COLONY MAINTENANCE OF *ANOPHELES ALBIMANUS* WIEDEMANN BY FEEDING PRESERVED BLOOD THROUGH NATURAL MEMBRANE

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ABSTRACT. Techniques were developed for using natural membranes to successfully feed a colony of *Anopheles albimanus* Wiedemann on bovine blood preserved by defibrination and storage at 5°C. Egg production by mosquitoes fed the preserved blood was 58% lower than production by those fed on live rabbits, but this reduction was not detrimental to colony maintenance and productivity. The advantages associated with membrane feeding make that technique much more desirable than maintaining live animals for that purpose.

INTRODUCTION

In controlling human disease through control of the vectors much research is needed to determine efficacy of control procedures. That research, in turn, often requires maintenance of laboratory colonies of the vectors. This is particularly true in studies of mosquito-borne diseases, and many laboratories around the world now maintain colonies of many species of mosquitoes.

As the necessity for research has increased, the need for more efficient rearing techniques also has increased, so that over the years investigators have experienced increasing efficiency in mosquito rearing. Thus, techniques for feeding blood to colony female mosquitoes, a prerequisite to viable egg production by most species, have undergone several evolutionary changes through the years.

Most early workers in mosquito rearing (Boyd et al. 1935, Rozeboom 1936, Crowell 1940) depended on human blood donors to feed the adult females. In fact some allowed the adult mosquitoes the freedom of the room so they could feed upon the insectary technician (Crowell 1940). Other early workers used animals as sources of blood (Shute 1936), and the majority of current colonies are provided live host animals as a blood source (Gerberg 1970).

There are definite advantages to using human or animal blood donors for mosquito colonies, e.g., the blood is available in a natural condition and is at the correct temperature for good feeding response. There are also obvious disadvantages such as accidental disease transmission from one host to another, and with human donors there is always the danger of hypersensitivity to mosquito bites. In addition, when laboratory animals are used, there is the expense of caging, feed-

ing, and caring for them. Also, severe stress is placed on the animals by the feeding, thereby increasing the potential for induced illness due to the feeding stress or to subsequent crowding in holding cages. There is also the necessity for shearing and preparing the animal for feeding and special equipment is needed for holding the animal during confinement in the mosquito cages.

As a result of these disadvantages, recent workers have evaluated techniques for preserving animal blood and methods for feeding mosquitoes through natural and artificial membranes (Tarshis 1956; Rutledge et al. 1964). Because of our continuing interest in the routine maintenance of mosquito colonies for laboratory research and in the efficient mass production of *Anopheles albimanus* Wiedemann for field studies of the sterile-insect technique, their results encouraged us to develop a method for feeding preserved blood that would eliminate the need for keeping donor animals.

PRELIMINARY TESTS. To determine the suitability of preserved blood for routine colony use for *An. albimanus,* adults were fed on bovine blood preserved by different methods. Blood obtained at a slaughter house was preserved chemically by the addition of 0.3% sodium citrate or by a combination of 0.3% sodium citrate and 0.06% formaldehyde or by physically removing the fibrin (a fibrous, elastic protein produced in the coagulation of blood) by stirring the fresh blood with metal rods and then removing the clotted material. Following storage at about 5°C, the different blood formulations were fed to the caged adult females using a Parafilm® membrane. The membrane was stretched to about twice its original size and secured to a 10-cm hoop over a supporting layer of netting material. The hoop was then placed on top of the cage, and preserved blood that had been heated to about 40°C was poured onto the membrane. The mosquitoes feeding on the blood were subsequently "egged" individually in vials. Females fed on a live rabbit using the system of Ford and Green (1972) served as controls. Blood suitability was determined by the percentage of females ovipositing and the number of eggs per female that oviposited.

