DETERMINATION OF THE QUANTITATIVE RELATIONSHIP OF OUTER AND INNER MEMBRANE PROTEINS IN RAT LIVER MITOCHONDRIA BY MEANS OF ENZYMOLOGY AND ELECTRON MICROSCOPY

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Received 21 November 1969

1. Introduction

Since the first publications on separation of outer and inner mitochondrial membranes by Parsons et al. [1, 2], Sottocasa et al. [3] and Schnaitmann et al. [4] numerous reports on the properties and composition of mitochondrial membranes have appeared. So far no attempt has been made to determine the quantity of each membrane in respect to the whole mitochondrion. This question became of interest, when the groups of Green [5, 6] proposed extremely different views on the qualities and quantities of mitochondrial subparticles from those widely accepted elsewhere. They stated the outer membrane of mitochondria to amount to 33% of the total mitochondria protein on a basis of enzyme- and phospholipid content. There is a special need to determine the quantity of mitochondrial membranes because it has been shown that enzymes long believed to be microsomal marker enzymes are also present in outer mitochondrial membranes [2, 7]. Electron microscopic studies on the weight and volume of subcellular particles have been carried out by Bahr and Zeitler [8] and Weibel et al. [9] and in a recent study Loud [10] measured by stereological calculations of electron microscopic pictures of the rat liver not only the volume of mitochondria, peroxysomes, lysosomes, lipid and glycogen but also tried to determine the volumes of three mitochondrial regions: envelope (13.8%), cristae (25.5%) and matrix (60.7%).

In this paper the quantity of outer and inner membrane of rat liver mitochondria has been determined by biochemical methods and electron microscopy and a combination of the two methods resulting in the following values, varying with the different methods applied: outer membrane 6–10%, inner membrane 25–29% and matrix near 65% of total mitochondrial protein.

2. Materials and methods

Rat liver mitochondria were prepared according to Schneider [11]. Pure outer and inner mitochondrial membranes were prepared as recently described [12, 13]. Monoamine oxidase was determined according to Tabor et al. [14]. Succinic dehydrogenase was determined as described by Brdiczka et al. [15]. Electron micrographs were carried out by standard osmium tetroxide fixation, Maraglas embedding and Pb-staining. A Zeiss electron microscope EM-9 was used. Planimetric measurements were carried out on 200 enlarged electron micrographs of mitochondria in native rat liver. Soluble and insoluble mitochondrial proteins were determined by the following procedure: mitochondria were suspended in 10 ml of 0.1 M phosphate buffer at pH 7.3 and sonicated (Branson sonifier step 6) in an ice bath for 40 sec, 10 sec at a time and 30 sec interval. The suspension was then centrifuged at 144,000 × *g* for 60 min; the supernatant was decanted and the pellet resuspended in 10 ml of 0.1 M phosphate buffer. The sonication and centrifugation steps were repeated as above. The fractions obtained were then analysed for their

Table 1
Content of monoamine oxidase (MAO)in whole mitochondria and pure outer membranes.

	Experiment	1	2	3
Mitochondria	Spec. activity of MAO	320	700	580
	% of pure outer membrane	7.5	8.7	8.0
Outer membrane	Spec. activity of MAO	4800	8000	7200

Table 2
Content of succinic dehydrogenase (SDH) in whole mitochondria and pure inner membrane.

	Experiment	1	2	3
Mitochondria	Spec. activity of SDH	13.6	16.0	14.5
	% of pure inner membrane	27.8	27.0	25.5
Inner membrane	Spec. activity of SDH	50.0	59.3	57.0

