Cuticular Hydrocarbon Synthesis in Relation to Feeding and Developmental Stage in Nymphs of *Blattella germanica* (Dictyoptera: Blattellidae)

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ABSTRACT The patterns of hydrocarbon synthesis and transport to the epicuticle were examined in males and females of the German cockroach, *Blattella germanica* (L.), during the last nymphal stadium. Methods used to extract hydrocarbon from insects were validated in detail. A double hexane extraction for cuticular hydrocarbons and a triple chloroform-methanol extraction of homogenized insects for internal lipids removed essentially all hydrocarbon from the respective compartments, whereas the external extraction did not remove hydrocarbon from the interior of the insect. Synthesis in vivo was measured by the incorporation of [1-^{14}C]propionate into methyl-branched hydrocarbon at 2-d intervals throughout the stadium. In both sexes, hydrocarbons were synthesized at increasing rates during the first 2/3 of the stadium, then synthesis ceased 2 d before the imaginal molt. Hydrocarbon synthesis was related to stage-specific food intake in both male and female nymphs. A declining proportion, but relatively constant quantity, of newly synthesized hydrocarbon was transported to the epicuticle as the nymph progressed through the intermolt period. The majority of the newly synthesized hydrocarbon at all ages was retained internally, however, suggesting that they serve as a source of epicuticular and ovarian hydrocarbon in the adult. Gas-liquid chromatography confirmed the patterns of hydrocarbon synthesis and showed a greater accumulation of hydrocarbons internally than on the nymphal epicuticle. Early in the last stadium, the majority of internal hydrocarbon is in the hemolymph, whereas late in the stadium the fraction of internal hydrocarbon in the hemolymph declines, presumably as newly synthesized hydrocarbon begin to associate with fat body and the developing imaginal cuticle.

KEY WORDS *Blattella germanica*, cockroach, cuticular lipids, hydrocarbons, feeding

CUTICULAR HYDROCARBONS ARE a vital component of insect cuticle. They serve as barriers to moisture and therefore provide waterproofing (Hadley 1981, Blomquist et al. 1993). As chemical messengers, hydrocarbons mediate communication among insects (review: Howard 1993) and between insects and plants; surface hydrocarbons of plants are an important determinant of their acceptability to insect herbivores. In tritrophic interactions, the surface chemistry of plants as well as the hydrocarbon profile of insect hosts determine acceptability of host insects to predators and parasitoids (see Espelie et al. 1991). Recent studies show that hydrocarbons also serve as kairomones for entomopathogenic fungi and bacteria; adherence to the cuticular surface is dependent on hydrocarbon-mediated distinction between hosts and nonhosts (Binnington 1993). As components of oocytes, hydrocarbons might serve an important maternal contribution by providing a water barrier for embryos and 1st instars (Gu et al. 1995). Despite excellent progress in the last 2 decades in the chemical characterization and biosynthesis of hydrocarbons (reviews: Blomquist et al. 1987, 1993; Lockey 1988; Nelson & Blomquist 1995), the storage, mobilization, and transport of hydrocarbons have received little attention. To this end, a necessary first step is to describe the patterns of synthesis and transport of hydrocarbon during development of model insects.

The developmental time course of hydrocarbon synthesis has been studied in several holometabolous insects, particularly in the Lepidoptera, Coleoptera, and Diptera. A common theme emerging from earlier work is that immature insects synthesize hydrocarbons while feeding and store them in internal sites for use primarily in the developmental stadium that follows the molt. In Lepidoptera, hydrocarbon synthesis in larvae is correlated with the period of active feeding, followed by cessation of synthesis during the wandering phase and through ecdysis (de Renobales and Blomquist 1983, Dwyer et al. 1986, Guo and Blomquist 1991). Hemimetabolous insects, specifically crickets, also have an age-dependent pattern of hydrocarbon synthesis (Cripps et al. 1988). In the adult female German cockroach, *Blattella germanica* (L.), hydrocarbon

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synthesis is related to the gonotrophic cycle; synthesis is high during an early feeding phase, followed by relatively low hydrocarbon synthesis during a fasting period while the ootheca is carried by the female (Schal et al. 1994). We now report an age-related pattern of hydrocarbon synthesis which varies throughout the last stadium of the German cockroach during preparation for the imaginal molt.

The hydrocarbons most often studied in insects are the most external, cuticular hydrocarbons. Because our studies of hydrocarbon synthesis, transport, and accumulation depend on exhaustive extraction of the outer surface of the insect, it is important to use extraction procedures that remove all of the external hydrocarbons, but none from internal compartments within the insect. Similar requirements are imposed in chemotaxonomic studies of insect hydrocarbons and in semiochemical investigations which depend on analysis of uncontaminated cuticular surfaces. Conversely, biochemical studies of hydrocarbon synthesis and in vivo transport require an exhaustive extraction of internal hydrocarbons which may contain much greater amounts of hydrocarbon than are present on the epicuticular surface. In this report we conduct an extensive analysis of extraction methods and validate that our procedure segregates external and internal pools of hydrocarbon.

