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SYNTHESIS AND RELEASE OF HYDROCARBONS BY THE OENOCYTES OF THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*

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Abstract—When incubated *in vitro*, the oenocytes in the peripheral fat body of the desert locust incorporate Na-^{14}C-acetate into hydrocarbons (paraffins). The presence of haemolymph in the incubation medium greatly stimulates the release of the ^{14}C-hydrocarbons into the medium. The labelled hydrocarbons appear to be rapidly released by the cells into the incubation medium as a function of time provided that haemolymph is present. The fact that the oenocytes not only synthesize ^{14}C-hydrocarbons but also release them into the medium supports the hypothesis that the oenocytes of the desert locust synthesize cuticle lipids.

INTRODUCTION

A VARIETY of functions have been attributed to oenocytes in the past (*cf.* WIGGLESWORTH, 1972). Based on light microscope observations, most workers suggest that the oenocytes produce structural lipoproteins for the cuticle, the egg shell, and the spermathecal sheath, or lipids such as paraffins and waxes which are part of the water-proofing wax layer (KRAMER and WIGGLESWORTH, 1950; WIGGLESWORTH, 1970, 1972). PIEK (1964) carried out biochemical and autoradiographical studies on the oenocyte-rich fat body of the honey-bee. She concluded, although not convincingly, that the oenocytes synthesize the hydrocarbons and wax acids found in beeswax. On the other hand, NELSON (1969) reported that in *Periplaneta americana* the epidermal cells are responsible for the synthesis of hydrocarbons rather than the oenocytes. This conclusion seems questionable because he assumed that the oenocytes are located in the fat body. According to KRAMER and WIGGLESWORTH (1950), however, the oenocytes in the cockroach are closely associated with the epidermis. Thus NELSON's results may support the hypothesis that hydrocarbons are synthesized by the oenocytes.

Recently, there has been a revival of an old hypothesis (KOLLER, 1929) that oenocytes might be the source of the moulting hormone. Several electron microscope studies have demonstrated that these cells are packed with smooth endoplasmic reticulum, thus resembling steroid-producing cells in vertebrates (DELACHAMBRE, 1966; EVANS, 1967; LOCKE, 1969; RINTERKNECHT *et al.*, 1969, 1973; GNATZY, 1970; CASSIER and FAIN-MAUREL, 1972; ROMER, 1972). For this reason it was suggested that the steroid hormone ecdysone might be produced by the oenocytes and not by the prothoracic glands as commonly assumed. This hypothesis was further supported by ligation experiments (WEIR, 1970; GERSCH and STUERZEBECHER, 1971) and by the fact that the conversion of cholesterol into α- and β-ecdysone can occur outside the prothoracic glands (GERSCH and STUERZEBECHER, 1971; NAKANISHI *et al.*, 1972). Furthermore, neither α- nor β-ecdysone could be demonstrated in the prothoracic glands of *Schistocerca gregaria* using gas–liquid chromatography (ELLIS *et al.*, 1972).

At present, however, there is no unequivocal evidence available to support any of the proposed hypotheses on the function of oenocytes. We therefore decided to reinvestigate the structure and the function of oenocytes in the desert locust *S. gregaria*, using cytological (GUPTA and DIEHL, in preparation) as well as biochemical techniques. The oenocytes of this insect are located in the peripheral fat body of the abdomen situated beneath the epidermis. They are associated with some small 'urate cells' and with ordinary fat body cells. The central fat body surrounding the gut contains only fat body cells. The fat body cells synthesize and store triglycerides as their main product (WALKER and BAILEY, 1970; WALKER *et al.*, 1970).

The outstanding cytological feature of the oenocytes in the desert locust is their richness in smooth endoplasmic reticulum (GUPTA and DIEHL, in preparation). This suggested that the oenocytes may synthesize lipids. *In vitro* studies on the incorporation of Na-^{14}C-acetate showed that the oenocytes synthesized large amounts of ^{14}C-hydrocarbons (DIEHL, 1973). Infrared spectroscopy and gas–liquid chromatography suggested that these hydrocarbons are saturated normal and branched paraffins. Similar paraffins could also be extracted

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from the haemolymph and from the cuticle where they constitute about 40 to 60 per cent of the chloroform-soluble lipids (DIEHL, 1973; DIEHL, in preparation). These findings strongly suggest that the oenocytes of the desert locust synthesize cuticle hydrocarbons which presumably participate in the formation of the wax layer. If this hypothesis is correct we would expect that the ^{14}C-hydrocarbons synthesized by the oenocytes would also be released into the haemolymph. In this paper we would like to present the results of some *in vitro* studies which indicate that the newly synthesized ^{14}C-hydrocarbons are indeed released by the cells into the incubation medium provided that haemolymph is present.

MATERIALS AND METHODS

Insects

The animals used were fifth instar female larvae of *Schistocerca gregaria*. They were reared as previously described (DIEHL, 1973). The fifth instar lasts about 9 days. In order to get reproducible results we used larvae of the same age (3½ days old). Parallel ultrastructural studies showed that the oenocytes at that stage have a fully developed smooth endoplasmic reticulum and appear to be active.

