Leishmania in phlebotomid sandflies

II. The insusceptibility of sandfly larvae to Leishmania*

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The susceptibility of fourth instar larvae of Lutzomyia flaviscutellata to Leishmania mexicana amazonensis was investigated by feeding them infected female sandflies or cultured promastigotes. Neither larvae nor adult flies reared from the larvae became infected. It is suggested that during evolution Leishmania has lost the ability, retained by the related genus Leptomonas, to be transmitted in nature from one invertebrate host to another.

The morphological similarity of promastigotes of Leishmania and Leptomonas has given rise to the reasonable assumption that these parasites arose from a common ancestral form (see review by Baker 1965). Leptomonas spp., monoxenous parasites of invertebrates, produce cysts by which transmission is effected by ingestion. We have investigated the possibility that a similar stage might be produced by Leishmania in the gut of infected sandflies. In nature, the omnivorous larvae of sandflies would then perhaps acquire infections by eating infected females dying at oviposition, in the way suggested for the transmission of a gregarine of sandflies (Lisova 1962). This would explain occasional infections of Leishmania in apparently nulliparous females. Other explanations are (i) that the parous state of the fly was misjudged (see Ward 1974), (ii) that the fly became infected by probing without taking blood (Williams 1966), or (iii) that Leptomonas was mistaken for Leishmania.

We infected sandflies with Le. mexicana amazonensis (strains M1861 and M1862) and 5–7 days later fed them to fourth instar larvae of Lutzomyia flaviscutellata, the natural vector of this parasite (Lainson & Shaw 1968; Ward, Lainson & Shaw 1973). Three species of sandflies (Lu. yuilli, Psychodopygus davisi and Ps. geniculata) coming to feed on man in the Utinga forest, Belém, Brazil, were caught and fed on lesions on the nose or feet of experimentally infected hamsters; the lesions contained masses of amastigotes of Le. m. amazonensis. Three and four days later, a total of 19 flies was dissected, identified and examined for the presence of promastigotes; 4/14 Lu. yuilli, 4/4 Ps. davisi and 1/1 Ps. geniculata were infected. Five to seven days after the infective feeds, 23 other flies from the same batch were

anaesthetized with CO₂ and their legs and wings were removed; they were then put in a Petri dish containing fourth instar larvae of *Lu. flaviscutellata*. In less than 24 h, the larvae had begun to eat the flies. One and two days after the first opportunity of feeding on the flies, a total of 22 larvae was dissected and the guts examined. Other larvae were permitted to pupate, and 1 male and 8 females which subsequently emerged were similarly examined. No parasites were found in larvae or adults. Napier & Smith (1926) similarly failed to infect second instar larvae of *Phlebotomus argentipes* by feeding them on infected females.

In a second experiment, we fed fourth instar larvae of *Lu. flaviscutellata* on cultured promastigotes of *Le. m. amazonensis*. Pieces of agar from heavily positive cultures (Senekjie’s medium) were readily eaten by larvae. One and two days later, when promastigotes on uneaten pieces of agar were still living, no parasites were found in the mid-guts of 6 fourth instar larvae; 8 female and 7 male flies reared from the dish were also negative. Christophers, Shortt & Barraud (1926) similarly found no infections in adult *Ph. argentipes* fed, as larvae, on cultured promastigotes of *Le. donovani*.

One factor possibly preventing the establishment of infections of *Leishmania* in the larvae is the rich bacterial flora in the mid-gut. Although promastigotes can develop in the adult fly in the presence of some bacteria (Killick-Kendrick, Molyneux & Ashford 1974), it is probable that heavy bacterial infections in the gut inhibit growth (Adler & Theodor 1927, Sherlock & Sherlock 1961) as they do usually, but not always (Disney 1968), in cultures.

Our results support the belief that, unlike *Leptomonas*, *Leishmania* produces no resistant cysts capable of development in the invertebrate host (Wenyon 1926). At some time during the evolution from the ‘hypothetical leptomonad stock’ (Baker 1965), *Leishmania* appears to have lost the ability to be transmitted in nature from one invertebrate host to another, and is now wholly adapted to a life-cycle alternating between invertebrate and vertebrate hosts. The amastigote stage is not, however, obligatory, since infections become readily established in females fed artificially on promastigotes from infected flies (Adler 1928).

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**References**


