Virchows Archiv A Pathol Anat (1989) 415:275–281

Virchows Archiv A
Pathological Anatomy and Histopathology

Fatal familial cholestatic syndrome in Greenland Eskimo children

A histomorphological analysis of 16 cases

K. Ornvold[1,2], I.-M. Nielsen[3], and H. Poulsen[1]

1 Department of Pathology, Hvidovre Hospital
2 Laboratory of Pediatric Pathology, Rigshospitalet, and
3 Department of Pediatrics, Rigshospitalet, University of Copenhagen, Denmark

Summary. We report the first detailed study of hepatic morphlogy in 28 biopsies from 16 Greenland Eskimo children with fatal familial cholestatic syndrome. The changes were categorized as early, intermediate and late. In the early stage, until 5 months of age, changes were restricted to zone 3, consisting of cholestasis and rosette formation without fibrosis. In the intermediate stage, from 5 to 14 months, cholestasis persisted and rosette formation increased, both with further extension into zone 2. Perisinusoidal fibrosis developed, first in zone 3 and later in zone 1. The late stage, from 17 to 60 months, showed a further increase in cholestasis and rosette formation, and fibrosis of zones 3 and 1 in nearly all biopsies. Portal to portal and portal to central fibrosis was evident with resulting cirrhosis in 2 of 7 patients. The morphological features can be summarized as pure cholestasis with prominent rosette formation followed by zone 3 fibrosis, zone 1 fibrosis, and, cirrhosis. Other characteristics are the virtual absence of inflammation and the lack of anatomical abnormalities such as paucity of bile ducts. The changes and their progression resemble those of Byler disease. Clinical and biochemical features are also largely similar, except for the presence of thrombocytosis in many of the Eskimo patients.

Key words: Intrahepatic cholestasis – Greenland – Eskimo – Cirrhosis

We have recently described the clinical aspects of the fatal familial cholestatic syndrome in Greenland Eskimo [13].

Offprint requests to: K. Ornvold, Department of Pathology, Hvidovre Hospital, DK-2650 Hvidovre, Denmark

Since 1970 we have seen 19 indigenous children with a syndrome leading to death in early childhood. From 1–3 months of age they presented with jaundice, bleeding episodes, steatorrhoea and pale stools. They failed to thrive, and hepatomegaly, pruritus and rickets developed. Biochemically, they showed hyperbilirubinaemia of conjugated type, profound hypoprothrombinaemia, thrombocytosis and secondary hyperparathyroidism. The aetiology of this syndrome is unknown and only symptomatic treatment is available. Ten children between $1^1/_2$ months and 3 years have died with haemorrhagic diathesis and infections. Nine children are alive, the oldest being 7 years. They are given a fat reduced diet (especially medium chain triglycerides) and supplementary vitamins K, D, E, A. Phenobarbital and cholestyramine are administered to relieve the itching. The pedigrees are compatible with autosomal recessive inheritance.

The purpose of the present paper is to give a detailed histomorphological analysis of the light microscopical hepatic changes in 16 children with this syndrome.

Material and methods

The material comprises 28 liver biopsies (20 needle and 8 surgical biopsies) from 16 patients. One patient from our first, clinical study [13] has been excluded because of inadequate histological material. Biopsies from three new cases, two of which are siblings to the original patients, have been included. Furthermore, additional biopsies from three of the patients already in the study have been included.

The biopsies were performed between the ages of 2 and 60 months. Two specimens were obtained post-mortem. During the course of the disease nine patients underwent biopsy twice, and in three a third biopsy was performed.

The following stains were applied: Haematoxylin & eosin (HE), van Gieson's picro fuchsin (VG), chromatotrope aniline blue (CAB), Gordon & Sweets' method for reticulin (GS), picro sirius (PS), periodic acid schiff reaction +/− diastase digestion,

orcein, ferricyanide reaction. Rhodamine staining for copper was performed when material was available [14].

The material was randomized and reviewed blindly by two of the authors (KO & HP).

