Cholesterol turnover in the American cockroach, *Periplaneta americana* (L.)

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SUMMARY Turnover of cholesterol in the American cockroach, *Periplaneta americana* (L.), was investigated by feeding cholesterol-4-C^{14} in the diet and examining periodically the specific radioactivity of the total carcass cholesterol. It was found that the movement of cholesterol into and out of the roach tissues was much slower than has been reported for mammals. The ester cholesterol pool was found to equilibrate slowly with the free cholesterol pool. At least 40% of the carcass cholesterol was found to be exchangeable with the dietary cholesterol.

IT HAS RECENTLY been demonstrated that certain sterols have "brain hormone" activity (1) and "juvenile hormone" activity (2) in insects. The molting hormone (ecdysone) of the silkworm, *Bombyx mori* (L.), has been shown to have a steroid structure (3). In addition, sterols are involved in the initiation of ovarian development (4). However, most insects apparently lack the capacity for the biosynthesis of the steroid nucleus and therefore show a dietary requirement for sterols (5). Thus, information on turnover of sterols in insects should be of value in the study of sterol metabolism of these organisms. Since it has been established (6) that cholesterol is the major sterol of cholesterol-fed adult American cockroaches, *Periplaneta americana* (L.), turnover of cholesterol in this insect was investigated.

METHOD AND MATERIALS

Newly emerged, adult *P. americana* males, reared on a nymphal diet of commercial dog food, were separated from a stock colony and placed on Noland's modified diet V (7) containing 0.1% cholesterol-4-C^{14}, specific activity 179,000 cpm/mg. The radiochemical purity of the cholesterol, examined by paper chromatography (8), was found to be greater than 95%. At intervals of 10 days, two groups of six roaches each were removed from the experimental group for analysis. The insects were anesthetized with CO_2 and rinsed with water and then acetone to remove any adhering particles. (The amount of sterol and sterol ester removed from the cuticular waxes by this procedure was found to be insignificant.) After the entire alimentary tract was removed from each roach, the carcasses were saponified in KOH–ethanol–water, heated short of boiling. The nonsaponifiable lipids were isolated into hexane and assayed for radioactivity. After chromatography of the nonsaponifiable lipids on alumina (8), the specific activity of the sterol fraction was ascertained by radioassay and mass determination by colorimetric measurement of the Liebermann-Burchard product (9).

The sterols of a similar group of roaches from the stock colony were examined by gas–liquid chromatography, and 99% of the sterols behaved as cholesterol. This percentage is in agreement with that of Vanden-Heuvel et al. (6).

An additional group of six roaches, which had remained on the radioactive diet for 60 days, was treated as above except that the total carcass lipids were extracted with chloroform–methanol 2:1 (v/v) (10), and the lipid fraction was chromatographed on silicic acid[1] after the method of Horning et al. (11). The specific activities of the ester sterol (after saponification) and of the free sterol were determined as described.

Fecal collections after 30 days and 60 days were each placed in a Soxhlet apparatus and extracted continuously with ethanol for 18 hr. After saponification, the nonsaponifiable lipids were extracted with hexane and chromatographed on alumina in the same manner as for the tissue lipids. Examination of the sterol fraction

[1] Unisil, Clarkson Chemical Co., Williamsport, Pa. Mention of this company does not necessarily imply an endorsement of this product by the U. S. Department of Agriculture.

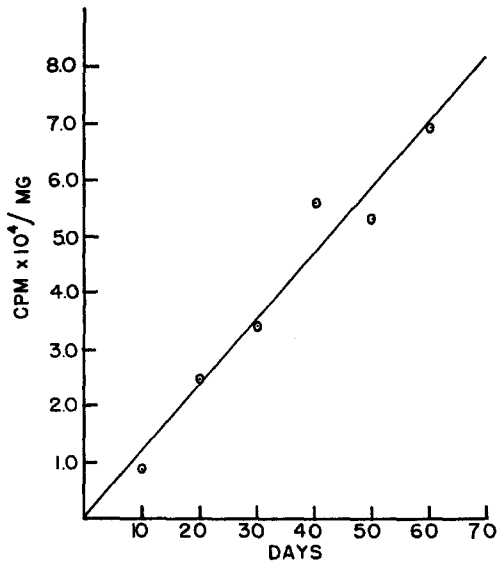

FIG. 1. Appearance of cholesterol in tissues of American cockroach.

by thin-layer chromatography (12) and gas–liquid chromatography revealed cholesterol and traces of compounds that behaved as coprostanone and coprostanol; the last two compounds were removed by further chromatography on alumina (13). Specific activities of the cholesterol were determined as described.

