Lipid Metabolism in the Mediterranean Meal Moth *Ephestia kühniella* Zeller During Its Life Cycle.[1]

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Introduction

The nature of the fuel reserves required for embryogenesis, metamorphosis and flight metabolism in many insect orders has been relatively well elucidated (14, 20). On the other hand fewer workers have investigated the energy reserves required for embryogenesis (2) and flight metabolism (3, 22) in the Lepidoptera, and those concerned with the relationship between the energy expended during the post-embryonic phase of the life cycle and the organic fuels catabolized during this phase have been indeed sparse. In this paper the Mediterranean meal moth, *Ephestia kühniella* Zeller, is studied. By the analysis of total lipids, non-esterified and total fatty acids, together with manometric determination of respiratory quotients, meal moth metabolism during development could be characterized.

Materials

Mediterranean meal moths were reared according to the directions of Whiting (19). The insects were fed degerminated yellow corn meal consisting of water, 12%; protein, 8.3%; fat, 1.2%; ash, 0.5%; carbohydrate, 78%; and fiber, 0.7% (13). Insects were kept in a constant temperature cabinet at 24 ±1° C. Relative humidity was maintained at 50-60 percent.

No attempt was made to use a genetically homogeneous meal moth population in this study. This heterogeneity may have been responsible for the considerable variations existing between the chronological age of the insects and their physiological and morphological development. Other investigators (cf. 10) have also noted an erratic time sequence of meal moth development despite the fact that external factors (temperature, light, relative humidity, population density, and availability of food) were carefully regulated. Therefore, the larvae of the insects were arbitrarily divided into stages according to fresh weight (4, 7).

Non-feeding prepupae were detected by the presence of an external cocoon and especially by the white color of the abdomen following complete elimination of fecal material (12). Pupae were considered as a single developmental stage. Adults were studied on the first, second, and ninth day after emergence.

Methods

Larval, pupal, and adult respiration were followed for periods of one hour in Warburg constant volume respirometers having a capacity of about 7 ml. The exact volume of the flasks containing insects and, in

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the case of larvae, their corn meal diet, was determined by the method of Hoopingarner and Beck (11). Data on oxygen consumption and carbon dioxide production were calculated by the "total uptake method" (18). All experiments were carried out at 25° C. in a constant temperature water bath. Animals at various stages of development were anesthetized with carbon dioxide (21), weighed on a Roller-Smith precision balance, and then placed in the main compartments of the respirometers; six vessels, not including the thermobarometer, were set up, three with 0.2 ml. of 10% KOH in the center wells and three without KOH in the wells. The average oxygen consumption calculated for the former was used in determining the CO_2 production of the latter. By comparison of these two parameters respiratory quotients were obtained which enabled the proportion of calories derived from fat and carbohydrate to be determined (6). The calorific values in Carpenter's Table were applied directly to the measured respiratory exchange without separately computing the protein catabolized.

Determination of changes in water concentration during development were made by weighing the insects before and after complete drying at 80° C. Batches of desiccated insects were subsequently used for lipid determination. Lipid, estimated as the total petroleum ether extractable material, included neutral fats, fatty acids and other ether-soluble material (8). The dried organisms were kept at 28° C. for 48 hours in tubes with 5 ml. of petroleum ether which was changed several times. They were then dried and reweighed. Thus, the weight of ether-soluble material was given by subtraction and not by direct weighing of the extract. Extraction for longer periods of time did not increase the amount of ether-soluble material.

Analyses for esterified (EFA) and non-esterified (FFA) fatty acids were carried out on larval and prepupal hemolymph. The insect was punctured with a glass needle and the fluid collected was added immediately to an extraction mixture: for EFA the extraction mixture was ethanol-ether (3:1); for FFA it was 1N H_2SO_4 (0.1 vol.), heptane (1 vol.) and isopropyl alcohol (4 vol.). Esterified fatty acids were determined by the method of Stern and Shapiro (16) using triacetin as the standard. Dole and Meinertz' (9) method, slightly modified, was used for the microdetermination of non-esterified fatty acids; recrystallized palmitic acid was the standard. In both analyses, EFA and FFA, 10-40 μl of hemolymph gave reproducible results.

