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Sites of Synthesis and Transport Pathways of Insect Hydrocarbons: Cuticle and Ovary as Target Tissues[1]

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SYNOPSIS. **The outer surface of insects is covered with a lipid layer that provides water-proofing and protection against environmental stresses. Hydrocarbons (HC) are major constituents of this epicuticular wax and they also serve as semiochemicals. In some insects HC are also exploited as biosynthetic precursors for pheromones. HC are synthesized by oenocytes which are situated in the integument or hemocoel. Shuttling of HC to the epicuticle, fat body, and gonads requires transport through an aqueous medium. Insects, unlike vertebrates, use a versatile lipoprotein to effect lipid transport and to selectively deliver lipids to specific tissues. A high-density hemolymph lipoprotein (lipophorin [Lp]) serves this function. In adult females of the German cockroach (*Blattella germanica*), Lp carries both HC and a contact sex pheromone. Lipophorin is a multi-functional lipid carrier serving also as a juvenile hormone binding protein in many insects. Studies of the interactions between Lp and HC are beginning to unravel the routes used in delivering HC to target tissues. We discuss the pathways and dynamics of loading of Lp with HC and HC-derived pheromones, their transport through the hemolymph, and deposition in various tissues, including the epicuticle, ovaries, and pheromone-emitting glands.**

INTRODUCTION

Insect cuticle is a complex of chitin fibrils in a matrix of proteins to which muscles, tracheae, and the alimentary canal are anchored and against which hydrostatic pressure can be exerted by the hemolymph. An epicuticular lipid layer covers the outer surface of the cuticle in insects. Hydrocarbons (HC), primarily *n*-alkanes, alkenes, and methyl-branched components, are by far the most common epicuticular lipids. Their functions, shown experimentally or inferred, are many. The hydrophobic characteristics of HC contribute significantly to water balance (Hadley, 1984), primarily by retarding water loss, but also by keeping water out of spiracles. In addition, epicuticular HC serve as chemical messengers (semiochemicals, infochemicals). In insects HC serve as pheromones that mediate intraspecific interactions, kairomones that mediate predator-prey and parasitoid-host interactions, and allomones that function in defense (Howard, 1993). HC also function in chemical mimicry and camouflage (Dettner and Liepert, 1994). Specific HC have been implicated in mediating recognition of plants by insects: surface HC of plants are an important determinant of their acceptability to feeding or oviposition by phytophagous insects (Eigenbrode and Espelie, 1995). In tritrophic interactions the surface chemistry of plants in combination with the HC profile of insect hosts can determine acceptability of host insects to predators and parasitoids. Cuticular HC also represent the first chemical barrier to the entry of pathogens, and HC also serve as kairomones for entomopathogenic fungi and bacteria whose adherence to the cuticular surface is dependent upon HC-mediated distinction between hosts and non-hosts (Lecuona *et al.*, 1991). Cuticular HC may also constitute a physical matrix for slow release of insect pheromones and as wax blooms (*e.g.*, scale insects) HC constitute a physical barrier against parasites and predators. The hydrophobic and lubricating properties of HC may also facilitate or impair the ability of insects to gain a foot-hold on smooth sur-

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faces. Interestingly, despite the ubiquitous role that HC play in insect-insect and insect-plant interactions, little is known about sensory transduction mechanisms for HC stimuli.

HC are synthesized in insects through elongation of fatty acyl-CoAs, fatty acid reduction to aldehydes, and fatty aldehyde conversion to alkanes that contain one less carbon (Blomquist *et al.*, 1987, 1993; Nelson, 1993; Nelson and Blomquist, 1995). In a few insect species HC are further modified into more polar constituents. In the German cockroach (*Blattella germanica*), for instance, 3,11-dimethylnonacosane, a major component of epicuticular HC, is hydroxylated to an alcohol and oxidized to yield a methyl ketone that functions as a courtship-inducing contact sex pheromone (Chase *et al.*, 1990, 1992; Schal *et al.*, 1991). A few of the enzymes that catalyze these biosynthetic steps have been partially purified in insects and excellent progress has been made in the last two decades in the chemical characterization, biogenesis, and chemotaxonomy of HC (Blomquist and Dillwith, 1985; Blomquist *et al.*, 1987, 1993; Lockey, 1988, 1991; Nelson, 1993; Nelson and Blomquist, 1995). However, the sites of HC biosynthesis and the pathway(s) by which HC are stored, mobilized, and transported have received relatively little attention.

