

THE ESSENTIAL CHOLESTEROL REQUIREMENT
OF THE ROACH EURYCOTIS FLORIDANA*

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The normal dietary cholesterol requirement of the hide beetle, Dermestes vulpinus, has been shown to be replaceable in part, though not in entirety, by a number of other sterols of the cholestane, ergostane and stigmastane series (Clark and Bloch, 1959). The most striking case of this sparing action involved the replacement of 97% of the normal dietary cholesterol requirement of this insect by sitosterol. The remaining 3% of the normal cholesterol requirement was indispensable. On the basis of this observation, the above authors suggested that the sterol requirement of the insect was twofold. The quantitatively major requirement (satisfied by sitosterol and other "sparing" sterols) was assumed to reflect a non-metabolic role of sterol in the organism's cellular and subcellular architecture. The irreplaceable, minimal requirement for cholesterol was taken to correspond to a metabolic need for a sterol precursor of hormones or bile acids.

According to this hypothesis an insect, when fed throughout its growing life on a diet in which the normal cholesterol requirement was replaced as nearly completely as possible with a sparing sterol, would be expected to reach maturity with only the sparing (structural) sterol present in its tissues in any appreciable concentration. The minimal cholesterol content of the diet would be expected to disappear as a result of its postulated metabolic transformation. This communication presents some preliminary results of experiments designed to test this expectation.

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Materials and Methods

The roach Eurycotis floridana was chosen for this study since it is of convenient size and is easily reared aseptically (Clayton, 1959). It has been shown that its normal cholesterol requirement (0.1% of the diet), like that of D. vulpinus, can be replaced to the extent of 95% by a sparing sterol, in this case cholestanol, but that 5% of the normal cholesterol requirement is essential for maturation (Clayton, unpublished).

Accordingly, insects were reared aseptically on the synthetic diet of Noland and Baumann (1949) containing either 0.1% cholesterol-4-C¹⁴ (894 d. p. m. / μ g) (Diet "A"), or 0.1% cholestanol-7 α -H³ (6,000 d. p. m. / μ g) and 0.005% cholesterol-4-C¹⁴ (3,788 d. p. m. / μ g) (Diet "B"). The mature insects were drowned in ice-water and dissected as quickly as possible in the cold. Total lipid extracts of weighed tissue samples were prepared by conventional procedures, assayed for C¹⁴ and H³ content by means of a scintillation counter, and separated by chromatography on alumina into two fractions containing ester and free sterols respectively.

Cholesterol-4-C¹⁴ was isolated from the tissues of insects reared on diet "B" by addition of non-radioactive cholesterol to the crude sterols and purification via the dibromide by the standard procedure. The recovered cholesterol had the correct specific activity assuming all C¹⁴ to have been present in the tissues as cholesterol. It contained no tritium.

Results

The results are recorded in the table and lead to the following observations:

- (1) All the tissues of insects reared on diet "A" contain cholesterol in concentrations which vary according to the tissue.
- (2) Insects fed diet "B" also contain cholesterol in all their tissues.
- (3) In only two tissues of the insects reared on diet "B", the gastric caeca and the mid-intestine, does cholesterol account for a smaller percentage of the total sterol content than in the diet itself, where it constitutes 5% of the total sterols. In the malpighian tubules it accounts for almost exactly 5%, and in all other tissues the percentage of cholesterol is considerably higher (fat body, ca. 9%; muscle, ca. 13%; cuticle, ca. 23%; ventral nerve cord, ca. 50%).

STEROL CONTENT OF TISSUES OF E. FLORIDANA

	Diet "A", 0. 1% Cholesterol			Diet "B", 0. 1% Cholesterol + 0. 005% Cholesterol				
	Ester	Free		Ester	Free		Ester	Free
	Cholesterol	Cholesterol	% ²	Cholesterol	Cholesterol	% ²	Cholesterol	Cholesterol
	µg/mg ¹	µg/mg	% ²	µg/mg	µg/mg	% ²	µg/mg	µg/mg
Crop	. 566	1. 018	69. 7 ($\pm 17. 5$)	. 0379	. 121	80. 5 ($\pm 4. 5$)	1. 658	. 821
Proventriculus	. 064	. 653	90. 2 ($\pm 3. 7$)	. 0027	. 090	97. 1 ($\pm 0. 1$)	. 115	. 516
Mid intestine	. 015	. 880	98. 2 ($\pm 1. 2$)	. 0085	. 071	88. 0 ($\pm 5. 0$)	3. 120	1. 590
Hind intestine	. 018	. 900	98. 1 ($\pm 0. 4$)	. 0075	. 109	94. 0 (± 0)	. 616	1. 078
Rectum	. 479	1. 243	69. 9 ($\pm 6. 4$)	. 0166	. 180	92. 5 ($\pm 2. 5$)	. 410	. 911
Gastric caeca	. 121	. 773	86. 8 ($\pm 0. 9$)	. 0032	. 044	92. 3 ($\pm 3. 7$)	. 610	. 590
Malpighian tubules	. 019	1. 143	98. 3 ($\pm 0. 6$)	. 0005	. 074	99. 5 ($\pm 0. 5$)	. 179	1. 306
Fat body	. 728	. 721	46. 6 ($\pm 0. 1$)	. 0037	. 105	96. 6 ($\pm 0. 6$)	. 464	. 712
Salivary gland	. 055	2. 443	99. 2 ($\pm 0. 4$)	. 0020	. 203	99. 0 (± 0)	. 143	1. 988
Muscle	. 038	. 268	88. 0 ($\pm 4. 9$)	. 0003	. 032	98. 1 ($\pm 1. 4$)	. 009	. 236
Ventral nerve	1. 185	1. 662	58. 5 ($\pm 3. 2$)	. 0059	. 779	99. 0 ($\pm 0. 5$)	. 281	. 501
Cuticle	. 067	. 313	82. 4 ($\pm 0. 1$)	. 0002	. 060	99. 8 ($\pm 0. 2$)	. 030	. 229
Remains	Total µg/mg 1. 22 ($\pm . 17$)			Total µg/mg 0. 0772 ($\pm 0. 004$)				
				Total µg/mg 0. 615 ($\pm . 009$)				

¹ All concentrations are expressed as µg sterol per mg fresh tissue.² Percentage of total cholesterol in free form.³ Percentage of total cholesterol in free form.

(4) The proportions of free and esterified cholesterol vary according to the tissue in the insects reared on diet "A", but the cholesterol in the tissues of insects given diet "B" is almost entirely unesterified. On diet "B" the esterified sterol fraction is made up almost wholly of cholestanol. This is especially obvious in the case of the nerve, the fat body and the rectum (figures underlined in the table).

It is clear from these results that, contrary to expectation based on the hypothesis of Clark and Bloch (1959), the minimal essential cholesterol requirement of this insect is not completely metabolized. It is found to be concentrated in preference to cholestanol in most of the tissues of the insect so that they come to contain a ratio of cholesterol to cholestanol which exceeds that of the diet. While these observations do not disprove the metabolic utilization of cholesterol by the insect, they provide good evidence for a specific structural role for cholesterol, which cannot be served by cholestanol. The evidence that these compounds serve separate functions is strengthened by the strikingly uniform occurrence of cholesterol almost entirely as the free sterol in tissues in which a considerable proportion of the cholestanol is esterified. The significance of this distinction is of great interest, and a more detailed analysis of the system is in progress.

This and related studies will be reported in full elsewhere.

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