Editorial

Bile Acids for Babies? Diagnosis and Treatment of a New Category of Metabolic Liver Disease

In this issue of HEPATOLOGY, Daugherty et al. (1) present convincing evidence for the resolution of liver biopsy alterations in three siblings after bile acid therapy of an inborn error of bile acid metabolism, Δ^4-3-oxosteroid-5β-reductase deficiency. Before we can place this study in perspective, some explanation is in order to help orient readers to the breathtaking advances that have been made in this new category of metabolic liver disease in the last few years.

Bile secretory failure and cholestatic jaundice are commonly associated with liver dysfunction in the neonate, regardless of the underlying cause (2, 3). Owing to immaturity of hepatobiliary function, the number of distinct disorders associated with cholestasis is arguably greater during the neonatal period than at any other time of life. Pediatric hepatologists have long been challenged by cholestatic disorders in the neonate that are collectively numerous, individually uncommon and, in many cases, idiopathic. For years it had been the experience of clinicians that many cases of neonatal liver disease remained undefined even after exclusion of extrahepatic biliary atresia, common perinatal viral infections and inborn errors of metabolism such as α_1-antitrypsin deficiency (2). In infants with severe familial liver disease, including forms of giant-cell hepatitis and so-called Byler's syndrome, it was clear that an inherited metabolic defect, possibly involving bile acid metabolism or transport, would eventually be defined as the underlying cause (1, 2).

Bile acids became an early focus of investigation into the pathogenesis of pediatric liver disease because of their central role in bile formation and because of the hepatotoxicity of certain bile acid species (3, 4). The pathways for the biosynthesis of bile acids from cholesterol are complex, involving multiple reactions catalyzed by more than 15 enzymes located in various subcellular compartments of the hepatocyte (5). In searching for metabolic errors that involve these pathways, many workers sought evidence for the overproduction or ineffective detoxification of known hepatotoxic bile acids such as lithocholic acid and its conjugates but found little of significance (6, 7). However, it has emerged that immaturity of bile acid synthesis, metabolism, detoxification and transport by the liver may place the infant at a greatly increased risk of cholestasis in association with a wide spectrum of liver disorders (8-10). Analysis of biological fluids by means of gas chromatography–mass spectrometry (GC-MS)—including serum, urine and bile from cholestatic and normal infants—yielded interesting findings such as the presence of a complex array of bile acids not typically found in the bile of healthy adults (particularly compounds hydroxylated at the C1, C4, and C6 positions) (11, 12). The significance of these unusual bile acids remains uncertain, but the increased hydrophilicity from the additional hydroxyl groups likely facilitates the excretion of these bile acids in urine during liver disease or as a result of the physiological immaturity of hepatic excretory mechanisms. In other cases, unusual long-chain bile acids, including trihydroxycoprostanic acid, were identified during early normal development and in cholestatic infants and adults (13). Such alterations involving the steroid side chain may reflect immaturity of bile acid synthesis, liver dysfunction or a primary defect (as is known to occur in disorders of peroxisomes) (14, 15). Although significant alterations in urinary serum and biliary bile acids may be found in liver disease, it has been difficult to determine whether such changes were primary or secondary to liver dysfunction. It was a great disappointment that, until recently, no alterations were indicative of a specific block in the biosynthesis of bile acids or in their transport through the hepatocyte. Indeed, I think many workers in the field were discouraged that bile acid analyses were no longer worth doing and would continue to show only nonspecific changes.

In searching for disorders of bile acid biosynthesis affecting enzymatic modifications of the bile acid steroid nucleus or side chain, one can anticipate the buildup of unusual bile acid precursors that should be detectable in serum and urine. Extremely low biliary bile acid concentrations are also to be expected. It is likely that many infants over the years with inborn errors of bile acid synthesis were missed because of inadequate methods for the qualitative and quantitative analysis of bile acids. The rather harsh, destructive hydrolysis and derivatization methods required to measure bile acids in biological fluids with GC-MS were probably responsible for the loss of diagnostic abnormalities (16).

A significant methodological breakthrough in the field of bile acid analysis came with the application of the technique of fast atom bombardment ionization–mass spectrometry (FAB-MS) (17). FAB-MS proved to be an

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ideal screening test in that it could be performed rapidly on a small amount (~10 to 50 μl) of a noninvasively obtained urine specimen, without the need for derivatization, and had an excellent level of sensitivity and specificity. Direct analysis of urine samples on FAB-MS can detect the urinary excretion of unusual bile acids such as unsaturated dihydroxy and trihydroxy cholenoates and other labile bile acid precursors, including conjugates of hydroxy-oxo-cholenoic and dihydroxy-oxo-cholenoic acids. The negative-ion FAB-MS spectrum has sufficient discrimination to differentiate cholestasis caused by a primary block in the biosynthesis of bile acids from other cholestatic disorders with normal primary bile acid production. However, it must be cautioned that the diagnosis of a possible inborn error must be confirmed by a careful and more definitive analysis with GC-MS (10, 18). Milder methods of hydrolysis of acid labile bile acid conjugates are now in use; they yield much more information than was available with antiquated analytical methods.

