BLOOD FEEDING ACTIVITY BY NEWLY EMERGED FEMALE MOSQUITOES[1]

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In studies on development of mosquitoes the age of 48 to 72 hours is frequently used as a criterion of maturity. This appears to have arisen from the idea that a mosquito is not "mature" until blood feeding commences, and that blood feeding does not take place until the age of 48 to 72 hours. Of the few cases where the age at the time of the first bite has been reported, Keener (1945) found that *Anopheles quadrimaculatus* Say would bite 18–20 hours after emergence; Howard (1923) reported a first bite by *Aedes aegypti* (L.) 18–24 hours after emergence; and Johnson (1937) reported a first bite 20–40 hours after emergence for the same mosquito. Seaton and Lumsden (1941) found a peak in the number of *A. aegypti* biting at 72–96 hours after emergence but did not state the age when biting started. Christophers (1960) found a majority of *A. aegypti* feeding on the second and third day after emergence but he did not give any indication as to their age when biting commenced.

As part of a study on the salivary glands of mosquitoes it was desired to study the development of the glands from emergence to the age at which biting commenced. Thus with only this general information of early biting activity available it was necessary to determine more accurately the age at which a first blood meal would be taken.

MATERIAL AND METHODS. The investigation was carried out using two species of colonized mosquitoes: *Aedes aegypti* (L.) and *Anopheles quadrimaculatus* Say, both maintained at the laboratory, and four local species, *Aedes vexans* (Meigen), *Aedes stimulans* (Walker), *Aedes trichurus* (Dyar), and *Aedes excrucians* (Walker). The *A. aegypti* colony was maintained following the method described by McKiel (1957); insects for study were reared in pans following the technique described by Trembley (1955). The *A. quadrimaculatus* were reared by the technique described by Armstrong and Bransby-Williams (1961). The local species were collected as third and fourth instar larvae in the field, returned to the laboratory for identification, and then reared in pans, by species, through to pupae and adults. These larvae were reared most successfully in water brought from their larval habitat with no extra food added.

The pupae were collected as required and placed separately in individual 3 inch by 1 inch vials containing about 2 ml. of water; the top of the vial was then closed with a piece of cheesecloth. These vials were checked at one hour intervals and as the adults emerged their time and date of emergence were recorded. After the adult had emerged, the water was carefully poured out through the gauze and the specimen was checked to determine its sex and condition. All males and any abnormal-looking females were discarded. The tubes containing the female mosquitoes were then placed in a darkened cabinet maintained at a constant temperature (26.5±0.5° C.) and a constant relative humidity (70–80 percent) and were only removed for the duration of a bite test which lasted about 10 minutes.

With the variation in availability of the different species and the variation in number emerging at any one time, it was not possible to expose the same number each hour for each species.

The mosquitoes were offered the chance

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to take a blood meal each hour from the age of 10 hours by pressing the gauze-covered mouth of the vial against the ventral surface of a volunteer's forearm. The exposure time was for 5 minutes; the time of biting was only recorded as a first bite or probe if the mosquito had not bitten or probed during the trial one hour preceding the positive trial. Although not all mosquitoes were tested at each hour of age, on completion mosquitoes had been tested at each hour from the age of ten hours. A mosquito was classed as having bitten when blood was visible in the gut after the exposure period. If there was no blood taken, but if the subject showed the existence of a wheal (McKiel, 1959) this was counted as a probe.

In the early stages of the investigation a relatively high mortality was noted in the adults of *A. stimulans, A. trichurus,* and *A. excrucians.* This was attributed to starvation and/or desiccation since these mosquitoes did not start feeding until they were at least 22 hours old. To overcome this mortality, nourishment was provided by placing a pad soaked in a 10 percent sucrose solution over the tops of the vials. This, however, presented the possibility that the mosquito might feed only on the sucrose and not take a blood meal, or delay taking a blood meal. To avoid the possibility of these erroneous results two series of biting experiments were set up, one with sucrose available at all times and the other with no sucrose available. In each case the times of first bite or first probe were recorded.

All experiments were continued until all the mosquitoes had bitten or probed or all had died. The results of this study are shown in Table 1.

