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Original Articles

An Autopsy Case of Erythropoietic Protoporphyria with Cholestatic Jaundice and Hepatic Failure, and a Review of Literature

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Summary. A 43-year-old woman with a history of photosensitivity died of hepatic failure following 3 and a half months of unexplained jaundice. The liver was black, showed mild fibrosis and conspicuous pigment deposition in the cytoplasm of the hepatocytes, Kupffer cells and portal macrophages, and within dilated lumina of bile canaliculi and of ductules. The pigment disclosed a striking birefringence and numerous slender electron-dense crystals on electron microscopy. Similar crystals were also found within the cytoplasm of the ductular epithelium. Despite absence of cirrhosis observed in almost all previously described fatal cases the diagnosis of erythropoietic protoporphyria was made at autopsy. Mild inflammatory changes of the liver with marked protoporphyrin deposition so far have not been described in the cases observed at autopsy.

Key words: Erythropoietic protoporphyria – Hepatic failure – Intrahepatic cholestasis

Introduction

The chief clinical manifestation of erythropoietic protoporphyria (EPP) is photosensitivity, usually beginning in early childhood (Bloomer 1976; Deleo et al. 1976; Hubler et al. 1976; Magnus et al. 1961; Mathews-Roth 1977; Reed et al. 1977). Hepatic disease may also be a clinical feature of EPP and sometimes a critical one (Barnes et al. 1968; Bloomer et al. 1972; Bruguera et al. 1976; Cripps et al. 1965; Cripps et al. 1971; Cripps et al. 1977; Donaldson et al. 1971; Hashimoto et al. 1970; Ibayashi et al. 1975; Klatskin et al. 1974; Matilla et al. 1974; Porter et al. 1963; Romslo et al. 1978; Scott et al. 1973; Singer

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et al. 1978; Thompson et al. 1973). A spectrum of hepatic lesions may be seen in patients with or without symptoms related to the hepatobiliary system, but patients with EPP and hepatic failure revealed liver cirrhosis at autopsy, usually of micronodular type (Barnes et al. 1968; Cripps et al. 1977; Donaldson et al. 1971; Hashimoto et al. 1970; Ibayashi et al. 1975; Scott et al. 1973; Singer et al. 1978; Thompson et al. 1973).

We present a fatal case, with progressive cholestatic jaundice and extensive deposition of brown pigment both in parenchymal and mesenchymal cells of the liver, which showed only a mild degree of inflammatory changes. Electron and polarization microscopic observations revealed an excessive deposition of protoporphyrin (Bloomer et al. 1972; Klatskin et al. 1974) in the liver. The mechanism of excessive deposition of protoporphyrin in the hepatocytes, and it's relation to cholestasis, is unknown. Its biological significance is discussed.

Materials and Methods

Autopsy specimens of the liver were fixed in 10% formalin, embedded in paraffin, and 5 μm thick sections were stained with H & E, Azan-Mallory, Gomori's reticulin stain, Perl's stain for iron, orcein stain (Shikata et al. 1974) and p-dimethyl-aminobenzyldine rhodamine stain for copper and periodic acid Schiff reaction before and after diastase treatment. Birefringence was studied under a conventional light microscope equiped with polarizing filters (Klatskin et al. 1974). The liver tissues obtained at autopsy were immediately fixed in buffered 3% glutaraldehyde, postfixed in buffered 2% tetraoxide and dehydrated with alcohol and embedded in EPON 812. Ultrathin sections were stained with uranyl acetate and lead citrate for electron microscope (JEM 100B).

Case Report

A 43-year-old housewife had photosensitivity since the age of 7, characterized by erythema and oedema of sun-exposed areas of the skin. She was admitted in May 1979 to the Koseiren Takaoka Hospital with jaundice of 5 week's duration. She did not report alcohol abuse nor drug addition. Her past medical history did not reveal episodes related to the hepatobiliary system. Her two daughters also suffered from photosensitivity. On admission she was deeply jaundiced. The edge of the liver was about 3 fingerbreadths below the costal arch. Burning, redness and oedema of the facies were noted, but other areas of the skin appeared normal, except for jaundice.

