Proc. Indian natn. Sci. Acad. **B51** No. 5 pp 461-467 (1985)

Ontogenetic Variations in Carbohydrate, Lipid and Protein Contents in *Trabala vishnou* Lef. (Lepidoptera: Insecta)

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(Received 15 October 1984; after revision 19 February 1985)

Day-to-day quantitative changes in carbohydrates, lipids and proteins contents during embryonic and post-embryonic development of *Trabala vishnou*, as also, the age and sex-related changes in these biochemical constituents have been studied. The female larvae, pupae and adults showed higher contents of carbohydrates and proteins as compared to their male counterparts. However, lipid contents were more in the various developmental stages of the male than of the females. The concentrations of all the biochemical constituents examined were more in the pre-moult than in the post-moult period. The carbohydrates, lipids and proteins, accumulated during the larval stages, were utilized during metamorphosis. The relationship between the quantitative changes of carbohydrates, lipids and proteins and various physiological and morphological events associated with development have been discussed.

Key Words : *Trabala vishnou* **Insecta Ontogenic variations, Carbohydrates, Lipids, Proteins**

Introduction

Studies on carbohydrates, lipids and proteins during insect development could indicate the level of activity and energy requirements during a given phase of development. The role of various biochemical constituents during insect development has been reviewed by Chen (1971). Most of the earlier studies pertain to a few arbitrarily chosen stages, like egg, mature larva, pupa and adult and further the studies on the sex-specific differences in these biochemical constituents have been restricted mainly to the adult stage.

In the present investigation, day-to-day changes in reducing carbohydrates, lipids and soluble proteins have been studied during the whole process of ontogeny of *Trabala vishnou*. In addition, the sex-specific differences in these biochemical constituents at larval, pupal and adult stages have also been studied. We have tried to correlate the chemical body composition to such phenomena as embryogenesis, acdysis, spinning, adult emergence, egg-laying and mating, etc.

Materials and Methods

The culture of *T. vishnou* larvae was maintained in an incubator on the leaves of castor, *Ricinus communis* at 28±2°C and 70% RH.

All the developmental stages of known chronological age were taken for analysis and were dried at 80°C to constant weight (48 hr), kept in a desiccator for 24 hr and weighed. No morphological distinctions were present to differentiate sex in the larval stages. However, in the final phase of Vth instar, larvae were sexed on the basis of their live weight (Gakhar 1983).

Total reducing sugars were estimated from ethanolic extract with the help of Dreywood's anthrone reagent using glucose as a standard (Morris 1948). For the quantitative determination of lipids, known dry weights of various stages were extracted with chloroform : methanol (2 : 1 v/v) for 24 hr (Folch et al. 1957). The proteins were determined by the method of Lowry et al. (1951).

All the data were subjected to students' 't' test to determine statistical significance.

Results and Discussion

Changes in the concentration of carbohydrates, lipids and proteins during the various stages of development are shown in figures 1–4.

Reducing Carbohydrates

The concentration of reducing sugars declined up to Day-3 by 33.8% ($P<0.01$) of embryonic development. It more than doubled on Day-4. Thereafter, it again declined continuously by about 75% ($P<0.01$). The breakdown of yolk reserve, which actively occurs during early embryogenesis requires utilization of carbohydrates, their depletion occurs initially (Chen 1971). The breakdown of yolk, however, results in the release of several products which, in turn, increase the carbohydrates on Day-4. The subsequent decline of carbohydrates may be related to the requirement of glucosamine in the formation of chitin for the pharate larva besides normal metabolic requirement (Chefurka 1965).

During the course of every larval instar, the sugar contents were more in the final phase of preceding instar as compared to the initial phase of the succeeding instar ($P<0.01$). The possible reason may be that carbohydrates are used for chitin synthesis or converted into reserve carbohydrates like trehalose or glycogen. Chang et al. (1964) observed a reverse correlation between the reducing carbohydrates and glycogen. Moreover, Tate and Wimer (1974) advocated that the soluble carbohydrates which accumulate in the larval stages, are utilized during metamorphosis.

