Table 2. Requirements for proline synthesis in the crude mitochondrial fraction of the blowfly. The complete incubation system contained 0.5 ml of crude mitochondrial fraction (1.318 mg protein/ml), 100 µl each of 10 mM NADPH, 10 mM NADH and 100 mM ATP, and 10 µl each of 1 M $MgCl_2$ and 6.5 µCi/ml U-^{14}C-glutamate in a final volume of 0.82 ml

Omission	None	NADPH	NADH	ATP	Mg^{++}	All cofactors
Radioactivity (dpm)	2464 ± 116	316 ± 32	1758 ± 78	56 ± 12	46 ± 8	32 ± 8

Values represent the mean ± SE for simultaneous triplicate incubations for 100 min.

roacetic acid (TCA). For determination of ^{14}C labeled CO_2 evolved during incubation, and on the addition of the acid, the gas was trapped with 0.1 ml of approximately 1 M methyl benzethonium hydroxide in methanol[5]. For the preparation of the sample for ^{14}C amino acid analysis, the reaction mixture containing 0.1 ml of 10% TCA was centrifuged. The precipitate was washed with 1 ml of 3% TCA 3 times, and the washings were combined with the supernatant. After extraction of TCA with ether, the remaining mixture was applied to a column (4 × 0.5 cm) of Dowex 50 (H^+ form). Washed with 1 ml each of 10 mM and 1 mM HCl and water, the amino acids were eluted with 2 N ammonia solution. The eluate was evaporated to dryness and dissolved in 0.5 ml of 80% ethanol. One half or one fifth of the solution was applied to a TLC plate after addition of 2 µl of a standard amino acid solution.

Thin layer chromatography. To separate proline from other amino acids, 2-dimensional TLC was performed[6], and the amino acids were localized with ninhydrin[7]. The amount of ^{14}C in the silica gel was determined by solubilizing the sample with 0.5 ml of methanol per 10 ml of toluene base scintillation fluid.

Protein determination. Protein was determined by the method of Lowry et al.[8].

Results and discussion. Table 1 shows the subcellular distribution of the decarboxylation and synthetic activity of proline from labeled glutamate in the homogenate of the abdomen. In the case of cell free extract the radioactivity incorporated into proline for 100 min corresponded to about 2.2% of that of ^{14}C glutamate incubated. It is clear that proline synthesis occurs only in the mitochondrial fraction. The amount of ^{14}C-incorporation into proline by the mitochondrial, microsomal and cytosol fractions accounted for only 54% of the total incorporation by the cell free extract, but the reasons remain obscure. The incorporation of ^{14}C into proline needs ATP, Mg^{++} and NADPH as the cofactors (table 2). Unlike the situation in the rat[9], NADH can partially replace NADPH in the blowfly. From the evidence obtained, we concluded that proline biosynthesis from glutamate in the blowfly occurs in the mitochondria through the reactions almost the same as those of *E. coli*[10].

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Lack of conversion of C_{29}-phytosterols to cholesterol in the khapra beetle, *Trogoderma granarium* Everts

J.A. Svoboda, A.M.G. Nair, Nita Agarwal and W.E. Robbins

Insect Physiology Laboratory, Agricultural Research, SEA, USDA, BARC, Beltsville (MD 20705, USA), and Department of Zoology, University of Delhi, Delhi (India), 29 November 1979

Summary. Metabolic studies in which ^{3}H-sitosterol, ^{3}H-stigmasterol, and ^{14}C-desmosterol were administered by feeding and injection to the khapra beetle, *Trogoderma granarium* Everts, provided strong evidence that this insect is unable to dealkylate phytosterols and convert them to cholesterol.

We recently reported the sterol composition of the khapra beetle, *Trogoderma granarium* Everts, reared on a diet consisting of a mixture of wheat and brewers' yeast[1]. The relative percentages of cholesterol in the pupae and adults were 1.2 and 1.3%, and the campesterol levels were 67.0 and 59.4%, respectively. Cholesterol comprised about 0.5% of the total sterols present in the diet, whereas the phytosterols campesterol (25.0%), stigmasterol (5.1%), and sitosterol (69.4%) together accounted for 99.5% of the dietary sterols. These data indicated that little, if any, of the C_{28}- or C_{29}-phytosterols ingested by the insect were converted to cholesterol and that the increase in cholesterol and campesterol content of the insect tissues, compared to the diet sterols, may have occurred through selective uptake from the diet. Previous studies had shown that 2 phytophagous insect species, the Mexican bean beetle, *Epilachna varivestis* Mulsant[2], and the milkweed bug, *Oncopeltus fasciatus* (Dallas)[3], are unable to dealkylate phytosterols such as sitosterol or campesterol and produce cholesterol. The Mexican bean beetle saturates the dietary C_{28}- or C_{29}-phytosterols before dealkylating and produces mainly stanols and some lathosterol (Δ^7-cholestenol)[2]; The milkweed bug directly incorporates unchanged dietary sterols into its tissues[3].

