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Fecundity, Metabolism, and Body Size in *Anopheles* (Diptera: Culicidae), Vectors of Malaria

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ABSTRACT In four *Anopheles* species, *An. albimanus, An. gambiae, An. stephensi,* and *An. quadrimaculatus* Say, total protein, lipid, and carbohydrate present at eclosion, after feeding on sucrose, and after extreme starvation were quantified to study the effect of teneral and maximal reserves on subsequent fecundity and to judge the extent of reserve mobilization and the minimal irreducible amounts required for survival. All parameters were regressed on body size, presented as the cubic value of wing length. Teneral reserves were isometric with body size, were considerably lower than previously reported for *Aedes aegypti* (L.) and were sexually dimorphic with respect to reserves and body size, all being slightly reduced in males. On the average, up to 70% of the teneral female lipids and up to 50% of their teneral protein could be mobilized during nutritive stress. Conversely, access to sucrose for a few days led to a pronounced glycogenesis (up to 509%) and lipogenesis (up to 450% of the teneral values), depending on the species. In absolute terms, lipogenesis prevailed over glycogenesis.

On a caloric basis, up to 30% of the blood meal protein was utilized for synthesis of yolk protein and lipid, and another 15% was deposited as an extra-ovarian, maternal protein and lipid store, perhaps compensating for the limited teneral reserves. A complete nitrogen budget revealed that in a given class of body size, roughly 80% of the blood meal protein was catabolized and excreted through the three major pathways of uricotely, ureotely, and ammonotely. Quantification of the hematin in fecal samples allowed a stoichiometrical determination of the amount of blood ingested. Eco-physiological aspects of larval feeding, teneral reserves, blood meal utilization, and possible behavioral adaptations to these physiological constraints are discussed and compared with previous data on culicine mosquitoes, stressing the invalidity of generalizations among these taxa.

KEY WORDS Insecta, *Anopheles* spp., reproduction, fecundity

BEGINNING WITH the pioneering work of Ross (1897), *Anopheles* mosquitoes have been known as the primary vectors of *Plasmodia* throughout the tropical and temperate regions of the world. However, most laboratory work centered around *Aedes aegypti* (L.) after that species was recognized as a vector of yellow fever in the Caribbean (Finlay 1886, Reed et al. 1900). It has become a very successful laboratory animal for the study of vector physiology, perhaps as a result of the ease with which colonies can be maintained. Despite their tremendous importance as vectors, mosquitoes in the genus *Anopheles* have been neglected in laboratory studies, and comparatively little is known about their basic reproductive physiology. Many assumptions have been made on the basis of occasional observations or have been extrapolated from work with *Aedes* or *Culex*.

An. stephensi Liston, among others, is known to extrude considerable amounts of red rectal fluid while ingesting blood. This is not the diuresis described by Boorman (1960) or Nijhout & Carrow (1978), but instead represents an efficient mechanism of concentrating the blood protein to allow the female to acquire a large amount of protein in a midgut anatomically limited to small blood volumes (Briegel & Rezzonico 1985, Briegel 1990). As a consequence of this limited distensability of the *Anopheles* midgut, multiple blood meals within a single gonotrophic cycle appear to be possible (Briegel & Rezzonico 1985).

Based on our previous work with anophelines (Briegel & Rezzonico 1985) and stimulated by questions posed by Reid (1982), a quantitative and comparative analysis of the reproductive physiology of four *Anopheles* species was initiated. In this report, data is presented on the relationship of female body size to teneral reserves, to the synthesis of reserves by females feeding on carbohydrates, and to their reproductive physiology following a blood meal.

Materials and Methods

Four species of *Anopheles* were used in this study. *An. (Nyssorhynchus) albimanus* Wiedemann, strain "San Diego del Norte," was obtained from W. E. Collins (CDC, Atlanta, Ga.); *An. (Anopheles) quadrimaculatus* Say, a strain originating from Florida, was acquired from the late A. B. Weath-

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ersby (Athens, Ga.); *An. (Cellia) stephensi* Liston originated in India and was obtained through R. W. Gwadz (NIH, Bethesda, Md.); and *An. (Cellia) gambiae* Giles, strain 16c55, was obtained from J. D. Gillett (London School of Hygiene and Tropical Medicine). The taxonomic status of *An. gambiae* s. str. was kindly determined by Dr. W. Maier (Bonn, Germany).

All larvae were reared at 27°C and long-day (14: 10 [L:D]) photoperiod. Three hundred larvae of *An. albimanus* or *An. stephensi* were reared in pans with 450 ml of distilled water, 300 *An. quadrimaculatus* larvae were maintained in 200 ml of distilled water, and 500 *An. gambiae* were kept in 150 ml of distilled water. These densities provided minimal mortality and synchronous eclosion. Larvae were fed 34–68 mg/d of a high-protein diet (Briegel 1990) until pupation occurred after 7–10 d. Imagoes were maintained in large (30 by 40 by 60 cm) cages at 27°C and 85% RH with access to sucrose ad lib. under long-day conditions that included a 20-min artificial sunrise and sunset. Stock colonies were fed weekly on 2 consecutive d for 20 min on a restrained but unanaesthetized guinea pig. For experimentation, all females were fed on a nonallergic human subject.

The wing length of individual mosquitoes was cubed to estimate body size and is a dimensionless number; actual wing lengths (in millimeters) were included below the abscissa. To examine a wide range of body sizes, the larval food supply was altered by counting either half or double the standard number of larvae per rearing pan and feeding the same amounts of food in the same volume of water as the standard conditions. In contrast to *Ae. aegypti* (Briegel 1990), the reduction of body size was more limited in the anophelines and was accompanied by considerable larval and pupal mortality.

Experimental females were collected on the day of eclosion. A major disadvantage of working with anophelines is that the quantitative enema technique (Briegel & Lea 1975) cannot be applied because these mosquitoes concentrate blood as they feed (Briegel & Rezzonico 1985). Therefore, we allowed mosquitoes to acquire their blood meals the second day after eclosion by "free-feeding" on a human host until they withdrew their mouthparts. Immediately afterward, the mosquitoes were individually placed into test tubes (10 by 75 mm) to collect the feces released during digestion. Fecundity was determined by dissecting females and counting mature oocytes or transferring them at 50–60 h after the blood meal into individual small round cages (25 mm diameter, 30 mm high) with screened tops and moist pieces of cellulocotton at the bottom for overnight oviposition. With respect to total caloric reserves and protein, mature oocytes and newly oviposited eggs (before embryogenesis had progressed) were considered identical. Under the circumstances of our experiments, most females had emptied their ovaries during overnight oviposition, which was routinely tested by dissections. Incomplete ovipositions were discarded.

The biochemical analyses were identical to the ones reported for *Ae. aegypti* (Briegel 1990): protein data were obtained from total nitrogen through Kjeldahl digestion and subsequent Nesslerization (Minari & Zilversmit 1963); total lipids were determined according to Van Handel (1985b); and total carbohydrates of whole mosquitoes were measured by applying the hot anthrone reaction (Van Handel 1985a), which did not distinguish between glycogen and oligosaccharides. This was considered irrelevant for most experiments reported herein because the carbohydrates consistently contributed only a minor fraction to the total caloric content per female mosquito. Only in sucrose-fed females was the sugar fraction first extracted with methanol and sodium sulfate and measured separately by anthrone (Van Handel 1985a) to eliminate the crop content from the analyses. All data were converted to calories to allow direct energetic comparisons to be made.