Abnormal laboratory data included: WBC, 11,100/cu mm with neutrophilia; serum total bilirubin, 12.9 mg/dl, direct reacting 10.4 mg/dl; serum alkaline phosphatase, 12.9 King-Armstrong units; SGOT, 198 Karmen units; SGPT, 134 Karmen units; serum gamma-GTP, 280 u/dl (normal: 0–40 u/dl); bilirubin in urine, positive; occult blood in faeces. Hepatitis B surface antigens, significant alpha-fetoprotein elevation, anti-nuclear, smooth muscle and mitochondrial antibodies were absent. Liver/spleen scan disclosed an enlarged left hepatic lobe and excess-radio-isotopes in spleen and bone marrow.

The hospital course was progressively downhill. Jaundice of cholestatic type increased gradually, and activities of serum alkaline phosphatase, gamma-GTP and cholesterol rose progressively. In June 1979, ascites, generalized oedema and haemorrhagic tendency developed, and serum ammonia level rose to 107 μg/dl (normal: 30 to 80 μg/dl). She died in hepatic failure following massive melena after 3 and a half months of jaundice. Unfortunately, protoporphyrin levels in the erythrocytes, plasma and stools were not examined.

Gross hepatobiliary findings: The peritoneal and cut surface of the liver, 1,250 g in weight, were black. The extrahepatic and main intrahepatic bile ducts were normal, and calculi were not found in the biliary system. The portal vein trunk and its tributaries were normal.

Observations

Light Microscopic Observation of the Liver. Although the lobular architecture was in principal intact, the portal tracts were enlarged by a variable, mostly

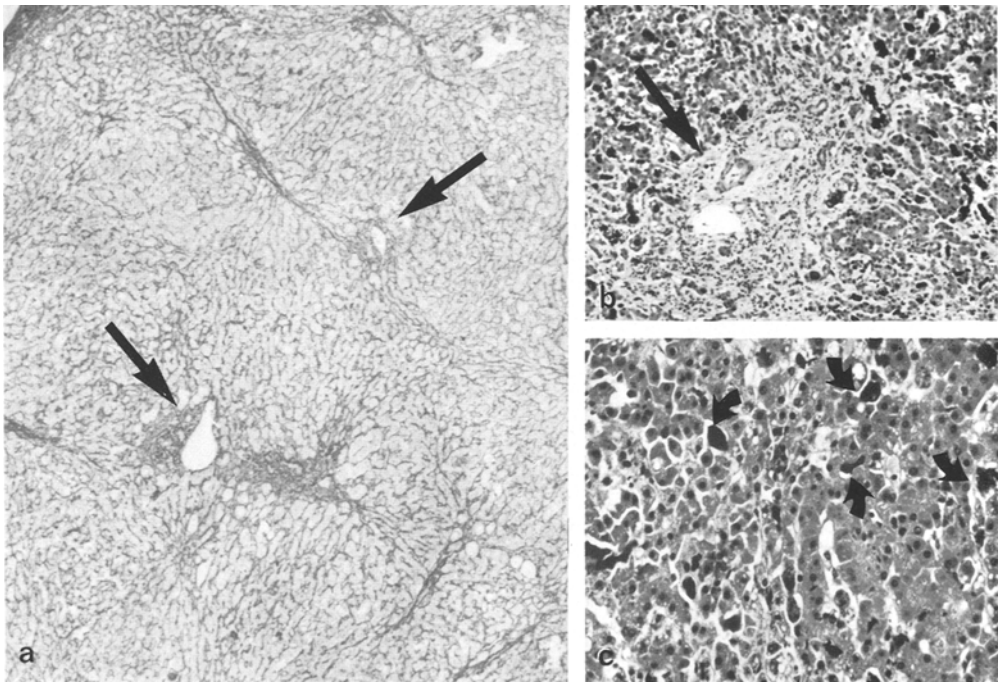

Fig. 1a–c. The lobular architecture is well preserved (**a**), but portal tracts (➔) are fibrotic with mild to moderate lymphocytic infiltration and ductular proliferation (**a, b**). Note massive pigment deposits in the hepatic parenchyma (**b, c**). Dark brown pigments are deposited as granules or globules (➘) in the cytoplasm of swollen Kupffer cells and within the distended bile canalicular lumina (**c**). Autopsy liver, Gomori's reticulum, ×70 (**a**), HE., ×100 (**b**) and ×150 (**c**)