In recent years these techniques have led to the discovery of two novel enzymatic defects affecting modifications of the steroid nucleus in the conversion of cholesterol to primary bile acids. The first of these defects to be identified involved a deficiency of 3β-hydroxy-Δ5-C_{27}-steroid dehydrogenase/isomerase (19). This disorder was found in a patient of Saudi Arabian origin who was the third of five siblings to be affected by progressive idiopathic neonatal cholestasis. More than 20 other patients have been subsequently identified (20, 21). Most exhibited progressive jaundice, increased transaminase levels, and conjugated hyperbilirubinemia during the first weeks of life. Cases are often familial in nature and are fatal if untreated. The spectrum of this disorder has now been expanded from infantile cholestasis to include progressive liver disease in a child 10 yr old (22). Serum bile acid concentrations are paradoxically normal or low in these patients when measured with routine methods and seemingly incompatible with a diagnosis of severe cholestasis. The diagnosis is strongly suggested by FAB-MS analysis of urine, which quantitatively indicates increased urinary bile acid excretion compatible with cholestasis but is remarkable for absence of normal glyco and tauro conjugates of the primary bile acids. The spectrum of ions present is consistent with sulfate and glycosulfate conjugates of unsaturated dihydroxy and trihydroxy cholenoic acids. These compounds have been definitively identified on GC-MS as 3β,7α-dihydroxy and 3β,7α,12α-trihydroxy-5-cholenoic acids. The lack of the microsomal 3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase, which catalyzes the conversion of 7α-hydroxy-cholesterol to 7α-hydroxy-4-cholesten-3-one explains the presence of these unusual bile acids.

More recently a second defect involving a deficiency of Δ^4-3-oxosteroid-5β-reductase has been defined with similar methodology (23, 24). This inborn error is also manifested as progressive neonatal cholestasis. The deficiency involves the cytosolic enzyme Δ^4-3-oxoy-steroid-5β reductase, which catalyzes the conversion of 7α-hydroxy- and 7α,12α-dihydroxy-4-cholesten-3-one into the corresponding 3-oxo-5β(H) intermediates. FAB-MS analysis in these patients reveals increased urinary bile acid excretion, reflecting cholestasis, and a predominance of bile acid conjugates with molecular weights consistent with unsaturated oxo-hydroxy and oxo-dihydroxy cholenoic acids. These predominant metabolites have been confirmed on GC-MS analysis. Small proportions of allo isomers of cholic and chenodeoxycholic acids were also found. The atypical bile acids in these patients account for up to 90% of total urinary bile acids. Thus far 14 patients have been identified with this disorder, including 2 infants with neonatal liver failure and presumed diagnoses of neonatal hemochromatosis (21, 25).

The mechanism for the progressive liver disease in these disorders remains uncertain, but a paradigm derived from other inborn errors of metabolism may be applied. In many enzymatic deficiency states tissue injury occurs because of the lack of an end product required for organ function and because of the continued, even accelerated, biosynthesis of potentially toxic metabolic precursors, which are unchecked by feedback inhibition. In the case of inborn errors of bile acid metabolism there is a profound deficiency of primary bile acids necessary for the generation of normal bile flow. This alone might be considered sufficient for the development of severe cholestasis and progressive liver disease. However, the uninhibited production of toxic bile acid precursors such as 3β-hydroxy-5-cholenoic acid presumably contributes to hepatic injury. Similar compounds have cholestatic effects in experimental animal models (18, 21, 24). There is also concern that unsaturated oxo bile acid intermediates, generated in the deficiency of Δ^4-3-oxosteroid-5β-reductase, may be relatively insoluble and also injurious on accumulation in the hepatocyte.

Inborn errors of bile acid metabolism may account for as many of 2% to 5% of the idiopathic cholestatic liver diseases in children (Setchell KDR, Personal communication). These estimates come from the major diagnostic reference laboratory for bile acid disorders in Cincinnati and thus may be somewhat biased by referral of patients most likely to be affected. Nevertheless, there is no doubt that bile acid analysis with FAB-MS should be included in the evaluation of any infant or child with undefined cholestasis. The increasing number of clinical presentations now includes neonatal liver failure, progressive cholestasis in infants and even chronic liver disease in one child. It would not be surprising if additional clinical variants were discovered, possibly even in adult patients with idiopathic cholestasis.

