CHAPTER V.

Biosynthesis of Cholesterol from acetate 2-C-14 in the Ovary and female accessory glands of the Cockroach.

(Qualitative study)

( This work was done at Bhabha Atomic Research Centre, Bombay, by the kind courtesy of Dr.G.B.Nadkarni, F.N.A., Head, Biochemistry & Food Technology Division).
Recent work of Shrivastava (1975, 1976 a,b, 1978) and Shrivastava and Shrivastava (1976), Shrivastava (1979) and Shrivastava (1980) fully confirms the view that some amino acids especially phenolic amino acids do take part in the biosynthesis of cholesterol while other amino acids influence this process in the negative direction in the vertebrate systems. Oser (1976) also mentions that any compound which is a precursor of acetyl CoA is also a precursor of steroids. Included in this category are carbohydrates, fatty acids and amino acids. As detailed in the previous chapters and in the above references, the capacity of production of cholesterol varies in different amino acids and in various organs. Many other compounds, specially vitamins also influence the biosynthesis of cholesterol from acetate (Eskelson et al., 1970, 1972). Since some of the amino acids increase the amount of cholesterol in in vitro studies in the Cockroach (Chapter, IV) it was considered necessary to examine the issue with the use of labelled acetate so as to confirm whether cholesterol synthesis at all takes place in the cockroach.

METHODS

Ovaries and female accessory glands from two cockroaches were dissected out and separately pooled in refrigerated insect saline (0.75%). Each of the material was homogenized in a glass homogenizer and the volume of the aliquot was made to 3 ml by adding
adequate saline. The homogenized material was divided into three portions of 1 ml. each in different test tubes marked 1, 2 & 3. Ovarian and female accessory gland material were treated similarly as follows:

**Test Tube No. 1:**

Ethyl alcohol and ether mixture (1:1) was immediately added to the homogenate.

**Test Tube No. 2:**

Ethyl alcohol and ether mixture (1:1) was added to the homogenate after 30 minutes.

**Test Tube No. 3:**

In this test tube sodium acetate 2-C-14 was added. The quantity of labelled acetate was 2 mg/2 x 10^6 DPM/0.2 ml. The incubation period was 30 minutes after which ethyl alcohol and ether mixture (1:1) was added to it.

The alcohol ether mixture was evaporated and cholesterol from each tube was extracted by 10 ml. of solvent ether 10 times. When only a little amount of solvent ether remained in the test tube after evaporation, it was taken up in a glass capillary and a spot was applied on silica gel plate for thin layer chromatography.

**Preparation of TLC plates and TLC:**

Glass plates of 0.25 mm thickness were utilized for this purpose. 40 gms. of silica gel 'G' (BDH) was mixed with 70 ml. of deionized water and the paste was spread on the plates with an applicator. The glass plates were dried for 10 minutes at room temperature and
activated for one and half hour in an oven at 110° C. Spots were applied after the plates were cooled down to room temperature. The entire method was based on Stahl (1969). Pure chloroform (BDH) was utilized as the solvent system for ascending chromatography in chromatographic jar. When the solvent run for 3/4 the distance of the plate, the plates were taken out and dried at room temperature.

The entire experiment was repeated thrice. In each case, a spot of pure cholesterol was applied along with the experimental material. The spots of one set were developed by keeping the plates in an iodine chamber. The spots of the other set were developed by spraying with sulphuric acid and keeping it in the oven at 80° C for 5 minutes. Radioactive spots were scrapped from the TLC plates under ultraviolet light.

Preparation of stock solution of labelled acetate and other details:

The labelled acetate (CH₃COONa) was supplied by isotope group, Bhabha Atomic Research Centre, Bombay. Its vacuum sealed package contained 0.53 mg of sodium acetate-2-C-14. Its radioactivity was 0.1 millicurie (mc) and specific activity 15.4 mc/millimole. It was dissolved in 1 ml of water and 9.50 mg of cold acetate was added to it. Thus the stock solution contained a total of 10 mg of acetate in 1 ml of distilled water. Out of this stock solution, 0.2 ml was taken out each time for in vitro incubation with 1 ml of homogenated tissue in insect saline. The radioactivity of the sample
can be estimated as follows:

10 mg sodium acetate/ml = 0.1 mc

The quantity of radioactive solution used in incubation = 2 mg / 0.2 ml

Hence its radioactivity = 2 mg/ 20 uc (microcurie) = $2 \times 10^6$
disintegrations per minute/ 0.2 ml.

Results:

All developed TLC spots gave positive evidence of existence of cholesterol in the ovarian and female accessory glandular tissue. The scintillation counter used for measuring the amount of radioactivity in the TLC spot gave the following average counts.

Initial counts in the substrate
acetate -2-C-14 = $2 \times 10^6$ DPM

Ovarian tissue = 751 counts/min.
259 background radiation (cpm)
492 net cpm.

♀ Accessory glands
90 counts/minute
35 background radiation (cpm)
55 net cpm

Thus the ovary appears to be the seat of some amount of biosynthesis of cholesterol from acetate. In chemical estimations also ovary gives an indication of the highest amount of synthesizing capacity of cholesterol.
among the various tissues of the body. Although this study was primarily conducted from a qualitative viewpoint, gross quantitative estimate gives a ratio of 80,000 to 4000 acetate units to one unit of acetate converted to cholesterol in the system (Ovary). Thus only doubtful amounts of cholesterol are found in this insect. Ordinary chemical methods are not sufficiently sensitive to give a true quantitative estimate and only studies with gas liquid chromatography and mass spectrometry can provide detailed data.

Discussion:

Detailed discussion is provided in the Chapter IV (page 122 to 145) of this thesis.
REFERENCES


Shrivastava, R.K. : Anaerobic biosynthesis of cholesterol from Dopa and significance of Ketoeno1 tautomerization.

Shrivastava, S. : Studies on oxygen consumption in the frog brain and its correlation with cholesterol and electrolyte concentration under the influence of amino acids.