MUCOCUTANEOUS LEISHMANIASIS – A REVIEW OF CLINICAL ASPECTS

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INTRODUCTION

In 1910 Gaspar Vianna suggested at a conference in Belo Horizonte that antimonial therapy was effective in the treatment of mucocutaneous leishmaniasis and this proved to be the case[98]. In 1926, Montenegro evaluated a skin test antigen which is of diagnostic value. It is some measure of the slow development of clinical reasearch in this important human infection that today both these discoveries are still being applied as standard practice and still the laboratory diagnosis and treatment is far from satisfactory.

We have written this review because in our clinical work we have been in doubt sometimes about what is the best line of management for our patients. Turning to the literature we did not always find the answer to our questions. This is, as the title suggests, a review concerned with the patients side of the problem and for this reason begins with the clinically important aspects. Later on we discuss the relevance of some recent biomedical research to our understanding of how this infection behaves in man.

CLINICAL FEATURES

The lesions of Mucocutaneous leishmaniasis are polymorphic and can closely resemble many other skin diseases. This has led to a number of classifications of skin and mucous membrane lesions by different workers. For example. Azulay[5] in his thesis in 1952 lists eight classifications. Although these classifications have value they have become extraordinary complex and since they are largely descriptive it is natural to search for a simpler one.

We shall use a simple classification in this discussion. The skin lesions will be considered under the general headings of closed and open since the usual evolution is from a closed lesion to an open ulcerated one. For mucosal lesions we will use the three evolutionary stages described by Klotz e Lindenberg[60], namely (1) nodulation without ulceration; (2) Early ulceration and (3) Late ulceration.

A marked feature of both our clinical observations and those in the literature as regards these lesions is the extreme variability in the course of the disease. For example in some patients severe mucosal damage appears relatively early and children may present irreparable facial damage. Other patients give a history of a minor skin lesion many years before the onset of mucous membrane involvement and yet others only have a minor skin lesion. Host immunity and the type of parasite probably plays an important part in this and the little we know about this subject is mentioned in our subsequent discussion.

CUTANEOUS LESIONS

CLOSED LESIONS

The site of the bite of the sandfly is generally regarded as the site of the initial lesion. The frequency with which innoculation of promastigotes by sandflies produces a lesion is not known. Determining factors may include the number of flagellates innoculated, the strain of Leishmania and the previous immunological experience and genetic makeup of the host. Quite frequently patients are seen with multiple early lesions all about the same stage and these probably represent the bites of several infected sandflies.

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Like Kala Azar the incubation period must be very variable. Lainson (personal communication) has well documented evidence it may be as short as 15 days. Azulay and Salgado, in 1966[8], noted 18 – 33 days in paratroopers dropped into the Amazonian forest. Guimarães, in 1955[53], calculated the average incubation period in a field study as being about 2 months.

If a lesion does develop it initially takes the form of an erythematous macule which develops into a papule and then grows into a nodule. The site of the lesions is most frequently the limbs or face probably because they are most frequently exposed. In Central America lesions of the pinna of the ear caused by *Leishmania mexicana* are very common[12].

These lesions can take a variety of forms and may resemble other infections. They can be listed as follows:

1) Papules with a pustular element – a rare form which could be confused with impetigo – therefore called impetiginous.

2) Follicular papules the induration being at the site of the follicles.

3) A small furuncular like nodule – has to be distinguished from a simple boil or a *Dermatobia hominis* infection.

4) Discreet nodules multiple and variable in size sometimes difficult to differentiate on sight from skin tuberculosis, sarcoid or leprosy. These are probably the result of blood stream dissemination and have been termed leishmanids[99] although this term has also been applied to lichenoid, hypochromic lesions appearing many years after the initial infection. Such lesions have a tuberculoid histology often without parasites[49]. Sometimes on a smaller scale they may resemble the apple jelly nodules of lupus vulgaris (more common in *L. tropica*) or give rise to a diffuse infiltration with a raised margin resembling the lesion of tuberculoid leprosy.

5) Hyperkeratotic lesions – such lesions with a histological appearance of marked epithelial activation can produce papillomatous lesions resembling the framboesia of secondary yaws. Condylome type lesions as well can be confused with the treponematoses. Verrucose granulomatous lesions may resemble histoplasmosis or chromoblastomycosis.

Many of these forms are uncommon and are presumeably governed by the tissue reaction to the invading parasite. The histopatology has been studied by several groups of workers[6, 58, 15]. and is essentially a parasite granuloma of the dermis with secondary changes in the epidermis. The sequence by which innoculated promastigotes result in intracellular amastigotes in tissue macrophages has not been directly observed in the dermis. It is not clear whether promastigotes penetrate tissue cells or whether they round up to form amastigotes which are then engulfed by macrophages.

The initial histology consists of parasitised macrophages and undifferentiated histiocytes. After a variable period lymphocytes appear indicating the appearance of a cell mediated immune response. The intensity of this response varies greatly and is a major factor influencing the chronicity of the lesion. Usually the lymphocytic infiltration becomes intense with associated plasma cells and eosinophils. Actual intradermal micro abscess formation can occur with polymorph invasion. In chronic lesions well defined granulomas with giant cells may be encounted. Vascular lesion are also important with endarteritis and new vessel formation. Fibrinoid necrosis of venules was regarded by Bittencourt and Andrade[15] to be a part of what they considered to be a histological picture suggesting a hypersensitivity reaction. The epidermis may show irregular acanthosis, pseudoepitheliomatous hyperplasia, hyperkeratosis, parakeratosis, keratin plugs or partial atrophy. On occasion hyperplasia may be so marked as to suggest carcinoma.