Fecal material was eluted in 1% lithium carbonate in which urate and hematin could be analyzed in a combined procedure (Briegel 1980). The amount of blood ingested was quantified retrospectively by measuring the hematin output of individual females based on the stoichiometric relationship between hemoglobin ingested and fecal hematin (Briegel 1986). By using this method, the loss of hemoglobin through blood meal concentration during feeding was not considered, because hematin was obtained only through digestion of the hemoglobin withheld by the midgut. The hemoglobin titer of the blood donor was monitored by using Drabkin's solution (Briegel et al. 1979); his total blood protein had to be recorded simultaneously (Kjeldahl procedure) to convert the hemoglobin readings to total protein input for each blood meal. The technical details for establishing total nitrogen budgets were described previously (Briegel 1986).

Extraovarian nitrogen and lipid deposits were revealed by deducting ovarian values from the total female contents; analyses of carcasses would have falsified such determinations through leakage of hemolymph or inevitable losses of fat body tissue.

Statistical analyses for significance were based on the t test for comparisons of means and on analysis of covariance (F test) for comparison of regression lines (Cavalli-Sforza 1972). Abbreviations used were P for total protein, L for total lipids, C for total carbohydrates, and WL^3 for body size.

Results

Teneral Reserves, Body Size, and Minimal Irreducible Amounts. Imagoes with a wide range of body size resulted when larval densities were varied. Newly eclosed females of all four *Anopheles* species were analyzed for their total amounts of protein, lipid, and carbohydrates after recording

their individual wing lengths. For female *An. gambiae*, all data (Fig. 1; left side, teneral) showed significant linear regressions with body size. For the three other *Anopheles* species, results were comparable to those of *An. gambiae;* only the linear regressions are presented in Fig. 2. Statistical comparison of these regression lines revealed significant, species-specific differences in body size and caloric content at eclosion (Table 1); in all but three cases, the regression coefficients were significant, thus the slopes differed significantly. Protein contributed 30–73% of the caloric content of newly eclosed females, 13–43% was lipid, and 7–26% were carbohydrates, depending on the species and the size of the female. Thus, total carbohydrates contributed only a minor fraction of the reserves available at eclosion for survival. The variable proportions are explained by the different slopes in the female regressions for caloric protein, lipid, and carbohydrates (Fig. 1 and 2). In *An. gambiae*, the caloric content of the smallest female was 30% protein and 43% lipid, whereas in the largest females, the content changed to 72% protein and 21% lipid. This change in caloric distribution was accompanied by a nearly 10-fold increase of total calories from about 0.2 cal per small female to 1.95 cal per large female.

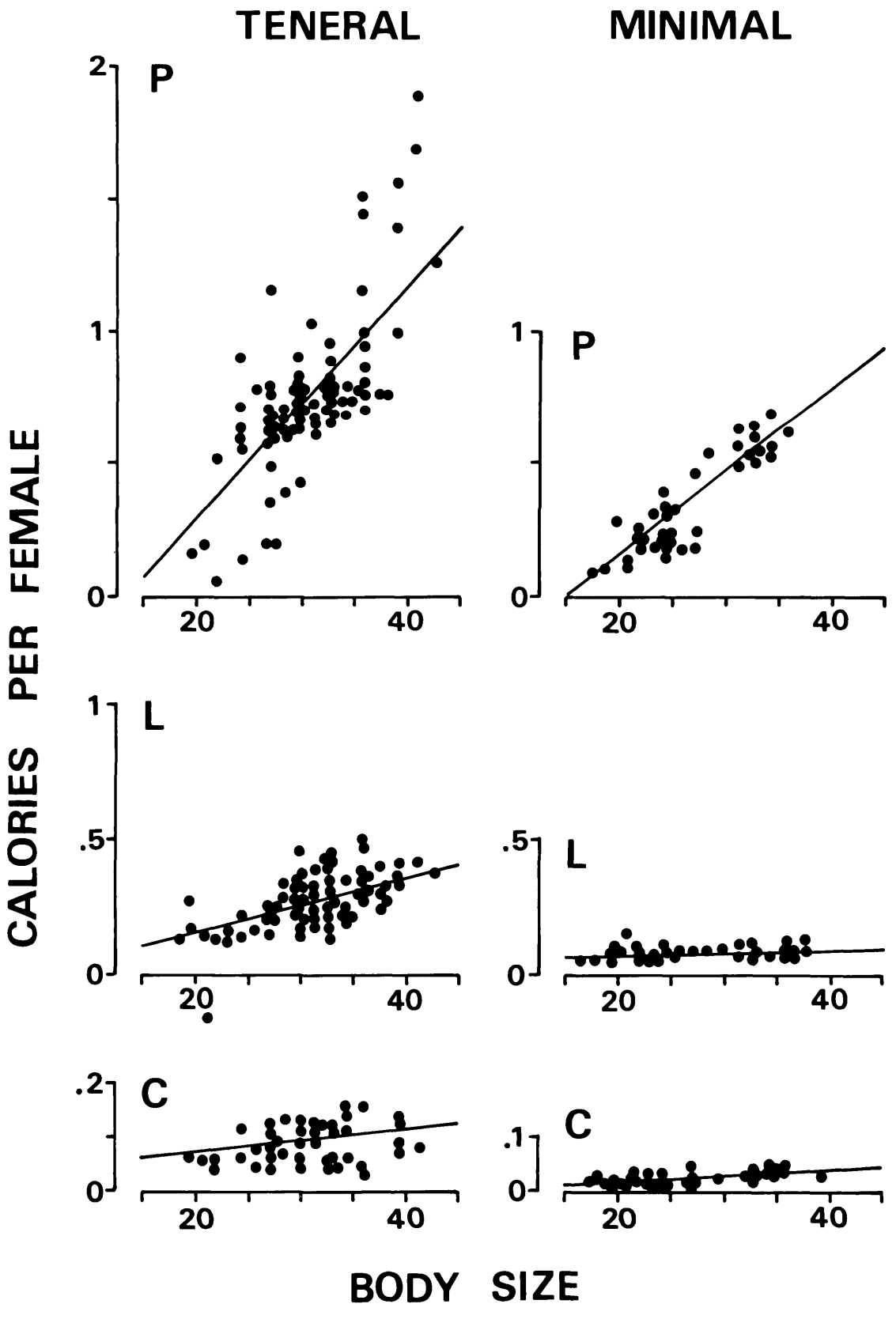
The average body size of males was always slightly (but significantly) smaller than that of females: males of *An. gambiae* were 97% of the female size, 94% in *An. albimanus* and *An. stephensi*, and 89% in *An. quadrimaculatus* (n = 42–219; t = 19.9–118.8; $P < 0.001$). Furthermore, the slopes of regression lines of male teneral reserves on body size differed significantly in all four species from the slopes in females (Table 2).

Mosquitoes analyzed for total nitrogen shortly after eclosion still contained their meconia, which were extruded within the next few hours. The extent of this nitrogen loss accounted for roughly 10% of the female and 15% of the male nitrogen carried over from the immature stages.

