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Vector compatibility of *Phlebotomus papatasi* dependent on differentially induced digestion

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Summary

Infection with *Leishmania tropica*, a strain specific to the sandfly *Phlebotomus papatasi*, was inhibited in sandflies fed on turkey blood. Reduction of the parasite number was correlated with the digestive process. A relatively high DNAase level was induced in the gut of the sandfly by the nucleated turkey erythrocytes. This is the first record of vector-pathogen incompatibility, thus induced, and of differentially triggered digestive processes.

Key words: *Leishmania; Phlebotomus papatasi;* vector capacity; insect digestion; DNAase.

Introduction

Reviews of the interaction between trypanosamatids and their vectors (Molyneux, 1977) or *Leishmania* and sandflies (Killick-Kendrick, 1979) include only a few experimental studies which demonstrate effects of the vector physiology on the cycle of transmission. For example, the pre-requisite of dietary components for the vector to host transmission of *Leishmania donovani* by *Phlebotomus argentipes* (Smith et al., 1940) and that of *Leishmania tropica* by *Phlebotomus papatasi* (Adler and Ber, 1941); and the nature of the establishment barrier for *Trypanosoma brucei* in the gut of *Glossina pallidipes* (Harmsen, 1973). This study demonstrates the involvement of an environmental factor, i.e., the choice of host animal, influencing the digestive processes of sandflies, which in turn inhibits the development of the pathogen they normally bear and transmit.

Field observations were made in that part of the Jordan Valley where cutaneous leishmaniasis is endemic, and where the zoonotic cycle of the *Leish-*

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mania tropica is maintained exclusively between the colony dwelling rodent *Psammomys obesus* and the sandfly *Phlebotomus papatasi*. The sandflies breed and find daily shelter in the rodent burrows. It was found that turkeys introduced into this biotope were very attractive to the sandflies, and that the sandflies collected in their vicinity were uninfected, compared to a usual rate of 20 to 50% infection (Schlein et al., 1982a, b). The laboratory study reported here shows that the turkey blood meals arrest the development of *L. tropica* in the sandflies and that a similar arrest occurs in nature.

The early work on artificial infection of sandflies was carried out using *Ph. papatasi* and *L. tropica* from the Jordan Valley, the same area from which the material for this study was obtained. Adler and Theodor (1929, 1930) showed that a feeding containing between 10 and 600 promastigotes infected 94% of the sandflies. They also showed that a second blood meal, either human or rabbit, did not reduce the rate of infection in *Ph. papatasi*. Such blood, even when it killed promastigotes in culture, lost its lytic quality in the gut of the sandflies. In addition, Adler (1938) demonstrated that the local strain of *L. tropica* caused high rates of infection even when it had been given in saline.

Materials and Methods

We used two local strains of *L. tropica*, LRC-L137 and LRC-323 and *Phlebotomus papatasi* reared in the laboratory, except for one series collected in the field.

The feeding and infecting methods were adapted from Adler and Theodor (1927). Cultured parasites were washed, counted and transferred to feeding medium to give 2×10^6 per ml, i.e., 200 parasites calculated per sandfly. The usual feeding medium was defibrinated rabbit blood that had been inactivated at 56° C for 30 min. Membranes, made of young chicken skin, were stretched over the wide end (diam. 3 cm) of plastic funnels that were filled with ether for sterilization and stoppered with cotton-wool. Sterile meals of 2 ml in such containers were put on the fine mesh cover of plastic feeding boxes ($10 \times 10 \times 7.5$ cm) and offered to approximately 50 *Ph. papatasi* in each box. Flies were starved for 24 h before and 48 h after meals. Normally, they were kept on water and 20% sugar solution. Ambient conditions were 80–100% r. h. and 28° C. Turkey blood meals were given by the direct application of a feeding box to the neck or head of a turkey. Sandflies were dissected and their guts were examined for promastigotes, using a phase contrast microscope. Each experiment included a control series whereby it was confirmed that a high level of infection could be obtained by each of the infective feedings. Each one of the experimental series was repeated at least twice.

