

Progressive intrahepatic cholestasis (Byler's disease): case report¹

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SUMMARY This paper reports the case of a child in which the clinical and laboratory data indicate a progressive intrahepatic cholestasis of the type described as Byler's disease. The histological and histochemical findings suggest an intrahepatic cholestasis. Electron microscopy reveals interruptions of the bile canalicular membrane, which have been described as characteristic of this disease. A striking feature in the present case is the remarkable increase of microfilamentous structures in the pericanalicular ectoplasm and in the hepatocytic cytoplasm. The findings suggest a primary disturbance in bile acid secretion as the cause of cholestasis, entailing a hypertrophy of pericanalicular microfilaments which supposedly play a role in the final step of biliary secretion.

In 1965, Clayton and his colleagues identified a disorder characterized by progressive intrahepatic cholestasis. Although eight out of 18 reported cases belong to the Amish kindred (Clayton *et al.*, 1969; Linarelli *et al.*, 1972) the affection seems not limited to one race or family (Gray and Saunders, 1966; Juberg *et al.*, 1966; Hirooka and Ohno, 1968; Williams *et al.*, 1972). The purpose of this paper is to report the clinical and morphological findings in a patient of Moroccan origin.

Case history

L.T., a girl of Moroccan origin, was first seen at the age of 7 months. The siblings, four girls and two boys, were clinically normal. The parents were consanguineous in the second degree. On admission, she weighed 5 kg 950 (< P3), her height was 64 cm (P3-P25) and head circumference 42 cm (P3-P25). The presenting symptom was constant scratching resulting in secondary infection and bleeding. Jaundice was inapparent, stools were intermittently loose and/or pale, urine was dark. Rachitic rosary

and craniotabes were present (Fig. 1). The liver was hard and palpable 2 cm below the costal margin. The spleen could not be felt. Over the two years of observation scratching and unexplained fever up to 38.39 °C were continuously noticed (Fig. 2). Subcutaneous haemorrhages with hypoprothrombin-aemia occurred at the age of 5 and 25 months respectively. Up to the age of 1 year total serum bilirubin remained below 1 mg % (Fig. 3). The stools were intermittently gray or white and the urine contained urobilinogen and on some occasions bilirubin. Thin layer chromatography of ethyl anthranilate azopigment extracts of the duodenal fluid showed presence of the β -compound and increase of the γ -fraction which is suggestive for cholestasis: α_0 13.1, α_1 1.0, α_2 0.9, α_3 3.3, β 6.1, γ 27.9 and δ 47.7 % (Fevry *et al.*, 1972). At the end of the first year, transaminases were moderately raised. Serum nucleotidase remained as low as 1.7-13 IU/l. At the age of 7½ months, bile salts in the serum and duodenal content were 82 μ g (normal value 1 μ g) and 150 μ g (normal value 3-5 mg) per ml respectively. On two more occasions, no bile salts were detectable in yellow stained duodenal fluid samples. The daily urinary excretion of bile salts was 24.7 mg of which chenodeoxycholic acid and cholic acid made up 12.6% and 87.4% respectively. No lithocholic acid could be detected. After the age of 1 year serum bile acids were studied only once and were 107 μ g/ml. The percentage of sulfobromo-

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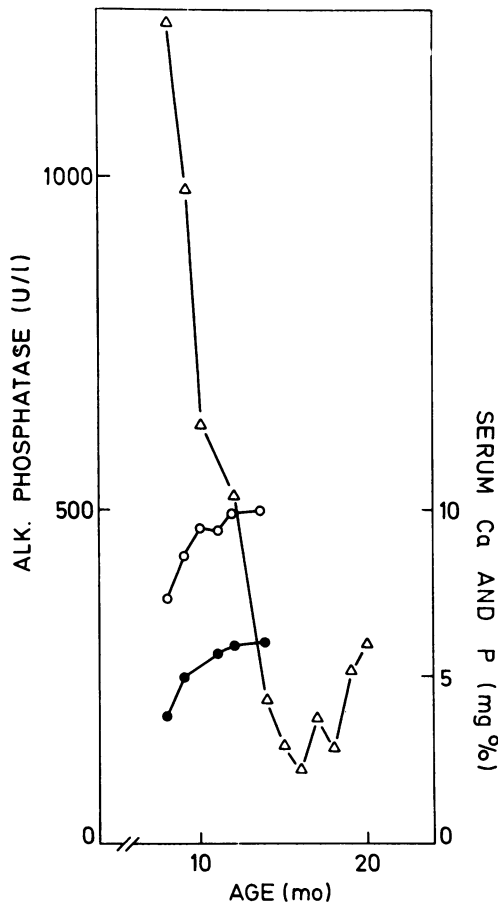


