Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 37 (1980)
Heft: 2

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DOI: https://doi.org/10.5169/seals-312645

Acta Tropica 37, 151–161 (1980)

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Mechanoreceptor – trypanosome interactions in the labrum of *Glossina:* fluid mechanics

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Summary

The fluid mechanics of the flow of blood through the labrum of *Glossina* is described in uninfected and trypanosome infected flies. The flow is characterised by the Reynolds number, and a frequency parameter for the pulsating flow and non-newtonian viscosity effects are considered. The effects of colonies of trypanosomes on the flow rate in the labrum and the interactions between colonies of *Trypanosoma (Nannomonas) congolense* and *Trypanosoma (Trypanozoon) brucei* and mechanoreceptors in the proximal third of the labrum are described. The direct association between trypanosomes and mechanoreceptors and the reduction in flow rate imposed by trypanosome colonies in the food canal distal to the mechanoreceptors will impair detection of stimuli by them. The epidemiological implications of these and earlier observations are discussed.

Key words: Glossina; Trypanosoma; Trypanozoon; Nannomonas; fluid mechanics; scanning electron microscopy; epidemiology; blood flow; viscous flow; pulsating flow.

Introduction

Recent studies have shown that salivarian trypanosomes during their development in the labrum of laboratory reared *Glossina m. morsitans* are associated with mechanoreceptors which have been described as monitoring flow in the food canal of *Glossina.* Molyneux et al. (1979) first demonstrated that rosettes of *Trypanosoma (Nannomonas) congolense* in *G.m. morsitans* were associated with LC1 mechanoreceptors (Rice et al., 1973); parasites were attached

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to the base of these sensilla and the hairs of the receptors were entangled in the colonies of parasites. It was also suggested that the presence of parasites distal to the LC1 sensilla which are localised in the proximal third of the labrum (Rice et al., 1973) could change the flow characteristics over the sensilla. Molyneux (1980) has demonstrated that in *T. (Duttonella) vivax* a similar situation to that described in *T. (N) congolense* pertains. Although *T. (Trypanozoon) brucei* is generally not associated with large colonies of parasites in the labrum of *Glossina* as are *Duttonella* and *Nannomonas* subgenera the study of Jenni et al. (1980) shows that a specific association of groups of apparently procyclic parasites of this subgenus are entwined with LC1 mechanoreceptors. The earlier suggestion that the parasite-receptor association in *Glossina* (Molyneux et al., 1979) may affect feeding behaviour was clearly demonstrated by Jenni et al. (1980) for *Glossina* infected with *Trypanozoon;* infected flies probed more frequently and fed more voraciously and frequently than uninfected flies. It was also reported that trypanosome colonisation of the labrum and hypopharynx would severely affect the flow of fluid in the labrum.

In this paper we report details of fluid mechanics of blood flow in the proboscis of infected and uninfected *Glossina m. morsitans* and discuss the epidemiological and epizootiological significance of the observations.

Materials and methods

The techniques used for the preparation of material for scanning electron microscopy (SEM) of trypanosomes within the proboscis of *Glossina* have been based on those used in an earlier SEM study of trypanosomes in an insect vector (Molyneux et al., 1978). The techniques of infection of *Glossina* with trypanosomes and the method of fly handling are described by Jenni (1977) and Jenni et al. (1980). *T. (T.) brucei* STIB 247 was isolated in 1971 in the Serengeti National Park (Tanzania) from a hartebeest *(Alcelaphus buselaphus). T. (N.) congolense* STIB 68 F-A is a derivative of STIB 228 which was isolated in 1971 in the Serengeti from a lion *(Panthera leo).*

Results

In order to estimate the effects of trypanosome colonies of *Duttonella* and *Nannomonas* on the flow of blood through the labrum of *Glossina* an examination of the available data pertaining to this system is required. The figures and sources which enabled calculations to be made are given below and expressed graphically in Fig. 1. Figs. 2–6 also illustrate the relationships between mechanoreceptors and parasites in mixed infections of *Trypanozoon* and *Nannomonas* in infected *G.m. morsitans* and of *Trypanozoon* alone in the same fly.

