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Freeze-fracture faces of inner and outer membranes of mitochondria

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SUMMARY

The concave and convex fracture faces of the inner and outer membranes of rat liver mitochondria have been studied. The patchwork pattern previously described from preparations of whole mitochondria was concluded to result from cleavage through sections of the outer membrane. The observed particle densities of concave and convex fracture faces of the inner and outer mitochondrial membranes differ and this difference may have functional significance. Thus, further studies of mitochondrial membrane surfaces using the technique of freeze-fracture will necessitate a prior separation of the inner membrane from the outer membrane.

Freeze-fracture and freeze-etch techniques have been employed to provide ultrastructural information on the hydrophobic interior of membranes[1-3] and the outer surface of membranes[4,5], respectively. With the exception of myelin[6] most membrane systems investigated so far have revealed fracture faces covered with particles. It has been proposed[7-9] that the particles represent globular protein molecules penetrating into or passing through the hydrophobic interior of the membrane and that they may be involved in catalytic functions of the membranes. In the case of the mitochondrion, Wrigglesworth *et al.*[10] have suggested that the particles may represent components of the respiratory chain.

In the earlier study from this laboratory[10] smooth mitochondrial fracture faces were observed and presumed to result from a fracture through the outer membrane. A patchwork-like pattern was also observed. It was suggested that this patchwork-like pattern could arise from a deflection of the fracture plane from either the inner membrane interior to the inner membrane surface, or alternatively, from the inner membrane interior to localized adhesions of the outer membrane. Freeze-fracture faces of purified inner and outer membranes of rat liver mitochondria were thus investigated in order to explain the formation of the patchwork-like pattern and also to determine the particle densities of these membrane systems.

Mitochondria were isolated from rat liver as previously described[11] and the inner and outer membranes were separated by the method of osmotic lysis described by Parsons *et al.*[12]. Inner and outer membrane preparations made by the methods of Schnaitman and Greenawalt[13] using digitonin, and Sottocasa *et al.*[14] using sonication, gave similar results. However, the osmotic lysis preparation was chosen since it minimizes membrane damage.

Membrane samples were treated with 20% glycerol before freezing. Replicas were prepared by the method of Moor and Muhlethaler[15] on a Balzers freeze-etch apparatus. The direction of shadowing is indicated by an encircled arrow for each figure. Replicas were examined under an Elmiskop IA (at the University of California, Berkeley, Calif.) and an RCA (at the Veterans Administration Hospital, Martinez, Calif.) electron microscope.

A typical pattern of activity for marker enzymes characterizing the inner and outer membranes and microsomal contamination is shown in Table I. Cytochrome oxidase activity[16] was measured for the inner membrane, rotenone-insensitive NADH-cytochrome *c* reductase[14] for the outer membrane and NADPH-cytochrome *c* reductase[14] to assess the degree of microsomal contamination. Protein concentration was determined using the Folin reagent[17]. From the enzyme activity pattern it was concluded that contamination of each membrane preparation was less than 10%. Conventional electron microscopy also indicated greater than 90% purity for these preparations.