Materials and Methods

Insects. Insects used in this study originated from an American Cyanamid (Princeton, NJ) insecticide-susceptible strain. They were raised in glass jars at 27°C, 50% RH, and photoperiod of a 12:12 (L:D) h. Insects were provided with Purina Rat Chow #5012 (Purina Mills, St. Louis, MO) and water ad libitum. All insects used in experiments were staged to within 2 h of ecdysis into the last nymphal stadium in midphotophase and were not allowed to eat their exuviae. Pairs of nymphs were placed in clean plastic petri dishes (90 by 15 mm) with distilled water and chow. Preliminary work showed that chow samples somewhat larger than an insect would consume in the last stadium contained no hydrocarbons detectable by gas-liquid chromatography (GLC).

Chemicals. Sodium [1-^{14}C]propionate (51 mCi/mmol) and [carboxyl-^{14}C]inulin (1.7 mCi/g) were obtained from NEN Research Products (DuPont, Boston, MA) or American Radiolabeled Chemicals (St. Louis, MO). [11,12-^{3}H]3,11-Dimethylnonacosane was provided by G. Prestwich (University of Utah, Salt Lake City) and prepared as described in Chase et al. (1992). All other chemicals were from Sigma (St. Louis, MO), Bio-Rad (Richmond, CA), or Fisher (Pittsburgh, PA).

Extraction, Separation, and Quantification of Hydrocarbons. All insects were killed by freezing at −20°C overnight. External lipids were extracted from the cuticle by immersing a pair of dead, thawed male or female insects in 2 ml *n*-hexane containing 15 μg *n*-hexacosane as internal standard, mixing gently for 5 min, decanting the solvent into a clean vial, repeating the wash, then rinsing the vial and insects with 1 ml hexane. Hexane extracts were blown to dryness under a gentle stream of nitrogen, taken up in 250 μl hexane, and loaded onto silica gel (Biosil A, Bio-Rad) in a Pasteur pipet minicolumn. The hydrocarbon fraction was eluted with 7 ml hexane, taken to dryness, and brought up to 150–250 μl for the injection of 1.0 μl into a splitless injector leading to an HP-1 capillary column 25 m by 0.32 mm by 1 μm (Hewlett-Packard, Avondale, PA). A Hewlett-Packard 3890II GLC was interfaced with a Hewlett-Packard Vectra 486/33T data acquisition computer. The temperature program held the column at 150°C for 2 min, then increased the temperature at 10°C/min to 245°C, followed by a 1°C/min temperature ramp to 260°C. The internal standard and all insect hydrocarbons eluted during this period. The temperature of the column was then taken at 10°C/min to 280°C and held for 2.5 min. This program resulted in baseline separation of all hydrocarbons. The injector and flame-ionization detector were held at 280 and 300°C, respectively.

Internal lipids, including those in any developing imaginal cuticle, were extracted by a modification of the procedure of Bligh and Dyer (1959). The pairs of insects previously extracted for epicuticular lipids were homogenized for 30 s (Polytron, Brinkmann, Westbury, NY) in chloroform/methanol/water (2:2:1.8 ml) containing 30 μg *n*-hexacosane as internal standard and the resulting homogenate vortexed for 30 s and centrifuged at 1,500 × *g* for 5 min. The chloroform layer was transferred to a clean vial, and the vortex centrifuge extraction of the methanol–water phase was repeated twice more with the addition of 2 ml chloroform each time. The chloroform was evaporated under nitrogen and the lipid extract was then taken up in hexane and fractionated and chromatographed as above.

Hydrocarbon Biosynthesis. Methylmalonyl-CoA, derived from propionate, acts as a methyl-branch donor in the synthesis of methyl-branched hydrocarbons in *B. germanica* (Blomquist et al. 1979, Chase et al. 1990). After being anesthetized by chilling on an ice bath for 10–20 min, insects were injected with 0.12 μCi sodium [1-^{14}C]propionate in 1 μl *B. germanica* iso-osmotic saline solution (Kurtti and Brooks 1976) using a 10 μl Hamilton syringe (Reno, NV) with a 33-gauge needle. The needle was inserted between the last 2 abdominal sternites; injections were monitored through a dissecting microscope. Insects were freeze-killed 8 h later, external and internal hydrocarbons were extracted as above, and the hydrocarbon fractions were dissolved in Scintillene cocktail (Fisher) and analyzed on a Beckman 5801 (Fullerton, CA) liquid scintillation spectrometer.

Extraction Method Development. We wanted to develop extraction methods that would remove all cuticular but no internal hydrocarbons and subsequently would extract all internal hydrocarbons. External and internal extracts were made as described above but with each 2-ml aliquot of solvent analyzed separately. Single insects in the radio labeling experiments were injected as above with either 0.08 μCi sodium[1-^{14}C]propionate in 1 μl saline, incubated for 24 h and frozen, or 0.017 μCi [11,12-^{3}H]3,11-dimethylnonacosane in 0.2 μl ethanol followed by freezing and extraction within 20 min. The individual insects were extracted 4 times for external hydrocarbons and 3 times for internal hydrocarbons. Extracts containing ^{14}C-hydrocarbons were prepared on silica gel minicolumns as described above before counting; samples containing ^{3}H-hydrocarbon were taken to dryness and counted. Samples for GLC were extracted as pairs, and each aliquot from the first 2 extractions was analyzed separately. The 3rd extracts were pooled and concentrated to 12 μl, 1.2 μl of which was analyzed by GLC.

