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Salivary Secretion in Three Species of Tsetse Flies *(Glossinidae)***

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Introduction

The study of the mechanism and process of salivation in tsetse flies is of paramount importance towards a fuller understanding of the transmission of trypanosomiasis and nagana diseases. GORDON, CREWE and WILLETT (1956) made direct observations, through a microscope, of the haustellum of *G. morsitans* as it penetrated into the ear of an anesthetised mouse and found that probing was accompanied by a copious but intermittent ejection of saliva from the hypopharynx. The outpouring of the saliva commenced during penetration of the stratum corneum and was maintained throughout probing of the tissues. During engorgement, blood is taken in through the labium while saliva was discharged from the hypopharynx at the same time. Besides this work, very little else seems to have been done on the salivary secretion by tsetse flies especially in relation to the hunger cycle of the fly and to the species of tsetse. These aspects were studied on three important species of tsetse and are reported upon in this paper.

Methods

(i) Collection of tsetse fly saliva

Three species of tsetse fly, namely, *Glossina austeni, G. morsitans,* and *G. pallidipes* were used in this study. They were obtained as newly emerged teneral flies from the ICIPE insectary and held in individual plastic tubes 42 mm × 32 mm with nylon gauze at both ends. In one experiment, the flies were given an initial blood meal by allowing them to feed on rabbit ear lobes before they were starved for varying periods. In another experiment, saliva was collected from them before they were given the first blood meal. Saliva was collected from the flies onto glass microscope slides by the bat wing membrane technique developed at ICIPE and described by YOUDEOWEI (1975).

(ii) Measurements of the quantity of salivary secretion

Estimation of the absolute quantities of saliva secreted by tsetse flies is difficult and complicated by three major characteristics of the saliva itself. Firstly the saliva is clear, transparent and produced in minute quantities. Secondly it is viscous and as soon as it is deposited on a slide it dries up within a few seconds even at ambient room temperatures. Thirdly, and most important of all, is the fact

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that there seems to be continuous synthesis of saliva by the secretory cells in the salivary gland, thus as saliva is ejected from the hypopharynx more is secreted into the lumen of the gland. The tsetse is therefore virtually capable of salivating continuously if appropriate stimuli are provided.

Because of these difficulties, only estimates of relative quantities have been made in this work by adopting two methods.

1. The number of salivary drops deposited on a microscope slide per minute was determined and compared in the three species of tsetse and for different intensities of starvation in *G. morsitans*.

2. The salivary drops on microscope slides were fixed with methanol and stained for 40 minutes with Giemsa's stain. The drops were examined under the microscope and all drawn to the same scale with a camera lucida. The area of each drop was then measured with an Allbrit plannimeter. Thus the areas of salivary drops secreted on microscope slides by the three species of tsetse was compared as well as the areas of the drops in relation to different levels of starvation in *G. morsitans*. It is believed that the area covered by each salivary drop is linearly related with its volume, and therefore measuring the area of the drop gives a satisfactory estimate of relative quantities.

Results

(i) Pattern of salivary secretion

Direct observations of salivation by tsetse flies under a microscope revealed that usually the first drop of saliva produced at the initial piercing of the bat wing membrane was the largest. Subsequent secretions at the following piercings were smaller. When the tsetse stopped probing and piercing for a short time and started after a brief rest, the same pattern was repeated, namely an initial large salivary drop followed by smaller drops at subsequent piercing of the membrane. This sequence was observed in all three species of tsetse tested. Table 1 presents estimates of the relative sizes of these drops obtained from *G. morsitans*. In this table, the size of the first drop has been separated from the other drops. It is clear from the data that the first drop of saliva was on the average 2.5 times larger than the others, and the difference between the mean size of the first drop and the others was highly significant ($t = 2.74$, $P < 0.01$).

Table 1. Comparison of the relative sizes of the first salivary drop and subsequent drops secreted by *G. morsitans*

Sequence of drop	Mean area ± S.E. (cm^2)	No. of drops measured	Difference	t
First drop	8.68 ± 0.88	40	5.27	2.74
Others	3.41 ± 0.46	40		$P < 0.01$

(ii) The relative sizes of salivary secretion in three species of tsetse flies

Teneral flies of *G. austeni*, *G. morsitans*, and *G. pallidipes* were given an initial blood meal by feeding them on the ear lobes of rabbits. They were starved for 2 days. After this period of starvation, they were induced to salivate on clean microscope slides through the wing membrane of either the fruit bat *Rousettus*

Table 2. The frequency distributions of the number of salivary drops secreted per minute by three species of tsetse flies

Salivary drops per min.	% Frequency of tsetse flies		
	G. austeni	*G. morsitans*	*G. pallidipes*
0–5	8	8	12
5–10	32	20	20
10–15	48	32	36
15–20	4	20	0
20–25	8	8	16
25–30	0	8	8
over 30	0	4	8

Table 3. Comparison of the numbers of salivary drops secreted per minute by three species of *Glossina*

Species	Mean No. salivary drops/min. ± S.E.	No of flies
G. austeni	11.2 ± 1.15	25
G. morsitans	13.72 ± 1.57	25
G. pallidipes	14.32 ± 1.66	25

Table 4. Comparison of the relative sizes of the salivary drops secreted by three species of *Glossina*

