J. Biosci., Vol. 1, Number 1, March 1979, pp. 27–33. © Printed in India.

Changes in carbohydrates and lipids during embryonic development of *Antheraea mylitta* (Lepidoptera)

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MS received 29 May 1978; revised 23 August 1978

Abstract. Changes in carbohydrate and lipid metabolism during embryonic development in *Antheraea mylitta* were studied. While carbohydrates were metabolized during early embryogenesis, lipids were catabolised at the later stages. A significant increase in both total carbohydrates and glycogen on days 5 and 6 suggested the concurrent occurrence of both gluconeogenesis and glycogenesis. As the development of the embryo proceeds, both lipids and carbohydrates were utilised, resulting in the increase in the concentration of citrate, pyruvate and lactate.

Keywords. Carbohydrates; lipids; metabolism; embryonic development; *Antheraea mylitta.*

Introduction

Despite several studies made on insects during periods of high energy demands like growth, locomotion, flight and metamorphosis, (Sacktor, 1965; Stevenson, 1969; Crompton and Birt, 1967), there is still dearth of information on energy requirement during embryogenesis for histogenesis of larval structures. Therefore, it was considered worthwhile to study some of the metabolites and enzymes of carbohydrate and lipid metabolism in the developing cleidoic egg of *Antheraea mylitta.*

Materials and methods

Preparation of homogenate of A. mylitta eggs

A. mylitta pupae were procured from Central Tassar Research Station, Ranchi, Bihar, and hatched in the laboratory. The fertilised eggs were collected, pooled, labelled with dates of oviposition and maintained at 27 ± 2° C in petri dishes kept in wire-netted wooden boxes.

Eggs (weighed, cleaned with distilled water and chilled) were homogenised in a pre-cooled Potter-Elvehjem type of homogeniser to 10% (w/v) tissue concentration. The homogenate was strained through nylon cloth to remove egg shells and was employed for the estimation of metabolites and for enzyme assays.

Estimation of metabolites and enzyme activities

Lipids were extracted and fractionated according to Pant *et al.*, (1973). Lipase (glycerol trioleate hydrolase, E.C. 3.1.1.3) activity was assayed by the method of Cherry and Crandall (1931) with slight modification. The free fatty acids liberated were estimated by Novak's method (1965).

Phosphorylase (E.C. 2.4.1.1). activity was assayed according to Green and Stumpf, (1942) as modified by Srivastava and Krishnan (1961). The liberated phosphate was estimated by Fiske and Subbarow's method (1925). Glycogen was isolated as described by Wiens and Gilbert (1967) and estimated according to Carrol *et al.* (1956). Total carbohydrates and reducing sugars were estimated by the method of Trevelyan and Harrison (1952) and Somogyi (1945), respectively.

Citrate, pyruvate and lactate were determined by the method of Weil-Malherbe and Bone (1949), Friedman (1957) and Barker and Summerson (1941), respectively. Total hexosamines were estimated colorimetrically (Elson and Morgan, 1933).

All assays were carried out in duplicates on three individual homogenates from 500 eggs each. Although a variation of 5–14% was observed among these three batches, the pattern of change with embryogenesis was nearly, the same in all the batches. The range of values are indicated in the figures.

Results and discussion

Total carbohydrates during embryogenesis of *A. mylitta* declined by about 72% through embryonic development till hatching on day 8 (figure 1). About 60%