A semiquantitative assessment (0: normal, 1: slight, 2: moderate, 3: severe) was attempted for each of the following parenchymal features: canalicular cholestasis, rosette formation, perisinusoidal fibrosis, perirosettal fibrosis, bridging fibrosis (portal-portal, porta-central), hepatocellular necrosis (focal, confluent), hepatocellular cytoplasmic content (bile, fat, iron, copper), giant cell formation of hepatocytes, inflammation, sinusoidal lining cell proliferation and lining cell accumulation of bile. Acinar zonal localization of these variables was determined. Portal tracts were evaluated in a similar fashion with regard to degree and type of inflammation, destruction of limiting plate, cholestasis and bile duct proliferation.

Results

The morphological changes seen showed a clear tendency to become more pronounced with age, allowing a chronological division into three stages: (*I*) Early changes (less than 5 months), (*II*) Intermediate changes (5 to 14 months), and (*III*) Late changes (above 17 months). The salient morphological features are summarized in Table I.

Table 1. Degree of cholestasis, rosette formation and fibrosis (0: normal; +: slight; ++: moderate; +++: severe) in 28 liver biopsies (16 patients) tabulated according to age

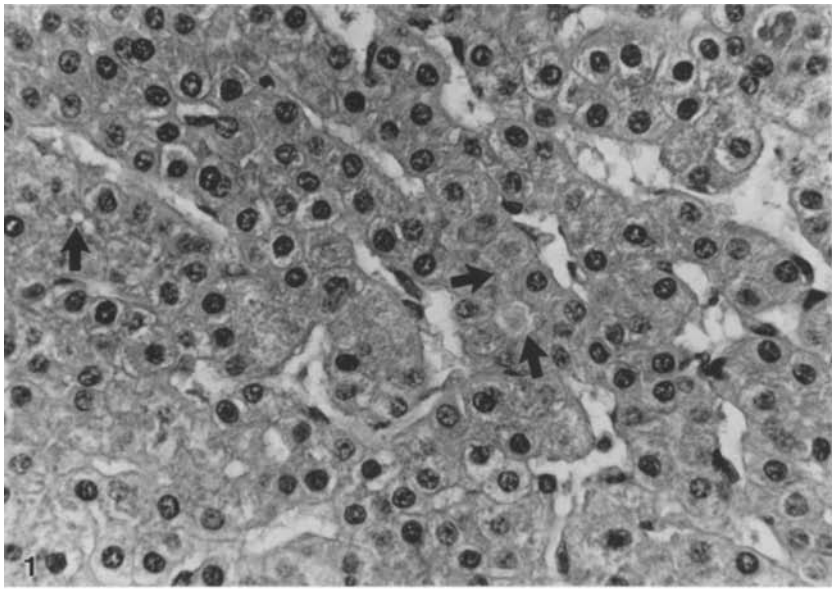
Biopsy no/ patient	Sex	Age of patient at the time of biopsy	Degree of cholestasis	Degree of rosette formation	Zone 3 fibrosis	Zone 1 fibrosis
1/PB	F	2 m	+	+	0	0
2/DB	M	$2^1/_3$ m	+	+	0	0
3/VM	F	3 m	++	+	0	0
4/AN	F	$3^1/_2$ m	+	+	0	0
5/FT	F	4 m	++	+	0	0
6/AP	M	4 m	+	+	0	0
7/KM	F	5 m	+	+	+	0
8/AP	M	6 m	++	++	+	0
9/AQI	F	6 m	++	++	0	0
10/KJ	F	$6^1/_2$ m	+	+	+	0
11/RM	F	$6^1/_2$ m	+	+	+	0
12/SI	F	8 m	++	++	+	0
13/KI	M	$8^3/_4$ m	++	++	+	+
14/FT	F	9 m	++	++	+	+
15/LM	M	10 m	+	+	+	0
16/KJ	F	$10^1/_2$ m	++	++	+	+
17/AQ	F	12 m	++	++	+	0
18/KMO	F	$12^1/_2$ m	++	++	+	0
19/SL	M	14 m	+	+	+	+
20/DB	M	17 m	++	+++	++	++
21/FT	F	21 m	+++	+++	++	++
22/KM	F	22 m	+++	+++	+	0
23/KMO	F	24 m	+++	+++	++	++
24/AQI	F	27 m	++	+++	+	++
25/SL	M	28 m	++	++	++	+
26/KM	F	35 m	++	+++	+++	++
27/SI	F	38 m	+++	+++	+	++
28/SL	M	60 m	+++	+++	+++	+++

