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Regional Differentiation of Fat Bodies in Larvae of the Indianmeal Moth, *Plodia interpunctella*

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Larvae of the Indianmeal moth, *Plodia interpunctella*, contain two morphologically distinct fat bodies. Tan-colored, highly tracheated fat body located posteriorly in the abdomen was the predominant fat body tissue during the early larval instars. White, sheet fat body located more anteriorly became the predominant type during the fifth (last) larval instar and eventually occupied most of the space of the hemocoel. Ultrastructural morphology of tan fat body showed the tissue to be composed of cells containing numerous, large, spherical mitochondria, with only few lipid, glycogen, or protein storage structures. In contrast, white fat body was composed of cells that in later larval stages had organelles typical of storage functions. Both fat bodies produced storage proteins during the late fifth instar, whereas only white fat body accumulated the storage proteins. Tan fat body dispersed and apparently autolyzed in pharate pupae, whereas the white fat body metamorphosed and persisted into the adult stage. These observations indicate that fat body of the Indianmeal moth is functionally and morphologically differentiated along the anterior-posterior axis into two regional subgroups of cells.

Key words: storage proteins, lepidopteran, mitochondria, metamorphosis, ultrastructure

INTRODUCTION

The fat body of insects is structurally diverse and performs a broad range of metabolic functions [1,2]. Fat body operates most importantly as the primary organ for intermediary metabolism serving as the principal source for hemolymph proteins, lipids, and carbohydrates. In addition to its role in intermediary metabolism, fat body also provides a major site for storage of lipids, proteins, and carbohydrates. These functional activities have been associated with one major cell type, called trophocyte [1] or adipocyte [2], that has a uniform mor-

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phology or only limited regional morphological differentiation of the metabolic and storage functions. Several other cell types, such as oenocytes, mycetocytes, urocytes, and chromatocytes, are also recognized as components of fat body tissue [1]. The various cell types can be heterogeneously distributed within the tissue or regionally segregated depending on the species. However, within the same insect order, the structure and distribution of fat body cells remains relatively constant [3].

Within the larvae of lepidopterans, the fat body is generally described as white sheets of trophocytes that have a uniform morphology, with only a size difference appearing between perivisceral and peripheral cells [1]. Although the morphology of the fat body cells remains essentially the same during most of larval development, fat body undergoes considerable morphological change during metamorphosis. The morphology of the fat body cells begins to change during the last larval instar in preparation for metamorphosis, when the fat body sequesters hemolymph proteins in granules, stores lipid droplets, and accumulates glycogen granules [1,4]. During pupation and adult development, the fat body undergoes metamorphosis, which leads to the dissociation of the larval tissue and reassociation as adult fat body [1,2].

This investigation reports the observation that, unlike the reported fat body structure in the six other lepidopterans examined [1], larvae of the Indianmeal moth, *Plodia interpunctella* (Hübner), have two morphologically distinct fat bodies that are regionally differentiated on an anterior to posterior axis within the abdomen.

MATERIALS AND METHODS

Insect Preparations

The *P. interpunctella* colony was reared according to Silhacek and Miller [5] in a 16 h:8 h light-dark cycle at 30°C and 70% relative humidity. Larvae were collected from the cultures and staged by external morphological characters and sexed by gonadal appearance.

The larvae were dissected in saline [6], and the fat bodies were removed for photography. Whole fat body was prepared for photography by placing the tissue in saline containing 50% glycerol. Immersion of the fat body in glycerol left the white fat body clear and the tan fat body translucent. Photographs were taken using a Zeiss SR stereomicroscope with a Zeiss MC 63 photographic system using Kodak Pan-X film.

To label the fat body proteins in vivo, early and wandering fifth instar larvae were injected with [^{35}S]methionine (>1,000 Ci/mmol; Du Pont NEN, Boston, MA) (2 μCi in 1 μl saline) and incubated for 6 h, and then the white fat body and tan fat body were dissected into separate samples. Labeled fat bodies were homogenized in 100 μl of sodium dodecyl sulfate (SDS)*-sample buffer [7] and boiled. To label the tissues in vitro, tan fat body and white fat body were dissected from early and wandering fifth instar larvae into Grace's insect medium (GIBCO, Grand Island, NY) and incubated for 24 h in Grace's medium without serum proteins plus 0.2 μCi/μl [^{35}S]methionine. The media and tissues were harvested and homogenized in SDS-sample buffer and boiled.

*Abbreviations used: RER = rough endoplasmic reticulum; SDS = sodium dodecyl sulfate.

Electron Microscopical Methods

Fat body tissues were fixed in 2.5% glutaraldehyde in cacodylate buffer (0.1 M sodium cacodylate, pH 7.5, 0.15 mM $CaCl_2$) at room temperature for 2 h and postfixed for 2 h in 1% OsO_4 in cacodylate buffer plus 4% sucrose. The tissues were dehydrated in a graded ethanol-acetone series and embedded in Epon-Araldite [8]. Ultrathin sections were poststained with 2% uranyl acetate followed by lead citrate [9]. Ultrastructural examination was performed on a Hitachi (H-600) transmission electron microscope operating at 50 kV.

