

**CHANGES OCCURRING IN THE CHOLESTEROL LEVEL OF THE  
THORACIC MUSCLES OF THE STARVED AS WELL AS DIFFE-  
RENTLY REARED *LUICLIA CUPRINA* (CALLIPHORIDAE :  
DIPTERA)**

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**Abstract :** Cholesterol metabolism of starved as well as those *Lucia cuprina* (Wied.) reared on different diets was studied during the first 40 hours of their life. In the starved insects the amount of cholesterol increased from 4 to 12 hours (from 0.325 to 0.395 mg/100 mg) then decreased at 16 hours but started to increase till 28 hours when it was found to be 0.690 mg/100 mg. A decrease was noted afterwards which continued till the end with the contents falling to 0.31 mg/100 mg. In the beef extract-fed flies cholesterol level increased from 4 till 12 hours (from 0.345 to 0.391 mg/100 mg) but then a slight decrease occurred at 16 hour-stage followed by an increase till 28 hours, when the amount was estimated to be 0.518 mg/100 mg. A steady decrease occurred till the end when the contents were found to be 0.34 mg/100 mg. In glucose-fed flies contents increased from 4 hours to 12 hours (from 0.343 to 0.384 mg/100 mg) followed by a slight decrease at 16 hours. After 28 hours they reached the maximum which was 0.482 mg/100. Then the level started to fall down till at the 40 hour it was 0.35 mg/100 mg. In mixed diet-fed flies cholesterol level increased till 12 hours (from 0.353 to 0.403 mg/100 mg), then decreased slightly at 16 hours and started to increase and reached its maximum at 28 hours which was 0.512 mg/100 mg. Later on a depletion in the values occurred, the level started to fall down and had become 0.35 mg at the end of 40 hours.

**INTRODUCTION**

Unlike other organisms insects can not synthesize sterols 'de novo', so they need an external source (Clayton, 1964 ; Robbins *et al* ; 1971 ; Svoboda *et al* ; 1975 ; Katra and Nes ; 1981). This can be dietary or in the form of intra-or extracellular symbionts (Houk *et al* ; 1976). Sterols are utilized in the structure of the cell membranes in all types of organisms (Nes and Mckean, 1977). Sterols are the precursors of ecdysones which are the moulting hormones in insects, without the sterol nucleus no moulting hormones can be synthesized. Apart from that they are an important component of the insect cuticle (Gilby and Mokellar, 1970). In the haemolymph it is a constituent of the lipoproteins (Gibert *et al*; 1977). Cholesterol as such is important for some carrion feeding

insects like Dermestidae, but phytophagous insects can metabolically transform the phytosterols into cholesterol (Richards and Davies, 1977). Cholesterol is the principal sterol in the haemolymph of many insects such as *Acheta domestica* (Florkin and Jeuniaux, 1974). Many insects can use cholesterol if they are denied an exogenous source of sterol.

In order to study the role of cholesterol during normal flight activity *Lucilia cuprina*, a carrion-feeder diptern was chosen.

#### MATERIALS AND METHODS

The Australian blowflies, *Lucilia cuprina* used during the present work were taken from a colony maintained in the laboratory at  $28 \pm C^{\circ}$ , relative humidity ranging from 70% to 75% and a 12 hours photoperiod. Females were used only to avoid discrepancy in the results. The newly emerged females were divided into 4 groups, each group containing several batches. 20 female flies were put in each batch and kept in separate glass jars covered with muslin. To the first group belonged the flies which were starved throughout. The remainder three groups were given the following diets respectively :

- group 1: Beef extract.
- group 2: glucose.
- group 3: mixed diet (Beef extract + glucose).

To determine the cholesterol contents the flies were first chilled and then their thoracic muscles were dissected out at 4 hourly intervals, starting from the newly emerged and upto the 40th hour of their life. Total cholesterol was estimated according to the method of Bowman and Wolf (1962). Three replicates were used for each test and the results were statistically analysed.

