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Defence reactions of *Glossina morsitans morsitans* against different species of bacteria and *Trypanosoma brucei brucei*

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Summary

Tsetse flies, *Glossina morsitans morsitans,* fed on rats infected with *Trypanosoma brucei brucei* showed wide fluctuations in total and differential haemocyte counts. Similar fluctuations occurred in controls fed on non-infected rats and also between the two groups without showing any difference which could be attributed to the infection. Trypanosome infection of the tsetse haemocoel occurred in 16.25% of the flies, starting from the second day after feeding on the infected rats, but salivary glands and proboscis became infected only after the eleventh day. About 2% of bloodstream forms of *T. b. brucei* injected into tsetse haemocoels completed their developmental cycle successfully. Injection of tsetse homogenates into teneral *G. m. morsitans* prior to exposure to trypanosome-infected feed increased *T. b. brucei* infections in the flies significantly.

Injection of live *Escherichia coli*, Enterobacter cloacae and *Acinetobacter calcoaceticus* into tsetse induced a remarkable increase in two pre-existing haemolymph proteins with molecular weights of about 70 and 17 kilodaltons, while live *Bacillus subtilis* and *Micrococcus luteus* induced a very weak response or sometimes none at all. *T. b. brucei* also failed to induce any increase in these proteins. Inoculation of *G. m. morsitans* with live *E. coli* und *T. b. brucei* prior to feeding on trypanosome-infected rats had no effect on the salivary gland and proboscis infection rates by *T. b. brucei.* Injection of live *T. b. brucei* into the haemocoels of tsetse caused no change in total haemocyte counts, but the trypanosomes disappeared from the haemolymph so rapidly that by 48 h post-injection, only about 1% were left.

Key words: *Glossina morsitans morsitans; Trypanosoma brucei brucei;* defence; haemocytes; electrophoresis.

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Introduction

The relationship between the pathogenic African trypanosomes and their tsetse fly vectors is not well understood. Several interesting observations, which obviously require further investigations, have been reported. For instance, the infection rate of *T. brucei* and *T. congolense* in various species of tsetse have been shown to be unusually low (Harley and Wilson, 1968; Elce, 1971, 1974; Harley, 1971). Furthermore, the infection rates have been shown to be higher in males than in females in several species of *Glossina* (Hoof, 1947; Harley, 1971; Distelmans et al., 1982), and in young teneral flies than in old flies (Wijers, 1958; Harmsen, 1973; Distelmans et al., 1982). One of the possible factors which may influence the infectivity of tsetse by trypanosomes, and which forms the basis of our present investigations, is the immune state of the fly (Jordan, 1976; Molyneux, 1980). As stated by Maudlin (1980), the relationship between tsetse and trypanosomes is an ancient one and it seems only natural that in the course of the trypanosome adapting to life within the tsetse, the fly would also have evolved mechanisms to deal with this invasion. Indeed, more recent observations by Maudlin (1982) and Distelmans et al. (1985) have shown that susceptibility of *G. m. morsitans* to *T. congolense* infection is genetically controlled. With the tsetse haemocytes now fully classified (East et al., 1980; Kaaya and Otieno, 1981; Kaaya and Ratcliffe, 1982), it should be possible to conduct detailed investigations on cellular and humoral defence reactions of tsetse against trypanosomes. Recent reports on humoral factors show that an antitrypanosomal factor occurs naturally in the haemolymph of tsetse (Croft et al., 1982; East et al., 1983). Injection with live *E. coli* has also been shown to stimulate production of some haemolymph proteins in tsetse (Kaaya et al., 1986).

The main objectives of these investigations were therefore to determine the role played by the haemocytes in defending tsetse against trypanosome infection, to determine whether the proteins induced by live *E. coli* could also be evoked by other microorganisms, and whether these induced proteins and/or other unknown factors produced against other antigenic materials confer any protection against trypanosome infections.

Materials and Methods

Tsetse flies

The flies, *G. m. morsitans* used in these experiments were obtained from the ICIPE insectary and were fed on rabbits 3 times a week. They were kept in an insectary maintained at 25° C and 80% relative humidity.

Infecting tsetse with trypanosomes

Rats were injected intraperitoneally with a stabilate of *T. b. brucei* (EATRO 1969). During the first peak of parasitaemia, teneral *G. m. morsitans* were allowed to engorge on the rats. After this the flies were maintained on uninfected rabbits for the rest of the experimental period. They were examined for trypanosome infection after 30 days.

