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Research Articles

Drosophila melanogaster does not dealkylate [^{14}C]sitosterol[1]

J. A. Svoboda[a], R. B. Imberski[b] and W. R. Lusby[a]

[a] *Insect Hormone Laboratory, Agricultural Research Service, USDA, Beltsville (Maryland 20705, USA), and* [b] *Department of Zoology, University of Maryland, College Park (Maryland 20742, USA)*
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Summary. Drosophila melanogaster was unable to dealkylate and convert [^{14}C]sitosterol to cholesterol and no evidence was found for conversion of [^{14}C]desmosterol to cholesterol. Therefore, *D. melanogaster* is incapable of dealkylating and converting C_{28} and C_{29} phytosterols to cholesterol.
Key words. Drosophila melanogaster; sterol metabolism; phytosterols; dealkylation; desmosterol; sitosterol; radiolabeled sterols.

Many insects can dealkylate the C-24 alkyl group from the side chain of dietary sterols and so convert phytosterols to cholesterol. Generally, those insects that can dealkylate grow about equally well on either sitosterol or cholesterol as well as on a number of other C_{28} and C_{29} phytosterols, such as campesterol (C_{28}) or stigmasterol (C_{29})[2]. Extensive biochemical studies using radiolabeled sterols supported by extensive sterol analyses, e.g. by gas-liquid chromatography (GLC) and mass spectrometry (MS), have verified that desmosterol is a common intermediate in the dealkylation and conversion to cholesterol of all phytosterols studied[2–5]. Consequently, desmosterol, as a dietary sterol, is readily converted to cholesterol in those insects that can dealkylate and supports normal growth and development[2,6]. In a study on sterol utilization by *Drosophila melanogaster*, Cooke and Sang concluded, based on the ability of various sterols to support development, that the *tu bw; st su-tu* strain of this species could dealkylate C-24 alkyl groups from the side chain and also convert phytosterols to cholesterol[7]. However, it was reported that phytosterols such as sitosterol (C_{29}) were superior to cholesterol (C_{27}) in supporting growth and development, and that desmosterol (C_{27}) very poorly supported growth[6]. The only analysis of dietary or insect sterols presented by these investigators was by thin-layer chromatography (TLC) which does not satisfactorily resolve cholesterol from other neutral sterols. Recently, in an investigation of ecdysteroid biosynthesis in *D. melanogaster*, several dietary sterols were utilized in aseptic media, and it was concluded that the C_{28} sterol, ergosterol, apparently was dealkylated by the insect, but no analyses of insect neutral sterols

were made[8]. In another recent study, sterols of *D. melanogaster* reared on mutant strains of the yeast *Saccharomyces cerevisiae* were analyzed by gas-liquid chromatography which demonstrated some metabolism of the yeast sterols, but from these analyses it is difficult to conclude that no cholesterol was present in the insects reared on the wild type strain[9]. Thus, in light of these and other studies, we felt that prior to undertaking studies on ecdysteroid metabolism and the effects of steroid metabolism inhibitors and ecdysteroid analogs in *D. melanogaster*, it was necessary to determine, unequivocally, the extent to which a wild type strain of this insect could dealkylate phytosterols. In addition, there are no previous reports in the literature of metabolic studies with this species using radiolabeled dietary sterols.

Materials and methods

Drosophila melanogaster of the wild type strain Oregon-R were cultured axenically on a casein-based defined medium described by Sang[10] with lecithin substituted by choline chloride and either cholesterol, [^{14}C]sitosterol, or [^{14}C]desmosterol as the sterol component. To minimize endogenous sterol contamination, the casein used in the medium was extracted 3 × with $CHCl_3$-MEOH(2:1).