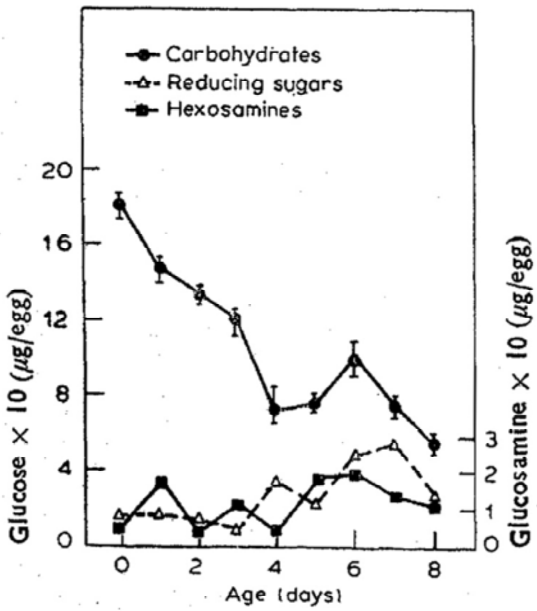

Figure 1. Total carbohydrates, reducing sugars and hexosamines during embryonic development of *A. mylitta.*

is utilised during early stages (days 0–4) while 12% is utilised from day 6 onwards. Reducing sugars with an initial low concentration, increase gradually during later developmental stages of the embryo (figure 1). Similar results have been reported during embryogenesis of *Philosamia ricini* (Lacy, 1967) and other insects (Urbani, 1959). The fluctuations in hexosamines all through embryogenesis (figure 1) suggests their continuous formation and utilisation for the synthesis of polysaccharides which are involved in the formation of tissues like mandibular teeth, bristles and cuticular chitin. Total lipids were depleted by about 60% during days 3–7 (figure 2). This suggests that lipid catabolism occurs mainly during later developmental stages (histogenesis, tissue differentiation and growth) whereas the catabolism of carbohydrates is more during early development of the embryo (blastokinesis and gastrulation).

Total lipids and neutral lipids (77–88% of the total lipids) vary more or less alike during embryonic development and are present at the highest concentration on day 3 (figures 2 and 3). Occurrence of such high concentration of neutral lipids with subsequent gradual depletion through days 4–7 suggests that lipids may be serving as one of the energy sources (Allias *et al.*, 1964; Kinsella and Smyth, 1966).

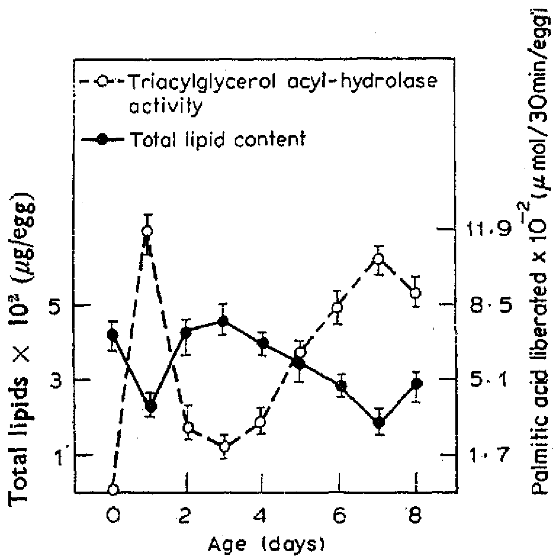

Figure 2. Total lipid content and lipase activity during embryonic development of *A. mylitta.*

The changes in free fatty acids (figure 3) and lipase activity (figure 2) follow a similar pattern. A significant decrease in free fatty acids and total carbohydrates with concurrent increase in total and neutral lipids suggests that lipogenesis may be taking place at the expense of carbohydrates during days 2, 3 and 8.

The amount of phospholipids remain essentially unchanged up to day 5 and gradually increase up to day 7 and then decrease on day 8. This suggests that the increase in phospholipids meets the requirements for the formation of cellular and subcellular structure (Allias *et al.,* 1964).

