LABORATORY STUDIES ON THE BIOLOGY OF *ANOPHELES STEPHENSI* LISTON[1]

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Anopheles stephensi Liston is the urban vector of malaria in the Near East and in Southwest Asia, and has been used in the laboratory for the experimental transmission of human, simian and rodent malaria. Although a number of studies have been published dealing with the bionomics of this species in relation to malaria, little has been published on its biology under laboratory conditions. Meller (1962) compared some aspects of the laboratory biology of *A. stephensi* with *A. atroparvus* and some work has been done on specific aspects of laboratory rearing. Because of its importance as a disease vector, and because of its potential as a research tool in other types of mosquito studies, a detailed study was undertaken on those aspects of the biology of *A. stephensi* which relate to laboratory rearing.

SOURCE AND MAINTENANCE OF COLONY. The mosquitoes used were obtained from Mr. P. G. Shute, Malaria Reference Laboratory, Horton Hospital, Epsom, England. This colony originated from material collected in the vicinity of Delhi, India. The work described was carried out in the Department of Entomology, Walter Reed Army Institute of Research, Washington, D.C. A temperature of approximately 27° C. and a relative humidity of 65–75 percent were maintained in the insectary. Illumination in the insectary was supplied by overhead lights and controlled by an electrical timer which provided a 14-hour light period preceded and followed by simulated dawn and dusk (Levin, *et al.*, 1958). Larvae were raised in 10 x 16 x 2.5 inch enamel pans. Adult mosquitoes in the stock colony were kept in a 2-cubic foot cage. In the stock colony and in all cages of the study not dealing with the effects of a maintenance diet, the mosquitoes were provided with 5 percent sucrose in sterile water and sliced apples.

EGG HATCH. Eggs from the stock colony were divided into lots of 100. The number of first instar larvae hatching in 72 hours was counted. Seven hundred eggs were taken from the stock colony and divided into 7 lots. One lot was placed in a pint jar of distilled water to determine the hatch rate. The remaining 6 lots were placed on moistened filter paper inside petri dishes sealed with masking tape and placed in a refrigerator at 0–2° C. At 2-day intervals, one petri dish was removed and the eggs allowed to hatch similar to the control.

The mean percent egg hatch of 1800 *Anopheles stephensi* eggs was 71.3±3.7. All hatching was completed within 92 hours from the time of oviposition. The egg hatch was not as high as that reported by Meller (1962) who obtained an 89.2 percent hatch. Differences in fertility and the formation of scum in two of the samples may have led to a lower hatch rate. When cold storage was attempted as a method of storing eggs a decreased hatch occurred after 4 days. The percentage of the eggs hatching in each of the 100 egg samples after different periods of cold storage varied. The hatch rate was 70 percent after 2 days, 74 percent after 4 days, 42 percent after 6 days, 5 percent after 8 days, 2 percent after 10 days and no hatch after 12 days of cold storage as compared with a 75 percent hatch on the day the eggs were collected. It appears from this limited trial that cold storage is effective for holding eggs of this species for short periods up to 4 days.

EFFECT OF LARVAL CROWDING ON ADULT

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[1] This paper is contribution number 411 from the Army Research Program on Malaria.

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WEIGHT. First stage larvae of less than 24 hours age were placed in three pans at density levels of 150, 300 and 600 larvae per pan. Each crowding level was replicated 3 times and each pan was filled with 2000 ml. of distilled water. The larvae were fed a yeast-water solution prepared by mixing one 0.5 gm dried yeast tablet in 50 ml. distilled water. On alternate days 2.5 ml. of yeast-water was added for each 75 larvae in the pans. The position of pans on insectary shelves was changed daily. Upon pupation, the pupae were placed in bowls within four 9 x 12 inch cylindrical cages corresponding to the different crowding levels, and the adults allowed to emerge. Nine days after pupation had begun, 15 female adults were removed from each cage, anesthetized and weighed on an electrobalance. These weights were used as an indicator of the effect of larval crowding. The mean weights of 15 females were 1.49 $\pm$ .06 mg for the 150 level, 1.31 $\pm$ .05 mg for the 300 level and 1.13 $\pm$ .04 mg for the 600 level. Statistically the F value for the linear response in adult weights was significant at the 0.1 percent level. For stock maintenance of the colony the 150 larvae per pan level was adopted.