Diffuse induration of the dermis with little apparent surface skin lesion have been described. Lymph gland enlargement is common, usually in primary nodes draining the site of the lesion. Since spread by the lymphatics to the circulation is believed to be the method of dissemination, such enlargement is not suprising even in closed lesions. In open lesions secondary bacterial infection plays an important role.

The lymph gland histology exibits the same basic processes already described in the skin lesions. An initial leucocytic infiltration is rapidly followed by a marked cellular activation of lymphocytes, plasma cells and histiocytes. Often leishmania can be seen in the latter. Giant cells are less common than in skin lesions but well defined tuberculoid granulomas may be present and these may even go on to caseation.

Special mention must be made here of diffuse cutaneous leishmaniasis (leishmaniasis

tegumentaria diffusa) which is usually a closed type of lesion and only rarely ulcerates. The lesions take the form of erythematous nodules which closely resemble lepromatous leprosy both in their individual appearance and their symmetrical distribution with prominent ear involvement. For this reason patients of diffuse cutaneous leishmaniasis have been interned in leprosaria. Similar ear lobe nodulation is also seen in Lobo's Keloidal mycosis[96]. Histology shows histiocytes rich in leishmania with little lymphocytic infiltration. The Montenegro test is negative. Usually these patients do not respond to treatment but if they do lymphocytic infiltration of the skin lesion has been noted to begin and Montenegro test has converted in some instances from negative to positive. The significance of this rare form of leishmaniasis is discussed later.

OPEN LESIONS

The process of ulceration is probably dependant on such factors as tissue oedema, foci of necrosis in areas of heavy inflammatory cell infiltration and vascular occlusion due to endarteritis. Ulceration is the usual sequel to the dermal infiltration and usually the ulcer is small with a surface crust. However such ulcers may be large, multiple and severe. In an analysis of 184 patients in Vila Queiroz São Paulo State Pessoa and Barreto[81] found a single ulcer in 46.8%, two in 25,7%, 3 in 9.7% and in 6.6% multiple ulcers up to 9 were seen in smaller numbers of patients.

In hospital series the proportion of patients with ulcerated lesions is high[97] probably because patients only presented at a relatively late stage.

Though the size, site and configuration of the ulcer is variable it often has a characteristic margin. This is infiltrated, raised and erythematous. In forest yaws (pian bois) for instance the lesion iis clearly punched out with steep sides and because of this can be confused with the tertiary stage of a treponematosis. Ulcers may also resemble those of varicose (stasis) ulcers, tropical ulcer associated with vincents organisms, and veld sore (cutaneous diphtheria). An epidermoid or basal cell carcinoma may mimic cutaneous leishmaniasis.

Occasionally leishmanial ulcers may follow the line of the lymphatic drainage and resemble sporotrichosis. Some skin ulcers can persist for years and become large deep and fibrotic. At his stage they may resemble Buruli ulcer (*Mycobacterium ulcerans* infection). This entity has yet to be reported in Brazil but the overhanging edge of skin so characteristic of Buruli ulcer is not a feature of such leishmanial ulcers. A scrofula like lesion should be distinguished from true tuberculous scrofula or actinomycosis.

MUCOSAL LESIONS

A variety of mucous membranes may be affected. Pessoa and Barreto, in 1948[81], summarising Barbosa's large series produces the following incidence figures – Nasal lesions, 1790 patientes; buccal lesions, 209; pharyngeal, 170 and laryngeal, 50. Rarer sites include the conjunctiva and genitals. The over all incidence of mucosal involvement in different series varies widely and Azulay[5] cites 7 sources with percentages varying from 8% – 80.9%. Since the nasal area is by far the most commonly affected the following discussion relates to this site. Specific complications at other sites are considered later.

1 – *Nodulation without ulceration* – The septum of the nose is the commonest site for this lesion which may be very small. It may also occur on the inferior turbinates[56]. Because of this it is essential that every patient have a careful examination of the nose or mouth otherwise a significant complication may be missed. Villela *et al*[103] encountered leishmania in apparently normal nasal mucosa of patients and it is likely that if a phase of blood stream dissemination occurs during the course of the disease organisms reach the nose where they then lie dormant. This aspect is further discussed in the last section. Walton *et al*[107] record the onset of mucosal lesions in 4 patients residing in a non endemic area for 11, 18, 19 and 24 years respectively before the appearance of mucosal disease. The mucosal granuloma may not be well defined but just take the form of a diffuse infiltration. Frank polyps may occur[69] which have to be differentiated from benign polyps or rhinosporidosis. Patients often present complaining of nasal obstruction. The lesion may be entirely intranasal.

2 – *Early ulceration* – If the granuloma is on the septum the thin cartilaginous wall of the septum will be fairly quickly destroyed by extension of the granuloma. Thus it is common to encounter patients with a septal perforation or without a septum. Similar ulcerations may occur on the alae and often in addition there is

a generalised infiltration of the nose which may be erythematous and succulent in appearance. The differential diagnosis here includes leprosy and Goundou of secondary yaws. A combination of these processes may give rise to a broad drooping nose, the so called tapir nose.

Late ulceration – These advanced cases present with marked tissue loss often causing hideous facial distortion. The nose may be totally lost and the patient present with a roughly circular hole making the nasal passages and mouth a common cavity. The differential diagnosis includes trauma, gangosa (tertiary yaws), lethal midline granuloma and a large basal cell carcinoma. The advancing border of the lesion may threaten the lower eyelids and if this is the case no time should be lost in instituting vigorous treatment.

