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Paraffin Synthesis in the Oenocytes of the Desert Locust

OENOCYTES are one of the least understood types of insect cells. Several workers have suggested that the oenocytes produce structural lipoproteins for the formation of the cuticle or the paraffins and waxes which are involved in water-proofing the cuticle[1-4]. Electron microscope studies[5-9], ligation experiments[10,11] and biochemical studies[11-13] suggest that the oenocytes rather than the prothoracic glands synthesize the moulting hormone ecdysone. At present, however, there is no direct and conclusive evidence to support either of these hypotheses.

We have investigated the cytology and function of the oenocytes in the desert locust *Schistocerca gregaria*. The animals used were fifth instar female larvae fed on bran and wheat seedlings and exposed to a day of 12 h (35° C) followed by a night of 12 h (25° C). The instar lasts about 9 days. The oenocytes are found only in the peripheral abdominal fat body in association with ordinary fat body cells and with some small "urate cells". The central fat body surrounding the gut contains only ordinary fat body cells. The extensive smooth endoplasmic reticulum which we have observed in the oenocytes suggests that these cells synthesize lipids. We therefore carried out incorporation studies using ^{14}C-acetate with both types of fat body and with hypodermal cells from the tergum of the first thoracic

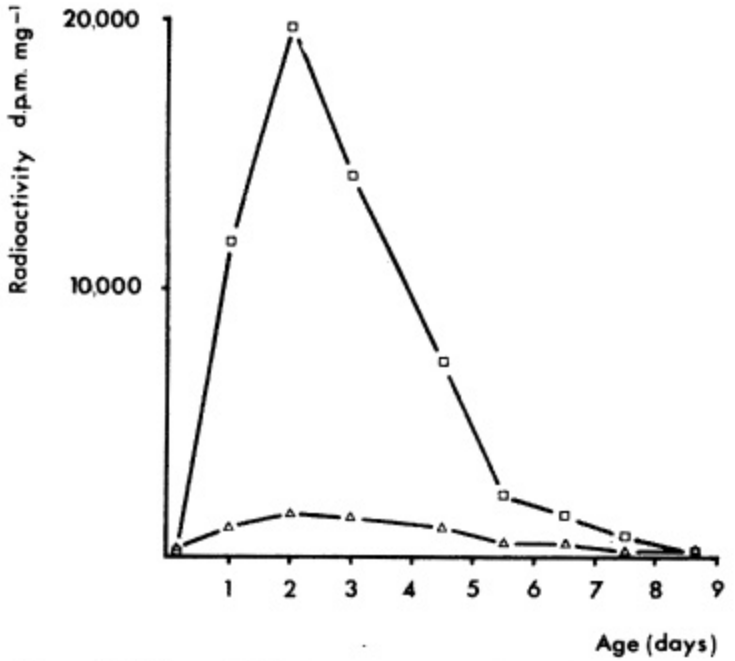

Fig. 1 ^{14}C-labelled lipids from the central yellow fat body during the development of the fifth instar in *Schistocerca gregaria*. Radioactivity (in discharges per minute, d.p.m.) in phospholipids (△) and triglycerides (□) per mg tissue (wet weight). The instar lasts about 9 days.

segment. Dissections and incubations were carried out in a buffered Ringer solution (20 mM PIPES-buffer at *p*H 6.8 containing 125 mM Na^+, 10 mM K^+, 2 mM Ca^{2+}, 2 mM Mg^{2+}, 143 mM Cl^-, and 2.5 mM glucose). In each experiment 100 mg of fat body or 4 terga, freed from other tissues, were incubated for 1 h at 35° C in 2.5 ml. of the Ringer containing 1.6 μCi of uniformly labelled ^{14}C-Na-acetate (0.03 μM). After homogenization the lipids were extracted with chloroform-methanol (2:1 v/v)[14], separated by thin-layer chromatography on silica gel G using several solvent systems of differing polarity and identified by comparison with lipid standards. Radioactive spots were located with a plate scanner, removed from the plate and counted by liquid scintillation counting.

Incorporation of the radioactive precursor was followed daily throughout the instar. Figs. 1 to 3 show that both phospholipids and triglycerides were labelled extensively in all three tissues. In both types of fat body the high rates of incorporation into triglycerides confirm previous observations[15] that triglycerides are the principal lipids synthesized

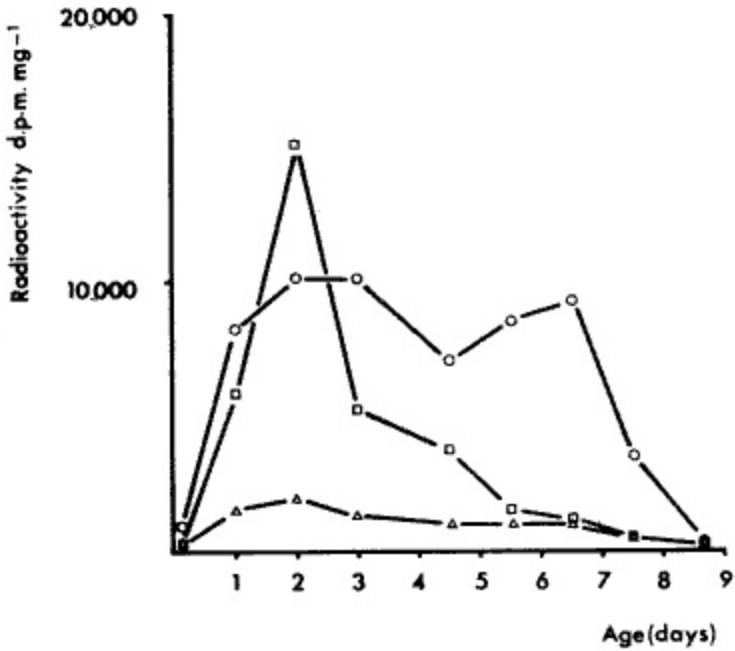

Fig. 2 ^{14}C-labelled lipids from the oenocyte-rich peripheral fat body. Radioactivity (d.p.m.) in phospholipids (△), triglycerides (□) and paraffins (○) per mg tissue (wet weight) determined at various stages of the fifth instar.

