A STUDY OF THE PROTEIN, FAT AND GLYCOGEN CONTENTS OF
NORMAL AND DIAPAUSING LARVAE OF T. GRANARIUM

Several insects are known to undergo diapause during various stages of their development. Diapause in the egg condition and subsequent arrest of the embryonic development is reported in Melanoplus (Slefer, 1958) and Bombyx mori (Chino, 1958). Diapause at the larval stage is recorded in the gall fly, Eurosta solidaginis, the web worm, Laxostege sticticalis (Salt, 1961), Gastrophilus (deGeer) (Levenbook, 1951), some of the parasitic wasps and in few species of dermestids such as T. parable (Burges, 1961) and Anthrenus verbasci (Blake, 1958) apart from T. granarium. A large number of insects are known to enter into a quiescent state during pupal and adult stages. Pupal diapause is recorded in Hyalophora (Harvey, 1961), Antherea (Demyanovsky et al., 1952; cf. Domroese and Gilbert, 1964) and Sphinx (Brieteux-Gregoire et al., 1957). The boll weevil, Hypera postica (Tombes, 1966), Anthonomus grandis (Lambremont et al., 1964, Nettles and Betz, 1965), Coccinella (Hodek and Crekasov, 1961) and Leptinotarsa (deWilde and deBoer, 1961) undergo diapause in the adult condition.

In diapause, insects store large amounts of lipids and carbohydrates as reserve materials. These metabolites are gradually depleted during the prolonged dormant period. Among the metabolites lipids constitute the major reserve food. In
the diapausing adult boll weevil the fat content amounts to 20% of the fresh weight (Brazzel and Newsom, 1959, Lambremont et al., 1964). Accumulation of fat and its gradual depletion during diapause was reported in *Hypera postica* (Bennett, 1963, Tombes, 1964). Similarly *Culex tarsalis* builds up large triglyceride reserves for hibernation (cf. Takata and Harwood, 1964). In *Coccinella* there is an increase in the fat content during diapause which is three times the non-diapausing individuals (Hodek and Cerkasov, 1961).

Carbohydrate accumulates in the form of glycogen, glycerol and sorbitol, prior to the initiation of diapause. Insects which undergo aestivation during winter, accumulate large amount of glycerol and sorbitol. A physiological significance of such an accumulation of glycerol is to protect the tissues from low temperature (Salt, 1958, 1959). Salt (1958, 1959) had shown that the larvae of *Bracon cephi* contain high concentration of glycerol and could withstand temperatures as low as -40°C. Chino (1957, 1958) had noted an increase in glycerol and sorbitol contents in the diapausing eggs of the silk worm, *Bombyx mori*. As the embryos enter diapause, the glycogen disappears producing an equivalent amount of glycerol and sorbitol which remain in high concentrations until the diapause is ended at which time they were reconverted into glycogen (Chino, 1957, 1958). In the pupae of *Cecropia* and a related species *Telia polyphenus*, glycerol accumulates gradually during diapause and disappears rapidly when diapause is broken.
(Wyatt and Kalf. 1958). In adult diapausing beetles, such as Coccinella (Hodek and Cerkasov, 1961) and Anthonomus (Nettles and Betz, 1965) carbohydrate accumulates in the form of glycogen during diapause.

No data is available regarding the protein levels during diapause. However, Demyanovsky et al. (1952) had reported the incorporation of labelled methionine into both blood and tissue proteins in diapausing pupae of Antheraea pernyi Guerin. In diapausing Trogoderma larvae the proteinaceous globules in the fat body have already been described (Chapter. 3). It has also been shown (Chapter. 5) that they break down and apparently supply material to the developing tissues during metamorphosis. Since the protein reserves of the diapausing larva is considerably more than that of the normal, it should be interesting to know whether it has any significance in diapause. A quantitative study on the levels of fat, glycogen and protein in the normal and diapausing larvae is reported in the present chapter. In diapause larvae, the estimations were made at different intervals of diapause with and without food.

