mine inhibit the same in cotton roots[21]. The variations possibly reflect the differences in either uptake and metabolism of individual amino acids or in the regulatory nature of the enzyme itself, in different species. Stimulation of in vivo nitrate reductase activity by alpha ketoglutarate is possibly due to an increased reductant (NADH) level through the stimulation of the tricarboxylic acid cycle.

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Definitive evidence for lack of phytosterol dealkylation in honey bees

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Summary. Neither developing honey bee larvae nor queens were able to convert dietary ^{14}C-desmosterol to cholesterol, nor was larval development of sterol metabolism affected when the inhibitor, 25-azacoprostane, was included in the worker bee diet. These results provide strong evidence that this phytophagous insect is unable to dealkylate phytosterols at the C-24 position in order to produce cholesterol.

Results from previous studies[1-3] on sterol utilization in the honey bee, *Apis mellifera* L., indicated that this phytophagous insect is incapable of dealkylating C_{28} and C_{29} phytosterols to produce cholesterol. In recent years, several species of phytophagous insects[4-6] have been found to be unable to make this conversion, although it had previously been held that the dealkylation and conversion of C-24 alkylated phytosterols to cholesterol was a trait common to phytophagous as well as many omnivorous insects[7]. The constant occurrence of low levels of cholesterol (from trace to 2.2% of total sterols) and desmosterol (from trace to 2.5% of total sterols), found in previous honey bee studies, regardless of the dietary sterol[1-3], suggested that a more definitive study should be carried out to determine whether conversion of the C_{28} and C_{29} phytosterols might occur at a very low level to produce these small quantities of desmosterol and cholesterol. Two experiments would lend themselves well to providing this information. First, feeding ^{14}C-desmosterol as the sole added dietary sterol in an artificial diet would result in easily measurable amounts of ^{14}C-cholesterol if the biochemical pathway for conversion of 28- or 29-carbon phytosterols to cholesterol is present in the insect[8,9]. Second, the addition of a potent inhibitor of the Δ^{24}-sterol reductase enzyme to the diet in combination with a 24-alkylated sterol would cause an accumulation of desmosterol in the insect sterols and perhaps adversely affect larval development if this pathway exists[10-12].

Test colonies were set up with 400 g of newly emerged 'Italian' bees (about 4000 bees) plus a mated laying queen and maintained in small hives in flight cages as previously described[13]. The chemically defined artificial diet was also prepared as in prior studies[13]. The [26(27)-^{14}C] desmosterol was purchased from Amersham Corporation, Arlington Heights, IL, USA[14] and purified by argentation column chromatography of the acetate and recrystallization. The sterol was then examined for radiochemical purity by counting areas of adsorbent scraped from thin-layer chromatography (TLC) plates in a Packard Tricarb-Liquid Scintillation Spectrometer and by trapping and counting fractions from the gas-liquid chromatography (GLC) effluent. For one test diet, ^{14}C-desmosterol (> 97% pure, sp. act. 209 cpm/µg) was coated at 0.1% (dry weight) on the dry dietary components with dichloromethane. A second test diet was coated with 0.1% (dry weight) of 24-methylenecholesterol in combination with 0.01% (dry weight) of 25-azacoprostane (prepared as published[11]). 24-Methylenecholesterol is converted to cholesterol through the intermediate desmosterol by insects having the capacity to convert 24-methyl sterols to cholesterol[15]. Two units were set up on each diet; 2 samples of prepupae (130 and 89, respectively) from the ^{14}C-desmosterol-fed colony, and 2 samples (127 and 129 prepupae, respectively) from the 24-methylenecholesterol plus 25-azacoprostane-fed colony were weighed and stored frozen until work-up for sterol analysis. The 2

queens from each diet regimen were similarly stored for analysis after the prepupal samples were collected.

