INTRODUCTION

In vitro culture techniques for parasitoids have contributed knowledge to physiological and biochemical studies on parasitic insects and indicate that mass production of natural enemies is promising for biological pest management (Thompson, 1986a, 1999; Grenier et al., 1994; Vinson, 1994; Cônsoli and Parra, 1999). Successful in vitro rearing of parasitic hymenoptera remains restricted to egg-, pupal-, and ecto-parasitoids. However, no larval endoparasitoids have been reared from embryo to adult due to complex interactions between the hosts and wasp larvae. Endoparasitoids have probably coevolved with their hosts and use host substances both for nutrition and as regulatory signals (Thompson, 1999). Many artificial media have been developed for a wide range of parasitoid species, which often require insect-derived materials as sources of essential nutrients and physiological cues (Nettles, 1990).

Venturia canescens (Hymenoptera: Ichneumonidae) is a larval endoparasitoid of pyralid moths (Salt, 1976). Their 1st larval ecdysis is synchronized with the host ecdysis to the final instar (Corbet, 1968). Our previous studies have shown that V. canescens development to the 2nd larval instar is arrested in a basic medium, although 2nd larval instar larvae developed to the final larval instar in the same medium (Ohbayashi et al., 1994; Yamamoto et al., 1997). Nakahara et al. (1999, 2000) showed that lipophorin, one major insect hemolymph protein, is necessary for the first larval ecdysis. The main function of lipophorin is transport of lipid throughout the insect hemocoel as a reusable shuttle (Chino, 1985; Shapiro et al., 1988; Blacklock and Ryan, 1994; Soulages and Wells, 1994; Ryan and van der Horst, 2000). It is assumed that host lipophorin act as a cue for V. canescens development to the 2nd larval instar and provides dietary lipids to support larval growth.

In this study, first, the effect of lipoproteins from various animals that contain quantitatively and qualitatively different lipids was compared and second, the effects of lipophorin lipid-depleted by lipase-treatment and liposome containing host lipids were examined to clarify whether the growth promotion effect of lipophorin

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Insect lipophorin and vertebrate lipoproteins support larval development of the endoparasitoid Venturia canescens (Hymenoptera: Ichneumonidae) as dietary lipid sources

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Abstract

We previously reported that lipophorin, an insect lipoprotein, is essential for in vitro development of the larval endoparasitoid Venturia canescens from the 1st larval instar to the 2nd instar. In this study, we compared the effect of various lipoproteins from insects and vertebrates to clarify whether the growth promotion effect of lipophorin is caused by its lipid moiety. As well as the permissive host (the greater wax moth) lipophorin, lipoproteins from a non-permissive host (the silkworm) and vertebrates (chicken and human) also promoted V. canescens development to the 2nd larval instar, suggesting that essential factors for the first larval ecdysis are ubiquitous in a wide range of animals. When total lipids from the permissive host were emulsified in a lipoprotein-free medium with multilaminar vesicles of phosphatidylcholine (liposome), V. canescens larvae underwent ecdysis in the medium. In contrast, lipophorin lipid-depleted by lipase treatment promoted larval growth less than intact lipophorin. These results indicated that the growth-promotion effect of lipophorin is mainly due to the lipid moiety of lipophorin.

Key words: Larval endoparasitoid, artificial rearing, lipophorin, lipoprotein, liposome

INTRODUCTION

In vitro culture techniques for parasitoids have contributed knowledge to physiological and biochemical studies on parasitic insects and indicate that mass production of natural enemies is promising for biological pest management (Thompson, 1986a, 1999; Grenier et al., 1994; Vinson, 1994; Cônsoli and Parra, 1999). Successful in vitro rearing of parasitic hymenoptera remains restricted to egg-, pupal-, and ecto-parasitoids. However, no larval endoparasitoids have been reared from embryo to adult due to complex interactions between the hosts and wasp larvae. Endoparasitoids have probably coevolved with their hosts and use host substances both for nutrition and as regulatory signals (Thompson, 1999). Many artificial media have been developed for a wide range of parasitoid species, which often require insect-derived materials as sources of essential nutrients and physiological cues (Nettles, 1990).

