CHICKEN EGG CHORIO-ALLANTOIC MEMBRANE FOR MOSQUITO FEEDING

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Blood feeding of mosquitoes presents special problems in the laboratory where viruses or test chemicals must be administered with the food. Techniques in current use have certain disadvantages. Extensive handling of infected animals is necessary if birds or mice are utilized as the source of virus for infection. Routine blood feeding to obtain eggs requires caging and maintenance of animals, and immobilization during the feeding period. While direct feeding of non-blood inoculum from a saturated cotton pad has been successful (1, 2, 3), it subjects the mosquito to external contamination. In addition, uniform engorgement of specimens is difficult to obtain without special attractants (4). To overcome such disadvanages, trials were conducted in the laboratory to find a convenient procedure for blood feeding and for obtaining engorgement with non-blood test substances.

PROCEDURE. Adult *Aedes aegypti* females, 5 days old, were maintained 24 hours without sugar feeding and then distributed in groups of 20 to individual cages for test feeding.

A hole (window) was cut with a small scissors through the shell over the air sac of a 9-day old embryonated chicken egg. This was enlarged by cutting away the shell without breaking the chorio-allantoic membrane. The egg was then turned over and the exposed membrane placed on the screen mesh covering of the mosquito cage. A small hole was punched in the top of the egg shell to permit entry of air, allowing the embryo to "drop" against the screen. Heat and light were provided by placing a 60-watt electric lamp at a distance of 3 inches above the egg. During a 2-hour period, six groups were given a twenty-minute feeding period. Two mosquitoes from each group were weighed before and after feeding to determine the amount of blood ingested, following the method of Lang and Wallis (4).

The second procedure was designed to introduce experimental chemicals into the mosquito without a blood feeding and subsequent egg development. A window was cut in the shell of an infertile chicken egg to expose the shell-chorio allantoic membrane adjacent to the air sac. Then a second window was cut in the opposite end to permit removal of the contents. The hollow egg shell was washed with sterile distilled water and placed on the screen wire covering of the mosquito cage with the membrane in contact with the wire. An experimental chemical solution of two percent dexedrine sulphate was prepared in five percent sucrose solution. This was warmed to 27° C. and poured into the egg shell. Light and warmth were obtained from a 60-watt electric light bulb placed over the egg. The inoculum was offered to mosquitoes in six cages.

RESULTS. In each of six cages of mosquitoes, the 20 female *A. aegypti* engorged with blood within a 20-minute feeding period from the time the embryo was placed on the cage. The mosquitoes were readily attracted to the warm chorio-allantoic membrane. They probed through the wire mesh of the cage into the membrane and engorged without interruption. The rich blood supply in the capillary network and the numerous arterioles and venules in the mesodermal layer of the membrane provided an attractive, easily-obtained source of blood. All female mosquitoes become completely engorged. Average weight of blood meal determined by weighing before and after feeding was 2.05 mg. (range 1.70 mg. to 2.13 mg.) for a series of 12 specimens weighed individually.

In trials for administration of a solution of dexedrin sulphate, a satisfactory number of mosquitoes engorged in each of the six test cages. Of the total number tested (120), 94 engorged completely. Nineteen fed (ingested sufficient inoculum to distend the abdomen, but less than taken in complete engorgement) and only seven did not feed sufficiently to distend the abdomen.

DISCUSSION. The feeding of blood meals to mosquitoes in the laboratory and techniques for oral administration of non-blood solutions, e.g., suspensions of virus inoculum and solutions of chemosterilant compounds, have become important as work expands in the area of virus-vector experiments with arthropod-borne viruses and on the effects of various chemical substances when fed to adult mosquitoes. Since the emphasis in such work has shifted to quantitative experimentation, it is necessary to utilize more reliable techniques that provide uniformity in the quantity of material fed to individuals in a series. It is desirable to do this in a manner that minimizes determinant error due to external contamination of the mosquitoes and, at the same time, permits as nearly as possible a natural mode of feeding. For this purpose, the egg shell chorio-allantoic membrane provides an effective barrier between the mosquito and the inoculum but is easily pierced by the mosquito.