Hemolymph Volume. We followed the procedure described and validated by Gu et al. (1995). Chilled nymphs were injected with 26,000 dpm [carboxyl-^{14}C]inulin and maintained at 27°C for 2 h. Hemolymph samples were collected in a 5-μl capillary tube from a clipped cercus and subjected to liquid scintillation spectrometery.

Body Mass and Daily Food Intake. Fresh body mass was measured on a Sartorius 1712 MP8 balance (Westbury, NY). Food intake by pairs of nymphs was determined by daily changes in mass of dishes containing ground chow.

Statistical Analysis. All measures of deviation around means are represented by the standard error of the mean. Single variate data were analyzed by the Student *t*-test (Microsoft 1995). Correlation analysis used Pearson's correlation (Microsoft 1995). To examine time lags between food intake and hydrocarbon synthesis, and between hydrocarbon synthesis and its accumulation (mass), we performed successive correlation analyses after shifting the output variable by 2-d intervals relative to the input variable (e.g., hydrocarbon synthesis relative to food intake).

Results

Validation of Hydrocarbon Extraction and Hydrocarbon Synthesis Methods. The efficiency of our cuticular extraction was determined by analyzing each successive 2-ml hexane wash separately. The first 5-min hexane wash removed almost all (98.33 ± 0.58%, $n = 5$) of the available hydrocarbons, whereas the second 5-min rinse extracted the remainder (1.67 ± 0.58%) of quantifiable hydrocarbon. A third 5-min wash failed to produce GLC peaks larger than baseline noise even in pooled, concentrated samples representing 1–2 nymph equivalents. Thus our 3-step extraction, consisting of two 5-min hexane washes, followed by a rinse of the extraction vial and sample, is reliable and simple. The efficiency of the cuticular extraction procedure was confirmed using radio-labeled hydrocarbon. Nymphs were injected with 0.08 μCi sodium[1-^{14}C]propionate, incubated for 24 h and freeze-killed. Some newly synthesized methyl-branched hydrocarbon was transported to the epicuticular surface within 24 h (see below). The 1st two 5-min washes recovered 99% of the labeled methyl-branched external hydrocarbon (Table 1). Similar results (99%) were obtained with insects extracted 20 min after they were injected with tritiated 3,11-dimethylnonacosane, the major component of *B. germanica* hydrocarbon (Table 1).

Table 1. Validation of the extraction method: mean ± SE recoveries of radiolabeled hydrocarbon from external (cuticular) and internal extractions

Extraction	^{14}C-labeled hydrocarbon[a]	^{3}H-labeled hydrocarbon[b]
Cuticular		
First 5-min wash	94.43 ± 0.32	92.15 ± 0.89
Second-5 min wash	4.61 ± 0.27	5.84 ± 0.51
Third 5-min wash	0.59 ± 0.04	1.27 ± 0.37
Fourth-5 min wash	0.37 ± 0.05	0.74 ± 0.10
Internal		
First extraction	86.98 ± 2.78	89.65 ± 1.06
Second extraction	11.97 ± 2.72	9.44 ± 1.04
Third extraction	1.05 ± 0.12	0.91 ± 0.10

Successive procedures on the same sample add up to 100%.

[a] Sodium [1-^{14}C]propionate (0.08 μCi) was injected in 1 μl saline; cuticular and internal hydrocarbons were extracted 24 h later; $n = 6$.

[b] [11,12-^{3}H]3,11-Dimethylnonacosane (0.017 μCi) was injected in 0.2 μl ethanol; cuticular and internal hydrocarbons were extracted 20 min later; $n = 9$.

The extraction of internal hydrocarbons by the method of Bligh and Dyer (1959), after the 3-step external extraction, recovered 99% of the internal ^{14}C-labeled hydrocarbon in the first 2 chloroform extractions (Table 1), a 3rd extraction adding the remaining 1% to the total recovered (214 of 21,500 dpm). Similar results were obtained with insects injected with tritiated 3,11-dimethylnonacosane and whose exterior had been extracted 4 times with hexane before homogenizing the nymphs. Of recovered hydrocarbons, 99% of the internal ^{3}H-labeled hydrocarbon was found in the first 2 internal extractions and 1% in the 3rd (309 of 33,700 recovered dpm) (Table 1). These results clearly validate the efficiency of the 3-step external and internal extractions. Hexane proved to be a suitable, mild solvent for cuticular extraction; it did not extract internal lipids.

