

Mechanoreceptors, feeding behaviour and trypanosome transmission in *Glossina*

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Scanning electron microscope (SEM) studies of salivarian trypanosomes of the subgenera *Duttonella*, *Nannomonas* and *Trypanozoon* in the labrum of the tsetse fly *Glossina m. morsitans* have been carried out. These studies have shown that the colonies (rosettes) of *Trypanosoma (D.) vivax* and *T. (N.) congolense* occur in the proximal third of the labrum where groups of sensory receptors (sensilla) responsible for monitoring the blood flow rate occur. RICE *et al.* (1973) have described these sensory hairs as LC1 mechanoreceptors on structural grounds; 25 to 30 such sensilla are found in each labrum. They have a basal collar and emerge from discreet pits (Figs. 1, 2). The receptor hairs extend across the food canal and, according to RICE *et al.* (1973), "are certain to be stimulated by the passage of food during ingestion".

SEM studies of *T. (N.) congolense* (Fig. 2) (MOLYNEUX *et al.*, 1979) and *T. (D.) vivax* (Fig. 3) (MOLYNEUX, 1980) in the labrum of *G. m. morsitans* have demonstrated the close association between the colonies of parasites and these mechanoreceptors. The rosettes of both these subgenera are known to be attached to the labral wall (VICKERMAN, 1973; EVANS *et al.*, 1979) and the extent of rosette formation and colonization of the labrum was illustrated by CLARKE (1965) and HOARE (1972). SEM studies show that the rosettes, whilst firmly attached, are in association with the LC1 sensilla in the proximal part of the food canal. The parasites in this region are frequently attached around the base of the sensilla and sensilla hairs are entangled in the rosettes. RICE *et al.* (1973) reported LC1 mechanoreceptors to be "fluid flow meters" which, if they receive insufficient stimulus would activate probing and pre-feeding behaviour. We suggested (MOLYNEUX *et al.*, 1979) that the presence of colonies of parasites in the labrum may effect probing behaviour.

This hypothesis was confirmed by JENNI *et al.* (1980) who observed that *G. m. morsitans* infected with *T. (T.) brucei* (ST1B 247) probed more frequently and fed more voraciously than uninfected flies. JENNI *et al.* (1980) starved 40 flies infected with *T. (T.) brucei* and 40 uninfected flies for 24 hours and 48 hours. After 24 hours, only three of the 40 infected flies offered a blood meal on mice fed on the first probe and required a mean of 5.08 (± 0.40) probes before engorgement, and all 40 flies engorged. After 48 hours starvation of the infected flies, only four engorged after the first probe and the mean number of probes before feeding was 4.68 (± 0.37) and, again, all 40 flies in the batch fed. The 40 uninfected

flies which were subjected to the same conditions behaved as follows: 28 fed after 24 hours' starvation, 13 fed on the first probe and the mean number of probes before feeding was 1.89 (± 0.21); after 48 hours, starvation 38 out of 40 fed, 24 on the first probe, and the mean number of probes before engorging took place was 1.53 (± 0.13). The flies used in this experiment were 20 to 35 days old and had fed as teneral on an infected Swiss mouse.

Ten flies infected with *T. (T.) brucei* (5 male, 5 female) were then offered a series of mice on which to probe; if a fly failed to obtain a blood meal after withdrawing its proboscis it was offered another mouse. The ten infected flies infected a total of 38 mice after probing 41 times. The highest number of infective probes by a single fly was six before taking a blood meal (JENNI *et al.*, 1980). Infected flies are thus capable of infecting mammals by successive probes before taking a blood meal.

Although *Trypanozoon* infections in *Glossina* are not usually associated with heavy labral infections, our SEM studies have revealed that groups of apparently procyclic trypomastigotes are found closely associated with LC1 mechanoreceptors in the labrum (Fig. 1). The parasites are seen attached to the bases of the sensilla or are entwined around them (see JENNI *et al.*, 1980); clearly such interference could effect receptor function during probing behaviour and would explain the observed differences in feeding behaviour between *G. m. morsitans* infected with *Trypanozoon* and uninfected flies.

The food canal of *Glossina* represents a suitable structure on which to apply the principles of fluid mechanics. We have therefore (JENNI *et al.*, 1980; LIVESEY *et al.*, 1980) investigated the effects of trypanosome infections in the labrum on blood flow rate and the required increase in suction ($-\Delta\bar{p}$) to maintain the same rate of blood flow under conditions of trypanosome infection experience by *Glossina*.

