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Fatal copper storage disease of the liver in a German infant resembling Indian childhood cirrhosis

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Summary. A female child of non-consanguineous, healthy German parents fell ill at the age of 7 months with a progressive liver disease leading to irreversible hepatic failure 3 months later. Histological examination revealed severe liver cell necrosis, excessive Mallory body formation and veno-occlusive-like changes associated with massive storage of copper, similar to Indian childhood cirrhosis (ICC). Chronic copper contamination of drinking water was the only detectable aetiological factor. The study illustrates that ICC most probably is an environmental disease, also occurring outside the Indian subcontinent, and is likely to be underdiagnosed in the Western world.

Key words: Liver – Copper storage – Indian childhood cirrhosis

Introduction

Liver damage in early infancy encompasses a diverse group of disease entities of varying aetiologies and morphological characteristics (Thaler 1964; Hardwick and Dimmick 1976; Ishak and Sharp 1979; Mowat 1979). Among these, significant metal storage is a rather rare event. However, besides perinatal haemochromatosis (Goldfischer et al. 1981; Blisard and Bartow 1986), there are at least two other disorders showing severe disturbance of copper metabolism in early infancy: Menkes' disease (Danks 1983) and Indian childhood cirrhosis (Portmann et al. 1978; Tanner et al. 1978, 1979, 1983; Popper et al. 1979; Goldfischer et al. 1980; Bhandari et al. 1981; Marwaha et al. 1981; Mehrotra et al. 1981). The latter resembles Wilson's disease manifesting later in life especially with respect to copper storage, but differs among other features in that it is nearly always fatal. Indian childhood cirrhosis (ICC) is endemic throughout the Indian subcontinent (Nayak and Ramalingaswami 1975; Nayak 1979; Bhave et al. 1982). Recently it has also been described in an American sibship (Lefkowitch et al. 1982). Various studies assume that nutritional copper intoxication may be of aetiological importance in the development of the disease (Sharda and Bhandari 1980; Bhandari and Sharda 1982; Tanner et al. 1983). This paper reports the morphological findings resembling ICC in a German infant that died of liver failure as a result of toxic copper storage. The pathogenesis of the disorder is discussed with regard to the fact that the copper intoxication was caused by chronic contamination of drinking water. A similar case has previously been described (Walker-Smith and Blomfield 1973).

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Case report

K.S., a girl, the only child of healthy, non-consanguineous German parents, was delivered after an uneventful 38-week gestation and developed normally until the age of 7 months, when progressive enlargement of the abdomen and occasional vomiting was noted. Two months later, the clinical examination revealed severe hepatosplenomegaly and disturbance of liver function, especially elevation of transaminases and of bilirubin and a prolongation of prothrombin time; no firm diagnosis could be established. A liver biopsy (see below) disclosed severe copper storage disease, the copper content being 228 µg per gramme wet weight (normal 5 µg/g). The level of serum copper and ceruloplasmin were in a high normal range. At the age of 45 weeks the child died of progressive irreversible liver failure. Postmortem epidemiological investigations revealed that the drinking water which was obtained from a well via copper-containing pipes, was severly contaminated by copper. The child was breast fed only for a short period of 5 weeks. Further clinical details and results of laboratory investigations are to be published elsewhere (Weiß et al. 1987).

Material and methods

Postmortem tissues were fixed in 10% formol solution embedded in paraffin and examined with haematoxylin-eosin, PAS with diastase digestion, van Gieson, Prussian-blue iron stain, Ladewig stain for alcoholic hyalin, orcein and rhodanine stain for copper binding protein and for copper ion respectively.

Quantitative assays for copper were performed on frozen stored or formaline-fixed unembedded tissues by ICP-emission spectroscopy (Schramel et al. 1982).

Enzymehistochemical studies included the demonstration of acid phosphatase, Mg-ATPase (Müller-Höcker et al. 1984) without addition of an uncoupler for visualization of canalicular ATPase, and cytochrome-c-oxidase as previously described (Müller-Höcker et al. 1983) but without H_2O_2. In addition, the indirect immunoperoxidase technique was applied for the detection of hepatitis-B-antigens and for alpha-fetoprotein.

