Utilization of Human Blood and Sugar as Nutrients by Female *Aedes aegypti* (Diptera: Culicidae)

AMARA T. NAKSATHIT,[1] JOHN D. EDMAN,[2] AND THOMAS W. SCOTT[3]

Department of Entomology, University of Maryland, College Park, MD 20742

Downloaded from academic.oup.com/jme/article/36/1/13/875542 by Univ. Of Lake Alfred Crec user on 06 November 2026

J. Med. Entomol. 36(1): 13–17 (1999)

ABSTRACT We examined the utilization of sugar and human blood as nutrient sources for small and large female *Aedes aegypti* (L.) when they were fed blood 2 or 5 d after emergence. Laboratory-reared mosquitoes were fed human blood alone or sugar plus human blood and assayed at 4, 12, 24, and 48 h after the blood meal. Starved and well-fed mosquitoes were obtained by holding teneral females (≤1 d old) with 0, 5, 10, and 15% sucrose solutions ad libidum from emergence. Both small and large mosquitoes increased their glycogen and sugar levels significantly by feeding on blood only or on blood plus sugar when they imbibed a human blood meal on day 2 after emergence. Mosquitoes only fed blood on day 2 had the highest lipid levels of any treatment group. Both size classes and all feeding regimes failed to increase the total amount of glycogen, lipid, or sugar when they fed on blood 5 d after emergence. We conclude that there is an energetic advantage to *Ae. aegypti* when they feed on blood early in adult life (≤day 2 after emergence).

KEY WORDS *Aedes aegypti*, blood feeding, sugar feeding, energy reserves, size

BLOOD IS ESSENTIAL for egg production by anautogenous female mosquitoes. Most mosquito species feed on mammals other than humans or birds to satisfy their protein–reproductive requirement (Clements 1992). However, *Aedes aegypti* (L.) prefer to feed on human blood (Scott et al. 1993a), even though it produces fewer eggs than feeding on similar quantities of the blood of other animal hosts (Woke 1937). Although nutrients from ingested blood are used principally for egg development, there is evidence that they also are used as an energy source (Nayar and Sauerman 1975, Edman et al. 1992, Hancock and Foster 1993, Van Handel et al. 1994, Scott et al. 1997, Costero et al. 1998a, Naksathit and Scott 1998b).

Ingestion of carbohydrates versus starvation can modify significantly the effects of a blood meal on mosquito reproduction and maintenance reserves (Klowden et al. 1988, Clements 1992). Because sugar-fed mosquitoes already have reached a plateau of maximum glycogen accumulation, energy obtained from blood meals should be used to increase lipid reserves unless it can be used for vitellogenesis (Van Handel 1965, Clements 1992). Nayar and Sauerman (1975) found that starved *Ae. aegypti* accumulate triglycerols for 1–2 d after a blood meal. This accumulation begins to decline within 2–3 d and stabilizes by 5 d after feeding when all detectable triglycerols are associated with eggs. At that time, if the mosquito does not feed soon, it will die of starvation. Female mosquitoes can replenish their energy reserves by feeding on blood, but the process is metabolically less efficient than if they imbibe sugar (Van Handel 1965, Clements 1992, Foster 1995).

Our objective in the current study was to determine how large and small female *Ae. aegypti* that are fed sugar or blood as adults utilize nutrients in those foods during a single gonotrophic cycle. To examine the effect of diet, mosquitoes were offered human blood at 1 of 2 times, 2 or 5 d after emergence. An improved understanding of accumulation of energy reserves after feeding on blood and sugar may help explain why this species appears to forego sugar feeding (Edman et al. 1992, Van Handel et al. 1994, Costero et al. 1998b) to preferentially (Scott et al. 1993a) and frequently feed on human blood (Scott et al. 1993b).