To examine the extent of reserve mobilization under conditions of extreme nutritive stress and to reveal the minimal irreducible amounts necessary for survival (Van Handel 1984), females were starved to death in the presence of a source of drinking water. The original data are given for *An. gambiae* (Fig. 1, right side), together with the corresponding regressions. In all four species tested, a linear relationship resulted when these data were related to body size (Fig. 3). Compared with the teneral reserves present at eclosion, there was a significant reduction of 46–58% of the caloric content in all species examined before death occurred (n = 44–103, all $t > 15.4$). Most of the teneral carbohydrates (69–89%), lipids (68–75%), and some of the protein (28–53%) was reduced, depending on the species. Similar reductions were found in males (not shown).

Carbohydrate Feeding and Lipogenesis. Starvation or access only to water is probably unnatural, and as a common laboratory practice, mosquitoes are routinely offered sugar solutions as a source of energy. Therefore, the effect of feeding on sucrose solutions was examined with respect to the synthesis of glycogen and lipid reserves. Females were mounted on tooth picks, offered 10% sucrose from a calibrated pipette, and the glycogen and lipid contents were analyzed at 24-h intervals. Because the volumes ingested by these females rarely exceeded 1 μl, the caloric input (up to 0.5 cal) per single session was too small to enhance the reserves convincingly. Alternatively, females in groups of 10–20 per small cup (approximate volume, 350 ml) were offered cotton wicks soaked with 20% sucrose solution for up to 12 d, and the reserves were analyzed at various intervals. In the latter experiment, the soluble sugar fraction was extracted before glycogen was determined by hot anthrone, thus crop contents were excluded. Results at 1, 2, 6, and 12 d after eclosion with continuous access to 20% sucrose are given for *An. gambiae* and *An. stephensi* in Table 3. In both species, the maximum glycogen content was reached between 1 and 2 d, followed by a gradual decrease thereafter. However, total lipids reached their maximum within 6 d, exceeding the glycogen level by almost two to four times. By 12 d, all values had decreased; obviously, catabolism now was faster than synthesis. Evidently, glycogenesis was faster, but lipogenesis prevailed over glycogenesis. This led to the conclusion that young female *Anopheles* must feed on the sucrose rather frequently to compensate for the small volumes ingested (as encountered when feeding from the pipette) and to store large amounts of lipids. A more general comparison is presented in Table 4, where the observed lipid and carbohydrates or glycogen maxima were related to their minimal irreducible amounts, and both were expressed as a percentage of the female content at eclosion (100%). Obviously, female *Anopheles* are capable of an efficient lipogenesis from sugar meals. But these changes were accompanied by a gradual decrease of protein by roughly 10–14% in *An. albimanus* and *An. quadrimaculatus*, whereas *An. stephensi* and *An. gambiae* kept their protein fairly constant at the teneral level.

Bloodmeal Size, Body Size, and Fecundity. Experimental analyses of gonotrophic cycles were confined largely to *An. albimanus*, *An. gambiae*, and *An. stephensi*. Females of these three species attempted to feed on a host within the first 6–10 h of imaginal life and often were successful within the next 6–8 h; in *An. quadrimaculatus*, this took longer. The volume of the first blood meal, ingested under undisturbed conditions, was estimated by computing hemoglobin consumption from fecal hematin. In all three species, the hematin output was correlated significantly ($P < 0.001$) with body size. Individual hematin determinations showed that the largest females ingested up to 6.4 μl (5.1 cal protein) in *An. stephensi*, 5.3 μl (4.3 cal protein) in *An. albimanus*, and 3.5 μl (2.4 cal protein) of


TENERAL
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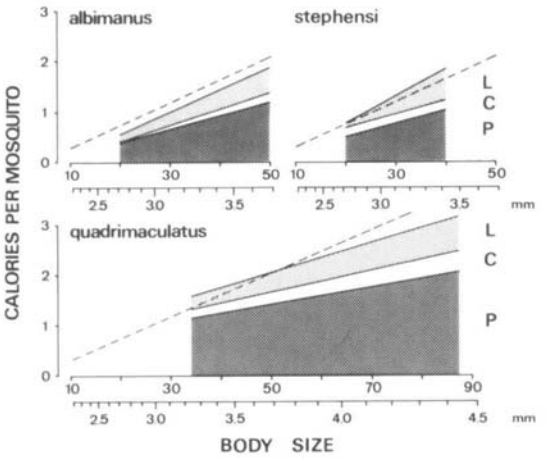

Fig. 2. Summation of caloric content of protein (P), lipid (L), and total carbohydrates (C), plotted as a function of body size for teneral *Anopheles*. The corresponding regression lines were added and female body sizes are given as cubic wing length; actual wing lengths (millimeters) are indicated below the abscissa. For comparison, the regression line for teneral protein in *Ae. aegypti* (Briegel 1990) was plotted as a broken line. Regression equations were as follows. *An. albimanus*: protein, $Y = 0.03X - 0.14$ ($n = 103$, $r = 0.74$, $P < 0.001$); lipid, $Y = 0.01X - 0.09$ ($n = 102$, $r = 0.74$, $P < 0.001$); carbohydrates, $Y = 0.005X - 0.10$ ($n = 64$, $r = 0.595$, $P < 0.001$). *An. stephensi:* protein, $Y = 0.03X - 0.04$ ($n = 46$, $r = 0.47$, $P < 0.001$); lipid, $Y = 0.03X - 0.43$ ($n = 45$, $r = 0.58$, $P < 0.001$); carbohydrates, $Y = 0.001X + 0.14$ ($n = 26$, $r = 0.07$; n.s.). *An. quadrimaculatus:* protein, $Y = 0.02X + 0.57$ ($n = 48$, $r = 0.82$, $P < 0.001$); lipid, $Y = 0.01X + 0.03$ ($n = 48$, $r = 0.61$, $P < 0.001$); carbohydrates, $Y = 0.005X - 0.04$ ($n = 45$, $r = 0.68$, $P < 0.001$).

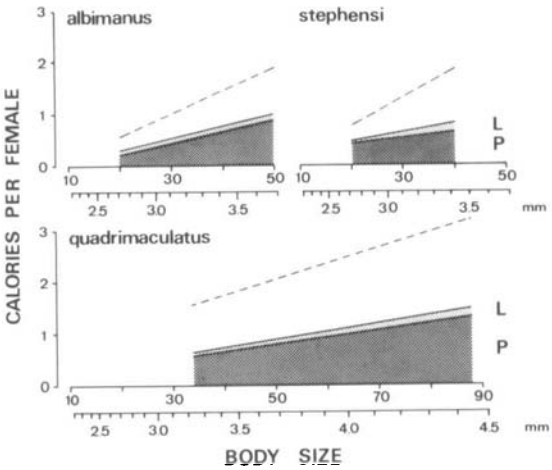

Fig. 3. Summation of caloric content of minimal, irreducible protein (P) and lipid (L) reserves in female *Anopheles* as a function of body size (cubic wing length) and plotted in the same way as Fig. 2. The broken line is the total caloric content of teneral females taken from Fig. 2. The total carbohydrates always were below 0.05 cal, too low to be included in the figure. The regression equations were as follows. *An. albimanus:* protein, $Y = 0.02X - 0.22$ ($n = 92$, $r = 0.74$, $P < 0.001$); lipid, $Y = 0.001X + 0.06$ ($n = 44$, $r = 0.27$, n.s.). *An. stephensi:* protein, $Y = 0.01X + 0.2$ ($n = 34$, $r = 0.32$, n.s.); lipid, $Y = 0.01X - 0.11$ ($n = 32$, $r = 0.43$, n.s.). *An. quadrimaculatus:* protein, $Y = 0.01X + 0.35$ ($n = 69$, $r = 0.61$, $P < 0.001$); lipid, $Y = 0.001X + 0.08$ ($n = 69$, $r = 0.20$, n.s.).

human blood in *An. gambiae* if undisturbed during feeding; for *An. quadrimaculatus*, the corresponding values were only 3.9 μl (3.1 cal) of human blood despite its larger size. These observations represent maximal values for blood consumption; smaller volumes are more likely under field conditions.

