The epidemiology of dermal leishmaniasis in British Honduras

III. The transmission of *Leishmania mexicana* to man by *Phlebotomus pessoanus*, with observations on the development of the parasite in different species of *Phlebotomus*

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In previous papers (LAINSON and STRANGWAYS-DIXON, 1962; 1963; 1964) we discussed dermal leishmaniasis in British Honduras, with particular reference to the human disease (“chiclero’s ulcer”) and the demonstration of reservoir hosts among the forest rodents. We now wish to record our studies on the transmission of *Leishmania mexicana*, commencing with a brief review of some of the previous literature on the role of phlebotomine sandflies as vectors of New World leishmaniasis in general.

Earlier work leading to the incrimination of *Phlebotomus* species as vectors of visceral and cutaneous leishmaniasis in the Old World has already been extensively reviewed (ADLER and THEODOR, 1957; ADLER, 1964) and need not be repeated here. It was assumed that these insects would ultimately be shown to transmit these diseases...
in the New World, and attention was soon drawn to the coincidental distribution there of leishmaniasis and the presumed vectors (PESSÔA and BARRETTO, 1944; HERRER, 1951; RODRIGUEZ and AVILÉS NUGUÉ, 1953; FLOCH and SUREAU, 1953; DEANE, 1956).

In the New World, however, the situation is more complex and was well summarized by ADLER (1964), who wrote:

[...] The leishmanias of mammals have speciated more in the New than in the Old World and each species may well have its own vectors, reservoir and spectrum of host infectivity. Speciation in the genus Phlebotomus has also been more extensive and more complex. [...] The bionomics of forest species [...] will not be easy to elucidate. A few important species appear to have no particular food preferences, e.g. P. longipalpis [...] feeds readily on birds, rodents, equines, dogs and man. [...] Some thirty species of sandflies have been found associated with Cuniculus paca [a large forest rodent]; this illustrates the complexity of the problems in South America.

A further serious obstacle has long been the extreme difficulty in breeding, maintaining and feeding many of the New World phlebotomines.

Until recently, evidence of transmission of Leishmania by Phlebotomus in the Americas was based on the finding of leptomonads in wild-caught insects (PIFANO, 1940, 1943 and 1958; DEANE 1956; JOHNSON et al., 1963; McCONNELL, 1963), or the experimental infection of different species of sandflies by feeding them on infected animals (PESSÔA and COUTINHO, 1941; DEANE, 1956; PIFANO, 1958; GORGAS MEMORIAL LABORATORY REPORT, 1962; COELHO and FALCÃO, 1962). PIFANO (1958) produced stronger evidence of transmission of cutaneous leishmaniasis, by Phlebotomus panamensis, in Venezuela; 17 specimens of this sandfly were taken from a man acting as bait for the collection of sandflies, and four weeks later he developed two dermal nodules containing Leishmania. Unfortunately it is not clear if those nodules arose exactly where the sandflies had bitten, and none of the insects were dissected for leptomonads.

Finally, in British Honduras, we demonstrated the transmission of L. mexicana to man by Phlebotomus under controlled
experimental conditions (STRANGWAYS-DIXON and LAINSON, 1962), and in the same year the Brazilian workers (COELHO and FALCÃO, 1962a) transmitted both *L. mexicana* and *L. braziliensis* by *Phlebotomus* species to hamsters.

**PRESENT INVESTIGATIONS**

Investigations on both parasite and vector were necessary before any transmission experiments could be attempted. These may be listed as follows, before each is discussed in detail:

- a) systematics of the vector;
- b) identification of man-biting species;
- c) source of sandflies for experimental purposes;
- d) maintenance of *Phlebotomus* in the laboratory;
- e) source of infection for sandflies: the parasite;
- f) transmission of *L. mexicana* to man; the susceptibility of different species of *Phlebotomus* to infection;
- g) the development of *L. mexicana* in the insect host;
- h) natural infections of *Phlebotomus* species with *L. mexicana*, or other flagellates;
- i) mechanical transmission.

a) Systematics of the vector

Phlebotomines collected in different parts of the country were returned to the laboratory, either dried or in 70% ethyl alcohol, and 18 known species were identified as follows:

*Phlebotomus apicalis* Floch; *P. beltrani* Vargas and Diaz;
*P. bispinosus* Fairchild and Hertig; *P. carpenteri* F. and H.;
*P. cayennensis maciasi* F. and H.; *P. cruciatus* Coquillett;
*P. deleoni* F. and H.; *P. galindo F.* and H.; *P. geniculatus* Mangabeira;
*P. hamatus* F. and H.; *P. ovallesi* Ortiz; *P. panamensis* Shannon;
P. permirus F. and H.; P. pessoanus* Barretto; P. shannoni Dyar;
P. trinidadensis Newstead; P. undulatus F. and H.;
P. ylephiletor F. and H.

Identifications were made from descriptions by FAIRCHILD (1955) and FAIRCHILD and HERTIG (1947, 1947a, 1948, 1948a, 1950, 1951, 1952, 1953, 1953a, 1956 and 1958), and LEWIS and GARNHAM (1959). Most of these species have previously been described from British Honduras by LEWIS and GARNHAM (1959) and WILLIAMS et al. (1965) but, as far as we know, our records of P. carpenteri, P. galindoi, P. pessoanus and P. undulatus remain to be confirmed.

In addition, we collected specimens of seven other sandflies which we have so far not identified. Some of these, at least, may represent new species and we hope to publish further details later.

b) Identification of man-biting species

To establish which species might feed on man collections were made in widely different areas of forest, with man as bait. Trapping was continuous over 24 hour periods, with members of the team sitting or standing at ground level and stripped to the waist. At night complete darkness was maintained, flashlights being used only when flies were removed.