Preparation of tsetse homogenates

Thirty 6 h- or 4-week-old male *G. m. morsitans* were killed by freezing at –20° C for 30 min and then thoroughly homogenized in 7.50 ml of *Aedes aegypti* saline (Hayes, 1953), centrifuged at 9,000 *g* for 10 min at 4° C, and the supernatant filtered through 0.45 µm Millipore filters and protein concentration determined using the method of Lowry et al. (1951). Protein concentration was then adjusted to 10 µg/µl und stored at –20° C until required for use.

Preparation of bacterial cultures

Bacillus cereus (T2), *E. coli* (Kl2), *B. subtilis* (NCIB 3610), *E. cloacae* (NCIB 10101), *Pseudomonas aeruginosa* (NCIB 8295) and *Micrococcus luteus (lysodeikticus)* (NCIB 9278), obtained from the National Collection of Marine and Industrial Bacteria, Aberdeen, Scotland, and *Acinetobacter calcoaceticus* (ACll) from the Department of Microbiology, University of Stockholm, Sweden, maintained on agar slants at 4° C, were subcultured in nutrient broth (Oxoid Ltd., London) for 24 h, centrifuged at 12,000 *g* for 5 min at 24° C, and then washed twice in *A. aegypti* saline. Prior to injecting into insects, bacterial concentrations were determined using a Helber counting chamber (Weber Scientific International Ltd., England), after which the required concentrations were prepared in *A. aegypti* saline, and injected using an Arnold hand microapplicator (Kaaya et al., 1986).

Determination of total haemocyte counts (THC), differential haemocyte counts (DHC), and trypanosome infection rates in G. m. morsitans

A group of 200 teneral (newly emerged) male *G. m. morsitans* were fed on rats infected with *T. b. brucei*, while an identical group engorged on uninfected rats. All flies were subsequently maintained on uninfected rabbits and 10 flies from each group sacrificed on days 1, 2, 3, 6, 11, 14, 21 and 27 post-infected meal and their THC, DHC and infection rates determined. Haemolymph for haemocyte and trypanosome counts was collected by limb amputation as described by Kaaya and Ratcliffe (1982), and THC and DHC were determined in diluted haemolymph (Kaaya and Otieno, 1981; Kaaya et al., 1986). Trypanosome infections in the gut, salivary gland and proboscis were determined by dissection.

In a separate experiment, 200 one-week-old male *G. m. morsitans* were each injected into the haemocoel with 1×10^3 live *T. b. brucei* in 2 µl of phosphate-buffered saline containing 1% glucose (PSG) and thereafter 10 flies were bled at 6, 24 and 48 h to determine THC and trypanosome counts.

Injection of India ink and sheep red blood cells (SRBC)

India ink (Pelikan, Fount India) was diluted 1:30 with *A. aegypti* saline after which 3 groups of 30 male teneral *G. m. morsitans* were injected with 2 µl of the diluted ink, and another 3 identical groups with 2 µl of the saline alone. The flies were allowed to engorge on rats infected with *T. b. brucei* the following day and thereafter maintained on uninfected rabbits until they were killed and examined for trypanosome infection 30 days later.

In another experiment, groups of 75 teneral male *G. m. morsitans* were inoculated with either 1×10^5 SRBC previously fixed in 5% formalin and washed 3 times in saline, 2 µl of India ink (1:15 dilution), or with 2 µl of *A. aegypti* saline alone. Twenty-four hours later, all flies received intrahaemocoelic injections of 200 live *T. b. brucei* suspended in 2 µl of PSG and then maintained on uninfected rabbits and dissected 30 days later to determine whether the injected trypanosomes would complete their maturation cycle and whether blocking of the phagocytic haemocytes with SRBC or India ink would have any effect on the infection rates.

Injection of tsetse homogenates

Five groups of 50 male teneral *G. m. morsitans* were injected with either 5 µl (containing 50 µg protein) of the young tsetse homogenate, 5 µl (50 µg protein) of old tsetse homogenate or with 5 µl of *A. aegypti* saline alone. These flies were fed initially on rats infected with *T. b. brucei* showing high parasitaemia and subsequently maintained on uninfected rabbits. They were examined for trypanosome infection as stated above.