The formaldehyde-citrate mixture gave poor results, and testing with that formulation was discontinued. The citrated and defibrinated bloods were more promising, however. The mean results of 2 replicates with 50 females each were as follows:

	% Females ovipositing	No. eggs/female
Live rabbit	97	136
Citrated bolld	99	96
Defibrinated blood		100
100		

Thus, females fed on citrated and defibrinated bloods oviposited as readily as those fed on the live rabbit but produced only 71 and 74% as many eggs, respectively. Although the results suggest that neither preserved blood is equal to blood from the live rabbit, the 26 to 29% reduction in egg productivity was not sufficient to rule out the use of preserved blood for colony purposes. The ease of membrane feeding vs. the continual maintenance of live host animals could readily outweigh the reduction in productivity in both small and large scale rearing operations. That, coupled with the hope that some modification in techniques might improve the results with preserved bloods, prompted us to pursue studies of membrane feeding on a larger scale.

TESTS WITH NATURAL MEMBRANES. In personal communication with Drs. A. D. Flynn and S. G. Breeland of the Center for Disease Control (DHEW) in Atlanta, GA, we learned that they were successfully using natural animal membranes for small-scale feeding of mosquitoes. In attempting to use their natural membrane feeding technique on a much larger scale, we found that the females fed readily on blood through a natural membrane prophylactic (condom) made from sheep intestine. The females also

were attracted to this membrane at an earlier age than when Parafilm membrane was used.

In the early studies with prophylactics, and in the initial colony maintenance, we used a lubricated and packaged product purchased from a local pharmacy. The cost averaged about $1 each, and the lubricant had to be removed before use by washing in warm water. Also, sometimes during the washing process small holes would appear in the membranes rendering them useless. These disadvantages led to inquiries, which resulted in obtaining from the manufacturer a supply of prophylactics that had been cured and dried, and tested for holes, but not lubricated or packaged. The cost of that product ranged between 17 and 28¢ each, depending on the manufacturer and the length and thickness of membrane. These products were superior in quality for our purposes, being longer-lasting and developing fewer holes than those purchased over the counter.

DEFIBRINATION AND STORAGE OF BLOOD. For routine colony maintenance bovine blood is collected once a week from a slaughter house and is defibrinated with a mechanical device developed for that purpose (Fig. 1). No suitable units were available commercially. The defibrinator consists of a series of eccentric rollers powered by a 1/3 HP DC motor that operates from the 12V battery of the transport vehicle, which allows the blood to be defibrinated while in transit from the slaughter house to the laboratory. The blood is collected in 8-liter largemouth plastic jugs containing 8 aluminum rods about 1 cm in diameter and 25 cm long. The jugs of blood are placed on the rollers of the defibrinator and agitated for about 30 min. The fibrin in the blood collects around the metal rods during this process and can be removed with the rods. The defibrinated blood can then be stored for at least a week at 5°C without the addition of anti-coagulants and without apparent deterioration of quality.

FEEDING PRESERVED BLOOD. The use of natural membrane prophylactics has now

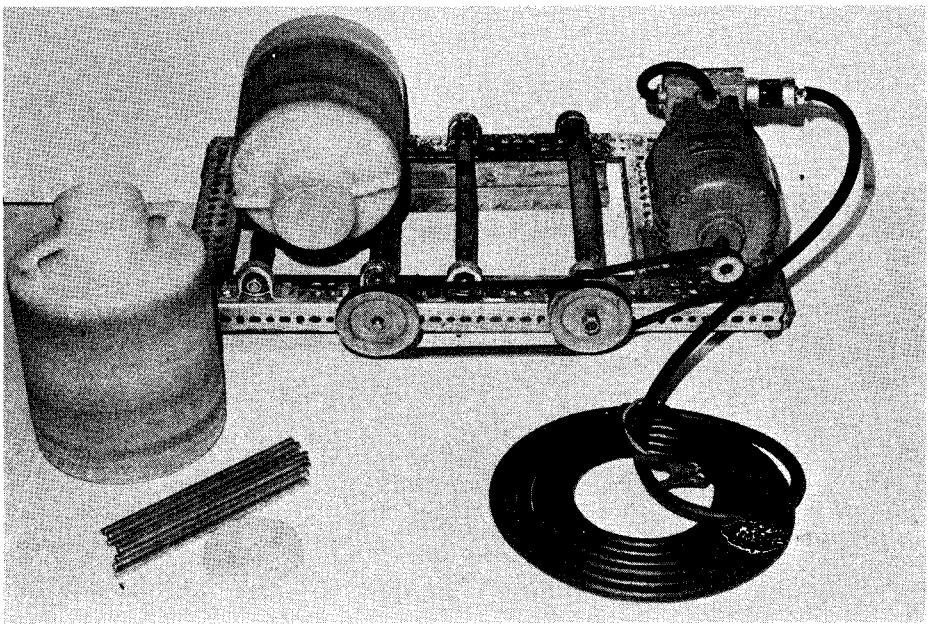

Figure 1. Mechanical defibrinator.

