Hormonal control of protein synthesis in the fat body of *Leptinotarsa decemlineata*

A biochemical and histological study

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J.F. Dortland

Hormonal control of protein synthesis in the fat body of *Leptinotarsa decemlineata*

Proefschrift

ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
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Druk: Pudoc, Wageningen, Nederland, 1978

STELLINGEN

I

Het blijkt op grond van de gemeten synthesesnelheid van eiwit wat door het vetlichaam wordt vastgehouden, niet mogelijk te zijn een voorspelling te doen over de synthesesnelheid van eiwit wat door het vetlichaam aan de haemolymph wordt afgegeven.

Dit proefschrift

II

Het relateren van de snelheid van eiwitsynthese aan de hoeveelheid eiwit, welke aanwezig is in het vetlichaam, kan tot een verkeerde interpretatie van de reële eiwitsynthese leiden.

Turner A.E. en Loughton B.G. (1975) Insect Biochem. 5, 791-804.
Hill L. en Goldsworthy G.J. (1968) J. Insect Physiol. 14, 1085-1096.

III

De conclusie van Ferenz dat stimulatie van de vitellogene eiwitsynthese door toevoeging van juveniel hormoon aan vetlichaam van *Locusta migratoria in vitro* bewezen zou zijn, berust op een misverstand.

Ferenz H.J. (1978) J. Insect Physiol. 24, 273-278.

IV

De door De Loof en Lagasse in hun onderzoek met *Leptinotarsa decemlineata* waargenomen uitgebreide intercellulaire kanaalstructuur is niet kenmerkend voor het vetlichaam van reproduktieve vrouwtjes.

De Loof A. en Lagasse A. (1970) Z. Zellforsch. 106, 439-450.

Wissenschaft ist gar nichts anderes als eben das "Versessensein auf das Finden von Unterschieden".

H.Hesse in: Narziss und Goldmund

Voorwoord

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3. A histological survey of the adult development of the fat body of *Leptinotarsa decemlineata*. Submitted to *Cell Tiss. Res.* 35

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General introduction

Reproduction and diapause in the Colorado potato beetle are influenced by a complex of environmental factors such as photoperiod, temperature, and food quality (De Wilde, 1969).

The effect of the photoperiod has been especially well-investigated. Treatment of females with long day conditions (18 hours light: 6 hours dark) results in reproduction, while short day conditions (10 hours light: 14 hours dark) induce the diapause syndrome. Photoperiodic induction probably takes place by the direct effect of light on the brain (De Wilde *et al.*, 1959). Histological experiments suggest that long day treatment results in an activation of the neurosecretory cells (Schooneveld, 1970). In their turn, the corpora allata (which produce the juvenile hormone) are probably activated by cerebral neurosecretion (De Wilde and De Boer, 1969). Under short day treatment, neurosecretory activity fails to be induced. Thus the length of the photoperiod has an indirect effect on the activity of the corpora allata.

In addition, it has been shown that a long day photoperiod results in high juvenile hormone titres in haemolymph, while only moderate amounts of juvenile hormone have been detected in short day treated insects (De Wilde *et al.*, 1968). Recently it was established that treatment with long day conditions does indeed coincide with active corpora allata, while under short day treatment only limited amounts of juvenile hormone are produced (Kramer, 1978).

The influence of daylength on the protein composition of the haemolymph is investigated by De Loof and De Wilde (1970a, 1970b). Vitellogenin predominates in the haemolymph of long day females, while specific short day proteins are observed in the haemolymph of short day females. In addition, it is shown that juvenile hormone influences the protein composition of the haemolymph. Juvenile hormone induces the appearance of vitellogenin in the haemolymph, while in the absence of juvenile hormone three short day proteins (called diapause proteins in this thesis) appear in the haemolymph. However, De Loof and De Wilde conclude that the protein composition of the haemolymph is also under the control of the

neurosecretory cells.

In the fat body of ovipositing females, the electronmicroscope reveals the presence of much rough endoplasmic reticulum. The fat body of a short day female is used for the storage of fat, protein, and glycogen (De Loof and Lagasse, 1970). The fat body seems a likely candidate for vitellogenin synthesis (Pan *et al.*, 1969), but definite proof is lacking in the case of the Colorado potato beetle. A biochemical characterization of the development of the fat body of females reared under these two photoregimes seems necessary.

In relation to the preceding experiments, attention is only paid to protein metabolism in this study. Article 1 deals with the storage and synthesis of proteins by the fat body of the adult Colorado potato beetle. In this connection, Article 2 describes what kind of proteins are stored and synthesized. The results of these two papers show a change in function during development, suggesting that a more extensive, histological study is needed. The correlation between the histological and biochemical properties of the female fat body is described in Article 3. Following this description, an attempt is made to obtain a better insight into its regulation of protein synthesis. Finally, in Article 4 special attention is paid to the relationship between juvenile hormone and vitellogenin synthesis.

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Protein synthesis and storage in the fat body of the Colorado potato beetle, *Leptinotarsa decemlineata*

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ABSTRACT

Protein metabolism in the fat body was investigated in females of the Colorado potato beetle *Leptinotarsa decemlineata*, reared under different photoregimes. After adult ecdysis the RNA content and the *in vitro* incorporation of lysine into retained protein increased in females reared under both long-day conditions as well as under short-day conditions. However, a significant difference in the rates of lysine incorporation into released protein was observed under the two photoregimes. Under SD conditions incorporation remained low, but under LD conditions there was a 55-fold increase between day 0 (adult ecdysis) and day 8 (ovipositing females). The RNA content and the rate of incorporation of lysine into released and retained protein in LD males was low compared with females. Protein storage started in haemolymph and fat body of SD beetles 6 days after adult ecdysis.

INTRODUCTION

Protein metabolism in larval fat body has been the subject of many investigations. During development the function of larval fat body changes from an active protein synthesizing tissue in the young last larval instar to a storage organ for protein and other reserve materials in the late last larval instar (review in PRICE, 1973).

The reverse can be expected during the transition from pupa to adult. THOMSEN and THOMSEN (1974) studied the ultrastructural changes during the development from pupal to adult fat body in relation to egg maturation in *Calliphora erythrocephala*. Furthermore, DE LOOF and LAGASSE (1970) described the ultrastructural properties of the adult fat body of *Leptinorarsa decemlineata*. The female Colorado potato beetle starts oviposition under long-day (LD) conditions 5 days after adult emergence. Under short-day (SD) conditions the female does not show oviposition and enters diapause 11 to 12 days after adult emergence (DE WILDE *et al.*, 1959). Thus, marked differences in the development of the fat body can be expected in animals reared under these two photoregimes with regard to protein synthesis.

PRICE (1966) investigated the difference in incorporation kinetics of released and retained protein in an *in vitro* system. Moreover, recent studies also suggest that *in vitro* systems might be valuable tools in studies of protein synthesis (HAGEDORN *et al.*, 1973; CHEN *et al.*, 1976). Therefore the synthesizing capacity of the fat body can be characterized by the incorporation of labelled amino acid into released and retained protein.

In this paper the incorporation of lysine into released and retained protein and the protein- and RNA-content of the female fat body will be investigated at different stages of development and under two different photoregimes.

MATERIALS AND METHODS

Insects

Leptinotarsa decemlineata Say were reared on fresh potato foliage according to DE WILDE (1957) and DE KORT (1969) under two different photoregimes: 18 hr

photophase (LD conditions) or 10 hr photophase (SD conditions). The animals were aged precisely as follows: one or two days before adult ecdysis pharate adults were removed from the soil, insects which ecdysed within 6 hr were taken as one group. Each group consisted of 30 to 40 animals (females and males). The beetles emerge from the soil 1 day after adult ecdysis. In former publications this was called day 0. In this publication adult ecdysis is called day 0.

Determinations of protein, RNA, and DNA

The dorsal part of the abdominal cuticle with the attached fat body was dissected. All dissections were performed in ice-cold *Leptinotarsa* Ringer (KCl: 131 mM, NaCl: 2 mM, $CaCl_2$: 1 mM, $MgCl_2$: 5 mM). After dissection the fat bodies were washed in *Leptinotarsa* Ringer. Groups of 10 to 15 fat bodies were homogenised in Ringer and processed according to TANGUAY and CHAUDHARY (1971). The final residues were extracted with 0.5N PCA at 90°C for 30 min. DNA was determined by the diphenylamine test (BURTON, 1956). Standard fish sperm DNA was also subjected to this 90°C PCA-treatment. The RNA content was measured by the orcinol method as described by SCHNEIDER (1957). Protein was measured by the method of HARTREE (1972) with BSA (fraction V) as a standard.

Protein measurements in haemolymph

Blood protein was determined by the Folin method (LOWRY *et al.*, 1951). BSA (fraction V) was used as a standard. Haemolymph volumes were measured using [^{3}H]-inulin (WHARTON *et al.*, 1965).

In vitro *experiments*

Before dissection, the beetles were chilled at 4°C for 30 min to prevent formation of excreta during dissection. Scissors and forceps were cleaned in 70% alcohol. The abdomens were surface sterilized with 70% alcohol. Two groups of 5 to 10 fat bodies were carefully excised and washed in *Leptinotarsa* Ringer. The fat bodies were incubated for 0.5 hr (measurement of incorporation into retained protein) or 6 hr (measurement of incorporation into released protein)

under constant shaking in 550 µl of incubation medium (Table 1).

Table 1. Incubation medium for fat bodies of *L. decemlineata*

Constituents	mM
KCl	119
NaCl	1.8
$MgCl_2$	4.6
$CaCl_2$	0.9
Tris-HCl	9.1
L-alanine	1.8
L-arginine	1.4
L-asparagine	1.4
L-aspartic acid	1.4
L-cysteine-HCl	0.5
L-glutamine	3.6
L-glutamic acid	1.8
L-glycine	2.7
L-histidine-HCl	1.9
L-isoleucine	0.9
L-leucine	0.9
L-methionine	0.5
L-phenylalanine	0.9
L-proline	9.1
L-serine	5.4
L-threonine	1.8
L-tryptophan	1.4
L-tyrosine	0.9
L-valine	2.3

The amino acid concentrations were according to the amino acid composition of the whole beetle (DE KORT and KRAMER, 1976). The pH was adjusted to 6.5 with HCl. The incubation-temperature was 25°C. The incorporation was started by the addition of

2 µCi (117 mCi/mmol) L-[^{14}C]-lysine-HCl.

For the measurement of incorporation into released protein samples of 75 µl were removed after 0,4, and 6 hr and immediately centrifuged for 2 min in an Eppendorf centrifuge (Type 3200). Two samples of 25 µl of the resulting supernatant were processed for liquid scintillation counting. After 6 hr the incubation was terminated by freezing in liquid nitrogen and stored for analysis of the DNA content. The incorporation in retained protein was measured as follows: The medium with fat bodies was frozen in liquid N_2 30 min after the start of the incubation. The 5 to 10 fat bodies were then processed for measurement of DNA content and of incorporation of [^{14}C]-Lysine into retained protein.

Assay of radioactivity

For the measurement of the incorporation into released protein samples of 25 µl supernatant were added to 50 µl 10% TCA and 25 µl of a BSA-solution (20 mg/ml) served as coprecipitate. After 10 min the protein fractions were transferred to Gelman glass fibre filters (Type AE, diameter 20 mm) and washed with 5% TCA, ethanol-ether (1:1) and ether in this order. Measurement of radioactivity was carried out by adding 10 ml of scintillation mixture, 1.0% PPO, 0.05% POPOP, and 5.0% naphtalene in dioxane/methylcellosolve, 5:1 (BRUNO and CHRISTIAN, 1961), to these glass filters. The counting efficiency was 75 to 76% for ^{14}C and 26 to 27% for ^{3}H.

For the assay of incorporation into retained protein 250 µl samples of protein hydrolysed in 1 or 2 ml 1N NaOH, were neutralized with 250 µl 1N HCl and counted in 10 ml of scintillation mixture, 0.3% PPO in xylol/Triton X-100, 3:1 (ANDERSON and McCLURE, 1973). The counting efficiency was 85-86%. Radioactivity was measured with a Nuclear Mark I liquid scintillation counter.

Measurements of oöcyte volumes

Lenght and width of all oöcytes in an ovariole were measured. The volume was calculated using the formula for a prolate spheroid (BELL, 1969): $V = (W/2)^2(L/2)(4\pi/3)$. Each point is the mean of 80 ovarioles taken from 4 females of the same age. Because more than one oöcyte developed in one ovariole we calculated the sum of all developing oöcytes per ovariole.

Chemicals

Cycloheximide, RNA, DNA and BSA were purchased from Sigma. Streptomycine was obtained from Mycofarm, Delft, The Netherlands. L-[U-^{14}C]-Lysine-HCl (287mCi/mmol) and [^{3}H]-inulin (300 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, U.K. All other reagents were of analytical grade and dissolved in double distilled water.

RESULTS

Changes in ribonucleic acid- and protein-content

The fat body of the Colorado potato beetle is a diffuse organ built up of small lobes connected by tracheae. Therefore it is not possible to collect the whole fat body. For this reason RNA-, protein-content and the rates of lysine incorporation are expressed on the basis of DNA content of the tissue.

Figure 1a shows the RNA- and protein-content based on fat body DNA from animals reared under LD conditions. The protein content decreases slightly within the first 24 hr after ecdysis. It increases at day 2 and becomes constant at day 4. The RNA content also shows a decrease within 24 hr but rises sharply between day 1 and day 2. The RNA- and protein-content of fat bodies from females reared under SD conditions are shown in Fig. 1b. The protein content also decreases during the first 24 hr after ecdysis, but at day 6 protein storage occurs which lasts till day 9. The RNA content under SD conditions roughly follows the same pattern as under LD conditions. However, at day 13 (diapause) a sharp decrease in RNA content is observed.

Protein synthesis of fat body in vitro

We developed an *in vitro* system for amino acid incorporation with fat bodies derived from the Colorado potato beetle. With this system we found high rates of incorporation. The time courses of lysine incorporation into released and retained protein under optimal conditions are shown in Fig. 2.

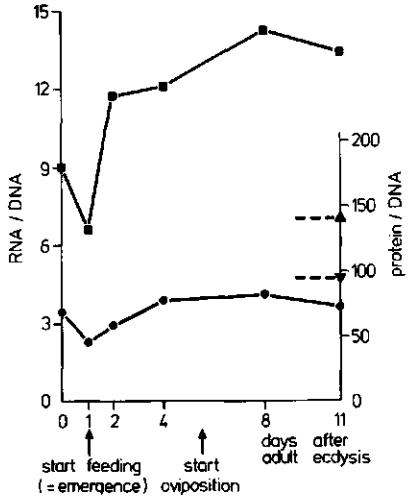

Fig. 1a. Changes in the protein and RNA content in the fat body of females or males reared under LD conditions. Each number represents the mean of duplicate samples taken from two replicates of 10 to 15 insects. Solid circles, protein/DNA females; solid squares, RNA/DNA females; solid inverted triangle, protein/DNA males; solid upright triangle, RNA/DNA males.

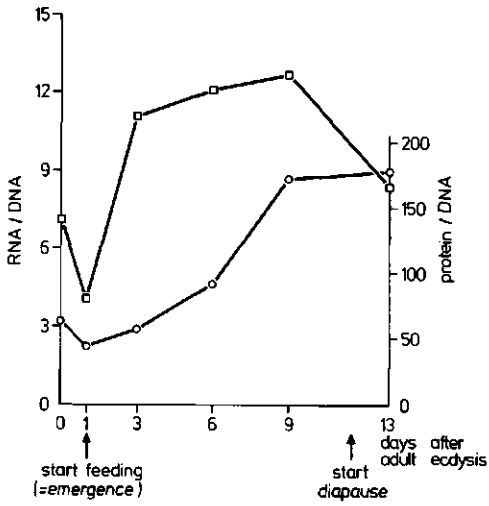

Fig. 1b. As Fig. 1a but for fat bodies of females reared under SD conditions. Open circles, protein/DNA; open squares, RNA/DNA.