Collection of haemolymph

Haemolymph was always freshly collected immediately before an experiment. The tip of the abdomen was cut off and the head with the attached alimentary canal gently pulled away from the body. The carcass was placed in a conical centrifuge tube, thorax downwards. The tubes were then centrifuged at 5 to 10 *g* for 1 min. The animals were removed and the tubes with haemolymph recentrifuged for 5 min at about 800 *g* to remove haemocytes and tissue debris. The samples of clear supernatant were then pooled. The whole procedure was carried out at 2 to 4°C. This prevented coagulation and darkening of the haemolymph.

Tissues

In this study both the oenocyte-free central fat body and the oenocyte-rich peripheral fat body from the ventral site beneath the nerve cord were dissected out and carefully freed from other tissues. The whole process was carried out in a modified culture medium TC 199 (VAGO, 1971). Medium TC 199 based on Earle's salts with 20 mM HEPES buffer (Flow Laboratories, Irvine, Scotland) was adjusted to a pH of 6·8 and an osmolarity of about 320 mOsm by adding NaCl (1·75 g/l.), $NaHCO_3$ (100 mg/l.), and 2 N HCl.

Release studies

All *in vitro* studies on the release of the ^{14}C-hydrocarbons by the oenocytes into the medium were carried out with intact oenocyte-rich peripheral fat body rather than with isolated oenocytes. Thus we avoided possible damage to the cells by enzymatic and mechanical dissociation procedures. The tissues were labelled in 2 ml of a simple Ringer solution (DIEHL, 1973) containing uniformly labelled Na-^{14}C-acetate (1·2 μCi/ml, sp. act. 57 mCi/m-mole, Radiochemical Centre Amersham). The labelling was carried out for 10 min at 30°C in a shaking water-bath. This tissue will subsequently be referred to as 'prelabelled tissue'. The samples were then rinsed for 1 min in the modified medium TC 199 and further incubated in 1 ml of the medium at 30°C. In some experiments the medium contained varying amounts of either fetal calf serum or of locust haemolymph collected from animals of the same age. At certain times during the incubation period, aliquots of 25 μl were removed from the medium and the radioactivity of the lipids determined. At the end of the experiment the tissues were rinsed several times in fresh medium TC 199 before the tissue lipids were extracted.

Extraction of lipids

Lipids from homogenized tissue and from the incubation media were extracted with chloroform–methanol (2 : 1 v/v) and purified according to FOLCH *et al.* (1957).

Thin-layer chromatography (TLC)

Lipids were separated on factory-made 20 × 20 cm glass plates coated with silica gel-G (E. Merck, Darmstadt, Germany). The plates were activated for 30 min at 110°C. For the separation of the main lipid classes we used the double-development system of FREEMAN and WEST (1966) with diethyl ether–benzene–ethanol–acetic acid 40 : 50 : 2 : 0·2 followed by diethyl ether–hexane 6 : 94. This was sometimes followed by a third development with hexane to obtain a better separation of the hydrocarbons. For the separation of hydrocarbons and the wax esters from the rest of the lipids we used a double-development system with chloroform–carbon tetrachloride 5 : 95 followed by hexane. Hexane alone was used to separate hydrocarbons from the other lipids. The lipids were visualized by exposing them to iodine vapours. They were identified by comparison with commercially available lipid standards run on the same plate. The hydrocarbon standards used were squalene and paraffins (C_{16}-, C_{18}-, C_{28}, and C_{36}-n-alkanes).

Radioactivity measurements

After separation, the radioactive lipids were localized by scanning the plates with a radiochromatogram scanner (Berthold/Frieseke, Wildbad, Germany). The silicic acid containing the radioactive lipids was scraped off into counting vials.

Table 1—The effect of different incubation media on the release of ^{14}C-hydrocarbons by prelabelled peripheral fat body into the medium

incubation medium	^{14}C-hydrocarbons in counts/min		% of ^{14}C-hydrocarbons released into the medium
	in tissue	in medium	
TC 199	67'960	2'810	4 %
TC 199 + 20 % fetal calf serum	82'610	8'570	9.4 %
TC 199 + 20 % haemolymph	13'458	32'874	71 %

Fifteen ml of toluene (scintillation grade) containing 2·7 g/l. PPO and 26·7 mg/l. POPOP were added to each vial. The ^{14}C-radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer (Model 3320). When necessary, the results were corrected for quenching by means of an external standard or by the channel ratio method. The counting efficiency was 82 per cent.

RESULTS

Dependence of the release of ^{14}C-hydrocarbons on the presence of haemolymph in the incubation medium

In order to study *in vitro* the effect of haemolymph on the release of the ^{14}C-hydrocarbons into the incubation medium prelabelled oenocyte-rich peripheral fat body was incubated for 1 hr at 30°C in 1 ml of medium. Three different kinds of media were used: medium TC 199, medium TC 199 with 20% haemolymph, and medium TC 199 with 20% fetal calf serum. Table 1 shows that the tissues synthesized ^{14}C-hydrocarbons which cochromatographed with the paraffin standards in all solvent systems used in thin-layer chromatography. The addition of haemolymph led to a release into the incubation medium of about 71 per cent of the ^{14}C-hydrocarbon radioactivity. In contrast less than 10 per cent of the hydrocarbon radioactivity was released from the tissues when they were incubated in medium TC 199 (4 per cent) or in medium TC 199 with 20% fetal calf serum (9·4 per cent).