The [26(27)-^{14}C]desmosterol and [4-^{14}C]sitosterol were purchased from Amersham Corporation, Arlington Height, IL, USA. The [^{14}C]desmosterol was purified by argentation column chromatography of the acetate and recrystallization; the [^{14}C]sitosterol was purified on an alumina column and both sterols were examined for radiochemical purity by counting areas of adsorbent, scraped from TLC pates, in a Packard Tricarb-Liquid Scintillation Spectrometer and by trapping and counting fractions from the GLC effluent. The radiolabeled sterols ([^{14}C]sitosterol, sp. act. 300 dpm/μg; [^{14}C]desmosterol, sp. act. 600 dpm/μg) were coated on the dry diet components with dichloromethane to achieve a final concentration of 0.03% (wet wt) in the total medium. Media coated with the same amount of unlabeled sitosterol or cholesterol were also prepared for comparative growth studies. Pupae and prepupae reared on the radiolabeled sterols were collected, weighed, and stored in MeOH at −15 °C until sterol analyses were carried out. We obtained 1.942 g fresh weight of mixed prepupae and pupae (0–16 h post pupariation) from the [^{14}C]sitosterol-coated and 0.712 g fresh weight of similar material from the same preparation using [^{14}C]desmosterol-coated diet. Thus, overall development on desmosterol was much inferior to development on sitosterol. In comparative studies using media coated with unlabeled sitosterol or cholesterol, 20% heavier puparia were obtained from the sitosterol-reared insects and these achieved pupariation in ca 14 h less time than cholesterol-reared insects.

The insect samples and portions of media were homogenized in $CHCl_3$-MeOH (2:1), and following saponification of the crude lipids, the sterols were isolated and purified by previously published methods of column chromatography on alumina (Woelm, ICN Pharmaceuticals, Cleveland, OH, USA) and column fractions were monitored by TLC[11]. The purified sterol fractions from [^{14}C]desmosterol-fed *Drosophila* also were acetylated in pyridine/acetic anhydride (2:1, v/v), and the acetates fractionated via argentation chromatography on 20% $AgNO_3$-impregnated Unisil (Clarkson Chemical Company, Williamsport, PA, USA)[12]. Argentation chromatography effectively separates cholesteryl and desmosteryl acetates, and provides a useful method to monitor conversion of [^{14}C]desmosterol to cholesterol.

Sterol and steryl acetate fractions were quantitatively and qualitatively analyzed by GLC on a J&W DB-1 fused silica capillary column, 15 m × 0.25 mm i.d. (0.25 μm film), at 235 °C, helium carrier gas at 25 cm/s linear velocity, 23:1 split ratio, in a Varian model 3700 gas chromatograph interfaced with a Shimadzu C-R1B chromatopac data processor. GLC identifications were made by comparing retention times (RRTs) relative to cholestane as an internal standard. In addition, the sterols from [^{14}C]sitosterol-fed *Drosophila* were fractionated on 15 m × 0.53 mm i.d. J&W DB-1 Megabore GLC column (1.5 μm film) and fractions were trapped from the effluent for counting in a Packard Tri-Carb 460CD Liguid Scintillation System. Insect and media sterols were also analyzed by gas chromatography-mass spectrometry (GC-MS) with a Finnigan model 4510 automated instrument equipped with a J&W DB-1 column.

Relative percentage of sterols from unfortified medium, sitosterol-fortified medium, [^{14}C]sitosterol-fed *Drosophila*, and [^{14}C]desmosterol-fed *Drosophila melanogaster*

Sterol	Medium with no sterol added*	Sitosterol fortified medium‡	[^{14}C]-Sitosterol-fed *D. melanogaster*	[14C]-Desmosterol-fed *D. melanogaster*
Cholesterol	95.4	0.5	1.2	4.1
Desmosterol	-	-	-	90.3
Campesterol	TR**	1.2	2.1	0.5
Sitosterol	4.6	98.3	96.7	2.1
Unknowns	-	-	-	3.0

* < 0.3 mg total sterol from 50 ml of medium.
‡ > 15 mg available in 50 ml of medium.
** < 0.5%.