Fig. 1 On admission, the severe rickets was successfully treated with 10 000 IU vitamin D per day for two months. From the age of 9 months, maintenance therapy was 2 000 IU of vitamin D per day.

phthalein retained at 45 minutes was 33%. The ¹³¹I Rose Bengal excretion test showed normal liver captation (49%) and blood half life (17 minutes) but low faecal excretion of the isotope (11.8% in 72 hours). Cholecystography with the use of intravenously administered Biligraphin visualized neither gall bladder nor bile ducts. Infectious hepatitis could not be shown; HBsAg and Rubella antibodies were not detectable. When vitamin K was given orally, routine coagulation tests were normal. Blood ammonia was 1 µg/ml, α₁-anti-trypsin 275 mg %, and blood amino acids within normal limits.

Faecal fat excretion was 4.7 g/day (normal value less than 2.5 g/day). Stool cultures were repeatedly negative and parasites could not be identified. The duodenal fluid was sterile and free from parasites. Pancreatic enzymes in the duodenal content were normally active. Stereomicroscopic and histological examination of an intestinal biopsy specimen showed no lesions. The other laboratory data, including those on urine and renal function were normal. Figure 4 shows that the disease severely impaired growth and psychomotor development, although weight gain was favourably influenced by the administration of Vitamin C (500 mg/day), phenobarbitone (2 × 10 mg/day), and cholestyramine (4 × 1 g/day).

The needle biopsy liver specimen was divided into three parts. One fragment was fixed in Bouin's fixative for routine paraffin sections and staining (Pearse, 1960). A second fragment was frozen from which cryostat sections were cut for the histochemical detection of total and conjugated bilirubin (Hall, 1960; Desmet *et al.*, 1968). The third fragment of the liver specimen was fixed in a mixture of glutaraldehyde-formaldehyde (Töro and Joo, 1966) postfixed in osmium tetroxide (Millonig, 1961) dehydrated, and embedded in Epon (Luft, 1961).

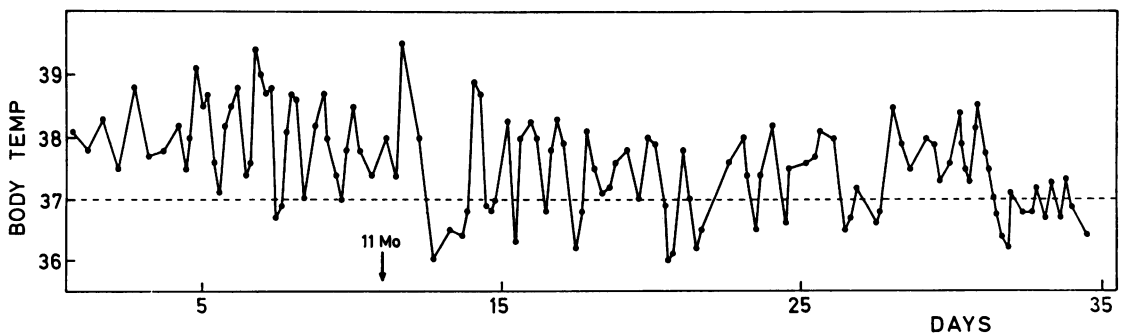


Fig. 2 Fever of unknown origin and with no reflection upon the general condition was observed over the course of several weeks.

