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Hepatic Ultrastructural Findings of Familial Hyperbilirubinemia Syndrome

Sung Hye Park*, M.D. and Je G. Chi, M.D.

Department of Pathology, Chung-Ang Gil Hospital and*
Seoul National University College of Medicine

= Abstract = **Seven liver biopsies of congenital/familial hyperbilirubinemia were studied ultrastructurally including 3 cases of Dubin-Johnson syndrome, 2 cases of Rotor syndrome, one case of Gilbert syndrome and one case of type 2 Crigler-Najjar syndrome. All five cases of Dubin-Johnson syndrome and Rotor's syndrome had conjugated hyperbilirubinemia and both cases of Gilbert's syndrome and a Crigler-Najjar syndrome had unconjugated hyperbilirubinemia. In Gilbert's syndrome, the microvilli of the sinusoidal membrane of hepatocytes showed decreased height and number with collagen lay down in the sinusoidal spaces, Megamitochondria, mild proliferation of smooth endoplasmic reticulum, and dilated rough endoplasmic reticulum were also noted. Lipofuscin bodies were seen, but they were less numerous than characteristic Dubin-Johnson bodies. In Crigler-Najjar syndrome, bile canalicular and ductular cholestasis were noted both light microscopically and ultrastructurally. Most bile canaliculi are filled with ovoid homogeneous electron dense material (bile pigments). Widening of the intercellular spaces with increased number of microvilli on the lateral surface of hepatocytes were present. All three cases of Dubin-Johnson syndrome revealed characteristic abundant lysosomal bodies and dilatation of bile canaliculi. These bodies were numerous and membrane bound in round, oval or pleomorphic shapes with variable degrees of electron densities. Dilated bile canaliculi showed expanded lumen with decreased number of microvilli. In Gilbert and Rotor syndromes, the hepatocytes contained lipofuscin-like lysosomal bodies. In both cases of Rotor's syndrome, we found reduced number of microvilli along the sinusoidal side of hepatocyte, like Gilbert syndrome, immature bile canaliculi and pleomorphic megamitochondria and lipofuscin-like lysosomal bodies. We concluded that hepatocytic hyperbilirubinemia syndromes could be differentiated by ultrastructural study along with clinicopathologic correlation.**

Key Words : *Congenital/familial hyperbilirubinemia, Gilbert syndrome, Crigler-Najjar syndrome, Dubin-Johnson syndrome, Rotor syndrome, Ultrastructural study*

서울대학교 의과대학 병리학교실 : 지제근
중앙길병원 해부병리과 : 박성혜

INTRODUCTION

Congenital/familial hyperbilirubinemia syndromes are rare inherited disorders of bilirubin metabolism, and include unconjugated hyperbilirubinemia, as in Gilbert (Muraca et al.; 1987, June & Benjamine; 1984) and Crigler-Najjar syndromes (Crigler & Najjar; 1952, Huang et al.; 1970) and conjugated hyperbilirubinemia, as in Dubin-Johnson (Dubin & Johnson; 1954, Seymour et al.; 1977) and Rotor syndromes (Rotor et al.; 1948, Blanckaert & Schmid; 1982). They present as asymptomatic jaundice since youth except Crigler-Najjar type I. Asymptomatic icterus may be exacerbated by intercurrent illness, pregnancy or oral contraceptives and it is often discovered incidentally during investigation for an intercurrent illness. Type I Crigler-Najjar usually presents with a severe icterus, often with kernicterus and death in early infancy. The diagnosis of these syndromes are made by clinical findings and laboratory data, and are confirmed by biochemical studies and liver biopsy. However, in conventional histopathological studies, the liver appears normal in Gilbert syndrome and Rotor syndrome. Bile canalicular cholestasis in otherwise normal parenchyma is the only abnormality in Crigler-Najjar syndrome. Characteristic golden brown pigments are found in Dubin-Johnson syndrome and yet they are not definitive.

We studied ultrastructurally 7 cases of liver biopsies of congenital/familial hyperbilirubinemia syndromes to elucidate the ultrastructural characteristics of these syndromes.

MATERIALS AND METHODS

Materials consist of 1 case of Gilbert's syndrome, 1 case of Crigler-Najjar syndrome, 3 cases of Dubin-Johnson syndrome, and 2 cases of Rotor's syndrome. Clinical findings and laboratory data are summarized in Table 1. All cases except one were obtained from the file of the Department of Pathology of Seoul National University Children's Hospital from 1989 to 1991 and one case of Crigler-Najjar syndrome was obtained from the file of Chung-Ang Gil Hospital in 1992.

The liver studied included six percutaneous needle biopsies and one (Dubin-Johnson syndrome) wedge biopsy. All fresh hepatic tissue samples were divided into two part, and one was fixed in 10% formaldehyde solution and the other in 2.5% glutaraldehyde solution for 4 hours. The former was processed routinely for light microscopy and stained with hematoxylin eosin, Masson trichrome, reticulin, Fontana-Masson, acid fast blue, Periodic Acid Schiff and Prussian blue for iron. The latter samples fixed in glutaraldehyde were washed in cold 0.1 mol/L phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide, in 0.1 mol/L phosphate buffer. These sections were then embedded in eposy resin. Thin sections were stained with uranyl acetate-lead citrate, and examined under a Hitachi 600 transmission electron microsope.

RESULTS

Ultrastructural remarkable findings seen in our

Table 1. Summary of clinical and laboratory findings

	Number	Age / Sex	TB / DB (mg / dl)
Gilbert syndrome	1	7Y / M	4.8 / 0.6
Crigler-Najjar syndrome	1	25Y / M	15.4 / 0.5
Dubin-Johnson sybdrome	1	2Y / M	8.5 / 6.1
	2	3M / M	7.7 / 7.5
	3	1M / M	13.1 / 6.9
Rotor syndrome	1	6Y / M	3.4 / 2.1
	2	12Y / M	5.3 / 3.2

TB: Total bilirubin, DB: Direct bilirubin

cases are summerized in Table 2.

Gilbert syndrome: Light microscopically, the liver shows excessive accumulation of coarsely granular, golden brown pigment. Ultrastructurally, they are irregular, heterogeneous aggregates of mixed electron-dense and electron-lucent globules similar to lipofuscin granules (Fig. 1). The granules ranges from 500 to 5560 nm in diameter. In the space of Disse, increased collagen fibers are seen (Fig. 1). The sinusoidal membrane of hepatocytes shows reduced number and stunting of microvilli (Fig. 2). The smooth endoplasmic reticulum is increased number and dilated (Fig.1). The mitochondria are enlarged and some of them contain crystalloid inclusion bodies (Fig. 1). No consistent ultrasturctural alterations of bile canaliculi, sinusoidal pores, Golgi apparatus or glycogen amount are noted.

