

Crystalline Inclusions in Hepatocyte Mitochondria of a Patient with Porphyria Cutanea Tarda

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Intramitochondrial crystalline inclusions were found in hepatocytes of a patient with porphyria cutanea tarda (PCT). They were composed of parallel filamentous structures which measured approximately 12 nm in diameter. Each filament was separated from an adjacent filament by a space measuring approximately 5 nm. The mitochondria containing such inclusions were usually elongated and enlarged. It seemed likely that these changes are not particular in PCT, but indicate one reversible pathological finding in the liver.

Key words: intramitochondrial crystalline inclusions; liver; porphyria cutanea tarda; transmission electron microscopy

Hepatic intramitochondrial inclusions, referred to as crystalline (Svoboda and Manning, 1964), crystalloid (Haust, 1968), paracrystalline (Ruffolo and Covington, 1967), or filamentous inclusion (Mugnaini, 1964), have been thus far reported. The ultrastructure of the inclusion is characterized by the presence of closely packed filamentous structures in the enlarged mitochondria.

The inclusions were found in human liver in various conditions such as Weil's disease (Sandborn et al., 1966), chronic viral hepatitis (Ruffolo and Covington, 1967), Dubin-Johnson syndrome (Ruffolo and Covington, 1967), Wilson's disease (Sternlieb, 1968), mucopolysaccharidosis (Haust, 1968), diabetes mellitus (Bhagwat and Ross, 1971), metastatic hypernephroma (Djaldetti et al., 1974), porphyria cutanea tarda (PCT) (Waldo and Tobias, 1973; Blekkenhorst et al., 1976), hyperthyroidism (Mandel et al., 1976), alcoholism (Uchida et al., 1984), morbid obesity (Boon et al., 1988), and so on. The inclusions were also found in experimental animals (Watrach, 1964; Simpson et al., 1974; Reid et al., 1978; Wilson-Martino et al., 1980). In addition, they were reported even in normal human livers (Mugnaini, 1964; Wills, 1965) but their pathological significance remains controversial.

PCT, clinically characterized by cutaneous fragility, photosensitivity and hepatic dysfunction, is due to reduced activity of the enzyme uroporphyrinogen decarboxylase. We found intramitochondrial crystalline structures in the liver biopsy from a patient with PCT. In this paper, we describe ultrastructures of the crystalline inclusions and compare ultrastructural features of the inclusions thus far reported.

Materials and Methods

Liver biopsy in a 57-year-old male with PCT, chronic hepatitis type C and alcoholic intake for about 30 years, was performed. The tissues were immediately fixed in a fixative solution containing 1.0% glutaraldehyde, 2.0% paraformaldehyde, 0.03% CaCl₂ and 0.02% MgCl₂ in 0.1 mol/L cacodylate buffer (pH 7.4). A part of the specimen was cut into small pieces, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanols, and finally embedded in Epon 812 through propylene oxide. Ultrathin sections (60 nm) were cut by an ultramicrotome (Ultracut UCT, Leica, Austria), stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (JEM-100CXII, JEOL Ltd., Tokyo, Japan).

Results

Characteristic findings of the biopsy specimen were as follows: The smooth endoplasmic reticulum in some hepatocytes was hyperplastic with dilation of the cisternae. The peroxisomes were sometimes increased in number, and their limiting membranes were partially continuous with the smooth endoplasmic reticulum. Needle-like structures were often observed (Fig. 1). The core of the structure was electron-translucent and surrounded by lysosomal substances. The shape of the mitochondria was not spherical as observed in the normal liver, but was somewhat enlarged, elongated and partially narrowed. Some matrix granules contained electron-translucent area (Figs. 2a and 3a).

Crystalline inclusions were often observed in the enlarged mitochondria (Figs. 2 and 3). They were composed of filamentous structures oriented parallel to the longitudinal axis of the

mitochondria (Fig. 2). In cross sections, the filaments were arranged in equilateral triangle pattern, forming a hexagonal appearance as a whole (Fig. 3b). Each filament measured approximately 12 nm in diameter and was separated from an adjacent filament by a space measuring approximately 5 nm. At high magnification, the filaments were composed of massive electron-dense substances without hollow structures (Figs. 2b and 3b).

