

# Absorption and Tissue Distribution of Cholesterol in *Manduca sexta*

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In *Manduca sexta* larvae, radioactive free cholesterol is absorbed directly from the midgut into mucosal cells where it is stored both in the free form (87% in males and 93% in females) and esterified form (13% in males and 7% in females). Subsequently, cholesterol is transported to fat body via lipophorin in the hemolymph exclusively in the free form. In fat body, the distribution of cholesterol between the free and esterified form varied significantly between genders and developmental stages. Except for the larval stage, males and females were able to store cholesterol in both free and esterified forms in the fat body and in the adult stage cholesterol ester accounted for more than 75% of the stored cholesterol. Placement of radioactive cholesterol at different locations in the gut—foregut, midgut, and hindgut—demonstrated that the midgut is the site where cholesterol is absorbed and released into the hemolymph. Cholesterol-labeled lipophorin injected into larval hemolymph was cleared from the hemolymph with a half-life of 10.2 h. After 17 h, most of the cleared radioactivity was recovered in the fat body (38%). Arch. Insect Biochem. Physiol. 49:167–175, 2002. © 2002 Wiley-Liss, Inc.

KEY WORDS: *Manduca sexta*; lipophorin; cholesterol; absorption; transport

## INTRODUCTION

Insects cannot make cholesterol via de novo synthesis because they lack the enzyme(s) squalene synthase and/or lanosterol synthase (Grieneisen, 1994). In insects, cholesterol serves both as a component of cell membranes and as a precursor for the molting hormones, ecdysones (Svoboda, 1999; Behmer and Elias, 2000; Canavoso et al., 2001), that are essential for the molecular and cellular events that lead to molting and metamorphosis (Sehnal, 1989; Gilbert et al., 1996). Thus, for normal growth, development, and reproduction, insects require a dietary source of cholesterol. Although plants contain little or no cholesterol, most phytophagous and omnivorous insects are able to metabolize phytosterols and dietary sterols to fulfill their requirement for cholesterol (Svoboda, 1999). Absorption of cholesterol or other sterols has been investigated in only a few

insects, including the silkworm larvae *Philosamia cynthia* (Chino and Gilbert, 1971), the *Heliothis zea* larvae (Kuthiala and Ritter, 1988), and the dragonfly larvae *Aeshna cyanea* (Komnick and Giesa, 1994). On the other hand, the metabolism of cholesterol has been studied in many insects including *M. sexta* (Sakurai et al., 1989; Warren and Gilbert, 1996; Svoboda, 1999). This study focuses on (1) absorption of cholesterol in *M. sexta* larvae, (2) tissue distribution of dietary cholesterol throughout the life span of the insect, and (3) the localization of cholesterol absorption and release from the midgut lumen of the *M. sexta*. Since absorption of cholesterol in vertebrates is influenced by many factors including adaptation, a single dose of radioactive cholesterol was used in order to eliminate potential adaptive changes associated with its continuous feeding. Some of the unique features of sterol metabolism in insects could provide potential targets for control.

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## MATERIALS AND METHODS

### Materials

Glutathione, dithiothreitol, phenylmethylsulfonyl fluoride, benzamidine, diisopropyl fluorophosphate, and Grace's medium were purchased from Sigma;  $^{14}\text{C}$ -cholesterol (specific activity of 50 mCi/mmol) and (1,2n)- $^3\text{H}$ -cholesterol in 99.9% ethanol (specific activity of 52 Ci/mmol) were purchased from Amersham Pharmacia Inc. (Piscataway, NJ).

### Insects

*M. sexta* were reared according to Bell and Joachim (1976) on a high wheat germ diet, in an incubator at 26°C, with an 18 h light/dark cycle. All animals were synchronized at the end of the fourth larval instar by the appearance of head capsule apolysis.

### $^{14}\text{C}$ -Cholesterol Absorption Studies

Five groups (7 insects/group) of second day fifth instar *M. sexta* (wt = 3.1–3.2 g) were fasted for 20 min, then fed a small piece of diet (0.3 × 0.3 × 0.3 cm) containing either 0.25, 0.5, 1, 2, or 4  $\mu\text{Ci}$   $^{14}\text{C}$ -cholesterol. Following consumption of the labeled diet (about 5 min), the animals were placed on unlabeled diet. Twenty-four hours later, insects were weighed and hemolymph was collected, as described below. The total volume of the hemolymph in the larva was calculated as previously described (Meyer-Fernandes et al., 2000). Midgut contents were collected and midgut tissues were washed and all washes were collected as part of the carcass (muscle plus cuticle). The entire midgut was dissected out under cold PBS buffer (phosphate buffered saline: 50 mM phosphate, 150 mM NaCl, pH 6.5) containing 0.5 mM benzamidine hydrochloride, 2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 mM glutathione, and 0.1 mM diisopropyl fluorophosphate, dried on tissue-paper and its weight recorded. The fat body was carefully dissected and washed extensively

with PBS, dried and its weight recorded. The fat body, midgut, carcass, and frass were homogenized in PBS and were either used immediately for lipid extraction or frozen for later usage.