#### RESULTS

##### *Starved Insects* (Fig. 1, Tables I-III).

The cholesterol level of the thoracic muscles of the starved females flies was 0.33 mg/100 mg after the 4th hour of emergence. It increased about 6% at 8 hours and became 0.35 mg/100. After 12 hours the content was 0.36 mg/ 100 mg (nearly 3% increase as compared to 8 hours). No change occurred after 16 hours and at 20 hours of their life nearly 3% increase, with the contents reaching 0.37 mg/100 mg, was noted. It reached 0.44 mg/100 mg (nearly 19% increase) at 24 hours, and 0.48 mg/100 mg after

TABLE I : THE CHANGES IN THE CHOLESTEROL CONTENTS OF THE THORACIC MUSCLES OF THE STARVED AND DIFFERENTLY REARED *LUCILIA CUPRINA*

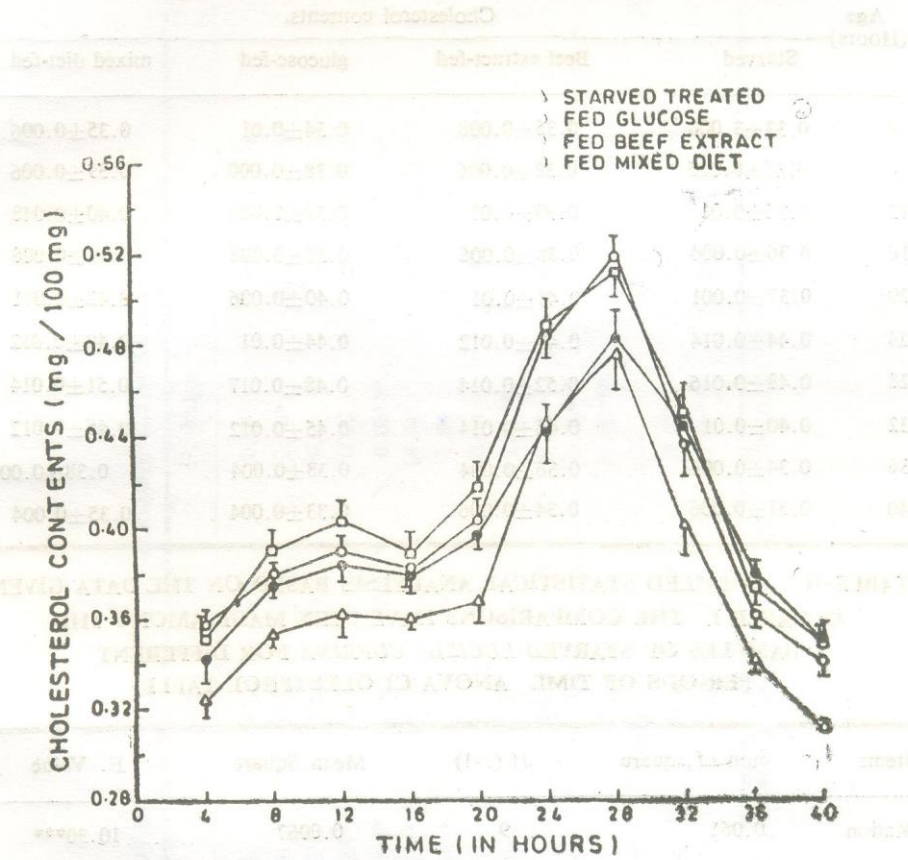


FIG.1 : CHANGES IN THE CHOLESTEROL CONTENTS IN THE THORACIC MUSCLES OF *Lucilia cuprina* AT DIFFERENT TIME INTERVALS REARED ON DIFFERENT DIETS.

TABLE I : THE CHANGES IN THE CHOLESTEROL CONTENTS OF THE THORACIC MUSCLES OF THE STARVED AND DIFFERENTLY REARED *LUCILIA CUPRINA*

