

## THE OBESE MOSQUITO

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When food is withheld from animals, caloric reserves are catabolized to provide energy; when re-fed, the food absorbed in excess of immediate metabolic needs is stored as glycogen and fat (triglycerides are often called fat in this paper). The available data on quantitative relations between food consumed and caloric reserves produced are not in proportion to the present interest in obesity. In warm-blooded animals a single meal provides hardly enough energy for a measurable increase of total body fat and glycogen and this may explain the lack of information. Certain cold-blooded animals take meals in excess of their body weight and, without the obligation to maintain constant temperature, may convert a measurable portion of the chemical energy in a single meal to caloric reserves.

Certain mosquito species appeared to be uniquely suitable for such energy-balance studies. The fat content of the female *Aedes sollicitans* increases from almost zero to more than 50% of her dry weight, when she is maintained on sugar for a week (Van Handel & Lum, 1961). When starved until glycogen and fat have disappeared, this mosquito can ingest a single dose of sugar solution or a blood meal, the dry weight of which is several times her own dry weight. New glycogen and fat pools are produced and grow until the rates of utilization exceed the rates of synthesis. The quantitative relations between a massive dose of sugar or protein and the sizes of the glycogen and fat pool resemble the relations between a tracer dose of precursor and the specific activity of its products in steady-state experiments.

The present study describes the growth and decline of the total glycogen and fat pools, produced at constant temperature by meals of sugar, ranging from 0.075 to 3.3 mg per mosquito.

### METHODS

Several large collections of female *A. sollicitans* were brought from the field, as adults do not mate well under laboratory conditions. Their eggs were collected and stored for hatching as needed. A standardized larval rearing method was used throughout (Lea, 1963). Soon

after the adults emerged from the pupae, unmated individuals of each sex were isolated and maintained, with water available, in groups of twenty in plastic cups of 400 ml., with 12 hr light and 12 hr dark. Males were starved for 5 days, female for 7 days at 27° C. They were fed individually by easing the proboscis into a micropipette calibrated in  $5 \times 1 \mu\text{l}$ . The sugar used in all experiments was glucose/fructose 1:1. All females received 3  $\mu\text{l}$ . of solutions of various concentrations, but 4 and 5  $\mu\text{l}$ . sugar solutions were given for the 10.5 and 13 cal levels. Males were fed on 1.5  $\mu\text{l}$ ., because they would not take more in one dose. Blood was given by letting starved females (weighing 3.6–4.2 mg) engorge on fasted white rats. Only mosquitoes that took 12–14 mg were used. After feeding they were maintained as during starvation. The mosquitoes, when not disturbed, sit rather still and make only occasional short flights across the cup. At appropriate intervals, at least six mosquitoes were killed and analysed individually for sugar, glycogen, and triglycerides (Van Handel, 1965a).

In some experiments total lipids were determined in the phospholipid-free chloroform extract (Pande, Khan & Venkitasubramnian, 1963) in addition to triglycerides, lipid phosphorus (Bartlett, 1959) in the methanol eluate from silicic acid, and protein after extraction of lipids (Lowry, Rosebrough, Farr & Randall, 1951).

All results are presented in calories per mosquito in order to make fat comparable to carbohydrate and protein. One milligram carbohydrate or protein was calculated as 4 cal and 1 mg triglyceride as 9 cal. Zero values were determined immediately after feeding, so that sugar at time zero was the amount recovered by actual analysis, and allowance could be made for the small amounts of carbohydrate and triglycerides in blood, when protein was given. In the curves representing net changes zero time values of glycogen (0.00–0.05 cal) and triglycerides (0.00–0.10 cal) were subtracted. The ease with which populations of *A. sollicitans* can be raised, starved, fed, maintained, and analysed under standard conditions greatly helped to minimize biological variation. Even with different collections of eggs, experiments showed a high degree of reproducibility.