Stimulation of protein production

Groups of 75 one-week-old male *G. m. morsitans* were injected with either 1×10^5 live *B. cereus, E. coli, B. subtilis, E. cloacae, P. aeruginosa, M. luteus, A. calcoaceticus* or with 500 live *T. b. brucei* per fly, while a control group received 2 µl of *A. aegypti* saline alone. Haemolymph was collected 48 h post-injection and proteins separated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (Kaaya et al., 1986). Electrophoretic mobilities of phosphorylase, bovine serum albumin, ovalbumin and cytochrome C were used as markers for molecular weight (mw) determinations.

In order to determine changes in protein levels in the tsetse haemolymph, 250 one-week-old male *G. m. morsitans* were injected with 1×10^5 live *E. coli* and haemolymph from groups of 30 flies collected at 3, 6, 18, 24, 30 and 48 h post-injection and changes in protein levels determined.

Inoculation of G. m. morsitans with live E. coli and T. b. brucei

Three groups of 50 male teneral *G. m. morsitans* were inoculated with live *E. coli* (1×10^3 per fly), while three identical groups received 2 µl of *A. aegypti* saline alone. After 24 h, the flies were allowed to engorge on rats infected with *T. b. brucei* and thereafter maintained on rabbits until they were examined for trypanosome infection.

In another experiment, 6 groups of 30 male teneral *G. m. morsitans* were inoculated with live *T. b. brucei* (1×10^2 per fly), while 3 identical control groups received 2 µl of PSG alone. Three of the 6 trypanosome-inoculated groups were fed on uninfected rabbit throughout, while the remaining 3 and the 3 PSG-injected groups were initially exposed to *T. b. brucei* infected blood meal and thereafter maintained on uninfected rabbits. All flies were dissected 30 days later to determine the infection rates.

Statistical analysis

Analysis of data was conducted using the 't' test at 95% confidence limits.

Results

Both THC and DHC in *G. m. morsitans* fed on rats infected with *T. b. brucei* showed wide fluctuations between the different sampling times and between the experimental and control groups, without any difference which could be attributed to trypanosome infection (Table 1). Haemocoelic infections by the trypanosomes occurred in 13 out of 80 (16.25%) flies examined, starting from the second day after ingestion of the infected blood meal. On the sixth day, haemocoelic infections reached a peak of 5 out of the 10 (50%) flies examined. Thereafter, the incidence of haemocoelic infections decreased so that on days 21 and 27, none were found in any of the flies examined. In the flies with haemocoelic infections, the haemolymph did not appear different from that of uninfected flies. Salivary gland and proboscis infections were observed only after the 11th day following ingestion of the infected blood meal.

In *G. m. morsitans* injected into the haemocoel with 1×10^3 live *T. b. brucei* and in PSG-injected controls, THC were not significantly different from each other (see Table 2). However, a rapid and significant ($P<0.01$) drop in the number of trypanosomes in the haemolymph occurred so that 48 h post-injection, only about 1% of the injected trypanosomes were present. Furthermore, the trypanosomes became progressively sluggish as they remained in the haemolymph. Phagocytosis, nodule formation and encapsulation of trypanosomes by the haemocytes were not observed at any time.

Table 1. Total haemocyte counts, differential haemocyte counts and infection rates in male *G. m. morsitans* at different times after feeding on rats infected with *T. b. brucei*. Means of 10 flies are presented.

Days post-exposure	THC (µl)	DHC (%)			Infected out of 10 dissected			
		PLs	GRs	THs	Gut	Haemo.	S. gland	Prob.
1	Controls 7718±1094[a]	55.20 ±3.40	14.40 ±3.24	30.80 ±5.84	–	–	–	–
	Exposed 9009±1224	59.40 ±4.07	32.30 ±3.39	8.60 ±3.80	10	–	–	–
2	Controls 5054±695	38.20 ±5.88	11.70 ±1.82	50.10 ±6.41	–	–	–	–
	Exposed 6380±689	37.40 ±6.68	21.10 ±4.95	41.60 ±8.95	10	3	–	–
3	Controls 5838±716	28.10 ±3.97	18.30 ±2.74	53.60 ±5.97	–	–	–	–
	Exposed 7060±900	18.90 ±2.91	9.00 ±2.16	72.10 ±4.63	10	3	–	–
6	Controls 8307±1164	16.62 ±3.11	16.00 ±6.51	67.25 ±6.87	–	–	–	–
	Exposed 1771±1449	15.75 ±4.66	7.62 ±2.58	76.62 ±7.07	5	5	–	–
11	Controls 3408±394	28.75 ±4.45	24.62 ±4.21	46.75 ±5.01	–	–	–	–
	Exposed 8417±1250	9.25 ±2.07	29.12 ±3.96	61.62 ±5.15	2	1	–	–
14	Controls 6656±1131	19.00 ±4.75	14.70 ±3.73	66.30 ±6.99	–	–	–	–
	Exposed 5250±814	19.60 ±3.94	18.10 ±5.14	62.10 ±6.82	5	1	3	3
21	Controls 6595±1181	12.37 ±3.57	13.75 ±5.87	73.87 ±8.45	–	–	–	–
	Exposed 8708±1409	13.30 ±3.14	20.80 ±7.22	65.90 ±8.61	4	–	5	5
27	Controls 4050±387	25.00 ±4.65	32.50 ±4.55	42.50 ±6.21	–	–	–	–
	Exposed 6905±1507	12.37 ±2.17	7.00 ±1.87	80.75 ±3.60	3	–	3	3

