AN ELECTRICALLY HEATED MEMBRANE BLOOD-FEEDING DEVICE FOR MOSQUITO COLONY MAINTENANCE[1]

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The maintenance of most mosquito species in the laboratory requires periodic feeding of blood to females. The expense and inconvenience of maintaining laboratory animals for this purpose has led to the development of methods for feeding preserved blood through various natural and artificial membrane materials (Tarshis 1958, Bailey et al. 1978, Wirtz and Rutledge 1980, Savage et al. 1980, Thomas et al. 1985). The resulting convenience and cost reduction can more than compensate for the reduced egg production reported by some of these workers, especially in laboratories where mass rearing is done. In our mosquito rearing facility, where production demands are usually low, this technique proved to be worthwhile. To decrease handling time, devices for holding and filling the membrane, and for maintaining a constant blood temperature were constructed.

Initially, a coiled glass tube was inserted into the blood-filled condom, and 40°C tap water circulated through it. This device required proximity of hot and cold running water and, due to unpredictable changes in hot water temperature, needed constant adjustment. Aquarium heaters have been used to maintain the temperature of a water bath for a specialized membrane blood-feeding device described by Tarshis (1958), and for directly warming blood held in a condom (D. A. Dame, personal communication). In the latter case, use of the heater was discontinued because it frequently malfunctioned; however, we recently have obtained satisfactory results from an electric heater that was modified as described below.

As a base for holding both the condom and the heater, four disks were cut from acrylic sheet (Lucite™, DuPont Co., Methacrylate Products Division, Wilmington, DE 19898) and laminated with methylene chloride to the configuration shown in Fig. 1. Three holes were drilled through the resulting stack: a 2.54 cm (1 in.) diam. hole through the center to hold the heater tube and two 5 mm (3/16 in.) diam. holes on opposite sides of the center hole for filling and draining the device. The resulting collar has a groove formed by the 6 x 36 mm diam. disc in which the opening of the condom can be held by a rubber band. A 20 cm (8 in.), 50 watt, 110 VAC automatic aquarium heater (Fritz Pet Products, Div. of Fritz Chemical Co., Dallas, TX 75217) was modified as follows: The plastic screw-clamp assembly was removed and discarded allowing removal of the printed circuit board and heating element from the glass tube. The pilot light and resistor were removed and two 20 gauge, insulated wires were soldered in their place. These wires were threaded through the same holes in the rubber plug as the AC power leads and wrapped to the AC cord for a distance of 1 m, at which point the remaining AC cord and plug were clipped off. The wires were threaded into a plastic electrical outlet box in which a 600 watt, 120 VAC incandescent dimmer switch (Lutron Corp., Coopersburg, PA) and one miniature 120 VAC neon lamp (Radio Shack #272-707) were mounted. The 20 guage wires were connected to the neon lamp, and the power cord was wired to an additional 2 m of lamp cord with the dimmer switch interposed in one of the lines.

The heater was then mounted in the plastic collar with silicone cement (General Electric Silicone Windshield and Glass Seal™) such that the top 2 cm of the glass tube projected above the top of the collar. An additional 3.6 cm diam. collar cut from a single thickness of 3 mm (⅛th in. nominally) acrylic sheet was cemented 1.5 cm from the bottom tip of the tube to prevent the membrane from contacting the heated glass. Sixteen holes of 5 mm (3/16 in.) diam. were drilled around the periphery of this collar to allow blood to flow past it.

A feeding port similar to that described by Bailey et al. (1978) was added to the top of each of our 30 x 30 x 30 cm aluminum screen cages by replacing the screen on half of the top with 3 mm acrylic sheet. A 22 cm long by 3.8 cm diam. sleeve of mosquito netting material extended into the cage from a 4.5 cm diam. hole in the sheet to support the membrane feeding device and prevent escape of mosquitoes.