Venturia canescens (Hymenoptera: Ichneumonidae) is a larval endoparasitoid of pyralid moths (Salt, 1976). Their 1st larval ecdysis is synchronized with the host ecdysis to the final instar (Corbet, 1968). Our previous studies have shown that V. canescens development to the 2nd larval instar is arrested in a basic medium, although 2nd instar larvae developed to the final larval instar in the same medium (Ohbayashi et al., 1994; Yamamoto et al., 1997). Nakahara et al. (1999, 2000) showed that lipophorin, one major insect hemolymph protein, is necessary for the 1st larval ecdysis. The main function of lipophorin is transport of lipid throughout the insect hemocoel as a reusable shuttle (Chino, 1985; Shapiro et al., 1988; Blacklock and Ryan, 1994; Soulages and Wells, 1994; Ryan and van der Horst, 2000). It is assumed that host lipophorin act as a cue for V. canescens development to the 2nd larval instar and provides dietary lipids to support larval growth.
is caused by its lipid moiety.

**MATERIALS AND METHODS**

**Insects.** The parasitoid *V. canescens* (Hymenoptera, Ichneumonidae) was maintained on the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera, Pyralidae) as described by Nakahara et al. (1997). A permissive host, the greater wax moth (*Galleria mellonella*; Lepidoptera, Pyralidae), was reared on a semiartificial diet as described by Wani et al. (1994). A nonpermissive host, the silkworm (*Bombyx mori*; Lepidoptera, Bombycidae), a hybrid race of N601-2 and C602-3, was reared on a commercial artificial diet (Silkmate®, Nihon Nosan Kogyo Co., Yokohama, Japan) as described by Hirayama et al. (1998).

**Lipoprotein.** *G. mellonella* lipophorin was isolated from pupae as described by Nakahara et al. (1999), and designated GmHDLp. *B. mori* lipophorin was isolated from male or female pupae using the same procedure as for GmHDLp, and designated mBmHDLp and fBmHDLp. Lipophorins were stored at 4°C in darkness and used within a week.

Human low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were purchased from Chemicon International, Inc. (Temecula, CA). Very low-density chicken egg yolk lipoprotein (CEY-VLDL) and low-density chicken egg yolk lipoprotein (CEY-LDL) were isolated from newly laid eggs purchased commercially as follows: 10 ml of chicken egg yolk was mixed with 170 ml of the extraction buffer (20 mM Tris, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 2 mM (p-amidinophenyl) phenylmethanesulfonyl fluoride (p-APMSF), 0.3 mM NaH₃, pH 7.5). Insoluble materials were removed by centrifugation (18,000×g, 10 min, 16°C). The supernatant (density = ca. 1.03) was divided into 20 ml aliquots in centrifugation tubes, onto which 2 ml of the low-density solution (85.5 mM NaCl, 2.7 mM EDTA, 0.3 mM NaH₃, density = 1.005) was layered. It was centrifuged (18,000×g, 30 min, 16°C), and 2 ml of the top fraction containing chylomicron and VLDL (density < 1.01) was collected. The remainder was subjected to ultracentrifugation (200,000×g, 18 h, 16°C), and the yellow batter-like layer of sticky lipoprotein covering the top of the samples was removed with a spatula. The liquid phase was adjusted to density = 1.06 by adding high-density solution (2.6 mM NaCl, 3.0 mM KBr, 2.7 mM EDTA, 0.3 mM NaH₃, density = 1.35), on which 2 ml of the low-density solution was layered. After ultracentrifugation (200,000×g, 18 h, 16°C), 2 ml of the top fraction containing LDL (density < 1.06) was collected. Each fraction was stored at 4°C in darkness and used within a week.

