# CRYSTALLINE STRUCTURES IN THE MITOCHONDRIA OF NORMAL HUMAN LIVER PARENCHYMAL CELLS

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Inclusions have been observed in the mitochondria of human liver parenchymal cells in a number of pathological states and have been variously described as "crystalline," "para-crystalline," "myelin figures," or "myelin degeneration" (1–12). It is generally assumed that these structures represent an abnormal change, little emphasis having been placed on their occurrence in normal specimens (3, 7). The present brief note reports the finding of such intramitochondrial inclusions in parenchymal cells from normal human liver.

## MATERIALS AND METHODS

### *Biopsies*

Material was obtained from patients under general anaesthesia during laparotomy. The three cases selected were without liver or biliary tract disease and without general conditions known to affect these systems. Livers were judged to be normal on the basis of the clinical history and examination, laboratory investigations, macroscopic appearance, and histological examination of portions of the same biopsy as that examined in the electron microscope.

### *Preparation for Electron Microscopy*

On removal, the biopsies were cut up in fixative into blocks of less than a ½-mm cube. Methods of fixation were as follows:—

1. Iced veronal-buffered osmium tetroxide (13) for 2 hours.
2. Iced phosphate-buffered osmium tetroxide (14) for 2 hours.
3. Iced glutaraldehyde (15) for 4 hours with post-osmication in iced phosphate-buffered osmium (14) for 2 hours.

Following fixation, all blocks were dehydrated in graded alcohols and embedded in Epikote 812, essentially as described by Luft (16). Sections were cut with glass knives on a Porter-Blum microtome and were mounted on carbon-coated grids (17); they were stained either with a saturated alcoholic solution of uranyl acetate (18), or with lead tartrate (19), or a combination of both, and were then examined in a Philips electron microscope Model EM 200.

## OBSERVATIONS

### *General*

A small proportion of liver parenchymal cells contained mitochondria with characteristic crystalline inclusions (Figs. 1 to 3). Since the tissue was not orientated during processing, it is not known whether such cells had a specific distribution within the liver lobule.

Each affected cell usually contained several mitochondria with inclusions situated in any zone of the cytoplasm, but most commonly near the sinusoidal border.

The mitochondria were normal in size and shape, and contained one (Fig. 1) or sometimes several (Figs. 1 and 2) inclusions. Occasional giant mitochondria were present in which the inclusions were more frequent (Fig. 3) and sometimes larger than elsewhere.

### *Structure of Inclusions*

The inclusions were roughly square or rectangular in profile and commonly of the order of 200 to 350 mμ across. When sectioned longitudinally (Figs. 1 and 3), they showed a crystalline

structure of parallel repeating electron-opaque strands, approximately 8 to 10 mμ in diameter.

In cross-section (Figs. 1 and 2), the filamentous structure of the inclusions was evident in the appearance of parallel rows, in two dimensions, of electron-opaque dots.

In both longitudinal (Fig. 1) and transverse (Figs. 1 and 2) section, the component strands were separated by a gap which was usually constant at about 20 mμ. However, less regular spacing was observed (Figs. 2 and 3), and sometimes a fine band of moderate electron opacity lay between two arrays of rods (Fig. 2).

Apart from these fine bands, the space between the filaments had the same electron density and appearance as the remainder of the mitochondrial matrix (Figs. 1 to 3).

Inclusions with differing orientations to the plane of section were sometimes found in the same mitochondrion (Fig. 3).

## DISCUSSION

The inclusions described here can be regarded as cubes or polyhedra composed of parallel filaments which lie within the mitochondrial matrix.

Similar electron-opaque material arranged in a crystalline manner has been described in the mitochondria of liver parenchymal cells from patients with prolonged virus hepatitis (1), acute "alcoholic hepatitis" (2), alcoholic fatty liver (3, 4), obstructive jaundice of both extrahepatic (1, 5–7) and intrahepatic (7–9) origin, Waldenstrom's disease (9), chronic familial non-haemolytic acholuric jaundice (10), primary amyloidosis (11), diabetes (3), and from a case of hepatosplenomegaly (12). Nothing similar has been reported in mitochondria of animal liver cells, and the structures are generally assumed to reflect some manifestation of human disease.

