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SCHIZOTRYPANIDS: THE OCCURENCE OF DERMATITIS IN IMMUNODEFICIENT ANIMALS INFECTED WITH *TRYPANOSOMA CRUZI*

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Congenitally athymic nude Balb/c (nu/nu) and their phenothypically normal adult and neonate littermates (nu/+), the C_3H/HeN as well, were intraperitoneally infected with two strains (Y or CL) of Trypanosoma cruzi. *The nude mice and the neonates developed a severe parasitaemia, the susceptible C_3H/HeN also presented high level and adult Balb/c mice presented parasitaemia similar to that observed in outbred mice. Erythematous skin lesions were observed initially in infected athymic nude and neonates, being characterized by nests of amastigotes in the dermis; in C_3H/HeN infected mice no nest of parasite was observed but a low-grade inflammatory reaction was seen. In adult Balb/c or OF_1 outbred mice nest was found but discreet inflammatory reaction was observed in severe infections.*

Key words: *Trypanosoma cruzi* – dermatitis – immunodeficience – schizotrypanids – nude mice

Mazza & Miyara (1940) described a cutaneous lesion in patients in the acute phase of Chagas' disease that was characterized by an erythematous eruption, rosettiforme, not pruriginous, that received the name schizotrypanid by analogy with trypanids, dermatitis observed in European people suffering from sleeping sickness (Sicé, 1937). The latter, however, was tought to be due to the presence of a high number of parasites in the lesion. One year later, Mazza et al. (1941) after observing several patients presenting the acute form of Chagas' disease with cutaneous lesions found the colonization of the dermis by *Trypanosoma cruzi* associated with inflammatory cellular infiltration. Recently, it was observed that immunosuppressed chagasic patients, receiving suppressor and anti-inflammatory drugs for cardiac transplant presented cutaneous lesions that allow the diagnosis of the disease by the observation of amastigotes in the skin biopsy (Jatene, 1987). The present paper reports the observation that immunodeficient animals infected with *T. cruzi* show dermatological lesions associated with amastigote colonization.

MATERIALS AND METHODS

Animals – The following female animals were used: homozigous congenitally Balb/c inbred athymic nude (Nu/Nu) and littermate (Nu/+) mice, 6-8 weeks old; OF_1 outbred mice, neonates, from 24 hours, and 6-8 weeks old, purchased from IFFA-CREDO (Saint-Germain-Sur-l'Arbresle France); C_3H/HeN inbred mice from Oswaldo Cruz Institute, 6-8 weeks old. Experimental groups consisted of 6 mice.

Parasites – Two strains of *T. cruzi*, Y and CL, were used. The former was originally isolated from a patient in the acute phase of the disease (Pereira da Silva & Nussenzweig, 1953), and the latter from naturally infected *Triatoma infestans* bugs collected from a house in Rio Grande do Sul, Brazil (Brener & Chiari, 1963).

Infection – Each animal received a single dose of 10^4 trypomastigotes intraperitoneally. Two series of animals were infected, one with Y and the other with the CL strain.

Parasitaemia – Parasitaemia was measured by Pizzi's method (Talliaferro & Pizzi, 1955), with slight modifications. Briefly, 5 µl of tail vein blood were placed under a 22 x 22 mm cover slip. The parasites were counted in 100 fields and this figure was converted to the number of parasites per ml of blood. Blood was collected from the infected mice in a laminar flow hood using sterilized instruments.

Histopathology – Animals were sacrificed on 8th day after infection. Fragments of skin and organs were removed and immediately fixed in Millonig's fluid (Carson et al., 1973). This material was then routinely processed for

paraffin embedding. Five µm thick sections were stained with haematoxylin and eosin.

RESULTS

Comparative course of infection – High levels of parasitaemia were observed in Balb/c nude mice, newborn complete mice and in the susceptible C_3H/HeN mice, while in the heterozygous adult Balb/c mice (Nu/+) parasitaemia was similar to that of the swiss albino mice infected with the Y or the CL strain. Figure 1 shows the parasitaemia of Balb/c nude and littermate mice infected with 10^4 trypomastigotes of the Y strain (Fig. 1a) and the CL strain (Fig. 1b) of *T. cruzi*.