Thus the addition of haemolymph greatly stimulated the release of ^{14}C-hydrocarbons from the tissue into the incubation medium. On the other hand, neither ^{14}C-phospholipids nor ^{14}C-triglycerides were detectable in the medium although these lipids were extensively labelled in the tissue. It is therefore unlikely that the release of the ^{14}C-hydrocarbons was caused by cell damage.

Effect of haemolymph concentration on the release of ^{14}C-hydrocarbons into the medium

Fig. 1 shows that the ^{14}C-hydrocarbons were released from the prelabelled tissue at a greater rate as the haemolymph concentration was increased up to 20%. Addition of more haemolymph seemed to have no effect on the amount of ^{14}C-hydrocarbon radioactivity released.

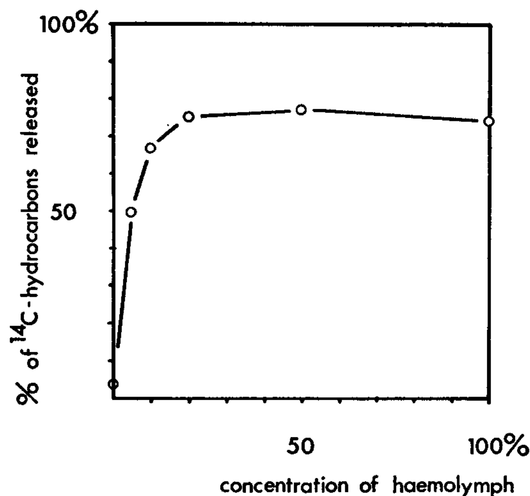

Fig. 1. The effect of different haemolymph concentrations in the incubation medium on the release of ^{14}C-hydrocarbons by peripheral fat body. Prelabelled peripheral fat body from 1 animal was cut into six pieces of about the same size. Each of the pieces was then incubated for 1 hr at 30°C in 1 ml of medium. The six incubation media were made up from medium TC 199 with the addition of different amounts of haemolymph (0, 5, 10, 20, 50, and 100 per cent). In the above figure the amount of ^{14}C-hydrocarbons released into the medium is expressed as a percentage of the total amount of ^{14}C-hydrocarbons produced by the tissue during the entire experiment.

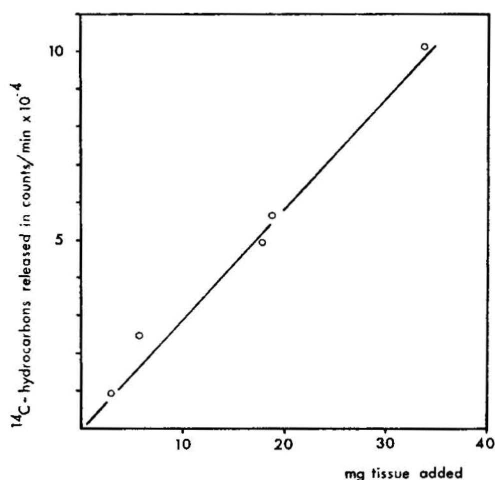

Fig. 2. The release of ^{14}C-hydrocarbons from prelabelled peripheral fat body as a function of the amount of tissue (wet weight) added to the incubation medium.

Fig. 2 indicates that when increasing amounts of prelabelled tissue were incubated in medium TC 199 with 20% haemolymph added, the ^{14}C-hydrocarbon radioactivity in the medium increased as a function of the amount of tissue added. This shows that under the experimental conditions the capacity of the medium to take up ^{14}C-hydrocarbons did not reach saturation.

Release of the ^{14}C-hydrocarbons as a function of time

Figs. 3 and 4 show the time course of the release of ^{14}C-hydrocarbons from prelabelled tissue into the medium containing 20% haemolymph. The initial rate of release was high. From Fig. 3 it can be estimated that the amount of hydrocarbon ^{14}C-radioactivity released into the medium during the first 10 to 15 min equalled the amount of hydrocarbon radioactivity which was originally present in the prelabelled tissue at the beginning of the release period. This suggests that at least in the initial stage of the experiment the ^{14}C-hydrocarbons were rapidly synthesized but also rapidly released. After about 15 min the amounts of hydrocarbon ^{14}C-radioactivity released into the medium gradually decreased. From Fig. 3 it appears that the ^{14}C-hydrocarbons released after 15 min were to a large extent synthesized during the incubation period in medium TC 199 with 20% haemolymph. About 60 per cent of all the ^{14}C-hydrocarbons synthesized during the entire experiment was produced during the 2 hr release period and about 40 per cent during the prelabelling period. A considerable amount of hydrocarbon radioactivity appeared therefore after the prelabelling period. Our experiments did not correspond to an ideal pulse-chase situation, where no appreciable labelling of the cell product occurs after the pulse period. Nevertheless, Figs. 3 and 4 demonstrate that most of the ^{14}C-hydrocarbons synthesized during the entire experiment (75–81 per cent of the total hydrocarbon radioactivity) were also released by the cells into the medium as a function of time.