One disease, again perculiar to South America which has not been mentioned but which enters into every differential diagnosis of mucosal leishmaniasis is South American blastomycosis (*Paracoccidiodes braziliensis*). The importance of this differential diagnosis cannot be overemphasized. Usually culture and biopsy will settle the question. As a short cut to diagnosis a P.A. chest X ray showing pulmonary infiltration usully indicates blastomycosis unless severe leishmaniasis is complicated by aspiration pneumonia or pulmonary turberculosis. Rhinoscleroma, a rare infection due to *Klebsiella rhinoscleromatosis* may also closely resemble nasal leishmaniasis[59].

Other sites – The sites after the nose most commonly affected are structures of the mouth such as palate, tongue or lip. Apart from blastomycosis mentioned above neoplasms are the chief differential diagnosis. More rarely the leishmanial granulomatous process may descend from the mouth to involve the pharynx and larynx the patients presenting with dysphagia, dysphonia or even aphonia. Genital lesions are relatively rare. Ulcers of the glans penis and the female perineum have to be distinguished from venereal infections such as syphilis, soft sore and particularly granuloma inguinale. Genital lesions may be the result of infected phlebotomus bites occurring when the genitals are exposed in the forest. Ocular lesions are also uncommon and have been reviewed by Andrade, in 1932[2].

COMPLICATIONS

1) Montenegro, in 1924[74] demonstrated that auto innoculation may occur in patients with an open lesion. This is not frequent and the usual explanation for multiple lesions is lymphatic or blood stream spread. Probably moist contiguous skin surfaces create optimum conditions for this type of spread.

2) Secondary bacterial infection of open ulcers is almost inevitable; although there have been no specific studies these are probably the usual skin flora (c. g. cocci).

Superinfection with vincents organisms (*Borrelia vincenti* and *Bacillus fusiformis*) is reported[81]. When the lesion is in the nose secondary bacterial infection may give rise to a purulent rhinitis. Sinusitis may also be a complication and in the throat aspirated secretion may be responsible for a secondary pneumonia.

3) Buccal and pharyngeal lesions may give rise to sialorrhea and difficulties in deglutition.

4) In advanced laryngeal lesions there may be not only loss of speech but also actual obstruction to the airway by granuloma or scarring. Tracheostomy may be necessary.

5) Conjuntival lesions may give rise to distortion of the palpebral fissure and in rare instances loss of the eye.

6) Severe cicatrization in the face area may reduce the mouth or nose apertures to such small proportions as to prejudice breathing or alimentation.

7) Large ulcers may be the site of a secondary myiasis.

8) Like all large infected ulcerated areas in the centre of the face cavernous sinus thrombosis may be a complication.

9) Extension to the base of brain with secondary bacterial meningitis is perhaps more likely if such an ulcer is the seat of myiasis.

10) Pupo, in 1946[87], described bone and joint complications as a result of leishmaniasis. These usually appear to be of a non specific nature and are a natural sequelae to any deep ulceration contiguous to a bone or joint. For example the periostal reaction of a tibia beneath the site of a deep ulceration can be found in a varicose ulcer. However rarely another type of bone lesion may be seen with absorption of the distal phalanges. Usually associated with diffuse cutaneous leishmaniasis[95] it resembles the distal bone changes of leprosy. The explanation is obscure.

PROGNOSIS

Although some patients pass on to mucosal involvement in a considerable proportion self

healing of the skin ulcer occurs without relapse. The scar of a previous leishmanial lesion is relatively characteristic. Usually on the limb it is slightly depressed, of smooth atrophic skin and often hypopigmented in the centre. It has to be distinguished from the scar of a mother yaw, burn, or varicose ulcer. Reactivation after healing is documented in *L. tropica* infections of the middle east[91] but is more frequent in South American leishmaniasis. Sometimes in a patient with mucosal disease no evidence of a previous skin sore can be detected. It is wise not to after a firm prognosis. Many patients are aware of the course of the disease and will go to great lengths to ensure healing of the skin ulcer in the belief this will prevent nasal complications. The authors have seen patients with renal damage due to prolonged, unsupervised self administered antimonial therapy for this reason.

LABORATORY DIAGNOSIS

Evidence for a diagnosis of mucocutaneous Leishmaniasis is provided by three types of laboratory examination, namely (1) direct demonstration of the parasite, (2) Immunological evidence by skin test or serology and (3) biopsy. The pathology has been described but the first two aspects are considered below.

Demonstration of the parasite – As Furtado[48] pints out the percentage of positive isolations by direct examination of material from the ulcer is inversely related to the duration of the lesion. Multiple aspirations with an intramuscular needle will provide material for smears. These stained with giemsa demonstrate leishmania with greater clarity than those identified in formalin fixed H. and E. slides. However impression smears made at the time of skin biopsy are better. Biopsy is best done from the border of the lesion using a skin punch. Several specimens are taken and one of these can be cultured using the method described by Herrer *et al*, in 1966[55]. We have had poor results with culture and have found it difficult to predict who might have leishmania demonstrable in smears or biopsy. Usually in late lesions with mucosal damage parasites are rare. The most effective way of isolating the parasite is to innoculate triturated biopsy material into the nose and feet of a hamster[63]. Often months later lesions appear at the site of innoculation containing parasites. If no visible lesion appears it is still worthwhile examining smears from the innoculation site at six months and a year after innoculation. Another possible tecnique for isolating leishmania is xenodiagnosis using specific phlebotomines[32]. The difficulties in rearing sandflies make it unlikely that this method can be widely used.