For electron microscopy the tissue was fixed in glutaraldehyde, (6.25% in phosphate buffer, pH 7.4) for two hours, washed for more than 24 h in 0.2 M buffered sucrose solution, postfixed with 2% osmic acid, embedded in epon and contrasted as usual with uranyl acetate and lead citrate.

Results

The surgical wedge liver specimen showed striking architectural distortion and liver cell damage on light microscopy. From the portal tracts a loose fibrous network encircling groups of hepatocytes extended into the liver parenchyma which was severly altered in the periportal region by piecemeal necrosis (Fig. 1a). Within the fibrous tissue neutrophilic and some eosinophilic granulocytes as well as histiocytic and lymphoid cells accumulated, spilling over into the lobules, especially near necrotic liver cells, which were widely distributed throughout the lobule. Most conspicuously, the hepatocytes showed severe ballooning of their cytoplasm; there was no storage of lipid or of glycogen, but abundant cytoplasmic hyalin, i.e. Mallory bodies were found in a panlobular distribution (Fig. 1a). Occasionally, mild intracellular cholestasis was present. The bile ducts, however, were essentially normal and free from bile plugs. In single central veins the intima was oedematous, but no veno-occlusive changes were detected. No regenerative nodules could be seen. There was no evidence of alpha$_1$-antitrypsin deficiency, of hepatitis B or of alpha-fetoprotein expression by immunohistochemical stains.

The iron stain was negative, but a light brown pigment could be observed in the cytoplasma of numerous hepatocytes, especially in the periportal region. This pigment stained positive on PAS with diastase pretreatment and above all with the rhodanine and orcein stain, indicating accumulation of copper and copper binding protein respectively (Fig. 1b).

Ultrastructurally the cytoplasmic hyalin in the liver cells was composed of typical randomly orientated intermediate filaments and of granular material (Fig. 1c, d). Less often a finger-print-like aspect could be discerned. Aggregates of microtubules could not be detected. There were numerous irregularly outlined electron-dense lysosomal bodies, apparently filled with storage material not typical of lipofuscin (Fig. 1c). The smooth and rough endoplasmatic reticulum was not markedly developed. The mitochondria varied somewhat in shape and size, but their finer structure seemed undisturbed.

A generalized icterus was noted at autopsy. Both the liver and spleen were severely enlarged and weighed 780 g and 200 g respectively (expected weights for age 240 and 25 g respectively). The liver was rather firm, its capsular surface was smooth and the cut surface did not show a nodular aspect. The gall bladder was devoid of bile fluid and the stool in the bowel was clay-coloured.

Light microscopy of the liver revealed in addition to the biopsy findings a severe progression of liver necrosis which involved nearly the whole parenchyma, leaving only small islands unaffected (Fig. 2a). There was only a weak inflammatory response and the necrobiotic hepatocytes filled with Mallory bodies persisted throughout the lobule (Fig. 2b, c). Liver cell regeneration was not observed, but occasionally multinucleated hepatocytes were found. The bile ducts showed marked proliferation and fibrosis was massive. Focally moderate canalicular cholestasis could be seen. Various central veins showed changes similar to veno-occlusive disease (Fig. 2a). The histochemically detectable copper was markedly increased and especially concentrated in necrotic liver cells. Copper-containing granules were also found in Kupffer cells and occasionally in fibroblasts. In the draining hepatic lymph nodes, single macrophages also stained positive for copper, whereas in the spleen and in various other organs, for instance the kidneys, the heart, skeletal muscle and brain the ion remained histochemically undetectable. Quantitative measurements revealed strikingly elevated levels of copper per gramme dry weight in the liver, in the kidneys and to a lesser degree in other organs (Table 1). Interestingly, the iron content was highly reduced in the liver, being 70 µg/g dry weight (normal 520 µg). Enzyme-histochemistry showed normal activity of cytochrome-c-oxidase in intact liver cells, in the skeletal muscle, the diaphragm and the heart. The activity of acid phosphatase was very high in the liver, whereas canalicular ATPase reacted very weakly.

