CHAPTER 3

THE HISTOLOGICAL ORGANIZATION OF THE LARVAL FAT BODY OF *ANTHRENUS VORAX*

The insect fat body presents many structural diversities among and within the various Orders. In general, the fat body contains two cell types - trophocytes and urate cells. The urate cell may, however, be absent in some insects in which case the urates or uric acid is stored in the ordinary fat cells. Symbiont carrying cells are also known to occur in the fat body of some insects. The histological organization of the fat body is often more complicated by the incorporation of oenocytes which originate from the epidermal cells as in *Schistocerca* (Coupland, 1958) and *Poecilocera* (Hegdekar, 1963). The presence or absence of the cell membrane is also a variable character (Buys, 1923). In view of these structural diversities presented by the fat body of insects in general, it was thought necessary to study the histology of *Anthrenus* larval fat body as a first step in elucidating its role in the general metabolism of this insect. The fat body of any of the dermestid genera has not been studied so far.

MATERIAL AND METHODS

The insects were obtained from a laboratory culture maintained at 32 ± 1°C on dried and crushed pigeon breast muscle supplanted with 5% Brewer's yeast (Chapter 2).

Observations on the gross histology were made on fresh

fat body from the last instar larvae dissected in Clarke's insect saline (Hale, 1958). Histological observations were also made on paraffin sections after fixation of the larva in Bouin's, Zenker's, Carnoy's, Susa's and Ciaccio's fixatives. Fixation in Ciaccio's fluid (formalin, 20 ml., 5% potassium dichromate solution, 80 ml. and glacial acetic acid, 5 ml.) with prolonged post-chromation as recommended by Chou (1957) for vertebrate adipose tissue gave the most satisfactory preservation of the histological integrity. The entire larva was frozen hard, the anterior and posterior ends of the body chopped off with a blade, and the middle piece dropped into cold fixatives which were later brought to the desired temperature. This procedure obviated any tissue damage due to dissection (the liquid droplets of fat had a marked tendency to ooze out of the fat body when injured) and at the same time ensured good penetration of the fixatives.

For the demonstration of the nuclei the fat body was stained with haematoxylin - eosin or with the trichrome stain employing azocarmine G, orange G and methyl green as recommended for insect material by Lower (1955).

OBSERVATIONS

The full-grown larva contains numerous large and small lobes of fat body closely packed in the haemocoele (See Fig. , Chapter 4). They are many cells thick, have a whitish appearance and seem to be covered by a thin limiting membrane which when damaged causes the liquid drops of fat to flow out.

Fresh fat body, when examined under the microscope, showed numerous configurations of fatty globules, each consisting of a large central globule surrounded by numerous spherical globules (Fig. 1). For the sake of identity and description, the following terms will be used to denote these parts of the globular configuration - the central globule and the peripheral globules.

A similar histological pattern was obtained in paraffin sections after fixation in Ciaccio's fluid and post-chromation for a week at room temperature (Figs. 2 & 3). The peripheral globules were well preserved, but the central globules were represented by empty spaces. In Ciaccio fixed and acid-haematein-fixed gelatin sections, however, the central globule was well preserved and could be stained with the various stains for fat (Chapter 4). In paraffin sections of the tissue fixed in other fixatives such as Bouin, Zenker, Carnoy, and Susa, the histological structure appeared more distorted with poor preservation of fat and the peripheral globules often tended to become confluent and scattered.

The distribution of the nuclei in the fat body amidst the globular configurations could be seen in Fig. 4. They appeared more or less rounded and granular.

When fresh unfixed fat body from larvae which were nearing the prepupal stage were examined in saline solution, it showed a tendency to get dissociated into smaller units. These could be well separated by gentle maceration in glycerine. Each