Incorporation into released protein shows a sharp and reproducible increase in incorporation 3.5 hr after the start of the incubation. This increase is not due to the presence of bacteria. Incorporation experiments in the presence of 0.1mg/ml streptomycine show the same kinetics. In addition, there is no significant incorporation of label into protein if pieces of cuticle are used. Fat body preparations derived from diapausing females show very low rates of incorporation (Fig.3). Moreover, fat body incubated in the presence of 0.1 mg/ml cycloheximide does not show protein synthesis. These observations indicate that bacterial contamination does not contribute significantly to the rate of incorporation found in our *in vitro* system. We have taken the difference in incorporation between 4 and 6 hr as a measure for the rate of incorporation into released

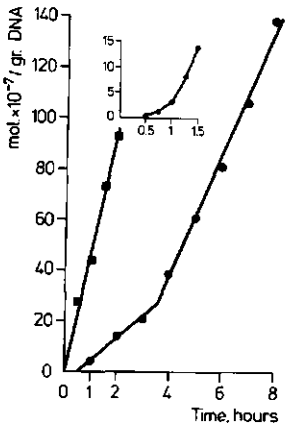

Fig. 2. Rates of *in vitro* incorporation of lysine into released protein (solid circles) and of lysine into retained protein (solid squares) by fat bodies from ovipositing females as a function of time. The inset shows the early time points for the incorporation into released protein. Each number represents the mean of duplicate samples taken from two replicates of 5 to 10 females.

protein. The inset of Fig. 2 shows a lagtime of 30 to 45 min.

The incorporation into retained protein starts immediately and is linear for 2 hr. These values were obtained by subtracting the amount of incorporation into released protein from the amount of incorporation into total protein. Measurements of the rates of incorporation into retained protein (Fig. 4) were performed by incubation experiments of half an hour.

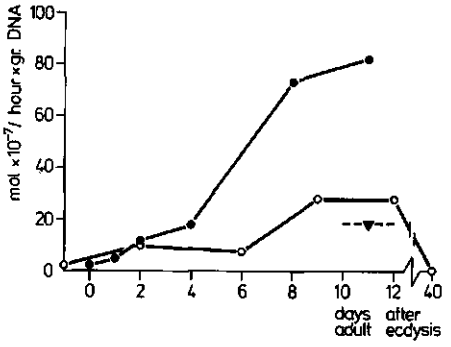

Fig. 3. Rates of *in vitro* incorporation of lysine into released protein as a function of the successive stages of females after adult ecdysis reared under LD conditions (solid circles), of females reared under SD conditions (open circles) and of males 11 days after adult ecdysis reared under LD conditions (solid inverted triangle). Each number represents the mean of duplicate samples taken from two replicates of 5 to 10 insects.

The effects of age and photoperiod upon incorporation of lysine into released protein are shown in Fig. 3. In LD females there is a sharp continuous increase in the rate of incorporation at day 4. At day 11 the rate of incorporation into released protein is 55 times higher than at adult ecdysis. Fat body derived from males shows a much lower incorporation. Under SD conditions the fat body never shows such high rates of incorporation into released protein as found under LD conditions. In diapause, the systhesis of released proteins is hardly measurable.

After the termination of the 6 hr experiments the incorporation into retained protein was also determined. The level of incorporation in fat body from LD- and SD-females of the same age is of the same magnitude. However, incorporation into retained protein is not linear over a 6 hr period. We, therefore, repeated these experiments with the aid of short-time (0.5 hr) incubations. These experiments were performed in winter when the potato foliage was in a bad condition. This resulted in an incorporation of only half or one-third of that found in summertime.

Figure 4 shows that the differences in the rates of incorporation into retained protein by fat bodies derived from animals reared under different photoregimes are much reduced. Fat bodies derived from LD- and SD-females both show high rates of incorporation. In contrast fat body from LD-males shows a lower incorporation, while protein synthesis in fat body from diapausing females is extremely low.

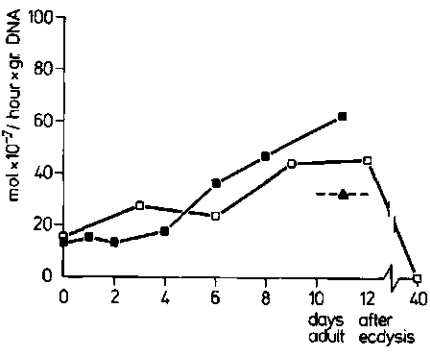

Fig. 4. As Fig. 3 but for the rates of incorporation of lysine into retained protein. Solid squares, LD-females; open squares, SD-females; solid upright triangle, LD-males.

Protein synthesis in relation to oögenesis and protein storage

The fat body plays a central role in protein metabolism. Apart from retained proteins, the fat body synthesizes haemolymph proteins, including the vitellogenins. The capacity of protein synthesis of fat body should therefore on one hand correlate with reproductive development under LD conditions and on the other with protein storage under SD conditions. Reproduction was followed by measuring the growth of oöcytes (Fig. 5). All oöcytes show a logarithmic growth, but the length/width ratio changes during development. We therefore monitored the total volume of the oöcytes. At 48 hr after ecdysis the terminal oöcytes start vitellogenesis. At day 5 each ovariole contains 5 vitellogenic oöcytes of different sizes. From day 5 to 6 onwards the beetle oviposits daily.

The amount of protein storage in the fat body is shown in Fig. 1b. However, significant amounts of protein also accumulate in the haemolymph (DE LOOF and DE WILDE, 1970). The amount of protein stored in haemolymph can be calculated from the protein content of the haemolymph and the haemolymph volume. Under LD conditions the volume is 60 µl at day 1, reaches a maximum of 75 µl at day 5, and reaches a constant level of 53 µl at day 7. Under SD conditions the volume is 50 µl at day 1, then rises to 84 µl at day 5 and subsequently decreases to a constant level of 65 µl at day 7. Figure 5 shows the changes in protein content of the haemolymph under LD and SD conditions.

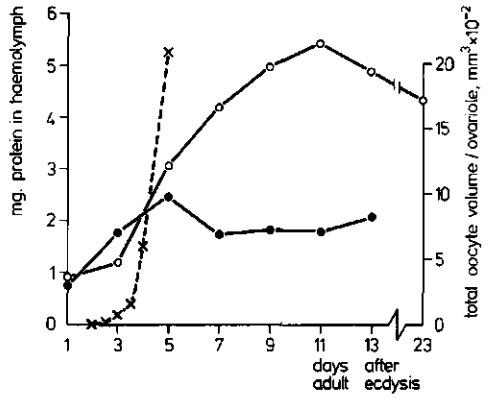

Fig. 5. The amount of protein in the haemolymph of females after adult ecdysis reared under LD (solid circles) or SD (open circles) conditions. Also the total oöcyte volume per ovariole (broken line with crosses) in females reared under LD conditions is represented.

DISCUSSION

Because the photoperiod has a drastic influence on the physiological state of the Colorado beetle (DE WILDE *et al.*, 1959, 1968), there should be significant differences in the development of the fat body after adult ecdysis between animals reared under different photoregimes. Females kept under LD conditions begin vitellogenesis at day 2 and oviposition starts at day 5. The maximal rate of oviposition is found on day 9 (DE LOOF and DE WILDE, 1970). At this age females produce 60 eggs daily, which is equivalent to 3.2 mg egg-protein. In contrast, females kept under SD conditions never oviposit. Such beetles enter diapause about 12 days after adult ecdysis. During this prediapause period they show normal feeding and accumulate large amounts of reserve materials, especially lipids and proteins.

The results presented here show biochemical changes in the fat body of the Colorado beetle in relation to reproduction and diapause. We followed the changes in RNA- and protein-content and in the rates of lysine incorporation into total fat body protein *in vitro*. The RNA- and protein-content and the rate of incorporation were expressed on the basis of fat body DNA, because the DNA content per cell in adult fat body is constant. Although polyteny occurs in larval fat body (THOMSON, 1973), to our knowledge it does not occur in that of the adult.

At adult ecdysis the fat body is rather inactive and functions mainly as a storage organ. Immediately after ecdysis marked changes occur in the fat body. Under both LD and SD conditions the fat body develops towards an active protein synthesizing tissue. This can be inferred from the fact that, after an initial fall, the RNA content increases significantly between day 1 and 2. In *Aedes aegypti* at the onset of vitellogenesis there is a nearly 3-fold increase in RNA content of fat body (HAGEDORN *et al.*, 1973). This increase in RNA content suggests an increase in the capacity for protein synthesis in beetles reared under the two different photoregimes. More direct proof that the fat body develops into a tissue active in protein synthesis came from the experiments concerning lysine incorporation by fat body *in vitro*. The capacity for lysine incorporation into retained protein is high in fat body from both LD and SD females, but this activity is even higher than in fat body derived from LD males. Only after the beetles have entered diapause does the rate of incorporation drop to very low levels.

The rate of incorporation into released protein did show significant differences in fat body from LD and SD females. Fat body from ovipositing females shows a 3 times higher rate of incorporation than 8 to 12 days old SD females and over 4 times the rate of incorporation by fat body derived from males. This high rate of incorporation into released protein by fat body from ovipositing females is compatible with the high protein demand for oögenesis. The steep increase in incorporation after day 4 coincides with the rapid growth of the oöcytes due to vitellogenesis.

In contrast fat body from SD females showed much less of an incorporation into released protein. During the pre-diapause period proteins accumulate in haemolymph and such an accumulation requires the production of only about 0.5 mg of protein per female per day. This is much less than the 3.2 mg of egg protein necessary for oviposition.

Fat body from SD females is very active in amino acid incorporation into retained fat body proteins. This high rate of protein synthesis can be correlated with the expected high metabolic activity of fat body during pre-diapause. During this pre-diapause period the large amounts of food eaten have to be converted and stored in the fat body cells, mainly in the form of lipids and proteins. Apparently, these high metabolic demands require a relatively high rate of protein synthesis. A significant amount of the stored protein of the beetle is present in the haemolymph. A female in diapause contains 17 mg of protein. About one third of the protein content is present in haemolymph. In larvae of *Calliphora stygia* the haemolymph serves as a temporary storage organ for protein. At the end of larval life blood proteins are sequestered and stored as protein granules in the fat body (MARTIN *et al.*, 1969). In the Colorado potato beetle protein storage by the fat body is not accompanied by a drop in the haemolymph protein content. This suggests that fat body and haemolymph both participate in storage of protein.

The results given in this paper indicate that protein synthesis by fat body has been directed to different functions, dependent on the length of the photoregime. Under LD conditions the fat body produces mainly proteins necessary for oögenesis and under SD conditions storage proteins in preparation for diapause. The nature of the proteins produced by the fat body should differ under these two physiological conditions. Whether the fat body from beetles reared under different

photoregimes is able to produce different proteins *in vitro*, will be subject of a subsequent paper.

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Synthesis of vitellogenins and diapause proteins by the fat body of *Leptinotarsa*, as a function of photoperiod

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ABSTRACT

During maturation of adult female *Leptinotarsa decemlineata*, synthesis of specific proteins by the fat body was investigated with the aid of *in vitro* incubations. Both vitellogenins and diapause proteins were present in all females. Under long days high rates of vitellogenin synthesis were observed, which were not reflected in the amount of vitellogenin present in the haemolymph. Under short days synthesis of three diapause proteins predominated. The highest rates of synthesis of these proteins occurred during the second half of the prediapause period. At the same time storage of two diapause proteins by the fat body was observed. Comparison of the synthesis of specific proteins *in vivo* and *in vitro* revealed the fat body as the main producer of haemolymph proteins.

INTRODUCTION

It is well established that during reproduction in insects vitellogenins are synthesized by the fat body of the adult female. For example, vitellogenin synthesis is low in non-reproductive *Aedes aegypti* females, but shows a rapid increase after a blood meal to reach its maximum within 28 h. However, 40 h after the blood meal vitellogenin synthesis has declined (HAGEDORN *et al.*, 1973). Similarly in *Leucophaea maderae*, the synthesis of proteins by the fat body is subject to marked changes; with vitellogenin synthesis greatly increased during oöcyte maturation, but diminished thereafter (WYSS-HUBER & LUSCHER, 1972).

In *Calliphora*, synthesis of specific proteins by the last larval instar (PRICE & BOSMAN, 1966) and during adult maturation (KINNEAR & THOMSON, 1975) is well documented. The protein calliphorin is selectively synthesized by the larval fat body and it seems that high rates of protein synthesis are specific to particular instars.

In the Colorado potato beetle, *Leptinotarsa decemlineata* Say, reproduction and diapause are under photoperiodic control. Beetles reared in long days reproduce, whereas beetles reared under short days enter diapause after 12 days. DE LOOF (1972) showed that these two kinds of females possessed different protein profiles in the haemolymph.

The rates of lysine incorporation into total protein released by fat bodies of females reared under long and short days have been described previously (DORTLAND & DE KORT, 1978). Under long days this rate increases from 12×10^{-7} mol/h x g DNA (see DORTLAND & DE KORT, 1978, for explanation of this unit) at day 2 after adult ecdysis to 73×10^{-7} at day 8. Under short days, however, during the first half of the prediapause period (days 0-6), rates of only up to 9×10^{-7} mol/h x g DNA are reached, and during the second half of the prediapause period (days 6-12) the rate increases to only 28×10^{-7}. The present paper reports on what kinds of proteins are involved in these rates of lysine incorporation into total released protein.

The amount of protein present in total haemolymph was also reported by DORTLAND & DE KORT (1978). During adult maturation the haemolymph of long day females contains about 2 mg protein, whereas that of short day females contains up to 5 mg. The present paper reports a further study of how the different

proteins are built up in the haemolymph during these periods.

MATERIALS AND METHODS

Leptinotarsa decemlineata Say were reared on fresh potato foliage according to the method of DE WILDE (1957) and DE KORT (1969) under two different photoregimes: long days (18 h light : 6 h dark) or short days (10 h light : 14 h dark). Adult ecdysis is considered as day 0.

Electrophoresis of haemolymph, fat body and egg proteins

Disc electrophoresis was performed at 4°C, in 7% acrylamide gels with a 4% spacer gel following the method of DAVIS (1964). The gels were 6 cm long and 7 mm in diameter. Haemolymph samples of 1-2 µl were diluted 10 times with '*Leptinotarsa* Ringer' (131 mM KCl, 2 mM NaCl, 1 mM $CaCl_2$ and 5 mM $MgCl_2$). Homogenates of dissected fat bodies or fresh-laid eggs were prepared in this Ringer and then centrifuged for 2 min at 8000 g in an Eppendorf Type 3200.

After addition of a few crystals of sucrose to all samples, gels were run at 4 mA per gel, stained overnight in 0.02% Coomassie Brilliant Blue (R 250) in methanol, water and acetic acid (45:45:10, v/v), and destained in methanol, water and acetic acid (15:75:10, v/v) for 3-6 h in a shaking water bath. They were then scanned at 570 nm on a Photovolt Model 520, the areas under the different peaks analysed by planimetry, and the area of each peak was expressed as a percentage of the total area of all peaks determined over the entire length of the gel.

Synthesis of labelled haemolymph proteins

For measurement of *in vitro* synthesis of the different proteins, eight to ten fat bodies derived from animals of the appropriate age were incubated for 7 h in 200 µl of medium (DORTLAND & DE KORT, 1978). The incubation was started with 2 µCi [^{14}C]-lysine. At the end, a sample of 30 µl of medium was centrifuged. A few crystals of sucrose were added to the supernatant and the proteins, now labelled, were applied to the gel.

