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MORPHO-DIFFERENTIATION AND GROWTH RELATED BIOCHEMICAL CHANGES IN THE DEVELOPMENT OF *DROSOPHILA ANANASSAE* (DOLECHELL) : CARBOHYDRATES, PROTEINS AND LIPIDS

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The morpho-differentiation and growth during metamorphosis of insects involve biochemical changes. The above phasic changes in the development of *Drosophila ananassae* were determined with regard to carbohydrates, lipids and proteins. The values of the above metabolites showed significant and temporal changes from 3rd larval stage upto 2-day adult. The result revealed that all these principal metabolites are involved in both the catabolic and anabolic events of these differentiation and growth in *D. ananassae*.

Key words : Tropical insects, differentiation, imaginal disc, larvae, pupae, *Drosophila*.

INTRODUCTION

Insect metamorphosis involves a series of developmental stages by means of which, the insect completes the development from larval to adult. In holometabolous insects such as *Drosophila* sp. metamorphosis involves the destruction of certain larval tissues and organs (histolysis) and organization of adult structures from primitive cell complexes known as imaginal discs (Bodensteins, 1950). The biochemical and physiological change concurrent to the various developmental stages are due to the differential expression of genes and endocrine interactions (Laufer, 1961; Marker & Ursprung, 1970). As morphological changes that occur during growth and development of insects are known to be heterogenic, so also the nature of biochemical changes that accompany them. The significance of nutrient reserves such as carbohydrates, proteins and lipids development has been established (Chen & Levenbrook, 1966; Wyatt, 1975). Though such studies have been carried out with reference to temperate species of *Drosophila* sp., they are meager with reference to the tropical forms where the environmental factors are of more limiting nature. In the present study, the biochemical characterization of development involving the major and essential nutrients such as carbohydrates, lipids and proteins in the different developmental stages of *Drosophila ananassae* was made.

MATERIALS AND METHODS

The fruit fly *D. ananassae* was the test insect in the present study. The banana baiting technique described by Patterson (1943) was used to trap the flies. Fermenting bananas with peels were crushed and placed in empty 250 ml bottles over a lining of coarse filter paper. The filter paper facilitated not only the removal of moisture but also the used-up baits. The bottles thus prepared were kept in kitchens for 4-5 days. Files attracted by fermenting bananas were quickly captured by keeping another inverted bottle over the mouth of the trap bottle. Flies gathered into upper bottle due to their positive phototaxic behaviour. The flies then etherized and transferred to medium vials individually. From a single inseminated female fly, F_2 progeny was raised and a pure line of the fly was established. Species identification was done after examination of various external and internal characters of both sexes as described by Vaidya & Godbule (1971). The pure stock of Chennai flies was obtained and maintained on food medium at 25 ± 2^0C under laboratory conditions. The MSY medium used for rearing *D. ananassae* contained :

Maize flour	: 50.0 g	Agar	: 15.0 g
Sucrose	: 20.0 g	Distilled water	: 1000 ml
Yeast powder	: 10.0 g	Propionic acid	: 1 ml

All the ingredients except propionic acid were added to water and cooked till a homogenous slurry was obtained. The boiled medium was allowed to cool for a few minutes and propionic acid was added. The medium thus, prepared was then distributed to culture bottles of 250 ml capacity, plugged with non-absorbent cotton and autoclaved at 15 lbs for 2 hrs.

Total carbohydrate was estimated following the method of Roe (1955) using anthrone reagent. Fifty milligrams of each developmental stage was homogenized in a hand homogenizer with 2.0 ml of 80% ethanol and the homogenate was centrifuged at 6000 rpm for 15 minutes. The precipitate was spared for protein estimation. The clear supernatant was used for the estimation of carbohydrates. To 0.5 ml of the supernatant, 5.0 ml of anthrone reagent was added and kept in boiling water bath for 15 minutes. Exactly after 15 minutes, the tubes were cooled and protected from light before the absorbance at 620 nm was read in Spectronic 21 spectro calorimeter (Bausch & Lomb). Glucose (AR) was used as the standard.