DIAGNOSTIC IMMUNOLOGY

The Montenegro reaction – Furtado[48] has tabulated many reports of the use of the Montenegro test and has discussed some of the difficulties in relation to what would appear to be a simple test of delayed hypersensitivity. Usually the antigen consists of a suspension of dead promastigotes obtained from culture in a preservative. The concentration of these flagellates per ml of culture is important and governs the sensitivity of the test[90]. A concentration of at least 1×10^6 is desirable and better results are achieved with even higher concentration (1×10^7). Unfortunately this information on the nature of the preparation of the antigen used in skin test studies is often missing from published studies thus limiting the value of the observations. More refined antigens obtained by sonication[34] polysaccharide extraction[44] or soluble extraction[79] have been tested but results have only been marginally more accurate. They do have the advantage however that they can be standardised by weight. Recently exoantigens have been prepared and tested against the standard leishmanin antigen. Such exoantigens produce an immediate hypersensitivity reaction in thirty minutes with wheal and flare. *T. cruzi* exoantigens produces a similar immediate response but in contrast to the leishmanial exoatingen a delayed hypersensitivity response after 48 hours was not seen indicating the greater specificity of the latter antigen[94].

As regards specificity of the Montenegro test, Furtado[48] also reviews a number of reports assessing cross reactivity with respect to other skin conditions. Diverse dermatose including leprosy, blastomycosis and pemphigus have been said to give positive reactions, also systemic or glandular tuberculosis. Further studies are needed to confirm or refute these observations as many consider leishmanin to be relatively specific. At Jacobina Pessoa and Sousa Lopes[83] found the skin test to be positive in 3 cases of cured Kala Azar but negative in untreated cases in the same study. They found an incidence of about 10% of positive skin tests in individuals (mainly school

children) without any clinical evidence of leishmaniasis. This raises the possibility that avirulent strains or subclinical infections may be responsible for skin test conversion.

Much work has been done in Africa along similar lines in areas of *L. donovani* infection of man but restricting our discussion to *L. braziliensis* there is strong evidence not only from Brazil but from other South American and Central American countries that a positive Montenegro test in individuals without signs of leishmaniasis is a common finding[3, 14, 42, 70]. A negative test has occurred in patients in whom *L. braziliensis* parasites have been found[72] and this finding is presumeably an indication of a poor cell mediated immune response. How soon after infection the Montenegro test becomes positive is also in doubt and is probably variable. There have been reports of positive conversion of the reaction in patients under treatment[27, 36]. A positive reaction persists after resolution of the clinical lesion[80] but its relation to parasite persistence in the body of man is unknown. Although the Montenegro test is a reflection of cell mediated immunity and has been employed for many years there is little information on other parameters of cell mediated immunity. In 1970, Tremonti and Walton[101] reported blast transformation of peripheral lymphocytes of patients with American cutaneous leishmaniasis. When the lymphoblast transformation test was assessed in 20 patients with cutaneous leishmaniasis[73] the percentage of blast transformation ranged from 19-27% but no correlation could be found with the intensity of the skin test. Convit and Pinardi[34] claim to have shown a clear difference in the behavior or cultured lymphocytes stimulated with leishmanial tegumentaria diffusa. Where as patients with a marked lymphocyte response and a strong Montenegro test showed a high percentage of blast formation.

Serology – There have been a number of studies on the value of a complement fixation test using both *L. braziliensis* and *T. cruzi* antigens[48, 49]. Furtado has reviewed early work on the indirect fluorescent antibody test which because of its comparative simplicity is more likely to be employed in routine diagnosis. However cross reactions with *T. cruzi* occur[24] and these authors have described a fluorescent inhibition procedure to overcome this problem. Cross reactions with Chagas disease have also been noted using the passive haemogglutination test[4].

Later studies by Chiari *et al*[31] and Guimarães *et al*[54] using cultural flagellate antigens did not find cross reactions such a serious problem as to negate the usefulness of the indirect fluorescent antibody test. *Trypanosoma cruzi* and *L. donovani* infections of man produce sero positivity with *L. braziliensis* patients. A slide of a 8 day old culture promastigotes treated with 2% formalin can be kept at – 20ºC for three years without loss of antigenicity[54]. Walton *et al*[106] have cut down the incidence of cross reactions to other infections by using an antigen prepared from amastigotes in tissue culture. The chief value of the IFA from a clinical point of view would be if it could be developed as a criterion of cure and used to evaluate chemotherapy. There is some indication this may be possible. Walton[105] and Chiari *et al*[31] have noted that the IFA test becomes negative after successful therapy.

THERAPY

In any discussion of this subject two important matters must be considered at the beginning. First we still have no accepted criteria of cure. Since this is such an unpredictable disease with frequent spontaneous healing ideally every drug trial should have an adequate control group and the trial terminated when statistically significant improvement is achieved in the group under therapy. For a variety of practical reasons this has not been possible to date. As mentioned elsewhere in this discussion persistence of leishmania after healing of the skin lesion has been demonstrated so that the presence of parasites does not correlate with tissue response. Azulay 1966[7] agrees there are no accepted criteria of cure and feels that healing of the lesions does not justify suspension of therapy. Possibily the presence of parasites after treatment does not correlate well with subsequent relapse and tissue breakdown. This question deserves study as does the value of the fluorescent antibody test in assessing cure.

A second consideration is that to date there is no good scientific observations to shed light on the undoubtedly true clinical impression that this is a disease characterised by frequent relapses, late mucosal involvement and in some instances marked tissue destruction. Although this is frequently the history in individual clinical cases how often such complications

occur in a community exposed to infection is unknown. It is not known whether such complications are a feature of reinfection. Most important of all it is not known how therapy modifies this disease pattern.

In short we know nothing of the evolution of the disease in a population with or without therapy and this makes it very difficult to devise a sound scheme of management of such patiests.

This is partly because of the difficulty of follow up of patients diagnosed in hospital. This problem is illustrated by an attempt by Castro[28] to follow up 23 patients treated with Amphotericin B. Only nine patients returned for follow up observations after one year and only 4 after 3 years.