Using known parameters and data the flow regime in the food canal of *Glossina* has been characterized. This is a pulsating flow characterized by a low Reynolds number and non-newtonian viscosity effects are considered. These considerations show the Hagen-Poiseuille theory is correct for the time average flow and time-averaged pressure gradient. The pressure drop or suck required to maintain the same meal flow rate would be inversely proportional to D^{-4} (=diameter of the labrum) and directly proportional to the

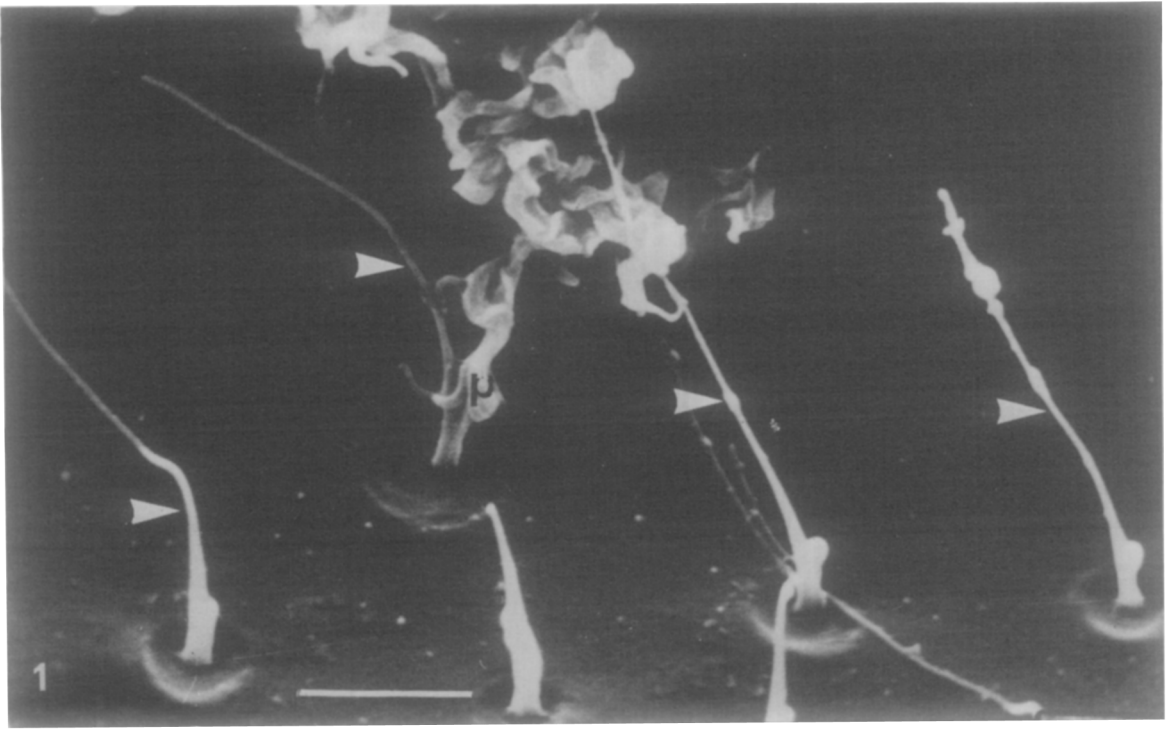


Fig. 1. Mechanoreceptors (= arrowed) of *G. m. morsitans* associated with *Trypanosoma (Trypanozoon) brucei* trypanosomes (possibly procyclic) (= P) in labrum of infected fly 19 days after an infective feed. Bar = 10 μm.