## RESULTS

### *Emptying and filling the male and female metabolic pools*

Mosquito pupae do not feed and the depots at emergence are carried over from the larval stage. At emergence males contain 0.7 cal glycogen and 1 cal triglycerides. Starving males mobilize fat more rapidly than glycogen, so that after 1 day they contain more glycogen than triglycerides. Starving females catabolize both products proportionally (Fig. 1, left). At 27° C, mortality begins to occur on the 5th day in the male and on the 7th day in the female and analysis of dead mosquitoes shows a virtual absence of glycogen and triglycerides. At the time mortality began to occur, survivors were fed on 3.3 cal sugar (in 1.5  $\mu\text{l}$ . portions to both male and female) and the synthesis of glycogen and fat was followed as a function of sugar disappearance. The female used 30% of this sugar for fat synthesis, but the male only 3% (Fig. 1, right). Neither by feeding *ad libitum* for 10 days nor by repeated pipette-feeding of concentrated sugar solution could the male be induced to synthesize more than a minute quantity of triglycerides. This could not be due to lack of storage capacity, as much larger amounts had been present at emergence (Fig. 1, top). In the male only small reserves (glycogen) are produced from sugar

so that an empty stomach is soon followed by death. When fed on sugar *ad libitum*, the female *A. sollicitans* may store fifty times more fat than the male (Van Handel & Lum, 1961).

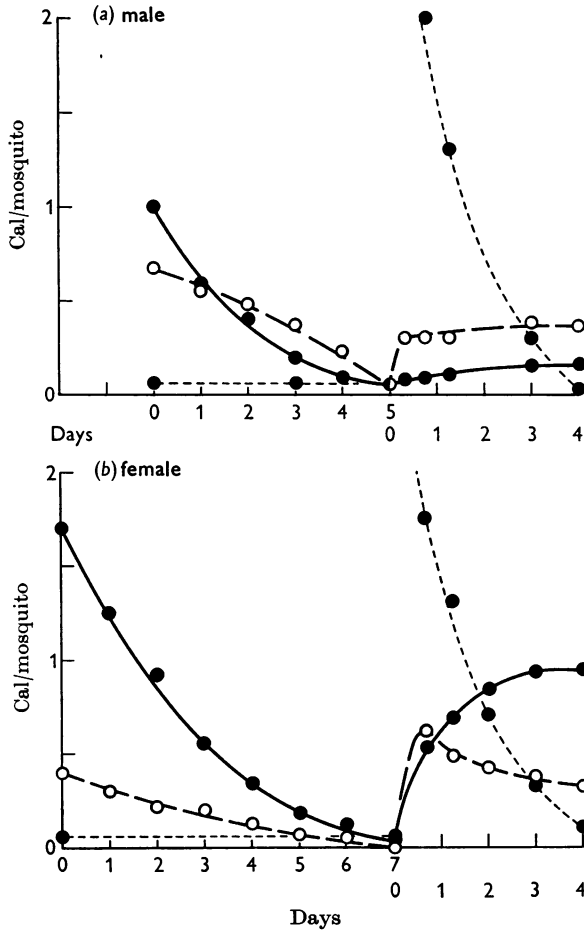


Fig. 1. Triglyceride, glycogen and sugar levels in male (a) and female (b) *A. sollicitans* at 27° C, maintained on water from emergence until the beginning of mortality (5 days in males and 7 days in females), then fed on a single dose of 3.3 cal (0.8 mg) sugar. 1 cal triglyceride = 0.11 mg. 1 cal sugar or glycogen = 0.25 mg. Filled circles, full lines, triglycerides; filled circles, interrupted lines, sugar; empty circles, glycogen.

