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ADAPTATIONS OF MEMBRANE FEEDING TECHNIQUES FOR FEEDING THE SQUIRREL FLEA, *ORCHOPEAS HOWARDI*, AND THE SQUIRREL LOUSE, *NEOHAEMATOPINUS SCIUROPTERI*, WITH NOTES ON THE FEEDING OF THE HUMAN BODY LOUSE, *PEDICULUS HUMANUS* VAR. *CORPORIS*[1]

Techniques for feeding hematophagous arthropods through membranes offer greater advantages in studies of vector potential than traditional methods involving feeding of the parasites on live hosts. An especially important advantage of membrane techniques is the ability to expose the arthropod to defined doses of pathogens, a virtual impossibility when live-host techniques are used. Secondly, loss of test arthropods through host self-grooming is eliminated with membrane techniques. Additional advantages of membrane feeding are the ability to control physical and chemical parameters that affect feeding (e.g., temperature, light, chemical stimulants, etc.) and the elimination of interference from other ectoparasites that might be present on a live host.

Despite the significant technical progress made in the development of membrane feeding techniques (Wade, 1976, Ann. Trop. Med. Parasitol. **70:** 113–20), none of the reported membrane techniques were successful for feeding the flying squirrel ectoparasites *Orchopeas howardi* (Baker) and *Neohaematopinus sciuropteri* (Osburn). These insects have been implicated in the transmission of the sylvan strain of *Rickettsia prowazeki* isolated from Southern Flying Squirrels, *Glaucomys volans* [Bozeman, Williams, Stocks, Chadwick, Elisberg, Sonenshine & Lauer, 1977, Folia Microbiol. (in press)]. To induce feeding through membranes, several innovations in the technique were required, particularly in the control of blood pressure, design of the blood chamber, and choice of membranes. This paper reports the adaptation of the technique to permit its use in transmission experiments with the sylvan typhus agent and the 2 parasitic insects. In addition, comparisons were made with the human body louse, *Pediculus humanus* var. *corporis* L., in view of its importance as the sole known vector to man.

Apparatus and parasites. The membrane feeder consisted of 3 primary units: a blood chamber, a circulating pump, and a temperature-controlled water bath. The blood chamber (FIG. 1) was made with a Sykes-Moore (Sykes & Moore, 1959, Proc. Soc. Exp. Biol. Med. **100:** 125–27) tissue culture chamber, 3 cm diam. A membrane, a 2 cm × 0.3 cm rubber "O" ring, and a 2.7-cm cover glass were used to contain the blood reservoir. A metal retaining ring was screwed into the threaded metal wall of the chamber, compressing the components and preventing leakage. Blood was introduced via a pair of 20-gauge hypodermic needles inserted through the rubber "O" ring and holes in the metal chamber wall. Polyethylene tubing was attached with metal adapters to the needles and to the Dekastaltic circulating pump (Buchler, Inc., Ft. Lee, New Jersey). Blood circulated at 3 ml/min. from the blood chamber (or chambers) through the pump, a coil of tubing immersed in a Plexiglas water bath (Tarshis, 1956, Proc. X. Int. Congr. Entomol. **3:** 767–84) and back to the blood chamber. The water bath was heated with an aquarium heater (Metaframe Corp., Elmwood Park, New Jersey). The small quantity of tubing used between the water bath and the blood chamber facilitated control of blood temperature. Maintenance of temperature gradients above the membrane surface was made possible by the fact that the blood chamber was separated from, rather than immersed in the water bath. The blood chamber was filled by drawing blood from an external reservoir with the aid of the circulating pump until the desired pressure on the membrane was achieved. The total blood volume in the system was ca. 3 ml, 1/3 of which was in the blood chamber at any moment. Continuous circulation of the blood prevented sedimentation. Fresh defibrinated rat blood was used to feed *O. howardi*, defibrinated rat blood plus flying squirrel blood for *N. sciuropteri*, and defibrinated rabbit blood for *P. humanus*. Blood temperature was maintained at 37° ± 1°C.

The parasite feeding chamber, a 2 cm diam. × 5 cm

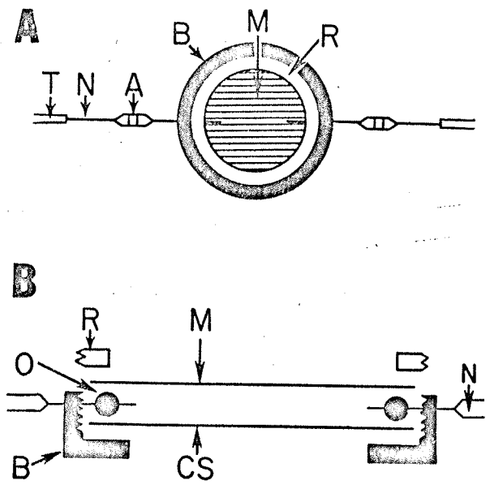

FIG. 1. Membrane chamber for feeding of flying squirrel ectoparasites: (A) dorsal view; (B) partially "exploded" cross section. A=♀-♀ adapter, B=base of Sykes-Moore chamber, CS=glass cover slip, M=membrane, N=20 gauge hypodermic needle, O=rubber "O" ring, R=retaining ring, T=polyethylene tubing.

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TABLE 1. Feeding of 3 species of ectoparasites through natural skin membranes and on live hosts.

