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ATYPICAL EPIDERMAL ALTERATIONS IN CHRONIC *LEISHMANIA MEXICANA MEXICANA* LESIONS OF C3H MICE

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C3H mice chronically infected with Leishmania m. mexicana, *and in some groups treated with BCG or levamisole, presented atypical epidermal alterations, including pseudoepitheliomatous hyperplasia, hyperkeratosis and dysplasia. These alterations increased in frequency and intensity during the course of infection, but were not related to lesion size or tissue parasite load. Age matched normal, BCG and levamisole treated control mice, examined simultaneously, did not show epidermal modifications. In infected mice the dermis and hypodermis presented an inflammatory infiltrate of histiocytes, lymphocytes and plasma cells, accompanied at times by neutrophils and eosinophils, which did not vary with duration of infection.*

In typical chronic experimental *Leishmania mexicana* lesions, the main histopathological finding is a proliferation of histiocytes, most of them containing amastigotes in large single vacuoles (Coutinho-Abath & Coelho, 1965; Alexander & Phillips, 1978; Wilson, Dieckmann & Childs, 1979; Grimaldi, Moriearty & Hoff, 1980a) and accompanied by an infiltrate of lymphocytes, plasma cells and polymorphonuclear leucocytes (Coutinho-Abath & Coelho, 1965; Wilson et al, 1979; Grimaldi et al, 1980a). Although epidermal alterations are relatively frequent and intense in human American cutaneous leishmaniasis (Pessoa & Barretto, 1948; Laison & Strangeways-Dixon, 1963), such changes have rarely been noted in experimental hosts. Thus, outbred CFLP and inbred CBA and NIH mice infected with *L. mexicana* did not show epidermal alterations (Alexander & Phillips, 1978); outbred albino mice, chronically infected with this parasite, showed squamous epithelium only slightly cornified, either normal or atrophic, sometimes with areas of acanthosis, in the skin covering the lesion (Coutinho-Abath & Coelho, 1965).

In contrast, in the C3H mice used in the present study, long-term *L. m. mexicana* infection resulted not only in chronic inflammatory infiltration of the dermis, but also in atypical epithelial modifications. This host-parasite combination thus may indicate potential models for the study of preneoplastic alterations associated with persistent infection by a eucaryotic organism.

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MATERIALS AND METHODS

Parasite and host. L. m. mexicana, strain 5, was used for infections. Promastigotes were obtained by culturing cutaneous lesions from mice in NNN blood agar medium with an overlay of Hanks balanced salt solution, then subculturing in modified LIT liquid medium (Gutteridge, Knowler & Coombs, 1969).

Young adult inbred C3H mice of both sexes were infected by subcutaneous inoculation in the perinasal region with 10^5 washed promastigotes. Designated groups were killed after 3, 5, and 8 months of infection.

Treatment. Mice were treated with BCG or levamisole regimens from 3 to 5 months after *L. m. mexicana* infection. Details of treatment schemes are described elsewhere (Grimaldi, Moriearty & Hoff, 1980b). Briefly, one group of mice received 8 weekly topical applications of 6×10^5 mycobacteria after scarification of the lesion site. Levamisole was given to another group as intermittent intraperitoneal doses of 5 or 12 mg/kg.

Pathology. Leishmanial lesions were classified according to diameter: large (more than 1 cm); medium (0.6 – 1 cm); small (0.2 – 0.5 cm); minimal (less than 0.2 cm). At sacrifice, the cutaneous lesions were entirely removed and fixed in Bouin's solution. Paraffin sections (5 μm) were prepared from central and peripheral zones of the lesion and stained with hematoxylin-eosin, Masson trichrome and Dominici stain (Litt modification) (Litt, 1963). Sections were scored on a scale of 0 – not observed; 1 – slight; 2 – moderate; 3 – marked; 4 – intense, for the following features; epidermis – erosion or necrosis, reepithelisation, atrophy, hyperkeratosis, parakeratosis, acanthosis, hyperplasia, dysplasia, anaplasia, exocytosis, spongiosis, and acatholysis; dermis – presence of histiocytes, vacuolated macrophages, macrophages with parasites, lymphocytes, plasma cells, necrosis, neutrophils, eosinophils and fibroblasts with fibrosis. Perinasal tissue from treated and normal controls was included in the series. Slides were examined in blind fashion by two pathologists.

RESULTS

Histological and immunological features of *L. m. mexicana* infection of C3H mice, nontreated or treated with the two immunostimulatory agents, BCG and levamisole, are described in detail elsewhere (Grimaldi et al, 1980a, 1980b). In this system, all animals inoculated with *L. m. mexicana* showed immunological and histopathological evidence of infection, without, however, demonstrating uniformity of lesion size. Macroscopically, lesions appeared as persistent cutaneous nodules, with ulceration appearing only in some of the treated animals.