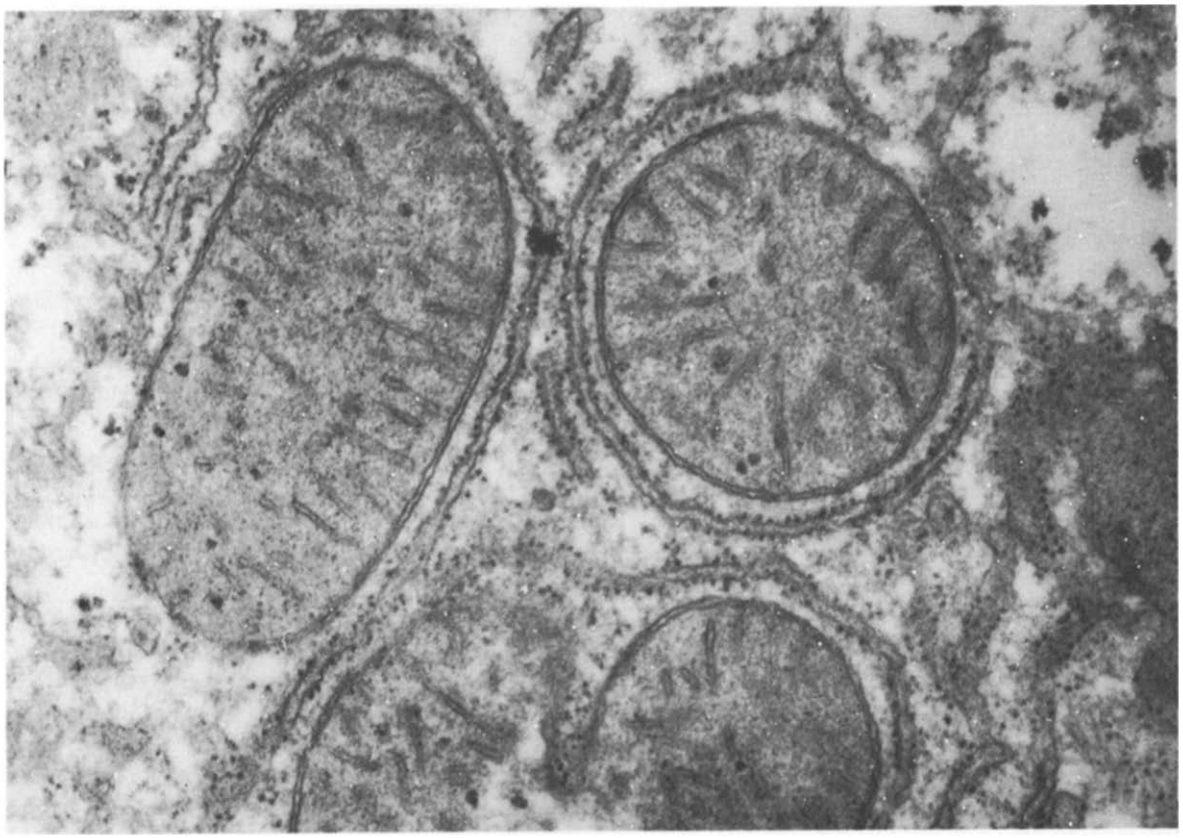

Fig. 1.

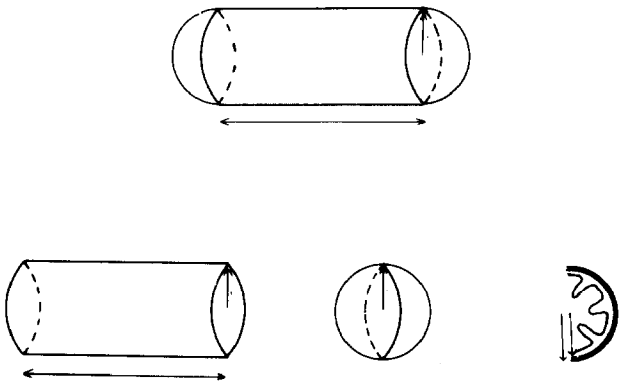

Fig. 2.

protein content. The supernatants decanted after each centrifugation are designated "soluble" protein and the pellet "insoluble" protein.

3. Results

3.1. *Enzymatic determinations*

In tables 1 and 2 the enzymatic activity of two typical marker enzymes for outer and inner membranes is shown in pure inner and pure outer mitochondrial membranes compared to whole mitochondria of the same preparation. In achieving pure membrane fractions, we applied an improved techique [6] leading to fractions with less than 5% contamination of other subparticles. Monoamine oxidase, a marker enzyme for the outer membrane of rat liver mitochondria, can be found in whole mitochondria to an extent of 7–10% of the specific activity of the corresponding purified outer membrane fraction. The amount of inner mitochondrial membrane was estimated by using succinate dehydrogenase as marker enzyme. Table 2 shows that by this approach the inner membrane amounts to 25–28% of the whole mitochondrion.

3.2. *Electron microscopy*

The following two sets of calculations were carried out on enlarged electron micrographs of mitochondria of native rat liver.

Calculation 1: In calculating the volume of the mitochondrion it was assumed that the mitochondrion within the cell is a sphere or a body geometrically composed of two half spheres plus a cylinder (figs. 1 and 2). Although mitochondria can have many different shapes, we noted that two predominate in native rat liver – the longitudinal and the spherical. By substracting the volume of the sphere or the cylinder with the radius r' (volume without outer membrane) from the total volume of the mitochondrion (radius r) the total content of the outer membrane can be obtained. Dividing this volume by that of the total volume results in the percentage of the outer membrane content:

$$\frac{\pi r^2 h - \pi r'^2 h}{\pi r^2 h} \text{ and } \frac{\frac{4}{3}\pi r^3 - \frac{4}{3}\pi r'^3}{\frac{4}{3}\pi r^3}.$$

Applying this calculation to a large number of mitochondrial pictures we have calculated the volume of the outer membrane assuming either a cylindrical plus spherical or a spherical shape to amount to 4–10% of the total mitochondrion.