An important variable in in vivo incubations is the duration of incubation. Fig. 1 shows the incorporation rates of [1-^{14}C]propionate into hydrocarbon at several incubation times ranging from 2 to 24 h in both females and males. Within 4 h the synthesis of radiolabelled hydrocarbons was complete; incubation times as long as 24 h did not increase either total incorporation of the isotope or

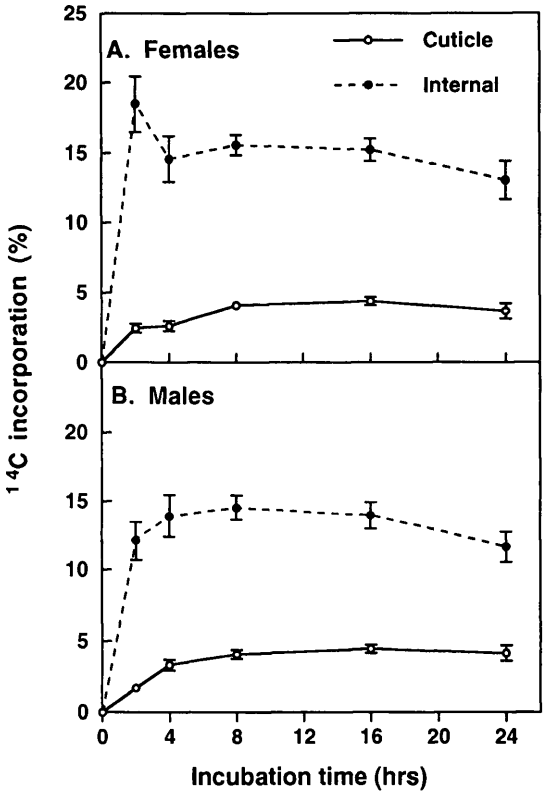

Fig. 1. Time-course of label incorporation from [1-^{14}C]propionate into methyl-branched cuticular and internal hydrocarbons in 6-d-old last instar *B. germanica*. (A) Females. (B) Males. Nymphs were injected 0.12 μCi and incubated in vivo for the times indicated. Data represent mean ± SE; n = 6–14 females, 6–9 males.

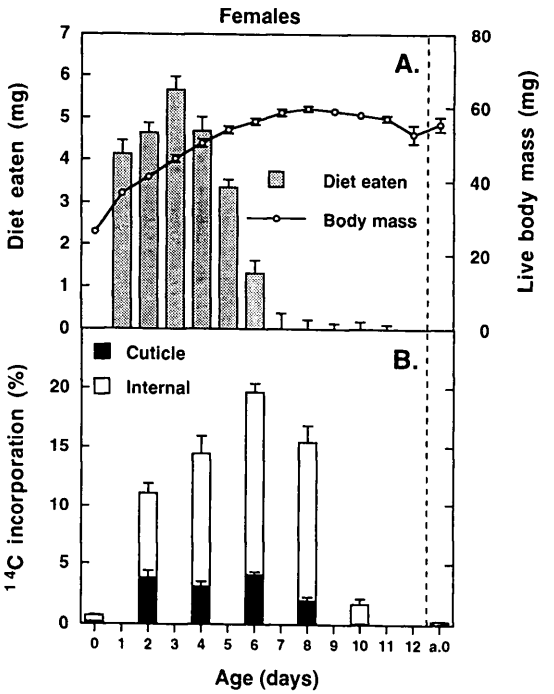

Fig. 2. Relationship between daily food intake and hydrocarbon synthesis over the course of the last nymphal stadium of female *B. germanica*. (A) Daily food intake and body mass. (B) Hydrocarbon synthesis was assayed by incorporation of [1-^{14}C]propionate into methyl-branched hydrocarbons. The top of each bar in B represents total hydrocarbon synthesis. Shown are means ± SE; n = 23. The dashed vertical line indicates the imaginal molt.

the relative distribution of labeled hydrocarbons between epicuticular and internal sites. This supports the conclusion that the cessation of incorporation was the result of depletion or excretion of the radiolabeled precursor. Transport of newly synthesized hydrocarbon to the cuticular surface changed little 8 h after injection. The incubation time used in our experiments (8 h) was chosen to allow completion of synthesis and distribution of hydrocarbons while remaining well within both the daily cycle and the 12 h of the photophase. One replicate performed under identical conditions during midscotophase showed no difference between the 2 phases of the photoperiod (data not shown).

Patterns of Feeding and de novo Synthesis of Methyl-Branched Hydrocarbon. Last instars exhibited cyclic feeding in relation to the molt cycle. The amount of food consumed increased for the first 3 d in females (Fig. 2A) and 4 d in males (Fig. 3A). Body mass increased concurrently. Subsequently, after the nymphs had doubled their mass, food intake rapidly declined to undetectable levels by day 7 in females and by day 8 in males. Thus, insects fed little during the latter half of the last stadium. Body mass remained relatively unchanged after day 7 in females and after day 8 in males.

Propionate selectively labels the methyl branches in *B. germanica* hydrocarbons (Chase et al. 1990) and because >80% of the hydrocarbons of the nymph are methyl-branched (Jurenka et al. 1989), this allows us to track overall hydrocarbon synthesis. Moreover, propionate labels total *B. germanica* hydrocarbons more efficiently (≈20% of injected label; Fig. 1) and more specifically than does acetate (see Chase et al. 1990). A distinct pattern of synthesis of methyl-branched hydrocarbons was evident in both male and female last instars (Figs. 2B and 3B). Synthesis increased in the first few days of the stadium to a peak in midstadium. It declined rapidly after day 8 in females (Fig. 2B) and day 10 in males (Fig. 3B) and ceased before eclosion. The pattern was asymmetrical in both sexes in that synthesis terminated more abruptly than it began. Newly eclosed adults, like newly ecdysed nymphs, exhibited very low rates of hydrocarbon synthesis.