Three important processes are central to all events involving HC (including molt-related recruitment of HC to the new cuticular surface, repair of the abraded wax layer, uptake of dietary HC, and transport of HC to internal sites): (1) regulated synthesis of HC, (2) its uptake from biosynthetic and storage sites, and (3) tissue-specific deposition of HC. Our objectives in this essay are thus to (1) review work on the sites of HC synthesis in selected representative insects; and (2) examine pathways of HC transport to sites of deposition, including the cuticular surface and gonads. The developmental patterns of HC synthesis are beyond the scope of this review and the reader is referred to Blomquist *et al.* (1993) and Nelson and Blomquist (1995) for recent summaries.

SITES OF HYDROCARBON BIOSYNTHESIS

Few studies have examined the precise site(s) of HC synthesis. Oenocytes, large cells that are rich in smooth endoplasmic reticulum and mitochondria and that appear to be restricted to abdominal tissues, have been implicated in HC synthesis in a number of insects (Romer, 1991). In the American cockroach (*Periplaneta americana*), the oenocytes are distributed below the epidermal cells of the abdominal tergites and sternites, and are separated from the hemocoel by a basal membrane (Kramer and Wigglesworth, 1950). Early results showed that the integument, but not the fat body, synthesized HC *in vitro* in this species (Nelson, 1969), and these results were subsequently confirmed (Katase and Chino, 1982). Similarly, in the housefly (*Musca domestica*), both epicuticular HC and HC sex pheromones are synthesized in the abdominal integument (Dillwith *et al.*, 1981), and in both *P. americana* and *M. domestica* microsomal preparations from the abdominal integument synthesize HC whereas preparations from the fat body do not (Blomquist *et al.*, 1993). Ultrastructure studies of integument from German cockroaches reveal that oenocytes are located in the epidermis (Liang and Schal, 1993). Radiotracer studies with various body parts of adult female *B. germanica* (Gu *et al.*, 1995) and last stadium nymphs (unpublished data, H.P.Y) incubated *in vitro* confirmed that only the abdominal integument synthesized appreciable amounts of HC. In these experiments, abdominal sternites synthesized more HC than abdominal tergites. Furthermore, HC-derived sex pheromone was also synthesized in the abdominal integument of adult females. A microsomal fatty acid synthetase has been isolated from *B. germanica* (Juárez *et al.*, 1992). It synthesizes methyl-branched fatty acids which are then presumably converted to methyl-branched HC. The abdominal integument was a rich source of this enzyme compared with other tissues. However, the precise cellular localization of HC synthesis in this insect will have to await purification of oenocytes from the abdominal integument.

In the locusts *Schistocerca gregaria* and

Locusta migratoria the oenocytes are found only in the peripheral fat body which is situated beneath the abdominal epidermis, and in these species the peripheral fat body synthesizes more HC than any other tissue (Diehl, 1973, 1975; Katase and Chino, 1984). HC synthesis by isolated oenocytes has been documented only in *Tenebrio molitor* in which the oenocytes are grouped along the upper side of the tracheal trunks, separated from the fat body (Romer, 1980).

It thus appears that the ectodermally-derived oenocytes synthesize HC in both hemimetabolous and holometabolous insects. Because HC biosynthesis is a cyclic event in many insects, it will be of interest to determine whether concomitant cyclicity can be detected in the morphology, ultrastructure, or degree of polyploidization of oenocytes. In this context, abrasion of the cuticular lipids of cockroaches presumably induced lipid secretion by the oenocytes (that is, reduced oenocyte volume) and increased deposition of epicuticular lipids (Kramer and Wigglesworth (1950). However, because oenocytes serve many roles, including cuticle and hormone synthesis, alterations in oenocyte size may be related to their other functions.

In some insects HC can be extracted from specialized glands. Examples include the abdominal pheromone glands of some moths (Yin *et al.*, 1991) and various exocrine glands in social insects (*e.g.*, postpharyngeal gland of ants, Vienne *et al.*, 1995). In these insects it is unclear whether HC are synthesized within these specialized tissues or transported to and concentrated within them.

