THE ISOLATION AND ANALYSIS OF A MITOCHONDRIAL MEMBRANE FRACTION

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There exists in the literature indication that some mitochondrial enzymes, particularly succinoxidase and cytochrome oxidase, are part of the mitochondrial structure and thus are insoluble when the mitochondria are disrupted. It has further been supposed in particular by Cleland and Slater (1) that this insoluble fraction consists of mitochondrial membranes. However, no direct test of the membrane hypothesis has been reported. The present note describes briefly findings which strongly support the idea that certain insoluble enzymes are structurally linked to membranes of mitochondria.

Rat liver mitochondria were isolated in 0.44 M sucrose and treated in suspension with 0.3 per cent deoxycholate. This treatment resulted in the solubilization of 80 to 90 per cent of the protein N. Of various fractions obtained in the centrifuge and examined in the electron microscope by sectioning techniques, the final pellet obtained at 105,000 *g* is pertinent here. Fig. 1 shows this fraction to consist mostly of systems of vesicles bounded by single or multiple layers of membranes. The dimensions of these systems are in the order of mitochondrial dimensions. We were not clear as to the source of these elements since they may have arisen not only from mitochondrial membranes, but possibly also from interactions between deoxycholate, the various non-membranous components present, and osmium tetroxide. They might also represent microsomal contamination.

In order to follow the interaction of mitochondria with deoxycholate, a pellet of isolated mitochondria was layered in the centrifuge tube with 0.3 per cent deoxycholate in sucrose, allowed to stand for a while, and then fixed and embedded. The pellet was then sectioned transversely through the depth of the pellet, and mitochondria in various stages of dissolution from the bottom to the top of the pellet could be followed. Fig. 2 shows, near the bottom of the pellet, mostly intact mitochondria plus a number of swollen ones. Microsomal contamination present in this particular preparation can be identified by the granules adhering to the membranes. In the middle of the pellet layered with deoxycholate are found numerous much swollen mitochondria (Fig. 3). These still contain most of their matrix. In Fig. 4 is shown the appearance of mitochondria at the top of the pellet. These elements have lost most of their matrix, and are somewhat smaller than those encountered in

TABLE I

		Ratio of amount in fraction to amount in intact mitochondria	
		100,000 *g* pellet	Supernatant
Protein N		0.059	0.83
Phospholipide phosphorus		0.15	0.68
μg. phospholipide phosphorus/μg. protein N		2.6	0.82
Succinoxidase	Total activity	0.38	0.053
	Activity/protein N	6.4	0.064
Cytochrome oxidase	Total activity	0.41	0.29
	Activity/protein N	6.9	0.35
DPNH-cytochrome *c* reductase	Total activity	0	0.37
	Activity/protein N	0	0.42
Adenylate kinase	Total activity	0	1.0
	Activity/protein N	0	1.2

Showing the ratios of amounts of protein nitrogen, phospholipide phosphorus, and enzymes present in the 105,000 *g* pellet of 0.3 per cent deoxycholate-treated mitochondria and in the supernatant from this fraction to the corresponding amounts found in intact mitochondria. In the 105,000 *g* fraction, succinoxidase and cytochrome oxidase show an increase in activity referred to protein nitrogen of 6 to 7 times over that in intact mitochondria. DPNH-cytochrome *c* reductase and adenylate kinase were absent from this fraction.

the middle of the pellet. What are interpreted as cristae show as numerous vesicles within the mitochondria. It is felt that this represents a fairly satisfactory demonstration that the elements isolated at 105,000 *g* from deoxycholate-treated suspensions are indeed derived from mitochondrial membranes. The biochemical studies of this fraction are presented briefly in Table I. Of the oxidative enzymes assayed, succinoxidase and cytochrome oxidase were found to have 6-fold concentrations in the membrane fraction, while DPNH-cytochrome *c* reductase and adenylate kinase were absent. Substantial inhibition by deoxycholate rendered the picture obscure for other enzymes.