Using sequential reordering of the time-course of hydrocarbon synthesis relative to the time-course

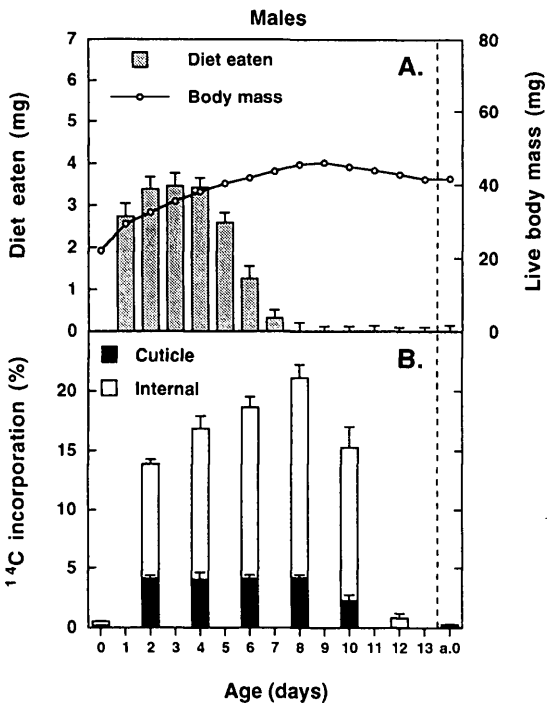

Fig. 3. Relationship between daily food intake and hydrocarbon synthesis over the course of the last nymphal stadium of male *B. germanica*. (A) Daily food intake and body mass. (B) Hydrocarbon synthesis was assayed by incorporation of [1-^{14}C]propionate into methyl-branched hydrocarbons. The top of each bar in B represents total hydrocarbon synthesis. Shown are means ± SE; error bars not visible are smaller than the symbol; n = 6–13. The dashed vertical line indicates the imaginal molt.

of food intake, we examined the time lag between food intake and hydrocarbon synthesis. Table 2 shows that the Pearson correlation coefficient, r, is maximal when the developmental course of hydrocarbon synthesis is accelerated by 2 d relative to the course of food intake. On either side, a 4-d lag, or no lag at all, result in diminished correlations,

Table 2. Pearson correlation coefficients (r), between stage-specific food intake and hydrocarbon synthesis, and between hydrocarbon synthesis and net change in hydrocarbon mass over 2-d intervals in last-stadium female nymphs

Time lag[a] 1st vs 2nd variable	Food intake vs total hydrocarbon synthesis	Total hydrocarbon synthesis vs net change in hydrocarbon mass
T_0 vs T_{-4}	0.59	0.21
T_0 vs T_{-2}	0.88	0.78
T_0 vs T_0	0.59	0.66
T_0 vs T_{+2}	−0.33	−0.54

[a] For each successive correlation, the 2nd (output) variable was shifted by ±2 d relative to the 1st (input) variable. For example, for T_0 versus T_{-2}, food intake between days 0 and 2 was correlated with hydrocarbon synthesis between days 2 and 4.

suggesting that hydrocarbon synthesis lags by ≈2 d relative to food intake.

Throughout the hydrocarbon biosynthetic period, a relatively small amount of new hydrocarbon was transported to the exterior of the nymphal cuticle. The proportion of hydrocarbon transported to the cuticle declined as hydrocarbon synthesis increased, whereas an internal reservoir received an increasingly larger proportion of newly synthesized hydrocarbon (Figs. 2B and 3B). The absolute amount of new hydrocarbon transported to the nymphal epicuticle remained relatively constant throughout the biosynthetic period. This pattern suggests that after directing hydrocarbon to the teneral nymphal cuticle early in the intermolt period, a greater proportion of newly synthesized hydrocarbon is directed to an internal reservoir, presumably for the newly synthesized imaginal cuticle. The hydrocarbon directed to the outer surface of the cuticle appears to be closely regulated, although our experiments shed no light on the origin or mechanism of allocation of newly synthesized hydrocarbon.

Patterns of Hydrocarbon Accumulation. There were definite patterns of accumulation of hydrocarbon both internally and on the cuticular surface over the last larval stadium of females and males. Fig. 4A shows the relatively monotonic increase in the amount of hydrocarbon on the exterior of the female cuticle, which doubled by the end of the stadium. The pattern seen in males (Fig. 4B) is similar.

The internal hydrocarbon of both sexes, however, increased ≈5 times during the intermolt period. Figs. 5A and 5B show the change during each 2-d interval of accumulated hydrocarbon internally and on the nymph's epicuticle. The net change in internal hydrocarbon appeared to lag by ≈2 d relative to the pattern of hydrocarbon synthesis shown in Figs. 2 and 3. Correlation analysis with sequential shifting of the output variable (hydrocarbon mass) relative to the input variable (hydrocarbon synthesis) showed a lag (1–2 d) between peak synthesis rates and maximal accumulation of hydrocarbon in female nymphs (Table 2).

