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INFECTIVE STAGES OF *LEISHMANIA* IN THE SANDFLY VECTOR AND SOME OBSERVATIONS ON THE MECHANISM OF TRANSMISSION

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Infective stages of Leishmania (Leishmania) amazonensis, *capable of producing amastigote infections in hamster skin, were shown to be present in the experimentally infected sandfly vector* Lutzomyia flaviscutellata *15, 25, 40, 49, 70, 96 and 120 hours after the flies had received their infective blood-meal. Similarly, infective stages of* Leishmania (L.) chagasi *were demonstrated in the experimentally infected vector* Lu. longipalpis *examined 38, 50, 63, 87, 110, 135, 171 and 221 hours following the infective blood-meal, by the intraperitoneal inoculation of the flagellates into hamsters. The question of whether or not transmission by the bite of the sandfly is dependent on the presence of "metacyclic" promastigotes in the mouthparts of the vector is discussed.*

Key words: *Leishmania (Leishmania) amazonensis* – *Leishmania (Leishmania) chagasi* – infective stages – sandfly vectors – *Lutzomyia flaviscutellata* – *Lutzomyia longipalpis*

Recent years have seen a re-awakened interest in the mechanism by which *Leishmania* is transmitted during the bite of the sandfly vector. Killick-Kendrick (1979, 1986) has given reviews of the pertinent literature, and concluded (1986) that "Infective forms of *Leishmania* arise in the midgut of an infected fly three or more days after an infecting feed. They are small promastigotes with a long free flagellum... and highly motile. These stages are the only parasites seen in the proboscis of the sandfly and are undoubtedly deposited in the skin when the sandfly bites".

Among the evidence he presented to support this conclusion, Killick-Kendrick (1986) discussed the recent observations of Sacks & Perkins (1985), demonstrating that infective stages of *Leishmania* (*L.*) *major* and *Leishmania* (*L.*) *amazonensis* appeared in the midguts of *Phlebotomus papatasi* and *Lutzomyia longipalpis*, respectively, "as early as 3 days" after an infective bloodmeal, but he himself felt that "Infective forms probably do not arise in the midgut before 3 days", as "Parrot and Donatien (1927) inoculated mice with large numbers of promastigotes of *L. major* from the midguts of *P. papatasi* with 34-48 hour-old infections with negative results".

Our own interest in this subject commenced with some observations made during our experimental transmission of *L.* (*L.*) *chagasi* by the bite of its natural vector, *Lu. longipalpis* (Lainson et al., 1977). It was noted that although transmission to hamsters was achieved on days 7, 11, 14 and 17 after the infective bloodmeal, promastigotes were detected in the probosces of very few flies, and *only* after 14 days following the infective bloodmeal. While sympathetic to the "metacyclic promastigote" hypothesis, we found it difficult to reconcile these findings with the view that proboscis infections are essential if transmission by bite is to be achieved, and we suggested that possibly there was "... a surge of parasites forward from the pharynx, into the mouthparts, during the feeding process". We were, consequently, stimulated to examine further the stage at which infective forms were first to be found in the infected sandfly, with some rather surprising results which form the subject matter of the present paper.

MATERIAL AND METHODS

We used two models:

1. *L.* (*L.*) *amazonensis* in *Lutzomyia flaviscutellata*

The parasite stock was MORY/BR/85/M10150, isolated from the skin of a rodent, *Oryzomys* sp., in the Serra dos Carajás, Pará State, north Brazil. The sandflies were from our laboratory colony (Ward, 1977), at the time in its 74th generation.

The flies were infected by feeding them on hamster skin lesions and subsequently maintained, at approximately 24°C, under conditions previously described by Ward (1977).