**Lipase treatment of lipophorin.** Lipid-depleted lipophorin was prepared as described by Kawooya et al. (1991). GmHDLp was dialyzed against Tris-HCl buffer (20 mM Tris, 150 mM NaCl, 7 mM CaCl₂, pH 7.5) to remove any traces of EDTA. Prior to lipase treatment, 30 mg of lipophorin was incubated with 1 g of fatty acid-free albumin in 10 ml Tris-HCl buffer containing 2 mM p-APMSF for 10 min at 35°C. One milligram of lipoprotein lipase (from *Pseudomonas* sp., 29.2 U/mg, Toyobo, Osaka, Japan) was then added to the reaction medium and incubated for 4 h at 35°C. The reaction was terminated by adding 0.5 ml of 100 mM EDTA solution. Control was incubated without the enzyme and EDTA, which were added at the end of incubation. Each reaction medium was then subjected to density-gradient ultracentrifugation (200,000×g, 18 h, 16°C), and the lipophorin fraction was collected. Lipophorin density increased from 1.11 to 1.14–1.18 after lipase treatment, indicating that HDLp was converted to a very high-density lipophorin (VHDLp).

**Culture media.** Culture medium for *V. canescens* was a mixture of MGM-450 (Mitsubishi and Inoue, 1988) containing 1.43 μg/ml of 20E, FBS, and lipoprotein solution (7:2:1) (Nakahara et al., 2000). Prior to addition to culture medium, each lipoprotein was desalted into Carlson’s solution (120 mM NaCl, 2.5 mM KCl, 1.4 mM CaCl₂, 0.5 mM MgCl₂·6H₂O, 1.7 mM NaH₂PO₄·2H₂O, 0.15 mM NaHCO₃, 44.4 mM glucose; Carlson, 1946) by passage over a PD-10® column (Amerham Pharmacia Biotech, Buckinghamshire, UK) to remove KBr, EDTA, and NaH₃, and concentrated to 10, 20 or 30 mg protein/ml. FBS purchased from Gibco BRL (Rockville, MD) contained less than 0.2 mg protein/ml of LDL and 0.12 mg protein/ml of HDL. The culture medium containing 20% FBS was estimated to harbor less than 64 μg protein/ml of FBS-derived lipoprotein, which is negligible because it cannot promote *V. canescens* development (Nakahara et al., 2000).
Preparation of liposome medium. Total lipids were extracted with a 10-fold volume of chloroform/methanol (2:1) from the pupal homogenate of *G. mellonella*. After the solvent was evaporated in a N₂ stream, total lipids were quantified gravimetrically using an electronic microbalance. Lipids with a 4-fold volume of phosphatidylcholine from chicken egg (Wako Pure Chem. Indus., Ltd., Osaka, Japan) were dissolved in chloroform/methanol (2:1), subjected to a rotary evaporator forming a thin layer of lipids on the inside wall of a flask, and left in vacuo overnight in darkness to remove the solvent completely. Lipids were emulsified in MGM-450 medium containing 20% FBS by intense shaking and sonication on ice for 10 s 6 times at 10 s intervals to form multilamellar vesicles of phosphatidylcholine (liposome) surrounding hydrophobic molecules. The emulsified medium was designated as liposome medium. As a negative control, sonicated medium containing 20% FBS without lipids was designated liposome-free medium. Prior to filtration (0.45 μm pore, Millex®-HV, Millipore, Bedford, MA), 20-HE (1 μg/ml) was added to each medium. Phosphatidylcholine concentration was determined with a Phospholipid C test kit® (Wako Pure Chem. Indus., Ltd.). Liposome medium contained 12–15 mg/ml of phosphatidylcholine, so host-derived lipid concentration in the medium was estimated at 2.8–3.5 mg/ml, comparable to the medium containing 3 mg protein/ml of GmHDLp.

In vitro culture. The 48 h-old embryos (germ-band-stage) of *V. canescens* were individually transferred to 96-well microplate wells (Falcon® 3072, Becton and Dickinson, Bedford, MA) with 30 μl of culture medium as described by Nakahara et al. (1997). Embryos and larvae were cultured at 25°C without medium replenishment. After 30 d, the larval instar was determined under a dissecting microscope. Tests for each lipoprotein were repeated at least 3 times.

In the test for the effects of liposome, embryos were cultured in 10 μl of MGM-450 medium without supplements for 2 d to allow hatching, and then 30 μl of test medium (with or without liposome) was added to the culture.

