

AGE RELATED DIFFERENCES OF SALIVA COMPOSITION IN *Aedes aegypti*

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ABSTRACT. Saliva composition in *Aedes aegypti* mosquitoes of differing ages was compared by acrylamide gel-electrophoresis. At 0 and 3 days after adult emergence the composition differed compared with 5- and 10-day old mos-

quitoes. Immediate type hypersensitivity reactions in a human volunteer were less pronounced towards 3- than 11-day old mosquitoes. Course and duration of the feeding process appeared not to be age-dependent.

INTRODUCTION. In our work on lymph node responsiveness to bites by the yellow fever mosquito, *Aedes aegypti*, (Vos and Mellink 1975; Mellink 1975) we observed variations in vertebrate host responses which seemed to be related, at least partially, to differences in the age of the mosquitoes used. As it is well established that products from the salivary glands are responsible for the immunological phenomena alluded to (c.f. Benjamini and Feingold 1970), and also that the development of these glands is not yet fully completed at adult emergence (c.f. Christophers 1960; Orr et al. 1961), we wondered whether the saliva composition of mosquitoes of different ages could be diverse. Saliva from mosquitoes of various ages was therefore subjected to acrylamide gel-electrophoresis.

Furthermore, to get an idea of the possible meaning of the differences found for feeding process and host reactions, a highly

hypersensitive human volunteer was challenged with bites by both very young and essentially older mosquitoes.

MATERIALS AND METHODS. The mosquitoes from a laboratory stock colony of *A. aegypti* were cultured under well defined and strictly standardized conditions (Mellink and Vos in prep.). Up to shortly before use they were maintained on a 25% sucrose solution.

Saliva from 0-, 3-, 5- and 10-day old female mosquitoes was collected on slides exposed to vigorously probing mosquitoes, from sucrose-wicks used for routine feeding or by incubation overnight of whole salivary glands in 1 M NaCl. The salivary secretions harvested by whichever method were taken up in a 1-2% sodium dodecyl sulphate (SDS) solution.

Protein separation was achieved with a Vitatron VAT100 disk electrophoresis apparatus using a 10% acrylamide gel in 0.1 M phosphate buffer (pH 7.0) contain-

ing 0.2% SDS at room temperature (Weber and Osborne 1969). Such gels are suitable for a detection range of molecular weights (MW) from about 15,000 to 80,000 daltons. The size of the samples was 500 μ l for those obtained by both probing methods or 50 μ l for those by extraction. They were applied directly above the gels. Electrophoresis was conducted at 8 mA. per tube for 1.5-3 hours until the tracking dye (brom phenol blue) was ca. 0.5-1 cm. from the bottom of the columns. To stain the proteins in the gels Coomassie brilliant blue in the method of Meyer and Lamberts (1965) was used. The columns were subsequently scanned with a Kipp Densitometer DD2 at 500-550 $m\mu$, and the results calibrated using proteins of known MW as references.

The mosquito bites were applied on the inside of the forearms of the human volunteer in two sessions with 1 week interval. At each session 5 bites by 3- and 11-day old mosquitoes each were given, alternatively and at least 4 cm. apart with intermissions of 20 minutes. Feeding process, skin reactions and subjective sensations were recorded. The different phases of the feeding process were defined as by Gillett (1966). The results were statistically analysed using Student's t-test.

RESULTS. With the acrylamide gel-electrophoresis up to 9 different fractions at a time could be discerned (Fig. 1). No qualitative or quantitative differences of any significance were observed between the results obtained by whichever method of saliva isolation. Leaving aside the differences presumably caused by differences in size of the samples, it appears that at least the fractions of MW 33,000, 60,000 and 40,000 daltons were relatively less well represented in the saliva from the younger (0- and 3-day old) mosquitoes, while inversely, the fraction of MW 12,500, and as far as the 0-day old mosquitoes are concerned also the fraction of MW 46,000, were found in relatively high quantities. The differences observed in the fractions of MW 100,000 daltons and over should be considered unreliable, being on the bor-

der or even well outside the detection range of the method. No differences were observed in the 5- and 10-day old mosquitoes.

The feeding process of 3- and 11-day old mosquitoes on the human volunteer did not show any significant differences in duration of penetration, feeding or safety periods. None of the mosquitoes completed its blood meal within the safety period, probably as a result of its short duration.

The immediate type hypersensitivity responses reached their maximum in 10 to 15 min. and lasted about 2 hrs; the concurrent itching started at about 2 min. after penetration and in general disappeared within half an hour after penetration. No differences were observed in this respect between the younger and older mosquitoes. Maximum wheal and flare size were smaller following bites by the younger than the older mosquitoes (Table 1), albeit at a somewhat higher probability level in the former than the latter ($0.05 < P_D < 0.02$, resp. $0.10 < P_D < 0.05$), probably because of the lower definability of flare sizes. Differences between the results of the two sessions proved to be insignificant.

Delayed type reactivity, although occurring, could not be quantitated, and no relevant differences between the two groups were observed.

