CHAPTER 5

A QUANTITATIVE STUDY OF THE CHANGES IN FAT AND GLYCOGEN CONTENTS DURING METAMORPHOSIS

The chemical changes during insect metamorphosis have been studied by several investigators. These studies have indicated fat and glycogen as the major chemical raw-materials occurring in holometabolous insects during the pupal period. That glycogen is utilized as a source of energy in a variety of insect species is now well established (Gilbert and Schniederman, 1961; Karlson and Sekeris, 1964). As reviewed by Wigglesworth, (1950), utilisation of fat during the pupal stage has been reported in Bombyx mori, Pyrausta nubilalis, Ophyra cadaverina, Lucilia and Calliphora. Decrease in fat content was also reported in Musca vicina (Levison and Silverman, 1954), M. domestica (Pearincott, 1960; Russo-Caia and Cecere, 1960) and the female Cecropia silkworm (Gilbert and Schniederman, 1961) during the pupal period. Among Coleoptera, a decrease in fat content was reported in the meal worm, Tenebrio molitor (Evans, 1934; Becker, 1934) and the Japanese beetle, Popillia japonica (Ludwig and Rothstein, 1949; Battista, 1954).

No such investigations have been carried out on dermestids though Sinoda and Kurata (1932) reported a high level of lipids in Dermestes sp. The presence of large amounts of neutral fat in the fat body of Anthrenus larva has already been noted (Chapter 4). It was also seen that glycogen granules were formed de novo in the prepupal fat body. In view of these findings,

it could be expected that these metabolites would play an important role as sources of energy during metamorphosis. A quantitative assessment of the levels of fat and glycogen in the larva and their changes during metamorphosis was therefore undertaken. Observations on the changes in total body weight and water content during metamorphosis were also made.

MATERIAL AND METHODS

Insects from a laboratory culture maintained on a diet consisting of a mixture of dried pigeon breast muscle and yeast was used. Last instar larvae were collected from a stock culture maintained at room temperature and transferred to an incubator at 32 ± 1 °C, for further development. The different stages of development used in the biochemical assay were characterised as follows:

Full grown larva - - - - - - - - - as distinguished by size

Late prepupa - - - - - - - - - characterised by cessation of feeding, shortening of the body and complete quiescence

1st day pupa - - - - - - - - - collected within 12 hours of the larval-pupal moult

The newly formed pupae were collected every morning from a group of prepupae separated previously into another container. Error due to the time of pupation was minimised by using only those pupated in the night. Subsequent stages were obtained from pupae thus collected and dated. In the case of the pupa and the pre-emergent adult, the persisting exuvia of the previous stages

were removed before the chemical determinations were made.

Fat content:

Estimations of fat were made on batches of 50 or 100 individuals. Insects of the appropriate stages were collected, weighed, killed and dried in a hot air oven at 80-100°C till constant weights were obtained. Extraction of fat was carried out in Soxhlet apparatus with a 3:1 ethanol-ether mixture in order to obtain the free (ether extractable) as well as the bound (extractable with ethanol ether mixture) lipids (George and Jyoti, 1955). The extract was centrifuged to remove the debris, if any, poured into tared weighing bottles, dried in an air oven to remove the traces of alcohol, kept in a desiccator for a few days and the weight determined.

Glycogen content:

Of the two forms of glycogen known to occur in tissues (desmoglycogen and lyoglycogen) the fraction which is soluble in cold trichloracetic acid (TCA) which represents the free glycogen or the lyoglycogen (Kugler and Wilkinson, 1959, 1960) was estimated according to the method outlined by Kugler and Wilkinson (1959, 1960). The method adopted was as follows. Five insects were dropped into a chilled mortar containing 2 ml cold 10% TCA and homogenised for 3 minutes. The homogenate was filtered into centrifuge tubes and washed again with an additional 3 ml of TCA. An equal volume of 90% ethanol was added to the filtrate and the glycogen allowed to precipitate overnight in a refrigerator. The tubes were then centrifuged, the supernatent

poured off and the precipitated glycogen washed with 4 ml of 95% ethanol. The washed glycogen was dissolved in 5 ml of distilled water and 1 ml of this solution analysed in duplicate for glycogen content by the anthrone method according to Seifter et al. (1950). The dilution was altered according to the glycogen present, which varied considerably in the different stages, so as to obtain a more or less uniform range of colour development. 4 ml of 0.2% anthrone reagent in 95% sulphuric acid were added quickly into 1 ml of the sample kept in an ice bath and mixed well by shaking. The colour was developed by heating the tubes in a boiling water bath for 5 minutes. The transmittance was read at a wave length of 620 mμ in a Bausch and Lomb spectronic- 20 colorimeter. Glucose was used as the standard.

