MASS REARING OF *ANOPHELES STEPHENSI* LISTON[1]

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In recent years *Anopheles stephensi* has become an increasingly popular mosquito in laboratories investigating human, simian and rodent malarias. Its rapid life cycle, readiness to feed on a variety of animals and ability to transmit malaria are factors contributing to its increased use.

Various techniques for the laboratory rearing of *A. stephensi* have been reported by Russell and Mohan, 1939; Russell, 1941; Knowles and Basu, 1942; Trembley, 1955; Meller, 1962; Thompson, 1964; and Shute and Maryon, 1966. These techniques, while being adequate for small scale rearing, are time-consuming and provide comparatively few mosquitoes.

During the course of investigations on simian malaria in our laboratories, it was found necessary to develop a mass rearing system in order to provide large numbers of adult mosquitoes on a daily schedule. The mosquitoes used to start our colony were obtained from Dr. Ronald A. Ward, Walter Reed Army Institute of Research, who in turn had obtained them from Mr. P. G. Shute, Malaria Reference Laboratory, Horton Hospital, England. Mr. Shute's colony came originally from Delhi, India.

The mass rearing techniques developed in our laboratories now provide adequate numbers of mosquitoes for our needs and the colony can be expanded easily if the necessity arises.

EGGS. The eggs are collected in a plastic container,[3] 5" x 7" x 2½" (12.70 cm. x 17.78 cm. x 6.35 cm.) in size, containing a layer of cotton pads covered with paper toweling. Approximately 300 ml of water are added to the container to the level required to wet, but not cover, the surface of the paper toweling. Oviposition containers are placed in the mosquito cages 48 hours after the females have been provided with a blood meal. The container is removed after 24 hours exposure to gravid females and the eggs are washed gently into plastic hatching containers 12½" x 9" x 4" (31.75 cm. x 22.86 cm. x 10.16 cm.) in size, half filled with well water maintained at 80° F. (27° C.). Approximately 72 hours later hatching is completed. The hatch rate varies from 70.1 percent to 90.9 percent.

LARVAE. The newly hatched first instar larvae, along with the water in which they hatch, are placed in an aliquot container (Fig. 1) constructed from a 3-liter glass battery jar to which spigots have been attached at 1¼" (3.16 cm.) intervals down the side. A stirring rod attached to a variable speed motor (250 r.p.m.), keeps the larvae evenly suspended for periods up to 45 minutes without apparent damage. While the larvae are thus suspended, aliquot samples of one ml each are withdrawn at various levels, the larvae per sample counted, and the mean number of larvae per ml of water calculated. The amount of water containing the number of larvae required for each rearing tray is then withdrawn into individual containers. Rearing trays are filled to a depth of ¾" (1.9 cm.) with well water 24 hours prior to introduction of larvae. This allows time for stabilization to the desired water temperature. Larvae are intro-

[1] This study was supported by the U.S. Army Medical Research and Development Command, Dept. of the Army, under Contract DA-49-193-Md-2771, under the auspices of Walter Reed Army Institute of Research. This paper is contribution number 387 from the Army Research Program on Malaria.

[2] The assistance of Thomas M. Hopkins is acknowledged.

[3] Tri State Plastic Molding Co., 400 North Wells Street, Chicago 10, Illinois.