Domroese and Gilbert (1964) on *Hyalophora cecropia* and Campbell et al. (1976) on *Sitophilus* suggested that the respiratory quotient (RQ) was more in the larval stages than in the pupal and the adult stage. It was, therefore, suggested that the larval stages use reducing carbohydrates as respiratory substrates, whereas, the pupal and the adult stages utilize non-carbohydrate sources (mainly lipids).

During metamorphosis, carbohydrate contents increased slightly during first four days, thereafter, it generally had a declining trend. This is in agreement with the findings of Singh and Sinha (1977). Tate and Wimer (1974) have shown that the reducing carbohydrates are utilized more for the synthesis of proteins and other carbohydrate moieties than for the process of energy production during pharate adult development.

During the adult life, carbohydrates concentration declined by about 45% in the male moth and by 70% in the female moth ($P<0.01$) up to the day before death. This may be due to active flight by the male in search of female and due to egg laying in the female. Domroese and Gilbert (1964) suggested that the adult metabolize both reducing carbohydrates and lipids.

The higher level of reducing sugars and soluble proteins in the female larvae, prepupae, pupae and adults than in the males ($P<0.01$) may be due to its necessity for

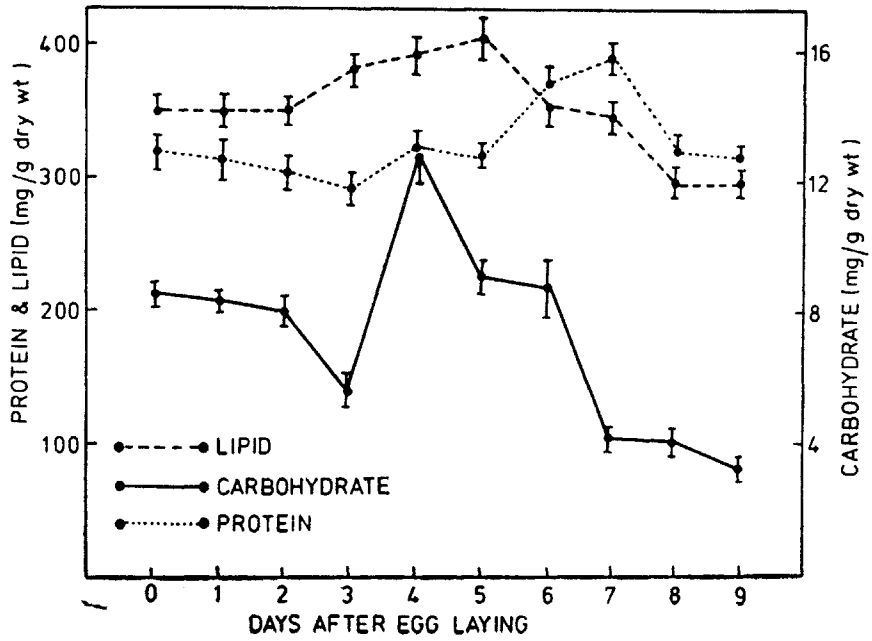

Figure 1 Changes in carbohydrates, proteins and lipids during embryogenesis of *T. vishnou*