^{14}C-diglycerides were also released into the medium as a function of time (Figs. 3, 4). It is reasonable to conclude that the fat body cells were responsible for their synthesis. Triglycerides are the predominant lipids synthesized and stored by the fat body cells of *S. gregaria* (Walker *et al.*, 1970; Diehl, 1973). It appears therefore that these lipids are released into the haemolymph in the form of diglycerides as this appears to be the case in the closely related species *Locusta migratoria* (Tietz, 1967; Peled and Tietz, 1973), in the grasshopper *Melanoplus differentialis* (Chino and Gilbert, 1965),

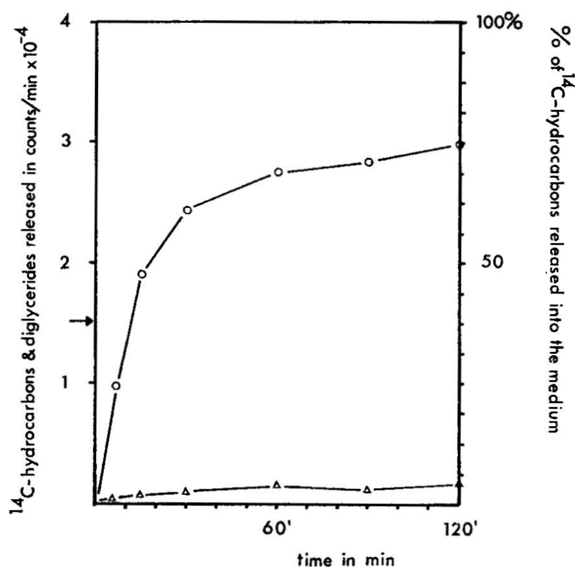

Fig. 3. Time course of the release of ^{14}C-hydrocarbons (○) and ^{14}C-diglycerides (△) from prelabelled peripheral fat body into the incubation medium. Peripheral fat body from 1 animal was prelabelled and then cut longitudinally into two halves of about equal size. One half of the tissue (2·8 mg dry weight) was immediately extracted with chloroform–methanol (14,050 counts/min in the hydrocarbon fraction). The other half of the tissue (3·2 mg dry weight) was further incubated for 2 hr at 30°C in medium TC 199 containing 20% haemolymph. The arrow (→) on the ordinate (left-hand side) indicates the amount of ^{14}C-hydrocarbons present in the tissue at the beginning of the 2 hr release period. This value was estimated by using the amount of ^{14}C-hydrocarbons present in the other half of the tissue extracted at that time. Ordinate on the right-hand side: ^{14}C-hydrocarbon radioactivity released expressed as a percentage of the total amount of ^{14}C-hydrocarbons synthesized during the entire experiment.

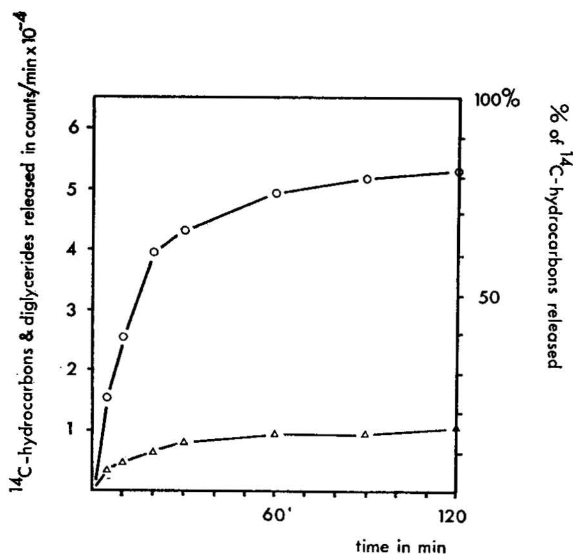

Fig. 4. The release of ^{14}C-hydrocarbons (○) and ^{14}C-diglycerides (△) from prelabelled peripheral fat body (18·3 mg wet weight) into the incubation medium as a function of time. In another experiment (not shown) very similar percentage values of released ^{14}C-hydrocarbons were obtained. At 10 min 39 per cent, at 20 min 58·9 per cent, at 30 min 69 per cent, at 60 min 74·7 per cent, at 90 min 81 per cent and at 120 min 81·2 per cent were the amounts released into the medium.