Davidson (1958) and Meller (1962) expressed their results in terms of a surface area requirement per larva; their findings being 3.2 and 1.8 cm^3 respectively. When expressed in this manner, the levels of larval crowding in our tests were 2.7 cm^2 at the 150 level, 1.4 cm^2 at the 300 level and 0.7 cm^2 at the 600 level.

High larval mortality was noted in two of the three pans at the 600 level. The percentage of larvae dying in each of these pans was 50.0 and 36.2. The third pan had a lower mortality rate of 28.9 percent. In only one pan was the mortality as high as that reported by Meller (1952) in which only 40.4 percent of 400 larvae reared in 35 x 35 x 5 cm pans reached the pupal stage.

EFFECT OF LARVAL DIET ON ADULT WEIGHT. The diets tested were yeast-water, dog chow and a combination diet in which yeast-water was fed during the first 2 instars and dog chow during the last 2 instars. Seventy-five first instar larvae were placed in pans containing 2000 ml. distilled water with four replicates per treatment. The dog chow (Pard Crunchers, Swift & Co., Pet Feed Div., Chicago, Ill.) was distributed at a rate of 100 mg per pan on alternate days. Samples of female mosquitoes were weighed. The mean weights of females were 1.29 $\pm$ .04 mg for the yeast-water diet, 1.51 $\pm$ .06 mg for the dog chow ration and 1.38 $\pm$ .04 mg for the combination diet. There was a significant difference at the 1 percent level between the weights of mosquitoes obtained from larvae fed on yeast-water as compared with those fed on dog chow alone. The combination diet did not show a significant difference from either separate diet.

LARVAL LONGEVITY AND DURATION OF PUPAL PERIOD. There were marked differences in larval mortality among the different larval diet groups. Among larvae receiving the yeast-water diet 45.3 percent mortality occurred, a 24.1 percent mortality rate among those fed the yeast-water for the first 2 instars and the dog chow fed larvae. These rates are higher than those reported by Meller (1962).

Dog chow and mixed diet reared larvae completed development faster than those fed on yeast-water alone. By day 3, 58 percent of the former had pupated while only 30 percent of the latter reached the pupal stage. On the 4th day pupation reached over 90 percent in the dog chow and mixed pans while the yeast-water rose to 76 percent. In the tests of different crowding levels, 85 percent of the larvae from the 75 and 150 pans pupated by day 3 while 69 percent and 51 percent pupated from pans with 300 and 600 larvae respectively. By the 4th day between 87–94 percent of the larvae had pupated at the three lower densities and 75 percent at the most crowded level. In these experiments a few larvae lagged and did not pupate until day 14.

In *Anopheles stephensi* the duration of the pupal period was very brief. All viable pupae became adults within a span of 24–48 hours.

EFFECT OF LARVAL REARING WATER ON ADULT WEIGHT. When the eggs of the stock colony were first received from the Malaria Reference Laboratory it was advised that the eggs should be hatched in distilled water and the larvae similarly reared. Initial rearing procedures followed this technique. Experiments, however, showed no significant differences in adult female weights when larvae were reared in distilled as compared with tap water. It should be emphasized that tap water varies from locality to locality and it must be tested before use.

EFFECT OF WATER LEVEL AND PUPAL CROWDING DURING EMERGENCE PERIOD. Crowding within the pupal container and the distance from the water surface to the top of the jar which the newly emerged mosquito must negotiate have been suggested as determinants affecting the death of newly emerged adults. Twelve wide mouth pint jars were placed in a 2-cubic foot cage. The jars were divided into two water level groups with three pupal density levels within each group. The water levels used were 1 and 3 inches, and the density levels were 75, 150 and pupae per jar. Jars with water 1″ from the surface had mean adult survival from pupal densities of 75, 150 and 300 as follows: 96 percent, 99 percent and 97 percent. Survival in jars with a span of 3″ between the water level and top had emergences of 98 percent and 97 percent in the above sequence of larval densities. None of these differences was significant.