*Quantitative relations between precursor (sugar) and the products (glycogen and triglycerides)*

Figure 2 represents the growth and decline of glycogen and triglyceride pools, synthesized by the female from ever larger amounts of sugar. When

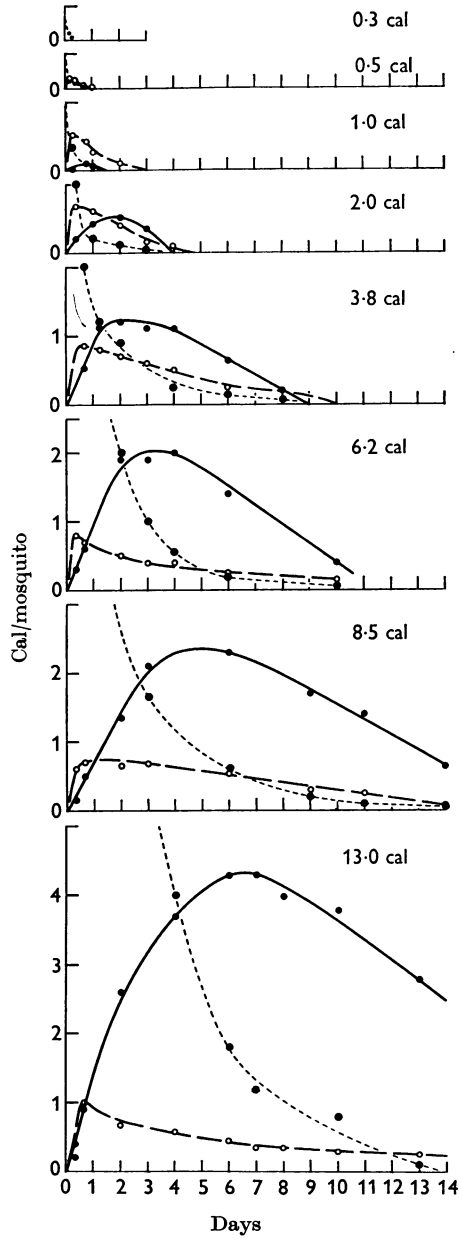


Fig. 2. Net changes of triglyceride, glycogen and sugar in starved female *A. sollicitans* at 27° C, fed on single doses of 0.3–13 cal (0.075–3.3 mg) sugar. Filled circles, full lines, triglycerides; empty circles, full lines, glycogen; filled circles, interrupted lines, sugar.

*A. sollicitans* is fed 0.3 cal (0.08 mg) sugar, neither glycogen nor fat is deposited, so that all sugar is used to provide energy. Between 0.5 and 1 cal sugar increasing amounts of glycogen accumulate until a plateau is reached. Increasing the sugar beyond 1 cal does not result in more glycogen, but induces fat deposition. After 2 cal sugar, fat reaches almost the same plateau as glycogen; after 3.8 cal sugar, fat surpasses the glycogen peak, and from there on, ever increasing sugar meals are reflected in ever increasing fatness. The largest single dose the mosquito would take may well justify the title of this paper; a starved female, receiving 13 cal (3.3 mg) sugar which is 4.5 times her own dry weight, will, within 6 days, accumulate 4.5 cal fat (0.5 mg) which is  $\frac{2}{3}$  of her own dry weight before feeding (0.75 mg).

During the first hours after feeding the rate of glycogen synthesis greatly exceeds the rate of fat synthesis, but deposition of fat takes place before glycogen has reached its peak, provided that the sugar meal exceeds 1 cal. This peak (0.7–0.9 cal) is reached for all sugar doses in 8–12 hr, after which time glycogen and fat diverge: while the fat pool continues to grow, the glycogen pool continues to shrink.

As the sugar is disappearing, the female becomes increasingly dependent on her reserves. Following the disappearance of 1 cal sugar she lives almost entirely on glycogen, but as the sugar doses increase, fat plays an ever more important role as a nutritional reserve until at the highest doses, after the disappearance of sugar, between 80 and 90% of the energy is supplied by fat and only a little by glycogen (Fig. 2).

#### *The utilization of sugar*

Absorption of sugar is a very rapid process: within minutes after a meal of sugar an exhausted mosquito can resume the highly accelerated energy expenditure of flight. In all cases sugar follows the exponential law of decline in which the rate of disappearance is proportional to the amount present, so that the logarithm of the sugar level plotted, as a function of time, yields a straight line (Fig. 3). This relation holds from a sugar meal of 0.3 cal, where no reserves are accumulated to a sugar meal of 13 cal, where at least 5.5 cal is transferred into glycogen and fat.