[a] One standard error of the mean; Haemo. = Haemolymph; S. gland = Salivary gland; Prob. = Proboscis; PLs = Plasmatocytes; GRs = Granular haemocytes; THs = Thrombocytoids

Table 2. Total haemocyte and trypanosome counts in the haemolymph of male *G. m. morsitans* at different times after intrahaemocoelic injection of 1×10^3 live *T. b. brucei* or 2 µl PSG per insect. Means of 10 flies are presented.

Hours post-injection	PSG	Trypanosomes	
	THC (µl)	THC (µl)	*T. b. brucei* (µl)
6	1644 ±92[a]	1840 ±192 Ns	245 ±32
24	1344 ±124	1312 Ns ±138	38 ±6.80 Sf
48	1344 ±170	1360 Ns ±93	2.60 ±1.33 Sf

[a] One standard error of the mean; Sf = Significantly different ($p<0.001$), Ns = Not significantly different from the control

Table 3. Infection rates in *G. m. morsitans* injected with saline, young tsetse homogenate and old tsetse homogenate before feeding on rats infected with *T. b. brucei*

Inoculum	Gut infection (%)	Haemocoelic infection (%)	Salivary gland infection (%)	Proboscis infection (%)
Saline	41.73 ±0.75[a]	2.75 ±0.70	25.05 ±1.39	25.50 ±1.39
Young fly homogenate	85.00 ±2.46 Sf	4.65 ±0.95 Sf	46.66 ±0.41 SF	46.66 ±0.41 Sf
Old fly homogenate	76.92 Sf ±2.51	3.30 ±0.33	34.60 ±0.30 Sf	34.60 ±0.30 Sf

[a] One standard error of the mean; Sf = Significantly higher than control ($p<0.05$)

Injection of India ink into tsetse to block the phagocytic haemocytes 6 h prior to ingestion of the infected blood meal had no effect on the trypanosome infection rates in the flies. Furthermore, intrahaemocoelic injections of India ink or SRBC into tsetse 24 h prior to injecting *T. b. brucei* also failed to alter the infection rates. The inoculated trypanosomes completed their developmental cycle in only 1 out of 60 inoculated flies.

Injection of tsetse homogenates into male teneral *G. m. morsitans* before feeding on *T. b. brucei*-infected rats caused a significant increase ($P<0.01$) in gut, haemocoel, salivary gland and proboscis infection rates with the homogenates from young flies causing a greater enhancement than those from old tsetse (Table 3).

All flies injected with live *B. cereus* and *P. aeruginosa* died by 18 h post-injection and therefore haemolymph for electrophoresis was not obtained. Hae-