Many drugs have been tried in the treatment of this disease but none have superceded antimonials as the mainstay of therapy. In cases resistant to antimonials the literature suggests that Amphotericin B has produced the best results. These two lines of therapy will be first discussed and other drugs subsequently considered. Before considering details of treatment with the main drugs used in therapy it is necessary to mention their main toxic effects. Pentavalent antimonials are much less toxic than trivalent but like all heavy metals they can produce cardiac, hepatic, renal or cerebral complications. Amphotericin B is a very toxic drug. The most important toxic effect is renal damage. In one study many years after treatment with this drug the majority or patients had evidence of renal impairment[23]. Ideally therefore patients should be treated with these drugs in hospital. A serum transaminase and bromosulphalein test done to assess hepatic function, an electrocardiogram to detect cardiac abnormalities and a urine examined and blood urea estimated to exclude the possibility of renal impairment before commencing treatment.

ANTIMONIALS

Azulay[7] lists 18 antimonial preparations that have been used in the treatment of this disease. Tartar emetic the trivalent antimonial had to be abandoned because of its serious toxic side effects and the necessity for intravenous administration. However pentavalent antimonials have proved to be effective in this as well as other forms of leishmaniasis. The two must widely used preparations have been antimony-n-methyl-glutamine, Glucantime (Rhodia) and sodium antimony gluconate, Pentostam (Burroughs Wellcome). Since the former is manufactured in Brazil it has been much more widely used in *L. braziliensis* infections though Pentostam has also given good results[45]. No controlled trial has been done on which is best but there is probably little difference. Both drugs have the advantage that they can be given intramuscularly as well as intravenously which is particularly helpful when treating small children.

Various treatment schedules have been recommended. The one we use is as follows. The dose must be related to the patients weight and is calculated on the basis of a total dose of drug of 1gm/kilogram. This is administered intravenously over a 10 days period with the patient resting in bed and receiving one injection a day. After 1 month's, rest this treatment is repeated and the same process repeated yet again to give a total of three treatment courses with a final total dose 3gm/kilo body wt. Glucantime is available in 5cc ampoules containing 1.5g of drug which contains 425 mg of antimony. The mechanism of action of such an antimonial on leishmania is still poorly understood. It is possible it is broken down in the body and acts in the trivalent form. Studies *in vitro* bear little relevance to the action in man. In view of the relatively low toxicity of glucantime and pentostam it is possible to administer therapy on an outpatient basis.

AMPHOTERICIN B (Fungizone)

This drug can never be used on an outpatient basis. Still the main drug effective in the deep systemic mycoses its value in the deep systemic mycoses its value in mucocutaneous leishmaniasis was verified simultaneously by *Furtado and Lacaz et al, in 1959. Since then it* has been used with success by various South American workers[11,93]. Practice varies widely both as regards the initial and subsequent doses of this drug but a common regimen is as follows.

Amphotericin B is dissolved in 500 ml of 5% dextrose and injected by slow intravenous drip over a period of 6 hours on alternate days. The initial dose is 0.5 mg/kg/day and depending on tolerance this is gradually raised to 1 mg/kg/day. Effective total doses are lower

than in systemic fungal infections. In one study such total doses varied between 4.4 mg/kg and 55.6 mg/kg with a mean of 28.7 mg/kg[28]. Cure without relapse has been reported in follow ups up to ten years.

Side effects however are considerable with fever, anorexia, nausea almost invariable. Venous thrombosis at the drip site is another problem. This can be lessened by slow diluted infusions and adding 25-50 mg of hydrocortisone sodium succinate to the drip bottle. Kidney toxicity is strictly dose dependent and renal function should be monitored twice a week until azotemia is stable. Bennett[13] prefers to keep the blood urea below 50 mg and the serum creatinine below 3.5 mg/100 ml. Hypokalemia occurs in 25% of patients and requires oral supplemention. Permanent reduction in glomerular filtration rate probably occurs in all patients receiving a therapeutic course of Amphotericin B for fungus infection. One electron microscopy study revealed tubular changes but the glomeruli appeared normal[26].

Patients must be carefully selected for Amphotericin B therapy as the treatment scheme involves a prolonged stay in hospital and a capacity to tolerate side effects. This situation should be explained to the patient and his collaboration ensured. This drug treatment while undoubtedly effective should be reserved for patients with progressive destructive lesions who have not responded to antimonial therapy.

OTHER DRUGS

A multitude of other drugs have been tried in this infection but none have produced consistent successful results such that they can be reviewed in the same way as the two previously mentioned. Azulay[7] in hisceview mentions a number of drugs which have been tried with little success among them sodium arsenite, bismuth, atebrin, yatren, germanine, sulphanilamide and furazolidine. One group of drugs he mentions, the aromatic diamidines should perhaps be reevaluated for use in certain cases in view of its success in treating diffuse cutaneous leishmaniasis in Ethiopia. Bryceson[20] reported cure in 7 out of 31 patients treated with lomidine (pentamidine dimethansulphonate). In drug resistant cases of *Leishmania braziliensis* infection this might be worth a trial. Especially as Lopes and Almeida[68] felt it was effective. However some newer drugs have also been tried and these will be briefly reviewed here.

Cycloguanil pamoate (Camolar) A depository antimalarial related to paludrine, it contains 140mg of active compound in each cc. combined with mineral oil and benzyl benzoate. Given in a single dose by deep intramuscular injection. For children of 4-6 years of age 140mg is sufficient, 280mg for those 5-10 and 350mg is the adult dose[19,30,57,104]. The first 3 authors used this drug in 50, 26 and 20 patients respectively in whom leishmania had been found. The criterion of cure was clinical healing of the lesion and cure rates of 88, 73 and 88% respectively were obtained. Bryceson *et al*[19] concluded, however, that the drug had no effect on *L. tropica* infections in Ethiopia. The drug has side effects at the side of innoculation with persistent muscle pain and occasionally abscess formation.