Immediately after the imaginal molt, female exuviae contained 72.6 ± 3.54 μg hydrocarbon (n = 17), slightly less than the 88.1 μg found on day 12 nymphal cuticle (Fig. 4A). This suggests that little of the epicuticular hydrocarbon is internalized before the molt, especially because additional hydrocarbons might be extracted with the exuviae as tracheal and other internal hydrocarbons normally not extracted with the epicuticle. The new imaginal cuticle is covered with 73.5 ± 3.07 μg hydrocarbon (n = 17), whereas the loss in internal hydrocarbon is ≈50 μg. Clearly, nymphal internal hydrocarbons comprise the hydrocarbon of the new adult cuticle, and the internal adult hydrocarbons decline from nymphal levels.

Hemolymph Hydrocarbon. Because the homogenization procedure did not resolve the internal

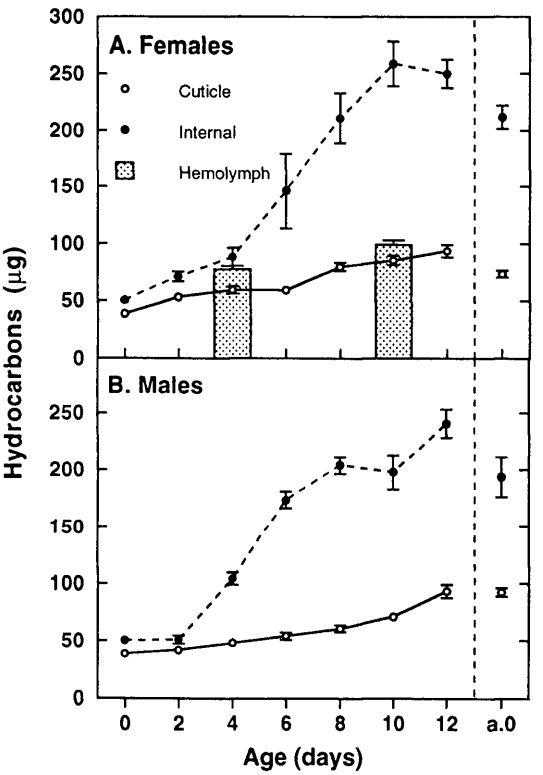

Fig. 4. Accumulated female cuticular and internal hydrocarbons over the course of the last nymphal stadium in *B. germanica* nymphs, as determined by GLC. (A) Accumulated hydrocarbon. (B) Change in hydrocarbon during 2-d interval. Hemolymph hydrocarbons on days 4 and 10 are indicated by bars. Dashed vertical line represents the imaginal molt. Data represent mean ± SE; n = 4 pairs of nymphs per data point.

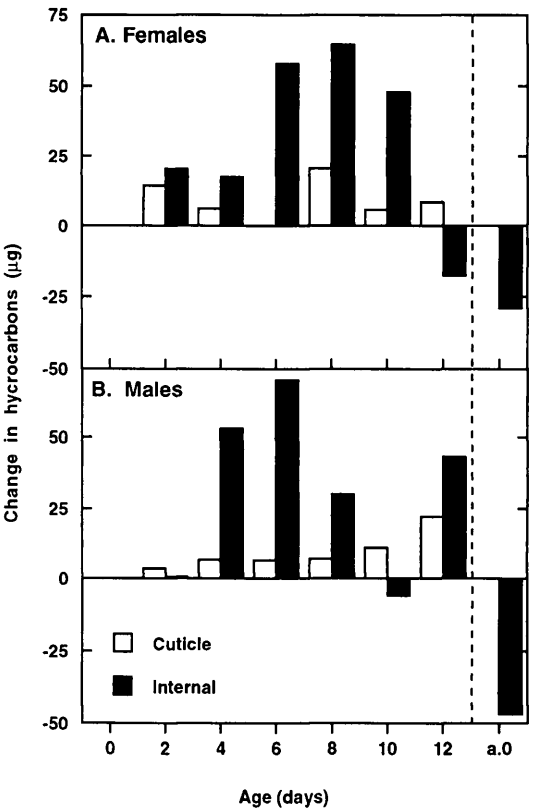

Fig. 5. Accumulated male cuticular and internal hydrocarbons over the course of the last nymphal stadium in *B. germanic* nymphs, as determined by GLC. (A) Accumulated hydrocarbon. (B) Change in hydrocarbon during 2-d interval. Dashed vertical line represents the imaginal molt. Data represent mean ± SE; n = 5 pairs of nymphs on days 2–10; 11 pairs on nymphal days 0 and 10 and adult day 0.

reservoir into various compartments, we conducted a separate study of hemolymph hydrocarbons. The hemolymph of 4-d-old last instar females contained 8.1 ± 0.26 μg/μl total hydrocarbon. Hemolymph volume, determined by the ^{14}C-inulin dilution method, was found to be 9.7 ± 0.56 μl (n = 19). Thus, nearly 100% of internal hydrocarbons were in hemolymph on day 4, presumably in association with lipophorin (Gu et al. 1995). Day 4 represents a stage before formation of the adult cuticle. In 10-d-old nymphs, hemolymph volume was 12.40 ± 0.91 μl (n = 12) and total hemolymph hydrocarbon was 98.8 μg, representing only 44% of total internal hydrocarbons. Thus, as expected, a smaller fraction of internal hydrocarbon is associated with the hemolymph as internal hydrocarbons presumably begin to associate with the imaginal cuticle.