Other findings included involution of the thy-

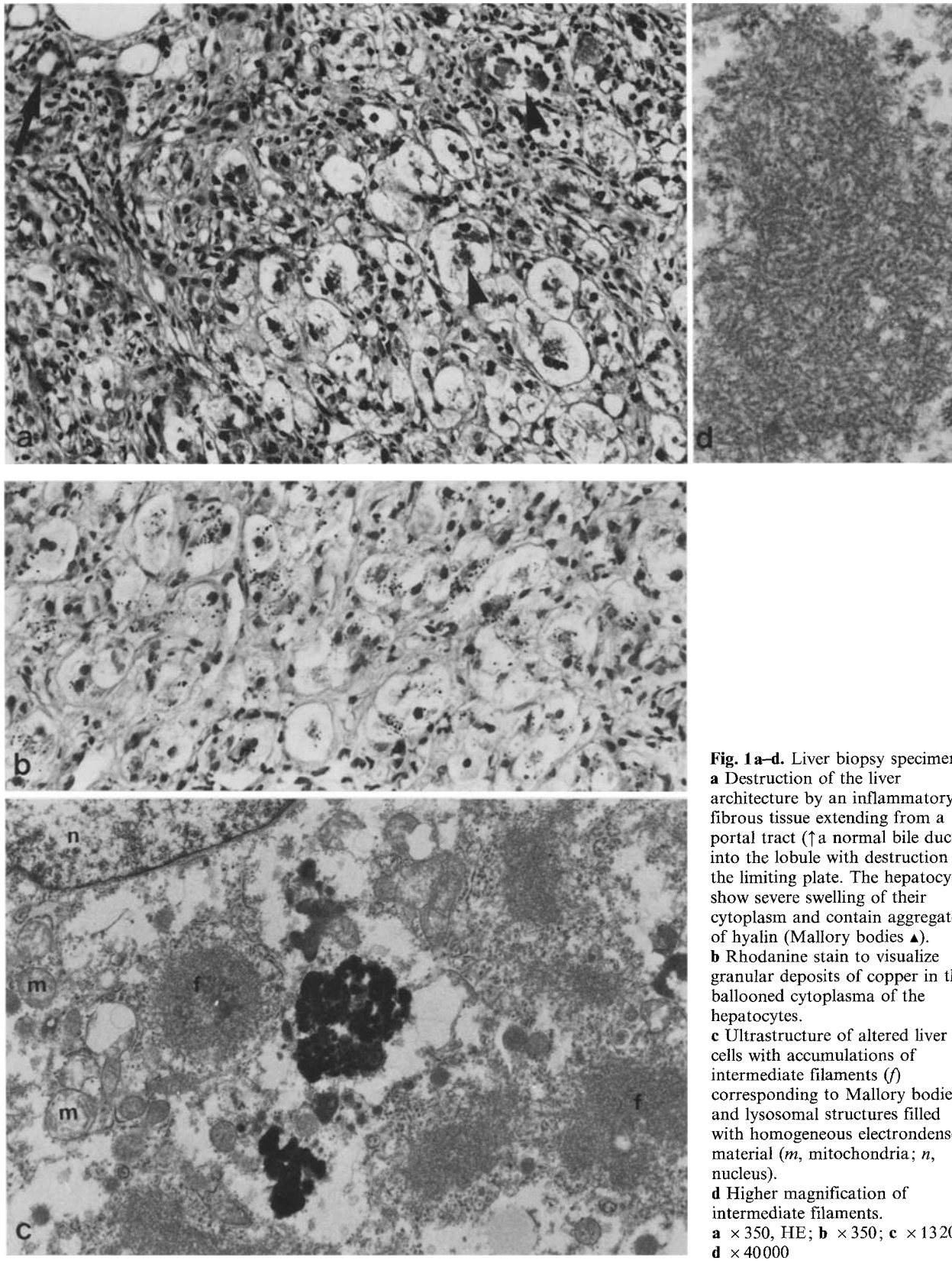

Fig. 1 a–d. Liver biopsy specimen.
a Destruction of the liver architecture by an inflammatory fibrous tissue extending from a portal tract (↑a normal bile duct) into the lobule with destruction of the limiting plate. The hepatocytes show severe swelling of their cytoplasm and contain aggregates of hyalin (Mallory bodies ▲).
b Rhodanine stain to visualize granular deposits of copper in the ballooned cytoplasma of the hepatocytes.
c Ultrastructure of altered liver cells with accumulations of intermediate filaments (*f*) corresponding to Mallory bodies, and lysosomal structures filled with homogeneous electrondense material (*m*, mitochondria; *n*, nucleus).
d Higher magnification of intermediate filaments.
a ×350, HE; **b** ×350; **c** ×13200; **d** ×40000

