American Cutaneous Leishmaniasis

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SUMMARY: This review outlines the present knowledge of American cutaneous leishmaniasis, a disease which, owing to the increase in international travel, is being seen with increasing frequency in Europe and North America.

A knowledge of this disease is of particular importance to the military medical officer as in recent years approximately one-hundred and fifty cases of leishmaniasis have been seen in British troops who have served in Belize. *Leishmania braziliensis* sp. have been isolated from a number of these cases. Organisms of this complex had not previously been recorded in Belize and the Army Medical Services are therefore in a unique position to study this disease, as well as having a responsibility to ensure its correct management.

Geography

The disease is endemic in a large area of the Americas on either side of the equator, from approximately 25°N to approximately 30°S¹. It has been reported from Mexico, all of the Central American countries and every South American country with the exception of Chile. Autochthonous cases were thought not to occur in the United States, but in 1976, three such cases were reported².

In man the incubation period for the disease is quite variable, usually between two and ten weeks, but occasionally several months. It is hence possible that individuals may present with the disease in countries where it is not endemic a considerable time after leaving the area where they acquired their infection.

Parasitology

The disease is caused by a protozoa of the *Leishmania* genus, a member of the Trypanosomidae family, and is transmitted by the bite of an infected female phlebotomine sandfly.

In the gut of the insect vector the parasite is in the promastigote form, a motile spiral organism 15-40µ in length, with a single anterior flagellum. Multiplication is by binary fission. Promastigotes migrate from the gut of the sandfly to the buccal cavity and can subsequently be injected into the vertebrate host when the sandfly takes another blood meal. The organisms then invade local tissue histiocytes, where they change into non-flagellate amastigote forms. Multiplication within the vertebrate host is also by binary fission to give further amastigotes which are round or oval, measuring 2-5µ in length by 2-3µ in width.

Sandflies feeding on an infected animal may

acquire infection with amastigotes which then develop into promastigotes in the gut and complete the parasite life cycle.

The species of *Leishmania* responsible for cutaneous disease do not cause American visceral leishmaniasis, which is due to the related species *L. chagasi* and is less common than the cutaneous forms. Numerous cases have been reported from Brazil and less from other American countries.

Insect vectors and animals hosts

Each species or sub species of *Leishmania* is transmitted between animals, and to man, by only a very limited number of phlebotomine sandfly species³, despite numerous other species of sandfly feeding on both man and the relevant animal hosts⁴. Sandflies usually bite between sunset and dawn⁵, although they may bite at other times if disturbed from their resting sites amongst the forest litter on the ground⁶. Sandflies have a limited flight range⁷ and within their forest habitat the number of sandflies varies markedly from place to place. There are also marked variations in their populations with season⁸ — in most areas the numbers are greatest during the rainy seasons although their numbers may decline with extremely high rainfall. Cutaneous leishmaniasis is a zoonosis, with the usual cycle being transmission of the disease from an infected animal to a healthy one by the sandfly. Man is only an 'accidental' host, when he is exposed to the bite of the infected sandfly and is usually the end of the transmission chain, playing no important part in the maintenance of the *Leishmania* species. Not all *Leishmania* can be transmitted to man. Small forest rodents such as the tree rat, white footed mouse and spiny pocket mouse⁹ are the principal

animal hosts for most species. *Leishmania braziliensis* infection of dogs has been described in Panama¹⁰, but the sloths are the major hosts to *Leishmania braziliensis panamensis*. In both rodent and mammal hosts leishmanial infection may produce only a few indistinct skin lesions¹¹, in rodents typically of the tail, and in other mammals around the ears or nose.

Clinical features of human disease

After an incubation period varying from two weeks to many months a small blue-red papule develops at the site of a sandfly bite. These lesions occur predominantly on exposed areas as the sandfly is unable to bite through clothing. Lesions of the head and neck, ears, arms and hands are therefore common, whilst lesions of the trunk are unusual. The initial lesions are single or few in number. The papule increases in size over ensuing weeks to form a nodule or plaque of between one half and twelve centimetres diameter. Superficial crusting may be present at the centre of relatively early lesions, but frank ulceration subsequently develops and a crust of dried exudate and pus, due to secondary bacterial infection, usually then forms over the granulomatous base. The ulcer has a raised, infiltrated blue-red border and is relatively painless. Not all lesions progress to ulceration, they may remain as papules, nodules, or plaques. Slight lymphangitis and lymphadenitis in the area draining the lesion is not uncommon. Some species of leishmania have a tendency to lymphatic or haematogenous spread to cause metastatic secondary lesions. In the majority of cases the natural course is for the skin lesion to eventually heal, leaving a characteristic scar which is slightly depressed, smooth, atrophic and dyschromic. Infections due to *L. braziliensis* may lead to the development of late nasopharyngeal lesions, espundia, as discussed below.