Changes in lipase activity observed during embryogenesis (figure 2) show significant utilisation of triglycerides during initial and final stages of development

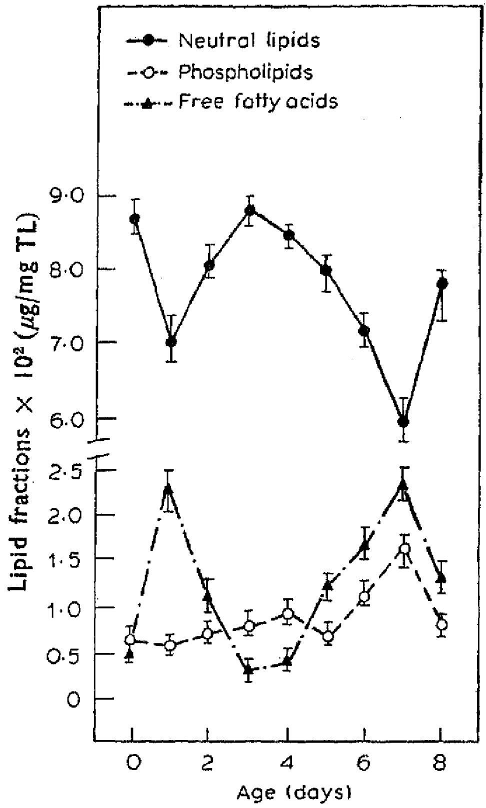

Figure 3. Free fatty acids, neutral lipids and phospholipid content during embryonic development of *A. mylitta*. TL–total lipids.

(days 1 and 4-8). This is in agreement with the observations made in *P. ricini* (Pant and Nautiyal, 1974a) and other insects (Allias *et al.*, 1964). However the absence of lipase activity in the freshly laid egg is contrary to the previous findings (Pant and Nautiyal, 1974a).

Glycogen content decreased gradually during early embryogenesis through days 0–4 and increased during days 5 and 6 and once again decreased on days 7 and 8 (figure 4). The utilisation of glycogen for various metabolic as well as for physiological functions such as energy source and substrate for chitin formation has been well recognised during development of insects (Wyatt, 1967) . The simultaneous increase both in total carbohydrates and glycogen between days 4 and 6 suggests concurrent occurrence of both gluconeogenesis and glycogenesis. High activity of both alanine and aspartate aminotransferases and proteases accompanied with significant protein depletion with no simultaneous appreciable increase in free amino acids has also been observed (Pant and Kumar, 1979) This suggests the possibility of the synthesis of carbohydrates at the expense of glycogenic amino acids. However, the simultaneous depletion of total and neutral lipids is rather intriguing in view of the controversy regarding the conversion of lipids to carbohydrates (Boell, 1935; George and Nair, 1964; Ludwig *et al.,* 1964).

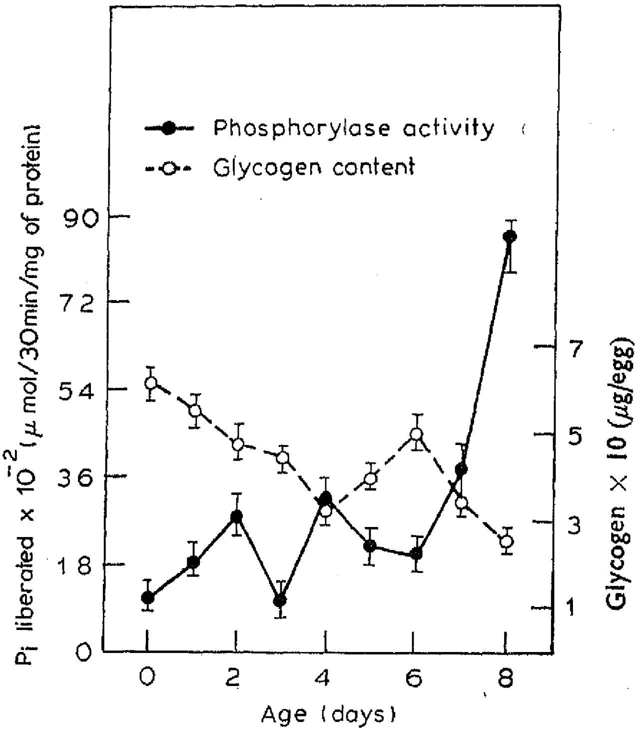

Figure 4. Active phosphorylase activity and glycogen content during embryonic development of *A. mylitta.*

Phosphorylase activity varies generally in concordance with glycogen content except on day 3 (figure 4). High phosphorylase activity with a relatively low glycogen level at the tail end of embryogenesis could be attributed to its participation in cuticular chitin synthesis, after day 6 (Pant and Nautiyal, 1974b).

