

The main lesion, located in the derma or in the subcutaneous layer, was a diffuse massive infiltrate of large univacuolated histiocytes parasitized by numerous leishmaniae. Those cells, pressed together, gave the lesion

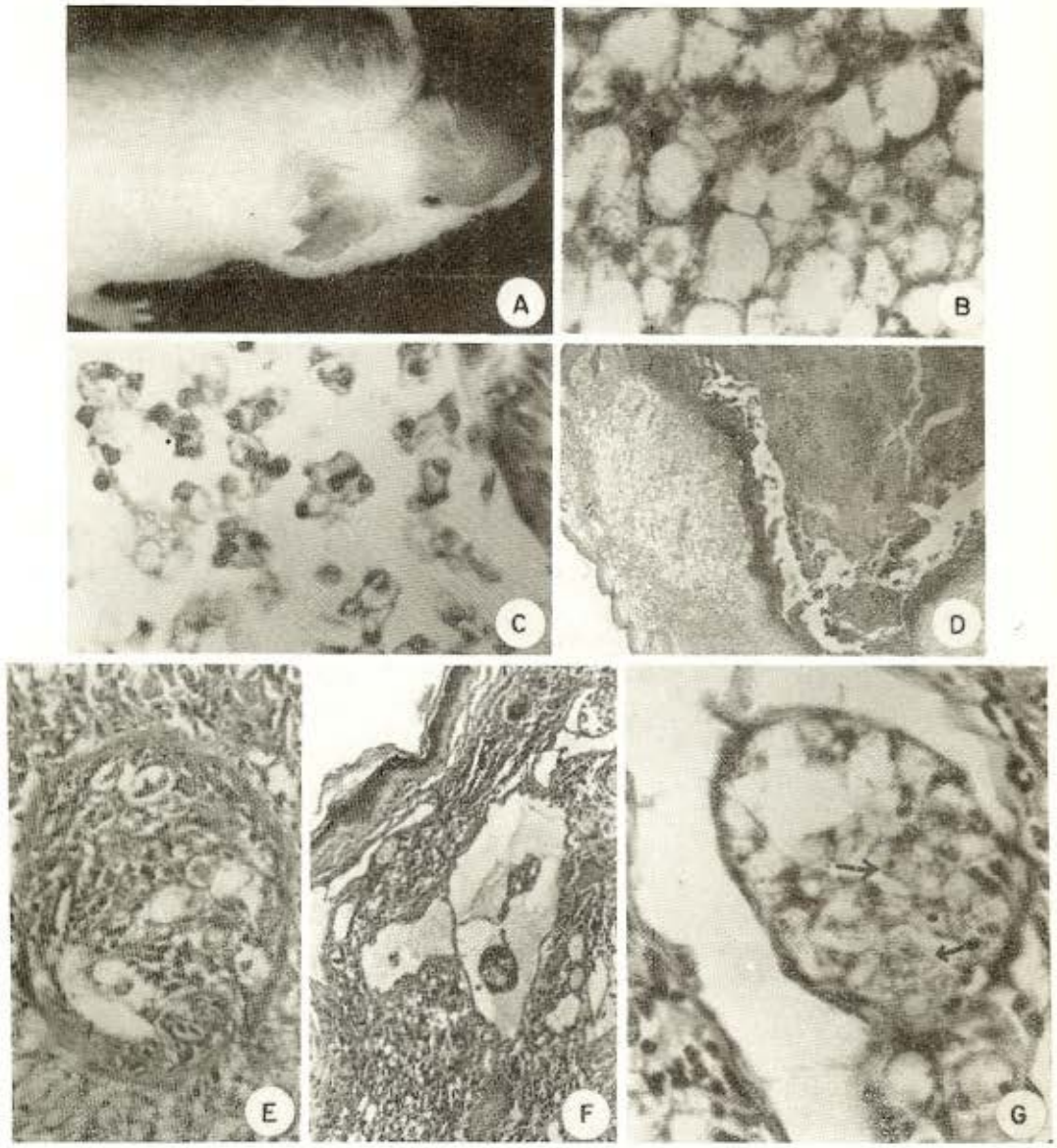

Fig. 1 — A) Lesion on the snout of a hamster, 6 months after infection with *Leishmania mexicana*. B) "Compact" type of lesion (skin of the snout) with big-sized histiocytes with a sole intracytoplasmic vacuole containing leishmaniae in a "garland" fashion (H.E. 160×). C) "Loose" type of lesion (skin of the scrotum), with marked interstitial oedematous infiltration and foam-cells containing leishmaniae (H.E. 160×). D) Extensive necrosis in the derma, the pus being eliminated through the epidermis (H.E. 10×). E) Obstructive endophlebitis caused by *Leishmania mexicana*. Hundreds of parasites are present in the cytoplasm of the endothelial cells (H.E. 82×). F) "Colonization" of parasitized macrophages on the valves of a lymph vessel (H.E. 40×). G) The same histological picture under higher magnification (H.E. 160×).