The basic histopathological picture, regardless of lesion size and consequently of the number of intracellular amastigotes, was of a chronic mononuclear infiltrate of histiocytes, lymphocytes, and plasma cells. Neutrophils and eosinophils were also encountered in some cases. There was no longitudinal modification of these inflammatory features at the dermal and hypodermal levels.

In infected mice, alongside this inflammatory process, modifications appeared in the epidermis, with hyperplasia, frequently pseudo-epitheliomatous, showing lengthening of epidermal crests and narrowing of dermal papillae; dysplastic alterations, with disorganization of the normal architecture and irregularities of form and volume of epidermal cells and their nuclei; and hyperkeratosis (Fig. 1). Development of these alterations with time is summarized in Fig. 2. Mice sacrificed at 3 months had received no treatment. Figures for 5 and 8 months represent both treated and control infected animais, since treatment did not significantly alter frequency of these findings. A total of 20 age-matched normal and treated uninfected control mice (6 normal, 6 BCG, 8 levamisole), divided into

corresponding groups and sacrificed at the same time as infected mice, did not show epidermal alterations.

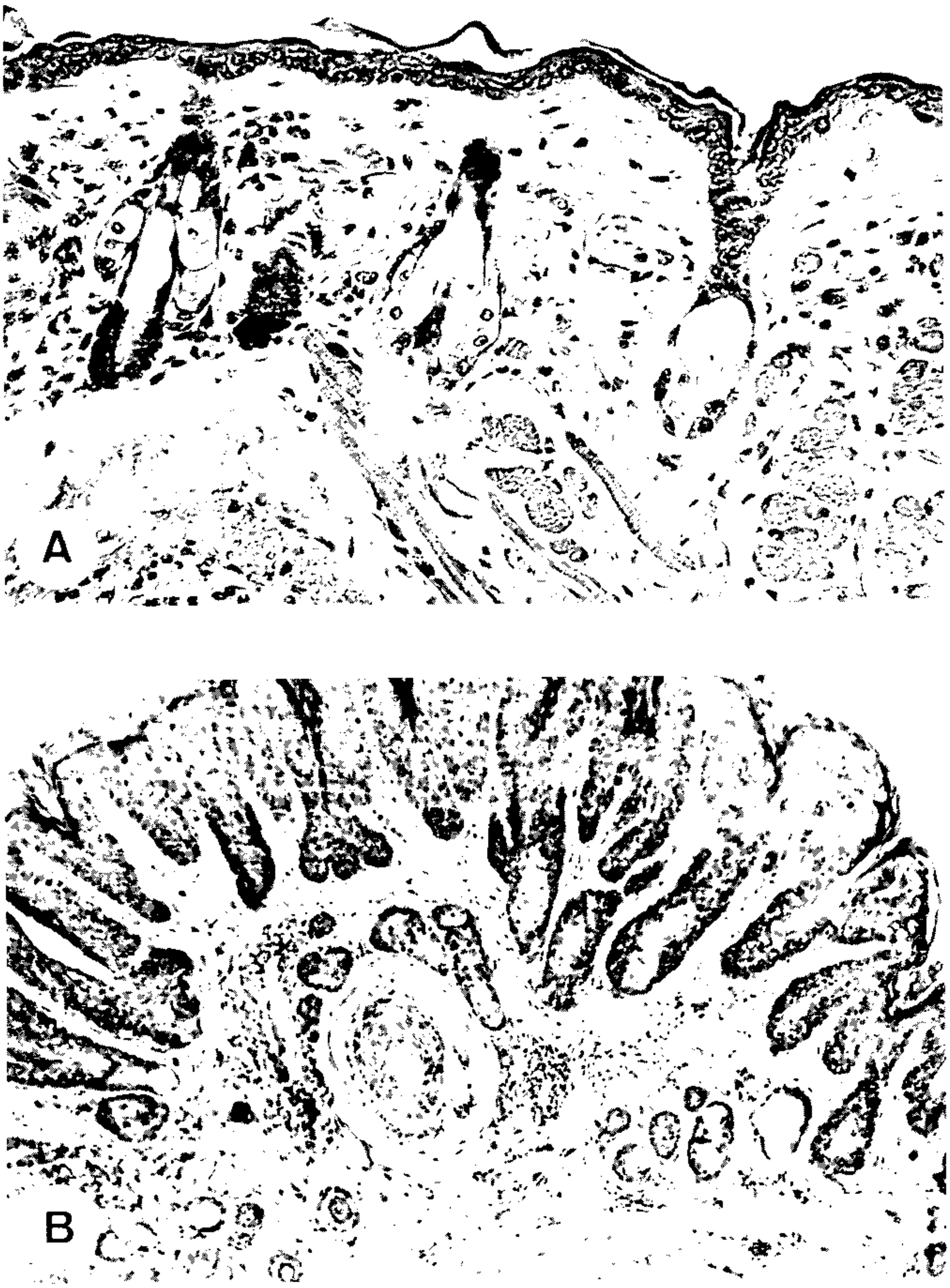

Fig. 1 – Photomicrographs of normal C3H mouse skin and epidermal alterations in chronic cutaneous lesions caused by *L. m. mexicana*. a. Histological appearance of normal perinasal skin of C3H mouse (H & E x 560). b. Large lesion, 8 months: Pseudo-epitheliomatous hyperplasia, showing lengthening of epidermal crests and narrowing of dermal papillae. Note also the hyperkeratosis, mainly in the left upper side (H & E x 350).