Lipid analysis. Lipids extracted from each lipoprotein with chloroform/methanol (2:1) were separated by thin-layer chromatography (TLC) on a silica gel glass rod (Cromarod®-S III, Iatron, Tokyo, Japan). Prior to TLC, a known amount of cholesterol acetate was added as an internal standard. TLC was conducted combining 2 solvent systems: first, chloroform/methanol/H₂O at 16:8:1 (v/v/v) to a 4 cm height 3 times and second, hexane/diethyl ether/formic acid at 120:10:1 (v/v/v) to a 12 cm height. Lipids were analyzed by a flame ionization detector (Iatroscanner TH-10, Iatron).

RESULTS

Lipid compositions of various lipoproteins

The lipid composition of lipoproteins from insects and vertebrates were analyzed (Table 1). In lipophorin from the permissive host (GmHDLp), diacylglycerol was dominant and the composition of other lipids basically coincided with the results of Thomas (1979). After lipase treatment (VHDLp), the relative percentage of total lipids decreased from 50.5% to 18.1%, mainly due to the disappearance of diacylglycerol. Lipophorin from the nonpermissive host (*B. mori*), irrespective of gen-

*Liposome medium contains host lipids (x mg), which include 30% phosphatidylcholine (0.3x mg), and a 4-fold volume of phosphatidylcholine (4x mg). Thus, the amount of phosphatidylcholine in the liposome medium (12–15 mg) is equal to 4x+0.3x mg. Thus, host-derived lipids are 2.8–3.5 mg.
der, also promoted the development of *V. canescens* dose-dependently (Fig. 1).

We also examined the effects of triacylglycerol-rich lipoprotein from chicken (CEY-VLDL and CEY-LDL) and cholesterol-rich lipoprotein from human (LDL and HDL) instead of the diacylglycerol-rich lipoprotein from insect (Fig. 2). All lipoproteins promoted *V. canescens* development to the 2nd larval instar. Similar to insect lipoprotein, vertebrate lipoprotein also supported development

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**Table 1. Relative percentage of lipid and relative amount of each component in lipoprotein per 1 mg of protein**

<table>
<thead>
<tr>
<th>Donor</th>
<th><em>G. mellonella</em></th>
<th><em>B. mori</em></th>
<th>Chicken</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>HDLp</td>
<td>VHDLp</td>
<td>HDLp</td>
<td>HDLp</td>
</tr>
<tr>
<td>Relative percentage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>49.5</td>
<td>81.9</td>
<td>52.9</td>
<td>49.5</td>
</tr>
<tr>
<td>Lipid</td>
<td>50.5</td>
<td>18.1</td>
<td>47.1</td>
<td>50.5</td>
</tr>
<tr>
<td>Relative amount (μg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>20</td>
<td>6</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>429</td>
<td>ND</td>
<td>282</td>
<td>234</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>ND</td>
<td>ND</td>
<td>282</td>
<td>234</td>
</tr>
<tr>
<td>Cholesterol ester/Hydrocarbon</td>
<td>30</td>
<td>23</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>30</td>
<td>10</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>37</td>
<td>5</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>139</td>
<td>50</td>
<td>122</td>
<td>152</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>310</td>
<td>123</td>
<td>357</td>
<td>499</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>1</td>
<td>ND</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Lisophosphatidylcholine</td>
<td>9</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unknown</td>
<td>10</td>
<td>3</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>(Total lipids)</td>
<td>1,016</td>
<td>221</td>
<td>885</td>
<td>1,019</td>
</tr>
</tbody>
</table>

*a* Relative amount of each component per 1 mg of protein.

*b* Not detected.

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**Fig. 1. Percentage of *V. canescens* larvae reaching each larval instar in media containing lipophorin (mean±SD); 48-h-old embryos were cultured for 30 d in MGM-450 medium (20-HE 1 μg/ml, FBS 20%) containing lipophorin (1–3 mg protein/ml) from the permissive host, *G. mellonella* (GmHDLp), and nonpermissive host, *B. mori* (mBmHDLp from males, fBmHDLp from females). Each test used 10 embryos and was repeated at least 3 times.**

**Fig. 2. Percentage of *V. canescens* larvae reaching each larval instar in media containing vertebrate lipoprotein (mean±SD); 48-h-old embryos were cultured for 30 d in MGM-450 medium (20-HE 1 μg/ml, FBS 20%) containing lipoprotein (1–3 mg protein/ml) from chicken egg yolk (CEY-VLDL and -LDL) and human blood (human-LDL and -HDL). Each test used 10 embryos and was repeated at least 3 times.**
to later stadia, although human HDL was inferior as compared with other lipoproteins.