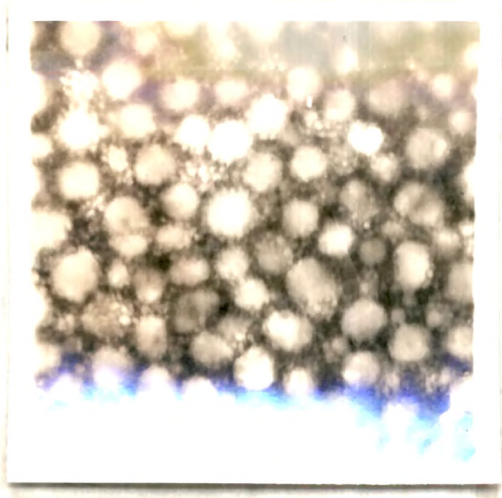

Fig. 1 A lobe of fresh unfixed fat body showing structure

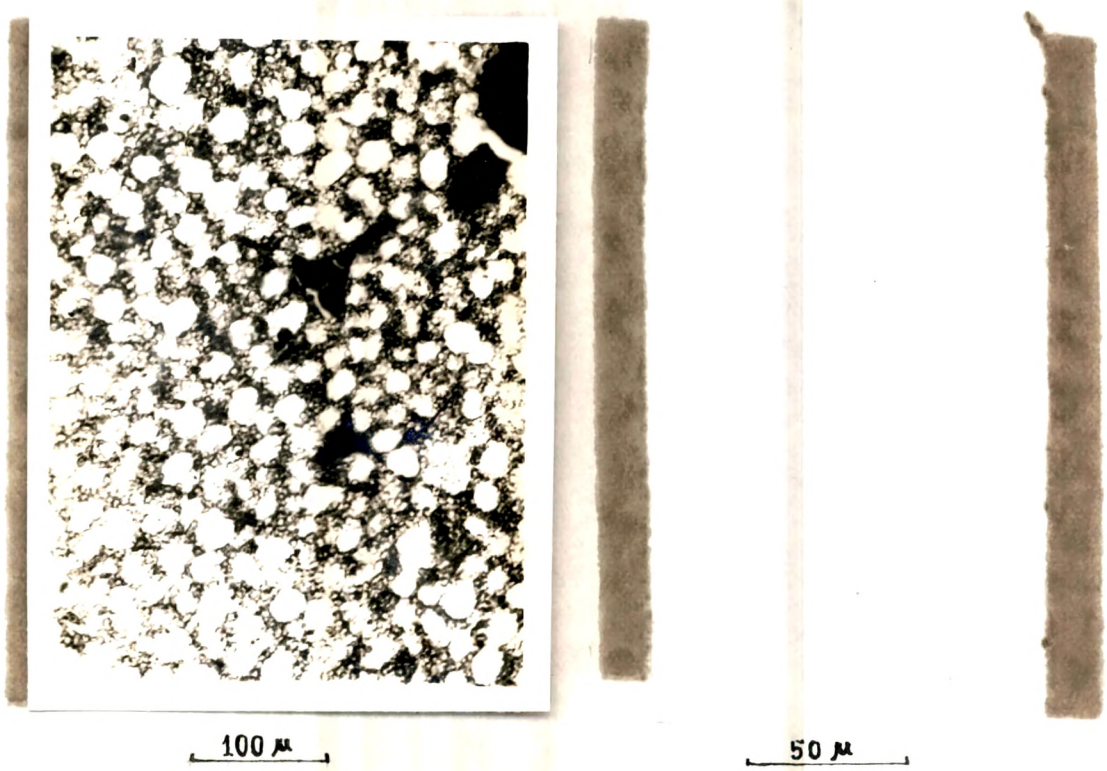

Fig. 2 A section of the fat body showing histological structure (Fixed in Ciaccio and stained with insect trichrome)

Fig. 3 Same as Fig. 2 at higher magnification. CG- Central globule PG- Peripheral globule, NU- Nucleus

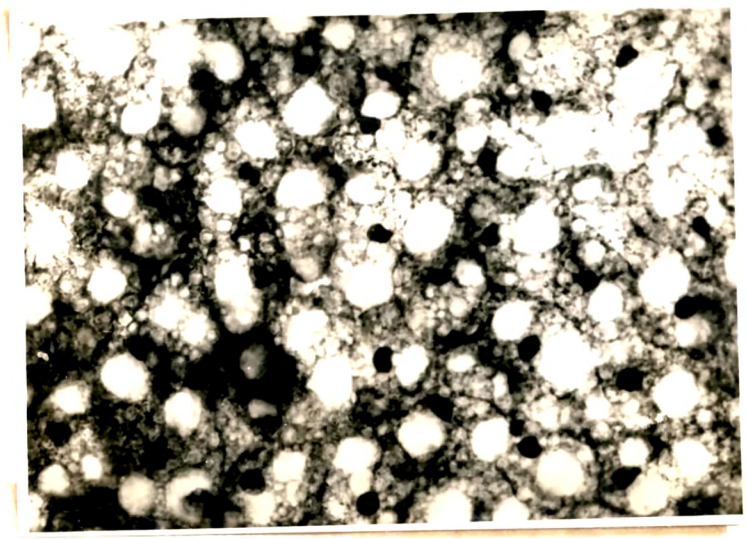

Fig. 4 A lobe of fat body showing the distribution of nuclei (Stined with haematoxylin)