To 100–200  $\mu\text{l}$  of the homogenized tissues, 1 ml of PBS was added and lipids were extracted three times with 3 ml chloroform: methanol (2:1, v:v). The organic layers were combined, dried under nitrogen, and then redissolved in 50  $\mu\text{l}$  hexane and radioactivity was counted using a scintillation counter. To account for recovery, an internal standard of  $^3\text{H}$ -cholesterol (50,000 dpm) were added prior to homogenization. Absorption and % recovery of  $^{14}\text{C}$ -cholesterol were determined using the following equations:

$$\text{Absorption}_{(\text{dpm})} = \text{Fed}_{(\text{dpm})} - [\text{midgut content}_{(\text{dpm})} + \text{frass}_{(\text{dpm})}]$$

$$\% \text{ Recovery} = [\text{midgut tissues}_{(\text{dpm})} + \text{fat body}_{(\text{dpm})} + \text{carcass}_{(\text{dpm})} + \text{corrected hemolymph}_{(\text{dpm})}] \times 100 / \text{Absorbed}_{(\text{dpm})}$$

### Isolation of Lipophorin

Hemolymph from 3rd day 5th instar larva fed 10  $\mu\text{Ci}$   $^3\text{H}$ -cholesterol 24 h earlier, was collected through an incision in the second pair of prolegs into PBS. Hemocytes were separated by centrifugation for 10 min at 2,000g. The supernatant was adjusted to a density of 1.31 g/ml with KBr; then 20 ml of this solution was overlaid with 20 ml of 0.9% NaCl, followed by ultracentrifugation at 50,000 rpm for 16 h (Shapiro et al., 1984) and densities were determined along the gradient. Fractions containing lipophorin were combined and dialyzed against a PBS. The purity of the lipophorin preparations was confirmed by SDS-PAGE analysis (Laemmli, 1970).

### Time Course Studies

Third day, fifth instar *M. sexta* (about 5 g) were fed a small piece of diet containing 4  $\mu\text{Ci}$  of  $^3\text{H}$ -cholesterol, then switched to the regular diet. Cholesterol distribution in various tissues and in

hemolymph was determined after 2, 4, 6, 24, 36, 620 (pupa), and 720 h (adults).

### Determination of Free and Esterified Cholesterol

Second day fifth instar *M. sexta* were fed 4  $\mu\text{Ci}$  of  $^3\text{H}$ -cholesterol and dissected after 24 h (larval), 7 days after pupation (pupa), and 2 days after eclosion (adult). Samples, 100–250  $\mu\text{l}$  of hemolymph or 200  $\mu\text{l}$  of midgut and fat body homogenates, were extracted as described above using  $^{14}\text{C}$ -cholesterol (70,000 dpm) as an internal recovery standard. Extracted lipids were evaporated under nitrogen, dissolved in hexane, and applied to TLC Silica plates and developed using petroleum ether:diethyl ether:acetic acid (95:5:0.5, v:v:v) as the solvent. Cholesterol and cholesterol ester bands were identified with iodine vapor, scrapped and counted for radioactivity. In some cases, total cholesterol content was determined by adding 2.5 ml KOH-methanol (20:70) to samples and saponifying them at 70°C for 2 h prior to extraction (Jouni et al., 1995). Extracted lipids were loaded on TLC Silica plates and treated as mentioned above. In this case, the amount of cholesterol ester was calculated as the difference between free and total cholesterol. The recovery of the added  $^{14}\text{C}$ -cholesterol was used to correct for procedural losses (average recovery 87%).

### Site of Cholesterol Absorption

To determine the site of cholesterol absorption in the gut of the insect,  $^3\text{H}$ -cholesterol was placed into the lumen at different locations, foregut, midgut, or hindgut, and radioactivity released into the hemolymph was measured after 2 h.

### Clearance of Cholesterol-Labeled Lipophorin From Hemolymph

To determine the rate of clearance of labeled lipophorin from hemolymph, second day 5th instar *M. sexta* were injected with  $^3\text{H}$ -cholesterol-labeled lipophorin (50  $\mu\text{l/g}$  body weight; 1,500 dpm/ $\mu\text{l}$ ). At the indicated time, fat body, midgut, and

hemolymph were collected and analyzed for radioactivity.

### Other Assays

Protein concentrations were determined by modified Lowery procedure using bovine serum albumin as standard (Markwell et al., 1978). For statistical analysis, Student's unpaired *t*-test was used to determine the significance of differences between means.