Gel Electrophoresis

One-dimensional 7.5% or 8–15% linear gradient SDS-PAGE was performed according to O'Farrell et al. [10]. Lanes were loaded with equal quantities of either total protein or incorporated radioactivity. Samples containing unlabeled proteins were homogenized in saline and centrifuged 2 min at 4°C and 12,000*g*, and an aliquot of the supernatant was assayed for protein before addition of SDS-sample buffer. Protein determinations were made in triplicate on the fat body homogenate supernatant using a bicinchoninic acid assay (Pierce Chemical Co, Rockford, IL), with bovine serum albumin as a standard. The amount of radiolabeled protein was determined by trichloroacetic acid precipitation as described previously [11]. Molecular weight standards (Sigma, St. Louis, MO) were coelectrophoresed for size estimation. Gels were either electroblotted to nitrocellulose or stained with Coomassie brilliant blue 250 or silver [12] and dried. Gels and immunoblots containing radioactive samples were autoradiographed for periods ranging from 12 h to 72 h using Kodak X-Omat AR film.

Immunoblots

Because antiserum to storage proteins of *P. interpunctella* is not available, the antisera to *Galleria mellonella* storage proteins were used to assess storage protein production in *P. interpunctella*. Immunoblots were prepared according to Towbin et al. [13] using fat body homogenates of *P. interpunctella* or purified *G. mellonella* 81K and 74K/76K storage proteins [14] resolved on SDS-PAGE. Proteins were electroblotted from the gel to nitrocellulose (BA-85; Schleicher and Schuell, Keene, NH) in transfer buffer (25 mM Tris, pH 8.3, 192 mM glycine, 20% methanol) at 20 V for 12 h using a Transblot cell (Bio-Rad, Richmond, CA). After transfer, the lanes containing the molecular weight standards were removed and stained as a reference for the blot. The electroblot was blocked with 3% gelatin in 20 mM Tris, pH 7.5, and 0.5 M NaCl and then reacted with a 1:1 mixture of whole antisera to the *G. mellonella* 81K and 74K/76K storage proteins [15], (Bean, Malone, and Silhacek, unpublished) diluted 1:2,000 with 1% gelatin, 0.05% Tween 20, 20 mM Tris, pH 7.5, and 0.5 M NaCl. When the fat body proteins of newly pupated *P. interpunctella* were cross reacted individually with the 81K storage protein antiserum and the 74K/76K storage proteins antiserum, the same single polypeptide band with a molecular weight of 81K was found to cross react with the two antisera identifying the 81K polypeptide as a putative storage protein in *P. interpunctella* (data not shown). The immunoreactive bands were visualized with an Immun-Blot color assay (Bio-Rad) using horseradish peroxidase-linked goat antirabbit IgG as the second antibody.

RESULTS

Identification and Distribution of Larval Fat Bodies

Wandering last instar larvae were dissected and examined. The last two abdominal segments contained a tan-colored, clustered fat body, whereas the remainder of the hemocoel contained a white, flat-sheet fat body. The fat bodies were removed intact and examined in 50% glycerol buffer (Fig. 1). Under these conditions, the white fat body became clear, and the tan fat body became translucent. Four major lobes of white fat body that joined with a central mass of tan fat body were observed. The gross morphology of the tan fat body appeared as a highly tracheated, clustered structure continuous with the sheets of white fat body (arrows in Fig. 1).

Examination of younger larvae showed that the tan fat body was the predominant form in larval instars 1–4 (data not shown). During the fourth instar, the amount of white fat body began to increase and in the fifth instar was the predominant type. The source of and the mechanism controlling the appearance of the increasing amount of white fat body was not determined. As the larvae became pharate pupae, i.e., at the time of head capsule slippage in the last instar, the tan fat body dispersed and the white fat body persisted into the pupae. When the tan fat body dispersed, the cells appeared to be undergoing autolysis, although this remains to be confirmed. No fat body cells with the tan fat body morphology were observed in the adult fat body (data not shown), suggesting that these cells either radically changed their morphology or they did not persist through metamorphosis.

Ultrastructural Characterization of Tan and White Fat Bodies

Based on the distinctive gross morphologies of the two fat bodies present in the larvae, a comparison was made of the ultrastructural morphologies of the tan and white fat body cells from late fourth and wandering fifth instar larvae. Although both female and male tissues were examined, no sex-specific ultrastructural characters were observed (data not shown), and the micrographs presented are represented for both females and males. In addition, the fat bodies of early fifth instar larvae were examined but had essentially the same morphologies as observed for the fourth instar larvae (data not shown).

The cellular morphology of tan fat body from both stages was remarkably distinct from previous literature descriptions of lepidopteran fat body in that the cytoplasm was filled with numerous large spherical mitochondria (Figs. 2A, 3A, 4). In addition to the distinctive mitochondria, the tan fat body cells of fourth instar larvae were observed to contain only infrequently lipid droplets or glycogen granules and limited amounts of extensive rough endoplasmic reticulum (RER) networks (Figs. 2A, 4A). Numerous lipid droplets, glycogen granules, and RER are structures previously reported as characteristic of lepidopteran larval fat body [1,2]. In the wandering fifth instar larvae, the tan fat body cells had essentially the same ultrastructural morphology as those of late fourth instar larvae except for the appearance of a few small lipid droplets (Figs. 3A, 4B).