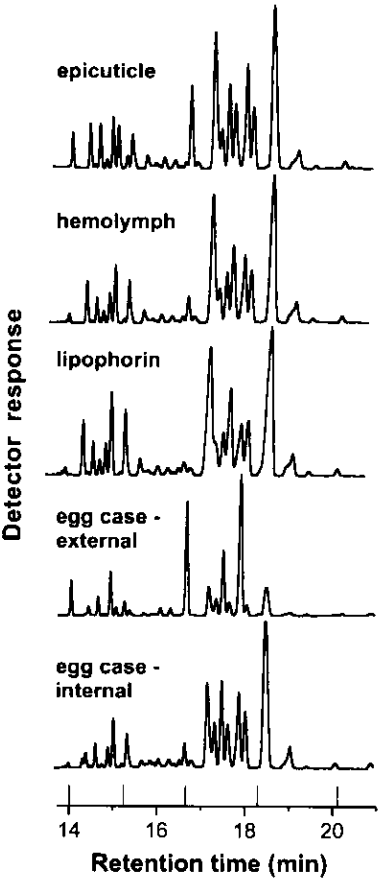

FIG. 1. Partial gas chromatograms of HC extracted from 6-day-old female *Blattella germanica*. HC are shown from the epicuticle, hemolymph, and lipophorin. HC were also extracted from the exterior and interior of the egg case from mated 25-day-old females. The vertical lines above the abscissa represent retention times for n-C_{27}, n-C_{28}, n-C_{29}, n-C_{30}, and n-C_{31}, respectively from left to right.

"INTERNAL" AND EPICUTICULAR HYDROCARBONS ARE SIMILAR

In a number of insects an internal HC pool has been characterized after the epicuticular lipids had been extracted, and in most insects the profile of internal HC appears qualitatively similar to cuticular HC (Fig. 1). Examples include members of the Orthoptera (locust, Chino and Kitazawa, 1981; house cricket, Cripps *et al.*, 1988), Dictyoptera (American cockroach, Katase and Chino, 1982; German cockroach, Gu *et al.*, 1995), Diptera (house fly, Blomquist *et al.*, 1987; fruit fly, Pho *et al.*, 1996), Hymenoptera (honey bee, Francis *et al.*, 1989), Lepidoptera (tobacco hornworm, Coudron and Nelson, 1981; cabbage looper, Dwyer *et al.*, 1986; southern armyworm, Guo and Blomquist, 1991), and Coleoptera (Japanese beetle, Bennett and Shotwell, 1973; Colorado potato beetle, Dubis *et al.*, 1987). The internal HC might represent pools of HC in the oenocytes, epidermis, hemolymph, fat body, and/or gonads. In insects with oenocytes in the hemocoel a hemolymph transport pathway(s) would clearly be required to deliver HC to the epicuticle.

By contrast, in insects whose oenocytes are bound within an integumental basal membrane hemolymph transport of HC to the epicuticle might not be necessary; HC might pass from the oenocytes to adjacent epidermal cells and then through pore canals to the epicuticle. Yet, large amounts of HC can be extracted from the hemolymph of all species examined to date, suggesting that an indirect, internal transport pathway may be universal among insects.

The integument is not the only target tissue for HC deposition. HC are found in other internal tissues, most notably the fat body and gonads (Schal *et al.*, 1994; Gu *et al.*, 1995). However, few studies have separated internal HC into various tissue compartments. Quantitative radio-inulin determinations of hemolymph volume showed that a major fraction of the internal HC in adult females (Gu *et al.*, 1995) and nymphs (Young and Schal, 1997) of the German cockroach was associated with the hemolymph. The hemolymph contains approximately as much HC as does the epicuticle and it thus serves not only as a transport medium but also as a HC reservoir. Early in the last stadium of this cockroach all internal HC is associated with the hemolymph, but as more HC is synthesized and the imaginal cuticle begins to form, hemolymph HC titer remains relatively constant and HC becomes associated with the pharate integument and fat body (unpublished data, H.P.Y). These results highlight the dynamic nature of internal HC and emphasize the need to identify and distinguish among tissues that synthesize, store, transport, and receive HC.

