

ARTIFICIAL BLOODFEEDING OF *ANOPHELES SACHAROVII* ON A MEMBRANE APPARATUS

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ABSTRACT. *Anopheles sacharovi*, the main human malaria vector in Turkey, has been maintained in our laboratory by feeding on anesthetized rabbits for about 20 years but it is a difficult species to colonize and bloodfeed. To eliminate the need for keeping and using live rabbits to supply blood meals, artificial bloodfeeding methods with suitable membrane apparatus were investigated. The feeding apparatus designed by the World Health Organization and 3 other types designed by us (for feeding on preserved human blood) were tested. Artificial membranes (latex and paraffin film) and locally produced and dried calf intestine were used. The calf intestine membrane gave the best feeding results and a modified apparatus designated type III was the most successful. This apparatus was preferable for the artificial feeding of *An. sacharovi* because it has a small reservoir, is easy to use, is adaptable to different feeding conditions, and supports reasonably high bloodfeeding rates 44.4–50.5% as compared to 35% on live rabbits.

KEY WORDS Artificial bloodfeeding, membrane apparatus, *Anopheles sacharovi*

INTRODUCTION

Short-term colonization of *Anopheles sacharovi* Favre, the main human malaria vector in Turkey (Kasap 1990), was previously attempted in Italy (Coluzzi 1964) and Germany (Weyer 1965). In Çukurova University, a colony (TBADA) was started in 1979 with collections from Hıdırlı Village, Adana, Turkey (Kasap and Kasap 1983), and was sustained successfully until the present by feeding on anesthetized rabbits.

For most mosquito species, including *An. sacharovi*, bloodfeeding is essential for the production of viable eggs. Although it is possible to bloodfeed mosquitoes on human volunteers (Bailey et al. 1978), bloodfeeding on laboratory animals has some definite advantages. The use of live animals means that the blood is available in a natural condition, at the correct temperature, and with natural feeding cues. Disadvantages include accidental disease transmission and hypersensitivity to mosquito bites (Bailey et al. 1978). In addition, animal maintenance can be costly.

Because of these disadvantages, mosquito colonies have been fed blood via artificial membranes, which vary from bat wing skin to latex condoms (Weyer 1965, Bailey et al. 1978, Wirtz and Rutledge 1980, Benzon and Apperson 1987, Hagen and Grunewald 1990, Novak et al. 1991). Feeding success depends on the variables inherent to the artificial feeding systems and the mosquito species.

We designed 3 different membrane feeding systems and used different human blood types to explore the most suitable conditions for artificially feeding *An. sacharovi* on a small volume of preserved human blood.

MATERIALS AND METHODS

Test mosquito specimens were from the TBADA colonies and heparinized human blood was

provided from the Blood Bank at Çukurova University Hospital. Because of difficulties encountered in stabilizing the temperature of both the blood and the membrane with the World Health Organization feeding apparatus (WHO 1975), 3 types of glass apparatus were generated and evaluated.

Apparatus type I (Fig. 1A): The support base of a graduated glass cylinder of 1-liter volume was cut off, then a glass funnel with a rim the same size as the circumference of the cylinder was placed inside the cylinder, and the overlapping rims of the funnel and the cylinder were joined by melting. The neck of the funnel was extended with a glass tube to the top of the cylinder. The mouth of the funnel was covered with an artificial membrane.

The extended neck of the funnel served to fill the funnel reservoir with blood and to measure the temperature of the blood and of the membrane. Temperature of the membrane was measured by touching the membrane with the thermometer inserted through the blood. Blood was agitated with plastic tubing inserted through the neck and connected to an aquarium air pump (Trumpf®; Tropikal Galeri, Izmir, Turkey). The reservoir was filled with 100 ml of heparinized human blood and the surrounding cylinder was filled with water and heated with an aquarium thermostatic heater (Rena®; Tropikal Galeri). Thus, a water jacket was created to heat the blood reservoir inside the funnel. It proved difficult to maintain a homogeneous temperature, for example, when the membrane temperature reached 36°C, the water jacket surrounding the funnel reservoir was 37.5°C, and upper parts of the water surrounding the funnel neck were 42°C. Membrane temperature did not remain stable.