The susceptible C_3H/HeN mice infected with 10^5 trypomastigotes showed a time course similar to nude mice, since after reaching levels over 10^7 parasites, the parasitaemia was maintained in a plateau without the decrease tendency observed normally in outbred OF_1 mice or in intermediate Balb/c mice (Fig. 2).

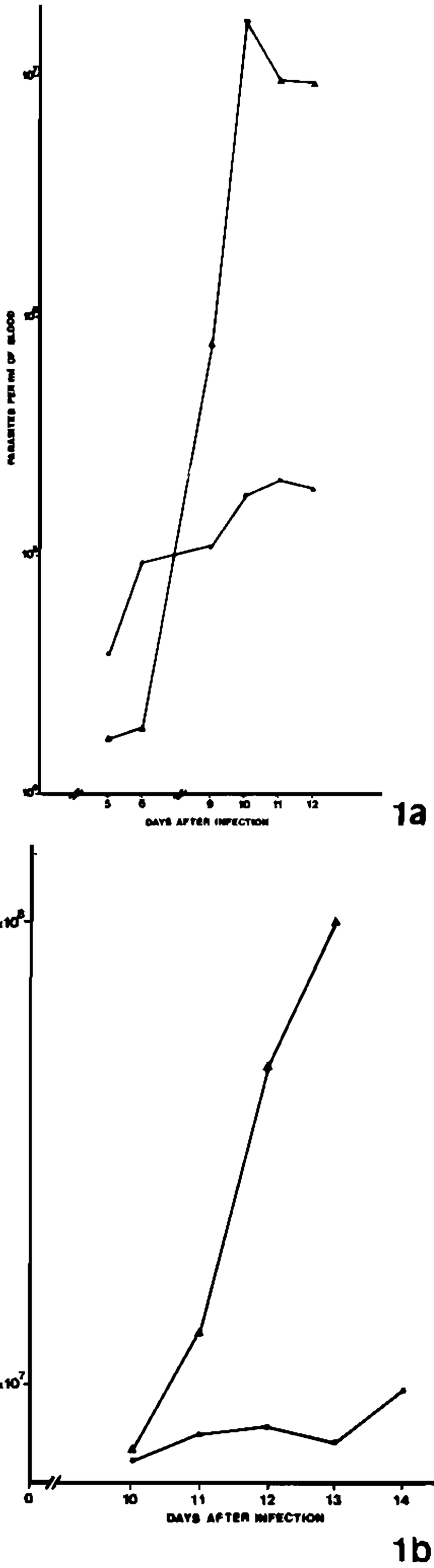

Fig. 1: kinetics of parasitaemia in athymic and complete Balb/c mice infected with 10^4 trypomastigotes. 1.a: show the parasitaemia observed in nude (▲ – ▲) and littermates (● – ●) infected with *Trypanosoma cruzi* Y strain. 1.b: parasitaemia of infected nude (▲ – ▲) and littermates (● – ●) with the CL strain.