RESULTS. From the data it can be seen that mosquitoes will take a blood meal at an earlier age than has been previously reported or accepted. It is interesting to note that the three species which did not take a blood meal until they were more than 23 hours old were all members of the subgenus *Ochlerotatus.* The early feeding of *A. quadrimaculatus* and *A. aegypti* may be partially due to the fact that these mosquitoes have been maintained as laboratory strains and it may be that we were dealing with a group of insects which have been selected for their ability to feed readily under artificial conditions. However, *A. vexans,* collected from the field as were *A. stimulans, A. trichurus,* and *A. excrucians,* took its first blood meal at a much earlier age than these other mosquitoes. The early blood feedings by *A. quadrimaculatus* and *A. aegypti* are in general agreement with Keener's (1945) report that *A. quadrimaculatus* will take a first bite at 18–20 hours, and Howard's (1923) observation that *A. aegypti* were capable of biting at 18–24 hours of age. For the other species the times of first bite (with the exception of *A. vexans*) tended to be more in agreement with the idea of mosquitoes not biting until they are at least 24 hours old.

TABLE 1.—The age, in hours, of virgin female mosquitoes at the time of the initiation of biting activity.

Species	Sucrose not available		Sucrose available	
	Age at first bite	Age at first probe	Age at first bite	Age at first probe
A. quadrimaculatus	14(27)*	no probe	NO TEST	NO TEST
A. aegypti	16(6)	no probe	NO TEST	NO TEST
A. vexans	17(15)	no probe	NO TEST	NO TEST
A. stimulans	36(11)	16(11)	38(12)	18(12)
A. trichurus	23(7)	16(7)	24(7)	no probe
A. excrucians	41(5)	no probe	no bite	61(9)

* The number in parentheses is the average number of mosquitoes exposed at each hour.

In comparing the "sucrose" series with the "no sucrose" series some differences do appear. With *A. stimulans* the availability of sucrose resulted in a 2-hour delay in the onset of both probing and blood feeding. With *A. trichurus* the first bite was recorded one hour earlier when sucrose was not available; there was no indication of any probing activity in the "sucrose" series. For *A. excrucians* there was no probing activity with the "no sucrose" series, and no blood feeding with the "sucrose" series. The availability of sucrose did result in a delay of 20 hours in the initiation of any interest in the host as a source of nourishment. Since the experiment had been set up primarily to ascertain the age at which a mosquito would start blood feeding, and since there had not been any problem of mortality in the three species which took a blood meal at an early age *(A. quadrimaculatus, A. aegypti* and *A. vexans)*, a series of these mosquitoes with sucrose available was not studied.

One interesting observation was made with respect to the biting habits of the mosquitoes studied. The three species which took blood at an early age with no indication of any probing activity *(A. quadrimaculatus, A. aegypti,* and *A. vexans),* all bit within the first minute or two of being applied to the host. In general they followed the same pattern of flying or crawling fairly quickly to the end of the tube applied to the host and after one or two preliminary "samplings" a blood meal was taken.

The species which took their first blood meal at an older age *(A. stimulans, A. trichurus* and *A. excrucians)* followed a different pattern of activity before biting or probing. In each case these mosquitoes did not approach the end of the tube applied to the host as quickly, nor did they bite within as short a time as the others. Also these mosquitoes would frequently touch the proboscis to the gauze or surface of the skin several times and then fly to the other end of the tube without biting or probing.

The short time interval between application of the tube to the host and engorgement by *A. quadrimaculatus* and *A. aegypti* may be attributed to the history of these mosquitoes as being from laboratory strains, but *A. vexans* was not maintained as a colony and had the same history of collection and rearing as did *A. stimulans, A. trichurus* and *A. excrucians.* For this reason the pattern of activity of *A. vexans* can be compared and contrasted with that of *A. stimulans, A. trichurus* and *A. excrucians.* Again the three members of the subgenus *Ochlerotatus* showed a similar pattern of activity prior to probing or blood feeding and they probed before feeding.