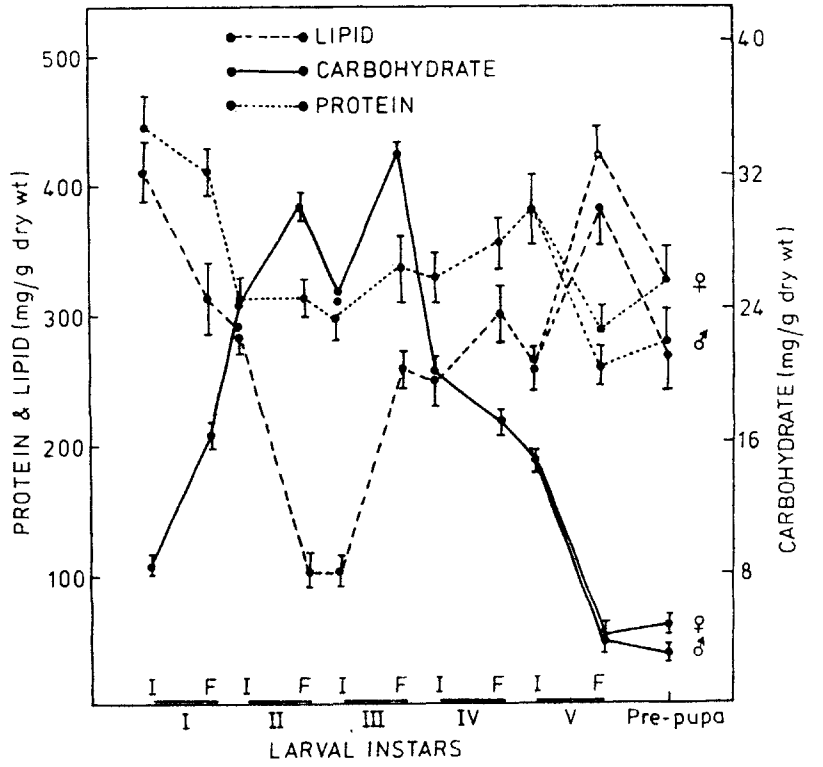

Figure 2 Changes in carbohydrates, proteins and lipids in various larval stages of *T. vishnou*

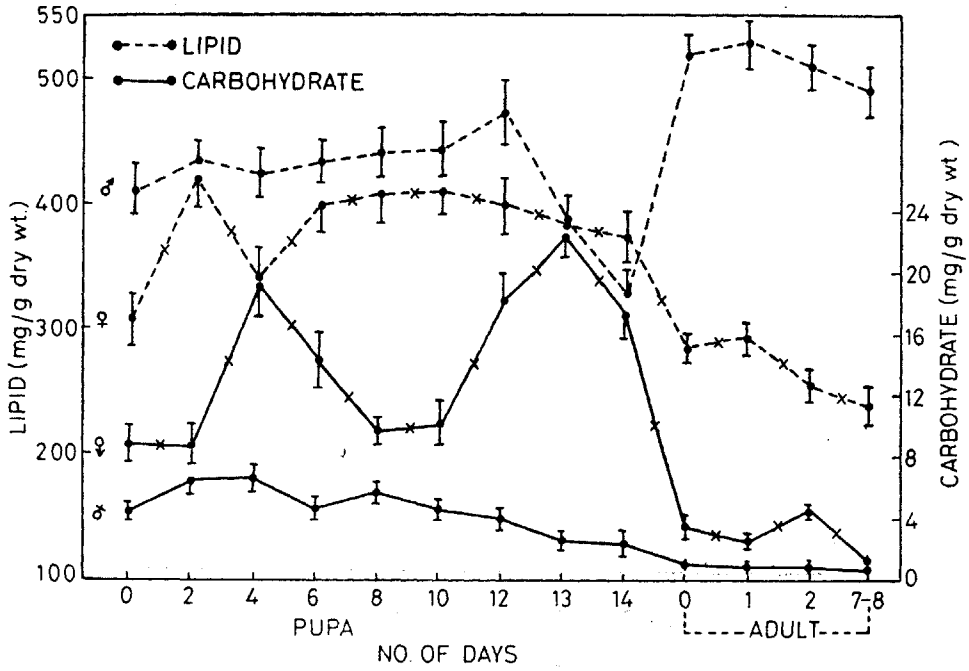

Figure 3 Changes in carbohydrates and lipids during metamorphosis and adult life of *T. vishnou*