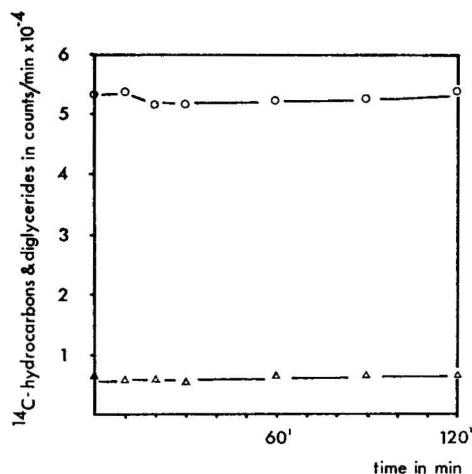

Fig. 5. Incubation of peripheral fat body with medium containing ^{14}C-hydrocarbons (○) and ^{14}C-diglycerides (△) prepared from prelabelled peripheral fat body: 18·2 mg (wet weight) of peripheral fat body were prelabelled (step 1) and incubated for 30 min at 30°C in 1 ml of medium TC 199 with 20% haemolymph (step 2). After removing the prelabelled tissue fresh unlabelled peripheral fat body (19·6 mg wet weight) was added to the radioactive medium and incubated for 2 hr at 30°C (step 3). The figure shows the amounts of ^{14}C-hydrocarbons (○) and ^{14}C-diglycerides (△) in counts/min $\times 10^{-4}$ present in the incubation medium during step 3. At the end of the experiment the originally unlabelled tissue was washed several times in medium TC 199 and then extracted with chloroform–methanol (2 : 1). It contained 1206 counts/min in the hydrocarbon fraction (2 per cent of the total hydrocarbon ^{14}C-radioactivity). During step 3 the total amount of ^{14}C-radioactivity in all extractable lipids from tissue and medium increased by about 6·3 per cent compared with the amount present in the lipids of the medium at the beginning of step 3.

and in some other insects (cockroach: CHINO and GILBERT, 1965; DOWNER and STEELE, 1972; Lepidoptera: CHINO and GILBERT, 1965; CHINO *et al.*, 1969; BHAKTAN and GILBERT, 1970).

Possible contribution of ^{14}C-diglycerides and other labelled compounds in the medium to the synthesis of ^{14}C-hydrocarbons

Fig. 3 shows that about 60 per cent of the hydrocarbon radioactivity was synthesized during the 2 hr release period although the precursor Na-^{14}C-acetate was no longer present in the medium. Two experiments were therefore designed to determine if the ^{14}C-diglycerides or other labelled compounds present in the medium were taken up again by the oenocytes and subsequently converted into ^{14}C-hydrocarbons.

In the first experiment (Fig. 5) labelled incubation medium was prepared by prelabelling peripheral fat body (step 1). The tissue was then incubated for 30 min at 30°C in medium TC 199 with 20% haemolymph (step 2). Fresh peripheral fat body was then added to this medium and incubated for 2 hr (step 3). Fig. 5 shows that the levels of radioactivity of the hydrocarbons and the diglycerides did not change to any great extent during step 3. At the end of the experiment the tissue contained only about 2 per cent of the total hydrocarbon radioactivity. From this experiment it is not clear how much of these ^{14}C-hydrocarbons found in the tissue after 2 hr were in fact taken up from the medium and how much of these lipids were synthesized *de novo* from ^{14}C-diglycerides or other labelled compounds present in the medium. A synthesis of labelled lipids certainly occurred during step 3 because the total amount of radioactivity in all extractable lipids from tissue and haemolymph increased by 6 per cent during this period. This could be caused by water-soluble ^{14}C-compounds released during step 2 from the prelabelled tissue into the medium and taken up by the unlabelled tissue during step 3.

In the second experiment (Fig. 6), labelled medium was prepared which contained ^{14}C-diglycerides but no ^{14}C-hydrocarbons. Central fat body (without oenocytes!) was prelabelled (step 1) and incubated for 30 min in medium containing

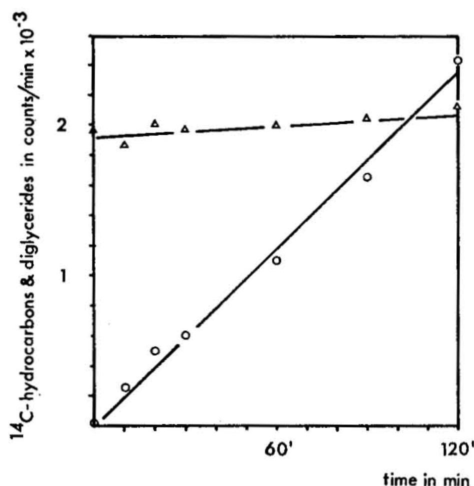

Fig. 6. Incubation of peripheral fat body with medium containing ^{14}C-diglycerides (△) prepared from prelabelled central fat body: 12·4 mg (wet weight) of central fat body were prelabelled (step 1) and incubated for 30 min at 30°C in medium containing 20% haemolymph (step 2). Fresh peripheral fat body (11·8 mg wet weight) was incubated for 2 hr at 30°C in the radioactive medium (step 3). The figure shows the levels of ^{14}C-hydrocarbons (○) and ^{14}C-diglycerides (△) in counts/min $\times 10^{-3}$ present in the medium during step 3. At the end of the experiment the tissue was washed several times with medium TC 199. It contained 260 counts/min in the hydrocarbon fraction (9·6 per cent of the total hydrocarbon ^{14}C-radioactivity). During step 3 the total amount of ^{14}C-radioactivity in all extractable lipids from tissue and medium had increased by about 135 per cent compared with the amount of radioactivity present in the lipids of the medium at the beginning of step 3.