Crigler-Najjar syndrome: Liver biopsy shows canalicular and bile ductular cholestasis. Ultrastructurally, most bile canaliculi are filled with amorphous homogeneously electron dense bile pigment and their microvilli appear degenerated and are flattened (Fig. 4 & 5). Widening of intercellular spaces with microvilli proliferation on the lateral surface of hepatocytes are present (Fig. 6). Mitochondria containing paracrystalline inclusions with slight variability in size and shape are noted. Presence of bile pigments in Kupffer cell cytoplasm is also seen (Fig. 7).

Dubin-Johnson syndrome: Light microscopically, the hepatocytes reveal accumulation of golden brown pigments especially in acinar zone 3. These pigments were positive for Fontana-Masson stain (Fig. 8). Ultrastructurally, numerous characteristic granules were found in virtually all hepatocytes (Fig. 9). They are membrane bound, round or oval to irregular shaped characteristic granules. The granules range from 700 to 5800 nm in diameter and contain a number of fine granular material of varying electron density. They lack the lobulation typical of lipofuscin granules. Some of them show pleomorphic or scalloped appearance (Fig. 10). Also present is dilatation of bile canaliculi characterized by expanded lumen and reduction in number and height of microvilli (Fig. 5 & 9). Mitochondria, hepatocytic sinusoidal membrane, smooth endoplasmic reticulum, the Golgi apparatus and glycogen content are unremarkable.

Rotor Syndrome: Light microscopically, the liver was essentially unremarkable, however, ultrastructurally, remarkable abnormalities are noted. Mitochondria (Fig. 3) are enlarged with occasional paracrystalline inclusions. Bile canaliculi show immature and simplified feature, i.e., reduction in size and number of microvilli (Fig. 5). Variable sized lipofuscin granules show two-tone type electron densities and less dense fine granular appearance (Fig. 11). Hepatocytic sinusoidal membrane revealed flattening and decreased number of microvilli along with collagen lay down similar to Gilbert's syndrome (Fig. 2). Cytoplasmic glycogen particles appeared increased in amount.

Table 2. Ultrastructural changes in familial hyperbilirubinemia syndromes

	Gilbert	C-N	D-J	Rotor
Lipofuscin granules	+	+	−	+
D-J type lysosomal bodies	−	−	+	−
Cholestasis	−	+	−	−
Size of mitochondria	giant	enlarged	N	giant
Mitochondrial inclusions	+	+	−	+
Bile canaliculi	N	N	dilated	immature
Sinusoidal microvilli	reduced'	N	N	reduced'
Sinusoidal collagen	+	−	−	+

C-N: Crigler-Najjar syndrome, D-J: Dubin-Johnson syndrome, +: presence, −: absence, N: normal, reduced': reduced number and height

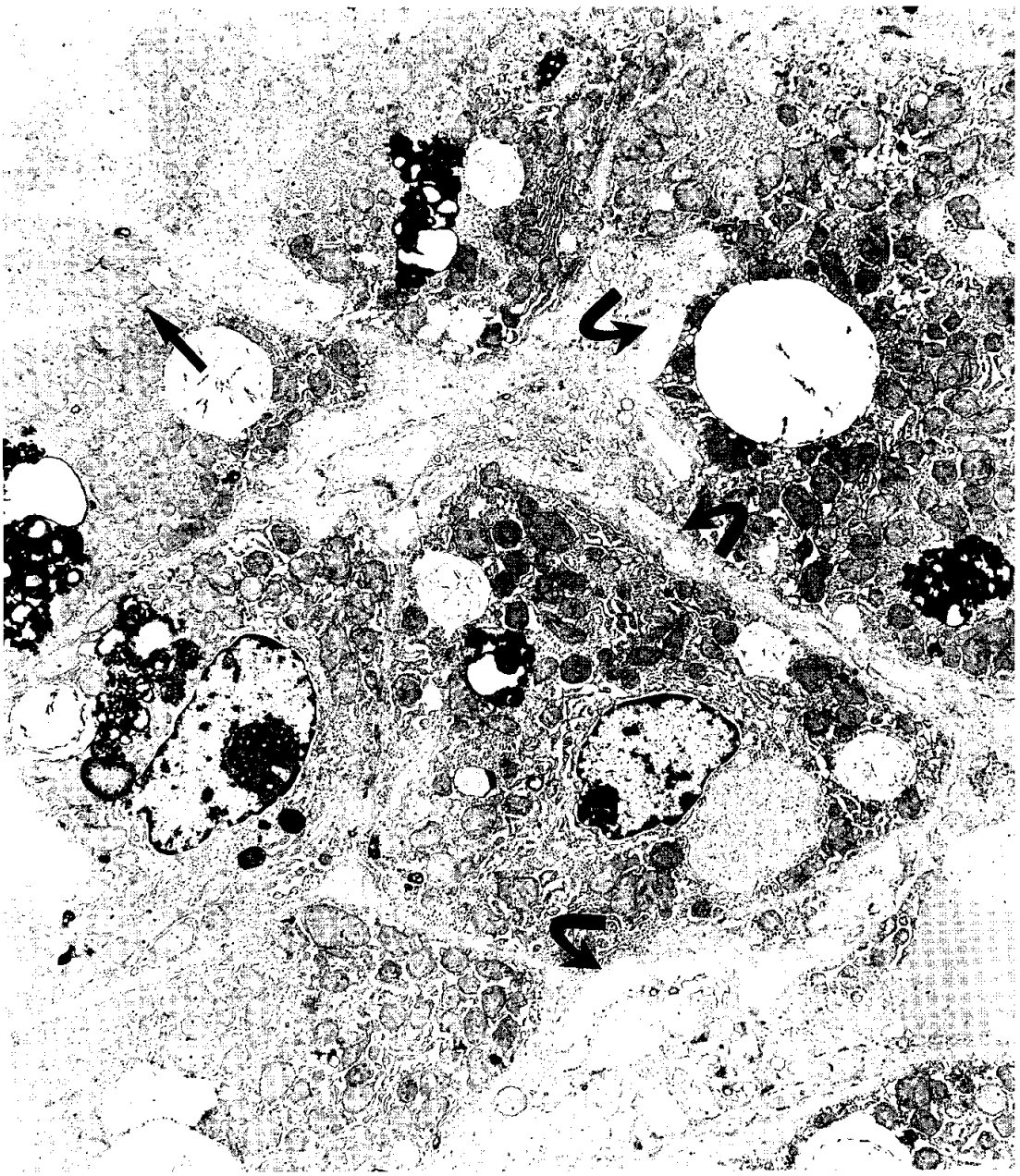

Fig. 1. Gilbert syndrome: The pigment granules are irregular, heterogeneous aggregates of mixed electron-dense and electron-lucent globules with a similar structural pattern of lipofuscin grnules (open arrows). In the space of Disse, increased collagen-lay down are seen (curved arro-ws). The sinusoidal membrane of hepatocytes shows recuced number and flattening of microvilli. The smooth endoplasmic reticulum shows slightly increased number. The mitochondria are enlarged and some of them contained crystalloid inclusion bodies (arrows) (Uranyl acetate and lead citrate, × 5,650).