This is the 2nd of 3 publications on the effect of diet on glycogen, lipid, and sugar levels in *Ae. aegypti.* The 1st paper concerns utilization of sugar alone (Naksathit et al. 1998a) and the 3rd examines partitioning of nutrients from a human blood meal between body remnants (reserves that can be used for maintenance activities) and ovaries (nutrients directed toward reproduction and that are presumably not available as maternal reserves) (Naksathit et al. 1998b).

Materials and Methods

Two 2 × 4 factorial experiments were carried out in randomized complete block designs with repeated measures (SAS Institute 1989). Blocks were the 3 repetitions of each experiment. The 2 factors were mosquito size (2 levels: small [low energy reserves at

[1] Current address: Department of Biology, Mahidol University, Bangkok, Thailand.

[2] Department of Entomology, University of Massachusetts, Amherst, MA 01002.

[3] Current address: Department of Entomology, University of California, Davis, CA 95616.

0022-2585/99/0013–0017$02.00/0 © 1999 Entomological Society of America

Table 1. *F* values from the ANOVA for the effect of size, sucrose concentration, and time on the amount of glycogen, lipid, and sugar per female *Ae. aegypti* fed human blood on day 2 after emergence

Source of variation	df	*F* values		
		Glycogen	Lipid	Sugar
Replicate	2	1.21	0.06	2.75
Size	1	37.74**	57.55**	0.01
Sucrose concentration	3	38.83**	3.37*	16.94**
Size × sucrose	3	5.80**	4.69**	1.08
Time	3	7.38**	0.20	4.71**
Size × time	3	1.59	0.08	0.96
Sucrose × time	9	1.58	0.87	1.75
Size × sucrose × time	9	0.65	1.07	1.13

*, $P \leq 0.05$; **, $P \leq 0.01$.

Table 2. Means and LSD values for the amounts of glycogen, lipid, and sugar for small and large female *Ae. aegypti* fed ad libidum different concentrations of sucrose and a blood meal 2 d after emergence

Size + % sucrose[a]	Glycogen	Lipid	Sugar
Small + 0	31	46	26
Small + 5	64	67	34
Small + 10	57	65	33
Small + 15	57	64	90
Large + 0	32	444	3
Large + 5	91	217	37
Large + 10	75	244	49
Large + 15	98	198	96
LSD	11	95	22

Values are milligrams per female.
[a] $n = 60$ for each size category + sucrose concentration.

emergence] and large [high energy reserves at emergence]) (Briegel 1990), and sugar feeding (4 sucrose concentrations: 0, 5, 10, and 15%) measured 4 times (4, 12, 24, and 48 h after blood feeding). In experiment 1, mosquitoes were allowed to feed on human blood on day 2 after emergence, whereas in experiment 2, the same treatments were used except mosquitoes were not allowed to feed on human blood until day 5 after emergence. The *Ae. aegypti* colony, methods for rearing small and large mosquitoes, and conditions under which adult mosquitoes were held were described in the 1st article of this series by Naksathit et al. (1998a).

Groups of 50 teneral female mosquitoes (≤1 d old) in the 2 different size classes were introduced into a cage (30 by 30 by 30 cm) supplied with water or one of the above sugar solutions, and allowed to feed ad libidum for the duration of the experiment. Each cage was treated as an experimental unit. Blood meals on day 2 or 5 were obtained from the arm of a human volunteer (Institutional Review Board protocol number A-3433-01 for use of human subjects). Mosquitoes that did not imbibe blood were removed from the cage and excluded from analysis.

Five representative adult females from each cage were collected at the 4 time intervals (see above) and held in a freezer (−4°C) overnight. The next morning, a wing was removed for size determination, and specimens were dried in a 100°C oven for 1 h, after which they were held at room temperature (≈26°C) until they were analyzed individually for glycogen, lipid, and sugar as described by Naksathit et al. (1998a).

Results from both experiments were examined by multivariate analysis as randomized complete block design with split plot in time using general linear model procedures (SAS Institute 1989). Sources of variation are presented in Table 1. Means within factors found to vary significantly ($P \leq 0.05$) were separated using a least significant difference (LSD, Sokal and Rohlf 1981) procedure ($P = 0.05$).