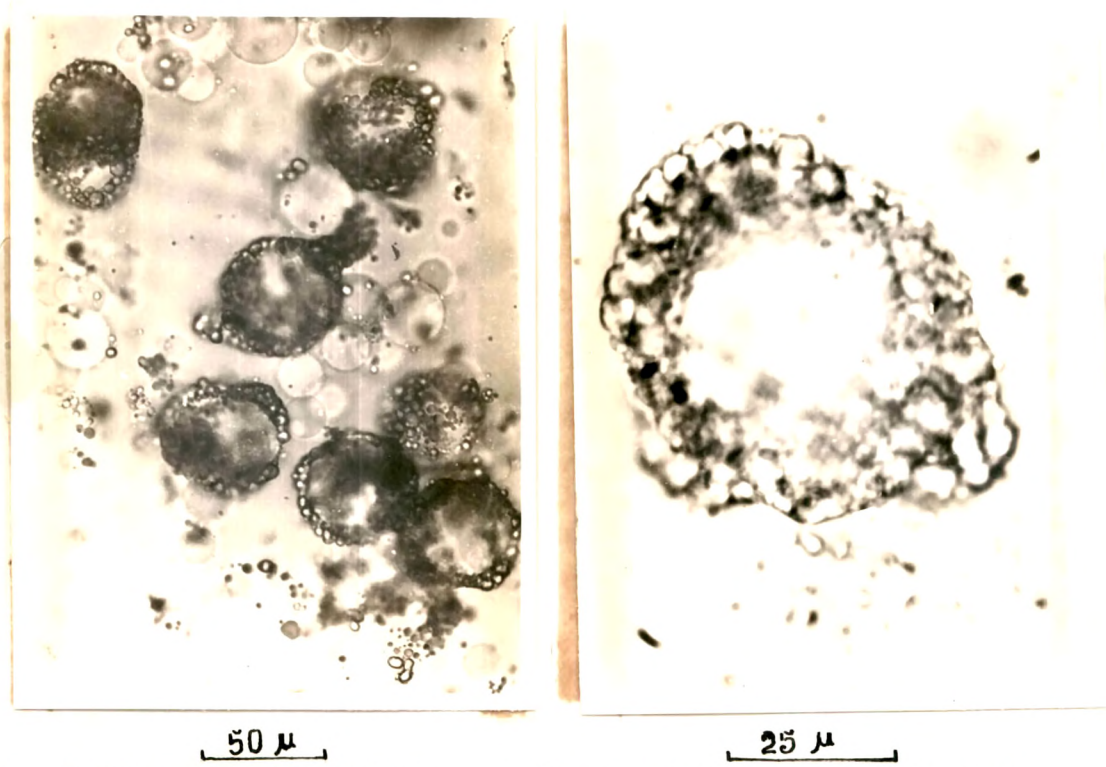

Fig. 5 Dissociated globular configurations of the fat body from early prepupa (unstained)

Fig. 6 A single configuration enlarged (unstained)

of these represented the globular configuration consisting of the central fat globule surrounded by the halo of tiny peripheral globules (Figs. 5 & 6). Though in most cases, the peripheral globules were found to cover the entire surface of the central globule, in some they were distributed sparsely.

Attempts to demonstrate the cell boundary in the larval fat body employing an adaptation of Recklinghausen's method (Nayar, 1954) were not successful. It may be mentioned here that loose entities of the fat body consisting of the central and peripheral globules were observed inside the everted wing rudiments during the pupal period. At the time of pupation, when the wing rudiments became everted, no such units were noticeable, but they had later migrated from the dissociating fat body and could be seen through the transparent wing-folds. However, complete dissociation of the larval fat body did not occur during the pupal period.

DISCUSSION

Buys (1923) showed that the fat body of insects could be classed into two groups: one in which the cell boundaries are completely lost early in development and the tissue appearing in the form of a syncytium (eg. Ephemeroptera, Trichoptera, the lower Diptera such as Tipulidae and Chironomidae, some Coleoptera, and the anterior region in Hemiptera) and the other in which the cell boundaries are maintained at least in part, until the insect is nearly mature or ready for pupation (eg. Orthoptera, Lepidoptera, Hymenoptera, some Coleoptera and the posterior region in

Hemiptera). The present study shows that the fat body of Anthrenus may be considered as a syncytium and that it could be classed under the first category. The absence of oenocytes and of separate urate cells may also be pointed out.

Each globular configuration in the fat body comprising of the central globule and the surrounding peripheral globules appeared to possess a nucleus situated amid the latter. However, the identity of a nucleus to correspond to each configuration was not always possible from histological sections. This appears to be due to the possibility that in thin sections all the nuclei could not be observed in the same plane.

The dissociation of the fat body in the prepupal stage into the globular configurations points to the fact that every such configuration represents a basic unit of organization of the fat body. As noted earlier, such units were also observed in the living insect inside the everted wing rudiments during the pupal period. In all probability, they could be regarded as specialized units of organization that have evolved from the delimitations of the primitive (embryonic) fat cells which lost their cell membrane early in development. However, in the fat body of those insects in which the cell membrane is maintained there exist a large number of fat globules of varying sizes within a single cell seems paradoxical to this concept. But it may be pointed out that the yellow adipose tissue of vertebrates possesses cells with a single large central fat globule.

The syncytial type of fat body which occurs in many

insect Orders marks a specialized line in the evolution of the fat body of insects which may well have significant physiological implications. The peripheral globules have a resemblance to the so-called albuminoids described in the fat body of other insects. Their spatial distribution in the fat body of Anthrenus is very characteristic and rather unique. They are considered in greater detail with reference to their homology, composition and probable functions in next chapter.