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Periodic dissections of sandfly guts were made (Table I) in drops of sterile, normal saline (0.85%) and examined briefly, under a small cover-slip, by phase-contrast microscopy. After removal of the cover-slip, infected guts were broken up with fine needles and all the material on the slide taken up into a 1.0 ml syringe containing a further 0.1 ml of normal saline. Pools of such material, from the batches of sandflies examined at the different times indicated in Table I, were inoculated into the dorsal surface of the back feet of hamsters, which were subsequently examined for evidence of infection by making Giemsa-stained smears from the site of inoculation.

2. *L. (L.) chagasi* in *Lutzomyia longipalpis*

The parasite stock was ILON/BR/84/M8188, isolated from a naturally infected specimen of *Lutzomyia longipalpis* from a focus of visceral

TABLE I

Infective stages of *Leishmania (Leishmania) amazonensis* in experimentally infected *Lutzomyia flaviscutellata* as shown by the inoculation of the parasites into the skin of clean hamsters

Duration of Infection	Sandfly infections: Number of infected flies used for inoculum and general observations	Hamster infections: First detection of amastigotes: type of lesion
15 hours	Pool of 7 flies. No parasites visible in blood-meal in midgut	14 days: small nodule
25 hours	Pool of 5 flies. No parasites visible in blood-meal in midgut	32 days: inapparent
40 hours	Pool of 4 flies. Variable number of flagellates, from a few (up to 10) to over 100	31 days: inapparent
49 hours	Pool of 3 flies. Variable number of flagellates, from a few (up to 10) to over 100	30 days: inapparent
70 hours	Pool of 3 flies. Vast numbers of flagellates, restricted to the blood-meal in abdominal midgut	16 days: small nodule
96 hours	Pool of 3 flies. Heavy thoracic midgut infection and entering oesophageal diverticulum	29 days: inapparent
120 hours	Pool of 2 flies. Heavy thoracic midgut infection	13 days: small nodule

TABLE II

Infective stages of *Leishmania (Leishmania) chagasi* in experimentally infected *Lutzomyia longipalpis* as shown by inoculation of the parasites intraperitoneally into clean hamsters

Duration of Infection	Sandfly infections: Number of infected flies used for inoculum and general observations	Hamster infections: Nature of infection on autopsy (4-5 months)
38 hours	Pool of 5 flies. Large numbers of elongate promastigotes in residual blood-meal in abdominal midgut only	Abundant amastigotes in spleen and liver
50 hours	Pool of 4 flies. Increasing numbers of elongate promastigotes in residual blood-meal only	" " "
63 hours	Pool of 4 flies: Increasing numbers of elongate promastigotes in residual blood-meal only	" " "
87 hours	Pool of 5 flies. Extremely heavy midgut infections, with flagellates now in thoracic midgut. Blood-meal digested in 3 flies	" " "
110 hours	Pool of 4 flies. Extremely heavy infections and mostly in thoracic midgut, at stomodeal valve. Blood-meals digested in all flies	" " "
135 hours	Pool of 4 flies. As for 110 hours	Very abundant amastigotes in spleen and liver
221 hours	Pool of 2 flies. As for 110 hours	" " " "

leishmaniasis in Santarém, Pará State, Brazil (Lainson et al., 1985). The sandflies were from our laboratory, Ceará colony (Ward, 1974), at that time in its 97th generation. The flies were infected by feeding them, through a chick-skin membrane, on infected hamster spleen triturated in defibrinated, inactivated rabbit blood (Lainson et al., 1977), and maintained at approximately 24°C. Triturated guts from the batches of flies dissected at the different times indicated in Table II, were inoculated into single hamsters by the intraperitoneal route, and the spleens and livers of these animals were examined for parasites in Giemsa-stained smears of these organs when the hamsters were killed 4-5 months later.

