STUDIES ON THE FEEDING RESPONSE OF MOSQUITOES TO NUTRITIVE SOLUTIONS IN A NEW MEMBRANE FEEDER

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Introduction

Studies of the nutrition, feeding behavior, vector potential and salivary secretion of suctorial arthropods often require a convenient, standardized technique for feeding defined meals in the laboratory. The membrane feeding technique is one approach to this problem.

The first feeding of a blood-sucking arthropod (*Glossina palpalis*) by the membrane technique was accomplished by Rodhain in 1912 (Tarshis, 1958). Devices for membrane-feeding mosquitoes are remarkably varied and ingenious. The apparatus may be a simple floating cage (Tarshis, 1958), a food cup with a membrane floor (Collins *et al.,* 1964a and 1964b) or more sophisticated models with machined parts and a self-contained water bath (Tarshis, 1958) or incubator (Bar-Zeev and Smith, 1959). Certain components appear to be common to all feeders: cage, membrane, the food and its container and a temperature control system.

This paper describes a new membrane feeder specifically designed for feeding mosquitoes infectious materials. It also discusses certain factors that influence the feeding response of mosquitoes to a feeder. Some information on fecundity and destination of meal in artificially-fed mosquitoes is also given.

Materials and Methods

The feeder (Fig. 1) [3] is made of heat-resistant glass or stainless steel to permit autoclaving. It is readily attached by rubber tubing to a source of warm water for temperature control and it can be used with any mosquito cage that has a screened horizontal top. The feeder is in the form of a hollow cone with a tube extending from its vertex; the cone and lower part of the tube are surrounded by a cylindrical water-jacket provided with a lower inlet and an upper outlet for connecting rubber tubing. The bottom of the cylinder has a circular lip over which the membrane can be securely tied with surgical silk to close the base of the cone. A thermometer and cork or rubber stopper close the top of the feeder after the food has been introduced.

The area of the feeding surface is 57 ± 4 cm^2. The capacity of the cone to its truncation is approximately 10 ml. When conservation of the food is important, 5 ml. or less may be used. A clamp and ring stand support the feeder and hold it in contact with the screened top of a mosquito cage. The inlet and outlet tubes are connected to a constant temperature circulator [4] (Fig. 2). Several feeders can be connected to the same pump in series or in parallel if care is taken to regulate the feeder temperatures.

Several membranes were tested for their performance with this feeder. These were the Baudruche membrane (a bovine intestine preparation),[5] chick skin (fresh or frozen), Saran Wrap [6] and Parafilm "M".[7] Membranes were discarded after a single feeding except in two experiments.

The materials fed were chick blood, erythrocyte extracts and bovine serum. Chick blood was withdrawn by cardiac

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[3] All figures: U. S. Army photographs, by Medical Audio Visual Dept., Walter Reed Army Inst. of Medical Research.

[4] Haake Circulators, Series F, Brinkmann Instruments, Inc.

[5] Long and Long Co., Belleville, N. J.

[6] Dow Chemical Co., Midland, Mich.

[7] American Can Co., Menosha, Wis,

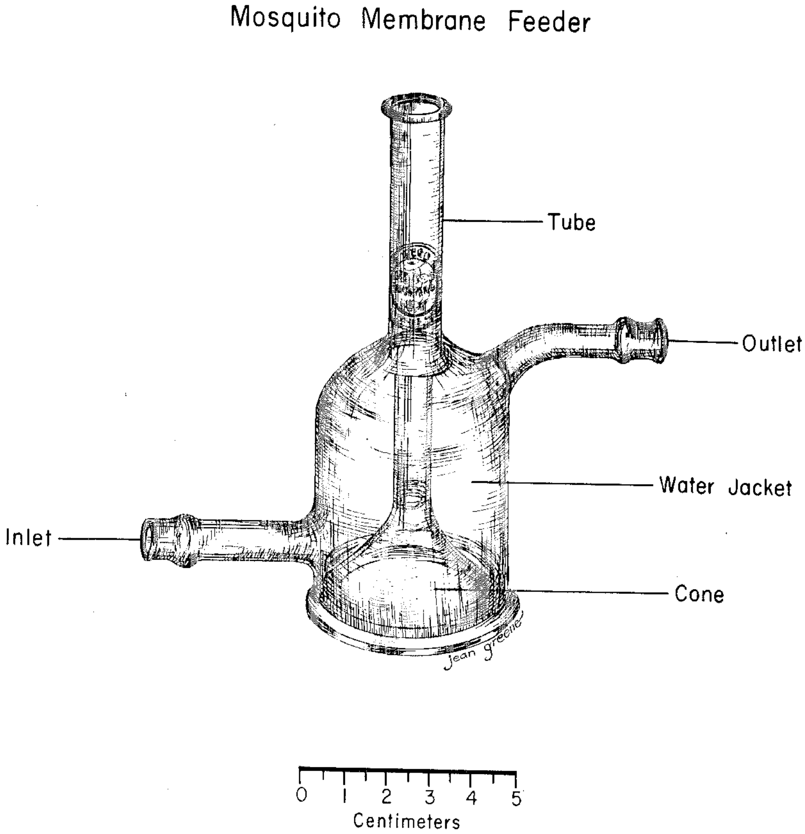

FIG. 1.—Glass mosquito membrane-feeder.

puncture, and coagulation was prevented by addition of one ml. heparin solution per 10 ml. of blood. Chick erythrocyte extract (C.E.E.) was prepared according to Whitman (1948) and frozen until used. Human erythrocyte extract was similarly prepared from outdated, citrated whole blood for transfusion. Bovine serum was diluted to 10 percent with normal saline solution and frozen until used. Food portions were discarded after a single feeding except in two experiments.