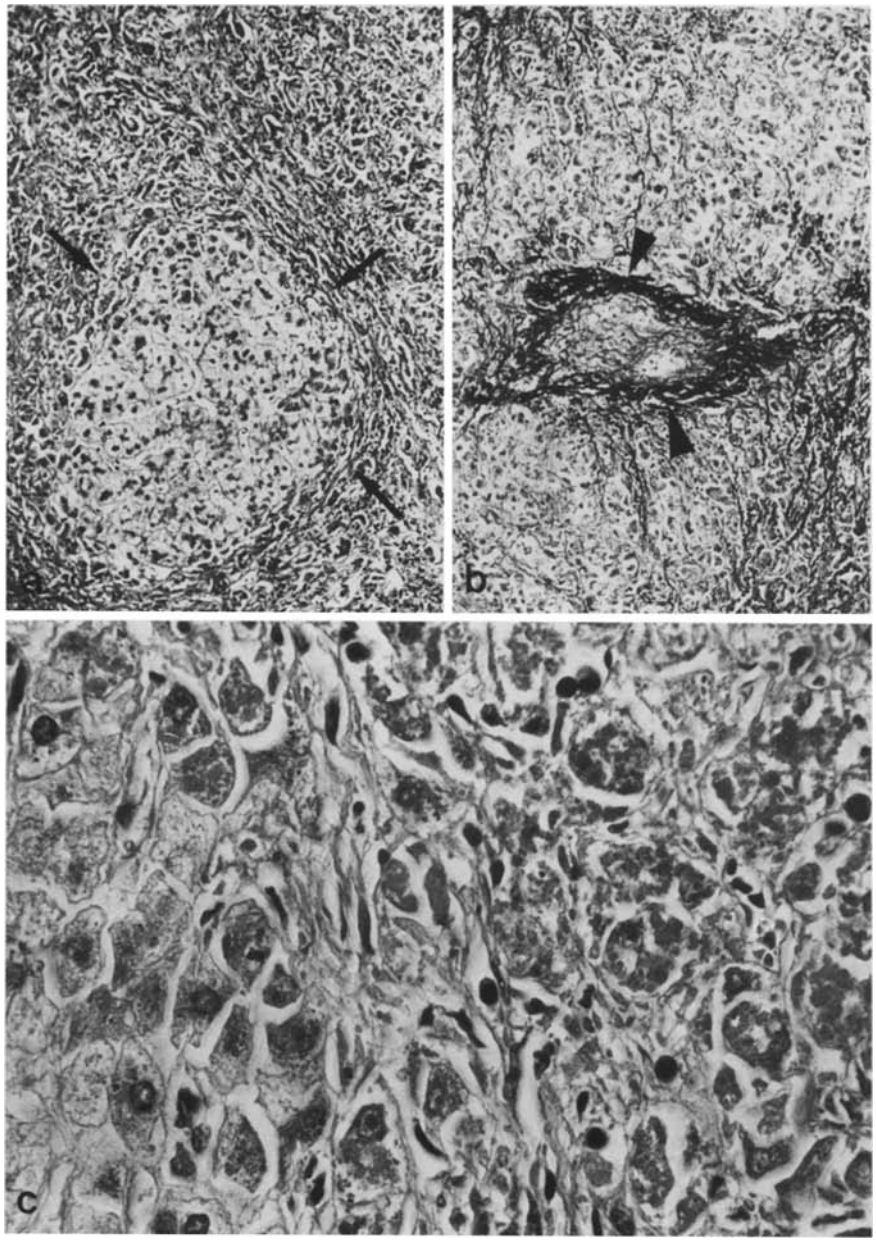

Fig. 2a–c. Postmortem liver specimen. **a** Panlobular liver cell necrosis with severe septal fibrosis and ductular proliferation. Only a small island of parenchyma is preserved (↑). **b** A central vein is severely narrowed (▲). **c** Higher magnification to show residual intact hepatocytes (*on the left*) and necrotic liver cells with numerous Mallory bodies (*on the right*). **a** ×350; **b** ×140, v. Gieson; **c** ×840, HE