## RESULTS AND DISCUSSION

### Tissue Distribution of Absorbed Labeled-Cholesterol

Figure 1 shows that feeding different amounts of labeled cholesterol to 3rd day 5th instar *M. sexta* larva resulted in a linear increase in the amount of radioactive cholesterol absorbed. At all levels of radioactivity fed, an average of  $52 \pm 6\%$  of cholesterol was absorbed (Table 1). This corresponds with the results in vertebrates where

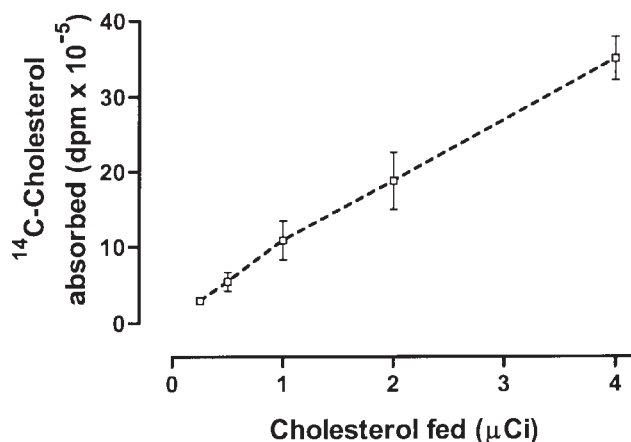


Fig. 1. Effect of increasing the amount of radioactive cholesterol on absorption in *M. sexta*. Five groups of second day fifth instar *M. sexta* were fasted for 20 min, then fed on a small piece of diet containing either 0.25, 0.5, 1, 2, or 4  $\mu\text{Ci}$ . After 24 h, hemolymph and different tissues were collected and measured for radioactivity (refer to experimental procedures for more details). Absorption of  $^{14}\text{C}$ -cholesterol was determined using the following equations:  $\text{Absorption (dpm)} = \text{Fed (dpm)} - [\text{midgut content (dpm)} + \text{frass (dpm)}]$ . Values represent averages  $\pm$  SE of 7 determinations.

TABLE 1. Tissue Distribution of Absorbed Cholesterol in *M. sexta* Larva\*

$\mu\text{Ci}$ fed	% Absorbed	Fat body	Midgut	Hemolymph	Carcass
0.25	50 $\pm$ 4	22 $\pm$ 3	18 $\pm$ 12	17 $\pm$ 6	43 $\pm$ 11
0.5	46 $\pm$ 6	25 $\pm$ 9	14 $\pm$ 2	12 $\pm$ 3	49 $\pm$ 4
1	62 $\pm$ 5	17 $\pm$ 5	13 $\pm$ 5	11 $\pm$ 3	51 $\pm$ 9
2	47 $\pm$ 8	18 $\pm$ 4	20 $\pm$ 8	10 $\pm$ 5	52 $\pm$ 7
4	53 $\pm$ 4	19 $\pm$ 6	12 $\pm$ 6	13 $\pm$ 6	56 $\pm$ 11
Av $\pm$ SD	52 $\pm$ 6	20 $\pm$ 3	15 $\pm$ 3	13 $\pm$ 3	50 $\pm$ 4

\*Five groups (7 insects/group) of second day fifth instar *M. sexta* were fed different amounts of  $^{14}\text{C}$ -cholesterol. After 24 h, hemolymph, midgut content, midgut tissue, fat body, carcass, and frass were collected. All tissues were homogenized and lipids were extracted and counted for radioactivity. To account for recovery, internal standards of  $^3\text{H}$ -cholesterol were added. Absorption and % recovery of  $^{14}\text{C}$ -cholesterol were determined using the following equations:  $\text{Absorption}_{(dpm)} = \text{Fed}_{(dpm)} - [\text{midgut content}_{(dpm)} + \text{frass}_{(dpm)}]$ .  $\% \text{ Recovery} = [\text{midgut tissues}_{(dpm)} + \text{fat body}_{(dpm)} + \text{carcass}_{(dpm)} + \text{corrected hemolymph}_{(dpm)}] \times 100 / \text{Absorbed}_{(dpm)}$ . Values represent averages  $\pm$  S.D. for 6–7 determinations.

only half of the dietary cholesterol is normally absorbed, compared to other nutrients that are almost completely absorbed (Ostlund et al., 1999). In vertebrates, cholesterol absorption is influenced by many factors but nothing is known about what influences efficiency of sterol absorption in insects.

The majority of the absorbed cholesterol was recovered in the carcass (50  $\pm$  4%) (Table 1), where free cholesterol accounted for more than 93% (data not shown). Midgut, hemolymph, and fat body accounted for 15  $\pm$  3, 13  $\pm$  3, and 20  $\pm$  3%, respectively, of the absorbed cholesterol.

### Lipophorin Is the Sole Hemolymph Carrier of Cholesterol

Transport of cholesterol from sites of absorption to sites of utilization is of significant importance for the insects, because, unlike vertebrates, insects are unable to synthesize cholesterol via the de novo pathway, and require a dietary sterol source. Fractionation of larval hemolymph proteins by KBr gradient ultracentrifugation 24 h after feeding radioactive cholesterol is shown in Figure 2. The peak of radioactivity (92%) was associated with lipophorin peak (d = 1.14–1.16 g/ml), as confirmed by immunoblotting (data not shown). These data demonstrate that lipophorin is the sole hemolymph carrier of cholesterol. These results in *M. sexta* correspond with results obtained in the silkworm *P. cynthia* (Chino and Gilbert, 1971).

