FAT BODY PROTEIN GRANULES AND STORAGE PROTEINS IN THE SILKMOTH, *HYALOPHORA CECROPIA*

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**ABSTRACT**

Fat body cells of silkmoth pupae (*Hyalophora cecropia*) contain granules, showing a less dense outer zone and a denser, often crystalline, inner portion appear after cocoon spinning and increase until the larval-pupal ecdysis; more granules are formed in females than in males. Urate granules, appearing fibrous in internal structure, first form about the same time, but their accumulation is more gradual, and continues in the pupa. Both types have been isolated by centrifugation. Protein granules dissolve in buffers to yield proteins 1 and 2, with distinct electrophoretic and antigenic properties. These proteins have been isolated individually from pupal fat body extracts by using their different thermal stabilities in phosphate buffer containing MgCl₂ and (NH₄)₂SO₄, respectively, and purification was completed by gel chromatography. Protein 1 has a molecular weight of 480,000 and a subunit of 85,000 daltons, while protein 2 gives values of 530,000 and 89,000, respectively. Their amino acid compositions are similar but distinct. Proteins 1 and 2 accumulate in the hemolymph, beginning 3 days before spinning, reach maximal levels at spinning, and then decline in the hemolymph while granules are formed in the fat body, although the total hemolymph protein concentration does not decline at this time. It is concluded that the fat body of the late, feeding larva synthesizes two related "storage proteins" and secretes them in partially crystalline granules as protein reserves for metamorphosis.

**KEY WORDS** silkmoth · fat body · hemolymph · protein · granules · storage · metamorphosis · urate

The fat body of insects is a center for intermediary metabolism and the synthesis of hemolymph components which has been compared to the liver of vertebrates (24, 40, 52). It also serves for storage of nutrient reserves, a function which is most pronounced during the metamorphosis of holometabolous insects, when the fat body cells hypertrophy and become loaded with lipid, glycogen,
and protein. Since the end of the last century (3), dense proteinaceous granules have repeatedly been described as accumulating in the fat body cells of various insects at the beginning of metamorphosis (40, 46). Since they disappear during the pupal-adult transformation, a role as a reserve for the growth of adult tissues has been generally accepted. Their origin, however, has been the subject of diverse speculation (50) but, until recently, little evidence.

In the skipper, Calpodes ethlius, Locke and Collins (28, 29) distinguished with the electron microscope several types of protein-containing granules, and presented evidence for their formation by a combination of processes including isolation of intracellular organelles within membranes derived from the Golgi complex, and pinocytotic uptake of protein from the hemolymph. Protein uptake was demonstrated with injected plant peroxidase as a tracer, and its natural occurrence was suggested by a marked decline in hemolymph protein level at the same time as the build-up of granules in the fat body. Thomasson and Mitchell (45) isolated granules from mature Drosophila larvae and showed that they contained radioactivity from previously injected 3H-labeled hemolymph protein, but not from injected 14C-leucine. In the blowfly, Calliphora, a protein called calliphorin, which makes up some 60% of the total soluble protein in the mature larva, is believed to have a storage role (36). Granules appear in the fat body at the same time as uptake of calliphorin from the hemolymph (32), and the formation of the granules from this protein has been inferred (46). The composition of isolated insect protein granules and its relationship to specific proteins of the hemolymph and fat body have not, however, heretofore been described.

We have studied the fat body of the silkmoth, Hyalophora cecropia, by light and electron microscopy, developed a procedure for isolating from it two classes of granules, characterized the major components of the protein granules, and followed changes in the distribution of these proteins, as well as some associated biochemical changes, during the larval-pupal transformation. Some of the results have been reported in preliminary form (52).

MATERIALS AND METHODS

Animals

Cecropia silkmoths, H. cecropia, were reared outdoors on wild cherry trees, and in the laboratory on an artificial diet (41). Mature larvae (1 day before spinning) were recognized by the empty midgut. The time from onset of spinning to the larval-pupal ecysis was 8 days, at 25°C. Male and female larvae were distinguished by abdominal markings (22).

Tissue Preparation for Light and Electron Microscopy

For light microscopy, portions of fat body were fixed in 95% ethanol, dehydrated, and embedded in paraffin. Sections 5 μm thick were generally stained first by the argentaffin (methenamine silver nitrate) method for uric acid (18), and then with mercuric bromophenol blue for protein (33). Nucleic acids were detected by either methyl green-pyronin, Feulgen (21), or azure B reagent (15). The periodic acid-Schiff reaction (PAS) was used to demonstrate glycogen (21).