After staining and destaining, the gels were sliced according to position of

the protein bands (twenty to twenty-three portions). All slices were digested in 0.5 ml 30% H_2O_2 at 90°C for 2-4 h and then 10 ml of scintillation mixture (0.3% PPO in xylol/triton X-100,3:1 (ANDERSON & McCLURE, 1973)) were added. The counting efficiency was 82-85%. Radioactivity was measured with a Nuclear Chicago Mark I liquid scintillation counter. For each protein the radioactivity of the individual band was expressed as a percentage of total radioactivity over the whole gel.

In the *in vivo* measurement each female was injected with 2 µl (0.1 µCi) [^{14}C]-lysine. After 1 h the haemolymph was collected, centrifuged and the labelled proteins were separated on a gel. The L-[U-^{14}C]-lysine-HCl (287 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, U.K. All other reagents were of analytical grade and dissolved in double distilled water.

The results are quoted as the means of two duplicate samples taken from two pooled replicates of eight to fifteen females, the two replicate mean points being shown as two small dots in the figures.

RESULTS

Comparison between in vitro *and* in vivo *synthesis*

Several proteins can be distinguished in the female haemolymph. The occurrence of the three diapause proteins (DP1, DP2 and DP3) in the haemolymph of prediapause (= short day) females and the occurrence of one vitellogenic protein in the haemolymph of ovipositing females has been described previously (DE LOOF & DE WILDE, 1970). Table 1 shows, however, that two vitellogenins (V1 and V2) as well as the three diapause proteins occur in both long day and short day females. Although I have found more than twenty different proteins, I considered these five to be the most suitable for the present study. In egg-homogenates, the two vitellogenins predominate, but they also contain traces of the diapause proteins.

From 3.5 to 8 h *in vitro*, incorporation of labelled lysine into total released protein is linear (DORTLAND & DE KORT, 1978), and *in vivo* the incorporation into haemolymph proteins is linear between 0.5 and 1.5 h (S.J. KRAMER, personal communication).

TABLE 1. Percentages of the different Ringer-soluble protein species in an egg-homogenate and in the haemolymph of long day and short day female *Leptinotarsa* 8 days after adult ecdysis.

Protein	Eggs	Haemolymph	
		Long day	Short day
V1	32.4	12.4	3.2
V2	26.3	17.4	3.9
DP1	3.9	3.7	13.9
DP2	0.2	2.2	11.7
DP3	0.2	0.7	8.3

V1 and V2 = vitellogenins; DP1, DP2 and DP3 = diapause proteins.

Moreover, there are no changes in the percentages of total incorporation into the five proteins during either form of incubation (unpublished), so that the experiments were performed during the linear phase of incorporation for all five proteins. The relative incorporation into the different proteins is similar *in vitro* and *in vivo* (Fig. 1).

The relative incorporation of [^{14}C]-lysine into the different proteins *in vitro*, together with the *in vitro* rates of lysine incorporation into total released protein (see Introduction) provide a basis for the quantitative comparison of the rates of lysine incorporation into the different proteins.

In vitro *incorporation into released protein and appearance in haemolymph of the two vitellogenins*

Fig. 2a shows the rates of lysine incorporation into the vitellogenins by fat bodies derived from long day females. At adult ecdysis, incorporation was too low to determinate accurately the rates in V1 and V2, but 2 days later 12% of

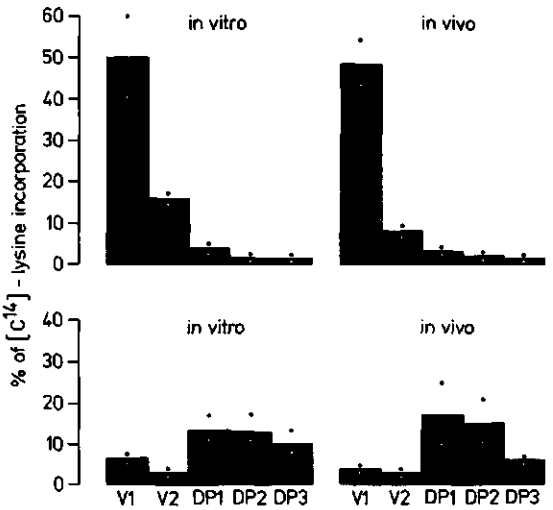

Fig. 1. Comparison between the percentage [^{14}C]-lysine incorporation into the different haemolymph protein species *in vivo* and *in vitro*, measured by disc electrophoresis. The fat bodies were derived (a) from 8-day-old females reared under long days (upper histograms), and (b) from 9-day-old females reared under short days (lower histograms). V1 and V2 = vitellogenins; DP1, DP2 and DP3 = diapause proteins.

total incorporation was in V1 and 7% in V2; 8 days after adult ecdysis these values were 56% and 16%, respectively. Thus the steep increase in synthesis of total released protein is largely based on the increase in synthesis of V1 and V2. In short day females, incorporation into the two vitellogenins by the fat body was consistently low during the prediapause period, and approached zero during diapause (Fig. 2b).

An additional parameter to these rates of synthesis is the amount of the specific protein present in haemolymph. This value can be calculated from the amount of protein present in the total haemolymph volume, together with the percentages of the different proteins present in haemolymph. In long day females, the percentages of V1 and V2 showed a small but steady increase, rising from 6% to 12% for V1 and from 5% to 17% for V2. With no major changes in the total protein content of the haemolymph, this results in a slow increase in the amounts of proteins V1 and V2 in haemolymph (Fig. 3a). In short day females, however, since the absolute

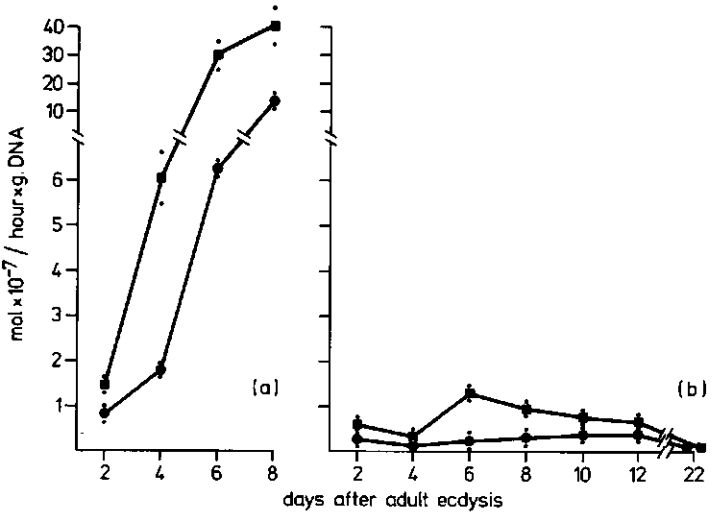

Fig. 2. Rates of *in vitro* incorporation of lysine into released vitellogenins proteins by fat bodies of females of different age reared under (a) long days and (b) short days. Solid squares, V1; solid circles, V2.

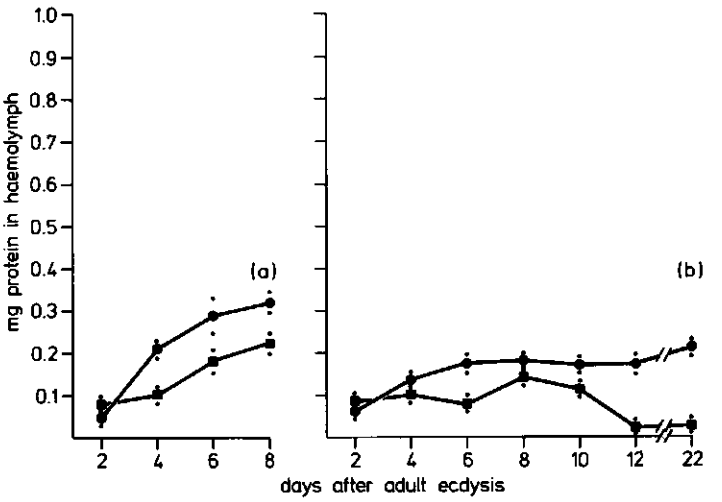

Fig. 3. The amounts of the two vitellogenins present in the haemolymph of females of different age reared under (a) long days and (b) short days. Solid squares, V1; solid circles, V2.

amounts of V1 and V2 remained roughly the same (Fig. 3b) while the total amount of protein increased, a marked decrease occurred in their percentage contribution to haemolymph protein during maturation (for V1 from 9% to 1%, and for V2 from 7% to 3%).

In vitro *incorporation into released protein and appearance in haemolymph of the three diapause proteins*

Using the same methods, the *in vitro* synthesis of the three diapause proteins by the fat body was investigated. In spite of high rates of lysine incorporation into total released protein, the fat body of long day females showed low incorporation into the three diapause proteins (Fig. 4a), except for protein DP1, the synthesis of which increased by day 8. In contrast, after day 6 fat body from short day females showed substantial rates of incorporation into all three diapause proteins (Fig. 4b), with relatively much more diapause proteins being produced than vitellogenins. DP2 reached its highest rate of incorporation into released protein at day 8 after adult ecdysis, DP1 and DP3 at day 12. The rate of incorporation into total released protein by fat body from females 10 days into diapause (day 22) was very low.

The absolute amounts of the three diapause proteins in the haemolymph of long day females were low (Fig. 5a). This can be inferred from the fact that the percentages of total protein of the three diapause proteins never exceeded 7%. Fig. 5b shows the increase in the absolute amounts of the three diapause proteins in the haemolymph of short day females. The highest amount of DP2 was observed at day 8 after adult ecdysis. By day 2 only 11% of the haemolymph proteins was diapause protein, but 12 days after adult ecdysis the diapause proteins accounted for more than 35% of the protein content of the haemolymph. During the first 10 days of diapause the amount of DP1 and DP3 decreased significantly.

Protein storage in the fat body

There are no major changes in the protein/DNA-ratio of the fat body when the females are reared under long days, but in short days a three-fold increase in this ratio is accompanied by protein storage in the haemolymph (DORTLAND &

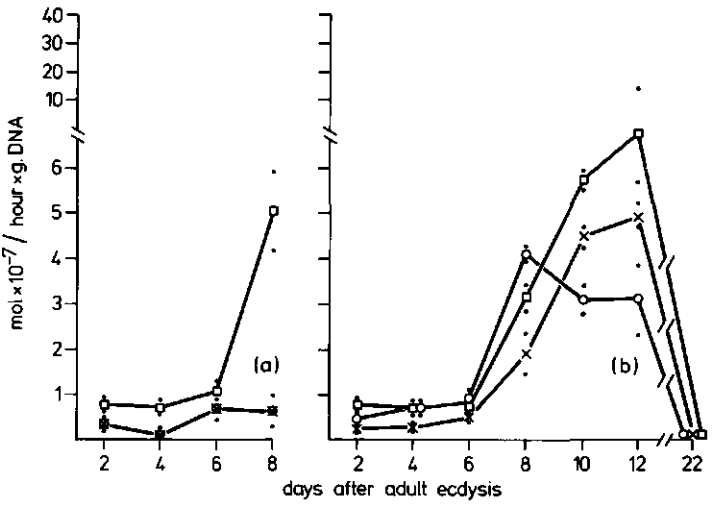

Fig. 4. Rates of *in vitro* incorporation of lysine into released diapause proteins by fat bodies of females of different age reared under (a) long days and (b) short days. Open squares, DP1; open circles, DP2; crosses, DP3.

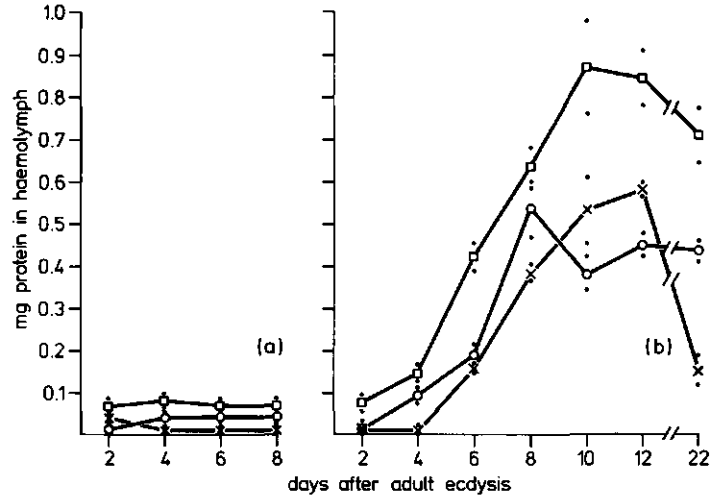

Fig. 5. The amounts of the three diapause proteins present in the haemolymph of females of different age reared under (a) long days and (b) short days. Open squares, DP1; open circles, DP2; crosses, DP3.

DE KORT, 1978). It is therefore interesting to know which proteins accumulate in the fat body. Disc electrophoresis and subsequent scanning of the Ringer-soluble protein fraction of the fat body homogenates derived from prediapause females revealed that it is especially DP3 and DP1 that are stored (Table 2). In females at the onset of diapause the diapause proteins accounted for nearly 40% of the Ringer-soluble proteins of the fat body.

TABLE 2. Percentages of the three diapause proteins present in the Ringer-soluble fraction of homogenates of fat bodies derived from short day females, 4, 8 and 12 days after adult ecdysis.

Protein	Day 4	Day 8	Day 12
DP1	0.1	9.6	15.8
DP2	0.9	2.8	4.2
DP3	0.3	6.2	19.7

DISCUSSION

There are important differences in the blood protein pattern of the haemolymph of Colorado potato beetles reared under long and short days. DE LOOF & DE WILDE (1970) observed vitellogenins in reproducing (i.e. long day) females, but found that three diapause proteins predominated in short day females. The present paper shows, however, that there are two vitellogenins and three diapause proteins present in both short day and long day females.

I was able to measure the rates of lysine incorporation into these five proteins synchronously. Comparison between the *in vitro* and *in vivo* data with respect to the percentage incorporation into the released proteins showed that the fat body *in vitro* synthesizes the haemolymph proteins in the same proportions as occurs *in vivo*. Changes in the lysine pool in the haemolymph are of no importance in the determination of these percentages *in vivo*. When we take into account that the different proteins are synthesized in each tissue in a pattern

characteristic for that tissue (as has been demonstrated for *Calliphora stygia* by KINNEAR *et al.*, 1971), we can conclude from Fig. 1 that the fat body synthesizes most of the haemolymph proteins. This is in agreement with the findings of TURNER & LOUGHTON (1975) in the fifth instar of *Locusta migratoria migratoroides*.

When females are reared under long days, a steep increase in their synthesis of the vitellogenins occurs. I found a 32-fold increase in V1 synthesis, which is comparable with the 20-fold increase reported in *Leucophaea* (WYSS-HUBER & LUSCHER, 1972); the increase in V2 synthesis was only slightly lower.

This increase in vitellogenin synthesis is not reflected in the amounts of vitellogenin present in haemolymph, however, apparently a massive uptake of vitellogenins takes place during oöcyte maturation. Two days after adult ecdysis the terminal oöcyte starts vitellogenesis, and electrophoresis of egg-homogenates revealed the accumulation of both V1 and V2, with V1 predominating. The higher level of V2 than V1 in the haemolymph is therefore not surprising. The release of vitellogenins from the fat body must be rapid, since total incorporation into the two vitellogenins present in the Ringer-soluble fraction of the fat body homogenates never exceeded 6% (unpublished). This is in agreement with the finding that vitellogenins are released immediately after systhesis (BROOKES, 1969).

In the fat body of short day females the *in vitro* synthesis of the vitellogenins was low but detectable. This is in accordance with the occurrence of vitellogenesis in the terminal oöcytes. However, these oöcytes never mature completely and are subsequently resorbed. The low rates of synthesis in combination with limited uptake of V1 and V2 by the oöcytes leave vitellogenin in the haemolymph in comparable amounts to that occurring in long day females. Thus the amount of a protein in the haemolymph certainly does not reflect the rate of its synthesis.

When females are reared under long days the synthesis of the three diapause proteins by the fat body is low. This is in agreement with the low amounts these proteins in the haemolymph. However, an unexplained, but reproducible increase in the synthesis of DP1 alone can be observed at day 8 after adult ecdysis, an increase that is not reflected in its amount in the haemolymph.