### Time Course Distribution of Cholesterol in Different Tissues

Figure 3 shows the in vivo distribution of a single dose of labeled cholesterol in hemolymph, midgut content, midgut, and fat body tissues from larvae (2nd day, 5th instar) to pupae and adult stages. Over the period of the experiment, the percent recoveries of absorbed cholesterol ranged between 83 and 102%. The amount of absorbed cholesterol in midgut mucosal cells peaked within the first hour of feeding and was followed by a sharp decrease within 4 h and finally by a steady decrease until late pupation when the tissue starts to disintegrate (Fig. 3A).

In the fat body, labeled-cholesterol increased steadily up to 36 h after feeding (Fig. 3B). However, during the pupal and adult stages radioactive

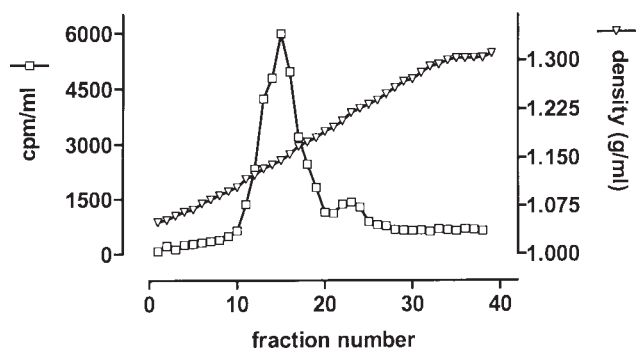


Fig. 2. Fractionation of *M. sexta* larval hemolymph. Third day 5th instar *M. sexta* were fed  $^3\text{H}$ -cholesterol diet. After 24 h, the hemolymph was collected and subjected to density gradient ultracentrifugation for 17 h at 4°C. One-milliliter fractions were collected, density (open triangles) and radioactivity (open squares) were determined.

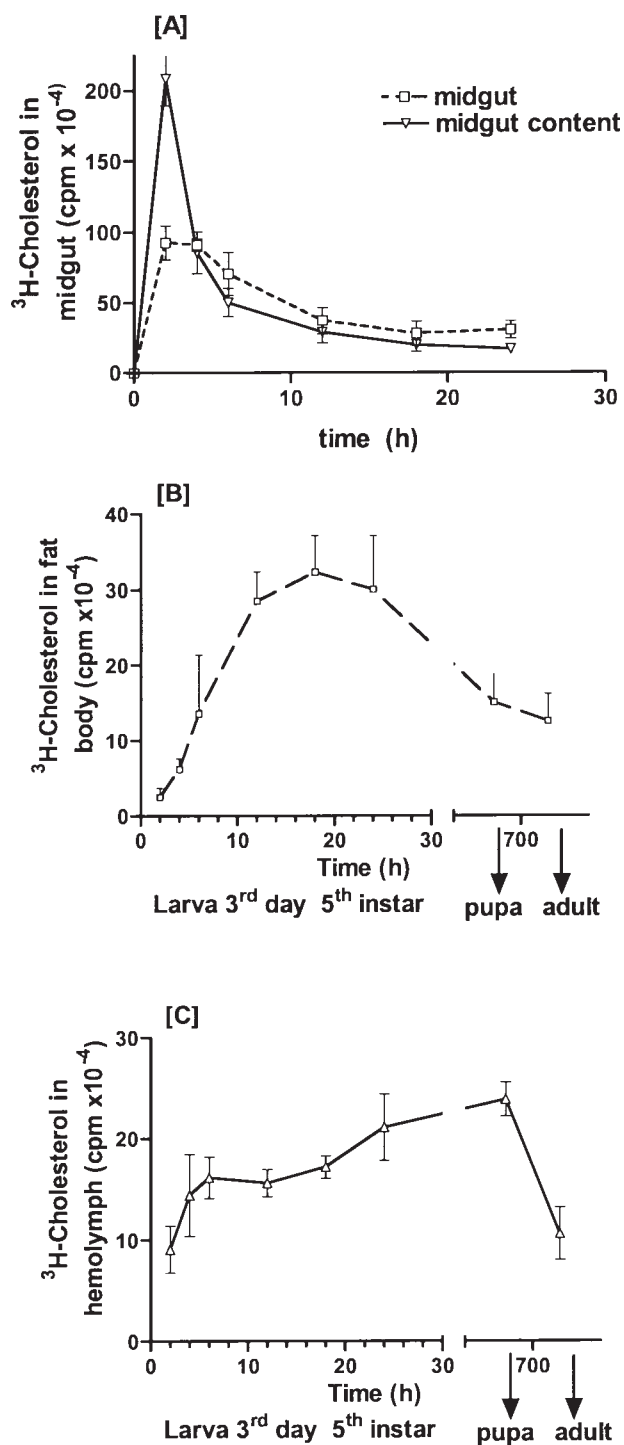


Fig. 3. Distribution of radioactive cholesterol throughout the life span of *M. sexta*. Third day, fifth instar larva were fed a small piece of diet containing 4  $\mu\text{Ci}$  of  $^3\text{H}$ -cholesterol, then switched to the regular diet. Cholesterol distribution in various tissues and in hemolymph were determined at the indicated time interval. A: Distribution of labeled cholesterol in midgut (open squares)

cholesterol dropped significantly, 54% and 61%, respectively, compared to the larval stage. The decrease of cholesterol could be accounted for by the needs of cholesterol by other tissues, including newly formed muscle tissue, the brain, reproductive organs specifically ovaries for oocyte formation, as well as for the biosynthesis of ecdysteroids, the molting hormone, which have been shown to be at the highest concentration during the first 4 days following pupations in male *M. sexta* (Lozana et al., 1989; Svoboda and Weirich, 1995).

