FEEDING ADULT MOSQUITOES ON PRESERVED BLOOD TO MAINTAIN EGG PRODUCTION[1]

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The laboratory mass-rearing of mosquitoes has become a matter of routine for a number of species, and providing a blood meal for the adult females is generally an indispensable part of maintaining egg production. Even before the beginning of this century, entomologists began feeding adult mosquitoes in various ways and on different substances. In general, the problem of feeding many adults simultaneously has been solved in one of three ways: (1) offering a live animal, e.g. chick, guinea pig, dog, rabbit, or human arm; (2) using a membrane, e.g. Baudruche membrane, or a prepared rat, chick, or bat skin stretched over a source of preserved blood; (3) absorbing preserved blood on a cotton pad. As far as egg production is concerned, the first method, using a live host, appears to be used almost exclusively in the regular laboratory mass-rearing of most species. The membrane technique has been used largely in special feeding experiments for parasite transmission studies (Bishop and Gilchrist, 1944), where egg production was not important, and in nutritional studies (Woke, 1937) and Greenberg (1951). However, in many cases it has not been easy to induce large numbers of mosquitoes to probe or take a meal through a membrane, and this technique has not been applied to general laboratory rearing. The third method, the use of a blood-soaked pad, has seldom been employed in mass-feeding mosquito colonies to maintain egg production, although there are a number of references to this technique in connection with parasite and virus transmission work. MacGregor and Lee (1929) found that *Aedes argenteus (-aegypti), Culex pipiens,* and *Anopheles maculipennis* would feed on citrated blood if honey was added. Russell (1931) in malarial transmission studies, induced *Culex fatigans, Aedes aegypti,* and *Anopheles ludlowi* to feed on blood spread on gauze. Knowlton and Rowe (1935) fed equine encephalomyelitis virus to several species of mosquitoes by soaking a cellucotton pad with a mixture of glycerine-brain emulsion and defibrinated horse blood. In these examples too, the production of eggs from these meals was not the primary aim.

Considering the number of reports of mosquitoes successfully fed from blood-soaked cotton pads, it is surprising that this method has not been reported in connection with routine mass-rearing in the laboratory of such a commonly cultured species as *Aedes aegypti.* Recently, Schiavi and Franco (1949) recommended feeding a mixture of defibrinated blood and honey on cotton to maintain colonies of *Anopheles albitarsis, Anopheles strodei,* and *Culex fatigans.* McLintock (1952) has used a blood-sucrose mixture on a cotton pad in the continuous rearing of *Culiseta inornata.*

It has been the standard practice in the entomology laboratory at The Ohio State University to keep several rabbits as host animals for the stock colonies of *Aedes aegypti* and *Anopheles quadrimaculatus* adults. It was difficult to keep the rabbits healthy because of the large number of mosquitoes which fed on them regularly. However, it was primarily the time required in their care and feeding, and the problem of putting a rabbit into a stock cage and taking it out, that prompted the search for a faster and simpler method of feeding the stock colony.

Since it was customary to provide all test and holding cages with a 10 per cent

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honey solution on a cotton pad as a source of food for the males and for the females between blood meals, it seemed likely that female mosquitoes would ingest enough of a blood-honey mixture from a cotton pad to lay viable eggs.

In the first experiments, using *A. aegypti,* a cotton pad soaked in citrated beef blood with 10 per cent honey added was placed on the floor of a test cage. The females engorged on the mixture and laid an abundant supply of eggs. A large colony of *A. aegypti* was then established, and egg production was maintained for over a year by feeding the adults only the citrated blood and honey mixture.

Several experiments indicated that hanging the blood-pad in the cage rather than placing it on the floor would give better results. Tests were also made to determine the importance of heat as a factor in feeding. Thus, it was found that a high rate of oviposition could be obtained from blood without the honey added if the blood-saturated pads were heated to 42°–45° C. before hanging them in the cage.

Most investigators agree that *A. aegypti* which have just ingested whole blood in the course of "biting" an animal or piercing a membrane always have the midgut full and the ventral diverticulum containing rarely more than a trace. When whole blood alone was fed from a pad, dissections showed that, by this method too, the midgut was distended with blood, only traces being found in the ventral diverticulum, whereas a blood-honey mixture was dispatched primarily to the ventral diverticulum. Therefore, the use of whole blood alone will result in more "natural" ingestion.

However, at the suggestion of Dr. C. R. Philip of the Rocky Mountain Laboratory, freshly citrated blood was frozen for storage purposes. Because of hemolysis during freezing, it appeared that the females did not feed as well on the hemolyzed blood alone, and therefore, it was necessary to add honey to the frozen blood in order to induce feeding and maintain egg production. Frozen blood plus 10 per cent honey has been used successfully for the past four months in our stock colony.

The results obtained with *Aedes* served as a basis for developing an artificial feeding method for *Anopheles quadrimaculatus.*

Preliminary experiments with *Anopheles* were with citrated beef blood and honey mixtures absorbed on cotton pads and hung in the cages. The response to the food was poor. The resulting eggs were not sufficiently abundant to be used in culturing the insects, and investigations were initiated to find a more satisfactory method of feeding blood.

Observations by many workers indicated that heat was a powerful attrahent to many species of mosquitoes, and in this laboratory it had been noted that *Anopheles* would swarm to warmed surfaces. Therefore, attempts were made to induce the mosquitoes to feed on blood heated to human body surface temperature (34–35° C.).

In preliminary trials, test tubes wrapped with blood-soaked cotton were inserted through a hole in the tops of small cages. The temperature was raised by placing hot water within the tubes. It was observed that the female *Anopheles* were strongly attracted to the heated blood, and it was not necessary to add honey to the blood. A large percentage engorged within a very short time, and egg production was found to be abundant.