Results

Mosquito Size. Small (i.e., nutritionally deprived as larvae) mosquitoes had an average wing length of 2.47 ± 0.01 (mean ± SE) mm, which was significantly less ($F = 771.26$, df = 1, $P < 0.0001$) than that of large, well-fed mosquitoes, which had a wing length of 2.92 ± 0.01 mm.

Experiment 1: Mosquitoes Fed Blood on Day 2 After Emergence. *Glycogen.* The effects of the 2 treatments (size and sucrose) on the amount of total glycogen in each female were significant (Table 1). There was no significant difference between total glycogen in small and large mosquitoes fed only on human blood (0% sucrose group), but all large mosquitoes fed blood plus sugar had a significantly more glycogen than small mosquitoes fed blood plus sugar for all sucrose concentrations (Table 2). Glycogen levels increased significantly 48 h after feeding on blood only (Table 3). Overall, large mosquitoes contained more glycogen per female than small mosquitoes (Table 4). Mosquitoes fed sugar contained more glycogen than those fed only blood (Table 2). However, total glycogen in mosquitoes fed only blood increased over time, indicating that part of the digested blood meal was converted to glycogen (Fig. 1).

Lipid. The effects of size, sucrose diet, and size × sucrose interaction were significant for lipids (Table 1). Small mosquitoes had significantly less lipids per female than large mosquitoes (Table 4). Overall, mos-

Table 3. Means and LSD values for the amount of glycogen, lipid, and sugar in female *Ae. aegypti* fed only human blood on either day 2 or day 5 after emergence and collected for analysis at different times after blood feeding

Time, h[a]	Glycogen	Lipid	Sugar, μg/♀
	Blood-fed on day 2		
4	62.4	154.3	46.3
12	56.5	167.2	35.8
24	61.5	176.7	44.1
48	71.9	174.3	58.0
LSD	7.5	66.8	15.2
	Blood-fed on day 5		
4	55.7	139.0	59.3
12	51.3	121.1	55.0
24	51.6	151.5	61.6
48	54.6	143.4	72.1
LSD	11.1	69.1	17.3

Values are milligrams per female.
[a] $n = 120$ for each collection time.

Downloaded from academic.oup.com/jme/article/36/1/13/875542 by Univ. Of Lake Alfred Crec user on 06 November 2026

Table 4. Means and LSD values for the amount of glycogen, lipid, and sugar in small and large female *Ae. aegypti* fed a blood meal on either day 2 or day 5 after emergence

Size[a]	Glycogen	Lipid	Sugar, μg/♀
	Blood-fed on day 2		
Small	52.2	61	46.3
Large	73.9	276	45.9
LSD	5.3	47	10.8
	Blood-fed on day 5		
Small	48.2	136.4	46
Large	58.4	141.2	78
LSD	3.9	1.3	10

Values are milligrams per female.
[a] $n = 240$ for each size category.

quitoes fed only blood had more total lipids than those in any of the groups fed sucrose (Fig. 1). Lipids in small mosquitoes did not change according to the sucrose treatment (Table 2). However, large mosquitoes fed only blood had significantly more lipids than mosquitoes fed blood plus sucrose solutions. Lipids did not change significantly during the time periods following blood feeding (Table 3; Fig. 1).

Sugar. The only significant effects on sugar in mosquitoes were sucrose concentration and time (Table 1). Mosquitoes fed human blood alone had significantly lower total sugars than mosquitoes fed blood plus sugar (Fig. 1). Mosquitoes fed human blood plus 5% and 10% sucrose solutions had higher total sugars than mosquitoes fed blood alone, but there was no significant difference in the amount of sugar between these 2 treatments. The most sugar was observed in mosquitoes fed the 15% sucrose solution (93 μg per mosquito); a significantly higher amount than mosquitoes in any other treatment. The average amount of total sugar per female did not change significantly up to the 24-h collection period and increased significantly thereafter (Table 3; Fig. 1).