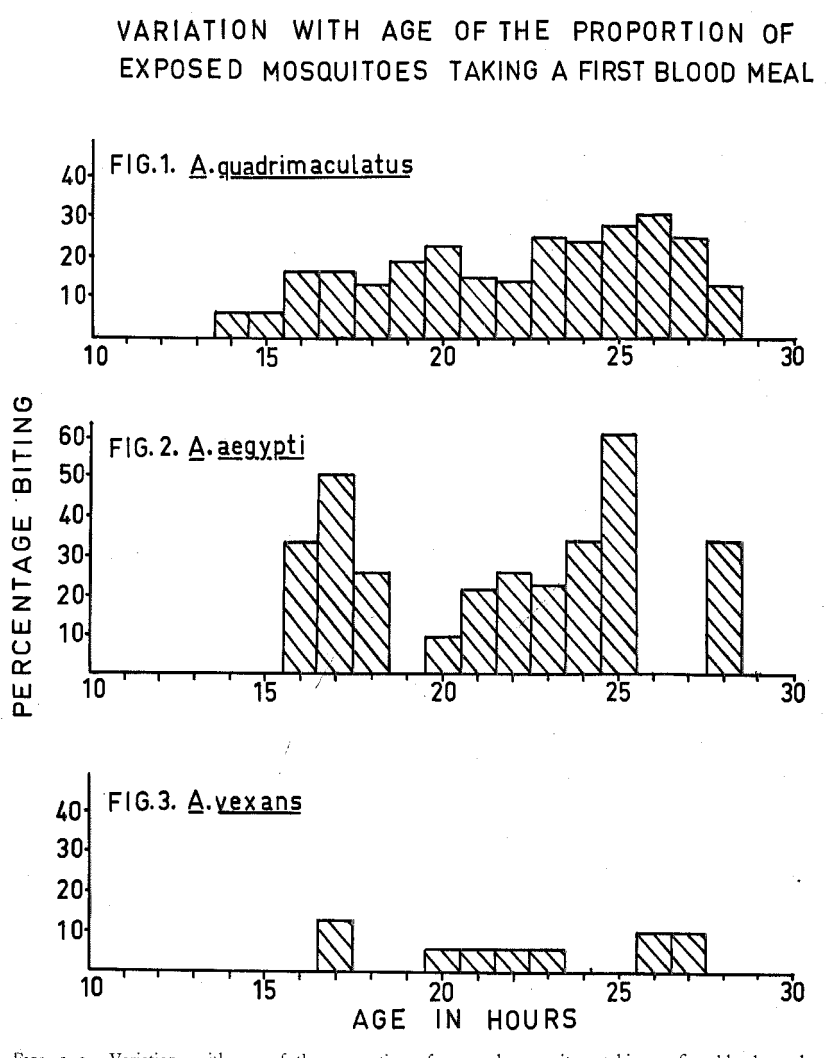
The design of the experiment, with the variation in numbers tested and the fact that tests were carried out at different times of the day did not permit the comparison of results on a statistical basis; however, some observations can be made using the actual numbers tested as a guide.

1. *A. quadrimaculatus.* (See Fig. 1.) With a first bite at 14 hours of age (2 took a blood meal) the number biting each hour increased steadily to a maximum number biting at the age of 26 hours. There was then a decrease in the number biting and from the age of 29 hours to the completion of the experiment at 38 hours the number available for exposure had dropped to the extent that only two per hour were tested and of these one took a blood meal at the age of 35 hours. The period 31 to 38 hours is not shown on the histogram.

2. *A. aegypti.* (See Fig. 2.) This series was terminated at the age of 30 hours. The first bite was recorded at the age of 16 hours (2 bit). After a first peak at 17 hours the number biting decreased to no bites at 19 hours followed by a steady increase in the number biting to the age of 25 hours when 3 of the 5 mosquitoes exposed took a blood meal. There was then a second cessation in biting activity until the age of 28 hours when the last of the first bites was recorded with 2 of 6 biting. With the design of the experiment no significance can be

attached to these increases and decreases in numbers biting.

3. *A. vexans.* (See Fig. 3.) At the age of the earliest blood meal (17 hours) 2 mosquitoes took blood. None bit for the period 18 and 19 hours. There was then constant biting activity from 20 to 23 hours inclusive, and followed by a cessation in biting activity for the period 24 and 25 hours, mosquitoes bit again at 26 and 27 hours of age. No blood meals were taken during the period 28 to 37 hours. After the age of 37 hours single mosquitoes bit at irregular intervals up to the age of 53 hours. No bites were recorded from the age of 53 hours to the age of 72 hours at which time the experiment was stopped. With this sporadic



FIGS. 1–3.—Variations with age of the proportion of exposed mosquitoes taking a first blood meal.

biting after the age of 28 hours this portion is not shown in the figure.

4. *A. stimulans.* The presence of sucrose as an alternative food source did not affect the probing/biting activity pattern when compared with the pattern exhibited by the mosquitoes in the "no sucrose" series. The only difference was, as has been stated, a delay of 2 hours in the time of the first probe and first blood meal. In the "no sucrose" group a single mosquito probed at the age of 16 hours. There was then no activity until the age of 36 hours when one mosquito took a blood meal. After this time there were only single bites at intervals up to the age of 41 hours and no biting from this age to the completion of the experiment at 51 hours. In the "sucrose" group a single mosquito probed at 18 hours; there was no activity until the age of 38 hours when again only a single mosquito took a blood meal. There were only individual mosquitoes biting at irregular intervals from the age of 38 hours to the completion of the experiment at 51 hours.