Adenosine triphosphate (ATP), sucrose, alanine and lysine were added to the foods at various levels to assess their influence as phagostimulants.

Six mosquito species from laboratory colonies at the Walter Reed Army Institute of Research were used in these studies. These were: *Aedes aegypti* (Bangkok, Thailand), *Aedes togoi* (unknown), *Culex tritaeniorhynchus* (Japan), *Culex pipiens pallens* (Japan), *Armigeres subalbatus* (West Pakistan) and *Anopheles stephensi* (Delhi, India). Age ranges of the first four species were 3–26, 4–13, 7–12 and 12–13 days from day of pupation, respectively. The last two species were taken from stock cages of unknown age.

For feeding trials, female mosquitoes were placed in cylindrical plastic cages (12.6 cm high and 9.5 cm diameter) with nylon mesh tops and paper-covered bottoms. Between 15 and 146 (mean=60) females were used in each cage. After experience had accumulated, 70 was taken as the standard number. Prior to use, the diet of the mosquitoes was a 4 percent sucrose solution; *Aedes aegypti* were ordinarily starved 6 to 24 hours before use. Except for *Armigeres subalbatus* and *Anopheles stephensi* no mosquito used had

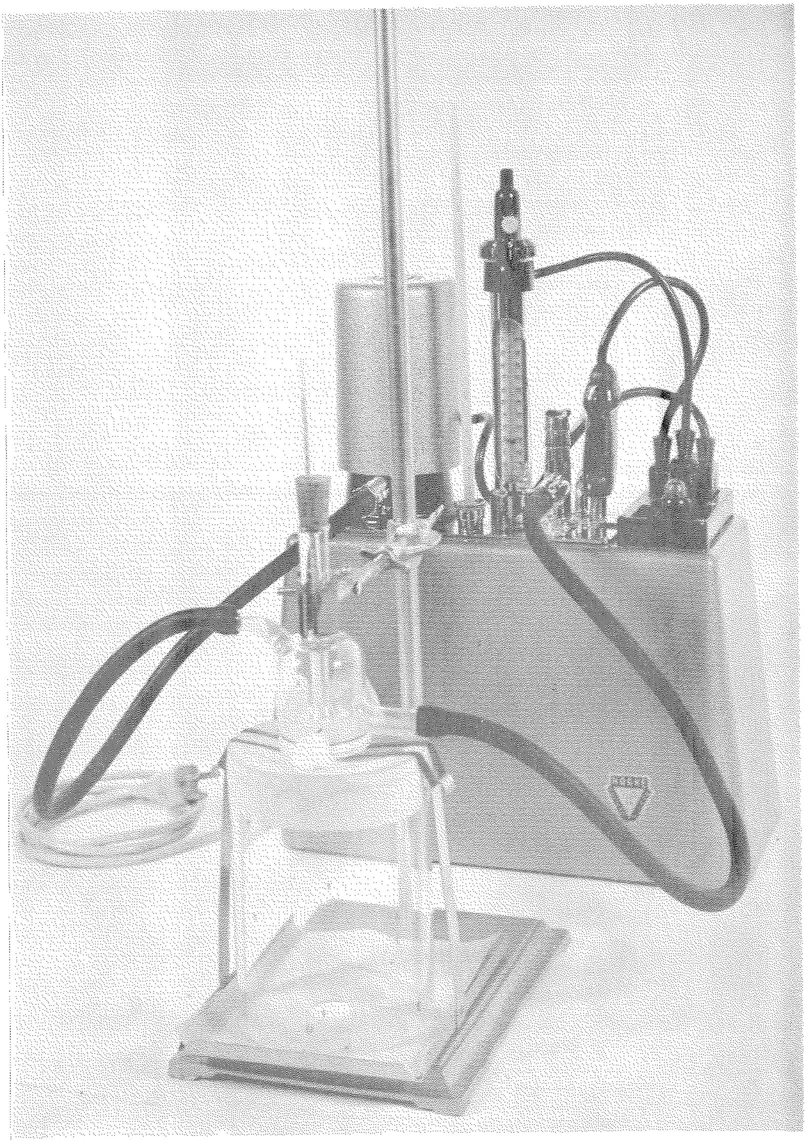

FIG. 2.—Membrane—feeding apparatus.

been offered a blood meal prior to use. The feeder temperature was 38±1° C. while the ambient temperature range was 23—38° C. (mean=26.1° C.). Relative humidity varied from 18—60 percent (mean=41.5 percent). Feeding trials were conducted between 1130 and 1630 hours. Unless otherwise indicated the test period for each cage was 15 minutes.