The present studies were carried out to determine whether *T. granarium* larvae converted either of the radiolabeled C_{29}-phytosterols, ^{3}H-sitosterol or ^{3}H-stigmasterol, incorpo-

rated in a semi-defined diet, to the C_{28}-sterol campesterol or to cholesterol[4]. In addition, ^{14}C-desmosterol, the final intermediate in the conversion of phytosterols to cholesterol in insects, was fed to *T. granarium* larvae[4]. The diet and rearing conditions were as previously described[5]. The concentration of the labeled sterols (all > 98% purity) was 0.2% dry weight of the diet, and the specific activities of ^{3}H-sitosterol, ^{3}H-stigmasterol, and ^{14}C-desmosterol were 313, 220, and 215 cpm/µg, respectively. The mature larvae were collected from the diet, counted, weighed, and refrigerated in methanol until needed for analysis. The insects were extracted with $CHCl_3$-MeOH (2:1) in a Ten-Broeck homogenizer and the sterols were isolated and purified, after saponification of the crude lipids, by column chromatography on alumina (Woelm, ICN Pharmaceuticals, Cleveland)[6] and digitonin precipitation, as previously described[7]. The sterols were identified by GLC analysis on a 1% OV-17[8] and an 0.75% SE-30[9] system. Possible conversion of labeled dietary sterols to cholesterol or other sterols was determined by trapping the GLC effluent at appropriate intervals and counting the trapped material by liquid scintillation spectrometry. Also, the sterols from larvae fed ^{14}C-desmosterol were acetylated and the acetates were chromatographed on 20% $AgNO_3$-impregnated Unisil[7]. Desmosterol acetate is readily separable from cholesterol acetate by this method. The column fractions were monitored on 20% $AgNO_3$-impregnated silica gel H chromatoplates developed in benzene-hexane (1:1). Areas of the chromatoplates were scraped and counted in scintillation vials to further check for possible conversion to cholesterol. The relative distribution of radioactivity recovered from the GLC-effluent fractions is reported in the table. The amount of radioactivity trapped in the cholesterol peak for each diet was: sitosterol, < 1%; stigmasterol, 2%; desmosterol < 1%. Clearly, there was not sufficient radioactivity associated with cholesterol in samples from insects fed the labeled dietary sterols to prove that any of these sterols were converted to cholesterol during larval development. On the contrary, the bulk of the radioactivity was associated with the dietary sterol in each case. By contrast, in previous studies with insects that are capable of converting these 3 sterols to cholesterol[9,10], the bulk of the labeling was associated with cholesterol in mature larvae fed the labeled sterol throughout larval development. Since ^{3}H-campesterol was the major impurity of the dietary ^{3}H-sitosterol, and since previous results[1] indicated there was some selective uptake of campesterol from the dietary sterols, it was not surprising to find as much as 4.7% of the radioactivity associated with campesterol in the sample from ^{3}H-sitosterol-fed *T. granarium* larvae. The radioactivity trapped in the campesterol and sitosterol GLC peak effluents from ^{3}H-stigmasterol-fed larvae occurs because campesterol and stigmasterol are not well separated on these GLC systems and, in addition, some of the labeled stigmasterol tails into the sitosterol peak area.

Comparison of relative percentages of radioactivity distribution in sterols from *Trogoderma granarium* larvae fed labeled sterols (determined by GLC trapping)

Sterol peak	% Radioactivity*		
	^{3}H-Sitosterol diet	^{3}H-Stigmasterol diet	^{14}C-Desmosterol
Cholesterol	< 1.0	2.0	< 1.0
Desmosterol	–**	–	93.4
Campesterol	4.7	5.6	–
Stigmasterol	–	85.9	–
Sitosterol	92.3	5.4	–

* Radioactivity percentages do not total 100, because the remainder was trapped in prepeak and postpeak areas. ** Recovered radioactivity < 0.1% of total.

Further proof that *T. granarium* lacks the ability to convert any of the 3 dietary sterols to cholesterol was obtained by injecting individual insects of 3 groups of *T. granarium* last-instar larvae with 1 µl suspensions (in olive oil) of one of the respective labeled sterols containing the following radioactivity: ^{3}H-sitosterol, 3500 cpm; ^{3}H-stigmasterol, 4800 cpm; ^{14}C-desmosterol, 2175 cpm. After 48 h, the insects were stored refrigerated in methanol and the sterols were isolated and examined for radioactivity distribution in the same manner as the sterols from insects fed the labeled sterols. Regardless of which sterol was injected, the radioactivity associated with cholesterol in the sterols recovered from the insects was even less than that found in the samples from insects fed the labeled sterols. These results further support the conclusion that *T. granarium* does not dealkylate and convert C_{29}-phytosterols to cholesterol. In this regard, this species is similar to another dermestid, *Dermestes maculatus* De Geer, which has been previously studied extensively with respect to sterol metabolism[11].

The khapra beetle is the 3rd species of phytophagous insect found to lack the mechanism for dealkylating plant sterols to produce cholesterol. It is quite different from the Mexican bean beetle which does dealkylate but produces mainly stanols and lathosterol from dietary phytosterols[2]. With respect to sterol utilization, *T. granarium* appears to be very much like the milkweed bug[3] except that the khapra beetle does seem to be capable of some selective uptake or retention of both campesterol and cholesterol from the diet[1]. By contrast, the milkweed bug is only able to increase slightly the relative concentration of cholesterol in its tissues over the dietary concentration. However, *T. granarium* is very unlike another beetle that is also a stored products pest, the tenebrionid, *Tribolium confusum* Jacquelin duVal; in the latter insect, 7-dehydrocholesterol comprises 50% or more of the total sterols and a nearly equivalent amount of cholesterol is also found[8]. Since these 2 species occupy fairly similar ecological niches, one might expect more similarity in sterol metabolism. However, more diversity of sterol utilization and metabolism is being found between insect species as an increasing number of in-depth studies in this area of insect biochemistry are completed. There will undoubtedly be more examples reported of insect species that possess unique mechanisms of sterol utilization and metabolism.

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