The bite of *Phlebotomus* is usually accompanied by a severe stinging sensation, ensuring collection of only those insects actually probing. A hunting-light strapped to the forehead was found particularly useful, as this leaves both hands free for manipulation of collecting apparatus.

* This species was identified from the description of FAIRCHILD and HERTIG (1951) as *P. paraensis*, and was recorded as such in our preliminary note (STRANGWAYS-DIXON and LAINSON, 1962). These authors have since informed us that the species is in fact *P. pessoanus*. 
We collected nine species biting man. They were: *P. apicalis*, *P. bispinosus*, *P. cruciatus*, *P. geniculatus*, *P. ovallesi*, *P. panamensis*, *P. pessoanus*, *P. shannoni* and *P. ylephiletor*.

c) Source of sandflies for experimental purposes

Ideally, laboratory-bred insects are the choice in any transmission experiment. Phlebotomines, however, are difficult to breed and maintain in the laboratory, and of the relatively few insects bred at a given time a proportion are males, useless for experimental purposes. Furthermore, laboratory-bred females, in our hands, could rarely be induced to feed in numbers sufficient for transmission studies.

In contrast, wild-caught flies taken from human bait were (obviously) all females, all man-biters, and all physiologically eager to feed. Given the right conditions, many hundreds of such sandflies could quickly be obtained for use in transmission experiments, and these collections inevitably included most of the man-biting species listed above. This was to prove useful in assessing the susceptibility of different species to infection with *L. mexicana*, and wild-caught insects were therefore used in all the following studies. The possibility that naturally infected sandflies were present in our catches does not appreciably affect the present findings. Firstly, the proportion of naturally infected insects was found to be very low, while our experimental infection rate was nearly 100%. Secondly, we were simply attempting to demonstrate transmission of *L. mexicana* by *Phlebotomus*, and for this purpose it would matter little if the sandflies were infected naturally or experimentally.

To ensure maximum supplies of sandflies we studied both the immediate feeding habits of the man-biting species in relation to time, temperature, humidity and other environmental factors, and seasonal fluctuations of phlebotomine populations in general. For a period of just over two years, weekly collections were made of phlebotomines resting in selected tree buttresses in neighbouring forest. Data obtained indicated, as expected, that populations were high during the wet season and low during the dry months. We found, however, that an exceptionally heavy rainstorm during the wet season would temporarily reduce the usually
high population, while conversely, rain during the dry season would lead to a temporary rise in the numbers of sandflies collected. Our transmission experiments could therefore be planned to coincide with conditions producing maximum phlebotomine density.

For our main trapping area we selected an area of forest yielding consistently high catches of man-biters. This was situated at Roaring river, about five miles South along the Humming-Bird Highway (see map, LAINSON and STRANGWAYS-DIXON, 1964), and only some 100-200 yards into high forest flanking this road. The selected site was to yield both naturally infected rodents and infected sandflies. Although the higher forest here was practically levelled to the ground during hurricane “Hattie” in 1961, the area is still successfully used as a major trapping site today.

d) Laboratory maintenance of *Phlebotomus*

Attempts to maintain wild-caught sandflies in a wide variety of cages were unsuccessful. In one cage, made large enough for a man to enter as bait, the natural habitat was imitated as closely as possible by using soil and plants from the forest.

By comparison, keeping single sandflies in glass or Perspex tubes gave much better results. In addition, these tubes enabled more easy handling and the subsequent identification of single insects whose individual experimental histories could be recorded from the moment of capture.

The insects were isolated in corked, 3 x 1 inch glass tubes or 2½ x ½ inch Perspex tubes, each cork with a vertical groove cut in it to allow air exchange. All tubes were kept at 100% relative humidity and in continual light, and each contained a single, green, hairless leaf which was replaced when showing signs of drying. The surrounding temperature was kept at about 25°C.

Under these conditions our experimental sandflies survived up to a maximum of 18 days from the date of capture, the usual survival period being 4-8 days. Though we were by no means satisfied with this brief life-span, we felt that it afforded sufficient time in which to conduct
transmission experiments, and that these should take preference over further attempts to improve on vector maintenance.

e) Source of infection for sandflies: The parasite

Two strains of *L. mexicana* were used throughout the following work: H 4, of human origin, and M 379 isolated from a wild rodent *Nyctomys sumichrasti* (LAINSON and STRANGWAYS-DIXON, 1963; 1964)

In hamsters and mice *L. mexicana* produces large, tumour-like skin lesions which contain vast numbers of Leishman-Donovan (LD) bodies, and such lesions were obvious sites on which to feed sandflies. Consistent failure to demonstrate parasites in peripheral blood, viscera and normal skin in fact suggested that they are normally restricted to the primary lesion, and infection rates for sandflies fed directly on lesions, at the periphery and on normal adjacent skin were 95%, 48% and zero respectively.

f) Transmission of *L. mexicana* to man: The susceptibility of different species of *Phlebotomus* to infection

We had already pin-pointed an area of forest where *Phlebotomus* species were to be found naturally infected with *L. mexicana*, the parasite being isolated in a hamster which had been inoculated with a suspension of some 300 wild-caught sandflies triturated in Locke’s solution (LAINSON and STRANGWAYS-DIXON, 1964)

Man-biters were collected from the trapping site, throughout the night, and the tubed insects at once taken to a temporary laboratory outside the forest, where they were immediately fed on the infected animals. Usually the sandflies, so recently interrupted in their attempts to feed on human bait, resumed feeding at once, or not at all. Those not feeding at once were rejected.

This trapping and feeding of *Phlebotomus* was continued until the early hours of the morning when the fed flies were taken to our main laboratory at Baking Pot, maintained under the conditions described
above, and given the opportunity to re-feed on volunteers on each succeeding day.