5. *A. trichurus.* In the "no sucrose" series single mosquitoes probed at intervals for the period 16–23 hours of age. From the age of 24 to 29 hours single mosquitoes took blood meals at each hour. There was then no more biting activity until the age of 43 hours when a single mosquito took a blood meal. In the "sucrose" series mosquitoes did not show any probing activity prior to engorgement, and the first bite by a single mosquito was noted one hour later than in the "no sucrose" series (at the age of 25 hours). Other blood meals were taken at 25 to 42 hours, again by individual mosquitoes. There was some probing activity, also by single mosquitoes, at 44 to 48 hours. Both series were terminated at the age of 50 hours.

6. *A. excrucians.* These mosquitoes showed the least desire to either probe or bite the subject, and in the attempt to get records these two series were continued for the longest times. The "sucrose" series was finished at 108 hours, and the "no sucrose" series was finished at 74 hours due to mortality of the test insects. In the "sucrose" series no blood meals were taken and only two mosquitoes probed, at the ages of 61 and 62 hours. In the "no sucrose" series the earliest recorded bite was at the age of 41 hours when one mosquito took a blood meal. Three mosquitoes probed during the period 51 to 54 hours of age and one mosquito took a blood meal at the age of 65 hours. There was no biting or probing activity after this time.

CONCLUSIONS

1. The age at which virgin female mosquitoes would take a blood meal was determined for two laboratory strains and four local species of mosquitoes. The earliest age at which a mosquito would bite was 14 hours for the laboratory species, and 17 hours for the local species.

2. A pattern of activity prior to blood feeding was noted. *A. quadrimaculatus* and *A. aegypti* (both laboratory strains), and *A. vexans* (local species) all approached the host quickly and took a blood meal after a minimum time involved in touching the tip of the proboscis to the host. There was no probing activity before engorgement. *A. stimulans, A. trichurus* and *A. excrucians* (local species, all members of the subgenus *Ochlerotatus*) all took a longer time to approach the host and spent more time "investigating" the host by frequent touching of the proboscis to the host or to the gauze on the mouth of the vial before probing or feeding.

3. There was some indication of a pattern of blood feeding activity. *A. quadrimaculatus, A. aegypti* and *A. vexans* showed the same general pattern of activity in that after the first bite was recorded fairly large numbers bit at regular intervals up to the age of about 28 hours. After this time there was a decrease in biting activity. It is acknowledged that this decrease in biting activity after the age of 28 hours was primarily due to a decrease in numbers available. So many mosquitoes took a blood meal at the

earlier ages that there were few left for tests at the older ages. *A. stimulans, A. trichurus* and *A. excrucians* also showed a similar pattern of biting activity that was not affected by the presence of a permanent alternative food source. With these mosquitoes there was probing activity first followed by engorgement several hours later. Once biting had started, only individual mosquitoes took blood meals at irregular intervals.

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THE EFFECT OF ODORS RELEASED BY VARIOUS WATERS ON THE OVIPOSITION SITES SELECTED BY TWO SPECIES OF *CULEX*

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Some mosquitoes are known to choose specific types of water for oviposition. Wallis (1954) showed that *Aedes aegypti* (L.) preferred a 0.25 percent salt solution to distilled water. O'Gower (1963) found that the choice of oviposition site by this species was influenced by at least five different stimuli. Gjullin and Johnsen (1964) showed that distilled water could be made more attractive to *Culex quinquefasciatus* Say by adding different chemicals.

Culex quinquefasciatus and *C. tarsalis* Coquillett usually lay their eggs on waters containing grass, logs, sewage, and other organic matter. These materials give off various gases as they break down. This paper reports the results of tests conducted to demonstrate the effects of gases recovered from such waters and of other gases known or suspected of occurring in these waters on these two mosquito species.

MATERIALS AND METHODS. Waters used as a source of odors for these tests were obtained from log ponds or from grass infusions. The infusions were prepared from dried grass and tapwater in the ratio of 1 gram of grass to 82 ml. of water.

Odors were recovered from a glass vessel partially filled with the grass infusion or log pond water. The vessel was closed except for a small air inlet at the top and a glass tube which terminated an inch or two above the water surface. Air drawn through this tube passed through a fritted glass tube placed in a test tube of distilled water.

The 75-ml. distilled water samples