Viscosity

Kinematic viscosity (ν) is derived from

$$\frac{\text{Dynamic viscosity}}{\text{Density}} = \frac{\mu}{\rho}$$

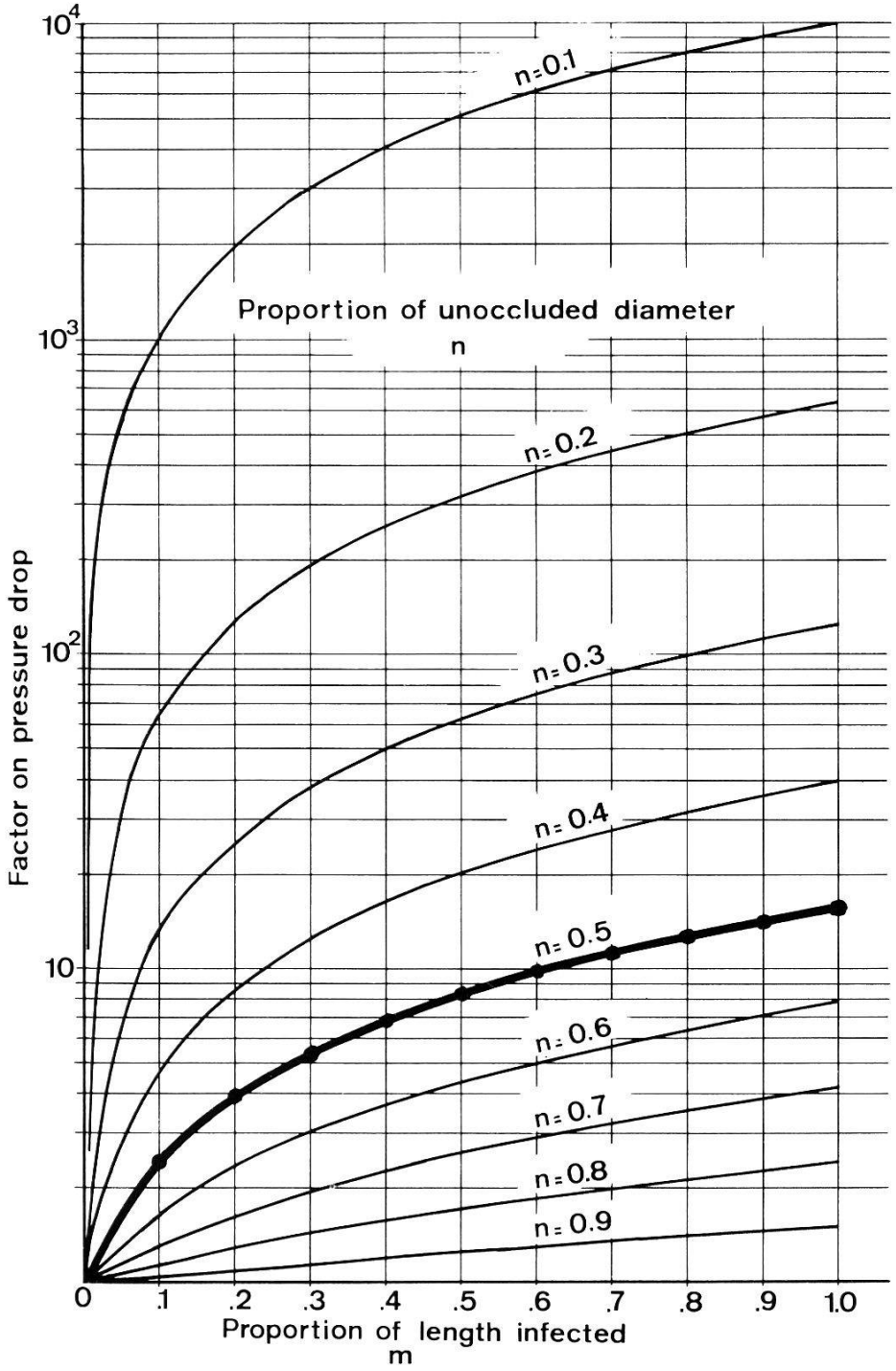


Fig. 1. Factors by which the required pressure drop $-\overline{\Delta p}$ would need to be increased for a given proportion of length colonised by trypanosomes (m) and for a given proportionate reduction in diameter (n) as a result of trypanosome infection if the same flow rate in the labrum was to be maintained.