mild, fibrosis (Fig. 1a, b), and some adjacent portal tracts were connected. Rarely, bridging fibrous bands enclosed parenchymal nodules, resulting in focal loss of the normal architecture. Fibrous septa and portal tracts contained lymphocytes, monocytes, pigmented macrophages and proliferating bile ductules, and in some areas the limiting plates were lysed, but as a whole the hepatic inflammation was mild. Occasionally hepatocytes had been lost in centrolobular zones. They were replaced by fibrous tissue containing pigmented macrophages and lymphocytes. Centro-portal fibrous bridging were rare.

Throughout the hepatic parenchymal areas much dark brown pigment was seen in the cytoplasm of hepatocytes, Kupffer cells and portal macrophages, and with in distended lumina of bile canaliculi and ductules. In the last two they formed dark bile plugs (Fig. 1c, 2a). The cytoplasm of duct epithelium had coarse granules. Some of the luminal plugs had a green hue suggesting a mixture of protoporphyrin with bile. Many pigment deposits, especially in Kupffer cells, were PAS positive after diastase treatment. Stains for iron and copper revealed negative results.

The pigment deposits exhibited striking birefringence (Fig. 2b). Many small cytoplasmic and canalicular pigment granules, not visible under routine microscopy, were seen to be birefringent, often red. Maltase cross formation was noted

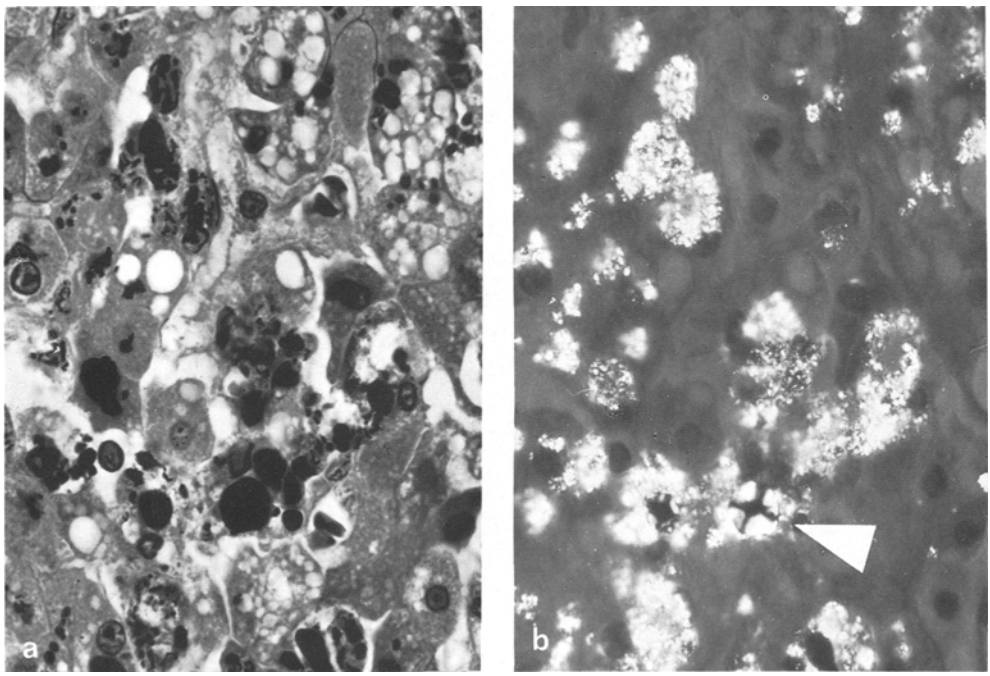

Fig. 2a, b. a and **b** are the same part observed under light microscope (**a**) and the polarized light microscope (**b**). The large globules in the Kupffer cells and in the distended canalicular lumina revealed striking birefringence, some of them showing a central Maltase cross (◁). Other pigment granules in the hepatocytes also birefringent, Autopsy liver, HE., ×700

(Fig. 2b). Occasionally, larger deposits with relative light hue within the lumina of ductules and canaliculi failed to show birefringence or exhibited only a narrow bright red halo.

