

THE THERMAL DEPENDENCE OF THE RATES OF GLYCOGEN AND TRIGLYCERIDE SYNTHESIS IN THE MOSQUITO

By EMILE VAN HANDEL

*Florida State Board of Health, Entomological Research Center,
Vero Beach, Florida*

(Received 29 November 1965)

In poikilothermic animals the dependence on temperature of physiological processes such as growth, respiration, heart-beat, wing-beat, motility, etc., has been studied, but no information seems to be available for the temperature dependence of the synthesis of a defined product. An opportunity to measure rates of synthesis of specific products, namely, the accumulation of caloric reserves, is offered by the female mosquito. When mosquitoes are starved they catabolize all glycogen and triglycerides; after subsequent feeding on a single, measured meal of sugar, quantitative relations can be established between this precursor and its products, the new glycogen and triglyceride pools (Van Handel, 1965*a*). In the present investigation, this biological technique has been used to determine the velocity with which these two products accumulate at different temperatures.

MATERIAL AND METHODS

Eggs obtained from *Aedes sollicitans* brought from the field were stored and hatched when needed. Larvae were reared on a liver-yeast dog biscuit diet (Lea, 1963). A light cycle of 12 hr. was maintained throughout. Unmated females were collected within 6-12 hr. after emergence, and maintained in groups of twenty in plastic cups of 400 ml. with access to water only. The mosquitoes, when not disturbed, sit rather still and make only occasional flights across the cup. The experiments were carried out in incubators with temperatures held constant to $\pm 0.5^{\circ}\text{C}$.

Females were starved for 7 days and then fed on 1 mg. of sugar by easing the proboscis into a calibrated micropipette filled with 2 μl . of a 50% solution of glucose/fructose 1:1. Within minutes after feeding (zero values) and at appropriate time intervals thereafter, depending on the temperature of incubation, three pools of three mosquitoes each were analysed for glycogen, triglycerides and sugar (Van Handel, 1965*b*). Zero values for glycogen and triglycerides did not exceed 0.01 mg. for either product. The experiments were repeated 4 to 8 times, until the standard errors of the means of the pooled data were smaller than 5%.

When glycogen content and triglyceride content are plotted against time the curves have a flat maximum (Van Handel, 1965*a*) so that the time at which this maximum (0.2 mg. for glycogen and 0.13-0.14 mg. for triglycerides) is reached can not be determined with sufficient accuracy to serve as the rate constant, k . Therefore the time at which 75% of the maximum (0.15 mg. for glycogen and 0.10 mg. for trigly-

cerides) is reached was obtained from the experimental curves by interpolation and used as the rate constant. The activation energy was calculated from the equation

$$\mu = 4.58 \times \left(\frac{\log k_2 - \log k_1}{1/T_1 - 1/T_2} \right).$$

Medial neurosecretory cells were surgically removed as described previously (Van Handel & Lea, 1965).

RESULTS

(a) Glycogen synthesis

The rate of glycogen synthesis in *A. sollicitans* increases with temperature from 10 to 22.5° C. Above 22.5° C. the rate of glycogen synthesis is independent of temperature (Table 1). The relationship between the reciprocal of the absolute temperature and the logarithm of the rate constant (time in which the mosquito synthesized 0.15 mg. glycogen) can be plotted as two intersecting lines, one with $Q_{10} = 0$ or $\mu = 0$ and one with $Q_{10} = 5$ or $\mu = 30$ Cal./mole (Fig. 1a).

The maximum amount of glycogen produced from 1 mg. of sugar was 0.2 mg. for each of the seven experimental temperatures between 10 and 35° C.

Table 1. Time required for net synthesis of 0.15 mg. glycogen and 0.1 mg. triglycerides at different temperatures, after feeding 1 mg. of sugar to starved female *Aedes sollicitans*

Temperature (°C.)	Glycogen (hr.)	Triglycerides (hr.)
35	7	
30	7	24
25	8	36
22.5	7	
20	14	60
15	36	108
12.5	—	156
10	72	240

(b) Triglyceride synthesis

The rate of triglyceride synthesis in *A. sollicitans* increases with temperature from 10 to 30° C. (Table 1). At 35° C. maximum synthesis remained below 0.1 mg. and the rate of synthesis was the same or slightly less than at 30° C. It is therefore not certain whether 30° C. is the transition point beyond which the rate of triglyceride synthesis does not increase or whether the result is due to heat damage. The relationship between the reciprocal of the absolute temperature and the logarithm of the rate constant (time in which the mosquito synthesized 0.10 mg. of triglycerides) can be plotted as a straight line with an estimated $Q_{10} = 3$ or $\mu = 20$ Cal./mole (Fig. 1b). The points can also be plotted as two straight lines ($Q_{10} = 2.5$ between 30 and 20° C.; $Q_{10} = 3.5$ between 20 and 10° C.), but the experimental error is so great that the existence of different slopes cannot be regarded as established.