Statistical analysis followed Steel and Torrie (1960). The 95 percent confidence level was adhered to in all statistical analyses. Confidence limits for percentages were computed by the method of the normal approximation. Data classified according to more than one variable were analyzed as contingency tables or by the analysis of variance after imposing the inverse sine transformation.

RESULTS

PHAGOSTIMULANTS. Adenosine triphosphate (ATP) was shown by Hosoi (1959) to be a phagostimulant for female mosquitoes. Figure 3 plots our results obtained in feeding 24 cages of *A. aegypti* through chick skin and the Baudruche

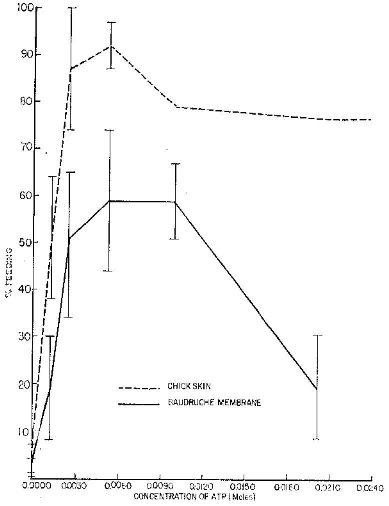

FIG. 3.—Percent *A. aegypti* feeding on CEE+ATP through Baudruche membrane and chick skin.

membrane on CEE to which was added 0.00, 0.0012, 0.0025, 0.0050, 0.010 or 0.020 M. ATP. The feeding period was 30 minutes. Mosquito ages were 5 to 26 days from pupation, and number of mosquitoes per cage was 15 to 75 (mean=42.4).

Confidence limits for the chick skin feedings at 0.010 and 0.020 M. ATP are not given in Figure 3 because each estimate is based on a pool of data from 3 replicates, one of which is statistically heterogeneous.

The phagostimulant effect of ATP is clearly shown by the steepness of the two curves at concentrations below 0.005 M., which appears to be near the optimum. We may consider the decline in percent feeding at the higher concentrations of ATP to be analogous to the repellent effect of chemical insect attractants at high concentrations (Dethier, 1947).

The phagostimulant effect of ATP extends to feedings of 10 percent bovine serum, but no significant effect was detected when ATP was added to fresh blood (Table 1). It seems reasonable to think that the natural food would be at least as acceptable as its adulteration.

In the chick blood experiment, a second feeding on each feeder was attempted immediately without replacing either food or membrane. Percent feeding was depressed by 38 percent and 22 percent. An examination of the membranes revealed that the cells had settled, forming a crust, dry below and viscous above. This phenomenon has been reported by previous workers and is a serious drawback to the use of cellular foods in the feeder. In other experiments we have found that shaking the feeder at five-minute intervals does not visibly retard the process of crust formation.

Sucrose solutions are commonly used in insectaries to sustain mosquitoes between blood meals. Table 2 shows results obtained with six cages of *Aedes aegypti* fed CEE with and without sucrose for 30 minutes. Mosquito age was 4 to 10 days from pupation (mean=6.5). Analysis of covariance for the randomized

TABLE 1.—Percent feeding of *Aedes aegypti* through the Baudruche membrance on chick blood and 10 percent serum with and without ATP. Feeding period was 15 minutes, and 67–70 mosquitoes were used in each cage. Membrane and food were not replaced for trial 2.

Experiment	Age range	Food	% Feeding	
			Trial 1	Trial 2
1	4–6 days	Chick blood	47%	9%
		Chick blood+0.010 M. ATP	31%	9%
2	4–18 days	10% bovine serum	0%	..
		10% bovine serum+0.0050 M. ATP	46%	..

complete-block design with missing observations was performed on the transformed data. Chi square is significant based on the conservative figure of 49 mosquitoes per cage. We conclude that sucrose is a low-order phagostimulant for *A. aegypti*.

Brown and Carmichael (1961) have shown that alanine and lysine are weak attractants for *A. aegypti*. L-alanine and L-lysine were tested in our laboratory for possible phagostimulant effect on this species. A 2 x 2 factorial experiment with 70 mosquitoes per cage was used. Mosquito age was 9 to 11 days in each cage. No significant phagostimulant effect was detected (Table 3).

MEMBRANES. Chick skin is clearly superior to the Baudruche membrane (Fig. 3 and Table 2). Further data substantiating this finding and extending its applicability to feedings of chick blood are presented in Table 4. Chi square is significant for comparison of the data pools. In this experiment the feeding period was 30 minutes and mosquito ages were 6–10 days for the chick skin trials and 8–9 days for the Baudruche membrane trials.

TABLE 2.—Percentages of *Aedes aegypti* feeding on CEE plus sucrose in four concentrations.

