The American leishmaniases: some observations on their ecology and epidemiology

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Summary
As the first species of Leishmania encountered were the agents of human visceral and cutaneous leishmaniasis, it is understandable that studies on these parasites for a long time concentrated on those organisms commonly causing disease in man. Epidemiological studies over the past 20 years or so, however, have led to the inescapable conclusion that the genus Leishmania is comprised of numerous species of well adapted parasites, in a wide range of mammals, throughout most of those tropical and subtropical regions of the world where phlebotomine sandflies exist (Diptera: Psychodidae). Many of the leishmanias probably never gain entrance into man: due either to an incapacity to survive in his tissues, or (more likely) because the natural sandfly vectors do not feed on him. The leishmanias that do infect man are, nevertheless, among the greatest protozoological scourges of mankind, and a better understanding of their life-cycles may well help in future prevention or control of the diseases they cause. With few exceptions the leishmanias are zoonoses, with a major source of infection in wild or domestic animals. In the Americas, the disease is essentially a rural one, and most commonly acquired by those penetrating forested or wooded regions. The following paper deals with the better known human leishmaniasis of the New World, and some new ones, and discusses the major historical events in the laborious task of elucidating their ecology and epidemiology.

Introduction
Although some authorities have suggested that visceral leishmaniasis may have been introduced into the Americas in post-Columbian times, there remains little doubt regarding the indigenous nature of the different forms of American cutaneous leishmaniasis (ACL). Ancient Peruvian and Ecuador pottery from the period 400-900 A.D. shows human faces with mutilations extraordinarily similar to those caused by present-day cutaneous and mucocutaneous leishmaniasis. The alternative view (SALAMAN, 1949) that they depicted sacrificial victims of a 'potato cult', who had their noses cut off to represent the potato 'eyes', seems much less likely. Some of the ceramics show perfect reproductions of the characteristic, septumless and collapsed nose ('tapir nose') so well known in the clinical records of mucocutaneous leishmaniasis, and early Spanish historians, at the time of the 'conquistadores', had written of prominent ulcers seen on the faces of Peruvian indians. Finally, the discovery that such primitive South American mammals as sloths, anteaters and armadillos are the natural hosts of a number of different leishmanias has removed any reasonable doubt as to the antiquity of South American leishmaniasis.

The first clinical recognition of the disease was probably in 1859 (MATTA, 1918) when a Dr. L. Villar wrote that "... the disease (Peruvian "uta") is very like the Aleppo button .." (i.e. "oriental sore" due to Leishmania tropica), but a link between ACL and the phlebotomine sandfly vectors goes back as far as 1764, when there was a written account implicating sand-flies in the transmission of "uta" (Leishmania braziliensis peroviana) by a gentleman named Cosme Bueno (see HERRER & CHRISTENSEN, 1975).

Proof of the nature of the aetiological agents of ACL was not forthcoming, however, until 1909, when LINDENBERG and CARINI & PARANHOS independently demonstrated amastigotes of Leishmania in human skin lesions in Brazil; and VIANNA (1911) later referred to the parasite as L. braziliensis.

After this date, studies on the leishmaniasises in the Americas were for a long time limited to case-reports of the cutaneous and mucocutaneous disease, principally by physicians who were largely interested in the clinical aspects. From these reports, however, the fact did emerge that ACL extended from Mexico in the north down to Argentina in the south: furthermore, it became obvious that the various forms of the disease were mostly associated with the forested regions and that they almost certainly represented zoonoses.

From the early 1950s onwards, there began a search for the reservoirs and vectors of the different neotropical leishmanias. Much has subsequently been writ-
ten on this subject and it would be foolish to attempt to review all of this literature here. Rather, I will concentrate on the major historical events in this long and arduous task which were to most profoundly influence our progress and thinking with regards the American leishmaniases. Particular attention has been paid to the Amazon basin, not only because this is where the author has had most experience, but because it is here that we can still see what are presumably the ancient sandfly/mammalian-host cycles which have remained relatively uncomplicated and unchanged by man's intrusion into these great forests.

More detailed discussions on the epidemiology of the New World and Old World leishmaniases have already dealt with geographical distribution, mode of spread, control and treatment, and with the taxonomy of the causative parasites (LAINSON & SHAW, 1979; LAINSON, 1982b, 1983a and 1983b, in press).

Subspecies of L. mexicana

Subspecies of L. mexicana are widespread among a variety of wild rodents in the neotropics, and may less frequently be found in some other animals sharing the same habitat, such as marsupials and foxes.

In this respect these parasites seem to form the counterpart of Leishmania major of the Old World (probably also comprised of a number of closely related parasites) which is, too, predominantly found in rodents. L. mexicana and L. major share other features: development in their respective sandfly vectors is of the 'supravyarian' type (LAINSON & SHAW, 1979; KILLICK-KENDRICK, 1979), in that the flagellate stages are restricted to the midgut and foregut of the insect, and do not invade the hindgut; they are morphologically similar in their possession of large amastigotes and promastigotes; both are relatively easy to maintain in simple blood-agar media, and both tend to produce large, histiocytoma-like lesions, containing very abundant amastigotes, in the skin of laboratory hamsters and mice.

Differential Characters: L. mexicana or L. braziliensis?

This is, of course, the main differential diagnosis which concerns those working in the Americas. It is not just an academic exercise for the protozoologist, for the isolation and identification of species and subspecies of the parasite from man has assumed much importance to the physician, due to our increasing awareness of a multiplicity of leishmaniases causing human disease. It is no longer sufficient to simply say that a patient has 'cutaneous leishmaniasis', for prognosis greatly depends on which organism is involved. Thus, in many parts of Brazil mucocutaneous leishmaniasis is a common sequel to infection with L. braziliensis braziliensis, sometimes several years after the primary lesion has healed, whereas L. b. guyanensis ('pian-bois') is not known to be associated with spread to the naso-pharyngeal mucosae. Although skin lesions due to L. mexicana amazonensis are relatively mild, and unassociated with metastases to the naso-pharyngeal tissue in immunologically competent patients, infection may frequently progress to incurable diffuse cutaneous leishmaniasis in persons with a defective cell-mediated immune system. While it will be beyond the scope of most clinicians to bring diagnosis down to the specific or subspecific nature of the Leishmania involved, attempts should at least be made to have the organism isolated and passed on to experts for more accurate identification.

Current methods and criteria used in the identification of subspecies of L. mexicana and L. braziliensis have been discussed at length elsewhere (CHANCE, 1979; LAINSON & SHAW, 1979; MILES et al., 1980, 1981; McMAHON-PRATT & DAVID, 1981; McMAHON-PRATT et al., 1982; LAINSON 1982a, 1982b; JACKSON et al., 1982; BARKER & BUTCHER, 1983) and will not be repeated here.

1. Leishmania mexicana mexicana: 'Chiclero's Ulcer' of Central America

In the north of Central America, in the Yucatan Peninsula, Belize and Guatemala, there is a form of cutaneous leishmaniasis long known as 'chiclero's ulcer' (Fig. 2). The chicleros, as their name implies, are the men who collect the 'chicle' or chewing-gum latex from the sapodilla trees (Achras sapota L.), and for this purpose they are accustomed to spend up to six months of the year living in the forest. As this period coincides with the rainy season and, therefore, with the maximum sandfly density, it is not surprising that there has always been an extremely high infection-rate among the chicleros. BIAGI (1953) studied the epidemiology of chiclero's ulcer in the Yucatan, and felt that the clinical aspects of the disease suggested the causative agent to be more closely related to Leishmania tropica, of the Old World, than to L. braziliensis. Accordingly, he gave the name of L. tropica mexicana to the parasite: this was later amended to Leishmania mexicana by GARNHAM (1962), while LAINSON & SHAW (1972) referred to the organism as L. mexicana mexicana, following their use of a new subspecific name, L. mexicana amazonensis for a closely related parasite discovered in man in Brazil (see below).

The first evidence that silvatic animals acted as reservoirs of any form of ACL was obtained by Hertig and colleagues (ANON., 1957, 1959), in Panama. They isolated a Leishmania after culturing heart-blood from the forest rodents Proechimys semispinosus and Haplomys gymnurus. Later workers, in Brazil, found amastigotes in skin lesions of the rodents Dasyprocta azarae and Kannabateomys amblyonyx, and obtained promastigotes in cultures made from the heart-blood of another forest rodent, Agouti paca (FORATTINI, 1960). Although the identity of these Panamanian and Brazilian parasites remained doubtful, their discovery certainly did much to arouse the interest of other workers regarding the role of wild animals in the epidemiology of New World leishmaniasis.

With this information to hand, Lainson & Strangways-Dixon started studies on chiclero's ulcer in the forests of Belize (then British Honduras). They found the reservoir of infection among forest rodents; namely the tree-rat Ototylomys phyllotis (Fig. 1), the spiny pocket-mouse Heteromys desmarestianus and the vespert-rat Nyctomys sumichrasti, with infection-rates of 40 0%, 10 3% and 12 5% respectively (LAINSON & STRANGWAYS-DIXON, 1962, 1964). The parasite was found to be restricted to the skin, and associated with small, inconsiderable lesions located principally on the base of the tail. This time the organism was isolated in both hamsters and blood-agar culture: it was shown to
Fig. 1. The cricetid rodent Otomys phyllois, a major host of Leishmania mexicana mexicana. (From LAINSON & STRANGWAYS-DIXON, 1964).

Fig. 2. 'Chiclero's ulcer', due to L. m. mexicana: almost total destruction of the external ear. (From LAINSON & STRANGWAYS-DIXON, 1963).

Fig. 3. The echimyid rodent Proechimys sp., major host of Leishmania mexicana amazonensis.

Fig. 4. Incubable, diffuse cutaneous leishmaniasis caused by L. m. amazonensis. (From LAINSON & SHAW, 1973).

Fig. 5. 'El Bosque de Macuto', an isolated pocket of riverine forest on the outskirts of the town of Barquisimeto, Lara State, Venezuela: type-locality of Leishmania mexicana venezuelensis.

Fig. 6. Pylorus of the sandfly Lutzomyia umbratilis, naturally infected with Leishmania braziliensis guyanensis: typical growth of rounded promastigotes and paramastigotes attached to the cuticular wall. (From LAINSON et al., 1977).
Fig. 7. "Tapir-nose": mucocutaneous leishmaniasis due to *Leishmania braziliensis* (sensu lato). Pará State, Brazil.

Fig. 8. Além Paraiba, Minas Gerais State, Brazil: type-locality of *L. b. braziliensis*: extensive de-forestation for farmland, but still with isolated pockets of primary and secondary forest (1983).

Fig. 9. Some remaining primary forest Além Paraiba: arrowed person gives indication of the height of the trees.

Fig. 10. An "Agro-Vila" (agricultural settlement) in the midst of Amazonian forest along the TransAmazon Highway, Pará, Brazil. The colonies are in constant contact with the high, primary forest.

Fig. 11. The sloth, *Choloepus didactylus*, a major reservoir-host of *Leishmania braziliensis guyanensis* in the Guyanas, and Brazil north of the Amazon River.

Fig. 12. Multiple ulcers on the arm of a forest-worker, due to *L. b. guyanensis* ('pian-bois').
produce typical infections in volunteers, and one isolate, from *N. sumichrasti* (M379) still remains in use as the 'standard' of *L. m. mexicana* for biochemical characterization and other comparative studies.

Lainson & Strangways-Dixon (1964) concluded that transmission among the animals, and to man, was by the bite of sandflies at ground level, and they isolated *L. m. mexicana* in a hamster which had been inoculated intradermally with a saline triturate of sandflies caught off human bait. Finally (Strangways-Dixon & Lainson, 1962, 1966) the parasite was transmitted from hamster to man by the bite of an experimentally infected sandfly identified as *Psychodopygus pessosanus* (Baretto). Although this gave no good indication of the natural vector, the achievement was of historical note as the first experimental transmission of a neotropical *Leishmania* by the bite of a phlebotomine sandfly, and it removed what little doubt that may have existed regarding the role of this insect in the epidemiology of leishmaniasis in the New World.