When the females are reared under short days, the synthesis of the three

diapause proteins is low for the first 6 days after ecdysis, though there is a steady increase in their level in the haemolymph, suggesting low rates of protein turnover. After day 6 the synthesis of all three increases sharply, with the peak of DP2 synthesis at day 8 coinciding with its peak in the haemolymph. The peaks of DP1 and DP3 synthesis, between day 10 and day 12, are not so perfectly reflected in the haemolymph. However, during the second half of the prediapause period, protein storage takes place (DORTLAND & DE KORT, 1978) and it was demonstrated that the proteins DP1 and DP3 are stored in the fat body. I think that proteins are taken up by the fat body from the haemolymph, in particular DP1 and DP3. This uptake by the fat body is also suggested by the fact that both DP1 and DP3 showed a significant drop in the haemolymph after day 12, while DP2 did not (Fig. 5b). However, the direct storage of newly synthesized proteins within the fat body cannot be ruled out.

It is evident that in prediapause females of *Leptinotarsa* the synthesis and storage of proteins takes place simultaneously. Apparently the fat body needs no transformation from a synthesizing to a storage function. This is in contrast with the situation in the third instar larvae of *Calliphora stygia*, where high rates of synthesis and protein storage do not occur at the same time. Why proteins are released by the fat body into the haemolymph and subsequently taken up, is not fully understood. Possibly some conformational change in the structure of the proteins occurs in the haemolymph, and is necessary before uptake by the fat body can take place (MARTIN *et al.*, 1971). The existence of such a process, for example in the processing of vitellogenins by haemolymph enzymes, has been demonstrated by KOEPPE & OFENGAND (1976).

The assumption has been made for *Leptinotarsa* that juvenile hormone stimulates the synthesis of the vitellogenins, while inhibiting the synthesis of the diapause proteins (DE LOOF & DE WILDE, 1970). However, this theory now requires some modification. When the females are reared under long days a rapid increase in the synthesis of the vitellogenins can be observed, but 8 days after adult ecdysis there is also a considerable increase in the synthesis of DP1. This stimulus of DP1 synthesis seems to occur in the presence of juvenile hormone. Moreover, an increase in the synthesis of DP1 is possible, while the synthesis of DP2 and DP3 remain low. When the females are reared under short days, DP2 reaches its highest level of incorporation into released protein 8 days after

adult ecdysis, while DP1 and DP3 do so 10 and 12 days after adult ecdysis. Thus, separate stimulation of the synthesis of the different diapause proteins is likely, and the regulation of their synthesis seems rather complex.

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A histogical survey of the adult development of the fat body of *Leptinotarsa decemlineata*

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SUMMARY

The fat body of a female *Leptinotarsa decemlineata* at adult ecdysis contains a large number of protein granules which are composed of light and dark zones. Some of these granules comprise a light amorphous zone which is believed to be urate. During the first two days after adult ecdysis, fat body development is not essentially different whether the females are reared under long or short day conditions. Protein granules and large watery vacuoles disappear and the first cell organelles are regenerated. The effect of the photoperiod on the histological structure of the fat body is expressed after these events. When the females are reared under long day conditions, the fat body develops into a tissue specialized for vitellogenin synthesis. When the females are reared under short day conditions, the fat body stores massive amounts of lipid until day 6 after adult ecdysis. Then the first electron-dense protein granules develop near the nucleus, and on day 10 the first autophagic protein granules can be seen. The histological results of these events are discussed in connection with the known biochemical properties of the adult fat body of the Colorado potato beetle.

INTRODUCTION

The fat body of insects is the major site of metabolism and storage (e.g. lipid, glycogen, and protein). Much research has been done on the synthesis in adult females of specific proteins in relation to oögenesis. During reproduction, the fat body produces large amounts of vitellogenins which are subsequently stored in the oöcytes (see reviews of Price, 1973, and Keeley, 1977). For some insect species, the biochemical evidence for vitellogenin synthesis of the female fat body is supported by its ultrastructural properties. High rates of vitellogenin synthesis correlate with large amounts of rough endoplasmic reticulum (RER) in the fat body of *Locusta migratoria* (Chen *et al.*, 1975). At the end of the reproductive cycle, different inclusions of lysosomal origin are found. These inclusions probably have a function in the remodeling of the cells. Comparative studies of vitellogenin synthesis and the ultrastructural properties of the female fat body have also been performed with *Aedes aegypti* (Behan and Hagedorn, 1978). A total regression of the ultrastructural machinery is observed after the peak of vitellogenin synthesis in this insect. This correlates with the period during which the fat body is not responsive to ecdysterone (Bohm *et al.*, 1978). Reproduction in the above insect species is cyclic, with short peaks of vitellogenin synthesis during oögenesis. No important protein storage (e.g. protein granules) is observed during the inactive period. This is in contrast to the situation found in the viviparous cockroach *Diploptera punctata*: the fat body of this insect contains many protein granules during gestation (Stay and Clark, 1971).

In the female Colorado potato beetle under study here, reproduction is not cyclic. When females are reared under long day (LD) conditions, oviposition starts five days after adult ecdysis and egg-masses of 50-60 eggs are produced daily. Oviposition coincides with high rates of vitellogenin synthesis (Dortland, 1978). A short day (SD) photoperiod induces the preparation for diapause which lasts 10 to 12 days. During this time low rates of vitellogenin synthesis are observed; and after day 6, synthesis of three diapause proteins predominates and two of these are accumulated by the fat body.

The development of larval fat body, and the change to pupal fat body, was

investigated previously by means of light microscopy (Labour, 1970) and electron microscopy (Labour, 1974). Development of the larval fat body was shown to be a dynamic process. The built-up of protein granules was observed in the fourth larval instar and in the pupae, and part of the fat body was lysed during pupation. In addition, the ultrastructural properties of the fat body of ovipositing and prediapause females have been compared previously (De Loof and Lagasse, 1970).

In view of the above-mentioned results, we felt it necessary to study the developmental changes during the pupal to adult transition in females reared under two different photoregimes. It will be shown here that development of the adult fat body under long and short day conditions is equally dynamic, with important histological changes at well-defined points.

MATERIALS AND METHODS

All experiments were performed with females of the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera, Chrysomelidae), which were bred either under long day conditions of 18 hours photophase or under short day conditions of 10 hours photophase. Further rearing conditions have been described previously (De Wilde, 1957; De Kort, 1969). Development is described starting from the moment of adult ecdysis (= day 0).

For light microscopy, a part of the abdomen (sternite 4, 5 and 6) was isolated at 4°C in Bouin's fluid, and fixed at room temperature for three days in the dark. It was then dehydrated in alcohol, methylbenzoate, methylbenzoate containing 2% celloidin, and embedded in paraplast. Sections of 7 µ were cut on a Leitz microtome and stained by a modified (0.05% instead of 0.5% toluidine blue) Dominici method (1902).

For electron microscopy, small abdominal parts of the cuticle with attached tracheae and fat body were dissected at 4°C in *Leptinotarsa* Ringer (Dortland and De Kort, 1978). Each piece was fixed in glutaraldehyde (2% with 0.1 M Na-cacodylate and 0.03 M $CaCl_2$, pH 7.2) at 4°C for 2 hours, and stored for 22 hours at 4°C in 0.1 M Na-cacodylate and 0.03 M $CaCl_2$ at pH 7.2 .The tissue was post-fixed at room temperature for 2 hours in the dark in 1% OsO_4 with 0.1 M veronal acetate,

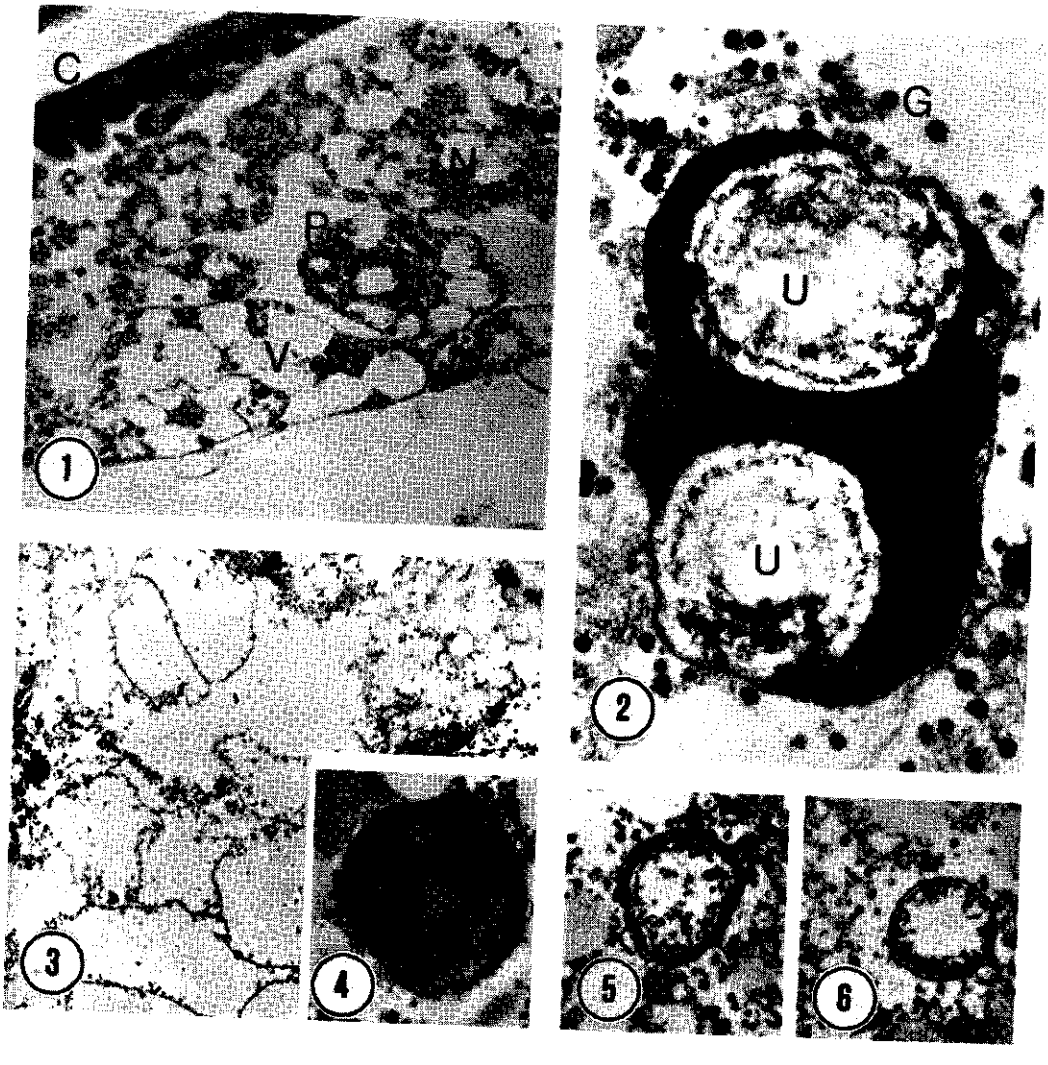


Fig. 1. Fat body of a LD-female at adult ecdysis. Lobes situated directly under the cuticle (C). Protein granules (P), watery vacuoles (V), nucleus (N).Dominici. x 440

Fig. 2. Protein granule with two amorphous zones of urate (U). Rosettes of glycogen (G). One day old LD-female. x 22 500

Fig. 3. Part of adult fat body, which is subject to lysis. One day old LD-female. x 3200

Fig. 4. One day old LD-female. Protein granule with light and dark zones. x 12 800

and block-stained for 0.5 hour in 1% uranyl acetate in double distilled water. The tissue blocks were dehydrated in alcohol and embedded in Epon 812. Sections were cut on a LKB Ultramicrotome III. Thin sections were mounted on formvar grids and these were stained with lead citrate (Reynolds, 1963) and examined on a Siemens Elmiskop 101 transmission electron microscope (accelerating voltage 80 kV).

For additional light microscopy, 1 μ thick Epon-sections were routinely stained in toluidine blue (2%).

RESULTS

General appearance of the fat body

The fat body of the Colorado potato beetle is built-up by a loose association of separate parts which are connected by tracheae. It depends on the condition of the female whether the fat body is floating freely in haemolymph, or is compressed as a result of an increase in size of the ovaries or the fat body itself. The whole tissue has a yellow appearance.

Two types of arrangements of fat body cells can be distinguished: a) thin strands of fat body which are found between the aorta and the ovaries (Fig. 9 and 13); and b) small lobes which are predominantly situated directly under the cuticle (Fig. 4). These two types occur simultaneously in females reared under long as well as short day conditions. Whole cross-sections of the abdomen show no obvious difference in cytological structure of the fat cells in lobes or strands. Each cell normally contains one nucleus and penetrating tracheoles. Oenocytes, sometimes two or three per fat lobe, are mostly observed in regions just beneath the cuticle.

Adult development of fat body from females reared under long day conditions

The fat body of a female at adult ecdysis contains numerous protein granules (Fig. 1). There is little cytoplasm and the cells contain large vacuoles. Pro-

Fig. 5. Remnants of a protein granule. One day old LD-female. x 12 800
Fig. 6. As Fig. 5 but further digested. x 12 800

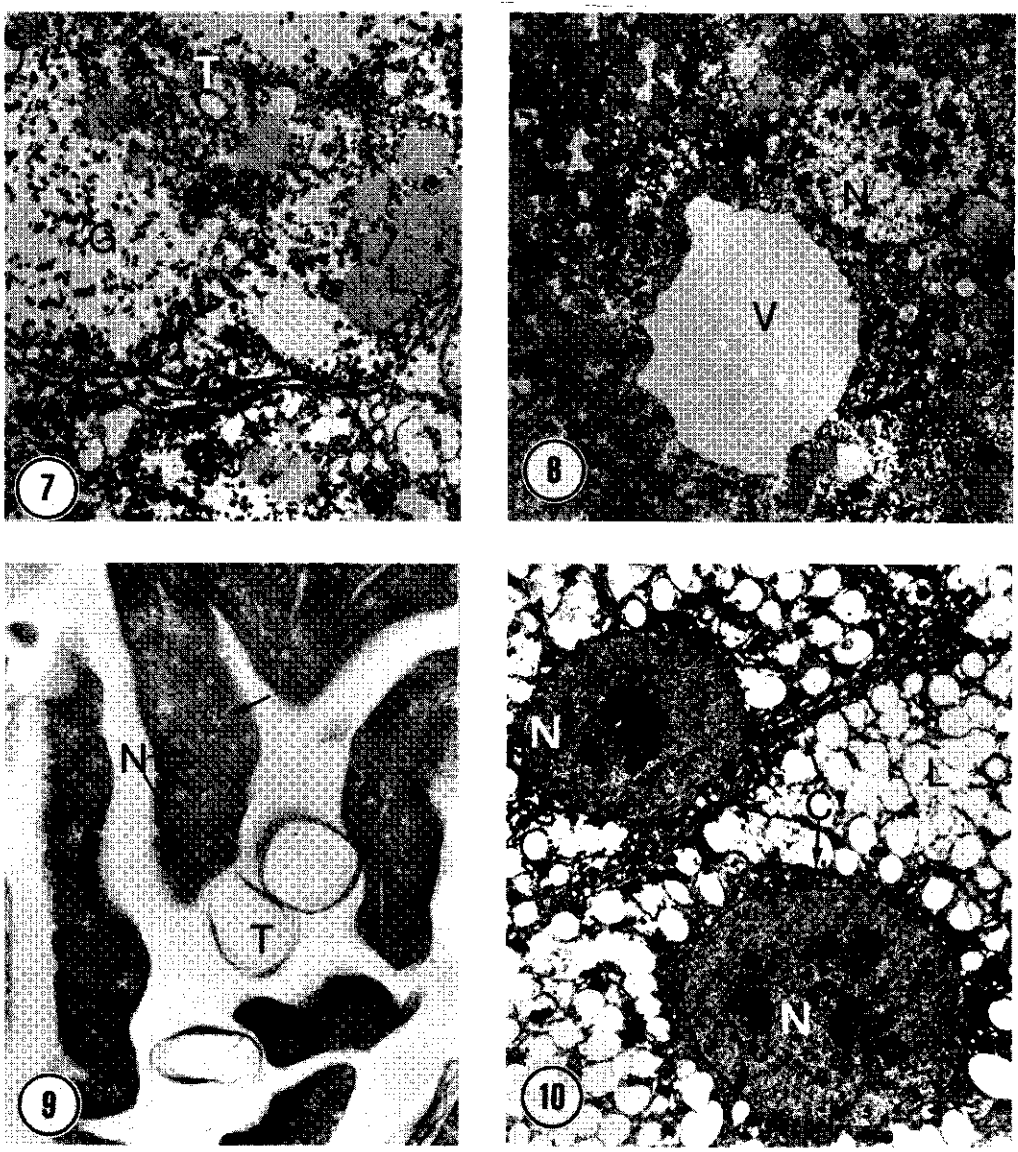


Fig. 7. LD-female one day after adult ecdysis. Fat body with small lipid droplets (L), glycogen rosettes (G). A tracheole (T) invades the cell. x 6400

Fig. 8. Expanding cytoplasm (arrows) in a fat body cell with nucleus (N). The largest part of the cell is rebuilt. Watery vacuole (V). The fat body was derived from a LD-female two days after adult ecdysis. x 3200

tein granules are inhomogeneous and contain scattered light and dark zones (Fig. 4). Part of the granules contain electron light zones (Fig. 2) which are believed to be urate (Labour, 1974).