For electron microscopy, tissues were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 4-5 h and post-fixed in 1% OsO4 in 0.1 M cacodylate buffer for 1 h, all at 0-4°C. Dehydration and embedding in Epon 812 followed according to Luft (31). Some specimens were block-stained in 0.5% uranyl acetate in 0.05 M veronal acetate buffer at pH 5.0 for 1 h at 0-4°C before dehydration. Thin sections of 60-70 nm were cut with a diamond knife and stained with uranyl acetate and lead citrate. Observations were made with either a Philips 300 or an RCA EMU-4 electron microscope.

Chemical Analyses of Tissue Components

Fat body was rinsed with Ringer's solution (KCl 154 mM, NaCl 5.6 mM, CaCl2 2.3 mM, brought to pH 7.0 with about 0.1 mM NaHCO3), and drained. 1 g portions were homogenized with 5 ml of water in a motor-driven, all-glass Potter-Elvehjem homogenizer, and duplicate 0.5-ml samples of the homogenate were dried to constant weight at 90°C, to give tissue dry weight.

Portions of the homogenate were fractionated for determination of uric acid, nucleic acids and protein (27). An equal volume of cold 1 N HClO4 was added, and the precipitate collected by centrifugation was washed twice with 0.5 N HClO4, the extracts being combined for the determination of soluble uric acid. The residue was washed twice with ethanol-ether (3:1) and twice with ether. To extract undissolved uric acid, the residue was suspended in 0.01 M Tris-HCl buffer, pH 7.0, and dialysed at 50°C against the same buffer, which was changed hourly until absorpt. of the dialysate dropped below 0.01. The dialysates were combined for uric acid determination. Then the residue was extracted three times with 0.5 N HClO4 at 70°C for 20 min, and the combined extracts were used for determination of DNA and RNA. From the final residue thus obtained, protein was extracted three times with 0.25 N NaOH.

Uric acid was determined by absorbance at 290 nm before and after treatment with uricase (1). The acid
extracts were first neutralized with KOH, and KClO₄
was removed by centrifugation. Combined values from
the acid extracts and dialysates gave the total uric acid.

DNA was determined by the diphenylamine method
(7), with calf thymus DNA as a standard. RNA was
estimated from A₂₆₀nm of the hot-acid extract, corrected
for DNA (8, 27).

Protein in the alkali extract was determined by both
the method of Lowry et al. (30) and the biuret reaction
(13), with bovine serum albumin as a standard. In
hemolymph samples, protein was measured by the biuret
reaction directly after twofold dilution and centrifuga-

**Isolation of Urate and Protein Granules**

Fat body (2 g) from female pupae was homogenized
with 20 ml of 0.3 M sodium-potassium phosphate buffer-
0.01 M MgCl₂, pH 6.8, by 15 strokes in a motor-driven
Teflon homogenizer (Dupont Instruments-Sorvall, Wil-
lington, Del.). The granules were centrifuged down at
600 g for 10 min, resuspended in buffer, and filtered
through four layers of cheese cloth, which retained most
of the nuclei. The filtrate was layered on 10 ml of 1.8 M
sucrose in the same buffer and centrifuged at 600 g for
10 min, which caused urate granules to sediment to the
bottom, while protein granules remained at the inter-
face. The pellet was twice suspended in buffer and
centrifuged down, yielding the urate granule fraction.

The interface fraction was diluted with phosphate buffer,
layered on a discontinuous gradient containing 7 ml each
of 1.2 M, 1.5 M, and 1.8 M sucrose, and centrifuged at
22,500 g for 20 min (SW-25 rotor, Spinco L centrifuge
Beckman Instruments, Inc., Spinco Div., Palo Alto,
Calif.). The protein granules were retained partly at the
interface between 1.2 and 1.5 M sucrose (fraction I),
but mainly between 1.5 and 1.8 M sucrose (fraction II).

Both were collected, sedimented, and washed. The isolated
fractions were analyzed for protein, RNA,
DNA, and uric acid by the methods described above.

**Protein Extraction from Fat Body
and Hemolymph**

Rinsed, blotted fat body was homogenized with 10
vol of 0.05 M phosphate buffer, pH 6.8 (without
MgCl₂), and centrifuged at 12,000 g for 20 min. The residue
was reextracted in the same manner at least
twice, to allow protein granules to dissolve, and the extracts were combined. Hemolymph was collected into
a tube containing a known amount of buffer and a few
crystals of phenylthiourea, then diluted to fivefold by
adding buffer, and centrifuged at 12,000 g for 20 min.