During subsequent work in Belize, between 1964 and 1966, Disney (1966) perfected a simple but remarkably efficient trap to catch sandflies attracted to the rodent reservoirs. With this he showed that the species most commonly coming to these animals was *Lutzomyia olmeca olmeca* (Vargas & Díaz Nájera), and this vitally important observation led directly to the incrimination of this sandfly as the vector of *L. m. mexicana*. In October, 1965, Disney (1968) found his first naturally infected *Lu. olmeca* and, armed with this knowledge, Biagi et al. (1965) confirmed the role of this insect as the vector in neighbouring Yucatan, in December of the same year.

2. *Leishmania mexicana amazonensis*: Amazonian cutaneous and diffuse cutaneous leishmaniasis

With the role of rodents firmly established as the reservoirs of *L. m. mexicana* in Central America, it was logical that they should be high on the list of suspects in the epidemiology of cutaneous and mucocutaneous leishmaniasis in South America, especially as the presence of *Leishmania* had already been signalled in a few of these animals in Panama and South Brazil.

The author discussed this possibility with virologist Dr. Otis Causey, during a visit to his laboratory at the Instituto Evandro Chagas, Belém, Pará, north Brazil, in 1963. Causey had noted lesions on the tails of some of the rodents he had examined for arboviruses, but had dismissed them as due to simple injuries or bacterial infections. A subsequent examination of Giemsa-stained smears of exudate from a number of such lesions of the 'rice-rat', *Oryzomys capito*, however, soon revealed their true nature: they were teeming with amastigotes of *Leishmania*. At first it was thought that Causey had uncovered a vast reservoir of *L. braziliensis* and the parasite was, in fact, referred to by this name (Guimarães & Costa, 1966). A lengthy study of the organism, however, made it quite clear that this was not so.

In 1965 the Wellcome Parasitology Unit was established at the Instituto Evandro Chagas, specifically to study the epidemiology of leishmaniasis in the Amazon Region of Brazil. Work started with the comparison of a large number of isolates of *Leishmania* from rodents and man obtained from the same forested regions, and a very significant fact emerged. In almost all cases the parasite from the wild animals (that found by Causey) was very different from the one most commonly recovered from man, principally in its behaviour in the sandfly, laboratory animals and *in vitro* culture. The organism, so commonly found in *Oryzomys* and other small forest mammals, proved to be so similar to *L. mexicana* of Central America that it was given the name of *L. mexicana amazonensis*. Lainson & Shaw, 1972: the causative agent of chilciero's ulcer thus became *L. m. mexicana*.

*L. m. amazonensis* was found to have a remarkably wide range of hosts among wild animals, and thirteen different species have been found infected to date, including rodents, marsupials and foxes. The primary host was shown to be the echimyid rodent, *Proechimys guyannensis* (Fig. 3), and not the cricetid *Oryzomys* as originally thought (Lainson & Shaw, 1973). Infection is usually inapparent, with the parasites restricted to the skin where they seem to cause little or no ill-effect.

Lainson & Shaw (1968) used Disney-traps baited with *Proechimys* to pinpoint the vector of *L. m. amazonensis*. It proved to be *Lutzomyia flaviscutellata*, a rodent-loving species which is, significantly, closely related to *Lu. olmeca olmeca*, the vector of *L. m. mexicana*. It also proved the first time that the female of this species had been identified, the insect previously having been described only on the male forms (Mangabeira, 1942)—another indication of the great value of Disney’s trap. During this and later work, a total of 45 heavily infected *Lu. flaviscutellata* was recorded among 7,498 females dissected (0·6%). There was a total absence of infection with *L. m. amazonensis* in any other species of sandfly captured in the same areas of forest (Lainson & Shaw, 1979). Finally, the parasite was transmitted from hamster to hamster on four occasions by laboratory-bred specimens of *Lu. flaviscutellata* (Ward et al., 1977), and there remains no doubt in our minds that this fly is the major vector, and probably the only one, of *L. m. amazonensis*.

*Proechimys* is an extremely common animal in South American forests and the infection-rate with *L. m. amazonensis* is frequently in the region of 25·0% or more. It came as a surprise, therefore, to find that human infection with this parasite was rare. This fact was explained by later studies which showed *Lu. flaviscutellata* to be a nocturnal feeder, not much attracted to man, and most abundant in low, wet forests which are little frequented by man (Shaw & Lainson, 1968). Up to the time of writing only about 3·0% of the many hundreds of cases of cutaneous leishmaniasis which have passed through our hands in Belém, during the past 18 years, have been due to *L. m. amazonensis*: there remains the sombre fact, however, that 6 of these (30·0%) have progressed to incurable diffuse cutaneous leishmaniasis (Fig. 4)—an indication that although South American DCL is undoubtedly linked with a deficient cell-mediated immunity in the patient, it so far seems to be associated only with parasites of the *L. mexicana* complex, and we have never recorded this condition in the many hundreds of patients with *L. braziliensis* that we have examined.

The principal mammalian hosts of *Lu. flaviscutellata* are terrestrial rodents, and this insect is essential-
ly low-flying and unassociated with the forest canopy. Although found in primary forest, it is most abundant in low, secondary growth resulting from deforestation, and can often be found in isolated pockets of vegetation which no longer merit the description of forest, but which most closely approximate the English “copse” (i.e., ‘capoeira’, in Portuguese). Recent studies (Ready et al., 1983) have shown *Lu. flaviscutellata* to have even adapted remarkably well to the vast plantations of non-indigenous trees (pine and gmelina) that have been established in north Brazil, after the complete removal of the native forest habitat. Such artificial forests now support large populations of *Proechimys, Oryzomys* and other small terrestrial mammals, and animals infected with *L. m. amazonensis* have been found deep within such plantations. On the other hand, other sandflies, such as *Lu. umbratilis* Ward & Fraiha, vector of another form of cutaneous leishmaniasis of man in the same region, have not been able to adapt to these new ‘forests’ (see under ‘pian-bois’, below).

3. *Leishmania mexicana pifanoi*: Venezuelan diffuse cutaneous leishmaniasis

**Medina & Romero** (1959) described a bizarre form of cutaneous leishmaniasis in Venezuela, in which large histiocytoma-like nodules were scattered over the patient’s body. They were found to contain very abundant amastigotes, and the condition would not respond to treatment with drugs which were effective against other clinical forms of leishmaniasis. Principally for this reason the parasite received the new name of *L. brasiliensis pifanoi*, and has later been referred to simply as *L. pifanoi*. As discussed above, we now know American DCL to be the relatively rare result of an unhappy coincidence of infection with *L. mexicana* or related parasites (see also, subsequent pages) in persons with a defective cell-mediated immunity. A similar condition may be caused by the parasite referred to as *L. aethiopica* Bray, Ashford & Bray in Ethiopia (Bryceson, 1969). Both the African and the American forms of the disease remain seemingly incurable by present methods of treatment, although the infection can be subdued quite efficiently by a combined drug and heat treatment, which gives the patient a somewhat less unpleasant appearance (Lainson, 1982b).

The epidemiology of DCL due to *L. m. pifanoi* remains obscure. The human infections have been recorded from the States of Yaracuy, Lara and Miranda, and the inoculation of isolates from these patients into hamsters has consistently produced infections of the classical *mexicana* type, as described by Lainson & Shaw (1979), with the production of large metastatic lesions of the animal's extremities (Medina & Romero, 1959). One parasite sharing similar characteristics was isolated from the forest rodent *Heteromys anomalus*, captured in the same general region of Venezuela (Carabobo State) by Torrealba et al. (1972), and this may possibly have been *L. m. pifanoi*. Another, from the sandfly *Lu. flaviscutellata*, however, came from primary forest in the Sierra Parima region of Amazonas State, Venezuela (Pifano et al., 1973). This region is geographically very distant from the type locality of *L. m. pifanoi*, and approximating that of *L. m. amazonensis* in the Amazon basin of Brazil. As discussed above, *Lu. flaviscutellata* is the proven vector of *L. m. amazonensis* and it is likely that the isolate of Pifano et al., was this parasite, too. Cutaneous lesions containing amastigotes have been recorded from the rodents *Proechimys* sp. (Convit, 1968) and *Zygodontomys microtus* (Kerdal-Vegas & Essendfield-Yahr, 1966), but it would seem that these parasites were not isolated and their true nature remains even more doubtful than the ones already discussed.

As far as the author is aware, parasites identified as *L. m. pifanoi* have still to be recovered from patients showing simple uncomplicated skin lesions, as opposed to DCL. As in the case of *L. m. amazonensis*, however, the human infection is uncommon and such infections may easily go unnoticed or dismissed as being due to *L. braziliensis*.

**Scorza & Delgado** (1982) compared isolates of *L. m. pifanoi*, obtained from four cases of DCL in the States of Aragua, Anzoátegui and Sucre, Venezuela, with a parasite they referred to as ‘*L. mexicana mexicana*’ from the rodent *Oryzomys capito* in Panama (Herrer et al., 1971). They drew attention to the failure of Gardener et al. (1974) to differentiate the Panamanian parasite from one of the *L. m. pifanoi* stocks by enzyme electrophoretic mobility patterns, using the single enzyme MDH (malate dehydrogenase), and to similarities they themselves had noted in the morphology of amastigotes of the two organisms and the behaviour of the two leishmaniasis in hamsters and sandflies. From these observations, they concluded that the name *L. m. pifanoi* seems not to be tenable and that the isolates from the Venezuelan DCL patients ‘may belong instead to *L. mexicana amazonensis*’.

I feel that these conclusions are somewhat premature, for the following reasons. In the first place, the use of a single enzyme is no longer considered adequate procedure for the identification of the leishmanias on enzyme profiles, and Miles et al. (1980) were able to differentiate one stock of *L. m. pifanoi* from other subspecies of *L. mexicana* using up to 18 different enzymes. Secondly, all the presently recognized subspecies of this parasite are so similar in their morphology and behaviour in hamsters and sandflies, that these characters are of little use in distinguishing them, and recoupe must be made to biochemical or serological methods. Thirdly, the subspecies of *L. mexicana* in Panama is neither *L. m. mexicana* nor *L. m. amazonensis*: it is clearly differentiated from these parasites by a different kinetoplast DNA buoyant density (stock LV41, in Chance et al. (1974) and was named *L. mexicana aristides* by Lainson & Shaw (1979). Finally, if any foundation is found for the suggestion of synonymy, the name *L. m. pifanoi* Medina & Romero, 1959 would have priority over *L. m. amazonensis* Lainson & Shaw, 1972.

4. *L. mexicana garnhami*: Venezuelan Andean cutaneous leishmaniasis

Scorza et al. (1979) gave the name of *Leishmania garnhami* to a parasite associated with cases of cutaneous leishmaniasis in the Venezuelan Andes, based principally on the presence of a ‘unique’ refractile granule seen in the cytoplasm of the amastigote. A very similar structure has, however, already been recorded in a stock of *L. braziliensis*...
was the "main anthropophilic sandfly" found in the sandfly. This is so in the case of Lu. o. olmeca (L. m. endemic area. It has frequently been shown, however, intensified.

found in wild animal hosts, and the search should be has yet been recorded, but it should be remembered mexicana complex, one would expect to find a local representative of this group of sandflies there. None the Venezuelan Andes are due to a parasite of the mexicana complex (LAINSON & SHAW, 1979), with massive multiplication restricted to the abdominal and thoracic midgut and the attachment of vast numbers of flagellates at the stomodaeal valve as early as 4-5 days after the infective meal. On the other hand, MORENO & SCORZA (1981) claimed a braziliensis-like development of L. garnhami in the hindgut of wild-caught specimens of the sandfly Lu. townerendi (the suspected vector) which had been fed on hamsters infected with a number of isolates referred to as L. garnhami. In view of these observations that "L. garnhami can not be allocated within mexicana or braziliensis complexes'. It may be that L. garnhami is indeed the first leishmanial parasite shown to share those characters attributed to the peripylarian and suprapylarian leishmaniasis, as defined by LAINSON & SHAW (1979). These conflicting results also lead one to suspect, however, the existence of two different parasites causing cutaneous leishmaniasis in the Venezuelan Andes, and that observations on one of them may have been intercalated with observations on the other. In this respect, it may be noted that MORENO & SCORZA (1981) mention that the growth of L. garnhami in NNN medium was inconsistent, with two strains growing well (like L. mexicana) and five others badly (like some subspecies of L. braziliensis).