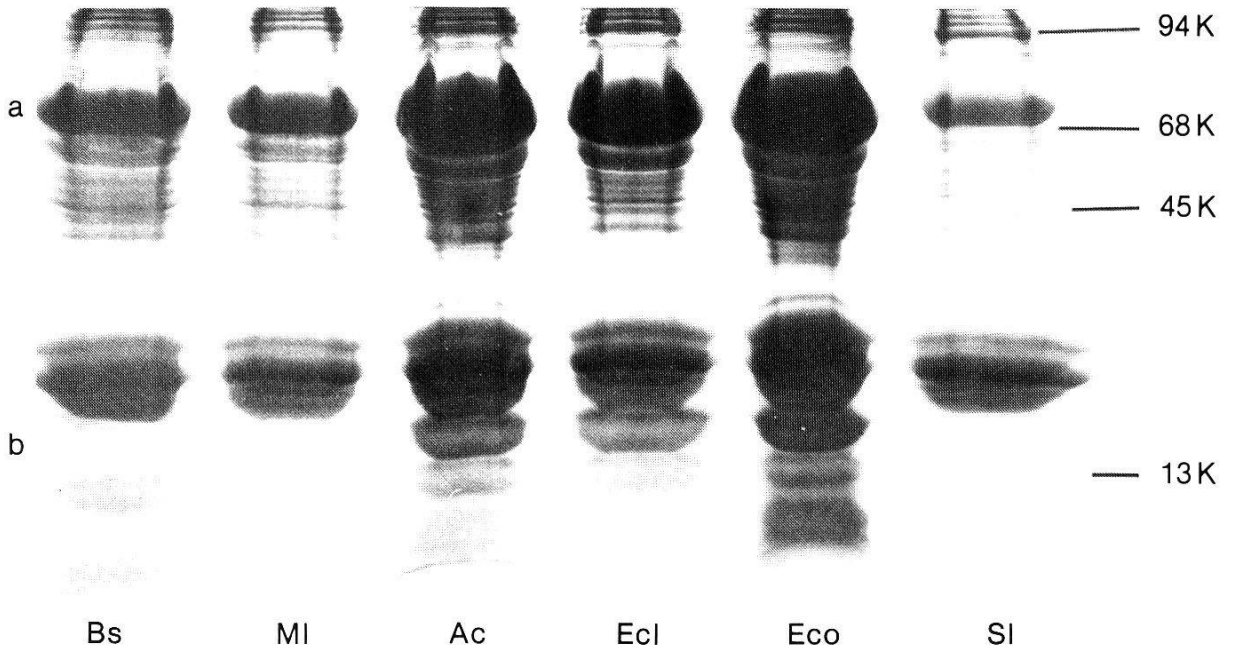

Fig. 1. Electrophoresis of haemolymph proteins from *G. m. morsitans* 72 h after injections of live *B. subtilis* (Bs), *M. luteus* (Ml), *A. calcoaceticus* (Ac), *E. cloacae* (Ecl), *E. coli* (Eco) and saline (S1). Note the remarkable increase in proteins (a and b) in Ac, Ecl and Eco and its slight increase in Bs, and Ml compared to S1.

molymph collected from flies 72 h after injection of live *E. coli, E. cloacae* and *A. calcoaceticus,* showed a remarkable increase in two proteins with mw of approximately 70 and 17 K, but haemolymph from flies injected with *M. luteus,* and *B. subtilis* showed no response (Fig. 1), furthermore, no increase occurred in the haemolymph of flies injected with *T. b. brucei.* Haemolymph collected at different time intervals following injection of live *E. coli* revealed that the increase in these proteins begins at approximately 18 h post-injection (Fig. 2).

G. m. morsitans inoculated with 1×10^3 live *E. coli* or 200 *T. b. brucei* 24 h prior to feeding on rats infected with *T. b. brucei* showed no difference in infection rates when compared with PSG-injected controls. The control groups inoculated with *T. b. brucei* but not subsequently fed on infected rats did not develop salivary gland or proboscis infections.

Discussion

In our present investigation, *G. m. morsitans* fed on rats infected with *T. b. brucei* showed no consistent change in THC or DHC, which could be attributed to the infection, irrespective of whether the haemocoels became infected or not. Furthermore, no evidence of phagocytosis, nodule formation or encapsulation of trypanosomes by the haemocytes was observed at any time. Flies inoculated into the haemocoel with *T. b. brucei* also showed no change in THC or any other