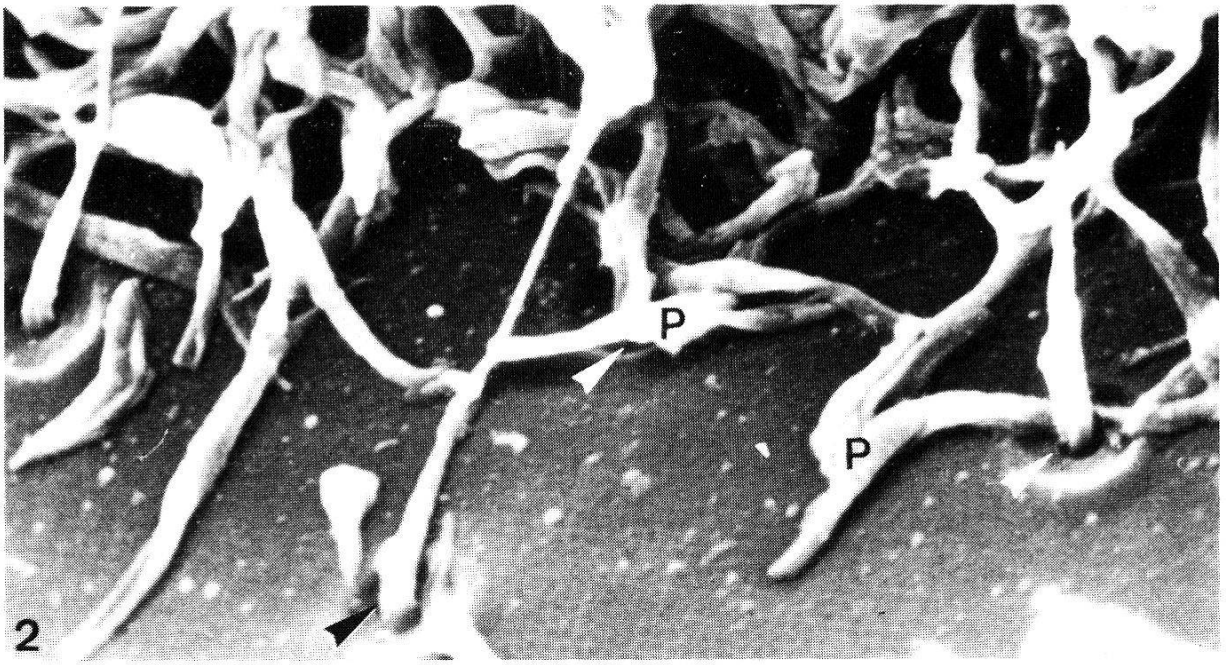
Fahraeus and Linquist (1931) give values of kinematic viscosity as 3×10^{-2} cm^2 s^{-1}

