

1969-03

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The Ohio Journal of Science. v69 n2 (March, 1969), 70-73

<http://hdl.handle.net/1811/5452>

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SEXUAL DIMORPHISM IN NEUTRAL LIPID METABOLISM
IN THE INDIAN-MEAL MOTH,
PLODIA INTERPUNCTELLA (HÜBNER)¹

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ABSTRACT

Of last larval instar Indian-meal moths, *Plodia interpunctella* (Hübner), males contain slightly more total neutral lipid per gram live weight than do females. The qualitative composition of the various neutral fractions is similar in both sexes. During the pupal period, the female catabolizes considerably more triglyceride than the male, resulting in a much lower triglyceride content in the newly emerged adult female. There is an almost equal rate of lipid utilization in both sexes during the first three days of adult life.

INTRODUCTION

Sex appears to influence lipid metabolism during development of some Lepidoptera (Gilbert, 1967). Niemierko *et al.* (1956) reported that male *Bombyx mori* (L.) have a slightly higher lipid content as early as the fourth larval instar. Also the male utilizes only 30 percent of its stored lipids between larval spinning

¹Manuscript received October 3, 1968.

and adult emergence, while the female uses 50 percent. Similarly, Demyanovsky and Zubova (1957) found a higher lipid content in fifth (last) instar male *Antheraea pernyi*, and Gilbert and Schneiderman (1961) state that, during the pupal-adult transformation of *Hyalophora cecropia* (L.), males utilize virtually no lipid, whereas females catabolize more than 20 percent of their reserves. I have investigated neutral lipid metabolism during development of the Indian-meal moth, *Plodia interpunctella* (Hübner), in an attempt to observe metabolic differences between the sexes.

MATERIALS AND METHODS

Plodia interpunctella cultures were reared in glass containers at 30°C, 60% RH, and a 12:12 hr light-dark cycle, using the culture medium described by Kirkpatrick and Harein (1965). Lipids were extracted with chloroform/methanol, 2:1 v./v. (30 ml/g live wt), and purified by the method of Folch *et al.* (1957). The material remaining after lipid extraction was retained and dried to constant weight. Chloroform containing the total lipid extract was dried at 40°C under a stream of nitrogen. The extract was then redissolved in chloroform and fractionated by silicic-acid column chromatography into neutral lipids (plus free fatty acids) and phospholipids. Neutral lipids were eluted from the column with chloroform, and the retained phospholipids were eluted with methanol/chloroform, 9:1 v.v.

Known amounts of neutral lipids were separated into individual fractions by chromatography on glass fiber-silica gel sheets (Gelman Instant Thin-Layer Chromatography) (Yurkiewicz, 1968), as described previously (Yurkiewicz and Oelsner, In press). A solvent system of petroleum ether (BP 30–60°C) and glacial acetic acid, 99.6:0.42 v.v., was employed. The individual fractions were identified by co-chromatography with known samples (Applied Science Labs., State College, Pa.), by gas-liquid chromatography, and by specific color reactions (Stahl, 1965).

The lipids were located in iodine vapor and eluted from the chromatogram with chloroform/methanol, 2:1 v.v. Recovery of known amounts of lipid averaged 94 percent. Quantitation was by the sulfuric-acid colorimetric method of Marsh and Weinstein (1966). Carefully measured amounts of high-purity lipids (Applied Science Labs.) were used to establish separate quantitative curves for each neutral lipid fraction. Duplicate samples were generally replicable within a ± 5 percent margin of error. Gravimetric determinations of a number of the neutral lipid samples confirmed the findings obtained by the colorimetric method. At least four samples were determined for each point in development and the mean values plus or minus the standard deviation are reported.

For tracer experiments, injections of tripalmitin-1-C¹⁴ (Nuclear Chicago, Chicago) were made laterally in the abdomen of last-instar larvae with a sharpened microliter syringe. Each injection of 0.5 μ l in volume consisted of enough labeled triglyceride in a cotton-seed oil carrier (Wesson Sales) to produce 100,000 counts/min. Total lipids were extracted from newly emerged adults (0–12 hr old) as above and fractionated by thin-layer chromatography on silica-gel plates (Merck), using a solvent system of petroleum ether-diethyl ether-glacial acetic acid, 85:15:1 v.v.v. The area of silica gel containing each fraction was scraped into a funnel plugged with glass wool and neutral lipids were eluted with chloroform/methanol, 2:1 v.v. The recovery of known amounts of lipid averaged 93 percent. Counting was done on a liquid scintillation spectrometer using a scintillation fluid consisting of 5 g. PPO, 0.3 g dimethyl POPOP, and toluene to 1 liter.

RESULTS

The neutral lipid extract was fractionated into mono-, di-, and triglyceride, free fatty acid, sterol, sterol ester, and the hydrocarbon fractions on the chromatography media. This is the same as reported for various other insects (Gilby, 1965; Gilbert, 1967), and the same as found in the Indian-meal moth embryo

(Yurkiewicz and Oelsner, In press). No qualitative differences were found between male and female insects.