Age (Hours)	Cholesterol contents.			
	Starved	Beef extract-fed	glucose-fed	mixed diet-fed
4	0.33±3.000	0.35±0.008	0.34±0.01	0.35±0.006
8	0.35±0.006	0.38±0.006	0.38±0.000	0.39±0.006
12	0.36±0.01	0.39±0.01	0.39±0.006	0.40±0.048
16	0.36±0.004	0.38±0.006	0.38±0.008	0.39±0.008
20	0.37±0.001	0.41±0.01	0.40±0.006	0.42±0.011
24	0.44±0.014	0.49±0.012	0.44±0.01	0.49±0.012
28	0.48±0.016	0.52±0.014	0.48±0.017	0.51±0.014
32	0.40±0.01	0.44±0.014	0.45±0.012	0.45±0.012
36	0.34±0.006	0.36±0.004	0.38±0.004	0.38±0.006
40	0.31±0.006	0.34±0.006	0.33±0.004	0.35±0.004

TABLE II : DETAILED STATISTICAL ANALYSIS BASED ON THE DATA GIVEN IN TABLE I. THE COMPARISONS HAVE BEEN MADE AMONG THE SAMPLES OF STARVED *LUCILIA CUPRINA* FOR DIFFERENT PERIODS OF TIME ANOVA CHOLESTEROL TABLE

Items	Sum of square	df (n-1)	Mean Square	F. Value
Ration	0.061	9	0.0067	10.30***
Error	0.013	20	0.00065	
Total	0.074	29		

28 hours (9% increase as compared to 24 hours). After this the contents started to decrease and at 32 hours the decline was about 17% as compared to 28 hours and the contents were noted as 0.4 mg/100 mg. After 36 hours the cholesterol level showed a further decrease of 15% and the amount became 0.34 mg/100 mg while at 40 hour stage the drop in the level was about 9% and the amount measured was 0.31 mg/100 mg.

TABLE III : DETAILED COMARISON BETWEEN RATIONS

	F. Value		F. Value		F. Value
4-hrs Vs 8-hrs	1.13	8-hrs Vs 36-hrs	0.27	20-hrs Vs 24-hrs	7.66***
4-hrs Vs 12-hrs	1.96	8-hrs Vs 40-hrs	2.68*	20-hrs Vs 24-hrs	16.25***
4-hrs Vs 16-hrs	1.66	12-hrs Vs 16-hrs	0.01	20-hrs Vs 32-hrs	1.27
4-hrs Vs 20-hrs	2.72*	12-hrs Vs 20-hrs	0.06	20-hrs Vs 36-hrs	1.21
4-hrs Vs 24-hrs	19.49***	12-hrs Vs 24-hrs	0.08***	20-hrs Vs 40-hrs	4.92**
4-hrs Vs 28-hrs	32.27***	12-hrs Vs 28-hrs	18.31	24-hrs Vs 28-hrs	1.60
4-hrs Vs 32-hrs	7.71***	12-hrs Vs 32-hrs	1.89	24-hrs Vs 32-hrs	2.68*
4-hrs Vs 36-hrs	0.29	12-hrs Vs 36-hrs	0.73	24-hrs Vs 36-hrs	14.97***
4-hrs Vs 40-hrs	0.32	12-hrs Vs 40-hrs	3.88**	24-hrs Vs 40-hrs	24.86***
8-hrs Vs 12-hrs	0.11	16-hrs Vs 20-hrs	0.12	28-hrs Vs 32-hrs	8.42***
8-hrs Vs 16-hrs	0.04	16-hrs Vs 24-hrs	9.76***	28-hrs Vs 36-hrs	26.36***
8-hrs Vs 20-hrs	0.33	16-hrs Vs 28-hrs	19.82***	28-hrs Vs 40-hrs	4.98***
8-hrs Vs 24-hrs	11.21***	16-hrs Vs 32-hrs	2.21	32-hrs Vs 36-hrs	33.07***
8-hrs Vs 28-hrs	21.28***	16-hrs Vs 36-hrs	0.55	32-hrs Vs 40-hrs	11.21***
8-hrs Vs 32-hrs	2.92*	16-hrs Vs 40-hrs	3.46**	36-hrs Vs 40-hrs	1.24

Key \* = P<0.05 ; \*\* = P<0.01 ; \*\*\* = P<0.001

*Flies fed on beef extract* (Fig. 1, Tables I, IV, V)

TABLE IV : DETAILED STATISTICAL ANALYSIS BASED ON THE DATA GIVEN IN TABLE I. THE COMPARISONS HAVE BEEN MADE AMONG THE SAMPLES FED ON BEEF EXTRACT FOR DIFFERENT PERIODS OF TIME. ANOVA CHOLESTEROL TABLE