The maximum amount of triglycerides produced from 1 mg. of sugar was 0.13–0.14 mg. for each of the six experimental temperatures between 10 and 30° C.

(c) *Synthesis of glycogen from injected glucose*

The sugar fed to mosquitoes is stored in the diverticulum (crop) and absorbed from the midgut. Even if emptying the diverticulum and absorption are independent of temperature, these processes may be rate-limiting. Furthermore, the rates at which the crop is emptied or the sugar absorbed could in themselves be dependent on temperature. Injection of the precursor directly into the haemolymph bypasses these potential rate-limiting factors.

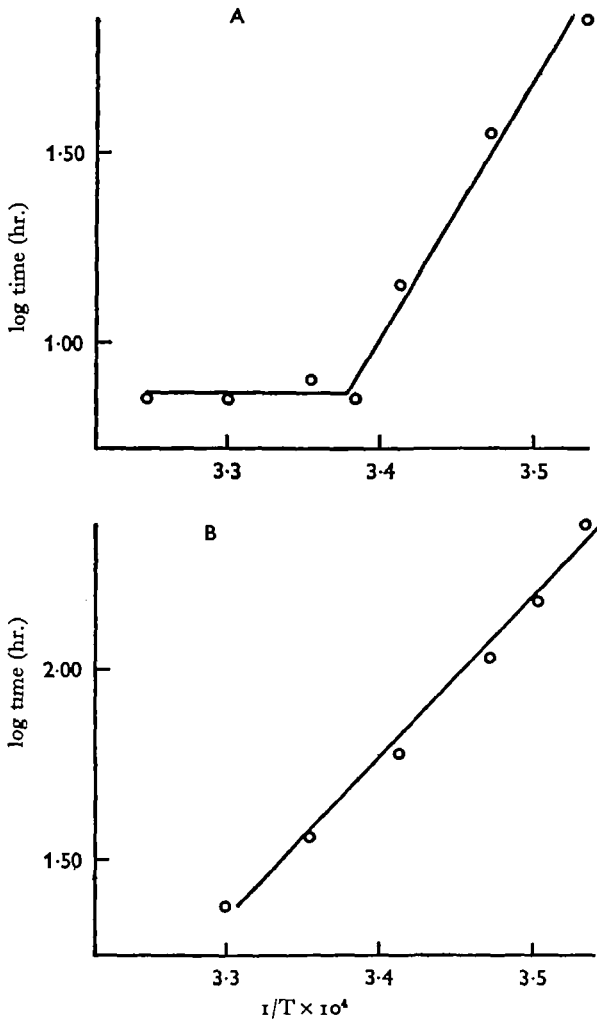


Fig. 1. Arrhenius plot of the temperature-dependence of glycogen synthesis (A) and triglyceride synthesis (B) in starved female *Aedes sollicitans* fed on a single meal of 1 mg. sugar (see Table 1).

One $\mu\text{l.}$ of 50% glucose was injected into the abdomen with the aid of a drawn-out glass capillary, the controls being fed on the same amount with a micropipette. Before feeding, the starved mosquitoes contained less than 0.01 mg. of glycogen

and less than 0.01 mg. of sugar. The insects were analysed individually after 4 and 8 hr. at 30°C, and after 8 and 16 hr. at 15°C.

In injected insects the rate of glycogen synthesis was not increased at 30°C, where synthesis was independent of temperature fluctuations of at least 5°C, or at 15°C, at which glycogen synthesis is strongly temperature-dependent (Table 2). In both injected and fed insects the sum of the synthesized glycogen and the remaining sugar was the same, so that injection did not affect the metabolic rate (Table 2).

These results justify the conclusion that emptying of the diverticulum and absorption of sugar are not rate-limiting factors in glycogen synthesis and, implicitly, in the much slower triglyceride synthesis.