RESULTS AND DISCUSSION

During the 60 day period the tissue cholesterol had not reached equilibrium with the dietary cholesterol, but the incorporation of radioactivity into the carcass cholesterol continued to increase (Fig. 1). In order to obviate the possibility that the sterol was ingested and merely stored by the insects, the total carcass cholesterol was calculated from the specific activity of the cholesterol and the total radioactivity in the lipid extract (since losses may have occurred in the purification of the cholesterol). It can be seen from Table 1 that the average amounts of cholesterol found in each group of insects remained relatively constant over the 60 day period.

Since an equilibrium had not been attained between the carcass and dietary cholesterol, both linear and semilogarithmic graphs of radioactivity incorporation are straight lines. If the logarithmic rate of incorporation is assumed, then the half-life of the carcass cholesterol is about 90 days. This rate of movement is much slower than that found in mammals, which may be a reflection of low rates of tissue growth and repair in adult roaches. The ratio of the specific activity of the carcass cholesterol at the end of the experiment to the specific activity of the dietary cholesterol indicates

TABLE 1 RADIOACTIVE CHOLESTEROL IN ROACH GROUPS

Time on Diet	Group	Total Activity per Group	Specific Activity	Total Carcass Cholesterol
days		*cpm*	*cpm/mg*	*μg per roach*
10	A	4.05×10^4	$8.93 \times 10^3 \pm 240$*	760
	B	3.50×10^4	$8.99 \times 10^3 \pm 190$	650
	Average		8.96×10^3	705
20	A	1.09×10^5	$2.47 \times 10^4 \pm 350$	740
	B	1.04×10^5	$2.45 \times 10^4 \pm 240$	710
	Average		2.46×10^4	725
30	A	1.45×10^5	$3.35 \times 10^4 \pm 170$	720
	B	1.54×10^5	$3.52 \times 10^4 \pm 880$	730
	Average		3.43×10^4	725
40	A	1.71×10^5	$5.54 \times 10^4 \pm 1940$	†
	B	2.13×10^5	$5.65 \times 10^4 \pm 3500$	630
	Average		5.60×10^4	
50	A	2.43×10^5	$5.30 \times 10^4 \pm 3290$	760
	B	2.60×10^5	$5.31 \times 10^4 \pm 420$	820
	Average		5.30×10^4	790
60	A	3.05×10^5	$7.01 \times 10^4 \pm 1260$	720
	B	2.93×10^5	$6.87 \times 10^4 \pm 0$	710
	Average		6.94×10^4	715

Total activity was determined on the crude lipid extract. The specific activity of cholesterol was determined after alumina column chromatography of the lipid extract. Total carcass cholesterol was calculated from the total activity and the specific activity. Each group consisted of six roaches.

* Standard error.

† Because of spillage only specific activity could be determined.

that at least 40% of tissue cholesterol is exchangeable with the dietary cholesterol.

When the specific activities of the total, free, and esterified cholesterol are compared (Table 2), it can be seen that those of the total and free cholesterol are similar, but the specific activity of the ester cholesterol is equal to 68% of that of the free cholesterol. Sterol ester represents 29% of the total carcass sterol pool. Ishii et al. (14) reported the ester pool to be about 11% of the total sterol after 20 days of equilibration. Since this value was calculated from the radioactivity of each fraction, it might be too low if the ester sterol had not

TABLE 2 TOTAL, FREE, AND ESTER CHOLESTEROL AFTER 60 DAYS SPECIFIC ACTIVITIES

Sterol	Total Activity	Specific Activity*	Mass*	% of Total Sterol
	cpm	*cpm/mg*	*mg*	
Free	2.01×10^5	6.93×10^4	2.90	71
Ester	5.53×10^4	4.75×10^4	1.16	29
Total	2.56×10^5	6.30×10^4	4.06	

Total activity was determined on the lipid fractions after silicic acid column chromatography. Specific activity was calculated from the total activity and the mass determinations.