Agitating both the water and blood with air bubbles from the aquarium air pump helped to stabilize

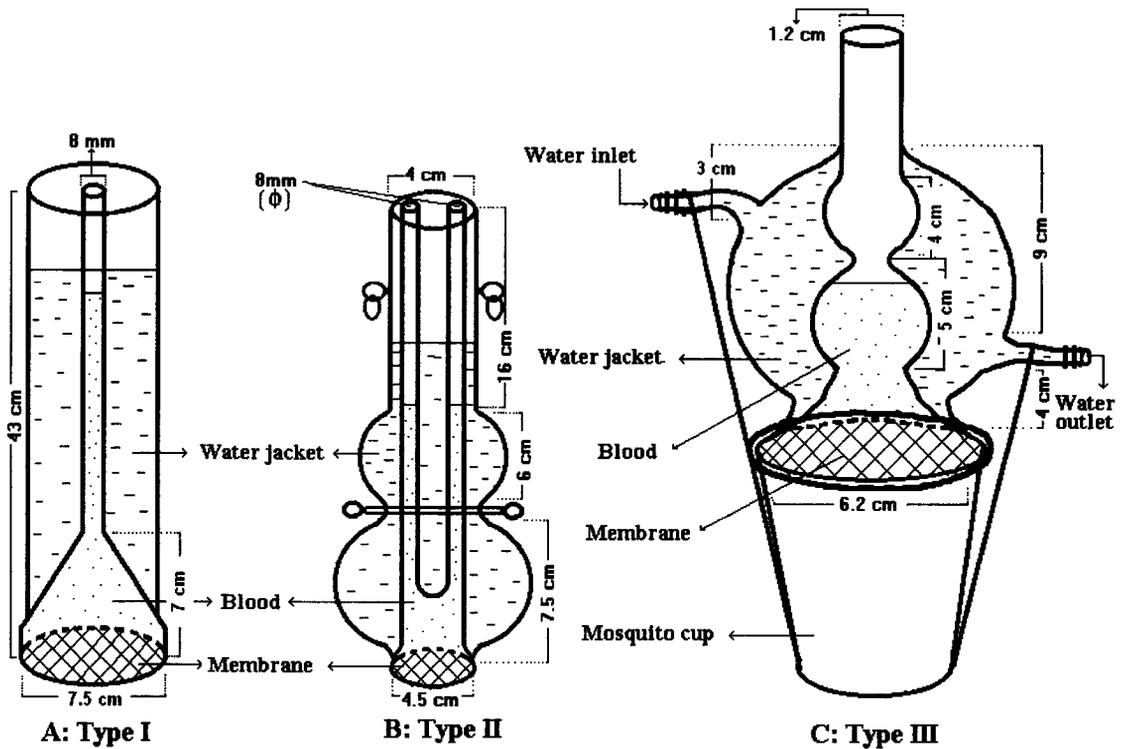


Fig. 1. Three types of glass feeding apparatus.

the temperature. The membrane temperature was maintained at $\sim 37.5^{\circ}\text{C}$ when the water jacket temperature reached $\sim 41\text{--}42^{\circ}\text{C}$. This required $\sim 45\text{--}60$ min.

Heat loss from the calf intestine was about 5 times higher than with the other membranes. Thus, to shorten the warming time, the blood was pre-heated at 37°C in an incubator for 30 min before pouring into the funnel (blood reservoir). This process shortened the stabilized warming time by about 50%.

Apparatus type II (Fig. 1B): The apparatus was made of heat-resistant glass and contained a small reservoir connected to 2 tubes, with one serving as the inlet for blood filling and as the inlet for blood agitation tubing from the air pump, the other as the inlet for a thermometer to measure the temperature of the blood and membrane. The reservoir was filled with 10 ml of heparinized human blood. Water in the water jacket was heated with an aquarium heater. Stabilization of membrane and blood temperature was obtained as with the type I apparatus.

Apparatus type III (Fig. 1C): In this system the water inlet tube was located near the top of the water jacket and the outlet tube was near the bottom. The inner blood reservoir consisted of 3 chambers with a larger inlet tube at the top for filling with blood and measuring the temperature. The water temperature and circulation were maintained

with a water bath (Nüve BM 102[®]; Nüve Sanayi Malzemeleri, Ankara, Turkey) that had a built-in thermostatic heater and a circulation pump (Fig. 2). This apparatus was used in series to bloodfeed the colony in large cages (Fig. 2) or to bloodfeed a small experimental group in a paper cup (Fig. 1C). The reservoir of each apparatus was filled with 10 ml of heparinized human blood. To feed mosquitoes in large cages, the top of the cage was covered with Plexiglas[®] in which 3 holes were cut and that were equipped with sliding doors. During feeding, the sliding doors were open and the apparatus was placed over the holes and connected to each other.

