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HUMAN MUCOCUTANEOUS LEISHMANIASIS IN TRÊS BRAÇOS, BAHIA – BRAZIL. AN AREA OF *LEISHMANIA BRAZILIENSIS BRAZILIENSIS* TRANSMISSION. II. CUTANEOUS DISEASE. PRESENTATION AND EVOLUTION

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The clinical records of 182 patients with cutaneous leishmaniasis probably due to Leishmania braziliensis braziliensis *are analysed. 68% had a single lesion which was usually an ulcer on the lower anterior tibial third. Many had short histories of one to two months and all age groups were represented. 13% had closed lesions of a verrucose or plaque like nature.*

Evolution of these skin lesions after treatment was related to the regularity of antimony therapy. Although healing usually occurred in three months, the time to scarring after commencing treatment was variable and related to the size of the lesion ($p \leq 0.01$). Usually if sufficient antimony treatment was given the lesion closed.

Seven of the ten patients with initially negative leishmanin skin tests converted to positive after treatment. A significant decline of indirect fluorescent antibody titres occurred in patients followed, during and after therapy.

Key words: Cutaneous leishmaniasis. *Leishmania braziliensis braziliensis*. Clinical presentation. Evolution. Treatment.

New knowledge of the taxonomy of American human Leishmania[11] and new techniques for identification of infecting species implies further clinical studies. Up to the present such studies have lacked the necessary technology to precisely identify the causative organism. The many reviews of clinical presentation rely on impressions of how *L. braziliensis braziliensis* (Lbb) behaves in man rather than in facts[17 20]. Furthermore, since such clinical studies are usually based on short term observations of hospitalised patients, without adequate follow up, they have limited value.

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Here we describe our findings in a total of 182 patients with cutaneous leishmaniasis observed and followed in our field clinic at Três Braços, Bahia, Brazil, from 1976 to 1982.

MATERIAL AND METHODS

Diagnostic criteria and methods of parasite detection have already been described in the first paper of this series[5]. Apart from a clinical diagnosis and compatible histology the great majority of patients had a positive Montenegro reaction (93%). In the analysis a simple classification into single or multiple cutaneous lesions is used.

Assessment of cure was based on clinical examination and a decline in the levels of antibodies detected by immunofluorescence, when these were present. Clinical healing was defined as total scarring of the initial lesions without induration or satellite nodules and without the appearance of other lesions at least six months and usually a year after the end of treatment, depending on follow up.

We attempted to examine indirect immunofluorescent antibody in patients at intervals after healing.

For many patients due to the terrain and difficulty of access in the rains this was not possible.

Recommended treatment since 1978 has consisted of three courses of glucantime, each course delivering 1 gram/kg body weight of drug in each series. This implies a daily dose of 28 mg/kg body weight of pentavalent antimony. This dose is based on our hospital experience[22]. The Brazilian Ministry of Health provides the drug without cost.

The majority of injections have to be given intramuscularly since they cannot be given under medical supervision. Inspite of extensive checking in some patients we cannot be sure whether the full dose was taken. These have been classified as irregular (IR) when both individual dose and series was intermittent, partially regular (PR) where the interval between series was more than one month, and regular (R) when at least two series in the manner prescribed were completed.

When failure occurred, patients were offered hospital treatment either with further antimony or amphotericin B. Before 1978 glucantime was not available and nifurtimox was used. Dosage schedules for this drug have been published elsewhere[9 15].

It was possible to follow up 146 patients with cutaneous disease for a minimum of six months and a maximum of 80 months. Mean follow up time was 24 ± 18 months. 89% were followed for more than one year, 43% for two years and 7% for more than 5 years.