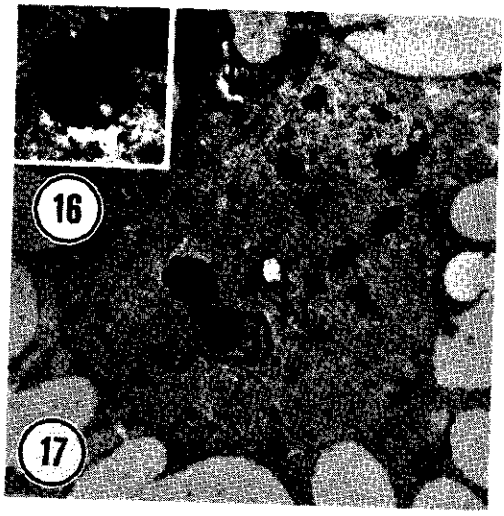
The protein granules rapidly decrease in number on day 1 after adult ecdysis It is possible that these granules are digested from the inside (Fig. 5 and 6). Some parts of the fat body are subject to lysis (Fig. 3); cell membranes are destroyed and the cellular content is released to the haemolymph while other parts of the fat body are rebuilt (Fig. 7). This is substantiated by the observation of newly-formed glycogen (predominantly in the rosette or α-form), and small lipid droplets. In some cases short strings of RER appear again.

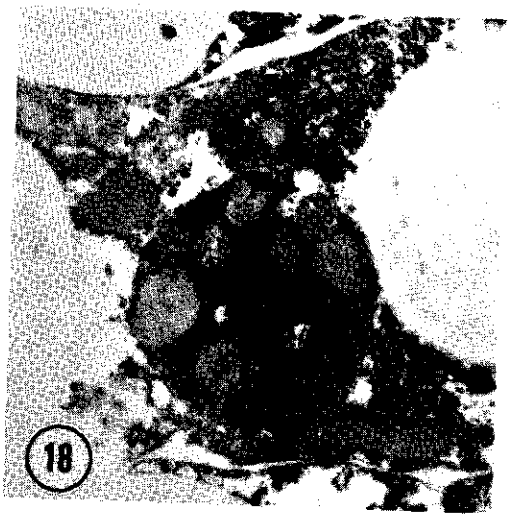
On day 2 after adult ecdysis, the cytoplasm of the surviving cells expands itself and pushes the remainder of the large vacuoles together (Fig. 8). These vacuoles have a watery content (Labour, 1970). All protein granules have by now disappeared. One day later all vacuoles have disappeared and the whole cell is filled with cytoplasm and additional lipid droplets (Fig. 9).

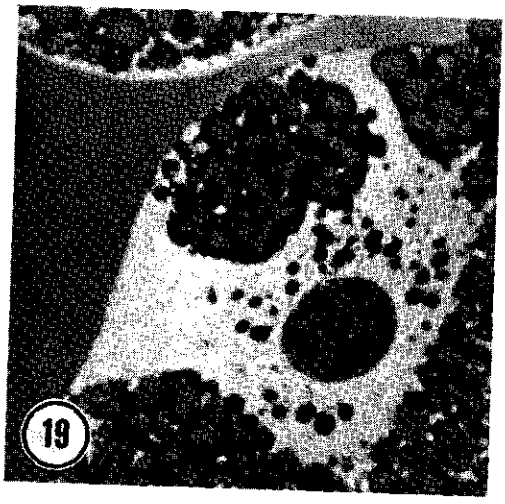
Vitellogenesis starts on day 2, and the first egg-masses are deposited on day 5. The ovaries increase tremendously in size and the abdomen of a 5-day-old female has a swollen appearance. Fat body is situated only between the ovaries and directly under the cuticle at this time. Fig. 10 shows two regions of RER and mitochondria in each cell: around the nucleus and beneath the plasma membrane. Lipid droplets can be seen between these two regions. A sheath of RER often borders the lipid droplets, as can be seen in Fig. 11. The plasma membrane at the boundary of the fat cell and haemolymph forms many invaginations which result in an increase of the cell surface. Fig. 12 shows a large amount of RER between these infoldings.

Fig. 9. Strand of fat body. The nuclei (N) are rather large; watery vacuoles are no longer observed. Tracheae (T). LD-female three days after adult ecdysis. Dominici. x 440

Fig. 10. EM-survey of fat body from a LD-female five days after adult ecdysis. Thin strands of cytoplasm (C) are seen around the nucleus (N) and at the periphery of the cell. Small lipid droplets (L) fill a large part of the cell. x 3200







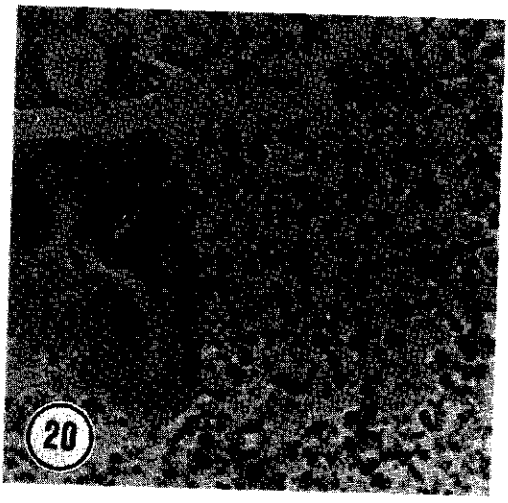


Fig. 16. Detail of an electron-dense granule. x 12 800

Fig. 17. EM-section through a nucleus of a fat body cell of a six day old SD-female. The first electron-dense protein granules(DP) appear in the vicinity of the nucleus (N). x 6400

Fig. 18. Detail of an autophagic granule, which was observed in the fat body of a ten day old SD-female. x 12 800

Fig. 19. Semi-thin section of a large oenocyte. SD-female ten days after adult ecdysis. Toluidine blue. x 450

Fig. 20. Light microscopic section of the fat body of a diapausing female. A malpighian tubule (Mt) and a trachea (T) are inserted between the fat body. Dominici. x 440

origin (Fig. 18). The nucleus is now surrounded by large lipid droplets, but RER and mitochondria are still dispersed between the lipid droplets. At this time oenocytes have a swollen appearance and contain several little droplets (Fig. 19). The significance of these droplets is not known, but they do not occur in the oenocytes of females reared under long day conditions.

The beetles enter diapause around day 12. Fig. 20 shows part of the fat body of a female after two days of diapause. All cells throughout the abdominal fat body are filled with numerous protein granules and lipid droplets.

DISCUSSION

The results in this paper show development of the fat body in the adult Colorado potato beetle. The fat body of a female at adult ecdysis contains a large number of protein granules. Some of these granules contain one or two amorphous zones which are mostly located in the centre. The pupa has a closed system with only gaseous exchange; therefore, it seems that a part of the excretory products is stored in solid state at a place which formerly served to store protein. Storage of urate in granules is also reported in the pupal fat body of *Hyalophora cecropia* (Tojo *et al.*, 1978), while in the female of *Nauphoeta cinerea* (Wüest, 1978) and *Diploptera punctata* (Stay and Clark, 1971) urate is stored in fat body cells specialized for this purpose. In these urocytes, urate is stored in spherocristals which are enclosed in a membrane.

In the Colorado potato beetle, granules with and without urate inclusions are soon broken down. This depletion starts in the disintegrating cells of the early pupa (Labour, 1970) and is completed in all surviving cells within 36 hours after adult ecdysis. These granules provide material for histogenesis of imaginal tissue. The observations presented in this paper give the impression that, during breakdown, granules are digested from within. On the other hand, urate inclusions might be dissolved during fixation, suggesting that the partly-digested granules are possibly remnants of granules which contained urate. The breakdown of the protein granules is demonstrated by a decrease in the protein/DNA-ratio of the female fat body. There is also a decrease in the RNA/DNA-ratio which suggests

that RNA is released from the fat body (Dortland and De Kort, 1978).

Concurrent with the breakdown of protein granules is the disappearance of the large vacuoles. In the fat body of a 3-day-old female, these vacuoles can no longer be observed. The disappearance of protein granules and vacuoles suggests total lysis of the pupal fat body. A large proportion of the fat body is subject to cell remodeling (a process whereby one population of cell organelles is destroyed and replaced by another during development) as was reported for *Calpodes* (Larsen, 1976). Some glycogen rosettes and lipid droplets are soon regenerated. In addition, the first membranes studded with ribosomes can be seen. The subsequent increase in protein/DNA- and RNA/DNA-ratio correlates with the rebuilding of cytoplasm (Dortland and De Kort, 1978). Thus it appears that the pupal fat body persists into the adult stage, although the cytoplasm is newly-reconstructed. This transforming process is also reported for the fat body of *Hyalophora cecropia* (Bhakthan and Gilbert, 1972) and *Trogoderma granarium* (Nair and Karnavar, 1968); but not for *Calliphora erythrocephala* (Thomsen and Thomsen, 1974), *Sarcophaga bullata* and *Drosophila melanogaster* (Whitten, 1964). In the Diptera, the larval fat body is replaced by a completely newly-built adult fat body.

When *Leptinotarsa* females are reared under long day conditions, the fat body develops into a tissue highly specialized for protein synthesis. The fat body of an ovipositing female contains large amount of RER and mitochondria. This observation correlates with the observed high rates of vitellogenin synthesis (Dortland, 1978). The plasma membrane is deeply invaginated, which facilitates the uptake of the precursors needed for protein synthesis and the rapid release of the synthesized vitellogenins. The basement membrane is not invaginated. Golgi-systems are very rarely observed, which is in agreement with the results of De Loof and Lagasse (1970). This suggests that the synthesized vitellogenins are not packed in secretion granules but are directly released into the haemolymph.

The fat body of an ovipositing female also stores lipid in the form of small droplets which have no membrane. The absence of a limiting membrane is also reported for *Nauphoeta cinerea* (Wüest, 1978) and *Calliphora erythrocephala* (Thomsen and Thomsen, 1974). In some cases, the lipid droplets are surrounded by a sheath of RER as described for *Aedes aegypti* (Behan and Hagedorn, 1978). It is not known

whether this arrangement has any significance.

The development of fat bodies in short day females younger than two days (after adult ecdysis) does not show any histological difference from those reared under long day conditions. Thus the effect of rearing the insects *ab ovo* under different photoregimes is expressed in a difference in fat body structure *after day* 2. Massive storage of lipid occurs in the fat body of the prediapause female, and this results in a large increase in size. Since no mitotic figures are observed, it is believed that the increase in size is due to enlargement of the cells. Lipid is mainly stored during the first half of the prediapause period (i.e. before day 6). This fat storage is nearly complete just prior to the appearance of protein granules (Locke and Collins, 1965).

A sharp increase in protein/DNA-ratio is observed between day 6 and day 9 (Dortland and De Kort, 1978). This corresponds with the reappearance of the first protein granules on the sixth day. These electron-dense granules first show up in the surroundings of the nucleus as reported for *Pieris rapae* (Kim *et al.*, 1977), which suggests that they are not formed by pinocytotic uptake of haemolymph proteins.

The first autophagic protein granules appear on day 10. They contain several inclusions which are probably enveloped mitochondria. With the onset of diapause, protein synthesis diminishes (Dortland and De Kort, 1978) and we may assume that at this time fat body organelles (e.g. mitochondria and RER) are isolated in autophagic granules. Therefore, a protein reserve is formed which can be used for the rebuilding of imaginal tissue during postdiapause.

It is not known whether the dense or autophagic granules in the adult fat body contain specific proteins. A selective accumulation in the fat body of two diapause proteins is observed during the second half of the prediapause period. Since these proteins first appear in the haemolymph and later decrease there, it appears that they are taken up by the fat body again. This finding would explain the invaginations found at the periphery of the fat cell which occur to a moderate extent during the second half of the prediapause period. It is likely that the accumulated diapause proteins are stored in protein granules, as has been reported for the larval fat body of *Hyalophora cecropia* (Tojo *et al.*, 1978).

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Vitellogenin synthesis in the fat body of the female Colorado potato beetle in the presence and absence of juvenile hormone

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ABSTRACT

Vitellogenin synthesis in the fat body of the female Colorado potato beetle is affected by juvenile hormone (JH), as was demonstrated by its stimulation after topical application of 50 µg JH to allatectomized prediapause females. The peak of vitellogenin synthesis was reached 5 days after the administration of JH. Allatectomy in 5-days-old long day females resulted in decreased rates of vitellogenin synthesis. However, 4 days after allatectomy rather large amounts of vitellogenins are still produced. In postdiapausing long day females, which were allatectomized during diapause, vitellogenin synthesis increased in the absence of JH. It is concluded that other factors than JH are involved. β-ecdysone does not seem to participate in the regulation of vitellogenin synthesis.

In virgin long day females (12 days after adult ecdysis) JH-synthesis and protein synthesis are as high as in mated long day females, although a virgin produces 1/6 of the amount of eggs, laid by a mated female. In the virgin female

the rate of vitellogenin synthesis is uncoupled from the rate of egg-laying, which suggests a rather autonomous role in the accumulation of proteins by the ovary.

INTRODUCTION

In most insects reproduction is under the control of juvenile hormone. This hormone has a stimulating effect on vitellogenin synthesis in the fat body as was demonstrated for *Leucophaea maderae* (ENGELMANN, 1971) and *Locusta migratoria* (CHEN *et al.*, 1976). In the Colorado potato beetle, *Leptinotarsa decemlineata* Say, DE LOOF and DE WILDE (1970) showed that the haemolymph of prediapause females contained more vitellogenin after injection of JH.

In all studies the positive effect of JH on vitellogenin synthesis was obtained by *in vivo* addition of the hormone. In some cases head-ligatured or even ovariectomized abdomens of females were used. Until now the stimulation of vitellogenin synthesis was not achieved for any insect if JH was added to the incubation medium in which fat body was kept *in vitro*. However, it is possible that for the induction of vitellogenin synthesis by JH also other factors are required (e.g. binding proteins). Thus it is not certain whether vitellogenin synthesis is affected directly or indirectly by juvenile hormone.