All the proven vectors of the subspecies of L. mexicana are members of the Lu. flaviscutellata group of sandflies, and if some of the human infections in the Venezuelan Andes are due to a parasite of the mexicana complex, one would expect to find a local representative of this group of sandflies there. None has yet been recorded, but it should be remembered that Lu. flaviscutellata and other related sandflies may often be difficult to locate for much of the year, even when parasites of the mexicana complex can readily be found in wild animal hosts, and the search should be intensified.

Lu. townerendi (Ortiz) was regarded by SCORZA et al. (1979) as the probable vector of L. garnhami because it was the "main anthropophilic sandfly" found in the endemic area. It has frequently been shown, however, that the vector of the local form of leishmaniasis in a given area is not always the most anthropophilic sandfly. This is so in the case of Lu. o. olmeca (L. m. mexicana), Lu. flaviscutellata (L. m. amazonensis) and Lu. umbratilis (L. braziliensis guyanensis), none of which can be described as highly anthropophilic compared with other sandfly species in the same localities. Further studies seem indicated to locate a significant number of infected Lu. townerendi in the endemic areas, to compare any parasites found in this species with isolates from man, and finally to attempt transmission experiments with laboratory-bred sandflies: biochemical methods should be applied in the comparison of all parasites involved.

The natural mammalian hosts of L. garnhami have yet to be indicated, although a similar parasite has been found in a single opossum (Didelphis marsupialis) (Prof. J. V. SCORZA, personal communication).

From the evidence discussed, the writer prefers to use the name L. mexicana garnhami for the time being.

5. L. mexicana venezuelensis

BONFAnte-GARRido (1980, 1983) isolated a parasite from a patient who acquired cutaneous leishmaniasis in a small, isolated pocket of forest ('el bosque de Macuto') on the margin of the River Turbio, State of Lara, Venezuela (Fig. 5). It has been differentiated from other subspecies of L. mexicana on enzyme profiles for ALAT, PGi, GPI, G6PD, MDH and MPI (MILES, 1983; BONFAnte-GARRido, 1983) and on monoclonal antibodies (DR. J. J. Shaw, personal communication). In hamster skin the parasite quickly produces huge histiocytoïd-like lesions containing very abundant, large amastigotes: its development in the sandfly Lu. longipalpis, in the writer's laboratory, was found to be typical of that seen in members of the L. mexicana complex. Interestingly, L. m. venezuelensis can also be differentiated from other known subspecies of L. mexicana by the difficulty with which it is maintained in blood-agar (Difco) medium (as defined by WALTON et al., 1977): it grows very well initially, but dies out on sub-culture.

Dr. Bonfante-Garrido (personal communication) has now recorded some 20 or more cases of infection with L. m. venezuelensis in the same locality, with lesions ranging from a single focus to a multiple ulceration of a nodular and disseminated nature: as far as I am aware, all have responded well to antimonial treatment.

The writer examined the area of forest in question, during a recent visit to Venezuela, and was impressed by its close similarity to that associated with most cases of cutaneous leishmaniasis due to L. m. amazonensis in Brazil, discussed above: namely, a low, wet riverine vegetation. The frequency of human infection with L. m. venezuelensis is striking, compared with that of L. m. amazonensis, however, and this suggests that the vector is much more anthropophilic than Lu. flaviscutellata. In this respect it is of interest that Disney-traps baited with rodents in the Macuto forest have made abundant catches of what appears to be a new subspecies of Lu. olmeca (DRS. C. Arredondo & Bonfante-Garrido, personal communication). Subspecies of Lu. olmeca are known to be more attracted to man than is Lu. flaviscutellata (BIAGi et al., 1965; Young & Arias, 1982).

6. Leishmania mexicana aristedesi: another possible parasite of man?

Workers in Panama (HERRER et al., 1971) found
what is clearly a subspecies of L. mexicana in rodents and an opossum: it was named L. m. aristedesi Lainson & Shaw, 1979, and is biochemically distinct (Chance, 1979). The vector is unknown, but Lu. o. bicolor Fairchild & Theodor was shown to be much attracted to rodents and is highly suspected. To date L. m. aristedesi has not been found in man but, in the light of what we know of the other, closely related leishmanias of the mexicana complex, it is probably only a question of time before human infection is diagnosed.

7. Leishmania mexicana enrietti: leishmaniasis of guinea-pigs.

Strictly speaking this parasite is even more out of place in the present discussion than L. m. aristedesi, as attempts to infect man experimentally with the organism have failed and it would seem unlikely to be a natural cause of human leishmaniasis. The parasite has achieved so much fame as a laboratory model for immunological studies, however, that it seems appropriate briefly to discuss its history.

It was discovered (Medina, 1946) in nodular skin lesions found in laboratory guinea-pigs in Curitiba, Paraná State, Brazil, and attempts to infect experimentally mice, dogs, monkeys, man and (surprisingly) wild guinea-pigs (Cavia aperea) all failed (Muniz & Medina, 1948). New isolates of the organism from further guinea-pigs were made by Luz et al. (1967). Muniz & Medina (1948) gave the parasite its name, and Lainson & Shaw (1972) included it in the mexicana complex by virtue of its large amastigotes and ease of culture in simple NNN medium. Subsequent studies on the parasite’s behaviour in sandflies (Lainson et al., 1977) and its biochemical studies (Chance, 1979) supported this decision: the organism is biochemically so close to L. mexicana, in fact, that Chance felt it was best regarded as a subspecies of this parasite, and the present author has tended to agree (Lainson, 1982a).

The wild animal host, and the manner by which the parasite gained entry into domestic guinea-pigs are not known. Luz et al. (1967) suspected a local sandfly of the Paraná pine forests as the vector (Lu. monticola Costa Lima), as heavy experimental infections were produced in this fly.

8. Cutaneous and Diffuse Cutaneous Leishmaniasis in Northern Mexico and Southern USA: L. m. mexicana?

The northern limit of chilchero’s ulcer was at one time thought to be the forested region of the Yucatan Peninsula in Mexico, and it came as somewhat of a surprise, therefore, when Stewart & Pilcher (1945) recorded cutaneous leishmaniasis in a boy from a ranch near Alice, Texas, who was said to have never travelled more than 60 miles from there. None of his family had visited neighbouring Mexico. Little attention was paid to this report at the time, and doubt was cast on the autochthonous nature of this case.

In 1968, however, Simpson et al. (1968) reported a case of DCL in a 64-year-old woman who had lived in San Benito, Texas, all her life, and whose visits to Mexico had been limited to the border States of Tamaulipas and Nuevo Leon; she had travelled to no other countries. This case was particularly interesting in that DCL had, up to now, not been recorded in infections with L. m. mexicana.

Shaw et al. (1976) described two further cases of apparently autochthonous cutaneous leishmaniasis from Texas. The first was a 74-year-old school teacher who developed three lesions on her face: she lived in Dilworth, an almost deserted village in east Gonzales county, where she owned a 500 acre cattle ranch, and was accustomed to take walks in the early morning in a small forested area bordering a stream that ran through her land. Small mammals (rodents and opossums) were said to be frequently seen on her property. The second patient, a man of 56 years, lived in Kenedy, Karnes county, and he developed a lesion of the nasal mucosa some time after receiving radiotherapy, ten years previously, for carcinoma of the tongue.

Hamsters inoculated with biopsy material from the first case developed skin lesions containing amastigotes, but the exact nature of the Leishmania remained undetermined. A Leishmania was isolated in hamsters and culture from the second case: its behaviour in the hamster was consistent with that of L. mexicana, and subsequent biochemical study of the parasite (DNA buoyant densities and enzyme profiles for malate dehydrogenase) failed to distinguish it from L. mexicana (quoted as a personal communication from Prof. W. Peters, Liverpool School of Tropical Medicine).

Ramos-Aguierre (1965) demonstrated amastigotes in an ulcer on the cheek of a 6-year-old girl from the Mineral La Llave de Aurora region, Coahuila State, north Mexico, and in a later publication (Ramos-Aguierre, 1970) described two cases of diffuse cutaneous leishmaniasis in two young men from Múzquiz and La Cuchilla, Coahuila State. No information is to hand regarding the identification of the causative parasite, but Dias-Najera (1971) records the presence of Lu. diabolicus (Hall) in the Múzquiz area. This sandfly is known to readily feed on man, and is the only anthropophilic species so far found in Texas.

Young (1972) lists six species of sandflies from southern Texas. Lu. anthophora (Addis), which was caught feeding on domestic rabbits and is known to be associated with nests of the rodent (woodrat) Neotoma micropus; Lu. texana (Dampf) known to be associated with armadillo burrows, although it is not certain if it feeds on these animals; Lu. cruciata (Coquillet) (Syn. Lu. diabolicus (Hall)); Lu. californica (Fairchild & Hertig); Lu. oppidana (Dampf) and Lu. vexator (Coquillet). Of these, both Lu. cruciata and Lu. anthophora have been successfully infected with L. mexicana (Dr. David G. Young, personal communication): finally, Dr. Young informs me that he considers Lu. diabolicus (Hall) to be distinguishable from Lu. cruciata (Coquillet).

Clearly epidemiological observations are needed in greater depth in the various areas where these cases of cutaneous leishmaniasis have been recorded in north Mexico and Texas. The identification of one isolate as L. mexicana mexicana is perhaps not very conclusive as it was based on enzyme profiles for only one enzyme (MDH). A sandfly of the Lu. flaviscutellata complex has not yet been recorded in the case areas, but the ecological features described for one case (Shaw et al., 1976) are so reminiscent of those discussed above for the other known subspecies of L. mexicana, that one wonders if a search for such a sandfly in the wooded areas, near the stream, might
not reveal its presence. It is particularly significant, I feel, that 21 different rodent species in this area are listed by Shaw et al., in addition to the common opossum. Examination of these by culture and hamster inoculation, using methods currently employed for the isolation of *Leishmania* (LAINSON, 1982a), together with the use of rodent-baited Disney-traps for the capture of sandflies attracted to these animals, might provide important new information.

9. Enzootic Cutaneous Leishmaniasis of Rodents and Marsupials on the Island of Trinidad, West Indies.

An undoubted subspecies of *L. mexicana* was discovered in the skin of small mammals in the dwindling forest of Trinidad, W.I., by TIKASINGH (1969). He regarded the parasite as *L. m. amazonensis* (1974), but LAINSON & SHAW (1979) felt that as Trinidad is some 2,000 km from the type locality of this subspecies and only some 700 km from that of *L. m. pifanoi*, it was unwise to assign a subspecific name until the organism had been studied in more detail. The sandfly incriminated as the vector was identified as *Lu. flaviscutellata*.

Preliminary study of what appear to be the sole remaining isolates of the Trinidad parasite (by courtesy of Dr. D. A. Evans) in the author's laboratory, suggest the organism to be distinct from *L. m. pifanoi* and *L. m. amazonensis* but, nevertheless, closer to the latter on monoclonal antibody tests (Dr. J. J. Shaw, personal communication).

No cases of human cutaneous leishmaniasis have been reported in Trinidad since 1930, and in the absence of isolates from man it is impossible to say if they were due to *L. mexicana* or *L. braziliensis*.