Results and discussion

Relative percentages of identified sterols from media and insect samples are listed in the table. Note that the major endogenous sterol in the non-supplemented diet components is cholesterol (95.4%), but the total endogenous sterols comprise only 0.0006% of the fresh weight of the medium. Medium not supplemented with sterol will support very little *Drosophila* growth[13]. When sterols from sitosterol-fortified medium and [^{14}C]sitosterol-fed *Drosophila* are compared, the GLC and GC-MS analyses indicate that the dietary sterols are changed little, if any, before incorporation into insect tissues. Results from counting fractions tapped from GLC from

[^{14}C]sitosterol-fed insects indicated that extremely little of the 72,400 dpm of recovered radioactivity was associated with cholesterol ($< 1\%$ of the total). Sterols from an insect capable of dealkylating and converting phytosterols to cholesterol would have a major portion of the radioactivity in the cholesterol fraction[3,6]. In addition, fractionation of the steryl acetates (129,000 dpm total) from [^{14}C]desmosterol-fed insects by argentation chromatography provided no evidence for conversion of [^{14}C]desmosterol to [^{14}C]cholesterol. There was no detectable radioactivity associated with cholesterol acetate in these fractions, and insects that are capable of dealkylating readily convert desmosterol, the terminal intermediate in this pathway, to cholesterol[3,6]. It does appear that some selective uptake of the endogenous cholesterol in the diet occurred, as there was a higher percentage of cholesterol in the sterol samples from both insect samples than was found in the sterols from sitosterol-fortified medium. Selective uptake and lack of dealkylation of phytosterols were reported from studies using unlabeled sterols in which GLC analyses were done with several cactophilic *Drosophila* species by Kircher et al.[14]. The somewhat higher level of cholesterol in the sterols from [^{14}C]desmosterol-fed insects in our study is probably a reflection of the inability of the insect to utilize desmosterol as well as sitosterol and the overall inferior growth and development on desmosterol. Kircher et al. also reported poor development of several cactophilic species of *Drosophila* as well as *D. melanogaster* fed desmosterol-coated diets in growth studies using unlabeled sterols[15]. Apparently, with respect to dietary sterol utilization and metabolism, *D. melanogaster* is more similar to the house fly, *Musca domestica*, a more advanced member of the order Diptera, (which is unable to dealkylate phytosterols)[16] than it is to *Aedes aegypti*, a more primitive dipteran (which can dealkylate phytosterols)[6]. However, in agreement with Cook and Sang[7], sitosterol supported growth and development of *D. melanogaster* somewhat better than cholesterol in comparative studies that we conducted. This is contrary to what would be expected in an insect that cannot convert phytosterols to cholesterol. Perhaps, the very small amount of endogenous cholesterol in the diet components is adequate for specific needs such as ecdysone (molting hormone) synthesis to provide a sparing situation, or, alternatively, C_{28} or C_{29} phytosterols may serve as precursors for other ecdysteroids. Also, sitosterol must be taken up and/or incorporated as a membrane component more efficiently than is cholesterol in this insect. It is noteworthy that in vitro studies on ecdysteroids produced by *D. melanogaster* ring glands using high pressure liquid chromatography and radioimmunoassay analyses to determine ecdysteroid profiles showed variations in ecdysteroids secreted that correlated with different dietary sterols[17]. It will be of interest to examine the relative utilization of several radiolabeled C_{27}, C_{28}, and C_{29} dietary sterols as precursors of ecdysteroids in *D. melanogaster*.

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Toxic effects of methylmercury on spermatozoa in vitro

M. V. Rao

Department of Zoology, School of Sciences, Gujarat University, Ahmedabad-380009 (India)
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Summary. In an in vitro investigation, methylmercury (MeHg) reduced the motility of rat spermatozoa probably by the inhibition of succinate dehydrogenase and ATPase activities. Concomitant morphological changes observed in the spermatozoa were coiled tails and kinks in midpiece and tail regions.
Key words. Methylmercury; sperm motility; enzymes; morphology; rats.