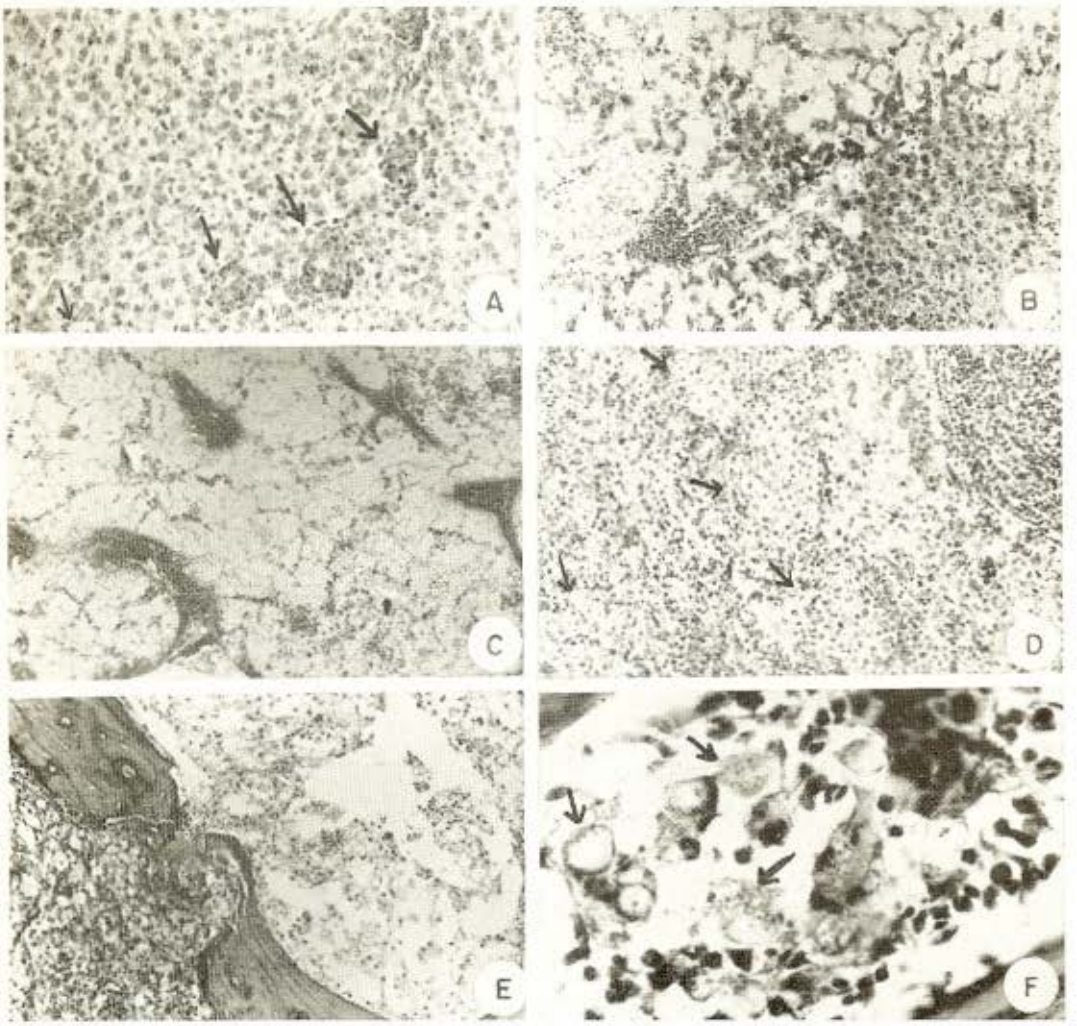

Fig. 2 — A) Nodules of histiocytosis scattered in the liver parenchyma in a "visceralized" hamster (H.E. 40×). B) Marked deposition of amyloid substance with atrophy of the hepatic cords; inflammatory infiltration of a portal space (H.E. 40×). C) Diffuse amyloidosis of the spleen, leading to atrophy of the Malpighian follicles (H.E. 10×). D) Nodules of histiocytosis (reticular hyperplasia) in the spleen of a "visceralized" hamster (H.E. 40×). E) Migration of parasitized macrophages from the granulomatous lesion on the snout into the bone marrow, after focal destruction of the skull bone by the growing "tumor" (H.E. 40×). F) Parasitized macrophages in the bone marrow of a skull bone of a mouse (H.E. 205×).

a compact look (Fig. 1B). In several animals, however, the skin lesion showed a loose aspect and the histiocytes were smaller, foamy, and seemed to be "floating" within the intensive interstitial oedematous infiltration (Fig. 1C). In this instance the parasites were less numerous than in the preceding type.

In either of the two types of cutaneous lesion, the histological picture was an infiltrate of lymphocytes, plasma cells and a few polymorphonuclear cells, generally located around small blood vessels and nerves.

No PAS-positive substance could be detected within those large-vacuolated histiocytes and only a few fat droplets were observed after staining.

The fragmentation of the reticulin framework was constant in all animals with the "loose type" of skin lesion.

In one case parasitized chromatophores were seen by the side of leishmaniae within the cytoplasm of histiocytes and fat cells in the corium of a scrotum with metastatic lesion.

Parasitic granuloma sometimes grow deep, there remaining a small band of superficial derma at the basis of the epidermal appendages. Only when the lesion becomes extensive and destroys the superficial derma does the

