Selective Sterol Transfer in the Honey Bee: Its Significance and Relationship to Other Hymenoptera

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The honey bee, *Apis mellifera*, is one of only a few species of phytophagous insects known to be unable to convert C-24 alkyl phytosterols to cholesterol. Regardless of the dietary sterols available to worker bees, the major tissue sterol of brood reared by the workers is always 24-methylenecholesterol, followed by sitosterol and isofucosterol. Normally, little or no cholesterol is present in honey bee sterols. The maintenance of high levels of certain sterols is accomplished through a selective transfer of sterols from the endogenous sterol pools of the workers to the developing larvae through the brood food material secreted from the hypopharyngeal and mandibular glands and/or the honey stomach of the workers. The selective uptake and transfer of radiolabeled C_{27}, C_{28} and C_{29} sterols have been studied to correlate these aspects of sterol utilization with the discovery of an unusual molting hormone (ecdysteroid) in honey bee pupae as the major ecdysteroid of this stage of development. The phylogenetic implications of this selective transfer phenomenon in the honey bee and comparison with sterol metabolism in certain other hymenopteran species emphasize the diversity of sterol biochemistry in insects.


Phytophagous species belonging to three insect orders are unable to dealkylate and convert C_{28} and C_{29} phytosterols to cholesterol. These include two species of Coleoptera, the Mexican bean beetle, *Epilachna varivestis* (1), and the khapra beetle, *Trogoderma granarium* (2); several species of Hemiptera including the milkweed bug, *Oncopeltus fasciatus* (3,4) and the cotton stainer bug, *Dysdercus fasciatus* (5); and the honey bee, *Apis mellifera*, of the order Hymenoptera (6). The inability to convert C-24 alkyl sterols to cholesterol is reflected in the ecdysteroid (molting hormone) metabolism of several of these species, since a C_{24}-ecdysteroid, makisterone A, is the major ecdysteroid during certain stages of development. This is true of last stage nymphs of *O. fasciatus* and four other species in the Pentatomomorpha group of *Hemiptera* (5,7) as well as the pupa of the honey bee (8). Thus, a C_{24} dietary sterol (e.g., campesterol) serves as a precursor for an ecdysteroid in these phytophagous species, and the usual C_{27}, C_{28} ecdysteroids, such as ec dysone or 20-hydroxyecdysone, are replaced by makisterone A.

We have studied in depth the utilization and metabolism of dietary sterols in the honey bee and discovered a fascinating aspect of sterol utilization in this species. The honey bee is capable of selectively transferring certain sterols to the developing larvae through the brood food. Regardless of the dietary sterols available to the worker bees, 24-methylenecholesterol was always the major component of the sterols isolated from prepupae or adults of the next generation (9). In addition, sitosterol and isofucosterol always were present as the next most abundant of the sterols from prepupae or adults of the next generation, with lesser amounts of campesterol (9).

This phenomenon occurred when a chemically defined diet coated with a highly purified sterol or no sterol (9) was fed, when the workers were fed pollen and sucrose in a cage study (Svoboda, J. A., and Herbert, E. W. Jr., unpublished data) and when samples were obtained from free-flying colonies (10). Studies with dietary radiolabeled campesterol, sitosterol or 24-methylenecholesterol coated on the chemically defined diet verified that none of these sterols was metabolized to cholesterol or any other sterol and that the majority of the sterols of prepupae or adults of the next generation was derived from endogenous sterol pools of the nurse bees (6). Thus, it is well-established that this selective transfer of specific C_{28} and C_{29} sterols is functional in the honey bee under all conditions studied.

Several recent studies discussed in this paper have provided additional information on the transfer of sterols from one generation to the next in honey bees. We have examined the sterols of hypopharyngeal glands, mandibular glands and honey stomachs, all of which are involved in the complex feeding system whereby the royal jelly is produced for the brood food (11). The sterols from the corresponding whole insects, royal jelly and pollen samples were compared with those of the previously mentioned organs. Studies on the incorporation of radiolabeled C_{27}, C_{28} or C_{29} dietary sterols coated on a chemically defined diet fed to workers provided additional information on selective uptake and transfer of dietary sterols to the next generation. Finally, we compared the sterols of another phytophagous hymenopteran, the alfalfa leafcutter bee, *Megachile rotundata*, and two species of omnivorous *Hymenoptera* with those of the honey bee to obtain data on sterol utilization in other species of *Hymenoptera*.

MATERIALS AND METHODS

Hypopharyngeal glands, mandibular glands and honey stomachs were carefully dissected from 100 adult bees at least two weeks old that had been reared by workers fed an artificial, chemically defined diet (12) coated with 24-methylenecholesterol (>99% pure) at a concentration of 0.1% dry weight and provided with 50% sugar syrup solution ad lib. The glands and honey stomachs were blotted dry and weighed, then stored frozen until analysis for sterol content. The colony was established with about 4,000 newly emerged yellow bees (400 g) plus a mated mating Midnite® queen to provide a genetic marker to easily identify newly emerged dark-colored progeny. The colony, in a small hive, was maintained in a 2 × 2 × 2 m screened flight cage. The adults were held overnight without access to the diet to clear the digestive tract of dietary sterol before dissection. Samples of 25 prepupae and 21 newly emerged “dark” adults were weighed and stored frozen prior to sterol analysis.