Items	Sum of square	df (n-1)	Mean Square	F. Value
Rations	0.082	9	0.0091	18.2***
Error	0.01	20	0.0005	
Total	0.092	29		

The cholesterol contents of the flies fed on beef extract were 0.35 mg/100 mg after the 4th hour of emergence. It increased to 0.38 mg/100 mg (9%) at 8 hours and 0.39 mg/100 mg (3%) at 18 hours, followed by a slight decrease (2%) with the level at 0.38 mg/100 mg at 16 hour-stage. It was followed by a gradual increase, and at 20 hours the value was 0.41 mg/100 mg, while it was 0.49 mg/100 mg at 24 hour stage. The maximum amount observed was 0.52 mg/100 mg after 28 hours of emergence, (nearly 6% increase as compared to 24 hours). After 32 hours, the level dropped to 0.44 mg/100 mg, at 36 hours to 0.36 mg/100 mg and at 40 hours it decreased about 5.5% as compared to 36 hours and the amount was 0.34 mg/100 mg.

*Glucose-fed flies* (Fig. 1 : Tables I, VI, VII).

The cholesterol content of glucose-fed females of *Lucilia cuprina* was 0.34 mg/100 mg after 4 hours of emergence. It had increased about 12% in the 8 hour-old flies and was 0.38 mg/100 mg. After 12 hours its level was found to be 0.39 mg/100 mg (nearly 5% increase). It was followed by a slight decrease and at 16 hours it was 0.38 mg/100 mg. Then at 20 hours it was 0.40 mg/100 mg and 0.44 mg/100 mg at 24 hours. The highest amount was observed at 28 hours, when it was 0.48 mg/100 mg with an increase of 9% as compared to 24 hours. At 32 hours a drop to 0.45 mg/100 mg was noted (6%). At 36 hours, the contents showed a further 14% decrease and had come down to 0.38 mg/100 mg and 0.33 mg/100 mg at 40 hours (8% less).

TABLE V : DETAILED COMPARISON BETWEEN RATIOS

	F. Value		F. Value		F. Value
4-hrs Vs 8-hrs	0.45	8-hrs Vs 36-hrs	0.74	20-hrs Vs 24-hrs	11.90***
4-hrs Vs 12-hrs	3.92**	8-hrs Vs 40-hrs	2.88*	20-hrs Vs 28-hrs	23.25***
4-hrs Vs 16-hrs	2.46*	12-hrs Vs 16-hrs	0.16	20-hrs Vs 32-hrs	1.84
4-hrs Vs 20-hrs	6.33***	12-hrs Vs 20-hrs	0.28	20-hrs Vs 36-hrs	3.59**
4-hrs Vs 24-hrs	35.61***	12-hrs Vs 24-hrs	15.90***	20-hrs Vs 40-hrs	7.44***
4-hrs Vs 28-hrs	53.87***	12-hrs Vs 28-hrs	28.72***	24-hrs Vs 28-hrs	1.88
4-hrs Vs 32-hrs	15.01***	12-hrs Vs 32-hrs	3.59**	24-hrs Vs 32-hrs	4.38**
4-hrs Vs 36-hrs	0.38	12-hrs Vs 36-hrs	1.83	24-hrs Vs 36-hrs	28.57***
4-hrs Vs 40-hrs	0.04	12-hrs Vs 40-hrs	4.80**	24-hrs Vs 40-hrs	38.19***
8-hrs Vs 12-hrs	0.24	16-hrs Vs 20-hrs	0.89	28-hrs Vs 32-hrs	12.00***
8-hrs Vs 16-hrs	-	16-hrs Vs 24-hrs	19.34***	28-hrs Vs 36-hrs	45.12***
8-hrs Vs 20-hrs	1.06	16-hrs Vs 28-hrs	33.29***	28-hrs Vs 40-hrs	57.03***
8-hrs Vs 24-hrs	20.09***	16-hrs Vs 32-hrs	5.31***	32-hrs Vs 36-hrs	10.58***
8-hrs Vs 28-hrs	34.27***	16-hrs Vs 36-hrs	0.89	32-hrs Vs 40-hrs	16.70***
8-hrs Vs 32-hrs	5.71***	16-hrs Vs 40-hrs	13.17*	36-hrs Vs 40-hrs	0.69