Niridazole (Ambilhar) This antischistosomal drug has been used in the dosage it is used for this infection namely 25 mg/kilogram daily for 10 days in the form of oral tablets[22,47]. Baranski[22] repeated this treatment up to 5 times, a dangerous policy in view of the side effects of this drug. Although the authors felt it had some activity clearly today this drug has no place.

Daraprim (Pyremethamine) Another antimalarial and a folic acid antagonist it has been tried without success in mucosal lesions by Viegas and Furtado[102] and Solano and Vargas[100].

Flagyl – A 5 nitroimidazole with good activity against Trichomonas vaginalis and intestinal protozoa this has been given in high doses without much effect by Furtado and Viegas[46]. Walton *et al*[110] have confirmed this finding.

Bayer 2502 (Lampit, nifurtimox) A current treatment for Chagas disease 6 patients have been treated with a dose of 480 mg per day for 25 – 30 days. In the only patient with mucosal involvement the treatment was continued until the 70th day. A good clinical response was noted in all patients[89]. Further trials are indicated.

Rifampicin (Rifaldin) A new antituberculous agent, good success was reported in *L. tropica* infections by El Din Selim and Kandil[41], using a dose of 1.200 mg per day for adults and 20 mg/kg/day for children. Recently Dourado (personal communication) has obtained good clinical healing with this drug in patients will *L. braziliensis* infections with only skin lesions in a

similar dosage sometimes continued for several months. Although neither investigator mention side effects these are known to be severe and include hepatitis, acute renal insufficiency, coagulation defects and a curious type of auto immune response[16]. These and the cost of the drug are likely to limit its usefulness.

Other drugs which have been tried without effect include Thiabendazole and Nagoxin[92, 111]. It is obvious from this short discussion that workers have been trying to find better drugs and the search will continue. However it is difficult to evaluate these results since the number of patients treated is so small, there is no comparison with standard therapy, and the criteria of cure is often clinical healing which could be spontaneous. It can safely be said to date that no new drug has been so successful as to become standard therapy.

HEAT THERAPY

It is well established that leishmania are heat sensitive and that small alterations in temperature can profundly influence the bahavior of leishmania in culture[67]. It has been suggested that the common sites of secondary lesions in *Leishmania braziliensis* infection, namely the nose or external ear, is determined by the lower temperature at these skin surfaces[1]. In animals healing of lesions has been produced by increasing the room temperature[114] yet suprisingly little work has been done on using heat or fever therapy in treatment in man. Baker and Gutierrez Ballesteros[9] used a vapour heat process used for the destruction of insect larvae in fruit and reported success in *L. braziliensis* infection in man and *L. enrietti* in the guinea pig. Bryceson[20] in his studies on diffuse cutaneous leishmaniasis in Ethiopia noted that an attack of measles dramatically improved three patients with marked skin involvement and suggests that the fever during the measles attack could be responsible. Bray and Ashford[17] following up this suggestion induced a *Plasmodium vivax* infection in a patient with diffuse cutaneous leishmaniasis and obtained a response. Recently success has been reported with the use of hot water or ultraviolet and infrared rays in the treatment of east African cutaneous leishmaniasis[77].

SURGERY

Cicatrization of oronasal lesions may require plastic surgery after medical treatment with reconstruction of the upper lip and nose. Very successful results have been obtained though little has been published to date[5]. One difficulty is that a relapse will result in loss of the graft. Because of this before undertaking reconstructive surgery a waiting period of 3 months to a year after the termination of successful medical treatment is desirable.

ASPECTS OF RESEARCH OF CLINICAL RELEVANCE

Involvement of the mucous membranes in cutaneous leishmaniasis is characteristic of the South American infection (*L. braziliensis*). However there have been isolated reports of mucosal involvement associated with visceral or dermal leishmaniasis from the Sudan[61], Yugoslavia[52] and Iran[112]. Cutaneous leishmaniasis occurs in most Central and South American countries with the exception of Chile[33]. The proportion of patients in clinical studies with mucous membrane involvement is very variable from 80% in some parts of Brazil to less than 5% in Guyana. Lainson (Personal Comunication) explains this on the basis of overlapping of different species. "As you travel southward in Brazil the incidence of espundia due to *L.b. braziliensis* increases as pian bois (*L.b. guyanensis*) decreases". Early reports of the geographical distribution have been reviewed by Pessoa and Barreto[81]. These authors in their extensive monograph document the disease in many Brazilian States. The disease probably exists in States where it has yet to be documented. For example 107 cases of mucocutaneous leihmaniasis were collected in Goias by one hospital service over a period of 4 years[10]. Previously there were no records from this state. Mucosal involvement seems to be rare in some surveys. For example in field studies of patients in Amapa[43] and indians in the Mato Grosso[25] no mucosal lesions were observed. It has been suggested that the Indian is more resistant to this type of lesion. Since we have little information on the actual incidence of mucosal lesions in communities it could be that they do exist but are sufficiently rare not to be represented in studies of small rural populations. Convit and Pinardi[33] note that in Venezuela in areas where mucosal forms are not usually seen if an epidemic occurs with a large number of cases muocutaneous lesions appear in a few patients.

There have been few studies of the clinical evolution of lesions on settled communities and such studies would provide valuable

information relating to prognosis. Guimarães[53] describes a community where a small epidemic was associated with tree felling. Of 306 persons examined 39 had characteristic lesions and of these 34 out of 36 had positive Montenegro reactions. Only two patients had nasal involvement and these proved resistant to treatment with tartar emetic and fuadin. In general the initial response to these drugs was good but there is no information on long term follow up. In three patients spontaneous cure was noted.