In the hemolymph, radioactive cholesterol increased sharply within the first few hours of feeding, and continued to rise gradually until a first peak was reached at 24 h (Fig. 3C). At the second day of wandering (72 h after feeding radioactive cholesterol), labeled-cholesterol increased again and remained high during the pupal stage (1.5-fold higher than larval hemolymph). Since no dietary cholesterol was consumed during the transition from larvae to wanderer stages, we suggest that the source of this increase is the fat body. Previous reports from our laboratory (Prasad et al., 1986; Fernando-Warnakulasuriya et al., 1988) have suggested a fundamental change in *M. sexta* fat body lipid metabolism during the wandering stage where the fat body is converted from a lipid storing to lipid mobilizing organ.

At the adult stage, the amount of radioactive cholesterol in hemolymph dropped sharply compared to the pupal stage (Fig. 3C). The observed decrease may result in the utilization of cholesterol for synthesis of tissues during the late pupal stage.

#### Localization of Cholesterol Absorption in the Gut

In order to determine the major site for absorption of cholesterol from the gut,  $^3\text{H}$ -cholesterol was placed into the foregut or midgut via the mouth and the hindgut via the anus, and the amount of

and midgut content (open triangles). B: distribution profile of cholesterol in hemolymph. C: Distribution of labeled cholesterol in fat body. Values represent averages  $\pm$  SE of 3–5 determinations.

radioactivity appearing in the hemolymph after 2 h was determined (Fig. 4). The amount of radioactivity found in hemolymph when cholesterol was introduced directly into the midgut was 5-fold and 9.5-fold higher than when paced in the foregut and the hindgut, respectively. These data demonstrated that the midgut is the major site of cholesterol absorption and release into the hemolymph. Other lepidopterans have also been shown to absorb sterols in the midgut (Joshi and Agarwal, 1977).

### Fate of Injected $^3\text{H}$ -Cholesterol Labeled-Lipophorin

To investigate the fate of lipophorin-cholesterol, cholesterol-labeled lipophorin was injected in *M. sexta* larva and its clearance from hemolymph and uptake by midgut and fat body was determined (Fig. 5). Injected cholesterol was cleared from the hemolymph with a half-life of 10.2 h (Fig. 5A). After 17 h, 30% of the radioactivity remained in the hemolymph and most of the injected cholesterol was recovered in the fat body (38%), with 11% in the midgut, demonstrating the preferential uptake of cholesterol by the fat body.

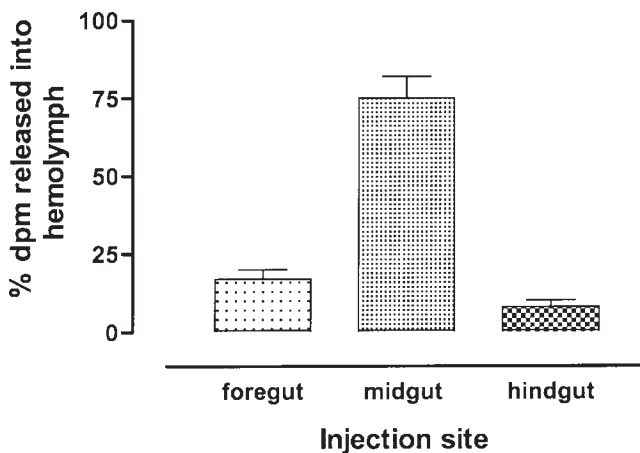


Fig. 4. Determining the major site for the release of cholesterol from the lumen to hemolymph.  $^3\text{H}$ -cholesterol (100,000dpm/5 $\mu\text{l}$ ) was injected into different locations of the lumen, the foregut and midgut, and the hindgut; then the amount of radioactivity appeared in the hemolymph was measured after 2 h. Values represent averages  $\pm$  SE of 4 determinations.

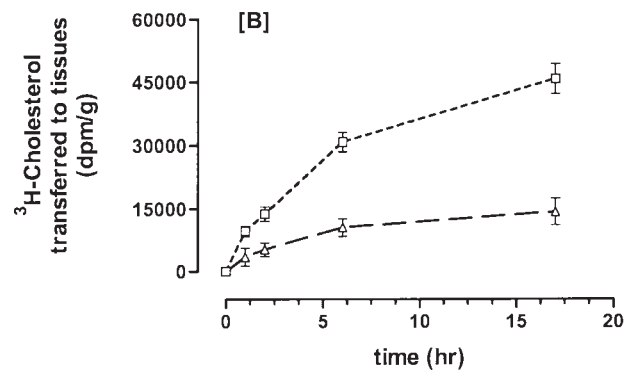
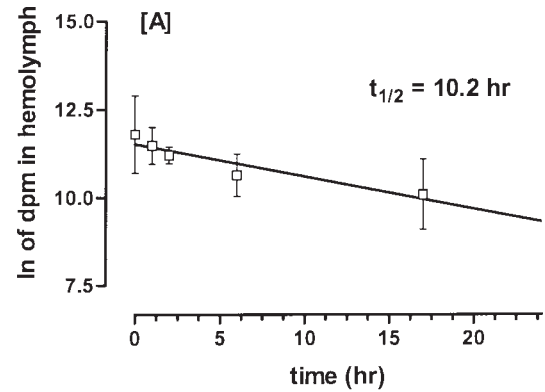