The morphology of the tan fat body cells (Figs. 2A, 3A) contrasted sharply with that of the white fat body cells (Figs. 2B, 3B) in both stages; the cellular morphology of the white fat body was more characteristic of previous reports

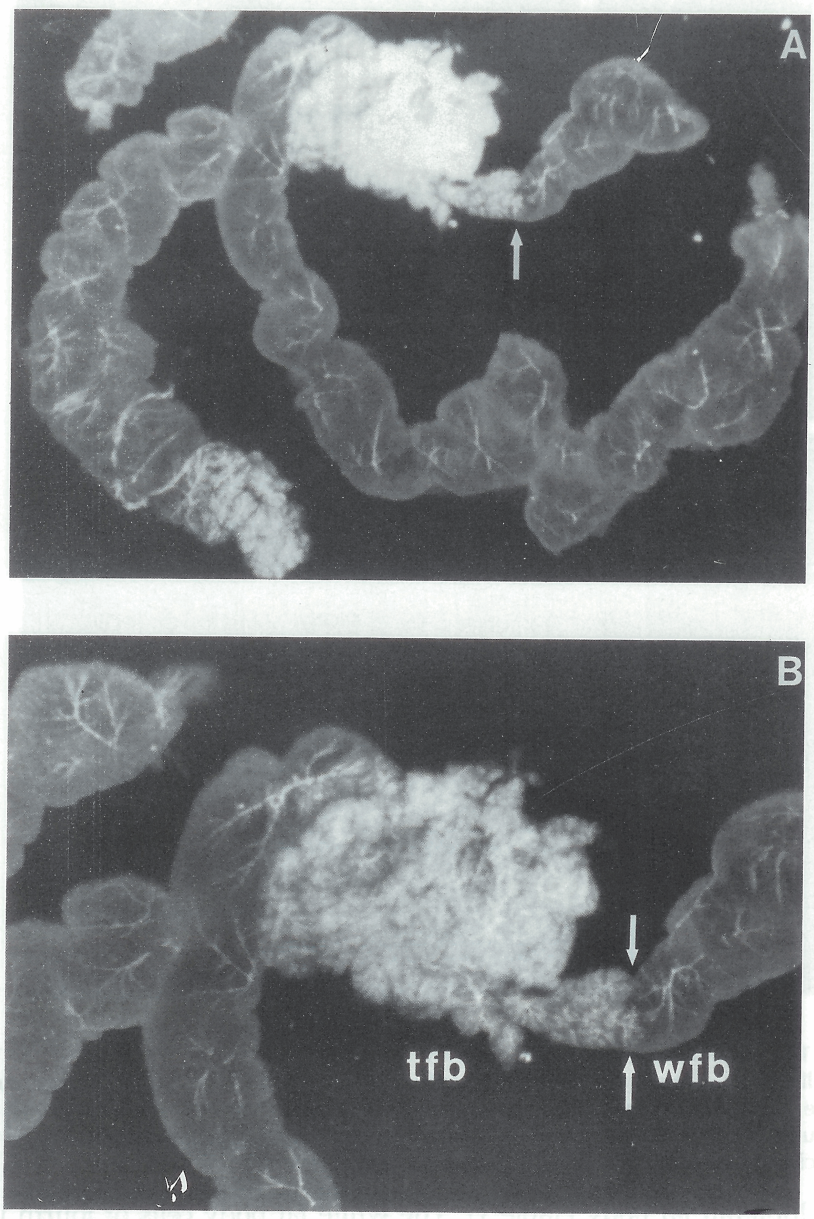

Fig. 1. Fat bodies from a wandering last instar larvae. **A:** Total fat bodies were photographed as described; white fat body appears as a transparent tissue in the preparation; the tan fat body is transluscent. × 20. Arrow shows area of transition between the two fat bodies. **B:** Transition between tan fat body and white fat body (arrows). × 50. Tan fat body appears as the highly tracheated, transluscent tissue while the white fat body is transparent. tfb, Tan fat body; wfb, white fat body.

