Research Note

A convenient mosquito membrane feeding method

Masakazu HARADA,¹⁾ Hiroyuki MATSUOKA²⁾ and Setsuo SUGURI¹⁾

¹⁾Department of Medical Zoology, Kagawa Medical School,
Kita-gun, Kagawa 761-07, Japan

²⁾Department of Medical Zoology, School of Medicine, Mie University,
Tsu, Mie 514, Japan

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Maintenance of mosquito colonies in the laboratory requires human or animal blood sources. Membrane feeding systems have many advantages over the use of live animals during mosquito rearing, and many devices have been developed. Tarshis (1958) used a simple floating cage and Collins *et al.* (1964a) and Collins *et al.* (1964b) replaced the bottom of a half-pint ice cream cup with a Baudruche membrane (natural intestine). Rutledge *et al.* (1964) devised a glass membrane-feeder, Wirtz and Rutledge (1980) used collagen sausage casings, Benzon and Apperson (1987) placed a heating device within a condom, and Hagen and Grunewald (1990) created a heating chamber with nylon gauze. Beier *et al.* (1992) covered a micro-tube with a Baudruche membrane and Cosgrove *et al.* (1994) developed an electronically controlled heating device covered with collagen membrane. In all of these methods, temperature is either not regulated or is controlled by specially designed instruments.

This report describes a convenient membrane feeding method using a cooler pot and Parafilm (American National Can, Greenwich, CT 06836, U.S.A.). Procedure.

1. Prepare an autoclaved 1 l glass pot with a concave bottom. A water cooler about 8 cm in diameter and 26 cm in height works well.
2. Turn pot upside down.
3. Load several hundred microliter of heparinized human or animal blood on the outer bottom of the pot (Fig. 1).
4. Wrap the blood on the pot bottom with a piece of thin, twice (in area) expanded Parafilm and seal tightly (Fig. 2).
5. Turn the pot upside down again and place it on the stainless steel mesh (0.9 mm opening, 170 mesh) of a mosquito cage (30×30×30 cm). Pour temperature-adjusted water into the pot. Mosquitoes will feed through the Parafilm membrane (Fig. 4). Blood temperature can be regulated by changing the temperature of the pot water, which is easily monitored by a thermometer. When 37°C water is added to the pot at a room temperature of 28°C, the water temperature remains above 35°C for 15 min. Water temperature in the pot can be maintained by adding hot water at intervals of a few minutes. On rearing mosquitoes requiring longer feeding period, blood can be kept to feed for several hours by controlling water temperature within suitable range and the use of anticoagulated blood.

About 50 females of *Anopheles stephensi* fed on blood meal from a live hamster or heparinized human blood by this method for 30 min and they laid 59.7 and 34.5

¹⁾ 原田正和, 村主節雄: 香川医科大学病理学講座医動物学 (〒761-07 香川県木田郡三木町池戸 1750-1)

²⁾ 松岡裕之: 三重大学医学部医動物学教室 (〒514 三重県津市江戸橋 2-174)

Fig. 1. An autoclaved glass pot (about 8 cm in diameter and 26 cm in height) is placed upside down. Several hundred microliters of human or animal blood are loaded onto the concave bottom of the pot.

Fig. 2. The blood is sealed by a thin membrane of expanded Parafilm.

Fig. 3. After the pot is turned upside down again, it is placed on top of a mosquito cage so that the membrane makes contact with the stainless steel mesh of the cage. Temperature-adjusted water is poured into the pot.

Fig. 4. Mosquitoes feeding on blood through the Parafilm membrane.

eggs/feeding female, respectively. The reduction rate in egg production was 42.2% in this method. This reduction may be partly due to the restricted feeding area and short feeding period. In 8 hr membrane feeding of *An. albimanus*, egg productivity dropped to 71% and 74% (Bailey *et al.*, 1978). In the case of *Aedes aegypti*, there were no differences in egg productivities when live animals were used as blood source and 30 min membrane feeding were employed (Benzon and Apperson, 1987).

In this method, although the egg productivity was lower than those of the methods described by Bailey *et al.* (1978) and Benzon and Apperson (1987), by the repetition of feeding and the elongation of feeding period, the sufficient eggs to maintain colony are obtained.

We have maintained a colony of *An. stephensi* for several generations with this membrane feeding device. The merits of this method are; 1) it requires no compli-

cated instruments or temperature regulators, 2) experimental animals used as blood source are released from the feeding stresses and pains, and 3) blood from a variety of animal sources can be used in the device as well as cultured red blood cells infected with malaria. The simplicity of this method makes membrane feeding possible in almost every laboratory.

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REFERENCES

- Bailey, D. L., D. A. Dame, W. L. Munroe and J. A. Thomas (1978) Colony maintenance of *Anopheles albimanus* Wiedemann by feeding preserved blood through natural membrane. *Mosq. News*, **38**: 403-408.
- Beier, J. C., M. S. Beier, J. A. Vaughan, C. B. Pumpuni, J. R. Davis and B. H. Noden (1992) Sporozoite transmission by *Anopheles freeborni* and *Anopheles gambiae* experimentally infected with *Plasmodium falciparum*. *J. Am. Mosq. Control Assoc.*, **8**: 404-408.
- Benzon, G. L. and C. S. Apperson (1987) An electrically heated membrane blood-feeding device for mosquito colony maintenance. *J. Am. Mosq. Control Assoc.*, **3**: 322-324.
- Collins, W. E., A. J. Harrison and J. C. Skinner (1964a) The use of a membrane feeding technique to determine infection and transmission thresholds of Semliki Forest virus in *Anopheles quadrimaculatus* and *Anopheles albimanus*. *Mosq. News*, **24**: 25-27.
- Collins, W. E., G. M. Jeffrey and J. C. Skinner (1964b) Comparative infectivity of a strain of *Plasmodium falciparum* from Panama to three species of *Anopheles* as studied by membrane feeding. *Mosq. News*, **24**: 28-31.
- Cosgrove, J. B., R. J. Wood, D. Petrić, D. T. Evans and R. H. R. Abbott (1994) A convenient mosquito membrane feeding system. *J. Am. Mosq. Control Assoc.*, **10**: 434-436.
- Hagen, H. E. and J. Grunewald (1990) Routine blood-feeding of *Aedes aegypti* via a new membrane. *Opera. Sci. Notes*, **6**: 535-536.
- 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.
- Tarshis, B. (1958) Feeding techniques for bloodsucking arthropods. *Proc. 10th Internatl. Congr. Ent.*, **3**:

767-784.

Wirtz, R. A. and L. C. Rutledge (1980) Reconstituted collagen sausage casings for the blood feeding of mosquitoes. *Mosq. News*, 40: 287-288.

摘 要

簡便な蚊の膜吸血法

蚊の膜吸血を簡単に行う方法を考案した。どこでも手に入るガラス製のポットを洗浄、滅菌した後、底面を上

にして置き、その上に血液を数百 μ l 載せ、約 2 倍に延ばしたパラフィルムで覆う。これを再び底面を下にして、ステンレス製の蚊飼育ケージの上に載せ、ポットの中に 37°C (または任意) の湯を入れる。これによりケージ中の蚊は膜を通して吸血することが出来、また血液の温度は中の湯の温度を変えることにより調節できる。この方法により人血液を吸血させた蚊の 1 雌当たり産卵数は 34.5 個で、ハムスターを吸血させた蚊より 42.2% 少なかった。しかしこの簡便な方法によりどこでも蚊の膜吸血を実施することができ、蚊のコロニーを維持することができる。