Phlebotomines are generally reluctant to re-feed in captivity and this constituted our greatest barrier in these investigations. Attempts to re-feed the infected sandflies were often continued throughout 24 hour periods. One useful inducement was to hold the reluctant sandfly in the current of cool air from an air conditioner, to the point when the insect was immobilized. After transfer to the skin of volunteers, such insects often probed vigorously on recovery, and sometimes completed a blood meal. All bites were plotted, whether or not they resulted in ingestion of blood.

Five such transmission experiments were made, with a total of 332 sandflies including all the known man-biters from British Honduras. As shown in Table 1****, 52 flies were induced to re-feed on volunteers. Of these, eight fed a second time and one a third time. Occasionally insects probed more than once before taking blood: the 52 insects concerned thus re-fed on 61 occasions, inflicting a total of 90 probes.

Transmission was achieved on one occasion. A small papule, about 3mm in diameter, appeared at the site of a bite on the abdomen of one volunteer, 17 days after the insect had probed. LD bodies were found in smears from the incised lesion, and NNN culture of the exudate quickly produced flagellates.

The vector was identified as P. pessoanus Barretto (see footnote, p. 286) and transmission took place at the insect’s second "blood meal", just three days and 23 hours after it had fed on the infected hamster. The infective bite was in fact only a 30 second probe resulting in no actual ingestion of blood.

From dissections during this and other experiments we found that all nine man-biting species of Phlebotomus (p. 287) were readily infected with L. mexicana, and all these insects must therefore be

**** N. ed.: A tabela mencionada não consta no original.
regarded as potential vectors. In this respect it may be noted that, in each species, massive development of the flagellates took place 3-5 days after the infective feed, with steady migration of the leptomonads to the anterior station of the insect’s gut. Active leptomonads were seen throughout the length of the proboscis in one specimen of *P. ylephiletor* during these experiments, and in a recent personal communication, Dr. Paul Williams, in British Honduras, tells us that he has now transmitted *L. mexicana* to man by the bite of *P. cruciatus* on two occasions.

**g) The development of *L. mexicana* in the insect host**

These observations were made after a study of smears of gut contents and sections of entire insects, which were fixed at three, six, 12, 24, 36, and 48 hours and 3-5 days after the infective feed on hamster or mouse lesions. Two strains of *L. mexicana* were studied (H1, human origin, and M 379, rodent origin; see LAINSON and STRANGWAYS-DIXON, 1963; 1964) and their development in *Phlebotomus* was found to be similar in all respects.

Smears of dissected guts were wet-fixed in aqueous Bouin fluid for 20 minutes, stained for 1-2 hours in Giemsa stain (two drops per 1ml of buffered distilled water, pH 7.4), differentiated in graded acetone-xylene mixtures, and then mounted in green Euparal. Whole insects were fixed in Carnoy fluid for 20-30 minutes before double-embedding by the usual celloidin method. Serial sections were prepared at 3-5µ and stained by the Giemsa-colophonium method.

The cutaneous lesions on which the sandflies fed contained very large numbers of LD bodies and it might be argued that this might influence the infection in the insect, in particular the time taken for leptomonads to reach the mouth parts and achieve transmission. We were surprised to find, however, that few leishmaniae are in fact ingested during the blood meal on the hamster lesion, and a long search was needed to find LD bodies in smears of guts from insects fed between three and six hours previously. The scanty parasites seen at this time (Fig. 1) measured 3-4µ x 2-3µ and were morphologically similar to leishmaniae seen in the vertebrate host.
The first indication of development of the ingested LD body appeared to be between 12 and 24 hours, when a few aflagellate forms were seen measuring 6-10µ and even up to 16µ in diameter (Figs. 2-4); they possessed a highly vacuolated cytoplasm, staining pale blue, and a rod-shaped kinetoplast, sometimes 2µ long. We have gained an impression that this early growth phase of the LD body is followed by a period of binary fission (Figs. 4-5) before the development of the flagellum, for in some smears made at 24 hours we have seen clusters of such aflagellate bodies, some of which have two nuclei and two kinetoplasts (Fig. 4).

Transformation into leptomonads by the development of the flagellum appears to commence 24-36 hours after the infective feed (Figs. 6-7). From this time onward development is rapid and from 4-5 days after the infective feed the entire midgut and foregut is packed with vigorously active flagellates, and the sandfly, with leptomonads now invading the proboscis, is capable of transmitting the infection (Figs. 11, 13-15). The speed of development of \textit{L. mexicana} in the local man-biting phlebotomines is thus surprisingly rapid, being approximately twice that recorded for Indian strains of \textit{L. donovani} (SHORTT et al., 1928) and \textit{L. tropica} (ADLER and THEODOR, 1927).

On no occasion did we see development of \textit{L. mexicana} in the hind-gut, although “back-wash” of flagellates into this region was sometimes seen to follow the more violent peristaltic movements of a heavily infected midgut under a cover-slip. In contrast, dr. Hertig and his colleagues have shown that Panamanian strains of \textit{Leishmania} undergo partial or even complete development in the rear station of the gut in both naturally and experimentally infected \textit{Phlebotomus} species (HERTIG and McCONNELL, 1963; JOHNSON et al., 1963; McCONNELL, 1963). Only rarely, too, (Fig. 15) did we see evidence of attachment of the leptomonads of \textit{L. mexicana} to the gut wall of the infected sandflies, forming the orderly “pallisades” of flagellates described by other authors (ADLER and THEODOR, 1927; HERTIG and McCONNELL, 1963; JOHNSON et al., 1963; and others). Perhaps
owing to the somewhat explosive development of *L. mexicana* in its insect hosts, the flagellates usually appeared to be scattered freely throughout the digesting blood meal in the midgut, and later appeared merely as a disorganized mass (Figs. 11-14)