Distilled water was used to prevent calcification in the water jacket. Before feeding, blood temperature was adjusted to $37 \pm 0.5^{\circ}\text{C}$ and membrane temperature was adjusted to 35°C . Feeding time was limited to 1 h.

RESULTS AND CONCLUSIONS

Preliminary experiments with apparatus type I

Apparatus type I was tried with 3 different membranes and 3 blood groups to artificially feed *An. sacharovi* and *Anopheles superpictus* Grassi. Colonies of these 2 mosquitoes already were adapted to feed on cotton pads soaked in 10% sugar solution. In the early experiments, 10% sugar was available along with different membranes (latex, paraffin

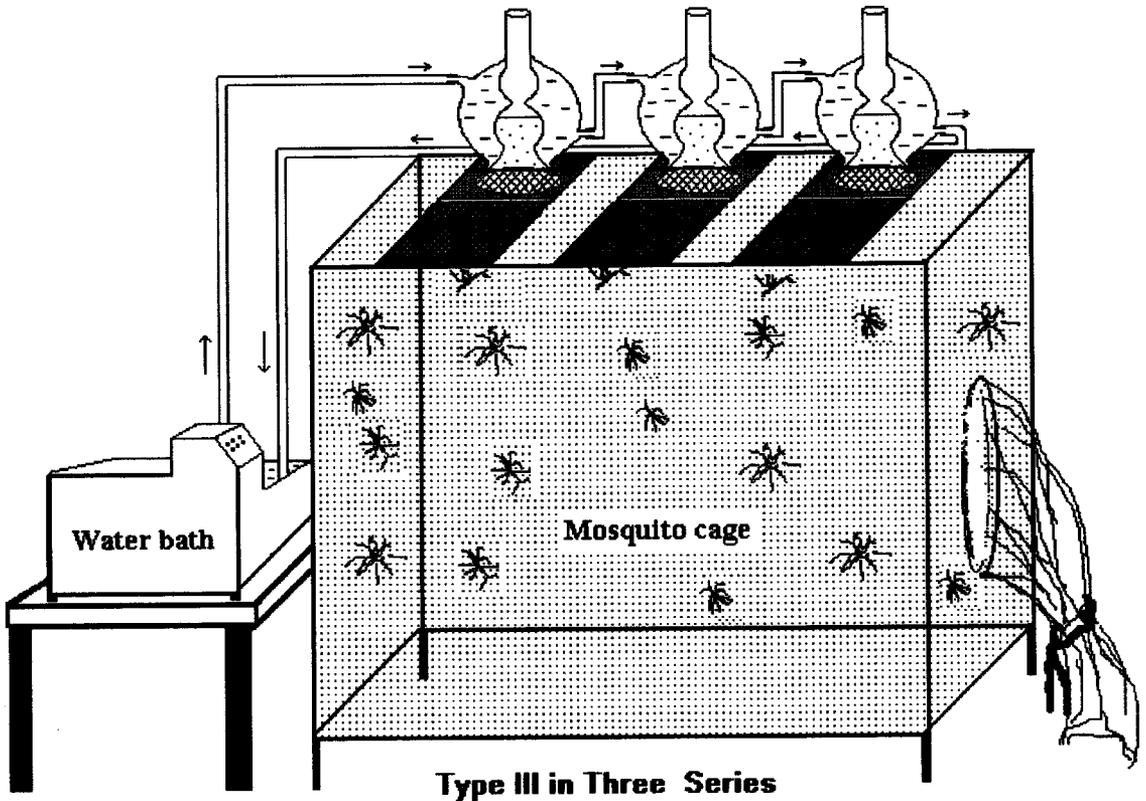


Fig. 2. Type III bloodfeeding apparatus used in 3 series in breeding cages.

film, and calf intestine). Latex and paraffin film were directly stretched over the basal orifice of the apparatus, whereas the calf intestine was softened and disinfected in absolute alcohol for 30 min before stretching. It was observed through repeated trials that 100% of both species (held with 10% sugar) fed on calf intestine, whereas none fed on latex and paraffin film. Therefore, in the following trials only calf intestine and heparinized human blood were used and a mosquito with a full meal was considered positively fed.

To understand the effect of age, cohorts of 3-, 4-, and 5-day-old females were selected and fed

a 10% sugar solution before bloodfeeding. For comparison (control) mixed-age females were sampled at random from the colonies. These females may have been previously fed on either a rabbit or 10% sugar solution. Calf intestine membrane feeding results with these groups with apparatus type I show differences but the overall mean feeding rate (67.2%) was significantly higher than the mean feeding rate on a live rabbit (35%; Table 1).