Table 1 – *Comparison of 182 patients with cutaneous disease as regards age and number of lesions.*

Age (in years)	*Cutaneous disease*				*Total*	
	Single Lesion		*Multiple Lesions*			
	N.º	*%*	*N.º*	*%*	*N.º*	*%*
≤ 9	18	14.5	19	32.7	37	20.3
10 – 19	54	43.5	18	31.0	72	39.6
20 – 29	14	11.3	4	6.9	18	9.9
30 – 39	8	6.5	5	8.6	13	7.2
40 – 49	14	11.3	7	12.2	21	11.6
50 – 59	12	9.7	2	3.4	14	7.6
≥60	4	3.2	3	5.2	7	3.8
Total	124	100.0	58	100.0	182	100.0

RESULTS

Clinical presentation

The distribution of active lesions in patients related to age and number of lesions is given in Table 1. 60% of patients were under 20 years of age and the disease was uncommon under the age of five. The youngest patient affected was 18 months old and the oldest 73 years of age. Patients under 10 years of age had multiple lesions more frequently ($p < 0.01$). Government survey data gives the male/female ratio for the area as 48/52[10]. The sex ratio of disease namely 57/43 was not significantly different.

Duration of the disease in relation to single and multiple skin lesions is shown in Table 2. In 50% of patients this was two months or less and in 71% four months or less. More single lesions were noted in the first month of the disease ($p < 0.05$) but otherwise evolution times were similar for single or multiple lesions. Of the 182 patients 68% had a single lesions, 15% two lesions, and 17% three or more lesions. One patient had 56 lesions of only two months duration. Most of the multiple lesions were localised on the same body segment or on adjacent segments. In 83% of patients the lesions were on the limbs. The commonest area was the lower anterior tibial third.

The morphology of the lesions was remarkably uniform 87% presenting with open ulcers on first consultation. Usually these ulcers had a raised indurated erythematous border. Early closed skin granulomas were only seen in multiple lesions where still ulceration was the main feature. Closed lesions were of two types – either a warty verrucose lesion with hyperkeratosis histologically (8.5%) or a rarer multiple plaque like, nodular infiltrative lesion (4,5%). We have isolated Lbb from both these types of lesions[5] (Figure 1).

Table 2 – *Analysis of 182 patients with cutaneous leishmaniasis as regards duration of disease and number of lesions*

Duration of Disease (months)	*Number of lesions*					
	Single lesion		*Multiple lesions*		*Total*	
	N.º	*%*	*N.º*	*%*	*N.º*	*%*
≤ 1	39	31.5	9	15.6	48	26.4
2	27	21.8	16	27.6	43	23.6
3 – 4	21	16.9	17	29.3	38	20.9
5 – 6	19	15.3	6	10.3	25	13.7
7 – 12	6	4.8	6	10.3	12	6.6
≥ 13	12	9.7	4	6.9	16	8.8
Total	124	100.0	58	100.0	182	100.0