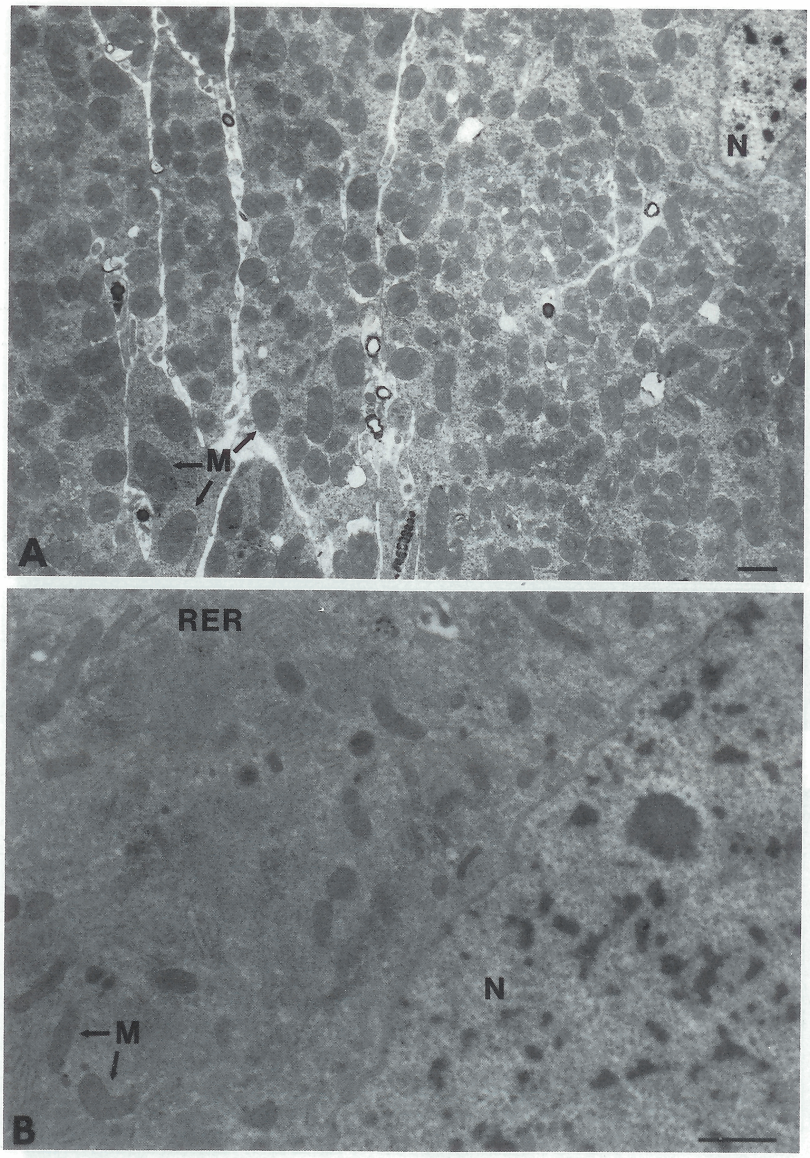

Fig. 2. Transmission electron micrographs of fat bodies from fourth instar larvae. **A:** Tan fat body cells showing the large, numerous, spherical mitochondria. ×8,000. The nucleus is in the upper right. **B:** White fat body cells showing few, elongate mitochondria and a well developed rough endoplasmic reticulum. ×13,500. The nucleus is in the lower right. Bars = 1 μm. M, mitochondria; N, nucleus, RER, rough endoplasmic reticulum.

of lepidopteran fat body (Table 1). The white fat body cells of fourth instar larvae had few small elongate or irregular mitochondria, along with few lipid droplets, apparent RER, and large irregular nuclei (Fig. 2B). This morphology was maintained essentially the same into the fifth larval instar except for an increase in the size and number of lipid droplets and the presence of numerous glycogen granules (Fig. 3B).