Discussion

The ultrastructures of liver porphyria have been described by Waldo and Tobias (1973), Biempica and colleagues (1974) and Siersema and colleagues (1992). The most characteristic feature of the porphyria is the presence of needle-like structures in the hepatocytes. The structures represent uroporphyrin crystals, since uroporphyrin, crystallized in vitro, displays the same

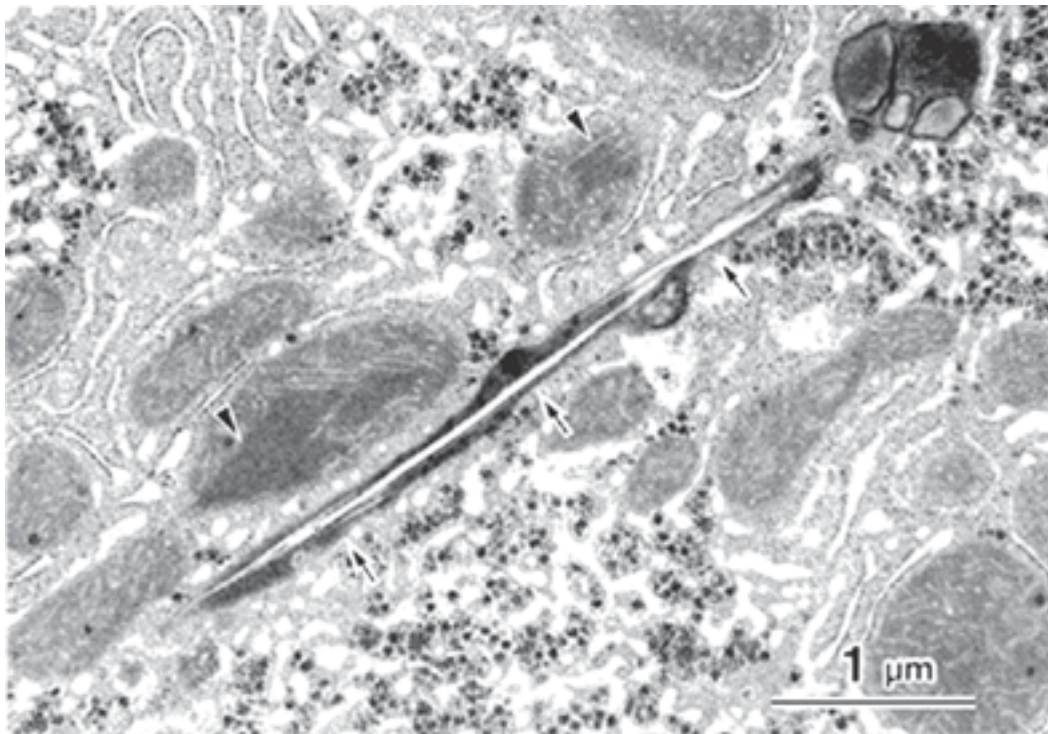


Fig. 1. Transmission electron micrograph of a needle-like structure in a hepatocyte of a patient with porphyria cutanea tarda. The needle is electron-translucent and surrounded by lysosomal substances (arrows). Arrowheads show crystalline inclusions in the mitochondria.