LIPOPHORIN, A MULTI-FUNCTIONAL LIPID CARRIER, TRANSPORTS HYDROCARBON

Hemolymph transport of hydrophobic ligands, such as HC, requires plasma lipoproteins. Chino and co-workers (see below) showed that in several insect species hemolymph HC associated only with a $M_r \sim$ 600 kDa lipoprotein, lipophorin (Lp). Lp in most insects is characterized by two constituent apoproteins, apoLp-I and apoLp-II, with approximate masses of 240 kDa and 80 kDa, respectively (Chino *et al.*, 1981; Chino, 1985; Kanost *et al.*, 1990; Blacklock and Ryan, 1994; Soulages and Wells, 1994). Lipids may comprise up to 50% of the mass of Lp, but the lipid composition varies greatly among species. In all insects examined to date Lp is well loaded with phospholipids. Flying insects generally have larger amounts of diacylglycerol, whereas the HC content of Lp is highly variable among insects and probably related to the amount of HC on their cuticular surface. An important feature of Lp is its dynamic nature. It can range from a low-density particle (LDLp), resulting from an exchangeable association of apoLp-III ($M_r \sim$ 17–20 kDa) and diacylglycerol with high-density Lp (HDLp), to a very high-density complex (VHDLp) that is depleted of lipids. HC-carrying Lp is generally characterized as a high-density Lp (density of 1.09 to 1.18 g/ml). However, in some insects a higher density lipid transfer particle (LTP; see below) also carries HC (Chino, 1985). Unlike vertebrate lipoproteins, insect Lp is generally considered to be a reusable particle that shuttles lipids among tissues without entering cells (Chino and Kitazawa, 1981; Van Heusden *et al.*, 1991). An exception to this is some insect eggs, which endocytose Lp (see below). High-density Lp serves multiple transport functions in insects. It has specific, high-affinity binding sites for juvenile hormone III in Coleoptera, Isoptera, Diptera, Hymenoptera and Dictyoptera (Trowell, 1992; Sevala *et al.*, 1997). It also carries carotenoids and other lipid ligands. The multiplicity of its functions and its use by different developmental stages suggest that the lipid composition of Lp is probably related to the stage-specific function that it serves. Recent reviews of Lp structure, physico-chemical properties, and function include Chino (1985), Kanost *et al.* (1990), Law *et al.* (1992), Van der Horst *et al.* (1993), Blacklock and Ryan (1994), and Soulages and Wells (1994).

UPTAKE OF HYDROCARBON BY LIPOPHORIN

The hemolymph of *P. americana* contains large amounts of HC and virtually all newly synthesized HC that enters the hemolymph is Lp-bound. Other hemolymph proteins are devoid of HC (Chino *et al.*, 1981). HC accounts for 28.3% of the total

lipids in Lp and 14.0% of the total mass of Lp. Moreover, hemolymph Lp that was purified by DEAE-cellulose chromatography contains the same three HC components that are found in the hemolymph and epicuticle, indicating that Lp mediates the transport of HC to the integument. *In vitro* studies confirmed that Lp was required for the incubation medium to be able to accept HC from ^{14}C-HC-labeled integument; a Ringer solution and native hemolymph proteins of higher density than Lp (probably vitellogenin) failed to accept HC (Katase and Chino, 1982). Moreover, radio-gas chromatography of HC that were extracted from the purified Lp established that the three HC components were labeled (Katase and Chino, 1984). Similarly, purified Lp from locust and Colorado potato beetle also contain HC identical to the respective hemolymph and epicuticle (Chino and Kitazawa, 1981; Katagiri and de Kort, 1991), indicating that Lp serves as a vehicle for transport of species-specific HC from sites of synthesis to the cuticle in all three species.

Oenocyte-containing tissues from both locust and cockroach can exchange their HC with both native and heterospecific Lp, indicating lack of species-specificity of HC loading by Lp (Katase and Chino, 1984). The HC composition of Lp thus appears to be determined largely by the types of HC biosynthesized by the oenocytes. This appears to be the case for diacylglycerol as well. Cockroach midgut containing labeled diacylglycerol was incubated in a Ringer solution only or in Ringer solutions that contained purified Lp from the cockroach, locust, or silkworm (Chino and Kitazawa, 1981). Lipophorin of all three species equally loaded diacylglycerol from cockroach midgut, indicating lack of molecular specificity of diacylglycerol loading by Lp.

