A SMALL VOLUME, THERMOSTATICALLY CONTROLLED APPARATUS FOR FEEDING RADIOACTIVE DIETS TO MOSQUITOES AND OTHER SUCKING ARTHROPODS

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ABSTRACT. With this apparatus, 1 ml of diet will provide about 7 cm^2 of feeding surface. The use of such small volume of diet reduces the expense and the hazards associated with the feeding of radioactive isotopes. The diet is held between two membranes, and so it can easily be removed and discarded after use, a feature which minimizes the chance of radioactive contamination. Temperatures are controlled to ± 0.1°C through the use of a proportional controller which supplies power to micro-immersion heaters in proportion to the difference between the actual and the desired temperature.

The feeding responses of suctorial insects and other arthropods are complex; most will not feed normally from a free liquid surface but must feed through a membrane (Tarshis 1958; Rutledge et al. 1964; Galun 1971; Friend and Smith 1972). Haematophagous arthropods also require a heat signal to initiate feeding (Friend and Smith 1977a). Many different types of artificial feeding apparatus have been developed to facilitate studies of the feeding behavior and nutrition of these animals. These feeding devices have many features in common. They usually consist of a diet container covered with a suitable membrane; when necessary, a method of controlling the temperature of the diet; and a cage to contain the animals.

The diet containers can vary from the simple Parafilm® packages used to feed the green peach aphid *Myzus persicae* (Mettler and Dadd 1964) to much more complex systems, some of which allow the diet to be exchanged rapidly during feeding (Friend and Smith 1977b) or others that will change the diet gradually in response to a pre-established program as the insects develop (Akey and Beck 1975). Diet containers which allow microscopic examination and filming of the mouthparts during feeding have also been developed (Smith and Friend 1971).

A wide variety of artificial and natural membranes have been tested and their advantages and disadvantages have been reviewed by Tarshis (1958), Galun (1971), and Friend and Smith (1972). The most effective artificial membranes tested to date are Parafilm[1], gutta percha rubber[2], and silicone rubber[3]. The most efficient natural material seems to be Baudruche membrane[4], a preparation of ox caecum, which can be obtained in sheets 24 x 90 cm. This membrane can be stored for many years without deteriorating. It is much easier to use than the skins, intestines or bladders of such animals as bats, chickens, or mice (Tarshis 1958).

Parafilm M membranes, a mixture of rubber and wax, have been used successfully with aphids (Mettler and Dadd 1964), body lice (Haddon 1956), and soft ticks (Galun and Kindler 1968), and less successfully with mosquitoes (Rutledge et al. 1964) and tsetse flies (P. Langley personal communication). *Rhodnius prolixus* (Friend 1965) and *Triatoma infestans* (Shimamune et al. 1965) will feed without difficulty through gutta percha rubber membranes. Recently, membranes made of silicone rubber have been used to feed tsetse flies. These are more practical than the agar membranes used previously (Baur and Wetzel 1976).

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[1] American Can Co., Neenah, Wisconsin.

[2] Julius Schmid, New York, N. Y.

[3] Stauffer-Wacker Silicone Corp., Adrian, Michigan.

[4] Long & Long Co., Belleville, New Jersey.

Rutledge et al. (1964) studied the effects of 4 different membranes on the feeding behaviour of 6 species of mosquito and found that the synthetic membranes Parafilm M and Saran Wrap were not as effective as chick skin or Baudruche membrane. We have recently determined that the mosquito *Culiseta inornata* will not feed through gutta percha rubber membranes and does not feed nearly as well through silicone rubber as it does through Baudruche membranes.

Various techniques have been used to control the temperatures of the diet. These range from having the diet surrounded by a water jacket through which thermostatically controlled water can be circulated (Rutledge et al. 1964), using brass blocks which have been heated on a slide warmer to surround the diet containers (Friend and Cartwright 1963), using a strong light shining on a piece of black tape attached to a micro diet container (Smith and Friend 1971) to the use of incubators (Bar-Zeev and Smith 1959) or immersion heaters in water baths (Tarshis 1958). Until recently these immersion heaters were connected to a thermostat and a power switch which turned the heaters on and off, an all-or-none response that usually caused overshoots. The regulation of water temperature has been improved by the development of a device which supplies power to the heater in proportion to the difference between the actual and the desired temperature (Thiel and Harvey 1970). Such proportional controlling of water temperature permits the use of micro-immersion heaters in very small water baths, a feature incorporated into the design of our feeding apparatus.