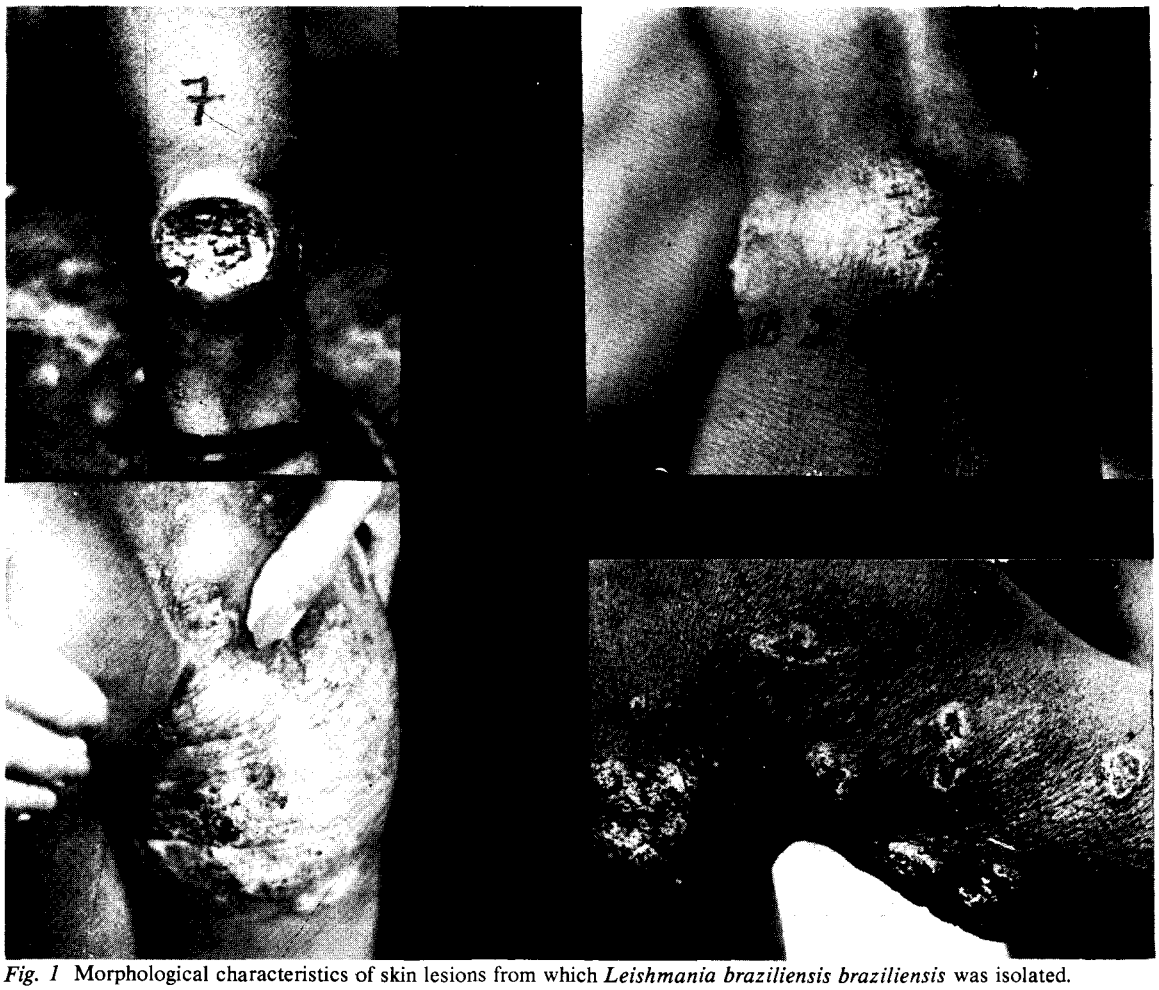

Fig. 1 Morphological characteristics of skin lesions from which *Leishmania braziliensis braziliensis* was isolated.

A. Typical leishmanial ulcer (4cms) on the lower tibial third of six weeks duration. Cure with glucantime (LTB 178).
B. Nodular infiltrative lesion of the wrist of six months duration. Cure with glucantime (LTB 263).
C. Plaque like infiltrative lesion of 24 months duration. Failed to respond to pentavalent antimonial (LTB 133).
D. Verrucose multiple lesions of 24 months duration. Cured with pentavalent antimonial (LTB 206).

Table 3 shows that a relationship exists between the size of the skin lesion (measured by its greatest diameter in cm) and the evolution time. It is possible to find patients with ulcers of 4cm and only a 1-2 month history. The diameter of the lesions grows rapidly in the first two months. Lesions of more than six months duration were significantly larger than the others ($p < 0.01$).

Table 3 – *Comparison of 146 patient as regards the duration of disease and their size at time of initial consultation.*

Duration of disease (months)	*Number of patients*	*Size of lesion (Mean ± 1 SD) (cm)*	*p Value*[(a)]
≤ 1	42	2.7 ±1.43	0.01
2	29	3.25±1.33	N.S.
3 – 4	20	3.18±1.21	N.S.
4 – 5	23	3.35±1.44	0.01
≥ 6	32	4.46+2.92	
Total	146		

N.S. = Not significant.
(a) Student's t test.