Density of blood 1.06 g cm^{-3}

Hence $\nu \times \rho = \mu$

$3 \times 10^{-2} \times 1.06$ cm^2 s^{-1} g cm^{-3}

$= 3.18$ centipoise $\frac{1}{100}$ g cm^{-1} s^{-1}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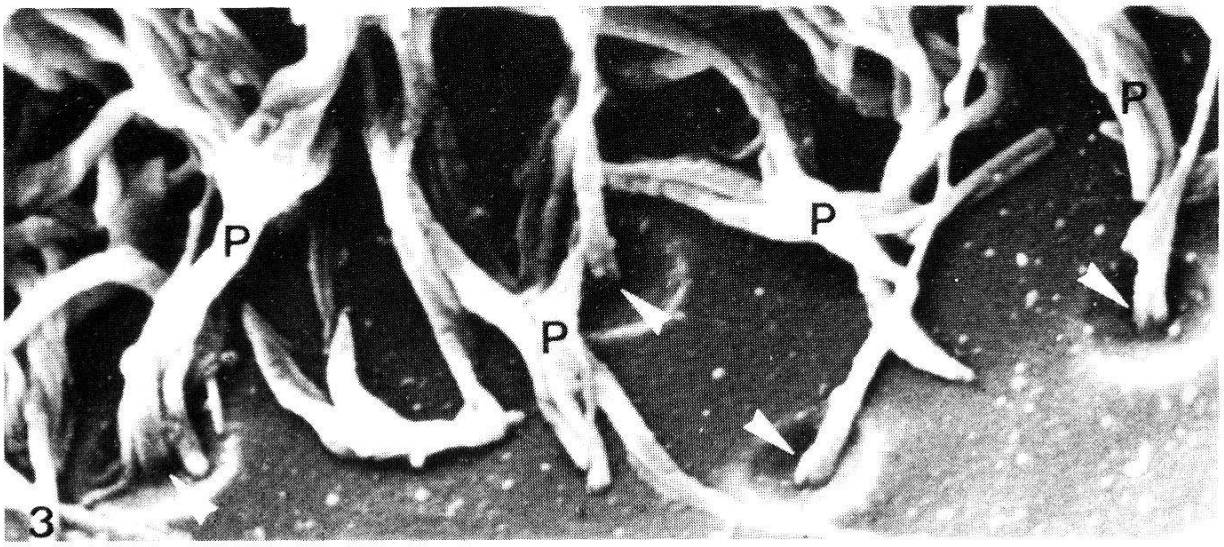
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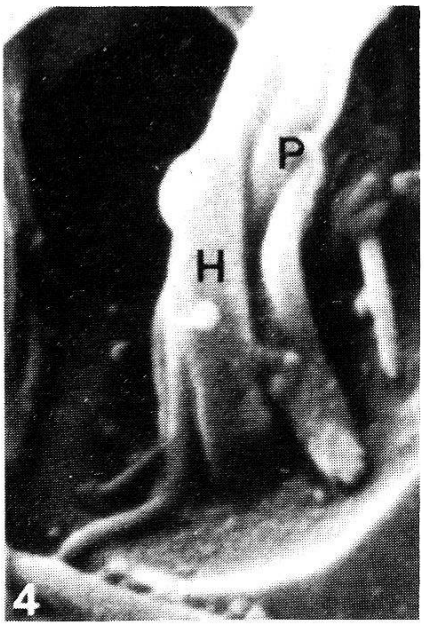
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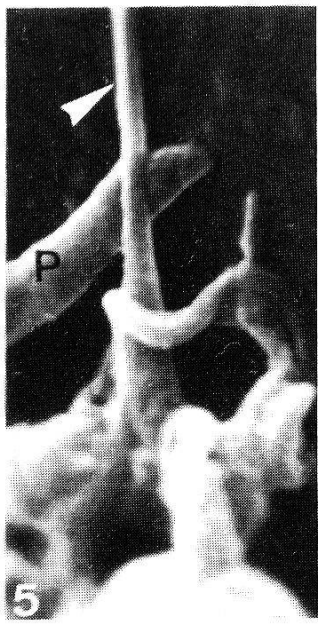
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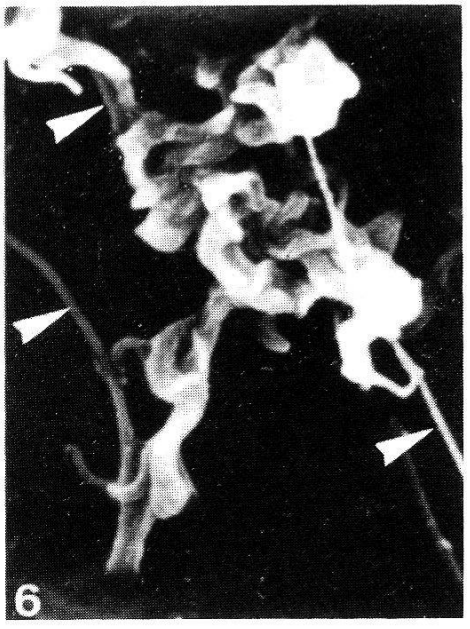
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Proboscis internal diameter

We have calculated from our SEMs and transmission Electron Micrographs an average diameter of 0.0035 cm in *G.m. morsitans.* Earlier calculations by Langley and Pimley (1973) we believe were not in accordance with our observations. Although the figure is likely to vary from one species of *Glossina* to another, this can be adjusted if necessary in calculations for an individual species.