In adapting the technique to the stock culture, a large Pyrex test tube (32×200 mm.) was fitted with a Masonite collar (2×3×⅛ inches) with a hole which would admit the bore of the test tube but not its lips (Fig. 1-A). This collar fitted into a frame in the top of the stock cage so that the tube was suspended within the cage. The tube was wrapped with blood-soaked cotton, and the temperature was maintained by circulating hot water from a tap or a reservoir, through the tube.

More recently, it has been found that an electric heating unit can conveniently replace circulating water. The heating units in use are either a 20 or 40 watt,

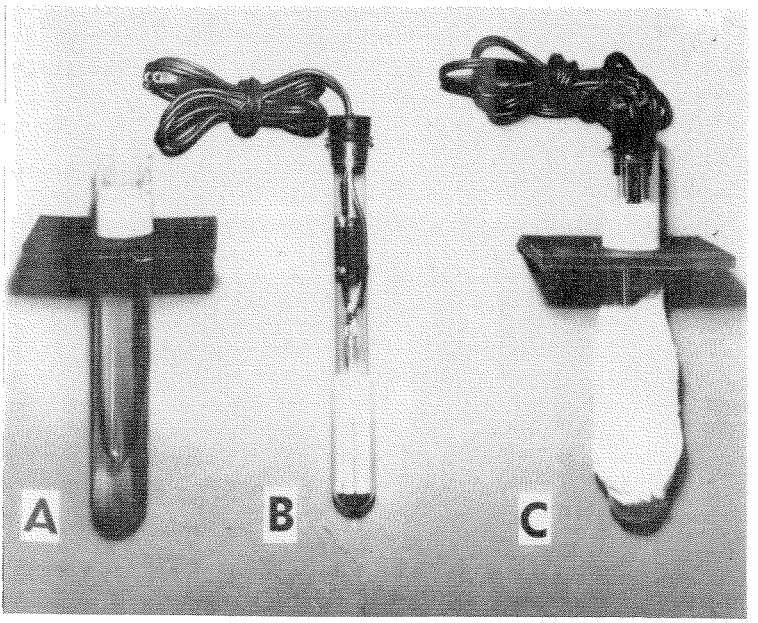

FIG. 1. A—A large Pyrex test tube (32×200 mm.) fitted with a Masonite collar. The tube contains the water necessary for operation of the heating unit. B—A 20 watt, single unit, thermostatically controlled aquarium heater. C—The heater in place within the large test tube which has been wrapped with cotton.

single unit, thermostatically controlled, electric aquarium heater (Fig. 1-B). These heaters consist of a thermoregulator and heating element contained in a 25×200 mm. Pyrex test tube, and are available at any acquarium supply store at very low cost.

The test tube containing the heating unit is placed in the larger tube around which is wrapped the blood-soaked cotton. Since the heaters will not function properly unless in a liquid medium, the space between the two tubes must be completely filled with water. The thermoregulator should be set to interrupt the current when the surface temperature of the blood pads reaches 35–38° C.

Several minutes are required for the temperature of the food to reach equilibrium after which the feeder may be allowed to run as long as insects are attracted. Feeding on alternate days appears to be satisfactory for maintaining a large supply of eggs from *Anopheles*.

Using the feeding method outlined above, the culture of *Anopheles* has been maintained in our laboratory for over nine months without the necessity of keeping mammalian hosts. Frozen blood has not been tested with this species.

With this method, it has been possible to feed the anophelines proteinaceous foods other than blood. Attempts have been made with skimmed milk, a 10 percent solution of egg albumin, and a 10 percent solution of proteose-peptone, and in all cases oviposition has resulted. The mosquitoes feed better on these foods if 1 part of honey is added to 9 parts of the protein solution.

These procedures for mass-feeding have numerous advantages over that of introducing a live animal into the stock cage. Blood is usually available at the local slaughterhouse and can be citrated and stored under refrigeration for at least two weeks or for several months if frozen. The blood-pad can be left in the cage

overnight, and few or no mosquitoes are lost in changing to a fresh feeder. These techniques also offer an excellent means of studying the nutritional requirements of the adult mosquito. The results of feeding milk to *A. aegypti* adults have been reported by Lea *et al.* (1955), and the preliminary results of feeding mixtures of amino acids by Dimond *et al.* (1955).

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THE BREEDING OF *HAEMAGOGUS ARGYROMERIS* D. AND L. IN SALINE WATER (DIPTERA, CULICIDAE)

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Haemagogus argyromeris D. and L. 1921 is a widespread and common mosquito in the Panama Canal Zone and adjacent Panama. In its choice of breeding-places, it is perhaps less restricted than any other species of *Haemagogus.* Dyar (1) records the larvae from tree holes. Galindo *et al.* (2) record the larvae from "a greater variety of breeding habitats than any other *Haemagogus* in this area [Panama], including tree holes, cans, coconut hulls, tires, rock holes, terrestrial bromeliads and occasionally ground pools" (p. 118), and from "5-gallon cans which had been left on the slopes of the island" [Flamenco, near the Pacific entrance to the Panama Canal].

In 1949 the writer recorded *H. argyromeris* larvae from terrestrial bromeliads in the Panama Canal Zone and the Republic of Panama, and once from an arboreal bromeliad in El Valle de Anton, in Panama (3). He now reports the larvae of this species breeding in definitely saline water contained in rock holes on the Pacific shores of the Republic of Panama.

Punta Paitilla is a low, isolated, rocky

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