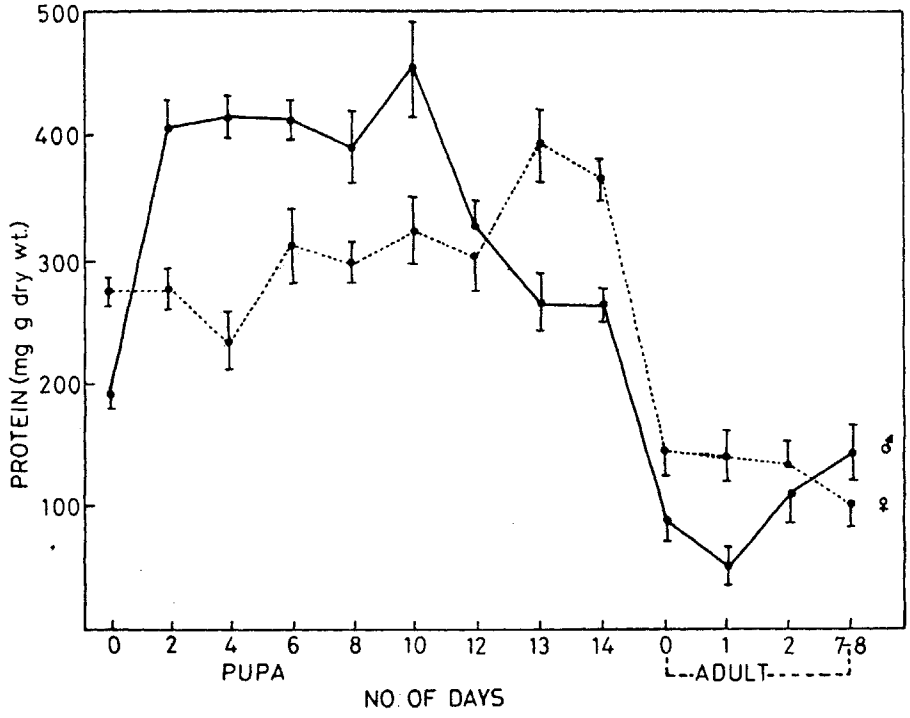

Figure 4 Changes in proteins during metamorphosis and adult life of *T. vishnou*

higher rate of metabolism owing to the maturation and development of oocyte in the female.

Lipids

The concentration of lipids increased by 15.7% ($P<0.05$) during first five days of embryogenesis. However, it declined by 25% ($P<0.01$) during the following days of embryonic development because lipids provide the primary source of metabolic energy during embryogenesis (Gilbert 1967). These findings are in agreement with those of many workers (Babcock & Rutschky 1961, Fast 1964, Gilbert 1967).

During larval growth, the lipids decreased by 75% up to the initial phase of Instar III. Thereafter, it increased by four-fold during subsequent larval instar ($P<0.01$). This is in conformity to the earlier reports of D'Costa and Birt (1966), Janda et al. (1966), Mukerji and Guppy (1973) and Singh and Sinha (1977). Such a high concentration and increase in the lipid contents during the successive larval instars could be due to its formation through inter-conversions from free amino acids, proteins and carbohydrates which show decline during this period (Wyatt 1967, McCabe & Bursell 1975, Gakhar 1983, Gakhar & Maleyvar 1984a,b). Increased concentration of lipids before moulting appears to be related with the increased rate of oxygen consumption during this phase (Gilbert & Schneiderman 1961). Declining level of lipids in the post-moult period may be associated with their utilization in the complex biochemical process related to moulting.

The results of the present study indicate a drop in lipids during pre-pupal development ($P<0.01$). This is similar in other Lepidopterans, *Hyalophora cecropia* (Bade & Wyatt 1962) and *Lambdina fascellaria* (Dunphy et al. 1977). This phenomenon is, in general, a characteristic of holometabolous insects where the energy for pupal transformation is derived from lipids, especially reserves of triglycerides accumulated during the larval stages (Fast 1964).

The quantity of lipids increased during the early pupa ($P<0.05$) which supports the findings of Pant et al. (1974) who reported that the biosynthesis of lipids takes place in the pupa but at a slower pace. Thereafter, it declined sharply in both the sexes ($P<0.01$). This may be owing to its utilization as respiratory substrate.

During the adult life, there was a continued decline in the lipid contents with ageing in both the sexes. These results are in agreement with the findings of Nowosielski and Patton (1965) in *Acheta*; Sharma and Sharma (1979) and Sidhu et al. (1980) who also reported decline in lipid contents with advancing age of Bruchids.

The reason attributed for higher concentration of lipids in the males ($P<0.01$) is said to be correlated with their mating behaviour. As the male moth has to fly great distances in search of the females, it needs more reserves (Gilbert 1967).