Pumping frequency

Rice (1970) gives a value of 10 Hz for the cibarial pump of *Glossina austeni.*

Blood meal

We have estimated that a 40 mg blood meal is taken up in 60 s

$$\text{Volume} = \frac{\text{Mass}}{\text{Density}} = \frac{40\text{ mg}}{1.06\times 10^{3}\text{ mg cm}^{-3}} = 4\times 10^{-2}\text{ cm}^{3}$$

Regime

Reynolds Number (a measure of the damping effects and the interaction of inertia and viscous forces) is obtained from

$$\text{Re} = \frac{\bar{V}\,\bar{D}}{\nu} = \frac{\text{Velocity}\times\text{Diameter}}{\text{Kinematic viscosity}}$$

For $\bar{V}$ cross sectional area of proboscis passage

$$\frac{\pi\, d^{2}}{4} = \frac{\pi}{4}\times(3.5\times 10^{-3})\ 2\text{ cm}^{2} = 9.62\times 10^{-6}\text{ cm}^{2}$$

Area $\times$ velocity = volume flow rate

$$A\bar{V} = Q$$
$$(9.62\times 10^{-6})\,\bar{V} = 4\times\frac{10^{-2}}{60}$$

$$\bar{V} = 0.64\times 10^{2}\text{ cms}^{-1} = 0.64\text{ ms}^{-1}$$

Figs. 2–6. Labra of *Glossina m. morsitans.*

Fig. 2. SEM of labrum infected with *T. (N) congolense* and *T. (T) brucei.* Mechanoreceptors LC1 (▶) are associated with parasites (P) attached around their bases. 2500×.

Fig. 3. As in Fig. 1; note close association between parasites (P) and all LC1 mechanoreceptors (▶). 2500×.

Fig. 4. Enlargement of part of Fig. 2 showing mechanoreceptor hair (H) with parasites (P) attached within pit from which mechanoreceptor emerges. 6000×.

Fig. 5. Parasites (P) in mixed infection with *T. (N) congolense* and *T. (T) brucei* entwined around LC1 mechanoreceptor hair (▶). Other parasites are at the base of the hair. 6000×.

Fig. 6. *T. (T) brucei* parasites from pure infection entwined around three LC1 sensilla (▶). 1600×.

This figure agrees with that of Langley and Pimley (1973) when adjustment is made for the diameter used in their calculations.

$$\text{Hence Re} = \frac{0.64\ \text{ms}^{-1} \times 35 \times 10^{-6}\ \text{m}}{3 \times 10^{6}\ \text{m}^2\ \text{s}^{-1}}$$

$$\text{Re} = 7.46$$

This is a low Re and confirms that we are dealing with a viscous or laminar flow regime; with a figure of Re of 2000 or more the flow would be turbulent. However, this low figure establishes the type of flow without doubt. This flow would give "entrance effects" at the ends of the food canal but they would be confined to a length of the tube equal to a fraction of the diameter of the tube: 0.03 D Re, say less than D/3 (Schlicting, 1960; Fargie and Martin, 1971).

Pulsating flow

Uchida (1956) has studied the unsteady flow superposed on a steady laminar motion of an incompressible fluid in a circular pipe. The non-dimensional group equal to $\bar{D}\sqrt{\frac{w}{\nu}}$, where w is the radian or circular frequency (not Hz) was introduced by Uchida (1956) and Womersley (1955). Circular frequency is 2π Hz, hence for *Glossina*

$$\bar{D}\sqrt{\frac{w}{\nu}} = \frac{0.035}{10}\sqrt{\frac{20\pi}{3 \times 10^{-2}}} = 0.16$$

This figure, which is markedly less than 1, establishes the pulsating viscous flow regime as one in which the velocity distribution is in phase with the pressure fluctuation and retains the parabolic velocity distribution of Hagen-Poiseuille flow. If a value of more than 10 (to ∞) existed then the velocity distribution would be greatly altered, the viscous wall flow responding nearly in phase with the pulsation and the centre line flow lagging in phase by 90°. A nearly exact theory was established (on the assumption of vanishingly small radial velocities) which yields the result that the Hagen-Poiseuille theory is correct for the time averaged flow and time averaged pressure gradient i.e.

$$\text{mean flow rate}\quad \bar{Q} = \frac{\pi D^{-4}}{8\mu} \qquad \frac{(-\overline{\Delta p})}{L}\ .$$

The assumption of small radial velocity in the nearly exact theory will be very precise for the low frequency regime characterised by $\bar{D}\sqrt{\frac{w}{\nu}} \ll 1$ for the case of the type of flow anticipated by cibarial pumping in which reverse flow will be unlikely to occur or will be an occurrence of negligible time scale in the pulse cycle.

It is clear that the pressure drop or suck $-\overline{\Delta p}$ below atmosphere required to obtain the same flow rate $\bar{Q}$ for the meal is proportional inversely to $\bar{D}^4$ and directly to the length L if the viscosity remains the same. The pressure drop

$-\overline{\Delta p}$ may be viewed as a measure of the local tissue loading on the anterior wall of the cibarium and of the magnitude of the force applied by the cibarial dilator muscles for a given insect.