Species	Mean area of drop ± S.E. (cm^2)	No. of drops measured
G. austeni	2.61 ± 0.32	50
G. morsitans	5.10 ± 0.36	50
G. pallidipes	7.16 ± 0.87	48

aegyptiacus or the insectivorous bat *Hipposideros commersoni.* The number of salivary drops secreted per minute was recorded and the areas of 50 salivary drops from each species of tsetse was measured. The results were as follows: Table 2 gives the frequency distributions of the number of salivary drops secreted per minute by the three species of tsetse. Tables 3 and 4 show the means and standard errors of the numbers of drops secreted per minute and the mean areas of the drops. For the two criteria used to assess the quantity of saliva produced by the flies, *G. pallidipes* seemed to secrete the greatest quantity while *G. austeni* secreted the least. Statistical analysis by the KRUSKAL-WALLIS H-Test (1952) showed that there was no significant difference ($X^2 = 1.93$, $P > 0.05$) between the numbers of salivary drops secreted per minute by the three species of tsetse flies

tested. There was a highly significant difference ($X^2 = 45.39$, $P < 0.01$) between the sizes of the salivary drops secreted by the three species of tsetse flies. It was thus concluded that *G. pallidipes* secreted the largest quantity of saliva while *G. austeni* secreted the least. The quantity secreted by *G. morsitans* was between the other two.

(iii) Salivary secretion by starving G. morsitans

G. morsitans was exclusively used for this experiment. They were made to salivate onto clean microscope slides and then given an initial blood meal by feeding them on the ear lobes of rabbits. They were then starved for 1 day, 2 days and 3 days and salivary drops obtained from them at the end of each starvation period. The number of salivary drops deposited on slides per minute was recorded for each level of starvation and the sizes of the drops were also determined by the method described earlier. A total of 25 flies were used for these tests and 42 or 70 salivary drops were measured for each level of starvation. The frequency distributions of the numbers of salivary drops secreted per minute is given in table 5. The mean number of salivary drops deposited per minute in relation to hunger is presented in table 6. Table 7 presents the means and standard errors of the salivary drop sizes. The results may be summarised as follows: The mean numbers of salivary drops secreted per minute and the size of the drops increased progressively in flies starved for less than 1 day to flies starved for up to 2 days. Analysis by the use of the KRUSKAL-WALLIS H-test (1952) performed on the data

Table 5. The frequency distributions of the number of salivary drops secreted per minute by starving *G. morsitans*

Salivary drops per min.	% Frequency of *G. morsitans*			
	starved less than 1 day	starved 1 day	starved 2 days	starved 3 days
0–5	40	16	4	8
5–10	36	4	20	28
10–15	20	32	32	24
15–20	4	32	20	12
20–25	0	12	12	20
25–30	0	4	8	8
over 30	0	0	4	0

Table 6. The number of salivary drops secreted per minute by starving young *Glossina morsitans*

No. of days starved	Mean No. salivary drops/min. ± S.E.	No. of flies
Less than 1 day	6.08 ± 0.80	25
1 day	13.60 ± 1.28	25
2 days	14.48 ± 1.50	25
3 days	13.88 ± 1.36	25

Table 7. The mean sizes of salivary drops secreted by starving young *G. morsitans*

No. of days starved	Mean size ± S.E. (cm^2)	No. of drops measured
Less than 1 day	3.90 ± 0.47	42
1 day	4.53 ± 0.38	70
2 days	5.38 ± 0.38	70
3 days	4.35 ± 0.29	70

showed that the number of drops secreted per minute at the different levels of starvation were significantly different ($X^2 = 28.14$, $P < 0.01$). There was also a highly significant difference between the mean sizes of drops secreted at the different levels of starvation ($X^2 = 13.99$, $P < 0.01$). After 2 days starvation there was a reduction of the number of drops secreted per minute as well as the size of the drops (tables 6 and 7).

Discussion

The use of the bat wing membrane technique to collect tsetse fly saliva (YOUDEOWEI, 1975) has proved to be a very useful method for studying the salivation behaviour of tsetse flies. The results reported there have shown clearly that the first salivary drop is the largest and subsequent salivary drops are smaller. This finding suggests that even if the fly does not succeed in engorging with blood the very first salivation would transfer a relatively large quantity of trypanosomes into the host. If the fly rested for a short time and pierced again a large quantity of saliva would be secreted. This pattern of salivation suggests a mechanism of rapid and continuous synthesis of saliva by the cells of the salivary glands.

The quantity of saliva secreted was related to the size of the tsetse. *G. pallidipes* which was the largest of the three species studied secreted the largest quantity while *G. austeni,* the smallest fly, secreted the least.

The amount of saliva secreted by flies was increased as the flies were starving but after 2 days of starvation, the amount of saliva secreted began to decline. YOUDEOWEI (1973, 1975) had shown that the proportion of *G. morsitans* salivating increased as flies were starved for up to 3 days. Further starvation lead to a decline in the proportion of flies salivating and to high mortalities. These findings correlate with those of BRADY (1972, 1973a, 1973b) who showed that the more a fly was starved the more it responded to stimuli which brought it to its host, the more it probed, and the more it fed. He correlated these behavioural changes to nutritional changes in the fly (BRADY, 1972).

There is therefore considerable evidence which suggests that an infected fly which has been starving for up to 2 days will be a most efficient transmitter of trypanosome parasites. Under this physiological state, the probing, salivating and feeding responses of such a fly are at a maximum. Increased starvation leads to a rapid reduction in the amount of salivation.

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