Pig blood was collected at a local slaughter house and sodium citrate (3.3 g in 25 ml water per liter of blood) was added to prevent coagulation. Since blood treated in this way deteriorated in 2–5 days under refrigeration, all but a 2 day supply was immediately frozen in 200 ml aliquots. Fresh blood was used as is, but our *Aedes* spp. fed poorly or not at all on blood that

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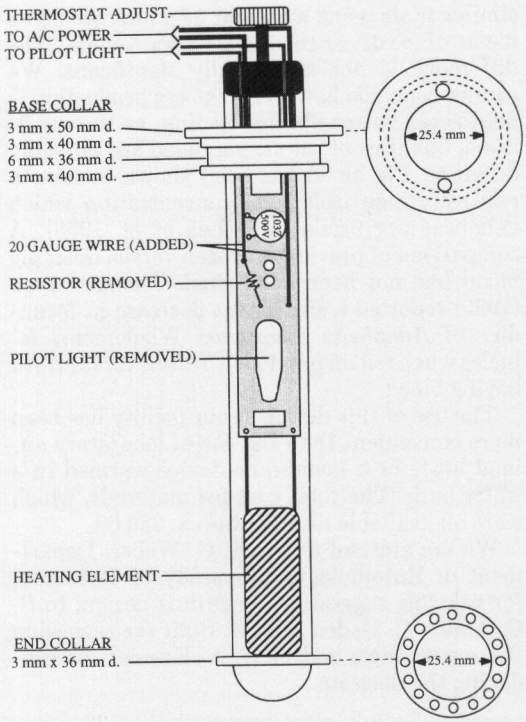

Fig. 1. Diagram of blood heating device showing configuration of plastic collars and modifications to circuit.

had been previously frozen. Adenosine 5′ triphosphate sodium salt (Sigma Chemical Co. #A-3377), at a concentration of 2.5 mg/ml of thawed blood (Wirtz and Rutledge 1980), stimulated rapid and complete engorgement (Hosoi 1959).

Prior to feeding mosquitoes, a natural membrane condom is rinsed in warm water and slipped over the heating device. The elastic string bonded to the opening of the condom is secured into the groove in the plastic collar with a rubber band. The dimensions of the device as given in Fig. 1 are most suitable for use with Kling-Tite Naturalamb™ condoms (Carter Products, Div. of Carter-Wallace, Inc., New York, NY 10153). Other brands tested were often irregular in shape, size and thickness. The basic design can be adapted for use with the collagen sausage casings described by Wirtz and Rutledge (1980). To fill the device, it is first placed in a stand fabricated from 3 mm acrylic sheet (Fig. 2). Blood is poured in through a small funnel set into one of the holes in the collar while the other hole acts as a vent. Filling the membrane to within 3 cm of the collar requires 150 ml. The condom should not be filled to the top or blood will be forced out of the fill holes when it is placed into the feeding port. The holes can be stoppered with microvial corks if desired.

The thermostat adjustment knob projecting from the top of the heater is used to set the