Fig. 1 was calculated on the basis of the above theory and indicates the factor by which the required 'suck' would be increased for a proportion of length mL (for m, 0.1 to 1.0) infected by trypanosomes and the proportionate reduced diameter $n\bar{D}$ (for n, 0.1 to 1.0) if the same mean flow rate obtained. Alternatively, if the same 'suck' were applied then the mean flow rate would be inverse to the factor in Fig. 1.

Taking a conservative view of the effect of the restriction due to the infection one might draw attention to the row of the table for $n = 0.5$ (colonies of flagellates even though localised may readily be estimated as significantly invasive hydrodynamically to an extent greater than $0.5\bar{D}$; Hoare, 1972; Molyneux et al., 1979). For $m = 0.1$ i.e. representing only a 10% infected length the factor is 2.5 and for a 60% infected length the factor is 10.

It should be noted that the average pressure difference and average flow rates are considered here. More precise indication of peak suction pressures would require a knowledge of the pressure pulse shape p(t) as a function of time. It is confidently anticipated that the likely pulse shape changes with constriction would imply greater peak suctions reinforcing the trends described.

Finally, although there is some uncertainty in relation to the magnitude of viscosity assumed it can be stated directly

i) the regime characterisation would not be affected at all by the known possible variation in viscosity,

ii) the comparative study represented by the table is independent of the viscosity,

iii) any suggestion that the Fahraeus-Lindquist (1931) effect (preferential tendency for red blood cells to 'shun' the wall and concentrate axially reducing the apparent viscosity) might invalidate the regime discussion as the tube constriction increases can be easily resisted (see Lighthill, 1975, p. 279 and 4, p. 274) on the basis that only a localised minimum in viscosity is implied with a variation less than that covered by (i) above and eventually an increase in viscosity occurs.

Fig. 1 is applicable to the labrum of infected *Glossina* but has the same validity if applied to an infected hypopharynx. In that situation a greater proportionate reduction in diameter due to the presence of parasites and the smaller internal diameter of the hypopharynx would apply. The pressure applied by the contraction of the salivary gland muscles to expel saliva would thus need to be much higher in infected compared with uninfected flies and the calculations would be entirely valid for this system.

Examination of the degree of infestation in terms of the occluded diameter by colonies of *Duttonella* and *Nannomonas* is indicated from figures given by Hoare (1972; figure 96). Epimastigotes of *T. (D) vivax* attached in the labrum

measure 18–35 μm and, according to Hoare (1972), "multiply intensively and give rise to clusters or colonies of flagellates attached to the walls of the labrum". *Nannomonas* epimastigotes measure 14.3–36 μm in the same site (Figs. 2–5). The degree of comparative occlusion of the diameter of the hypopharynx with *Nannomonas* is illustrated by Hoare (1922; figure 96) which is reproduced from a description of Bruce et al. (1913). These measurements and drawings as well as SEMs of Molyneux et al. (1979) and Figs. 2–5 indicate the degree of invasiveness of the labrum and hypopharynx which occurs in experimental and natural trypanosome infection in *Glossina.* The extent of colonisation by *Nannomonas* and *Duttonella* of the labrum in natural infection has been studied by Clark (1965) who demonstrated that the percentage of length of labrum colonised can reach 81% of the total. This figure is calculated from diagrams of Clark (1965 Drawing f). In a series of diagrams Clark (1965) also illustrates the gradual colonisation of the labrum with time but shows clearly that the first region to be colonised in both *T. (D) vivax* and *T. (N) congolense* is the proximal third of the labrum where LC1 mechanoreceptors are situated (see Figs. 2–6).

Discussion

We have indicated briefly in earlier papers (Molyneux et al., 1979; Jenni et al., 1980) the implication of our findings in epidemiology and epizootiology. In view of the described differences in behaviour of *Trypanozoon* infected compared with uninfected flies, the utilisation of infection rates as a measure of trypanosome risk would seem to be no longer valid. The important factors are the increased frequency of probing and the increased voracity of infected flies (Jenni et al., 1980). Indications also exist that infected flies live longer than uninfected flies (Baker and Robertson, 1957) and, although no recent published data are available, laboratory studies associated with our recent work do tend to confirm these observations. These findings suggest that the risk of acquisition of trypanosome infection ("Challenge") by susceptible animals must be measured in the field, after accounting for a variability in the behaviour of infected flies.