Table 3
Proportion of soluble and insoluble protein in mitochondria isolated from rat liver.

Fraction	Protein content (mg)
Original suspension	13.6
First soluble fraction	8.8
Second soluble fraction	0.88
Pellet	4.62

Soluble and insoluble mitochondrial proteins were determined by the procedures given in section 2.

Recovery 100%

Ratio: $\frac{\text{Insoluble protein}}{\text{Total protein}} = 4.62 : 13.6 = 34\%$.

Calculation 2: In this approach we first determined the amount of soluble and insoluble proteins of rat liver mitochondria (table 3). We found the soluble proteins to amount to 66% and the insoluble proteins to 34% of the total mitochondrial proteins. In order to determine the ratio of outer and inner membranes in the mirochondrion, we measured in 200 enlarged electron micrographs of native rat liver the sum of the length of the outer membrane and the inner membranes. A ratio of outer to inner membrane of 1 : 2.5 was obtained. Dividing the value for the membranous

protein (34%) by the sum of the ratio of outer and inner membranes 3.5 (1 + 2.5) results in the percentage for each fraction of the total mitochondrial protein content: 9.6% for outer membranes and 24.4% for inner membranes.

4. Discussion

Bearing in mind the assumption made and the completely different methods and techniques applied there is a close agreement in the values obtained for the relationship of mitochondrial subfractions. Our findings coincide with the values of Schiefer [16] who on a protein basis roughly estimates the outer membrane to amount to approximately 10% and the inner membrane to amount to 40% of the total mitochondrial protein. These findings are in contrast to the findings of Green et al. [6] who claim the outer membrane to amount to 30—40% of the total mitochondrial protein. This latter value becomes more doubtful if one considers that the calculations were carried out on bovine heart mitochondria which are known to contain far more inner membrane than do liver mitochondria. Differences in the values for the quantity of inner mirochondrial membranes from the rough estimates by Schiefer [16] may easily be explained by different preparation techniques. Sonic oscillations of different strength and duration will detach different amounts of matrix protein from the inner membrane, more than if treated by osmotic shock. These results may be helpful in calculating contamination of mitochondrial membrane fractions by other subcellular particles, especially microsomes, since recently it was shown that some microsomal enzymes are also present in the outer mitochondrial membrane.

Acknowledgements

The authors are grateful to Dr. F.L.Bygrave for critical advice. This work was supported by the Deutsche Forschungsgemeinschaft.

References

[1] D.F.Parsons, G.R.Williams and B.Chance, Ann. N.Y. Acad. Sci. 137 (1966) 643.
[2] D.F.Parsons, G.R.Williams, W.Thompson, D.Wilson and B.Chance, in: Mitochondrial Structure and Compartmentation, Eds. E.Quagliariello, S.Papa, E.C.Slater and I.M.Tager, Vol. 29 (Adiatrica Editrice, Bari, 1967.
[3] G.L.Sottocasa, B.Kylenstierna, L.Ernster and A.Bergstrand, J. Cell Biol. 32 (1967) 415.
[4] C. Schnaitman, V.G.Erwin and J.W.Greenawal, J. Cell. Biol. 32 (1967) 719.
[5] D.W.Allmann, E.Bachmann, N.Ohme-Johnson, W.C.Tan and D.E.Green, Arch. Biochem. Biophys. 125 (1968) 981.
[6] D.E.Green, D.Allmann, R.A.Harvis and W.C.Tan, Biochem. Biophys. Res. Commun. 31 (1968) 368.
[7] G.Brunner and F.Bygrave, European J. Biochem. 8 (1969) 530.
[8] G.F.Bahr and E.Zeitler, J. Cell Biol. 15 (1962) 489.
[9] E.R.Weibel, G.S.Kistler and W.F.Scherle, J. Cell Biol. 30 (1966) 23.
[10] A.V.Loud, J. Cell. Biol. 37 (1968) 27.
[11] W.C.Schneider, J. Biol. Chem. 176 (1948) 259.
[12] G.Brunner and W.Neupert, FEBS Letters 3 (1969) 283.
[13] G.Brunner and W.Neupert, FEBS Letters 1 (1968) 153.
[14] C.W.Tabor and S.M.Rosenthal, J. Biol. Chem. 208 (1954) 645.
[15] D.Brdiczka, D.Pette, G.Brunner and F.Miller, European J. Biochem. 5 (1968) 294.
[16] H.G.Schiefer, personal communication.