In *Aedes aegypti* a direct effect of 8-ecdysone on vitellogenin synthesis *in vitro* was found (FALLON *et al.*, 1974). However, it is suggested that during adult maturation females of *Aedes aegypti* do need juvenile hormone. Treatment with JH makes the fat body responsive to 8-ecdysone (FLANAGAN and HAGEDORN, 1977). Also in the Cecropia silkworm (*Hyalophora cecropia*) the role of JH in the regulation of vitellogenin synthesis is questionable. JH injected into isolated abdomens of diapausing pupae did not increase the percentage incorporation into vitellogenin (PAN, 1977).

As was demonstrated by PRICE (1966) and several others short-term incubations of fat body cultured *in vitro* are extremely useful for the investigation of protein synthesis. Using such a system the synthesis of the proteins released by fat bodies of *Leptinotarsa* females was measured by DORTLAND and DE KORT (1978). In this species the *in vitro* synthesis of the different proteins by the fat body was exactly in the same relative proportions as *in vivo*

(DORTLAND, 1978). With the aid of such an *in vitro* system, the influence of juvenile hormone on the synthesis of vitellogenins and diapause proteins was investigated.

MATERIALS AND METHODS

Insects

Leptinotarsa decemlineata Say were reared on fresh potato foliage according to DE WILDE (1957) and DE KORT (1969) under two different photoregimes: long days (18 h light: 6 h dark) or short days (10 h light: 14 h dark). Adult ecdysis is called day 0.

Measurement of the rates of synthesis of the different released proteins

For the *in vitro* incubations, the dorsal part of the abdominal cuticle with attached fat body was dissected and washed in ice-cold *Leptinotarsa* Ringer (DORTLAND and DE KORT, 1978). The composition of the culture medium and the measurements of the *in vitro* incorporation of [^{14}C]-lysine into total released protein were described elsewhere (DORTLAND and DE KORT, 1978). The relative incorporation of [^{14}C]-lysine into the different proteins *in vitro* (using disc-electrophoresis for the separation of the different proteins) was also described previously (DORTLAND, 1978). The method was slightly changed for the measurements of the relative incorporation of diapausing females, because these females have low rates of protein synthesis. Therefore, the fat bodies from 10-12 individuals were incubated for 8 h in 200 µl of medium. The experiment was started by the addition of 4 µCi [^{14}C]-lysine. At the end of the incubation samples of 60 µl centrifuged medium were applied to the gels. The rates of synthesis of the different proteins were calculated from the rate of incorporation into total released protein and the relative incorporation values of the different proteins.

Determination of protein and DNA

After termination of the *in vitro* incubations the fat bodies were homogenized

in *Leptinotarsa* Ringer. The homogenates were processed according to TANGUAY and CHAUDHARY (1971). DNA was measured with the diphenylamine test (BURTON, 1956). Fish sperm DNA was used for calibration. Protein was assayed with the method of HARTREE (1972) with BSA (fraction V) as a standard.

Protein measurement in haemolymph

The amount of protein present in total haemolymph was calculated by multiplying the protein concentration by the haemolymph volume as measured with the [^{3}H]-inulin method (WHARTON *et al.*, 1965). Haemolymph samples of 1-2 µl were diluted 10 times with *Leptinotarsa* Ringer and the different proteins were separated on gel according to DAVIS (1964) and stained, destained and scanned as reported previously (DORTLAND, 1978). The area under each peak was expressed as a percentage of the total area of all peaks determined over the entire length of the gel. The amount of the specific protein present in haemolymph was derived from the amount of protein present in total haemolymph and from the percentages of the specific proteins.

Juvenile hormone metabolism

The JH-synthesis of the corpora allata was determined with *in vitro* incubations, measuring the incorporation of methyl -[^{14}C]-methionine into juvenile hormone (S.J. KRAMER, 1978). The JH-III esterase activity was assayed *in vitro* by measuring the production of [^{3}H]-methanol as described previously (KRAMER and DE KORT, 1976). The determination of the JH-titre by the *Galleria*-test followed the procedure as described by DE WILDE *et al.* (1968).

Measurement of radioactivity

All samples were counted in 10 ml of scintillation mixture, 0.3% PPO in xylol/triton X-100, 3:1 (ANDERSON and McCLURE, 1973). Radioactivity was measured on a Nuclear Chicago Mark III liquid scintillation counter.

Application of juvenile hormone

JH-I (cecropia juvenile hormone) in acetone (50 µg/µl/beetle) was applied topically on the dorsal part of the abdomen. Before application the females were anaesthesized with CO_2. A high dose of 50 µg JH per beetle was used, since in comparison to other insect species high rates of JH break down were found in haemolymph of the Colorado potato beetle (KRAMER and DE KORT, 1976).

Surgical techniques

The corpora cardiaca - corpora allata complex was removed through a slit in the neck membrane (DE WILDE and STEGWEE, 1958). This operation is called allatectomy, since regeneration of the corpora cardiaca was observed within one week after the operation. Sham operation was performed by removing small pieces of fat body.

Chemicals

DNA and BSA were purchased from Sigma. L -[U - ^{14}C]-lysine - HCL (287 mCi/mmol) and [^{3}H]-inulin (300 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, U.K. The sources of other chemicals were described previously. All chemicals were dissolved in double distilled water. Each number quoted in the results represents the mean of duplicate samples taken from two replicates of 8 to 10 females.

RESULTS

Allatectomy in reproducing long day females

It is important to know to what extent vitellogenin synthesis decreases after removal of the corpora allata. Therefore, the effect of allatectomy was investigated. For this experiment five day old females reared under long days were selected. At this age egg-laying just started and vitellogenin synthesis is rather high, but still increased afterwards (DORTLAND, 1978).

Table 1. Rates of *in vitro* incorporation of lysine (x 10^{-7}mol/h x g DNA) into the released proteins by fat bodies of control (C), sham operated (S) and allatectomized (A) long day females at various times after the start of the experiment (= day 5 after adult ecdysis). V1 and V2: vitellogenins; DP1, DP2 and DP3: diapause proteins.

	V1			V2			DP1			DP2			DP3		
Days after operation	C	S	A	C	S	A	C	S	A	C	S	A	C	S	A
0	9.0			3.9			3.3			0.3			0.5		
1		2.8	2.4		1.9	1.2		0.9	0.9		0.1	0.2		0.2	0.5
2		5.9	4.7		3.6	3.0		1.1	2.6		0.7	0.5		0.7	3.2
4	12.8	6.1	2.7	9.6	3.3	1.4	4.7	1.7	0.8	0.5	0.1	0.4	0.8	0.6	0.7

The effect of allatectomy and sham-operation on the rates of protein synthesis are shown in Table 1. Operations have a severe effect on the rate of protein synthesis by the fat body. All females did not feed for 1 or 2 days after the operation and this resulted in decreased rates of protein synthesis. As shown in Table 1 allactectomy affected the rates of synthesis of V1 and V2. This was clearly demonstrated at day 4 after the operation. Nevertheless rather large quantities of vitellogenins were synthesized. It was also observed that 4 days after allatectomy 50% of the operated females still laid masses of 20-30 eggs. The synthesis of the diapause proteins was not reduced by allatectomy. A peak in synthesis of DP1 and especially DP3 was observed at day 2.

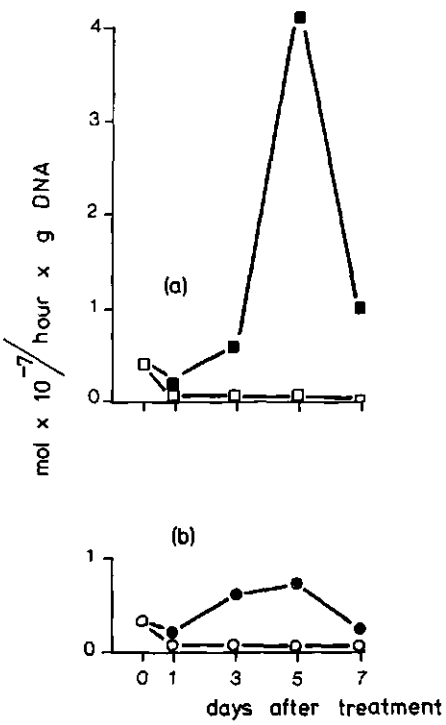

Fig. 1. The effect of application of 50 µg JH to allatectomized prediapause females on the rates of *in vitro* incorporation of lysine into released vitellogenin by the fat body. a. synthesis of V1 in JH-treated (solid squares) and control (open squares) females. b.synthesis of V2 in JH-treated (solid circles) and control (open circles) females.

Stimulation of vitellogenin synthesis in allatectomized short day females by juvenile hormone

The effect of a single application of JH was investigated in females, which had rather low rates of vitellogenin synthesis. These low rates were observed in short day females (DORTLAND, 1978). These females don't reproduce, but they prepare themselves for diapause. As an extra precaution the females were allatectomized two days after adult ecdysis, in order to avoid interference of juvenile hormones produced by the endogenous corpora allata.

At day 9 after adult ecdysis (in the experiment called day 0) all allatectomized females were either treated with acetone (AA) or 50 µg juvenile hormone (AJH), but kept under short days. The behavioural response of the acetone treated females was the same as of the normal prediapause females. At day 3 all acetone treated females entered diapause. The JH-treated females did not enter diapause, but started excessive feeding at day 2 and produced masses of 10-30 eggs on day 5 and 6.

Table 2. Rates of *in vitro* incorporation of lysine ($\times 10^{-7}$ mol/h x g DNA) into the released diapause proteins by fat bodies of control (A), acetone treated (AA) and juvenile hormone treated (AJH) short day females; DP1, DP2 and DP3: diapause proteins. All females were allatectomized at day 2 after adult ecdysis and either treated with acetone or juvenile hormone at day 9 after adult ecdysis.

	DP1			DP2			DP3		
Days after treatment	A	AA	AJH	A	AA	AJH	A	AA	AJH
0	2.1			0.9			1.8		
1		0.8	0.3		0.3	0.3		0.7	0.3
3		0.8	0.7		0.2	0.5		0.5	0.9
5		1.1	0.5		0.1	0.3		0.7	0.4
7		0.3	0.2		0.1	0.1		0.1	0.2

The effect of JH on the rate of V1 and V2 synthesis is shown in Fig. 1a and 1b respectively. The rate of V1 synthesis is stimulated, but the rate of V2 synthesis increased to a lesser extent. The peak of V1 synthesis (a 32 fold increase compared with day 1) was reached five days after the JH-application, when V1 synthesis accounted for nearly 40% of the labelled released proteins. The rate of synthesis of both proteins declined sharply at day 7.

The effect of the JH- and acetone-treatment on the rates of synthesis of the three diapause proteins are shown in Table 2. These rates were not greatly affected by the JH-treatment. The control at day 0 produced modest quantities. Later in the experiment synthesis of the diapause proteins in acetone- and JH-treated females decreased constantly, although, as stated above, their behaviour was not the same. Apparently in both cases production of diapause proteins is not longer required.

Stimulation of vitellogenin synthesis in postdiapause females in the absence of JH

Although the positive effect of juvenile hormone on vitellogenin synthesis was demonstrated, it is not certain whether also other factors are involved in the regulation of vitellogenin synthesis. Therefore the possibility of vitellogenin synthesis in the absence of JH was investigated.

An ideal stage for the stimulation of vitellogenin synthesis in the absence of JH is the diapausing female. As shown before, vitellogenin synthesis is very low in these females (DORTLAND, 1978), while the JH-titre is zero (DE WILDE *et al.*, 1968) and, in addition, JH-synthesis of the corpora allata is hardly measurable (KRAMER, 1978). Females that had been in diapause for 6 weeks were allatectomized immediately after removing the animals from the sand. These females were transferred to long days and reared separately. Four days after the operation a long day male was added to each female. These males copulated frequently with the allatectomized females. Thus all positive stimuli (mating and long day photoperiod), except the supply of JH, are present for the stimulation of vitellogenin synthesis.

At the start of the experiment (diapausing females) incorporation into total

released protein is very low (0.42 x 10^{-7} mol/h x g DNA), but 4 days later incorporation into total released protein has increased 10 fold and 9 days after the start of the experiment incorporation into total released protein has a rate of 14.25 x 10^{-7} mol/h x g DNA. The percentual incorporation into V1 and V2 increased from 3.4% to 16% and from 2.8% to 10.9% respectively. This results in a nearly 160 fold increase in V1 synthesis and in a 120 fold increase in V2 synthesis (Fig. 2a). At the end of the experiment the absence of the corpora allata was checked in all females, while as an extra check their haemolymph was assayed for JH. No JH was detected in any female.

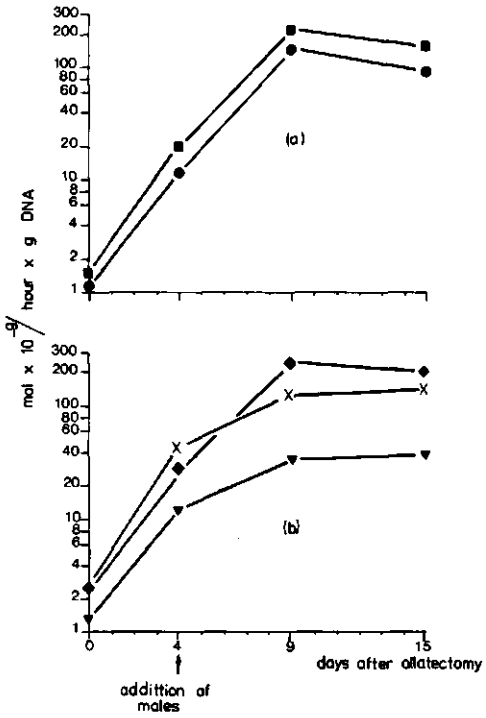

Fig. 2. The effect of transfer to long day conditions of allatectomized postdiapause females on the rate of *in vitro* lysine incorporation (in log scale) into different released proteins by the fat body. a. synthesis of the vitellogenins V1 (solid squares) and V2 (solid circles). b. synthesis of the diapause proteins DP1 (solid diamonds), DP2 (solid inverted triangles) and DP3 (crosses).

In addition the rate of oviposition of these allatectomized females was measured. Egg-laying started about 9 days after the beginning of the experiment and, at day 21, 70% of the females had produced eggs, which were all fertile. Egg-laying was irregular. In most cases one day of oviposition of 10-20 eggs was followed by one or two days without oviposition.

Fig. 2b shows a stimulation of the synthesis of the three diapause proteins as well. The percentual incorporation increased for DP1 from 5.6% to 17.9% and for DP3 from 5.6% to 9.4% respectively, while the percentual incorporation of DP2 (3.0%) did not change.

β-ecdysone and the regulation of vitellogenin synthesis

The involvement of β-ecdysone in the regulation of vitellogenin synthesis was also tested. Amounts of 5 and 10 µg β-ecdysone dissolved in *Leptinotarsa* Ringer were injected in females reared under short days. No increase in vitellogenin synthesis was observed. Injections of 20 µg β-ecdysone per female resulted in the death of the animals. Injections of 10 µg β-ecdysone into diapausing females did also not stimulate vitellogenin synthesis. Thus it seems that β-ecdysone takes no part in the regulation of vitellogenin synthesis in the female Colorado potato beetle.

Vitellogenin synthesis and its regulation by JH in mated and virgin females reared under long days.

Different patterns of protein synthesis are found in females reared under long days or short days. In long day females vitellogenin synthesis predominates while in short day females mainly diapause proteins are produced by the fat body. In long day females two groups behave differently, the mated and the virgin female.

