FUNCTIONAL ASPECTS OF MOSQUITO SALIVATION IN BLOOD FEEDING OF *AEDES AEGYPTI*

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ABSTRACT. Feeding processes and reproductive capacities of salivating and non-salivating yellow fever mosquitoes, *Aedes aegypti* (L.), were compared. Salivation was shown to facilitate blood imbibement and possibly, but less clearly, skin penetration. The assumed presence of an anaesthetic component in the saliva could not be substantiated. Egg production and egg hatching rates were not affected by the absence of salivation. Normal blood meal utilization appeared therefore to be present. It was concluded that the primary function of salivation lies in promoting the feeding process in this mosquito. Salivation thereby constitutes a definite survival value.

INTRODUCTION

It is well known that bites by blood-sucking insects may result in rather unpleasant skin reactions, or even worse. In general these reactions initially consist of allergic responses incited by the insect's oral secretions, probably virtually always of salivary origin (cf. Benjamini and Feingold 1970). While clearly detrimental to the host and potentially prejudicial to the insect itself (cf. Gillett 1967) the importance of salivation in haematophagy has largely remained an enigma.

In at least 2 mosquito species normal blood meal ingestion has been claimed to take place after the severing of salivary ducts (Hudson et al. 1960, Orr et al. 1961). Moreover, normal blood meal utilization as measured by reproductive capacity estimation has been shown to occur (Hudson 1964). The suggestion that salivation should here be marked as a relict from plant-feeding ancestry (De Meillon 1949), however, seems premature in view of salivary gland structure and cyclical activity (Orr et al. 1961).

In one of the forementioned series (Hudson et al. 1960) indications, though of a rather subjective character, were obtained for a greater painfulness of bites by non-salivating mosquitoes. The presence of an anaesthetic component in the saliva was therefore postulated. Although other interpretations of the original results are equally likely (cf. Clements 1963), all imply that salivation would contribute to the efficiency of the feeding process.

The present work was undertaken to further investigate the latter possibility. In our experiments we compared the feeding processes and reproductive capacities of salivating and non-salivating yellow fever mosquitoes, *Aedes aegypti* (L.). In contrast to previous reports detailed observations were made of the feeding process. Reproductive capacities were quantitated as well.

MATERIALS AND METHODS

The mosquitoes used originated from our laboratory stock which was maintained under well-defined standardized conditions (see Mellink 1980). Selected mosquitoes were 10–14 days old and had no blood feeding history. Non-salivating mosquitoes were obtained by cutting open the neck and severing the salivary ducts as described by Hudson et al. (1960). A third of the mosquitoes thus treated survived for up to at least 2 weeks. Prior to utilization the mosquitoes were starved for 2 days to ensure a sufficient biting eagerness.

All subsequent observations of the feeding process were made on the flexor side of the lower arm of a highly immediate (bite) hypersensitive human volunteer. The bites from the 2 categories of

Table 1. Comparing the biting processes and reproductive capacities of salivating and non-salivating mosquitoes; (geometric) means with 95% confidence intervals

parameters	time since operation					
	24 hr		4 days		8 days	
	blanks	non-salivating	controls	non-salivating	controls	non-salivating
penetration period in sec	42 (36–49)	185 (126–272)	47 (40–55)	78 (69–93)	40 (38–42)	62 (58–66)
feeding period in sec	103 (93–115)	124 (111–138)	118 (109–127)	150 (135–167)	122 (116–129)	227 (213–242)
egg production in nr/mosq	91 ± 12	81 ± 17	73 ± 9	84 ± 7	81 ± 8	62 ± 15
larval hatching rate in % of eggs produced	82 (60–97)	82 (68–93)	70 (54–83)	81 (57–93)	75 (40–97)	62 (29–89)

mosquitoes were applied in a randomized order unknown to the test person. The biting sessions took place at ambient laboratory conditions during the hours around noon.

With respect to each bite, alighting (cf. Mellink 1980), penetration, feeding and, where applicable, safety times (Gillett 1967) were recorded, as well as the subjective sensations experienced by the test person. Moreover, the subsequent egg production and egg hatching rates of the individual mosquitoes were determined.

None of the non-salivating, but all of the other mosquitoes induced definite wheal and flare (type I) reactions, denoting the effectiveness of the operations. The feeding process related parameters were found to answer a logarithmic normal distribution as before (Gillett 1967, Mellink 1980). The egg hatching rates were subjected to angular transformation prior to statistical analysis. Only mosquitoes taking a full blood meal (stage 5+ of Pilitt and Jones 1972) and laying eggs were included in the final evaluations. No differences between the categories were found in the latter respects.

RESULTS

In a 1st series, 10 normal mosquitoes (i.e. blanks) were compared with an equal number of non-salivating ones at 24 hr post operation. All bites were applied in 1 session on a single day. No differences in alighting times were observed. In contradistinction to the bites by the blanks, those by the operated mosquitoes were always clearly felt, i.e. were more painful, and unequivocally discerned as such. Penetration times were found to differ when subjected to a t-test ($P_D < 0.01$), but the feeding times were not ($P_D > 0.10$). Neither were differences met with in respect to egg production and egg hatching rates (both also $P_D > 0.10$ cf., Table 1).

Similar results were obtained from comparisons of small numbers of mosquitoes of both categories on ourselves (being practically hyposensitive to bites). The outcome of pilot experiments of the latter kind, however, indicated that the trauma inflicted by the operation could have affected the skin piercing capacities of the mosquitoes.