10. Cutaneous Leishmaniasis in the Serra do Roncador, Mato Grosso State, Brazil

RASSI et al. (1972) recorded cases of ACL among men clearing forest for cattle-farming in the foothills of the Serra do Roncador, Mato Grosso State, and a single isolate from there was studied by CHANCE et al.
likely ecological niche in which such a sandfly might occur would appear to be the low ‘waterlogged’ woodland referred to by MAYRINK et al. (1979). The most abundant sandfly recorded in the Caratinga area was Lu. whitmani (Antunes & Coutinho), which was captured in both peri-domestic and silvatic habitats. As previously discussed, however, the most common anthropophilic species in a given area of leishmaniasis may not necessarily be the vector.

12. Diffuse Cutaneous Leishmaniasis in the Dominican Republic of the West Indies

As human visceral leishmaniasis had been recorded on the island of Guadeloupe (COURMES et al. 1966) and cutaneous leishmaniasis in Trinidad (TIKASINGH, 1974), it was not particularly surprising when BOGAERT DIAZ et al. (1975) published the first records of cutaneous leishmaniasis in the Dominican Republic, in the same chain of Caribbean islands. It was surprising, however, that all three cases were brothers, four, eight and nine years old, and that they were all suffering from the anergic, diffuse cutaneous form of the disease. Since that time, some 18 further cases have been recorded, all in the form of DCL, and seemingly in the absence of patients with simple, uncomplicated cutaneous leishmaniasis.

The frequency of DCL, and especially the occurrence of three cases in a single family, suggested some genetic, immunological defect within a closely knit population, and all the infected persons certainly showed suppressor-cell problems (PETERSEN et al., 1982). Intradermal skin tests (leishmanin test) and serological investigations (IFAT) on non-infected persons in the same localities revealed numerous positive reactions, but in the apparent absence of any evidence of infection, past or present. In this respect, the situation is perhaps not so far removed from that seen regarding DCL in other parts of South America as it would first seem. A similar ‘absence’ of uncomplicated skin lesions has been recorded in Venezuela, where infection with L. m. pifanoi has so far been found only in cases of DCL. 20 cases of infection with L. m. amazonensis in Pará State, north Brazil, were divisible into 13 with simple, curable lesions and seven (35-0%) with DCL: it should be remembered that Pará is approximately 30 times the size of the Dominican Republic, but with about one third of that country’s population. Finally, an active search for cases of ACL has not been made in Pará, in the same way that has been possible in the relatively tiny area of the Dominican Republic. It remains likely that it is but a question of time before uncomplicated cutaneous leishmaniasis is found in both the Venezuelan and the Dominican Republic situations.

I have been unable to find published information on any epidemiological studies in the Dominican Republic, but am informed that natural forest is limited to mountain ridges and valleys, and that the number of indigenous wild mammals is very small (Dr. Bryce Walton, personal communication). The sandfly population is believed to be limited to two species: Lu. cayennensis hispaniolae (Fairchild & Hertig), which feeds on reptiles, and Lu. christophei (Fairchild & Trapido) which is known to feed on man (MARTINS et al., 1978).

The nature of the parasite causing DCL in the Dominican Republic has caused considerable specula-
tion, but there is little published information available. To date, New World DCL has been shown to be associated with subspecies of *L. mexicana* (LAINSON & SHAW, 1972; 1979), and in particular with *L. m. pifanoi* in parts of Venezuela and *L. m. amazonensis* in Brazil. It seems likely, therefore, that the parasite from the Dominican Republic may be another subspecies of *L. mexicana* or a related parasite. Its development in experimentally infected sandflies, *Lu. longipalpis*, is certainly of the *L. mexicana* type ("supravarian") (Shaw & Lainson, unpublished observations). Clearly much work remains to be done to elucidate the full details regarding this fascinating focus of cutaneous leishmaniasis.

In conclusion, it would appear that parasites of the *mexicana* complex not only have a wide distribution throughout the Americas, but they can be expected to be found in almost any woodland, however degraded, in which sandflles of the *flaviscutellata* group are known to exist in the presence of small, terrestrial mammals.

**Subspecies of *L. braziliensis*, and related leishmanias**

There are many gaps in our knowledge concerning the natural mammalian hosts and sandfly vectors of these interesting parasites, but it is significant that two of the best studied subspecies, *L. braziliensis panamensis* and *L. braziliensis guyanensis*, both have edentates (sloths and/or anteaters) as their primary vertebrate hosts. The major mammalian reservoir of *L. braziliensis braziliensis* has yet to be indicated, although the epidemiological features of the human infection and the occasional isolation of very similar organisms from a number of rodent species does suggest that these, or other terrestrial mammals, may prove to be the principal hosts, at least in the primitive silvatic situation (Lainson, 1982a for review).

Subspecies of *L. braziliensis*, and some related organisms, comprise a distinct group of leishmanias which can readily be recognized by their "peripylarian" type of development (Fig. 6) in their sandfly hosts (Lainson & Shaw, 1979; Killick-Kendrick, 1979). They first establish themselves in the hindgut of the insect as rounded or pear-shaped promastigotes and paramastigotes, firmly attached to the cuticular wall of the pylorus and/or the ileum. This attachment is effected by flagellar hemidesmosomes formed in the greatly expanded tip of this organelle: from here the flagellates migrate to the midgut, where multiplication takes place as free, elongate promastigotes, before the final invasion of the biting-mouthparts.

Amastigotes and promastigotes of *L. braziliensis* are notably smaller than those of *L. mexicana*: references to other criteria separating the two species have already been given in the discussion on subspecies of *L. mexicana*.

The presently recognized subspecies of *L. braziliensis* are undoubtedly responsible for the greater part of cutaneous leishmaniasis in the Americas and at least one subspecies is associated with the highly mutilating mucocutaneous form of the disease (Fig. 7). It is generally assumed that *L. braziliensis* (sensu lato) is distributed throughout the forested or wooded regions of most of Latin America. This generalization is often based only on clinical evidence, however, and in some areas local cutaneous leishmaniasis may be principally due to *L. mexicana* or related parasites, as discussed above.

1. *Leishmania braziliensis braziliensis*: an unsolved problem.

It is becoming abundantly clear that the name *L. braziliensis* has been loosely used for a number of different parasites which almost certainly will be shown to merit their own subspecific or even specific status. Furthermore the name has somehow or other become irretrievably linked with the clinical condition of mucocutaneous leishmaniasis, or "espundia", which was not that presented by the patient from which the parasite was first described.

Vianna (1911) gave the name of *L. braziliensis* to an organism causing disseminated cutaneous leishmaniasis in a patient from the small rural town of Alem Paraiba, on the border between the States of Minas Gerais and Rio de Janeiro, Brazil. Not surprisingly, the type material no longer exists, and present-day workers on ACL are in the difficult position of attempting to define leishmanias regarded as *L. b. braziliensis* (sensu Lainson & Shaw, 1979) in the absence of the original parasite and, by modern standards, any adequate description of it.

The problem would best be resolved by the designation of a neotype, which could serve as a firm basis on which to describe, differentiate and classify related parasites of the *braziliensis* complex in the Americas. Ideally, of course, the neotype should come from a human infection, in the type locality of Alem Paraiba, and in a recent discussion on this subject (Lainson, 1982b), I suggested that this might prove difficult in view of the considerable ecological upheaval that has resulted after many years of deforestation and agricultural development in that area of Brazil (Fig. 8). In February of this year, however, I paid a visit to Alem Paraiba and was surprised by the extent of some surviving patches of natural forest, within a few kilometers of the town and mostly jealously guarded by private land-owners.

A capture of sandflies in one such forest (Fig. 9), between the hours of 18.00 and 20.30 (Rangel & Lainson, unpublished observations), provided 165 female sandflies, the catch comprising 75.6% *Psycho doygus c. carreri* (Barretto), 22.4% *Ps. h. hirsutus* (Mangabeira) and 1.8% *Ps. davosi* (Rooti) (kindly identified by Dr. Habib Fraiha, Instituto Evandro Chagas). Two nights later a further catch produced approximately 350 females (between 18.45 and 22.40 hours). These were not identified, as they were triturated in saline and inoculated into hamsters in the hope of isolating a *Leishmania*: the catch, however, appeared to have a similar composition. All of these three species of sandfly are known man-biters, and *Ps. c. carreri* avidly attacks man not only at night but also during the daytime, especially in overcast weather.

It remains possible, of course, that none of these sandflies is, or was, a vector of Vianna's parasite from Alem Paraiba, and less adaptable vector species may well have disappeared from the region long ago, together with some unknown mammalian host of *L. b. braziliensis*. It would seem well worthwhile, however, to re-investigate the area more fully in terms of sandfly species and the present-day mammalian fauna. We were told by our guide, the farm manager,
that the forest in which we worked was about 2 km² in area and still contained much wild-life. This included howler monkeys ("Alouatta sp."), the characteristic roaring of which we certainly heard during our visit, armadillos, and larger 'game' rodents such as the 'paca' and 'agouti' ("Agouti spp."). This being the case, the presence of an abundance of other, smaller, rodents known to be hosts of Leishmania in other Brazilian forests, is almost a foregone conclusion. Clinicians questioned at the local hospital claimed that cutaneous leishmaniasis was non-existent in the Além Paraíba district, but admitted that they were without a dermatologist on their staff. The local population at large has little contact with the forested areas, which are strictly out of bounds to all except the farmers and their workers, and hunting is prohibited. Anyone developing skin lesions in these communities would be more likely to be taken to private laboratories in Rio de Janeiro, where most of the richer land-owners live, rather than to the local hospital in Além Paraíba.

Information regarding the incrimination of wild mammalian hosts and sandfly vectors of L. b. braziliensis (sensu lato) remains deplorably scanty and largely comes from areas of southern Brazil which are far distant from Além Paraíba, and where it remains likely that the parasite causing the local form of cutaneous leishmaniasis is not the same as that described by Vianna. The nearest areas of epidemiological research have been in nearby Rio de Janeiro State, where Aragão (1922) was impressed by the frequency of the sandfly Lutzomyia intermedia (Lutz & Neiva) in and around houses of the rural suburbs. He obtained living, engorged specimens brought to him by patients with cutaneous leishmaniasis who were instructed to select those flies feeding on the inflamed edges of their lesions, when possible. Aragão found scanty flagellates in a saline triturate of two of these sandflies, and material from another suspension of five flies produced a lesion containing amastigotes when inoculated intradermally into the nose of a dog. As pointed out by Pessoa & Barreto (1944), this evidence was highly suggestive but by no means conclusive, although it has been frequently cited in subsequent literature as firm proof of the role of Lu. intermedia as a vector of L. braziliensis in the Rio de Janeiro foci. None the less, this very abundant sandfly remains a prime suspect due to its greater abundance over all other man-biting species in that region, and the fact that DDT spraying programmes in and around houses have resulted in a sharp drop in the numbers of Lu. intermedia and the cases of cutaneous leishmaniasis (Guimarães 1955; Guimarães & Bustamente 1954; Sabroza et al., 1975; Lima et al., 1980a, 1982). Final incrimination of Lu. intermedia by the isolation and characterization of the parasite from naturally infected flies, and experimental transmission, however, has still to be achieved.

Evidence regarding the role of animal reservoirs of L. b. braziliensis (sensu lato) in southern Brazil remains very scanty: dogs have been found with skin lesions containing amastigotes and this has created a tendency to assume that domiciliary transmission is taking place between these animals and man. This may indeed be so, but pockets of nearby forest or woodland need to be investigated as a possible primary source of the parasite among silvatic mammals and sandflies. A small number of wild rodents and one domestic cat have been found infected with Leishmania in the State of Rio de Janeiro (Barbosa et al., 1970; Araújo, 1978), but as far as I am aware the parasites have not been characterized and they could well have been L. mexicana.