**Immunodiffusion Analysis**

To prepare antisera, 0.5-ml portions of the soluble
fraction from pupal fat body homogenized with 2 vol of
phosphate buffer were emulsified with equal volumes of
Freund's complete adjuvant and injected subcutaneously
into rabbits. Two additional similar injections were given
at weekly intervals, and the rabbits were bled 2 wk after
the final injection. To render the antiserum specific for
storage proteins, it was absorbed with hemolymph from
early fifth instar larvae, which lack these proteins. Anti-
sera were also prepared to the individual proteins by
cutting the zones of proteins 1 and 2 from 12 acrylamide
disc electrophoresis gels of fat body extracts, macerating
them with 0.05 M phosphate buffer, and injecting them
into rabbits without adjuvant.

Relative concentrations of the antigens were deter-
mined by the quantitative immunodiffusion technique of
Oudin (44), with pooled hemolymph from male dia-
pause pupae as a standard. When most of these analyses
were carried out, the pure proteins as standards for
absolute concentration were not available.

**RESULTS**

**Light Microscopy**

In sections or homogenates of fat body, two
types of granule were observed: protein granules, which
appeared orange by double staining with
methenamine silver nitrate and mercuric bromophenol blue observed by phase microscopy, and
urate granules, which stained bright yellow under
these conditions (Fig. 4). With methenamine
silver nitrate alone, the former were unstained,
while the latter stained black, which is specific
for uric acid. They could also be distinguished
without staining by observation between crossed
polaroid filters, which revealed strong optical
activity in the urate granules but very little in the
protein granules.

Sections of fat body from larvae up to the day
of spinning (Fig. 1) showed roughly spherical
nuclei and cytoplasm with many lipid droplets,
but no dense granules. Beginning 2-3 days later,
the nuclei became compressed and misshapen
and the cytoplasm increasingly filled with storage
granules (Fig. 2). The number and size of protein
granules reached a maximum about the time of
the larval-pupal ecdysis (Fig. 3). In diapause
pupae (Fig. 4), the granules ranged from 1-8
μm, most commonly 1.5-3 μm, in diameter.

Urate granules could be observed in smeared
fat body homogenate almost as early as the
protein granules, although, being small at this
stage, they were difficult to detect in sections.
Thereafter, they gradually increased in size and
number, becoming distinguishable among the
protein granules in sections (Fig. 4).

By counting the granules in sections of pupal

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fat body, we found that the number of protein granules per unit area was almost twice as great in females as in males, whereas the urate granules were much more numerous in males. Some cells in male diapause pupae were packed almost exclusively with urate granules. The number of lipid droplets was about 50% greater in males.

**Electron Microscopy**

These developmental changes were further clarified at the fine-structural level. Sections of fat body from fully-grown feeding larvae (Fig. 5) showed cytoplasm with abundant mitochondria and ribosomes (apparently aligned on membranes), and scattered glycogen deposits, between the numerous large lipid droplets. By the day of spinning, ribosome-containing cytoplasm was largely restricted to zones around the lipid droplets, and glycogen had become generally distributed. Four days later (Fig. 6), cytoplasm was still more reduced, and dense protein granules were present, some of them still in the process of formation. Some of the marginal vesicles in the granules appeared to contain ribosomes. In pupae in diapause (Fig. 7), the fat body was packed with protein granules that showed considerable variation in form, along with urate granules, lipid droplets and glycogen, the total picture representing conversion of the tissue to a predominantly storage role.

The protein granules in pupal tissue were typically more or less oval in shape, with an electron-dense inner portion, often showing flat faces, and a less dense outer zone of variable thickness surrounded by a thin membrane (Figs. 7 and 8). In some granules, the dense inner part was clearly crystalline, with a regular lattice spaced at about 18 nm, and apparent addition of molecular layers at the surface (Fig. 8). The urate granules (Fig. 9) were also membrane-bounded, and showed a curiously fibrous internal structure.

