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Point mutations in the ileal bile salt transporter cause leaks in the enterohepatic circulation leading to severe chronic diarrhea and malabsorption.

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Editorial

The enterohepatic circulation (EHC) is an in vivo ecological system for the conservation of bile salts allowing them to be used over and over for the absorption of fat (1–4). Conjugated bile salts are secreted from the liver and concentrated in the gallbladder where they are stored during fasting. When partly digested food products, especially fatty acids and amino acids, are released into the intestine cholecystokinin is released, the gallbladder contracts and empties bile salts into the duodenum where they aid in the digestion and absorption of fat. Bile salts are efficiently reabsorbed in the terminal ileum and return to the liver through the portal vein. A 70-kg human secretes ~ 30 g (range 15–45 g) of bile salt per 24 h (~ 1 mmol/kg per 24 h). Of that 30 g, ~ 0.3–0.5 g (~ 1 mmol) is lost in the feces and this loss is compensated by the hepatic synthesis from cholesterol (3). Thus, 98% of the bile acid is reabsorbed, passes into the portal vein, and returns to the liver. However, the total bile acid pool (mainly residing in the gallbladder and the gut) is only ~ 3 g (~ 100 μmol/kg). This means that on average the pool must circulate ~ 10 times every 24 h to give a secretion rate of 30 g. Thus, a small synthetic rate (0.3–0.5 g/d) is adequate to maintain a 3-g pool and the EHC assures the pool to circulate repeatedly allowing us to absorb copious fat.

The discovery of the EHC of bile is ascribed to M. Schiff in 1870 (1) but it was later realized that not all bile constituents recirculated, only the bile salts do. The EHC confines the bile salt pool to the liver, bile ducts, gallbladder, small intestine, and the portal vein. This led investigators to postulate that there was an active transport system in the intestines to remove bile salts from the intestinal lumen and a second active transport system in the liver to extract bile salts from portal blood. A number of investigators in the early 1960s located the active transport system in the ileum and noted its dependence on sodium. The active removal of bile salts from portal blood was recognized even earlier (4) because of the virtual absence of bile acids in the systemic circulation. Recently, some of the key molecules of the bile salt EHC have been discovered (5) and Oelkers et al. (6) show conclusively that mutations of one of these molecules, the ileal sodium-bile salt transporter (ISBT), can lead to interruption of the EHC.

A minimum of six (known or postulated) molecules (three in the ileal mucosal cells and three analogous molecules in the hepatocytes) constitutes the active players in the EHC (5). The polar ileal enterocytes and the hepatocytes each have three unique molecules, a receptor which binds bile salts on one surface and translocates them into the cell, a cellular bile salt binding protein which moves them across the cell, and an exit molecule which moves bile salts out of the other side of the cell. In the intestine ISBT is present on the brush boarders of the ileum but not the jejunum. It binds bile salts in the gut lumen and transports them across the brush boarder membrane and hands them to the ileal lipid-binding protein (ILBP) which binds the bile acid in the cytoplasm of the cell. This protein has a beta clamshell structure and is part of the fatty acid–binding protein family, except the cavity is somewhat larger and more loosely organized (7). ILBP allows the bile salts to move through the cytoplasm to the basolateral membrane of the ileal intestinal epithelial cell, where a sodium-independent organic ion exchange system secretes bile salts into portal capillaries. Bile salts in portal capillaries bind to albumin and flow to the liver. There they are recognized by a transporter with high homology to ISBT, the sodium taurocholate cotransporting polypeptide (NTCP). NTCP transports bile acids across the sinusoidal plasma membrane into the liver cell where they are bound by cytosolic carrier proteins and shuttled to the canalicular membrane. Bile acids conjugated with taurine or glycine are directed for immediate secretion into bile by an ATP-dependent transporter located in the canalicular membrane. Free bile acids (unconjugated) are first directed to systems which conjugate them with taurine or glycine and then are secreted by the same mechanism. They then pass down the biliary ducts into the gallbladder for storage and ultimate expulsion into the duodenum. Thus, the six key steps are mediated by the ISBT, ILBP, an organic anion exchanger in the ileum, and the NTPC, cytosolic carrier(s), and an ATP-dependent canalicular transporter in the liver. These molecules allow the bile acids to be maintained within the EHC so that they can be used repeatedly in the digestion process without being lost from the intestine into the feces or distributed into the systemic circulation. NTCP has been cloned from a number of species and is highly conserved. Rat NTCP has 349 amino acids, is 77% homologous to humans, and is expressed solely in the liver. It first appears in the livers of higher vertebrates. It has five glycosylation sites and seven putative transmembrane helices (5). The ileal transporter ISBT is a glycosylated protein of 348 amino acids with a 35% homology to NTPC. It also has seven putative membrane-spanning helices (5).