In pathological states, particularly cholestasis and chronic alcoholic fatty metamorphosis, the inclusions are numerous and are sometimes seen in the majority of mitochondria in all sections (3). The affected mitochondria are mostly enlarged, often bizarre in outline, and may have abnormal numbers of dense bodies. Cristae may appear sinuous, attached to the limiting membrane, or in parallel stacks.

Both the nature and origin of the crystalline structures are disputed; it is usually thought (1, 3, 9) that they are identical with "myelin figures" such as may be found *in vivo* after various non-specific alterations to phospholipids within cells, or following hydration of more or less purified phospholipid *in vitro*. However, a distinction should be made between "myelin figures" or "myelin degeneration," which consist of parallel rows of altered membranes (1, 2, 10), and "crystals" or "paracrystals" made up of regularly disposed filaments (3); the inclusions described here are of the latter type.

The pathogenesis of the inclusions is even less well understood. No one theory has satisfactorily explained their formation, and their presence in so many diseases has generally been regarded as a non-specific degenerative phenomenon.

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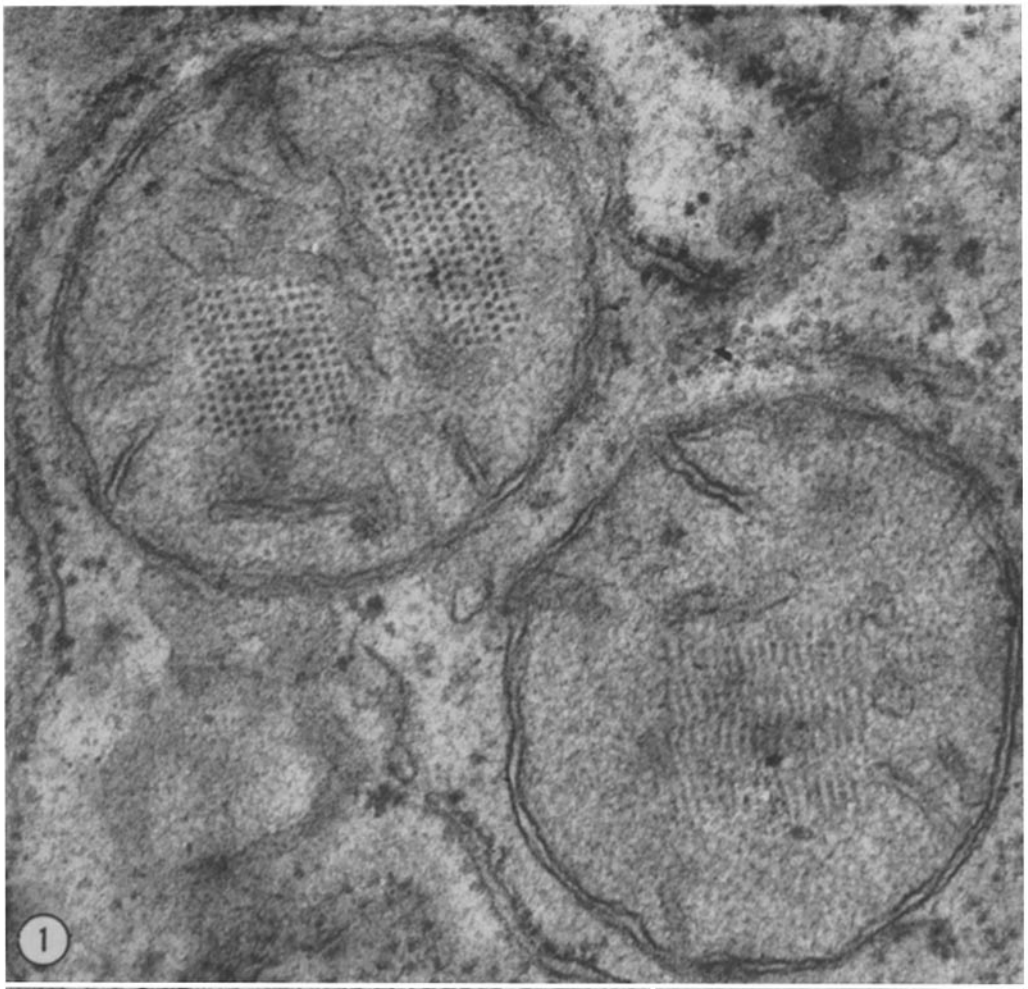
All the figures are electron micrographs of thin sections cut through liver parenchymal cells fixed with veronal-buffered (Fig. 1) or phosphate-buffered (Figs. 2 and 3) osmium tetroxide, embedded in epoxy resin, and stained in the section with lead tartrate.