Electron Microscopic Observation. The deposits consisted of masses of crystalline material in form of closely packed, relatively short, curved or straight bars with tapered or blunt ends, and relatively long wavy rods of varying diameter, arranged randomly or in a star-like pattern (Figs. 3, 4). Furthermore, granular amorphous materials and/or short curved fibrils of uniform size and shape, smaller than the above mentioned crystals, were in the hyaloplasm adjacent to the crystalline masses, some of which connected with the fibrils (Fig. 5a, b). All deposits were free in the cytoplasm of many hepatocytes and bile ductular epithelium (Fig. 6): they were not enclosed by a membrane, but displaced the organelles. Crystalline masses were also seen in the canalicular and ductular lumina, intermingled with amorphous masses of bile. Some bile thrombi exhibited an inner electron lucent, granular and fibrillar core and an outer electron dense shell containing crystalline material (Fig. 4). The same crystalline masses were also found in Kupffer cells and portal macrophages, but neither a limiting membrane nor any structural relationship to secondary lysosomes, described in other reports (Bloomer et al. 1972; Klatskin et al. 1974), were found.

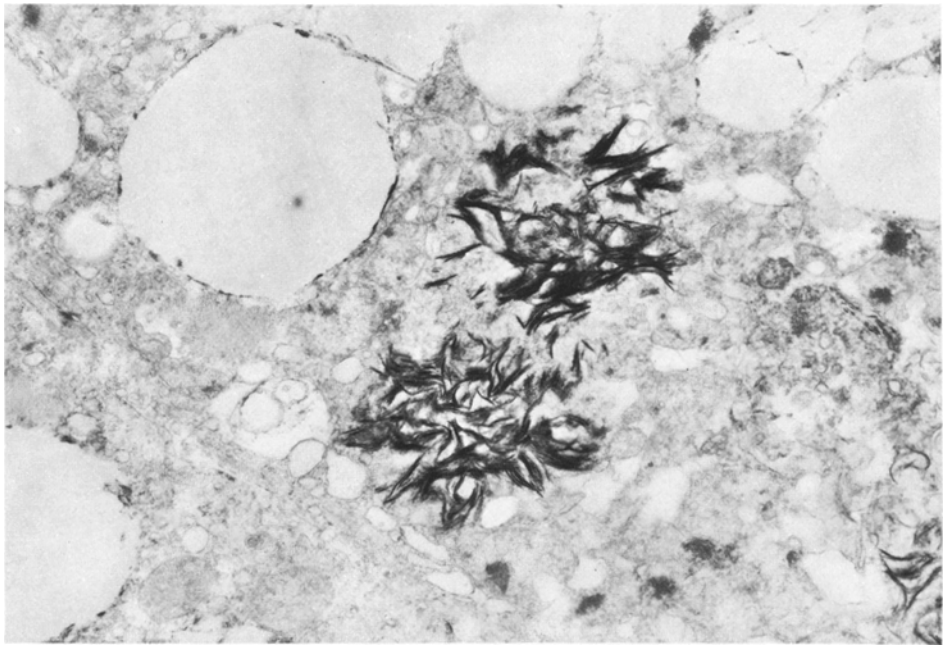

Fig. 3. Two masses of crystalline material showing many wavy or straight slender electron-dense bars in the cytoplasm of liver cells. Autopsy liver. ×15,000