Table 2. *Net synthesis of glycogen and sugar content (mg./female) of starved Aedes sollicitans, either fed on or injected with 1 µl. 50% (0.5 mg.) glucose. Average of twelve mosquitoes ± standard error.*

		30° C		15° C.		
		Fed	Injected	Fed	Injected	
4 hr.	{ glycogen	0.12 ± 0.01	0.10 ± 0.01	8 hr.	{ 0.11 ± 0.01	0.065 ± 0.005
	{ sugar	0.25 ± 0.01	0.24 ± 0.01		{ 0.25 ± 0.01	0.27 ± 0.025
8 hr.	{ glycogen	0.18 ± 0.01	0.14 ± 0.01	16 hr.	{ 0.11 ± 0.01	0.10 ± 0.006
	{ sugar	0.14 ± 0.01	0.19 ± 0.01		{ 0.20 ± 0.01	0.20 ± 0.012

(d) *Synthesis of glycogen after removal of medial neurosecretory cells*

In the female mosquito medial neurosecretory cells (MNC) restrict synthesis of glycogen from sugar. Removal of these cells greatly increases the storage capacity for glycogen at the expense of triglyceride storage (Van Handel & Lea, 1965). It would therefore be possible that MNC affect the rate of synthesis as well as the pool size. In order to investigate this possibility rates of glycogen synthesis were determined in mosquitoes from which the MNC were removed. *A. taeniorhynchus* was used, because in this species the maximum amount of glycogen produced by the unoperated control from a single dose of sugar is only 0.05 mg., as compared to 0.2 mg. for *A. sollicitans*. Almost all glycogen produced from sugar in operated *A. taeniorhynchus* is therefore due to the removal of the MNC (Van Handel & Lea, 1965).

Table 3. *Net synthesis of glycogen (mg./female) from 1 mg. of sugar in starved Aedes taeniorhynchus from which the medial neurosecretory cells were removed at emergence**

Time after feeding (days)	35°C.	20°C.	10°C.
0.5	0.08 ± 0.01	0.06 ± 0.015	—
1	0.18 ± 0.02	0.17 ± 0.008	—
2	0.26 ± 0.02	0.24 ± 0.01	0.10 ± 0.01
3	—	0.32 ± 0.01	0.20 ± 0.02

* Each point represents the average of seven to ten individual mosquitoes ± standard error. Before feeding they contained 0.01 ± 0.002 mg. glycogen. Unoperated controls fed on sugar do not store more than 0.05 mg. of glycogen at the maximum (Van Handel & Lea, 1965).

Removal of the MNC increased the storage capacity for glycogen at all temperatures, but the rate of synthesis at 35°C. did not exceed that at 20°C. (Table 3). Since the failure of the rate of glycogen synthesis to increase above a critical temperature

persists after removal of MNC, this failure in unoperated controls is not due to the MNC. At 10° C. the rate of glycogen synthesis is greatly depressed, in controls as well as in operated mosquitoes.

DISCUSSION

The process most frequently studied at different temperatures in intact poikilotherms is oxygen consumption. Some Q_{10} data for processes which plot approximately as straight lines on Arrhenius-type graphs are listed in Table 4. When a range is given for the Q_{10} , the higher value applies to the lower value of the given temperature range. This may indicate a gradual transition to diminished activity, chill coma, diapause or irreversible changes leading to death. Heart-beat frequency (from 12 to 40° C.) and wing-beat frequency (from 17 to 30° C.) in roaches vary proportionally rather than logarithmically with the temperature (Richards, 1963*a, b*). The same author has studied the temperature dependence of enzyme systems that might be pacemakers for some physiological functions (Richards, 1958).

The present investigation is concerned with the rate of accumulation of caloric reserves from ingested food. The maximum amount of glycogen (0.2 mg.) and triglycerides (0.13-0.14 mg.) synthesized from 1 mg. of sugar by the female *A. sollicitans* appeared to be the same at each temperature. This is 50% of the caloric value of the meal. Since the maximum pool size did not depend on the temperature, the observed changes in the rates of synthesis were directly due to changes in temperature, and not indirectly to changes in pool size.

Table 4. Thermal dependence of physiological processes that follow an Arrhenius-type plot in the indicated temperature range

Species	Process measured	Q_{10}	Temperature range (°C.)	Author(s)
<i>Thermobia domestica</i>	Oxygen consumption	3	5-50	Edwards & Nutting (1950)
Cockroach		2-2.5	3-35	Richards (1963 <i>c</i>)
Embryos (eggs) of five different insects		2-3	10-35	Richards (1964)
Goldfish	Flagellar activity	2-2.5	5-35	Fry and Hart (1948)
<i>Strigomonas oncopelti</i>		2.5	4-28	Holwill & Silvester (1965)
<i>Aedes taeniorhynchus</i>	Pupal duration	2-3	16-32	Nielsen & Evans (1960)
<i>A. sollicitans</i>	Larval development	2-3	15-30	Van Handel (1966)
<i>A. sollicitans</i> (adults)	Triglyceride synthesis	2.5-3.5	10-30	This article
	Glycogen synthesis	0	22.5-35	
	Glycogen synthesis	5	10-22.5	