Fig. 5. Clearance of  $^3\text{H}$ -cholesterol-labeled lipophorin. Second day 5th instar *M. sexta* were injected with  $^3\text{H}$ -cholesterol-labeled lipophorin (50  $\mu\text{l/g}$  body weight, 1500 dpm/ $\mu\text{l}$ ). A: At the indicated time, hemolymph was collected and analyzed for radioactivity. B: Fat body (open squares) and midgut (open triangles) were isolated, washed, homogenized, and used for the determination of radioactivity. Values represent averages  $\pm$  SE of 4–5 determinations.

### Cholesterol and Cholesterol Esters Profile in *M. sexta*

Figure 6 shows the distribution of radioactivity between free and esterified cholesterol in male and female larva (24 h after feeding), pupa (7 days after pupation and before oocyte formation) and adults (2 days after eclosion). In the larval stage, the midgut of both males (87  $\pm$  5%) and females (94  $\pm$  3%) stored cholesterol mainly in the free form but cholesterol esters were significantly higher in males (13  $\pm$  4%) than in females (6  $\pm$  1%) (Fig. 6A). Similar to *M. sexta* larva, many other insects (Turunen and Chippendale, 1977; Kuthiala and Ritter, 1988)

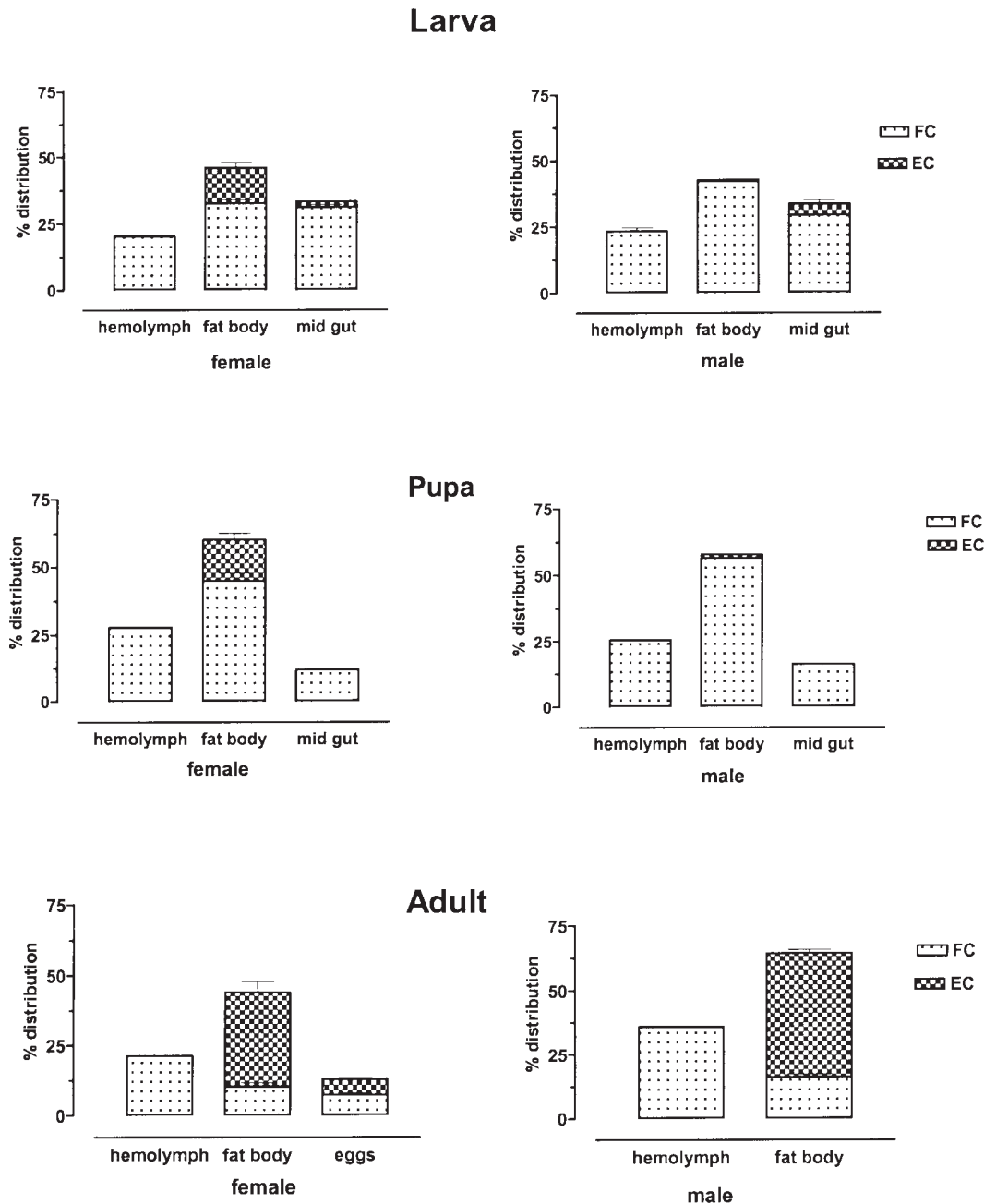


Fig. 6. Distribution of cholesterol and cholesterol ester in larva, pupa, and adult male and female *M. sexta*. Second day fifth instar *M. sexta* were fed  $^3\text{H}$ -cholesterol and dissected out after 24 h of feeding (larva), 7 days after pupation (pupa) and 2 days after enclosure (adult). Samples of hemolymph, midgut, and fat body homogenates were

extracted as described in Materials and Methods. Extracted lipids were applied to TLC Silica plates. Cholesterol and cholesteryl ester bands were identified with iodine vapor, scrapped and counted for radioactivity. Values represent averages  $\pm$  SE of 5 determinations.

and vertebrates (Thomson and Dietschy, 1981) have been shown to absorb free cholesterol in the epithelial cells and store excess cholesterol as chole-

sterol ester. Following absorption, cholesterol was released into the hemolymph where it was exclusively found in the free form (99%) (Fig. 6B).

In the fat body, the distribution of radioactivity between cholesterol and cholesterol ester varied significantly between genders and stages of development (Fig. 6C). In the larval stage, females had significantly higher cholesterol esters ( $29 \pm 3\%$ ) compared to males ( $1 \pm 1\%$ ). This ratio remained relatively constant during the pupal stage for both genders. The ratio of cholesterol ester to cholesterol significantly increased in adult males (31.2-fold) and females (3.1-fold), compared to pupa. Cholesterol ester accounted for 75 and 78% of total cholesterol in fat body from males and females, respectively. The increase of labeled-cholesterol ester at the adult stage appears to represent its storage form.