Pyruvate concentration gradually increased with intermittent fluctuations prior to hatching (figure 5). Lactate concentration did not appreciably change upto day 3, increased up to day 6 and decreased thereafter. However, citrate concentration steadily increases with development till emergence of larva (figure 6). This is in agreement with the findings in *P. ricini* (Pant and Sharma, 1965).

The main energy source for growth and development in the cleidoic egg is usually lipids which provide about 66% of the total energy requirement (Farkas, 1903). In some insects (Urbani, 1959; Allias *et al.,* 1964; Kinsella and Smyth, 1966; Lacy, 1967) as in the present case, marked depletion of both lipids and carbohydrates occur. This suggests that as development of the embryo proceeds both lipids and carbohydrates are utilised resulting in the elevation of citrate, pyruvate and lactate, the main metabolic products of carbohydrates and fat metabolism.

In mammalian liver, citrate has been reported to play an important role in the regulation of glycolysis via inhibition of phosphofructokinase (E.C. 2.7.1.1) and in lipogenesis via activation of acetyl coenzyme A carboxylase(E.C 6·4.1.2., Underwood and Newsholme, 1967; Harper *et al.,* 1977). Although the role of citrate in the regulation of glycolysis is yet to be established in insects,

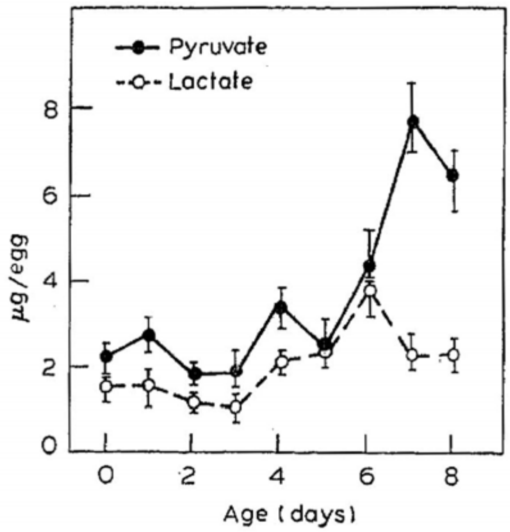

Figure 5. Pyruvate and lactate level during embryonic development of *A. mylitta*

the relatively high citrate content observed during later stages of embryogenesis of *A. mylitta* suggests its role in the regulation of glycolysis. Cuticular chitin formation occurs just before hatching; citrate probably diverts utilisation of carbohydrates for chitin synthesis rather than for production of energy.

Patterson (1956) reported the maximal accumulation of citrate at the time of minimal utilisation of oxygen in the *Tenebrio molitor* pupa and its decrease at the time of increased oxidative metabolism. In view of this, studies on the relationship between citrate content and the rate of oxygen utilisation during embryogenesis of this insect may be worth undertaking.

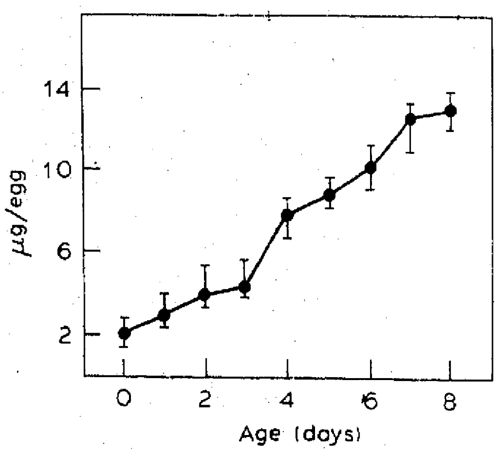

Figure 6. Accumulation of citrate during embryonic development of *A. mylitta*,

Acknowledgement

This research has been financed in part by a grant made by the United States Department of Agriculture, Agricultural Research Service, under PL 480.

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