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Disruption of mitochondrial membrane by acetone extraction

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SUMMARY

Lipid extraction from mitochondrial electron transport particles results in the breakdown of membrane structure. Membranous structure and succinate cytochrome *c* reductase activity are restored by the addition of lipid. The fragility of the electron transport particle membrane is in contrast to the inner mitochondrial membrane which retains its structure upon lipid extraction. The results suggest a stabilizing role for lipid in cristae membrane structure which is best revealed by extraction of the isolated membrane vesicles.

It has been reported that mitochondrial membranes depleted of more than 95% of their lipid retain the unit membrane structure[1,2]. We wish to report the breakdown of membrane structure following the removal of only 72% of the lipid from mitochondrial electron transport particles by treatment with aqueous acetone.

Electron transport particles are prepared from isolated beef heart mitochondria[3] by sonic disruption[4]. The particles, at a protein concentration of 25 mg/ml in 0.25 M sucrose–0.01 M Tris–HCl (pH 7.4) are extracted as previously described[2] with the following concentrations of aqueous acetone: 4% water in acetone, 10% water in acetone, and 10% water in acetone *plus* ammonia.

The phospholipid content and the succinate cytochrome *c* reductase activity of the acetone-extracted electron transport particle vesicles are summarized in Table I. The various treatments remove up to 84% of the total phosphorus and result in a dependence upon added lipid for succinate cytochrome *c* reductase activity[2,5,6]. Restoration of mitochondrial lipid to the acetone-extracted vesicles results in full or partial recovery of enzyme activity[2,7].

The fine structure of the electron transport particle vesicles before and after acetone extraction and after restoration of mitochondrial lipids to the extracted vesicles are observed in the electron microscope using the techniques of negative staining and thin sectioning. Fig.1 shows the untreated electron transport particle vesicles. Negative staining reveals the 90-Å inner membrane particles at the edges of the flattened vesicles. The sectioned vesicles clearly demonstrate unit membrane structure.

TABLE I

PHOSPHOLIPID CONTENT AND ENZYMATIC ACTIVITY OF ELECTRON TRANSPORT PARTICLES AFTER ACETONE EXTRACTION

Succinate cytochrome *c* reductase activity was measured spectrophotometrically at 30° by the method of Fleischer and Fleischer[9]. Specific activities of the acetone-extracted fractions were assayed in the absence and presence of added coenzyme Q or extract. The light petroleum-soluble lipids were recovered from the acetone extract and added to the assay mixture as an ethanolic solution. Protein was determined by the method of Yonetani[10] and phosphorus by a modified Fiske–SubbaRow method[11] following ashing of the sample[12]. ETP, electron transport particles; ML, mitochondrial lipids.

Sample	*Treatment*	*μg P/mg protein*	*Succinate cytochrome c reductase activity (%)*		
			No addition	*+ CoQ**	*+ Extract***
I ETP	None	13.4	100 ***	124	–
II	4% water in acetone	8.5	22	126	108
III	10% water in acetone	3.8	14	24	134
IV	10% water in acetone + NH_3	2.1	0	0.6	70
I + ML	–	26.2	100	–	–
III + ML	–	9.8	118	–	–
IV + ML	–	9.1	82		

* CoQ, coenzyme Q.
** Neutral and phospholipids recovered from the acetone extract.
*** 100%, 0.50 μmole cytochrome *c* reduced/min per mg protein at 30°.