RESULTS

The results obtained are presented in Table I. There was a gradual decrease in body weight resulting in a reduction of 12.32% of the initial pupal weight on emergence as the adult. The average weight of the fully grown larva was higher than that of the 1st day pupa. The average weight of the larva was found to be 7.08 mg as against an average of 6.41 mg for the 1st day pupa. The larval weight, however, included the weight of the ingested food also.

The water content of the larva was 47.44% of the body weight, of the 1st day pupa 47.06% and of the newly emerged adult 50.99%. There was a distinct decrease in fat content during metamorphosis - from 177.33 mg per 100 insects in the larva to

TABLE I

CHANGES IN BODY WEIGHT, FAT AND GLYCOGEN CONTENTS DURING METAMORPHOSIS

Stage of development	Body weight in mg. *	Fat content @		Glycogen content £	
		mg./100 insects	percentage by dry wt.	mg./100 insects	percentage by dry wt.
Larva	-	177.333 ± 5.558	53.70 ± 1.52	5.40 ± 0.69	0.812 ± 0.07
Late prepupa	-	-	-	10.29 ± 1.46	1.542 ± 0.13
1st day pupa	6.41	150.825 ± 5.978	51.06 ± 1.75	7.33 ± 0.99	1.375 ± 0.11
3rd day pupa	6.355	-	-	7.73 ± 0.54	1.410 ± 0.12
5th day pupa	6.318	-	-	7.82 ± 1.50	1.410 ± 0.23
7th day pupa	6.228	-	-	6.06 ± 0.53	1.108 ± 0.15
1st day pr. em. adult	6.057	-	-	6.08 ± 0.46	1.148 ± 0.08
3rd day pr. em. adult	5.954	-	-	4.95 ± 0.38	0.8996 ± 0.07
Emerged adult, 1st day	5.620	117.600 ± 2.400	51.27 ± 1.475	3.73 ± 0.25	0.6945 ± 0.04
Late adults collected at random	-	-	-	3.71 ± 0.08	0.7977 ± 0.06

*Body weight in the different stages was obtained by using the same group of individuals. Data represent the average of 3 groups of 10 insects each. @ Data obtained from a minimum of 3 sets of expts. £ Data represent the mean of at least 5 determinations on 5 insects each.