DNA digestion in the *Ph. papatasi* gut was measured by capillary feeding of sandflies with labelled DNA and comparing the radioactivity of the non-digested DNA with the total, in samples of homogenised flies. Capillary feeding was a simplified version of the method of Hertig and Hertig (1927) and Hertig and McConnell (1963). ^{3}H-DNA was extracted from (methyl-^{3}H) thymidine (5 Ci/mmole, Nuclear Research Centre, Negev, Israel) labelled *E. coli*. DNA was extracted using chloroform isoamyl alcohol, dissolved in 10 mM Tris Cl, pH 7.5, 1 mM EDTA and 0.15 M NaCl and then sheared by sonication to reduce its viscosity. The final solution contained 850 μg DNA/ml. An average fly meal was 0.5 μl, which is half of a normal blood meal.

To measure radioactivity, the flies were homogenized and sonicated in 0.5 ml of 50 mM Tris Cl, pH 7.4, to which 100 mM EDTA (pH 8) were added. Samples of clear sonicates were either dried on glass-fibre filters or precipitated using 10% TCA and filtered through the filters. The TCA precipitable material was washed with 1 N HCl containing 0.1 M sodium pyrophosphate and dried with ethanol. Radioactivity was measured in a scintillation counter.

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Table 1. A comparison between the levels of infection with *L. tropica* in *Ph. papatasi* and the digestion of turkey blood: 1st meal turkey blood; 2nd meal infective rabbit blood after 24 h

Time after 2nd meal	Level of blood digestion	Level of infection			
		high[1]	medium[2]	low[3]	none
48 h	Unbroken erythrocytes	11	5	1	0
	Half digested blood	0	5	1	1
	Empty midgut	0	3	2	23
	Total %	21.1	25	7.7	46.2
6 days	All with empty midgut	2	3	3	23
	Total %	6.4	9.7	9.7	74.2

[1] hundreds or more promastigotes   [2] 10 to 100 promastigotes   [3] 1 to 10 promastigotes

Table 2. The effect of turkey blood 24 h after a meal containing saline and promastigotes or saline, promastigotes and 5% rabbit blood, on infections of *L. tropica* in *Ph. papatasi*

Infective meal medium	Level of infection			
	high	medium	low	none
5% rabbit blood in saline	6 (1*)	13 (10)	19 (8)	6 (8)
	13.6%	29.5%	43.2%	13.7%
Saline only	0	0	2 (2)	36 (3)
	0	0	5.3%	94.7%

* No. of specimens containing large numbers of disintegrating promastigotes

Results

The *L. tropica* infection rate in the accumulated, laboratory reared control series, was 85.5% (118 flies) of 138 *Ph. papatasi* females. A series of flies that had been caught near the turkey sheds and given an infective meal one week later was only 17.5% infected (11 flies out of 63), when dissected after a further week. Similarly, laboratory reared sandflies that had been given a turkey meal and, a week later, a second meal of rabbit blood and promastigotes, showed a 25% infection (12 out of 48 flies). Since *Ph. papatasi* can be fed twice in succession before the beginning of the gonotrophic cycle refractory period, the flies were first given a turkey blood meal and then an infective meal after a 24-h interval. When examined 48 h later, the flies could be classified according to the degree of digestion of the blood meal, which correlated with the leishmanial infection. It was found that the highest infections were those in flies with the least digested blood. Most of the flies that had completed digestion were clear of parasites

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(Table 1). A group of flies of the same series that was examined after 6 days, when the guts of all the flies were empty of blood, showed an increase in the number of uninfected flies. In another experiment, the infective meal was given 24 h prior to the turkey meal, either in saline alone, or in saline containing 5% rabbit blood (Table 2). More than 90% of the flies that had been fed parasites in saline were uninfected; whereas the majority of flies of the other series had at least some live promastigotes in their guts.