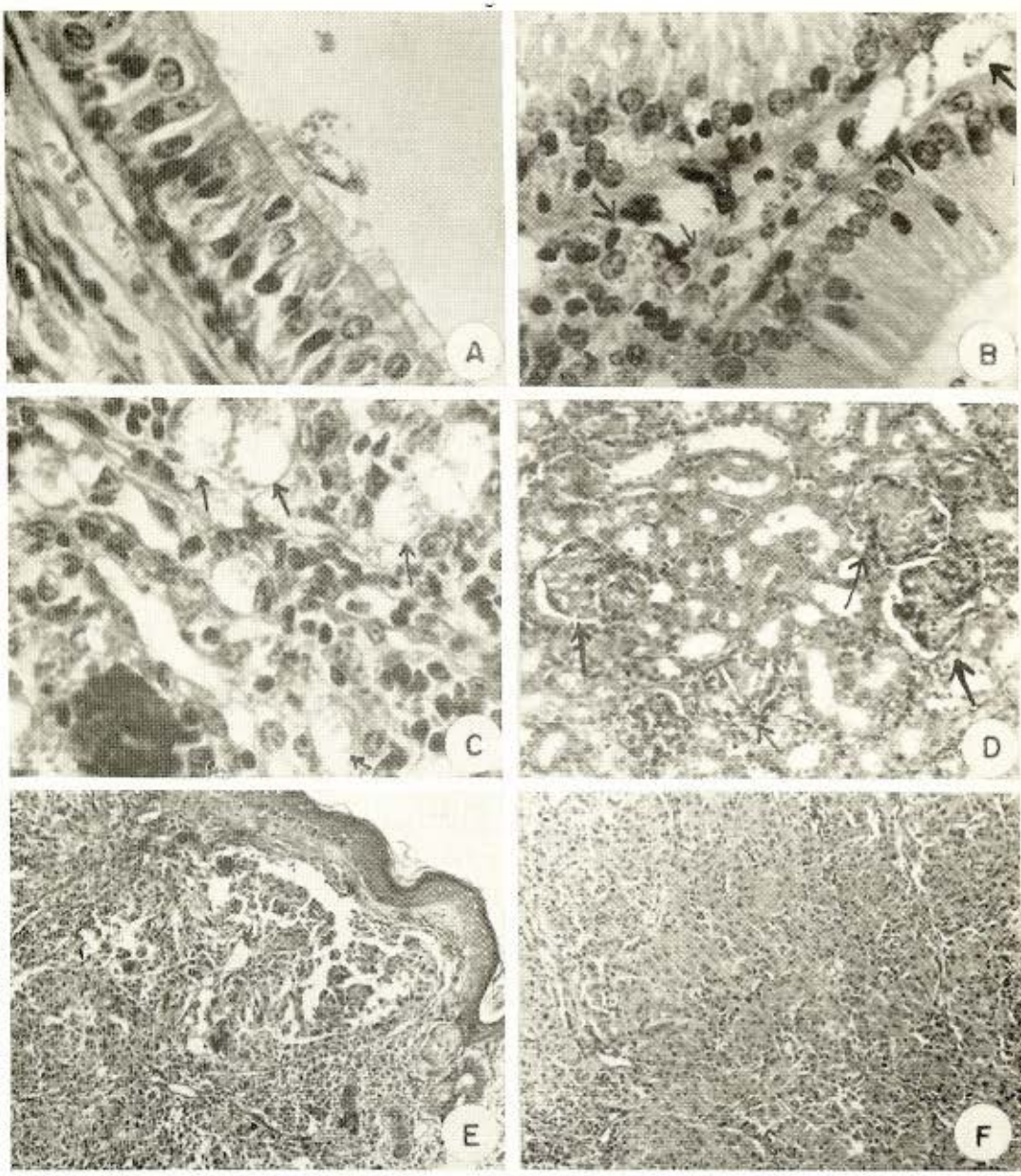

Fig. 3 — A) A parasitized macrophage being eliminated through the respiratory epithelium (H.E. 205×). B) Several parasitized macrophages in the interstitium of the epididymis (H.E. 160×). C) Parasitized histiocytes in the *lamina propria* of the rectal mucosa (H.E. 160×). D) Amyloidosis of the Malpighian glomeruli, in the kidney (PAS 40×). E) Microscopic appearance of the skin lesion in hamsters infected with *Leishmania tropica* (H.E. 40×). F) The same picture under higher magnification, showing epithelioid cells in a tuberculoid-like arrangement (H.E. 40×).

involvement of the cutaneous appendages become apparent and, then, the granuloma appears limited by the covering epithelium. In a few cases, wandering parasitized histiocytes were detected within the Malpighian layer, in the spaces resulting from a local spongiosis.

Micro-abscesses were frequently seen within the granuloma, especially in areas of heavier parasitic concentration. They sometimes coalesced and gave rise to extensive areas of purulent necrosis, the pus being eliminated through the epidermis (Fig. 1D). In the neighbourhood of those necrotic foci there could be found histiocytic cells containing Sudan III positive material, probably resulting from tissue breakdown.

Sometimes the inflammation spread out to the underlying muscles and then, diffuse interstitial myositis with parasitized histiocytes could be observed. The muscle fibre, however, did not keep its integrity when surrounded by heavily parasitized granulomatous tissue; in this case it was destroyed by mechanical compression but underwent subsequent regeneration. Pseudo-aspects of local destruction of the sarcolemman sheath by compression of parasitized histiocytes were seldom observed.

The small and medium-sized blood vessels often showed endothelial hyperplasia and then numerous leishmaniae were seen within those cells. Histological pictures of obstructive or semi-obstructive phlebitis (caused by the parasitism and reactional hyperplasia of the vascular endothelium) in the vessels within the granulomatous lesion were very frequently seen (Fig. 1E).