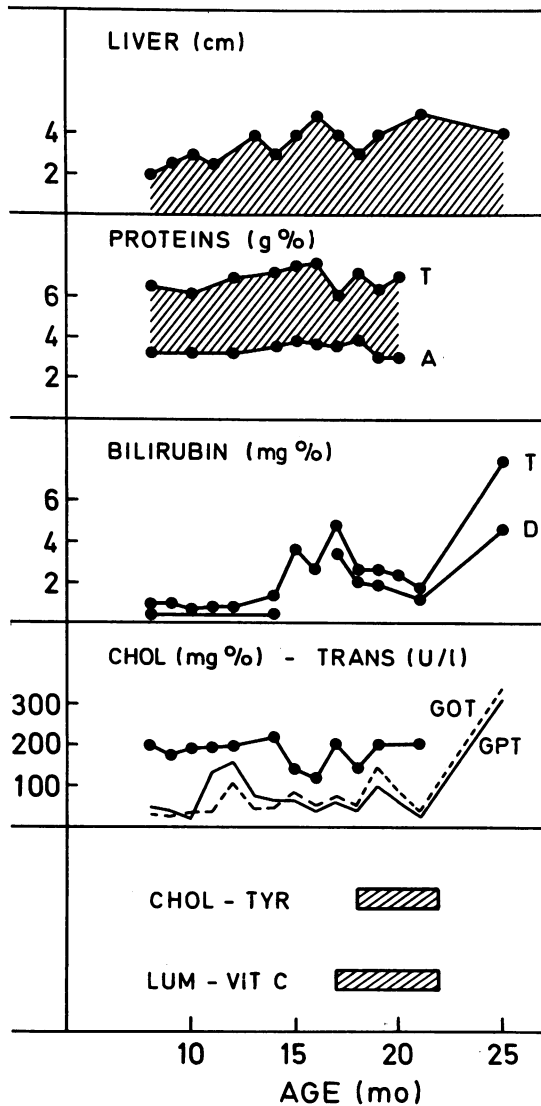


Fig. 3 Evolution of liver size, serum proteins (T) and albumin (A), serum bilirubin total (T) and direct (D), serum cholesterol (chol) and transaminases (GOT and GPT).

Ultra-thin sections were cut and stained for morphological study (Watson, 1958; Reynolds, 1963).

Results

OPTICAL MICROSCOPY

The lobular architecture was preserved. The portal tracts showed a slight periportal fibrosis and a moderate mononuclear cell infiltrate. There was slight proliferation of interlobular bile ducts. The

hepatocytes showed a normal appearance, with some anisokaryosis. Some groups of centrilobular liver cells occasionally showed tubular or acinar arrangement containing inspissated bile in the lumen. Intracellular bile granules were present in a few hepatocytes. Cryostat sections stained for bilirubin showed that most bile pigment was of the conjugated type.

ELECTRON MICROSCOPY

As with previously reported data (Clayton *et al.*, 1969; Linarelli *et al.*, 1972; Williams *et al.*, 1972), the most striking changes were found at the bile canaliculi. Most of them showed dilatation of the

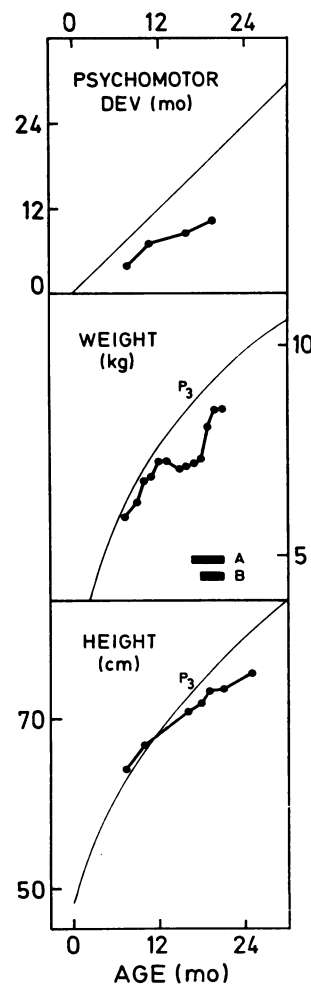


Fig. 4 Growth and psychomotor development. Treatment with phenobarbital and vitamin C had no almost no influence (A). The simultaneous administration of cholestyramine induced a net weight gain (B).