Evolution

In a group of 77 patients where their treatment was closely supervised only 35 (45%) took the two courses of glucantime in the prescribed manner (R). These patient healed in 3.25 ± 2.51 months (mean ± 1 s.d). This is a significantly shorter healing time ($p < 0.01$) than those PR patients in whom there was a long delay between series (4.76 ± 2.51 months) or the IR patients who only took their glucantime irregularly (9.72 ± 5.21 months).

Figure 2 shows graphically that most patients whose lesions remained active used the drug irregularly. Although we always recommended three series due the danger of metastasis in actual fact only 18% took this advice. Eighteen percent of patients accepted only one series, 45% two series and 13% four or more series. Invariably the incentive to continue therapy was when the lesion failed to heal rather than any medical advice. Regular therapy during the series (PR) was sufficient for healing in the great majority of cases if only one or two series were given although it had to be prolonged to three treatment series in five cases and four in two cases to achieve this result. The senior

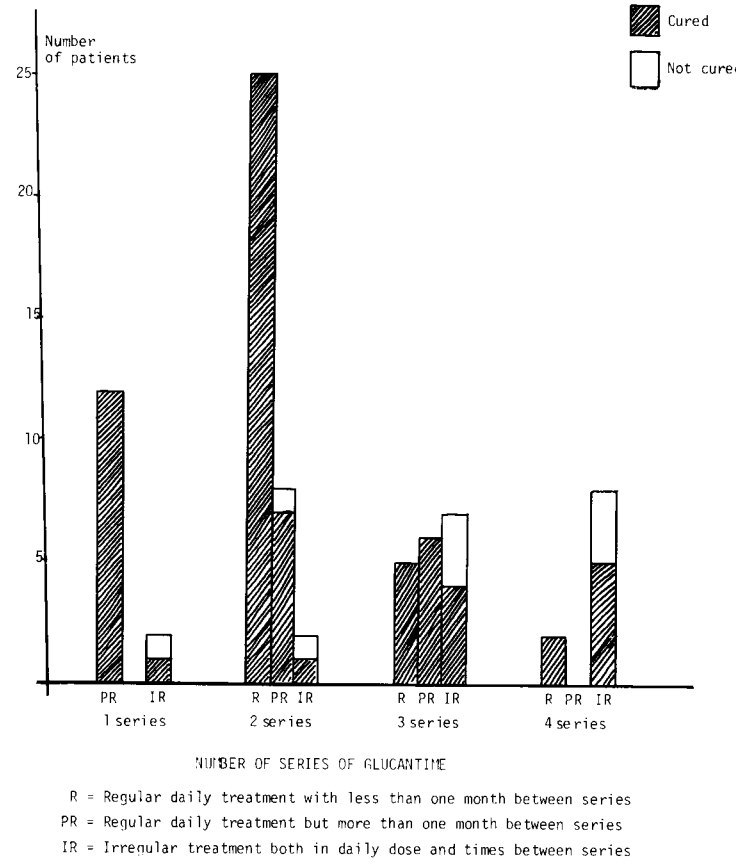

Figure 2 – Distribution of 77 patients with cutaneous leishmaniasis as regards treatment with glucantime.

author personally observed healing in 66 patients (Table 4). As might be expected smaller lesions healed quicker ($p < 0.01$). This holds good if patients with irregular treatment are excluded.

Lesions on the head, trunk and arms tended to heal quicker although this did not reach statistical significance ($p = 0.07$). The following factors did not influence healing: age of the patient, sex, duration of disease, number of lesions and histology.

Sixteen (11%) of 146 patients in whom follow up was possible either failed to close their initial lesions (9) or relapsed (7). Only six had received glucantime treatment and in all cases this drug was taken irregularly. The other patients had been treated with oral drugs such as nifurtimox or allopurinol. One patient (LTB 216) after regular glucantime treatment has continued with an open ulcer for 21 months. In another patient with nipple leishmaniasis after five series of glucantime without success the nipple was excised (LTB 250). Both had positive leishmanin skin tests. Six of the seven patients who had skin relapses did so in the original scar usually within a year of initial healing. The four patients who developed mucosal lesions are discussed in the third paper in this series.