There could also be observed emboly of parasitized histiocytes and dilatation (or even colonization) of the valves in superficial lymph vessels with macrophages phagocytizing leishmaniae, which simulated parasitic micropolyps in the vascular lumen (Fig. 1F and 1G). Periarteritis was present in only a few cases.

As the lesion became chronic, there could be noticed very marked local fibroelastic activity with the formation of collagen fibres and fibrotic areas that gradually replaced the primary lesion.

2) *Scrotum* — The primary lesion in the scrotal sack generally reproduced the same histological features of the skin elsewhere, but it often disclosed the "loose type" of lesion, with intense oedematous interstitial infiltration. The inflammatory process spreads deep to the cremaster. Sometimes the tunica vaginalis showed fibrous thickening and was infiltrated with parasitized histiocytes. The normal histological pattern of the testis was always preserved.

In a few cases, there occurred involvement of the epidimys, and histiocytes phagocytizing leishmaniae were seen in the interstitium (Fig. 3B).

Necrosis and infiltration with round cells were also present in the histological picture, although these aspects were then less conspicuous than in the skin of the snout.

3) *Liver* — In the liver of 52 (out of 89) animals (52.7 per cent of mice and 70.5 per cent of hamsters), were found nodules consisting of histiocytes and round cells without parasites. In 6 hamsters infected by intraperitoneal and/or subcutaneous route, the histiocytic granulomas showed tiny leishmaniae, very similar to *L. donovani* bodies (Fig. 1E). Besides the portal infiltration by polymorphonuclear cells, lymphocytes and plasmocytes there could also be observed amyloidosis in 32.7 per cent of mice and 23.5 per cent of hamsters. Extensive deposition of amyloid substance on the walls of sinusoids and blood vessels, with resulting partial atrophy of the hepatic cords, was sometimes evident (Fig. 2B). In a few cases, megakaryocytes were found within the sinusoids and small foci of necrosis were then also present in the liver parenchyma.

4) *Spleen* — The involvement of the spleen was indicated by the atrophy of the lymphatic follicles, which were but poorly delimited from the surrounding tissues. Sometimes diffuse lymphatic hyperplasia of the red pulp, with marked blurring of the lymphatic follicles, was evident. Amyloidosis, which occurred in 34.5 per cent of mice and 29.4 per cent of hamsters, began as small deposits of amyloid substance on the walls of the splenic sinuses around the Malpighian corpuscles and rapidly progressed to massive diffuse deposition, when only some remnants of submerges lymphoid tissue could still be identified (Fig. 2C).

Besides congestion and infiltration by polymorphonuclear and reticular cells, there could be observed heavily infected histiocytes in granulomatous formations and/or free ones within the venous sinuses or beneath the splenic capsule. The parasites, however, were very small-sized and poorly stained with the usual histological basic dyes, despite their capacity for inducing the formation of several histiocytic nodules (Fig. 2D) irregularly distributed in both the liver and the spleen, conferring those organs a very peculiar histopathological pattern.

5) *Bone-marrow* — The presence of both free and phagocitized leishmaniae within macrophages in the bone-marrow was relatively frequent in mice and in hamsters (Fig. 2E). The invasion of the bone-marrow took place not only in the skull bones (in cases of inoculation with primary lesion at the snout), but also in the feet, which frequently occurred when the skin lesion was not conspicuous (Fig. 2F).

6) *Intestine* — The presence of granulomatous lesion with histiocytes phagocytizing leishmaniae in the *lamina propria* of the mucosa and submucosa layers of the large intestine (rectum) was observed in only two animals inoculated in the scrotal sack (Fig. 3C). In some animals there was also observed unspecific inflammatory reaction, with or without amyloid deposition on the connective tissue of the submucosa of the intestinal villi.