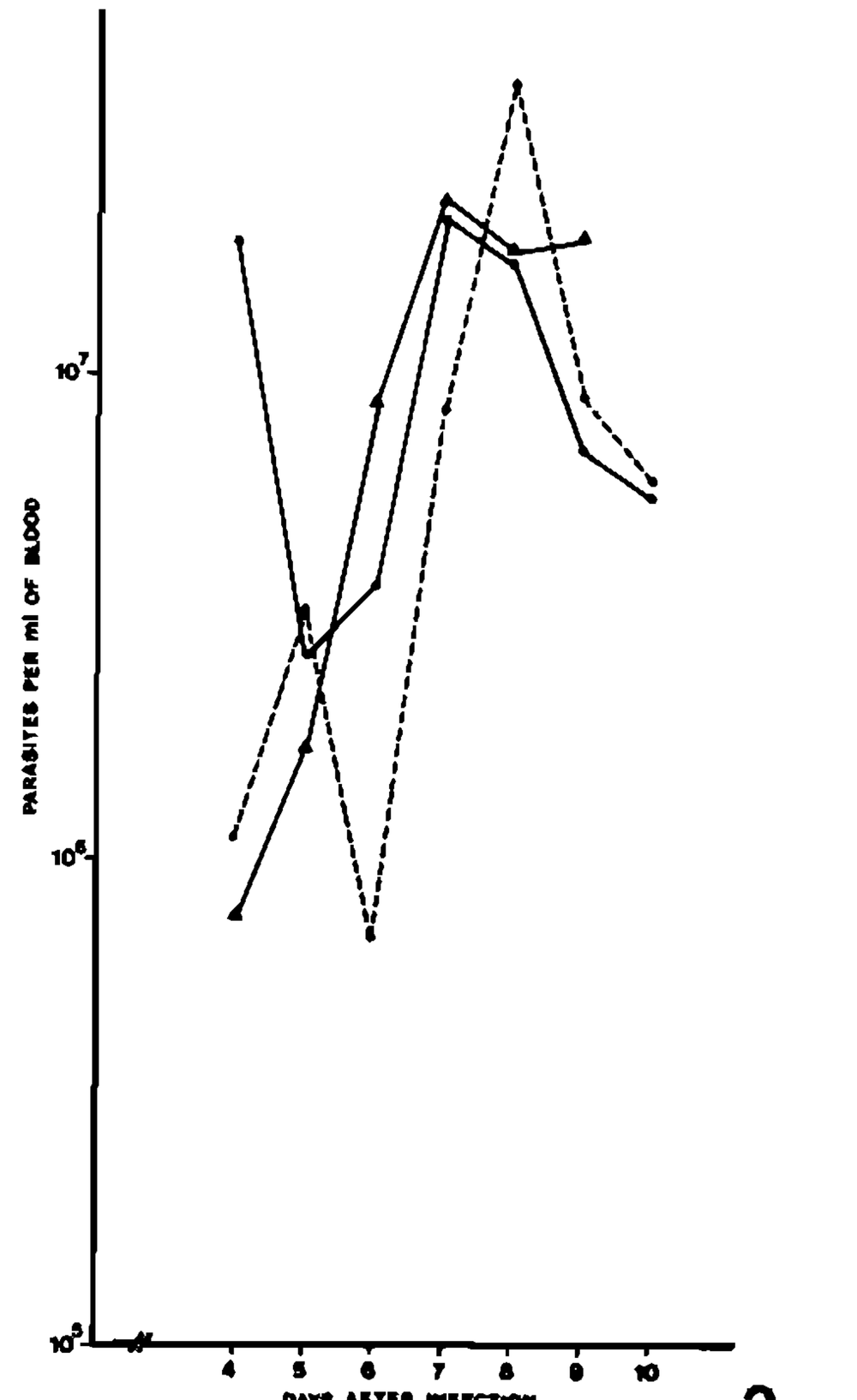

Fig. 2: time course of parasitaemia of different groups of mice, C3H/HeN (▲ – ▲), Balb/c (● – ●) and OF_1 (● - - - ●) receiving an infective dose of 10^4 trypomastigotes of the *Trypanosoma cruzi* Y strain intraperitoneally.

Fig. 3: histopathology of skin colonized by the Y strain of *Trypanosoma cruzi*. Nests of amastigotes in nude Balb/c mouse skin in a dermal papila (arrows). Haematoxilin and eosin staining. Magnification 160 X.