**Changes in the Composition of Fat Body and Hemolymph**

Since the granules appeared sufficiently abundant to influence tissue gross composition, the total protein, RNA, DNA, and uric acid were determined in fat body from a series of developing stages. The results (Figs. 10–12), based on dry weight, must be influenced by changes in other major tissue components which were not measured. Lipid and glycogen accumulate rapidly in fat body just before the beginning of metamorphosis and then decrease somewhat between spinning and the larval-pupal ecdysis, and there is further gradual loss of fat-body glycogen (due to conversion to glycerol) in the cecropia pupa (2, 17). These changes may account for the apparent fall in nucleic acids and protein just after spinning.
A protein granule in a section of fat body from a 40-day old pupa. The granule shows a surrounding membrane, an amorphous outer zone containing particles which may be storage protein (small arrows), and a dense core showing periodic structure and layers of crystallization at its surface (white triangles). The white-trimmed arrows point to the plasma membrane of the fat body cell, and glycogen granules are visible in the cytoplasm. × 60,000. Bar, 0.5 μm. (a) A growing protein granule, from an 8-day-old pupa. The arrows indicate the outer membrane of the granule extending beyond the region of dense protein deposition. The portion enlarged in b is outlined. × 13,000. Bar, 1 μm. (b) A portion of the growing granule from a enlarged to show crystalline structure. The repeat period in both directions 1 and 2 (arrows) is 18 nm. × 88,000. Bar, 0.1 μm.
due to uptake of degraded DNA from the degenerating silkgland, as already demonstrated in *Bombyx mori* (8).

The protein content of the fat body rose (much more in females than in males), during the week after spinning, indicating accumulation of protein, while RNA continued to decrease (Figs. 10 and 11). Uric acid increased greatly after spinning and continued to rise in the pupa, especially the male (Fig. 12). The results with respect to RNA, protein, and uric acid are therefore in accord with the microscope observations, and depict the transition of the fat body from a biosynthetic to a principally storage role.

The hemolymph (Fig. 13) showed a sharp rise in protein concentration, beginning in the mature, feeding larva, and continuing until about 4

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**FIGURE 9** A urate granule (UrG) in a section of fat body from a 180-day old pupa. The interior of the granule appears fibrous. x 34,000. Bar, 0.5 μm.

**FIGURE 10** Content of nucleic acids in fat body, as a function of developmental stage. ML, mature larva. A, total RNA; B, DNA. In Fig. 10–13, points represent determinations on individual animals.

**FIGURE 11** Protein content of fat body, as a function of developmental stage, determined by the biuret method.

**FIGURE 12** Uric acid content of fat body, as a function of developmental stage.
days after spinning, after which the level remained essentially constant. The protein concentrations averaged slightly higher in female than in male hemolymph.

**Isolation of Urate and Protein Granules**

After homogenization of fat body in most buffers, the protein granules tended to dissolve, leaving only membranes together with urate granules, which were more stable. Magnesium ions helped stabilization, and the best recoveries were obtained in sodium-potassium phosphate buffer, 0.3 M, pH 6.8, containing 10 mM MgCl₂, which was therefore used routinely. Nevertheless, repeated washing caused loss, and granules had to be prepared as rapidly as possible.

In addition to the urate granules, the isolation procedure (see Materials and Methods) yielded two fractions of protein granules. Protein granule fraction II was the cleaner, and under the microscope appeared virtually pure, as did the urate granule fraction (Figs. 14 and 15). The isolated protein granules stained orange with mercuric bromphenol blue, and the urate granules stained black with methenamine silver nitrate. Staining tests with PAS for glycogen, and Fuelpen, methyl green-pyronin and azure B for nucleic acids were negative. Under the electron microscope, protein granule fraction II showed intact granules with membranes, together with some ruptured granules (Fig. 16). The urate granule pellet was too hard for thin sectioning.

Chemical analyses showed, in the protein granule fractions, that protein made up about 98% of the substances assayed (Table I). The content of nucleic acids was very low. The urate granule fraction contained 75% uric acid and 24% protein. The homogeneity of the urate granule preparation under the microscope suggests that this protein is endogenous, and not due to contaminating protein granules.

**Solubilization and Identification of Protein Components**

Protein could be dissolved from the isolated

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**Figure 13** Protein content of hemolymph, determined by the biuret method, as a function of developmental stage.

**Figure 14** Isolated protein granules: fraction III prepared from the fat body of a female pupa. Stained with mercuric bromphenol blue; phase contrast. × 1,000. Bar, 10 μm.