Some workers have felt that “infective forms” of leptomonads, in cultures or in the insect host, can be distinguished morphologically from earlier division forms. Though such a suggestion is somewhat speculative, we have noted numerous unusually long slender forms (Figs. 8-10), particularly in the sandflies with older infections. Such forms are not met with in earlier infections and are less commonly seen in *in vitro* cultures: Their significance remains doubtful. Similar stages have been reported in sandflies infected with *L. tropica* (ADLER and THEODOR, 1927)

h) Natural infections of *Phlebotomus* species with

*L. mexicana* or other flagellates

This important study is one which time, unfortunately, did not permit us to pursue sufficiently. Now that reservoir hosts and many potential natural vector species have been indicated, however, this aspect of the epidemiological studies should receive major attention. *L. mexicana* develops readily in all the known man-biting phlebotomines in British Honduras and has actually been transmitted by two of them in the laboratory. In nature, however, the vectors responsible for maintaining the disease among the rodent population must be rodent-feeders, and all of them may not commonly bite man on the rare occasions when he presents himself in the forest. A study of the associations of forest phlebotomines with the infected rodents, and the incidence of *L. mexicana* infection among them, is thus of the utmost importance.

Four hundred and thirty-nine wild-caught female sandflies were dissected and the guts examined for flagellates. All were trapped, off human bait, in high forest some five miles north of Central Farm (Baking Pot), and represented the following species:
Eight *P. apicalis*; 30 *P. bispinosus*; six *P. cruciatus*; one *P. geniculatus*; 188 *P. ovallesi*; 110 *P. panamensis*; 48 *P. shannoni*; 48 *P. ylephiletor*.

Only two insects harboured flagellates. In one specimen of *P. ovallesi* a cluster of about 20 parasites was seen in the hind-gut (immediately behind the Malpighian tubules). In the other insect (*P. cruciatus*) very scanty flagellates were detected only when the gut was ruptured by the pressure on the cover-slip, and we are uncertain of their exact location in the insect.

As we had not yet established a hamster colony we were unable to check the infectivity of the flagellates for these animals. *L. mexicana* infects hamsters so readily (with as few as ten leptomonads) that, in future, animal inoculation would probably be the best method to adopt in identifying this parasite within the insect host.

From their scanty numbers and position in the rear-gut, however, we felt that the parasites in *P. ovallesi* were not leptomonads of *L. mexicana*. Of those in *P. cruciatus* we can say little, but as the flies were trapped in the same area of forest it is likely that the flagellates in the two insects had a common source. We have no clue concerning this source, but it is interesting to recall the high incidence of similar rear-gut infections described in wild-caught Panamanian sandflies (JOHNSON et al., 1963), the origin of which is again obscure. Clearly, the best areas from which to examine individual sandflies are those in which infected wild rodents have been trapped. We have already mentioned the isolation of *L. mexicana* from a batch of some 300 sandflies caught in such an area, after inoculating a suspension of the pooled insects into a hamster. Disappointingly, the individual dissection of flies from this area has so far produced no infected species (WILLIAMS et al., 1965). This at first seems curious in view of the abundance of infected rodents there, but may be a reflection of our previous suggestion that the common vectors are predominantly rodent-feeders. They infrequently attack man and are, therefore, infrequently caught.
Development of *Leishmania mexicana* in sandflies of British Honduras
Fig. 1 – Unaltered Leishman-Donovan (LD) body as seen in mid-gut smears from specimens of *Phlebotomus cruciatus* and *P. ylephiletor* fed on a hamster lesion, 3-6 hours previously.

Figs. 2-5 – Growth phase of LD bodies of *L. mexicana* in *P. cruciatus* and *P. ylephiletor*, from 12-24 hours after these sandflies had fed on infected hamsters. Aflagellate parasites are undergoing rapid binary fission.

Figs. 6-7 – Development of the flagellum from the enlarged LD body, as seen in smears of the mid-gut of specimens of *P. cruciatus*, *P. ylephiletor* and *P. geniculatus*, 24-36 hours after the sandflies had fed on infected hamsters.

Fig. 8 – Fully developed, slender form of leptomonad of *L. mexicana* as seen in the midgut, foregut and mouthparts of *P. cruciatus* and *P. ylephiletor* from 4-5 days after the infecting feed on hamsters.
Development of *Leishmania mexicana* in sandflies of British Honduras

(legenda no verso)
Fig. 9 – Developing and dividing leptomonads in a smear of the midgut of a specimen of *Phlebotomus cruciatus*, 36 hours after the insect had fed on the lesion of an infected hamster (oil immersion objective, further enlarged)

Fig. 10 – Fully developed leptomonads in a smear of the midgut contents of a specimen of *P. ylephiletor*, four days after the sandfly had fed on an infected hamster (oil immersion objective, further enlarged)

Fig. 11 – Low power view of a section of an entire specimen of *P. cruciatus*, five days after the insect’s infective feed on a hamster lesion. Leptomonads (arrowed) are packed throughout the midgut and foregut and also can be seen associated with the remains of the infecting blood meal.

Fig. 12 – Smear of midgut of *P. cruciatus*, low power view, to show density of leptomonads, four days after the fly had been fed on the infected hamster.

Fig. 13 – Section of *P. cruciatus* fed five days previously on lesion of infected hamster. Low power view showing leptomonads packing the cardia.

Fig. 14 – High power view of the same section, showing leptomonads (arrowed) passing through the oesophagus into the biting mouthparts. Further sections of the same sandfly showed parasites extending to the tip of the proboscis.

Fig. 15 – Section of cardia of same insect. High power view showing free leptomonads and a mass of others detaching from the intestinal wall (arrowed)
Mechanical transmission

By this we refer to the possibility of direct transfer of parasites by the contaminated mouthparts of a fly which, interrupted while feeding on the infected animal, at once recommences feeding on another, uninfected host.