We have recently established that newly synthesized integumental HC, as well as topically applied radiolabeled HC, rapidly enter the hemolymph and are translocated to various tissues in the German cockroach (Gu *et al.*, 1995). High-density Lp (density = 1.109 g/ml) was isolated from adult females of *B. germanica* and purified by density gradient ultracentrifugation (Fig. 2A).

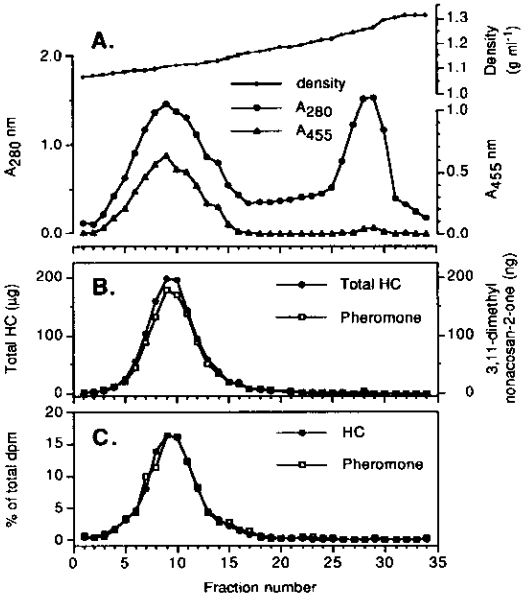

FIG. 2. Potassium-bromide gradient ultracentrifugation of hemolymph from adult female *Blattella germanica*. (A) Absorbance at 280 (protein) and 455 nm (carotenoids) and densities of KBr fractions. (B) Amounts of total HC and 3,11-dimethylnonacosan-2-one, a pheromone component, associated with each fraction. (C) Percentage of injected [11,12-^{3}H]3,11-dimethylnonacosane and methyl ketone pheromone, to which the HC was converted, recovered from each fraction. Figure re-drawn from data in Gu *et al.* (1995).

All hemolymph HC in this cockroach is associated only with Lp and no other hemolymph protein (Fig. 2B). Furthermore, injected radiolabeled HC was recovered from high-density Lp, as was the radiolabeled contact sex pheromone to which the HC was converted in the adult female (Fig. 2C; Gu *et al.*, 1995). These results showed for the first time that pheromone was transported by Lp. A time-course of incorporation of ^{14}C-propionate showed that newly synthesized HC first appears in the epidermal fraction and hemolymph and later on the epicuticle. These results support the hypothesis that Lp loads newly synthesized HC and pheromone from the abdominal oenocytes and transports them to the epicuticle.

Hemolymph HC of male and female German cockroaches are qualitatively identical to the respective integumental HC and Lp-bound HC (Fig. 1; unpublished data, C. S.).

Notably, the methyl-ketone contact pheromone that is derived from a HC in the integument of adult females is carried by high-density Lp and deposited on the epicuticle (Gu *et al.,* 1995). Adult males do not produce this pheromone and their Lp lacks this component (unpublished data, C.S.). In *Drosophila,* too, male and female high-density Lp carry different HC that serve as sex-specific sex pheromones (Pho *et al.,* 1996). Therefore, the sex-specific composition of Lp also supports the notion that its HC load is determined by the types of HC synthesized by sex-specific oenocytes.

The lack of species-specificity in uptake of HC by Lp is interesting in view of observations that Lp-antibodies show immunologic specificity and do not cross-react with Lp from other species. Locust-Lp-antibodies detected the presence of both male and female locust Lp and hemolymph, but not of purified Lp from cockroach or silkmoth (Chino and Kitazawa, 1981). We have shown recently that polyclonal antibodies directed against German cockroach Lp cross-reacted with hemolymph of *Supella longipalpa,* which is in the same family (Blattellidae) as *B. germanica,* but did not recognize hemolymph from cockroaches in other families (unpublished data, V.L.S.).

An important uninvestigated question is the role of low-density Lp in HC transport. In insects that are capable of lipid-fueled flight adipokinetic hormone, a peptide released from the corpora cardiaca, stimulates conversion of stored triacylglycerol to diacylglycerol. Concomitant with the uptake of diacylglycerol by high-density Lp, apoLp-III associates with high-density Lp to form a low-density Lp particle. Interestingly, in *L. migratoria* adipokinetic hormone also stimulates the loading of HC and phospholipids (Surholt *et al.,* 1992). These observations conflict with earlier results (Chino and Yazawa, 1986) and neither the source of the HC nor its function in sustained flight are understood.