It is clearly tempting to suggest that the frequent finding of animal trypanosomiasis in areas where *Glossina* densities are very low can be explained by the differences in behaviour and vector capability we have described. The results of England and Baldry (1972) and Moloo et al. (1978) are of interest in this respect. England and Baldry (1972) found infection rates of *T. (T) brucei* in *Glossina pallidipes* in three different areas of the Lambwe and Roo Valley areas of Kenya were 4.0% and 3.7% near two small dams in the Roo Valley whereas in part of the Lambwe Valley the rate was only 0.3%. In cattle in the same area the infection rates of *T. (T) brucei* was 39.8% (Robson and Askar, 1972) but 9 out of 10 bushbuck *(Tragelaphus scriptus),* which was the preferred host of *G. pallidipes,* were infected with *T. (T) brucei* and *T. (T) brucei* was the most common

trypanosome of other game animals (Allsop 1972). Moloo et al. (1973) failed to find any *T. (T) brucei* type infections in 6344 *G. swynnertoni* and 623 *G. pallidipes* although *T. (T) brucei* infection occurred in game (Geigy et al., 1971) and cattle (Mwambu and Mayembe, 1971) in the same area. Although the inadequacy of dissection techniques and the value of trituration and animal inoculation for the detection of trypanosome infections of *Trypanozoon* in *Glossina* is recognised (see review by Molyneux, 1977) a likely and alternative explanation for the observations of these workers in East Africa is the different behaviour of infected flies although we recognise that this remains to be shown in the field.

In human trypanosomiasis similar phenomena have been recognised. Intense man-fly contact has been recognised as being an important feature of transmission of the disease particularly in the West Africa Guinea Savanna at the end of the dry season where man and flies come into contact around water holes where humidity is highest. Nash (1978) reviews the entomological aspects of transmission of human trypanosomiasis and describes the situation in Sambo, Nigeria where man and fly share the same waterhole habitat. The range of the fly is restricted due to extreme environmental conditions and it is confined to localities where humidity is highest, being frequently forced to feed on man in an area of intimate personal man-fly contact. In Sambo 30 of 43 inhabitants had trypanosomiasis and it is likely in view of our findings that they could all have been infected by a single fly for in a situation, such as a water-hole, where many people may be gathered together, interrupted probing could occur and a fly could move to another individual before probing again; experimentally we have shown (Jenni et al., 1980) that a single fly can infect 6 mice by probing prior to engorgement if offered a susceptible mouse after each probe.

The observations of Laveissière (1976) of a group of cases of human trypanosomiasis in a leprosarium in Ouahigouya, Upper-Volta also suggest (outside known range of *Glossina*) that transmission by a single infected fly could occur. Localised resurgence of sleeping sickness in Gamadan, near Kano Nigeria in the apparent absence of *Glossina* has also been observed (see Thomson, 1969; Molyneux et al., 1979). In this focus 24 cases were discovered who were in the same stage of the disease. Their habits could have resulted in transmission by a single infected fly at a site of intimate man fly contact. Frezil (1971) has evoked the likelihood of mechanical transmission of sleeping sickness by mosquitoes in view of the finding of family cases in the same dwellings. Our observations of increased probing and more voracious feeding by infected flies could again explain this phenomenon.

The observation of parasite-host interaction which effects behaviour of a vector at a time when transmission would occur is, of course, of biological significance. The parasites display an adaptation which will certainly enhance their chances of survival. This association would also seem to have advantages for the host as our findings indicate that infected flies feed more frequently than uninfected flies. If, as Baker and Robertson (1957) suggest, they also live longer the

trypanosome tsetse fly association would clearly be advantageous to both parties.

Acknowledgments. We are grateful to Dr. A. M. Jordan for the supply of *Glossina* pupae and to Mr. K. F. Schell, Miss M. Kauffman, Mr. D. R. Lavin and Mr. D. V. Roscoe for technical assistance. We also thank the Swiss NSF for support to L. Jenni (Grant No. 3346-0.78) and from the UNDP/World Bank/WHO Special Programme and Overseas Development Administration (R.3495) for grants to D. H. Molyneux.

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