Preston, in 1974[86], has pointed out that the innoculation of leishmania into man is followed by three types of sequelae namely subclinical, self healing lesion, or a non healing lesion. Non healing appears to be associated with two types of host response, either anergic forms characterised by a supressed immonogical response as characterised by the rare diffuse cutaneous leishmaniasis or more commonly allergic forms with an enhanced immunological response on the part of the host. The two main suggestions to explain these different responses are:

1. An innate property of the host in terms of his ability to mount an immune response.
2. The type of leishmania innoculated.

There is much evidence to support both suggestions but at the time of writing it is too early to conclude which is the more important. Indeed it is likely that while both may be relevant the role they play may vary with the individual case.

The immunological response of the host has been recently reviewed by Bryceson[21] and Convit and Pinardi[33] and these authors seem largely in agreement. Bryceson has pointed out that the histological picture of leishmaniasis can be classified along the lines of the Ridley Jopling scale for leprosy and used as an index of the host's cell mediated immunity. His table is reproduced in table I illustrating these histological variations. There is usually a close correlation between skin sensitivity to leishmanin and the histological picture. The immunological defect in diffuse cutaneous leishmaniasis appears to be specific for such patients respond normally to tuberculin and lepromin. Also both Convit and Pinardi[33] in Venezuela and Bray[18] in 1974, in Ethiopia have demonstrated defective lynphocyte transformation in diffuse cutaneous leishmaniasis. Bray's group have also shown defective leucocyte migration, an absence of lymphocyte activation products containing a mitogenic factor and inhibition of macrophage migration. The poor capacity of the host to respond to the parasite in diffuse cutaneous leishmaniasis is also shown in the bad response to treatment. Bryceson[20] in Ethiopia found pentamidine dimethanesulphonate was the most effective drug and under the influence of treatment the leishmanin test frequently became positive suggesting the beginings of an cell mediated immune response. The cause of the defect in cell mediated immune response in diffuse cutaneous leishmaniasis is unknown. At one time a specific type of leishmania (*L. pifanoi*) was held responsible. However it appears the same organism can produce simple cutaneous leishmaniasis[18] and the isoenzyme structure of Ethiopian isolates from diffuse cutaneous leishmaniasis (D.C.L.) is similar to those from simple cutaneous leishmaniasis[84]. Zeledon[115] on these grounds has suggested that *L. mexicana pifanoi* is a species inquerenda. Lainson and Shaw (personal communication) have shown D.C.L. in Brazil to be due to *L. mexicana amazonensis* which produces uncomplicated cutaneous leishmaniasis in immunologically competent persons in the same geographical area. It is possible *L. pifanoi* is synonymous with *L. m. amazonensis* but since they have not examined the Venezuelan material designated *L. pifanoi* they prefer to use the name *L. mexicana pifanoi* for the moment, but evidence is building up against considering *L. pifanoi* as a distinct species.

Bryceson[20] noted in Ethiopia that the primary lesion of D.C.L. is frequently on the leg in contrast to oriental sore where 99% were on the face. Half the patients with leg lesions had in addition defective lymphatic function in the leg. He argues that the induction of an immune response in a lymph node which is damaged or preoccupied with competing antigens might be reduced or allow the development of a state of tolerance. Bray[18] reviewing his experience of the failure of transfer factor to activate immune response suspects that the relevant clone of lymphocytes no longer exists in D.C.L. At the opposite and of the spectrum is lupoid leishmaniasis[39]. The extreme tuberculoid picture representing a failure to eliminate the parasite despite exaggerated sensitivity to leishmanial antigens. In our experience of *L. braziliensis* infections in man this form is uncommon.

In South American mucocutaneous leishmaniasis all variants of the histological types set out in Bryceson's table can occur. Cutaneous leishmaniasis of the new world has

complex epidemiological aspects with a large animal reservoir particularly in small rodents. Lainson and Shaw[64, 65, 66] have emphasised that such leishmaniasis is predominantly a zoonosis with man only an incidental host playing little role in the transmission of the parasite in nature. The same authors have produced a classification of the organisms producing cutaneous and mucocutaneous leihmaniasis which is an extension of the trinomial system used by Pessoa[82] and considers geographical distribution, clinical features and growth in culture and the hamster. This classification is set out in an abbreviated form in table II and it represents an advance in our attempts to understand the varied clinical presentations of cutaneous and mucocutaneous leishmaniasis in man. It remains to be seen to that extent this classification will be confirmed by future experience. Unfortunately antigenic characterisation of such a complex does not appear feasible due to the number of common antigens but some progress has been made with a biochemical approach to taxonomy using isoenzyme and D.N.A. analysis. The results tend to support Lainson and Shaw's classification[29, 50].

Turning to another problem the question arises as to why the mucosal involvement has its unusual anatomical pattern with a preference for the nose and how nasal lesions arise. Probably the dissemination of leishmania from the site of innoculation is initially by the lymphatic system and then haematogenous spread. As mentioned earlier leishmania have been found in normal nasal mucosa in this infection[103] and in normal skin in Kala Azar[85]. There seems little doubt that leishmania can lie dormant in the tissues for many years and the onset of cutaneous leishmaniasis has been reported as long as 13 years after leaving the endemic area[71]. Walton has been mainly responsible for raising a number of interesting speculations regarding the manner in which a mucosal lesion might be precipitates. Walton and Valverde[108] have noted in Bolivia that necrotising lesions of the face are much more common in patients of negro descent than in Amerinds and that such negro patients have a greater sensitivity to leishmanin suggesting an exaggerated cell mediated immune response presumeably of genetic origin Further Walton *et al*[107] have published case histories of Bolivian patients who developed mucosal lesions years after the initial skin infection associated with the development of malnutrition or tuberculosis. Walton and Valverde[109] have also encountered patients who developed lesions at the site of skin injury. This last suggestion seems to us to be worth further investigation. The nose is frequently blown or picked – it is the commonest site of a mucosal lesion. Toothpicks are in common use and the mouth is the next commonest site. In guinea pig leishmaniasis (*L. enrietti*) one could investigate the suggestion that mechanical injury can precipitate leishmanial lesions in the presence of chronic leishmanial infection. Finally it must be noted again that temperature is probably important in the development of metastatic lesions as demonstrated in the infected hamster[113].