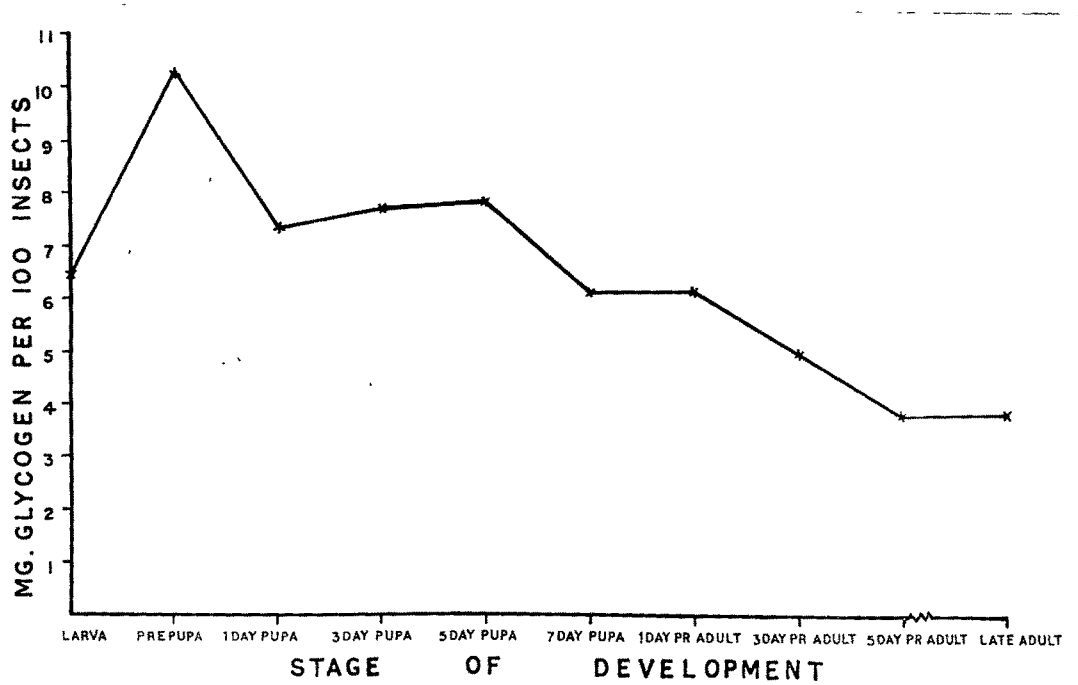

Fig. 1. Graph showing the changes in glycogen content during metamorphosis

117.6 mg per 100 insects in the newly emerged adult (Table I). The percentage value of fat did not show this reduction clearly due to the simultaneous decrease in the dry weight of the insect.

The glycogen content of the different stages as expressed in milligrams of glycogen per 100 insects and as percentage of glycogen on wet weight basis are presented in the table. On the analysis of variance the differences in the mean glycogen content of the different stages was found to be highly significant ($p < 0.01$). Further analysis was made to test the difference in the mean glycogen value between the particular stages employing the 't test' where the estimate for the error variance obtained from the analysis of variance table, which takes into consideration the overall variation in the whole set of experiments was used. The larval glycogen content was 5.396 mg/100 insects which increased significantly to 10.291 mg/100 insects in the late prepupa. There was a significant decrease in the glycogen content in the 1st day pupa ($p < 0.01$). From the 1st day to the 5th day pupa the mean glycogen content showed a trend for increase, the value reaching 7.824 mg/100 in the 5th day pupa from 7.328 mg/100 in the 1st day pupa. However, this increase was not found to be significant statistically ($p > 0.05$). A significant reduction was noted from the 5th day to the 7th day pupa ($p < 0.05$). From the 1st day pre-emergent adult, it decreased gradually till emergence reaching the adult value of 3.729 mg/100 insects ($p < 0.05$). There was no clear difference in the glycogen content of the newly emerged and the older adults.

DISCUSSION

The decrease in body weight during metamorphosis could be regarded as the result of utilisation of stored reserve materials for energy purposes. The decrease at the time of emergence is largely due to the loss of the larval and pupal exuviae. In Musca domestica a gradual decrease in weight occurred during the pupal period, the decrease being more marked in the pre-metamorphosis and the early pupal periods (Pearincott, 1960; Russo-Caia and Cecere, 1960). A significant reduction in weight was also reported in Popillia japonica during the transformation of the larva to adult (Ludwig, 1931).

The water content of different insects ranges from less than 50% to more than 90% of the total body weight (Wigglesworth, 1950). In Anthrenus larva it was found to be considerably low (47.77%). This is in conformity with the observation that the proportion of water is influenced to some extent by the quantity of fat present (Wigglesworth, 1950). There was an increase in the percentage of water content in the newly emerged adult. This increase could be only apparent, due to the reduction in total solids in the body, or if real, could indicate an increased oxidation of metabolites, particularly of fat which yields the highest amount of metabolic water.

The fat content varies considerably among the different species of insects, from 0.94 % of the wet weight in the cut worm, Lycophatia margaritosa to 28% in the larva of the weevil, Beloninus elephos (Timon-David, 1930). Compared to many insects, Anthrenus

larvae have a high percentage of fat. This is particularly interesting in view of the fact that their food consists of dry proteinaceous materials which contain very little fat. The high fat content might be correlated with the habits and habitats of the larva. As they live in dry habitats and as the food contains hardly any water they have to depend mainly on metabolic water. Therefore, the storage of an enormously high amount of fat could be considered as a mechanism for water conservation since fat produces more metabolic water on oxidation than carbohydrate and protein. A high percentage of fat (47% of the dry weight) was also reported in the larva of Dermestes (Sinoda and Kurata, 1932). One could also expect a high amount of fat in other members of the family Dermestidae since most of them live in dry habitats and thrive on dry food-stuffs. Anthrenus larvae are also capable of withstanding a long period of starvation during which, as in many insects, fat could be the chief reserve substance that is drawn upon.

In the 1st day pupa the fat content had decreased from 177.33 mg/100 insects in the larva to 150.82 mg/100 insects. The reduction in fat during this period amounted to nearly 15% of the larval fat content. It is to be concluded that the prepupal stage which intervenes the larva and the pupa utilises fat for energy purposes or converts it into other substances. That fat is being hydrolysed at this period is evident from the lipolytic activity noticed (Chapter 6).