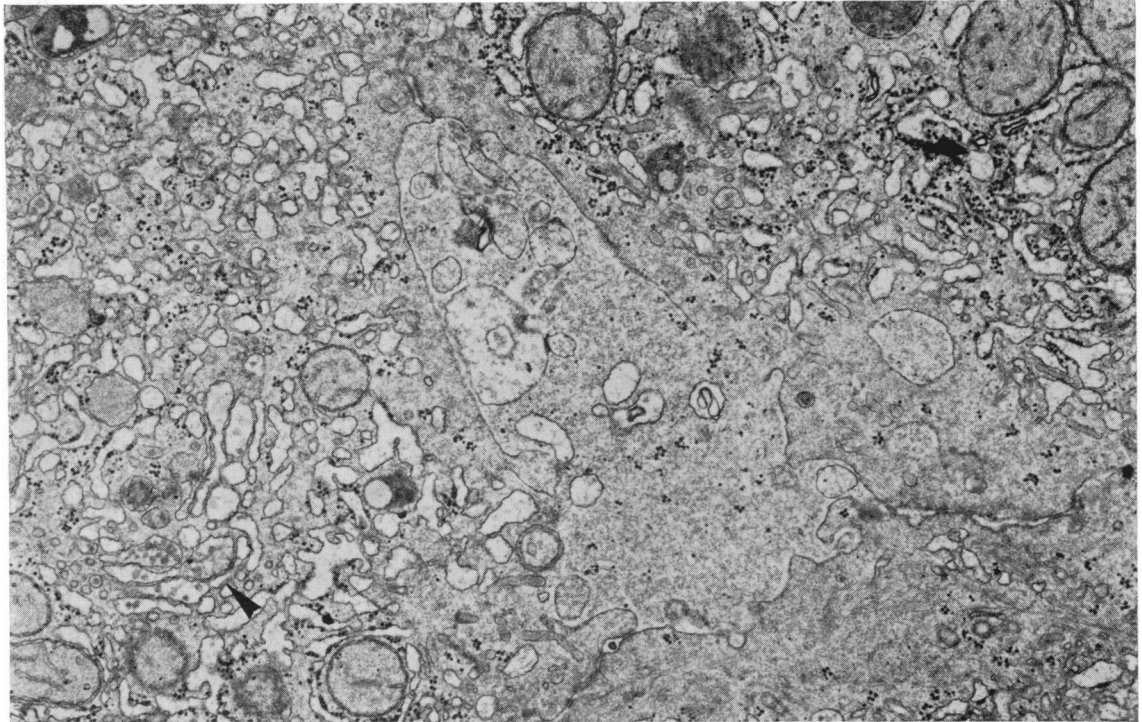


Fig. 5 Liver biopsy: thin section prepared for morphological study and stained with uranyl acetate and lead citrate. Liver canaliculus with focal interruptions of the limiting membrane and presence of cell organelles in the canalicular lumen. Note the prominent Golgi apparatus with VLD lipoproteins (▶). $\times 21\,900$.