Proteins

The level of soluble proteins declined by about 6% ($P < 0.05$) during the first three days of embryogenesis. Thereafter, it generally increased up to Day-7 and then declined by 19.9% ($P<0.01$) on the last two days of embryonic development (figure 1). The best explanation for the decrease in the concentration of protein with the onset of embryogenesis is the rapid increase in free amino acids pool during early days of embryogenesis (Maleyvar & Gakhar 1982). There must be breakdown of yolk proteins to release free amino acids, then this is followed by the active phase of histogenesis and differentiation. So for the protein synthesis, this would use the amino acids pool and cause a rise in the amount of soluble proteins. This must be the reason for such rise in soluble proteins during Days 5, 6 and 7 of embryogenesis. It could well be coincidental that most of

the free amino acids pool appears to be mopped up during this period (Maleyvar & Gakhar 1982). A similar trend of protein use and resynthesis has been noted in *Locust* and *Sphaerodema molestum* (Indira 1963, McGregor & Loughton 1974).

The level of proteins was generally high prior to moulting in all, but the last instar ($P<0.01$). This may be due to the appearance of new proteins associated with the moulting process only (Chen 1971, Firling 1977). Declining level of all the biochemical constituents in the post-moult period could also be partly attributed to their loss in the body fluids during moulting, exuviae and faecal waste (Gakhar 1983, Gakhar & Maleyvar 1984c).

The concentration of soluble proteins broadly increased during metamorphosis. Protein synthesis has also been reported in several species with different food habits, such as *Lucila* pharate adults of *P. regina* (Dinamarca & Levenbook 1966). The soluble protein build up during pupal stages of *T. vishnou* may be attributed to the metabolism of glycogen or chitin as suggested by Tate and Wimer (1974) in case of *P. regina*.

During adult life the concentration of soluble proteins declined within 24 hr of emergence in both the sexes. Thereafter, the increase in the protein contents in male moth may be due to the process of degradation during senescence (Gakhar & Maleyvar 1984d). However, the decline in the protein contents in the female moth on Day-8 may be attributed to the process of egg laying. The female larvae, pre-pupae and adults had more quantity of soluble proteins than their male counterparts ($P<0.01$). However, no conclusion could be drawn in this regard during metamorphosis.

References

Babcock K L and Rutschky C W 1961 Lipids in insect eggs: A review with new evidence from the milkweed bug *Oncopeltus fasciatus* (Hemiptera: Lygaedae); *Ann. Ent. Soc. Am.* **54** 156–164

Bade M L and Wyatt G R 1962 Metabolic conversion during pupation of the *Cecropia silkworm*.I. Deposition and utilization of nutrient reserves; *Biochem. J.* **83** 470–478

Campbell A, Singh N B and Sinha R N 1976 Bioenergetics of the granary weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae); *Can. J. Zool.* **54** 786–798

Chang C K, Lin F and Feng H 1964 Metabolism of eri-silkworm during metamorphosis. I. The properties of trehalose and its effect on metabolism; *Acta Ent. Sin.* **13** 494–502

Chefurka W 1965 Intermediary metabolism of carbohydrates in insects; in *The Physiology of Insecta* Vol. 2 pp 581–667 ed. M Rochstein (New York: Academic Press)

Chen P S 1971 Biochemical aspects of insect development; in *Monograph in Developmental Biology* Vol. 3 ed. A Wolsky, N Y Tarrytown (Basel: Karger S) 230 pp

D'Costa M A and Birt L M 1966 Changes in the lipid content during the metamorphosis of the blowfly, *Lucilia*; *J. Insect. Physiol.* **2** 1377–1394

Dinamarca M L and Levenbook L 1966 Oxidation, utilization and incorporation into protein of alanine and lysine during metamorphosis of the blofly, *Phormia regina* (Meigen); *Arch. Biochem. Biophys.* **117** 110–119

Domroese K A and Gilbert L I 1964 The role of lipid in adult development and flight muscle metabolism in *Hyalophora cecropia; J. expl. Biol.* **41** 573–590

Dunphy G B, Kelvin M, Keough W and Nolan R A 1977 Fatty acid and lipid content of late larval and pupal stages of the eastern hemlock looper, *Lumbdina fiscellaria fiscellaria* (Lepidoptera: Geometridae); *Can. Ent.* **109** 347–350

Fast P G 1964 Insect lipids—A review; *Mem. Ent. Soc. Can.* **37** 1–50

Firling C E 1977 Amino acid and protein changes in the haemolymph of developing fourth instar of *Chironomus tentans; J. Insect Physiol.* **23** 17–22