* Average of three determinations.

TABLE 3 SPECIFIC ACTIVITIES OF FECAL AND DIETARY CHOLESTEROL

Sterol	30-Day Specific Activity*	60-Day Specific Activity*
	cpm/mg	*cpm/mg*
Fecal	1.31×10^5	1.42×10^5
Diet	1.79×10^5	1.79×10^5

Feces were collected for two 30-day periods (days 1 to 30 and days 31 to 60). Specific activity was determined after alumina column chromatography.

* Average of three determinations.

reached equilibrium with the free sterol pool. From our data at 60 days it would be expected that the 20-day free and ester sterol pools would not be in equilibrium. It should be mentioned also that the gut sterols were not included in our determinations, which may further account for the discrepancy.

Apparently the establishment of equilibrium between free and ester sterol was slow, since the specific activity of the ester sterol was not near that of the free sterol after 60 days. Ishii et al. (14) also showed that after 20 days, in some tissues, free and ester sterol had not reached equilibrium. It appears, then, that in *P. americana* cholesterol is chiefly unesterified and that the free sterol equilibrates slowly with the esterified sterol pool.

Fecal sterols were studied to determine whether unchanged cholesterol was excreted by the roach. It was reasoned that if the fecal cholesterol represented only unabsorbed dietary cholesterol, the specific activity of the fecal cholesterol would be unchanged from that of the labeled cholesterol administered in the diet. However, if unchanged cholesterol were excreted from the body pool, which was less radioactive than the dietary cholesterol, then the fecal cholesterol would have a lower specific activity than the dietary cholesterol. It can be seen from Table 3 that the fecal cholesterol did have a lower specific activity ($P<0.01$ for both periods).

The role of sterols in certain insects has been investigated in several different laboratories. Robbins and Shortino (4) demonstrated that cholesterol exerts an effect upon the ovarian development in the house fly, *Musca domestica* L. In addition, Monroe (15) reported that cholesterol is essential for egg hatch in the house fly. Thus, it appears that cholesterol is intimately involved in the reproductive function of the house fly.

In an investigation with the hide beetle, *Dermestes maculatus* De Geer (= *vulpinus* F.), Clark and Bloch (16) showed that several sterols can replace a major portion of the dietary cholesterol requirement and have ascribed an apparently dual role to sterols in this insect. They postulated a structural role for the macronutrient requirement, satisfied by cholestanol and certain other sterols, and a metabolic role for the micronutrient requirement, satisfied by cholesterol. Furthermore, the cholesterol can be replaced completely only by the closely related desmosterol (24-dehydrocholesterol) or by 24-methylenecholesterol, while approximately 10 different sterols have been found to satisfy the macronutrient requirement (17). In similar studies with the house fly, Robbins (18) reported that only a minute quantity of cholesterol is needed when the remainder of the dietary sterol requirement is fulfilled by cholestanol. This feature of sterol metabolism, in which a major portion of the cholesterol requirement is replaced by another sterol, has been labeled by Clark and Bloch (16) as the "sparing" effect.

In further studies on the sparing effect of cholestanol, Lasser et al. (19) report that under sparing conditions three sterol pools exist in the cockroach, *Eurycotis floridana* (Walker). These investigators also reported that the primary sterol turnover occurred in the free macronutrient pool. Results of the present investigation show conclusively that replacement of tissue cholesterol by dietary cholesterol is a cause of the flux of cholesterol in the American cockroach. However, the flux reported here also may be due in part to conversion of cholesterol to some metabolic product, which possibly has hormonal activity. This latter mode of utilization is suggested, at least in the house fly, by the minute quantities of cholesterol required for growth and reproduction (18). Indeed, Kobayashi et al. (1) demonstrated that cholesterol exhibits "brain hormone" activity in "debrained" silkworm pupae, and Karlson et al. (3) showed that the molting hormone, ecdysone, has a steroid structure. In this light, sterol metabolism in insects takes on a greater importance.

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