

CHOLESTEROL AND PHOSPHOLIPID COMPOSITION OF MITOCHONDRIA AND MICROSOMES ISOLATED FROM MORRIS HEPATOMA 5123 AND RAT LIVER

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1. Introduction

The major component of membranous lipids is represented by phospholipids [1]. It is well known that this class of lipids plays an important role in regulating the biochemical properties of subcellular particles [2]. Moreover, there exists a cholesterol:phospholipid ratio peculiar for several kinds of membranes [3]. Cholesterol is important in stabilizing arrays of phospholipids in cellular and cytoplasmic membranes [4, 5], and in regulating the permeability to small molecules of artificial [6, 7] and natural [8] membranes. Lipid-lipid interactions play an important role in the interaction between lipid and protein [3]; the protein conformation is greatly influenced by their association with the lipid environment [9]. Alterations in the protein components of mitochondrial membranes have been observed in hepatoma mitochondria [10, 11]. They could be, at least in part, explained by changes in the lipid composition of membranes. In hepatoma mitochondria, we have also found functional changes, which could be related to high fragility and to an increased resistance to deformation and stretching [12, 13]. Similar alterations have been observed also in cholesterol-enriched mitochondria [5].

The knowledge of lipid composition of membranes isolated from tumors is lacking and, sometimes, contrasting [14, 15]. As a first approach to this problem we have studied the phospholipid and cholesterol content of mitochondria and microsomes isolated from rat liver and Morris hepatoma 5123.

2. Methods

Female Buffalo rats (150–180 g) were used to transplant Morris hepatoma 5123, and as a source of normal liver. Mitochondria were isolated as described previously [13] and washed three times. Some mitochondrial preparations were further purified as suggested by Neubert et al. [16]. In order to isolate microsomes the post-mitochondrial supernatants were first centrifuged at 250,000 *g*-min and washed once. Microsomes were sedimented from the collected supernatants, at 6×10^6 *g*-min. Soluble and insoluble fractions of sonicated mitochondria were separated as described by Mitchell [17]. Glucose-6-phosphate dehydrogenase was determined according to Lohr and Waller [18], cytochrome *c* oxidase according to De Duve et al. [19], malate dehydrogenase according to Schnaitman and Greenawalt [20], glucose-6-phosphatase according to Swanson [21]. Total lipids were isolated according to Folch et al. [22]. Neutral and polar lipids were separated by silicic acid columns (Unisil, Clarkson, Pa., USA); the different classes of phospholipids were separated and determined according to Skipski et al. [23]. Cholesterol was isolated by thin-layer chromatography using as a solvent *n*-heptane:isopropylether:formic acid (60:40:2), and determined according to Bowman and Wolf [24]. A biuret method was used to determine the protein [25].

3. Results

The data in table 1 show that the glucose-6-phosphatase activity is about 13–14 times higher in

Table 1

Glucose-6-phosphatase and cytochrome *c* oxidase activities in mitochondrial and microsomal fractions from liver and hepatoma.

Fraction	Glucose-6-phosphatase (nmoles of P _i /min/mg)	Cytochrome <i>c</i> oxidase (nmoles of cyt. <i>c</i> oxidized/min/mg)
Liver		
Mitochondria	16.4 ± 4.8	1,100 ± 158
Microsomes	234 ± 16	10.0 ± 5.8
Hepatoma		
Mitochondria	5.2 ± 1.5	1,097 ± 90
Microsomes	61 ± 4	8.4 ± 4.1

Mean values of 3 expts. ± S.D.

microsomal than in mitochondrial fractions from both liver and hepatoma, indicating that the extent of microsomal contamination is about 7%. The cytochrome *c* oxidase is 110–130 times higher in mitochondria than in microsomes from both tissues; this corresponds to a concentration of mitochondria in the microsomal fraction lower than 1%. The contamination of mitochondria by red cells, whose high content of cholesterol is known [3], was considered. The activity of glucose-6-phosphate dehydrogenase, a marker for erythrocytes, was almost absent in mitochondria. As shown in table 2, the cholesterol and phospholipid contents in normal liver mitochondria are, respectively, about 50% and 70% lower than in microsomes. In

hepatoma, the cholesterol content is higher in mitochondria than in microsomes. This arises from a significant increase in cholesterol per mg of mitochondrial protein, the cholesterol content per mg of protein being unchanged in microsomes. The phospholipid content is greatly decreased in both mitochondria and microsomes from hepatoma, as compared with the same particles from liver. If we consider the molar ratio cholesterol:phospholipid, we observe a 5-times increase in hepatoma mitochondria and a 2.7-times increase in hepatoma microsomes. In some experiments the mitochondria from both liver and hepatoma have been submitted to a further purification according to Neubert et al. [16]. Differences have not been found between the not-purified and the purified preparations in the content of both cholesterol and phospholipids. As this purification removes essentially nuclei and nuclear fragments [16], our data indicate that the latter contaminants do not contribute to the observed differences between the particles from liver and hepatoma.