There was a highly significant correlation ($P < 0.001$) between hematin output and the number of eggs matured for *An. albimanus*, *An. gambiae* and *An. stephensi* (Fig. 4); for *An. gambiae*, single data points are given but, for the others, regressions only. In all species, females ingesting <about 200 μg of hemoglobin (equivalent to 1.5 cal of total protein) failed to start oogenesis. The distribution of the single data points in *An. gambiae* (as well as in the other two species) suggested a "yes-or-no" relationship between protein input and egg maturation. The requirements for a minimal blood volume to stimulate oogenesis were examined by interrupting females at various times after they had initiated feeding, resulting in a wide range of suboptimal blood meal sizes. In *An. albimanus* fed human blood, no eggs matured unless at least 1.1 cal of protein were ingested. These thresholds were 1.0 cal for *An. stephensi*, 1.3 cal for *An. gambiae*, and 2.4 cal for *An. quadrimaculatus*, as indicated in Fig. 4 by the lower end of the respective regression lines.

In *An. albimanus*, in addition to the blood volume, there was evidence for body size as a threshold level for the stimulation of oogenesis. The number of eggs matured with human blood (Y) was clearly correlated with body size (X): larger females ingested larger meals and consequently had

←

Fig. 1. Caloric content of total protein (P), lipids (L), and carbohydrates (C) in teneral females (left side) as well as their minimal irreducible amounts (right side) of *An. gambiae*. The single data points (Y, cal) are given together with the following linear regressions on body size (X, WL^3). For teneral conditions: protein, $Y = 0.04X - 0.59$ ($n = 93$, $r = 0.71$, $P < 0.001$); lipid, $Y = 0.01X - 0.05$ ($n = 78$, $r = 0.60$, $P < 0.001$); carbohydrates, $Y = 0.002X + 0.03$ ($n = 48$, $r = 0.27$; n.s.). For minimal irreducible amounts: protein, $Y = 0.03X - 0.47$ ($n = 39$, $r = 0.91$, $P < 0.001$); lipid, $Y = 0.001X + 0.05$ ($n = 42$, $r = 0.44$, $P < 0.01$); carbohydrates, $Y = 0.001X + 0.0001$ ($n = 44$, $r = 0.58$, $P < 0.001$). The wing lengths ranged between 2.5 and 3.5 mm.

Table 1. Statistical comparisons of regression coefficients shown in Fig. 1 and 2 for the caloric contents of female *Anopheles* at eclosion

Species	Coefficient and number[b]	Protein[a]			Lipids[a]			Carbohydrates[a]		
		G	S	Q	G	S	Q	G	S	Q
An. albimanus	*F*	2.25*	6.07*	1.05	1.82*	1.76*	1.18	7.00*	320.0*	3.36*
	n	103/93	103/46	103/49	102/78	102/45	102/49	64/48	64/26	64/24
An. gambiae	*F*	—	13.66*	2.37*	—	1.04	2.16*	—	45.71*	23.5*
	n	—	93/46	93/49	—	78/45	78/49	—	48/26	48/24
An. stephensi	*F*	—	—	5.76*	—	—	2.08*	—	—	1,074.2*
	n	—	—	46/49	—	—	45/49	—	—	26/24

*, *F* value significant at $P < 0.05$.
[a] G, *An. gambiae*; S, *An. stephensi*; Q, *An. quadrimaculatus*.
[b] *F*, *F* value; *n*, sample sizes of the two cohorts compared.

a greater fecundity ($Y = 2.95X - 41.79$; $n = 126$; $r = 0.60$, $P < 0.001$). But more strikingly, when *An. albimanus* over a wide range of body sizes were fed to repletion, among the females with wing lengths <2.95 mm ($n = 62$), only 12% were oogenic, whereas among those with wing lengths >3.05 mm ($n = 65$), 91% developed eggs. Similarly, oogenic *An. gambiae* had body sizes of 3.03 ± 0.12 mm ($n = 26$), whereas non-oogenic *An. gambiae*, which also fed to repletion, had a significantly smaller ($P < 0.001$) mean body size of 2.90 ± 0.13 mm ($n = 26$). Obviously, a body size threshold corresponding to wing lengths of 3 mm in *An. albimanus* and *An. gambiae* appears to be crucial for oogenesis with the first blood meal, partially explaining the "yes-or-no" relationship recognized previously.

Efficiency of Bloodmeal Utilization for Oogenesis. Ovaries containing mature eggs were analyzed for their caloric protein and lipid content. This was compared with the caloric protein input by blood meal (X, cal) as calculated from the hematin output of individual females; all three species tested showed significant linear regressions ($P <$ 0.001). For ovarian protein (Y, cal), the following regressions were obtained: *An. albimanus*, $Y = 0.14X - 0.20$ ($n = 45$, $r = 0.77$); *An. gambiae*, $Y = 0.185X - 0.26$ ($n = 88$, $r = 0.86$); and *An. stephensi*, $Y = 0.17X - 0.23$ ($n = 41$, $r = 0.87$). For ovarian lipid (Y, cal), the regressions were as follows: *An. albimanus*, $Y = 0.12X + 0.05$ ($n = 54$, $r = 0.69$); *An. gambiae*, $Y = 0.15X + 0.02$ ($n = 101$, $r = 0.72$); and *An. stephensi*, $Y = 0.21X + 0.01$ ($n = 46$, $r = 0.79$). From these regression equations, ovarian protein and lipids were computed as percentages of the caloric input and compiled as shown in Fig. 5. In all three species, 1–13% of the blood meal protein were utilized for the synthesis of yolk protein and an additional 12–22% of the caloric input was utilized for the synthesis of yolk lipids. This result confirmed that the failure to initiate oogenesis after small blood meals probably was caused by limited protein utilization and not by limited lipid synthesis.

This observation was further substantiated by relating the ovarian protein and lipid contents to fecundity. The protein content per oocyte was significantly increased with the blood meal input in all three species (Fig. 6, all $P < 0.001$). In contrast, the average lipid content per oocyte was constant for each species examined but differed significantly from each other ($P < 0.001$), irrespective of the size of the blood meal. Therefore, with larger meals, maturing oocytes obtained increasing amounts of yolk, which comprised a constant, species-specific quantity of lipid and a meal-dependent amount of protein. In energetic terms, utilization of the dietary protein for yolk production clearly remained <0.1% per single oocyte.

In addition, the efficiency of blood meal utilization for oogenesis was affected profoundly by the source of the blood. When fed to repletion on a guinea pig, fecundity was considerably higher than when fed on a human host. The data are assembled in Fig. 7 for *An. albimanus*, *An. gambiae*, and *An. stephensi*, where fecundity is expressed as the percentage of blood meal protein recovered from mature ovaries.

