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Studies on the Fine Structure of the Rat Liver Inner Mitochondrial Membranes Negatively Stained

by

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Morphologically, mitochondria are described as tiny spheres, short rods, or filaments in which the outer membrane, inner membrane, and matrix are present as ultrastructures. Folds of the inner membrane called *cristae mitochondriales* protrude into the interior of the mitochondria as incomplete septum-like structures. On the other hand, plant mitochondria show tubular structures rather than *cristae*. The matrix, enclosed by the inner membrane, often has a filamentous appearance.

This classical image of the mitochondria, obtained from observations of tissue embedded and sectioned in the conventional, way, is notably different from that derived from negatively stained preparations.

Negative staining has brought into view new structures of the mitochondria over which there is no unanimous agreement. Some would interpret these ultrastructures as artifacts produced during the isolation procedure of the membranes (3, 4, 7).

The aim of this paper is to report our findings on the structure of inner mitochondrial membranes after negative staining.

Materials and Methods

The inner membranes of mitochondria from rat liver were isolated as described in the previous paper (6) and thoroughly washed by resuspension in 0.02 M phosphate and recentrifugation at 1,900 × g for 15 minutes and once more resuspended in 0.25 M sucrose and resedimented at 8,500 × g for 10 minutes. They were then fixed in buffered osmium tetroxide at 3° C and negatively stained as in the same paper (6) and then examined with a Siemens Elmiskop IA. The objective lens was carefully compensated and equipped with 50 or 30 μ aperture diaphragms; electron micrographs were taken at plate magnifications between 15,000 and 60,000.

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Results *

Negatively stained mitochondria show an abundance of interior structures. Fig. 1 corresponds to a mitochondria after hypotonic rupture of its outer membrane and before the usual centrifugation to separate inner membranes from the matrix and outer membranes. One can observe the delicate outer membrane (o.m.) in the form of an empty bag leaving behind a large quantity of tubular structures to its right.

In our negatively stained inner membrane preparations we find two types of structures: one tubular and another lamellar. Fig. 2 shows a typical tubular structure with PARSONS' projecting subunits (5), first described by FERNÁNDEZ-MORÁN (1) under the mane of elementary particles. They cover the entire outer surface of the tubules. Lamellae represent a minor component and they completely lack projecting subunits. Frequently tubular material is found to join lamellar areas forming long chains of alternating tubular and lamellar links. Fig. 3. At times though the smallest lamellar links do show projecting subunits.

Occasionally two or more tubules are seen as continuations of the lamellar structure and at the same time show bifurcation. The point of junction between the tubules and lamellae is ofter funnel shaped as seen in the lower center portion of Fig. 4.

The tubules are from 200 to 375 Å in diameter and the center, being less dense, permits the contrast material to penetrate. The length of the tubules varies considerably and at times reaches more than 7μ.

The tubular component in the inner membrane preparations from rat liver mitochondria is more abundant than the lamellar one.

Discussion

Negative staining of mitochondrial inner membrane gives a quite distinct picture from that obtained from ultrafine sections of embedded tissue. The embedded sections show a comparatively low number of *cristae* whereas negatively stained preparations give a number of lamellar structures and many tubules. Some tubules are found to be longer than the actual length of mitochondria.

The tubular material is definitely such, since in the osmium tetroxide fixation previous to negative staining they are seen lying down on the supporting film and studded with projecting subunits over its entire surface area. This excludes the possible «cristae viewed in profile» interpretation proposed by FERNÁNDEZ-MORÁN (1, 2) and STOECKENIUS (8). The fixation with osmium tetroxide does not eliminate the projecting subunits as suggested by STOECKENIUS (8).

One of the most interesting aspects about these tubular structures, and even about projecting subunits themselves, is whether they are structural elements really existing as such in the mitochondria or, on the contrary, are only artifacts as Mitchell and Sjöstrand point out (3, 4, 7). The main reason for not accepting that such structures are present in intact mitochrondia is that they have not yet been convincingly demonstrated in sections of embedded tissue. At the same time it is difficult to picture as an artifact such an orderly and regular structure and one that is unique to inner mitochondrial membranes. It is not possible that their formation is induced by phosphotungstate on making the contrast, since the membranes were previously fixed with osmium tetroxide. They would

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have to be formed during the short time of hypotonic rupture.

The behavior of inner mitochondrial membranes in the presence of the lytic agent ascorbate, studied by us (6), favors their actual existence, but further work is necessary to clarify this point.

If lamellar and tubular structures are present as such in intact mitochondria a renewed view of the ultrastructure of the mitochondria would have to be considered.

A comparative study of mitochondria from different sources is at present being completed in our laboratories.

Summary

Isolated inner membranes of rat liver mitochondria were fixed with osmium tetroxide and examined by electron microscopy after negative staining. Two types of structures were observed: tubules and lamellae. The tubules showed the typical projecting subunits covering their entire outer surface. Lamellae represent a minor component and they completely lack projecting subunits. Frequently tubular material is found to join lamellar areas. The point of junction is ofter funnel shaped.

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