Parsons and Yano [26] have reported that mitochondrial cholesterol is essentially located in the membrane fraction, mainly in the outer membrane. In order to determine to what extent the membranes contribute to the cholesterol increase in hepatoma mitochondria, we have separated the soluble from the insoluble compartment of mitochondria as described by Mitchell [17]. As shown in table 3 the strong sonication releases 40% of protein in liver

Table 2

Cholesterol and phospholipid content of mitochondria and microsomes from liver and hepatoma.

Fraction	Cholesterol (μg/mg protein)	Phospholipid (mg/mg protein)	Cholesterol (μg/mg phospholipid)	Molar ratio* (Cholesterol:phospholipid)
Liver				
Mitochondria	12.3 ± 3.3 (8)	0.187 ± 0.015	65 ± 10.8	1:8.5
Microsomes	25.9 ± 5.1	0.283 ± 0.079	91 ± 30.3	1:6.0
Hepatoma				
Mitochondria	40.8 ± 8.2 (6)	0.115 ± 0.035 (6)	355 ± 129.0	1:1.6
Microsomes	25.8 ± 3.2 (4)	0.102 ± 0.010 (4)	258 ± 40.8	1:2.2

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

Mean values ± S.D. Number of experiments in parentheses. The S.D. of the ratio cholesterol:phospholipid (μg/mg) was calculated according to Worthing and Jeffner [33].

Table 3
Cholesterol content of insoluble fraction of liver and hepatoma mitochondria.

Source of mitochondria	Protein		Malate dehydrogenase		Cholesterol Insoluble
	Soluble	Insoluble	Soluble	Insoluble	
Liver	40 ± 2	60 ± 4	80 ± 15	16 ± 6	82 ± 10
Hepatoma	29 ± 2	71 ± 2	75 ± 12	21 ± 3	76 ± 4

Mitochondria (30 mg of protein) were suspended in 20 ml of water and submitted to 3 min of sonication at 0° using a Biosonik III sonifier at its maximum output; 9 ml aliquots were then centrifuged at 21×10^6 g-min. The supernatant and sediment are referred to as soluble and insoluble components, respectively. The data (mean values of 4 expts. ± S.D.) are expressed as percent of the total content measured in sonicated, unfractionated mitochondria.

mitochondria and 29% in hepatoma mitochondria, and respectively, 80% and 75% of the matrix enzyme malate dehydrogenase in both liver and hepatoma mitochondria. The main fraction of cholesterol is clearly bound to the insoluble material in both types of mitochondria.

As far as the phospholipid composition of mitochondria and microsomes is concerned, data in fig. 1 show that no appreciable differences occur between hepatoma and liver subcellular particles. In both tissues we have found the same difference in the distribution of the phospholipid classes between microsomes and mitochondria. In fact, cardiolipin is high in mitochondria and almost absent in microsomes; on the other hand, sphingomyelin is higher in microsomes than in mitochondria.

4. Discussion

Our data indicate that a significant increase of cholesterol occurs in mitochondria from Morris hepatoma 5123. It is unlikely that such results depend on the impurity of the subcellular fractions. This is shown by the distribution of the activities of the marker enzymes in our fractions. Moreover, a supporting datum of an acceptable separation between mitochondria and microsomes in liver and hepatoma is given by the distribution of cardiolipin and sphingomyelin, which specifically characterizes the two subcellular particles. Moreover, the molar ratio cholesterol:phospholipids in our liver organelles is in agreement with that calculated on the basis of the cholesterol content observed by others [5, 27, 28].

It has been shown [5, 29] that the *in vivo* cholesterol enrichment of liver mitochondria renders them more resistant to the large amplitude swelling and to the changes occurring in the conformation of the particles associated with changes of the metabolic state. This probably arises from an increased strength of the membranes and/or from a reduced ability to exchange ions [5, 8]. A very high resistance to large amplitude swelling and to conformational changes was also found in hepatoma mitochondria [12, 30], so that we might suggest the hypothesis that these alterations could be related to the high cholesterol content in hepatoma mitochondria. In this case our data could give some insight to the problem of the existence of alterations of the mitochondrial dynamics in the intact tumor cell, as it is probable that the increase in mitochondrial cholesterol is present in the unfractionated hepatoma cells. An increase in cholesterol per phospholipid molecule has also been found in microsomes; van Hoeven and Emmelot [3] have observed

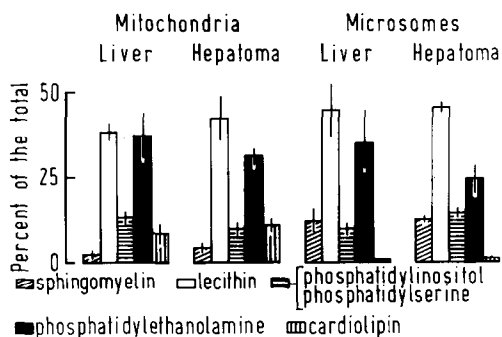


Fig. 1. Phospholipid classes in mitochondria and microsomes from liver and hepatoma. The data (mean values of 5 expts. ± S.D.) are given as percent of the total phospholipids.

an increase of cholesterol content in hepatoma plasma membrane. The high cholesterol levels in different subcellular particles from hepatomas could reflect the loss of feedback inhibition of cholesterol biosynthesis in these tumors [32].