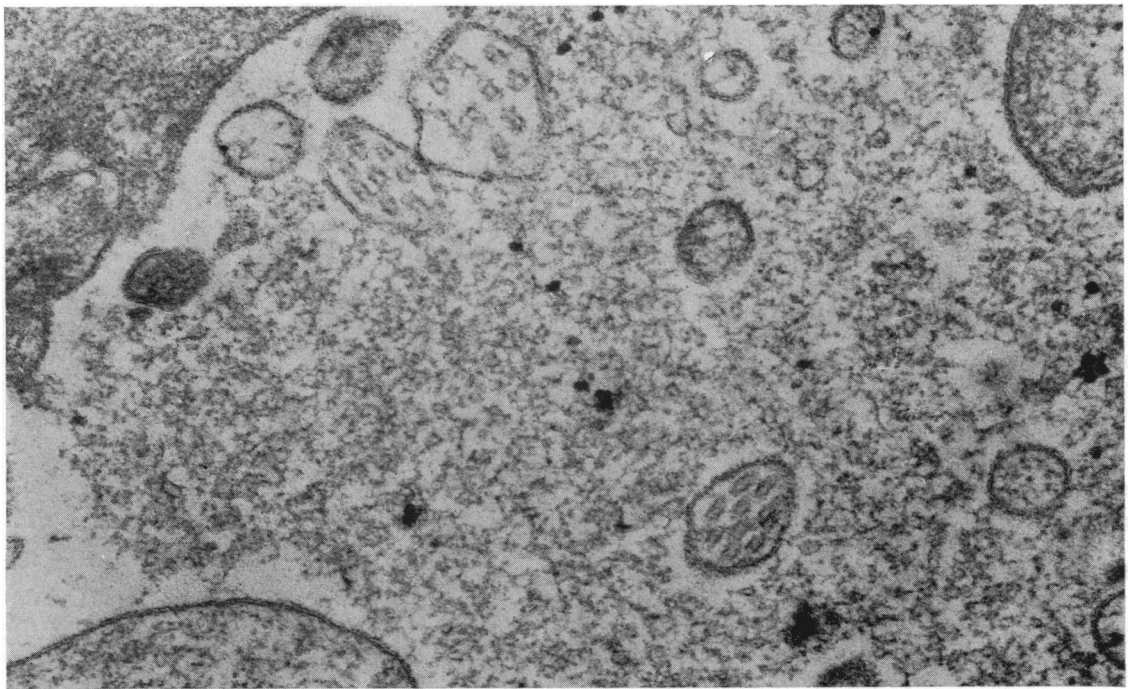


Fig. 6 Liver biopsy: thin section prepared for morphological study and stained with uranyl acetate and lead citrate. Part of a liver canaliculus with transsections of cilia-like microvilli in the lumen. $\times 76\,200$.

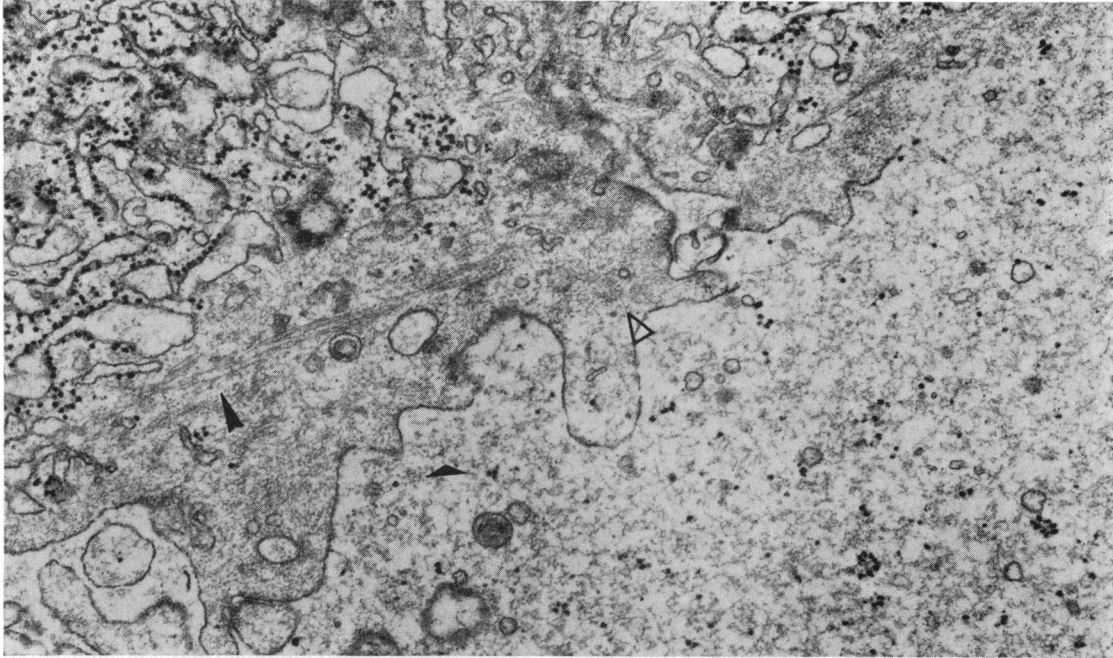


Fig. 7 Liver biopsy: thin section prepared for morphological study and stained with uranyl acetate and lead citrate. Part of a liver canaliculus with filaments in the ectoplasm (▶) and with fibrillar, granular material in the swollen microvilli (▷) and granular material in the canalicular lumen (◀). × 33 100.