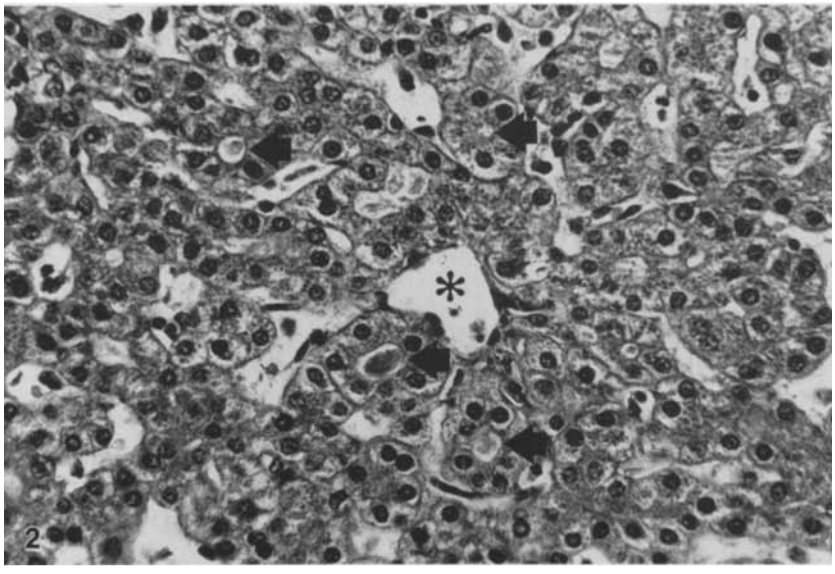
Six biopsies were examined from the period from 2 to 4 months, showing early changes. The normal infantile acinar architecture with double layered liver cell plates converging towards terminal hepatic veins (THV) was preserved. Cholestasis was demonstrable in four different manifestations: In bile canaliculi, in rosettes, in hepatocellular cytoplasm and in sinusoidal lining cells. The earliest detectable canalicular cholestasis was seen as a granular demarcation between adjacent liver cells with minimal dilatation of the canaliculi. Beyond this, an array of transitional changes was demonstrable in the Stage I biopsies; there were canaliculi with accumulations of bile communicating with fusiform or irregular ectasias. Often, these structures showed bulbous dilatation towards one end (Fig. 1). Occasionally, a transition could be demonstrated from the dilated canaliculi, still with the normal two layered architecture, to the complete rosette with several hepatocytes surrounding a dilated canaliculus containing a bile thrombus (Fig. 2). All six biopsies showed cholestasis of slight to moderate degree in the canaliculi and rosettes in the zone 3 areas. Cytoplasmic bile was present in the hepatocytes of some zone 3 areas in two biopsies. Occasional bile laden sinusoidal lining cells were noted in one biopsy.

Fibrosis judged by VG and PS staining was absent in Stage I. Focal necrosis of hepatocytes was present only to the slightest degree. Confluent necrosis was not seen. The iron content of the liver cells was within normal limits. Copper was present in slight to moderate amounts in the hepatocytes adjacent to the portal tracts in all six biopsies. Small fat vacuoles were present in the hepatocytes of one biopsy. Giant cell formation of hepatocytes was demonstrable to a slight degree in zone 3 areas in three biopsies. Inflammation was absent apart from very occasional neutrophils and lymphocytes in relation to focal necrosis. Moderate sinusoidal lining cell proliferation was present in 2 biopsies.