mus, lymphocytic depletion of lymph nodes and of the spleen, which additionally showed chronic congestion, and extramedullar hematopoesis. In the bone marrow myelopoesis, granulopoesis in particular was moderately increased.

Discussion

The rapid course starting at the age of 7 months with insiduous abdominal distention, obviously caused by hepatosplenomegaly, and the culmination in fatal hepatic failure approximately 3 months later are the main clinical features of the child presented in this report.

Morphological and laboratory investigations revealed a severe copper storage disease of the liver. There was a panlobular ballooning of hepatocytes, without steatosis but with large amounts of Mallory bodies, combined with liver cell necrosis, severe fibrosis and moderate inflammatory reaction in the biopsy. Within one month panlobular persisting liver cell necrosis with a further increase in Mallory body formation and an absence of regeneration had developed in the autopsy specimen.

Table 1. Tissue copper levels (μg/g dry weight)[a]

	Liver	Kidney	Muscle	Brain	Adrenal	Heart	Pancreas	Spleen
Pat. K.S.	1485	355	46	42	65	39	73	13.9
2 controls	22	8.4	6.1	9.6	5.1	10.8	4.7	5.8
	41	13.1	7.8	8.8	13.2	14.8	23	7.9

[a] Formaline fixed unembedded tissue

Additionally, pronounced proliferation of bile ductules as well as veno-occlusive-like changes of central veins were present. Copper was stored not only in hepatocytes but also, to a lesser degree, in mesenchymal cells.

With regard to these alterations, secondary copper storage associated with biliary disorders (Reed et al. 1972; Jain et al. 1978; Nakanuma et al. 1979; Goldfischer et al. 1980; Sumithran and Looi 1985) could be dismissed. Furthermore, neither the early manifestation, the rapid and fatal course nor the pathomorphology are typical of Wilson's disease. In this autosomal recessive disorder liver symptoms have never been reported before the age of six years (Sternlieb 1980). In contrast to our case, serum copper and ceruloplasmin levels are characteristically low and urinary copper is increased (for review see Sternlieb 1980; Danks 1983; Epstein 1983; Walshe 1984; Winge 1984). Pathological examination in Wilson's disease shows, early in the course, fatty metamorphosis and nuclear glycogen accumulation, especially of periportal hepatocytes, which was lacking in our liver specimens. Later on, chronic active hepatitis with regeneration, eventually leading to micro-macronodular cirrhosis with periportal Mallory body formation may develop (Sternlieb and Scheinberg 1979a; Ishak and Sharp 1979).

However, Wilson's disease cannot be completely excluded as the disorder may pose diagnostic difficulties, particularly in children when typical clinical and laboratory parameters are lacking (Epstein 1983; Danks 1983) and where no radiocopper assay measuring the incorporation of copper into ceruloplasmin (Sternlieb and Scheinberg 1979b) has been performed. Notwithstanding this, both the clinical aspect and the peculiar pathomorphology of our case show typical features of another copper storage disease, namely Indian childhood cirrhosis (Nayak and Ramalingaswami 1975; Nayak 1979; Tanner and Portmann 1981). In Indian childhood cirrhosis (ICC), clinically overt liver disease manifests most often between 1 and 3 years of age and in most cases is fatal within one to eight months after onset. Familial occurrence is seen approximately in 30%, but definitive Mendelian pattern has not been established, however, although a genetic mode of transmission has been assumed (Lefkowitch et al. 1982). The highest copper levels known up to now in human liver disease (more than 6000 μg) have been measured in ICC (Bhave et al. 1982); single therapeutic trials with penicillamine were unsuccessful (Bhave et al. 1982).