been adopted as a standard procedure in our *An. albimanus* colony. For feeding, the blood is removed from the refrigerator and about 200 ml is poured into each of the prophylactics needed. (The number depends on the number and size of the cages in the colony.) In our colonies we use 1 prophylactic per cage, in cages that are 60 × 60 × 75 cm high and contain approximately 25,000 mosquitoes. First an enameled pan is filled with hot tap water (about 70°C) and the prophylactics (sealed with a clip at the top to prevent spillage) are suspended in the water. When the blood reaches 45°C (4–5 min), the prophylactic is removed from the water and suspended in a cage through a feeding port, the port consists of a hole in the top of the cage in which a sock made of surgical tube gauze is suspended by a retaining ring made of PVC pipe and plexiglass. The prophylactic is supported by this sock within the cage so the mosquitoes can probe through the cloth and feed through the membrane. This procedure eliminates the need to open the sleeve of the cage to hang the prophylactic inside, thus accidental escape of mosquitoes into the colony room is greatly diminished.

Most feeding occurs in the first 10–15 min after the warmed blood is introduced, but a few mosquitoes continue to feed longer. The membranes are exposed in the feeding ports all day beginning at 8 am; however, they are removed at 10 am and at 1 and 3 pm, rewarmed and replaced for a total of 4 feedings per day. At 4 pm the membranes are removed, the blood is discarded, and the prophylactics are rinsed several times in warm, clear water. The membranes are then stored in water in a sealed container in a refrigerator at 5°C until the next day. A membrane can be used daily for 1–2 weeks when it is handled in this manner.

RABBIT VS. PRESERVED BLOOD. Since converting the *An. albimanus* colony to membrane feeding, egg production has been satisfactory, even though production from the membrane-fed mosquitoes has been somewhat less than from those fed on rabbits.

To determine the difference in egg production with the 2 methods, we set up four 61 × 61 × 61 cm cages in a room where the temperature is controlled at 28-29°C. The cages were stocked at the rate of 2000 pupae of mixed sexes each day and the locations of the cages were rotated daily to eliminate any influence of cage position. Mosquitoes in 2 of the cages were allowed to feed on a rabbit from 8 to 10 am daily. A membrane containing warm bovine blood was placed in each of the other 2 cages at 8 and 10 am and at 1 and 3 pm daily. After 7 days and for 14 days thereafter an oviposition pan containing well water was placed in each cage overnight. Each morning the pan was removed. The eggs were poured through 20-mesh screen to remove debris, aged for 24 hr on the surface of water, dried for 30 min with forced air passed through a cloth containing the sample of eggs, sifted through 70-mesh screen to break up clumps, and then measured volumetrically by using techniques of Dame et al. (1978).

Table 1 shows the results of the experiment. There were only 2 days during the test period that egg production from the membrane-fed mosquitoes equalled or exceeded that of the rabbit-fed mosquitoes. The daily average egg production was 1.2 ml/cage for those fed on rabbits and only 0.7 ml (58%) for those fed with membranes. The egg hatch was very good from both treatments, averaging 92% from the rabbit-fed mosquitoes and 93% from those fed with membranes.