Results

At 25° C. and 50-60 pecent relative humidity the weight range of the meal moth is from 0.021 mg. for the egg to about 27 mg. for the late sixth instar larva (Table I). The earliest larval stage weighs little more than the egg but weight gain is rapid, the early instars almost doubling their weight between molts. Non-feeding prepupae lose weight, as do the non-feeding pupae and adults. From the first day of emergence until the ninth day, both male and female adults lose 50 percent of their fresh weight; females, at all times, weigh more than males of corresponding age.

TABLE I

Changes in Weight and Water Concentration During the Meal Moth Life Cycle.

Stage	Number of Insects	Weight Average Weight per Insect mg.	Average Water Concentration No. of expts.	% wet weight
Egg	715	0.021	1	69.5
Larva				
Hatchling	45	0.025	1	62.5
Instar 1	21	0.080	1	61.7
Instar 2				
Early	62	0.256	2	65.5±2.21[1]
Late	88	0.752	1	69.1
Instar 3	82	1.94±0.013[1]	5	69.4±1.87
Instar 4				
Early	32	3.52±0.195	3	69.5±2.92
Late	31	5.43±0.174	3	74.6±1.03
Instar 5				
Early	75	12.27±0.321	14	68.9±0.505
Middle	35	16.37±0.482	11	67.3±1.33
Late	44	19.09±0.187	13	66.8±0.878
Instar 6				
Early	68	20.93±0.300	4	66.9±0.364
Late	10	26.81±0.480		
Prepupa	13	19.45±0.308	7	66.3±0.538
Pupa	32	15.31±0.806	15	68.4±1.83
Adult				
1 day old				
virgin female	3	13.39	1	68.0
male	3	9.11	1	69.2
2 day old				
female	3	9.30	1	65.7
male	3	7.04	1	56.3
9 day old				
female	3	6.04	1	63.1
male	3	4.88	1	61.1

[1] Standard error of the mean.

The average water concentration of the meal moth is rather constant throughout the life cycle (Table I), varying from about 66-69 percent of the fresh weight. Only the two earliest larval instars, the two-day-old adult male, and the nine-day-old adult male and female show values considerably lower than these.

Table II illustrates the changes in oxygen consumption and carbon dioxide production during the life cycle of the meal moth. The QCO_2 of the early fifth instar larva is relatively high, having a value which is about four times that of the prepupa and seven times that of the pupa. A similar relationship between the early fifth instar larva and the prepupa and pupa is noted for the QO_2. For both metabolic parameters the gas exchange values of the other developmental stages are not significantly different from each other.

TABLE II

Oxygen Uptake, Carbon Dioxide Production, Respiratory Quotients, and Approximate Contribution of Carbohydrate and Fat to the Total Amount of Energy Transformed During the Meal Moth Life Cycle.

Stage	No. of expts.	Gaseous Exchange CO_2[1]	Gaseous Exchange O_2[1]	Respiratory Quotient	Energy Derivation Fat %	Energy Derivation Carbohydrate %
Larva						
Instar 4	1	3.91[2]	3.62	1.08		
Instar 5						
Early	16	3.63 ± 0.42[3]	4.23 ± 0.61	0.906 ± 0.027	30.6	69.4
Middle	25	2.19 ± 0.24	2.56 ± 0.29	0.858 ± 0.023	47.6	52.4
Late	29	1.73 ± 0.15	1.97 ± 0.17	0.888 ± 0.019	37.4	62.6
Instar 6						
Early	14	2.14 ± 0.42	2.50 ± 0.46	0.841 ± 0.012	54.4	45.6
Late	10	1.51 ± 0.19	1.59 ± 0.17	0.941 ± 0.040	20.4	79.6
Prepupa	15	0.792 ± 0.07	1.10 ± 0.13	0.720 ± 0.018	95.2	4.8
Pupa	16	0.520 ± 0.04	0.640 ± 0.07	0.782 ± 0.033	74.8	25.2
Adult	3	1.62 ± 0.15	1.90 ± 0.16	0.854 ± 0.041	51.0	49.0

[1] μl./mg./hr.