7) *Kidney* — The most important histological lesions were concentrations of round cells around blood vessels (perivascularitis) interstitial focal infiltration of lymphoid cells and deposition of amyloid substance in the glomerulli and vessels walls (Fig. 3D). Dilatation of the convoluted tubules containing hyalin casts was present in some areas.

8) *Lung* — Most animals (60.6 per cent) showed pulmonary interstitial infiltration (pneumonitis) as well as congestive and hemorrhagic phenomena of secondary importance.

9) *Heart* — Foci of interstitial acute and chronic myocarditis, focal endocarditis or epicarditis and fibrous patches without leishmanial bodies were seldom found.

10) *Pancreas* — Rarely showed foci of inflammatory infiltration with mononuclear cells.

11) *Upper respiratory tract* — In cases of extensive primary lesion in the snout, with involvement of the bone-marrow of the skull bones, there could be observed propagation of the granulomatous skin lesion to the epithelium of the upper respiratory tract and, in some instances, the elimination of parasitized macrophages through the mucous membrane of the nasal cavity (Fig. 3A).

12) *Central nervous system* — In only one animal were two glial nodules seen in the brain cortex and the pons, close to the blood vessels, but no parasite was detected locally or in the neighbourhood.

IV — *Histopathology of animals infected with* L. tropica

The main lesion in all animals consisted of conglomerates of epithelioid cells usually phagocytizing leishmaniae present in the derma and thus tending to form tuberculoid-like granulomata (Fig. 3E, F). There were not found large macrophages with a sole vacuole containing leishmaniae in a garland-like fashion nor oedematous interstitial infiltration, characteristic of the pathology of *L. mexicana*.

The granuloma caused by *L. tropica* sometimes discloses giant cells, which are never found in the lesion by *L. mexicana*.

The infiltrate of mononuclear cells around local vessels occurs in the histological picture of both *L. mexicana* and *L. tropica*.

DISCUSSION

Although the cutaneous lesions observed in the present group of animals displayed the same general aspect of the skin of humans with "ulcera de los chicleros" [24, 20], some features however, are still well worth discussing.

The lesions seen in both mice and hamsters were similar, but they seemed to be more florid in mice.

The main lesion, consisting of a parasitic histiocytic granuloma, gave the disease a very peculiar histological pattern and, in

massive infections, disclosed a true histiocytoma, as previously remarked by MARTINEZ BÁEZ & ALEMÁN [24]. Acanthosis was not always present, as referred by LAINSON & STRANGWAYS-DIXON [20] and WILCOCKS [41] with regard to patients with cutaneous leishmaniasis.

The presence of leishmaniae in the epidermis was relatively frequent, which is in accordance with what has been reported about biopsies of human and experimental cases [20, 21]. Parasites, probably migrated from the underlying dermal lesion, were always located in the cytoplasm of macrophages. In only one case were parasitized chromathophores seen in the derma of a hamster inoculated in the scrotal sack.

There has been noticed that these leishmaniae have a tendency to be grouped in a garland-like fashion in the cytoplasm of histiocytes, as described by other investigators [13, 20] from both human and experimental material; this, however, was not an invariable aspect since, in massive infections, the parasites formed clumps in the center of the macrophages and, in some instances, were found free in the interstitium.

No extensive fatty metamorphosis has been detected in such parasitized histiocytes as reported by GARNHAM & LEWIS [13], but just a few droplets of sudanophilic material. The fact of the material used in this experiment having been kept in 10% neutral formalin for a certain length of time and of most tissues having been fixed in Bouin's fluid containing acetic acid, may partially account for the negative results [5]. It seems, however, that a condition of intracellular oedema (hydropic degeneration) has been somewhat responsible for that aspect.

The presence of leishmaniae in the bone marrow, already reported in human Kala-azar [38, 3], and in dogs [24], as well as in guinea-pigs infected with *L. enriettii* [34], was frequent in hamsters and mice infected with *L. mexicana*. There has been detected infection in the marrow of the skull bones (below the lesion at the snout) and of the feet, apparently in the absence of a cutaneous local lesion. In the head, the parasites reached the bone marrow through the expanding granulomatous lesion which, causing the focal destruction of the outer ond inner tables of the bone, let parasitized cells migrate into the marrow. The parasites probably reached the marrow of the feet through the blood stream.