Wilson's disease and ICC have a toxic accumulation of copper in common. The severity of liver cell necrosis combined with the absence of cell regeneration in our case, the amount of Mallory body formation, the bile duct proliferation and veno-occlusive-like-changes – the latter may also occur in Wilson's disease (Stromeyer et al. 1980) – reflect predominantly the severity of toxic liver cell damage by the accumulated copper ion. The exact mechanism of liver damage by copper is not known (see Evans 1973; Sternlieb 1980; Walshe 1984). Mallory body formation is probably the consequence of copper-induced impairment of microtubular function which leads to the accumulation of the prekeratin-like intermediate filaments in the Mallory bodies (see Popper et al. 1979).

In Wilson's disease copper accumulates in the liver in the first five years of life, reaching levels of approximately 3000 μg (Danks 1983) due to defective biliary excretion and insufficient incorporation of the metal into ceruloplasmin. Once saturation of the liver occurs, liver cell necrosis ensues and copper accumulates in other organs, especially the brain, the kidneys, cornea, the muscle etc. (Sternlieb and Scheinberg 1979a; Danks 1983; Epstein 1983).

Obviously, this process of saturation is accomplished far earlier in ICC than in Wilson's disease but also leads, as in our case, to copper storage in extrahepatic sites, especially in the kidneys and to a lesser degree in other organs.

The mechanism of copper accumulation and its pathophysical significance in ICC has been disputed (Sternlieb 1980; Mehrotra et al. 1981). How-

ever, various epidemiological studies indicate that in India the development of ICC is associated with the storage of milk in brass and copper household utensils (see Tanner et al. 1983).

In our case, a chronic nutritional copper overload by contamination of drinking water from the use of copper-containing pipes has been firmly established, the copper measuring 430 µg/l (normal value 10 µg). A similar case has previously been reported in an Australian boy (Walker-Smith and Blomfield 1973) the histology being indistinguishable from ICC (Portmann et al. 1980).

In this respect it is important to note that the parents of our child, although being exposed in the same way to an increased dietary load of copper, are clinically healthy (measurements of copper metabolism unfortunately could not be performed). Therefore susceptibility to exogenic copper intoxication and to the development of ICC would appear to be highest in early childhood.

Interestingly, even during normal development the copper content in the fetal and neonatal period is 6 to 10 times the normal value of the adult liver (normal value of the adult 5 µg/g wet weight 30 to 50 µg/g dry weight), which is reached only after 3 to 6 months (Brückmann and Zondek 1939; Emery and Hilton 1961; Goldfischer and Bernstein 1969; Reed et al. 1972; Evans 1973; Bloomer and Lee 1978; Goldfischer et al. 1980; Epstein 1983). The mechanism of this nontoxic physiological copper storage is not known, but immaturity of biliary excretion (Shenker et al. 1964) is likely to be at least partly responsible for it (Epstein and Sherlock 1981).

Concerning the development of ICC, a too-short period of breast feeeding with the early introduction of copper-contaminated food (Tanner et al. 1983; Bhandari and Sharda 1982) during the phase of physiologically impaired copper metabolism of the liver, appears to be an important individually predisposing factor, the more so as no other influences (especially metabolic influences) have yet been detected.

In Wilson's disease, a gene mutation resulting in the persistence of the fetal mode of copper metabolism has been assumed (Epstein and Sherlock 1981). It is therefore tempting to speculate that in ICC alimentary copper intoxication during the perinatal period might exert a similar effect on the liver cell.

Up to now ICC has been described in Indian immigrants (Tanner et al. 1978; Klass et al. 1980) in an Australian and in a North American family (Walker-Smith and Blomfield 1973; Lefkowitch et al. 1982). The occurence of ICC in a further German infant indicates that this probably environmental disorder may occur more often than assumed and may occasionally have been overlooked in the past. From a morphological-diagnostic point of view, special attention should therefore be paid to the presence of lipofuscin-like pigment in the liver of children with problematic liver disease, indicating the need for further investigations, especially of copper metabolism.

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