The comparison of these two groups was started with the measurement of their oviposition rates (Fig. 3). The numbers denominated in the figure are the mean oviposition rates of ten females. In *Leptinotarsa* oviposition began at day 5 in both mated and virgin females at the same rate. However, at day 10 mated females produced 3 times more eggs than virgins and this difference in oviposition

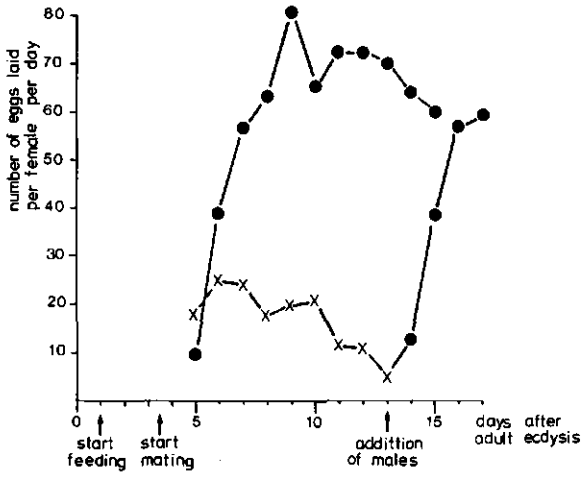

Fig. 3. Rate of oviposition in mated (solid circles) and virgin (crosses) females reared under long day conditions. At day 13 long day males were added to the virgins.

rate increased during the three following days. Virgin females showed a different behaviour of egg-laying. They produced no egg-masses as mated females do, but oviposition was more scattered. Another effect of the virgin condition is the size of the abdomen. The abdomen of a mated female is swollen by large amounts of eggs, while the abdomen of a virgin is not. When males were added to virgin females the rate and behaviour of egg-laying were restored to their normal level within three days.

It is in relation to the preceding experiments of particular interest what rates of vitellogenin synthesis occur in virgin females and whether the JH-titre is affected by mating. Vitellogenin synthesis in mated and virgin females was measured at three stages of the life cycle. The first measurement of vitellogenin synthesis was performed just before mating normally occurs (day 3 after adult ecdysis). The following measurements were carried out on day 7 (when the first difference in oviposition rate occurred) and day 12 (when mated females lay a 6 fold of the amount of eggs produced by virgins).

Fig. 4a shows that the rates of synthesis of V1 and V2 are the same in mated and virgin females. Moreover, the synthesis of the diapause proteins DP1 and DP2 (Fig. 4b) and synthesis of DP3 (Fig. 4c) also reach the same rates. This appeared from the fact that lysine incorporation into total released protein and the

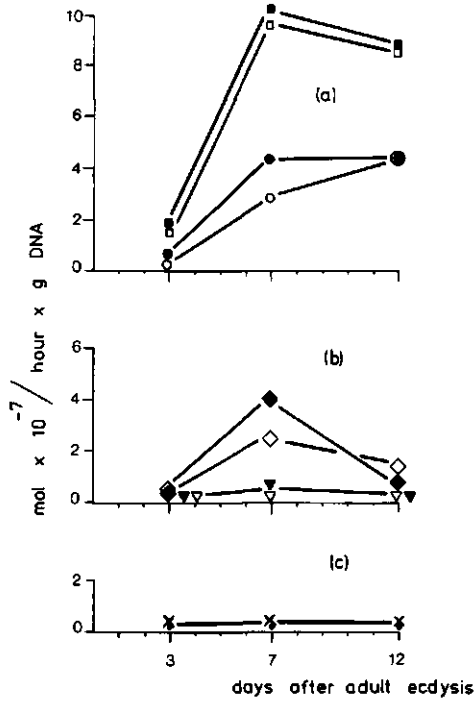

Fig. 4.
a. Rates of *in vitro* incorporation of lysine into released vitellogenin by fat bodies of mated and virgin females of different age reared under long day conditions. Symbols: V1 synthesis in mated females (solid squares); V1 synthesis in virgin females (open squares); V2 synthesis in mated females (closed circles); V2 synthesis in virgin females (open circles).
b. Synthesis of DP1 in mated(solid diamonds) and virgin (open diamonds) females and the synthesis of DP2 in mated (solid inverted triangles) and virgin (open inverted triangles) females.
c. Synthesis of DP3 in mated (crosses) and virgin (dots) females.

percentage incorporation of the different proteins in mated and virgin females were equal.

In addition, juvenile hormone metabolism in mated and virgin females was investigated (Table 3). Although there is more JH-III esterase activity in the haemolymph of virgins, breakdown is rather low in both groups compared with high rates (28 µmol/ml x h) of JH-III esterase activity observed in the haemolymph of short day animals (KRAMER and DE KORT, 1976). The activity of the corpora allata is the same in mated and virgin females. There was also no difference observed in the JH-titre in haemolymph. Moreover, gifts of 50 µg JH did not raise the egg-production in virgin females (unpublished observation). This clearly demonstrates that the rate of oviposition is affected by more factors than only JH.

Table 3. JH-metabolism in mated and virgin long day females 12 days after adult ecdysis.

	Mated	Virgin
JH titre (Galleria units/ml haemolymph)	3600	3000
synthesis of JH (pmol/h x pair CA)	1.2	1.1
JH-III esterase activity (µmol JH acid/h x ml haemolymph)	0.6	1.6

Although oösorption was observed in the ovarioles of virgin females, this took place only to a limited extent. The fat body of virgins produced massive amounts of vitellogenin, while there was probably a reduced accumulation of vitellogenin by the oöcytes, which resulted in a low rate of oviposition. Protein storage in haemolymph and fat body was therefore expected. Table 4 shows that virgin females stored more vitellogenins and diapause protein 1 in haemolymph,

Table 4. Amounts (in mg) of the five investigated proteins present in total haemolymph of mated and virgin females 12 days after adult ecdysis.

Protein species	Mated	Virgin
V1	0.23	0.32
V2	0.26	0.43
DP1	0.13	0.33
DP2	0.03	0.05
DP3	0.01	0.01

although these differences are not impressive. In addition, the protein storage in the fat body was investigated. Although storage of vitellogenin in the fat

body is not likely, it would seem possible that the remainder of the produced vitellogenins would be converted to other proteins, which are subsequently stored in the fat body.

Table 5. Effect of mating on the protein/DNA-ratio of fat bodies derived from long day females of different age.

Days after adult ecdysis	Mated	Virgin
3	70	65
7	66	67
12	69	62

Comparison of the protein/DNA-ratio in mated and virgin females (Table 5) reveals no difference in protein storage. It appears therefore that the proteins synthesized but not accumulated in the oöcytes are stored only partly in the haemolymph.

DISCUSSION

The control of vitellogenin synthesis

Much work has been done about the endocrine regulated synthesis of vitellogenins in amphibians (WALLACE and BERGINK, 1974) and birds (GRUBER *et al.*, 1976). In both groups of animals synthesis of vitellogenin by the liver is directly stimulated by estradiol *in vitro*. Vitellogenin is a sexlimited protein, normally found in mature females. However, also in males vitellogenin synthesis can be induced by estradiol administration (WANGH and KNOWLAND, 1975).

The regulation of vitellogenin synthesis in insects is much less understood (KEELEY, 1978). Control of vitellogenin synthesis seems to differ between insect species even within the same insect order. It is still an open question whether insects differ fundamentally in the regulation of vitellogenin synthesis or that the reported evidence is underlying to a common system. JH applied *in vivo*

stimulates vitellogenin synthesis in females of most insect orders: *Locusta migratoria*, Orthoptera (CHEN *et al.*, 1976), *Leucophaea maderae*, Dictyoptera (ENGELMANN, 1971), *Danaus plexippus*, Lepidoptera (PAN and WYATT, 1976) and *Leptinotarsa decemlineata*, Coleoptera (DE LOOF and DE WILDE, 1970). In *Hyalophora cecropia*, Lepidoptera (PAN, 1977) injection of JH into isolated abdomens did not stimulate vitellogenin synthesis. It is yet unknown which factor in this species is involved in the induction of vitellogenin synthesis.

As shown here JH is involved in the regulation of vitellogenin synthesis in *Leptinotarsa* females, because JH-application stimulated whereas allatectomy somewhat reduced vitellogenin synthesis. Moreover, under short days when the JH-titre is low (DE WILDE *et al.*, 1968) vitellogenin synthesis is depressed (DORTLAND, 1978), while under long days a high JH-titre coincides with high rates of vitellogenin synthesis. The effect of JH seems rather specific. When JH was applied topically to allatectomized prediapause females, only vitellogenin synthesis increased, whereas the synthesis of the diapause proteins was not affected. This specific stimulation of vitellogenin synthesis by JH was also reported for *Danaus plexippus* (PAN and WYATT, 1976) and to a lesser extent for *Leucophaea maderae* (ENGELMANN, 1971).

Stimulation of protein synthesis in allatectomized postdiapause females is not specific. The synthesis of the diapause proteins, especially DP1 and DP3 increased significantly (Fig. 2b), while the largest increase was observed for the synthesis of the two vitellogenins. This conclusively proves that vitellogenin synthesis can increase in the absence of JH in the female Colorado potato beetle. However, neither the rate of vitellogenin synthesis nor the rate of oviposition is as high as in normal reproducing females. This is not surprising, since one of the stimuli, which has a positive influence on vitellogenin synthesis, is missing! Vitellogenin synthesis in allatectomized queens was observed in *Apis mellifera* (ENGELS and RAMAMURTY, 1976). However, these females were allatectomized 3 days after adult emergence, thus it is possible that induction of vitellogenin synthesis had already taken place.

The increase in vitellogenin synthesis in the absence of JH in *Leptinotarsa decemlineata* suggests that other factors are also involved in the regulation. For *Aedes aegypti*, Diptera (FALLON *et al.*, 1974) vitellogenin synthesis is under the control of an ovarian hormone. Stimulation of vitellogenin synthesis in the

fat body *in vitro* was achieved through the addition of β-ecdysone to the incubationmedium. Detailed studies revealed that α-ecdysone is produced by the ovary and subsequently converted to β-ecdysone (HAGEDORN *et al.*, 1975). Massive synthesis of ecdysone by ovaries has also been reported in other insects i.e. in *Locusta migratoria* (LAGUEUX *et al.*,1977) and *Galleria mellonella* (HSIAO *et al.*, 1977; BOLLENBACHER *et al.*, 1978). In these two species the majority of the produced ecdysone remains within the ovary. Moreover, LAGUEUX *et al.*, showed that in the locust induction of vitellogenin synthesis is clearly not the role of this hormone since ecdysone production occurs in the terminal oöcyte when vitellogenin synthesis is virtually completed. In the Colorado potato beetle, HSIAO *et al.* (1976) could not detect moulting hormone activity in 3 days old long day females and they suggested that moulting hormome production may not be essential for ovarian activity in this species. Indeed, our injection experiments using β-ecdysone showed that this hormone is not able to induce vitellogenin synthesis in short day beetles. We can conclude that the regulation of vitellogenin synthesis in *Leptinotarsa* is a complex phenomenon. Although more factors than JH are involved, β-ecdysone does not seem to participate in this process.

Vitellogenin synthesis and JH-metabolism in virgin and mated females

Mating affects reproductive activity in many insects. For *Rhodnius prolixus* the difference between mated and virgin females was thoroughly investigated. Virgins had a slower rate of egg-production and they started oviposition ± 6 days later than the mated females (DAVEY, 1965). The virgin female of the Colorado potato beetle started oviposition on the same day (day 5 after adult ecdysis) and at the same rate as the mated female. Seven days later virgins oviposit only 1/6 of the rate of that of mated females.

In some insects mating seems to affect the activity of the corpus allatum (CA). In most of these studies CA-activity was not measured directly, but rather indirect criteria were used. Recently, WEAVER and PRATT (1977) studied the effect of enforced virginity and mating on the activity of the CA in *Periplaneta americana*, using an *in vitro* radiochemical assay. Enforced virginity causes the normal appearance of a small peak of CA-activity, but inhibits the large peaks

associated with the different cycles of ovarian development. Increased JH-synthesis after mating was also reported for *Diploptera punctata* (STAY and TOBE, 1977).

The results described in this paper differ significantly from those in *Periplaneta*. As shown before (DORTLAND and DE KORT, 1978), female Colorado potato beetles do not show cyclic changes in ovarian development. Vitellogenesis occurs continuously in the 4-5 oöcytes in each ovariole. In addition, it was shown that the CA from female Colorado potato beetles, when reared under long days, do not show cyclic changes in activity. Activation of the CA occurs rapidly and is completed three days after adult ecdysis (KRAMER, 1978). In contrast to *Periplaneta*, mating in the Colorado potato beetle occurs after the onset of vitellogenesis. We observed mating between day 3 and 4 i.e. during the large increase of vitellogenin synthesis in the fat body. Apparently, activation of the CA is independent of the mating stimulus (Table 3). In addition, we did not find any effect of mating on the JH-titre or JH-esterase activity.

The amount of vitellogenin present in the haemolymph of virgin females is higher than in mated females. Thus shortage of vitellogenins can not explain the low rate of oviposition. On the contrary, all five investigated proteins are synthesized at the same rate in virgin and mated females. Apparently, the synthesis of vitellogenins in virgins is uncoupled from egg-laying. It was not expected that a virgin female produces the same amount of vitellogenins as a mated female. Only a small proportion of the produced vitellogenin was accumulated in the oöcytes of virgins. Furthermore, as illustrated in Table 4 and 5, very little is stored in haemolymph and fat body. This suggests rapid breakdown of the synthesized vitellogenins in virgin females. The high rates of vitellogenin synthesis in virgins are probably related to the daily process of egg-laying in this insect and this is not comparable with insects, which have cyclic patterns of oviposition. In these insects, after each batch of eggs the fat body needs some time to restore its functionality (BOHM *et al.*, 1978). The fat body of the virgin female beetle can produce high amounts of vitellogenin for at least 12 days. When vitellogenin synthesis is high, the virgin female can react very fast on the mating stimulus, since it does not have to built up an active machinery for protein synthesis. Indeed, one day after the addition of adult long day males to virgins the behaviour of egg-laying is normal again e.g. mostly

large egg-masses are produced, while after three days the rate of oviposition is restored to 60 eggs per day per female.

The CA in mated and virgin females of *Rhodnius prolixus* were of the same size (PRATT and DAVEY, 1972), suggesting that equal amounts of JH were circulating in haemolymph. DAVEY and HUEBNER (1974) showed a positive, direct effect of JH on the patency index of the follicle in the mated *Rhodnius* female, which resulted in the accumulation of proteins by the oöcytes. This increase in space between the follicle cells was in the virgin *Rhodnius* female counteracted by an antigonadotropin, which was produced by the ovaries. In addition, it was suggested that this antigonadotropin is only produced in ovarioles with retained oöcytes. Ovulation apparently inhibits the production of antigonadotropin. Only little oösorption was observed in the ovary of a virgin *Leptinotarsa* female. This suggests that the reduced rate of oviposition is probably a result of a reduced rate of accumulation of proteins by the ovary. The ovary of the virgin *Leptinotarsa* female can inhibit the accumulation of proteins by the oöcytes independent of the JH-titre. This resembles the situation in the virgin *Rhodnius* female. However, more proof is needed for the excistence of such an antigonatropin in the ovary of the virgin *Leptinotarsa* female.

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General discussion

The measurement of protein synthesis with an in vitro *system*

In this report, protein synthesis in the fat body of the female Colorado potato beetle is measured with the aid of a short-term *in vitro* system, as was done previously for other insect species (Price, 1966; Hagedorn and Judson, 1972). The question arises as to how we may compare the measured rates of protein synthesis by fat bodies *in vitro* with their presumed activity *in vivo*. In particular we need to know whether or not the fat body, which has been deprived of its normal humoral and neural stimuli and is isolated in chemically-defined culture media, is capable of expressing its predetermined level of physiological activity.