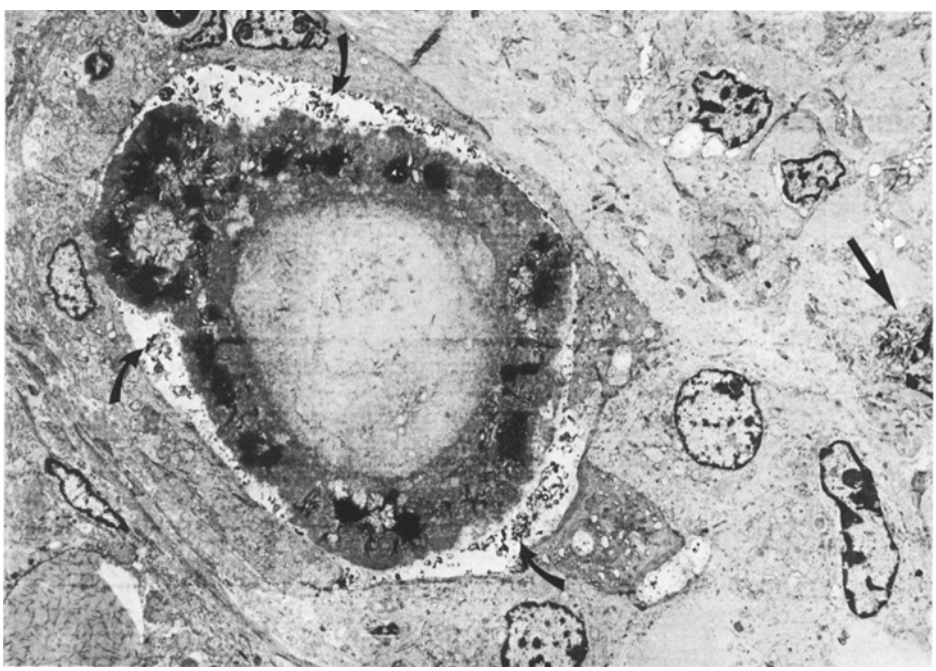

Fig. 4. Several masses of electron dense crystalline material intermingled with amorphous dense substance at the periphery of bile thrombus in the distended lumen of bile ductule. The central part shows an electron-lucent flocculent or partially membranous matrix. At the periphery of this thrombus there are many osmophilic membranes (↖) suggesting biliary phospholipids. A crystalline mass is also seen in a portal macrophage (→). Autopsy liver, ×30,000

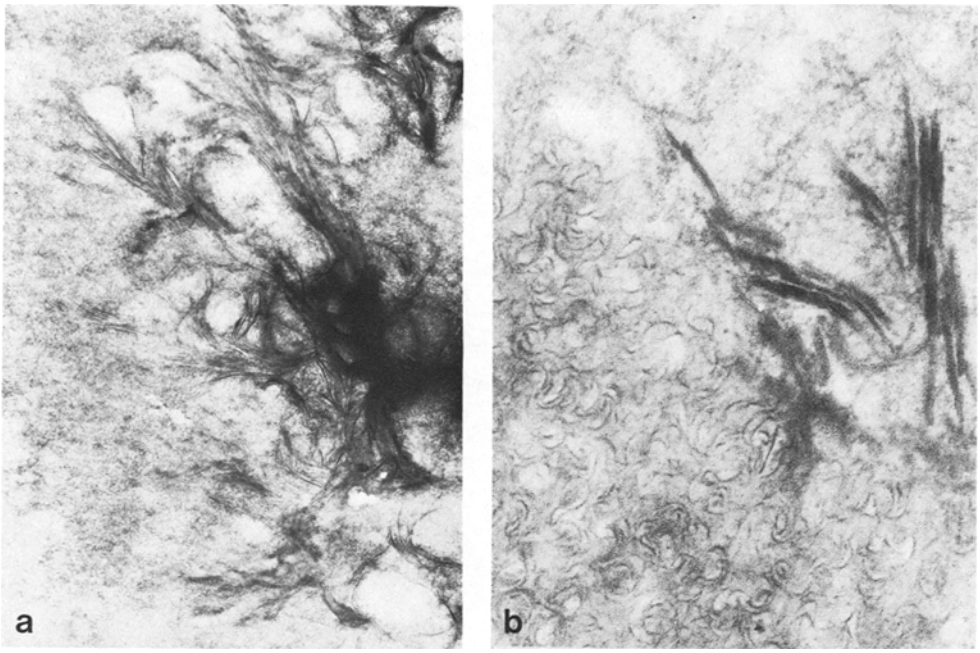

Fig. 5a, b. Granular amorphous substance (left side of **a**) and curved fibrils of regular size and shape (**b**) intermingled with electron dense crystallines in the hepatocytes. Autopsy liver. ×18,000 (**a**) and ×120,000 (**b**)