BIBLIOGRAPHY

1. Cleland, K. W., and Slater, E. C., *Biochem. J.*, 1953, **53**, 547.

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EXPLANATION OF PLATE 129

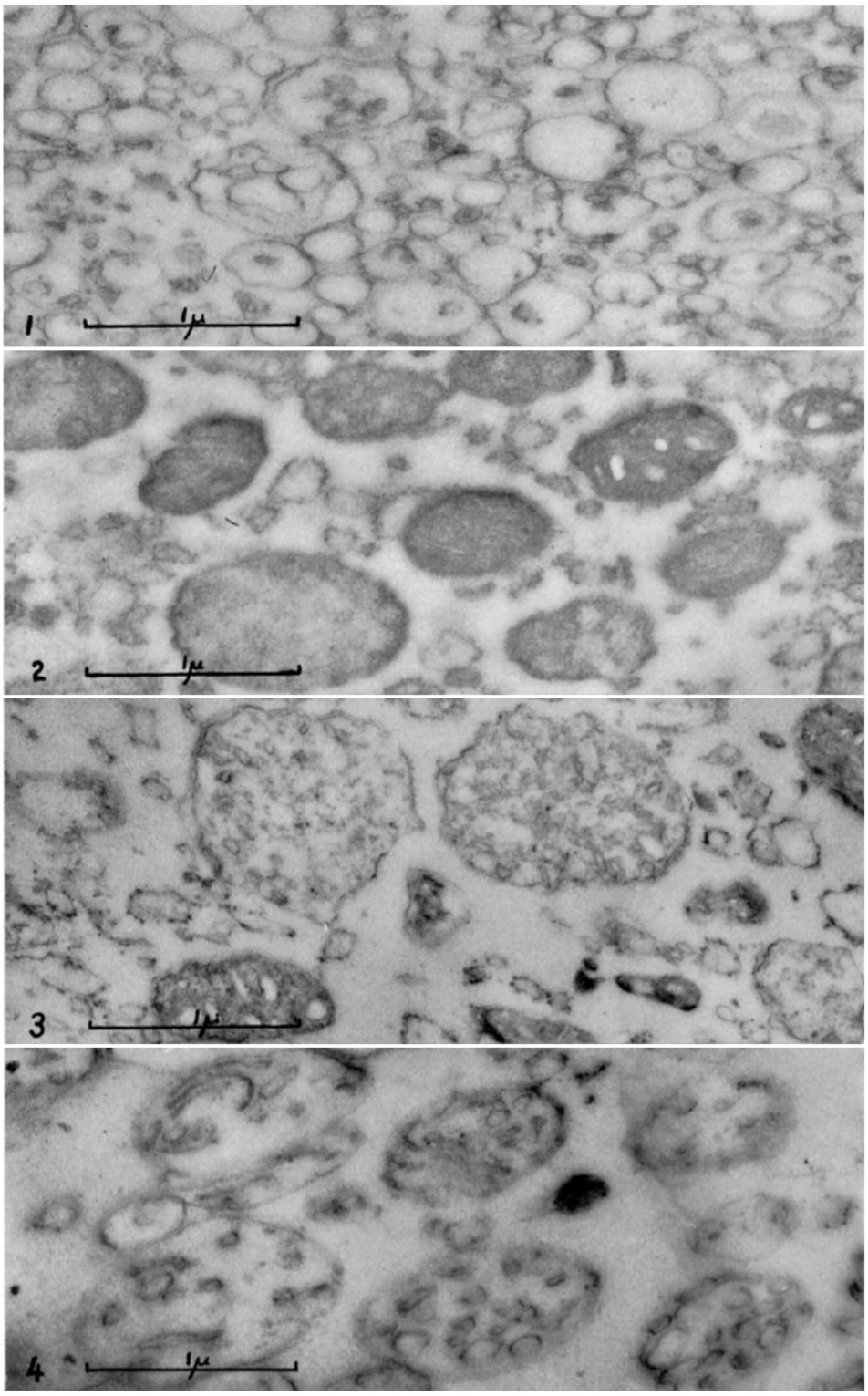
FIG. 1. Rat liver mitochondria, isolated in 0.44 M sucrose, and treated in suspension with 0.3 per cent deoxycholate adjusted to pH 7.5. This fraction was obtained in the centrifuge after 1 hour at 105,000 *g* from the supernatant left after 20 minutes at 2,500 *g*. The fraction consists of membrane-enclosed vesicles of various diameters ranging from 100 A to 1 μ. The interior of the vesicles appears no denser than the exterior, so that the vesicles are considered essentially free of matrix. In the figure, the two large elements are compound, having one or two outer membranes enclosing a number of swollen vesicles. They therefore present a distorted view of intact mitochondria without matrix. × 31,000.

FIGS. 2, 3, and 4. Mitochondria, isolated from rat liver in 0.44 M sucrose, were centrifuged at 5,000 *g* for 10 minutes to form a pellet. The supernatant was replaced by 0.3 per cent deoxycholate in 0.44 M sucrose and the pellet was exposed to this in the cold (*ca.* 5°C.) for 30 minutes. The tube was then placed in the bucket head of the centrifuge, brought to 150,000 *g* for 10 minutes to pack the surface layer, fixed, and embedded intact, and sectioned perpendicular to the surface of the pellet. All stages of dissolution of mitochondria in deoxycholate could be seen proceeding from intact mitochondria at the bottom of the pellet to membranes at the top.

FIG. 2. Mitochondria at some distance from the top of the pellet are beginning to react to the deoxycholate. Note that some elements are essentially intact while others show swelling. Unavoidable microsomal contamination is also present. × 31,000.

FIG. 3. Nearer the upper surface of the pellet most of the mitochondria have swollen substantially; however, a few elements are still intact. Cristae are observed in the swollen mitochondria, many of them having the same general dimensions as they have in intact mitochondria. The matrix is present as a loose network. × 31,000.

FIG. 4. At the surface exposed to deoxycholate are found vesicular elements somewhat smaller than the much swollen mitochondria seen in Fig. 3. These elements are in many cases enclosed by two membranes. In the interior are numerous smaller vesicles which are considered to be derived from the cristae. Such elements may be compared with the two larger elements discussed in Fig. 1. × 31,000.



(Watson and Siekevitz: Isolation of mitochondrial membrane fraction)