Membranes	Concentration of sucrose			
	0.000 M.	0.075 M.	0.150 M.	0.300 M.
Baudruche	0/52=0.0%	..	7/49=14.3%	..
Chick skin	1/59=1.7%	6/59=10.2%	3/96=3.1%	3/53=5.7%

Mosquito feeding response to the Baudruche membrane was also compared with that to Parafilm "M" and Saran Wrap. Before use the Parafilm was stretched to 1½ its original length and width. The stretching, however, was seen to be very uneven, so that the final thickness varied from original thickness to near-

TABLE 3.—Percentages of *Aedes aegypti* feeding on 10 percent bovine serum±alanine and lysine through the Baudruche membrane

Level of lysine	Level of alanine	
	None	0.24%
None	0%	3%
0.88%	1%	1%

TABLE 4.—Percentages of *Aedes aegypti* feeding on chick blood through two membranes. Data of trial 3 with the Baudruche membrane are statistically heterogeneous and are excluded from the data pool

Trial	Percent feeding	
	Chick skin	Baudruche membrane
1	44/44=100%	19/26=73%
2	37/39=95%	23/37=62%
3	18/19=95%	6/30=20%
Pooled data	99/102=97%	42/63=67%

TABLE 5.—Percentages of *Aedes aegypti* and *Anopheles stephensi* feeding on C.E.E.+0.005 M. ATP through three membranes.

Species	Experiment 1		Experiment 2	
	Baudruche	Parafilm "M"	Baudruche	Saran Wrap
Aedes aegypti	16%	1%	37%	0%
Anopheles stephensi	86%	67%	79%	0%

transparency. Experimental design and results are indicated in Table 5. Percent feeding is significantly higher in both comparisons for each species tested. There were 70 mosquitoes per cage. *Aedes aegypti* age ranges were 4–5 days for experiment 1 and 4–16 days for experiment 2. *Anopheles stephensi* ages were 4 days for experiment 1; mosquitoes from stock cages were used in experiment 2.

A great variety of other membranes have been used by previous workers (Tarshis, 1958). In general, the literature indicates that animal membranes are superior to those of vegetable or synthetic origin, and this principle is supported by our experiments. The physical and chemical basis for the relative effectiveness of the different membranes is, however, still unknown, and this field offers a great promise for future investigation. In this connection we may point out, qualitatively, some of the differences between the 4 membranes that we have used (Table 6).

FOODS. Dissimilar feeding responses to whole blood (Tables 1 and 4) and its non-cellular components, erythrocyte extract (Tables 2 and 5, and Fig. 3) and serum (Tables 1 and 3), have already been noted. Study of Figure 3 and Tables 1–5 indicates that blood is usually more acceptable than erythrocyte extract or serum whenever the membrane and phagostimulation are equivalent. Comparisons were also made of the feeding responses to two pairs of comparatively similar foods.

An experiment was set up to compare feeding responses of *A. aegypti* to erythrocyte extracts prepared from outdated human blood for transfusion and fresh chick blood. The experiment was done in two replicates on separate days. On each day two cages of 70 mosquitoes each were simultaneously fed, one on each extract, through the Baudruche membrane. Mosquitoes used the first day were aged 6–7 days; those used the second day were 10–14 days old. ATP (0.005 M.) was added

TABLE 6.—Qualitative judgments of the properties of four membranes.

Property	Chick skin [1]	Baudruche membrane [2]	Parafilm "M" [3]	Saran Wrap
Performance [4]	Excellent	Good	Fair	Poor
Origin	Animal	Animal	Synthetic	Synthetic
Thickness	Thick	Thin	Variable	Thin
Texture	Rough	Smooth	Smooth	Smooth
Color	Yellowish	Grayish	Grayish	Transparent
Light transmissibility	Translucent	Translucent	Translucent	Transparent
Water permeability	Slow	Immediate	Impermeable	Impermeable
Relative elasticity	Elastic	Inelastic	Elastic	Inelastic
Relative hardness	Soft	Intermediate	Soft	Hard
Salient chemical components	Protein+fat	Protein	Wax	Plastic

[1] Feathers removed by plucking.
[2] Wetted.
[3] Stretched as described in text.
[4] Based on performance of membrane in feeding trials with *A. aegypti* and C.E.E.+0.005 M. ATP.

to each extract immediately before feeding.

Percent feeding was significantly higher for human erythrocyte extract (Table 7). This response may be related to the question of anthrophilism versus ornithophilism in *Aedes aegypti*. On the other hand, it is not known how efficient the cell-washing process employed is in removing the additives, notably glucose and sodium citrate, in human blood for transfusion. It is possible that glucose, which is a normal constituent of blood, is more effective as a phagostimulant than sucrose.