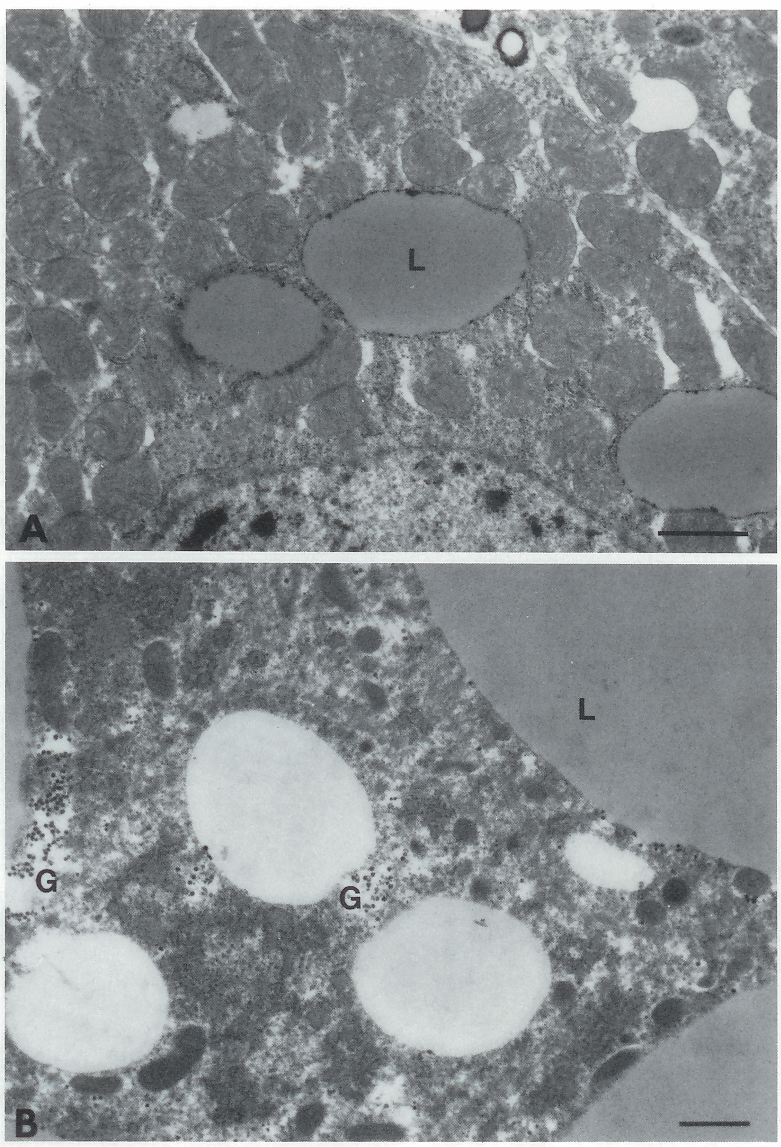

Fig. 3. Transmission electron micrographs of fat bodies from wandering fifth instar larvae. **A:** Tan fat body cells showing the large, numerous, spherical mitochondria and the appearance of small lipid droplets. ×20,000. The nucleus is in the bottom center. **B:** White fat body cells showing irregular, few, elongate mitochondria, large lipid droplets, and glycogen granules. ×13,500. Bars = 1 μm. L, lipid droplet; G, glycogen granules.

Characterization of Proteins Present in Tan and White Fat Bodies

The proteins contained in the fat bodies of fourth instar larvae, wandering fifth instar larvae, and pharate pupae were resolved by SDS-PAGE (Fig. 5). White fat body of both female and male wandering fifth instar larvae and pharate pupae contained two major protein constituents with apparent molecular weights between 76K and 81K (Fig. 5A, B, lanes e, g, i, j). These polypeptides

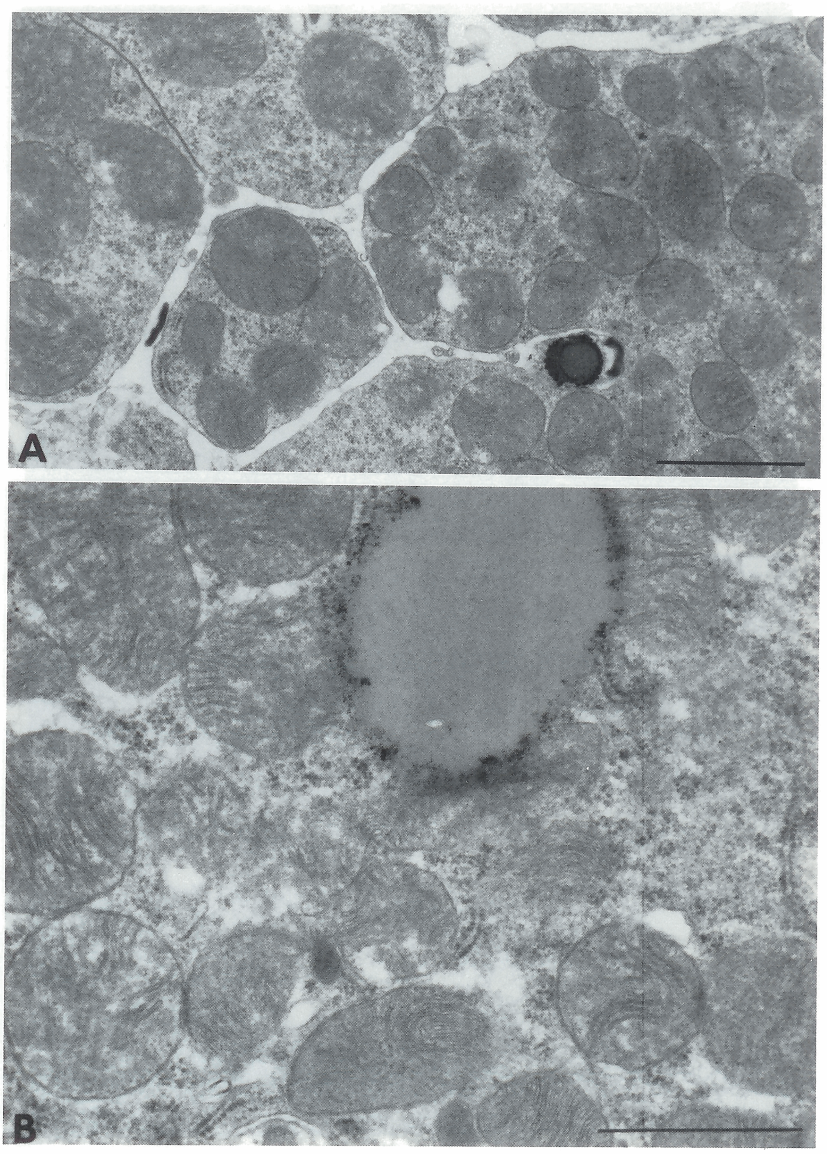

Fig. 4. High-magnification transmission electron micrographs of tan fat body cells from fourth instar larvae (**A**) (×29,000) and wandering fifth instar larvae (**B**) (×45,000). RER, rough endoplasmic reticulum. Bars = 1 μm.