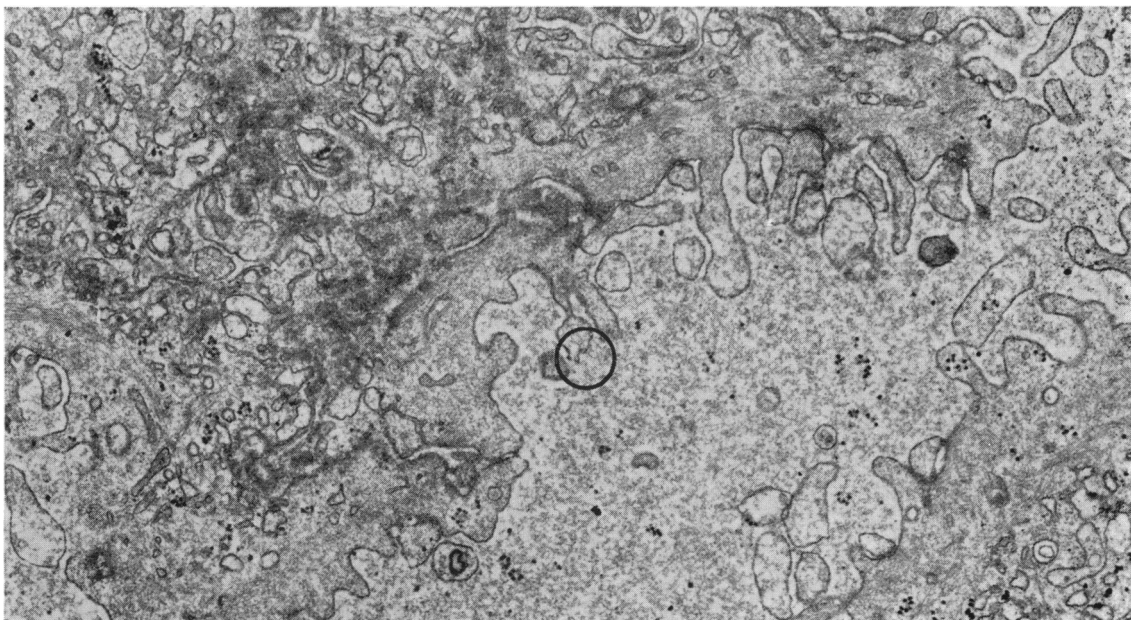


Fig. 8 Liver biopsy: thin section prepared for morphological study and stained with uranyl acetate and lead citrate. Part of a liver canaliculus with an interruption of the limiting membrane of a swollen microvillus with presence of apparently identical material inside the ruptured villus and in the canalicular lumen. × 24 600.

lumen and reduction in the number of microvilli, which often appeared swollen. Some canaliculi showed focal interruptions of the limiting membrane and presence of cell organelles in the canalicular lumen (Fig. 5). Besides the dilated canaliculi type 3, we also found canaliculi type 1 and type 2 with irregular lumen and few irregular microvilli as well as normal type 4 canaliculi (De Vos *et al.*, 1975). At the luminal border of a few canaliculi cilia-like microvilli were seen (Fig. 6).

Remarkable findings were filaments, of variable thickness—the majority measuring about 50 Å—in the normal microvilli and fibrillar and granular material in the swollen microvilli. Furthermore, there was a striking analogy between the material in the swollen microvilli and in the luminal content of dilated canaliculi (Fig. 7). On a few electron micrographs, an interruption of the limiting membrane of a swollen microvillus could be observed, with continuity of apparently identical material on both sides of the membrane (Fig. 8). Occasionally, a more electron dense amorphous or granular material of heterogeneous composition was found in the canalicular lumen. This finding was restricted to the lumen of the acinar/or tubular formations, and apparently corresponded to inspissated thrombi also observed in light microscopy. Numerous microfilamentous structures were present in the prominent and large pericanalicular ectoplasm. Their appearance was similar to the filaments described in the remaining normal microvilli. They were also observed more inside the vicinity of the Golgi apparatus.

The appearance of the other components of the hepatocyte—that is, the sinusoidal pole, the Golgi apparatus, the cytosomes, and the endoplasmic reticulum—was analogous to the data reported by Clayton *et al.*, 1969; Williams *et al.*, 1972; Linarelli *et al.*, 1972.