MATERIAL AND METHODS

Different stages of the insect were obtained from cultures maintained on crushed wheat, the normal larvae at 35±1°C and 70% R.H. and the diapause larvae at 30±1°C and 70% R.H. The fully mature larvae could not be staged accurately because of the considerable differences in size which exist among the same batch of larvae reared under the same conditions.
Moreover, there is considerable difference in the body size between the male and female larvae. In a group of mature larvae, the smaller ones are always males and the larger ones females, but the intermediates may be either male or female. Externally the body size is the only criterion by which the larvae could be separated into males and females. In the present study the normal larvae were collected a few days after pupation had begun in the culture which was started with a large number of eggs. Since the males would pupate earlier (Chapter 1) most of the remaining larvae should be females, from which the comparatively bigger ones of more or less of the same size were collected. These would consist mostly of female larvae in the last instar.

Larvae which failed to pupate at the end of 40 days at 30°C were regarded as diapause larvae (Chapter 2). Sex in the diapause larvae could be easily distinguished as the diapausing male larvae were distinctly smaller than the females. This was confirmed initially by taking a sample of 25 larvae each from groups of small and large larvae and breaking diapause by raising the temperature and examining the resulting pupae. The diapause larvae also showed more uniformity of size among individuals of either sex.

Studies on the changes in body weight, fat and glycogen during diapause were made in two batches of larvae. The cultures were made at 30°C and 70% R.H. from a large number of freshly hatched larvae. At the end of 40 days the
diapause larvae were separated and maintained with enough food for three months. The body weight was determined at the end of 1, 2 and 3 months in diapause. Batches of larvae were sacrificed at the end of 1, 2 and 3 months in diapause for the estimation of fat and glycogen. In one culture (Batch II), at the end of 3 months in diapause with food, the larvae were maintained without food for a period of two months and the changes in body weight, fat and glycogen were determined.

Batches of 20-25 females and 40-50 males were used for the estimations of fat and glycogen. The values presented are the average of two determinations except otherwise indicated in the Table. The method employed was that of Chino and Gilbert (1965) as described in Chapter 4.

Protein content was estimated in normal larvae, larvae after 1 month in diapause with food and the latter maintained without food for a period of another month. Protein was estimated by the biuret method (Colowick and Kaplan, 1957 in Methods in Enzymology Vol. III). 7 to 18 insects depending on the body weight were homogenized in enough water so as to obtain a homogenate containing approximately 15 mg tissue per ml. To 1 ml of the homogenate was added 4 ml biuret reagent. After 30 minutes the contents were shaken with 1.5 ml of diethyl ether and centrifuged so as to remove the lipids. The bottom layer was read on a Klett-Summerson colorimeter. Serum albumin was used as the standard.
RESULTS

Body weight: The average body weight of the mature larva (female) was found to be 2.91 mg. The changes in body weight during diapause in the two batches, are presented in Table I. It may be seen that in Batch II the body weight of both the sexes registered a gradual increase during the first 3 months in diapause with food. In Batch I, the body weight in both sexes showed alternate increase and decrease. It must be mentioned here that the changes in body weight experienced by the same group of larvae could not be determined as part of them were sacrificed for the estimation of fat and glycogen at different intervals. However, the diapause larvae do not show much individual variation in their body weight. The fluctuations in the body weight noted here must be largely due to the fact that the larvae are known to feed occasionally during diapause, a small part of the variation could also be attributed to the possible slight variations between sets of larvae. In another set of experiments a particular batch of 50 insects, at the end of 3 months in diapause were used to study the fluctuations in body weight when food was available. The data are presented in Table II. Some of the larvae entered pupation during the experimental period of 4 months as can be seen from the number of insects given in the Table in parenthesis.

The data show that there is considerable fluctuation in the body weight evidently due to the fact that the larvae feed intermittently. That the occasional increase in weight
Table I. Changes in body weight during diapause

<table>
<thead>
<tr>
<th></th>
<th>3 months in diapause</th>
<th>4 months in diapause</th>
<th>5 months in diapause</th>
<th>7 months in diapause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2.2</td>
<td>2.4</td>
<td>2.26</td>
<td>1.91</td>
</tr>
<tr>
<td></td>
<td>(50)</td>
<td>(35)</td>
<td>(35)</td>
<td>(25)</td>
</tr>
<tr>
<td>Female</td>
<td>5.1</td>
<td>7.7</td>
<td>7.46</td>
<td>6.71</td>
</tr>
<tr>
<td></td>
<td>(50)</td>
<td>(45)</td>
<td>(45)</td>
<td>(32)</td>
</tr>
</tbody>
</table>
Table II. Changes in body wt. and contents of glycogen and fat during successive intervals of diapause with and without food.