were present in small quantities in the tan fat body of these two stages of the fifth instar larvae and in the white fat body of fourth instar larvae. When the electroblot of protein samples from these stages was reacted with antisera to *G. mellonella* storage proteins, the only protein to cross-react with the antisera was the major 81K protein apparent in the white fat body of female and male wandering fifth instar larvae and pupae. A major 83K molecular weight protein that had similar temporal synthesis and accumulation with 81K did not cross-react with the storage protein antisera. No other proteins were found to

TABLE 1. Morphological Features of Tan and White Fat Bodies in Developing *P. interpunctella* Larvae

Instar	Tan fat body	White fat body
Fourth	Large, numerous, spherical mitochondria; sparse RER.	Small, few, elongate mitochondria; well defined RER; some Golgi bodies.
	No lipid droplets, protein granules, or glycogen granules.	No lipid droplets, protein granules, or glycogen granules.
Fifth	Large, numerous, spherical mitochondria; sparse RER; occasional, small, lipid droplets, and few glycogen granules; cell size larger than fourth instar.	Small, few, elongate mitochondria; well-defined RER; some Golgi bodies; some Golgi bodies; large, numerous lipid droplets, protein granules, and glycogen granules; cell size larger than fourth instar.
	No protein granules.	

cross-react with the storage protein antisera in the stages tested. On the basis of the apparent molecular weights, the temporal accumulation in the fat body, and the cross reactivity with antisera to storage proteins, the major 81K and 83K polypeptides in the white fat body of pharate pupae were considered to be storage protein subunits of *P. interpunctella*.

Protein Synthesis by Tan and White Fat Bodies

Early and wandering fifth instar larvae were injected with [^{35}S]methionine and the labeled proteins were resolved by SDS-PAGE. Comparison of the labeled protein pattern (Fig. 6B, lanes g–l) and the stained protein pattern (Fig. 6A, lanes g–l) between the tan and white fat bodies from early fifth instar larvae

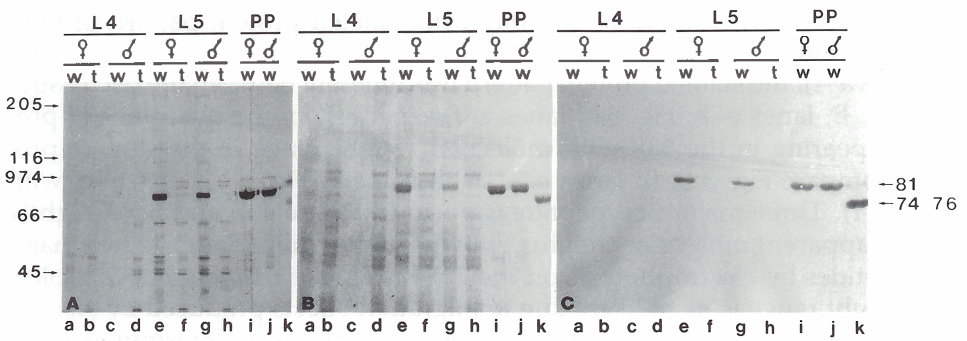

Fig. 5. Proteins contained in the tan and white fat body tissues of fourth instar larvae, wandering fifth instar larvae, and pharate pupae. The gels (7.5%) were stained with either silver (**A**) or Coomassie blue (**B**). To establish the identity of some proteins, a gel was electroblotted and reacted with antisera to *G. mellonella* storage proteins as described (**C**). Lane designations: a, white fat body from female fourth instar larvae; b, tan fat body from female fourth instar larvae; c, white fat body from male fourth instar larvae; d, tan fat body from male fourth instar larvae; e, white fat body from female wandering fifth instar larvae; f, tan fat body from female wandering fifth instar larvae; g, white fat body from male wandering fifth instar larvae; h, tan fat body from male wandering fifth instar larvae; i, white fat body from female pharate pupae; j, white fat body from male pharate pupae; k, *G. mellonella* storage protein standards (a mix of 81K and 74K/76K). Lanes a–d contain 5μg protein, and lanes e–k contain 2.5 μg protein. Positions of the molecular weight markers ($\times 10^3$) are shown on the left. The positions of the 81K and 74K/76K storage protein markers are shown on the right. L4, fourth instar larvae; L5, wandering fifth instar larvae; PP, pharate pupae; w, white fat body; t, tan fat body.