Classification of *Leishmania* and features of the different species

In the past various classifications¹² based on clinical features, geographic location and epidemiological findings have been employed. This has led to a proliferation of frequently overlapping and confusing terms which are all the more inappropriate as the disease is a zoonosis with man only an 'accidental' host. Most workers now accept Lainson and Shaws¹³ trinomial system of classification which divides the organisms into the *mexicana* complex and the *braziliensis* complex, each of which is then further divided into related species and sub species. Recent laboratory studies of the detailed morphology of the organisms^{14, 15} of their DNA buoyant densities and electrophoresis of their enzymes^{16, 17}

support this classification and give more objective assessments of the parasites.

The *L. mexicana* complex

The parasites of this complex multiply rapidly in the fore and mid gut of vector sandflies. On hamster inoculation they multiply rapidly¹⁸ to produce histiocytomata, with frequent metastatic spread. Growth on culture media is easy and rapid. Human disease due to members of this complex is frequently self-limiting.

L. mexicana mexicana

Infection with this organism occurs in Belize, Guatemala, parts of Mexico and probably other Central American countries. The principal insect vector is *Lutzomyia olmeca olmeca*.

The rodent hosts and infected sandflies have a habitat confined to forest areas, hence human infection is confined almost exclusively to those who work and, or, live in the forest¹⁹. Amongst native Belizeans infection occurs mainly in mahogany cutters and chicleros. Chicleros tap the 'sapodilla' trees (*Achras sapota*) for the crude chewing gum latex 'chicle'. Both groups of workers spend many months of each year living and working in the forest. Clinically apparent infection is not common among women and children, even those living in villages adjacent to the forest. The reason for the high incidence of disease amongst the chicleros and its low incidence in the villagers is that the vector sandfly *Lutzomyia olmeca olmeca* does not readily bite man and prolonged exposure in the forest is therefore required for infection to occur.

The classic lesions due to this species of *Leishmania* are the bay sore and the chiclero's ear²⁰. The term bay sore was first used during the reign of Queen Elizabeth I to describe the ulcerated skin lesions which severely affected the early European colonists opening up the logging industry around the Bay of Honduras. Chicleros ear refers to the chronic cartilagenous destructive lesions which may develop when the infection involves the ear. Lainson and Strangways Dixon²¹ report that 40% of the lesions they studied in Belize affected the ears, whilst 28% involved the hands and arms. Lesions of the legs and torso are uncommon. The ear is an area which is seldom covered when other areas of the body are so protected, this will increase the risk of sandfly bites occurring on the ear rather than elsewhere, as the sandfly is unable to bite through clothing. Ear lesions may be diagnosed more frequently than lesions on other parts of the body as, unlike lesions of other areas, they do not tend to heal spontaneously, but rather progress to chronic destruction of the pinna causing considerable deformity. The

relatively poor blood supply to the cartilage of the ear may facilitate the development of chronic lesions by leaving organisms here relatively inaccessible to the effects of cell mediated immunity.

The lesions are usually single and in sites other than the ear are usually self limiting. An ulcer may develop to several centimetres in diameter before starting to resolve and although most lesions start to heal after a period of weeks, some persist for months or years. The author has seen one case in a former mahogany cutter where extensive ulceration of the arm had been present for seventeen years and showed no sign of healing. If more than one lesion is present they are usually few in number and morphologically very similar, such multiple lesions are thought to be due to multiple bites within a short time causing separate primary lesions rather than to spread from a single primary lesion. Neither nasopharyngeal nor visceral lesions develop.

L. mexicana amazonensis

This species occurs in the Amazon basin, certain other parts of Brazil and in Trinidad. The vector *Lutzomyia flaviscutellata* seldom bites man²², but when human infection does occur it usually follows a similar pattern to that due to *L. mexicana mexicana* with the exception that there is no predilection for the ears. Diffuse cutaneous leishmaniasis developed in six of twenty patients infected with this species who were studied in Brazil²³. In this form of the disease numerous papules, nodules and plaques develop over the body surfaces, ulceration is uncommon. Parasites are exceedingly numerous within the lesions and there is specific anergy to the intradermal Montenegro test (*vide infra*). The multiple lesions are due to haematogenous spread of the parasite which may be found in the blood. Internal organs are not involved and nasopharyngeal lesions do not occur. Response to therapy is poor, with frequent relapses²⁴.