DEPOSITION OF HYDROCARBON IN THE INTEGUMENT AND GONADS

When lipophorin that contains labeled HC is injected into cockroach or locust hemocoel labeled HC appear on the cuticular surface in a time-dependent manner (Katase and Chino, 1982, 1984). However, Lp that is loaded with labeled diacylglycerol fails to release diacylglycerol to the integument, showing that different Lp-bound ligands are unloaded at different target sites. Experimental support for Lp-mediated delivery of HC to the epicuticle was provided in *B. germanica* by severing the veins to the fore-wings and thus blocking hemolymph transport to the wings (Gu *et al.,* 1995). The amount of newly-synthesized radio-labeled HC that appeared on the wings was significantly lower than on intact fore-wings. In this species the wings do not biosynthesize HC. These results, and the abundance of epicuticular HC on non-synthesizing body parts (*e.g.,* wings, legs), clearly show that in the German cockroach HC and its derivative sex pheromone are transported by high-density Lp, which shuttles lipids from the abdominal oenocytes to epicuticular deposition sites. However, while Lp must deliver HC to integument that does not synthesize HC, its role in delivery of HC to integumental tissue that synthesizes HC (*i.e.,* sternites and tergites) is unknown. Nor is it known whether transfer of HC to target cells is cell surface-mediated or whether high-density Lp enters the cell, unloads HC, and is then resecreted out of the cell.

HC is common in insect eggs. For example, complex mixtures of branched HC, including mono-, di- and tri-methyl HC were identified from eggs of *M. sexta* (Nelson and Sukkestad, 1970). We have shown that eggs of *Blattella* contain the same types of HC as the hemolymph, Lp, and cuticle of the adult female (Fig. 1). In *B. germanica* only 150 μg HC accumulate on the adult female epicuticular surface while up to 450 μg accumulate within the female during the pre-oviposition period of egg maturation (Schal *et al.,* 1994). The internal HC are divided primarily between the ovaries and 150 μg of Lp-bound HC in the hemolymph. During oocyte maturation ovarian HC increase from 3.5 μg on day 1 to 232 μg on day 8, the day before ovulation (unpublished data, C.S.). After ovulation on day 9, HC in the ovaries decline to only 8.2 μg. Thus, HC are clearly associ-

ated with oocytes and appear to serve as the cuticular HC of the embryos and first instars.

Delivery of HC to maturing oocytes involves hemolymph Lp, but no direct evidence has been published in support of this pathway. Thomas and Gilbert (1969) showed the presence of high-density Lp in silkmoth oocytes, but Chino *et al.* (1977) argued that Lp must act as a shuttle, unloading lipid at the oocyte, because the insect egg contains larger amounts of lipid than can be accounted for by the amounts of Lp and vitellogenin. Indeed, in some insects Lp is not detected in oocytes, or it represents only a minor fraction of total egg protein (*e.g., Rhodnius,* Gondim *et al.,* 1989), indicating that Lp acts primarily as a shuttle (although rapid post-endocytotic metabolism of Lp must be considered as well). In some insects, however, ovarian apoLp-I is physically and immunologically identical to hemolymph apoLp-I, indicating a common origin (Chino *et al.,* 1977). Furthermore, immunocytological observations and tracking of [^{3}H]diacylglycerol-labeled Lp and [^{35}S]apoLp-labeled Lp showed that high-density Lp from adult hemolymph is sequestered by oocytes without recycling the Lp back to the hemolymph (Kawooya and Law, 1988; Kawooya *et al.,* 1988; Van Antwerpen *et al.,* 1993). In *Hyalophora cecropia* high-density Lp and vitellogenin enter the oocytes through the same endocytotic mechanism, presumably involving receptor-mediated processes; high-density Lp is then converted into very high-density Lp particles in lipid droplets within the eggs (Kulakosky and Telfer, 1990; Telfer and Pan, 1988; Telfer *et al.,* 1991). In *M. sexta* lipid transfer particle might play a role in the delivery of lipid to the developing oocytes (Liu and Ryan, 1991).

