OBSERVATIONS ON THE NORMAL HISTOLOGY AND HISTOCHEMISTRY OF THE FAT BODY OF THE LOCUST (SCHISTOCERCA GREGARIA)

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(With Plate 5)

INTRODUCTION

The existence of intracellular inclusions of bacteria, rickettsia and virus particles in insects has been recognized for many years, and the subject has been reviewed by Steinhaus (1946). Micro-organisms have been described in the wall of the gut, the Malpighian tubules, the genital organs, the blood and the fat body of a variety of insects. They may exist in these sites as a result of a disease process which may result in the death of the host or as normal symbionts, as in the case of the inclusions in the fat body of the cockroach (Gier, 1936). D'Herelle (1911), investigating the high mortality rate of the locust Schistocerca pallens, reported the presence of the micro-organism Coccobacillus acridiorum in the intestinal canal of the dead locust. Pospelov (1926) observed the same micro-organism in the blood of the locust L. migratoria, but considered that it was a normal inhabitant which only became pathogenic when the insects were reared in unfavourable conditions of temperature and humidity. The recent work of Kilby & Neville (1957) has indicated that the fat body of the locust possesses marked enzymic activity, and the present work has been undertaken in an attempt to find out whether this activity is the result of the presence of symbiotic micro-organisms or to some normal cell constituent.

MATERIALS AND METHODS

Healthy specimens of the desert locust Schistocerca gregaria (1st- to 5th-instar stages and adults) have been examined as fresh preparations and after fixation. Material has been fixed in 10% formalin, Carnoy (6:3:1), Flemming, Champy, absolute alcohol, formol-dichromate, formol-calcium-cobalt (McManus, 1946), alcoholic Bouin and aqueous Bouin. Some paraffin embedded sections (cut at 4–8 μ) have been stained by a variety of histological methods, including haematoxylin and eosin, iron haematoxylin, Giemsa, Gram's stain, Bensley's acid-aniline methyl green (Cowdrey, 1948), Meyer's haemalum and ammoniacal silver nitrate; other sections have been stained for protein by the Millon and Danielli methods (Lillie, 1954), for glycogen by the P.A.S. technique (Hotchkiss, 1948), with saliva and acetylation controls (Gersch, 1949), for DNA and RNA by the Feulgen and methyl
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green pyronin methods (Pearse, 1953) using perchloric acid as a control, and for phospholipids by the Sudan Black B method of McManus (1946).

Fresh tissue was examined by phase contrast and by polarized light, and also after being stained supravitally by Janus Green B and neutral red.

FINDINGS

Observations on fixed tissues

(a) General morphology and histology

The fat body extends throughout the abdominal and thoracic cavities. It can be divided into a peripheral portion which is firmly attached to the overlying epidermis, and a more central mass which exists as a loose meshwork of anastomosing lobes in the space between the gut and body wall (Pl. 5, fig. 1). The peripheral and central masses of fatty tissue are continuous at many points and are intertwined with genital and Malpighian tubules. The fat body exists as a single or double layer of cells in the 1st-instar stage; at this time a few small discrete fat droplets may be seen scattered in the cytoplasm which, after staining with haematoxylin and eosin or Giemsa, is both eosinophilic and basophilic—the eosinophilia being diffuse, the basophilic staining reaction being confined to a fine reticular stroma. Fat cells increase in number by mitotic division and by the 2nd-instar stage are engorged with fat droplets so that the non-fatty cytoplasm is mainly confined to the perinuclear region, from which protoplasmic strands radiate towards the cell membrane. The fat cells contain intensely chromatic nuclei 10–12 μ in diameter; the cell membrane is often difficult to define. The fat cells are intimately associated with oenocytes, which are most numerous in the peripheral parts of the fat body but are also occasionally observed in the more central region. The oenocytes are large cells of 60–75 μ in diameter, each having a single rounded nucleus 15–20 μ in diameter whose chromatin is in smaller clumps and is less dense than that of the fat cells; the cytoplasm is diffusely eosinophilic but is permeated by a fine basophilic network. A variable number of small vacuoles may be observed in the cytoplasm of the oenocytes (Pl. 5, figs. 4, 5). The association between fat cells and oenocytes persists throughout all developmental stages and the two types of cell are present in the mature adult. Oenocytes are always most numerous in the subepidermal region and they retain their typical form throughout the life cycle of the locust; in the old adult some yellow granular pigment and birefringent uric acid deposits, which are stained by haemalum, are often observed in the cytoplasm of these cells.

Tissues fixed in formol dichromate and stained with Bensley's acid fuchsin-methyl green (Cowdrey, 1948) show the presence of small (0.1–0.5 μ) red granules, which may be rounded or ovoid, in the cytoplasm of the fat cells (Pl. 5, fig. 2), the Malpighian tubules, the gut and the follicular cells of the ovaries; these structures have not been observed in the oenocytes, but this may be due to the heavy background staining of the cytoplasm which masks the reaction. In addition to these small structures larger cellular inclusions in the Malpighian tubules and gut are also stained by acid fuchsin; these larger elements are probably secretion granules
as similar structures may be observed in the lumen of the Malpighian tubules. The 'albuminoid' plaques as described by Hollande (1914) in the fat body of Vanessa have not been observed in the locust.