The effect of replacement of the plasma of chick blood with CEE was then investigated. Ten milliliters of blood were drawn from a chick and centrifuged. The plasma was removed and the cells were resuspended in normal saline solution sufficient for 10 ml. of suspension. After a second centrifugation, the saline solution was thrown off and replaced with enough to give 20 ml. of red cell suspension. After thorough mixing the suspension was divided into 10 ml. portions and centrifuged for a third time. The supernatant was discarded, and the washed cells were diluted to the original volume of blood (5 ml.) for each portion. The diluents were, on the one hand, chick plasma, and on the other, C.E.E. ATP (0.005 M.) was added to each portion. The reconstituted and altered portions were simultaneously offered to cages of 70 *A. aegypti* (age: 5–8 days) using the Baudruche membrane. A second feeding was immediately done without replacement of food or membranes.

The results are given in Table 7. The differences in percent feeding on the two foods is not significant; however the depression in percent feeding due to crust formation, as discussed above, was confirmed.

TABLE 7.—Percentages of *A. aegypti* feeding for 15 minutes on pairs of similar foods through the Baudruche membrane.

Experiment	Food	% Feeding	
		Trial 1	Trial 2
1	Chick erythrocyte extract+0.005 M. ATP	16%	46%
	Human erythrocyte extract+0.005 M. ATP	31%	71%
2	Reconstituted chick blood+0.005 M. ATP	71%	27%
	Altered chick blood+0.005 M. ATP	74%	20%

CROWDING. The problem of exposing an adequate sample of mosquitoes to the membrane-feeder, while at the same time ensuring that the feeding conditions for each individual are identical, argues for the use of a single cage and feeding set-up for each experiment. On the other hand, it was felt that crowding—as related to volume or surface area of the cage or to the feeding surface available—might inhibit percent feeding. Accordingly, four cages, containing 24, 48, 94 and 136 (5 to 26 day-old) *A. aegypti* were fed on chick blood through the Baudruche membrane. Cages were fed, two at a time, in random order. The slope of the linear regression line (Fig. 4) obtained from the results is statistically significant, and its estimate is −0.45 percent per mosquito.

SPECIES. A comparison of feeding responses of different species requires meticulous control of other factors such as membrane quality and food constituents that also affect the feeding response. One way to reduce experimental error is to cage the species together in equal numbers for simultaneous exposure to the same feeder. The possibility exists that an interaction may exist between species caged together, *i.e.* each species may either encourage or inhibit the feeding of the other.

Nine cages of mosquitoes were pre-

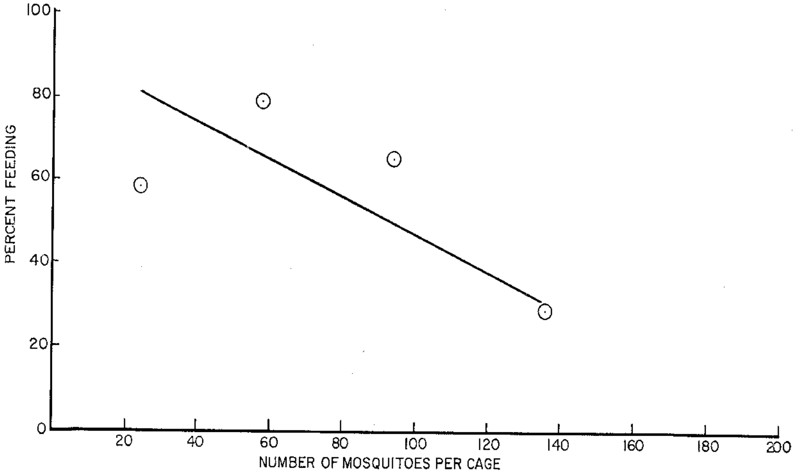

FIG. 4—Effect of crowding on artificial feeding of *A. aegypti.*

pared as follows: one cage of 80 *Aedes aegypti*; one of 80 *Armigeres subalbatus*; one of 80 *Anopheles stephensi*; two of 40 *A. aegypti* plus 40 *A. subalbatus*; two of 40 *A. aegypti* plus 40 *A. stephensi;* and two of 40 *A. subalbatus* plus 40 *A. stephensi*. *A. aegypti* were 9 to 14 days old; *A. subalbatus* and *A. stephensi* were taken from stock cages. The cages were exposed, two at a time, in random order, to the feeding apparatus for fifteen minutes. Freshly drawn chick blood and an unused Baudruche membrane were provided for each feeding. Six feedings were completed on one day, the remainder on another. After each feeding, the number of each species fed in each cage was recorded.

Thus, for each species our data were three percentages, each based on counts of 80 mosquitoes—one for the species caged alone and two for that species caged with another species (Fig. 5). The number of mosquitoes per cage was constant at 80. The data were subjected to the inverse sine transformation, and orthogonal, single degree of freedom, comparisons were made between the two feeding responses of a species when caged with another species and also between its response when caged alone versus the mean of its responses when caged with other species.

