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The cholesterol content of the outer and inner membranes of guinea-pig liver mitochondria

A better understanding of the role of cholesterol in membrane structure[1,2] could be obtained if cholesterol determinations were made on a wider variety of pure preparations of cell membranes. This report gives the results of cholesterol determinations on the purified outer and inner membranes of guinea-pig liver mitochondria[3,4].

Cholesterol was estimated by extracting 1–5 ml of the membrane suspension containing 1–50 mg/ml of membrane protein with chloroform–methanol (2:1)[5]. The washed chloroform–methanol extract of total lipids was then evaporated to dryness. The residue was re-dissolved in 5 ml of hexane and passed through a silicic acid (Unisil, 200–325 mesh activated silicic acid, Clarkson Chemical Co. Inc., Williamsport, Pa.) column (1 cm × 5 cm). After passing the hexane solution of lipids through the column, the column was washed with 2 × 5 ml of hexane. The neutral lipids (mainly cholesterol) were eluted with 50 ml chloroform (containing 2% ethanol preservative). Silicic acid column separation was necessary because of strong interference in the Liebermann–Burchard color reaction when the reaction was carried out on total lipid fractions from mitochondria. The neutral lipid fraction was evaporated to dryness in a water bath and the residue re-dissolved in chloroform. The cholesterol was determined in the chloroform solution using the Liebermann–Burchard color reaction following the method of STADTMAN[6]. The color was read in a cell of 1-cm light path using a Zeiss spectrophotometer (Model No. M4Q III) at a wavelength of 625 mμ. All determinations refer to total cholesterol, without preliminary hydrolysis of cholesterol esters. Proteins were determined by the method of LOWRY *et al.*[7].

The results (Table I) include whole mitochondria (as isolated[3,4]). The low-speed pellet obtained after swelling of the mitochondria contains mainly inner membrane but some ruptured outer membrane still remains attached to the inner membrane.

TABLE I

CHOLESTEROL CONTENT OF GUINEA-PIG LIVER MITOCHONDRIA MEMBRANE FRACTIONS

Number of experiments in parentheses.

Fraction	*Cholesterol (μg/mg protein) (A)*	*Phospholipid* (mg/mg protein) (B)*	*Cholesterol (μg/mg phospho-lipid (A/B)*	*Molar ratio** cholesterol: phospholipid*
Whole mitochondria	2.28 ± 0.68*** (10)	0.159 ± 0.031 (4)	14.3 ± 5.0§	1:29 to 1:59
Low-speed pellet after swelling	1.97 ± 1.04 (10)	0.142 ± 0.027 (3)	13.9 ± 8.3	1:25 to 1:99
Inner membrane	5.06 ± 2.22 (5)	0.301 ± 0.072 (4)	16.8 ± 8.4	1:22 to 1:66
Outer membrane	30.1 ± 12.8 (14)	0.878 ± 0.132 (4)	34.3 ± 15.4	1:11 to 1:29
Microsomes	30.2 ± 2.3 (8)	0.391 ± 0.092 (3)	77.2 ± 18.9	1:6 to 1:9

* Results by THOMPSON[4].

** Mol. wt. of phospholipid = 700, cholesterol = 387.

*** Standard deviation = $\sqrt{\Sigma \frac{(x - \bar{x})^2}{n - 1}}$, $\bar{x}$ = mean, n = number of determinations.

§ Standard deviation[19] of ratio A/B = $\pm$ A/B · $\sqrt{\frac{(\text{S.D.}_A)^2}{(A)} + \frac{(\text{S.D.}_B)^2}{(B)}}$.

Of the sucrose gradient fractions, Fraction B consists of the detached outer membrane and Fraction P the inner membrane "ghosts" without outer membrane attached. The phospholipid to protein ratios were determined on the same or similar fractions by Dr. W. THOMPSON. In general agreement with past reports[8], we found a low content of cholesterol in whole mitochondria. For whole guinea-pig liver mitochondria the range was 1.0 to 1.8% of total lipids. This range was slightly lower than reported for other types of mitochondria (2–5%)[8]. Since most isolated mitochondria preparations are contaminated by significant amounts of microsomes, it is possible that this small amount of cholesterol was associated with microsomal membranes rather than with the mitochondrial membranes. Table I indicates that only 8% microsomal contamination (in terms of membrane protein) would be required to give the observed cholesterol content. In our experience most isolated mitochondria preparations are contaminated to about this extent. However, the outer membrane fraction contained more cholesterol than the inner membrane. Determinations of microsomal content of the outer membrane fraction[4] indicated that the apparent cholesterol content of the outer membrane was not due to contamination by microsomes. On comparing (Table I) the cholesterol contents for the outer membrane and inner membrane, it is clear that the outer membrane contained significantly more cholesterol (measured in relation to protein) than the inner membrane. The data of Table I do not establish that the molar ratio of cholesterol to phospholipid is different for the two membranes. However, taking together the results for whole mitochondria, low-speed pellet and inner membrane it appeared likely that there was a smaller proportion of cholesterol to phospholipid in the inner membrane as compared with the outer membrane. The results of Table I, while of limited accuracy, emphasize the different chemical composition of the outer and inner membranes. The ratios of protein:phospholipid:cholesterol were 1:0.878:0.030 for the outer membrane and 1:0.301:0.005 for the inner membrane. Making the approximation that these were the main components of the membranes, the outer membrane contained 1.6% by dry weight of cholesterol and the inner membrane 0.4% of cholesterol. As previously reported[4], the phospholipid composition of the two membranes was quite different. The cholesterol content of the outer membrane resembled that of microsomes. Other similarities in DPNH–cytochrome b_5 and phosphatidyl inositol content have been reported previously[4].

Previously reported values of the molar ratio of cholesterol to phospholipid for whole mitochondria vary widely[9], as follows: rat liver 1:9 (ref. 10), 1:16 (ref. 11), 1:22 (ref. 12); pig heart 1:21 (ref. 13); bovine adrenal medulla 1:3 (ref. 14). Our own results give for guinea-pig liver mitochondria a mean ratio of 1:39 (1:29 to 1:59). For microsomes the ratios were: rat liver 1:2 (ref. 15), 1:9 (ref. 10), 1:23 (ref. 12); pig heart 1:4 (ref. 13); bovine adrenal medulla 1:2 (ref. 14). Our results of guinea-pig liver microsomes give a mean ratio of 1:7 (1:6 to 1:9). Thus the cholesterol to phospholipid molar ratios for mitochondria and microsomes are not established at a constant value even for a single tissue such as rat liver. These variable results may be ascribed to inaccuracies in the assay[16], impure membrane preparations and possible variations in membrane cholesterol with level of cholesterol synthesis.

The present cholesterol determinations reinforce our previous suggestion[17] that structurally, the outer and inner membranes of mitochondria are very different from one another with respect to protein, phospholipid, cholesterol and enzymatic composition. These observations give no support to the proposal that a single lipid model,

such as the DAVSON–DANIELLI model[18], can be applied to all cell membranes, even though this model may well be suited for the structure of myelin. The inner membrane of mitochondria is of a unique type, amongst the cell membranes and resembles more a bacterial plasma membrane. The outer membrane of mitochondria resembles smooth endoplasmic reticulum. We conclude that it remains unestablished whether cholesterol exists in fixed stoichometry with respect to phospholipid in mitochondria and microsomes.

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