A number of sandflies were interrupted while feeding on hamster lesions and then allowed at once to complete their blood meal on volunteers. No transmissions were achieved, though 21 specimens of P. ylephiletor, one P. shannoni and one P. ovallesi were used, which altogether inflicted 39 probes. LAINSON and SOUTHGATE (1965), however, succeeded in transmitting L. mexicana to a clean hamster by exactly the same method, using Stomoxys as the vector. The surprisingly high rate of three transmissions followed single bites from only four flies. The efficiency of Stomoxys in this mode of transmission may be due to its much larger mouthparts and the more severe bite inflicted.

A much more likely mode of mechanical transmission, as far as man is concerned, would be the squashing of feeding sandflies on the skin and resulting contamination of the bite wound with the vast numbers of flagellates freed from the infected insect (Fig. 12). Only a strong-willed person can resist taking a slap at a biting insect and a subsequent scratch of the irritated part. The sandfly often inflicts a painful bite and, as it displays a remarkable tenacity while feeding, it probably rivals the mosquito as the most frequently “swatted” biting insect of the forest.

In conclusion, the question has frequently been asked – can man act as a source of L. mexicana for sandflies, in nature? As far as we can see this is most unlikely. Although we have on one occasion infected six out of eight sandflies (P. cruciatus and P. ylephiletor) by feeding them on man, this proved possible only when the insects were placed directly on a newly acquired, non-ulcerative lesion which contained unusually large numbers of parasites (Case W. F., Fig. 23,
LAINSON and STRANGWAYS-DIXON, 1963). Sandflies show a marked reluctance to feed on the scabbed, horny layers of older lesions which, in any case, contain only scanty parasites in the deeper layers beyond the reach of the insect’s proboscis.

A lengthy chain of circumstances would be needed for man-to-man transmission – constant exposure of a suitable lesion; the choice of such a lesion as a biting site in preference to the rest of the exposed body; survival of those very few insects that have fed on this lesion; presence of a further human being in that immediate area of the forest 4-5 days later, when the insects are infective; and, finally, the choice of these persons as a source of food.

**SUMMARY**

During 1960-62 a study on the epidemiology of dermal leishmaniasis due to *Leishmania mexicana* in British Honduras revealed 18 species of *Phlebotomus* in the enzootic forest areas. In addition, seven other sandflies remain to be identified and may represent new species.

Nine species of *Phlebotomus* were found commonly to bite man. All were predominantly nocturnal in their feeding habits and, as they were readily infected with *L. mexicana*, all must be regarded as potential vectors in nature. The infection rates for sandflies fed directly on hamster lesions, at the periphery, and on normal adjacent skin were 95%, 48% and 0% respectively.

Maintenance of wild-caught *Phlebotomus* species is discussed. Best results were obtained by keeping single flies in corked tubes containing a fresh green leaf. The corks had a groove cut throughout their length, to allow air exchange, and all tubes were kept in constant light and at approximately 100% relative humidity.

Three hundred and thirty-two sandflies, including all the known man-biting species from British Honduras, were fed on the
lesions of hamsters and mice infected with both human and rodent strains of *L. mexicana*. Fifty-two flies were induced to re-feed on volunteers (eight fed a second time and one a third time), in all inflicting a total of 90 probes. Transmission of *L. mexicana* to man was achieved, by *Phlebotomus pessoanus*, on one occasion. This insect had fed on the infected hamster only three days and 23 hours previously.

The development of *L. mexicana* in the insect host has been followed by a study of the gut contents and sections of entire sandflies which were fixed at three, six, 12, 24, 36, and 48 hours and 3-5 days after their infective feed. The development is to an anterior station, the leptomonads reaching the proboscis as early as four days after the infecting blood meal.

Four hundred and thirty-nine wild-caught female sandflies were dissected in an attempt to find insects naturally infected with *L. mexicana*. Flagellates were seen in the hind-gut of one specimen of *P. ovallesi* and others in the crushed gut from a single *P. cruciatus* (exact position in the gut uncertain). In the absence of animal inoculation the nature of these flagellates remains uncertain, but they are not thought to be leptomonads of *L. mexicana*.

Attempts to demonstrate mechanical transmission of *L. mexicana* were made by interrupting sandflies feeding on hamster lesions and immediately allowing such insects to renew feeding on volunteers. No transmission was achieved and it is felt that such a mode of transfer plays little or no part in the transmission of the parasite in nature. On the contrary, the killing and squashing of infected sandflies feeding on human skin may well facilitate entry of the parasite.

Although sandflies were successfully infected with *L. mexicana* after feeding on a newly acquired and non-ulcerative human lesion, man-to-man transmission is considered most unlikely in nature.
REFERENCES


DISCUSSION

This discussion took place on 16 May 1963, after dr. Lainson had described Part I of the series, and given a summary of Parts II and III. As the discussion largely concerned the reservoir hosts and transmission of L. braziliensis, it was postponed to the end of Part III.

Professor P. C. C. Garnham

Anybody who has worked on the epidemiology of leishmaniasis knows how difficult it is to get a break-through, and when drs. Lainson and Strangways-Dixon went to British Honduras three years ago, I thought that in these rough and primitive conditions, they would have a particularly hard task. The story that dr. Lainson has just told us shows that the task was hard enough – but the investigation proceeded like clockwork and it is clear that these workers have a flair for getting to the heart of the matter.

They have uncovered the existence of a beautiful zoonosis, and it is interesting perhaps to speculate a little further on the mechanism of this process. Dr. Lainson has shown that at least three wild rodents are concerned. Probably other species also play a part.

More by intuition than luck, the British Honduras team chose a little place in the forest, just off the Humming-Bird Highway, where everything unfolded before them – or perhaps I should say instead,
where they unravelled everything. May I ask dr. Lainson if he thinks the same intensity of infection prevails throughout the forests of British Honduras? Probably the condition is bound up with the presence of the special reservoirs and that is why *L. mexicana* is confined solely to regions where these rodents are found. Further South, conditions change: the forest gives way to cultivation, specialized arboreal rodents are driven off, and “bay-sore” is replaced by another form of leishmaniasis which requires less exacting conditions. Probably the cases of leishmaniasis in Spanish Honduras are of the latter type.