The portal tracts contained normal bile ducts, portal veins and hepatic arteries. Paucity of interlobular bile ducts was not seen. There was slight portal inflammation in one and moderate inflammation in two biopsies. The predominant cells were lymphocytes and macrophages. The limiting plate was intact and no ductal cholestasis or bile duct proliferation were observed.

Intermediate changes were seen in the period from 5 to 14 months in the 13 biopsies included. Normal hepatic architecture was preserved in all





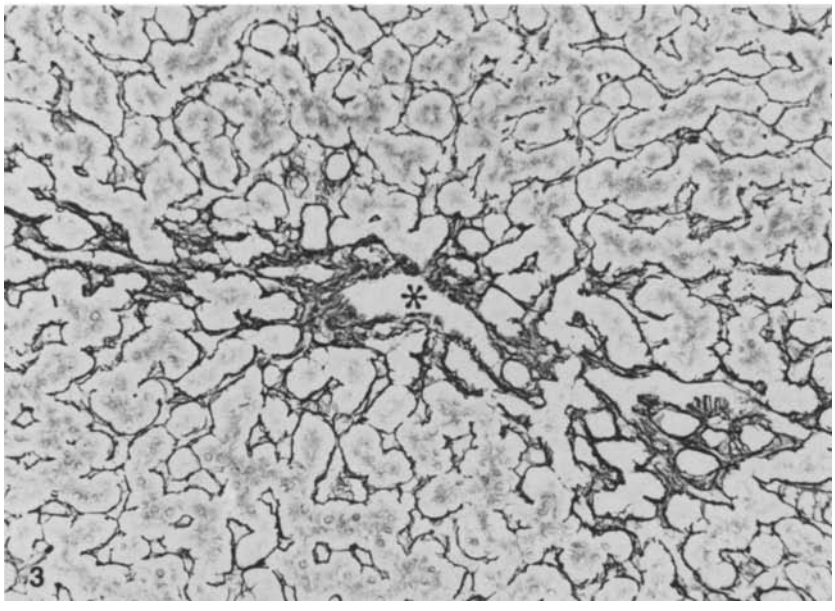
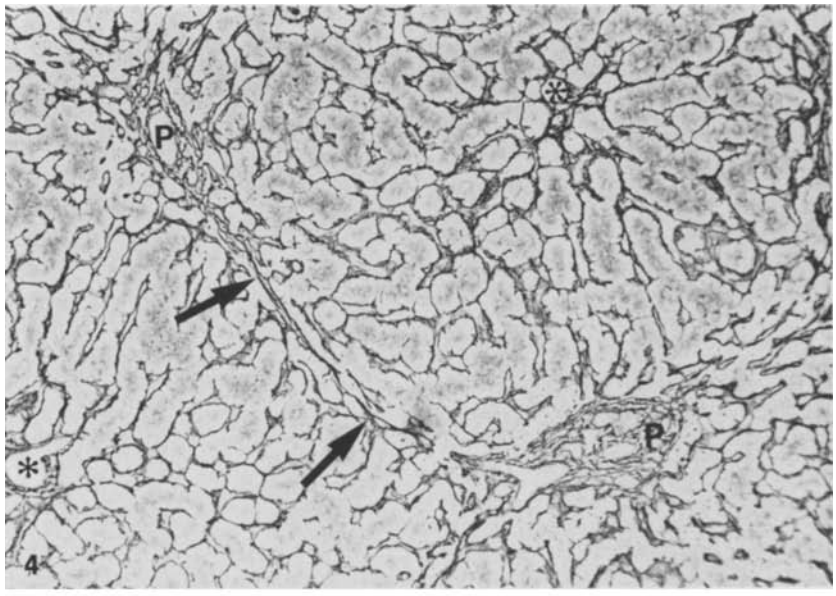
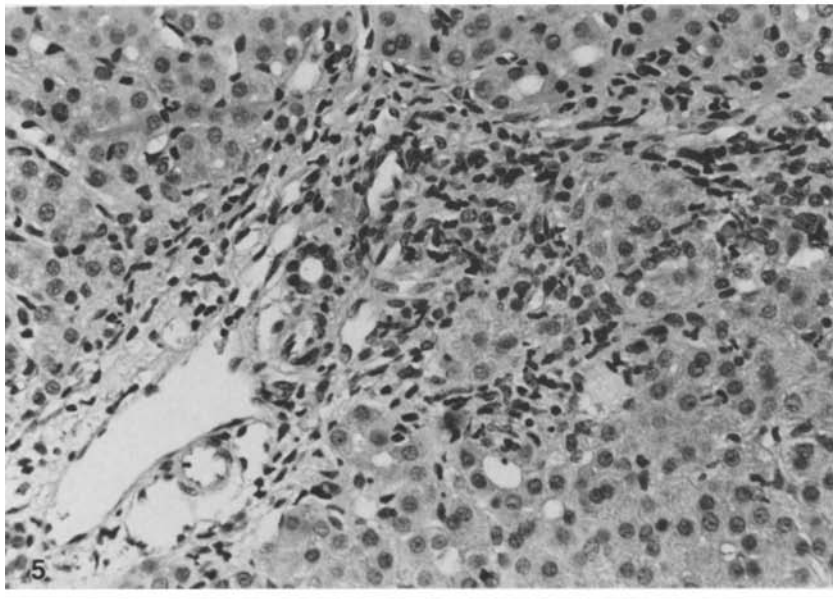