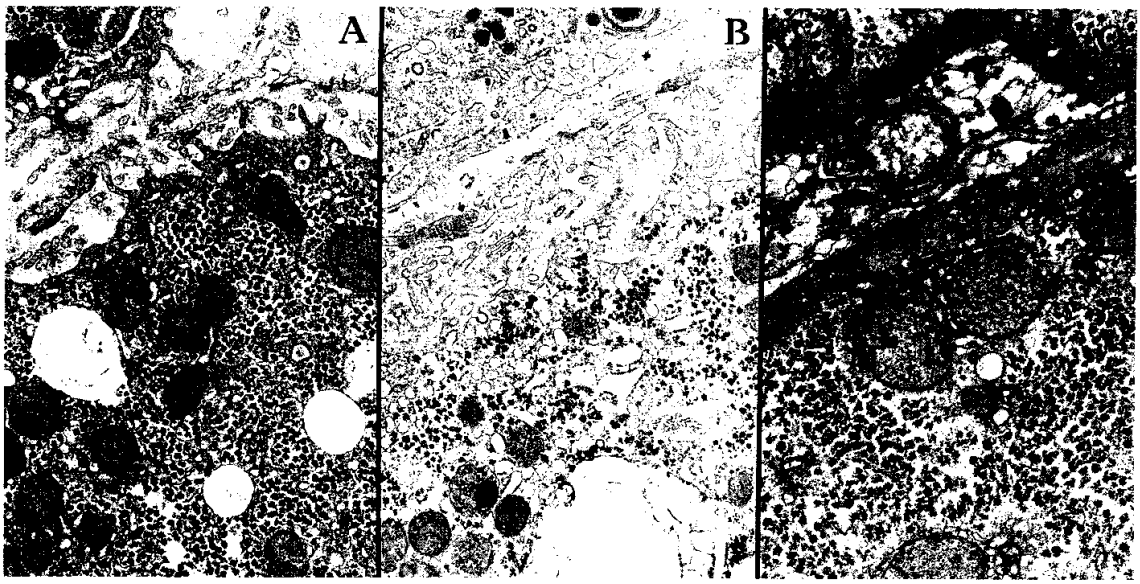

Fig. 2. A : Gilbert syndrome, B : Dubin-Johnson syndrome, C : Rotor syndrome. In both Gilbert and Rotor syndrome, sinusoidal microvilli show reduced number and flattening in contrast with relatively normal long and numerous sinusoidal microvilli of Dubin-Johnson syndrome (Uranyl acetate & lead citrate, A, B, C: × 18,400).

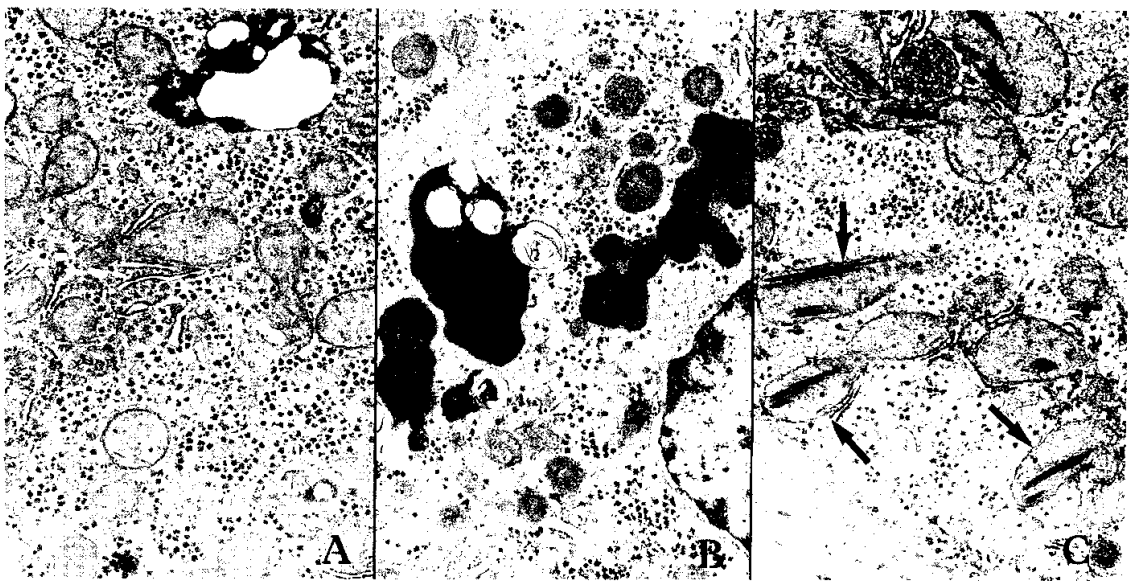

Fig. 3. In both Gilbert and Rotor syndrome, the mitochondria are enlarged with paracrystalline inclusions (arrows). Especially in Rotor syndrome, Giant mitochondria are seen. In Dubin-Johnson syndrome, the mitochondria are relatively normal or smaller size. A : Gilbert syndrome, B : Dubin Johnson syndrome and C : Rotor syndrome (Uranyl acetate & lead citrate, A, B & C : × 18,400).

