AN INTRAMITOCHONDRIAL CRYSTALLOID IN ELEMENT III OF RAT CHORIOALLANTOIC PLACENTA

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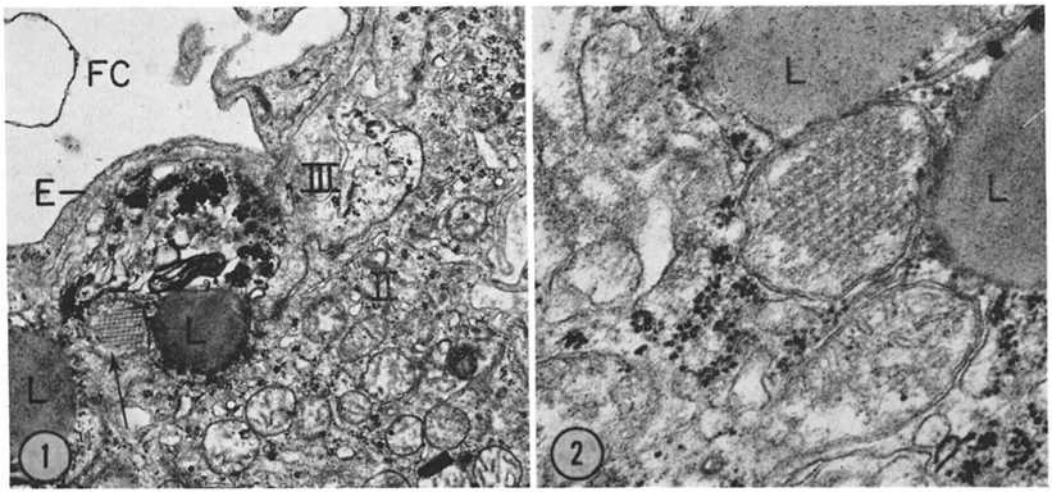
INTRODUCTION

Mitochondrial inclusions of a filamentous or crystalline nature have been reported in a number of cell types such as hepatic cells (10), cells in the thick segment of Henle's loope (9), and follicular cells of the thyroid (2). Suzuki and Mostofi (9) have recently reviewed the various forms and possible explanations of mitochondrial inclusions. The present report describes a hitherto unreported intramitochondrial "crystalloid" in element III of rat chorioallantoic placenta.

MATERIALS AND METHODS

Placentae were taken from rats of the Holtzman and Sprague-Dawley strains during the 15th, 16th, 17th, 18th, and 20th days of gestation. Gestation was timed by the examination of saline vaginal lavages taken the morning after rats had been paired for breeding. Day zero of gestation was indicated for animals showing sperm in the lavage. One pregnant rat was utilized at each time period. Four placentae were removed from each animal under sodium pentobarbital anesthesia. Tissue slices were initially fixed for 2 hr in cold 3% glutaraldehyde buffered at pH 7.4. Subsequent osmication of suitably minced tissue was carried out for 2 hr in 1% OsO_4 buffered at pH 7.4 with Millonig (6) phosphate buffer. Tissue blocks were dehydrated in ethanol and propylene oxide and embedded in Epon 812 (4). Thin sections were cut and stained with lead citrate (11) or double stained with uranyl acetate and lead citrate. Observations were made with RCA-EMU-3F and Philips EM-200 electron microscopes.



Abbreviations

FC, Fetal capillary	*C*, Crista
E, Endothelium	*III*, Element III
L, Lipid	*II*, Trophoblast II

FIGURE 1 The arrow indicates an intramitochondrial crystalloid in element III. Degenerative changes are obvious in the cytoplasm near the crystalloid while other areas of element III, the endothelium and trophoblast II, appear well fixed. 20-day labyrinth, double stained. × 16,000.

FIGURE 2 A lipid droplet abuts directly upon the outer mitochondrial membrane of this crystalloid-containing mitochondrion. The cytoplasm surrounding the crystalloid does not show the severe degenerative changes illustrated in Fig. 1. 20-day labyrinth, double stained, × 46,000.

OBSERVATIONS

Crystalloids were present in the mitochondria of element III in the chorioallantoic labyrinth (Fig. 1). They were present at all placental ages examined except those of 15-days' gestation. The frequency of intramitochondrial crystalloids seemed to increase as the gestation age increased. The crystalloid revealed a well ordered, gridlike lattice of symmetrical quadrilaterals which measured 370 A center-to-center, on the average (Figs. 3 and 4). The quadrilateral elements of the lattice appeared to be formed by two major arrays of parallel electron-opaque lines which intersected at angles varying from 90° to 117°. The points of intersection possessed increased electron opacity and evidence of substructure suggestive of a cluster of tightly packed subunits with lucid centers (Fig. 5). A less evident array of parallel lines composed of similar lucid subunits was also discernible crossing the lattice (Figs. 3, 6). Lucid subunits were also found associated with the membranes of the cristae and the inner mitochondrial membrane (Figs. 5, 6).