Table 4 – *Relation between of extension of lesion (area) and scarring time in 66 patients with cutaneous leishmaniasis*

Area of lesion (cm^2)	*N.º of patients*	*Time of scarring(a) (mean ± 1 SD) months*	*p Value (b)*
< 4	21	3.59±3.24	< 0.01
≥ 4	45	5.68±4.67	
Excluding patients with irregular treatment:			
< 4	9	1.96±0.95	< 0.01
≥ 4	23	3.69±2.78	

(a) Time in months from start of treatment to healing.
(b) Single Student's t test.

Ten of 12 patients with a negative Montenegro reaction recorded in Table 1 of the paper on diagnosis[5] had the leishmanin skin test repeated after treatment and in 7 the reaction become positive. The three negative patients (in two parasites were isolated) were still negative one year later and two had healed their skin lesions. In one patient (LTB 179) despite adequate treatment lesion activity persisted for 15 months before scarring and five series of glucantime were needed. All were well nourished.

Table 5 – Evolution of titres of indirect immunofluorescent (IgG) antibody in 36 patients with cutaneous leishmaniasis after two series of Glucantime

Examination period	*Number of Patients*	*Titres of IFA – IgG*							*% with positive titres*	*MGT**	*p Value(a)*
		< 20	*20*	*40*	*80*	*160*	*320*	*640*			
Pre treatment	36	2	4	10	9	8	3	—	94.5	65.93	> 0.50
During treatment	36	4	4	7	7	10	4	—	83.9	67.27	< 0.001
Termination	36	11	6	3	7	7	1	1	69.5	41.18	< 0.001
Scarring	36	12	10	6	2	5	1	—	66.5	27.74	< 0.001
3-6 months after scarring	36	21	7	7	1	—	—	—	41.7	15.87	

* MGT = Mean geometric titre.
(a) Unpaired student's t test.

A group of 16 patients with an initially positive leishmanin skin test were followed up for 34 ± 14 months (range 18-72 months) after scarring and the test repeated. The difference between two readings was 6.4 ± 5.7 mm (confidence limits 95%: 3.56 to 9.19) suggesting a significant diminution in cutaneous hypersensitivity.

Table 5 shows the serological titres in a group of 36 patients who took two series of glucantime with resultant scarring. A significant decline in titres of the group as a whole occurred after this treatment. Although only 30% had negative serology at the time of scarring there is a significant decline in titres after treatment ($p < 0.001$). Forty percent of patients 3-6 months after scarring had titres at a low level.

Another group of patients had serological data over a two year period although numbers of late follow up sera are very small and sera sampling was done at irregular times. We observed that 3 out of 23 initially negative sera became positive with treatment. Two of them remained positive after two years. Fourteen out of 27 patients with initially positive sera persisted with circulating antibodies after a 7 to 24 month period of observation. We have a few patients in whom anti-

bodies are still present after 3 or 4 years. Patients using irregular treatment are common in this cohort.

We have observed four different patterns of titres rise and fall in our patients over the years in Três Braços. Two common ones are initial positive titres falling to negative with treatment and persistence of positive titres inspite of treatment. Rarer are persistently negative serology (which can occur in the presence of recoverable parasites) and rarer still negative turning to positive with treatment.

DISCUSSION

Patients with simultaneous cutaneous and mucosal disease are considered in the third paper[16] in this series since mucosal disease is more important as treatment is more difficult.

Of our 62 stocks characterised by monoclonal antibody immunofluorescent or isoenzyme analysis to date only two have been of the Mexicana group. Neither of these patients are included in this analysis although their skin lesions (one single, one multiple) were not remarkably different from what we generally see in Três Braços. The task of identifying all our stocks will take years particularly as the value of the various taxonomic methods is still under discussion. It is possible that other Mexicana infections are present in the cohort discussed here but they will be few based on our results. We have never seen a case of diffuse cutaneous leishmaniasis in Três Braços which has only been documented in Brazil with Mexicana infections.