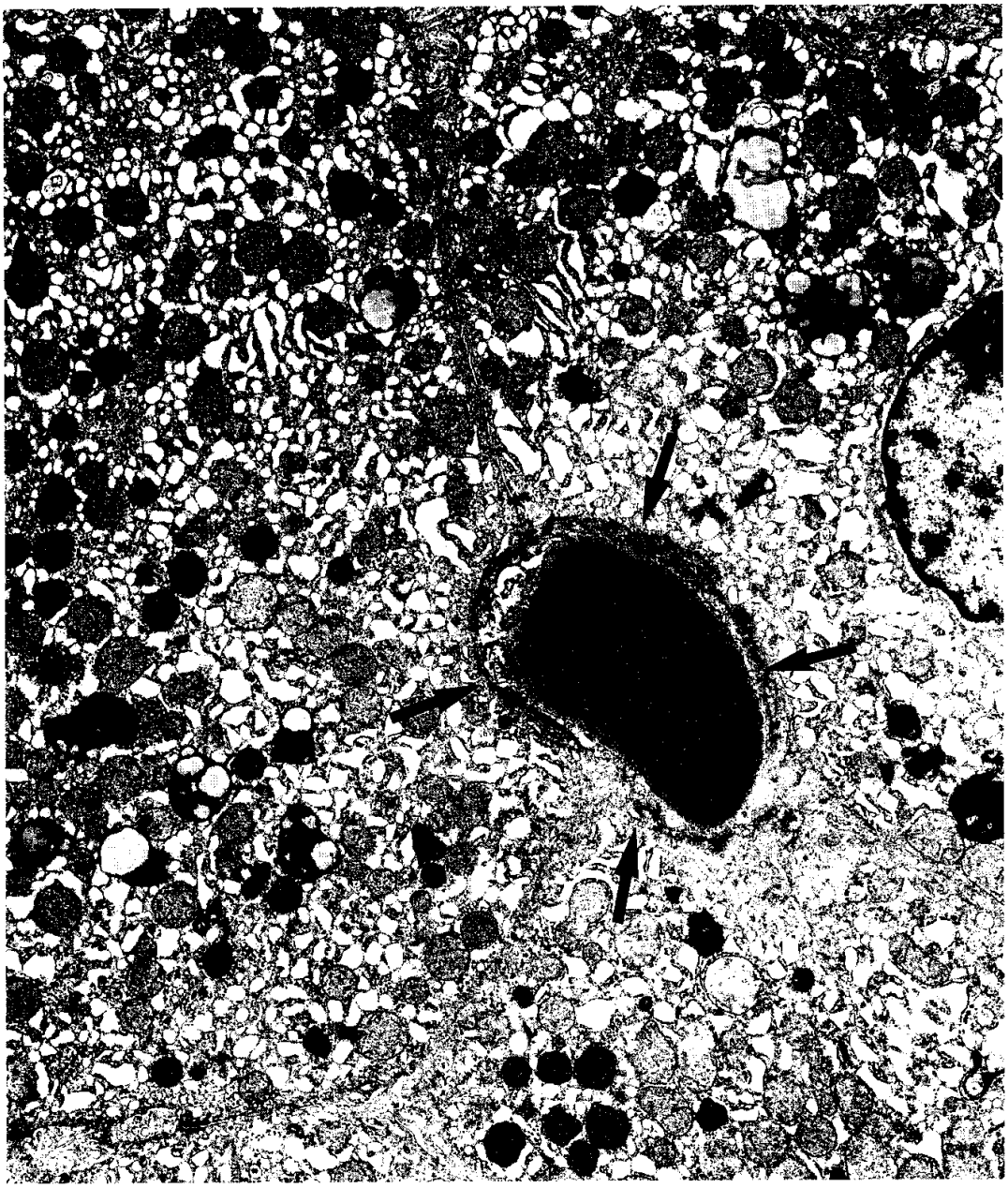

Fig. 4. Crigler-Najjar syndrome: Bile canaliculus is filled with amorphous homogeneously electron dense bile pigment and their microvilli shows degenerated and flattened apparance (arrows). Lipofuscin-like bile pigments are also seen in hepatocytic cytoplasm (Uranyl acetate & lead citrate, × 8,000).

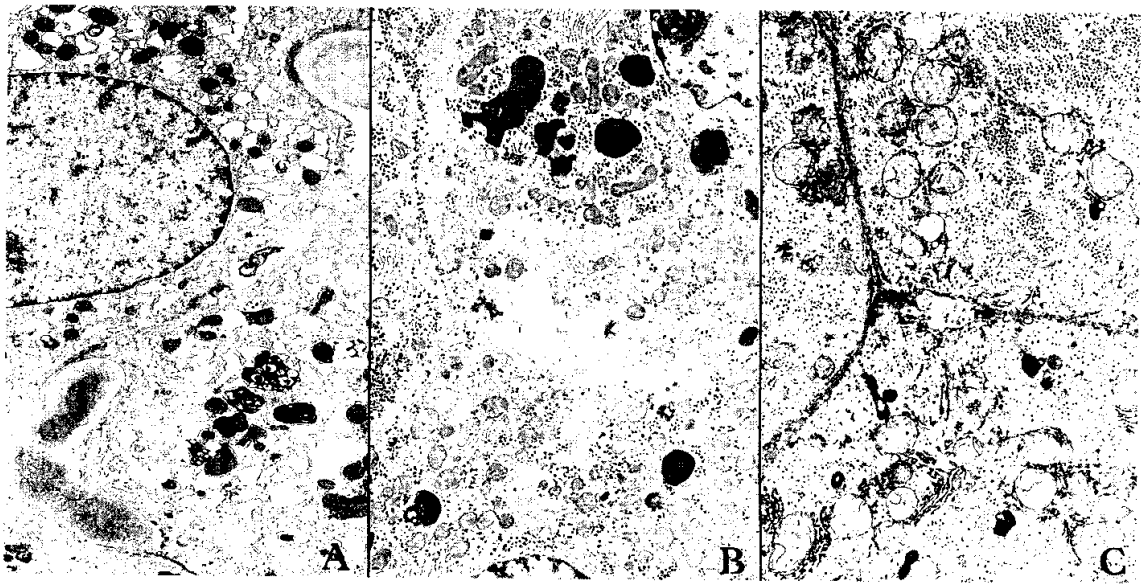

Fig. 5. A: Crigler-Najjar syndrome: Bile canaliculi are dilated and filled with amorphous electron dense bile pigment. B: Dubin-Johnson syndrome: Bile canaliculus shows roundly expanded lumen with reduced number and height of microvilli. C: Rotor syndrome: Bile canaliculi are small size with immature and simplified feature (Uranyl acetate & lead citrate, A, B & C: × 9,200).

Remaining organelles were unremarkable (Fig. 11).

Bilirubin is derived predominantly from heme metabolism. In humans, bilirubin is taken up by the liver, conjugated with glucuronic acid and then excreted into the bile (Rosenthal et al. 1987). Novikoff and Essner (1960) reported that uptake of material into hepatocyte occurs at hepatocyte membrane on the side of space of Disse and excretion of material is at the bile canaliculi.