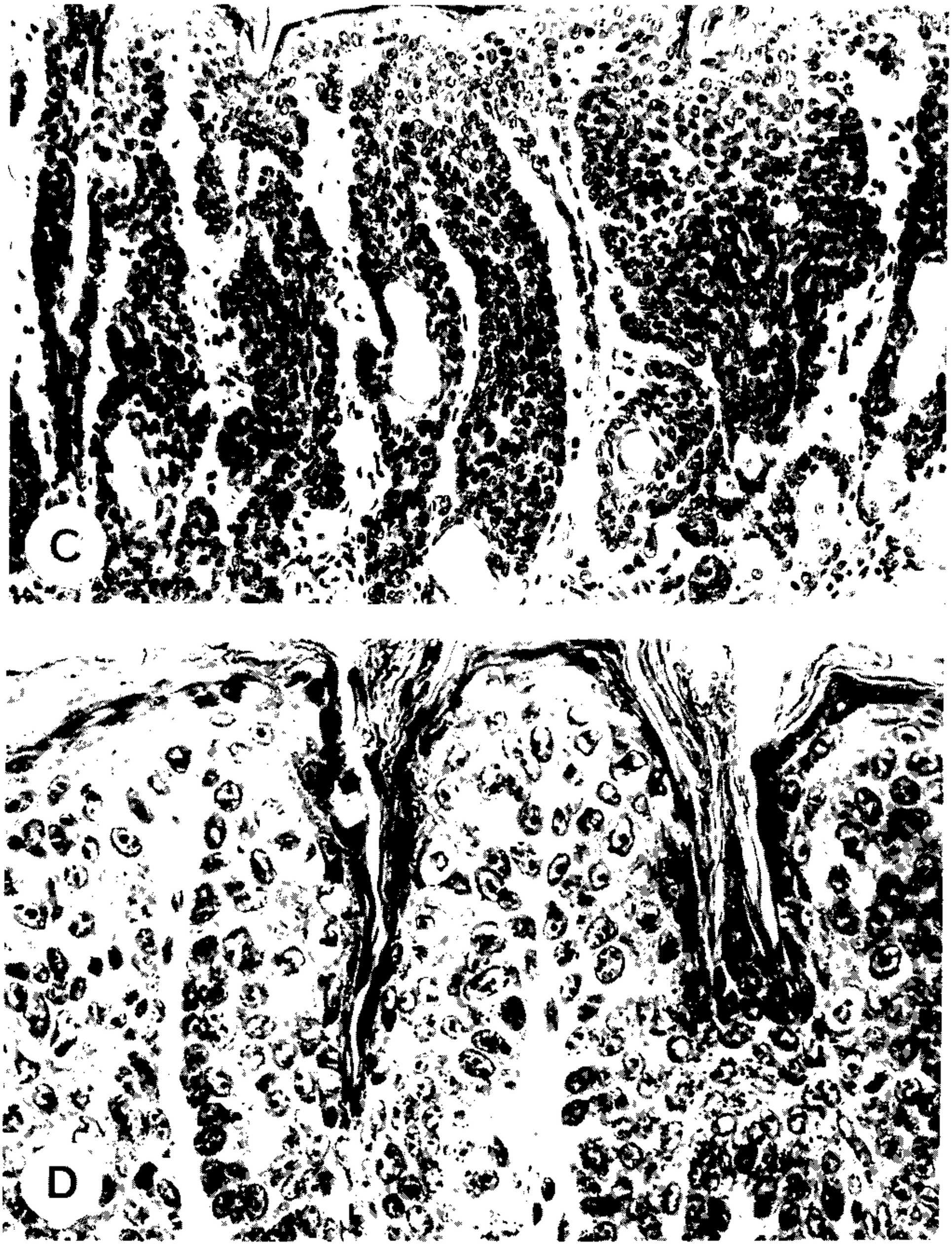

Fig. 1. c. Small lesion, 8 months: In the epidermal crests there is cellular hyperplasia with dysplastic alterations and disorganization of the normal architecture (H & E x 560). d. Large lesion, 8 months. Detail of dysplastic alterations, with irregularities of form and volume of epidermal cells and