In an ovoviviparous cockroach, *Leucophaea maderae,* monoclonal antibodies specific to hemolymph apoLp-I can detect apoLp-I in both ovarian and egg case extracts (Rayne and Koeppe, 1988). Lipophorin content of the oocytes increases in this species throughout vitellogenesis until ovulation. However, the Lp content of recently ovulated eggs is low (7.5 μg per egg) compared to their vitellin content (2,250 μg per egg) (Engelmann, personal communication). By contrast, in the viviparous cockroach *Diploptera punctata* Lp content increases up to 23 μg per ovary pair early in vitellogenesis, but declines rapidly to nondetectable levels in ovulated eggs (King and Tobe, 1993). This pattern indicates either that Lp shuttles out of the oocytes or it is rapidly metabolized within the oocytes. In *Blattella,* non-vitellogenin proteins with high lipid content, presumably Lp, have been isolated from ovaries (Kunkel and Pan, 1976). Polyclonal antibodies against hemolymph-Lp react with ovarian proteins in this species, but as in *L. maderae,* the amount of Lp in ovaries appears to be low relative to the amount of vitellin (unpublished data, V.L.S.). In *Blattella,* hemolymph-Lp is the sole vehicle for HC delivered to the ovary and Lp appears to act as a HC shuttle. The function(s) of the minor amounts of Lp that enter the ovary remains unknown, as is the fate of ovarian HC.

While delivery of HC to the gonads clearly requires Lp mediation of HC transport in females, this need is less apparent in adult males. Adult males, like other life stages, store HC in order to repair damage to the epicuticular surface. However, our preliminary evidence shows little accumulation of HC in testes or associated reproductive accessory glands of *B. germanica.* Less than 1 μg of HC can be extracted from the reproductive system of males and less than 4 μg is delivered to the female in the spermatophore (unpublished data, C.S.). Other functions of the large reservoir of hemolymph HC in adult males thus remain unknown. Notably, Lp has been detected in insect testes, but its function is also unknown (Yun *et al.,* 1994).

SPECIFICITY OF HYDROCARBON DEPOSITION

Lack of either species- or molecular-specificity of HC uptake by Lp might cause problems for insects. Phytophagous insects encounter large amounts of plant HC in their diet and through contact, and predacious insects encounter heterospecific HC, which might be available for Lp to accept at the midgut or integument. Work on grasshoppers (Blomquist and Jackson, 1973) and *M. sexta* (Nelson *et al.,* 1971) indicates that

a fraction of the dietary HC (alkanes) is transported to the cuticular surface, presumably by Lp. If this pathway prevailed in nature, species-specificity of epicuticular HC would be greatly compromised. In the honey bee, however, neither HC from pollen nor individual HC added to ingested pollen are incorporated appreciably in hemolymph HC (Francis *et al.,* 1989).

Different lipid ligands (HC, diacylglycerol, phospholipids, juvenile hormone) are deposited by Lp at different tissues. Although Lp appears to lack molecular specificity for the types of HC it binds (see above), unloading of HC from Lp to target tissues might also involve greater specificity. Disparities in some insects between the chromatographic profiles of epicuticular and internal HC (*e.g.,* de Renobales *et al.,* 1988) suggest some degree of selective unloading of Lp-bound HC. In social insects, in which individuals within a colony but in different castes (workers, soldiers) or engaging in different tasks (brood-tenders, nest builders, foragers) might synthesize different HC, selective Lp-mediated transport might serve to re-distribute HC among colony members. We can conceive of at least two situations in which certain Lp-associated HC might be selectively deposited at particular tissues: (1) Specific HC, or greater amounts of HC, might be deposited in tissues that require special attention (*e.g.,* water-proofing of joints and bases of setae, wax blooms), and (2) Deposition of semiochemical HC in specialized glands from which emission is coordinated with physiological and behavioral events.

There are many examples among insects of specific types of HC occurring on particular locations on the body (Gibbs and Crowe, 1991; Howard, 1993; Nelson and Blomquist, 1995). In the German cockroach, the HC profile on the exterior of the egg case differs significantly from the exterior of the female, her hemolymph, Lp, or the interior of the oviposited eggs within the egg case (Fig. 1). Interestingly, the egg case HC also melt at an almost 15°C higher temperature than the epicuticular HC, presumably affording the embryos added protection against water loss (unpublished data, H.P.Y). Nevertheless, while the mechanism(s) by which these insects effect tissue-specific HC profiles might involve specific Lp-target tissue interactions, it remains to be elucidated how this is accomplished. Certainly, post-deposition processes such as selective grooming and resorbtion of certain HC should not be discounted.