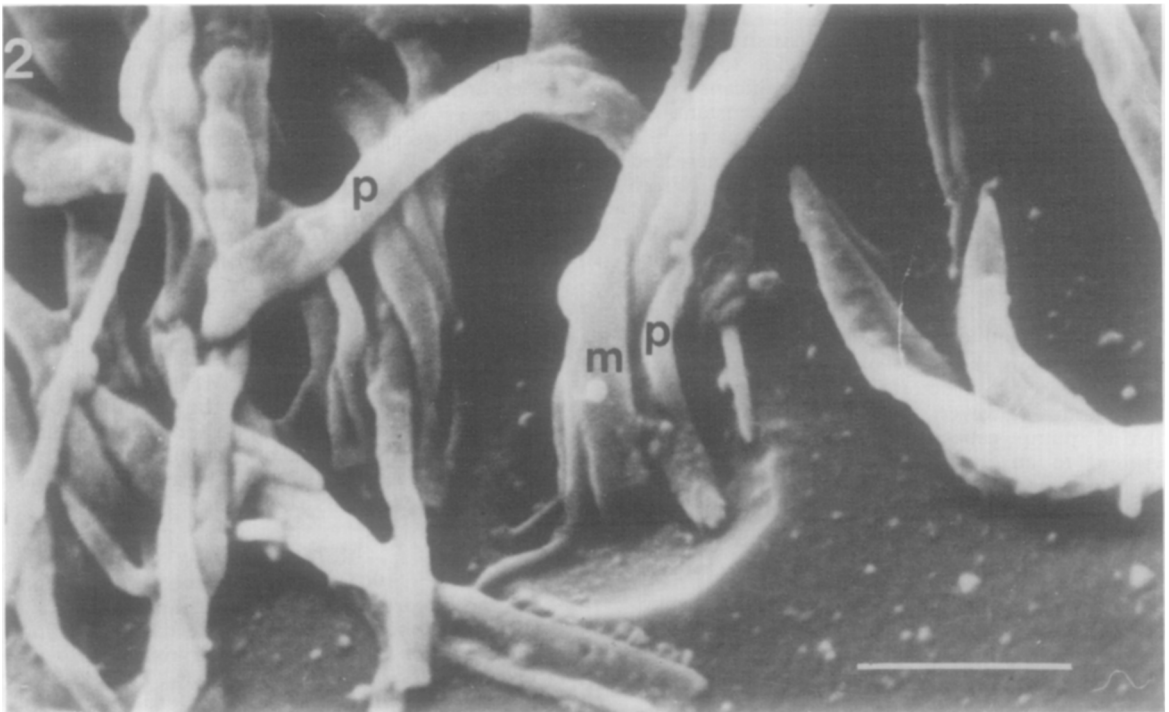


Fig. 2. *Trypanosoma (Nannomonas) congolense* parasites (P) associated with mechanoreceptor (m) in labrum of *G. m. morsitans*. Bar = 4 μm.

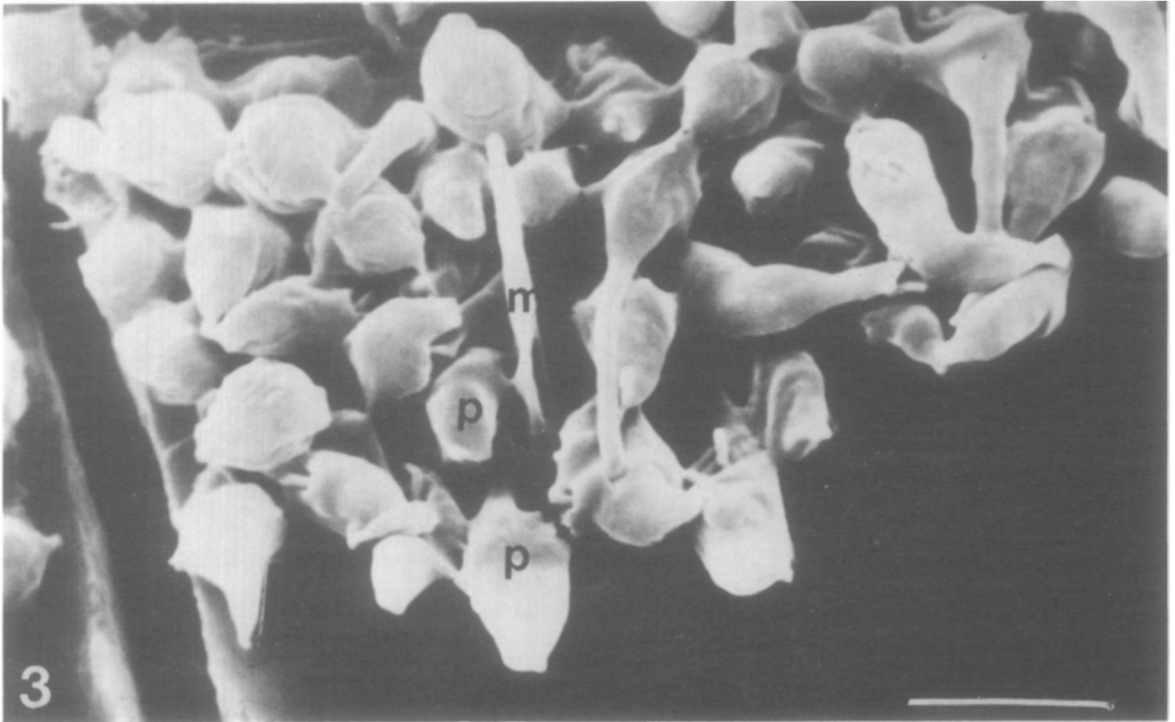


Fig. 3. Mechanoreceptor (m) emerging from a colony of *T. (D) vivax* (=P) in labrum. Bar = 4 μ m.