TABLE I

PATTERN OF MARKER ENZYMES FOR RAT LIVER MITOCHONDRIA AND DERIVED INNER AND OUTER MEMBRANE PREPARATIONS

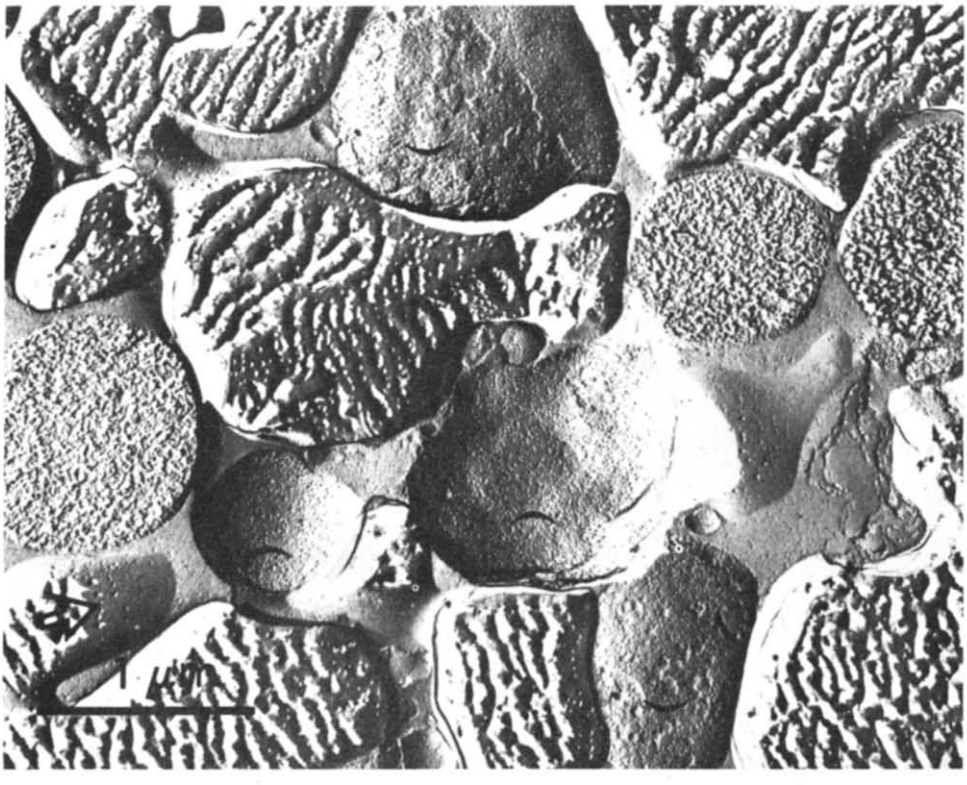
	Specific activity (nmoles/min per mg protein)		
	Cytochrome oxidase	*Rotenone-insensitive NADH-cytochrome c reductase*	*NADPH-cytochrome c reductase*
Mitochondria	988	332	7
Inner membrane	1000	239	19
Outer membrane	41	3980	84

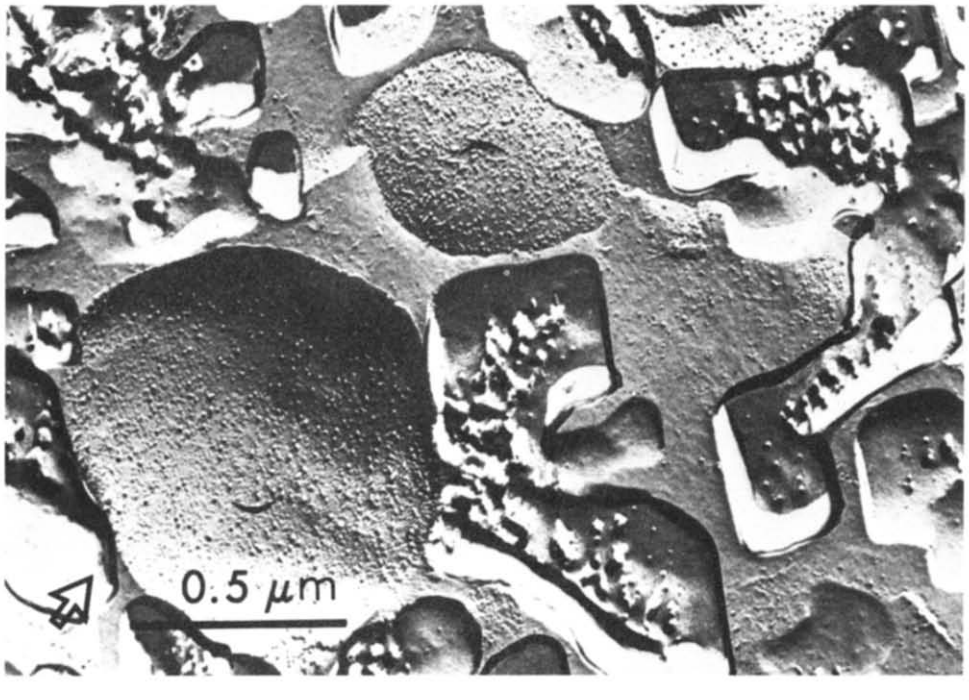
Replicas of freeze-fracture faces of rat liver mitochondria and the inner and outer membrane preparations shown in Figs. 1-3 reveal:

(a) The convex fracture face of the outer membrane is relatively smooth in appearance by comparison with the concave fracture face of the outer membrane or either the concave or convex fracture face of the inner membrane.