Royal jelly samples were obtained from three colonies (1500 g each) of newly emerged yellow bees maintained in five frame swarm boxes in 3 × 3 m screened flight cages. The colonies were fed sugar syrup and a freshly
collected mixture of tulip poplar, blackberry and clover pollens. Royal jelly was collected daily from queen cells to coincide with the first six days that queen larvae would be fed. Samples of royal jelly ranging from 1.47 to 4.35 g were collected each day and frozen prior to analysis. Corresponding samples of nurse bees and pollen from this experiment were weighed and stored frozen.

To examine utilization and incorporation of radiolabeled sterols, [4,4-^3H]cholesterol (236 cpm/μg sp act) (The Radiochemical Center, Amersham, England), [2,4-^3H]campesterol (380 cpm/μg sp act) (13), 24-[2,4-^3H]methylenecholesterol (392 cpm/μg sp act) (13) or [4-^4C]sitosterol (258 cpm/μg sp act) (Amersham Corp., Arlington Heights, Illinois) were coated on the chemically defined diet to achieve a concentration of 0.05% dry weight. Experimental duplicate colonies for each diet were established with 300 g of newly emerged bees plus a Midence® mated laying queen per colony and maintained in hives and flight cages similar to those previously described. Samples of 20 and 15 prepupae from each test were weighed and frozen three and four weeks, respectively, after colony establishment and from 50 to 89 “dark” adults per test were weighed and frozen at the latter time.

For the comparison of sterol utilization in phytophagous and omnivorous hymenopteran species, samples of 25 honey bee prepupae reared in a free-flying field colony foraging primarily on cucumber pollen, 30 newly emerged adult alfalfa leafcutter bees, seven balfaced hornet (Dolichovespula maculata) workers and 31 yellowjacket (Vespula maculifrons) workers were weighed and stored frozen until analysis. These samples were all field-collected in the vicinity of our laboratory.

Samples to be analyzed for sterols were homogenized in CHCl3-MeOH (2:1, v/v) with a Virtis or Polytron homogenizer. After partitioning, the CHCl3 phases were combined, dried over Na2SO4, and filtered, and the solvent was removed on a rotoevaporator. The crude lipids were saponified and the sterols in the nonsaponifiable fraction were isolated by column chromatography on Acid Grade silica (Woelm, ICN Pharmaceuticals, Cleveland, Ohio) as described previously (9). Fractions from column chromatography were monitored by thin layer chromatography. Gas liquid chromatographic analysis of sterols was accomplished with a Varian model 3700 gas chromatograph equipped with a J & W DB-1 fused-silica capillary column (240 C, 15 m × 0.25 μm film). A Shimadzu C-RIB Chromatopac processor provided information for qualitative and quantitative analyses of the sterol mixtures. Cholestane was the internal standard used in the determination of relative retention times (RRT) for identification of unknown sterols by comparison of their RRTs with those of authentic standards.

RESULTS

Table 1 summarizes the results of sterol analyses from hypophysaryngeal and mandibular glands and honey stomachs of adult bees reared in brood provisioned by workers fed the chemically defined diet coated with 24-methylenecholesterol. The data from sterol analyses of prepupa and intact adults collected at the same time as the glands and honey stomachs are included for comparison. The major sterol in each sample was 24-methylenecholesterol, which comprised >60% of the total in the sterols of hypophysaryngeal glands, prepupae and new adults and >50% of the total in the mandibular glands and honey stomachs. Sitosterol and isofucosterol were the next most predominant sterols in each sample, and 5.3-7.7% campesterol was present in each sample. The relative percentages of cholesterol in the sterols from the glands and honey stomachs were several times greater than those from the prepupae and adults.

The sterols of royal jelly samples (Table 2) collected over a six-day period contained 49.0–58.4% 24-methylenecholesterol, whereas the nurse bee sterols had 39.1% and the pollen sterols only 5.9%. Sitosterol was the major pollen sterol and isofucosterol was next in abundance; however, sitosterol and isofucosterol were most predominant after 24-methylenecholesterol in all other samples. Campesterol comprised 4.0-7.7% of the total sterols in the samples summarized in Table 2. No cholesterol was detected in any of the royal jelly, nurse bee or pollen samples analyzed in this experiment.

The sterol content of prepupae and adults reared by workers fed chemically defined diet coated with radiolabeled sterols is summarized in Table 3. The dietary sterol accumulated noticeably in each of the respective prepupal and adult samples. However, 24-methylenecholesterol was the major sterol (59.3–73.5% of total) in every sample, followed by sitosterol and isofucosterol in all but the prepupal and adult samples from the 2-H-campesterol-fed colonies. Cholesterol comprised 1.4% or less of the

<table>
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<th>Sterol</th>
<th>Hypophysaryngeal glands</th>
<th>Mandibular glands</th>
<th>Honey stomachs</th>
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<th>New adults</th>
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*Columns do not total 100%; only values for these five sterols are included for comparison.*