Pessôa & Pestana (1940a) questioned the validity of so-called 'urban' transmission of L. b. braziliensis following their studies in certain parts of São Paulo State, and drew attention to frequent and often forgotten visits that patients may make to neighbouring woodlands. As a public health problem, they maintained, ACL is characteristic of new zones of human penetration and habitation. A series of studies on the sandfly fauna of highly endemic rural areas in Villa Queiroz, Pompeia (west São Paulo State, or 'Alta Paulista') and neighbouring localities showed Lu. whitmani (Antunes & Coutinho), Lu. migonei (França), Lu. ficheri (Pinto) and Lu. pessoi (Coutinho & Barreto) to be the most common anthropophilic species (Pessôa & Pestana, 1940b, 1940c; Pessôa & Coutinho 1940; Galvão & Coutinho, 1940; Coutinho, 1940; Pessôa & Coutinho, 1941).

Examination of large numbers of these sandflies by serial sections or direct dissection of fresh specimens, revealed promastigote infections in 8 out of 3,742 Lu. migonei (0.21%), 10 out of 4,940 Lu. whitmani (0.20%) and 8 out of 2,711 Lu. pessoi (0.29%): in some instances the flagellates were shown to be in the pharynx. No parasites were isolated, and it remains doubtful as to their exact nature: nevertheless, these species of sandflies must remain the most highly suspected vectors in the regions of São Paulo studied by the above-mentioned authors. The species Lu. ficheri has never been found infected but, due to its highly anthropophilic nature and occasional abundance in deforested areas where sporadic cases of AVL occur, Coutinho & Barreto (1941) felt that it might have importance as a secondary vector which is possibly adapting to a domestic or peridomestic habit. Pessoa (1941a) found Lu. intermedia to be rare in the Vila Queiroz area and, although more common in other areas as far as I am aware: isolation from the viscera would have any role as reservoirs of infection for man.

Forattini et al. (1972a, 1972b and 1973) isolated a parasite referred to as L. b. braziliensis from three species of rodents (Oryzomys capito, O. nigripes and Akodon arviculoides), and from single specimens of the sandflies Lu. pessoi and Lu. intermedia, captured in patches of forest in the Moi-Guaçu river valley, southern São Paulo: the same workers (1976) found Lu. intermedia to be a major man-biting species in and around houses in another focus of ACL in the Serra do Mar region, again in southern São Paulo State. Finally, Yoshida et al. (1979) isolated a Leishmania from the viscera of the opossum Didelphis marsupialis captured in the Município de Conchas, São Paulo State. The nature of the parasite remains uncertain, as far as I am aware: isolation from the viscera would suggest L. braziliensis but, on the other hand, the behaviour of the organism in the skin of hamsters seems to be more like that of L. mexicana.

Whether or not the Leishmania causing past and present foci of ACL in Minas Gerais, Rio de Janeiro
and the various parts of São Paulo is the same remains uncertain. There appear to be natural, ecological barriers restricting leishmaniasis to certain sandfly vectors, however, (see Conclusions) and on this basis one might suspect different parasites to be involved, for the suspected vectors in these foci are representatives of three distinct sandfly groups (MARTINS et al., 1978): the sub-genera Nyssomyia (Lu. intermedia and Lu. whitmani) and Pintomyia (Lu. pessoi) and the migonei group (Lu. migonei). On the other hand, all the areas in southern Brazil, discussed above, have suffered drastic ecological upheavals following deforestation and increasing agricultural development. Sandfly-mammal-parasite relationships will have changed: in many instances, perhaps, primitive sandfly vectors will have disappeared, or will have become so uncommon that they no longer function efficiently in transmission. Other more robust species will have adapted to the new ecological conditions, and some have even become peridomestic or domestic in habit. This seems to be the case, in variable degrees, for Lu. pessoi, Lu. migonei, Lu. whitmani, Lu. fischeri, Lu. intermedia and Lu. longipalpis. The first four of these sandflies are possibly involved in the maintenance of infection among wild animals still persisting in the remaining patches of degraded or secondary forest. The latter two species appear to have successfully adapted and are able to thrive in a peridomestic or domestic habitat. Lu. longipalpis is now undoubtedly responsible for a dog—man transmission of visceral leishmaniasis (see later pages) under these conditions, and it remains possible that Lu. intermedia may become a peridomestic vector of cutaneous leishmaniasis involving dogs and man.

It is reasonable to suppose that in the densely wooded regions of the Amazon basin (Fig. 10), we are likely to encounter the primitive sandfly/wild mammalian host situation for L. braziliensis. Here there is no doubt whatsoever of the zoonotic nature of human leishmaniasis.

In the early 1970s, mining operations in the range of hills known as the Serra dos Carajás, Pará State, north Brazil, resulted in the penetration of an extensive labour-force into primary forest for the construction of roads and mining camps. Cutaneous leishmaniasis soon became a serious problem, and the author's laboratory was asked to investigate the epidemiological situation.

A survey of the sandfly fauna rapidly indicated one particular species, Psychodopygus wellcomei Praiha, Shaw & Lainson, as the predominant man-biting insect (WARD et al., 1973), and this sandfly was later incriminated as the major vector of the parasite referred to as L. b. braziliensis in north Brazil (LAINSON et al., 1973). Ps. wellcomei belongs to the squamiventris group of sandflies, which is notable for the inclusion of a number of species or sub-species the females of which are morphologically indistinguishable: identification depends on the morphology of the males.

In our earlier studies in the Serra dos Carajás, lack of roads restricted our observations to the higher parts of the hills (at about 500 m), and there was no means of working on the descent or at the foothills. Recently, however, extensive roadbuilding has enabled us to collect sandflies from these areas and breeding-out from gravid females in the laboratory revealed the fact that our 'Ps. wellcomei' population was actually composed of two species: true Ps. wellcomei, and a sympatric species Ps. complexus (Mangabeira). Above 300 m (where the majority of human infections are acquired) the proportion was found to be about 95·% Ps. wellcomei to 5·% Ps. complexus: below this altitude, however, the position became reversed, so that in the foothills the catch was predominantly Ps. complexus. This interesting finding (Dr. P. D. Ready, personal communication) does not basically invalidate our previous incrimination of Ps. wellcomei as the major vector of L. b. braziliensis in this region of Brazil, as all our observations at that time were made at approximately 500 m. Whether or not Ps. complexus acts as a vector of cutaneous leishmaniasis in the lowlands has yet to be decided. If it is, then it remains very likely that it is transmitting a different leishmanial parasite.

The search for the wild mammalian reservoir of L. b. braziliensis in the Serra dos Carajás still continues. Observations on the vertical distribution of Ps. wellcomei clearly indicate this fly to be predominantly a forest-floor species which is not commonly found in the canopy (WARD et al., 1973; Dr. P. D. Ready, personal communication): this does tend to suggest that the mammalian reservoir is some terrestrial or semi-terrestrial animal.

Our interest became aroused as to whether or not the distribution of Ps. wellcomei extended to ecologically similar ranges of forested hills in other parts of north and northeast Brazil, where cutaneous leishmaniasis is common. It was particularly significant, we felt, that parasites isolated from man in the Serra dos Carajás, and the hilly regions of the northeastern States of Ceará and Bahia have proved to be indistinguishable on present-day means of identification (MCMAHON-PRATT & DAVID, 1981; CUBA CUBA et al., 1982; Shaw & Lainson, unpublished observations), and that 'Ps. squamiventris' was reported to be taken from human bait on the forested slopes of hills at Pacou, on the Serra de Batutiré, Ceará (LUCENA, 1953).

READY et al. (1983) discussed a visit to this region in May, 1982, when a 75 minute capture of sandflies in a Shannon trap near Guaramiranga produced 379 females and 71 males of Ps. wellcomei: it was, in fact, the only species captured.

It is almost certain, then, that the sandfly recorded as Ps. squamiventris in this area by Lucina in 1953 was actually Ps. wellcomei. This same author also recorded 'Ps. squamiventris' from ecologically similar localities in the nearby States of Paraíba, Sergipe and Pernambuco. These and other regions of hillside forest at about 300 m and higher may, therefore, form part of a single enzootic of L. b. braziliensis s.l. over a far greater area of the Brazilian Shield than we previously suspected. With this in mind, Dr. Paul Ready visited a highly endemic area for cutaneous and mucocutaneous leishmaniasis in an area known as Três Brasóis, Bahia State, where the disease is associated with mountainous coastal forest (BARRETO et al., 1981). Although an exceptionally delayed rainy season was most unfavourable for sandfly captures, and Ps. wellcomei was not encountered, he did find other species commonly associated with this sandfly (Ready, personal communication). This, the altitude, ecology, and the indistinguishable nature of the
parasite from Três Brasos and the Serra dos Carajás, Pará, all suggest a common enzootic.

Elsewhere in Brazil there is scanty information on cutaneous and/or mucocutaneous leishmaniasis, in particular regarding vectors and mammalian reservoir hosts. LAINE & SHAW (1969, 1970) studied a focus of both forms of the disease in a sparsely inhabited region of the Serra do Roncador, Mato Grosso State, and regarded the causative parasite as *L. b. braziliensis* based on its morphology and behaviour in hamsters and blood-agar culture. A single isolate of what appeared to be the same organism was made from a small tail-lesion of the rodent *Oryzomys concolor*. Human infection was clearly associated with penetration of forest, but the authors were unable to conclude if this was the riverine, "gallery"-forest, or the much more extensive, neighbouring dry forest. From knowledge gained since that time it is much more likely to have been in the latter. The same authors (LAINE & SHAW, 1979; LAINE, 1982a, for reviews) have also isolated a similar parasite from a limited number of rodents (including a domestic rat), marsupials and the sloth *Choloepus didactylus*: these isolates have been very poorly studied, however, due to the great difficulty in maintaining them in laboratory animals and culture, and their significance regarding human leishmaniasis remains in doubt.

An overall picture is slowly emerging that the parasite referred to as *L. b. braziliensis* (sensu LAINE & SHAW, 1979) may be restricted to the higher altitude forests, as discussed above, and that isolates from man, donkeys and dogs during work in the plantations. Isolates from man, dogs and donkeys have been characterized in the author's laboratory and appear to be indistinguishable from each other on isoenzyme profiles and monoclonal antibodies. Furthermore, the Venezuelan parasite is extremely close, if not identical, with *L. b. braziliensis* from the Serra Dos Carajás region of north Brazil, discussed above (Dr. M.A. Miles and Dr. J.J. Shaw, personal communication). AGUILAR (1981) examined a very similar focus of cutaneous leishmaniasis in El Caserío Las Rosas, in the District of Pa1vecino and Urdaneta, particularly in the Districts of Pa1vecino and Urdaneta. Sporadic cases were first seen in 1974, again associated with agricultural development of forest land: by 1978 21 out of the 124 inhabitants had acquired the disease. At first the lesions were purely cutaneous, but by 1978 four of the patients had developed nasopharyngeal metastases. No infections were detected in 118 wild animals examined, but lesions containing amastigotes were found in three out of 43 dogs and six out of 28 donkeys. Sandflies were captured in the peridomestic environment, off man, dogs and donkeys: *Ps. panamensis*, *Lu. atroclavata* (Knab), *Lu. trinidadensis* (Newstead) and *Lu. gomezi* proved to be the most common of 17 different species encountered. None, however, was found to be infected.

Finally, MÁRMOL LEON et al. (1975) found that *Lu. gomezi*, *Lu. ovalesi* (Ortiz) and *Ps. panamensis* were the sandflies most commonly attracted to man in a focus of cutaneous leishmaniasis in the district of Miranda, Zulia State, Venezuela. Human infections were more commonly associated with communities in wooded areas rather than those in agriculturally developed land.

Although the possibility of peridomestic or domestic transmission cannot be excluded in these foci of Venezuelan cutaneous and mucocutaneous leishmaniasis, no infected sandflies have yet been found in houses: infections of man, the dog and the donkey almost certainly are acquired when they accompany one another through the wooded areas very close by, on their way to the plantations.