Seven days after pupation, the midgut still exists, although the insect is undergoing metamorphosis and is in the process of losing this tissue. The disappearance of esterified cholesterol in the midgut of both genders suggests that the cholesterol ester pool is the first to be depleted, as cholesterol is shuttled out of the midgut to the fat body where it is stored as cholesterol ester. This observation is supported by the fact that in the fat body, labeled cholesterol ester slightly increased during pupation and far exceeded free cholesterol in adults. Similar results were obtained in the larval *Aeshna cyanea* (Komnick and Giesa, 1994). The absence of cholesterol ester in lipophorin in the hemolymph probably reflects the specificity of export of free cholesterol from tissues to lipophorin, although the mechanism is unknown.

In oocytes, the percentages of radioactive cholesterol ( $57 \pm 5$ ) and cholesterol esters ( $43 \pm 5$ ) were distributed almost equally. Incorporation of cholesterol into the eggs has been shown to be required for the developing embryo (Saxena, 1978; Behmer et al., 1999). In addition, cholesterol in the meconium, the metabolic waste products discharged at adult emergence, accounted for  $22.5 \pm 4\%$  and  $20.4 \pm 3.8\%$  of the radioactivity in females and males, respectively. Thompson et al. (1974) have shown that *M. sexta* meconium contains mainly derivatives of cholesterol, 3-epiecdysone and 3-epi-20-hydroxyecdysone.

## SUMMARY

Cholesterol is the precursor of the membrane sterols and the molting hormones, ecdysones, which are essential for driving the molecular and cellular events that lead to molting and metamorphosis (Sehnal, 1989; Gilbert et al., 1988, 1996). In *M. sexta* absorption of this sterol is relatively slow compared to triacylglycerol (Tsuchida and Wells, 1998). Cholesterol is absorbed into the midgut lumen mainly in the free form and transported via lipophorin exclusively in the free form. Lipophorin, the sole carrier of cholesterol in hemolymph, distributed its sterol to different tissues, including the fat body where cholesterol is stored both in the free and the esterified forms. Mobilization of cholesterol demonstrates the importance of lipophorin in the transport process and its selective uptake by different tissues.

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## LITERATURE CITED

- Behmer ST, Elias DO. 2000. Sterol metabolic constraints as a factor contributing to the maintenance of diet mixing in grasshopper (Orthoptera: Acrididae). *Physiol Biochem Zool* 73:219–230.
- Behmer ST, Elias DO, Grebenok RJ. 1999. Phytosterol metabolism and absorption in the generalist grasshopper, *Schistocera americana* (Orthoptera: acrididae). *Arch Insect Biochem Physiol* 42:13–25.
- Bell RA, Joachim FG. 1976. Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann Entomol Soc Am* 69:635–673.
- Canavoso LE, Jouni ZE, Karnas JK, Pennington JE, Wells MA. 2001. Fat metabolism in insects. *Annu Rev Nutr* 21:23–46.
- Chino H, Gilbert LI. 1971. The uptake and transport of cholesterol by haemolymph lipoproteins. *Insect Biochem* 1:337–347.
- Fernando-Warnakulasuriya GJP, Tsuchida K, Wells MA. 1988. Effect of dietary lipid content on lipid transport and stor-