Microscopic examination of Giemsa stained smears of *Phlebotomus* guts with dead and disintegrating parasites, showed many promastigotes with two kinetoplasts. Kinetoplast division occurs early in cell division and it seems that this was the stage at which the life cycle was interrupted. These results led to the hypothesis that differences in the digestion of meals of turkey and rabbit blood may account for the differences in the viability of *Leishmania* infection. Indeed, it was found, in two experiments, that the labelled DNA digested by *Ph. papatasi*, fed 24 h earlier on turkey, amounted to 54.2%; whereas that of flies similarly fed on rabbits was only 29.7% of the total labelled DNA ingested. These results are after the subtraction of the proportion of DNA digested in a control series, where unfed flies were homogenized with labelled DNA. The TCA soluble fraction of samples of labelled DNA that had been incubated with sonicated rabbit or turkey blood for up to 3 h showed no significant increase in radioactivity.

Discussion

Summing up the results of the experiments on rates of infection, it is clear that turkey blood meals significantly reduce *Leishmania tropica* infections in *Ph. papatasi*. These series comprise 117 flies that were given turkey and infective meals and subsequently showed infection rates of 3.5% to 25.8%, some of the flies harbouring only a few parasites. The infection rate in the control series was 85.5% of 118 flies, with most specimens containing many parasites.

The low infection rates observed in the series where flies had an infective meal at a week's interval shows that the reduction of vector capacity persists even after the causative turkey meal had been completely digested. The fate of the infections in the series of wild *Ph. papatasi* caught near the turkey sheds that received the same treatment were similar, supposedly for the same reasons. Table 1 demonstrates that there is an inverse correlation between the digestion of turkey blood and the intensity of leishmanial infection, as most of the flies without blood meal remnants in their guts did not show infections.

The differences of enzyme quantity in the digestion of turkey nucleated and rabbit non-nucleated erythrocytes was demonstrated by the much higher DNAase activity in the guts of *Ph. papatasi* containing turkey blood. The actual DNAase quantity in the gut must be much higher than expressed by the 54% DNA digestion, as the labelled DNA was mixed with erythrocyte DNA in a

calculated proportion of 1:10. According to Spector (1956), turkey blood contains 5.2 mg DNA per ml.

The administration of an infective meal in saline containing 5% rabbit blood to sandflies permitted greater viability of parasites than the series that had the parasites in saline solution prior to the turkey blood meal, where 94% uninfected flies were observed (Table 2). In view of the differences in the DNAase activity it is suggested that rabbit non-nucleated erythrocytes and turkey nucleated erythrocytes trigger different levels of enzymes in the sandfly stomach, and the conditions elicited by the turkey blood are unsuitable for leishmanial parasites. 5% rabbit blood, given prior to the turkey meal seemed to improve the conditions for infection above the expected value. The conditions required for success in the vector, by the local strain of *L. tropica* that were disturbed by the ingestion of nucleated erythrocytes are not necessarily shared by other *Leishmania* and trypanosomes types. Reptilian leishmanias and trypanosomes (Adler, 1964) are capable of thriving in their respective sandfly vectors, even though they are ingested with reptilian nucleated erythrocytes. This only means that they develop under different conditions and not that their requirements are less specific.

Blood of different animal species is lethal to leishmanias in vitro and *L. tropica* in cultures were killed within the first hour by the addition of a few drops of turkey blood (unpublished data). A similar phenomenon has been described for *Trypanosoma cruzi* killed in culture by chicken blood. It was demonstrated that the capacity to lyse the trypanosome blood forms was complement dependent (Kierszenbaum et al., 1976). The lysis of leishmanias in culture was also caused by some human and rabbit blood samples, but the mortality ceased immediately after the promastigotes and blood had been ingested by sandflies (Adler and Theodor, 1930). The cause of death of leishmanias in vitro is undoubtedly not the same as that causing death in sandfly gut.

The killing effect of the rabbit and human blood was completely lost in the gut of the sandfly (Adler and Theodor, 1930). The turkey blood also loses its initial lethal quality in the gut of the sandflies and the death occurs slowly, after most of the blood has been digested. Further evidence for the endogenic nature of the fate of leishmanias in the sandfly gut comes from a study by Adler (1938), who demonstrated that the same rabbit serum triggered selective killing of non-specific leishmanias in the gut of different sandfly species.