Experiment 2: Mosquitoes Fed Human Blood on Day 5 After Emergence. *Glycogen.* The only effect of sucrose that was significant on glycogen occurred when mosquitoes fed on blood 5 d after emergence (Table 5). Glycogen did not significantly change during the 48-h mosquito collection period (Table 3). There was no effect of size on the amount of glycogen (Table 4). The least amount of glycogen was observed in mosquitoes fed human blood alone (30 μg per mosquito), whereas the highest amount was in mosquitoes fed on a human blood meal plus 5% sucrose (Table 6).

Lipid. There was no effect of size or sucrose on the amount of lipid per female fed blood on day 5 (Tables 4–6). Although the effect of sucrose was not significant, there was a substantial difference in the average amount of lipid among mosquitoes fed blood plus different sucrose solutions (Table 6). The lowest amount of lipid was observed in mosquitoes fed on blood alone and the highest was in mosquitoes offered the 10% sucrose solution. Amounts of lipid per female did not change during the 48 h following feeding on blood (Table 3).

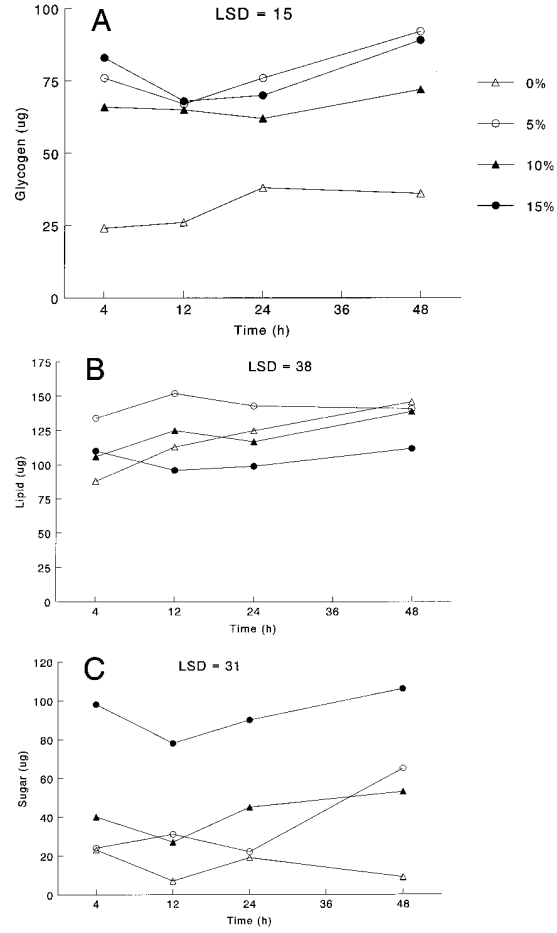


Fig. 1. Mean amounts of (A) glycogen, (B) lipid, and (C) sugar (μg/female) for female *Ae. aegypti* fed ad libitum different percentages of sucrose concentrations and human blood on day 2 after emergence, and collected for analysis at different times after blood feeding ($n = 30$ for each sucrose concentration + collection time).

Sugar. Both the effects of size and sucrose treatment were significant for amounts of sugar per female, although the effect of time was not (Table 6). Large mosquitoes contained more total sugars than small ones (Table 4). The lowest amount of sugar was in mosquitoes that were fed only human blood (Table 6). Mosquitoes fed human blood plus a 10% and 15% sucrose solution had higher amounts of sugar than mosquitoes fed blood plus a 5% sucrose solution.