DISCUSSION AND CONCLUSIONS. Differ-

Table 1. Mean maximum wheal and flare sizes (\pm S.D.) following bites by *A. aegypti* of different ages on a hypersensitive individual.

mosquitoes		wheal size in mm. ²	flare size in cm. ²
age in days	n		
3	10	47 \pm 21.8	8.6 \pm 1.47
11	10	70 \pm 22.5	11.5 \pm 1.87

ences between younger and older mosquitoes in saliva composition and size of immediate type skin reactions induced by their bites were established. Whether this correlation is linked to a causal relationship remains to be ascertained as no observations were made on the amount of salivary secretions introduced. Our results,

however, clearly indicate that mosquito age—at least for the period shortly after adult emergence—should be taken into account when speaking about host reactions to mosquito bites. No changes in saliva composition were found to occur after about 5 days following adult emergence.

Duration of penetration and feeding

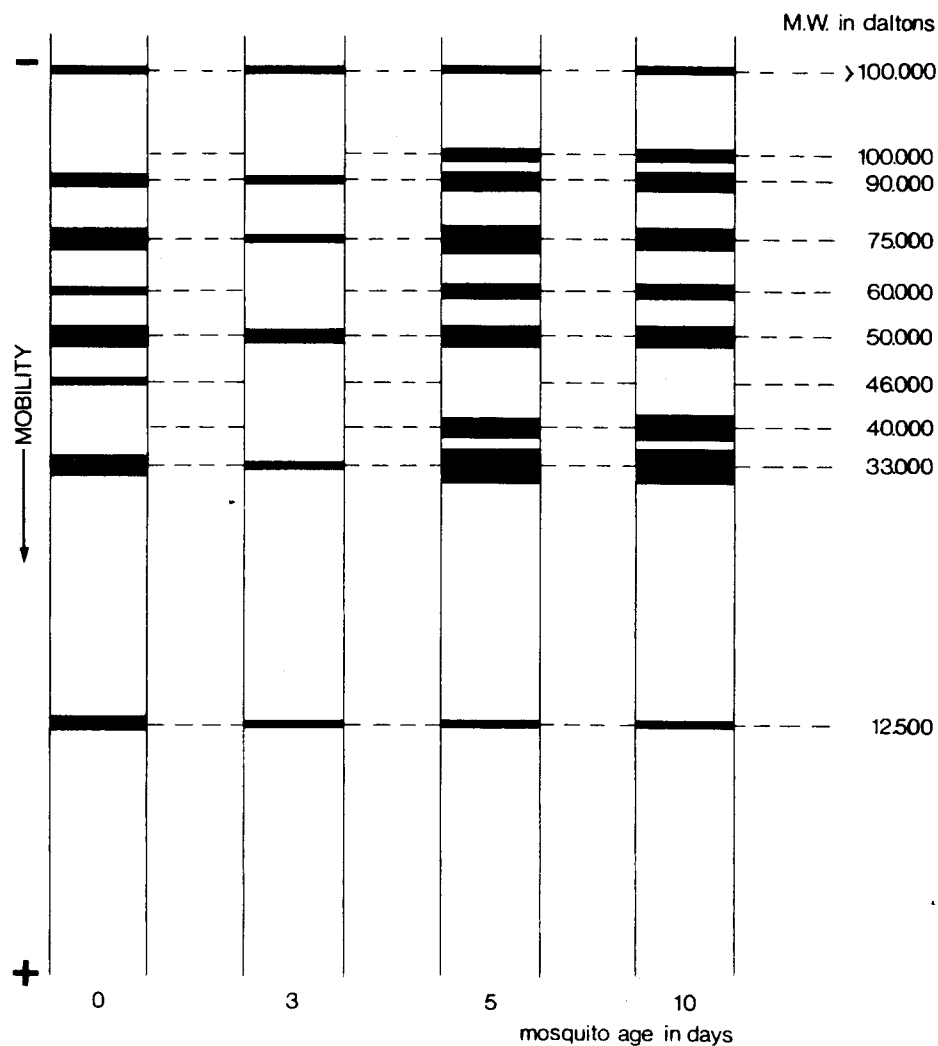


Fig. 1. Acrylamide gel-electrophoretic separation of saliva from female *A. aegypti*. Protein staining. Band width indicating densitometric intensity (semi-quantitative).

periods of both the 3- and 11-day old mosquitoes on the human volunteer were of the same order as reported by Gillett (1966) for "unsuccessful" *A. aegypti* (resp. ca. 1 and 3 min.), while the safety periods were on the average evidently shorter (by ca. 1 min.). The latter divergence may of course be host dependent.

Although our figures for the duration of penetration, feeding and safety periods did not warrant statistical analysis, they seemed to indicate a positive correlation between penetration and safety periods, but not between feeding period and the latter. This is in contrast to the results of Gillett (1966) with colonized *A. aegypti* and wild caught *Aedes africanus* where no such relationship or even the reverse was found. Our finding, which of course needs further confirmation, would agree with the results of Hudson et al. (1960) by which evidence for the presence of an anaesthetic component in the saliva of *A. aegypti* was obtained, as a longer penetration period would mean a prolonged introduction of this component in the tissues and therefore a delay in the onset of irritation. As we observed no differences in this respect between the younger and older mosquitoes, this component would not only already be present in the very young mosquitoes, but would be secreted in as effective quantities as in the older.

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