Table 2. Demonstration of sexual dimorphism in regressions of teneral reserves on body size for four *Anopheles* species

Species	Coefficient and number[a]	Protein	Lipids	Carbohydrates
An. albimanus	*F*	2.14	19.83*	3.50*
	n	55/14	70/15	12/12
An. gambiae	*F*	2.19*	1.30	3.00*
	n	93/59	78/44	48/44
An. stephensi	*F*	302.0*	1.84*	45.71*
	n	46/28	45/28	26/22
An. quadrimaculatus	*F*	3.31*	3.48*	2.72*
	n	49/29	49/28	24/21

*, *F* value significant at $P < 0.05$.
[a] *F*, *F* values; *n*, sample sizes for females/males.

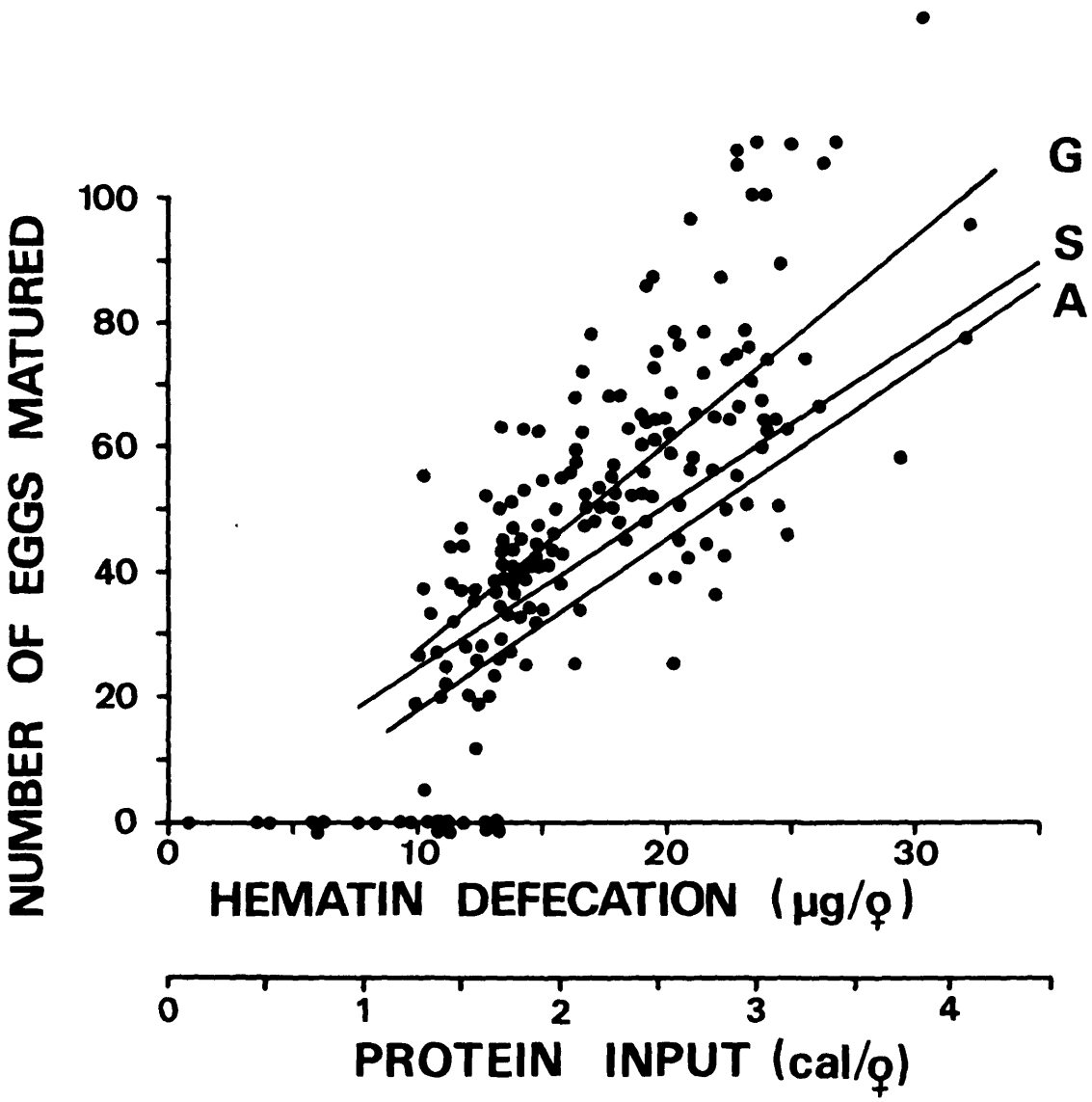

Fig. 4. The number of eggs matured (Y) by three *Anopheles* species as a function of hematin output (X, µg). The blood consumption is indicated on the lower axes after conversion of the hematin values stoichiometrically to hemoglobin and finally to total caloric blood protein. Individual values are plotted for *An. gambiae* only; the regression equations were as follows. (A) *An. albimanus:* $Y = 2.72X - 9.28$ ($n = 115$, $r = 0.74$, $P < 0.001$). (G) *An. gambiae:* $Y = 3.31X - 5.53$ ($n = 169$, $r = 0.74$, $P < 0.001$). (S) *An. stephensi:* $Y = 2.61X - 1.7$ ($n = 182$, $r = 0.85$, $P < 0.001$).

Table 3. Effect of feeding female *Anopheles* 20% sucrose ad lib. during several days on the synthesis of glycogen and lipid reserves

Days	Cal/♀ ± SE (n)			
	An. gambiae		*An. stephensi*	
	Glycogen	Lipids	Glycogen	Lipids
1	0.41 ± 0.08 (12)	0.28 ± 0.06 (12)	0.34 ± 0.08 (12)	0.23 ± 0.04 (12)
2	0.38 ± 0.06 (11)	0.47 ± 0.08 (12)	0.39 ± 0.07 (12)	0.44 ± 0.08 (15)
6	0.28 ± 0.09 (10)	0.66 ± 0.18 (10)	0.33 ± 0.11 (15)	1.12 ± 0.16 (15)
12	0.09 (2)	0.57 ± 0.16 (9)	0.12 ± 0.03 (7)	0.45 ± 0.15 (11)

Table 4. Comparison of lipid and carbohydrate reserves in female *Anopheles* between teneral conditions, the minimal irreducible amounts, and their synthesis (referred to as maximal) after feeding 20% sucrose ad lib. for several days

Species	Body size	Cal/♀ (% loss or gain)		
		Teneral (100%)[a]	Minimal	Maximal[b]
An. albimanus	31.10	L 0.25	0.14 (54)	1.12 (450)
		C 0.11	0.02 (20)	0.55 (509)
An. gambiae	21.75	L 0.17	0.07 (41)	0.66 (395)
		C 0.08	0.02 (32)	0.28 (373)
An. stephensi	27.59	L 0.28	0.08 (30)	1.12 (396)
		C 0.16	0.02 (12)	0.33 (201)
An. quadri-maculatus	61.35	L 0.50	0.12 (25)	1.57 (315)
		C 0.21	0.04 (21)	0.67 (320)

Because data were computed for an average body size (WL^3) for each species from regression equations, no standard errors could be calculated. The percentages of the respective teneral values (in parentheses) indicate the drastic differences between starvation and sugar-feeding.