**Figure 15** Urate granules isolated from the fat body of a female pupa. Treated with mercuric bromphenol blue; the granules do not stain. Phase contrast. × 1,000. Bar, 10 μm.
FIGURE 16 An electron micrograph of isolated protein granules, fraction II, prepared from the fat body of a 30-day-old female pupa, embedded and sectioned. Some granules seem to have swollen and some ruptured their outer membranes. × 9,400. Bar, 1 μm.

### TABLE I

<table>
<thead>
<tr>
<th>Chemical Composition of Urate Granule and Protein Granule Fractions Isolated from Fat Body of Female Pupae</th>
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<tbody>
<tr>
<td>Percent of total substances determined</td>
</tr>
<tr>
<td>Fraction</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Urate granule</td>
</tr>
<tr>
<td>Protein granule I</td>
</tr>
<tr>
<td>Protein granule II</td>
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</tbody>
</table>

granules by suspending them in 0.05 M phosphate buffer, pH 6.8. The urate granules dissolved only slowly, and protein was recovered from them by dialysis overnight at 4°C against phosphate buffer. The electrophoretic patterns of proteins from both types of granules are shown in Fig. 17, along with those from hemolymph and fat body extracts from different developmental stages. The protein granules yielded two major components, which migrate slowly and very close together, and are designated proteins 1 and 2. The urate granules yielded chiefly one broad, much faster-moving band.

In the hemolymph (Fig. 17a) of early fifth instar larvae, proteins 1 and 2 were not detectable; a band in this region had significantly different mobility. Proteins 1 and 2 first appeared in the hemolymph of late fifth instar larva, and increased in concentration until the time of spinning. Protein 2 decreased markedly by five days after spinning, and both bands declined to minor status by the pupal stage. Electrophoretic bands 3 and 4 were additional major hemolymph proteins: band 3, which increased greatly in concentration from the late fifth instar, probably included the vitellogenin (38, 42). Other plasma proteins (44, 53) were poorly resolved or detected by the techniques used.

In fact body extracts (Fig. 17b), only traces of proteins 1 and 2 were detected before spinning, but by five days after spinning they became very prominent, and they remained so in the pupal stage. Proteins 3 and 4 appeared in the fat body in only relatively small amounts.

### Purification and Characterization of Proteins 1 and 2

For purification of proteins 1 and 2, extracts of whole female fat body were used, as to avoid the losses involved in isolation of granules. Pupal fat body was rinsed, drained, and homogenized in four times its weight of 0.1 M sodium phosphate buffer, pH 7.5, at 0°C. The homogenate was centrifuged at 10,000 g for 20 min, the residue was twice re-extracted, and the combined
Acrylamide-gel electrophoretic patterns of proteins from female cecropia of different stages, a, Hemolymph; b, extract from fat body. 

FIGURE 17

The patterns show several electrophoretic bands of protein 1 or 2, respectively (Fig. 19). To each solution, (NH$_4$)$_2$SO$_4$ was then added to make 60% saturation and the pH was adjusted to 7.5. Precipitated protein was collected and redissolved in 10 ml buffer (0.05 M Na phosphate, pH 7.5, for protein 1, and the same plus 0.05 M NaCl for protein 2); undissolved material was centrifuged off. The solutions were chromatographed on columns of Ultrogel ACA 22 previously equilibrated with the respective buffers (Fig. 20). Analyses showed that peaks A, B, and C contained predominantly glycogen, protein 1 or 2, and urate, respectively.

Recoveries from these procedures are shown in Table II. No increase in immunochemical purity is indicated by steps after the heat treatments, and the ammonium sulfate precipitation and Ultrogel chromatography served chiefly to concentrate the proteins and remove nonprotein contaminants. The estimates in the crude extracts indicate that proteins 1 and 2 together represented some 80% of the total protein in the soluble extract, or almost 60% of the total tissue.
Immunochemical Analysis of Proteins 1 and 2 in Hemolymph and Fat Body

Antiserum from rabbits immunized with fat body extract exhibited two zones of precipitation in the Oudin test when antiserum in agar was overlaid with pupal hemolymph, pupal fat body extract, or purified proteins 1 plus 2. This indicates that these two proteins are immunologically distinct. By a mutual dilution test (44), the two antigens in pupal fat body extract were shown to

Figure 19 Polyacrylamide-gel electrophoresis of fat body extract and purified storage proteins. A, crude extract of female pupal fat body; B, protein 1 obtained by treatment of extract for 6 min at 63°C in 0.05 M Na phosphate buffer, pH 7.5, 0.05 M MgCl₂; C, protein 2 obtained by treatment of extract for 6 min at 72°C in 0.05 M Na phosphate buffer pH 7.5, 0.2 M (NH₄)₂SO₄; D, mixture of proteins 1 and 2. Acrylamide concentration, 5%.

