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NON-DEPENDENCE OF THE SATURATION OF DEPOT FAT ON TEMPERATURE AND PHOTOPERIOD IN A HIBERNATING MOSQUITO

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The opinion that fat cells can sense and respond to temperature so as to provide a triglyceride mixture with a melting point just below the environmental temperature (Lands, 1965) is widely held. This view disregards the fact that the body fat of most marine animals, including homeotherms, is highly unsaturated and has melting points much below environmental temperatures, whereas the final melting point of beef tallow and lard may be considerably above body temperature. In the bat, brown adipose tissue fat is less saturated in the summer than during hibernation (Wells, Makita, Wells & Krutzsch, 1965). In hamsters, body fat becomes less saturated after cold exposure, but this effect may be only indirectly due to temperature, because the same effect results from semi-starvation or cortisone treatment at normal temperature (Kodama & Pace, 1963). Furthermore, the fatty acid composition of the diet may greatly affect the body-fat composition and influence the results obtained by variation of temperature.

In the present experiment the hypothesis that cold exposure stimulates the synthesis *de novo* of a more unsaturated depot fat in a hibernating poikilothermic animal was tested in the mosquito *Culex pipiens*, which overwinters in the adult stage in arctic regions.

METHODS

Biological

Culex pipiens L. larvae were raised at 27° C. on a yeast and 'lab chow' diet. Emerging females were isolated and maintained with only water available (*a*) at 25° C. with controlled periods of 14 hr. light and 10 hr. darkness (long-day experiment), or (*b*) at 15° C. with 8 hr. light and 16 hr. darkness (short-day experiment). When 20–25% had died of starvation, survivors were fed on a single dose of 2 μl. of 55% invert sugar and maintained at 5, 10, 20 and 30° C. with 14 hr. light (long-day experiment) or 8 hr. light (short-day experiment). At appropriate time intervals samples were analysed in duplicate pools of at least fifteen mosquitoes each. Fatty acid composition between duplicates did not differ more than a fraction of 1%.

Analytical

The mosquitoes were extracted with chloroform–methanol 2:1, the methanol was removed with water, and the chloroform phase was passed through a silicic acid column; a portion of the chloroform eluate was assayed for triglycerides (Van Handel, 1965).

The remainder of the chloroform eluate was saponified with alcoholic KOH, water was added, and the hexane-extractable material was discarded. The soaps were acidified with dilute sulphuric acid and the hexane-extractable fatty acids were methylated with 3 N dry methanolic HCl at 65° C. for 30 min. Two volumes of chloroform were added and excess methanolic HCl was removed by repeated extraction with water. The chloroform was removed with a stream of nitrogen and the methyl esters were dissolved in hexane. Fatty acid composition was determined by gas–liquid chromatography, with ethylene glycol adipate polyester as the liquid phase and flame-ionization detection. The identity of saturated fatty acids was established by comparing retention time with that of a standard mixture and that of unsaturated fatty acids by the retention time after hydrogenation.

As reported previously (Van Handel, 1966), the lipids in the chloroform eluate were almost exclusively triglycerides.

RESULTS AND CONCLUSIONS

In both experiments the starved mosquitoes contained 0–0·005 mg. of triglycerides, just before feeding. After feeding they were maintained for 3 days at 30° C., 6 days at 20° C., 14 days at 10° C. and 20 days at 5° C. At the end of these periods triglyceride levels had increased to 0·15–0·20 mg., so that more than 95% of the triglycerides were the result of new synthesis.

Table 1. *Triglyceride fatty acids synthesized from sugar at different temperatures and photoperiods by female* Culex pipiens

Temp. (°C.)	30	20	10	5
	Percentage of total fatty acids*			
Saturated				
Palmitic, 16:0				
Long day	32	27·5	28	30·5
Short day	35	27·5	26	31·5
Unsaturated				
Palmitoleic, 16:1				
Long day	49	54	52·5	48
Short day	42	53	51	45·5
Oleic, 18:1				
Long day	13	14·5	14·5	14·5
Short day	18·5	15	18	15·5

* All samples contained in addition 2–3% 14:0, 1–2% 18:0 and 1–2% 14:1.

Saturation of fatty acids was practically independent of the temperature and the photoperiod (Table 1). Actually, the percentage of palmitic acid, the only saturated fatty acid present in quantity, increased from 27 to 31% when the temperature decreased from 20 to 5° C. At 30° C., slightly more oleic acid and slightly less palmitoleic acid was found in the short-day experiment. At the extremes (30° C. and 14 hr. photoperiod; 5° C. and 8 hr. photoperiod) the triglycerides contained the same proportion (35%) of saturated fatty acids.

DISCUSSION

Irrespective of photoperiod and temperature the triglyceride fatty acids of sugar-fed *Culex pipiens* consisted of 30% palmitic, 50% palmitoleic, 15% oleic acid, the balance being a few per cent of myristic, myristoleic and stearic acid. This temperature-independence of the fatty acid composition of depot fat has been demonstrated previously in a non-hibernating mosquito, *Aedes sollicitans* (Van Handel, 1966). From 10 to 35° C. the fatty acids synthesized by females of *A. sollicitans* consisted of 28% palmitic, 32% palmitoleic, 33% oleic, 4% stearic and a few per cent of myristic and myristoleic acid (Van Handel, 1966). The degree of saturation of the fats of these two species is quite similar, but *A. sollicitans* synthesized more than twice as much C_{18} acids as *Culex pipiens* (37% against 16% of the total). Polyunsaturated fatty acids were synthesized by/neither species.

At 5° C. mortality among *Culex* was low and fat synthesis was considerable, whereas in *Aedes sollicitans* mortality approached 100% within a few days. Obviously, *Culex pipiens* is more cold-hardy than *Aedes sollicitans*. The fatty acids present at emergence are much more unsaturated than those accumulating after sugar feeding. At emergence they include a considerable proportion of C_{18}:2, C_{20}:4 and C_{20}:5, carried over from the larval stage, mostly as phospholipids and triglycerides (Van Handel, 1966). In order to prevent mixture of fatty acids accumulating after feeding with those already present before feeding, the latter were excluded from the analysis. This was achieved with tryglycerides by starvation prior to feeding, and with (non-metabolizable) phospholipids by column chromatography. If the (highly unsaturated) fatty acids present at emergence are not excluded, they become diluted with the (more saturated) triglyceride fatty acids synthesized from the sugar meal. Since triglycerides are more rapidly synthesized at higher temperatures (Van Handel, 1966), the degree of dilution and therefore the fatty acid composition will vary with the interval between feeding and analysis, and with temperature. Consequently, failure to exclude the fatty acids already present before feeding could lead to the erroneous conclusion that more unsaturated fatty acids accumulate at low temperatures. This is one of a number of reasons why the claim that the hibernating mosquito *Culex tarsalis* accumulates a more unsaturated fat at short photoperiods and low temperature (Harwood & Takata, 1965) is unfounded.

SUMMARY

1. The fatty acid composition of the triglycerides synthesized from sugar was determined in female *Culex pipiens*, a mosquito that overwinters in the adult stage.

2. The fat had approximately the same composition when synthesized at 5, 10, 20, or 30° C.

3. At each of these temperatures the fat composition was the same, whether a photoperiod of 8 hr. or of 14 hr. was used.

4. The main fatty acids were palmitic (30%), palmitoleic (50%) and oleic acid (15%).

5. These results do not support a hypothesis that adipose tissue of poikilothermic animals synthesizes a more unsaturated fat under winter conditions.

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