**Effect of lipid-depleted lipophorin**

In the medium containing intact HDLp, all larvae developed to the 2nd larval instar and some to the final instar (Table 2). Lipid-depleted lipophorin (VHDLp) containing little diacylglycerol also promoted *V. canescens* development to the 2nd larval instar. The yield of 2nd instar larvae, however, declined to 75% and the developmental period was significantly prolonged as compared to those in the medium containing intact HDLp (ANOVA, *p* < 0.01). Furthermore, the yields of 3rd and 4th instar larvae became markedly reduced, and no final instar larvae were obtained.

**Effect of liposome without lipoprotein**

A medium containing multilaminar vesicles (liposome) containing host lipids without lipophorin was used to determine the contribution of the lipid moiety of lipoproteins to parasitoid development. The liposome-containing medium appeared to be deleterious to *V. canescens* embryos because most embryos were damaged and died before hatching in this medium. In the basic medium (MGM-450 medium) without any supplements, most embryos developed and hatched, but 1st instar larval growth stopped. When liposome was provided to the halted larvae, development restarted and they underwent ecdysis to the 2nd larval instar (Table 3).

**DISCUSSION**

In a series of studies, we showed that lipophorin promotes larval development of the endoparasitoid *V. canescens* (Nakahara et al., 1997, 1999, 2000). Lipophorin transports lipids throughout the insect hemocoel as a reusable shuttle and appears to be exploited by parasitoid larvae as diet in a parasitized host. Host lipophorin taken up orally is likely digested into small molecules, i.e. amino acids, sterols, fatty acids, and glycerol in the alimentary canal and used as nutrients and physiological cues.

This study showed that a growth promotion effect of lipophorin is caused mainly by the lipid moiety, since parasitoid larvae developed in the liposome medium without lipoprotein (Table 3). However, the nutritional value of the protein moiety is obscure. Although it is natural that the protein moiety of lipophorin would be assimilated as a nitrogen source, the MGM-450 medium we used as a basic medium is rich in nitrogen, namely albumin (10 mg/ml) and free amino acids (approximately 300 mM), which are probably sufficient to mask the nutritive effects of the protein moiety of lipopro-

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**Table 2. Effect of lipid-depleted lipophorin on in vitro development of *V. canescens***

<table>
<thead>
<tr>
<th>Lipophorin</th>
<th>n</th>
<th>2nd % days±SD</th>
<th>3rd % days±SD</th>
<th>4th % days±SD</th>
<th>5th % days±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>100.0 13.2±1.8 a</td>
<td>76.7 17.5±1.9 a</td>
<td>40.0 22.4±2.1</td>
<td>6.7 26.0</td>
</tr>
<tr>
<td>Lipase-treated</td>
<td>30</td>
<td>75.0 15.7±3.0 b</td>
<td>35.7 21.7±3.4 b</td>
<td>3.6 26.0</td>
<td>0.0 —</td>
</tr>
</tbody>
</table>

*a 48-h-old embryos were cultured for 30 d in MGM-450 medium (20-HE 1 μg/ml, FBS 20%) containing either control-treated lipophorin (GmHDLp) or lipase-treated lipophorin (GmVHDLp). In both cases, lipophorin was supplied at a concentration of 3 mg protein/ml.

*b Developmental period from set of culture to attainment of each instar. Values (mean±SD) within a column followed by different letters indicate a statistically significant difference (ANOVA, *p*=0.01).