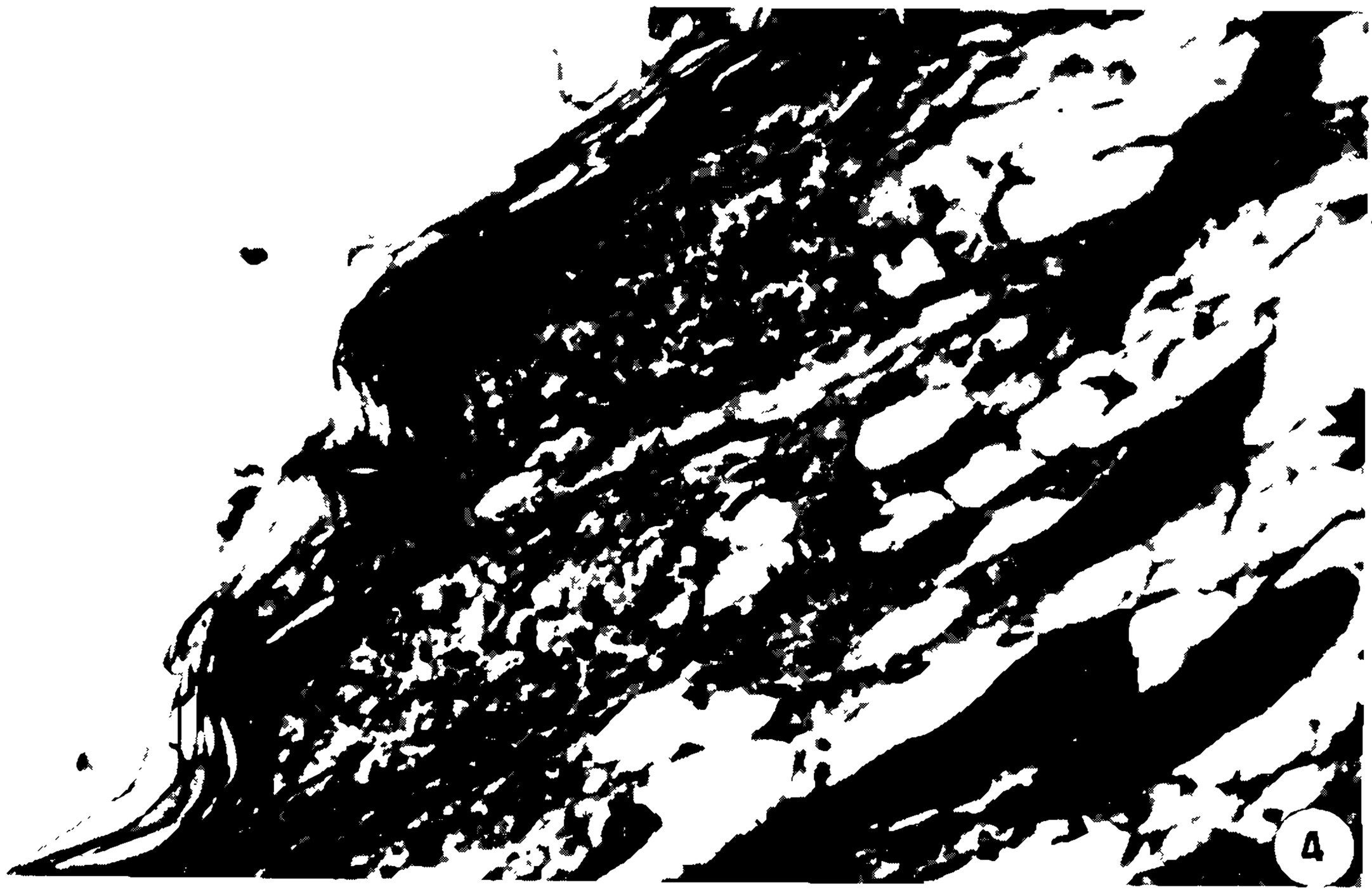

Fig. 4: histopathology of skin in newborn complete C3H/HeN mice infected with the Y strain on day 9 post-infection, showing a relatively intense inflammatory infiltrate associated with nest of *Trypanosoma cruzi* amastigotes (arrows). Haematoxylin and eosin staining. Magnification 160 X.

Histopathology of skin lesions – Tissue sections were prepared from Nu/Nu, and Nu/+ adult Balb/c as well C_3H/HeN mice 8 and 13 days after infection with *T. cruzi* Y strain. Sections of newborn Balb/c mice were prepared from necropsy 9 days after infection. It was observed that on 13th day post infection, the skin of nude mice present a more or less diffuse erythematous lesions that histologically showed a great number of disperse amastigotes

in the dermis (Fig. 3). The dermatitis in nude mice showed the presence of mucilagenous substance in the external surface of epidermis.