[a] L, lipid reserves; C, carbohydrate reserves.

[b] Sugar-fed for 6 d, except for *An. albimanus*, which was fed for only 3 d. In this column, the carbohydrates refer to glycogen only, because sugars were first extracted and were thus excluded from this column.

Nitrogen Budgets and Maternal Deposits During a Gonotrophic Cycle. The low percentage of blood meal protein recovered from mature oocytes led to an examination of the fate of the surplus nitrogen. A nitrogen budget that contained the principal catabolites was established for *An. albimanus*, *An. stephensi*, and, in part, for *An. gambiae*. Surplus nitrogen was degraded to various catabolites: uric acid, urea, ammonium, and hematin. Their average distribution is illustrated in Fig. 8 as the percentage of the blood meal nitrogen input and was compared with previous data for *Ae. aegypti* (Briegel 1986). In *An. stephensi*, urea showed an unusually high portion in nitrogen excretion, contrasting with the prevalence of uricotely in the other mosquito species. In *An. albimanus* and *An. gambiae*, an appreciable segment of the ingested nitrogen (11% in *An. albimanus*, 15% in *An. gambiae*) was deposited outside the ovaries, presumably as a maternal deposit (Fig. 8, MD). In the latter species, even nonoogenic females retained approximately 10% of the blood meal nitrogen, suggesting that the acquisition of maternal protein deposits had priority over the synthesis of yolk protein.

To explore maternal metabolism and the fate of its reserves during the gonotrophic cycle and as a function of body size, several regression lines for *An. albimanus* were compiled in Fig. 9 for protein and in Fig. 10 for lipids, all on a caloric basis. The segment of the blood meal protein that was retained by the female after digestion and excretion had been completed was revealed by comparing female protein content before the blood meal, which was grossly equivalent to teneral levels, with that just before oviposition (Fig. 9, BOP). This segment,

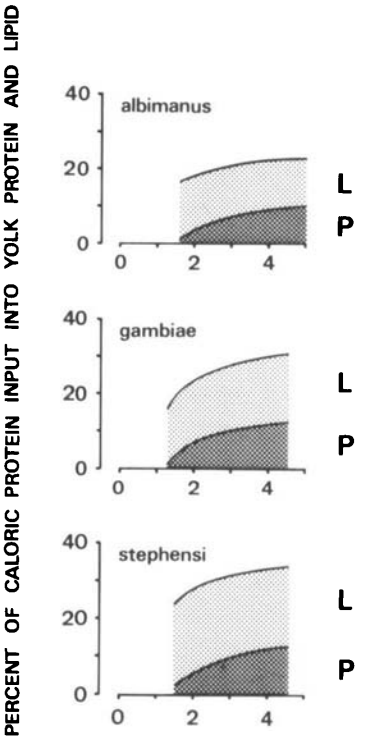

Fig. 5. Utilization of blood meal protein for the synthesis of yolk protein (P) and lipids (L) by three *Anopheles* species. The percentage was computed on a caloric basis from linear regressions between caloric yolk content and protein input; the lines were drawn for best fit of the points. For each species fed on a human host, the approximate caloric protein threshold required for initiation of oogenesis can be extrapolated from the protein lines approaching the abscissa, coinciding with the results in Fig. 4.

however, was segregated into two areas by the line for ovarian protein (OV): the yolk protein (dotted) and the remaining protein (vertically hatched), which represents the net gain in the form of maternal deposits. Lipid metabolism, on the other hand, differed in two ways (Fig. 10). First, females of all sizes reached a higher lipid level just before oviposition than was present when fed sucrose before the blood meal (compare BOP and MAX in Fig. 10). Second, a more complicated situation was established by the line for ovarian lipid (OV). In large females, it constituted a substantial fraction of the preblood meal lipid (heavily dotted area), which the females were converting to yolk and ultimately loosing through oviposition, whereas small females invested a smaller portion of lipid into yolk, thus earning a considerable amount of extraovarian, maternal lipid deposit (vertical hatching). Therefore, depending on body size, there appeared to be a complementary correlation between maternal reserves and yolk deposition. In large females, more protein and lipid was contrib-

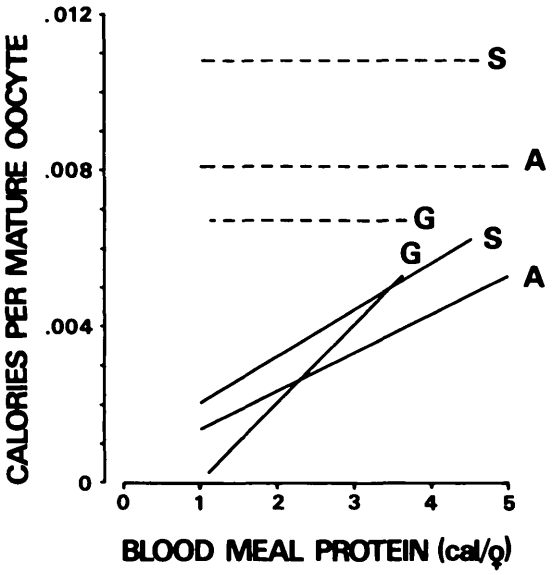

Fig. 6. Caloric lipid and protein content per mature oocyte plotted as a function of the protein input for three *Anopheles* species. The caloric input by blood meal protein was determined from fecal hematin values. For lipid yolk (broken line), the mean calories (± SE) per oocyte were constant and significantly different from each other (all $P < 0.001$). (A) *An. albimanus:* 0.008 ± 0.001 ($n = 56$). (G) *An. gambiae:* 0.007 ± 0.001 ($n = 102$). (S) *An. stephensi:* 0.011 ± 0.001 ($n = 44$). For protein yolk (solid lines) linear regressions were as follows. (A) *An. albimanus:* $Y = 0.001X + 0.0004$ ($n = 47$, $r = 0.51$, $P < 0.001$). (G) *An. gambiae:* $Y = 0.002X - 0.002$ ($n = 53$, $r = 0.89$, $P < 0.001$). (S) *An. stephensi:* $Y = 0.001X + 0.008$ ($n = 42$, $r = 0.58$, $P < 0.001$).

uted to oogenesis, but in small females, more protein and lipid was allocated to extraovarian maternal storage.

Evidence for this trend also was found in the two other *Anopheles* species analyzed for maternal and ovarian protein and lipids. In *An. gambiae*, 19% of the total caloric blood meal protein was utilized for yolk production (4% protein plus 15% lipid), and 33% for maternal deposits (15% protein plus 18% lipid). In the group of female *An. gambiae* that failed to develop eggs from the blood (see above), 36% of the calories were transferred to extra-ovarian deposits (10% protein plus 26% lipid), and 64% of the protein ingested was degraded, 13% more than in oogenic females; this 13% resembled the 19% of mean caloric yolk found in oogenic females.

In *An. stephensi*, maternal protein occasionally was observed to be slightly higher after oviposition than it was before the blood meal. This insignificant increase has been omitted from Fig. 8 because of its inconsistent appearance. With respect to lipids, maternal stores declined (−0.3 cal per female) with small blood meals, but with large meals there was a net gain of lipids (+0.2 cal per female).