The overall clinical picture fits with what is often cited as the characteristic cutaneous lesions of *L. braziliensis braziliensis* infection[2]. Single, destructive, extensive and chronic ulcerative lesions being the most common presentation. The predominance of such lesion on the lower anterior leg suggests that a low flying Phlebotomine vector is biting a frequently exposed site. It is tempting to relate the rapid ulceration to the frequent necrosis found on histology[14 21]. Ridley[21] is of the opinion that this necrosis is due to the presence of the parasite. This type of response could be the mechanism of destruction of the parasite and the surrounding tissues. Why verrucose or plaque like lesions occur in some patient from whom we recover Lbb is not clear although these lesions are usually of long duration[20] (Figure 1).

There is some indication of early lymphatic spread in our cases[2]. Multiple lesions are rarer in the first month of the disease and commoner in children. We have rarely detected indurated lymphatic around the site of the lesion or marked local lymphadenopathy but these are crude measures. We have recovered parasites in individual cases from both these sites. Clinically the size and duration of multiple lesions may be a guide as to whether multiple infected sandfly bites are responsible. For instance, in our patient with 56 lesions of two month duration two localised near the upper thoracic vertebral appeared in the first month and were larger than the other 54 lesions some of which were of pimple size. The conclusion was haematogenous spread. To date in this and other patients with evidence of blood stream dissemination we have not detected circulating parasites by inoculating peripheral blood into hamster or concentrating white cells and seeding culture and hamsters. How frequently and when metastasis occur awaits clarification using more sensitive techniques for isolating Lbb than those currently available.

Another aspect of multiple lesions is that they do not always follow local lymphatic drainage unlike *Leishmania braziliensis guyanensis*[12] but can appear in widely disseminated sites (12% of our patients). There is no sure way of telling if this is due to multiple infected bites or rapid blood stream dissemination. The sporotrichoid picture of ulcers following lymphatic drainage we have only seen in three patients.

That single early closed skin granulomas were not seen is due probably to a patient factor since they only seek treatment when an ulcer is established. Such early lesions must occur, since we see them in multiple lesion infections.

A high incidence in patients under 20 years of age might suggest a high risk of transmission. However 58.6% of the general population in our unpublished field survey were in this age group and disease expression appears to reflect the age distribution in our population. Does the occurrence of leishmaniasis in children and occasionally in house bound women who claim no contact with the cacao farms indicate peridomiciliary transmission? We still know so little about the epidemiology of the transmission of leishmaniasis in Três Braços that this remains a speculative question. Despite much field activity we have still to incriminate the suspected vector (*Lutzomyia whitmani*) or find an infected animal with Lbb apart from the domestic dog[3].

The application of glucantime under field conditions is a persistent problem even in supervised programmes. Its efficacy cannot be doubted. In this

large series we have had no absolute failure, but its application in patients living in remote rural areas requires great effort. We have provided glass syringes, taught sterilisation procedures and attempted to install a network of people who can give an intramuscular injection in such areas. For a sixty kilo man to accept 20ml of drug intramuscularly daily for ten days is painful. With children such injection regimes can be even more difficult. It is not suprising that patients often abandon treatment when they see a response. In our clinic we give glucantime by slow intravenous injection (no diluent is necessary). We ask all patients to rest for 30 minutes before walking home. We are fortunate to have had no serious side effects in the area.

The main conclusion of our detailed analysis of such therapy is that if injections are taken as prescribed the response in terms of clinical healing is good enough in cutaneous disease to use only two treatment series. A distinct possibility exists that in the future we could given continuous therapy for 20 days at a dose of 20mg Sb^v/kilo per day[2]. However as demonstrated by our brief descriptions of some of our patients in the results section, the response to therapy is variable and some patients only respond to much more antimony therapy than is normal.

Our data also show that irregular therapy with glucantime increases significantly ($p < 0.01$) the risk of relapse, of scarring time and the possibility of therapeutic failure. Also that our decision to abandon nifurtimox alone or nifurtimox plus glucantime given in one series is justified since the majority of relapses have occurred in this group. With no alternative drug in sight we must concentrate on more efficient glucantime schedules for field use.