Histopathology of the skin of infected newborn mice on the 9th day of infection showed the same picture observed in nude mice, but some inflammatory cells were present. When skin of adult susceptible C_3H/HeN mice was observed, a small colonization of the dermis was seen associated with high infiltrate (Fig. 4). The intense inflammatory reaction observed in the dermis of susceptible mice consisted of lymphoid cells and histiocytes. The same results were observed with the CL strain.

DISCUSSION

Histological features and clinical forms of infectious diseases, are the results of several factors, as invasiveness or toxic action of parasite, and the immune reactions of the host as well. Frequently, the host-parasite relation-ship leads to typical spectral disease as a result of a balance between effector and suppressor mechanisms which are modulated by various factors, includind age, nutritional status, concomitant infections and the action of immunosuppressive and steroid drugs. The lesions observed in the skin of man and experimental animals, infected with *T. cruzi* are a good example of these mechanisms leading to spectral pathology.

Studies using athymic nude mice had showed that *T. cruzi* can parasitize almost all tissues of mice; however, the T-dependent immune response controls parasite invasion, leading to inflammatory infiltrate and consequently undesirable immunopathological effects (Gonçalves da Costa et al., 1984; Russo et al., 1988). Athymic Balb/c mice, being nude and presenting a white skin, allow to observed the dermatitis associated with acute Chagas' disease being, with the outbred OF_1 nude mice, suitable models for observing this condition. The observation of the trypanids was made in European people, in which the erythematous lesions were easier to be seen. The colonization of the skin occuring in the *T. cruzi* infected mice seems to be dependent on parasite load and deficiency of immune response. The results verified in experiments using two strains of inbred mice were similar to those obtained by Trischmann et al. (1978). The course of parasitaemia in C_3H/HeN was similar to that seen in nude Balb/c mice, but the skin lesions present inflammatory reactions associated to parasite nests, in contrast with those of nude mice in which the parasites proliferate in the derme without inflammatory reaction.

After the observation of Mazza in the years 40, only recently the skin lesions in patients with Chagas' disease received attention by its occurence in heart transplant. In these patients it became an element of diagnosis in the case of reacutization of the infection as consequence of suppressive therapy (Jatene, 1987; Bocchi, 1987). Subcutaneous nodular lesions showing panicutilis were observed in cases of heart transplantation (Bocchi, 1987). In reality, only under immunosuppressive action, a second acute phase with outbreaks of high parasitaemia can be observed during chronic Chagas' disease (Brener & Chiari, 1971; Krettli, 1983).

The present studies show that in the absence of T dependent immune response, skin lesions occur and parasite load is the base of induction of the exanthema. When T dependent immune response is present, as in the case of susceptible C_3H/HeN mice, heavy inflammatory reaction occurs in the dermis, and paniculitis is observed; the lesions in neonates appear intermediary, with low inflammatory reaction associated. In the early descriptions of schizotrypanids, Mazza et al. (1941) discuss the nature of these lesions induced by haematological dissemination of the parasite with concomitant allergic reaction. As stated in the literature, the trypanosomid of African trypanosomiasis is a result of immunological alterations of the infected patient (see Stedman Medical Dictionary); our results in experimental American trypanosomiasis suggest, by the mononuclear inflammatory reaction observed in C_3H/HeN and other mice, that immunopathological involvement of schizotrypanids is based on the delayed type hypersensitivity occured after the colonization of the skin by the parasite. But in athymic mice the exudative erythematous lesion is a result of parasite load in the skin. Using nude, newborn and C_3H/HeN, we can observe the polar spectrum of the disease at the level of the skin. Besides immunosuppression by drugs we may expect that other situations of immunodepression like AIDS or other infectious diseases, age and so forth, that may lead to skin lesions, in chagasic patients. One example of this is a case report of a 62-year man with a 2-year history of erythematous skin lesions which developed neurological symptoms. After his death the au-

topsy revealed a tumor like lesion of the brain caused by colonization of the central nervous system by *T. cruzi* (Queiroz, 1973). No histological studies were made from erythematous skin lesion, which may perhaps be related to the presence of amastigotes.

We maintained the long stablished term schizotrypanids proposed by Mazza 1941, despite the fact that the suffix "ide" is related to cutaneous allergides.

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