- age during larval development of *Manduca sexta*. *Insect Biochem* 18:211–214.
- Gilbert LI, Combest WL, Smith WA, Meller VA, Rountree DB. 1988. Neuropeptides, second messengers and insect molting. *Bioassays* 8:153–158.
- Gilbert LI, Rybczynski R, Tobe S. 1996. Endocrine cascade in insect metamorphosis: Post-embryonic reprogramming of gene expression in Amphibian and insect cells. San Diego: Academic Press. p 59–107.
- Grieneisen ML. 1994. Recent advances in our knowledge of ecdysteroid biosynthesis in insects and crustaceans. *Insect Biochem Mol Biol* 24:115–132.
- Joshi M., Agarwal HC. 1977. Site of cholesterol absorption in some insects. *J Insect Physiol* 23:403–404.
- Jouni ZE, Winzerling JJ, McNamara DJ. 1995. 1,25-Dihydroxyvitamin D<sub>3</sub>-induced HL-60 macrophages: regulation of cholesterol and LDL metabolism. *Atherosclerosis* 117:125–138.
- Komnick H, Giesa U. 1994. Intestinal absorption of cholesterol, transport in the haemolymph, and incorporation into fat body and Malpighian tubules of the larval dragonfly *Aeshna cyanea*. *Comp Biochem Physiol Comp Physiol* 107:553–557.
- Kuthiala A, Ritter KS. 1988. Uptake of cholesterol and cholestanol by intestine, hemolymph, and fat body of *Heliothis zea*. *Arch Insect Biochem Physiol* 7:225–236.
- Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T<sub>4</sub>. *Nature* 227:680–685.
- Lozano R, Thompson MJ, Svoboda JA, Lubsy WR. 1989. Profiles of free and conjugated ecdysteroids and ecdysteroid acids during pupal-adult development of *Manduca sexta*. *Arch Insect Biochem Physiol* 12:63–74.
- Markwell MAK, Hass SM, Bieber LL, Tolbert NE. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* 87:206–210.
- Meyer-Fernandes JR, Gondim KC, Wells MA. 2000. Developmental changes in the response of larval *Manduca sexta* fat body glycogen phosphorylase to starvation, stress and octopamine. *Insect Biochem Mol Biol* 30:415–422.
- Ostlund RE Jr, Bosner MS, Stenson WF. 1999. Cholesterol absorption efficiency declines at moderate dietary doses in normal human subjects. *J Lipid Res* 40:1453–1458.
- Prasad SV, Rayan RO, Law JH, and Wells MA. 1986. Changes in lipophorin composition during larval-pupal metamorphosis of an insect, *Manduca sexta*. *J Biol Chem* 261:558–562.
- Sakuari S, Warren JT, Gilbert LI. 1989. Mediation of ecdysone synthesis in *Manduca sexta* by a hemolymph enzyme. *Arch Insect Biochem Physiol* 10:179–197.
- Saxena YP. 1978. Variation in the total sterols, free sterols and sterol esters concentration during embryogenesis in *Dysdercus similis* (Freeman). *Biochem Exp Biol* 14:385–387.
- Sehnal F. 1989. Hormonal role of ecdysteroids in insect larvae and during metamorphosis. In: Koolman J, editor. *Ecdysone: from chemistry to mode of action*. New York: Thieme Medical Publishers. p 271–278.
- Shapiro JP, Keim PS, Law JH. 1984. Structural studies on lipophorin, an insect lipoprotein. *J Biol Chem* 259:3680–3685.
- Svoboda JA, Weirich GF. 1995. Sterol metabolism in the tobacco hornworm, *Manduca sexta*: a review. *Lipids* 30:263–267.
- Svoboda JA. 1999. Variability of metabolism and function of sterols in insects. *Crit Rev Biochem Mol Biol* 34:49–57.
- Thompson MJ, Kaplains JN, Robbins WE, Dutky SR, Nigg HN. 1974. 3-Epi-20-hydroxyecdysone from meconium of the tobacco hornworm. *Steroids* 24:359–366.
- Thomson ABR, Dietschy JM. 1981. Intestinal lipid absorption: major extracellular and intracellular events. In: Johnson LR, editor. *Physiology of the gastrointestinal tract*. New York: Raven Press. p 1147–1220.
- Tsuchida K, Wells MA. 1988. Digestion, absorption, transport and storage of fat during the last larval stadium of *Manduca sexta*. Changes in the role of lipophorin in the delivery of dietary lipid to fat body. *Insect Biochem* 18:263–268.
- Turnern S, Chippendale GM. 1977. Lipid absorption and transport: sectional analysis of the larval midgut of the corn borer, *Diatraea grandiosella*. *Insect Biochem* 7:203–208.
- Warren JT, Gilbert LI. 1996. Metabolism in vitro of cholesterol and 25-hydroxycholesterol by the larval prothoracic glands of *Manduca sexta*. *Insect Biochem Mol Biol* 26:917–929.