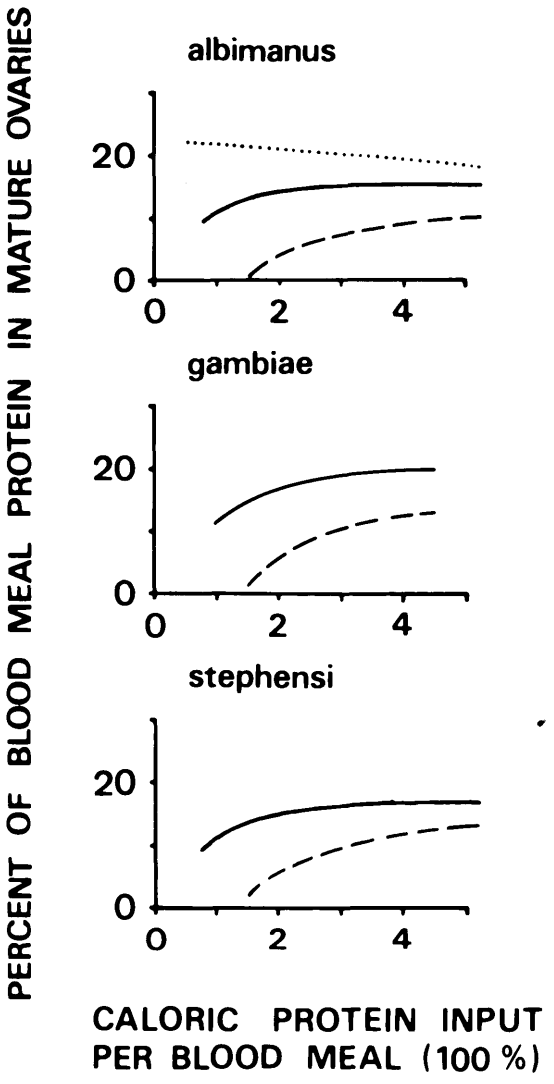

Fig. 7. Effect of the source of the blood on enhanced protein utilization for vitellogenesis by three *Anopheles* species, fed on guinea pig (solid lines) or human hosts (dashed lines). Ovarian protein is expressed as percentage of the input by blood meal. The underlaying linear regressions for human blood were the same ones used for protein in Fig. 4, whereas for guinea pig blood, they were as follows: *An. albimanus*, $Y = 0.17X - 0.05$ ($n = 87$, $r = 0.80$, $P < 0.001$); *An. gambiae*, $Y = 0.22X - 0.11$ ($n = 30$, $r = 0.8$, $P < 0.001$); and *An. stephensi*, $Y = 0.18X - 0.07$ ($n = 69$, $r = 0.69$, $P < 0.001$), where X is blood meal protein in calories per female and Y is ovarian caloric protein. For comparison, protein utilization by *Ae. aegypti* fed on a human host was included as a dotted line (adopted from Briegel 1985).

Discussion

Female and male *Anopheles* appear to eclose as "undernourished" imagoes when compared with the more robust *Aedes*, and the term "obese mosquito," coined correctly for *Aedes* by Van Handel

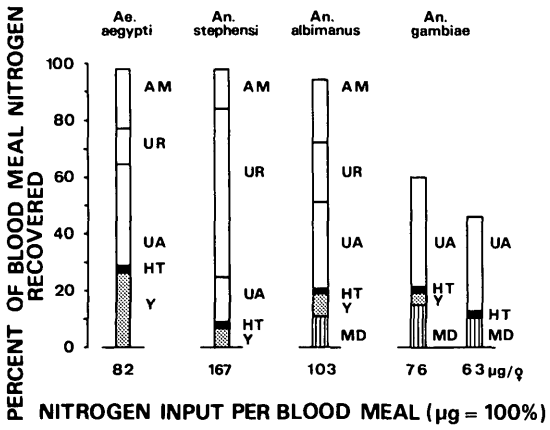

Fig. 8. The nitrogen budget and its partitioning at the end of the gonotrophic cycle of *Anopheles* fed human blood to repletion, expressed as average percentage (n = 5–25) of the nitrogen input by blood meal. For comparison, data for *Ae. aegypti* fed human blood were adopted from Briegel (1986). The mean nitrogen content of the blood meal is given in micrograms per female below the columns (100%). The budget for *An. gambiae* is incomplete, but a nonoogenic cohort is included to underscore the apparent priority of producing and retaining a maternal nitrogen deposit over vitellogenesis. AM, ammonium ions; HT, hematin; UA, urate; UR, urea; Y, yolk; MD, maternal nitrogen deposit.

(1965), may not be appropriate for *Anopheles*. The levels of total caloric reserves in teneral anophelines, which have been carried over from the larval stages, were considerably lower than those in teneral culicines (Briegel 1990), particularly when considering individuals of the same body size. Furthermore, in contrast to *Ae. aegypti*, male and female *Anopheles* carry similar, size-dependent amounts of lipids, and in this respect there is no extreme sexual dimorphism other than the slightly (but significantly) smaller size and reserves of males. Evidently, anopheline larvae are incapable of accumulating plentiful reserves, even when food is abundant, which, in turn, may be a consequence of their feeding behavior. Harvesting primarily the water surface for floating food particles appears to be less rewarding nutritionally than browsing on bottom detritus or suspension feeding (Dahl 1988).

The limited synthesis of reserves by larval anophelines, as opposed to the large amounts of lipid and protein accumulated by culicine larvae, bears profound consequences for imaginal metabolism and behavior. When *Anopheles* fed on sucrose as imagoes, the low lipid levels clearly were increased, thus partially compensating for the poor larval lipogenesis. Carbohydrates play a minor role. Therefore, *Anopheles* appear to follow principles similar to those described for *Aedes* by Van Handel & Lea (1965, 1970); namely, hormonal suppression of glycogenesis in favor of lipogenesis, as supported by decapitation experiments (unpublished data). Under nutritive stress, and as long as there was access to drinking water, female *Anopheles* mobilized substantial portions of their teneral reserves: most of their carbohydrates and up to 75% of their lipids; surprisingly, up to 53% of their proteins had disappeared during 1–2 days of starvation. A similar mobilization of 80% of teneral lipids but only 15% of proteins had been reported for *Ae. aegypti* (Briegel 1990). This was less surprising in the latter species because there is plenty of each available per size unit. Mobilization of glycogen and lipids for producing energy is common in insects, but it is unusual for anautogenous species to catabolize protein for nonreproductive purposes. Therefore, it is assumed that feeding on carbohydrate sources, which are readily available and visited in the tropics (McCrae et al. 1969, El-Akad et al. 1989), may be one way by which anophelines minimize protein catabolism, contrary to the view of Muirhead-Thomson (1951) that sugary foods are an unnatural diet for *Anopheles*. Clearly, this behavior does not preclude the preference of females to feed on blood donors as soon as they are accessible.