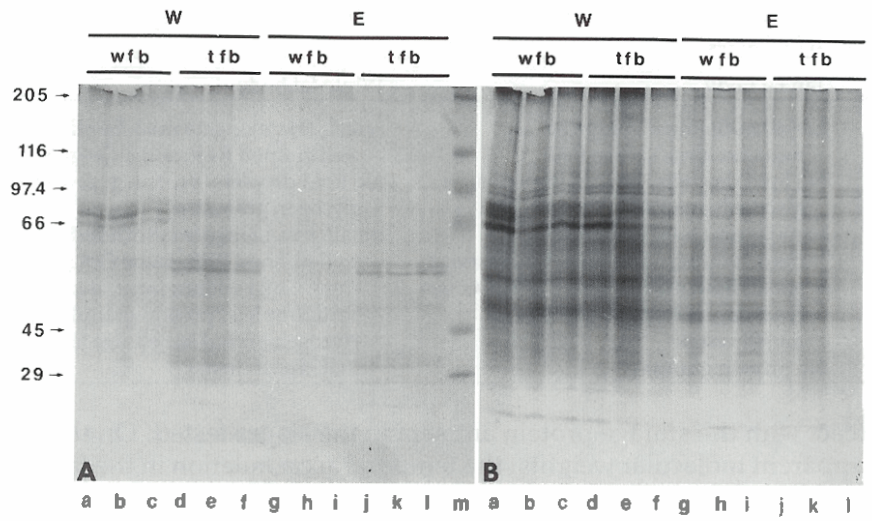

Fig. 6. In vivo radiolabeling of proteins in tan and white fat bodies of early and wandering fifth instar larvae. A: Coomassie blue stained SDS gel (8–15% gradient). B: Corresponding autoradiogram of the gel. Lane designations: a, b, c, white fat body from wandering fifth instar larvae; d, e, f, tan fat body from wandering fifth instar larvae; g, h, i, white fat body from early fifth instar larvae; j, k, l, tan fat body from early fifth instar larvae; m, molecular weight standards [corresponding weights ($\times 10^3$) designated on left side of figure]. W, wandering fifth instar larvae; E, early fifth instar larvae; wfb, white fat body; tfb, tan fat body.

showed that the labeling pattern and the staining pattern for both tissues were qualitatively almost identical. Although the labeled protein pattern of both tan and white fat bodies of wandering fifth instar larvae were identical (Fig. 6B, lanes a–f), the stained protein pattern was different between the two tissues (Fig. 5A, B, lanes e–h; Fig. 6A, lanes a–f) especially in the quantities of proteins appearing in the 66K–90K molecular weight range of the storage proteins (compare Fig. 5A, B, lanes e, g, with f, h and Fig. 6A, lanes a–c, with lanes d–f). Three major polypeptides appear in this region and have slightly smaller apparent molecular weights than those determined for the two major polypeptides by electrophoresis on the 7.5% gels. The discrepancy in molecular weight range is most likely due to migration effects produced by electrophoresis on gradient gels, but one of the proteins was determined to be identical in the two gel systems by other criteria (see below). The storage protein bands were heavily labeled in both the tan and white fat bodies of wandering fifth instar larvae whereas little biosynthesis of these proteins was observed in tan and white fat bodies of early fifth instar larvae.

As the labeled 66K–90K proteins appearing in the fat bodies of the wandering larvae were considered to be storage proteins, antisera to *G. mellonella* storage proteins were used to determine the nature of the labeled proteins by comparison of radiolabeled proteins on an immunoblot. A single band of cross-reacting protein was observed in the tissues and medium of cultured tan and white fat body from wandering fifth instar larvae on the immunoblot (Fig. 7, lanes c, d, g, h). The storage protein cross-reacting band in the culture media of fat bodies from wandering fifth instar larvae was identical to a highly radio-

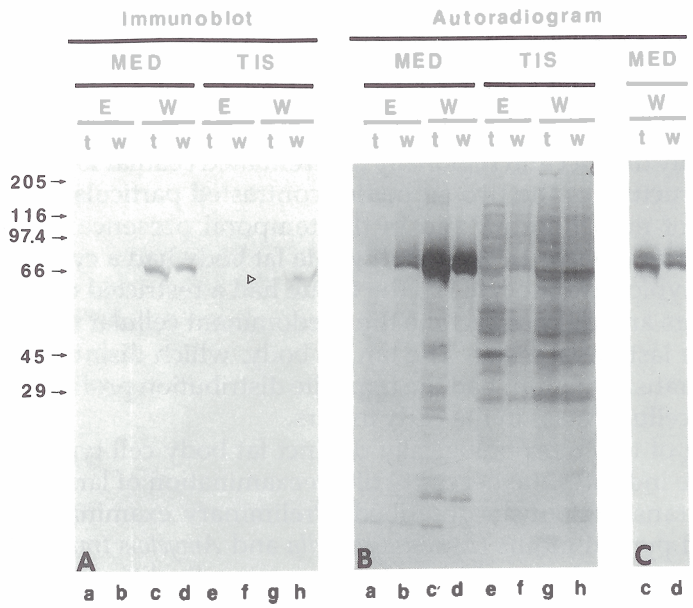

Fig. 7. Immunoidentification of labeled proteins contained in the tissues and secreted into the media of cultured tan and white fat bodies of early and wandering fifth instar larvae. After culturing, the proteins were resolved by SDS-PAGE (8–15% gradient), electroblotted to nitrocellulose, probed with antisera, and autoradiographed. **A:** Immunoblot of proteins in tissues and media probed with antisera to *G. mellonella* storage proteins. Open triangle marks the position of a faint cross-reacting band. **B:** Autoradiogram of the immunoblot (72 h exposure). **C:** Reduced exposure (12 h) of lanes c and d of the immunoblot. Lane designations: a, medium of tan fat body from early fifth instar larvae; b, medium of white fat body from early fifth instar larvae; c, medium of tan fat body from wandering fifth instar larvae; d, medium of white fat body from wandering fifth instar larvae; e, tissue of tan fat body from early fifth instar larvae; f, tissue of white fat body from early fifth instar larvae; g, tissue of tan fat body from wandering fifth instar larvae; h, tissue of white fat body from wandering fifth instar larvae. MED, medium; TIS, tissue; E, early fifth instar larvae; W, wandering fifth instar larvae; t, tan fat body; w, white fat body. Positions of the molecular weight markers ($\times 10^3$) are designated on the left side of the figure.