The temperature-dependence of the rates of synthesis can be presented as an Arrhenius plot. From 10 to 30° C. the rate of triglyceride synthesis increases with an approximate $Q_{10} = 3$ ($\mu = 20$ Cal./mole). The rate of glycogen synthesis increases with an approximate $Q_{10} = 5$ ($\mu = 30$ Cal./mole) from 10 to 22.5° C., while further increase in temperature does not result in further increase in the rate of synthesis. These two widely different Q_{10} values, observed in glycogen synthesis, do not occur during glycogen utilization; during starvation, the rate of disappearance of glycogen increases over the entire range from 10 to 35° C. (Van Handel, in preparation). It is not known why, above 22.5° C., the rate of glycogen synthesis is independent

of temperature, but neither crop emptying and sugar absorption (Table 2) nor the medial neurosecretory cells (Table 3) are rate-limiting factors.

To my knowledge, no other measurements have been reported for the thermal dependence of the rate of synthesis of specific products in an intact animal.

SUMMARY

1. Net synthesis of glycogen and triglycerides produced from 1 mg. of sugar by the female mosquito *A. sollicitans* was determined at different temperatures.
2. At each temperature 0.2 mg. of glycogen and 0.13–0.14 mg. of triglycerides was synthesized from 1 mg. of sugar.
3. The logarithm of the rate constant (the time required to synthesize 75% of the maximum) was proportional with the reciprocal of the absolute temperature.
4. For triglyceride synthesis the $Q_{10} = 3$ from 10 to 30° C.; for glycogen synthesis the $Q_{10} = 5$ from 10 to 22.5° C., but from 22.5 to 35° C. the rate of glycogen synthesis was independent of the temperature ($Q_{10} = 0$).
5. Crop emptying and sugar absorption are not rate-limiting factors.
6. Medial neurosecretory cells, which limit the maximum amount of glycogen that can be synthesized from sugar, do not affect the rate of synthesis.

The author thanks Rosetta Warren, Jose E. Guira and Marius H. Van Handel for technical assistance and Dr Roger W. Meola for performance of the brain surgery. This work was supported by grants (AI 05054 and AI 06587) from the National Institutes of Health.

REFERENCES

- EDWARDS, G. A. & NUTTING, W. L. (1950). The influence of temperature upon the respiration and heart activity of *Thermobia* and *Grylloblatta*. *Pryche*, **57**, 33–44.
- FRY, F. E. J. & HART, J. S. (1948). The relation of temperature to oxygen consumption in the goldfish. *Biol. Bull., Woods Hole*, **94**, 66–77.
- HOLWILL, M. E. J. & SILVESTER, N. R. (1965). The thermal dependence of flagellar activity in *Strigomonas oncopelti*. *J. Exp. Biol.* **42**, 537–44.
- LEA, A. O. (1963). Some relationships between environment, corpora allata, and egg maturation in aedine mosquitoes. *J. Insect. Physiol.* **9**, 793–809.
- NIELSEN, E. T. & EVANS, D. G. (1960). Duration of the pupal stage of *Aedes taeniorhynchus* with a discussion of the velocity of development as a function of temperature. *Oikos*, **11**, 200–22.
- RICHARDS, A. G. (1958). Temperature in relation to the activity of single and multiple physiological systems in insects. *Proc. 10th Int. Congr. Ent.* **2**, 67–72.
- RICHARDS, A. G. (1963a). The effect of temperature on heart-beat frequency in the cockroach, *Periplaneta americana*. *J. Insect. Physiol.* **9**, 597–606.
- RICHARDS, A. G. (1963b). The effect of temperature on wing-beat frequency in the male of the cockroach, *Periplaneta americana*. *Entom. News*, **74**, 91–4.
- RICHARDS, A. G. (1963c). The effect of temperature on the rate of oxygen consumption and on an oxidative enzyme in the cockroach *Periplaneta americana*. *Ann. Ent. Soc. Amer.* **56**, 355–7.
- RICHARDS, A. G. (1964). The generality of temperature effects on developmental rate and on oxygen consumption in insect eggs. *Physiol. Zool.* **37**, 199–211.
- VAN HANDEL, E. (1965a). The obese mosquito. *J. Physiol.* **181**, 478–86.
- VAN HANDEL, E. (1965b). Microseparation of glycogen, sugars and lipids. *Anal. Biochem.* **11**, 266–71.
- VAN HANDEL, E. (1966). Temperature independence of the composition of triglyceride fatty acids synthesized de novo by the mosquito. *J. Lipid Res.* **7**, 112–15.
- VAN HANDEL, E. & LEA, A. O. (1965). Medial neurosecretory cells as regulators of glycogen and triglyceride synthesis. *Science*, **149**, 298–300.