The exceptionally low response of *A. aegypti* caged alone is attributed to the fact that these mosquitoes had been etherized on the day of the experiment and had not been starved. The statistical analysis of the remaining data indicates that *A. subalbatus* feeds better when caged with another species than when caged alone, but that mixing has no effect on percent feeding of *A. stephensi*. Further, *A. aegypti* feeds better in the presence of *A. subalbatus* than in the presence of *A. stephensi,* and *A. subalbatus* feeds better in the presence of *A. aegypti* than in the presence of *A. stephensi*. The findings seem to suggest particularly aggressive, appropriative, gluttonous behavior on the part of *A. stephensi,* a picture supported by observation in our laboratory. They have been seen to pierce a taut rubber band to feed upon a monkey rather than step a millimeter or so to the side. We have also seen two individuals unsuccessfully probing an adult German cockroach (*Blatella germanica*). They are the best feeders of any used in our membrane feeding trials.

RESPONSES OF DIFFERENT SPECIES. Theoretically, only injured, diseased or defective mosquitoes—or those temporarily incapable of feeding by reason of being already fed, too young, etc.—will not feed under natural conditions. Survival of the genotype must often depend upon opportunistic behavior, and such behavior consequently has a selective advantage in nature. From this point of view it is doubtful whether "percent feeding" varies greatly from species to species in nature.

In the laboratory we are generally unable to elicit the maximum feeding response, particularly when the use of such artificial conditions as membrane-feeding is necessary for the experiment at hand. For any particular set of laboratory conditions one species will usually feed more freely than another. An approach in finding a suitable set of conditions for the membrane-feeding of a species is to compare its feeding response with that of a better-known species under the conditions that are used for the latter.

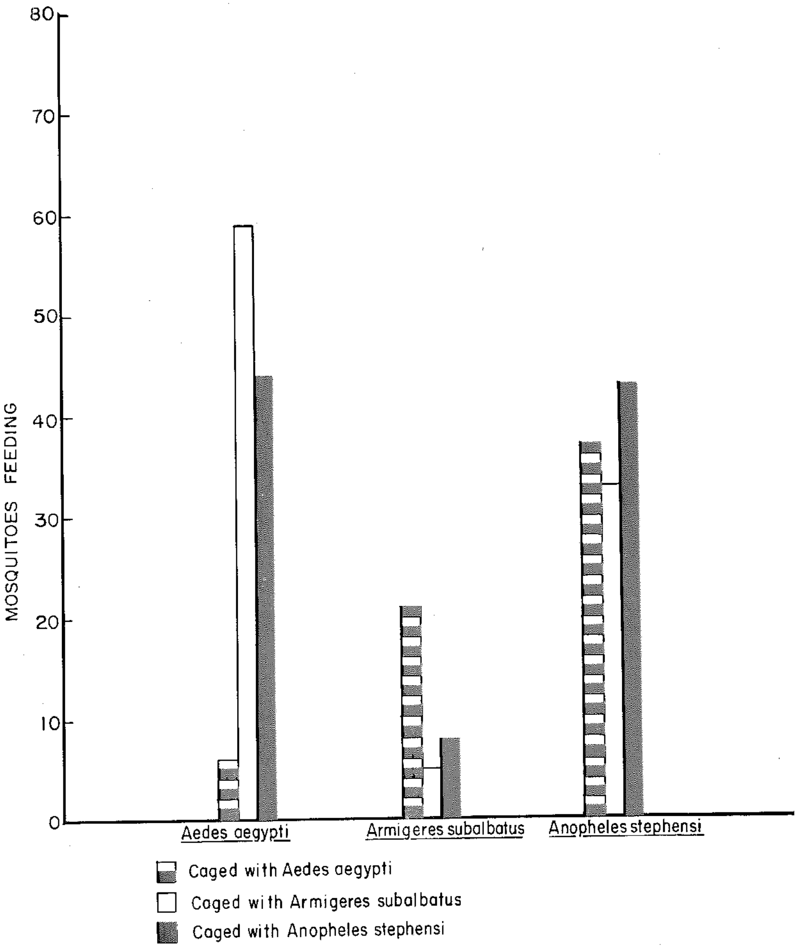

FIG. 5.—Numbers of mosquitoes (80 per cage) feeding on chick blood through Baudruche membrane.

In this way we have compared the feeding responses of five species with those of *Aedes aegypti* for the Baudruche membrane and chick blood, C.E.E. + 0.005 M. ATP and 10 percent serum + 0.005 M. ATP.

Each comparison was done in a continuous series of feedings, two cages at a time. Thus, three feeding were required for each comparison—the two species being fed simultaneously for 15 minutes on each of the three foods taken in random order. On one afternoon, however, two species were compared with *A. aegypti*; in this case nine cages were required to be fed, and a two-stage randomization was employed, *i.e.* first the feeding order for foods was randomized, then the feeding order for species was randomized for each food. The number of mosquitoes per cage was 70 except that the *A. aegypti* standard for the nine-cage comparison consisted of 35 mosquitoes per cage. Mosquito ages were 3 to 19 days for *Aedes aegypti*, 4 to 13 days for *Aedes togoi*, 12 to 13 days for *Culex pipiens pallens* and 7 days for *Culex tritaeniorhynchus*. *Anopheles stephensi* and *Armigeres subalbatus* were taken from the stock cages.