Discussion

Energy reserves accumulated in female *Ae. aegypti* vary according to the type of food they imbibe, their nutrient reserves at emergence (indicated by body size), their age when they feed on blood, and the time they are examined after feeding on blood (Van Handel 1965, Briegel 1990, Foster 1995). Our results indicated that it was advantageous, from the perspective of ac-

Downloaded from academic.oup.com/jme/article/36/1/13/875542 by Univ. Of Lake Alfred Crec user on 06 November 2026

Table 5. *F* values from the ANOVA for the effect of size, sucrose concentration, and time on the amount of glycogen, lipid, and sugar per female *Ae. aegypti* fed human blood on day 5 after emergence

Source of variation	df	*F* values		
		Glycogen	Lipid	Sugar
Replicate	2	27.62	15.57	0.54
Size	1	2.45	0.01	14.25*
Sucrose	3	9.65*	2.66	17.69*
Size × sucrose	3	0.71	0.62	0.70
Time	3	1.32	0.87	1.76
Size × time	9	2.90	1.15	0.82
Size × sucrose × time	9	1.39	1.01	0.23

*, $P \leq 0.01$.

cumulating energy reserves, for *Ae. aegypti* to feed on human blood early in adult life; that is, during the 2nd or perhaps 3rd d after emergence.

When fed blood on day 2 after emergence, large mosquitoes accumulated significantly more glycogen and lipid than did small mosquitoes. This result is consistent with the outcome from our study of mosquito fed only sugar (Naksathit et al. 1998a). The pattern of utilization of a sugar meal was dependent on teneral reserves which are correlated positively with size (Briegel 1990). There was no size effect in the current study on total sugars. Conversely, when fed blood 5 d after emergence, size did not effect the amount of glycogen or lipid, but was associated with more sugar in large than in small mosquitoes. These data indicated that if mosquitoes with relatively high energy reserves imbibed blood early in life (when ≤2 d old), they had an energetic advantage. If blood feeding was delayed (≈5 d old), energy reserves accumulated during larval development did not effect the synthesis of new energy reserves. It should be noted that differences associated with large and small mosquitoes may be caused by differences in body mass rather than inherent differences in the ways that large and small mosquitoes manage their energy reserves.

In all but 1 case, access to sugar resulted in greater accumulation of glycogen, lipid, and sugar regardless of mosquito size, sugar concentration, or when mosquitoes fed on blood. When mosquitoes fed only on blood early in life, regardless of their size, they accumulated significantly more lipid than cohorts that fed on blood plus sugar. Similarly, in a study of mosquitoes fed chicken blood, Nayar and Sauerman (1975) reported an increase in lipids within 24–72 h. In our study on partitioning of compounds between ovaries and body remnants, *Ae. aegypti* fed only human blood deposited similar portions of lipid reserves in ovaries regardless of size (≈55% at 72 h) (Naksathit et al. 1998b).

Table 6. Means and LSD values for the amount of glycogen, lipid, and sugar in female *Ae. aegypti* fed ad libidum different concentrations of sucrose and imbibed blood on day 5 after emergence

% sucrose concentration[a]	Glycogen	Lipid	Sugar
0	30	73	12
5	79	97	64
10	54	219	81
15	50	166	92
LSD	11	69	171

Values are milligrams per female.
[a] $n = 120$ for each sucrose concentration.

Increases over time were detected only for glycogen and sugar when mosquitoes fed on blood 2 d after emergence. For all other treatments and kinds of compounds, no differences were detected over the time periods we studied. Given that mosquitoes not fed sugar had no source of nutrition, we expected at least the 0% sucrose treatment to cause a significant change in mosquito nutrient levels. The lack of a detectable change could be caused by starved mosquitoes using all available nonreproductive reserves acquired from blood for their survival and maintenance, leaving no excess for accumulation.