In experiments with mosquitoes infected with viruses, where it is necessary to administer blood feeding, the embryonated egg provides a natural source of avian blood that is attractive to the mosquito. The embryonated egg is easily obtained, and is more easily maintained in the laboratory until time of use than are laboratory animals. In use, it does not require the restraining devices necessary for laboratory animals, and the exposure time may be extended into overnight periods without complications of fecal contamination and disturbance of the feeding mosquitoes by movement of the animals.

Administration of non-blood solutions by allowing mosquitoes to probe through egg membranes may be accomplished with the utilization of fresh chicken eggs that are inexpensive and

readily available. Maximum engorgement of the mosquito may be obtained on virus suspensions when this technique is used, without the complication of loss of virus in developing eggs and oviposition by the mosquito that occurs when blood from infected laboratory animals is used.

SUMMARY. Experiments were conducted with *Aedes aegypti* on blood feeding and on engorgement with non-blood solutions to find a procedure that was both clean and convenient for laboratory use. For this purpose, the chorio-allantoic membrane of chicken egg embryos, after 9 days' incubation, provided an excellent vital membrane suitable for mosquito feeding in administration of blood meals, for egg production and for oral administrations of test chemical solutions.

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PRESERVATION OF MOSQUITOES FOR MALARIAL OOCYST AND SPOROZOITE DISSECTIONS

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In the determination of malarial infection rates in mosquitoes, sample size is limited by the time and personnel available for the dissection of freshly collected material. Our laboratory uses a freezing technique which enables us to preserve, for periods of at least nine months, large quantities of mosquitoes infected with *Plasmodium gallinaceum*. This permits us to make dissections for plasmodial oocysts and sporozoites whenever time or personnel are available. Freezing techniques have been used by parasitologists (Jeffery and Rendtorff, 1955; Molinari, 1961) for the preservation of malarial parasites in whole blood or mosquito salivary glands. To our knowledge, this procedure has not been applied in the preservation of entire infected mosquitoes for dissection at a future date.

For processing, the mosquitoes are immobilized by chilling in a household refrigerator or by exposure to ether or carbon dioxide. They are transferred to serum bottles (5 ml. or larger, depending on the number of mosquitoes in the sample) containing a small wad of absorbent cotton moistened with water which is added to prevent drying of the mosquitoes. The bottles are capped with sleeve type rubber stoppers. Labels are typed on adhesive plaster and applied to the outside of each bottle. The bottled specimens are stored in a —20° C. freezer until needed for dissection.

The freezing compartment of a refrigerator is also satisfactory as a storage area. If serum bottles are not available, mosquitoes can be placed in a petri dish lined with filter paper moistened with 1 ml. of water. Masking tape is used to seal the dish. Prior to dissection of the stored mosquitoes, a serum bottle is removed from the freezer and is allowed to remain at room temperature for ten minutes before it is opened. The dissection technique is the same as that used with freshly killed material.

The oocysts and sporozoites of *Plasmodium gallinaceum* have been well preserved in their

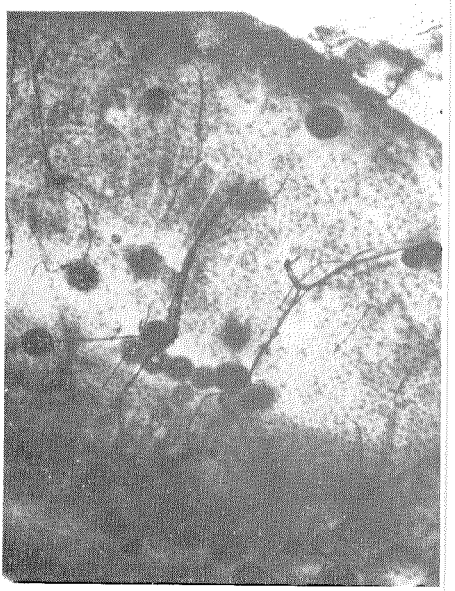

FIG. 1.—*Aedes aegypti* midgut with *P. gallinaceum* oocysts. Prepared from frozen material and stained with methylene blue. x 75

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