their nuclei. Note also some degree of hyperkeratosis (H & E x 1400).

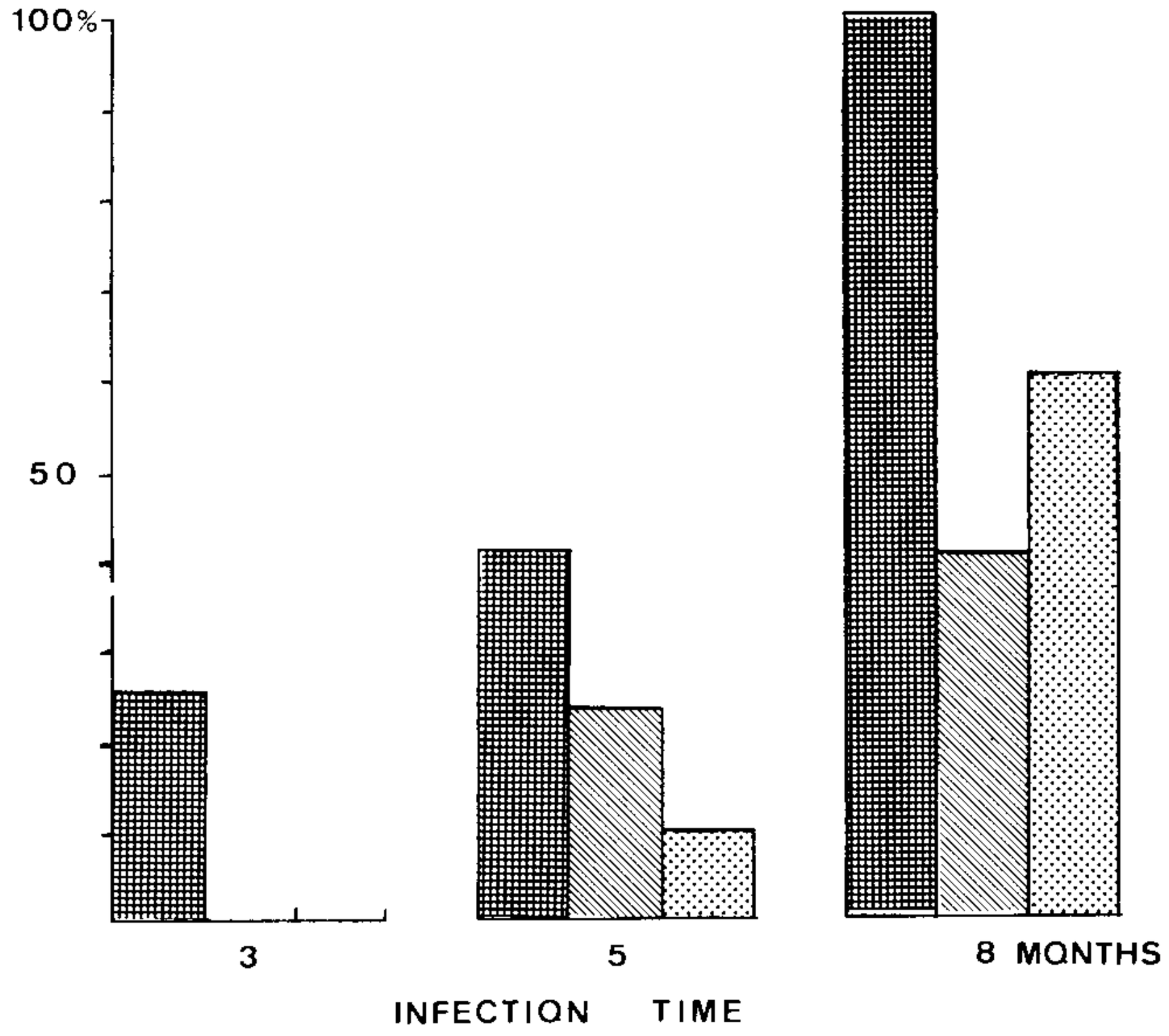

Fig. 2 – Development of epidermal alterations during *L. m. mexicana* infection in C3H mice. Cross hatched – hyperplasia; diagonal hatched – dysplasia; stippled – hyperkeratosis. 3 months, N = 4; 5 months, N = 22; 8 months, N = 20.