Fig. 1. Early changes. Liver biopsy (no. 5, 4 months) showing bulbous dilatation of bile canaliculi (*arrows*). H.E. original magnification: 400

Fig. 2. Early changes. Liver biopsy (no. 5, 4 months) with rosette formation of liver cell plates around dilated bile canaliculi (*arrows*) in zone 3 area adjacent to terminal hepatic vein (*asterisk*). CAB stain. Original magnification: 400

Fig. 3. Intermediate changes. Liver biopsy (no. 7, 5 months) with slight perisinusoidal and pericellular fibrosis in zone 3 around terminal hepatic vein (*asterisk*). GS stain. Original magnification: 250





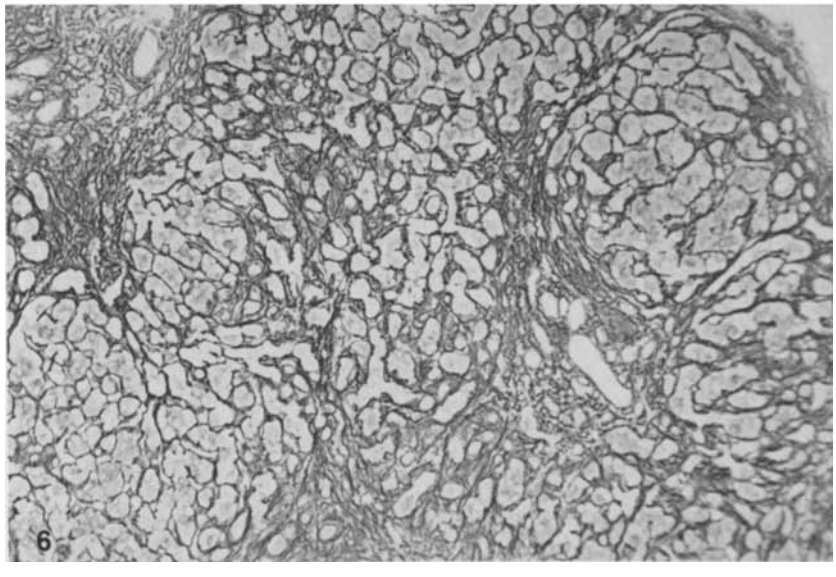


Fig. 4. Intermediate changes. Liver biopsy (no. 16, $10^1/_2$ months) showing zone 1 fibrosis with slender fibrous septa (*arrows*) bridging between portal tracts (P). Terminal hepatic veins in upper right and lower left corners (*asterisks*). GS stain. Original magnification: 250