It has been suggested that the development of diffuse cutaneous leishmaniasis is due to a failure of the hosts cell mediated immunity to the parasite^{25, 26, 27}. This hypothesis does not however explain why internal organs are spared when the parasite is present in the blood stream. There is increasing evidence that the normal vertebrate host response to infection with *Leishmania* requires the participation of both cell mediated and humoral immunity²⁸, and it is possible that failure of integration of these responses within the dermis leads to diffuse, anergic disease.

L. mexicana venezuelensis

L. mexicana venezuelensis is the organism responsible for diffuse cutaneous leishmaniasis in

Venezuela. It is very similar to *L. mexicana amazonensis* and almost certainly causes simple cutaneous lesions as well as the diffuse disease.

L. mexicana garnhami

This species occurs in the humid zone of the Venezuelan Andes up to a height of 1,800 metres. It produces spontaneously healing cutaneous lesions which are usually single. Late nasopharyngeal involvement does not occur. The vector is thought to be *Lutzomyia townsendi*.

The *L. braziliensis* complex

Parasites of this complex develop in the fore, mid and hind gut of the vector sandflies. Growth on hamster inoculation is slow, producing small swellings with few detectable parasites and no metastatic spread. Growth on culture media is slow and difficult.

Human disease due to this group may present with single or multiple skin lesions which may be very destructive, nasopharyngeal involvement may develop subsequently.

L. braziliensis braziliensis

Most cases due to this organism occur in Brazil. The principal vector *Psychodopygus wellcomei* bites man during the daylight hours as well as at night. The primary lesions are usually few in number but they are frequently large, chronic and destructive ulcers. It is usually possible to demonstrate only a few parasites in the lesion and there is a very vigorous tissue reaction. Late metastasis to nasopharyngeal tissues, espundia, is common; the incidence varying from one region to another. In Venezuela it is said to occur in between twelve and thirty-four per cent of cases whilst in Brazil an incidence of eighty per cent has been reported. The mechanism of development of these late lesions is not established — it presumably reflects haematogenous or lymphatic dissemination of the parasites but the reason for the localization of the lesions remains unclear. Leishmania are present in the nasopharyngeal lesions in their early stages but become progressively more difficult to demonstrate as the condition progresses. There is usually an asymptomatic period of three to ten years between the primary skin lesion and the late nasopharyngeal lesions developing. The reason for this delay is unknown. In Brazil some patients develop the nasopharyngeal lesions within one year of their primary lesion developing, but only some 10% of cases of espundia have an active primary lesion at the time of presentation with nasopharyngeal disease. The mucosa overlying the anterior cartilagenous part of the nasal septum becomes inflamed. The patient usually notices some nasal dis-

charge and small nose-bleeds may occur on blowing the nose. Ulceration usually follows within a short time, destroying mucosa, cartilage and soft tissues but initially leaving bone intact. The lesion then extends progressively more deeply into surrounding tissues causing massive destruction of the nasal fossae, palate, fauces, pharynx tongue, gums and floor of mouth. Further spread may involve the trachea, bronchi and larynx, involvement of which causes marked change in the voice. Secondary bacterial infection occurs almost invariably. Gross disfigurement develops and severely compromises normal speech, feeding and breathing. Wasting and malnutrition due to feeding difficulties develop and death due to respiratory infection may ensue. Late lesions of the genitalia and rectum occur, but less frequently than do nasopharyngeal lesions. These late lesions may prove very difficult to treat.

L. braziliensis guyanensis

This species occurs in Guyana, Surinam and Brazil. The principal vector is *Lutzomyia umbratilis*. There is a tendency for *Leishmania* from the primary ulcer to metastasise along the lymphatics draining that area to give lymphadenitis, nodules or further ulcers. Metastases to give numerous ulcers widely distributed over the body, 'pian-bois', is not uncommon. Only a few parasites can be isolated from lesions. The species does not cause nasopharyngeal disease.

L. braziliensis panamensis

Causes simple cutaneous leishmaniasis in Panama; sloths are the major animal host.