<table>
<thead>
<tr>
<th>Body wt.</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
<th>5 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2.17 mg</td>
<td>1.86 mg</td>
<td>2.26 mg</td>
<td>4.79 mg</td>
</tr>
<tr>
<td>Female</td>
<td>5.27 mg</td>
<td>5.53 mg</td>
<td>4.63 mg</td>
<td>7.02 mg</td>
</tr>
<tr>
<td>Male</td>
<td>0.9796%</td>
<td>0.8352%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>(1.823 mg/100 L)</td>
<td>(1.883 mg/100 L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27.29%</td>
<td>25.86%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>(151.2 mg/100 L)</td>
<td>(123.9 mg/100 L)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values presented for fat and glycogen are the average of 2 determinations. The differences between the two individual values varied between 0.25-2.03% for fat and 0.01-0.267% for glycogen. * Consisted of only one determination.
noted during diapause is due to the intake of food is further shown by the fact that there was a constant reduction in body weight when the diapausing larvae were denied access to food. Larvae belonging to Batch II (Table I) kept without food for a period of two months (at the end of 3 months in diapause) showed a reduction in their body weight which amounted to 23.91% in the female and 10.74% in the male.

Fat: The fat content of the normal larva (female) was 21.05% of the body weight (61.25 mg/100 larvae). The fat content at various intervals of diapause are presented in Table I. At the end of 2 months in diapause with food, in Batch I, the male had 30.23% (56.22 mg/100 larvae) and the female 27.35% (151.2 mg/100 larvae) fat. The corresponding values for Batch II closely approximate the figures obtained for Batch I. There was considerable increase in the fat content of the female diapause larva - more than twice that of the normal. It may be seen from Batch II larvae that the fat content had already reached this high value at the end of 1 month in diapause. During the 3 months in diapause with food, in both Batch I and Batch II, the absolute fat content was maintained at a more or less constant level with slight fluctuations.

In diapause larvae kept without food for a period of 2 months (at the end of 3 months in diapause with food, Batch II Table I) the fat content registered a considerable reduction both in absolute amounts and percentage values. The amount of fat utilized during this period was found to be
22.35 mg/100 larvae in the male (30.9% of the initial fat content).

It should be noted that the male in all stages possessed a higher percentage of fat though the female had more than twice the absolute amount of the fat present in the male, owing to its bigger size.

Glycogen: The glycogen content of the normal larva (female) was found to be $0.312 \pm 0.057$% of the body weight ($0.308 \text{mg/100 larvae}$). In Batch I larvae, two months in diapause with food, it amounted to $0.98\%$ in the male ($1.823 \text{mg/100 larvae}$) and $1.567\%$ in the female ($8.664 \text{mg/100 larvae}$) (Table I). It may be seen that there was a considerable increase in the glycogen content in both the sexes during diapause. In the female the increase amounted to about 9 fold the value of the normal larvae. During the 3 months in diapause with food, the glycogen content was maintained at a more or less constant level with occasional ups and downs.

In diapause larvae kept without food for a period of two months (at the end of 3 months in diapause with food, Batch II, Table I) the glycogen content reduced considerably both in absolute amounts and percentage values. The reduction in glycogen content during the two months in diapause without food was found to be $1.413 \text{mg/100 larvae}$ for the male ($51.35\%$ of the initial glycogen content) and $4.931 \text{mg/100 larvae}$ in the female ($51.1\%$ of the initial glycogen content).

It should be noted that in all stages the female
contained more glycogen both in absolute amounts and in percentage values.

Protein: In the normal larva (female) the protein content was found to be 19.30±0.316% of the body weight (46.54 mg/100 larvae), (based on 4 determinations of 18 larvae each). Diapause larvae used for the estimation of protein contents were not drawn from the same batches used for the estimation of fat and glycogen. Estimations were made only on female larvae. The diapausing females showed a protein content of 18.64±1.738% (103.9 mg/100 larvae) at the end of one month in diapause with food which shows that a considerable increase in the absolute amount of protein occurred during diapause. Diapause larvae, one month in diapause when kept without food for a period of one month showed a protein content of 20.74±1.119% (90.22 mg/100 larvae). The above values for protein during diapause were obtained from 3 determinations of 7 larvae each. It may be seen from the figures presented that there was considerable variation among the individual values obtained. No conclusion could therefore be drawn from the present data as to the changes in the protein contents during diapause. Apparently the absolute amount of protein remained more or less constant during the one month period of diapause without food.