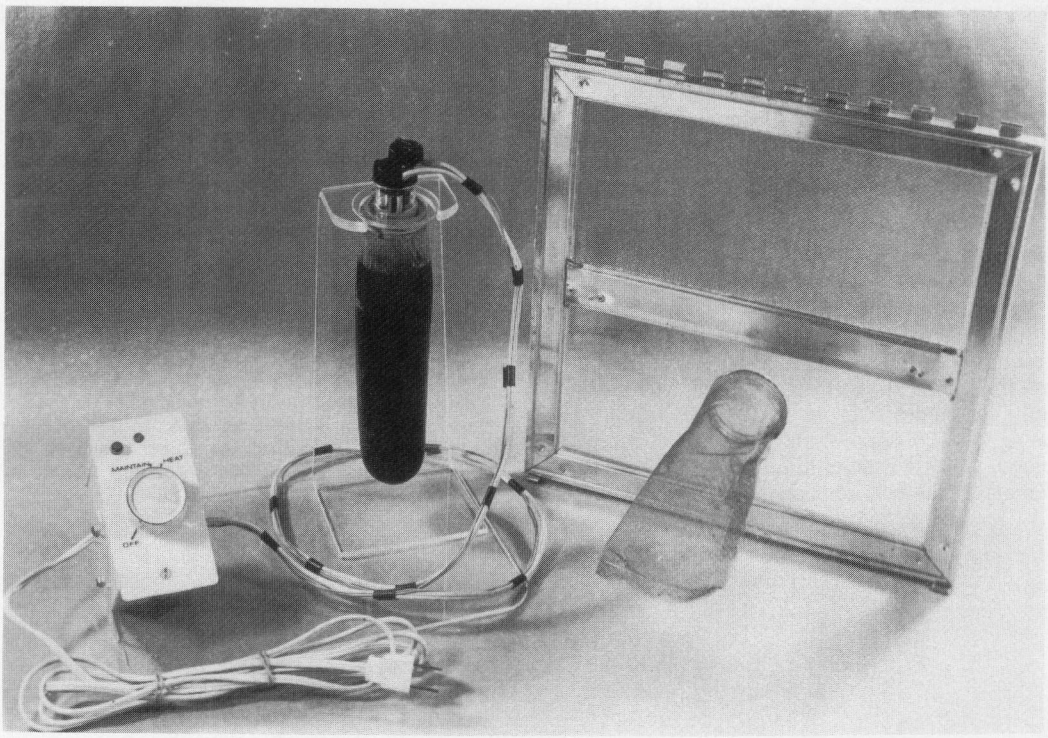

Fig. 2. The membrane blood-feeding device with temperature control, stand and modified cage top.

blood temperature. At the setting we use, the blood temperature oscillates between 37 and 39°C as the thermostat cycles on and off. The pilot light indicates when the thermostat contacts are closed and current is reaching the heating element. This is useful for determining when the set temperature has been reached so that it may be adjusted if necessary. The dimmer switch is used to limit the temperature of the heating element; otherwise, blood will cake onto the surface of the glass tube near the element. The device was calibrated with a condom full of water and two thermistor probes inserted through the fill holes to monitor the temperature of the water and the glass surface next to the heating coil. Two settings were marked on the dimmer switch. The higher setting limits the temperature of the glass surface to ca. 55°C, and is used only in the initial heating of the blood. About 15 min is required to heat the blood from 10 to 37°C. The lower setting is the lowest that will reliably activate the device when the thermostat contacts close, and is used to maintain the blood at the temperature set with the thermostat adjustment knob.

After reaching the desired temperature, the device is inserted into the feeding port of a screen cage. When fresh blood is used, the upper collar rests on two 150 ml specimen cups such that only the lower two-thirds of the membrane are inserted, thereby preventing some mosquitoes from engorging on serum after the cellular elements have settled. After use, the device is normally emptied and cleaned; however we have stored the device, filled with blood, at 2–5°C for up to 48 hr and reused it successfully. When not filled, the membrane is removed from the device, rinsed and then stored frozen. Individual membranes were used for up to six days of feeding.

The species of mosquitoes successfully maintained in our laboratory using this bloodfeeding technique are *Aedes aegypti* (L.), *Ae. triseriatus* (Say), *Anopheles quadrimaculatus* Say, and *Psorophora ferox* (von Humboldt). We have successfully fed over 1,000 *Aedes aegypti* within 30 minutes. Egg production data was accumulated only for *Aedes aegypti*, where five trials, each using ca. 150 females fed on fresh citrated pig blood, yielded a mean of 82.89 (±3.28 SD) eggs/female in the first four days of oviposition. Five similar trials using a live rat as a host yielded a mean of 88.33 (±16.56 SD) eggs/female. The difference is not statistically significant. We cannot conclude however, that egg production is unaffected by membrane feeding, as the nutritional qualities of rat vs. pig blood are probably different. Pig blood has been shown to have a relatively high isoleucine concentration which enhances egg production (Lea et al. 1958). A comparison of previously frozen versus fresh pig blood has not been conducted. Thomas et al. (1985) reported a significant decrease in fecundity of *Anopheles albimanus* Wiedemann females when fed on previously frozen versus fresh bovine blood.

The use of this device at our facility has been more convenient than the use of laboratory animal hosts or a membrane device warmed in a water bath. The total cost for materials, which were all available locally, was ca. $30.00.

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