

## ROUTINE BLOOD-FEEDING OF *Aedes aegypti* VIA A NEW MEMBRANE

HANS-E. HAGEN AND JÖRG GRUNEWALD

*Institute of Tropical Medicine of the University of Tübingen, Wilhelmstrasse 31, D-7400 Tübingen, F.R.G.*

**ABSTRACT.** A simple feeding system for the routine maintenance of mosquitoes with a new kind of membrane based on an acrylic wound dressing spray and commercially available nylon gauze is described.

A colony of *Aedes aegypti* (Linn.) has been maintained for several years at the Institute of Tropical Medicine of the University of Tübingen. Beside the costs and inconvenience of breeding rodents as blood hosts, an increasing restriction on the scientific use of vertebrates in compliance with the German law for the prevention of cruelty to animals has led to the development of a simple but effective way of feeding mosquitoes preserved blood.

The design of the feeding apparatus is based on the system of Grunewald and Wirtz (1978) for the maintenance of blackflies (Simuliidae). The mosquitoes of the colony are kept in a cage within a plastic box about 55 × 40 × 72 cm in size. Due to a simple (slot and key) sliding mechanism, the upper layer of the rearing cage can be exchanged partially for a gauze top. Thus the mosquitoes can be fed without the need of special feeding cages.

The membrane is produced by covering nylon gauze with a wound spray (Nobecutan Spray, Astra Chemicals, 2 Wedel/Holstein, F.R.G.). The spray is an acrylic resin dissolved in an acetic ester [Bis(dimethylthiocarbamyl)disulfid

(Thiram) to vulcanize and disinfect the acrylic material], and it is applied to the gauze several times until it is totally sealed. The gauze is a commercially available fabric for window screens. The width of the mesh is almost negligible as is the thickness of the membrane as long as the gauze can be sealed with this wound dressing. Once the spray has dried the gauze can be fixed on an aluminum feeding ring (Fig. 1B). The membrane is now ready to use. The blood is pipetted in 1.5-ml samples each into such a ring (Fig. 1C), which is then screwed onto a heating chamber (Fig. 1A). The temperature within this chamber is kept constant at 37°C by a constant water flow from a water bath. After the blood has reached the desired temperature, the feeding device can be lowered onto the gauze top of the rearing cage. Four feeding devices, fixed to a mobile bar, can be used per feeding trial (Figs. 1 and 2). Shading the top part of the cage may enhance the feeding activity of the females. Once feeding is finished, the membrane can be left on the feeding ring and should be stored, after careful rinsing, in deionized water. Leaks can easily be repaired by respraying the