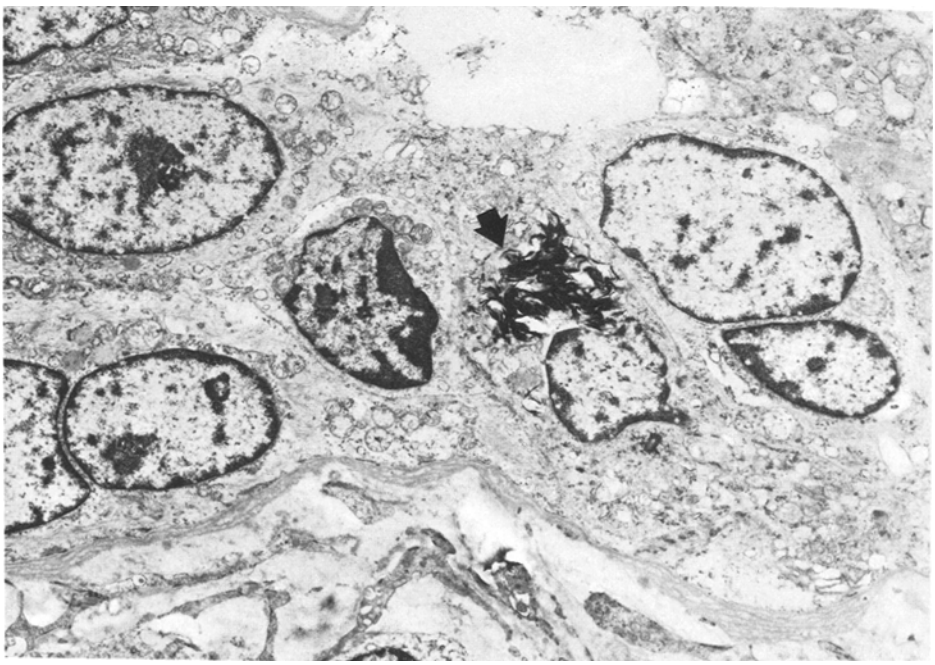

Fig. 6. Crystalline mass (⧫) with the same structures seen in the hepatocytes, is also found in the cytoplasm of bile ductular cell. Note loss of microvilli, proliferation of microfilaments and duplication of basement membrane of bile ductule. Autopsy liver. ×6,000

Dilated bile canaliculi contained occasionally membranous or granular materials, probably bile pigment. Their microvilli were lost or deformed and a thickened pericanalicular ectoplasm showed excess microfilaments, as indication of cholestasis (Adler et al. 1980; Desmet 1972; Popper et al. 1970). Also the bile ductular epithelium showed increased microfilaments and bile pigments in the lumina and cytoplasm, loss of luminal microvilli and duplication of the basement membrane.

The lesions and discussed polarization and electron microscopical findings, are interpretated as the effects of hepatic deposition of protoporphyrin (Bloomer et al. 1972; Klatskin et al. 1974). Thus, a diagnosis of EPP was made, despite lack of antemortem demonstration of protoporphyrin in the erythrocytes, plasma or faeces.

Discussion

While the clinical course of EPP usually is benign and the symptoms are restricted to the skin (Bloomer et al. 1976; Deleo et al. 1976; Hubler et al. 1976; Magnus et al. 1961; Mathews-Roth 1977; Reed et al. 1977), the liver may be affected slightly or severely (Barness et al. 1968; Bloomer et al. 1972; Bruguera et al. 1976; Cripps et al. 1965; Cripps et al. 1971; Cripps et al. 1977; Donaldson et al. 1971; Hashimoto et al. 1970; Ibayashi et al. 1975; Klatskin et al. 1974; Matilla et al. 1974; Porter et al. 1963; Romslo et al. 1978; Scott et al. 1973; Singer et al. 1978; Thompson et al. 1973). In most reported cases of EPP, with or without hepatobiliary symptoms, the liver contained brown pigment which reveals a striking birefringence, notably a red brilliance with a central Maltese cross and numerous slender electron-dense crystals in a star-like pattern on electron microscopy. Both of these features are explained by excess protoporphyrin in the liver (Klatskin and Bloomer 1974). Reported lesions revealed a spectrum from almost normal liver to micronodular cirrhosis.