by and stored in the fat body of *Schistocerca*. In the oenocyte-rich peripheral fat body, however, there is a third extensively labelled lipid fraction which is absent from the central fat body (Fig. 2). Thin-layer chromatography suggests strongly that these lipids are paraffins because they co-chromatograph in hexane with paraffin standards. Several lipids of unknown nature were also labelled to a small extent. (The data for these small fractions are omitted from Figs 1 to 3.) The hypodermis also synthesizes paraffins (Fig. 3), but the amount is so small that the incorporation of ^{14}C-acetate into paraffins by the entire hypodermis is less than 1% of that simultaneously incorporated by the oenocyte-rich fat body. Thus the synthesis of paraffins must take place primarily in the oenocyte-containing peripheral fat body.

The "paraffin" fraction isolated by thin-layer chromatography was analysed by gas-liquid chromatography on a SE-30 column. Most of the components showed retention times similar to those of the n-alkanes C_{21}–C_{33} with the odd-numbered C_{25}-, C_{27}-, C_{29}- and C_{31}-*n*-alkanes dominating.

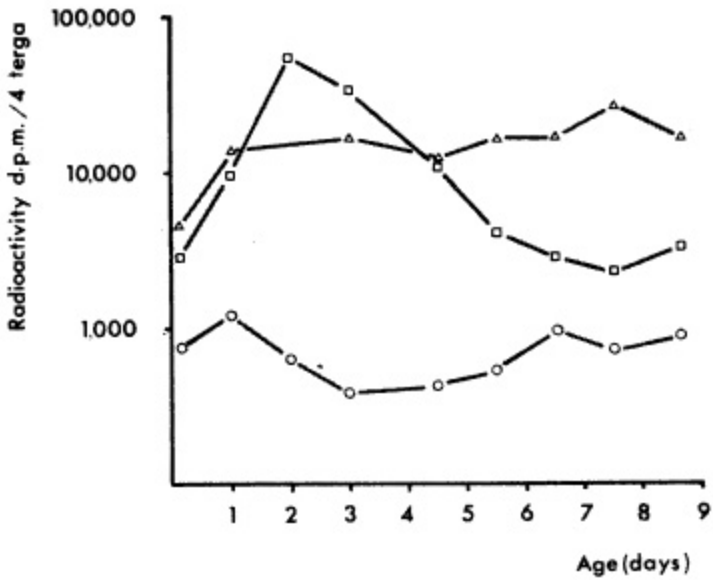

Fig. 3 ^{14}C-labelled lipids from the hypodermis of the tergum of the first thoracic segment. Radioactivity (d.p.m.) in phospholipids (△), triglycerides (□) and paraffins (○) determined at various stages of the fifth instar. Four terga were used in each experiment. Note that the vertical scale is logarithmic by contrast with those of Figs. 1 and 2.

Infrared spectroscopy also suggests that these lipids are saturated paraffins.

Our cytological and histochemical studies suggest that the oenocytes are responsible for the synthesis of paraffins. This is supported by a cell separation experiment. Oenocyte-rich fat body from 5-day-old larvae was incubated as above and then placed for 10 min in Ringer solution at 35° C containing 0.05% 'Pronase'. Cell separation was facilitated by repeatedly drawing the tissue sample through a pipette. Centrifugation at low speed (3–5*g*) for 1 min left most of the fat body cells in a froth at the surface. Most of the "urate cells" remained in the supernatant. The pellet contained an abundance of oenocytes with a few fat body cells and "urate cells". In the pellet the ratio between the radioactivity in the paraffin fraction and in the triglyceride fraction was 13 : 1 whereas in the intact control tissue the ratio was 2.3 : 1. This enhancement of the paraffin fraction following partial separation of the oenocytes strongly supports the view that these cells are respon-

sible for paraffin synthesis and that triglyceride synthesis is mainly associated with the ordinary fat body cells.

The paraffins synthesized in the oenocytes must be transported to the cuticle where they make up 40–60% (w/w) of the chloroform-soluble cuticle lipids. Indeed, after *in vivo* incorporation of ^{14}C-acetate, paraffins are the most extensively labelled of the cuticular lipids. From the above results, we conclude that in *Schistocerca* larvae a large proportion of the cuticular lipids is synthesized by the oenocytes and that this must be one of the main functions of these cells. The role of oenocytes in ecdysone synthesis, however, remains uncertain. ^{3}H-cholesterol injected into locusts without prothoracic glands is converted into α and β-ecdysone (identified by thin-layer chromatography of their trimethylsilyl derivatives[16]). At present one cannot exclude the possibility that oenocytes may be responsible for this conversion.

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