On emergence as the adult, the fat content decreased

again from 150.825 mg/100 insects in the pupa to 117.6 mg/100 insects. Thus the fat consumed during metamorphosis (from larva to newly emerged adult) amounted to 33.69% of the initial fat content of the full grown larva. However, a large percentage of fat was still present when the adult emerged. That the adults at emergence possess a high amount of fat reserve is significant in view of the autogenous egg production. The adults are non-feeding, at least in the present laboratory conditions, and in addition to the eggs already present in the ovary at the time of emergence are capable of producing more eggs after emergence (Chapter 2). The raw-materials for egg production must come, therefore, from stored reserve materials. In females, fat must be contributing the major raw-material for egg production and this pre-emergence fat store is to be considered a specific metabolic adaptation for its reproductive phase. Fat constitutes a major part of the yolk in terrestrial insect eggs and serves as the chief metabolic fuel during embryogenesis (Bunsel, 1937; Rothstein, 1952; Jura et al., 1957; Babcock and Rutschky, 1962). The fat reserves in the males are probably used for the production of sperms. In this context, it may be mentioned that it is now definitely established that fat is synthesized and mobilized in the rat testis and incorporated into the sperms through the different stages of spermatogenesis (George and Ambadkar, 1963). Fat could also serve as a major source of emergy in both males and females during adult life as they are nonfeeding and as the glycogen content is neither high nor is reduced considerably.

Free glycogen remained at a low level during larval and adult stages being high in the pupal period, especially the earlier part. A sharp rise was recorded in the prepupal stage indicating a definite synthesis. It should be recalled that a reduction in fat content is characteristic of this period. With an initial drop in the 1st day pupa the glycogen content remained at a high level till the 5th day pupa. From this time onwards glycogen had decreased progressively till the emergence of the adult. Since glycogen is known to be a metabolic fuel in many insect species, the increase in glycogen during the early pupal period and its depletion as the adult emerges would indicate the utilisation of this substance for metabolic energy. The possibility of glycogen being utilised for the synthesis of chitin cannot, however, be ruled out in the light of the recent studies on chitin synthesis (Candy and Kilby, 1962) which demonstrated that C^{-14} glucose injected into the haemolymph of the desert locust, Schistocerca gregaria gave rise to labelled chitin. Since the period between the prepupa and the 1st day pupa is a period when the chitin of the pupal cuticle will be laid down, the decrease in free glycogen at this period could also denote the above contention. It may be pointed out here that an increase in glycogen content was noticed in the epidermis of Rhodnius prolixus prior to the formation of the new cuticle (Wigglesworth, 1950). Also the utilisation of glycogen in the egg of Melanoplus seems to be correlated to the formation of chitin in the white cuticle of the egg and the embryonic

and first instar cuticles (Wigglesworth, 1950). The decrease in glycogen content from the 5th day to the 7th day pupa likewise could indicate the utilisation of free glycogen for the formation of the adult cuticle.

The increase in glycogen content takes place after cessation of feeding. Increase in glycogen at this time has also been noted in other insects such as Bombyx (Battaillon and Couverer, 1892), Prodenia (Babers, 1941), Popillia (Ludwig and Rothstein, 1949) and the blow fly (Frew, 1929). In Popillia japonica the increase in glycogen on the 5th and 6th day of pupation coincided with a decrease in fat (Ludwig and Rothstein, 1949; Battista, 1954). Since the decrease in weight of fat was sufficient to account for the increase in weight of glycogen which occurred at this period, these authors concluded that glycogen which was used as a source of energy during the pupal period was replenished at the expense of stored fat when it reached a low level. Conversion of fat to carbohydrate during the pupal period was also suggested in the silkworm (Bataillon and Couverer, 1892), and the blow fly (Frew, 1929). According to Buck (1953) the conversion of fat to carbohydrates seems to be definitely established for the second half of the pupal period in the blow fly Phormia. It would appear, therefore that in Anthrenus the glycogen is likewise produced from fat since an increase in the former coincides with a decrease in the latter. However, the long standing suggestion of the net conversion of fat to carbohydrate during the initial stages of pupation

still remains to be proved conclusively. It is possible that while fatty acids could be a source of energy at the initial stages of pupation, the glycerol part derived from the hydrolysis of fat could be used for building up carbohydrates. To what extent the proteins from histolysing larval tissues may contribute to the formation of glycogen is not known. Utilization of trehalose for glycogen synthesis was also suggested recently in Bombyx mori and Platysamia cecropia since the pupal blood trehalose level was found to be half that in the mature larva (Wyatt and Kalf, 1957).