On the pathogenesis of congenital/familial hyperbilirubinemia syndromes, there are several hyposthesis. Gilbert syndrome is characterized by only slight or inconstant unconjugated hyperbilirubinemia and light micoscopically, liver biopsy reveals normal histology. Black and Billing (1969) and Felscher et al. (1973) revealed decreased glucuronyl transferase after measurement of hepatic glucuronyl transferase. However, they described that there was no relationship between the level of measured glucuronyl transferase and the level of serum bilirubin and certain other factors might be operative. Arias et al. (1969) and Arias (1961) also described that hepatic cytoplasmic protein Y and Z transport the indirect bilirubin and were related to bilirubin uptake and there were two types of Gilbert's syndrome, one with and the other without defect of glucuronyl transferase. Recently, Watson and Gollan (1989) described that Gilbert syndrome is a part of a spectrum which includes the Crigler-Najjar syndromes. Molecular biology data suggested that there was an absence of one or even more glucuronyl transferase isoenzymes in these disorders. Based on ultrastructural study, Brown (1970) and Gentile et al. (1990) suggested disorder of hepatocytic uptake as an explanation for hyperbilirubinemia in Gilbert syndrome, while Barth et al. (1971) believed that disorder of lysosomal metabolism is the culprit based on the

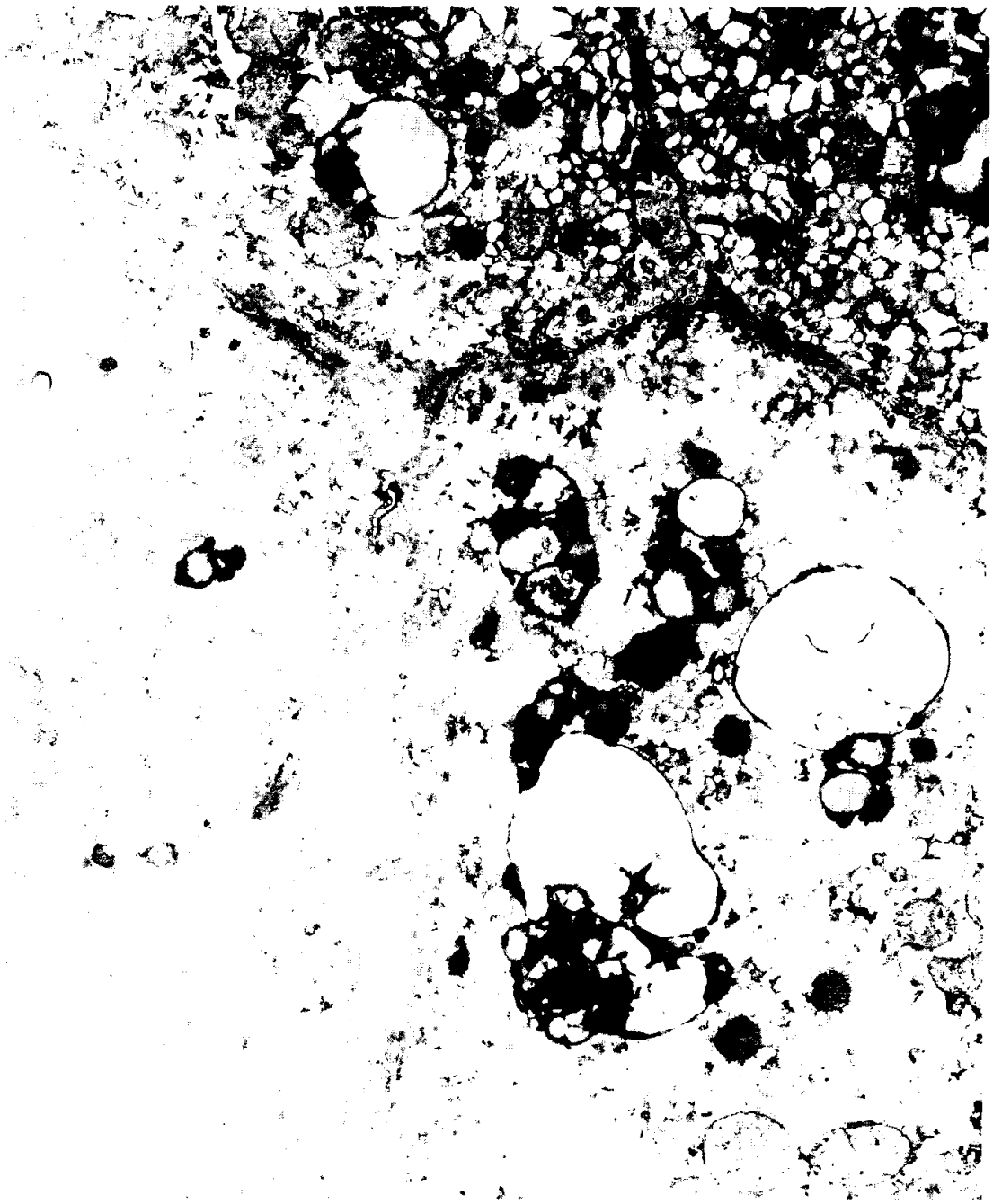

Fig. 6. Crigler-Najjar syndrome: Widening of intercellular spaces with increased microvilli on the larteral surface of hepatocytes are shown (Uranyl acetate & lead citrate. × 11,500)