It seems as if *L. mexicana* is thus a product of a special rodent host – the species of *Phlebotomus* on the other hand are ubiquitous, and much the same kinds extend from Panama in the South to Yucatan in the North. But if this is so, why are the peculiar ear lesions confined to the Northern countries? The lesion on the ear must be the site of the *Phlebotomus* bite, and nothing to do with the species of *Leishmania*. Is *P. pessoanus* a vector in Panama?

I understand that the disease in the wild rodents is principally confined to lesions on the tail. Is the blood ever invaded, or do the animals eventually recover without visceral metastasis? If the infection is entirely chronic in the rodents, this may indicate the antiquity of the zoonosis, dating back perhaps a thousand years ago, when the Maya civilization began to decline and the heavily populated country reverted to forest. On the contrary, in Ceará (N.E. Brazil) dr. and mrs. Deane suggested that the severity of the leishmaniasis in the local reservoir – a sort of fox – meant that the zoonosis of that region was of recent origin.

Both the chicleros and the Maya indians keep large numbers of dogs for hunting. Dr. Lewis and I examined dogs from a Maya village without finding *Leishmania*. I wonder if dr. Lainson discovered anything in these animals? And how do dogs respond to inoculation with *L. mexicana*? If they are susceptible, they should readily contract the infection in the forest, while hunting, and pass it on to the villagers. But the latter – the women and children – never get “bay-sore”.
There is a puzzle here, and perhaps the new team which has just gone to British Honduras may find a solution.

Dr. Lainson has shown that the disease is much more important in British Honduras than was originally thought. When we were there in 1958, the Governor asked us to assess the danger of “bay-sore” to N. American tourists who, he thought, might flood into the country at some time in the future. We said that the danger was slight, but having listened to Dr. Lainson’s story, this opinion seems to be wrong and I should like to hear his comments.

Dr. R. S. Bray

I should like to add my congratulations to those of the previous speaker on a remarkable piece of detective work. To me the successful solving of these epidemiological puzzles in the short space of time available to Drs. Lainson and Strangways-Dixon is little short of fantastic.

I am going to be unfair to them both by asking two questions; I fully realize they could have had little or no time in which to find the answers. However, to clarify my own mind on the general epidemiological picture, and perhaps to indicate where further work might be desirable, I would like to ask what information is available on the limits of the chiclero’s ulcer endemic to the South of British Honduras. The endemic area stretches into Yucatan in the North where it will peter out in savannah or montane. It is no doubt limited by montane conditions to the West, but is there a natural barrier to the South which could prevent its spread to Costa Rica, or indeed prevent the spread of Costa Rican dermal leishmaniasis to British Honduras? Is there any information on the disease in Eastern Guatemala, Honduras and Nicaragua which might help? I am prompted to ask this question as the name of the West coast of Honduras and Nicaragua – “Costa de Mosquitos” has a fine old tropical medicine ring to it.

My second question deals with the vector. I would like to ask Dr. Lainson if insects were captured, by using the reservoir hosts as
bait, and whether other insects coming to bite man or the reservoir hosts were identified and dissected.

Lastly I should like to make a plea for prophylaxis in view of the availability of the small population at risk. It would seem possible to cover a large proportion of the “first-time” chicle gatherers each year with a single injection. This might prompt attention to the possibility of immunization with an attenuated strain of *Leishmania mexicana* or the development of an effective immunizing antigen. In the meantime I believe the prophylactic action of the aromatic diamidines might be investigated. While they are ineffective in kala-azar they might nonetheless have some prophylactic effect with *L. mexicana*.

Dr. S. G. Browne

I should like to add my sincere congratulations to the authors of this fascinating paper.

My immediate interest in the problems of dermal leishmaniasis is clinical, and I wish to underline the differences in the clinical manifestations of leishmaniasis in the Old World and the New. In particular, I should like to ask Dr. Lainson if he observed any late or delayed cutaneous lesions in his patients who presented such well-marked and typical ulcerations at the sites of inoculation of the parasites. In Calcutta, recently, I saw a cross-section of the 57 patients attending Professor Sen Gupta’s Dermal Leishmaniasis Clinic, and I saw also another patient at Polambakkam, near Madras. In India, these examples of so-called dermal leishmaniasis arise characteristically 3-5 years after an attack of visceral kala-azar which has been successfully treated by standard procedures.

The differential diagnosis from either nodular lepromatous leprosy or atypical macular tuberculoid leprosy may at times present great difficulty, and in fact many patients have in the past been treated for leprosy and have suffered from the social stigma attaching to this disease. The succulent nodules follow the distribution of lepromatous nodules, viz., the helices and lobes of the ears, the cheeks, the chin and
the forehead. The hypopigmented macules are small and of uniform size; they are very numerous and very widespread over the trunk and limbs: like leprosy lesions, they tend to spare the axillae, the inguinal region, the scalp, the paravertebral region and areas covered by tight clothing. Leishman-Donovan bodies are extremely scanty in this macular eruption, which has many of the features of an allergic manifestation comparable with the -id rashes seen in other skin diseases. It would be of great immunological interest to know if a similar picture obtains in patients with true dermal leishmaniasis in British Honduras.

Colonel H. E. Shortt

From the lofty pinnacle of nearly 50 years’ acquaintance with *Leishmania* I am delighted tonight to look upon this latest research on this parasite. In the first place I have to congratulate dr. Lainson on a really magnificent piece of work, rendered the more creditable as this was his first experience of the tropics and the difficulties and frustrations of field work in the tropics which, though familiar to many of us, must have been novel and dishaertening to him. His energy and determination in overcoming these are promise of a brilliant research career still to come.