Key, \* = P<0.05 ; \*\* = P<0.01 ; \*\*\* = P<0.001

TABLE VI : DETAILED STATISTICAL ANALYSIS BASED ON THE DATA GIVEN IN TABLE-1. THE COMPARISONS HAVE BEEN MADE AMONG THE SAMPLES FED ON GLUCOSE FOR DIFFERENT PERIODS OF TIME. ANOVA CHOLESTEROL TABLE

Items	Sum of square	df (n-1)	Mean Square	F. Value
Rations	0.04	9	0.0044	11***
Error	0.009	20	0.0004	
Total	0.049	29		

*Flies fed on mixed diet.* (Fig. 1 : Tables I, VIII, IX)

The cholesterol level of the thoracic muscles of the female flies was 0.35 mg/100 mg after the 4th hour of their emergence. It increased to 0.39 mg/100 mg (nearly 5%) at 8 hours and to 0.40 mg/100 mg after 12 hours (2.5% increase). Then at 16 hours there was a slight decrease of about 2.5% and the cholesterol contents became 0.39 mg/100 mg. After that a gradual increase was noted upto 28 hours. At 20 hours the value was observed to be 0.42 mg/100 mg, 0.49 mg/100 mg at 24 hours, and it had reached 0.512 mg/100 mg at 28 hours (nearly 2.5% increase as compared to the 24 hours). After that a decline in the values was observed and at 32 hours when the level fell down to 0.45 mg/100 mg, at 36 hours to 0.38 mg/100 mg (15.5% less than 32 hours) and after feeding 40 hours on the mixed diet it was 0.35 mg/100 mg (6% less). Same trend in the values was observed in all categories of flies. The value increased in the beginning and was followed by a decrease. The maximum increase was observed after 28 hours in the beef extract-fed insects. It was 0.52 mg/100 mg, that is nearly 50% more as compared to what was found after 4 hours, although the mixed diet-feds also showed a comparable increase of 45% after 28 hours. The maximum decrease was found in the starved insects after 40 hours, where it was 0.31 mg/100 mg, thus showing a decrease of about 6% as compared to the initial amount. In all the other categories of the flies the amount towards the end of the experiment was found to be slightly less as to what that was present initially, except in the mixed diet-feds where it was noted to be the same.



TABLE VIII : DETAILED COMPARISON BETWEEN RATIOS

	F. Value	F. Value	F. Value	
4-hrs Vs 8-hrs	2.5	0.03	20-hrs Vs 24-hrs	4.76**
4-hrs Vs 12-hrs	3.78**	1.56	20-hrs Vs 28-hrs	16.00***
4-hrs Vs 16-hrs	3.08*	0.03	20-hrs Vs 32-hrs	4.83**
4-hrs Vs 20-hrs	6.72***	0.42	20-hrs Vs 36-hrs	0.72
4-hrs Vs 24-hrs	22.80***	8.01***	20-hrs Vs 40-hrs	5.11**
4-hrs Vs 28-hrs	43.47***	21.60***	24-hrs Vs 28-hrs	3.30*
4-hrs Vs 32-hrs	22.95***	8.1***	24-hrs Vs 32-hrs	—
4-hrs Vs 36-hrs	3.08*	0.04	24-hrs Vs 36-hrs	9.12***
4-hrs Vs 40-hrs	0.11	—	24-hrs Vs 40-hrs	19.74
8-hrs Vs 12-hrs	0.13	0.07	28-hrs Vs 32-hrs	3.24*
8-hrs Vs 16-hrs	0.03	9.12***	28-hrs Vs 36-hrs	23.40***
8-hrs Vs 20-hrs	1.02	23.40***	28-hrs Vs 40-hrs	39.20***
8-hrs Vs 24-hrs	10.20***	9.21***	32-hrs Vs 46-hrs	9.21***
8-hrs Vs 28-hrs	25.12***	—	32-hrs Vs 40-hrs	19.88***
8-hrs Vs 32-hrs	10.30***	2.02	36-hrs Vs 40-hrs	2.02