Fig. 1. Freeze-fracture replica of rat liver mitochondria. Note the patchwork, cross sections, and variable particle densities. ∩ represents a convex fracture face. ∪ represents a concave fracture face. x28 225.

Fig. 2. Freeze-fracture replica of a rat liver inner mitochondrial membrane preparation. Note the higher particle density on the convex (∩) fracture face in comparison to the concave (∪) fracture face. x56 450.

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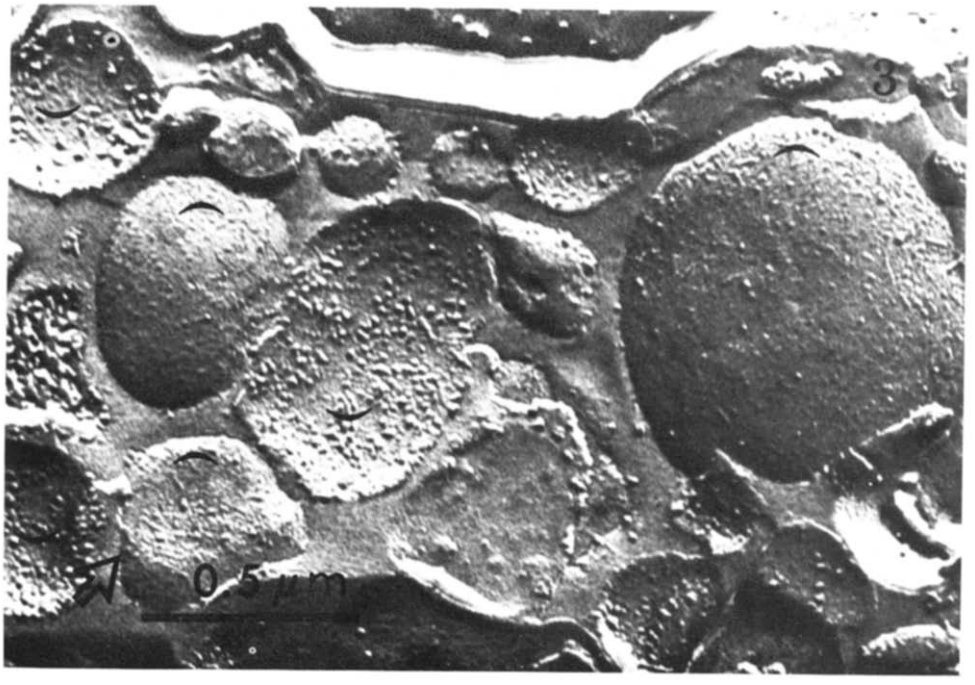

Fig. 3. Freeze-fracture replica of a rat liver outer mitochondrial membrane preparation. Note the higher particle density on the concave (∪) fracture face in comparison to the convex (∩) fracture face. ×56 450.

(b) The particle density (see Table II) of the concave fracture face of the outer membrane is similar to the particle density of the convex fracture face of the inner membrane.

(c) There is 4-fold greater particle density on the concave fracture face as on the convex fracture face of the outer membrane.

(d) The convex fracture face of the inner membrane has about twice as many particles as the concave fracture face of this membrane.

(e) The size range of most particles was between 60 and 100 Å on all fracture faces observed, similar to the distribution described by Wrigglesworth *et al.*[10].

It may be further noted that the patchwork pattern described by Wrigglesworth *et al.*[10] is seen only with intact mitochondria. If the patchwork was due to a deflection of the knife from the inner membrane interior to the outer surface of the inner membrane, then an equal amount of patchwork would be expected to be observed in the inner membrane preparations as in intact mitochondria. However, hardly any patchwork was observed after fracture of the purified inner membranes. The low particle density on the patchwork seen in intact membranes is similar to the particle density seen in fracture faces of the purified outer membrane preparations. The patchwork-like appearance therefore most likely represents cleavage through certain areas of the outer membrane. Table II summarizes the data in which the percent patchwork, cross sections and membrane fracture faces (other than patchwork) are tabulated. The particle densities for mitochondria, and the derived inner and outer membrane preparations are also given.