Folch J, Lees M and Sloane-Stanley G H 1957 A simple method for the isolation and purification of total lipids from animal tissue; *J. Biol. Chem.* **226** 497–509

Gakhar S K 1983 Studies on Development of *Trabala vishnou* Lef. (Lepidoptera: Insecta); Ph.D. Thesis Kurukshetra University, Kurukshetra 271 pp

—— and Maleyvar R P 1984a Qualitative sex-specific changes in free amino acids during metamorphosis of *Trabala vishnou* (Lep.: Insecta); *Comp. Physiol. Ecol.* **9** 185–188

——and——1984b Quantitative changes in free amino acids during metamorphosis of *Trabala vishnou* (Coleoptera: Insecta) and the sex-specific differences; *Proc. Indian natn. Sci. Acad.* **B50** 277–285

—— and —— 1984c Analysis of the excreta during the various larval instars of *Trabala vishnou* Lef. (Lepidoptera: Insecta); *Monitore Zool. Ital.* **18** 199–205

—— and —— 1984d Age and sex-related free amino acid changes in *Trabala vishnou* (Lepidoptera: Insecta); *Proc. Indian natn. Sci. Acad.* **B50** 362–367

Gilbert L I 1967 Lipid metabolism and function in insects; *Adv. Insect Physiol.* **4** 69–211

—— and Schneiderman H A 1961 The content of juvenile hormone and lipid in Lepidoptera: Sexual differences and developmental changes; *Gen. Comp. Endocr.* **1** 453–472

Indira T 1963 Biochemical and Cytochemical Studies during Development and Ovarian Growth in *Sphaerodema molestum* Duf.; Ph.D. Thesis, Annamalai University, Annamalainagar

Janda J r V, Sehnal F and Simek V 1966 Changes in some chemical constituents of *Galleria mellonela* larvae in relation to growth and morphogenesis; *Acta Biochem. Polon* **13** 329–335

Lowry O H, Rosebrough N J, Farr A L and Randal R J 1951 Protein measurement with the folin-phenol reagent; *J. Biol. Chem.* **193** 265–275

Maleyvar R P and Gakhar S K 1982 Changes in free amino acids during embryonic development of *Trabala vishnou* Lef. (Lasiocampidae: Lepidoptera); *Proc. Indian natn. Sci. Acad.* **B48** 199–206

McCabe C T and Bursell F 1975 Inter-relationships between amino acid and lipid metabolism in the tesetse fly, *Glossina morsitans; Insect Biochem.* **5** 781–789

McGregor D A and Loughton B G 1974 Yolk protein degradation during embryogenesis of African migratory locust; *Can. J. Zool.* **52** 907–917

Morris D L 1948 Quantitative determination of carbohydrates with Dreywood's anthrone reagent; *Science* **107** 254–255

Mukerji M K and Guppy J O 1973 Changes in the lipid and nitrogen content during post-embryonic development of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae); *Can. Ent.* **105** 471–478

Nowosielski J W and Patton R L 1965 Daily fluctuation in the blood sugar concentration of the house cricket *Gryllus domesticus* L.; *Science* **144** 180-181

Pant R, Nautiyal G C and Singh J B 1974 Variation in major lipid components during development of *Philosamic ricini; Indian J. Biochem. Biophys.* **10** 116–118

Sharma S P and Sharma G 1979 Age-related lipid studies in bruchids; *Curr. Sci.* **48** 955–956

Sidhu D S, Kaur S P and Kumar N 1980 Metabolic reserves and free amino acids during the adult life of *Collosobruchus maculatus* F. (Coleoptera: Bruchidae); *Entomon.* **5** 49–51

Singh N B and Sinha R N 1977 Carbohydrate, lipid and protein in the developmental stages of *Sitophilus oryzae* and *S. granarius* (Coleoptera: Cuculionidae); *Ann. Ent. Soc. Am.* **70** 107–111

Tate L G and Wimer L T 1974 Incorporation of ^{14}C from glucose into CO_2, chitin, lipid, protein and soluble carbohydrate during metamorphosis of blowfly, *Phormia regina*; *Insect Biochem.* **4** 85–98

Wyatt G R 1967 The biochemistry of sugars and polysaccharides in insects; *Adv. Insect Physiol.* **4** 287–360