Discussion

Hydrocarbon Extraction is Reliable and Complete. Our extraction methods remove the hydrocarbons on the exterior of the cuticle while leaving all hydrocarbons in the interior of the insect. Radiolabeling experiments (Table 1) show that 2 hexane extractions remove >99% of the cuticular hydrocarbon, presumably epicuticular hydrocarbon from only the outer surface and not hydrocarbon embedded or bound within the chitin-protein cuticular matrix. Our extraction method is nondestructive and noninvasive; specimens, even if mounted for museum preservation, can be stored safely and examined later for morphological characters.

Extraction of internal hydrocarbons is similarly reliable. Partitioning of hydrocarbons into the chloroform phase is excellent and the low water content of most insects allows the use of the final postdilution proportion of 2:2:1.8 chloroform/methanol/water (Bligh and Dyer 1959) throughout the entire extraction.

Developmental Pattern of Hydrocarbon Synthesis. Hydrocarbon synthesis and the amount and types of hydrocarbons on the cuticle may change with age, developmental stage, genetic strain, and

environmental conditions such as temperature, relative humidity and habitat. Although many studies have documented the presence of hydrocarbons in all life stages of insects, including on and in the oocytes, larvae, pupae, and adults, few studies have examined the physiological processing of hydrocarbons in relation to development. Periodic shedding of the cuticle in arthropods requires the synthesis or transport (or both) of water-proofing lipids to a new cuticle. *Spodoptera eridania* (Cramer) and *Trichoplusia ni* (Hübner) lose all their cuticular hydrocarbons during each molt because none of the external hydrocarbon is reabsorbed before the molt (Dwyer et al. 1986, Guo and Blomquist 1991). An as yet undetermined internal hydrocarbon pool that is 4–5 times greater than the mass of epicuticular hydrocarbons is used to provide cuticular hydrocarbons for the newly molted insect (de Renobales and Blomquist 1983, Dwyer et al. 1986, de Renobales et al. 1988, Guo and Blomquist 1991). Thus, although epicuticular hydrocarbons remain relatively constant throughout each stadium, internal hydrocarbons undergo distinct cycles of synthesis, accumulation, and depletion during each intermolt period. Accumulation of hydrocarbon corresponds to a pattern of hydrocarbon synthesis during early larval feeding stages. At the beginning of the wandering phase, the rates of biosynthesis decline, ceasing altogether at the time of the molt. Allocation of newly synthesized hydrocarbons to the larval epicuticle also ceases as synthesis declines (Dwyer et al. 1986, Guo and Blomquist 1991).

A different pattern of synthesis and hydrocarbon accumulation has been found in the house cricket, *Acheta domesticus* (L.) (Cripps et al. 1988) and the locust *Schistocerca gregaria* (Forsk.) (Walker et al. 1970). These species also cease hydrocarbon synthesis immediately before ecdysis but have a more constant level of hydrocarbon synthesis throughout the larval intermolt periods once synthesis resumes postecdysis. In the cricket, general lipogenesis follows a different pattern, peaking and then declining earlier in the stadium, presumably reflecting the pattern of food and water intake (Cripps et al. 1988).

The results of our experiments with [1-^{14}C]propionate show a pattern of hydrocarbon synthesis and deposition on the epicuticle more similar to the hemimetabolous than to the holometabolous pattern. Nymphs synthesize essentially no hydrocarbon immediately following ecdysis; then from day 2 to day 6 the rate of hydrocarbon biosynthesis increases in a linear fashion (Fig. 2B). Following a slight decrease over the next 2 d the rate of synthesis drops steeply to <10% of the peak rate. In contrast to the cricket, *Blattella* ceases synthesis completely during the last 4 d before eclosion, representing 1/3 of the time females spend in the last stadium.

The chromatographic data (Fig. 4) support this picture of synthesis and distribution for all hydrocarbons, including the normal alkanes which are not labeled by incorporation of propionate. Cuticular and internal pools are roughly equal (40–50 μg) in newly ecdysed nymphs. There is a slight increase in the internal pool to days 2–4, followed by a linear increase through day 10 in females and day 12 in males. In both sexes, internal hydrocarbons remain rather constant at 235–250 μg before the imaginal molt. This is more than quadruple the amount present on day 0 of the last stadium. In contrast, epicuticular hydrocarbons of last instars increase <2 times in amount.