TABLE II

CHARACTERIZATION OF FREEZE-FRACTURE FACES OF MITOCHONDRIA AND DERIVED INNER AND OUTER MEMBRANE PREPARATIONS

	Density (particle/μm²)		*Observed profiles (%)**		
	Concave	*Convex*	*Cross sections*	*Patchwork*	*Fracture faces*
Mitochondria			21	15	64
Inner membrane	990	2290	7	4	89
Outer membrane	2120	578	3	2	95

*250 profiles analyzed for each preparation.

If the observed particles are protein molecules penetrating into the hydrophobic interior of the membrane, then the functional properties of the membrane may result from the firm attachment of certain of these proteins to either the outer or inner half of the membrane. Thus, when the membrane is split by freeze-fracture, such proteins are observed associated with the half to which they were more firmly attached. In the case of myelin membranes, which seem devoid of enzyme activity, freeze-fracture faces are devoid of particles[6]. Several authors, including Branton[2], Meyer and Winkelmann[18], and Takacs and Holt[19], have also noted different particle densities on the two fracture faces of various biological membranes. Hence, the heavier particle densities of the outer half of the outer mitochondrial membrane (cytoplasmic side) and on the inner half of the inner mitochondrial membrane (matrix side) may be of biological significance.

At the present time neither the biological function nor the chemical composition of the particles observed on the fracture faces of the mitochondrial membranes have been characterized. It would seem imperative that in studies involving identification of these particles the outer membrane be removed prior to freeze-fracture and that both concave and convex fracture faces be examined.

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REFERENCES

1 D. Branton, *Proc. Natl. Acad. Sci. U.S.*, 55 (1966) 1048.
2 D. Branton, *Annu. Rev. Plant Physiol.*, 20 (1969) 209.
3 N. Naninga, *J. Cell Biol.*, 49 (1971) 564.
4 P. Pinto da Silva and D. Branton, *J. Cell Biol.*, 45 (1970) 598.
5 T.W. Tillack and V.T. Marchesi, *J. Cell Biol.*, 45 (1970) 649.
6 D. Branton, *Exp. Cell Res.*, 45 (1967) 703.
7 K. Muhlethaler, H. Moor and J.W. Szarkowski, *Planta*, 67 (1965) 305.
8 M.E. Toutellotte, D. Branton and A. Keith, *Proc. Natl. Acad. Sci. U.S.*, 66 (1970) 909.
9 R.W. Weinstein and V.M. Koo, *Proc. Soc. Exp. Biol. Med.*, 128 (1968) 353.
10 J.M. Wrigglesworth, L. Packer and D. Branton, *Biochim. Biophys. Acta*, 205 (1970) 125.
11 R.C. Stancliff, M.A. Williams, K. Utsumi and L. Packer, *Arch. Biochem. Biophys.*, 131 (1969) 629.
12 D.F. Parsons, G.R. Williams and B. Chance, *Ann. N.Y. Acad. Sci.*, 137 (1966) 643.
13 C. Schnaitman and J.W. Greenawalt, *J. Cell Biol.* 38 (1968) 158.

14 G.L. Sottocasa, B. Kuylenstierna, L. Ernster and A. Bergstrand, *J. Cell Biol.*, 32 (1967) 415.
15 H. Moor and K. Muhlethaler, *J. Cell Biol.*, 17 (1963) 609.
16 C. Schnaitman, V.G. Erwin and J.W. Greenawalt, *J. Cell Biol.*, 32 (1967) 719.
17 O.H. Lowry, R.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
18 H.W. Meyer and H. Winkelmann, *Protoplasma*, 68 (1969) 253.
19 B.J. Takacs and S.C. Holt, *Biochim. Biophys. Acta*, 233 (1971) 258.