The precipitate obtained by extraction with 80% ethanol was dissolved in known quantity of 1.0N NaOH and the resulting solution was used for the estimation of soluble protein following the procedure of Lowry *et al.* (1951). Bovine serum albumin (Sigma) was used as the standard.

The extraction of lipid was done as per the method of Folch *et al.* (1957) and estimation was done according to the procedure outlined by Barnes & Blackstock (1973). Ten milligram of dry mass of each developmental stage was homogenized with 5ml of chloroform : methanol (2 : 1 v/v). To this 0.2ml volume of 0.9% sodium chloride solution was added and the contents were mixed and centrifuged at 3000 rpm for 10 minutes. The lower phase alone was separated using a syringe free of fluff. The volume was then made to the original quantity of 5ml with chloroform. This method is based on the sulpho-phosphovanillium reaction of Charbol and Charonnat described by Zollner & Kirsh (1962) which depends on the reaction of lipids with sulphuric acid, phosphoric acid and vanillian to give a red complex. Known quantity (usually 0.5ml) of extract was measured into a clean test tube and dried in a vacuum desicator over silica gel and the dried lipid was dissolved in 0.5ml conc. sulphuric acid and mixed well. Then the tubes were plugged well with non-absorbent cotton-wool; they were then placed in a boiling water bath for 0 minutes. The test tube were then cooled to room temperature. From this, 0.2ml of aliquots were taken and 5ml of vanillin reagent (2g vanillin powder in 800ml of 88% phosphoric acid and 200ml of distilled water) was added. The tubes were shaken well and were allowed to stand for 30 minutes and the colour developed was read at 520 nm in a Bausch & Lomb Spectronic 21 spectrophotometer.

RESULTS AND DISCUSSION

The results on total carbohydrate content of *D. ananassae* during the developmental stages are presented in Table I. The total carbohydrates represent both reducing and non reducing sugars and also bound form of saccharides. The level of carbohydrates at different developmental stages appear to be low, when compared to the protein at the corresponding stages. In III instar larva, the level remained at 26.16 ± 0.794 µg/individual. In the first pupal stage the level increased to 31.79 ± 1.084 µg/individual, respectively. Subsequent to pupation, its concentration increased to 25.48 ± 1.179 µg/individual in the emerged, increased to 25.7 ± 0.883 µg/individual in the 2-day old adult. The difference of values in different developmental stages were found to be statistically significant ($p < 0.01$).

Table 1 : The values of major nutrient reserve metabolites in different developmental stages of *Drosophila ananassae* (µg/individual).

Nutrient reserves	III Larva	I Pupa	II Pupa	III Pupa	Emerged fly	2-day adult
Total carbohydrate	26.160 ± 0.795	31.793 ± 1.084	25.955 ± 0.958	19.892 ± 0.892	25.487 ± 1.180	25.777 ± 0.883
Total protein	91.667 ± 16.083	117.833 ± 13.848	84.333 ± 10.985	95.333 ± 11.843	90.500 ± 2.345	75.833 ± 3.125
Total lipid	105.141 ± 1.543	94.523 ± 1.728	76.590 ± 1.733	54.972 ± 1.946	44.473 ± 1.896	41.097 ± 1.695

The values in different developmental stages are statistically significant ($p < 0.01$).

The quantity of protein, initially in the III instar larval stage remained 91.66 ± 16.083 µg/individual. It increased to 117.83 ± 13.847µg/individual in the I pupa. In the II pupa it showed a decrease to 84.32 ± 10.984 µg/individual. But the protein content again increased to 95.33 ± 11.843 µg/individual in the III pupa. In the emerged fly and a 2-day adult the level decreased to 90.5 ± 2.345 µg/individual and 75.83 ± 3.125µg/individual, respectively.

The results reveal that protein content increased during two stages of the development of the fly. The first increase occurred at between III instar and I pupa and the second increase between II pupa and III pupa. The above increases are found to be statistically significant, compared to their respective previous stages ($p < 0.001$). The protein content also showed a decrease at two different stages. The decrease by 33 µg occurred between stages I pupa and II pupa. Secondly, the decrease by µg was noticed between emerged fly and 2-day adult. The above decreases are statistically significant ($p < 0.01$).