When the sugar meal increases and the amount of sugar absorbed per unit of time increases, the rate of glycogen synthesis is not accelerated at all and the rate of fat synthesis only very slightly accelerated (Fig. 2). The rate of disappearance of sugar from the female is similar to that from the male, in spite of their very different rates of synthesis of reserves (Fig. 1), so that the rapid fat synthesis in the female does not accelerate sugar absorption.

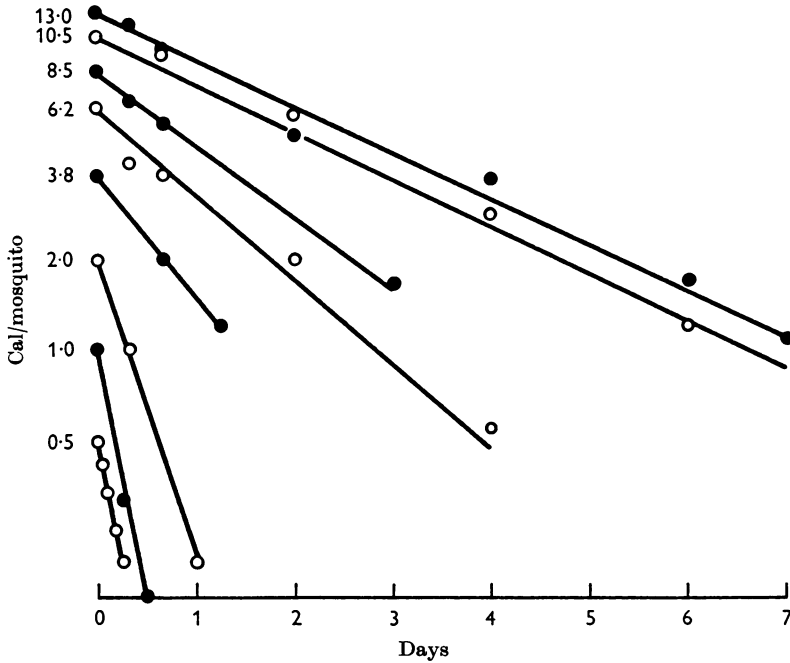


Fig. 3. Logarithmic disappearance of sugar (both empty and filled circles) from starved female *A. sollicitans* at 27° C after ingestion of 0.5–13 cal.

*Protein versus sugar as precursor*

As the natural food of the female mosquito is either nectar or blood, she can be used to test differences between sugar and protein as precursors for fat and glycogen. The females used in this experiment took an average of 13 mg of blood from a fasting rat. Rat blood contains about 17% protein and less than 0.5% lipids and carbohydrates. This small amount of non-protein was accounted for, along with the residual lipids and carbohydrates of the mosquito, by analysis immediately after blood feeding (zero time). Net synthesis was therefore due to  $13 \times 0.17 \times 4 \text{ cal} = 8.8 \text{ cal protein}$ , which was compared with the net synthesis after 8.8 cal sugar feeding (Fig. 4).

Blood has to be broken down by proteolytic enzymes and is undoubtedly absorbed more slowly than sugar. Yet, absorption is not an important rate-limiting factor, because the rate of fat synthesis after a blood meal is the same as after a calorically equivalent sugar meal. After the blood meal the rate of glycogen synthesis, at all times, is less than half the rate of fat synthesis. Sugar fills the glycogen pool to the same height, but ten times faster than protein (Fig. 4). This may mean that protein

and sugar lead to triglycerides through a common rate-limiting precursor, whereas they follow different rate-limiting pathways to glycogen.