DISCUSSION

Although egg production by the membrane-fed mosquitoes was only 58% that of rabbit-fed mosquitoes, the difference has not had any marked effect on overall colony size. Even though we made no direct comparison of egg production before and after beginning to use membranes routinely, we still maintain only 1 large cage of adults which supplies us

Table 1. Daily egg production and hatch of *Anopheles albimanus* fed on live rabbit or bovine blood through natural membrane prophylactics

	Egg production (ml)			Egg hatch (%)	
Day	Rabbit	Membrane	Mem./rab. (%)	Rabbit	Membrane
1	1.3	0.4	31	91	90
2	.5	.6	120	94	92
3	1.3	.7	54	90	97
4	1.7	.7	41	82	82
5	.6	.6	100	84	
6	1.5	.7	47	97	98
7	1.4	.9	64	95	95
8	1.0	.5	47	95	97
9	.9	.5	56	89	99
10	1.2	.8	67	97	97
11	1.0	.7	70	97	96
12	.8	.5	63	96	97
13	1.1	.9	82	92	88
14	1.4	.6	43	92	87
Avg.	1.2	.7	58	92	93

with more than enough eggs to replenish the colony as well as to supply the research needs in our laboratory, which was the case when we used live rabbits. At any rate, the advantages of membrane feeding adequately compensate for any reduced egg production.

Moreover, the rabbits used in this experiment were healthy, which would give every advantage to those mosquitoes fed on them, but that is not always the case, especially in mass rearing. In a mass rearing program where many rabbits are necessary, it is very difficult to keep the animals in good health unless a considerable surplus of rabbits is maintained. It also becomes quite expensive to feed, house and care for them. But if only a minimum number of animals is kept, there is such a stress on them that they become sickly, which in turn lowers the egg production in the mosquito colony.

It has been our experience that production of eggs in an actual mass rearing program in El Salvador (ca. 1 million mosquitoes produced daily), where membrane feeding has been used, has been quite comparable to production from the same colony previously fed on rabbits. In addition to the fact that unhealthy animals are no longer involved there, the egg production has also appeared to increase with time, probably due to selection of a strain adapted to membrane feeding.

This study shows that mosquitoes can be produced adequately by feeding the adult females on bovine blood through natural membrane, rather than using live host animals. This is a great advantage in mosquito production and greatly increases the efficiency of mass producing mosquitoes.

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DISTRIBUTION OF *AEDES TRISERIATUS* (SAY) AND *AEDES HENDERSONI* COCKERELL IN SOUTHWESTERN ONTARIO, 1975–76[1]

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ABSTRACT. Tree holes containing water suitable for mosquito larvae were examined throughout southwestern Ontario. Larvae of *Aedes triseriatus* (Say) and *Ae. hendersoni* Cockerell were collected from 165 and 6 tree holes respectively. Both *Ae. triseriatus* and *Ae. hendersoni* were widely distributed in southwestern Ontario with *Ae. triseriatus* more abundant than *Ae. hendersoni.* Larvae of *Anopheles barberi* Coq., *Culex restuans* Theob., and *Orthopodomyia signifera* (Coq.) occurred also in tree holes in Ontario. The majority of the tree holes were cavities between trunks of sugar maple trees (*Acer saccharum* Marsh) and were located less than a meter above the ground. The diameter of the tree holes ranged from 3.0 to 41 cm. and the pH values of the water varied from 4.0 to 8.3.

INTRODUCTION

Two species of tree-hole mosquitoes, *Aedes triseriatus* (Say) and *Aedes hendersoni* Cockerell, are closely related and were classified as one species, *Ae. triseriatus,* until Breland (1960) resurrected and elevated the name *Ae. hendersoni* to full species level. Zavortink (1972) indicated that *Ae. hendersoni* had been collected from nearly all areas of the United States while *Ae. triseriatus* was present in the Eastern United States from Minnesota to Maine south to Texas and Florida. Records indicate that *Ae. triseriatus* was more abundant over its range and that breeding between the 2 species produces hybrid forms (Truman and Craig 1968, Grimstad et al. 1974). Five reports indicated the presence of *Ae. triseriatus* in Ontario, at 2 locations in southeastern Ontario (James et al. 1969) and from 7 locations in southwestern Ontario (Beckel and Atwood 1959, McLaine 1943, Smith and Trimble 1973, Steward and McWade 1960). *Ae. hendersoni* has not been reported from Ontario except in personal communication with Woods (1976).

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