All figures are scanning electron micrographs of material fixed in 2.5% glutaraldehyde in 0.2M cacodylate buffer which were subsequently critically point dried, mounted on double-sided sellotape on aluminium stubs, gold coated in a Polaron sputter coater and viewed in a Cambridge Stereoscan IIA at accelerating voltages of 20-30 kV.

length of the labrum if the viscosity remains constant. Thus any constriction (due to trypanosome infection) could have a significant effect on flow rate if the suck remained constant or, alternatively, if the flow rate was to be maintained the pump pressure would need to be increased (JENNI *et al.*, 1980; LIVESEY *et al.*, 1980). From these calculations it can be shown that if 60% of the length of the labrum is infected and the diameter is reduced by 50% then the factor by which flow rate would be reduced, if $-\Delta p$ is constant, is a factor of 10 (JENNI *et al.*, 1980; LIVESEY *et al.*, 1980). To maintain the same flow rate in the above situation the $-\Delta p$ or "suck" would have to be increased by a factor of 10 also.

We know, however, that there is a limit to the cibarial pump capacity (JENNI *et al.*, 1980) to respond to an increase in viscosity and, under such conditions, flow rate is reduced; hence the pump would have difficulty in maintaining the same flow if the labrum is heavily infected and the mechanoreceptors can be expected to detect flow rate changes (RICE *et al.*, 1973).

We consider that evidence from the morphological and experimental studies described above, as well as theoretical fluid mechanics, demonstrate that trypanosome-mechanoreceptor interactions occur in the labrum of *Glossina* infected with salivarian trypanosomes and affect the feeding behaviour of infected tsetse. We suggest that the

effects on mechanoreceptor feedback occur as a result of both the direct trypanosome-mechanoreceptor interactions and the effects that colonies of parasites have on flow rate, which are detected by mechanoreceptors proximal to the rosettes in the labrum.

The implication can be anticipated to be relevant to the transmission and epidemiology of both human and animal trypanosomiasis transmission and epidemiology. Such considerations have already been discussed elsewhere (MOLYNEUX *et al.*, 1979; LIVESEY *et al.*, 1980).

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Characterization of antigens from erythrocytic stages of *Plasmodium falciparum* reacting with human immune sera*

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Epidemiological studies and the experimental data of MCGREGOR & WILLIAMS (1978) in the Gambia have shown that, in areas endemic for malaria, humans acquire a partial immunity to malaria infection after repeated infections, as reflected, for example, by a decrease in the number of individuals with patent parasitaemia with age. There is evidence that humoral factors play a major role in this process. It has been shown, for example, that passive transfer of immunoglobulins from adults living in hyperendemic areas of West Africa can suppress the parasitaemia of children suffering from *Plasmodium falciparum* and *P. malariae* infections (COHEN *et al.*, 1961). Some of these sera also inhibited the growth of erythrocytic forms of *P. falciparum* in vitro (WILSON & PHILLIPS, 1976). There have been a number of studies on the circulating malaria antigens recognized by the serum antibodies of individuals living in endemic areas (MCGREGOR *et al.*, 1968; WILSON *et al.*, 1975), but little is known about the antigens of *P. falciparum* erythrocytic forms which are recognized by these sera. The aim of this work is to identify the malaria antigens present in the various erythrocytic developmental stages of *P. falciparum* and which are recognized by the sera of individuals with varying degrees of immunity.

An isolate of *P. falciparum* prepared from

parasitized blood of a European patient returning from Senegal (SGE 1) was adapted to *in vitro* culture according to the method of TRAGER & JENSEN (1976). The culture was synchronized twice at 34-hour intervals by plasmagel sedimentation (REESE *et al.*, 1979) and by incubation in 5% manitol to lyse mature erythrocytic forms (LAMBROS & VANDERBERG, 1979). The synchronized culture showed more than 98% homogeneity for the successive developmental stages of *P. falciparum* (ring forms, trophozoites and schizonts).

Synchronized cultures were sequentially pulse-labelled for four hours with ³⁵S-methionine (specific activity: 239 TBq/mmol; 20 μCi/ml of culture medium; 4% suspension of human RBC with 9% parasitaemia). Labelled parasitized RBC were harvested, washed twice in phosphate saline buffer and lysed in seven volumes of 0.5% Nonidet-P40 in Tris NaCl-EDTA buffer. The lysate was centrifuged at 20,000 g for 20 min at 4°C. 85 to 90% of the label was present in the supernatant and 8 to 15% in the pellet. The supernatant of each stage-specific culture lysate was used as antigen source of the various *P. falciparum* erythrocytic stages. The total incorporation into TCA precipitable material was respectively 0.7%, 4.6% and 6.6% in ring forms, trophozoites and schizonts and less than 0.1% in normal RBC.

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