Key, \* = P<0.05 ; \*\* = P<0.01 ; \*\*\* = P<0.001

TABLE VIII : DETAILED STATISTICAL ANALYSIS BASED ON THE DATA GIVEN IN TABLE-I. THE COMPARISONS HAVE BEEN MADE AMONG THE SAMPLES FED ON A MIXED DIET FOR DIFFERENT PERIODS OF TIME. ANOVA CHOLESTEROL TABLE

Items	Sum of square	df (n-1)	Mean Square	F. Value
Rations	0.064	9	0.0071	12.90***
Error	0.011	20	0.00055	
Total	0.075	29		

### DISCUSSION

The cholesterol contents in the experimental insects showed variations in the pattern of fluctuation. In the starved insects the level increased upto 28 hours, thus showing that these insects were synthesizing continuously from the sterols which they had carried on from the pupal life. After exploiting that amount the continuous consumption led to a depletion in the cholesterol level. The glucose-fed insects also followed the same pattern as they also did not have a dietary source of sterols, although they displayed slight variations in the amount. The latter had always more amount, probably due to the reason that they had an alternative source of energy in the form of glucose.

The other two categories also followed the same pattern of increase and decrease but sometimes with higher levels of cholesterol at different stages. On the whole, the results show that cholesterol is synthesized continuously in these blowflies if a sterol source is present and it is utilized steadily throughout the life by the flight muscles. Its consumption rate increased with its continuous synthesis, thus indicating that it also provides some energy during flight when other fuels are in short supply.

TABLE IX : DETAILED COMPARISON BETWEEN RATIONS

	F. Value		F. Value		F. Value
4-hrs Vs 8-hrs	2.28	8-hrs Vs 36-hrs	0.45	20-hrs Vs 24-hrs	7.27***
4-hrs Vs 12-hrs	4.09***	8-hrs Vs 40-hrs	2.75	20-hrs Vs 28-hrs	14.25***
4-hrs Vs 16-hrs	2.2	12-hrs Vs 16-hrs	0.32	20-hrs Vs 32-hrs	1.60
4-hrs Vs 20-hrs	6.91***	12-hrs Vs 20-hrs	0.36	20-hrs Vs 36-hrs	3.21*
4-hrs Vs 24-hrs	28.36***	12-hrs Vs 24-hrs	10.91***	20-hrs Vs 40-hrs	7.71***
4-hrs Vs 28-hrs	41.02***	12-hrs Vs 28-hrs	19.20***	24-hrs Vs 28-hrs	1.16
4-hrs Vs 32-hrs	15.18***	12-hrs Vs 32-hrs	3.51**	24-hrs Vs 32-hrs	2.04
4-hrs Vs 36-hrs	0.69	12-hrs Vs 36-hrs	1.40	24-hrs Vs 36-hrs	20.16***
4-hrs Vs 40-hrs	0.02	12-hrs Vs 40-hrs	4.71**	24-hrs Vs 40-hrs	32.91***
8-hrs Vs 12-hrs	0.26	16-hrs Vs 20-hrs	1.37	28-hrs Vs 32-hrs	6.29***
8-hrs Vs 16-hrs	—	16-hrs Vs 24-hrs	14.97***	28-hrs Vs 36-hrs	31.01***
8-hrs Vs 20-hrs	1.25	16-hrs Vs 28-hrs	24.48***	28-hrs Vs 40-hrs	42.94***
8-hrs Vs 28-hrs	14.56***	16-hrs Vs 32-hrs	5.95***	32-hrs Vs 36-hrs	9.36***
8-hrs Vs 28-hrs	23.95***	16-hrs Vs 36-hrs	0.38	32-hrs Vs 40-hrs	16.36***
8-hrs Vs 32-hrs	5.69***	16-hrs Vs 40-hrs	2.57	36-hrs Vs 40-hrs	0.96

Key, \* = P<0.05 ; \*\* = P<0.01 ; \*\*\* = P<0.001

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