Anophelines showed a surprisingly low efficiency of blood meal utilization for oogenesis; their ovaries always contained less than one-third of the energetic input of the blood meal, significantly lower than observed for large culicines (Briegel 1985, 1990) but comparable to small culicines (Briegel 1990). Even with rodent blood, which is known to improve this efficiency, utilization never reached the level in culicines fed human blood (Fig. 7). The relatively poor utilization for vitellogenesis undoubtedly is related to the deviation of a substantial fraction of the blood protein to the synthesis of maternal lipid and protein deposits, at least in *An. albimanus* and *An. gambiae*. Together with the vitellogenic processes, this accounted for roughly one-third of the protein input, with all the rest catabolized and excreted. This percentage, then, is similar to protein utilization for vitellogenesis in large culicine mosquitoes (Briegel 1985). Increasing the maternal protein and lipid from the blood meal may be an adaptive consequence of the low teneral amounts. The fact that nonoogenic *An. gambiae* still showed an unaltered synthesis of extraovarian protein and lipids stressed the priority of improving maternal reserves over vitellogenesis. Altogether, female *Anopheles* seem to be in constant need for subsequent blood meals to improve reproductive efficiency (fecundity) or to further accumulate reserves, an assumption supported by field observations (Muirhead-Thomson 1951; Boreham & Garrett-Jones 1973; Burkot et al. 1988), our preliminary evidence for multiple feeding within the same gonotrophic cycle (Briegel & Rezzonico 1985; unpublished data), and our preliminary data on host-seeking (M. J. Klowden & H. B., unpublished data). The higher fecundity with rodent blood than with human blood, which is known for its low content of isoleucine (the limiting factor in mosquito vitellogenesis [Briegel 1985]), revealed

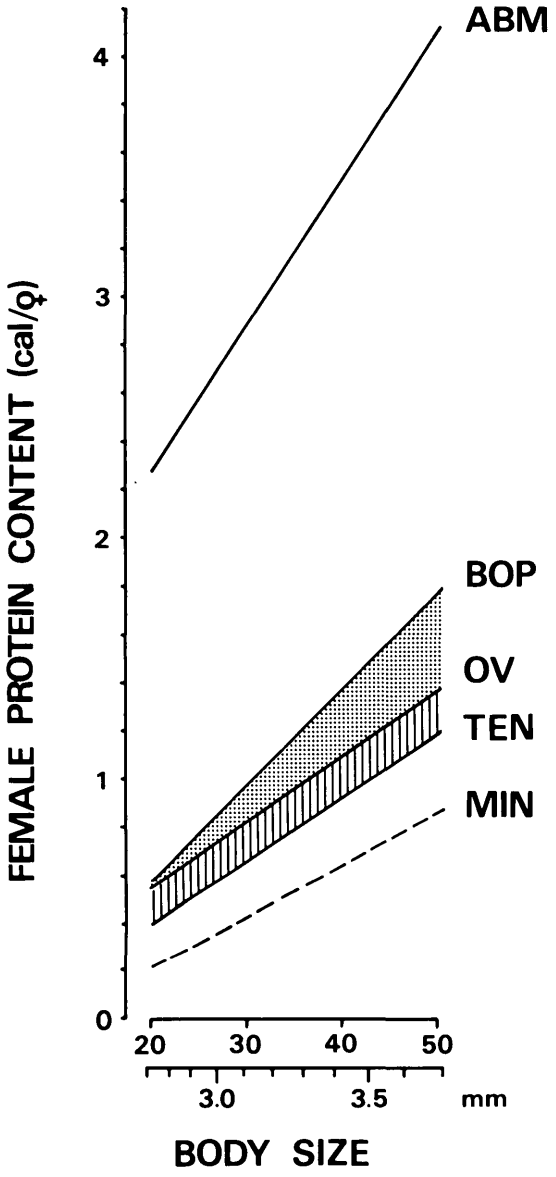

Fig. 9. Compilation of regressions for protein content against female body size for *An. albimanus*. The regression lines are drawn within the range of actual wing lengths (in millimeters, below abscissa) for the following conditions: teneral (TEN), minimal irreducible amounts (MIN), shortly after the blood meal (ABM), shortly before oviposition (BOP), as well as for the ovarian protein content (OV) present at this time, all in calories. The dotted area indicates the yolk protein, whereas the vertical hatching visualizes protein diverted from the blood meal protein but not used for synthesis of yolk, thus representing a net gain of extraovarian, maternal protein during the gonotrophic cycle.

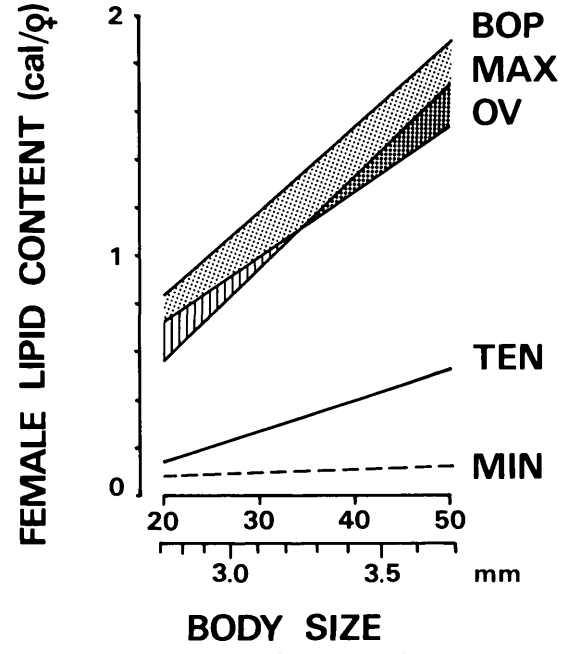

Fig. 10. Comparison of regressions between maternal and ovarian lipid against female body size in *An. albimanus*. The linear regressions are drawn within the range of actual wing lengths (millimeters) for the following conditions: teneral (TEN), minimal irreducible amounts (MIN), shortly before oviposition (BOP), ovarian lipid content present at this time (OV); here, the maximal lipid reserves (MAX) after sugar feeding are given instead of the values shortly after blood meal, because the caloric lipid content of the blood ingested was negligible. Both dotted areas represent yolk lipid; the heavy dotting reflects a part of yolk which appeared to be tapped from maternal reserves, possible only in larger females. The hatched area represents lipid derived from the blood meal but not present in the yolk; therefore, this indicates a maternal lipid deposit synthesized from the blood meal at the expense of yolk production, which seems necessary only in smaller females (wing length <3.2 mm).

another possibility of adaptive behavior. Human blood generally has a higher protein content than rodent blood, but because of its low isoleucine titer, only a limited fraction can be funneled into oogenesis, leaving appreciable amounts available for the synthesis of extraovarian deposits. This physiological principle might explain the attraction of malaria vectors to human hosts with their superior source of protein. With bovids, equally attractive to many anophelines (Horsfall 1972, Gillies & De Meillon 1968), this also seems true, because they have higher titers of blood protein than do rodents (unpublished data).

Although the reproductive physiology of selected anopheline species has been examined in this report only for their first gonotrophic cycle, a metabolic need and sufficient behavioral evidence has been found to postulate that females in the field are likely to seek multiple blood meals during a single gonotrophic cycle, especially the first one. It would be premature, however, to discuss aspects of gonotrophic dissociation. Nevertheless, under natural conditions, such as in a native hut where

families sleep together, there is the possibility for any blood-fed female to feed repeatedly during the same night on the various members, and the far-reaching epidemiological consequences could be underestimated by conventional attempts to determine gonotrophic age on the basis of parity. Finally, it should be stressed that physiological and behavioral findings cannot be extrapolated from culicines to anophelines because the underlying physiological mechanisms are different.

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