That larger lesions are slower to heal comes as no suprise. As we observed elsewhere[13] it is unfortunate that so many lesions are on the lower anterior leg since this is a site of poor vascularisation with impaired healing power.

In the first paper in this series we showed that 92% of patients had a positive leishmanin skin test[5] when first seen. Although the majority of those patients with negative delayed skin hypersensitivity converted after treatment, of the three patients with persistently negative skin test two healed. Although various factors such as parasite load, nutritional state etc. may interfere with this skin test[1] none of them could be implicated in these patients.

It is thought by some that the leishmanin skin tests remains positive throughout life permitting a retrospective diagnosis of infection in 100% of cases[7 20]. The group of 15 patients in whom we observed diminution of skin test reactivity after treatment is too small to draw firm conclusions but our results suggest that changes in the leishmanin skin test may occur more rapidly after treatment than has been noted by other workers[18 19]. The persistence of skin test positivity could indicate the presence of living parasites and this test might have value in the evaluation of cure. Further investigation is necessary.

Decline or disappearance of circulating antibodies is a better established criterion of cure[4 6 23]. We habitually use it in our treatment evaluations both in the hospital[22] and in the field[5] and as our results show titres fall over time after successful treatment ($p < 0.001$). However we have a relatively large number of patients in whom low titres persist. We need further studies to assess whether such titres represent parasite persistence. Serology is also useful in predicting relapse. We can offer no explanation for why some patients, even with detectable parasites, do not develop detectable antibodies although using the more sensitive Elisa test this number is reduced.

The differential diagnosis of American cutaneous leishmaniasis has been discussed in detail elsewhere[8 17]. Três Braços patients frequently confuse leishmaniasis with vascular or stasis ulcer although these rarely present difficulties for the clinician. Epidermoid carcinomas of both skin and mucosa have been misdiagnosed before histology became available. Fortunately *Paracoccidiodes brasiliensis* is easily seen in biopsies. Sometimes ulcers following trauma (insect bites, scratching, football) can mimic leishmaniasis especially as leishmanial ulceration can appear at the site of trauma. Practice in our clinic in Três Braços suggests that an experienced clinician will be righ in the diagnosis made on clinical grounds in the majority of patients.

The refusal of most patient to have a further biopsy after healing is understandable. Cutaneous leishmaniasis does not carry the stigma of leprosy and patients know that the chances of relapses or reinfection in Três Braços are small. In the few patients in whom we have attempted to isolate parasites from biopsies of healed lesions the results have been negative but we know from experimental studies that parasite can persist in healed lesions[11 24]. We briefly discussed the four possible criteria of cure in this

difficult disease some years ago[15]. Histological and parasitological cure assessment not being possible in view of the difficulty of biopsy we have to rely on clinical and serological evidence.

SUMÁRIO

Foram analisados os dados clínicos de 182 pacientes com leishmaniose cutânea, provavelmente causada por Leishmania braziliensis braziliensis. *Sessenta e oito por cento apresentavam uma única lesão, usualmente uma úlcera, na terça parte inferior anterior da tíbia. Todos os grupos etários estavam representados e muitos apresentaram histórico de um a dois meses. Treze por cento apresentavam lesões fechadas de natureza verrucosa ou em placa.*

Após tratamento, a evolução destas lesões foi relacionada à regularidade da terapia por antimônio. Embora a cura usualmente ocorresse em três meses, o tempo de cicatrização, após o início de tratamento, foi variável e relativo ao tamanho da lesão ($p < 0.01$). Em geral a lesão fechava quando era dado suficiente antimônio como tratamento.

Sete entre dez pacientes que apresentavam teste cutâneo negativo para leishmania tornavam positivos após o tratamento.

Observou-se por fluorescência indireta, um declínio significante nos títulos de anticorpos em pacientes acompanhados durante e após a terapia.

Palavras chaves: *Leishmania braziliensis braziliensis.* Apresentação clínica. Evolução. Tratamento.

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