20% haemolymph (step 2). Fresh peripheral fat body was then incubated in this medium for 2 hr (step 3). Fig. 6 shows that during step 3 a small amount of ^{14}C-hydrocarbons was synthesized by the peripheral fat body which was subsequently also released into the medium. As in the previous experiments (Figs. 3, 4), about 90 per cent of the hydrocarbon radioactivity was released into the medium. The amount of ^{14}C-diglycerides did not change to any significant extent. From these data we cannot decide whether ^{14}C-diglycerides or some other labelled compounds in the medium served as precursors for the synthesized hydrocarbons. Again as in the preceding experiment (Fig. 5), net synthesis of lipids was occurring during step 3 because the total radioactivity in all extractable lipids from tissue and medium increased by about 135 per cent mainly due to the synthesis of the ^{14}C-hydrocarbons. Water-soluble labelled compounds, released during step 2 by the prelabelled tissue, must have served as precursors for this lipid synthesis.

From these two experiments (Figs. 5, 6) we can conclude that non-lipid ^{14}C-labelled substances were released by the tissue (presumably the fat body cells) into the medium which subsequently could be taken up again by the cells. These water-soluble compounds were used to synthesize lipids. Therefore it appears that these compounds and also perhaps the ^{14}C-diglycerides in the medium could have been used by the tissue to synthesize at least part of the ^{14}C-hydrocarbons which were synthesized after the prelabelling period in Fig. 3. Figs. 5 and 6 seem to indicate that the labelled medium, after 30 min of incubation with prelabelled tissue, gave rise to the synthesis of only a small amount of ^{14}C-hydrocarbons compared with the amount of hydrocarbon radioactivity produced and released during the time course experiment (Fig. 3). But these experiments are of only limited value in so far as they show only the amount of potential hydrocarbon ^{14}C-precursors which were present in 30 min 'old' incubation medium. Therefore the actual contribution of these labelled compounds in the medium to the synthesis of ^{14}C-hydrocarbons during the 2 hr release period (Fig. 3) was presumably higher than what would be estimated from the two experiments (Figs. 5, 6).

Another possibility exists of explaining at least part of these ^{14}C-hydrocarbons synthesized after the prelabelling period in Fig. 3. It is likely that labelled precursors like acetate accumulated to a certain extent intracellularly in the oenocytes during the prelabelling period, which were later used for hydrocarbon synthesis during the 2 hr release period. But from our data we cannot estimate as to how much of these ^{14}C-hydrocarbons are accounted for by this storage. It is likely that both phenomena, uptake of labelled compounds from the medium and use of intracellularly stored precursors, contributed in Fig. 3 to the synthesis of ^{14}C-hydrocarbons during the release period.

Possible uptake of ^{14}C-hydrocarbons from the medium by the tissue

In the experiments on the time course of the release of the ^{14}C-hydrocarbons (Figs. 3, 4), about 19 to 25 per cent of the total hydrocarbon radioactivity was still associated with the tissue at the end of the 2 hr release period. One simple explanation could be that the tissue might have taken up ^{14}C-hydrocarbons from the medium. But Fig. 5 shows that this did not happen to any appreciable extent because the originally unlabelled tissue only contained 2 per cent of the total hydrocarbon radioactivity at the end of the experiment. The possible uptake of labelled hydrocarbons by fat body cells was investigated (Fig. 7). The central fat body contains no oenocytes and therefore shows no synthesis of hydrocarbons. This tissue was incubated for 2 hr in a labelled medium containing ^{14}C-hydrocarbons which was prepared from prelabelled peripheral fat body. As in Fig. 5, no appreciable uptake of labelled hydrocarbons took

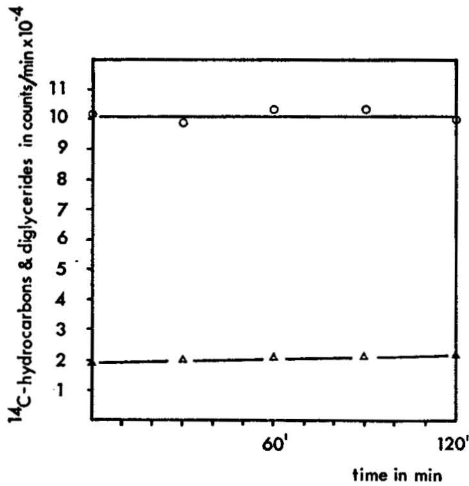

Fig. 7. Incubation of central fat body in radioactive medium containing ^{14}C-hydrocarbons (○) and ^{14}C-diglycerides (△) prepared from prelabelled peripheral fat body: 35 mg (wet weight) of peripheral fat body was prelabelled (step 1) and further incubated for 30 min at 30°C in medium containing 20% haemolymph (step 2). Fresh central fat body (15 mg wet weight) was added to the medium and incubated for 2 hr at 30°C. The figure demonstrates the amounts of ^{14}C-radioactivity in counts/min × 10^{-4} present in the hydrocarbons (○) and in the diglycerides (△) of the medium during step 3. At the end of the experiment the tissue was rinsed several times in medium TC 199. The originally unlabelled tissue contained 1769 counts/min in the hydrocarbon fraction (1·7 per cent of the total hydrocarbon radioactivity). At the end of step 3, 2·4 per cent more ^{14}C-radioactivity was found in all extractable lipids from tissue and medium compared with the amount of radioactivity present in the lipids of the medium at the beginning of the 2 hr incubation period