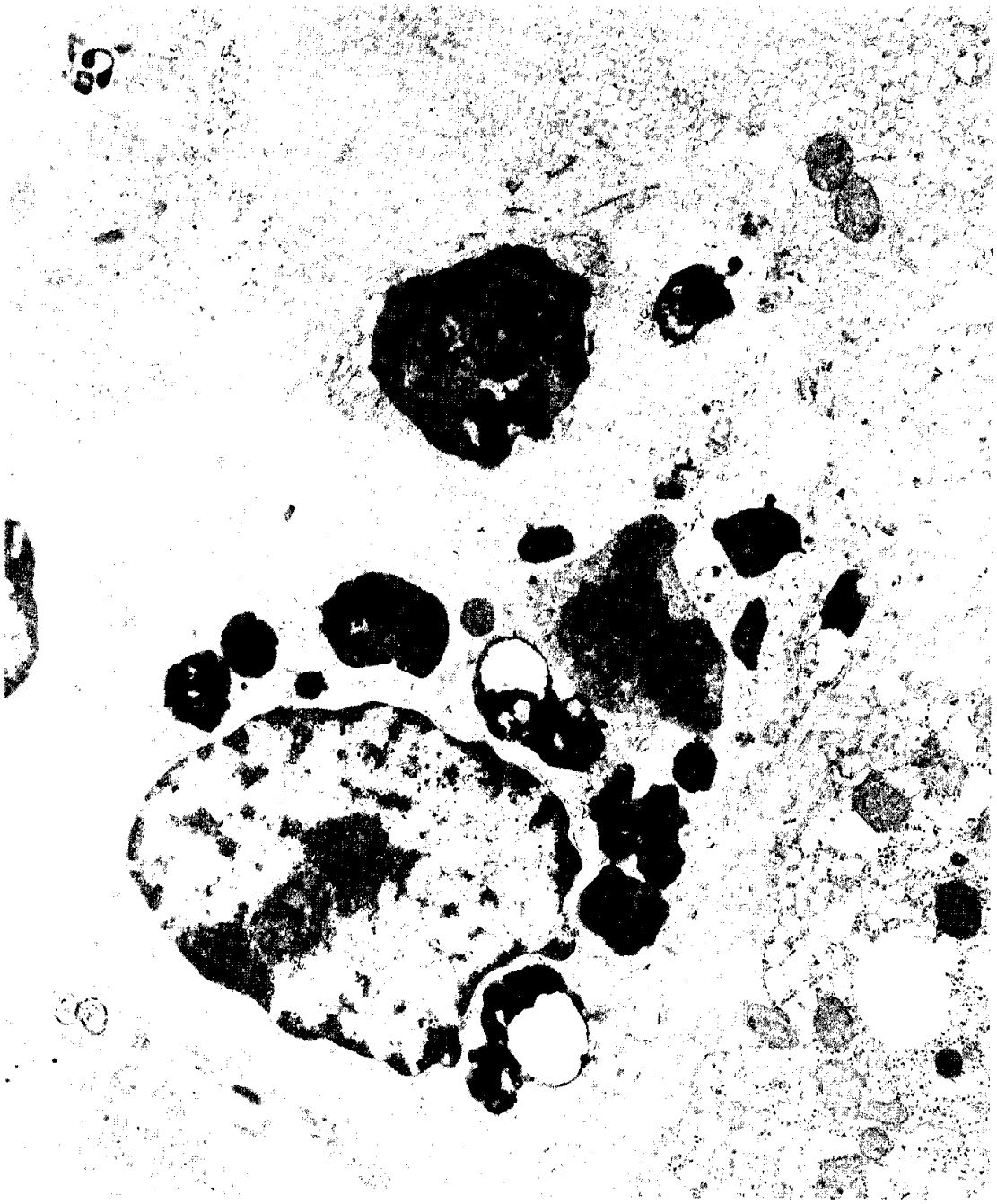

Fig. 7. Crigler-Najjar Syndrome: Presence of bile pigments in Kupffer cell cytoplasm are seen (Uranyl acetate & lead citrate, × 11,500)

Fig 8. Dubin-Johnson syndrome: Accumulated intrahepatocytic pigments are distributed in acinar zone 3 and positive for Fontana Masson stain (Fontana-Masson stain. × 100).

finding of lipofuscin accumulation. Barth et al. (1971) did not detect consistent ultrastructural alterations of the endoplasmic reticulum, mitochondria, bile canaliculi, sinusoidal pores or glycogen content. The difference of opinion proposed by Brown, Gentile and Barth might be explained by the fact that they are working on different case types. McGee et al. (1975) presented that ultrastructurally, their 8 cases with Gilbert's syndrome revealed gross hypertrophy of hepatocyte agranular endoplasmic reticulum, without other significant abnormality suggesting impairment of microsomal enzyme activity controlling bilirubin conjugation within liver cells. However, June and Benjamin (1984) did not find hypertrophy of endoplasmic reticulum in their 9 cases of Gilbert's syndrome. Therefore, reduced activity of

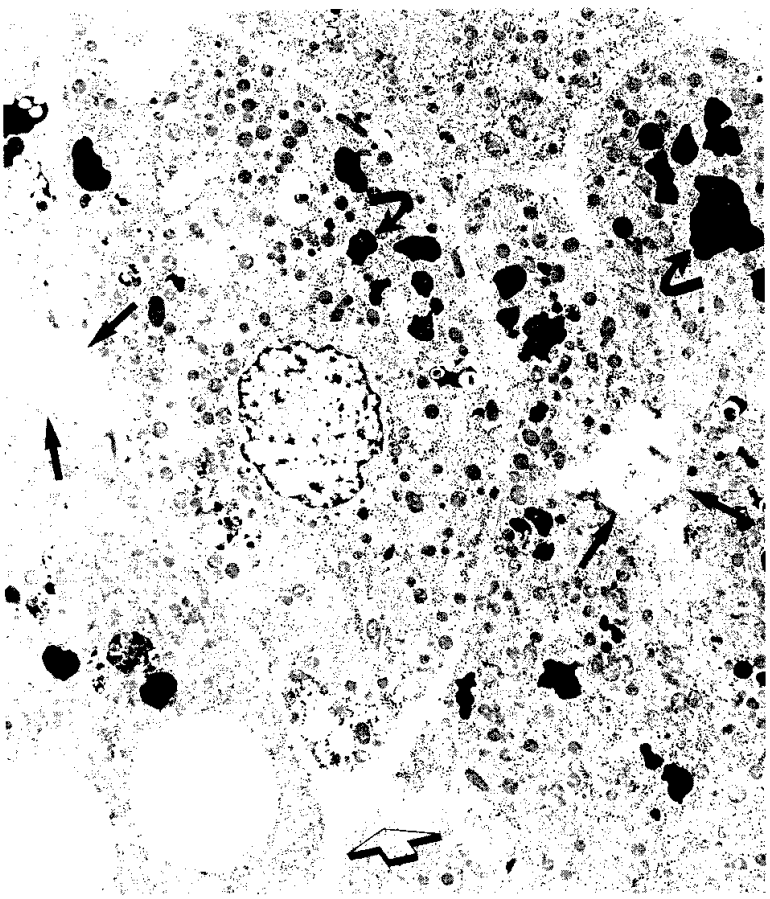

Fig. 9. Dubin-Johnson syndrome: Numerous characteristic granules are seen in virtually all hepatocytes (curved arrows). Bile canaliculi show roundly expanded lumina with reducted number of their mi-crovilli (arrows). In sinusoidal space, long and numerous hepatocytic microvilli and no collagen lay down are noted (open arrows) (Uranyl acetate & lead citrate, × 5,750)