Neutral-lipid changes during development are shown in table 1. In the last instar, male larvae contain slightly more total neutral lipid and triglyceride per gram live weight than do females. Day 12 is a period of wandering and non-feeding, and coincides with a drop in triglyceride content in both sexes. A significant difference in lipid metabolism occurs during the pupal period. The female catabolizes much more of its triglyceride reserve from day 13 to day 19 and, as a result, newly emerged adult females at day 20 have only about half as much triglyceride per gram live weight as do males. During the first three days of adult life, there is an almost equal rate of triglyceride catabolism in both sexes, with the male utilizing 14.1 mg per gram live weight and the female utilizing 12.3 mg. Because the adults were not in contact with any food source, feeding was not a variable needing to be considered. If the data in table 1 are expressed as mg of lipid per gram of lipid-free dry material remaining after extraction, the conclusions derived are not significantly different.

TABLE 1
Neutral lipid content during development of P. interpunctella

Stage	Age (days)	Mg. lipid per g live weight						
		Mono-Glyceride	Di-Glyceride	Tri-Glyceride	Free Fatty Acid	Sterol	Sterol Ester	Hydro-carbon
Larva (last instar)	9	♂ 1.4±0.3*	4.1±0.7	145.5± 8.7	1.6±0.8	0.8±0.6	0.5±0.2	<0.1
		♀ 1.7±0.4	4.2±0.5	135.3±10.1	1.4±1.2	1.0±0.5	0.4±0.2	<0.1
	11	♂ 0.8±0.3	2.4±0.3	168.0±10.2	0.9±0.6	0.6±0.5	0.2±0.1	<0.1
		♀ 0.9±0.2	2.1±0.2	157.7± 7.5	1.0±0.4	0.8±0.2	0.3±0.1	<0.1
	12	♂ 1.0±0.5	2.7±0.5	153.5± 5.9	1.0±0.5	0.8±0.3	0.3±0.2	<0.1
		♀ 1.2±0.3	2.5±0.5	141.4±11.8	1.5±1.2	1.0±0.5	0.3±0.1	<0.1
Pupa	13	♂ 1.1±0.3	2.6±0.3	151.2±12.6	1.3±0.7	1.0±0.2	0.2±0.1	<0.1
		♀ 1.5±0.4	3.1±0.5	143.3± 7.8	1.8±1.0	1.4±0.7	0.4±0.2	<0.1
	16	♂ 1.4±0.3	2.9±0.7	154.5±15.2	1.2±0.2	1.1±0.5	0.4±0.3	<0.1
		♀ 1.5±0.2	4.0±0.9	122.7±12.8	2.2±0.7	2.0±0.3	0.3±0.1	<0.1
	19	♂ 1.6±0.5	4.1±1.1	140.0± 9.8	1.7±1.0	0.8±0.4	0.3±0.1	<0.1
		♀ 2.0±0.2	7.3±1.8	87.9±10.7	4.2±1.6	1.7±0.1	0.5±0.2	<0.1
Adult	20	♂ 1.9±0.6	3.3±0.8	143.1±16.1	1.5±1.2	1.1±0.4	0.4±0.3	<0.1
		♀ 2.7±0.5	8.2±2.2	74.5± 8.8	3.5±0.8	1.5±0.3	0.3±0.2	<0.1
	23	♂ 1.7±0.4	3.5±0.2	129.0±10.4	2.0±0.6	0.7±0.3	0.3±0.1	<0.1
		♀ 2.3±0.7	7.8±2.3	62.2± 6.7	3.3±1.2	1.6±0.4	0.4±0.2	<0.1

*Mean ±standard deviation.

The data from the tracer experiments support the finding of a higher rate of triglyceride catabolism in the female pupa. Based on six replicate experiments, 62±6 (S.D.) percent of the injected label from tripalmitin-1-C¹⁴ was recovered in the lipid extracts of newly emerged adult males, while only 38±4 percent of the label was found in females. In every case more than 92 percent of the recovered label was still in the triglyceride fraction. Insignificant amounts of label were recovered in carbohydrate extracts or in the material remaining after lipid and carbohydrate extraction, thus eliminating the possibility of any extensive conversion of lipid to non-lipid components.

DISCUSSION

Neutral lipid metabolism during development of *Plodia interpunctella* is similar to reports for several other Lepidoptera in that (1) the male larva has a slightly higher lipid content, (2) the female catabolizes much more lipid during the pupal

period, and (3) the adult female has a much lower lipid content than does the male. This paper indicates that triglyceride is the major neutral lipid fraction catabolized by the female during the pupal period. The adaptive value for sexual dimorphism of lipid metabolism in Lepidoptera is not clear. Domroese and Gilbert (1964) state that the pupal-adult transformation of male *Hyalophora cecropia* is characterized by a lipid-sparing metabolism. They found a low respiratory quotient in females during most of this period, suggesting that females catabolize appreciable lipid, whereas males must utilize other substrates, which allows them to conserve their lipid store for adult life. Gilbert and Schneiderman (1961) attempted to determine, from experiments on castration and implantation of gonads, if egg development were the cause of the greater utilization of lipids in the female pupa of *H. cecropia*. Their data indicate that egg development only partly explains the difference in lipid metabolism. They propose that, because there is also a dimorphism in lipid content in the larvae, a genetic difference may be in evidence. More recently, Chino and Gilbert (1965) found that, during pupal-adult development of *H. cecropia*, there was no appreciable difference in the incorporation or breakdown of palmitate, acetate, or glucose between males and females, although a difference would be expected if the male were to have a lipid-sparing metabolism.

Perhaps further experiments involving the metabolism of labeled carbohydrates and amino acids in several different lepidopteran species could provide the answers, because present data do not yet allow an explanation for the sexual dimorphism in lipid utilization in Lepidoptera.

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