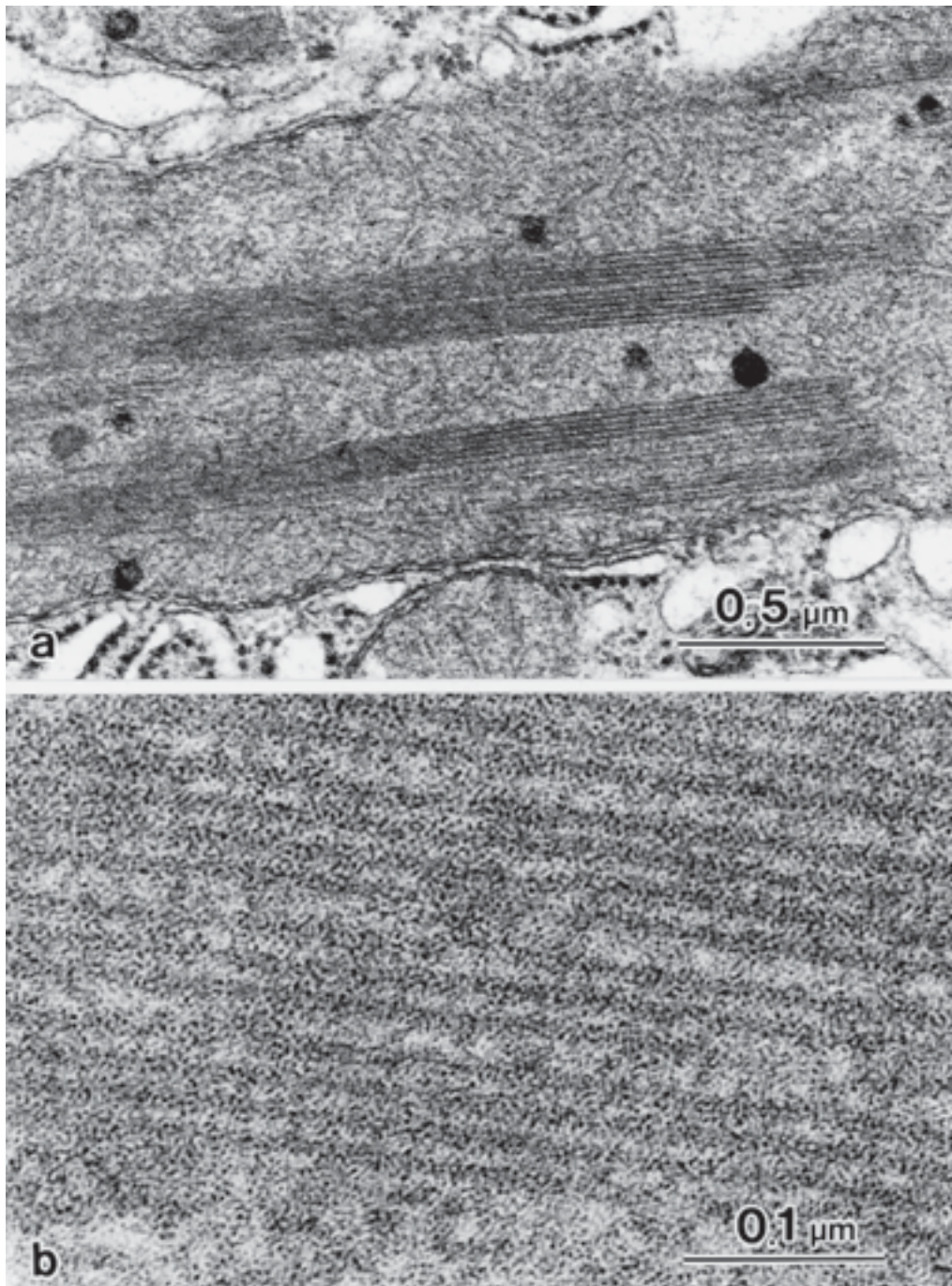


Fig. 2. Longitudinal sections of crystalline inclusions. **a:** The crystalline inclusions are composed of closely packed filamentous structures arranged parallel to the longitudinal axis of the mitochondria. **b:** Higher magnification of the intramitochondrial inclusion. Each filamentous structure comprises electron-dense substances of approximately 12 nm in diameter.