**Table 3. Effect of liposome on in vitro development of *V. canescens***

<table>
<thead>
<tr>
<th>Media</th>
<th>Larval yield (%)</th>
<th>2nd instar</th>
<th>3rd instar</th>
<th>4th instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposome-free</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Liposome</td>
<td>76.8±7.0</td>
<td>3.3±0.1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*a 48-h-old embryos were cultured in MGM-450 medium without supplements for 2 d, then test medium was added. The parasitoid stage was examined at 21 d of culture.
tein. In contrast, the MGM-450 medium is lacking in lipids. Lipoprotein may compensate for essential and beneficial lipids and support parasitoid development. The lipids that insects fail to de novo synthesize, such as polyunsaturated fatty acids and sterols, must be taken in the diet. Polyunsaturated fatty acid benefits have been reported in some ichneumonid wasps (Yazgan, 1972; Thompson, 1976; Ferkovich et al., 2000) and sterols are essential for larval development of 2 other parasitoids (Thompson, 1981).

V. canescens aborted growth at the 1st larval instar in the basic medium and lipid sources were needed for them to develop into the 2nd larval instar (Figs. 1, 2, Table 3). However, 2nd instar larvae developed to the final larval instar in a lipid-free medium, although yields of final instar larvae was low (Yamamoto et al., 1997). With regards to V. canescens lipid-requirements, the larval developmental stage can be divided into 2 phases: (1) from hatching to the 1st larval ecdysis where lipids are essential and (2) from the 2nd larval instar to the last instar where lipids are not essential.

In the V. canescens-host system, the parasitoid larva does not undergo ecdysis until host larvae reach the final instar (Corbet, 1968). A particular lipid may be a regulating factors for this developmental synchrony. Irrespective of donor, insect or vertebrate, all lipoproteins tested successfully induced the 1st larval ecdysis (Figs. 1 and 2), indicating that essential lipids for development to the 2nd larval instar are likely ubiquitous in a wide range of animals. Elucidation of the essential lipids for V. canescens is needed in future studies and will clarify the physiological mechanisms of developmental synchrony.

VHDLp containing only 18% lipid also promoted development to the 2nd larval instar, probably due to residual lipids. However, growth promotion into later stadia was much inferior than for intact HDLp (Table 2), suggesting that lipids are not essential but beneficial for later larval development. Comparing GmHDLp and VHDLp, CEY-VLDL and LDL, or human LDL and HDL, irrespective of the dominant lipid (i.e. diacylglycerol, triacylglycerol, and cholesterol), good results were observed in the lower-density lipoproteins. Therefore, lipid supply appears to be important for support of parasitoid development in later stadia. Even if using CEY-VLDL containing extremely large amount of lipids, only a few larvae attained the last instar. This might be caused by dispensation of medium replenishment. Usually, endoparasitoid larvae obtain fresh food in the living host. We thus expect that medium replenishment would raise the yield of final instar larvae.

Lipid composition also affected the growth promotion effect of lipoproteins (Table 1). Compared to other lipoproteins, a lower effectiveness of human HDL might be caused by lower amounts of fatty acid storage, such as acylglycerols, phosphatidylethanolamine and phosphatidylcholine. In the polyphagous parasitoids that adapt to a wide range of hosts, de novo synthesis of fatty acids is limited, and a large part of the fatty acids are from the diet (Thompson, 1986b). Therefore, dietary fatty acids appear to be extremely important for larval development of polyphagous V. canescens. However, excess free fatty acids are toxic, so they should be supplied in a bound form. Thus, diacylglycerols from insects, triacylglycerols from vertebrates, and phosphatidylcholine commonly detected from all lipoproteins can be utilized. Possibly, V. canescens also possesses flexible requirements for fatty acids, which allows nonspecific requirements of lipoproteins.

The greatest contribution of this study is that V. canescens underwent ecdysis in the medium containing emulsified lipids without lipoprotein. However, the liposome medium was inferior to the lipoprotein-containing medium, thus requiring further modifications to improve parasitoid development. Using liposome, which allows provision of chemically defined lipids to parasitoid larvae, the effects of each lipid can be elucidated by further studies. Knowledge of the lipid-requirements of V. canescens will give insight into the physiological interactions between parasitoid and host, and contribute to development of a chemically defined medium for rearing.

ACKNOWLEDGEMENTS

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REFERENCES

Carlson, J. G. (1946) Protoplastic viscosity changes in differ-