Energetic differences associated with feeding on blood early in life are consistent with the notion that human blood meals are used to fulfill energy as well as egg production requirements (Briegel 1985). Except in the case of lipids in mosquitoes that fed only on blood early in life, mosquitoes fed only blood accumulated relatively lower amounts of glycogen, lipid, and sugars than those fed blood plus sugar. An extension of this result is that when feeding only on blood, *Ae. aegypti* will need to feed early in adult life and/or more than once during each gonotrophic cycle to avoid starvation (Scott et al. 1993a, 1997; Costero et al. 1998a, b; Naksathit and Scott 1998; Naksathit et al. 1998b). Nayar and Sauerman (1975) pointed out that at ≈72 h after feeding on blood, energy reserves begin to decline in *Ae. aegypti*, and between 72 and 120 h, energy reserves are exhausted. Unless they feed on sugar or blood to replenish the reserves, female *Ae. aegypti* that fed only on blood die of starvation after 72–120 h. This indicates that imbibing multiple blood meals during a single gonotrophic cycle (Scott et al. 1993b) may overcome potential nutritional disadvantages when blood is the sole source of adult female nutrition.

About 90% of the dry weight of a blood meal is protein, and human blood contains a small amount of carbohydrate, mostly in plasma (Lehane 1991). Nayar and Sauerman (1975) suggested that when a blood meal is imbibed, the serum separates from the coagulated blood and is absorbed quickly by mosquitoes. Relatively more sugar at 4 h in mosquitoes fed no sucrose may be because of the sugar obtained from the blood meal. Digestion of the coagulated blood is thought to start ≈4 h after blood engorgement. The increase in sugar among mosquitoes fed only blood is consistent with the notion that part of the digested blood meal is converted to energy reserves (Briegel 1985).

The increase in glycogen after human blood feeding agreed with results of Nayar and Sauerman (1975), who used chicken blood. Absence of a significant time × sucrose interaction indicated that the increase in glycogen was not affected by sucrose feeding, although mosquitoes fed sucrose had more glycogen than those fed only blood. This observation indicated

Downloaded from academic.oup.com/jme/article/36/1/13/875542 by Univ. Of Lake Alfred Crec user on 06 November 2026

that a portion of the blood meal was converted to glycogen regardless of the level of energy reserves of mosquitoes at emergence. However, in experiment 2 when sugar-fed mosquitoes had access to sucrose ad libidum for 5 d, amounts of glycogen per female did not change from 4 to 48 h after blood feeding. As explained by Van Handel (1984), glycogen in sugar-fed mosquitoes reaches a maximum, and thereafter the accumulation of lipids increases and amounts of glycogen stay constant or decreases, if mosquitoes do not feed again. The observation that glycogen did not change in mosquitoes that had access to ad libidum sugar for 5 d may be because they had already accumulated the maximum glycogen levels from sugar meals.

Although there was a trend of increasing amounts of lipid with time, lipid per female did not change significantly during the course of our 2 experiments. This is contrary to results reported by Nayar and Sauerman (1975), Van Handel (1984), and Hancock and Foster (1993), who reported an increase in lipids after blood feeding. However, in their studies, mosquitoes were examined at 24-h intervals and for longer periods of time than 48 h after the blood meal. The lack of a statistically significant change in lipids in our study may be caused by the slow synthesis of lipid reserves from a human blood meal, significant change occurred after 48 h, and/or high variance in amounts of lipid among individual mosquitoes.

Acknowledgments

We thank Geoff Attardo and Leslie H. Lorenz for their expert technical assistance. This research was supported by Grant No. AI22119 from the National Institutes of Health.

References Cited

Briegel, H. 1985. Mosquito reproduction: incomplete utilization of the blood meal protein for oogenesis. J. Insect Physiol. 31: 15–21.

1990. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti.* J. Insect. Physiol. 36: 165–172.

Clements, A. N. 1992. The biology of mosquitoes, vol. 1. Development, nutrition and reproduction. Chapman & Hall, London.

Costero, A., J. D. Edman, G. G. Clark, and T. W. Scott. 1998a. A life table study of *Aedes aegypti* (Diptera: Culicidae) in Puerto Rico fed only human blood versus blood plus sugar. J. Med. Entomol. 35: 809–813.