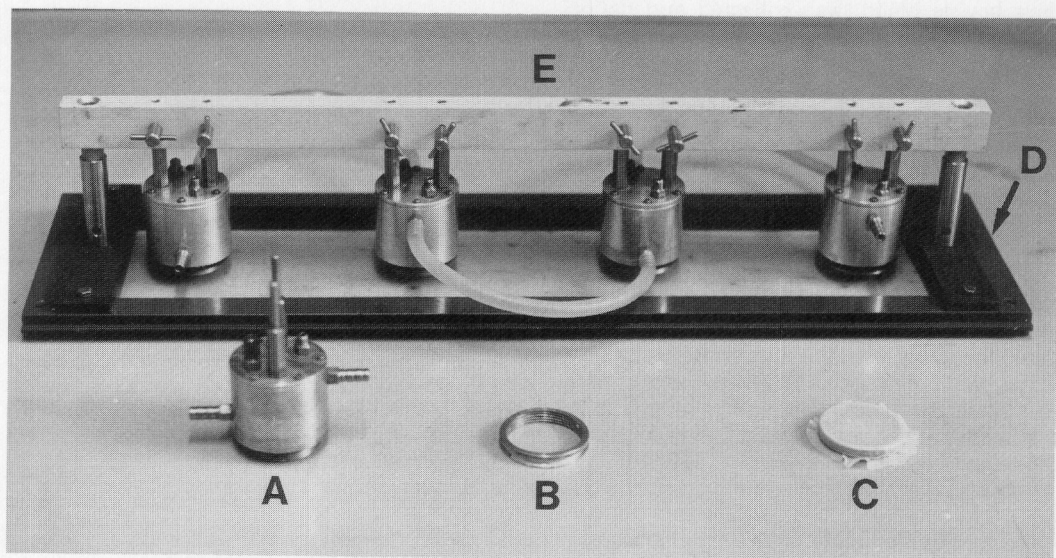


Fig. 1. Parts of the feeding apparatus: (A) Heating chamber, (B) feeding ring, (C) ring with membrane, (D) gauze top, and (E) bar with 4 feeding devices.



Fig. 2. Feeding system on the rearing cage.

corresponding spots. Thus the membrane can be used 2-3 times per week for up to several months.

Pig blood, which proved to be more suitable than bovine blood, was collected and defibrinated at the local abattoir in Tübingen. Before storage at  $-70^{\circ}\text{C}$  the blood was supplemented with one chicken egg yolk/100-ml blood and transferred in 1.5-ml aliquots to Eppendorf reaction tubes. After thawing, adenosine triphosphate (ATP) was added to each blood sample at a final concentration of  $100\ \mu\text{M}$ . The 10 mM ATP stock solution was stored in 100- $\mu\text{l}$  samples at  $-20^{\circ}\text{C}$ .

Although fecundity and fertility have not yet been exactly determined, the approximate yield of eggs of the membrane fed mosquitoes compared favorably with the number of eggs following a blood meal with *Meriones unguiculatus* (Rodentia: Gerbillinae) as a live host (Fig. 3). The membrane is rapidly found and accepted by the females; gorging starts within 15 min. Hav-

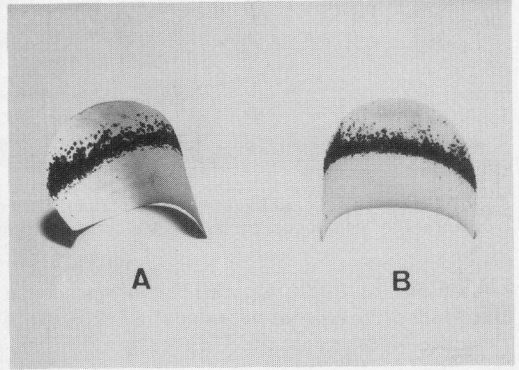


Fig. 3. Yield of eggs following a blood meal of a host (A) and via the membrane (B). The mosquitoes were fed alternately with preserved blood or on anesthetized rodents (30 min each) at 4-day intervals. The number of adults within the stock cage was approximately the same.

ing tried a whole range of different membranes (Rutledge et al. 1964), this new membrane proved to be most effective and simple. The yolk, originally used to supplement blood for feeding triatomine bugs (Heteroptera: Reduviidae) (Nunez and Lazzari 1990), seems also to be a promising supplement to compensate the reduced egg production of mosquitoes after feeding of preserved blood (Thomas et al. 1985). Thus, the feeding system described above provides a convenient way of bloodfeeding mosquitoes and is suitable for their routine maintenance.

We would like to thank Mr. H. Renner for manufacturing the equipment of the feeding system.

#### REFERENCES CITED

- Grunewald, J. and H. P. Wirtz. 1978. Künstliche Blutfütterung einiger afrikanischer und parläarktischer Simuliiden (Diptera). *Z. Angew. Entomol.* 85:425-435.
- Nunez, J. A. and C. R. Lazzari. 1990. Rearing of *Triatoma infestans* Klug (Heteroptera: Reduviidae) in the absence of a live host. II. Moulting and its dependence of blood diet composition. *J. Appl. Entomol.* (in press).
- Rutledge, L. C., R. A. Ward and D. J. Gould. 1964. Studies on the feeding response of mosquitoes to nutritive solutions in a new membrane feeder. *Mosq. News* 24:407-419.
- Thomas, J. A., D. L. Bailey and D. A. Dame. 1985. Maintenance of *Anopheles albimanus* on frozen blood. *J. Am. Mosq. Control Assoc.* 1:538-540.