Fig. 5. Late changes. Liver biopsy (no. 22, 22 months) showing irregular outline of the limiting membrane at the right hand side of the portal tract. H.E. Original magnification: 250

Fig. 6. Late changes. Liver biopsy (no. 23, 24 months) showing early cirrhosis with fibrosis and regenerative nodules. GS stain. Original magnification: 100

biopsies. The number of canalicular bile thrombi in Stage II did not differ notable from that of Stage I. However, it was obvious that the bile thrombi were not restricted to zone 3 but also showed variable extension into zone 2. The number of rosettes was larger in Stage II biopsies than in Stage I, and the rosettes were, like the cholestasis, extending into zone 2. Cytoplasmic bile in the hepatocytes or bile accumulation in the sinusoidal lining cells were not seen. Slight zone 3 fibrosis was present in 12 of the 13 biopsies. From 5 months of age a delicate mesh of perisinusoidal collagen was demonstrable, branching from the external border of the THV into the adjacent zone 3 parenchyma (Fig. 3). With increasing age there was a tendency for the collagen fibers to appear coarser. The rosettes, invariably present, were enveloped by the fibrous tissue except in two biopsies taken at $6^1/_2$ months in which the fibrosis was solely perisinusoidal.

As in Stage I only the slightest degree of focal hepatocellular necrosis was observed and confluent necrosis was absent. Periportal iron was present in minimal amounts in six biopsies. Copper was present in slight to moderate amounts in three of four examined biopsies. Fat vacuoles and giant cells were not seen.

Inflammation was absent apart from very occasional neutrophils and lymphocytes in the parenchyma. Sinusoidal lining cell proliferation was present to a slight degree in ten biopsies.

The bile ducts, veins and arteries of the portal tracts were normal as in Stage I. No paucity of bile ducts was noted. Portal inflammation with lymphocytes and histiocytes was slight in nine biopsies with an additional small component of neutrophils in one. The remaining four biopsies showed no portal inflammation. The limiting plate was intact in 11 biopsies and showed slight discontinuity in two. One biopsy showed slight cholestasis within the interlobular bile ducts, and in another there was slight marginal bile duct proliferation.

Slight zone 1 fibrosis was present from $8^3/_4$ months of age in four biopsies. In two of these, from $10^1/_2$ and $12^1/_2$ months, slender fibrous septa was demonstrable as portal to portal bridging fibrosis (Fig. 4). Portal to central fibrosis was not seen in Stage II biopsies.

Late changes were seen in the period from 17 months to 60 months where nine biopsies were included. The hepatic architecture was preserved in six and partially preserved in three biopsies. Cholestasis was severe in five and moderate in four biopsies. The degree of rosette formation was severe in eight, and moderate in one biopsy. Although present in a panacinar fashion these two features showed a tendency to diminish gradually towards zone 1. Zone 3 fibrosis was severe in two, moderate in four and slight in three biopsies and in all cases, was sinusoidal as well as perirosettal.

Focal hepatocellular necrosis was absent in two, slight in six and moderate in one biopsy. Confluent necrosis was absent. Cytoplasmic bile, iron or fat vacuoles were not demonstrated in the hepatocytes. Giant cells were not seen. Copper was present in slight amounts in one of three biopsies examined. Sinusoidal lining cell proliferation was demonstrable to a slight degree in all biopsies. Bile accumulation was seen in the sinusoidal lining cells of one biopsy.

The portal tracts showed slight inflammation with lymphocytes and histiocytes in all biopsies and additional neutrophils in one biopsy. The limiting plate was preserved in two biopsies and showed moderate destruction in five and slight destruction in two biopsies (Fig. 5). Slight diffuse bile duct proliferation was evident in six biopsies and absent in the remaining.

Zone 1 fibrosis was severe in one, moderate in six, slight in one and absent in one biopsy. Portal to portal bridging fibrosis was severe in one, moderate in four, slight in two and absent in two biopsies. Portal to central bridging fibrosis was moderate in three, slight in five and absent in one biopsy. Early cirrhosis was evident in two biopsies (Fig. 6). Cirrhosis was also suspected in a third biopsy due to severe fibrosis and partial loss of normal architecture.