In a 2nd and 3rd series of 2 sessions each, employing 10 mosquitoes of each category per session, sham-operated controls were therefore utilized. At the same time a 4- and 8-day recovery period was respectively allowed from the operation, after either of which wound-healing appeared to be complete. In contrast with the 1st series, bites by non-salivating mosquitoes were now incidentally felt, and if so faintly, and could no longer be distinguished from those by the controls (and blanks) before the immediate type reactions came up by the subject. Neither were difficulties at skin penetration or aberrant behavior of the operated mosquitoes any longer apparent.

In none of the variables determined were differences of significance found between the results of the 2 sessions in either series (two-way analyses of variance, $P_D>0.10$). Joint assessment of the respective sessions appeared therefore to be warranted (cf. Table 1). Alighting times did not differ. Again longer penetration times, but now also extended feeding times were encountered in the non-salivating mosquitoes (both $P_D<0.05$). Egg production and egg hatching rates were not affected ($P_D>0.10$ and $0.10>P_D>0.05$ respectively). The values obtained for the controls corresponded with those for the blanks.

DISCUSSION

Insect salivation in haematophagy is generally considered important in maintaining blood in a fluid state for transport to the gut. The presence of anticoagulins in the saliva of all blood-sucking species of insects so far examined (in respect to mosquitoes see Clements 1963 and Hudson 1964) and the occurrence of blood-uptake blockades in non-salivating tsetse flies (Lester and Lloyd 1928) seem to support this view. In contrast to the former opinion our results confirm the earlier findings of Hudson et al. (1960) and Hudson (1964) that at least in *Ae. aegypti* feeding to repletion is possible in the absence of salivation. Salivation therefore appears not necessarily to be a prerequisite to blood imbibement *per se*. Possibly the presence of anticoagulins is rather of importance to pool feeding than to vessel feeding species (Benjamini and Feingold 1970). As has previously been shown (Mellink 1980) *Ae. aegypti* essentially belongs to the latter category.

However, cutting the salivary ducts was not without effects. The most prominent of these was evidently on the feeding time (cf. Table 1), but only so at 4 and 8 days after the cessation of saliva egestion. This suggests a gradual diminishing capacity which may be related either to a dwindling of functional salivary reserves still left in the alimentary canal (cf. Hudson 1964) or to pathological effects from saliva spilling in the haemocoel. Although the latter may have played a role, they probably fail to explain the observations because host-seeking behavior (i.e. alighting times) and reproductive capacities went unaffected. Moreover, an exhaustion of salivary reserves constitutes a more likely alternative.

As Hudson (1964) has shown, premature blood-clotting may occur in the alimentary canal of non-salivating mosquitoes. These clots possibly result in partial blockades obstructing the free flow of blood and leading to prolonged feeding times. Such observations were made by Lester and Lloyd (1928) with regard to tsetse flies where the obstructions, moreover, progressed at each meal taken. Premature clotting of the blood was not observed in non-salivating mosquitoes in our series, but it was likely to be missed as a consequence of the superficiality of our examinations in this respect.

Other functions ascribed to salivation pertain to the preparation of the host's dermal tissues for piercing and blood withdrawal. Only for the first assumption some experimental evidence exists, in the form of the finding of a powerful spreading factor in flea saliva (Feingold and Benjamini 1961). In addition a role of salivary substances in lubricating the biting apparatus (Lester and Lloyd 1928, Orr et al. 1961, Hudson, 1964) or in holding the stylets together by surface tension (cf. Lee 1974) has been envisaged. Although penetration times were affected by the absence of salivation in our series, an operation artifact can not be excluded as being causative, because a strong tendency to return to normal piercing capacities was observed, if sufficient time for recovery was allowed. It may therefore be that the cuticular glands in the theca rather than the salivary glands do produce the substances that lubricate and hold together the biting apparatus in mosquitoes (Robinson 1939).

Bites by non-salivating mosquitoes were not more painful than from control animals if sufficient time for wound-healing was allowed. Our results thereby invalidate the arguments for the presence of an anaesthetic component in the saliva of this mosquito as put forward by Hudson et al. (1960). Neither were any indications for the presence of such substances encountered in previous experiments (Mellink 1980).

Although there is some evidence that relatively large amounts of saliva are sucked up together with the blood meal in several haematophagous insect species (Clements 1963, Yorke and MacFie 1924, Lester and Lloyd 1928, Hawkins 1966, Wanson 1950) its significance in blood meal digestion remains to be established. Digestive enzymes possibly related to the utilization of the blood meal appear to be lacking in all haematophagous insect species so far studied (cf. Gooding 1975). The only salivary components probably relevant in this respect are the agglutinins of many, but by no means all blood-sucking species examined, in general occurring in association with high levels of anticoagulins (Clements 1963, Yang and Davies 1974).

In our series egg production and egg hatching rates were not affected by the absence of salivation. Although long term effects could not be investigated, these have been reported to be absent by others (Orr et al. 1961, Hudson 1964). It therefore appears that substances facilitating digestion, as far as present in the saliva, are not essential for an efficacious blood meal utilization in this mosquito.

In conclusion it may be said, that the primary function of salivation in *Ae. aegypti* during haematophagy lies in promoting feeding speed. By shortening the hazardous sojourn on the host, salivation thus constitutes a definite survival value.

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