Following the extensive physiologic descriptions of ileal active bile acid transport some 30 yr ago by Lack and Wiener (8) and others, Dowling, Redinger, and I forecast in 1972 (3) that a genetic defect in the predicted bile acid receptor in the ileum would lead to diarrhea and/or steatorrhea and suggested that bile acid turnover and fecal bile acid excretion be studied in patients with unexplained diarrhea. Between 1973 and 1976 Thaysen and Pedersen (9) described several patients who had diarrhea and excessive bile acid loss, without other ileal pathology. In 1979 Heubi et al. (10) reported a case study of a boy who presented 48 h after birth with severe diarrhea, steatorrhea, and malabsorption. Intestinal absorption of bile acid was nearly absent and resulted in a small pool size, a low interluminal bile acid concentration, and severe malabsorption of water and fat. Ileal biopsies had no active bile acid transport (10). Parenteral nutrition was necessary to sustain the child. At the other extreme, a child with marked bile acid malabsorption, but with almost normal development, nearly normal fat absorption, and a moderately well-maintained bile acid pool, was described (11). This patient had a 15-fold increase in bile acid

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synthesis which was adequate to maintain pool size, interluminal bile salts, and fat absorption. Thus, the clinical phenotype apparently can vary from severe diarrhea, fat malabsorption, and malnutrition (10), to modest diarrhea without significant fat malabsorption (11). The bile acid malabsorption and the variable severity could represent mutations in any of the three main players in the ileal transport.

Dawson's group first cloned the hamster ISBT and, shortly after, human ISBT (5). They then looked for molecular defects in a patient (JB) described earlier (10). In addition to a splice junction mutation, the gene of the proband had two significant mutations of highly conserved amino acids, L243P and T262M. Both were expressed and transported to the plasma membrane in Cos cells, but neither supported bile salt translocation. The L243P mutation is predicted to occur in transmembrane helix 6. Thus, this mutation is not severe enough to be retained and degraded in the cell, but is severe enough to prevent bile salt translocation. Using an algorithm for the prediction of membrane proteins (12, 13), the L243P substitution would change the transmembrane helix 6 to a hydrophobic β strand. Using photoreactive bile acid analogs, Kramer et al. (14) have identified the ileal bile acid transporter as a complex of a 93-kD protein and membrane-bound ILBP. The 93-kD protein most likely represents a dimer of ISBT. The association of ILBP with ISBT would make sense in that the bile acid transported across the membrane could immediately be transferred to the membrane-bound ILBP and then released into the cytoplasm for movement to the basolateral membrane. The structural predictions (12, 13) for the transmembrane part of ISBT also suggest that transmembrane region 4 is more likely to be a long hydrophobic β strand than a helix. Thus, it is probable that the ISBT complex involves a nonroutine combination of hydrophobic α helices and β strands to constitute the bile acid translocator. Since ISBT must recognize, bind, translocate, and release bile salts, it is suggested that L243P affects translocation, while T262M, which is in a lumenal loop, affects the recognition and binding site.

The paper by Oelkers et al. (6) opens up a most interesting field concerning the structural mechanisms for the recognition, binding, and translocation of bile acids across the intestine (and the liver) and is thus an important landmark in pathophysiology of the EHC.

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