The cages which contain the test insects must allow the insects to assume their normal feeding posture and should not limit access to the feeding membrane. Many types of cages have been used (Tarshis 1958, Galun 1971). One ingenious solution to the cage-membrane problem was to confine the insects in a cage which had the feeding membrane as its bottom and then float the cage on the diet! (Tarshis 1958).

For our studies of the feeding behavior of female *Culiseta inornata* we needed to incorporate radioactive ^{14}C inulin or dextran into the diets so that we could measure the amounts of diet ingested. In order to reduce the cost and the hazards associated with such diets we developed a feeding apparatus that operates efficiently with diet volumes as low as 1 ml and yet is suitable for 6 or more mosquitoes to feed on simultaneously.

MATERIALS AND METHODS

Figure 1 illustrates the apparatus. The water bath consists of a piece of acrylic plastic tubing 5 cm long x 4.5 cm O.D., closed at the bottom end with a thin sheet of rubber. The rubber is attached to the tube by taping it to the outer wall of the water bath.

The water bath is inverted and the diet container constructed on the rubber bottom. A ridge of silicone rubber[5] is built up around the outer rim of the rubber bottom to a height of approximately 2–3 mm. Care is taken that the upper rim of the ridge is smooth and even and that there are no gaps that would allow leakage. The silicone rubber is allowed to cure for 24 hrs. The shallow cup formed by the silicone rubber ridge and the rubber bottom of the water bath will hold about 1 ml of diet. The diet is poured onto the rubber surface and a Baudruche membrane is then stretched over the diet and secured to the side of the tubing with an O-ring which fits into a groove in the water bath (see Fig. 1). The water bath is then turned, diet side down, and partially filled with about 15 ml of water. A micro-immersion heater of approximately 6 Ω resistance consisting of 45 cm of 26 gauge Nichrome® resistance wire wrapped around a ceramic tube 2.5 cm long 0.5 cm O.D. and inserted in a test tube 8 cm long 1 cm O.D. was used to heat the water and thus the diets to 38°C ± 0.1°C. The heater is regulated by a proportional controlling device which has 3 major cir-

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[5] Canadian General Electric Co., Toronto, Ontario.