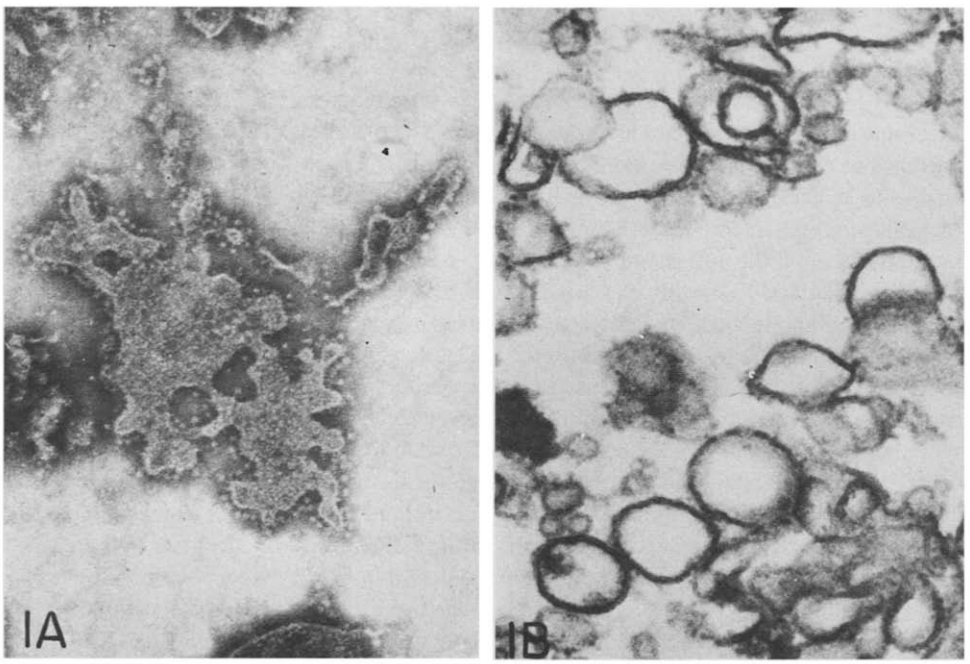

Fig. 1. Electron transport particle vesicles. A. Sample was negatively stained with 2% phosphotungstate (pH 6.9)[13]. B. Sample was fixed in 1% OsO_4 buffered in 0.028 M veronal acetate (pH 7.4) containing $CaCl_2$ and NaCl, washed, and soaked in 0.5% uranyl acetate[2]. Sample was dehydrated in acetone and embedded in Epon 812 [14]. Sections were post-stained with 2% aqueous uranyl acetate and Reynolds' lead citrate[15]. x 100 000.

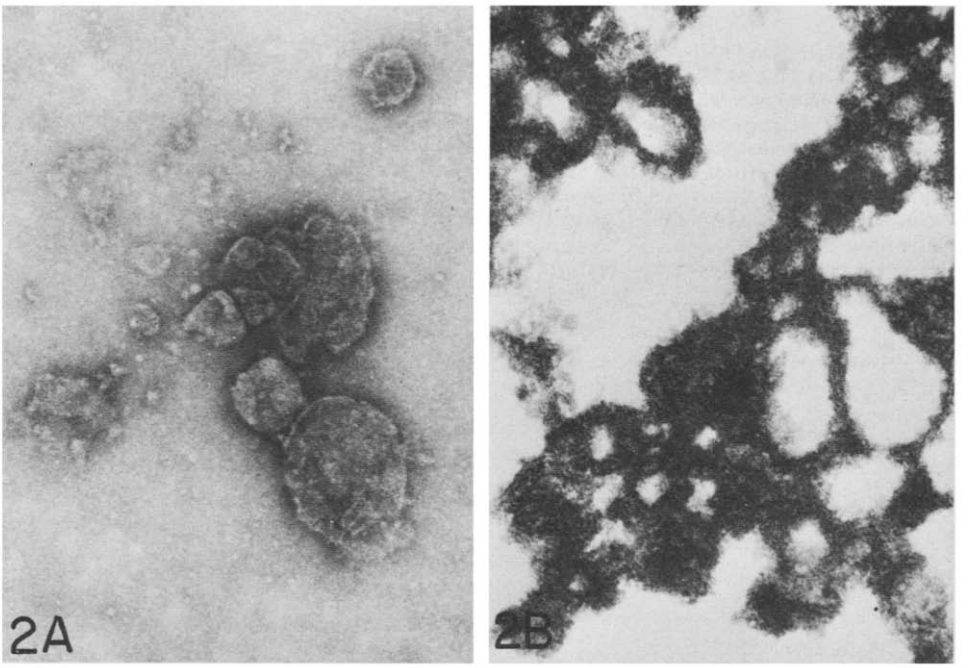

Fig. 2. Electron transport particle vesicles extracted with 10% water in acetone. A. Negatively stained. B. Thin section. x 100 000.

Fig. 2 shows electron transport particle vesicles extracted with 10% water in acetone. The inner membrane particles are no longer visible at the edges of the vesicles. However, particles of this size are detected in the background. The acetone-extracted vesicles tend to aggregate in electron-dense masses making observations of their morphology difficult. Thin sections of the extracted material reveals some vesicular outlines. However, not all of the membranes show the unit membrane structure observed in the untreated vesicles. Often the membranes appear uniformly electron-dense throughout their thickness. Considerable amounts of nonmembranous, electron-dense material is also present. This material is thought to represent protein or lipoprotein resulting from breakdown of the membranes. The extracted material appears more electron-dense than the membranes of the untreated vesicles. This suggests an alteration in the stainability of the membrane components or the exposure of new sites which are capable of reacting with the stain molecules.