The maximum fat pool produced by 8.8 cal protein is only  $\frac{2}{3}$  of that produced by 8.8 cal sugar (Fig. 4). This may be due to a less efficient conversion of protein into fat, but the two experiments are not entirely comparable, because after a meal of blood a portion of the protein is used for egg production. Only mosquitoes that had not laid eggs were used in

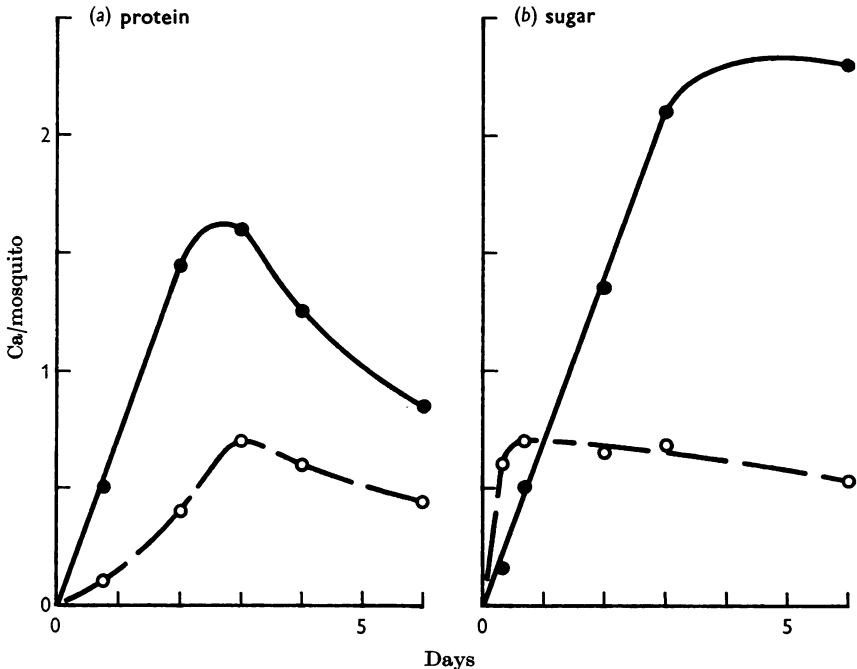


Fig. 4. Net changes of glycogen and triglyceride in starved female *A. sollicitans* at 27° C, fed on a single dose of either 8.8 cal rat blood (a) or sugar (b). Filled circles, triglycerides; empty circles, glycogen.

the experiment, so that the data include fat and glycogen in eggs, but the undetermined amount of blood that was used for synthesis of egg proteins could have reduced the size of the fat pool. The *A. sollicitans* fed on sugar does not develop eggs and all sugar was therefore available for energetic processes.

#### DISCUSSION

For convenience of discussing caloric reserves, the energy produced by complete oxidation of 1 mg fat is equated with 9 cal and that produced by complete oxidation of 1 mg hexose, glycogen, and protein with 4 cal. It does not mean that 1 mg sugar or protein can be transferred into 1 mg

glycogen or  $\frac{4}{3}$  mg of fat, as these conversions are less than 100% efficient and the difference must contribute to the heat production of the insect.

When fed mosquitoes were maintained in glass tubes, these tubes never accumulated any anthrone-positive material so that the mosquitoes do not excrete the sugar which has been fed to them. In the females fed on sugar, phospholipid ( $46 \pm 3 \mu\text{g}$ ) and protein ( $600 \pm 20 \mu\text{g}$ ) levels remained practically constant. The phospholipid-free chloroform eluate contains triglycerides and traces of sterols, free fatty acids and hydrocarbons (Van Handel & Lum, 1961). The small difference between total lipids and triglycerides was virtually constant so that triglyceride was the only lipid synthesized from sugar in quantity by the female mosquito. It may therefore be assumed that, at each time interval, the sum of sugar, glycogen and triglycerides subtracted from the amount of sugar given, represents the caloric expenditure or metabolic rate.