As regards the research work, his account leaves no doubt that he has succeeded in what he set out to do, i.e., to discover the mode of infection of the form of dermal leishmaniasis prevalent in the part of S. America where he was working. As species of *Phlebotomus* seem to be the vectors of *Leishmania* wherever the latter are found he naturally concentrated on these insects and succeeded in demonstrating here again that they are the local vectors.

There are a few points in his account on which I would like to comment or ask for further information. The distribution of lesions on parts of the body exposed to attack i.e., face and arms, which are normally uncovered, is similar to what one finds in the case of *L. tropica* and was to be expected, but the reason given for the special severity of ear lesions is interesting and certainly suggestive.
The lytic action of normal human serum on the leptomonads of *L. mexicana* is again duplicated by a similar action on those of *L. donovani*.

The large amount of work done by Dr. Lainson on the possible animal reservoirs, and the definite incrimination of some, will be of great help to future workers in this field.

Dr. Lainson has emphasized that the lesions of this form of *Leishmaniasis* are almost invariably single and that there is no cutaneous spread from an initial lesion. This would appear to be contradicted in his account of certain animals in which there were multiple lesions down the length of the tail.

I would like to ask Dr. Lainson if he had any evidence of visceral infection in any of the animals, apart from the one opossum, whether naturally or as the result of inoculation of parasites.

I have only one criticism to offer and that is a regret that no experiments were carried out with flies bred in the laboratory. This would have enabled vector species to be pin-pointed and will certainly have to be done in future work.

Thus, in the experiment where 100 assorted *Phlebotomus* were fed on animal lesions and fed for a second, third, or fourth time on volunteers, one fly caused a lesion at a second feed, and that merely a probe. As the flies were caught wild there seems a possibility that this fly may have fed previously and that, therefore, the infective feed may have been not the second but a third or even fourth, in which case the source of infection might even have been other than that offered in the laboratory.

In the same connexion it is to be noted that only in the fly which fed four times were flagellates found in the mouth parts.

With this single criticism, and I am sure that only limits of time prevented the breeding of flies, I end these remarks as I began them with congratulations to Dr. Lainson and his helpers for what I consider an outstanding piece of research which has laid solid foundations for the use of future workers in this field.
Dr. C. C. Chesterman

I would like to put to Dr. Lainson the same suggestion that I made to Professor Garnham after his paper in 1958, that it is probable that it is the lower temperature of the pinna which increases the viability of *L. mexicana* in that location. It grows at room temperature in NNN medium and Dr. Lainson remarks that lesions in the warmer areas of the body appear to heal more readily. If one wraps the tip of one’s ear round the bulb of a clinical thermometer the mercury will not rise above 95°F. Differing susceptibility to temperature of various leishmaniae is comparable to the predilection of *Mycobacterium leprae* for superficial tissues including nerves, as opposed to the viscerotropic tendency of *Mycobacterium tuberculosis*.

Sir Selwyn Selwyn-Clarke

Observed that Dr. Chesterman’s contribution had reminded him that when sitting at the feet of one of the President’s distinguished predecessors over 40 years ago, namely, the late Sir Leonard Rogers, he had learnt that it was Sir Leonard who had made the discovery some years earlier that Leishman-Donovan bodies could be cultivated in a medium kept at room rather than at body temperature.

Sir Selwyn said that he had listened to Dr. Lainson’s address with great interest but that he would be grateful if Dr. Lainson would indicate on what grounds he claimed that dermal leishmaniasis was a real problem in British Honduras, bearing in mind that the able Research Unit had encountered only 46 persons suffering from the condition during the three years’ investigation?

Dr. Lainson (in reply)

I would like to say how much we appreciate this opportunity of discussing our work before so distinguished a Society. As Professor Garnham mentioned, conditions were at times difficult and in this connection we must thank all those who helped smooth the way.

In particular we are indebted to Dr. G. V. A. Griffith, then D. M. S. for British Honduras, whose recent death has saddened us so much. Dr. Griffith played a major role in averting the threat to public...
health after the terrible hurricane of 1961 and his constant help and friendship will always be remembered.

Links with home are important. In this respect we would like to thank Dr. R. Lewthwaite and Dr. E. T. C. Spooner for hard work on our behalf and especially for the numerous visits they paid us. In London Professor P. C. C. Garnham and Dr. D. J. Lewis have been a constant source of advice and encouragement. The information gained from their pilot trip in 1959 proved most useful in our subsequent studies.

Just before we arrived in British Honduras we were fortunate enough to spend a few weeks with Dr. G. B. Fairchild and Dr. M. Hertig at the Gorgas Memorial Laboratory in Panama and we owe much to their extensive advice and information on the taxonomy of New World phlebotomines.

In British Honduras itself it is impossible to list all the good people who made our stay so profitable and enjoyable, but special mention must be made of our staff – Robert Reyes, Norris Wade, Eulalio Garcia and Liborio and Angel Gonzales. Finally, to our wives, all too seldom mentioned in our acknowledgements and yet on whose cheerful tolerance we so much depended.

In reply to your questions, first to Professor Garnham

Until further surveys are made it is impossible to be sure that L. mexicana exists throughout all the forests of British Honduras and the neighbouring countries. I think it likely, however, that the parasite will be found throughout this area wherever there is an association of the known reservoir hosts and the phlebotomine vectors. The infection appears to be restricted to the humid, medium to high forest and it is absent from coastal areas and the higher, open pine-forest in the interior. As Professor Garnham has suggested, we feel sure that L. mexicana is essentially a parasite of rodents, and man is only an accidental host.