Species (no. replicates)	Membrane feeding (37° ± 1°C)		Live host feeding
	% feeding success (no. fed/no. exposed)	Volume blood consumed (µl)	Volume blood consumed (µl)
Neohaematopinus sciuropteri (4)	95.9 (352/367)	0.003* (*n*=13)	—
Orchopeas howardi (6)	87.3*** (620/710)	0.026 ± 0.004 (SE)* (*n*=25)	0.085** (*n*=31)
Pediculus humanus (2)	77.1 (192/249)	0.043** (*n*=29) 0.077 ± 0.008 (SE)* (*n*=20)	0.974 ± 0.107 (SE)* (*n*=39)

*Determined by ratio assay; *O. howardi* and *P. humanus* were assayed individually; the *N. sciuropteri* assay was done with a single pool of 13 fed lice.

**Determined by measuring mean weight increase of the total population sample before and after feeding.

***Under reduced light; fleas starved 1 week.

high black plastic cylinder, was installed directly above the membrane. Ectoparasites were released into the darkened chamber 24–48 hr after preconditioning them at 27°C, 80–90% RH, without food (except *N. sciuropteri*, which were removed directly from their hosts). All ectoparasites remained on the membrane surface 1–5 hr.

Natural membranes composed of flying squirrel skin were used in most experiments. Animals were stored frozen until used. Skins were removed, scraped clean of fat, moistened and stretched tightly over the rubber "O" ring (dried skins were discarded since they tended to crack and leak blood). No detergents or disinfectants were used. The fur was cropped close to the membrane for feeding lice, but was left in its natural state when feeding fleas. Parafilm (Haddon, 1956, Am. J. Trop. Med. Hyg. **5:** 315–25) was used in attempts to feed lice and fleas, but was abandoned when uncontrolled leaks followed penetration by the arthropod mouthparts.

The *O. howardi* used in these studies were originally from a natural population near Ashland, Virginia, and were maintained in a laboratory colony of immune flying squirrels. *N. sciuropteri* were from wild-caught animals taken at the same locality; *P. humanus* were from a rabbit-adapted strain obtained from the School of Public Health, Harvard University, Boston, Massachusetts (courtesy of Dr Edward S. Murray), and maintained on laboratory rabbits. Feeding was verified by microscopic examination of each insect. Blood-volume uptake was determined by weight gain using a Cahn Model G Electrobalance (Ventron Instruments Corp., Paramount, Calif.) and by radioassay techniques using radio-labeled blood. Three radionuclides, ^{14}C, ^{36}Cl, and ^{32}P (ICN Corp., Irvine, Calif.), were used as tracers and they were assayed with a Beckman LS-250 liquid scintillation counter (Beckman Instruments Corp., Fullerton, Calif.). Comparisons were also made with blood volumes taken while feeding on normal hosts (omitted in the case of *N. sciuropteri*), including restrained mice, after the technique of Kyles (1973, unpubl. Ph.D. dissertation, University of Georgia, Athens, Georgia), and on laboratory rabbits.

Feeding success. All 3 ectoparasites fed through the membrane (TABLE 1). Success was highest with *N. sciuropteri* (95.9%) and lowest for *P. humanus* (77%). *O. howardi*, which had 87.3% feeding success under the conditions described in TABLE 1, also fed on human blood (6.6%). In addition, some feeding occurred (23.1%) when fleas were exposed to ambient light, ca. 2000 lm/m². Hunger was also important since feeding success for adult fleas starved 1, 3, and 7 days after emergence was 23.0%, 62.1%, and 87.0%, respectively.

Blood consumption was substantial when ectoparasites were fed via membranes, though less than when they were fed on live hosts (TABLE 1). Unfed *O. howardi* feeding via membranes imbibed 27.4% of the amount of blood taken while feeding on mice. Similarly, *P. humanus* feeding via membranes consumed 6.2% of the amount of blood taken from a rabbit. *N. sciuropteri* consumed 0.003 µl of blood/louse when fed by the membrane method. No comparisons were made with natural host feeding by these lice because their poor survival and frequent small blood meals minimized the reliability of the data.

Membrane feeding did not appear to affect parasite vitality. Membrane-fed *O. howardi* fed readily when offered a 2nd meal 1 week later. Moreover, these fleas demonstrated a 0.013-µl increase in average blood consumption, as compared to a 0.056-µl increase in blood consumption by fleas fed on mice after a similar interval. Membrane-fed individuals of all 3 species survived on hosts for at least 2 weeks. *O. howardi* were observed to mate, oviposit, and produce viable larvae following membrane feeding. Squirrel lice were observed to defecate during membrane feeding, as well as deposit their elongated eggs on the hairs.—**David M. Lauer** and **Daniel E. Sonenshine,** Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23508, U.S.A.

PERSIS

The biting gnat *Cu* (Ceratopogonidae) is a bluetongue and epizootic h animals (Foster, Jones & Res. **24:** 1195–200; Fost Metcalf, 1977, J. Wildl. D of any studies on the poten bacteria. Transmission o an important aspect of bacterial diseases. In a Utah, *Chrysops discalis* W major vector of the etiol (McCoy & Chapin), to l *Culicoides* species, and the (Kieffer) were also implica 1973, J. Am. Med. Assoc.

Usually, biting flies beco pathogens by contact wit superficial the contaminat the less likely it is that bacte is favored by interrupted However, contamination pathogens to persist longe pathogens for days rather Tabanidae but similar blood-feeding by *C. variip* gonadotropic cycle. This 3 or more days between b to mechanically transmit circumstances, the fly woul during the interval betwe In this study, we report th species of Enterobacteriace by adult female *C. variipen*

Flies were reared by (Jones, Potter & Baker, 1483–86). Three groups were removed from larval allowed to emerge in sepa after emergence, the flies w only. Then the water was 1 were provided with a 10% an initial concentration of *coli* (Migula); the flies in

TABLE 1. Percenta (group 2) af

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