L. peruviana

Differs markedly from other forms of American cutaneous leishmaniasis in that it occurs on the barren western slopes of the Andes in Peru up to a height of 2800 m, whilst all other forms are confined to lower, humid, forested areas. The domestic dog is the only known animal reservoir and transmission seems to be essentially domiciliary. It causes a single or limited number of self-healing lesions called 'uta', with no nasopharyngeal involvement.

Diagnosis

A history of having visited the relevant geographic area together with the clinical appearance should suggest the diagnosis. Differential diagnosis may include pyogenic infections, basal cell epithelioma, keratoacanthoma, phagedenic and 'tropical' ulcers, atypical mycobacterial infection, lymphoma, sporotrichosis, tungiasis and cysticercosis. Lepromatous leprosy closely resembles the anergic form and nasopharyngeal disease must be differentiated

from South American blastomycosis, lethal mid-line granuloma and rhinoscleroma.

Definitive diagnosis requires demonstration of the parasites microscopically. Superficial smears may be negative in the presence of secondary bacterial infection; it is therefore preferable to make a linear scalpel incision along the edge of a lesion and take the smears from the cut edge; a bloody smear is of no use. A fine bore pipette or needle may be used to obtain tissue fluid from the edge of a lesion or samples may be taken from the cleaned base of an ulcer. The ease with which *Leishmania* are isolated varies from species to species. Early lesions are more likely to yield a positive result than are late lesions, particularly if secondary bacterial infection has developed. In addition to the examination of smears stained with Giemsa's or Leishman's stains, culture of the specimens should also be carried out. *Leishmania* may sometimes be detected only by culture and this is also necessary to identify the species. The use of both artificial culture media and inoculation route into hamsters is recommended.

Novy, MacNeill and Nicholles (NNN) medium is one of the oldest and most frequently used media often with the addition of an antibiotic to inhibit bacterial growth. Initial culture may however be more successful on a semi solid blood agar medium with subsequent inoculation onto NNN medium which has a solid phase with liquid overlay.

Microscopic examination of a biopsy from the lesion is a less reliable means of diagnosis than the above methods. If a biopsy is taken an impression smear of the tissue should be taken prior to fixing, and a part of the tissue cultured for *Leishmania*.

Histologically early lesions frequently show hyperkeratosis and acanthosis. Liquefactive degeneration of the basal cell layer of the epidermis may develop in the presence of a contiguous dermal infiltrate. The dermal infiltrate is granulomatous with large numbers of epithelioid histiocytes, lymphocytes, plasma cells, polymorphonuclear leucocytes and Langhans' giant cells in varying proportions. Amastigotes may be seen within macrophages and are more easily visualised with Giemsa or Leishman stain than with haematoxylin and eosin. There is usually dilatation and reactive growth of dermal blood vessels in the affected areas and occasional extravascular erythrocytes may be seen. Chronic ulcerated lesions usually have an infiltrate consisting mainly of polymorphonuclear leucocytes.

Montenegro's intradermal leishmanin test may be used to give further evidence to support the diagnosis. The test consists of the intradermal injection of cultured, heat killed *Leishmania*. The standard dose is 0.1 ml, containing at least 100,000 promastigotes and with a nitrogen concentration of at

least 30 $\mu\text{g}/\text{ml}$. The injection site is marked and examined at 48 and 72 hours. A positive reaction is an indurated papule of 5 mm. or greater diameter, surrounded by erythema. A positive result indicates present or previous leishmanial infection. The rates of positive delayed hypersensitivity are higher than the rates of past or present clinical disease, as the test is specific and reproducible this suggests that subclinical cases of leishmaniasis occur²⁹. In residents of areas endemic for leishmaniasis this is not a definitive test as positive reactions may reflect previous infection and any current skin lesion could be of unrelated aetiology. In individuals who have not previously visited endemic areas the test is more reliable as previous leishmanial infection is unlikely. The test becomes positive within a few weeks of infection and remains so for many years, possibly for life. A positive reaction indicates the ability to mount a localised immune response at the site of injected antigen and does not indicate complete immunity to infection with the various species of *Leishmania*. There are several modifications of this test using different antigens³⁰.