When reviewing the material from the nine patients with multiple biopsies (patients DB, FT, AP, KM, QAI, KJ, SI, MOL, SL) it was evident (Table 1) that the individual patients showed progression of the morphological changes in each subsequent biopsy although the acceleration of the changes varied from patient to patient.

Discussion

The intrahepatic cholestatic syndromes of childhood are numerous and their aetiology varied. They can be divided into two major groups according to whether or not they possess an anatomically identifiable primary structural abnormality of the intrahepatic bile ducts. The first group with such abnormalities includes patients with paucity of interlobular bile ducts, originally described by Alagille and coworkers [2], and, patients within the spectrum of the ductal plate malformation [11].

Clearly, the absence of duct abnormalities in our patients exclude them from belonging to this group. The other major group, with no structural abnormalities of the ducts, is more heterogenous. Infections, primarily viral, are thought to be responsible for large subgroup under this heading. These patients are thought, initially, to have normal interlobular bile ducts. During the course either bile ductular hyperplasia or hypoplasia may develop. Rubella, coxsackie and cytomegalo virus are some of the etiological agents [8].

Metabolic errors such as galactosaemia, alpha-1-antitrypsin deficiency and tyrosinaemia constitute another subgroup where no anatomical abnormality of the ducts is evident [9].

The familial recurrent cholestasis reported in patients from the Faroe Islands by Summerskill [15] and Tygstrup [16] is a benign condition without accompanying fibrosis or cirrhosis. The cholestatic syndrome reported by Aagenæs et al. in Norwegian children [1] is also benign in its course with little or no fibrosis and cessation of the cholestatic episodes in early adulthood.

Neither the clinical [13] nor the morphological information about our patients indicate that they belong among any of these subgroups.

Byler disease was first described by Clayton et al. in 1965 [4]. A number of Byler-like conditions have been reported subsequently [3, 5, 6, 7, 10, 12, 18, 19]. Although there are minor clinical and morphological differences among these disease, they are all familial, chronic and progressive in their course. The common features are early onset, pure cholestasis, normal interlobular bile ducts and progressive fibrosis leading to cirrhosis during childhood or early adolescence. The mode of inheritance is autosomal recessive. The aetiology of these syndromes is uncertain. Abnormal bile metabolism [9] and ultrastructural abnormalities of pericanalicular ectoplasm [6, 18] have been implicated as possible explanations in some patients.

In the present report of fatal familial cholestatic syndrome in Greenland Eskimo children we found the hepatic morphological changes identical to those of the Byler-like conditions. The symptomatology is also similar except for the thrombocytosis seen in some of the Eskimo children, a feature not mentioned in other progressive familial cholestatic syndromes.

Because of the large number of biopsies in the present study, we were able to establish a chronology for the morphological events: In the early stage, the changes are exclusively parenchymal and restricted to zone 3. The cholestasis and rosettes observed in the biopsies from the first 4 months of life are not accompanied by any fibrosis. In the intermediate stage, from 5 to 14 months, perisinusoidal zone 3 fibrosis develops in nearly all biopsies.

In the late stage, above 17 months, fibrosis in both zone 3 and zone 1 is present in all biopsies except one. Furthermore, portal to portal and portal to central bridging fibrosis causes architectural irregularities in most biopsies, with cirrhosis in two of these.

The aetiology is unknown. The biopsy findings speak against inflammation, necrosis, or errors in iron or copper metabolism as causative factors in the development of cholestasis and fibrosis. Although the primary defect responsible for the initiation of the disease is unknown, a correlation between cholestasis and fibrosis does seem to exist in the present study, as the fibrosis appears only after cholestasis has persisted for a minimum of 5 months and the deposition of collagen fibers appears in close proximity to the bile containing rosettes.