After $CHCl_3$-MeOH (3:1) extraction of the frozen samples in a VirTis homogenizer, the sterols were isolated and purified by previously described methods[1] of column chromatography on alumina (Woelm, ICN Pharmaceuticals, Cleveland, OH, USA) and TLC. Qualitative and quantitative analyses were accomplished with GLC on 2 systems (2% OV-17 and 2% SE-30). Distribution of radioactivity in the sterol mixtures was determined by counting fractions trapped from GLC effluent. In addition, the sterols from the ^{14}C-desmosterol-fed bee samples were acetylated and the acetates fractionated by argentation chromatography on $AgNO_3$-impregnated Unisil (Clarkson Chemical Company, Williamsport, PA, USA) and 20% $AgNO_3$-impregnated Silica Gel H chromatoplates as previously described[15]. Argentation chromatography allows total separation of cholesterol and desmosterol acetates, and thus provides an additional method to monitor conversion of ^{14}C-desmosterol to cholesterol.

Analysis of the sterols from ^{14}C-desmosterol-fed honeybees failed to provide evidence for conversion of the dietary sterol to ^{14}C-cholesterol, either in the prepupae or queens. The total radioactivity in the purified sterols was 195,000 cpm, 112,500 cpm and 20,400 cpm for the 2 prepupal and the queen samples, respectively. The amount of recovered radioactivity associated with cholesterol in the GLC trapping experiments was less than the background prepeak in each case. Also, less than 1% of the total radioactivity was associated with the cholesterol acetate fraction from argentation chromatography of sterol acetates from prepupae or queens. In these samples, desmosterol comprised 9.2% of the total mass of sterols in the prepupae and 5.8% in the queens, whereas cholesterol was less than 1% of the total sterols in the prepupae and 2.1% in the queens. Thus, these results indicate that there is no measurable conversion of ^{14}C-desmosterol to cholesterol but that there is an accumulation of the dietary ^{14}C-desmosterol in the sterol pools of both prepupae and queens. In insects known to have the biochemical capability to dealkylate and convert C_{28} and C_{29} phytosterols to cholesterol, as much as 90% of recovered radioactivity in sterols from insects fed ^{14}C-desmosterol is associated with cholesterol[8].

The addition of 25-azacoprostane to the diet in combination with 24-methylenecholesterol had no measurable effect on larval development or on sterol metabolism in larvae or queens at the concentration tested. The levels of cholesterol and desmosterol (table) in the sterols isolated from prepupae and queens from the test colony are comparable to concentrations of these sterols found in other studies using several dietary sterols[1,2]. In other species capable of converting C_{28} and C_{29} phytosterols to cholesterol, an azasteroid such as 25-azacoprostane, at this concentration, causes a large accumulation of desmosterol from dealkylation of the dietary 24-methylenecholesterol and usually disrupts larval development[10-12]. Thus, new safe, selective control agents with these types of activities may be developed that are selectively potent against certain pest species and yet have no undesirable effects on a beneficial insect such as the honey bee.

These results provide definitive evidence that the low levels of cholesterol and desmosterol that have been commonly found in all stages of the honey bee that we have examined must originate from dietary sources available to foraging workers in the field. All of our sterol metabolism tests have been set up with adult bees that were reared in wild foraging situations, thus making it possible for small amounts of these sterols, which could come from pollen in the wild colonies, to be transferred to developing brood larvae reared under our test conditions. We have shown that cholesterol and desmosterol, along with 24-methylenecholesterol, sitosterol, and isofucosterol are selectively transferred from adult bees to developing brood under our experimental conditions using an artificial, chemically defined diet[1-3]. Evidence from this study further diminishes the likelihood that honey bees are capable of converting 24-alkylated phytosterols to desmosterol or cholesterol at detectable levels.

Percentages of cholesterol and desmosterol in total sterols from honey bee prepupae and queens from colonies fed diet containing 24-methylenecholesterol plus 25-azacoprostane

	Prepupae		Queens	
	Controls*	25-Aza-fed	Controls*	25-Aza-fed
Cholesterol	T-2.2**	0.6	0.3-1.0	1.8
Desmosterol	T-2.5	1.2	0.9-2.1	1.7

* Range of values obtained in previous studies[1,2], in percent of total sterols. ** T, trace amount detected (< 0.3%).

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