place. Only a small amount (1·7 per cent) of ^{14}C-hydrocarbons was associated with the tissue at the end of the experiment, which could not be removed by repeated washings in fresh medium TC 199. From Figs. 5 and 7 we can estimate that during the time course experiment on the release of ^{14}C-hydrocarbons (Figs. 3, 4) the peripheral fat body took up only a small amount of the ^{14}C-hydrocarbons present in the medium. The uptake of already-released ^{14}C-hydrocarbons could only to a small extent account for the hydrocarbon radioactivity which was associated with the peripheral fat body at the end of the experiment. Therefore the majority of these labelled hydrocarbons present in the tissue were synthesized but presumably not yet released.

DISCUSSION

If the hypothesis that oenocytes in the desert locust are involved in the synthesis of cuticle lipids is correct, one should expect that these lipids are released into the haemolymph in order to get transported from the site of synthesis to the target tissue (e.g. epidermis for cuticle). The results suggest that at least under *in vitro* conditions this was indeed the case. Although the time course of the release of the ^{14}C-hydrocarbons is rather difficult to interpret for want of estimations of specific activities and because a true pulse-chase situation was not achieved, it nevertheless appears that most of the hydrocarbon radioactivity was released by the oenocytes into the medium provided that haemolymph was present. At least the initial rate of release appeared to be high. The amount of hydrocarbon radioactivity which was released into the medium during the first 10 to 15 min equalled the total amount of hydrocarbon radioactivity originally present in the tissue at the beginning of the release period. This suggests that after their synthesis in oenocytes the ^{14}C-hydrocarbons were rapidly released by the cells.

Although one has to be careful in applying the results from *in vitro* experiments to the *in vivo* conditions, the finding of a rapid release of the cell product after synthesis would agree well with the ultrastructure of the oenocytes (GUPTA and DIEHL, in preparation). The hydrocarbons do not seem to be stored to any appreciable extent inside the cell in contrast to, for example, triglycerides in the fat body cells which are stored in large granules. As a working hypothesis it is therefore proposed that the hydrocarbons are synthesized in association with the smooth endoplasmic reticulum, further processed through numerous Golgi bodies, and that the product leaves the cells by exocytosis of vesicles, much in the same way as some lipoproteins in the mammalian liver (e.g. STEIN and STEIN, 1973).

It appears that the presence of haemolymph in the medium greatly stimulates the release of the ^{14}C-hydrocarbons. At present we do not know the reason for this stimulatory effect. It could be that the haemolymph contains hormone(s) or other substances which are needed for operating the release mechanism. A similar stimulation by haemolymph was noted for the release of diglycerides from the fat body cells in *L. migratoria* (TIETZ, 1967; PELED and TIETZ, 1973) and in *H. cecropia*, *P. americana*, and *M. differentialis* (CHINO and GILBERT, 1965). The release of phospholipids from fat body in *H. cecropia*, *L. maderae*, and *P. americana* also depended on the presence of haemolymph in the medium (THOMAS and GILBERT, 1967).

From the results on the release of other kinds of lipids in insects it is tempting to speculate that the presence of haemolymph lipoproteins is required for the functioning of the release mechanism. These lipoproteins act presumably as 'acceptors' for the lipids. In the silkworm *Philosamia cynthia* the release of diglycerides and of cholesterol seems to depend on specific lipoproteins which had to be present in the medium (CHINO *et al.*, 1969; CHINO

and GILBERT, 1971). From our results we cannot rule out the possibility that the oenocytes synthesize the hydrocarbon-carrying lipoproteins themselves, but do not release them in the absence of a blood-borne stimulating substance (e.g. hormonal). Nevertheless, at present we favour the hypothesis that the release of the ^{14}C-hydrocarbons was due to the presence of haemolymph lipoproteins which were capable of accepting the hydrocarbons. Consequently, this would raise the interesting question as to whether the haemolymph lipoproteins are taken into the cells, 'loaded' intracellularly with the hydrocarbons, and released again into the medium or whether the hydrocarbons are transported to the cell surface and only there combine with the lipoproteins.

Apart from acting as 'acceptors' for the hydrocarbons during the actual release, the haemolymph lipoproteins presumably serve also as 'transport vehicles'. It is reasonable to suggest that the highly hydrophobic hydrocarbons are transported in the haemolymph in the form of water-soluble lipoproteins. In other insects the haemolymph lipoproteins are known to carry lipophilic substances such as hydrocarbons, carotenoids, sterol esters, sterols, tri-, di-, and monoglycerides, fatty acids, phospholipids, the terpenoid juvenile hormone, and ecdysone (THOMAS and GILBERT, 1968, 1969; CHINO *et al.*, 1969; EMMERICH, 1970; WHITMORE and GILBERT, 1972; DWIVEDY and BRIDGES, 1973; EMMERICH and HARTMANN, 1973).