[2] Mean

[3] Standard error of the mean

Respiratory quotients are also given in Table II. The value for a single determination on fourth instar larvae is 1.08. Fifth and sixth instar larvae and the adults do not differ significantly. The prepupae and pupae, however, have considerably lower respiratory quotients, 0.720 and 0.782, respectively. Thus, from Carpenter's (6) Table 13, which gives the approximate proportion of calories derived from carbohydrate and fat for each respiratory quotient (Table II), it would seem that the prepupae and pupae draw most heavily on lipids as a source of energy.

The absolute lipid content of individual meal moths increases steadily during development until the sixth instar, being then followed by a decrease in the non-feeding prepupae, pupae, and adults (Table III).

TABLE III

Changes in Petroleum Ether-Soluble Material During the Meal Moth Life Cycle.

Stage	No. of expts.	Ether-Soluble Material mg./animal	% dry wt. (mean±S.E.)	% wet wt. (mean±S.E.)
Larva				
Instar 1	1	0.004	13.0	4.8
Instar 2				
Early	2	0.022	24.60±0.142	8.50±1.06
Late	1	0.033	17.80	5.52
Instar 3	4	0.099	18.53±3.35	5.97±1.62
Instar 4				
Early	2	0.311	24.15±3.35	7.86±2.48
Late	3	0.362	24.22±2.52	6.39±0.384
Instar 5				
Early	13	1.58	44.86±2.76	13.09±1.74
Middle	10	2.49	48.46±2.11	15.11±1.19
Late	12	2.49	49.44±1.50	17.33±0.690
Instar 6	4	3.78	47.80±2.98	15.91±1.22
Prepupa	7	2.56	38.16±1.69	12.94±0.678
Pupa	10	2.27	43.02±2.06	14.84±1.05
Adult				
1 day old				
virgin female	1	1.67	39.4	12.6
male	1	1.24	44.00	13.6
2 day old				
female	1	2.11	43.6	22.7
male	1	1.35	44.2	19.3

Instars 2, 3, and 4 have essentially the same percentage of total lipid. This percentage is doubled in Instars 5 and 6, in the pupa, and in the adults.

The esterified fatty acid concentration (Table IV) of the hemolymph of fifth and sixth instar larvae, 442 mg% and 392 mg%, respectively, is not significantly different, while that of the prepupa reaches a considerably higher level (820 mg%). Free fatty acids increase less markedly from the fifth instar larva to the prepupa; the concentration in the latter, however, is significantly different from that of the former.

TABLE IV

Changes in Hemolymph Free and Esterified Fatty Acids During the Meal Moth Life Cycle.

Stage	Esterified Fatty Acids mg. % No. of expts.	(mean ± S. E.)	Free Fatty Acids mg. % No. of expts.	(mean ± S. E.)
Instar 5	2	442.50 ± 76.50	5	68.82 ± 6.02
Instar 6	12	392.08 ± 34.01	1	90.80
Prepupa	4	820.25 ± 53.00	4	108.51 ± 16.32