EFFECT OF MAINTENANCE DIET ON ADULT LONGEVITY. Pupae from larvae reared on dog chow were placed in six cages for emergence over 6 consecutive days. The test was divided into two treatments; adults were provided with 5 percent sucrose-water in three cages and 10 percent honey-water in the remaining three cages. The same number of pupae was placed in each of the treatment cages daily so that cage 1 of each group had 300 pupae, cage 2 received 300 pupae and cage 3 contained 263 pupae. A record of emergence date, pupal and adult mortality reached the 50 percent level. The 50 percent mortality level occurred in 20.3 days in the sucrose-water fed cages while in the honey-water groups an equivalent level was reached at 19.8 days. It appeared that neither honey-water nor sucrose-water extended the life of adult females to any appreciable degree over the other treatment. However, throughout the test, the honey-water feeders developed mold 24 hours after preparation. Under the conditions in this laboratory, sucrose-water is recommended as the main portion of a maintenance diet.

EFFECT OF THE NUMBER OF BLOOD MEALS ON ADULT LONGEVITY. Pupae reared from larvae fed on a dog chow diet were collected over a 3-day period. Groups of 50 males and 50 females were placed in each of 12 cages. These cages were divided into four treatments of 0–3 blood meals per week. A rabbit was offered to the females as a blood source on Monday, Wednesday and Friday. A maintenance diet of sucrose solution and apples was provided to each cage during the test. The mean number of days required to reach the 50 percent mortality level on the females was the same in the 2, 3 blood meal group and the control (Table 1). Females that received only one blood meal weekly died a little sooner than the other groups. This difference may not be significant as one replicate was much lower than the others. The responses of the males with their associated

TABLE 1.—Days to the 50% mortality level in *Anopheles stephensi* that received different numbers of blood meals per week.

Blood meals weekly	Day of 50% mortality	
	Males	Females
0 (Control)	18.3	23.0
1	13.7	20.7
2	14.0	23.0
3	12.7	23.0

females were quite different. Males associated with control females lived 4–5 days longer (as determined by the 50 percent mortality level) than the males in cages with females provided with 1–3 blood meals.

EGG PRODUCTION. Approximately 750 pupae were placed in a 9 x 12″ cylindrical cage for emergence. A blood meal was offered to the mosquitoes 10 days later. One hundred engorged females were transferred to another cylindrical cage provided with a small water bowl for oviposition. An additional 36 engorged females were individually placed in pint jar cages (Eldridge & Gould, 1960). Each jar was provided with 5 percent sucrose solution and a small pill cup of water for oviposition. Egg counts were made daily from each group over a 10-day span. The mean number of eggs laid per female in the 36 randomly selected engorged females was 50.4 ± 6.5. When discounting those mosquitoes which did not oviposit, the mean egg count was 75.7 ± 3.3 per female. The mean of the 100 engorged females maintained in a single cage was 51.3. This did not vary significantly from the mean number of eggs derived from individual females placed in separate containers.

SUMMARY. The mean egg hatch of a colony of *Anopheles stephensi* was 71.3 ± 3.7 percent. The hatch rate of eggs stored at 0–1° C. was markedly reduced when storage time exceeded 96 hours. Larval crowding was shown to have an inverse linear effect on adult female weight. A larval diet of dog chow was found to be superior for larval rearing to either a yeast-water or combination diet. The duration of the pupal period was found not to exceed 48 hours. Neither different pupal crowding levels nor the water levels of the pupal emergence containers affected the mortality of pupae and emerging adults. A 5 percent sucrose solution was superior to a 10 percent honey solution as a maintenance diet for adults due to the formation of mold on the honey-water feeders. The number of blood meals had no apparent effect on the longevity of adult females. The mean egg production for females from the colony as a whole was 50.4 ± 6.5. This rose to 75.7 ± 3.3 when negative mosquitoes were discarded. The larval rearing density is probably the single most important factor in the successful laboratory rearing of *Anopheles stephensi*. Optimal rearing at 27° C. occurred with 150 larvae placed in a 10 x 16 x 2.5″ pan containing 2000 cc water.

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