The quantity of lipid in III instar larva was 105.14 ± 1.542 µg/individual. Thereafter, it decreased steadily in all the subsequent stages from I pupa upto 2-adult. The level of lipid content in the I and 2-adult pupal stages were 94.52 ± 1.728 µg/individual and 14.09 ± 1.695µg/individual, respectively. The decrease in lipid content between stages I and II was about 18µg, while its reduction between pupa II and III was about 21.6µg. The above decreases are statistically significant ($p < 0.01$).

The increase in carbohydrates beyond pupation in both emerged and 2-day adult stage is indicative of gluconeogenesis and this has been reported in other insects. Wigglesworth (1942) showed that glycogen stores of *Aedes aegypti* depleted by starvation could be restored by feeding casein, alanine and glutamic acid. Similarly, Nayar & Sauerman (1971) reported that glycerols, can be converted into glycogen in mosquitoes. Sang (1956) also reported that *Drosophila melanogaster* and *D. simulans* can use a variety of carbohydrate sources and can also survive to adulthood even when no carbohydrates are provided exogenously. The importance of carbohydrates during development and their temporal changes have also been demonstrated on other insects and other invertebrates (Ramalingam, 1989; Ramalingam *et al.*, 2004).

Srinivasan & Kesavan (1979) reported that in *Musca domestica* the protein content increased during embryogenesis at the at the first hour of development. In the present study, the protein content showed a peak in the I pupal stage and a deep fall in the II pupal stage. Again there is an increase in the total proteins at the last pupal stgae with a gradual reduction in both imago and 2-day adult stages. Fluctuations in the total protein content during the developmental stages have been reported by Jain & Parkash (1979) in *D. ananassae* and in *D. melano-*

gaster (Roberts *et al.*, 1977). In all these studies, the total protein content per individual steadily rises throughout the larval stage and reaches a maximum at puparium formation. The present study agrees with the above findings. The decrease in the II pupal stage may be attributed to a loss by way of pupal glue elimination as has been reported in *D. melanogaster* by Roberts *et al.* (1977) and the significant increase at the last stage of pupation may be due to synthesis of new proteins. The changes in the wet weight per individual as observed in the present study at the corresponding stage (III pupa) support the above suggestion. The decline of protein content after pupation may be on account of utilization of tyrosine in the adult cuticle. Church & Robertson (1966) reported that after pupation a considerable amount of protein is being utilized in the process of histogenesis. The phasic changes in tissue/organisms protein both quantitatively and qualitatively have been established among invertebrates.

In *Drosophila* sp., characterization of lipid has been made both by quantitative and qualitative methods, which revealed that lipids function both as a functional energy source and also as stored energy during development and growth (Finkel, 1948; Srinivasan *et al.*, 1993). Wren & Mitchel (1959) reported the lipid level as 6.1 ± 0.31% dry weight of adult *Drosophila* sp. Church & Robertson (1966) in their biochemical study on the growth of *D. melanogaster* observed that total lipids increased during larval development from about 6% in young larva to 15% at pupation.

In the present study the total lipid content showed uniform decrease right from the III larval stage upto 2-day adult stage, revealing thereby its utilization during growth and morphogenesis. The qualitative changes in different lipid components in conformation to the diminution of the total lipid content are obvious, in the present study The appearance and disappearance of the TLC lipid fractions during the course of metamorphosis reveal their functional involvement in either morphogenesis or in meeting that in insects. Factors such as nutrition, temperature, sex and diapause could influence the lipids both quantitatively and qualitatively. The various functional significance of lipids that have been reported in higher organisms include :

- The utilization of ketone bodies and free fattly acids as energy sources to the nervous system.
- The role of FAA in the transport of other lipids from the fat depots.
- The tryglycerides being the alternative energy source.
- Phospholipids and cholesterol becoming the membrane constituents of organisms thus representing the structural elements.
- The involvement of tryglycerides in spawning (Cahill *et al.*, 1966; Foster, 1967; Larsson & Lewander, 1973; Harper, 1971; Lewander *et al.*, 1974).

In the light of the above reports, the changes in the lipids content of *D. ananassae* suggest their contribution to both structural differentiation and functional utilization during morphogenesis and growth.

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