FIGURE 1 Detail of mitochondria showing crystalline inclusions. The crystals have a rectangular profile and are composed of parallel regularly repeating electron-opaque strands lying about 20 mμ apart. The strands appear as dots when cut transversely, as in the mitochondrion on the left, and as lines in longitudinal section, visible in the mitochondrion on the right. × 92,000.

FIGURE 2 Detail of mitochondrion showing several crystals lying close together, but with slightly differing orientations to the plane of sectioning. A fine band of moderate electron opacity can be seen between several of the arrays of rods. × 81,000.

FIGURE 3 Mitochondrion with four inclusions. The two at the top have been cut longitudinally and show electron-opaque strands separated by gaps of varying width and resembling *cristae mitochondriales*. The two at the bottom have been cut transversely. × 38,000.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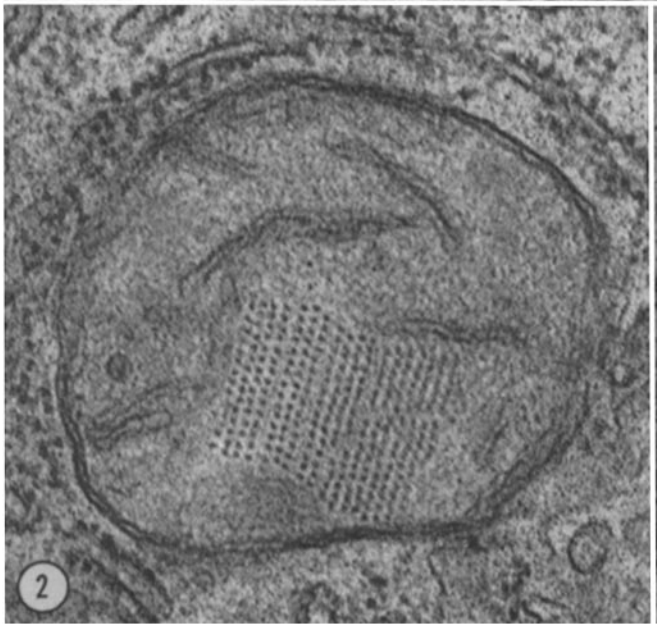
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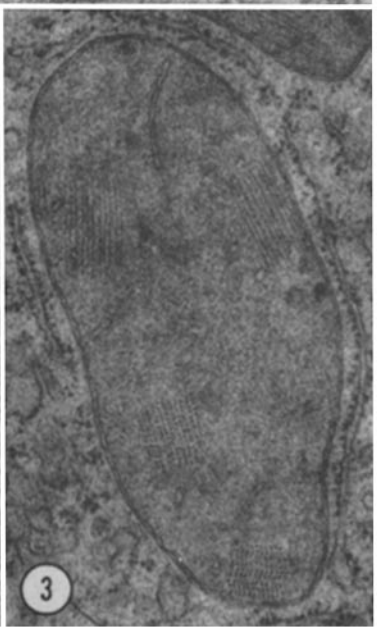
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The mitochondrial inclusions described in the present communication are morphologically identical to those known in pathological conditions, and they differ only in being less frequent and occurring in otherwise normal mitochondria. They should be considered as a variant of normal human hepatocyte mitochondrial structure. Their presence in some cells but not in others may be a reflection of a functional heterogeneity among the cells within each liver lobule, such as has been established for the distribution of certain enzymes (20).

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*Note Added in Proof:* Since this communication went to press, G. J. Roth, B. F. Trump, and E. A. Smuckler have reported (abstract in *J. Cell Biol.*, 1964, **23,** 79A) the occurrence of intramitochondrial inclusions in liver biopsy specimens from four subjects without clinical evidence of liver disease. This work confirms that the inclusions are composed of regularly arranged rod-shaped subunits.

In addition, E. Mugnaini has found (*J. Ultrastruct. Research*, 1964, **11,** 525) identical inclusions in the liver cell mitochondria of a 20-year-old male whose liver was normal apart from the presence of increased amounts of pigment in the parenchymal cells of the centrilobular zone.

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