In Colombia, WERNER & BARRETT (1981) considered both cutaneous and mucocutaneous leishmaniasis to be a public health problem of considerable magnitude. The diseases are considered to be endemic in the forested parts of almost all of the administrative sections of the country, and about 25% of the reported cases are said to be of the mucocutaneous form. The only information on the nature of...
the parasite(s) appears to be that of WERNER (1981), who studied the behaviour of 14 isolates from patients with the cutaneous disease in blood-agar culture and hamsters. He concluded that 11 of these from coastal regions (Pacific and Atlantic coasts) showed close relationship to L. b. braziliensis in their poor growth in culture and moderate growth in hamster skin. On the other hand, he felt that three inland isolates (San Carlos, Códoa; Nariño, Antioquia and Valdivia, Antioquia) “had a closer relationship with L. b. panamensis by having a better in vitro growth but poor or no development in hamsters”. In the writer’s experience, however, poor growth in hamster skin is more a characteristic of many isolates of L. b. braziliensis than of L. b. panamensis (LAINSON & SHAW, 1970; LAINSON, 1982a, b).

Apart from case histories and clinical surveys (DESJEUX et al., 1974; DE MUYNCK et al., 1978; LEÓN & LEÓN, 1976; RECACOECHEA, 1980; VILLA-LONGA, 1963) there is little or no information regarding the nature of the parasite(s) causing dermal leishmaniasis in Amazonian Peru, Ecuador, Bolivia, Paraguay and Argentina. Cutaneous and mucocutaneous leishmaniasis are known in all of these countries, however, and the presence of the latter has generally been taken to indicate the presence of L. b. braziliensis or closely related parasites.

Leishmaniasis has been said to be absent in Uruguay and Chile, although there is a report of five cases of the cutaneous disease in a hospital in the latter country (SAPUNAR et al., 1980). I have been unable to see the original report and am not sure, therefore, if these cases are autochthonous.

Until relatively recently, L. b. panamensis has remained the only known ‘peripylarian’ Leishmania in Central America, with its distribution seemingly limited to the northern parts of Colombia, Panama and Costa Rica (see below). There has been reason to suspect the existence of other members of the braziliensis complex for some time, however. Thus STRANGWAYS-DIXON & LAINSON (1962) reported promastigotes attached to the peritrophic wall of a specimen of the sandfly Lu. ovallesi in Belize: we now know that development in the hindgut is a characteristic of the L. braziliensis complex, and that the parasite could not have been L. m. mexicana, although the full significance of this finding was not realized at that time.

WALTON et al. (1968) discussed the treatment of patients with cutaneous leishmaniasis in Panama, and noted that it was impossible to culture the parasite from some patients in a blood-agar medium which supported copious growth of L. b. panamensis. It is particularly interesting that all these cases refractory to culture acquired their infection in the same area of forest, on the Caribbean coast (WALTON et al., 1977) in an area known as Fort Sherman. This, and the fact that rare cases of the mucocutaneous disease have been recorded in Panama strongly suggested that L. b. braziliensis was involved.

ZELEDÓN et al. (1982) examined five patients with cutaneous leishmaniasis acquired in the southeast of the Republic of Honduras in the Department of Paraíso, close to the border with Nicaragua. On enzyme profiles for glucose-6-phosphate dehydrogenase, and buoyant densities of kinetoplast and nuclear DNA, the parasite was considered as closest to L. b. panamensis. Finally, numerous isolates recently made from cases of cutaneous leishmaniasis among British troops carrying out jungle warfare training in Belize, have revealed the presence of a previously unsuspected subspecies of L. braziliensis (Dr. D. A. Evans, personal communication) which is remarkably close to L. b. braziliensis from the Serra Dos Carajás, Brazil, discussed above.

The failure of earlier workers to locate this parasite in Belize (LAINSON & STRANGWAYS-DIXON, 1963; WILLIAMS et al., 1965; WILLIAMS, 1970; DISNEY, 1968) seems surprising in view of the high rate of infection recorded in these soldiers, but there are two possible explanations. Firstly, most isolates made from man in the past were from ‘chicleros’ and other forest workers in the central and northern parts of the country, whereas the infected soldiers were carrying out their manoeuvres well down in the south of Belize, in forest which is rarely frequented by the local population. Secondly, it is possible that improved culture media of recent years has now permitted detection of the L. braziliensis parasite, which possibly failed to grow in the more simple NNN medium used by earlier workers. I think the first explanation is the most likely.

The vector and reservoir of L. braziliensis in Belize remain to be discovered. Lu. cruciata (Coquillet) must be highly suspected in view of its highly anthropophilic nature, and the fact that it has been found naturally infected with promastigotes which are highly likely to have been Leishmania (STRANGWAYS-DIXON & LAINSON, 1962). The record of attached promastigotes in the hindgut of the sandfly Lu. ovallesi in Belize certainly now takes on added interest.

In the meantime, the close similarity of L. braziliensis sensu lato from parts of Brazil, Venezuela and Belize suggests that the mammalian reservoir is likely to be an animal, or group of animals, which is particularly abundant and with a wide geographic range. Sloths would appear to be eliminated as suspects as they are not found further north than Nicaragua, and the evidence is against terrestrial mammals as a reservoir. The common opossum, Didelphis marsupialis is exceedingly common, is found throughout Central and South America, and has been found harbouring a parasite closely resembling L. b. braziliensis in Brazil on one occasion (LAINSON & SHAW, 1979): rodents also remain likely candidates.

2. Leishmania braziliensis panamensis: Panamanian cutaneous leishmaniasis.

Although natural flagellate infections had been recorded in a number of neotropical sandfly species (LAINSON & SHAW, 1979, for review), on no occasion were they proven to be Leishmania until the extensive studies carried out at the La Loma laboratory in Panama, by Hertz, Fairchild, Johnson and McConnell. Over many years of patient work they recorded natural promastigote infections in more than 400 anthropophilic sandflies (JOHNSON et al., 1963), including Lu. trapidoi (Fairchild & Hertig), Lu. ylephiletor (Fairchild & Hertig) and Lu. gomeesi. Ps. panamensis was also found infected by CHRISTENSEN et al. (1969).

It remains uncertain how many of these sandfly infections were Leishmania, and MCCONNELL (1963)
suggested that a great many were certainly not developmental stages of this parasite: it is likely that some were *Endotrypanum*, an endoerythrocytic flagellate of sloths which also develops as promastigotes in the gut of certain sandfly species (SHAW, 1969).

Others may have been infections with *Leishmania* species which are unconnected with human leishmaniasis, such as *L. hertigi* of porcupines (HERRER, 1971) or the *Leishmania* recently discovered in armadillos (LAISON et al., 1979b).

Nevertheless, the inoculation of volunteers with isolates from some sandflies did provide sound evidence that *Lu. trapidoi* frequently harboured promastigotes of *Leishmania* infective to man, and it is reasonable to suppose that they were the parasite now referred to as *L. braziliensis panamensis* LAISON & SHAW, 1972. The role of the other species of sandflies infected with promastigotes remains less clear.

The fact that *Lu. trapidoi* and *Lu. ylephiletor* are highly arboreal species of sandflies led the Panamanian workers to suspect an arboreal mammal as the reservoir of *L. b. panamensis* and HERRER & TELFORD (1969) and HERRER et al. (1973) finally found a high rate of infection in the two-toed sloth, *Choloepus hoffmanni*. The infections were all inapparent, with parasites localized in the skin and the viscera: occasional infections were found in other animals, including monkeys and procyonids, but were considered of minor epidemiological importance. A few dogs were also found infected (HERRER & CHRISTENSEN, 1976), but these are probably best regarded as "accidental" hosts, like man. Parasites considered as *L. b. panamensis* have been found in sloths (C. hoffmanni and Bradypus griseus) and the sandfly *Lu. ylephiletor* in Costa Rica (ZELEDON et al., 1975; ZELEDON & ALFARO, 1973), and in *Lu. trapidoi* in Colombia (MORALES et al., 1981). The epidemiological features of *L. b. panamensis* are similar to those of *L. b. guyanensis*, discussed below, and the two parasites are clearly closely related—as shown by the above-mentioned biological characters and by current biochemical methods of identification (MILES et al., 1981).

3. *Leishmania braziliensis guyanensis*: "Pian-bois" of the northern Amazon Basin.

Although the causative agent of Peruvian "uta" had received the separate name of *Leishmania peruviana* in 1913, the general assumption persisted that the various South American leishmaniasis were all due to a single parasite, "*L. braziliensis*": a view which was to virtually paralyse our progress in understanding their ecology and epidemiology for close on forty years. French workers studied one clinical form in French Guyana, however, and concluded that the causative parasite was different from that described by Vianna, in Brazil. Like Biagi, they preferred to regard it as a subspecies of *L. tropica*, and the agent of "pian-bois", "bosch-yaws" or "bush-yaws" (Fig. 12) was given the name of *L. tropica guyanensis* by Floch (1954) to distinguish it from the other two South American leishmanias known at that time, which were referred to as *L. tropica braziliensis* and *L. tropica peruviana*. It remained for PESSOA (1961) to correct these names to those in use today, namely *L. braziliensis braziliensis*, *L. b. guyanensis* and *L. b. peruviana*.

Little information was given concerning the ecological features of "pian-bois" by Floch, but attention was drawn to the possible role of one particular sandfly as a vector, namely *Luizomyia anduzei* (ROZEBOOM, 1942). Sandflies identified as this species were found to congregate in large numbers on the bases of the larger trees, from which they frequently attacked man when disturbed (FLOCH, 1957).

Nearly ten years were to elapse before further progress was forthcoming, this time in neighbouring Surinam, where WIJERS & LINGER (1966) dissected an unspecified number of *'Lu. anduzei'* from tree-trunks and found promastigotes in twelve of them. Unfortunately, the Dutch workers' attempts to infect a hamster inoculated with flagellates from two of the flies failed.

In 1975, workers in Brazil commenced studies in an area near Monte Dourado, Pará State, north of the Amazon River, where some 300 cases of 'pian-bois' were registered in one year among men engaged in clearing forest. Promastigote infections were found in 4 of 55 specimens of *'Lu. anduzei'* dissected, and on this occasion the parasites were successfully established in hamsters (LAISON et al., 1976). Suspicions were aroused during these studies that the sandfly referred to as *'Lu. anduzei'* in all of these epidemiological investigations in French Guiana, Surinam and Brazil was not in fact identical with the type material of that insect (ROZEBOOM, 1942), and WARD & FRAIHA (1977) described it as a new species, *Lu. umbratilis*.

The important role of this sandfly as a vector of "pian-bois" was further emphasized during subsequent studies in Monte Dourado (LAISON et al., 1979b): *L. b. guyanensis* was isolated from 16 more *Lu. umbratilis* and, of 77 sandflies caught attacking two men collecting from tree-trunks, 72 proved to be *Lu. umbratilis* (92.5%). Some idea of the efficiency of transmission may be gained by the fact that the two men developed a total of 13 leishmanial lesions on their arms; and all of 10 others who worked periodically in the same area became infected within a few months. The role of *Lu. umbratilis* as the major vector of *L. b. guyanensis* has since been confirmed in Amazonas State, north Brazil (ARIAS & FREITAS, 1978) and French Guiana (LE PONT & PAJOT, 1980).

Two other species of sandfly have been found infected: *Lu. anduzei*, by ARIAS & FREITAS (1978) and *Lu. whitmani* (Antunes & Coutinho) by LAISON et al. (1979b). Neither is considered an important vector to man, however, even if they do play some role in the maintenance of the parasite in nature. Infection in *Lu. anduzei* appears rare, and *Lu. whitmani* is seldom caught biting man in its silvatic habitat.