labeled band of the same molecular weight observed in the autoradiogram of the immunoblot. However, there was a highly radiolabeled band with a molecular weight of 83K appearing in these samples that did not cross-react with the storage protein antisera that was similar to the nonlabeled protein band observed on the immunoblots of 7.5% gels. The possibility that this protein represents a storage protein of *P. interpunctella* that does not share antigenic homology with the storage proteins of *G. mellonella* remains to be clarified. No detectable cross-reacting proteins were observed in either the tissue or media of the tan and white fat bodies of early fifth instar larvae.

DISCUSSION

Previous descriptions of fat body from other lepidopterans indicate that fat body in these species appears to be a relatively homogeneous tissue that undergoes characteristic and uniform morphological changes during metamor-

phosis [1,2]. The only regional differentiation noted in the moth *Calpodes ethlius* was a difference in cell size between the perivisceral and peripheral fat body cells [1]. We report here that the larval fat body of *P. interpunctella* is unlike the fat body of other lepidopterans based on the observation that there has been a segregation of the metabolic and storage functions of the fat body into two morphologically distinct and regionally differentiated cellular forms. The cytological ultrastructure of the two fat bodies contrasted particularly in the size and number of mitochondria and in the temporal presence of organelles involved in storage (Table 1). Although white fat body had a cytology similar to that of the typical trophocyte [1], the tissue had a restricted distribution in the early instars and did not become the predominant cellular mass of the fat body until the last larval instar. The tan fat body, which disintegrated at the larval–pupal transformation, had the opposite distribution profile and was the predominant cellular mass in the early instars.

The finding of two morphologically distinct fat body cell types in *P. interpunctella* larvae indicates the necessity of a reexamination of larval fat body in the lepidopterans previously described. Preliminary examination of other closely related pyralid moths (*Ephestia cautella* and *Amyelois transitella)* in this laboratory has shown the larval fat body to be regionally differentiated as well. However, the posterior fat body of *G. mellonella* was found to contain a mixture of the large, spherical and small, elongate mitochondria observed separately in the tan and white fat bodies, respectively, of *P. interpunctella* and more organelles associated with storage functions (data not presented).

Using antisera to storage proteins of *G. mellonella*, a single protein band with a molecular weight of approximately 81K was tentatively identified as a storage protein of *P. interpunctella*. Additional proteins with molecular weights between 66K and 83K met the criteria of storage proteins as well but did not cross react with the mixture of antisera to storage proteins of *G. mellonella* used to identify the 81K storage protein. Although all of these proteins have the correct temporal expression and accumulation in the fat body, they must be considered putative storage proteins of *P. interpunctella* and require further study before this can be confirmed.

Both the tan and white fat bodies of the late fifth instar larvae were capable of synthesizing storage proteins (Fig. 6B, lanes a–f; Fig. 7b, lanes c, d, g, h) although only the white fat body showed any significant storage protein accumulation during this period (Fig. 5, lanes e, g, i, j; Fig. 6A, lines a–c). The synthesis and accumulation of storage proteins has been demonstrated as a characteristic developmental phenomenon of fat body approaching metamorphosis in late last instar larvae of all lepidopterans examined [1(see Table 4),14,15]. The tan fat body of *P. interpunctella* has been the only lepidopteran fat body tissue reported that does not accumulate the storage proteins in any quantity.

The lack of an ability to accumulate storage proteins may be a consequence of the fate of tan fat body, which dissociates and apparently undergoes cell autolysis during the pharate pupal stage. This phenomenon suggests that this tissue may be a bona fide larval fat body in this lepidopteran. Previously, larval fat body cells of the silk moth, *Hyalophora cecropia*, have been shown to persist through metamorphosis and make up the cells of the adult tissue [16].

To determine the developmental history of fat bodies in *P. interpunctella*, the fate of the tan and white fat body cells will be followed during metamorphosis to confirm the larval nature of the tan fat body.

A possible explanation of the function of tan fat body comes from the niche that this insect fills; *P. interpunctella* feeds strictly on seeds, as do the other closely related pyralids, which is what places it in the position of a stored-products pest. The presence of numerous mitochondria may provide this moth with the ability to metabolize very efficiently the fats and carbohydrates of the seeds and to produce considerable amounts of metabolic water in the process. The ability to generate metabolic water would allow the larvae of the Indianmeal moth to feed on plant materials of very low water content, providing an advantage over other insects for utilizing seeds as a food source.

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