Leishmania enrietti of the guinea pig, discovered by Medina in 1946 in Panamá[76] has proved useful laboratory model of mammalian cutaneous leishmaniasis. Guinea pigs usually develop a single lesion on the ear which ulcerates and heals in a few months; like most human cutaneous leishmaniasis this is accompanied by delayed hypersensitivity and often followed by long lasting immunity to reinfection.

Paraense[78] studied the spread of this parasite through the body of the guinea pig in an attempt to establish how leishmania metastasise. He found that parasites are carried in macrophages from the innoculation site to local lymph glands where many are destroyed. Others however by post glandular lymphatic channels gain the blood stream and are thence widely disseminated. He found parasites in the feet, nasal mucosa, scrotum and eyelid of guinea pigs innoculated in the ear.

Bryceson[21] and his group (1972) have made many contributions to our understanding of the development of studies of the host immune response which may have relevance to equivalent human infections. In this model the incubation period depends upon the infecting dose of flagellates. Also and perhaps relevant to human South American leishmaniasis, the subsequent course of the disease is influenced by the size of the initial innoculum. Animals receiving large doses of flagellates developed large initial lesions and more metastases. The development of delayed hypersensitivity as measured by the skin test coincided with the appearance of the clinical lesion. The cell mediated immune response measured after 4 weeks (the longest incubation period) showed that lymph node cells transformed in the presence of leishmanial antigen. Migration of

macrophages was inhibited by this antigen. It was felt that two host mechanisms operated in the host tissues to eliminate the parasite – enhanced macrophage phagocytosis and lymphocytic cytotoxicity, and experimental evidence suggested the later was more important. Studies of antibodies using immunofluorescent techniques on the serum of infected guinea pigs[88] detected complement fixing antibody after the second week of infection. This antibody appeared to be unrelated to the healing process or to the pathogenesis of local tissue damage.

Unfortunately other animal models have not proved as helpful. In some rodent studies it has been difficult to differentiate strains of *L. braziliensis* although skin lesions can be readily produced[35]. Drug evaluation in rodents also fails to yield information applicable to man[40]. However these latter authors made the interesting observation that amastigotes can often be found in healed lesions. Rhesus monkeys have been successfully infected[37, 62] and more recently marmosets. It is likely primate models will provide useful information in the future.

CONCLUSION

It is obvious from this discussion that advances in our diagnosis and management of this important infectious disease of man are to be expected. Already much useful information has come from recent immunological and epidemiological studies. In view of the unsatisfactory nature of therapy clinicians will continue to test new drugs probably in better controlled trials. There is a need to understand more about the natural history of the disease with and without therapy.

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TABLE 1. The histological spectrum of Leishmaniasis After Bryceson, 1972

M.M.	Macrophage	Thin intact epidermis, clear subepidermal zone. Dermal infiltration with macrophages, often vacuolated, full of parasites† histiocytes, many vessels containing monocytes, absence of lymphocytes.
M.I.	Macrophage Intermediate	M.M. together with scanty lymphocytes scattered throughout or grouped deeply beneath the lesion.
I.I.	Intermediate	Epidermis thickened, intact early, may ulcerate later. No clear zone. Lymphocyte intimately mixed whith large, fleshy, histiocytes, moderate numbers of parasites or M.M. areas along side IT or TT areas.
IT	Intermediate Tuberculoid	Epidermis ulcerated; where intact, shows reduplication of the layers, nuclear damage, and hyperkeratosis. Lymphocytes predominate, early arrangement into tubercles around clumps of epitheloid cells, parasites scanty.
TT	Tuberculoid	Epidermis ulcerated. Tubercle formation, often with giant cells. Parasites rare or invisible.

TABLE 2. Aspects of a Classification of American Cutaneous Leishmaniasis as proposed by Lainson and Shaw, 1972

Leishmania Mexicana Complex	Behavior in culture	Behavior in hamster
L. mexicana mexicana Causes chiclero ulcer, bay sore usually single lesion often self healing ear lesions often chronic destructive no naso pharygeal involvment.	Luxuriant Growth	Rapid development of histocytomas in a few months.
L. mexicana amazonensis Single or limited lesions in man in Amazon basin.	Ditto	Ditto
L. mexicana pifanoi Only known from cases of anergid diffuse cutaneous leishmaniasis.	Ditto	Ditto
Leishmania Braziliensis Complex *L. Braziliensis* Few small to very large progressive lesions, frequent mucous membrane involvement.	Scanty growth	Slow growth few parasites
L. braziliensis guyanensis Pian bois, single or multiple ulcers metastases often seen as nodules along lymphatics.	Scanty growth	Slow growth few parasites
L. braziliensis panamensis Single or multiple ulcers lymphatic spread. Rarely naso pharyngeal involvement.	Grows reasonably well	Slow growth moderate number of parasites
L. peruviana Uta. single or limited lesions self healing no mucous membrane involvement.	Grows well	Little information

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