Enzyme secretion in the gut of *Ph. papatasi*, following the ingestion of a mammalian or an avian blood meal, is apparently achieved by differential triggering of the gut cells, by different factors in the blood. This seems to be a direct effect on the stomach cells, i.e., a secretogogue mechanism (Gooding, 1975).

The observations made in this study add to the understanding of disease transmission by insects. The enzymatic processes of the gut of the sandflies were shown to function differently, when triggered by different types of meals. The

conditions, thus elicited in the gut, can be unsuitable for the pathogen. This situation, whereby a vector is rendered unsuitable for harboring its specific pathogen, lasts in the sandfly for a relatively long time.

This flexible compatibility of the sandfly and its specific leishmanial parasite, which depends on the choice of host animals available, could be an important factor in the distribution of leishmaniasis. Similar physiological mechanisms may play a role in the prevalence of other insect borne diseases.

Adler S.: Factors determining the behaviour of *Leishmania* sp. in sandflies. „Harefuah" *14*, 1–5 (1938).

Adler S.: Leishmania. In: Advances in parasitology, p. 35–86. Academic Press, London/New York 1964.

Adler S., Ber M.: Transmission of *Leishmania tropica* by bite of *Phlebotomus papatasi*. Nature (Lond.), *148*, 227 (1941).

Adler S., Theodor O.: The behaviour of cultures of *Leishmania* sp. in *Phlebotomus papatasi*. Ann. trop. Med. Parasit. *21*, 111–134 (1927).

Adler S., Theodor L.: Attempts to transmit *Leishmania tropica* by bite: the transmission of *L. tropica* by *Phlebotomus sergenti*. Ann. trop. Med. Parasit. *23*, 1–18 (1929).

Adler S., Theodor O.: The behaviour of insect flagellates and *Leishmanias* in *Phlebotomus papatasi*. Ann. trop. Med. Parasit. *24*, 193–210 (1930).

Gooding R. H.: Digestive enzymes and their control in haematophagous insects. In: Blood digestion in haematophagous insects. Acta trop. (Basel) *32*, 96–111 (1975).

Harmsen R.: The nature of the establishment barrier for *Trypanosoma brucei* in the gut of *Glossina pallidipes*. Trans. roy. Soc. trop. Med. Hyg. *67*, 364–373 (1973).

Hertig A. T., Hertig M.: A technique for the artificial feeding of sandflies *(Phlebotomus)* and mosquitoes. Science *65*, 328–329 (1927).

Hertig M., McConnell E.: Experimental infection of Panamanian *Phlebotomus* sandflies with *Leishmania*. Exp. Parasit. *14*, 92–106 (1963).

Kierszenbaum F., Ivanyi J., Budzko D. B.: Mechanisms of natural resistance to trypanosomal infection. Immunology *30*, 1–6 (1976).

Killick-Kendrick R.: Biology of *Leishmania* in phlebotomine sandflies. In: Biology of kinetoplastida, ed. by W. H. R. Lumsden and D. A. Evans, Vol. 2, p. 395–460. Academic Press, London/New York 1979.

Molyneux D. H.: Vector relationship in the Trypanosomatida. Advanc. Insect Physiol. *15*, 1–82 (1977).

Schlein Y., Gunders A. E., Warburg A.: Leishmaniasis in the Jordon Valley. I. Attraction of *Phlebotomus papatasi* (Psychodidae) to turkeys. Ann. trop. Med. Parasit. *76*, 517–520 (1982a).

Schlein Y., Warburg A., Schnur L. F., Gunders A. E.: Leishmaniasis in the Jordan Valley. II. Sandflies and transmission in the central endemic area. Trans. roy. Soc. trop. Med. Hyg. *76*, 582–586 (1982b).

Smith R. O. A., Halder K. C., Ahmed I.: Further investigation on the transmission of kala-azar. Indian J. med. Res. *28*, 575–590 (1940).

Spector W. S. ed: Handbook of biological data. W. B. Saunders Co., Philadelphia 1956.