American cutaneous leishmaniasis does not lead to the massive increase in circulating gamma globulins seen in kala azar. Specific circulating antibodies are formed however and techniques are being developed to identify these in a reliable diagnostic test. At present these tests are available in only a few specialist centres and require further refinement, before becoming available for general use. Immunofluorescence^{31, 32}, radio-immunoassay, enzyme profiles³³ and enzyme linked immunosorbent assay³⁴ are among the techniques in use.

Treatment

The tendency to spontaneous healing of some forms of leishmaniasis has led to claims for the therapeutic efficacy of a wide variety of drugs, which subsequent investigations have shown to have no effect on the natural course of the disease. The drugs which have been proven to be effective are potentially toxic and therefore it is important to establish the diagnosis prior to commencing therapy in order that these substances are not given unnecessarily. It is also of use to establish the species of leishmania responsible for the infection. Cases due to *L. braziliensis braziliensis* all need to have a full course of treatment with an effective drug in order to prevent subsequent mucocutaneous lesions, whilst lesions due to *L. mexicana mexicana* may resolve completely, and lead to no late sequelae, without any specific drug therapy. Extensive lesions and lesions involving the cartilage of the ear or nose require effective treatment. If facilities for species identification are not available a decision on whether

to employ drug therapy should be based on knowledge of the pattern of disease previously experienced in the area where the disease was contracted. Although late mucocutaneous lesions have been reported from every endemic country south of Mexico the incidence is much higher in the southern areas than the more northerly ones.

Pentavalent antimony (Sb) compounds are the most widely used drugs, either sodium stibogluconate (Pentostam) or antimony-n-methyl glutamine (Glucantime). There is no evidence that either drug has advantages over the other in efficacy or toxicity. Sodium stibogluconate is more readily available in the UK, in the United States it is obtainable only from the National Communicable Diseases Centres, Atlanta, Georgia. Although intramuscular administration is possible a prolonged course of treatment is necessary and slow intravenous injection is therefore preferred. Therapy should be as a hospital in-patient whenever possible. The dose of pentostam is 10-20 mg. Sb/kg body weight with a maximum daily dose of 850 mg. Sb. This dose is given daily for at least three weeks. With daily injections antimony accumulates in the body³⁵. Toxic effects³⁶ include nausea, vomiting, anorexia, fever, joint pains, transient changes in the ST and T^{37, 38} segments of the ECG and bradycardia³⁹.

Lesions which have not responded to adequate antimony therapy may respond to a diamidine drug such as pentamidine⁴⁰. The drug is given by slow intravenous injection in intermittent seven to twelve day courses and may cause pruritus, exfoliative dermatitis, impaired glucose tolerance and neuropathies; too rapid injection can cause hypotension and collapse⁴¹. This drug should be used only in patients with normal hepatic and renal function.

Severe mucous membrane lesions unresponsive to the above agents may be treated with amphotericin B⁴² in a dose of 0.5 to 1.00 mg/kg body weight (maximum dose 50 mg) by slow intravenous infusion diluted in 5% dextrose solution to a concentration of drug of 100 mg/l or less. The drug should be given on alternate days over a three to seven week period. It is very toxic and treatment often has to be discontinued for this reason. Fever, nausea, and anorexia develop commonly, venous thrombosis may occur at infusion sites. Renal damage is not unusual and the dosage should be reduced if the blood creatinine rises.

There are several reports of the efficacy of rifampicin^{43, 44} but further controlled trials are required as treatment failures are also reported⁴⁵. Ketoconazole has been reported to be effective in a limited number of cases⁴⁶. Early reports of the efficacy of trimethoprim-sulfmethoxazole⁴⁷ or metronidazole⁴⁸ have not been borne out by subsequent investigators^{49, 50}. Intralesional injections of anti-

leishmanial agents are used in a few centres, they are often painful and may increase subsequent scarring and are not therefore recommended⁵¹. Amastigotes are only able to multiply within a narrow temperature range and locally applied heat appears to speed the resolution of lesions⁵². Cryotherapy is also reported to be effective^{53, 54} whilst cautery may result in unsightly scars⁵⁵.

Disease Control

Eradication of the animal hosts or insects vectors at present seems to be impractical. There are reports from other parts of the world that anti-malarial insect spraying reduces the incidence of leishmaniasis; the extent of the jungle habitat of the vectors in America would make such an insecticidal policy impossible to implement effectively. Of the insect repellents at present available N N-diethyl-m-toluamide is the most effective against sandflies, but the climate of Central and South America necessitate frequent applications.