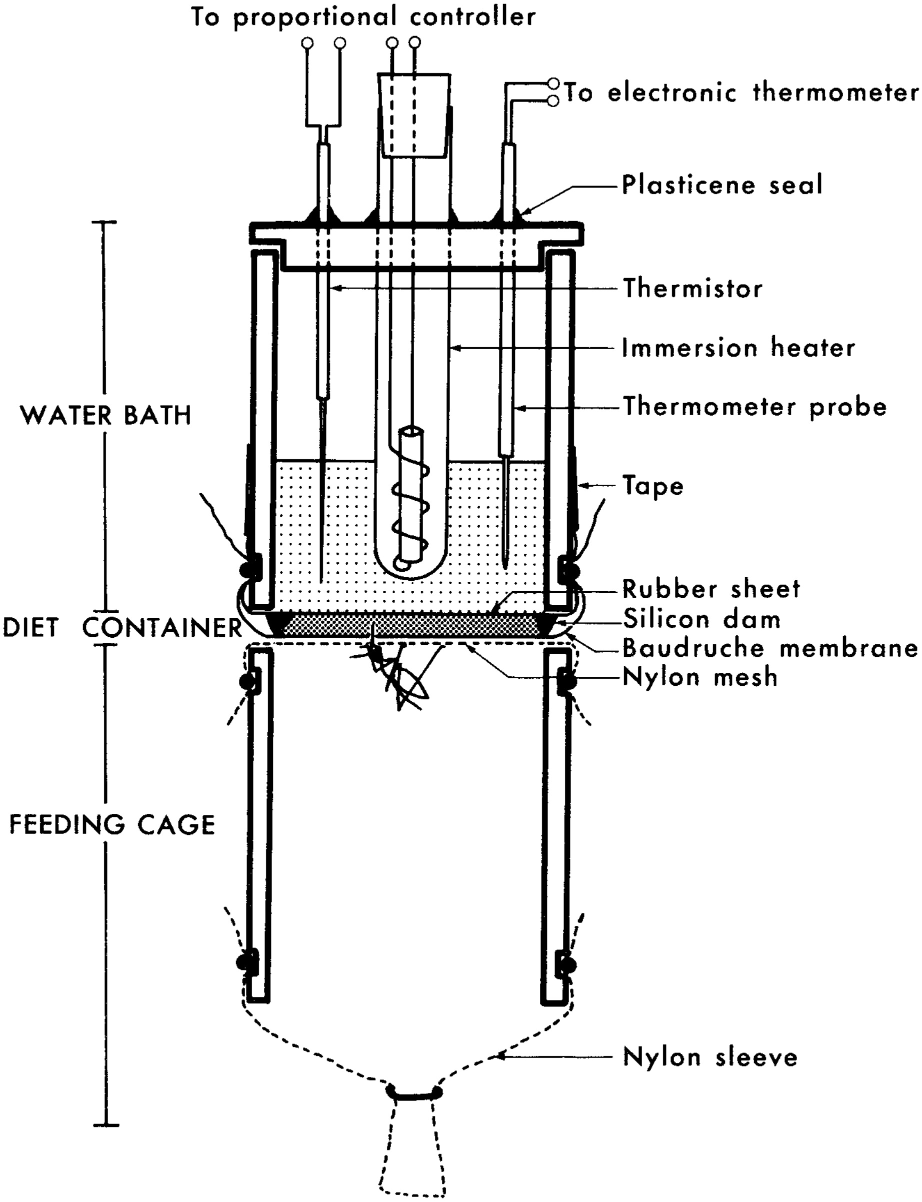

Fig. 1 Feeding apparatus.

cuit sections (see Fig. 2): (1) a Wheatstone bridge with a set-point control potentiometer and a thermistor, (2) a unijunction pulse circuit and, (3) a TRIAC. The TRIAC acts as the control element. It is an AC semi-conductor switch which is triggered into conduction by a gate signal from the unijunction circuit. The amount of power supplied to the heater is proportional to the difference in temperature between that set on the control and that measured by the thermistor (Thiel and Harvey 1970). Our proportional controllers were custom made, however suitable proportional controllers are available commercially.[6] Temperatures were monitored with an electronic thermometer, the probe of which was immersed in the water bath.

The cages used to contain the mosquitoes while they fed consisted of 5 cm long pieces of acrylic tubing 4.5 cm O.D. capped at one end by tightly-stretched nylon stocking mesh fastened to the tube by an O-ring. The other end of the cage was closed by a short sleeve cut from the leg of a nylon stocking which facilitated the loading of the mosquitoes into the cages. The female mosquitoes which were to be fed were collected from the stock colony with an aspirator. The aspirator was then inserted through the nylon sleeve which was gathered around the aspirator tube, the insects were then blown from the aspirator into the cage, and the sleeve twisted and closed with a rubber band. Usually 6 females were confined in each cage. These cages are then clamped under the diet chamber with the tightly-stretched nylon mesh in contact with the Baudruche membrane. The mosquitoes feed upward through the nylon mesh and the membrane.

We normally use 2 feeding apparatus simultaneously.

RESULTS AND DISCUSSION

The feeding apparatus described here has been used to determine the effects of the presence or absence of a membrane and the presence or absence of a heat signal on the feeding responses of the mosquito *Cs. inornata* to blood, and to artificial diets containing the phagostimulants ATP and sucrose (W.G. Friend manuscript in preparation). It has also been used in a study of the manner in which this mosquito eliminates water after feeding. This required us to observe and time the feeding responses of individual animals (Friend, Tobe, Hewson manuscript in preparation) and to capture them and treat them immediately after feeding.

Both these studies required accurate determinations of the amount of diet ingested. Ingestion was measured by incorporating either ^{14}C inulin or ^{14}C dextran into the diets and then, after feeding, determining the amount of isotope in each insect by scintillation counting.