Tissue fixed in absolute alcohol or Carnoy was stained for uric acid by the ammoniacal silver nitrate method (Hollande, 1914), and for urates by Mallory's haemalum method (Lillie, 1954). Scattered deposits of silver are observed in both oenocytes and fat cells at all stages, the number of granules increasing with age. No haemalum-positive granules were observed during the instar stage, but a few are present in the oenocytes of the adult locust.

No micro-organisms have been observed in sections stained by either Giemsa or Gram's method for tissue sections.

(b) Histochemical methods

(1) Nucleoproteins. Using Carnoy-fixed material stained by the Feulgen method DNA is confined to the nuclei in all cells.

In tissue fixed with 10% formol RNA-positive material is present in large amounts and scattered throughout the cytoplasm of the oenocytes and 1st-instar fat cells; in older specimens it is abundant throughout the oenocytes but is mainly confined to the perinuclear region of the fat cells. RNA is reduced in amount in the adult insect.

(2) Glycogen. The non-fatty cytoplasm of 1st- to 5th-instar fat body cells contains large amounts of glycogen; this is present in both fat cells and oenocytes (Pl. 5, fig. 3). The reaction is in part diffuse and in part granular. The glycogen content of the adult locust is less than that of the immature insect and usually exists as small granules.

(3) Proteins. Both the Danielli diazo reaction and the Millon reaction are positive throughout the non-fatty cytoplasm of the fat body cells and oenocytes (Pl. 5, fig. 4). The reaction is partly diffuse and is partly concentrated in small cytoplasmic granules; large plaques of protein are not observed. The reaction is less intense in the adult locust.

(4) Fat. In Champy-fixed material reduced osmic acid occupies practically the whole of the cytoplasm of the fat-engorged cells. In sections fixed with formol-calcium-cobalt and stained with Sudan Black show an intense staining of the cytoplasm of the oenocytes with only a slight reaction in the fat cells (Pl. 5, fig. 5).

Stains for bacteria

Blood smears and smears of crushed whole fat body and of homogenized centrifuged material have been examined, after heat fixation, for bacteria. Smears were stained by Gram's method and by Giemsa. No bacteria or other micro-organisms were observed in any specimen.*

* The author is indebted to Dr K. Zinneman of the Department of Bacteriology, University of Leeds, for performing Gram stains and examining the sections.
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Fresh tissue

Under the ordinary light microscope, and with phase contrast, the majority of the cells of the fat body are seen to be engorged with fat droplets of various sizes from less than 1–10 μ in diameter. In the immature forms the fat is pigmented and has a yellow colour; this colour disappears in a patchy fashion in the young adult and the old adult is practically devoid of pigment. In addition to fat droplets, small rounded granules of about 0.5 μ in diameter may be observed scattered throughout the cytoplasm of the cells; many of these show Maltese cross-birefringence and are considered to be uric acid spherules. Similar granules, together with birefringent rectangular crystals of up to 1 μ in long axis, are also seen in the cells of the Malpighian tubules. The rectangular crystals are never observed in the fat body. The fat droplets are monorefringent. The application of absolute alcohol to the tissue results in the disappearance of the Maltese cross-birefringent granules and the appearance of sheaves of uric acid crystals which can be seen by polarized light or by staining with Meyer's haemalum.

Supravital staining

Tissues were stained supravitally with 1:10,000 Janus Green B in 0.9% saline. Shortly after the application of the dye the nuclei of the haemocytes and the general cytoplasm of the cells of the Malpighian tubules become blue. The fat body has no affinity for the dye during the first 10–15 min. After this time the nuclei of the haemocytes may be seen stained as before, but diffuse coloration of the cells of the Malpighian tubules is no longer apparent and the dye is now seen to be concentrated in small round or ovoid perinuclear bodies which measure 0.1–0.5 μ in long axis, the remainder of the cytoplasm being either uncoloured or pale pink as a result of the formation of leuco Janus Green B or diethyl safranin (Lazarow & Cooperstein, 1953). In the cells of the fat body (fat cells and oenocytes) and the follicular cells of the ovary small discrete blue stained bodies 0.2–0.6 μ in diameter and round or ovoid in shape may now be observed. These are often most numerous in the perinuclear region, but are also scattered throughout the non-fatty parts of the cytoplasm. These granules are non-refractile and hence can readily be distinguished from small refractile fat droplets which at times appear to have a slight affinity for the dye; they are monorefringent. The Janus Green-positive granules are not stained by either Meyer's haemalum or an alcoholic solution of iodine. They are therefore distinct from uric acid granules, glycogen and small fat droplets. After about 40 min. the blue colour fades.