Before discussing this, however, it is necessary to point out that we use in this study short-term, not long-term, incubations. Our system is similar to the *in vitro* assay of corpus allatum (CA) activity as discussed by Tobe and Pratt(1976). The long-term organ culture of CA by Judy *et al.*(1973) and Dahm *et al.*(1975) yielded rather low amounts of synthesized juvenile hormone (JH) when compared with the short-term incubations by Tobe and Pratt(1976). In the latter case it was observed that the rate of biosynthesis of JH by CA remained constant for a period of three to five hours after removal from the animal, irrespective of the spontaneous rate exhibited at the time of extirpation (Pratt *et al.*, 1975). Even in an animal such as *Schistocerca gregaria*, in which nerve sectioning of the CA resulted in a rapid and predictable decline in the ability of the CA to synthesize JH, the short-term incubation period appeared to be suitable for measuring the CA activity (Tobe and Pratt, 1975). Apart from the constant rate of synthesis, the *in vitro* system revealed large changes in the spontaneous activity of the glands during development and reproduction for *Diploptera punctata* (Tobe and Stay, 1977) and *Periplaneta americana* (Weaver *et al.*, 1977). In the case of the Colorado potato beetle, the measured JH titres

in vivo (De Wilde *et al.*, 1968) correlated very well with the observed rates of JH synthesis *in vitro* (Kramer, 1978). All of the above observations indicate the likelihood that *in vitro* procedures faithfully reflect the changes in synthetic capability of the glands *in vivo*.

Using a similar short-term *in vitro* system, we study here the fat body and its capacity for protein synthesis. In the system we use, the rate of synthesis is constant for a certain period, which suggests that the tissue remains active after extirpation if placed in an appropriate medium. The incorporation of lysine into retained protein is linear for at least two hours, while the incorporation into released protein shows a rather long lag-period (4 hours) before reaching its highest rate.

The *in vitro* system of examining fat body reveals large changes in synthesis of released proteins in relation to photoperiodic treatment. These changes in activity correlate very well with the onset of reproduction under long day conditions and diapause under short day conditions (Article 1). In addition, the percentage incorporation of labelled lysine into the five studied proteins is, under both long and short day conditions, the same *in vitro* and *in vivo* (Article 2). Moreover, the *in vitro* system also shows changes in the rates of synthesis of the different proteins in relation to long and short day conditions. From these observations and the results obtained by other workers (Price, 1966; Hagedorn and Judson, 1972; Chen *et al.*, 1975) we are confident that the *in vitro* system is a suitable tool for the study of protein synthesis in the fat body.

The measured changes in protein synthesis during adult development correlate fairly well with the observed, histological changes in capacity for protein synthesis. Although the rates of protein synthesis measured with the *in vitro* system are much lower than *in vivo*, we have the impression that the measured *in vitro* rates are a good reflection of the situation *in vivo*.

Stimulation of vitellogenin synthesis is not successful when JH-I or the JH-analog ZR 512 is added to fat bodies of the Colorado potato beetle incubated *in vitro* (unpublished experiments). There are several possible reasons for this:

1. Juvenile hormone is the appropriate hormone, but the hormonal response is too slow. The *in vitro* system is useful until eight to ten hours after the start of the incubation. When the first increase

in vitellogenin synthesis is achieved later than ten hours after the addition of the hormone, it cannot be observed.

2. Juvenile hormone is the appropriate hormone, but an additional factor (such as the binding protein) is lacking in the *in vitro* assay.
3. Juvenile hormone is the appropriate hormone, but the composition of the medium is not good. Although the fat body synthesizes the investigated proteins *in vitro* in a proper reflection of the situation *in vivo*, it cannot respond to a hormonal stimulus.
4. Juvenile hormone is *not* the appropriate hormone for the stimulation of vitellogenin synthesis *in vitro*.

These four points show that more investigations are needed to see whether or not juvenile hormone is the appropriate hormone for the direct stimulation of vitellogenin synthesis in the fat body of the Colorado potato beetle.

Protein storage in the adult fat body

A second point which deserves additional discussion is the storage of proteins by the fat body of females (and probably also males) reared under short day conditions. During the first half of the prediapause period fat is stored. The histological data suggest the start of protein storage occurs when the storage of fat is nearly complete (Article 3). This seems to fit in with a marked increase in the protein/DNA-ratio observed during the second half of the prediapause period (Article 1).

Storage of proteins can be achieved in two ways: 1) they can be synthesized, retained, and directly stored by the fat body; and 2) they can be taken up from the haemolymph after previously being released from the fat body. The selective accumulation of proteins from haemolymph by fat body was successfully demonstrated for the last larval instar of several insect species (Collins, 1975; Cölln, 1973). The proteins (which later served for storage) were synthesized by the larval fat body and released into the haemolymph where they accumulated for temporary storage. Subsequently the larval fat body changed from a synthesizing into a storage tissue; and after this change in function, the fat body absorbed the proteins it secreted earlier.

Both events, synthesis and storage of proteins, take place at the same

time in the fat body of the Colorado potato beetle. Because of this, there is no need for temporary storage of the synthesized diapause proteins in the haemolymph. However, both the large amounts of DP1 and DP3 present in the Ringer-soluble fraction of a homogenate of fat bodies on day 12 and the decrease of DP1 and DP3 in the haemolymph, suggest that there is a selective uptake of proteins by the fat body.

A question arises about the aim of the observed events. As suggested in Article 3, it is possible that the sequestered proteins are subject to a change in conformation in the haemolymph as reported for *Calliphora stygia* (Martin *et al.*, 1971). Such a change might enable the specific uptake of these proteins. If in the beginning all the synthesized proteins are released directly into the haemolymph, the fat body might be able to choose (by means of the plasma membrane) which proteins it needs for storage.

However, we feel that the picture is more complicated than described above. The observed first appearance of electron-dense protein granules near the nucleus (Article 3) suggests direct storage of newly-synthesized proteins. It is obvious that evidence for both types of protein storage is present. However, it is not possible to calculate in what percentages both types contribute to the observed protein storage.

The regulation of responsiveness in adult fat body

The hormonal control of vitellogenin synthesis in egg-producing vertebrates is well-described. For example, in the claw toad, *Xenopus laevis*, estradiol stimulated vitellogenin synthesis *in vitro* in the liver of females. Although vitellogenin is a sex-specific protein and estradiol does not occur in males, vitellogenin synthesis was induced in the male liver *in vitro* by estradiol (Wangh and Knowland, 1975). Moreover, estradiol induced vitellogenin synthesis in *Xenopus* embryos (Knowland, personal communication). This shows that vitellogenin synthesis is possible in females, males, and embryos after the addition of estradiol, which suggests that the liver is nearly always receptive. In addition, this is the situation which seems to occur normally in the liver of all oviparous vertebrates.

Although studied for several years, the hormonal control of vitellogenin synthesis in insects is much less understood. There is one insect species from which we know the hormone that acted on the fat body to enhance vitellogenin synthesis. This is the female mosquito *Aedes aegypti* in which vitellogenin synthesis was found to be under the control of β-ecdysone (Fallon *et al.*, 1974). β-ecdysone is the well-known moulting hormone in larvae, and is mainly observed in haemolymph during the moult. However, vitellogenins have never been observed in larval haemolymph. Moreover, β-ecdysone also occurred in the male *Aedes* to a level of 100 pg ecdysone/mosquito, while in the *Aedes* pupae (obtained ten minutes after larval-pupal ecdysis) a level of about 200 pg ecdysone/mosquito occurred (Schlaeger *et al.*, 1974). But the male fat body cannot be induced by β-ecdysone to synthesize vitellogenins (Hagedorn, personal communication). Thus it seems that only the female fat body is responsive to β-ecdysone, which is a situation quite different from the male and embryo livers of oviparous vertebrates.

In a later report it was suggested for *Aedes aegypti* that the corpora allata had a positive influence on the responsiveness in the female fat body (Flanagan ana Hagedorn, 1977). This finding (i.e. the increase in responsiveness in the female fat body) is essential, and might explain the difference in responsiveness between males and females.

In oviparous vertebrates the regulation of vitellogenin synthesis occurs by the presence (in females) or the absence (in males and embryos) of the appropriate stimulus. This contrasts with the situation in the *Aedes* mosquito where the appropriate stimulus (β-ecdysone) does occur in the larvae, males and females. Regulation of vitellogenin synthesis is perhaps elaborated by the presence (in females) or absence (in males and larvae) of the appropriate receptor in the fat body.

In contrast to the above, juvenile hormone has a positive influence on the rate of vitellogenin synthesis in females of many other insect species. But males treated with juvenile hormone are incapable of synthesizing vitellogenins(Keeley, 1978). The occurrence of large amounts of vitellogenin in the haemolymph of females and males was, until now, only reported for *Rhodnius prolixus* (Chalaye, 1978). It is not known whether juvenile hormone is the hormone which acts on the *Leptinotarsa* female fat body to enhance vitellogenin synthesis. This makes it rather difficult to decide whether regulation of vitellogenin synthesis in these

females and males might indeed act by the absence or presence of the juvenile hormone receptor.

The fat body of long and short day females of the Colorado potato beetle can be induced, at any time after adult ecdysis, to synthesize vitellogenin *in vivo* with juvenile hormone. But juvenile hormone occurs in the haemolymph of long day males at a somewhat lower level than in long day females. Yet, no vitellogenins are found in male haemolymph (De Loof and De Wilde, 1970). The first measurable rates of vitellogenin synthesis in fat bodies derived from long and short day females are traced after adult ecdysis. Juvenile hormone did occur in the larval haemolymph, but at levels one order of a magnitude less than in the adult long day females (De Kort, personal communication). However, larval haemolymph does not contain vitellogenins. The histological data give evidence that the adult fat body of the Colorado potato beetle originates from the larval fat body. Thus it seems likely that its increase in responsiveness is indeed gained by the remodeling of the larval fat body. But much more evidence is needed to determine whether this remodeling in the future female is developmentally programmed or whether the increase in responsiveness is induced by a hormonal factor.

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Summary

This thesis deals with the hormonal control of protein synthesis in the female fat body of *Leptinotarsa decemlineata* Say. The results are discussed in relation to reproduction (obtained by rearing under long day conditions) and the preparation for diapause (obtained by rearing under short day conditions).

The effect of the photoperiod on protein synthesis and storage in the adult fat body is described in Article 1. Protein synthesis is studied with a newly-developed *in vitro* system which measures the rate of lysine incorporation into protein. The synthesis of the released and retained proteins is measured separately. The rates of lysine incorporation into the retained proteins are not affected by the photoperiod. In addition, the behaviour of the RNA/DNA-ratio is the same in long day and short day females. However, the synthesis of the released proteins differs significantly. Fat bodies derived from females reared under long day conditions synthesize many more released proteins. Females reared under short day conditions start storing proteins in the fat body on day 6 after adult ecdysis, but protein storage in haemolymph is also observed.

Article 2 deals with the synthesis of five specific released proteins (two vitellogenins and three diapause proteins) which are measured simultaneously. The five proteins are separated by disc-electrophoresis. The relative incorporation of the five investigated proteins produced by the fat body *in vitro* is the same as *in vivo*. This demonstrates the important role of the fat body in haemolymph protein synthesis in the entire insect. The large increase in synthesis of released proteins in females reared under long day conditions is mainly attributed to vitellogenin synthesis; vitellogenin synthesis is low in prediapause females. This difference, however, is not expressed in the amount of vitellogenin present in haemolymph. A single increase in the synthesis of one diapause protein is observed in ovipositing females. Massive synthesis of three diapause proteins occurs during the second half of the prediapause period, which results in high levels of these proteins in the haemolymph. The fat body stores

Daarentegen wordt door het vetlichaam van een korte dag vrouwtje juist weinig vitellogeen eiwit geproduceerd. Dit is in goede overeenstemming met de juist alleen onder lange dag omstandigheden waargenomen eileg. De grote verschillen in vitellogene eiwitsynthese worden evenwel niet weerspiegeld in de hoeveelheden vitellogeen eiwit aanwezig in de haemolymph.

Van de drie onderzochte diapauze-eiwitten wordt er slechts één in redelijke hoeveelheid door het vetlichaam van eierleggende vrouwtjes aangemaakt. Massale synthese van alle drie de diapauze-eiwitten wordt daarentegen in de tweede helft van de prediapauze periode waargenomen. Deze diapauze-eiwitten zijn op dat tijdstip ook in ruime mate in de haemolymph aanwezig, terwijl er slechts van twee diapauze-eiwitten accumulatie in het vetlichaam plaatsvindt.

In het derde artikel wordt de histologie van het vetlichaam behandeld. Tijdens de adulte vervelling worden in de cellen van het vetlichaam veel eiwitgranula waargenomen. Tot 2 dagen na deze vervelling blijken de veranderingen in het vetlichaam van korte en lange dag vrouwtjes niet wezenlijk te verschillen. Daarna treden er afhankelijk van de daglengte grote verschillen op. Het vetlichaam van een vrouwtje dat juist met de reproduktie start, is helemaal ingericht voor massale eiwitsynthese. Toch is er op dit tijdstip van de ontwikkeling ook opslag van vet te onderscheiden in de vorm van kleine druppels.

Wanneer de vrouwtjes onder korte dag omstandigheden worden gekweekt is er op dag 6 veel vetopslag waar te nemen; kennelijk als voorbereiding op de diapauze. Op dit tijdstip wordt ook de eerste eiwitopslag zichtbaar. Deze verschijnt dan in de vorm van zeer elektronen-dichte eiwitgranula rond de kern. Autophage eiwitgranula zijn daarentegen op dag 10 pas te onderscheiden. Er is nu, vergeleken met de situatie in het vetlichaam van eierleggende vrouwtjes, veel minder rough endoplasmatisch reticulum waar te nemen.

In het vierde artikel wordt ingegaan op de hormonale regulering van de vitellogene eiwitsynthese. Juveniel hormoon, dat normaal in grote hoeveelheden in de haemolymph van eierleggende vrouwtjes voorkomt, blijkt een positieve invloed op de vitellogene eiwitsynthese van het vetlichaam te hebben. Toch is het ook mogelijk onder bepaalde omstandigheden een aanzienlijke stijging van de vitellogene eiwitsynthese te induceren in de afwezigheid van juveniel hormoon. De synthese van dit eiwit wordt dus door meer faktoren gereguleerd dan alleen door juveniel hormoon. Daarom werd ook ß-ecdyson getest; maar dit hor-

moon lijkt niet in staat te zijn de vitellogene eiwitsynthese te beïnvloeden.

Verder is de relatie tussen juveniel hormoon en de vitellogene eiwitsynthese bij maagdelijke en bevruchte vrouwtjes onderzocht. De juveniel hormoon-spiegel en de snelheid van de vitellogene eiwitsynthese zijn in beide typen vrouwtjes even hoog. Dit is verrassend omdat gedurende de verdere ontwikkeling de maagdelijke vrouwtjes veel minder eieren leggen. De vrouwelijke Coloradokever blijkt in staat te zijn de eileg te ontkoppelen van de vitellogene eiwitsynthese. Het teveel geproduceerde eiwit wordt kennelijk weer afgebroken.

De belangrijkste conclusie uit dit proefschrift betreffende de hormonale regulering van de eiwitsynthese in het vetlichaam van de Coloradokever is dat er meer factoren dan alleen juveniel hormoon zijn, welke *in vivo* de vitellogene eiwitsynthese positief kunnen beïnvloeden. β-ecdyson blijkt bij deze regulering geen rol te spelen. Toekomstig onderzoek zal moeten uitwijzen of er één of juist meer hormonen in de Coloradokever voorkomen die, toegevoegd aan vetlichaam *in vitro*, de vitellogene eiwitsynthese stimuleren.

Curriculum vitae

Johannis Freerk Dortland werd geboren op 20 januari 1949 te Gouda. Na het behalen van het eindexamen HBS-b in 1967 aan de Rijks Hogere Burgerschool te Gouda begon hij zijn studie aan de Landbouwhogeschool te Wageningen. Het kandidaatsexamen Planteziektenkunde werd in januari 1972 afgelegd, waarna in januari 1975 het doctoraal examen Planteziektenkunde (met de vakken entomologie, biochemie, plantenfysiologie en informatica) werd behaald. In de periode april 1975 tot april 1978 was hij in dienst van ZWO werkzaam op het Laboratorium voor Entomologie, Binnenhaven 7, Wageningen.