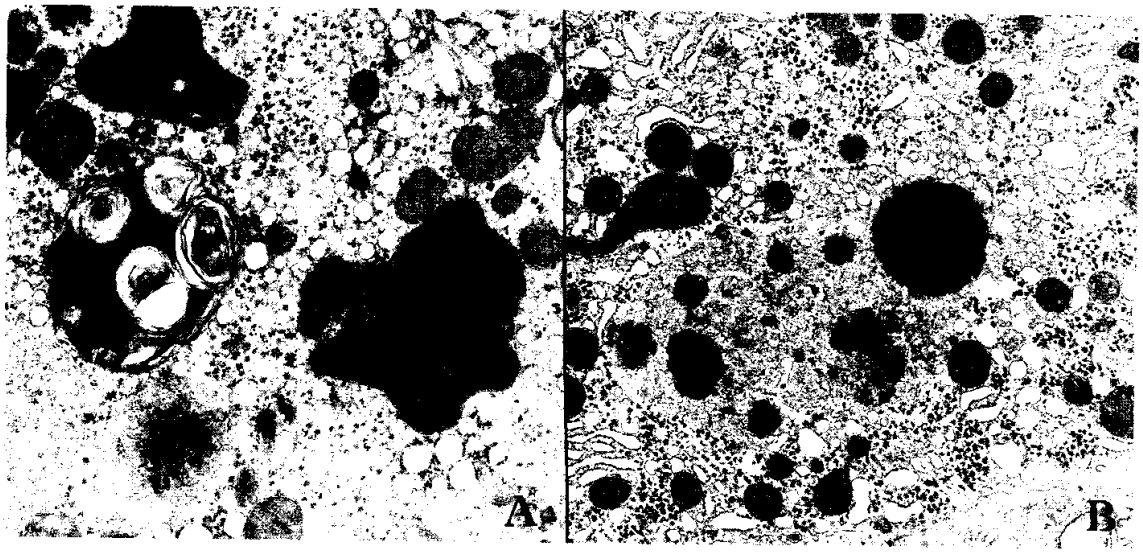

Fig. 10. Dubin-Johnson syndrome: The unique Dubin-Johnson type pigment bodies are membrane bound, round or oval to irregular shape and some of them shows scalloped surface (A). The content of these bodies shows a number of very dense and less dense fine granular appearance (B). The pigment bodies range from 700 to 5800 nm in diameter (Uranyl acetate & lead citrate, A: × 23,000, B: × 13,800).

bilirubin-uridine diphosphate glucuronyltransferase and disorders of hepatocytic uptake are considered as its pathogenesis.

Crigler Najjar syndrome has been thought to be due to a severe deficiency of the bilirubin conjugation enzyme, hepatic microsomal uridine diphosphatate glucuronosyltransterase (Chowdhury & Chowdhury; 1983, Schmid; 1978). Type I Crigler-Najjar syndrome is extremely rare and characterized by severe jaundice with kernicterus and death in infancy. Type II patients have lower serum bilirubin concentrations and survive into adulthood with no neurological damage. Type I appears to be transmitted as an autosomal recessive trait, however, the genetic background of type II disease is less well worked out but it is likely either an autosomal dominant trait or else inherited as two different abnormal genes (Sherker & Heathcote; 1987). In both types, light microscopically, liver biopsies reveal occasional bile plugs in an otherwise normal looking parenchyma.

Dubin-johnson syndrome is an uncommon but well described entity which manifests an autosomal recessive trait. The liver biopsy appears normal except dark, iron-free pigmentation, observed predominantly in zone 3 hepatocytes. Its pathogenesis is thought to be hepatic excretory defect and the unidentified brown granules in the liver cells is thus thought to be the result of hepatic excretory defect rather than its cause (Dubin; 1958, Dollinger & Brandborg; 1967, Ware et al.; 1972, Welan & Combes 1971).

Rotor syndrome is an autosomal-recessively inherited benign familial disorder resembling the Dubin-johnson syndrom (Rotor et al.; 1948, Blanmckaert & Schmid; 1982). Grossly and microscopically, the liver appears normal but its pathogenesis is thouht to involve both disorders in uptake and excretion of bile pigment (Miyakoda ; 1975).

There have been several reports on ultrastructural studies of liver with congenital/familial hyperbilirubinemia syndromes (Barth et al.; 1971,