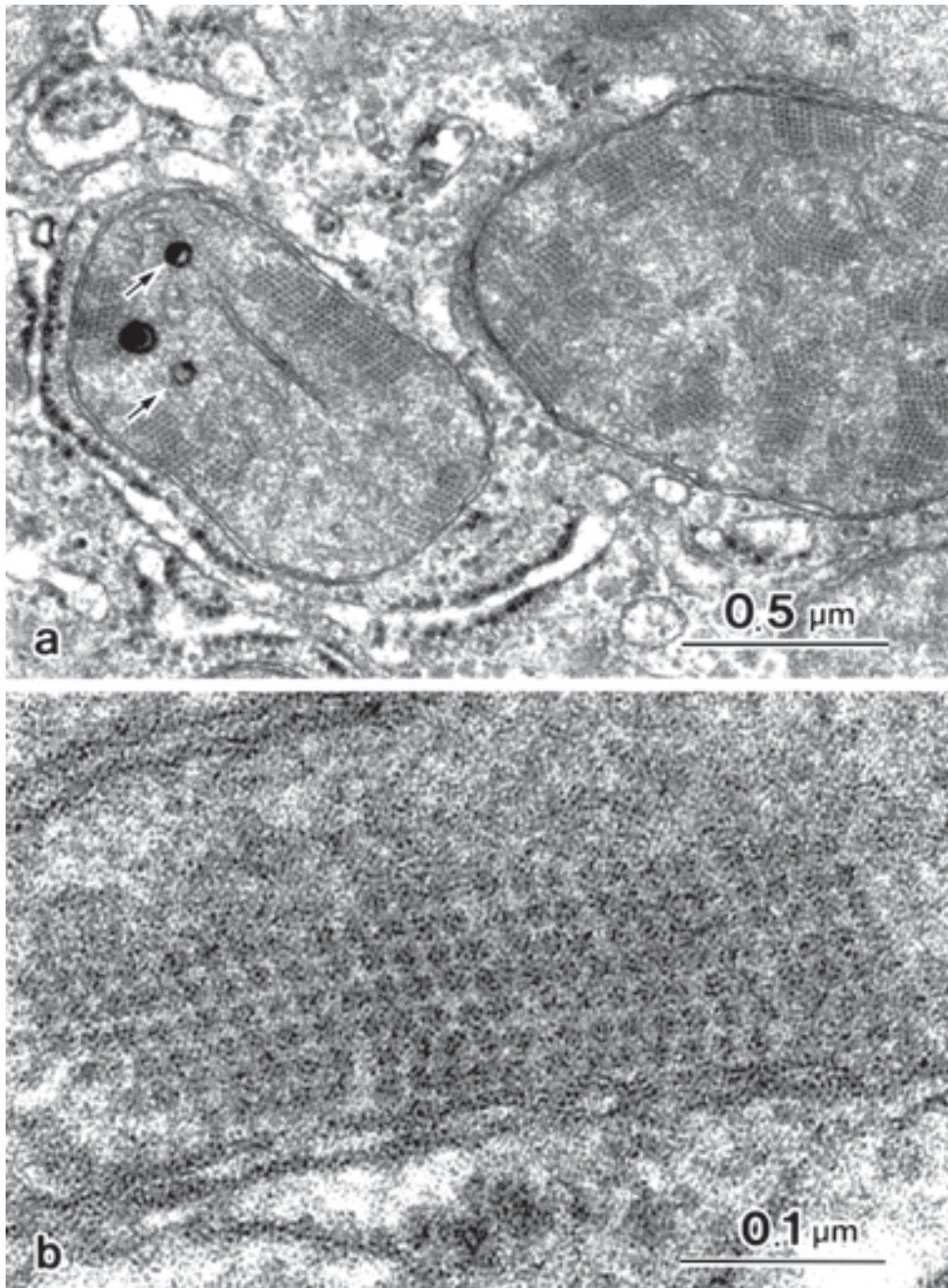


Fig. 3. Cross sections of crystalline inclusions. **a:** The inclusions are regularly spaced and closely packed. Arrows show matrix granules with electron-translucent area. **b:** Higher magnification of the intramitochondrial inclusion. Filaments are arranged in an equilateral triangle pattern, forming a hexagonal appearance as a whole. Each filament is separated from an adjacent filament by a space of approximately 5 nm.

Table 1. Intramitochondrial filamentous structures reported in the human liver

| Year | Author | Diameter* | Space† | Condition |
|------|-----------------------|-----------|----------|----------------------------------------------------------------------|
| 1964 | Mugnaini | 6 | 6 | Normal, alcoholic dyspepsia |
| 1966 | Sandborn et al. | 6 – 7 | 5 – 15 | Weil's disease |
| 1967 | Ruffolo and Covington | 6 – 8 | 10 | Dubin-Johnson syndrome, chronic viral hepatitis |
| 1970 | Lundbergh and Westman | 7 | – | Oral contraceptive use |
| 1974 | Djaldeiti et al. | 7.5– 8.5 | 7.5– 8.5 | Metastatic hypernephroma of the liver |
| 1968 | Sternlieb | 8 | 14.5–16‡ | Wilson's disease |
| 1965 | Wills | 8 – 10 | 20 | Normal |
| 1968 | Haust | 8 – 10 | 15 – 20 | Mucopolysaccharidosis |
| 1971 | Bhagwat and Ross | 8 – 10 | 20‡ | Infectious hepatitis, type 1 hyperlipoproteinemia, diabetes mellitus |
| 1969 | Panner and Hanss | 10 | 10–15 | Mushroom poisoning (<i>Amanita verna</i>) |
| 1964 | Svoboda and Manning | 12 | 20 | Chronic alcoholism |

* Diameter of the filament (nm).