DISCUSSION

The present study has thrown light on some of the metabolic and behavioural adaptations of the diapausing
Trogoderma larvae. They show an enormous increase in the body weight due to the accumulation of fat, glycogen and proteins.

Increase in the fat content is characteristic of diapause and is reported in many insects such as the boll weevil, Anthonomus grandis (Lambremont et al., 1964), in the alfalfa weevil, Hypera postica (Bennett, 1963; Tombes, 1964, 1966), in Culex tarsalis (Harwood and Takata, 1965) etc. In Trogoderma the female larva accumulates nearly 2.5 times greater amount of fat than the normal larva by the end of one month in diapause. In the present study, as the estimation of the fat content was made by the end of one month after the arbitrary period (40 days) separating the diapause larvae from the normal, it would be difficult to consider that the accumulation of fat took place entirely during the one month period after the larvae entered diapause. An accepted physiological characteristic of diapause is an accumulation of lipids prior to the period of reduced metabolism (cf. Tombes, 1966). However, in Trogoderma, most of the increase in body weight was noticed after the larvae completed the normal developmental period and entered diapause which suggests that at least part of the fat content was accumulated during the one month period in diapause as a result of occasional feeding. The fat content recorded a high titer by the end of one month, which was maintained with slight ups and downs during the three months period when food was
available. It may be noted that during two months in diapause without food a large percentage of the initial fat content (30.9% in the male and 40.35% in the female) was utilized to underwrite the energy demands. Brazzel and Newsom (1959) had noticed a similar decrease of fat content during overwintering of Anthonomus. In the boll weevil, Lambremon et al. (1964) recorded a drop in fat content from 20% to 3% during the diapause which lasted for seven months, about 3% of the lipid being utilized every month. In Trogoderma, 31% in the male and 40% in the female, of the initial fat content was utilized during two months when the diapause larvae were denied access to food. Considering the above values, 15.5% in the male and 20% in the female, of the initial fat content was utilized per month to meet the energy demands of the diapausing larvae. The total lipid reserves of the diapause larva would therefore suffice only for a period of six months for the male and five months for the female, even if the entire fat content could be utilized.

Accumulation of glycogen and its utilization during diapause was reported in a few insects such as Coccinella (Hodek and Cerkasov, 1961) and Anthonomus (Nettles and Betz, 1965). In Trogoderma there was a tremendous increase in the glycogen content in both sexes during the three months period in diapause with food. This stored glycogen also was utilized along with fat when the larvae were denied access to food. About 50% of the total glycogen was utilized by the end of two months. At this rate (25% per month), the glycogen store
should last only for about four months. However, in the course of the present investigation it was observed that diapause larvae denied access to food could survive for about 10-13 months, though no systematic studies were undertaken in this regard. Prolonged diapause without food results in occasional moulting and reduction in the body size. The present studies showed that the protein content also increased enormously during diapause. However, no definite conclusions could be drawn from the present data on the utilization of proteins during diapause. It appears that the protein content remains more or less unaltered during the one month in diapause without food. It may be expected that under extreme shortage of food, proteins might be mobilized for energy. However, under most natural conditions the diapausing larvae might enter pupation before their protein contents are considerably depleted with the result that the emerging adult are much bigger than the normal ones. A greater protein content would provide for a greater number of eggs, which should compensate, at least in part, for the absence of reproduction during the diapause period.

**Trogoderma** larvae are reported to undergo diapause which last for 3-4 years (Burges, 1959). This is possible because of a unique behavioural adaptation. Unlike most of the insects, when food is available, diapausing khapra larvae, often venture out to feed. This evidently helps in replenishing the spent up reserves. That this is so, is
clearly demonstrated in the present study by the levels of fat and glycogen during the three months in diapause with food. The fat and glycogen contents are maintained at a more or less steady level with occasional ups and downs. Occasional rise in the rate of oxygen consumption followed by feeding, in diapause larva was reported by Burges (1960). This behaviour of occasional feeding should be considered a definite adaptation for long periods of diapause.