Mitochondrial lipids can be restored to the extracted vesicles by the procedure described for whole mitochondria[7]. Restoration of lipid to the vesicles which were extracted with 10% water in acetone results in the full recovery of succinate cytochrome *c* reductase activity (Table I). This preparation is shown in Fig. 3. No detectable changes in the appearance of the vesicles as observed by negative staining are seen following the restoration of lipid. Thin sectioning of the lipid-restored electron transport particles reveals membranous vesicles and dense clusters of membranous fragments. The membranes of both vesicles and fragments show unit membrane structure.

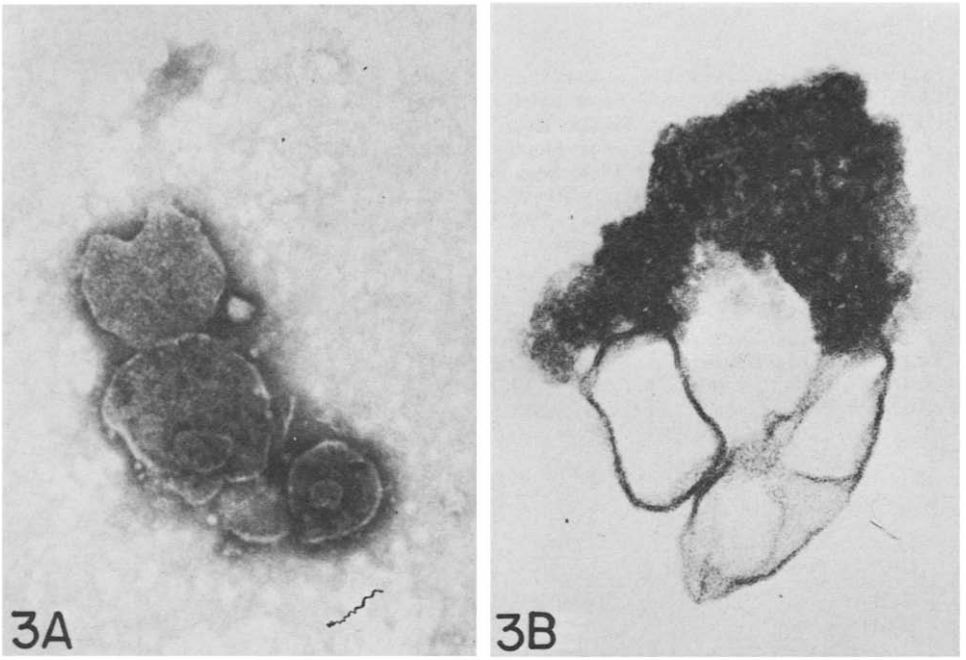

Fig. 3. Electron transport particle vesicles extracted with 10% water in acetone and restored by the addition of mitochondrial lipid. A. Negatively stained. B. Thin section. x 100 000.

Extraction of electron transport particle vesicles with 4% water in acetone results in less severe breakdown of the membranes as observed by both negative staining and thin sectioning. Many intact vesicles and only small amounts of nonmembranous material are seen. The stainability of the membranes appears unaltered. Vesicles extracted with 10% water in acetone *plus* ammonia are the most severely disrupted. Mostly nonmembranous material and only occasional vesicular structures are observed. This preparation appears more electron-dense than the untreated vesicles.

We find that acetone-extracted mitochondria tend to retain membranous structure as previously reported[1,2] while acetone-extracted electron transport particles lose membranous structure. Although this later observation is in contrast to previous findings[1,2], it should be noted that the electron transport particles used in previous studies were prepared in a French pressure cell whereas we have used sonic fragmentation. Possibly the greater fragility in our particles may be related to structural changes in the membrane during preparation of the vesicles. Chance *et al.*[8] have proposed structural reorganization during preparation of electron transport particles on the basis of changes in cytochrome localization Particles prepared by sonic fragmentation lose much of their energy-coupling capacity. This may also be related to membrane reorganization and increased damage by acetone.

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