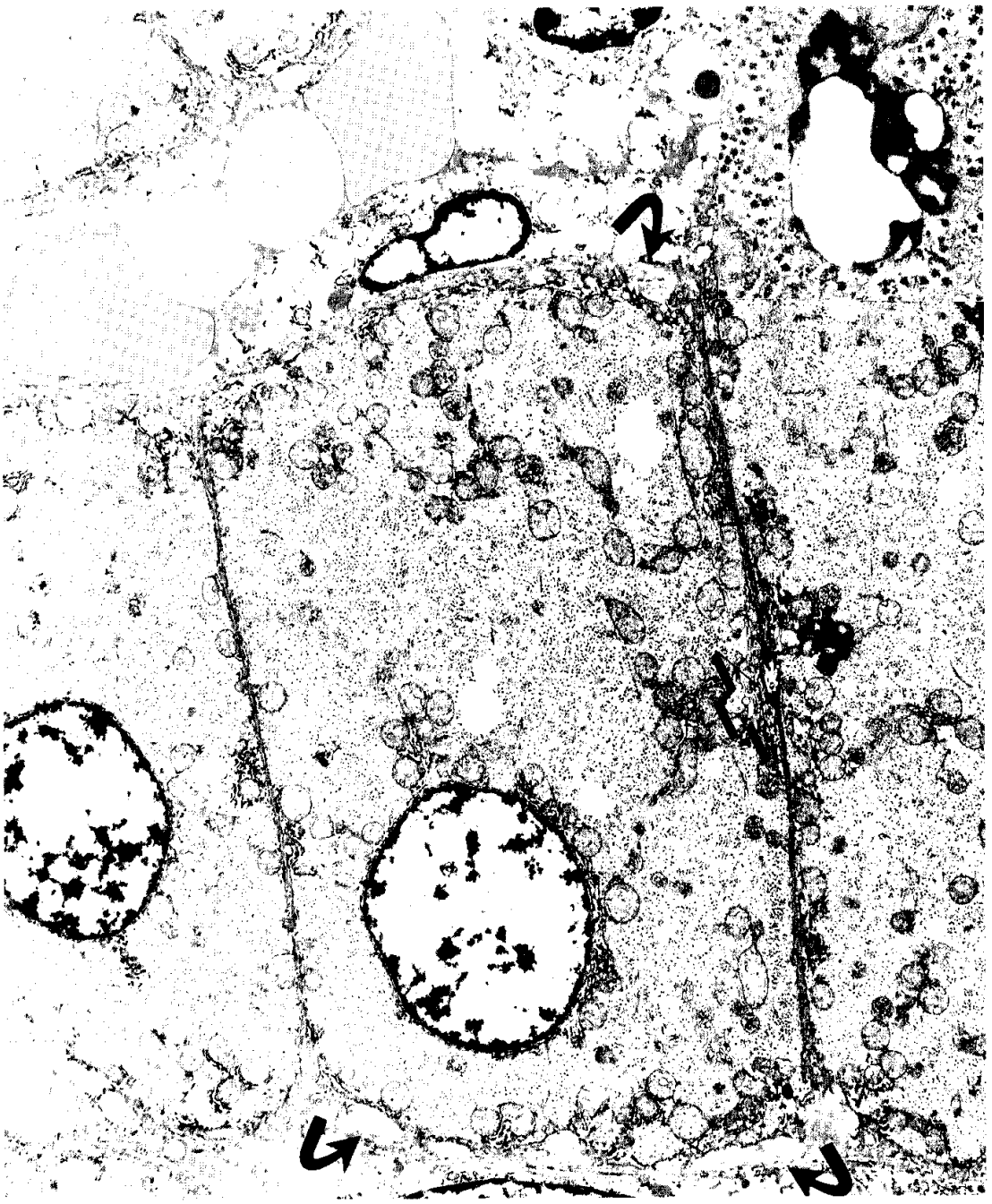

Fig. 11. Rotor syndrome: Hepatocytic sinusoidal membrane shows flattened and decreased nember of microvilli along with collagen lay down (curved arrows). Bile canaliculus is immature and simplified feature (open arrow). Variable sized liposfuscin granules are seen (inset). (uranyl acetate & lead citrate, × 5,750).

MacGee et al.: 1975, Miyakoda; 1975, Seymour et al.; 1977). Miyakoda (1975) reported hepatic ultrastructural morphometric analysis in 7 cases of Gilbert's syndromes, 7 cases of Dubin-Johnson syndromes and 3 cases of Rotor syndromes.

On ultrastructural study of our cases, liver with Gilbert syndrome revealed a decrease in number and flattening of microvilli along the sinusoidal membrane of hepatocytes, and collagen lay down in the space of Disse. These findings along with increased level of unconjugated bilirubin in the serum suggest disordered hepatocytic uptake rather than lysosomal abnormality. We found slightly increased number of smooth endoplasmic reticulum and this might indicate abnormality in bilirubin conjugation. As previous reports (Spronz & Nelson; 1954), the pigment seen in Gilbert and Rotor syndrome were typical lipofuscin granules composed of electron dense and less dense fine granular material with fat vacuoles. The excess accumulation of lipofuscins, which are products of degradation, may indicate that there is impaired elimination of accumulated lysosomes. There were no evidence of bile canalicular changes in our cases of Gilbert's syndrome, suggesting that there is no abnormality in bilirubin excretion.

Our case of Crigler-Najjar syndrome was in a 25 year old male and he had a persistent unconjugated hyperbilirubinemia (Total bilirubin/Direct bilirubin: 125.4/0.5 mg/dl) since his youth. He was diagnosed type II Crigler-Najjar syndrome. Ultrastructurally, there was definite intrahepatic and bile canalicular bile stasis, dilatation of bile canaliculi with increased number of microvilli, megamitochondria with paracrystalline inclusion bodies and widening of the interhepatocytic spaces with increased microvilli on non-sinusoidal surface of hepatocytes. Although all these findings are nonspecific and can be seen in the liver with non-familial hyperbilirubinemia, Crigler-Najjar syndrome can be diagnosed with these findings along with persistent unconjugated hyperbilirubinemia. Notably, we did not find any evidence of disorders of smooth endoplasmic reticulum. Koch et al (1978) also failed to confirm a hypertrophy of the smooth endoplasmic reticulum by electon microscopic study of the type II syndrome. Therefore, the pathogenesis cannot be analized by the ultrastructural features.

In our 3 cases of Dubin-Johnson syndrome, ultrastructural findings were identical and characterized by deposit of unique lysosomal bodies and changes of bile canaliculi (Table 2). The unique Dubin-Johnson pigments were mem-brane bound and shaped variably containing less electron-dense matrix with microparticles and larger dense granules. Morphologically, the pigments in Dubin-Johnson syndrome were different from lipofuscin pigments. The pigment bodies have the same biochemical characteristics as melanin pigment and some studies concluded that the pigment is of the melanin type (Wegman et al.; 1960), but later studies suggested a different chemical composition (Swartz et al.; 1979) The nature and origin of these bodies is still controversial. Since these bodies stained with acid phosphatase it is possible that pathogenesis of these bodies are originated from lysosome (Seymour et al.; 1977). We found no abnor-malities in mitochondria, Golgi apparatus, SER, and sinusoidal membrane of hepatocytes in our cases. Bile canaliculi show luminal dilatation which obliterated their microvilli. According to the morphometric analysis by Miyakoda (1975), the average luminal area of bile canaliculi was larger than those of normal liver, Gilbert's syndrome or Rotor's syndrome. This morphologic abnormalities of bile canaliculi suggest disorder of bile excretion. Clinically, defect in the hepatocellular secretion of conjugated bilirubin, bromosulphophthalein retention and nonvisualization of the gallbladder on oral cholecystography supported that idea. Ware et al. (1972) reported that no disorders were investigated in hepatocytic uptake and conjugation of bilirubin by their study of cases of Dubin-Johnson syndromes with 131I-BSP liver scintigram.

In our cases of Rotor syndrome, the characteristic ultrastructural findings were 1) decreased number and flattening of sinusoidal microvilli of hepatocytic membrane along with collagen lay down in space of Disse, 2) Megamitochondria with paracrystalline inclusion bodies, 3) simplified and immature bile canaliculi. Miyakoda (1975) observed the former two findings but bile canaliculi in their cases had dilated lumen with shorten-

ed microvilli. Therefore, we thought that both disorders of hepatocytic uptake and excretion of bile pigments are the pathogenesis of Rotor's syndrome based on the ultrastructural changes. Clinically, this pathogenesis was supported by BSP retention test and 131I-BSP liver scintigram. The meaning of megamitochondria seen in Gilbert, Crigler-Najjar and Rotor syndrome is not clear, but such mitochondria are indirectly related to the uptake of bilirubin in Gilbert and Rotor syndromes, since mitochondria are the site of energy production.

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