The frequency of obstructive or semi-obstructive endophlebitis, determined by hyperplasia of the lining endothelial cells phagocytizing leishmaniae, as seen in the infected skin, suggests parasitic hematogenic transportation, according to the findings of PARAENSE [34] on the cutaneous leishmaniasis of the guinea-pig. The parasitism of the vascular endothelium was accidentally seen [29, 30, 35], in humans with mucocutaneous leishmaniasis.

The presence of dilated lymph vessels with emboly of parasitized macrophages and the colonization of leishmaniae within macrophages on the valves of the lymph vessels (leading to the formation of intravascular micropolyps), strongly suggests possible dissemination of parasites through the lymph stream. However, as no histological examination of the regional lymph nodes has been performed, we have no grounds for estimating this possibility.

An interesting aspect deserving discussion is the presence of leishmaniae in the *lamina propria* of the respiratory portion of the epithelium of the nasal cavity with the elimination of parasitized macrophages through its mucous membrane. PARAENSE [33, 34], also referred to the involvement of the mucous membrane of the nose in guinea-pigs infected with *L. enriettii*. There has been reported the presence of leishmaniae in the nasal secretion of visceral leishmaniasis and there has been considered the possibility of contact without the interference of any vector [2]. VILLELA et al.[40] reported the presence of leishmaniae in the apparently normal nasal mucous membrane of patients with cutaneous leishmaniasis.

The dissemination of parasites (visceralization) in cutaneous leishmaniasis, though not constant, has already been reported. KOJEVNIKOV et al.[19], NATTAN-LARRIER & NOYER [31, 32], and ANSARI [4] reported this finding in livers and spleens of albino mice infected with *L. tropica* by dermal route. GUIMARÃES [17] refers to the visceralization of a strain of *L. braziliensis* (isolated from a human case mucocutaneous leishmaniasis) in hamsters inoculated by intraperitoneal and/or subcutaneous route.

LATYSHEV & KUYUKOVA [22] remark that cutaneous leishmaniasis does not visceralize in its normal hosts except when the parasites are introduced by an unusual route like, for instance, the peritoneal cavity. LAINSON & STRANGWAYS-DIXON [20] did not notice any visceral involvement in patients with "ulcera de los chicleros". The occurrence of histiocytosis nodules in 58.4 per cent of the animals (including animals without visceralization of strain No. 5), the deposition of amyloid substance and the portal infiltration were characteristic features frequently observed in the liver of mice and hamsters infected with *L. mexicana*. The hepatic involvement in visceral leishmaniasis, as evaluated by liver function tests [9, 33], or by histological examination, has been pointed out by several investigators [27, 38, 3]. The formation of histiocytosis nodules may be an immunoallergic reaction (of the R.E.S.) determined by the reabsorption of substances liberated after destruction of parasites from the primary cutaneous lesion and eventually conveyed by the blood stream to other departments of the body. The presence of leishmaniae in the mentioned nodules would occur only in cases of massive dissemination of parasites which, under special but still unknown conditions, would colonize in the phagocytes of the liver, spleen and bone marrow, where the R.E.S. is particularly developed.

Similar nodules of histiocytosis have also been described in human cases and in natural and experimental infection of laboratory animals with *Trypanosoma cruzi* [25, 26].

The histopathological changes found in the spleen (such as atrophy of the lymphatic Malpighian follicles, amyloidosis and presence of histiocytosis nodules containing leishmaniae) had been previously preported in human cases of Kala-azar [23, 39]. Amyloidosis of the liver, spleen and adrenal glands has also been detected in the course of experimental infection of hamsters with *L. donovani* [27, 15, 37], but so far no reference has been made about its occurrence in cutaneous leishmaniasis.