These experiments also show that *An. sacharovi* fed best on the natural calf intestine membrane. The freshness of the blood has no advantage, even outdated preserved human blood samples could be used provided cell lysis had not occurred.

Bloodfeeding rates achieved with the different types of apparatus with calf intestine and heparinized human blood groups were significantly different among the blood groups used with any type of apparatus or among the types of apparatus when using the same blood group except for O Rh+ (Table 2). Despite more than 20 years of adaptation to bloodfeeding on rabbits, the feeding rate of the colony of *An. sacharovi* on rabbits was only 35% (Table 1); whereas another study (Kasap 1990) reported that the bloodfeeding rate of the same colony on human volunteers was 79.2%. Even though appa-

Table 1. Feeding rates for different age groups of *Anopheles sacharovi* with apparatus type I. The blood used was heparinized human A Rh+.

Age	Feeding rate ¹ fed/total (%)
3 days	10/14 (71.6)
4 days	19/28 (67.9)
5 days	54/67 (80.6)
Subtotal	83/109 (76.1)
Mixed age and feeding history	44/80 (55.0)
Total	127/189 (67.2)

¹ Bloodfeeding rate on caged or anesthetized rabbit was 14/40 (35%).

Table 2. Feeding rates of 3- to 5-day-old female *Anopheles sacharovi* fed in 3 different membrane apparatuses with different heparinized human blood groups.

Blood groups	Feeding rates with apparatus ¹			
	Type I fed/total (%)	Type II fed/total (%)	Type III fed/total (%)	Type III in 3 series fed/total (%)
A Rh+	83/109 (76.1)	53/120 (44.2)	103/236 (43.6)	35/50 (70.0)
A Rh-	84/104 (80.7)	27/77 (35.1)	74/188 (39.4)	39/112 (34.8)
O Rh+	—	67/126 (53.2)	190/313 (60.7)	27/37 (73.0)
Total	167/213 (78.4)	147/323 (45.5)	367/737 (49.8)	101/199 (50.8)

¹ Values are significantly different among the blood groups used with any type of apparatus or among the types of apparatus when using the same blood group except for O Rh+ (Kruskal-Wallis test for 1 factor, $P < 0.05$).

ratus type I showed the overall highest membrane feeding rate (78.4%), we found that apparatus type III may be preferable because it is very handy and adaptable to various operations. Apparatus type III required only 10 ml of human blood and produced acceptable feeding rates (49.8% and 50.8%; Table 2) and allowed for easier maintenance of mosquito colonies.

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REFERENCES CITED

- Bailey DL, Dame DA, Munroe WL, Thomas JA. 1978. Colony maintenance of *Anopheles albimanus* Wiedemann by feeding preserved blood through natural membrane. *Mosq News* 38:403-408.
- Benzon GL, Apperson CS. 1987. An electrically heated membrane blood feeding device for mosquito colony maintenance. *J Am Mosq Control Assoc* 3:322-324.
- Coluzzi M. 1964. Maintenance of laboratory colonies of *Anopheles* mosquitoes. *Bull WHO* 31:441-443.
- Hagen HE, Grunewald J. 1990. Routine blood-feeding of *Aedes aegypti* via a new membrane. *J Am Mosq Control Assoc* 6:535-536.
- Kasap H. 1990. Comparison of experimental infectivity and development of *Plasmodium vivax* in *Anopheles sacharovi* and *An. superpictus* in Turkey. *Am J Trop Med Hyg* 42:111-117.
- Kasap M, Kasap H. 1983. Laboratory colonization of *Anopheles sacharovi*, the principal vector of human malaria in Turkey. *Mosq News* 43:498-499.
- Novak MG, Berry WJ, Rowley WA. 1991. Comparison of four membranes for artificially blood feeding mosquitoes. *J Am Mosq Control Assoc* 7:327-328.
- Weyer F. 1965. Biologische Beobachtungen an einer Laboratoriumszucht von *Anopheles sacharovi*. *Tropenmed Parasitol* 16:1-16.
- WHO [World Health Organization]. 1975. *Manual on practical entomology, part II: methods and techniques*. Geneva, Switzerland: World Health Organization.
- Wirtz RA, Rutledge LC. 1980. Reconstituted collagen sausage casing for the blood feeding of mosquitoes. *Mosq News* 40:287-288.