From the tree-trunk and canopy-loving habits of *Lu. umbratilis* it was logical to suspect an arboreal animal as the primary host of *L. b. guyanensis*. An examination of these in the Monte Dourado study area (LAISON et al., 1981b, 1981c) revealed 27 out of 59 (46%) sloths (*Choloepus didactylus*) (Fig. 11) and 6 of 27 (22-2%) anteaters (*Tamandua tetradactyla*) to be infected. Significantly, only one of 67 terrestrial rodents harboured the parasite (a specimen of *Proechimys guyannensis*), and one of 21 opossums (*Didelphis marsupialis*). It was concluded that neither rodents nor marsupials were of great importance as
reservoir hosts of *L. b. guyanensis* under natural conditions in primary forest.

Workers in French Guiana examined 74 mammals captured in an area endemic for 'pian-bois', by the inoculation of a pool of viscera and skin into hamsters. *Leishmania* was isolated from two out of 19 (10.5%) rodents (*Proechimys* sp.) (LE PONT et al., 1980), seven out of 15 (46.7%) sloths (*C. didactylus*), and one of 7 (14.3%) kinkajous (*Potos flavus*) (GENTILE et al., 1981).

One of the isolates from a sloth was identified in the writer's laboratory as *L. b. guyanensis*, and it is reasonable to suppose that the other 6 isolates from *C. didactylus* and the one from the kinkajou (which is also a canopy dweller) were also of this parasite. The isolates from the rodent *Proechimys* were unfortunately lost (GENTILE et al., 1981). They were quite likely *L. mexicana amazonensis* (discussed above), as *Proechimys* is the principal host of this very common parasite of rodents. One of the infected animals had a visible lesion of the ear which contained amastigotes: a situation not yet recorded for animals infected with *L. b. guyanensis*, but which is not infrequently seen in wild animals infected with *L. m. amazonensis*. Finally, the French workers were unable to find infections in a number of marsupials, including eight specimens of the opossum *D. marsupialis*.

ARIAS et al. (1981) investigated another area of 'pian-bois' near Manaus, Amazonas State, Brazil. They found no infections in 26 *D. marsupialis* captured in relatively undisturbed primary forest, but isolated *L. b. guyanensis* from three out of 15 (20%) in a biological reserve which was "much disturbed by man" and "poor in game". In later studies (ARIAS & NAIFF, 1981) they recorded five out of seven (71.4%) of these opossums infected in the same reserve, and eight of 14 (57.1%) in an area of primary forest ("Parque das Laranjeiras") which was under development as a future residential area, and where cases of cutaneous leishmaniasis had 'frequently been reported'. It was suggested that this very high infection rate resulted from the withdrawal of the primary hosts (sloths and anteaters) due to man's disturbance, and an increase in the opossum population of the area because these animals are scavengers by nature and are attracted by man's presence.

There are a number of epidemiological features in this interesting situation which warrant discussion. In the title of their paper (ARIAS & NAIFF, 1981) the authors refer to the 'Parque das Laranjeiras' as one of the "... urban areas of Manaus". This term is somewhat misleading as the area in question was located some kilometers outside the city and most of the houses constructed and inhabited at that time...
abutted almost directly on to primary forest with a high canopy (Dr. P. D. Ready, pers. comm., after a visit to the study area), as can be seen in one accompanying photograph. In the text the area is more aptly referred to as ‘... disturbed primary forest ...’, and ‘... where much of the primary forest was left standing’.

The peridomestic acquisition of infection under such circumstances is not, therefore, so surprising as at first it would seem, for \textit{Lu. \textit{umbratilis}} comes readily to light-traps; with such a small distance involved, it would be similarly attracted to the lights of the houses at night. This situation was also recorded by the workers in French Guiana (LE PONT & PAJOT, 1981), who described the peridomestic transmission of ‘pain-bois’ among persons living in a small group of houses on the edge of a forest village.

Precipitin tests on blood-meals of 975 engorged \textit{Lu. \textit{umbratilis}} from the developing residential area near Manaus showed that 64.0% had fed on sloths and only 1.1% on opossums (CHRISTENSEN et al., 1982), clearly indicating that sloths were still in the near vicinity.

ARIA S & NAIFF (1981) referred to some hosts, including man, as ‘dead end tangents’ for the parasite, presumably in that they played no role as a further source of infection for the sandfly vector. They did not include \textit{D. marsupialis} in this category, doubtless because sloths were thought to be absent in an area where many opossums were infected, and where active transmission was taking place to man. As the sandfly blood-meal analysis showed that sloths were still in the neighbourhood and that \textit{Lu. \textit{umbratilis}} does not commonly feed on opossums, it seems distinctly possible that \textit{D. marsupialis} might represent one of the “dead end tangents”. The infection-rate in this animal may simply have built up to a high level in an unnaturally large, forest-fringe population of this species which had resulted from this animal’s attraction to the area by man’s refuse. In undisturbed, primary forest this build-up does not take place, and opossums are rarely found infected (ARIA S et al., 1981; LAINESSON et al., 1981c; GENTILE et al., 1981).

4. \textit{Leishmania braziliensis peruviana}: “Uta” in the Peruvian Andes

In spite of the predominantly silvatic nature of ACL, the first concerted attempt to unravel its epidemiology was not made in the forested regions, but high up on the relatively barren, western slopes of the Peruvian Andes.

The initial studies were made during an expedition mounted by the Harvard School of Tropical Medicine (STRONG et al., 1913, 1915). These workers considered the disease to be of a peridomestic nature, occurring at altitudes between 900-3,000 m, and in areas where the vegetation was at most of a low, shrubby nature. They found that the dog could be infected with the parasite taken from human lesions.

Later investigators, for this reason, tended to concentrate their studies on dogs and other domestic animals, and HERRER (1951a) demonstrated amastigotes in inconspicuous skin lesions (and sometimes in apparently normal skin) in 46 out of 513 dogs examined. No infections were found in 78 cats, 22 donkeys, 7 pigs and one horse, all of which, like the dogs, were examined by means of stained smears of skin, usually taken from the nose. ‘Considerable numbers’ of wild rodents and opossums were trapped and examined: at this time, however, Herrer was unaware that parasites might be associated with apparently normal skin, and it was stated that smears of skin were examined only in ‘exceptional cases’. No mention was made of examination of the viscera, inoculation of laboratory animals, or the use of blood-agar cultures, and it remains highly likely that inapparent infections of wild animals were missed.

Recent support for this supposition comes from the use of ‘sentinel’ hamsters placed in cages in man-made caverns in endemic areas for ‘uta’ in the Santa Eulalia Valley, Peru (HERRER, 1982a). During a period of 8 weeks, 21 out of 47 (47.7%) of these animals developed leishmanial lesions on the ears, feet and nose: although the presence of infected dogs in the area cannot be ruled out, these observations do suggest the presence of a wild animal reservoir—most probably a rodent.

HERRER (1951b, c; 1957) and HERRER & BATTISTINI (1951) worked principally in rural areas in the valleys of the Province of Huarochiri and the prevalence of infection in some villages was remarkably high. Judging by active infections and the presence of typical, old scars, up to 94.0% of the school-children were affected. Infection took place at an early age, but there was usually spontaneous healing which provided firm protection against reinfection. Lesions, found principally on the face, were usually single or few in numbers, and there was no evidence of subsequent development of mucocutaneous leishmaniasis. Like Strong and his colleagues, Herrer & Battistini concluded that transmission was peridomestic.

The sandfly most highly suspected as the vector of ‘uta’ during these early studies was \textit{Lutzomyia verrucarum} (Townsend), which was found to frequent both houses and animal-sheds. It was shown to be a catholic feeder and seemingly equally attracted to man, dogs and other domestic animals including rabbits and guinea-pigs. Presumably, it was thought, it might equally well be1 a rodent feeder for wild animals, for it was found in fox-holes and other resting-sites far removed from human dwelling places. The only other suspect was \textit{Lu. peruviana} (Shannon) and although the habits of this insect were less well known than those of \textit{Lu. verrucarum}, there was evidence to suggest that it was less domesticated and more inclined to feed on wild animals. Recently, HERRER (1982b) inoculated a triturate of 97 \textit{Lu. peruviana} into the skin of seven laboratory hamsters and produced infection with a \textit{Leishmania} in two of them. It was concluded, therefore, that this sandfly may very well be the natural vector of \textit{L. b. peruviana}.

It is of considerable interest that ‘uta’ virtually disappeared in some Peruvian villages following the destruction of peridomestic sandflies after the DDT spraying of houses in the control of bartonellosis (‘Carrión’s disease’), which is also transmitted by these insects. There is recent evidence of a resurgence of cutaneous leishmaniasis, however, following a long suspension of such control measures, and HERRER et al. (1980) recorded that there were 46 new cases registered by the Huinico Hospital, in the Valley of Santa Eulalia, during the period 1974-1979. This again supports the suggestion of a wild animal reservoir. Further epidemiological studies are needed.
American Visceral Leishmaniasis

Leishmania donovani chagasi: indigenous, or introduced?

The first clinical diagnosis of autochthonous human visceral leishmaniasis in the Americas was not made until 1913, when Migone recorded a case in Paraguay. Since then the disease has been registered in Argentina, Brazil, Venezuela, Bolivia, Colombia, Guatemala, El Salvador, Mexico, Surinam, Ecuador, Honduras and Guadeloupe in the West Indies. Extensive reviews on the epidemiology have been given elsewhere (Deane 1956; Lainson & Shaw 1979).

The causative parasite of American visceral leishmaniasis (AVL) was given the name L. chagasi Cunha & Chagas, 1937, but for many years was referred to simply as L. donovani (sensu lato). Adler (1964) and Adler & Theodor (1957) favoured the view that the parasite of AVL was different from L. donovani (India) and L. infantum (the Mediterranean Basin), and Lainson & Shaw (1972) argued for retention of the name L. infantum. L. braziliensis had adopted the Lucius of subspecific names, referring to L. donovani donovani, L. d. infantum and L. d. chagasi: a practice generally followed by subsequent authors.

The relatively recent recognition of the human disease in the Americas led some to suggest a recent introduction into Latin America, probably with early European colonists, or their African slaves (Gardener, 1977). This implied, then, that the parasite of AVL is in fact L. d. infantum, now being transmitted by a totally different sandfly, Lutzomyia longipalpis.

Adler & Theodor (1957) and Adler (1964) were opposed to this hypothesis. They argued that in the Old World, L. d. infantum had not adapted to transmission by any sandfly other than those of the Phlebotomus major complex and, as a result, visceral leishmaniasis in the Mediterranean Basin has not been known to establish itself in new foci in recent times. It therefore remained unlikely, they felt, that L. d. infantum could have made such an abrupt adaptation from its natural vector to a completely different genus of sandfly.

Lainson & Shaw (1979) and Lainson (1982a) tended to the same view and pointed out that if AVL was indeed due to L. d. infantum introduced from Europe, an explanation was needed for the rapid spread of the disease throughout almost the whole of the Latin American continent, in the face of very poor means of communication between the affected countries. They argued that canids (the presumed primitive hosts of parasites of the donovani complex) were well established throughout Latin America long before the coming of the white man (the indians already had their own dogs), and there was no reason why they should not have their own leishmanial parasite(s).

Against these arguments, Killick-Kendrick et al. (1980) showed that a stock of L. d. infantum from France developed well in the midgut of laboratory-bred Lu. longipalpis and felt this to support the suggestion that this parasite could have been introduced into the New World in recent times. On the other hand, the all-important migration of the parasite to the foregut of the experimentally infected sandflies was not seen, and no evidence was produced indicating that Lu. longipalpis could actually transmit L. d. infantum. Perhaps the most convincing evidence for the introduction of L. d. infantum into the Americas from the Old World was the recent establishment of a small focus of canine visceral leishmaniasis in Oklahoma, USA (Anderson et al., 1980), involving at least four dogs with no history of foreign travel. As imported canine leishmaniasis has been registered on seven occasions, in dogs coming from Greece (Anderson et al., 1978; Adler, 1979), it seems likely that the Oklahoma focus originated from such a source. The apparent absence of mammal-biting sandflies in this part of the USA raises interesting speculations as to the mode of transmission in the Oklahoma kennels. Possibly it was by a direct, contaminative route. None of the infected dogs was believed to have had any previous connection with human or canine cases of leishmaniasis, but congenital transmission could possibly have played some role in the history of the outbreak.