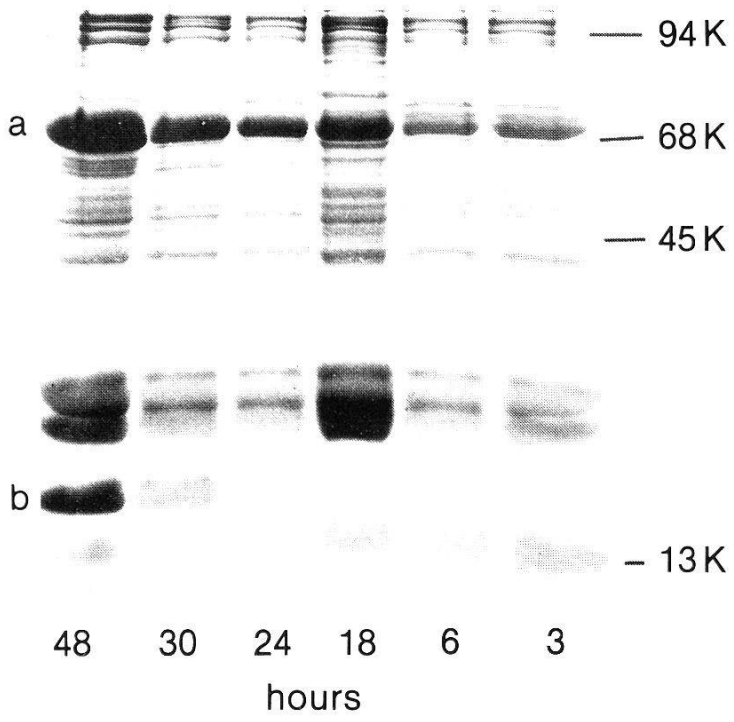

Fig. 2. Electrophoresis of haemolymph proteins from *G. m. morsitans* at different times following injection of live *E. coli*. Note the increase in proteins a and b starting from 18 h.

evidence of cellular response. Although injection of *Triatoma infestans* with India ink to block the phagocytic haemocytes prior to infecting with *Trypanosoma rangeli* has been reported to cause higher parasitaemias (Zeledon and Monge, 1966), our results show that blocking of tsetse phagocytic haemocytes with India ink or SRBC had no effect on the gut, haemocoel, salivary gland and proboscis infection rates by *T. b. brucei*, regardless of the route of infection. These observations strongly suggest that cellular defence reactions play no significant role in defending tsetse against trypanosome infections. Likewise, with bacterial infections, cellular defense appears to be of limited importance in the *Glossina* (Kaaya et al., 1986).

Mshelbwala (1972) reported that 40 (15.27%) out of 262 tsetse flies dissected after feeding on rabbits infected with *T. b. brucei* acquired haemocoelic infections, while Otieno (1973) reported that 2 (3.4%) out of 59 *G. m. morsitans* experimentally infected with *T. b. brucei* through the natural route acquired haemocoelic infections. Furthermore, Otieno and Darji (1977) found haemocoelic infections by *T. b. brucei* in 3 out of 955 *G. pallidipes* caught in the Lambwe Valley in Kenya. In our present investigations, 16.25% of the *G. m. morsitans* fed on rats infected with *T. b. brucei* developed haemocoelic infections. In these flies, the haemolymph appeared normal, without any contamination with red blood cells or haemoglobin, thus eliminating the possibility of gut rupture and subsequent migration of trypanosomes into the haemocoel. Although some reports (Evans and Ellis, 1983) show that trypanosomes may

take between 12 and 24 days to leave the ectoperitrophic space and cross the midgut cells into the haemocoel, our present observations show that this process can occur rapidly because one day after ingestion of the infected blood meal, 3 out of 10 flies examined had already acquired haemocoelic infections, and by the 6th day the proportion had increased to 5 out of 10 flies. Otieno (1973) also found haemocoelic infections in *G. m. morsitans* only 3 days after engorging on mice infected with *T. b. brucei*. He also observed that one of the flies was infected in the haemocoel by bloodstream forms of *T. b. brucei*, while another fly had a mixture of bloodstream forms and procyclics. His observations are therefore in agreement with our present findings, which indicate that trypanosomes may penetrate the gut wall and enter the tsetse haemocoel fairly rapidly.