Discussion

The clinical and laboratory data suggest that the present patient is affected by progressive intrahepatic cholestasis. Loose stools, pruritus, and severe rickets were already observed from the age of 4 months. On admission, at the age of 7 months, the hepatic excretion of bile acids, sulfobromophthalein, I^{131} Rose Bengal and of adipiodon-methyl-glucamine (Biligraphin, Schering) was already impaired and preceded by several months the other liver symptoms as jaundice and increase of plasma transaminases. The condition of our patient is very similar to that of patients affected by the progressive cholestasis called Byler's disease. Consanguinity of

the parents and the finding that the youngest sibling, aged 3 months at the time of writing, has developed identical symptoms are further arguments for an inherited metabolic disorder. In some of the reported patients (Linarelli *et al.*, 1972; Williams *et al.*, 1972), high levels of lithocholic acid, the bacterial degradation product of chenodeoxycholic acid, were present in all body fluids tested. The absence of this compound in our patient is tentatively attributed to the pronounced block of bile salt excretion into the intestinal lumen. Recently, lithocholic acid could also not be detected in other patients with Byler's disease (Banfield *et al.*, 1974).

The light microscopic changes are indicative of intrahepatic cholestasis, whereas the electron microscopic observations are almost similar to those reported in any type of cholestasis (Popper and Schaffner, 1970; Desmet, 1972). On analogy with findings in adult rat liver during bile duct ligation (De Vos *et al.*, 1975) and in fetal rat liver (De Wolf-Peeters *et al.*, 1974), the presence of canaliculi type 1 and type 2 might be interpreted as newly formed secretory biliary poles next to dilated and functionally inactive canaliculi type 3.

The morphological findings that are considered to be characteristic for the Byler syndrome as reported by Clayton *et al.* (1969), Williams *et al.* (1972), Linarelli *et al.* (1972) have also been found in our patient. The interruption of the canalicular membrane may explain the appearance of cell organelles in the canalicular lumen.

Particularly striking in the present case were the very high number of microfilamentous structures in the pericanalicular ectoplasm and even in the cytoplasm surrounding the Golgi apparatus. We were not able to identify such large amounts of microfilaments in normal human or rat hepatocytes. Oda *et al.* (1974) described two types of 'filaments' in the pericanalicular web of the normal rat hepatocyte, wherein numerous such filaments were closely associated with intracytoplasmic vesicles. The authors suggest that these structures may play a role in the integrity of the canalicular wall, in the contraction and relaxation of the microvilli, and in the intracellular transport mechanisms in the pericanalicular region. This interpretation is also supported by data from the literature on the physiology and function of microfilaments and microtubules in the cytoplasm of Kupffer cells (Carr, 1972; Singh, 1974). Recently, attention was also paid to these cell organelles in the pancreatic islet β cells where they would play a role in the mobilisation of secretory granules (Malaisse *et al.*, 1974). The significance of the hypertrophy of this presumed transport system in our patient with Byler's disease is not clear. A detailed study of this system in

different types of human and experimental cholestasis is being carried out.

The finding in the present patient of material in the canalicular lumen which was morphologically identical with material found in the swollen microvilli together with ruptures of the canalicular membrane—even a rupture of the microvillar membrane (see Fig. 7)—is a new observation, apparently indicating a lesion of the structural counterpart of the final biliary excretory mechanism of the liver cell.

The presence of single cilia with a pattern different from the normal pattern which shows nine peripheral and two central fibres, on the canalicular membrane of the hepatocyte, has, to our knowledge, not yet been described. Several reports have dealt with the appearance of cilia in different kinds of tissues in birds and mammals (Scherft *et al.*, 1967). From these findings, it was concluded that every cell type has the potentiality to form cilia, although the significance of this phenomenon remains unclear. In liver tissue, cilia have been described in bile duct epithelium in man (Enzan *et al.*, 1974) and in rats, both in normal and pathological conditions (Grisham and Porta, 1963). The appearance of cilia has been interpreted as reflecting a regression of the cells towards a more primitive state. By analogy, the cilia observed in this study on the canalicular pole of the liver cell may suggest a cholangiocytic or biliary metaplasia of the hepatocyte.

The overall findings point to a disturbed excretory function of the liver cell which, in the light of the clinical data, should consist in a primary disturbance of the secretion of bile acids (Popper and Schaffner, 1970). If the presumed interrelationship between the hyperplasia of the microfilamentous structures in the liver of the present case and the excretory defect of bile acids is correct, this would suggest that the microfilaments which have also been shown to be present, although in smaller amounts, in normal liver cells play a role in the still unknown final mechanism(s) of biliary excretion.

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