According to the descriptions by GOODWIN [16], GELHORN et al.[15], and RITTERSON [37] concerning hamsters infected with *L. donovani*, there had developed generalized oedematous infiltration (anasarca) in the final stages of the infections. Deposition of amyloid substance in the glomerulli and infiltration of the kidney's blood vessels were also observed. As already suggested by the aforementioned Authors, the oedematous infiltration might be ascribed to the loss of protein resulting from the injury of the glomerular loop. This explanation is acceptable at least for some of our cases.

The visceralization of *L. mexicana* did not seem to be correlated with the length of infection or the inoculation route.

In the "visceralized" animals the parasites were small-sized and poorly stained with the usual histological basic dyes and thus could hardly be visualized in the liver and spleen, under routine histological stainings. The best results were obtained after staining the section with Mayer's hemalum.

The involvement of the large intestine (rectum) and of the epididymis seems to have occurred through the expansion of the inflammatory process to those structures, since advanced cutaneous lesion was present in the scrotal sack of those animals.

Invasion of the cartilage by leishmaniae was never detected, even in cases of involvement of the marrow of local bones and of the mucous membrane of the nose.

No correlation was observed between the occurrence of the cutaneous lesion (either of the "compact" or "loose" type) and the type of host (mice or hamster), length of infection, inoculation route, degree of fibrosis or occurrence of necrosis.

Visceral amyloidosis took place after 86 to 580 days of infection (chronic phase).

The lesion caused by *L. mexicana* was easily differentiated from the one determined by *L. tropica* in the skin of experimentally infected animals. In infections with *L. tropica*, the histological pattern is very peculiar, consisting of conglomerates of usually parasitized epithelioid cells with a tuberculoid-like appearance. Though giant cells may also occur, the structure of the lesion is always very compact, and interstitial oedematous infiltration, characteristic of the infection with *L. mexicana* (loose type of lesion), is never observed. Our findings are not consistent with those of LAINSON & STRANGWAYS-DIXON [20], concerning human cases, where the histopathology of the skin lesions was similar to that described for Oriental sore.

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RESUMO

Leishmaniose cutânea experimental. II — Patologia da leishmaniose pela Leishmania mexicana

Os AA. estudam a anatomia patológica das infecções causadas por *L. mexicana* em camundongos albinos e hamsters sírios.

A lesão fundamental, de aspecto tumoral ou nodular, localizada nos tecidos subcutâneos, apresenta-se com dois aspectos fundamentais:

1) Lesão de tipo compacto, com grandes histiócitos, grande vacúolo citoplasmático, tendo, em sua periferia, leishmânias dispostas em forma de coroa.

2) Lesão de tipo frouxo, apresentando grau variável de edema intersticial, histiócitos pequenos e multivacuolados, ruptura e fragmentação do retículo e pequena quantidade de parasitas.

As lesões acima descritas são acompanhadas por infiltração linfoplasmocitária, de localização preferencial em tôrno dos vasos sangüíneos e dos filetes nervosos. São freqüentes os focos de necrose purulenta que tendem a coalescer quando o pus é eliminado para o exterior, através da epiderme.

É, ainda, assinalada a ocorrência freqüente de hiperplasia e parasitismo das células endoteliais de vasos sangüíneos, especialmente de capilares e veias, caracterizando-se histològicamente o quadro de endofiebites leishmanióticas, obstrutivas ou semi-obstrutivas. Foram observadas, ainda, micropólipos intravasculares, formados pela aglutinação de macrófagos parasitados nas válvulas de vasos linfáticos.

Tanto no fígado como no baço foram observados focos nodulares de histiocitose. Degeneração amilóide do fígado, baço e rins ocorreu em animais sacrificados do 86.º ao 580.º dia após a inoculação.

Em 76,4% dos hamsters e 60,0% dos camundongos observou-se atrofia dos folículos linfóides do baço.

A medula óssea, tanto nos ossos próximos à lesão inicial como nos focos metastásicos, apresentou-se parasitada em vários animais. Em ambos os animais, as lesões cutâneas causadas por *L. mexicana* apresentaram características que permitem, fàcilmente, diferençá-los da lesão causada por *L. tropica*.

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