Our experiments did not provide an ideal pulse-chase situation. A considerable amount of hydrocarbon radioactivity appeared after the initial radioactive pulse. This was presumably due to intracellular precursors which accumulated in the cells during the pulse and also due to labelled substances which were released into the medium and taken up again to synthesize lipids. One of the most likely possibilities is that the fat body cells released labelled ketone bodies such as acetoacetate and D-3-hydroxybutyrate. These compounds could readily acquire ^{14}C-radioactivity during the pulse in Na-^{14}C-acetate and during the subsequent mobilization of the labelled triglycerides. These substances have been shown to play an important rôle as a respiratory fuel in the desert locust (HILL *et al.*, 1972). In our *in vitro* experiments they may also have served as precursors for ^{14}C-hydrocarbons which were synthesized after the prelabelling period.

The *in vitro* results (DIEHL, 1973; the present paper) strongly support the hypothesis that the oenocytes are involved in the production of cuticle lipids (e.g. hydrocarbons). It remains to be shown that the hypodermal cells actually take up the hydrocarbons from the haemolymph and transport them to the cuticle. Preliminary *in vivo* studies suggest that this may indeed be the case. In specimens which had been injected with Na-^{14}C-acetate into the haemocoel, ^{14}C-hydrocarbons were readily removed from the cuticle by dipping the insects in hexane as shortly as 10 min after the injection (unpublished observations). The hypothesis is further supported by preliminary results obtained by gas–liquid chromatography. The hydrocarbons extracted from the oenocyte-rich fat body, from the haemolymph, and from the cuticle appeared to be qualitatively similar (DIEHL, in preparation). Furthermore, each hopper has an estimated 5 mg wet weight of oenocytes and produces about 500 μg of hydrocarbons during the instar. From this the rate of hydrocarbon synthesis appears to be of the order of 460 $\mu g/g$ of tissue per hr. This compares favourably with the estimated release of adrenocortical steroids of 120 to 3600 $\mu g/g$ per hr (GRANT, 1962) and with the release of lipids by the perfused rat liver of about $425 \pm 120{\cdot}9$ $\mu g/g$ per hr (MARSH, 1971). This comparison is admittedly only of limited value because we are dealing with different tissues and lipids. Nevertheless, it does show that the estimated rate of secretion of hydrocarbons is within the known synthetic capabilities of some lipid-producing cells.

In adults one function of the oenocytes is presumably to replace cuticle lipids which are lost due to abrasion. In adult females, however, the oenocytes seem to be bigger and undergo cyclical activation corresponding to the ovarian cycle (CASSIER and FAIN-MAUREL, 1972; WIGGLESWORTH, 1972). This may at least partly be explained by the fact that rather large quantities of hydrocarbons are associated with the egg yolk in the desert locust (about 10 per cent of the total egg lipids, unpublished observations). At present we do not know the function of these hydrocarbons. They may be utilized as cuticle lipids by the embryo or by the first instar larva.

It is possible that the oenocytes also synthesize other lipids which are released into the medium in a similar manner as the ^{14}C-hydrocarbons (unpublished observations). They have not yet been identified, but from their behaviour in thin-layer chromatography these lipids could be wax esters. This would be in agreement with the function of the oenocytes as cells producing cuticle lipids because wax esters are indeed part of the cuticle lipids (e.g. BLOMQUIST *et al.*, 1972).

At present we have no evidence to support the suggestion (WIGGLESWORTH, 1970) that oenocytes are involved in the synthesis of other structural lipids of the cuticle such as, for example, 'cuticulin'. Without the knowledge of the structure and the biosynthetic pathway of these lipids it is difficult to test this possibility. It may be that acetate is not an efficient precursor for such structural lipids. But one should not overlook the fact that appreciable amounts of bound lipids are present in the cuticle, the egg shell, and the spermathecal sheath (WIGGLESWORTH, 1970).

Recently, it has been demonstrated that the prothoracic glands of *Bombyx* and *Manduca* indeed produce α-ecdysone, at least *in vitro* (CHINO *et al.*, 1974; VEDECKIS *et al.*, 1974). But the conversion of cholesterol to ecdysones can also occur outside the prothoracic glands, perhaps in the oenocytes (GERSCH and STUERZEBECHER, 1971; NAKANISHI *et al.*, 1972; DIEHL, 1973). At present, it is not possible to decide if locust oenocytes are able to synthesize the moulting hormone. Nevertheless, in view of our results (DIEHL, 1973; the present paper) the synthesis of ecdysone, if indeed it occurs, cannot be the sole function of oenocytes in the desert locust. Therefore, one can conclude that in the desert locust the bulk synthetic activity of the oenocytes is directed to produce cuticle lipids, although at present we cannot rule out the possibility that the oenocytes may also be involved in the production of moulting hormones and possibly other complex lipid materials.

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Note added in proof—Prothoracic glands from *Tenebrio* larvae synthesize *in vitro* α-ecdysone. Isolated abdominal oenocytes synthesize mainly β-ecdysone, but only little α-ecdysone. (ROMER F., EMMERICH H., and NOWOCK J. (1974) Biosynthesis of ecdysones in isolated prothoracic glands and oenocytes of *Tenebrio molitor in vitro*. *J. Insect Physiol.* **20**, 1975–1987.)

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