TABLE 1. Position of triglyceride fatty acids synthesized from sugar by *Aedes sollicitans*

	(a)	
	Percentage in 1, 3-positions	Percentage in 2-position
Myristic	6	0.5
Palmitic	45	5
Palmitoleic	25	52
Stearic	5	0.5
Oleic	19	42

(b)

Structures in terms of saturated (S) and unsaturated (U) fatty acids (wt. %)

SSS	SUS	SSU	USU	UUS	UUU
2	27	3	1.5	46.5	20

After 2–13 cal sugar, maximum glycogen production is reached in 8–12 hr and limited to 0.7–0.9 cal. This maximum is not determined by storage capacity, but by the neurosecretory system. When the neurosecretory cells are removed glycogen synthesis continues beyond 0.9 cal, at the same rate as fat in the normal mosquito. Concurrently fat synthesis is reduced and not resumed even when repeated doses of sugar are given. Consequently, in the operated mosquito the glycogen curves resemble the fat curves and the fat curves the glycogen curves of the normal mosquito (Van Handel & Lea, 1965).

$C_{16}$  and  $C_{18}$  fatty acids comprise over 95% of the triglyceride fatty acids synthesized *de novo* by the female *A. sollicitans*. They include about 33% palmitoleic acid, but only a trace of linoleic acid (Van Handel & Lum, 1961). This composition appeared to be independent of the temperature at which the synthesis took place (Van Handel, 1965*b*).

The structure of the triglycerides, synthesized *de novo* from sugar by this mosquito, is not distinctly different from that of other animal and



vegetable fats; palmitoleic and oleic acid appeared predominantly in the 2-position and palmitic acid in the 1,3-positions (Table 1).

The quantitative relation between food intake and production of caloric reserves, demonstrated in this mosquito, may contribute to an understanding of energy balance in animals.

#### SUMMARY

1. In starved female mosquitoes (*Aedes sollicitans*) net synthesis of glycogen and triglycerides produced by sugar meals ranging from 0.075–3.3 mg was determined as a function of time.
2. Glycogen reaches a maximum in 8–12 hr, independent of the amount of sugar fed.
3. Triglycerides begin to accumulate before glycogen has reached its maximum, if sufficient sugar is fed to produce both products.
4. The triglyceride pool grows in proportion to the sugar meal.
5. The rate of triglyceride synthesis is similar, whether sugar or an equivalent amount of protein is fed, but glycogen is synthesized ten times faster from sugar than from protein.
6. The recently emerged, unfed male catabolizes triglycerides faster than glycogen and produces little or no triglyceride from sugar.
7. In the triglycerides synthesized *de novo* by the females, the unsaturated fatty acids appear predominantly in the 2-position and the saturated fatty acids in the 1,3-positions.

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#### REFERENCES

- BARTLETT, G. R. (1959). Phosphorus assay in column chromatography. *J. biol. Chem.* **234**, 466–471.
- LEA, A. O. (1963). Some relationships between environment, corpora allata, and egg maturation in aedine mosquitoes. *J. Insect Physiol.* **9**, 793–809.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin reagent. *J. biol. Chem.* **176**, 265–275.
- PANDE, S. V., KHAN, R. P. & VENKITASUBRAMNIAN, T. A. (1963). Microdetermination of lipids and serum total fatty acids. *Anal. Biochem.* **6**, 415–423.
- VAN HANDEL, E. (1965*a*). Microseparation of glycogen, sugars and lipids. *J. anal. Biochem.* **11**, 266–271.
- VAN HANDEL, E. (1965*b*). Non-dependence on temperature of triglyceride fatty acids synthesized *de novo* by the mosquito. *Fed. Proc.* **24**, 497.
- VAN HANDEL (1965*c*). Temperature-independence of the composition of triglyceride fatty acids synthesized *de novo* by the mosquito. *J. Lipid Res.* (In the Press.)
- VAN HANDEL, E. & LEA, A. O. (1965). Medial neurosecretory cells as regulators of glycogen and triglyceride synthesis. *Science*, **149**, 298–300.
- VAN HANDEL, E., & LUM, P. T. M. (1961). Sex as regulator of triglyceride metabolism in the mosquito. *Science*, **134**, 1979–1980.