Costero, A., G. M. Attardo, T. W. Scott, and J. D. Edman. 1998b. An experimental study of the detection of fructose in *Aedes aegypti* (Diptera: Culicidae). J. Amer. Mosq. Control Assoc. 14: 234–242.

Edman, J. D., D. Strickman, P. Kittayapong, and T. W. Scott. 1992. Female *Aedes aegypti* (Diptera: Culicidae) in Thailand rarely feed on sugar. J. Med. Entomol. 29: 1035–1038.

Foster, A. W. 1995. Mosquito sugar feeding and reproductive energetics. Annu. Rev. Entomol. 40: 443–474.

Hancock, R. G., and W. A. Foster. 1993. Effect of prebloodmeal sugar on sugar seeking and upwind flight by gravid and parous *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 30: 353–359.

Klowden, M. J. , J. L. Blackmer, and G. M. Chambers. 1988. Effects of larval nutrition on the host-seeking behavior of adult *Aedes aegypti* mosquitoes. J. Am. Mosq. Control Assoc. 4: 73–75.

Lehane, M. J. 1991. Biology of blood-sucking insects. Harper Collins, London.

Naksathit, A. T., and T. W. Scott. 1998. Effect of female size on fecundity and survivorship of *Aedes aegypti* fed only human blood versus human blood plus sugar. J. Am. Mosq. Control Assoc. 14: 148–152.

Naksathit, A. T., J. D. Edman, and T. W. Scott. 1998a. Amounts of glycogen, lipid, and sugar in female *Aedes aegypti* (Diptera: Culicidae) fed sucrose. J. Med. Entomol. 36: 8–12.

1998b. Partitioning of glycogen, lipid, and sugar in ovaries and body remnants of female *Aedes aegypti* (Diptera: Culicidae) fed human blood. J. Med. Entomol. 36: 18–22.

Nayar, J. K., and D. M. Sauerman. 1975. The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 2. Utilization of blood meal for survival. J. Med. Entomol. 12: 99–103.

SAS Institute. 1989. SAS/STAT user's guide for personal computers. SAS Institute, Cary, NC.

Scott, T. W., E. Chow, D. Strickman, P. Kittayapong, R. A. Wirtz, L. H. Lorenz, and J. D. Edman. 1993a. Blood-feeding pattern of *Aedes aegypti* (Diptera: Culicidae) collect in a rural Thai village. J. Med. Entomol. 30: 922–927.

Scott, T. W., G. G. Clark, L. H. Lorenz, P. H. Amerasinghe, P. Reiter, and J. D. Edman. 1993b. Detection of multiple blood feeding in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using a histologic technique. J. Med. Entomol. 30: 94–99.

Scott, T. W., A. Naksathit, J. F. Day, P. Kittayapong, and J. D. Edman. 1997. A fitness advantage for *Aedes aegypti* and the viruses it transmits when females fed only on human blood. J. Am. Soc. Trop. Med. Hyg. 57: 235–239.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. Freeman, San Francisco.

Van Handel, E. 1965. The obese mosquito. J. Physiol. 181: 478–486.

1984. Metabolism of nutrients in the adult mosquito. Mosq. News 44: 573–579.

Van Handel, E., J. D. Edman, J. F. Day, T. W. Scott, G. G. Clark, P. Reiter, and H. C. Lynn. 1994. Plant-sugar, glycogen, and lipid assay of *Ae. aegypti* collected in urban Puerto Rico and rural Florida. J. Am. Mosq. Control Assoc. 10: 149–153.

Woke, P. A. 1937. Comparative effects of the blood of different species of vertebrates on egg- production of *Aedes aegypti* Linn. Am. J. Trop. Med. 17: 729–745.

Received for publication 23 June 1997; accepted 14 May 1998.

Downloaded from academic.oup.com/jme/article/36/1/13/875542 by Univ. Of Lake Alfred Crec user on 06 November 2026