The application of a mixture of Janus Green 1:10,000 and neutral red 0.5% in saline to fresh tissue results after an interval of 10–15 min. in the staining of the small rounded or ovoid bodies, described above, with Janus Green, whilst larger cellular inclusions 0.5–1 μ show an affinity for neutral red. The nuclei of the fat cells and other tissues are stained intensely with neutral red.
DISCUSSION

The application of routine bacteriological stains to both smears and tissue sections has failed to reveal the presence of bacteria in the fat body of the locust. Virus particles of the type described by Williams & Smith (1957) in the crane fly *Tipula paludosa* can be excluded, as no DNA-positive material has been observed in the cytoplasm of the cells. It would therefore seem that the fat cells themselves, or the associated oenocytes, or both, must be responsible for the enzymic activity observed by Kilby & Neville (1957) in the carefully dissected fat body of the 5th-instar locust.

It has been shown by Lazarow & Cooperstein (1953) that in mammals supravital staining by Janus Green B is an oxygen-dependent reaction which depends upon the enzyme activity of the cell; the dye is thought to be reduced enzymically in the non-mitochondrial portion of the cell and more slowly in the mitochondria because of the presence of cytochrome oxidase in these structures. The application of a 1:10,000 solution of Janus Green B to portions of intact fat body, Malpighian tubules and follicular cells of the ovaries is followed after an interval of 10–15 min. by the staining of small rounded or ovoid structures (0.1–0.5 μ in diameter) in the cytoplasm of these cells. The structures are often most numerous in the perinuclear region and the colour fades after 30–45 min. These small granules are neither uric acid, minute fat droplets, nor glycogen, as judged by their birefringence and staining with Meyer’s haemalum, by their non-refractile nature or by staining with iodine. Similar small acid aniline-positive structures may be stained along with larger secretion droplets and cellular inclusions by Bensley’s acid aniline-methyl green; this stain is often considered to be relatively specific for mitochondria in tissue sections (Cowdrey, 1948). The fact that the cellular inclusions are stained by both Janus Green B and acid fuchsin (whilst not being identical in either microscopic appearance or staining with uric acids or urates, glycogen or fat) would suggest that these elements may be a form of mitochondrion; if so, they are more rounded or ovoid than the typical mammalian forms, as observed in the liver or pancreas, and more closely resemble the round or ovoid bodies described by Farrant, Potter, Robertson & Wilkins (1956) in the red beet.

Although accurate counts of the mitochondria-like bodies in the fat body cells cannot be made, because of the presence of overlying fat droplets, there does not appear to be any definite change in number in the successive developmental stages. This constancy in number would be more in keeping with the bodies being true organoids rather than inclusions, for both the uric acid and glycogen content of the cells vary in the different developmental stages.

The fat body of the locust is very rich in glycogen during the 1st- to 5th-instar stages. This is present not only in fat cells but also in oenocytes; the presence of glycogen in the latter type of cell is in contrast with the conditions found in the fat body of the mosquito larvae (Wigglesworth, 1942). Protein and phospholipids are very abundant in the oenocytes, while only relatively small quantities are present in the fat cells.
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In conclusion, it would seem, therefore, that the enzymic activity of the fat body of the locust is due to the presence of mitochondria-like bodies in both fat cells and associated oenocytes. As the great majority of the cells of the more readily dissected portion of the fat body are true fat cells, the oenocytes being most numerous at the periphery, it is probable that the activity observed by Kilby & Neville (1957) was mainly due to the presence of these structures in the true fat cells.

SUMMARY

The fat body of the locust *Schistocerca gregaria* is composed of both fat cells and oenocytes. The cells increase in number by mitotic division. Glycogen, proteins and phospholipids are present in all cells but are most abundant in the oenocytes; the amounts of these substances are less in the adult than in the immature forms, whereas uric acid is more abundant in the adult.

Small mitochondria-like structures (as judged by their staining reactions to Janus Green B and acid-fuchsin-methyl green) have been observed in the cytoplasm of the fat cells, the oenocytes, the Malpighian tubules and the follicular cells of the ovary.

No micro-organisms have been observed.

I am grateful to Mr K. R. Adkin for his assistance in preparing the histological material.

REFERENCES


EXPLANATION OF PLATE 5

Fig. 1. Fat body of 4th-instar locust. The body wall lies at the left and the gut at the right extremity of the photomicrograph. A few oenocytes lie in the peripheral zone. Haematoxylin and eosin, × 90.

Fig. 2. Fat cells of 4th-instar locust. Small discrete acid-fuchsin stained bodies are scattered throughout the cell. Acid-fuchsin-methyl green, × 1250.

Fig. 3. 4th-instar locust. Fat cells and oenocytes stained for glycogen. PAS, × 400.

Fig. 4. 4th-instar locust. Fat cells and oenocytes stained for protein. Danielli method, × 400.

Fig. 5. 4th-instar locust. Fat cells and oenocytes stained by McManus's method for phospholipid. Sudan Black B, × 400.
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