There is at present no effective, safe, way to immunize people living in endemic areas. 'Immunization' against *L. tropica* infections has been carried out in several countries by intradermal injection of live parasites into a cosmetically unimportant area^{56, 57, 58, 59}, producing clinical lesions at the injection site and subsequently immunity to re-infection. Such immunity can at present only be achieved with live organisms and requires careful control of the injected parasite to ensure that it is sufficiently immunogenic⁶⁰, but does not lead to severe disease. The several different species of *Leishmania* responsible for disease in the Americas makes the problem of immunization more difficult. It is essential that any induced infection will not lead to espundia, and detailed knowledge of the type of disease present in a locality is required before contemplating immunization. Induced or naturally acquired infection with *Leishmania* usually leaves the subject immune to re-infection with that particular species and there is evidence of some cross immunity between species⁶¹. Organisms of the *mexicana* complex appear to be poor immunizing agents against both other species of the same complex and against members of the *braziliensis* complex. This suggests that these species which usually produce self limiting disease, grow rapidly in culture and are easily maintained are unlikely to be of use for immunization against potentially more serious *L. braziliensis* infections. Species of the *braziliensis* complex appear to be more effective immunizing agents, but are more likely to cause serious disease.

There therefore remains a great deal of work to be done before routine human immunization is an acceptable procedure⁶². Long term chemoprophylaxis

is not feasible for residents of endemic areas, but if a suitable orally administered drug were found this would have potential use for visitors to endemic areas.

- 1 KERDEL VEGAS F. American Leishmaniasis-in Clinical Tropical Dermatology. London. Blackwell Scientific Publications, 1975.
- 2 SHAW P K. Autochthonous dermal leishmaniasis in Texas. *Am J Trop Med Hyg* 1976; **25**: 788-796.
- 3 ARIAS J R, de FREITAS R A. On the vectors of cutaneous leishmaniasis in the Central Amazon of Brazil. *Acta Amazonica* 1977; **7**: 293-294.
- 4 KILLICK KENDRICK R. Recent advances and outstanding problems in the biology of phlebotomine sandflies. *Acta Tropica* (Switz) 1978; **35**: 297-313.
- 5 STRANGWAYS DIXON J, LAINSON R. Dermal leishmaniasis in British Honduras. *Brit Med J* 1962; **1**: 297-299.
- 6 WILLIAMS P. Phlebotomus sandflies and leishmaniasis in British Honduras (Belize). *Trans R Soc Trop Med Hyg* 1970; **64**: 317-368.
- 7 PERFIT'ER P P. Phlebotomidae-in Fauna of the USSR, Vol III, Diptera. No. 2 Academy of Sciences of the USSR. New Serial No 93.
- 8 SHAW J J, LAINSON R. Leishmaniasis in Brazil VI. *Trans R Soc Trop Med Hyg* 1972; **66**: 709-717.
- 9 LAINSON R, STRANGWAYS DIXON J. Dermal leishmaniasis in British Honduras. *Brit Med J* 1962; **1**: 1596-1598.
- 10 HERRER A, CHRISTENSEN H A, BEUMER R. Epidemiological patterns of cutaneous leishmaniasis in Panama II. *Ann Trop Med Parasitol* 1976; **70**: 67-71.
- 11 HERRER A, CHRISTENSEN H A. Infrequency of gross skin lesions among Panamanian forest mammals with cutaneous leishmaniasis. *Parasitology* 1975; **71**: 87-92.
- 12 GARDENER P J. Taxonomy of the genus *Leishmania*. A review of the nomenclature and classification. *Trop Dis Bull* 1977; **74**: 1069-1088.
- 13 LAINSON R, SHAW J J. Leishmaniasis of the New World: Taxonomic problems. *Brit Med Bull*; 1972: 44-48.
- 14 SHAW J J, LAINSON R. Leishmaniasis in Brazil XI. Observations on the morphology of *Leishmania* of the *braziliensis* and *mexicana* complexes. *J Trop Med Hyg* 1976; **79**: 9-13.
- 15 GARDENER P J, SHCHORY L, CHANCE M L. Species differentiation in the genus *Leishmania* by morphometric studies with the electron microscope. *Ann Trop Med Parasitol* 1977; **71**: 147-55.
- 16 CHANCE M L, PETERS W, SHCHORY L. Biochemical taxonomy of *Leishmania* I. *Ann Trop Med Parasitol* 1974; **68**: 307-315.
- 17 GARDENER P J, CHANCE M L, PETERS W. Biochemical taxonomy of *Leishmania* II. *Ann Trop Med Parasitol* 1974; **68**: 317-325.
- 18 LAINSON R, SHAW J J. Leishmaniasis in Brazil V. *Trans R Soc Trop Med Hyg* 1970; **64**: 654-667.
- 19 GARNHAM P C C, LEWIS D J. Parasites of British Honduras with special references to leishmaniasis. *Trans R Soc Trop Med Hyg* 1959; **53**: 12-35.