† Space between the filaments (nm); ‡ center to center spacing.

ultrastructural characteristics (Siersema et al., 1992). The ultrastructure of needle-like structures observed in this study is identical to that reported by Siersema and coworkers (1992). The ultrastructural findings observed in the smooth endoplasmic reticulum and peroxisomes are characteristic features in alcoholic liver injury (Tanikawa, 1979).

The dimensions of the filamentous structures thus far reported are summarized in Table 1. The diameter of the individual filament ranges from 6 to 12 nm with the space of 5 to 20 nm. However, there are some confusions on the measurement of the space between filaments. Although the space was reported to be 20 nm (Wills, 1965) and 15 to 20 nm (Haust, 1968), the actual size measured from the micrographs was approximately 5 nm. Consequently, the ultrastructural dimension of the filaments in this study is identical to that described by Wills (1965) and Haust (1968).

The inclusions have been more frequently reported in diseased human livers than in normal human livers (Tange, 1981). Experimentally, Simpson and colleagues (1974) reported that the inclusions were produced in the normal dog after treatment with levamisole. The inclusions appear to be one of reversible pathological conditions: Svoboda and Manning (1964) reported that the inclusions found in chronic alcoholic patients diminished after 3 months on an adequate diet with elimination of alcohol intake.

The origin of the crystalline inclusions has been discussed. Svoboda and Manning (1964) speculated they are derived from mitochondrial cristae, based on the following morphological evidence: i) the continuity of the inclusions with the cristae; and ii) the decrease of the cristae. Sternlieb and Berger (1969) also showed a close relationship between the mitochondrial cristae and inclusions: the mitochondrial cristae are arranged in parallel with each other at an angle of about 130° in relation to the long axis of the filamentous inclusions. Furthermore, their optical diffraction studies indicated that they are phospholipid micelles or large protein molecules. In addition, according to Bhagwat and Ross (1971), electron microscopic cytochemistry showed the inclusions to be rich in cytochrome oxidase and succinic dehydrogenase activity.

Considering from some enzymatic activity on cell injury, Mugnaini (1964) speculated that the intramitochondrial inclusions are one type of non-specific reaction. Uchida and colleagues (1984) showed that the elongated mitochondria containing inclusions have a normal function from their histochemical studies. Recently, Daugherty and coworkers (1996) proposed a new idea on the formation of intramitochondrial inclusions based on hemodynamic alterations. They examined the ultrastructures of the liver obtained from patients with "cavernous transformation of the portal vein". Consequently, they considered that the deficiency of metabolic

substrates causes production of mitochondrial enzymes, thus producing the enlarged mitochondria containing inclusions. Although many researchers have regarded the crystalline inclusions as the expression of degeneration, it seems likely that they are adapting in response to the metabolic disorder as was discussed by Daugherty and colleagues (1996).