These disorders have generated considerable excitement, not only because a new category of liver disease has been identified but also because these disorders are eminently treatable if recognized before end-stage liver disease has developed. Although oral bile acid therapy is in vogue for several cholestatic disorders, in most cases treatment with the hydrophilic bile acid ursodeoxycholate results in improvement of liver bio-

chemical tests and possibly in liver morphology but does not usually lead to resolution of the liver disease (26, 27). The rationale for bile acid replacement in these inborn errors of metabolism is twofold. First, replacement of exogenous bile acids is necessary for the generation of bile acid–dependent bile flow. Second, the feeding of exogenous bile acids should inhibit bile acid synthesis at the level of 7α-hydroxylase and thus decrease significantly the production of potentially toxic bile acid precursors. It could have been predicted that treatment with ursodeoxycholate alone would not be effective; this bile acid does not inhibit 7α-hydroxylase. Indeed, in the first two infants with Δ^4-3-oxosteroid-5β-reductase deficiency, treatment with ursodeoxycholate alone failed, but combined treatment with cholic acid and ursodeoxycholic acid led to progressive improvement and normalization of liver biochemical parameters (28, 29). There is growing experience with the use of bile acid treatment for disorders involving the steroid nucleus as well as the bile acid side chain. Under the approach of feeding cholic acid or chenodeoxycholic acid, often with the addition of ursodeoxycholic acid, most patients have had a remarkable clinical and biochemical improvement, with normalization of liver function and resolution of jaundice (30, 31). Indeed, the only surviving patients with these defects are those in whom bile acid therapy was successfully initiated after diagnosis. Clayton has made the observation that treatment of 3β-hydroxy-Δ5-C27-steroid dehydrogenase/isomerase deficiency with chenodeoxycholic acid led to dramatic reduction in plasma and urinary concentrations of 3β-hydroxy-Δ5-bile acids but that the amount excreted in feces actually increased (18). This suggests that bile acid therapy of the disorder was more effective in promoting bile acid–dependent bile flow and excretion of bile acid intermediates rather than in completely inhibiting 7α-hydroxylase. The dose of bile acid required for treatment has been empirical but based on careful monitoring of urine and plasma samples from the patient on FAB-MS and GC-MS and titration of therapy according to the disappearance of the unusual bile acid metabolites from urine (18, 20, 24, 28).

The paper by Daugherty et al. (1) provides further evidence for the beneficial effects of bile acid replacement therapy in one of these inborn errors, Δ^4-3-oxosteroid-5β-reductase deficiency. Light and electron microscopic findings are presented from before and during treatment of three siblings with this disorder. The first described patients with this deficiency were identical male twins who were evaluated because of severe cholestatic liver disease at 3 wk of age. A previous sibling had died in early infancy of undefined liver disease. A subsequent sibling was born and was identified at birth on the basis of FAB-MS analysis of a urine sample. Pretreatment biopsy specimens from the two severely affected twins showed marked giant-cell transformation, pseudoacinar formation individual hepatocellular necrosis and bridging fibrosis. The findings were very similar to what is commonly described in the so-called neonatal hepatitis syndrome. However, ultrastructural findings were different from patterns found in other neonatal cholestatic disorders such as neonatal hepatitis, biliary atresia and Byler's syndrome (32). A mosaic of altered and unaltered bile canaliculi was observed. Characteristic findings included complex canalicular membrane contours consisting of diverticuli, numerous convoluted membranes and, on occasion, collapse of the canalicular space with granular electron-dense material outlining a portion of a membrane profile. The pathogenesis of these unusual changes is not known, but the deficiency of primary bile acids and the lack of normal bile flow might have been contributing factors. Alternatively, the accumulated potentially toxic intermediates of bile acid biosynthesis may produce the histological injury. Immature transport mechanisms on the basolateral and canalicular membranes for bile acids and other organic anions may have contributed. Indeed, the presence of primary bile acids may be required for normal expression of these systems and for normal morphological development of the bile canalicular membrane. It is also of interest that these changes probably begin *in utero*, as evidenced by similar but less severe changes that had already developed in the youngest sibling, who was identified at birth and underwent liver biopsy at 5 days of age. This is not surprising, however, because an immature form of bile acid secretion and enterohepatic circulation is found in the fetus in utero (33-35), and injury to the secretory apparatus might well occur before or after birth. The efficacy of early diagnosis and treatment of this otherwise fatal disease is supported by this study, which demonstrates over time and with continued treatment a progressive disappearance of the abnormal light and electron microscopic abnormalities such that, by 9 mo of age, the hepatic ultrastructure was near normal. Long-term follow-up in these and other affected infants is currently in progress. It would appear that treatment with oral bile acid replacement will be necessary for life because bile acids may cure the liver disease but not the enzyme deficiencies in these patients.

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