The mitochondria which contained the crystalloids were often found in an area of element III that had undergone obvious degenerative changes, while cytoplasmic structures immediately adjacent to element III were well preserved (Fig. 1). However, other crystalloids were surrounded by cytoplasm of element III that displayed little or no evidence of degeneration (Figs. 2, 4, 7). Lipid droplets were usually present near the mitochondria that contained crystalloids and, in some cases, abutted directly on the outer mitochondrial membrane (Fig. 2).

The crystalloid-containing mitochondria possessed an external double membrane and cristae (Fig. 4). Cristae were often observed in close approximation to the crystalloid, with their profiles parallel to one major array of the lattice and apparently connected to the crystalloid by extensions of the other major array (Fig. 4). Other points of possible connection were observed between the crystalloid and inner mitochondrial membrane (Fig. 4).

The uniformity of the quadrilateral components of the lattice was a persistent feature of the crystalloid. However, the gridlike nature of the lattice was not so evident in what appeared to be oblique sections (Fig. 8). Some lines of the array

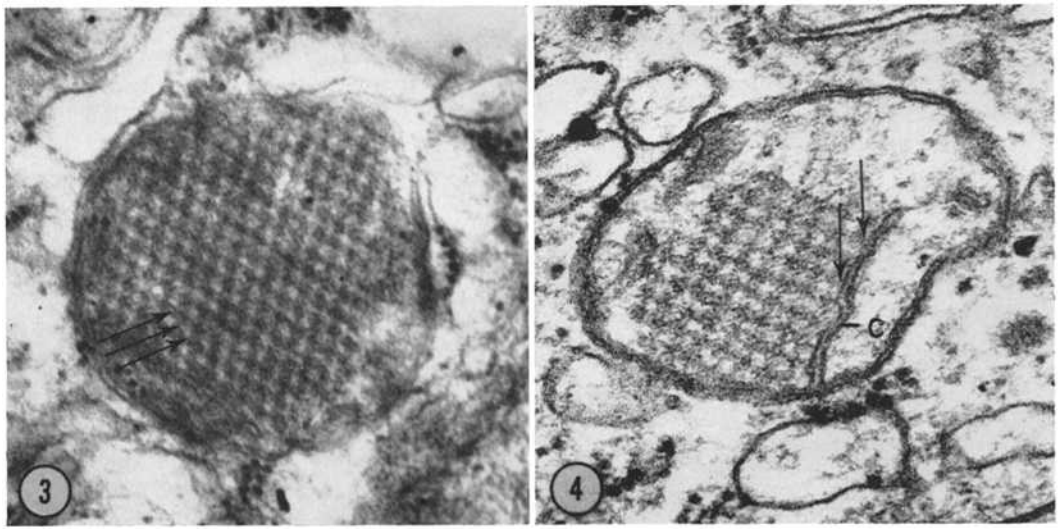


FIGURE 3 The two arrays of parallel electron-opaque lines of this crystalloid intresect at an angle of 110°. The arrows point in the direction of a third array of parallel lines composed of lucid subunits. 20-day labyrinth, double stained. × 60,000.

FIGURE 4 The two arrays of parallel electron-opaque lines of this crystalloid intersect at an angle of 90°. The vertical arrows indicate possible points of connection between the crystalloid and a crista. 20-day labyrinth, double stained. × 83,000.