References

- 1 Bhagwat AG, Ross RC. Hepatic intramitochondrial crystalloids. *Arch Pathol* 1971;91:70–77.
- 2 Biempica L, Kosower N, Ma MH, Goldfischer S. Hepatic porphyrias. Cytochemical and ultrastructural studies of liver in acute intermittent porphyria and porphyria cutanea tarda. *Arch Pathol* 1974;98:336–343.
- 3 Blekkenhorst GH, Pimstone NR, Webber BL, Eales L. Hepatic haem metabolism in porphyria cutanea tarda (PCT): enzymatic studies and their relation to liver ultrastructure. *Ann Clin Res* 1976;8:108–121.
- 4 Boon AP, Thompson H, Baddeley RM. Use of histological examination to assess ultrastructure of liver in patients with long standing jejuno-ileal bypass for morbid obesity. *J Clin Pathol* 1988; 41:1281–1287.
- 5 Daugherty CC, Yazigi N, Bove K, Balistreri W. Mitochondrial enlargement and crystalloid matrix arrays: distinctive finding in childhood portal hypertension due to cavernous transformation of the portal vein. *Pediatr Pathol Lab Med* 1996;16:263–274.
- 6 Djaldetti M, Mandel EM, Fishman P, Bessler H, Lewinski U. Intramitochondrial crystalline inclusions in metastatic hypernephroma of the liver. *Biomedicine* 1974;21:158–163.
- 7 Haust MD. Crystalloid structures of hepatic mitochondria in children with heparitin sulphate mucopolysaccharidosis (Sanfilippo type). *Exp Mol Pathol* 1968;8:123–134.
- 8 Lundbergh P, Westman J. Hepatic filamentous mitochondrial inclusions associated with oral contraceptives. *Scand J Infect Dis* 1970;2:105–109.
- 9 Mandel EM, Fishman P, Kissin E, Har-Zaav L, Djaldetti M. Intramitochondrial crystalline inclusions in the liver of a patient with hyperthyroidism. *Acta Hepato-Gastroenterol* 1976;23:182–186.
- 10 Mugnaini E. Filamentous inclusions in the matrix of mitochondria from human livers. *J Ultrastruct Res* 1964;11:525–544.
- 11 Panner BJ, Hanss RJ. Hepatic injury in mushroom poisoning. *Arch Pathol* 1969;87:35–45.
- 12 Reid IM, Donaldson IA, Heitzman RJ. Effects of anabolic steroids on liver cell ultrastructure in sheep. *Vet Pathol* 1978;15:753–762.
- 13 Ruffolo R, Covington H. Matrix inclusion bodies in the mitochondria of the human liver. *Am J Pathol* 1967;51:101–116.
- 14 Sandborn EB, Côté MG, Viallet A. Electron microscopy of a human liver in Weil's disease (leptospirosis icterohaemorrhagica). *J Pathol Bacteriol* 1966;92:369–374.
- 15 Siersema PD, van Helvoirt RP, Cleton-Soeteman MI, de Bruijn WC, Wilson JHP, van Eijk HG. The role of iron in experimental porphyria and porphyria cutanea tarda. *Biol Trace Elem Res* 1992;35:65–72.
- 16 Simpson CF, Bradley RE, Jackson RF. Crystalloid inclusions in hepatocyte mitochondria of dogs treated with levamisole. *Vet Pathol* 1974;11:129–137.
- 17 Sternlieb I. Mitochondrial and fatty changes in hepatocytes of patients with Wilson's disease. *Gastroenterology* 1968;55:354–367.
- 18 Sternlieb I, Berger JE. Optical diffraction studies of crystalline structures in electron micrographs II. Crystalline inclusions in mitochondria of human hepatocytes. *J Cell Biol* 1969;43:448–455.
- 19 Svoboda DJ, Manning RT. Chronic alcoholism with fatty metamorphosis of the liver. *Am J Pathol* 1964;44:645–662.
- 20 Tange T. Histopathological identification of hepatic intramitochondrial crystalloid inclusions. *Acta Pathol Jpn* 1981;31:747–754.
- 21 Tanikawa K. Ultrastructural aspects of the liver and its disorders. 2nd ed. Tokyo: Igaku-Shoin; 1979. p. 286–291.
- 22 Uchida T, Kronborg I, Peters RL. Giant mitochondria in the alcoholic liver diseases—their identification, frequency and pathologic significance. *Liver* 1984;4:29–38.
- 23 Waldo ED, Tobias H. Needle-like cytoplasmic inclusions in the liver in porphyria cutanea tarda. *Arch Pathol* 1973;96:368–371.
- 24 Watrach AM. Degeneration of mitochondria in lead poisoning. *J Ultrastruct Res* 1964;10:177–181.
- 25 Wills EJ. Crystalline structures in the mitochondria of normal human liver parenchymal cells. *J Cell Biol* 1965;24:511–514.
- 26 Wilson-Martino NA, Martino LJ, Millman-Feder NG, Benitz KF. The presence of hepatic intramitochondrial crystalline inclusions in polybrominated biphenyl-treated mice. *Arch Toxicol* 1980;45:233–239.

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