The latter has been reported in most fatal cases. In some reports, the liver had varying degree of portal fibrosis, inflammation, ductular proliferation and loss of periportal hepatocytes. The mechanism of the liver damage is not known. Excess protoporphyrin, a water-insoluble compound, accumulates in the liver cells with diminished activity of ferrochelatase which may be the primary biochemical defect of EPP (Bonkowdky et al. 1975; Bottomley et al. 1975; Romslo et al. 1978; Scholnick et al. 1971). Protoporphyrin, thus deposited within the liver cells, forms crystals which may be injurious to the liver cells, probably via mitochondrial damage (Romslo et al. 1978). Precipitation of protoporphyrin in the lumina of the bile canaliculi together with bile may cause prolonged cholestasis and contribute to hepatocyte injury. A genetic predisposition toward severe hepatobiliary damage may be responsible for a fatal outcome (Thompson et al. 1973).

The English and Japanese literatures (Table 1) (Bloomer et al. 1972; Barnes et al. 1968; Hashimoto et al. 1970; Donaldson et al. 1971; Scott et al. 1973; Thompson et al. 1973; Ibayashi et al. 1975; Cripps et al. 1977; Singer et al. 1978), reported 11 cases of EPP with hepatic failure and subsequent death. They include 6 males and 5 females, 10 are autopsy cases. The hepatic lesions

Table 1. Erythropoietic protoporphyria with hepatic failure and death reported in the English and Japanese literature

Author and reference	Age (y)	Sex	Weight (g)	Pathology of the liver			
				Color	Proto-porphyrin deposition	Hepatic lesion	Chole-stasis
1[a] Barnes et al.	42	M	2,170	black	present	cirrhosis	n.d.
2[a] Hashimoto et al.	58	F	780	n.d.	absent	pigmentary cirrhosis	present
3[a] Donaldson et al.	56	M	2,020	almost black	present	micronodular cirrhosis	present
4 Donaldson et al.	58	M			present	micronodular cirrhosis	absent
5[a] Scott et al.	43	F	1,330	black	present	micronodular cirrhosis	present
6[a] Thompsen et al.	31	F	1,850	deeply bile stained	present	coarse cirrhosis of mixed type	present
7[a] Thompsen et al.	29	F	n.d.	deeply bile stained	present	coarse cirrhosis of mixed type	present
8[a] Bloomer et al.	33	F	2,690	black	present	cirrhosis	present
9[a] Ibayashi et al.	26	M	1,830	greenish black	present	micronodular cirrhosis	present
10[a] Cripps et al.	11	M	1,700	dark brownish black	present	postnecrotic cirrhosis	present
11[a] Singer et al.	60	M	1,250	black	present	severe hepatic fibrosis	present

[a] Autopsy cases, n.d. = not described

were cirrhosis in 10 instances (micronodular type: 4 cases; coarse nodular post-necrotic type: 3 cases; not described: 2 cases) and severe hepatic fibrosis in 1 case. One case complicated by haemolytic anemia and treated with repeated blood transfusion had pigment cirrhosis. Excess brown pigment (protoporphyrin), was deposited in all livers. Thus mild alterations of hepatic lobular architecture with severe protoporphyrin deposition, as seen in the present case, have not been described previously in the fatal cases. Jaundice was noted in 10 out of 11 cases and usually developed progressively in the terminal stage of the disease. It occurred transiently in some non-fatal cases and clinically simulated large bile duct obstruction.

The mechanism of jaundice in EPP is unknown. Electron microscopic features such as loss of bile canalicular microvilli, luminal dilatation, and excess pericanalicular microfilaments, seen in the present case, are found in cholestatic livers from any cause (Adler et al. 1980; Desmet 1972; Popper et al. 1970). The pathogenesis of cholestasis is not yet established (Erlinger 1978; Phillips et

al. 1975; Popper et al. 1970; Tanaka et al. 1980) and the role of erythropoietic protoporphyrin is therefore only conjectural.

Several ultrastructural findings in this case have not been previously reported. The first are the crystals in the cytoplasm of the bile ductular epithelium, similar to those in the hepatocytes. Excess protoporphyrin was thought to be produced in the liver cells and erythroid cells (Scholnick et al. 1971). Thus, the ductal crystals may be absorbed from bile, containing protoporphyrin excreted by the hepatocytes. The second finding are the curved, hairlike fibrils of uniform size and shape. They were only seen within or close to crystalline material and are presumed to be associated with production of excess protophorphyrin in hepatocytes. Their exact nature and biological significance remain to be elucidated.

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