- 20 NOVALES J. Cutaneous leishmaniasis in Mexico. *Int J Dermatol* 1974; **13**: 283-286.
- 21 LAINSON R, STRANGWAYS DIXON J. *Leishmania mexicana*: the epidemiology of dermal leishmaniasis in British Honduras. *Trans R Soc Trop Med Hyg* 1963; **57**: 242-265.
- 22 SHAW J J, LAINSON R. Leishmaniasis in Brazil II. *Trans R Soc Trop Med Hyg* 1968; **62**: 396-405.
- 23 LAINSON R, SHAW J J. Epidemiology and ecology of leishmaniasis in Latin America. *Nature* 1978; **273**: 595-600.
- 24 CONVIT J, KERDAL VEGAS F. Disseminated cutaneous leishmaniasis. *Archs Dermatol* 1965; **91**: 439-447.
- 25 BRAY R S. Epidemiology of leishmaniasis. Ciba Foundation Symposium No. 20 (new series) Trypanosomiasis and leishmaniasis pp 88-105. Amsterdam. North Holland Publishing Company 1974.
- 26 CONVIT J, PINARDI M E, RONDON A J. Diffuse cutaneous leishmaniasis: a disease due to an immunological defect of the host. *Trans R Soc Trop Med Hyg* 1972; **66**: 603-610.
- 27 CONVIT J, PINARDI M E. Cutaneous leishmaniasis: the clinical and immunopathological spectrum in South America. Ciba Foundation Symposium No 20 (new series) Trypanosomiasis and leishmaniasis pp 159-169. Amsterdam. North Holland Publishing Company 1974.
- 28 MAUEL J, BEHIN R. Cell mediated and humoral immunity to protozoal infections. *Transplant Rev* 1974; **19**: 121-146.
- 29 PIFANIA F. La evaluacion de la leishmaniasis tegumentaria americana en el Valle de Aroa, Estado Yaracuy, Venezuela. *Arch Venezol Med Trop Parasitol Med* 1962; **4**: 25-36.
- 30 PELLEGRINO J, PEREIRA L H, FURTADO T A. Mucocutaneous leishmaniasis, intradermal test with a promastigote suspension and a crude extract from *Leishmania braziliensis*. *Rev Inst Trop Sao Paulo* 1977; **19**: 393-395.
- 31 BRAY R S, LAINSON R. The immunology and serology of leishmaniasis I; the fluorescent antibody staining technique. *Trans R Soc Trop Med Hyg* 1965; **59**: 535-544.
- 32 GUIMARAES M C S, GIOVANNINI V L, CAMARGO M E. Antigenic standardisation for mucocutaneous leishmaniasis immunofluorescence test. *Rev Inst Med Trop Sao Paulo* 1974; **16**: 145-148.
- 33 MILES M A *et al*. Leishmaniasis in Brazil XV. Biochemical distinction of *Leishmania mexicana amazonensis* L *braziliensis braziliensis* and *L braziliensis guyanensis*. *Trans R Soc Trop Med Hyg* 1981; **75**(4): 524-9.
- 34 ROFFI J *et al*. Detection of circulating antibodies in cutaneous leishmaniasis by enzyme-linked immunosorbent assay (ELISA). *Am J Trop Med Hyg* 1980; **29**(2): 183-9.
- 35 STEMMER K L. Pharmacology and toxicology of heavy metals: antimony. Pharmacology and Therapeutics Series A 1976; **1**: 157-160.
- 36 HARVEY S C. Heavy metals; antimony. In the Pharmacological basis of Therapeutics. London: Macmillan Publishing Co Inc, 1975: 929-930.
- 37 DEMPSEY J J. Leishmaniasis in the Sudan Republic XXVI. Electrocardiographic findings in Sudanese Kala-azar. *East Afr Med J* 1965; **42**: 131.
- 38 SAPIRE D W, SULVERMAN N H. Myocardial involvement in antimony therapy. *South Afr Med J* 1970; **44**: 948-950.