Why chiclero’s ulcer should be so frequently be found on the ear is uncertain, but we have made some suggestions in our previous publications. The local phlebotomines do not appear very specific in
their choice of a biting site on man and the locality of the leishmanial lesions may be governed by:

a) the parts of the body most frequently exposed during the night-time activity of the vectors;

b) the tendency of lesions on the body to spontaneous cure; and

c) an apparent predilection on the part of *L. mexicana* for ear tissue and a resulting severity and chronicity of lesions there.

As far as we know, *P. pessoanus* has yet to be incriminated as a vector of *Leishmania* in Panama, but the insect certainly does occur there.

In our experience *L. mexicana* produces only a chronic, dermal infection in the wild rodent* and I feel sure that Professor Garnham is right in suggesting that the rodent-parasite relationship is of great antiquity. We have not found natural infections in animals other than man and the wild rodents we have specified: in the laboratory, however, a number of animals can be experimentally infected, the dog included. Although some dogs do accompany the chicleros to the forest the number is insufficient to be of any great epidemiological importance, and certainly a dog-vector relationship does not appear to exist in the villages, where natural acquisition of *L. mexicana* infection is unknown. As to the importance of the disease to the tourist trade, this is negligible while the number of tourists to British Honduras remains at its present low level. Chiclero’s ulcer is sufficiently disfiguring, however, to deter many from working in the forests, and this is important in a country which depends on the forest for its main exports, chicle and timber.

Dr. Bray’s questions are difficult to answer and we are at present unable to indicate the southern limit of *L. mexicana*, apart from

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hearsay reports of bay sore in the Republic of Honduras. In this connection, however, we might do well to consider the distribution of the rodent hosts.

The genus *Ototylomys* apparently extends its range throughout the forests of Central America, down to Costa Rica; the actual species known to be a reservoir of *L. mexicana* (*O. phyllotis*) occurs in Guatemala, the Yucatan and Campeché areas of Mexico, Nicaragua, Honduras and Costa Rica. The genus *Heteromys* has an even wider range, from S. Mexico to as far as Ecuador; it is even found on the island of Trinidad. Our reservoir host *H. desmarestianus* is described from Costa Rica and S. W. Panama and is presumed to be found throughout Central American forested areas southwards from the Yucatan. *Nyctomys* is described in Vera Cruz, through S. Mexico, British Honduras, Guatemala, the East coast of Nicaragua, to Costa Rica. Sub-species of *N. sumichrasti* are known to occur throughout these countries as far South as Panama.

With regard to vectors, many man-biting species of *Phlebotomus* extend from Panama in the South to the Yucatan in the North. The geographical distribution of *L. mexicana*, however, may well be determined by the absence or presence of little known species of sandflies which maintain the parasite in the rodent population. Such species may, then, be restricted to the Yucatan, British Honduras and Guatemala, and chiclero’s ulcer consequently limited to this geographical area.

We have not yet ascertained the species of *Phlebotomus* and other biting insects commonly feeding on the rodent hosts of *L. mexicana*: clearly this is a most important aspect of study. The mere suggestion of a vector other than *Phlebotomus* is almost blasphemous to most workers on leishmaniasis and we must confess that we have not spent much time in search for alternative vectors. *Culicoides* sp., comes to mind, however, as another major man-biter in our immediate work area. Since my return to London I have attempted to infect large numbers of *Aedes aegypti* with *L. mexicana*, with no success.
The subject of prophylactic treatment of the chicleros before entry into the forest also remains to be investigated, and it would certainly be interesting to examine the possible prophylactic action of certain drugs*.

In reply to Dr. S. G. Browne I can only say that we have seen no manifestation of chiclero’s ulcer other than the single, dermal lesion we have described. It is always possible, of course, that isolated instances of disseminated cutaneous lesions or even visceral involvement may occur. If so, we feel that they are exceptional.

I thank you, Colonel Shortt, for your kind remarks. I think my comment in reply to earlier questions disposes of your first query concerning the predominance of ear lesions. With regard to the multiplicity of skin lesions on the tails of some of the rodents, we feel that this is most likely due to multiple bites from infected vectors, or interrupted feeding on the part of a single infected insect. Visceral infection can occur in animals infected with *L. mexicana*, but usually only in those which have been infected for long periods. We have mentioned such infections in our preceding work and will not repeat ourselves here. We have not seen evidence of visceral infection in any naturally infected rodent**. We agree entirely with Colonel Shortt’s criticism regarding the use of only wild-caught insects in our transmission experiments. As we stressed, however, we feel that this has not materially affected the result of our work. Natural infections in wild-caught flies were extremely rare, whereas the infection rate in our experimentally infected sandflies was almost 100%. Again, our primary object was to demonstrate transmission by way of *Phlebotomus* and for this it mattered little if the insect was already infected in nature or infected by us in the laboratory.

Dr. Chesterman has raised the much discussed suggestion that the lower temperature of the pinna may influence the viability of

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* See *Transactions* 58, p. 94.
** See *Transactions* 59, p. 103.
Strangways-Dixon, J.; Lainson, R. The epidemiology of dermal leishmaniasis in British Honduras. III. The transmission of *Leishmania mexicana* to man by *Phlebotomus pessoanus* ...

*L. mexicana* in this location. Unless one attributes this as a peculiarity of *L. mexicana*, however, it remains difficult to explain why ear lesions are not a predominant feature of other forms of cutaneous leishmaniasis in the New World (e.g. uta in Peru). We certainly need to explore this question further.

Sir Selwyn’s question of the importance of chiclero’s ulcer has largely been answered in my reply to Professor Garnham. I am sorry if we gave him a wrong impression of the incidence of the disease: certainly we only examined 46 cases in detail (i.e. by NNN culture, etc.), but these were selected from some 150 bay-sore patients coming to our laboratory. Presumably there were many more infected persons inaccessible to us.

ACKNOWLEDGEMENTS

We once more wish to thank all those who helped us in this work and whose names are mentioned in our preceding papers.

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