Radioactive diets are both expensive and hazardous; consequently it is advantageous to reduce the volume of these diets to a minimum. It is also advantageous to be able to discard unused portions of such diets efficiently and to be able to decontaminate or discard any parts of the apparatus that may become contaminated with radioactivity. This feeding apparatus has all of these advantages. Because of the way in which the diet is contained, sandwiched between membranes, only 1 ml or less of diet is required to provide a feeding target at least 7 cm^2 in area on which up to 6 mosquitoes can feed simultaneously. After feeding, the water bath can be emptied, and the diet chamber stripped off the bottom of the water bath with the diet still contained in its two membranes, and the entire packet discarded with no mess and little expense. The nylon mesh associated with the feeding cage which may have become contaminated due to the Baudruche membrane "sweating" or to liquid eliminated by the mosquitoes during or after feeding can also be discarded and the plastic tube which forms the remainder of the cage is easy to decontaminate by washing. Other advantages are: bub-

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[6] R.F.L. Industries, Boonton, New Jersey.

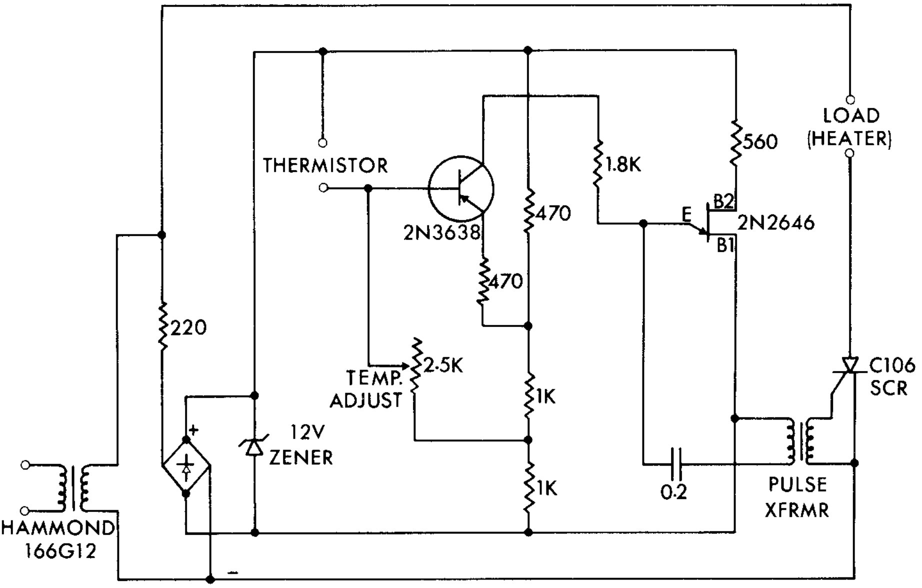

Fig. 2 Proportional controller—circuit diagram

bles in the diet present no problem because the insects feed upward; and the insects can be observed as they feed in this apparatus. As soon as an insect has completed feeding, the feeding cage can be quickly separated from the diet container and the fed insect captured by aspiration. The feeding cage can then be replaced and feeding resumed. These activities were necessary in our study of liquid elimination following feeding.

Cs. inornata is not an easy insect to feed. Under our laboratory conditions less than 40% of females which have emerged 5 to 8 days previously and were maintained on sugar cubes and water *ad libitum,* would take a blood meal when exposed to the ears of rabbits. The maximum amount of blood taken in a single meal is approximately 5μl (Owen and Reinholz 1968). Using our feeding apparatus 30 insects can be exposed to the diet and an average of 40% of these will feed in 30 min. The average amount of fresh, heparinized human blood taken was 3.4 μl and the maximum meal size was 5.1 μl.

The proportional heat control equipment should cost less than $50.00. Controlling the heat in this manner permits one to use very small water baths and consequently small diet containers. These small volumes, and the fact that only a thin sheet of rubber separates the water and the diet, allows rapid heat equilibration, which minimizes deleterious effects on heat-labile diets such as fresh heparinized blood. If the immersion heaters and the volume of water in a series of identical water baths were standardized so the temperature in each bath would fluctuate in the same manner as neighbouring baths a whole series of baths could be regulated from one master bath. The master bath would contain the thermistor and would supply the feedback signal to the proportional controller which would then feed the correct amount of power to all of the immersion heaters.

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