The relatively constant rate of transport of newly synthesized hydrocarbon to the nymphal cuticle (Fig. 2B and 3B) is of interest. The GLC results suggest that, at least at the constant temperature–humidity regime used in our experiments, the insects deposit a constant quantity of hydrocarbon to the existing nymphal cuticle. Kramer and Wigglesworth (1950) suggested that hydrocarbon deposition on the epicuticle is related to hydrocarbon synthesis; adsorbing lipids from the cuticle followed by exposure of *Periplaneta americana* (L.) to low relative humidity induced hydrocarbon deposition on the cuticle, based on observations that oenocytes shrank, presumably by discharging their contents, under the treated tergites and not under the control contralateral side in the same insect. Based on our results, it appears that the rate of transport of hydrocarbons onto the cuticular surface is independent of the rate of hydrocarbon synthesis. The total rate of hydrocarbon synthesis increases steadily through days 8–10, but most of the newly synthesized hydrocarbon is kept internally. This suggests that the mechanism allocating hydrocarbon between the 2 pools, external and internal, sends a constant amount, but a declining proportion, of newly synthesized hydrocarbons to the nymphal cuticle. Chromatographic profiles of cuticular and internal hydrocarbons show that the cuticular hydrocarbons are qualitatively identical to the internal hydrocarbon (data not shown), as in adult females (Gu et al. 1995).

Internal Hydrocarbon Depots. Our data support the hypothesis that hydrocarbons synthesized at any life stage are used primarily in the next developmental stage. Only a fraction of newly synthesized hydrocarbon is deposited on the nymphal cuticle (Fig. 2). Although some hydrocarbons are lost in feces (unpublished data), the great majority of newly synthesized hydrocarbon accumulates internally during the intermolt period. Early in the stadium, most internal hydrocarbon is hemolymph-bound (Fig. 3A), presumably associated with lipophorin, as in adults (Gu et al. 1995). Late in the stadium, however, internal hydrocarbon includes large amounts of hydrocarbon not associated with hemolymph (Fig. 3A). Hydrocarbon synthesized by *T. ni* pupae is deposited on the adult cuticle within 1–4 h of adult eclosion, but not before, although the adult cuticle had been formed for some time (de Renobales et al. 1988). In *B. germanica*, we

have preliminary evidence suggesting that deposition onto the pharate cuticle occurs throughout the last 2/3 of the last stadium. After the molt, a loss of internal hydrocarbon can be accounted for by hydrocarbon on the new adult cuticular surface (Fig. 4). Thus, hydrocarbon synthesized early in the last instar is used primarily for the adult cuticle. The remaining internal hydrocarbon in the newly eclosed adult is used as a pool for continued transport to the cuticular surface as well as to the maturing oocytes (Schal et al. 1994, Gu et al. 1995).

The temporal pattern of hydrocarbon synthesis is directly related to the developmental changes the nymphs undergo as they complete the last nymphal molt and prepare for the imaginal molt. The internal reservoir of hydrocarbon is of particular interest. Internal and external hydrocarbon could be derived from common or independent biosynthetic sites. Because the internal and external hydrocarbon spectra are qualitatively identical at both sites (data not shown), it is likely that they share a common origin. Our data from adult female cockroaches (Gu et al. 1995) implicate epidermal tissue of the abdomen, probably oenocytes, as the site of hydrocarbon synthesis, and preliminary experiments in vitro with nymphal tissues support identical conclusions. Thus, the model that emerges for nymphs is as follows; in early last instars, hydrocarbons newly synthesized by the integument are loaded onto lipophorin (unpublished data), as in young adult females (Gu et al. 1995). Only a minor but constant amount of newly synthesized hydrocarbon is transported to the nymphal cuticle, presumably to replace lost hydrocarbon and to cover exposed arthrodial membrane as the volume of nymphs increases with age. Midway through the stadium, the hemolymph is loaded with hydrocarbon to a plateau level and the imaginal cuticle begins to appear; the remainder of the internal hydrocarbon pool is then accumulated in other nonhemolymph internal tissues, including the developing adult cuticle. We are currently exploring the relative importance of various other internal tissues, particularly the fat body and the pharate adult cuticle to determine the principle site of hydrocarbon accumulation. Nothing is currently known about the kind of association, if any, that occurs between hydrocarbon and the developing cuticle.

Determinants of Hydrocarbon Synthesis. It is clear that biosynthesis and transport of hydrocarbons are closely regulated during molt cycles and during reproduction. To date, however, it remains unknown in any insect system how production of hydrocarbon is regulated. Armold and Regnier (1975) suggested that ecdysteroids stimulated hydrocarbon synthesis in postfeeding larvae of the flesh fly *Sarcophaga bullata* Parker. This idea is consistent with observations in immature insects, including *B. germanica* (current report), that hydrocarbon synthesis peaks at midstadium, presumably when ecdysteroid levels are high. Nevertheless, in adult female *B. germanica* and probably in many hemimetabolous adult females, hydrocarbon synthesis generally corresponds to periods of high JH titers and low ecdysteroid titers (see Schal et al. 1994).

In several larval and adult insects, patterns of hydrocarbon synthesis correspond to feeding patterns. In the current report, we show a high degree of correlation between the amount of food consumed by nymphs and rates of hydrocarbon synthesis 2 d later. Preliminary observations of in vivo as well as in vitro synthesis of hydrocarbon in starved nymphs support a causal relationship between food intake and hydrocarbon biosynthesis (unpublished results). In addition, alteration of the pattern of food intake by removal of the corpora allata in adult female *B. germanica* results in concomitant changes in hydrocarbon synthesis (Schal et al. 1994). We are currently examining this in nymphs with nutrient-deficient diets that provide ample substrate for hydrocarbon synthesis but do not promote a molt.

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