Epidermal abnormalities increased in frequency and intensity with time of infection, but showed no relation to lesion size, parasite load, or development of ulceration. Hyperplasia was apparent in one animal after 3 months of infection and by 8 months was seen in all of 20 lesions examined. Dysplasia and hyperkeratosis were observed at 5 months and, more frequently, at 8 months.

DISCUSSION

In human American cutaneous leishmaniasis, epidermal modifications have been described accompanying inflammation in deeper skin layers. These epidermal alterations are varied, and can include hyperplasia with proliferation of interpapillary cones, or acanthosis, dysplasia and formation of horny pearls similar to those observed in carcinoma (Pessoa & Barretto, 1948; Lainson & Strangeways-Dixon, 1963). Atypical epidermal alterations of this nature have not been described in animal models of the disease.

Hyperplasia of long standing, especially when associated with dysplasia, is of interest since it represents a frequent finding in preneoplastic skin lesions (Allen, 1977). Our findings of such alterations in chronic cutaneous leishmaniasis in C3H mice may have resulted from the fortuitous combination of use a host naturally susceptible to epithelial neoplastic changes together with a parasitic infection marked by chronic inflammation

without extensive tissue destruction. This strain of mouse demonstrates a particular genetically controlled susceptibility to various malignant tumors (Heston, Deringer & Dunn, 1956; Heston, Vlahakis & Deringer, 1960b). Tumors noted include mammary carcinoma (Andervont & McEleney, 1941; Deringer, 1959), hepatoma (Andervont & McEleney, 1941; Heston, Vlahakis & Deringer, 1960a) and ovarian tumors (Deringer, 1959). In contrast, other spontaneous tumors, such as primary pulmonary neoplasia, occur only rarely in animals of advanced age (Andervont & McEleney, 1941; Deringer, 1959).

We have not observed similar epidermal changes in chronic (7 – 9 months) infections by the same strain *L. m. mexicana* in outbred albino or inbred C57B1/10J mice. Interestingly, neither levamisole administration nor intermittent topical BCG application in C3H mice was associated with development of these abnormalities in the absence of *L. m. mexicana* infection.

Several two stage models of tumor production in mouse skin have been developed (Scribner & Suss, 1978; Diamond, O'Brien & Baird, 1980). These models utilize a single application of initiating agents such as hydrocarbons or UV irradiation, followed by repeated doses of a tumor promoter such as 12-0-tetradecanoyl-phorbol 13-acetate (TPA) (= phorbol myristate acetate, PMA), an active ingredient of the primary irritant croton oil. While the exact sequence of significant events in tumor promotion is not clear, Scribner & Suss (1978) conclude that ". . for efective promotion it appears that an agent must be an effective gene modulator that can induce hyperplasia, disrupt cell contact and inhibit immune surveillance". To date, studies of promotion have examined almost exclusively the role of chemical compounds. Possible promotion by infection with eucaryotic organisms has been virtually unexplored in the laboratory, although an association between chronic infection, e. g. with trematodes (Schwartz, 1980), and neoplasia has been suggested.

In appropriate circumstances, *L. m. mexicana* produces slow growing, non-ulcerating cutaneous lesions in mice which might fulfill the requirements for tumor promotion. This potential, with its implications for human pathology as well as its possible utility in studies of carcinogenesis, deserves further attention. Studies currently under way in our laboratories are aimed at further examining the role of inflammation associated with this infection in promoting epidermal tumors in mice.

RESUMO

Camundongos C3H cronicamente infectados com *Leishmania mexicana mexicana,* e em alguns grupos tratados com BCG ou levamisole, apresentaram alterações epidérmicas atípicas, incluindo hiperplasia pseudo-epiteliomatosa, displasia e hiperceratose. Estas alterações foram mais intensas em freqüência e intensidade durante o curso da infecção, porém não foram relacionadas com o tamanho da lesão ou com a carga parasitária tissular. Animais controles, respectivamente, normais com mesma idade, tratados com BCG e levamisole, examinados simultaneamente, não mostraram tais modificações epidérmicas. Nos camundongos infectados, a derme e a hipoderme apresentaram um infiltrado inflamatório contendo histiócitos, linfócitos e plasmócitos, acompanhado por vezes de neutrófilos e eosinófilos, o qual não variou com a duração da infecção.

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