The lack of any differences between hepatoma and liver in the phospholipid composition in mitochondria and microsomes contrasts with the results of Bergelson et al. [15], who found that the phospholipid classes of the different membranes are almost equalized in tumor cells and suggested the existence of a chemical dedifferentiation of tumor cellular membranes.

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References

- [1] S.K. Malhotra, *Progr. Biophys. Mol. Biol.* 20 (1970) 67.
- [2] S. Razin, *Biochim. Biophys. Acta* 265 (1972) 241.
- [3] L.L.M. Van Deenen, in: *Progress in the chemistry of fats and other lipids*, ed. R.T. Holman (Pergamon Press, Oxford, 1965).
- [4] K.W. Butler, I.C.P. Smith and H. Schneider, *Biochim. Biophys. Acta* 219 (1970) 514.
- [5] J.M. Graham and C. Green, *European J. Biochem.* 12 (1970) 58.
- [6] R.A. Klein, M.J. Moore and M.W. Smith, *Biochim. Biophys. Acta* 233 (1971) 420.
- [7] R.A. Demel, K.R. Bruckdorf and L.L.M. Van Deenen, *Biochim. Biophys. Acta* 255 (1972) 321.
- [8] J. Kroes and R. Ostwald, *Biochim. Biophys. Acta* 249 (1971) 647.
- [9] D.F. Wallach, in: *Membrane proteins, proceedings of a Symposium Sponsored by the New York Heart Association*. (Little Brown & Co., Boston, 1969) p. 3.
- [10] L.O. Chang, C.A. Schnaitman and H.P. Morris, *Cancer Res.* 31 (1971) 108.
- [11] F. Feo, G. Bonelli, R.A. Canuto and R. Garcea, *Cancer Res.*, in press.
- [12] F. Feo, *Life Sci.* 6 (1967) 2417.
- [13] F. Feo, R.A. Canuto and R. Garcea, *Europ. J. Cancer*, 9 (1973) 203.
- [14] S. Ruggieri and A. Fallani, *Lo Sperimentale* 118 (1968) (1973) 203.
- [15] L.D. Bergelson, E.V. Dyatlovitskaya, T.I. Torkhovskaya, I.B. Sorokina and N.P. Gorgova, *Biochim. Biophys. Acta* 210 (1970) 287.
- [16] D. Neubert, E. Oberdisse and R. Boss, in: *Biochemical aspects of biogenesis of mitochondria*, eds. E.C. Slater, J.M. Tager, S. Papa and E. Quagliariello (Adriatica Editrice, Bari, 1968) p. 123.
- [17] R.F. Mitchell, *Biochim. Biophys. Acta* 176 (1969) 764.
- [18] G.W. Lohr and H.D. Waller, in: *Methods of enzymatic analysis*, ed. H.U. Bergmeyer (Academic Press, New York, 1963) p. 744.
- [19] C.D. De Duve, B.C. Pressman, R. Gianetto, R. Wattiaux and F. Appelmans, *Biochem. J.* 60 (1965) 604.
- [20] C. Schnaitman and J.W. Greenawalt, *J. Cell Biol.* 38 (1968) 158.
- [21] M.A. Swanson, in: *Methods in enzymology*, Vol. 2, eds. S.P. Colowick and N.O. Kaplan (Academic Press, New York, 1955) p. 541.
- [22] J. Folch, M. Lees and G. Sloane Stanley, *J. Biol. Chem.* 226 (1957) 497.
- [23] V.P. Skipski, R.F. Peterson and M. Barclay, *Biochem. J.* 90 (1964) 374.
- [24] R.E. Bowman and R.C. Wolf, *Clin. Chem.* 8 (1962) 302.
- [25] A.C. Gornall, C.J. Bardawill and M.M. David, *J. Biol. Chem.* 177 (1949) 751.
- [26] D.F. Parsons and Y. Yano, *Biochim. Biophys. Acta* 135 (1967) 362.
- [27] L.A.E. Ashworth and C. Green, *Science* 151 (1966) 210.
- [28] R.F. Mitchell, *Biochim. Biophys. Acta* 176 (1969) 764.
- [29] B. Prasad, N.K. Carg and C.R. Krishna Murti, *Indian J. Biochem. Biophys.* 8 (1971) 167.
- [30] F. Feo and A. Matli, *Cancer Res.* 30 (1970) 2223.
- [31] R.P. van Hoeven and P. Emmelot, *J. Membrane Biol.* 9 (1972) 105.
- [32] M.D. Siperstein, V.M. Fagan and H.P. Morris, *Cancer Res.* 26 (1966) 7.
- [33] A.G. Worthing and J. Jeffner, *Treatment of Experimental Data* (Wiley, New York, 1943) p. 207.