- 39 COLTEN M de V, LOGAN M. Effects of antimony on the cardiovascular system and intestinal smooth muscle. *J Pharmac exp. Ther.* 1966; **151**: 7-22.
- 40 NIEMEL M P L A. Traitement de la leishmaniasis braziliensis cutanée par la lomidine (pentamidine). *Bordeaux medical* 1977; **10**/11: 701-703.
- 41 BRYCESON A D M. Diffuse cutaneous leishmaniasis in Ethiopia II — treatment. *Trans R Soc Trop Med Hyg* 1970; **64**: 369-379.
- 42 MARSDEN P D *et al*. Mucocutaneous leishmaniasis — an unsolved clinical problem. *Tropical Doctor* 1977; **7**: 7-11.
- 43 SELIM M M, KANDIL E. Rifampicin in the treatment of cutaneous leishmaniasis. *J. Kuwait Med Assoc* 1972; **6**: 159-166.
- 44 DOURADO H V *et al*. Leishmanione tegumentar americana: tratments com rifampicina. *Rev Bras Clin Ter (Brazil)* 1975; **4**: 1-6.
- 45 WHITE S W, HENDRICKS L D, CHULAY J D. Leishmaniasis: a case history and treatment failure with rifampicin. *Arch Dermatol* 1980; **116**(6): 620-1.
- 46 URCUYO, F G, ZAIAS, N. Oral ketoconazole in the treatment of leishmaniasis. *Int J Dermatol* 1982; **21**(7): 414-6.
- 47 KANDIL, E. Treatment of cutaneous leishmaniasis with trimethoprim-sulfamethoxazole combination. *Dermatologica* 1973; **146**: 303-309.
- 48 BELTRAN, H F, GUTIERREZ, M, BIAGGI, F F. Utilité du metronidazole dans le traitement de la leishmaniose cutanée Mexicaine. *Bull Soc Pathol Escot* 1967; **60**: 61-64.
- 49 SMIT, A M, VAN DER KAAG, H J. Cutaneous leishmaniasis. *Dermatologica* 1977; **154**: 56-57.
- 50 BRYCE, C *et al*. American cutaneous leishmaniasis: inefficacy of metronidazole in treatment. *J.A.M.A.* 1974; **228**: 1256-1258.
- 51 BRYCESON, A D M. Cutaneous leishmaniasis. *Brit Derm* 1976; **94**: 223-226.
- 52 MUTINGA, M J, MNGOLA, E N. Alternate treatment of cutaneous leishmaniasis. *East Afr Med J* 1978; **51**: 68-78.
- 53 DORMAN, G D. Treatment of cutaneous leishmaniasis by freezing. *J Am Med Assoc* 1974; **227**: 658-659.
- 54 BASSIOUNY, A *et al*. Cryosurgery in cutaneous leishmaniasis. *Brit J Dermatol* 1982; **107**(4): 467-74.
- 55 SAGHER, F. Cutaneous Leishmaniasis. In Clinical Tropical Dermatology. London. Blackwell Scientific Publications 1975: 186-191.
- 56 BERBERIAN, D A. Vaccination and immunity against oriental sore. *Trans R Soc Trop Med Hyg* 1939; **33**: 87-94.
- 57 GUNDERS, A E, NAGAN, L, MICHAELI, D. Follow up study of a vaccination programme against leishmaniasis I and II. *Trans R Soc Trop Med Hyg* 1972; **66**: 234.
- 58 KATZENNELLENBOGEN, I, LEVY, I. Prevention of leishmaniasis in a Kibbutz by inoculation. *Harefuah* 1968; **75**: 177.
- 59 SENEKJI, H A, BEATTIE, C P. Artificial infection and immunisation of man with cultures of *L tropica*. *Trans R Soc Trop Med Hyg* 1941; **34**: 415.
- 60 KOUFMAN, Z *et al*. Observation on immunisation against cutaneous leishmaniasis in Israel. *Israel J Med Sci* 1978; **14**: 218-222.
- 61 LAINSON, R, SHAW, J J. Studies on the immunology and serology of leishmaniasis III. *Trans R Soc Trop Med Hyg* 1966; **60**: 533-535.
- 62 LAINSON, R, SHAW, J J. Leishmaniasis in Brazil XII. *J Trop Med Hyg* 1977; **80**: 29-35.