Figure 20 Column chromatography on Ultrogel of heat treated extract from fat body. Extract was prepared and heat-treated for protein 2 as described in the text and Table II, and the solution containing 8.1 mg protein was applied to a 2 x 20 cm column of Ultrogel ACA 22. Eluting buffer: 0.05 M NaCl-0.05 M Na phosphate, pH 7.5; flow rate, 12 ml/h; temperature, 10°C; fraction size, 4 ml. Total protein was determined by the method of Lowry et al. (30), protein 2 by quantitative immunodiffusion, uric acid by the method of Brown (6) and glycogen by the phenolsulfuric acid reaction (14). Protein recovered in peak B was 7.0 mg. The elution pattern from extract heat-treated for protein 1 was very similar.

Table II
Purification of Storage Proteins from Cecropia Fat Body

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure for protein 1</th>
<th>Procedure for protein 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein mg</td>
<td>Protein 1* mg</td>
</tr>
<tr>
<td>Crude</td>
<td>49.9</td>
<td>25.5</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>8.8</td>
<td>10.6</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitation</td>
<td>4.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Ultrogel chromatography</td>
<td>4.3</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Fat body (3.0 g fresh wt) from female diapausing pupae was used. The crude extract was divided into two equal parts, which were processed separately for protein 1 and protein 2, as described in the text.

* Estimated by the immunodiffusion method of Oudin (see Materials and Methods), with male pupal hemolymph as a reference standard. Results were later converted to milligrams of each protein by assuming that the product from the final step was pure.
be common with those in the hemolymph. The slower and faster migrating precipitation zones corresponded to proteins 1 and 2, respectively.

Developmental changes in the concentrations of proteins 1 and 2 in hemolymph and fat body extracts were determined by quantitative immunodiffusion (Fig. 23). In the female, both proteins first appeared in the hemolymph 3 days before spinning, reached maximum concentrations about the time of spinning, and then declined. The fat body showed steady increase in content of both proteins from spinning to the early pupa, at the same time that they were decreasing in the hemolymph, until their concentrations were much greater in the fat body than in the hemolymph. These results support quantitatively the observations from gel electrophoresis. In the male, the hemolymph concentrations rose much less and then remained rather constant while there was a limited increase in the fat body.

**DISCUSSION**

In the fat body of pupating cecropia silkworms, we have recognized two types of dense cytoplasmic granules, and designated them, on the basis of their staining characteristics, their fine structure, and the analysis of isolated preparations, as urate granules and protein granules. Both are surrounded by membranes. The urate granules have a distinctive uneven, fibrous-appearing internal structure. The protein granules show two internal regions, an outer less dense zone and an inner electron-dense portion that is frequently crystalline.

Bodies showing fibrous structure in the electron microscope that may have been urate granules, although described as “myelin-like”, have been observed in the fat body of other silkmoths (34, 48, 49). In other insects, urate granules do not seem to have been recorded, possibly because attention was usually focussed on the early stages of metamorphosis, when little urate would have accumulated. The urate in cecropia fat body exists largely as the potassium salt (26), and our demonstration of one major protein in the urate granules suggests the presence of a protein-potassium urate complex (20).

Partially crystalline protein bodies have been previously described in cecropia fat body (4). Dense cytoplasmic granules, variously named and showing some diversity of structure but including types probably essentially similar to the protein granules of cecropia, have been observed with the electron microscope in other silkmoths.
Molecular weight determination of subunits of proteins 1 and 2. Proteins were denatured by heating to 30°C from 120 min in 1% SDS-5% mercaptoethanol, and subjected to electrophoresis in 5% polyacrylamide gel containing 0.1% SDS. TI, soybean trypsin inhibitor (23,000); α, β, β', E. coli RNA polymerase subunits (39,000, 165,000, 155,000); BSA, bovine serum albumin (67,000); C1, C2, cecropia storage proteins 1 and 2, respectively.