Percent feeding for each species on each food was tested by the chi square criterion with Yates' correction for continuity, the null hypothesis being that of no difference from the percentage observed for the corresponding *A. aegypti* control. Results are shown in Table 8, wherein (+) indicates percent feeding significantly higher than that of *A. aegypti*, and (o) and (—) have analogous interpretations. It is seen that the overall feeding responses of *Aedes togoi* and *Armigeres subalbatus* are roughly equivalent to those of *Aedes aegypti*. *Culex pipiens pallens* and *Culex tritaeniorhynchus* do not feed as well while *Anopheles stephensi* feeds better.

FECUNDITY AND DESTINATION OF MEAL IN ARTIFICIALLY FED MOSQUITOES. Day's (1954) hypothesis that only cellular or particulate fluids are passed to the mosquito's midgut came briefly under scrutiny during the course of our experiments. Ten *Aedes aegypti* were fed C.E.E. + 0.005 M. ATP and dissected to determine destination of the meal. All were found to have CEE in the midgut and three also in the diverticula. Similarly, of 11 *Aedes aegypti* fed 10 percent bovine serum + 0.005 M. ATP + methylene blue, all had serum in the midgut and nine also in the diverticula. These foods were clearly non-particulate, the C.E.E. having passed a Seitz filter.

Four cages of 8, 21, 40 and 64 C.E.E.-fed *A. aegypti* were provided with moist paper toweling for oviposition. After 2 to 4 weeks the eggs were collected and counted. Number of eggs per cage was 0, 493, 84 and 1759, respectively, and the

TABLE 8.—Percentages of 5 species of mosquitoes feeding on 3 foods, through the Baudruche membrane. Standard for comparison is percentage of *Aedes aegypti* feeding. See text for explanation of symbols in parentheses.

Species	Food		
	Chick blood	C.E.E.+.005 M. ATP	10% BS+.005 M. ATP
Standard	20%	69%	60%
Aedes togoi	54% (+)	54% (—)	64% (o)
Culex pipiens pallens	11% (o)	9% (—)	14% (—)
Standard	34%	50%	49%
Culex tritaeniorhynchus	17% (—)	19% (—)	29% (—)
Standard	76%	50%	57%
Armigeres subalbatus	21% (—)	57% (o)	83% (+)
Standard	39%	31%	14%
Anopheles stephensi	37% (o)	51% (+)	51% (+)

mean number of eggs per female was 17.4. Whitman (1948) considers C.E.E. to be a 20 percent concentration of the soluble fraction of the erythrocytes; this gives 17.4/.20=87 eggs per female for the 100 percent concentration. Greenberg (1951), by nearly identical methods, obtained 84.7 and 95.8 eggs per female fed chick blood and washed chick erythrocytes, respectively. It seems that the number of eggs per female is about the same for each of the three blood fractions.

In this connection also, we have obtained 146 eggs from a cage of 12 C.E.E.-fed *Armigeres subalbatus,* but none from a cage of 10 *Aedes aegypti* fed on 10 percent bovine serum.

DISCUSSION

A prime objective in developing this membrane-feeder was to achieve consistently high feeding responses for several species on experimental foods. Each species must be considered as a separate problem. Further, a remarkable number of factors influence percent feeding within any particular species.

In our experiments 20 cages of *Aedes aegypti* were fed on chick blood through the Baudruche membrane. The range in percent feeding was 6 to 82 percent. We are able to account for three of the lowest feeding proportions as caused by overcrowding, formation of a crust over the membrane, and effects of etherization. Table 9 is a frequency table for percent feeding in the remaining 17 cages.

Some two-thirds of the percentages are clustered around the expected value of 67 percent as given in Table 4, while nearly one-third are unaccountably lower. At the 95 percent confidence level not more than one of these can be expected to be due to chance.

Although number per cage, ambient temperature, and relative humidity are known to affect percent feeding, Spearman's coefficient of rank correlation reveals no significant effect due to any of these factors. The same test fails to detect any effect attributable to atmospheric pressure.

Spearman's coefficient of rank correlation for mosquito age and percent feeding is 0.45, and this value is significant by the *t* test. Significance is also shown by the quadrant sum test. For this analysis, the mid-point between extreme ages was taken as *the* age for each cage containing mosquitoes of mixed ages. The two variables are plotted, one against the other, in Figure 6. It is apparent that some, but not all, the aberrant percentages may be explained on the basis of the youthfulness of the mosquitoes used. There remain, evidently, other unknown influences on the feeding response that make predictions of percent feeding rather unreliable.

TABLE 9.—Frequency table for percent feeding in 17 cages of *Aedes aegypti* on chick blood through the Baudruche membrane.

Class value (%)	5	15	25	35	45	55	65	75	85	95
Frequency	0	0	3	2	0	3	4	3	2	0

With the membrane-feeder described herein it is possible to choose and standardize a number of variables that influence the feeding response such as food type and temperature, phagostimulants, membrane type, etc. The effects of external conditions such as crowding, species interactions, and ambient temperature, and conditions internal to the mosquito such as state of nutrition, age and species characteristics can then be investigated much more efficiently.