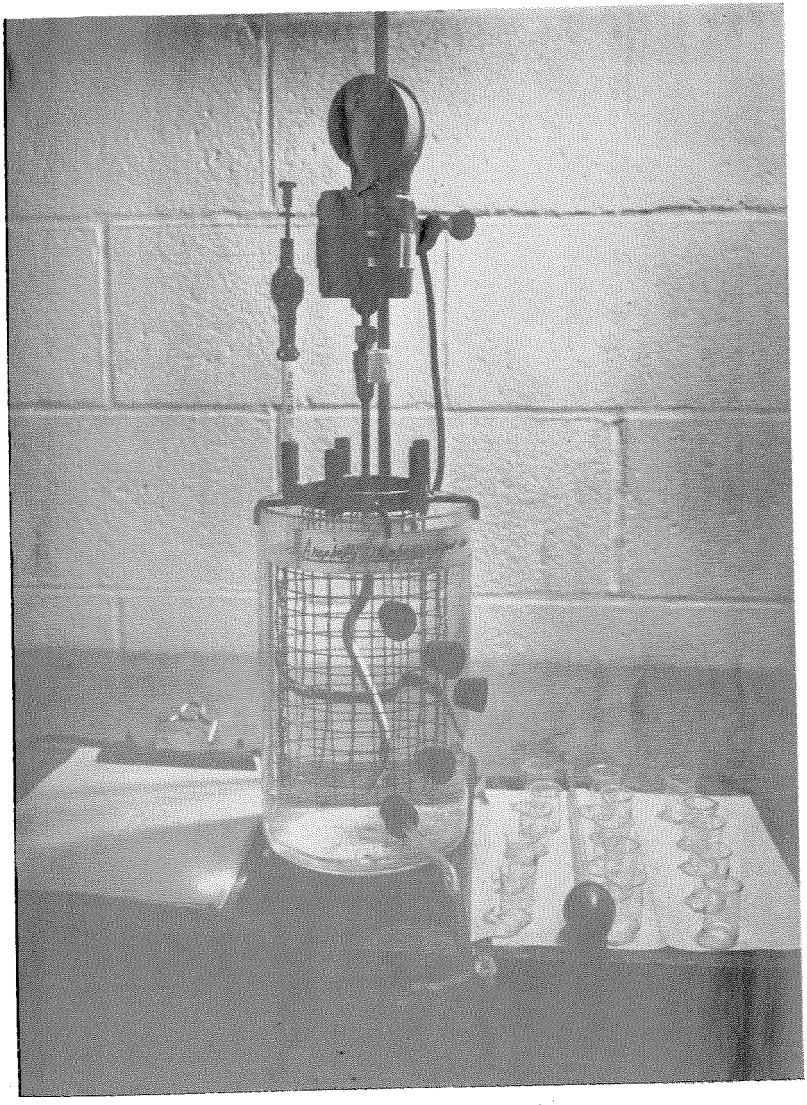

FIG. 1.—Mosquito larvae aliquot container.

FIG. 2.—Rack of mosquito rearing trays.

duced at a rate of 15,000 per tray (0.69 larvae per sq. cm. surface area).

The larval rearing trays (Fig. 2) are constructed of galvanized iron and are 54″ x 30″ x 2″ (137.6 cm. x 76.2 cm. x 5.08 cm.) in size. Each tray has a stoppered drain hole at the front corner. They are placed on metal racks with a space of 6 inches between trays. The metal racks, designed to hold ten rearing trays, are on casters to facilitate handling. The spacing of trays at this interval expedites filling, observation, feeding of larvae and draining, while utilizing space to the best advantage.

The larval food consists of 50:50 mixture of dog chow[4] and porcine liver powder[5] ground to a 40 mesh. The larvae are on the following schedule:

Day 0	(day of introduction) .13 mg/larva
Day 1	.13 mg/larva
Day 2	.13 mg/larva
Day 3	.20 mg+.045 mg Fleischmann's dried brewer's yeast/larva
Day 4	.26 mg/larva
Day 5	.40 mg/larva
Day 6–9	.53 mg/larva

With the above feeding schedule, and with the rearing medium maintained at 80° F. (27° C.), larval development is completed in six to nine days.

PUPAE. Pupation occurs over a 4-day period with approximately 5 percent pupation on day six, 35 percent on day seven, 50 percent on day eight and 10 percent on day nine. The pupae are drained into metal boxes with the overflow screened to prevent loss of specimens. The pupae may be separated by means of a pipette or small piece of wire screen. The ice water technique (Ramakrishnan *et al.*, 1963; Weathersby, 1963) can be used, or they can be separated by mechanical means based on size (Fay and Morlan, 1959). Approximately 1000 pupae are placed in a plastic tray, 5″ x 7″ x 2½″ (12.70 cm. x 17.78 cm. x 6.35 cm.) and introduced into a cage for subsequent adult emergence.

ADULTS. The cages for holding and maintaining adult mosquitoes are 1′ x 1′ x 1′ in size, constructed of aluminum framing and 20 mesh wire screening.[6] The front of the cage is fitted with a stockinette sleeve and the top with a nylon hammock.

The adults emerge within 36–48 hours at a temperature of 80° F. (27° C.), after which time the pupal containers are covered and removed from the cages. The newly emerged adults are furnished sucrose by placing cotton balls,[7] soaked in 10 percent sucrose, in the nylon hammock. Four well-soaked cotton balls are provided per cage, and are replaced daily. Sucrose is provided the adults continuously except during the short intervals required to furnish blood meals. Blood meals are provided by placing a restrained or anaesthetized rabbit, back down, on the nylon hammock for 30 to 60 minutes. Rabbits are prepared for feeding mosquitoes by clipping the hair from the back. Best egg production has been obtained by providing females with two blood meals. These blood meals are supplied at 72 and 84 hours after adult emergence.

Mating occurs readily in 1-cubic-foot cages maintained at 80° F. (27° C.), 80 percent relative humidity and a light schedule of 14 hours artificial light and 10 hours of darkness. No crepuscular period is required. When adult females are maintained on sucrose alone, 30 percent mortality occurs in approximately two weeks.

Eggs are laid 2–3 days after the first blood meal. The number of eggs laid per female varies from 90–158, with a mean of 117 eggs per female.

The best sex ratio and number of mosquitoes is 1,000 females and 500 males per 1 cubic foot of cage space.

[4] Purina dog chow—Ralston Purina Co.

[5] Cornell Chemical & Equipment Co., Inc., Baltimore, Md. 21228.

[6] Gerberg Mosquito Cage GH–1. Cornell Chemical & Equipment Co., Inc., Baltimore, Md. 21228.

[7] Jumbo Preptic Cotton Balls, Johnson & Johnson.

Due to the small size and ability of this species to escape, extra effort is required to prevent loss of living specimens. All precautions must be used when feeding, transferring or otherwise affording the slightest opportunity for escape.

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