Discussion

In developing insects changes in blood composition reflect the morphogenetic and biochemical transformations taking place in the tissue (5). Arnold (1), the only other worker that we are aware of who reported on blood lipids in relation to developmental processes in *Ephestia kühniella,* noted that the concentration of hemolymph lipid was low in the early larval stages; lipid concentration increased as pupation approached, reaching a maximal value in the prepupal period. Our data (Table IV) on EFA and FFA values in developing insects substantiate these findings. Feeding meal moths show relatively low hemolymph FFA and EFA concentrations, while non-feeding—or starving—animals (prepupae) show higher concentrations. Both FFA and EFA have been implicated as the transport form of fatty acids; hence, their concentration should rise when the starving insect is called upon to utilize lipid as its primary energy source. Arnold (1) further found that after the onset of pupation the lipid content of the hemolymph declined rapidly. His data suggest, as do ours, that *Ephestia* can synthesize fats from dietary carbohydrates and/or protein. Our animals were maintained on a diet consisting of 78 percent carbohydrates, 8.3 percent protein and only 1.2 percent fat. Still, the evidence indicates that meal moth larvae stored lipid for oxidation in the advanced developmental stages, the prepupae and pupae. Stages other than the prepupae and pupae oxidized both lipid and carbohydrate, with the greater proportion of energy being derived from the latter.

Total lipids constitute over 40 percent of the dry weight of Instars 5 and 6 and about 20 percent of the dry weight of Instars 2, 3, and 4. [Scroggin and Tauber (15), reporting on Timon-David's data for an unspecified larval instar of *Ephestia figuilella,* noted that 21.4 percent of the wet weight of this species was lipid; lipids account for 4.8 to 17.33 percent of the wet weight of *E. kühniella.*] About 43 percent of the pupal dry weight was lipid; in the prepupa the corresponding value was 38 percent.

The average Q_{O_2} for the inactive pupa was 0.640 μl./mg./hr., while the Q_{CO_2} was 0.520 μl./mg./hr. Using single meal moth pupae Taylor (17) measured oxygen consumption from 30 hours after pupal case

formation until the emergence of the adults (about 260 hours at 25° C.). He noted the typical U-shaped curve for pupal oxygen consumption, with a minimum Q_{O_2} of from 0.25 to 0.35 μl./mg./hr. for males and females to 0.700 to 0.800 μl./mg./hr. for males and 0.850 to 1.000 μl./mg./hr. for females. Thus, the Q_{O_2} of 0.640 μl./mg./hr. reported in this paper, while not taking into consideration developmental variation, represents an average pupal oxygen uptake which is in agreement with Taylor's (17) data.

The relation between CO_2 produced and O_2 consumed represents only the summation of the processes of synthesis and breakdown that are going on throughout metamorphosis. Hence, such analyses of the whole animal should not be interpreted uncritically. Still the R.Q. values of about 0.9 noted in meal moth larvae probably indicate that the bulk of the energy for development was derived from carbohydrates, with a lesser contribution from lipid; this is consistent with the efficient utilization of corn meal. Non-feeding prepupae and pupae, on the other hand, show a marked lowering of the R.Q. to 0.720 and 0.782, respectively, indicating increased oxidation of substantial amounts of lipid. Finally, the adult respiratory quotient of 0.854 may represent a mixed catabolism.

Summary

1. The average water concentration of the meal moth was rather constant throughout the life cycle, varying from 66 to 69 percent of the fresh weight.

2. The rates of oxygen consumption and carbon dioxide production followed similar U-shaped curves. Throughout larval development respiratory exchange, initially relatively high, declined, reaching a minimum when activity ceased during the last larval, or the prepupal, period. The rate then dropped further in pupal life, rising again to an adult level that was near the level attained by the early sixth instar larva.

3. The earliest larva studied had an R.Q. of 1.08. The prepupal and pupal respiratory quotients, 0.720 and 0.782, respectively, were considerably lower than the other stages observed.

4. Instars 2, 3, and 4 contained essentially the same percentage of ether-soluble material (about 20 percent of the dry weight); this value was doubled in Instars 5 and 6, in the pupa, the prepupa, and in the adults.

5. The esterified fatty acid content of the hemolymph of fifth and sixth instar larvae, 442 mg% and 392 mg%, respectively, was not significantly different, while that of the prepupa reached a considerably higher level.

6. Free fatty acids increased less markedly than EFA from the fifth instar larva to the prepupa.

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