The preliminary ultrastructural investigation of biopsies from three of our patients [13] have shown only nonspecific cholestatic features in contrast to the findings reported by De Vos et al. [6] and Weber et al. [18] concerning abnormal pericanalicular ectoplasm and possible microfilament dysfunction in Byler disease and in the severe familial cholestasis of North American Indians. Valencia-Mayoral et al. [17] have reported electron microscopic changes in Alagille's syndrome suggesting a block in the Golgi apparatus of the hepatocytes.

An ongoing, ultrastructural analysis currently involving approximately half of our patients may yield further information about the pathogenesis of the cholestatic syndrome in Eskimos. Bile acid analysis will also be carried out in an equal number of cases. Finally, DNA studies attempting to find the gene defect in patients, normal siblings and parents are in preparation.

References

1. Aagenæs O, Van der Hagen CB, Refsum S (1968) Hereditary recurrent intrahepatic cholestasis from birth. Arch Dis Child 43:647–657
2. Alagille D, Odievre M, GAutier M, Dommerguess JP (1975) Hepatic ductular hypoplasia associated with characteristic facies, vertebral malformations, retarded physical, mental and sexual development and cardiac murmur. J Pediatr 86:63–71
3. Ballow M, Margolis CZ, Schachtel B, Hsia YE (1973) Progressive familial intrahepatic cholestais. J Pediatr 51:998–1007
4. Clayton RJ, Iber FL, Ruebner BH, McKusick VA (1965) Byler's disease. Fatal familial intrahepatic cholestasis in an Amish kindred. J Pediatr 67:1025–1028

5. Clayton RJ, Iber FL, Ruebner BH, McKusick VA (1969) Byler disease. Am J Dis Child 117:112–124
6. De Vos R, De Wolf-Peeters C, Desmet V, Eggermont E, Van Acker K (1975) Progressive intrahepatic cholestasis (Byler's disease). Gut 16:943–950
7. Gray OP, Saunders RA (1966) Familial intrahepatic jaundice in infancy. Arch Dis Child 41:320–328
8. Heathcote J, Deodhar KP, Scheuer PJ, Sherlock S (1976) Intrahepatic cholestasis in childhood. N Engl J Med 295:801–805
9. Javitt NB (1976) Cholestasis in infancy. Gastroenterology 70:1172–1181
10. Juberg RC, Holland-Moritz RM, Henley KS, Gonzalez CF (1966) Familial intrahepatic cholestasis with mental and growth retardation. J Pediatr 38:819–836
11. Jorgensen MJ (1977) The ductal plate malformation. Acta Pathol Microbiol Scand [Suppl] 257:1–88
12. Linarelli LG, Williams CN, Philips MJ (1972) Byler's disease: Fatal intrahepatic cholestasis. J Pediatr 81:484–492
13. Nielsen I-M, Ornvold K, Jacobsen BB, Ranek L (1986) Fatal familial cholestatic syndrome in Greenland Eskimo children. Acta Paediatr Scand 1986; 75:1010–1016
14. Poulsen H, Christoffersen P (1979) Atlas of liver biopsies. Munksgaard, Copenhagen, pp 220–223
15. Summerskill WHJ, Walshe JM (1959) Benign recurrent intrahepatic "obstructive" jaundice. Lancet 2:686–690
16. Tygstrup N (1960) Intermittent possible intrahepatic cholestatic jaundice. Lancet 1:1171–1172
17. Valencia-Mayoral P, Weber J, Cutz E, Edwards VD, Phillips MJ (1984) Possible defect in the bile secretory apparatus in arteriohepatic dysplasia (Alagille's syndrome): A review with observations on the ultrastructure of liver. Hepatology 4:691–698
18. Weber AM, Tuchweber B, Yousef I (1981) Severe familial cholestasis in North American Indian children: A clinical model for microfilament dysfunction? Gastroenterology 81:653–662
19. Williams CM, Kaye R, Baker L, Hurwitz R, Senior JR (1972) Progressive familial cholestatic cirrhosis and bile acid metabolism. J Pediatr 81:493–500

Received March 28, 1989 / Accepted April 28, 1989