When the membrane feeding technique is to be used incidentally in experiments along other lines such as nutrition, repellency, toxicology, etc., the investigator must be prepared to do extensive prelimi-

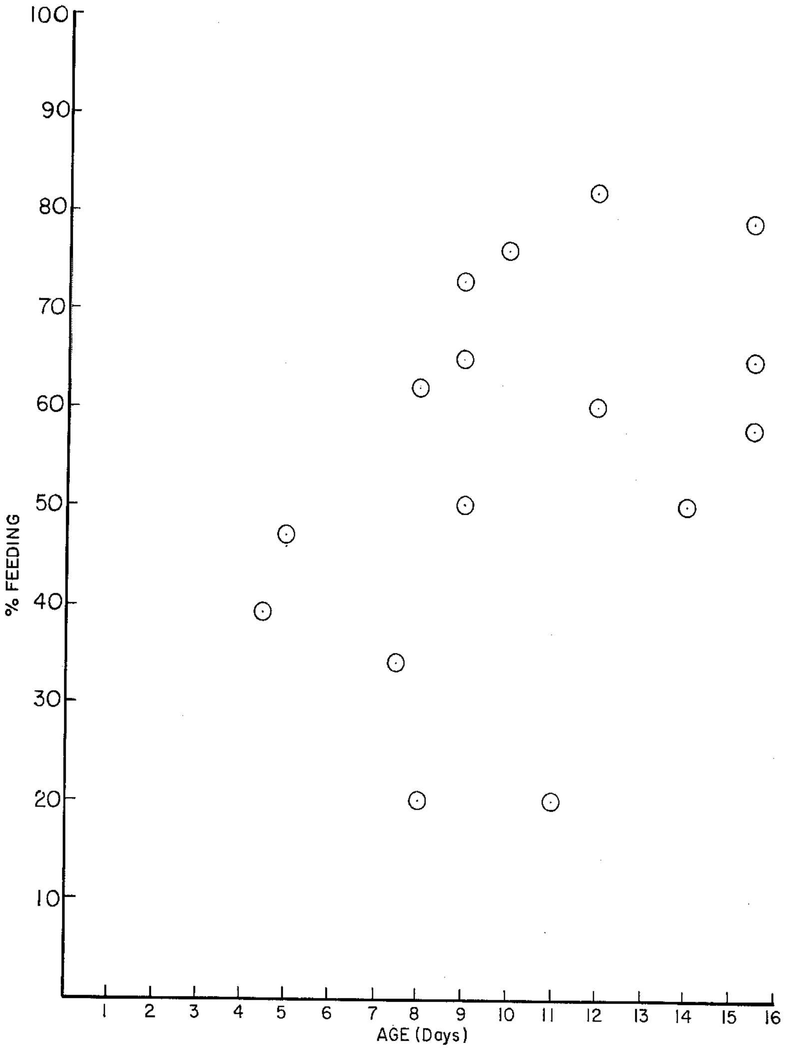

Fig. 6—Age and percent feeding of *Aedes aegypti* through Baudruche membrane on chick.

nary experimentation on the species chosen if he is to attain consistently usable results.

Summary

A new device for feeding mosquitoes is described. The feeder is made of heat-resistant glass or stainless steel to permit autoclaving. It is small and of single-piece construction for ease of handling; it can be used with any source of circulating warm water for temperature control and with any mosquito cage that has a screened horizontal top.

The usefulness of the new device is demonstrated by reports of some experiments relating to the effects of several

factors on proportion of mosquitoes feeding. Among the factors investigated were type of food, type of membrane, phagostimulants, species characteristics, species mixtures, crowding, and age. A note on egg production and destination of meal in artificially fed mosquitoes is included.

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SEVEN NEW RECORDS OF MOSQUITOES IN ALBERTA

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Forty-one species of mosquitoes, belonging to eight genera are known to occur in Alberta. These include 27 species of the genus *Aedes,* 5 of *Culiseta,* 3 of *Culex,* 2 of *Chaoborus,* and 1 of each of *Anopheles, Eucorethra, Mansonia,* and *Mochlonyx.* Summary lists of mosquitoes in Alberta were published by Strickland (10) and Twinn (11). Rempel (7, 8) includes information on the distribution of culicine larvae in Alberta and some adults. Information on the Albertan species of the subfamily Chaoborinae can be found in the work of Cook (3). Shemanchuk (9) also mentions Chaoborinae as well as species of the subfamily Culicinae. The latest published new record for Alberta is *Aedes* (*Ochlerotatus*) *melanimon* Dyar collected by Burgess (1) in a mosquito trap at Brooks.

After examining the mosquito collection in the Strickland Museum at the University of Alberta, a part of the mosquito collection at the Entomology Research Institute in Ottawa, as well as unpublished theses and reports of students who have studied mosquitoes in Alberta recently, seven new records for Alberta were found. The keys of Rempel (7, 8), Carpenter and LaCasse (2), and Cook (3)