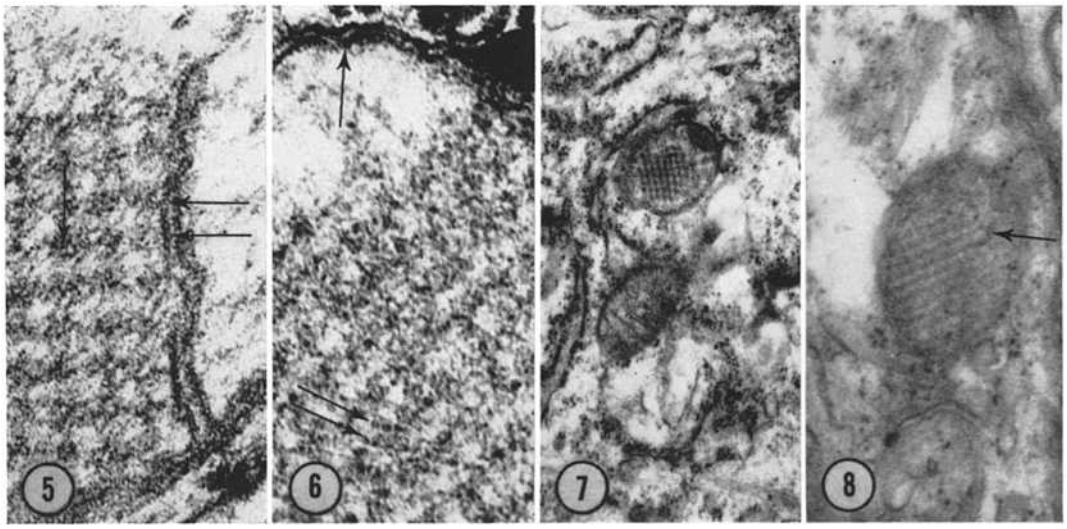


FIGURE 5 This micrograph is a higher magnification of a portion of Fig. 4. The horizontal arrows indicate lucid subunits associated with the membranes of the crista. The vertical arrow indicates a cluster of similar lucid subunits at a point of intersection. × 160,000.

FIGURE 6 The vertical arrow indicates lucid subunits associated with the inner mitochondrial membrane. The diagonal arrows indicate two series of lucid subunits that pass obliquely across the lattice. 20-day labyrinth, double stained. × 150,000.

FIGURE 7 A section of 17-day labyrinth that illustrates an intramitochondrial crystalloid and a mitochondrion with normal structure. The cytoplasm near the crystalloid demonstrates slight rarefaction, but otherwise appears well fixed. Double stained. × 19,000.

FIGURE 8 An oblique section of a mitochondrion that contains a crystalloid. The arrow indicates a hairpin-like bend in an electron-opaque component of the lattice. 20-day labyrinth, lead citrate. × 25,000.

appeared to be bent back on themselves in a hairpin-like manner. The bend of the opaque line possessed a less dense central region.

DISCUSSION

Cristae of element III mitochondria were frequently found with their profiles oriented parallel to one major array of the crystalloid lattice and directly connected to the lattice by extensions of the other major array. The crystalloid also appeared to be attached to the inner mitochondrial membrane. In addition, the cristae and the crystalloid lattice demonstrated substructure comprised of apparently similar lucid subunits. The lucid subunits may be the same as the "globular micelles" that Sjöstrand (7) reported to be associated with mitochondrial membranes. Malhotra (5) has also noted similar electron-transparent particles associated with mitochondrial membranes. Such evidence would seem to indicate either that the cristae are being incorporated into the lattice structure, or that the crystalloid lattice is being utilized to form the cristae. Mitochondrial inclusions have not been associated with developing mitochondria (9). Although this possibility cannot be completely discounted, the incorporation of cristae into the lattice would seem more likely, for the reasons discussed below.

Other investigators have noted the close relationship between cristae and mitochondrial inclusions (2, 9, 10). Svoboda and Manning (10) have postulated that formation of mitochondrial inclusions in liver cells of chronic alcoholics might be initiated by changes in the state of hydration of the cells or their mitochondria. This would result in rearrangement, condensation, and crystallization of mitochondrial phospholipid. They further suggested that the mitochondrial inclusions noted by other investigators in liver cells under different pathological conditions "probably represented a

nonspecific degenerative phenomenon." The mechanism suggested by them was based on investigations that have demonstrated formation of "myelin figures" after hydration of phospholipids (1, 8). Suzuki and Mostofi (9) pointed out that mitochondrial inclusions did not necessarily indicate a pathological process since there must be a constant turnover and natural cell death in normal organisms.

The placental tissue of the rat undergoes an entire life cycle during gestation that ends with parturition. Jollie (3) reported degenerative changes in trophoblast II and element III prior to delivery. He stated that such selective degeneration in the labyrinth may be caused by a fixity of life span of the structures involved that is equal to the gestation time of the species. In the present investigation, crystalloids were not observed in 15-day placentae and were found only with difficulty in 16-day specimens. This observation could be due to a sampling error. In addition, the method used to time gestation permitted a possible error of about 15 hr. Crystalloids seemed to be progressively more frequent as gestation age increased. The increase in frequency of intramitochondrial crystalloids would seem to be related to the progressive aging and degeneration of element III. Indeed, degenerative changes were often observed in the cytoplasm immediately adjacent to a mitochondrion that contained a crystalloid. Other placental tissue in the immediate area demonstrated well preserved morphological features. On the other hand, some crystalloid-containing mitochondria were surrounded by cytoplasm and organelles that showed little or no degeneration. This may indicate that mitochondrial changes initiate the degenerative process rather than being secondary to degenerative changes in the cytoplasm. Although trophoblast II has also been reported to undergo similar changes (3), the only crystalloids observed in the present study were in element III.

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Note Added in Proof:

Since this paper was submitted for publication it has come to my attention that Osvaldo and Latta (*J. Ultrastruct. Res.* 1966, **15**:589) illustrated, as an incidental observation in their paper on interstitial cells of the renal medulla, a similar structure in a mitochondrion. The dense layers were 360 A from center-to-center, as compared to my measurement of 370 A.

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