Most leishmanial parasites produce benign or inapparent infections in their natural hosts, in which there has developed a well balanced host-parasite relationship. If L. d. chagasi is indigenous to the Americas, then one might expect to find such infections in some wild animal(s). Early attempts to do so in the small, sporadic foci of AVL in the Amazon Region of Brazil (Pará) failed (Chagas et al., 1938; Deane, 1956), possibly because these workers relied on direct microscopic examination of tissue smears rather than culture and the inoculation of laboratory animals. Deane (1956) and Deane & Deane (1962) found a number of foxes (Lycalopex vetulus) infected in the highly endemic areas of AVL in Ceará, northeast Brazil, but these animals were mostly found with fulminating, fatal infections similar to those seen in dogs, and this led to the suggestion that this animal, like the dog, did not represent an ancient indigenous reservoir ... but that it is, on the contrary, a rather recent host of L. donovani. This observation has, in fact, been used by some as an argument in support of the suggestion that New World visceral leishmaniasis is due to recently introduced L. d. infantum. Alencar et al. (1974/75), however, felt that the natural sequence of infection
areas of Brazil and, as all three foxes showed no signs of illness, it is tempting to suggest that C. thous might be the long-sought, primitive host. The animal is widely distributed in the Americas, and shows a preference for forested or wooded areas associated with natural or man-made savanna. Clearly, it is necessary to examine many more of these foxes: if a significant infection-rate can be found it would suggest that the major, present-day endemic areas elsewhere in South America may have originated from ancient silvatic enzootics involving C. thous or related canids.

Current views regard wild members of the Canidae as the original hosts of L. d. infantum in the Old World (LYSENKO, 1971), but the possible role of other, non-canid hosts must always be borne in mind (LAINSON & SHAW, 1979). In this respect it may be noted that the rodents Arvicanthis niloticus, Acomys albigena and Rattus rattus, and the carnivores Genetta genetta and Felis serval, have been indicated as hosts of the parasite causing visceral leishmaniasis in the Sudan (HOOGSTRAAL & HEYNEMAN, 1969); domestic rats, R. rattus and R. norvegicus have been found infected with L. d. infantum in Yugoslavia and Italy (PETROVIC et al., 1975; BETTINI et al., 1978); ALENCAR et al. (1960) recorded the isolation of a Leishmania from the blood of R. rattus in Ceará, Brazil, which could have been L. d. chagasi; and what was fox-dog-man, and that the parasite had a silvatic origin. Human visceral leishmaniasis has remained relatively rare in the Amazon Region of Brazil, with only 30 cases having been reported up to 1966. All of these were in the northern State of Pará and principally on the Atlantic coast, at the mouth of the Amazon. After the last case record in 1966, on the Island of Marajó, there followed a long period which was seemingly free of the disease until 1980, when another isolated case was diagnosed, post-mortem, again on the eastern part of Marajó. Since that time seven more cases have come to light on that island, and two more on the mainland near the small town of Santarém.

This small outbreak gave a long-awaited opportunity to re-investigate the epidemiology of Amazonian visceral leishmaniasis, and has resulted in the isolation of L. d. chagasi from the skin and viscera of a fox (Cerdocyon thous) and the finding of undoubted L. longipalpis, in considerable numbers, in one house and numerous chicken-houses in villages where cases of AVL had occurred (SILVEIRA et al., 1982; LAINSON et al., 1983).

This represented the third occasion on which L. d. chagasi had been isolated from C. thous (LAINSON et al., 1969; LAINSON & SHAW, 1971). The parasite from the last animal was shown to be biochemically indistinguishable from that of man in various endemic areas of Brazil and, as all three foxes showed no signs of illness, it is tempting to suggest that C. thous might be the long-sought, primitive host. The animal is widely distributed in the Americas, and shows a preference for forested or wooded areas associated with natural or man-made savanna. Clearly, it is necessary to examine many more of these foxes: if a significant infection-rate can be found it would suggest that the major, present-day endemic areas elsewhere in South America may have originated from ancient silvatic enzootics involving C. thous or related canids.

Fig. 15. The ecology of visceral leishmaniasis due to Leishmania donovani chagasi in the Amazon Region. The vector, Lutzomyia longipalpis (v) may infest rural houses that have not been treated with insecticides, or unsprayed animal-houses: chickens are excellent maintenance-hosts (m). Infected foxes (I) invade villages in search of food, bringing a source of infection for L. longipalpis, which occasionally transmit the parasite to dogs or man (a). Where the transmission cycle among foxes (Cerdocyon thous) takes place is uncertain. Possibly it is in the open savanna (extreme right); or in wooded areas on the village periphery, where other wild animal hosts (?) could be involved.
appears to be this parasite has been found in the same animal in Honduras, Central America (personal communication, Dr. Bryce C. Walton).

Whatever animal host proves to be the most important, the presence of a wild reservoir of *L. d. chagasi* does much to explain the sporadic cases or periodic outbreaks of canine and human AVL, which are probably ‘spill-overs’ from the silvatic cycle into the domestic situation (Fig. 15), such as may occur after cessation or slackening of insecticide spraying programmes. During our investigations of the Marajó focus we were told that it was not customary to spray the chicken-houses during the anti-malaria campaigns; and this has enabled *Lu. longipalpis* to maintain a relatively high population in these refuges, in spite of being virtually banished from human living-quarters. Sporadic canine and, more rarely, human infections are presumably derived from sandflies which have fed on infected, marauding foxes which, we were told, very commonly raid the villages at night for chicken, ducks and other food. Whether or not any other species of sandfly is involved in the silvatic cycle is still uncertain.

Evidence for the existence of a feral reservoir of *L. d. chagasi* does not, of course, diminish the very important role of the dog as a source of infection for man. Dogs seem to become infected much more frequently than man (ALENCAR et al., 1962), presumably because the sandfly vector feeds more readily on these animals. Furthermore, dogs develop very heavy infections, with so many parasites in the skin and/or blood that they may provide a 100% infection-rate in sandflies fed on them (DEANE & DEANE, 1954a and b). Recent studies on the epidemiology of AVL in rural districts of the State of Rio de Janeiro, Brazil, have confirmed the classical *Lu. longipalpis*-dog-man association, with very high rates of infection in the dogs (MARZOCHI et al., 1983; COUTINHO et al., 1983; TRAMONTANO, 1983). To my knowledge, however, they have not included the examination of wild animals.

Although DEANE & DEANE (1954a) were able to find scanty amastigotes in the skin of some patients, they concluded that man could rarely serve as a direct source of infection for sandflies and that man-to-man transmission was unlikely. TOLEDO et al. (1983) suggested the existence of asymptomatic carriers of *L. d. chagasi* in a focus of AVL in Campo Grande, Rio de Janeiro but, as other authors have found the period of incubation to sometimes be as long as three years (CUNHA LIMA et al., 1979), the epidemiological significance of such infections remains difficult to assess. Clean, laboratory-bred *Lu. longipalpis* need to be fed repeatedly on such patients in order to settle this point.

WARD et al. (1983) have confirmed Mangabeira’s observations on slight morphological differences between *Lu. longipalpis* from Pará (Marajó) and Ceará and, following cross experiments with laboratory-bred flies, they concluded that ‘...there are at least two different sexually isolated forms of *Lu. longipalpis* which may represent members of a species complex ...’. Preliminary studies suggested that one ‘form’ was much more anthropophilic and more frequently found in houses than the other. This might be another explanation for the sporadic nature of AVL in some geographic areas (e.g., the Amazon Region) and the almost epidemic levels seen in others (e.g. Bahia and Ceará). Further studies to follow up this important new facet in the epidemiology of AVL are clearly indicated.

In conclusion, enzyme electrophoresis and serology have not yet proved of great use in distinguishing between *L. d. infantum* and *L. d. chagasi* (LAISON et al., 1981a; SCHNUR et al., 1981) and this has tended to perpetuate the belief that the two are identical. The author has discussed limitations of enzyme electrophoresis methods in distinguishing the leishmanias, however (LAISON, 1982a) and recent radiorespirometry and DNA analysis studies have, in fact, indicated significant differences between *L. d. infantum* and *L. d. chagasi* (JACKSON et al., 1982; Dr. J. E. D. Jackson, personal communication). It is to be hoped that extended investigations, in particular those using monoclonal antibodies, may help to clarify the situation further.

Conclusions

As the first species of *Leishmania* encountered were agents of visceral and cutaneous leishmaniasis of man, it is understandable that subsequent studies were for a long time concentrated on those leishmanias commonly associated with human disease. It has become clear, however, that these represent only the ‘tip of the iceberg’, and that the genus *Leishmania* is comprised of many more species of well adapted parasites in a wide range of mammals throughout many tropical and sub-tropical regions of the world.

A great many of these organisms probably have no access to man, due to host-specificity on their part or, more likely, on the part of their sandfly vectors. In the Americas we may mention *L. m. enriettii*, so far known only in guinea-pigs; *L. hertigi* in porcupines; the *Leishmania* recently discovered in the nine-banded armadillo; some other organisms found in other forest animals (and occasionally man); and a number of seemingly distinct leishmanias till now known only from certain species of sandflies (LAISON & SHAW 1979; LAISON et al., 1979b & 1981c; Lainson, Shaw, Ward & Ready, unpublished observations; ZELEDON et al., 1979).

There is strong evidence for natural barriers limiting the cycle of transmission of many leishmanial parasites to specific mammalian hosts (or to some mammalian groups) by certain sandfly species. The following associations, for example, appear to be rigidly maintained: *L. m. mexicana* (rodents and *Lu. olmeca olmeca*); *L. m. amazonensis* (rodents and *Lu. flaviscutellata*); *L. b. panamensis* (edentates and *Lu. traptoid*); *L. b. guyanensis* (edentates and *Lu. umbratilis*); *L. d. chagasi* (canids and *Lu. longipalpis*). Experimental evidence supports this natural host restriction, particularly with regards the sandfly vectors: thus, repeated attempts to establish subspecies of *L. braziliensis* in laboratory-bred *Lu. flaviscutellata* have failed, the parasites seemingly being unable to colonize the midgut and foregut of this insect (Lainson, Shaw & Ready, unpublished observations).

The sandfly vectors involved in the above-mentioned transmission cycles do bite man, to a variable degree, and in some cases this may result in high infection rates in man when he intrudes into the enzootic areas. As far as is known, however, such
human infections most probably represent a "dead-end" for the parasites concerned. On the other hand, it would seem that the vectors of some other leishmanias are much more selective with regards the animal species on which they will feed, which may restrict certain parasites to specific mammalian hosts. This would seem to be the case for *L. hertigi* of porcupines, and the *Leishmania* of the nine-banded armadillo, which have never been encountered in man, or any other animal, in forest areas in which up to 70% of porcupines and armadillos are infected. The continued isolation and comparison of *Leishmania* from man, wild animals and sandflies in the same localities is the only sure way of assessing the medical importance, and restriction certain parasites to specific mammalian hosts found in the South American forests.

Finally, throughout this discussion I have continued to use the trinomial nomenclature for the leishmanial parasites, as adopted by previous workers (Pessôa, 1961; Hommel, 1978; Lainson & Shaw, 1972 and 1979; Lainson, 1982a and 1982b). Suspicions have been aroused, however, that there may be geographical or ecological overlap of a number of the New World leishmanias, in particular those at present referred to as subspecies of *L. braziliensis* (Walton et al., 1977, in Panama; Lainson et al., 1981c, in Brazil). This being so, serious consideration must be given to the validity of at least some of the subspecific names currently in use, and a revision of the present classification of the leishmanias is clearly necessary.

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