Although East et al. (1983) are of the opinion that the presence of antitrypanosomal factor in the haemolymph of tsetse reduces the likelihood of an alternative pathway of trypanosome development, Otieno et al. (1976) observed that following intrahaemocoelic injections of *T. b. brucei* into *G. m. morsitans*, trypanosomes developed to the infective stage in 2–3% of the inoculated flies. Similarly, in our present investigation, approximately 2% of the flies injected with *T. b. brucei* in their haemocoels developed salivary gland and proboscis infections, indicating that some of the bloodstream forms of *T. b. brucei* are capable of completing their developmental cycle successfully once they gain access into tsetse haemocoel. These reports of haemocoelic infections and of successful completion of the developmental cycle in trypanosomes inoculated into tsetse haemocoels are very important because, as stated by Molyneux (1980), they throw doubt on the classical accounts of the life cycle of trypanosomes in tsetse, which is believed to take place in the midgut, proventriculus, and salivary glands, without penetrating through the haemocoel of the fly (Buxton, 1955). Indeed, there are several recent publications reporting penetration of tsetse peritrophic membrane and gut cells by trypanosomes during their developmental cycle (Ellis and Evans, 1977a, 1977b; Evans and Ellis, 1975, 1978; Evans et al., 1979) and even an alternative developmental pathway of trypanosomes from tsetse gut through the peritrophic membrane, midgut cells, haemocoel, to salivary glands has been proposed (Evans and Ellis, 1983). Doubtless, if trypanosomes have to cross the tsetse haemocoel during their developmental cycle, the environment of the haemocoel will be crucial to the development and transmission of trypanosomiasis.

Results from our experiments have shown that injection of tsetse homogenates into teneral tsetse prior to infecting with *T. b. brucei* enhances the infection rates of the flies significantly, and that homogenates from young flies give better results than those from old flies. It is possible that certain factors capable of enhancing trypanosome development are present in tsetse tissues and that they occur at higher concentrations in teneral flies. Indeed, teneral flies are known to be more susceptible to trypanosome infection than old flies (Wijers, 1958; Harmsen, 1973). Alternatively, the enhancement of the infection rates

might be a result of exhaustion of tsetse defence mechanisms due to injection of large amounts of antigenic material, thus allowing the trypanosomes to develop without much host resistance.

In a previous communication (Kaaya et al., 1986), we showed that inoculation of tsetse with live *E. coli* conferred protection against subsequent lethal doses of live *E. coli,* and that two proteins of mw of approximately 70 and 17 K were greatly increased in *E. coli*-injected flies. In the present investigations, it has now been shown that enhancement of these proteins begins at approximately 18 h after bacterial injections. These findings are similar to those of Hultmark et al. (1980) who showed that bacteriolytic substance appeared in the haemolymph of cecropia pupae 12–18 h after bacterial injection.

Inoculation of tsetse with low doses of live *T. b. brucei* and *E. coli* prior to feeding on rats infected with *T. b. brucei* failed to confer protection against trypanosome infection. Indeed, earlier investigations (Kaaya et al., 1986) and our present experiments have proved that live *T. b. brucei* do not stimulate production of the two proteins described in this paper. Moreover, although inoculation of tsetse with live *E. coli* stimulates production of the two proteins, it has been reported that insect immune proteins, cecropins, do not lyse eukaryotic cells (Steiner et al., 1981), hence, trypanosomes would presumably not be lysed by these proteins. This does not, however, rule out the possibility that tsetse may have inducible antitrypanosome substances.

Croft et al. (1982) reported that the motility of *T. b. brucei* and *T. dionisii* were greatly reduced when these trypanosomes were incubated in vitro with haemolymph from *G. m. morsitans* for 1–2 h at 28° C. They also demonstrated the presence of this antitrypanosomal factor in the haemolymph of *G. austeni, G. palpalis gambiensis* and *G. tachinoides.* East et al. (1983) reported that haemolymph from *G. m. morsitans* also immobilized *T. congolense* and *T. vivax.* Furthermore, it has been reported that haemolymph of *Periplaneta americana* and *Schistocerca gregaria* contain agglutinins against *T. brucei, Leishmania hertigi* and *Crithidia fasciculata* (Ingram et al., 1983) and that injections of *L. hertigi* and *T. brucei* cause increases in haemolymph agglutinin titres (Ingram et al., 1983, 1984). In both *S. gregaria* and *P. americana,* lysozyme levels were also increased following the injection of *L. hertigi* but not *T. brucei* (Ingram et al., 1983). Agglutinins of *T. brucei* and of calf, guinea pig, and chicken erythrocytes have recently been demonstrated in midgut and hindgut extracts of *G. austeni* by Ibrahim et al. (1984).

In our present investigation, *T. b. brucei* injected into the haemocoels of *G. m. morsitans* disappeared rapidly from the haemolymph so that by 48 h post-inoculation, only about 1% were present, and the trypanosomes became increasingly sluggish as they remained in the haemocoel. These observations strongly suggest the presence of an antitrypanosomal factor in tsetse haemolymph. Further experiments to characterize the antitrypanosomal factor and the bacteria-induced proteins are in progress.

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