The synthesis and deposition of epicuticular HC and comb waxes in honey bees is another example of possible discrimination of lipid components by Lp. Young and old worker bees produce primarily HC in epidermal cells that underlie the thorax and dorsal abdomen (Blomquist *et al.,* 1980). Middle-aged bees, which actively deposit comb wax, produce primarily monoesters in ventral wax glands. Thus, stage-specific maturation of and lipid production by wax glands (related to polytheism, or temporal division of labor) is responsible for temporal changes in epicuticular lipids (Blomquist *et al.,* 1980). Unfortunately, whether Lp is loaded with these lipids and whether Lp selectively deposits lipids at appropriate target sites remains unknown.

A clear example of selective deposition of a subset of the Lp-bound HC in specific tissues can be seen in a tiger moth, *Holomelina aurantiaca.* Our preliminary observations indicate that the adult female simultaneously synthesizes short-chain HC that serve as a volatile sex pheromone and long-chain HC that are deposited on the epicuticle (unpublished data, C.S.). All HC are synthesized in association with the abdominal integument and then loaded onto hemolymph high-density Lp. 2-Methylheptadecane and related pheromone homologs of similar chain length are specifically deposited by Lp into tubular pheromone glands that in turn open and emit the pheromone near the ovipositor. Long-chain HC, on the other hand, appear on the epicuticular surface; they are specifically deposited by Lp in the integument. This appears to be a unique system of spatial dissociation of short-chain and long-chain HC, probably requiring receptors for specific HC types at the appropriate target tissues. Structure-activity studies of the specificity of HC uptake by high-density Lp and the pheromone gland are now in progress.

CONCLUSIONS

Hydrocarbons serve a multiplicity of functions in insects. Over the last two decades considerable information has accumulated about the ecological functions of HC as well as the biosynthetic pathways involved in HC biogenesis. Regulation of HC production, HC transport pathways, and mechanisms of cellular discrimination among HC types have been little investigated, however. Therefore, the pathways by which HC reaches the epicuticular surface remain unknown. Does it follow an intraintegumental pathway in which newly synthesized HC is delivered from oenocytes to epidermal cells and then through cuticular pore canals to the surface? Or is an Lp-mediated, indirect pathway involved, whereby newly synthesized HC is shuttled from the oenocytes to the epidermal cells by hemolymph Lp, and then to the epicuticle through pore canals? Future investigations should provide evidence for the relative importance of these two pathways in HC-synthesizing tissues that are also sites of HC deposition (*e.g.,* abdominal integument) and for non-synthesizing tissues. The mechanisms of intracellular trafficking of HC and high-density Lp and identification of specific binding sites for both have not been investigated. Is HC, for instance, unloaded at the cell surface, or does Lp enter the cell? Although biochemical studies indicate that Lp plays an important role, a recent review states that "the relative importance of a direct route of HC from epidermal cells to the insect's surface or an indirect route via Lp is not known. The exact role of Lp in the formation of the wax layer on the newly synthesized cuticle is still an open question" (Nelson and Blomquist, 1995).

As in mammals, lipid transfer between lipoprotein molecules is mediated by a relatively species-nonspecific lipid transfer particle (Blacklock and Ryan, 1994). In addition to mediating HC transfer between Lp particles, lipid transfer particle may also play a role in the transport of HC through the hemolymph. In the American cockroach HC accounts for 40% of the lipids carried by lipid transfer particle (57% phospholipid) (Takeuchi and Chino, 1993).

An emerging theme in insects is that HC synthesized during one developmental stage is used primarily by the next stage (see Blomquist *et al.,* 1993; Howard, 1993). This hypothesis is supported by HC synthesis in adult females for deposition in the next developmental stage, the oocyte. Accumulation of HC, therefore, might be an important limiting factor in larval growth and in reproduction. The role of HC and Lp in embryonic development and mechanisms of sequestration of HC into oocytes have received little attention. Much benefit would be derived from such studies, including possible targets for disrupting these processes in pest insects.

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