TABLE III
Amino Acid Composition of Cecropia Storage Proteins, Compared with Calliphorin

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Cecropia protein 1</th>
<th>Cecropia protein 2</th>
<th>Calliphorin (reference 36)</th>
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</thead>
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<tr>
<td>Cysteine</td>
<td>trace</td>
<td>trace</td>
<td>0.5</td>
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<tr>
<td>Aspartate</td>
<td>13.3</td>
<td>13.4</td>
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<tr>
<td>Threonine</td>
<td>5.9</td>
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<td>Serine</td>
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<td>Glutamate</td>
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<tr>
<td>Proline</td>
<td>2.3</td>
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<td>Glycine</td>
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<td>Valine</td>
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<td>Methionine</td>
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<tr>
<td>Isoleucine</td>
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<td>Leucine</td>
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<tr>
<td>Tyrosine</td>
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<td>Phenylalanine</td>
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<td>Lysine</td>
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<td>8.1</td>
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<tr>
<td>Histidine</td>
<td>1.4</td>
<td>0.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.6</td>
<td>5.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>–</td>
<td>–</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Proteins were hydrolyzed in 6 N HCl at 110°C for 24 h, and amino acids were determined with a JEOL JLC 6 AH analyzer (JEOL Analytical Instruments, Cranford, N. J.)

* Excluding cysteine and tryptophan, which were not determined for cecropia storage proteins.
Changes in the content of proteins 1 and 2 in hemolymph and fat body of female (A) and male (B) cecropia during the larval-pupal transformation. Hemolymph and fat body extracts were prepared, and concentrations of antigens were determined by quantitative immunodiffusion, as described in Materials and Methods. Concentrations are expressed relative to a reference sample of pooled male pupal hemolymph. FB, fat body; BL, hemolymph (blood), D, diapause. Points represent determinations on individual animals.

they lack the exceptionally high tyrosine and phenylalanine of the latter but contain more of other hydrophobic amino acids. A common evolutionary origin and function for these proteins is likely.

The concentrations of individual proteins in insect hemolymph, and their rates of synthesis in the fat body, change characteristically with stage of development (40, 44, 46, 53). The changes in hemolymph concentrations of proteins 1 and 2 of cecropia correspond to those previously described for antigen 6 by Telfer and Williams (44). Proteins 1 and 2 first appear in the larval hemolymph three days before spinning and reach maximal concentrations about the time of spinning. Synthesis and secretion of these proteins by cecropia fat body incubated in vitro with \(^{3}H\)-leucine has been shown by Pan (37), who precipitated them from the medium with specific antiserum, and we have confirmed this (unpublished data). Production is active during several days before spinning, and falls off sharply by the day of spinning, to approach zero by the larval-pupal ecdysis. During the 8–10 days after spinning, the levels of the two proteins decline in the hemolymph and increase in the fat body at the same time as the appearance of visible intracellular protein granules. The observations indicate that these proteins are synthesized in massive amounts in the fat body during the final feeding period of the larva and secreted into the hemolymph for temporary storage, and then, during the first phase of metamorphosis, taken back into the fat body and deposited in semi-crystalline granules. Remarkable developmental regulation of fat body cell function is implied in the switch from an actively biosynthetic to a principally storage role. The capacity of the fat body to withdraw protein selectively from the hemolymph resembles the later activity of the ovary in taking up vitellogenins for deposition in yolk (43). Evidence indicating selective uptake of proteins from hemolymph into fat body has already been obtained for several other species on insects (9, 10, 11, 32).

The pattern just described is observed in the cecropia female. Males accumulate much less protein in the fat body, as previously reported for Calpodes (29), and the deposition of granules is not accompanied by a decrease in the levels of the specific proteins in the hemolymph. Possibly, the male fat body, not having to make vitellogenin, which the female fat body synthesizes from about the time of spinning (38), may continue to produce storage proteins for a longer time.

Although the fate of the granules and their constituent proteins has not been studied, it is probable that they provide reserves needed for synthesis in the developing adult tissues. Our observations, together with those of others on blowflies and other insects, indicate that the synthesis and deposition in the fat body of a class of storage proteins is a general major process in insect metamorphosis.

The authors are grateful to Dr. W. H. Teller for initially proposing this investigation, to Dr. M. L. Pan for suggestions and assistance, to Dr. Y. Nagata for performing amino acid analysis, and to Dr. P. Couble for help with photography.
The work was supported by grants to W. H. Teller from the National Science Foundation (GB-40154), and to G. R. Wyatt from the National Institutes of Health (HD-02176) and the Whitehall Foundation.

Received for publication 26 July 1977, and in revised form 15 May 1978.

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