RESULTS

1. *L. (L.) amazonensis* in *Lu. flaviscutellata*

The presence of infective stages of the parasite, producing amastigote infections in the skin of the inoculated hamsters, was demonstrated in flies examined 15, 25, 40, 49, 70, 96 and 120 hours following the infective bloodmeal (Table I). A few flies, left over after the experiment, were fed individually on clean hamsters, 7 days after the infective bloodmeal. Transmission was achieved on one occasion, the hamster showing a conspicuous nodule, containing abundant amastigotes, 73 days later. The sandfly which transmitted had spent some time in probing the skin of the hamster, but took no blood. On dissection, abundant promastigotes were seen in the abdominal and thoracic midgut (cardia) of the insect, but no flagellates were detected in the pharynx, cibarium or proboscis. Inoculation of the flagellates from the dissected gut into another hamster also produced infection.

2. *L. (L.) chagasi* in *Lu. longipalpis*

The presence of infective stages, producing visceral leishmaniasis in the inoculated hamsters, was demonstrated in flies examined 38, 50, 63, 87, 110, 135, 171 and 221 hours after the infective bloodmeal (Table II).

DISCUSSION

The combinations of parasite/sandfly-vector used in these experiments are *natural* ones (Lainson & Shaw, 1968; Lainson et al., 1985) and it is reasonable to suppose that, as experimental transmissions have been repeatedly achieved with them under similar laboratory conditions, the present results are likely to indicate the situation in naturally infected flies.

Although Sacks & Perkins (1985) clearly demonstrated an increasing degree of infectivity of midgut promastigotes of *L. (L.) amazonensis* with increasing duration of the parasite in *Lu. longipalpis* (*not*, incidentally, a natural vector of this organism), this was only studied at daily intervals for a period of 3-6 days. The authors nevertheless concluded that this "Sequential development of midgut promastigotes..." was "...from a *noninfective* to an infective stage..." (our italics). While we have made no attempt to demonstrate an increasing degree of infectivity of the midgut promastigote content during the present studies, our results leave no doubt that at least *some* of the flagellates in the sandfly possess the character of infectivity after a very short sojourn in the insect host – as early as 15 hours. Possibly these parasites have never lost this since the amastigotes stage.

The present observations do not invalidate the hypothesis that transmission by the bite of the sandfly depends largely on the inoculation of infective ("metacyclic") promastigotes in the proboscis of the fly. They are not consistent, however, with the view that "Infective forms probably do not arise... before 3 days", and they raise once more the vexed question as to whether or not other infective stages, undeniably present thoughout the parasite's development in the sandfly, might be equally efficient in producing infection, providing the mechanics of blood-taking allows their entry into the vertebrate host – a subject on which we are still very ignorant.

It is hoped that continuing observations on the morphology, antigenic nature and other features of the promastigotes in the various stages of the sandfly infection, may help to answer the many questions that still remain regarding the transmission of *Leishmania*.

RESUMO

Formas infectantes de *Leishmania* no vetor flebotomíneo e algumas observações sobre o mecanismo de transmissão – Foi demonstrado através de infecção experimental, que estágios infectivos de *Leishmania* (*L.*) *amazonensis*, capazes de produzir infecção na pele do hamster, encontram-se presentes no vetor flebotomíneo *Lutzomyia flaviscutellata* 15, 25, 40, 49, 70, 96 e 120 horas após o inseto ter recebido sua refeição sangüínea infectiva. Da mesma maneira, foi comprovada a presença de estágios infectivos de *L. (L.) chagasi* em exemplares do vetor *Lu. longipalpis*, examinados 38, 50, 63, 87, 110, 135, 171 e 221 horas após o repasto sangüíneo infectivo – através da inoculação em hamster por via intraperitoneal dos flagelados obtidos desses flebotomíneos. A questão sobre a transmissão do gênero *Leishmania* pelo flebotomíneo ser ou

não dependente da presença de promastigotos "metacíclicos" na proboscis do vetor, é discutida.

Palavras-chave: *Leishmania (Leishmania) amazonensis* – *Leishmania (Leishmania) chagasi* – formas infectantes – vetores flebotomíneos – *Lutzomyia flaviscutellata* – *Lutzomyia longipalpis*

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