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The enterohepatic circulation of bile acids in mammals: form and functions

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TABLE OF CONTENTS

1. ABSTRACT

The features of the enterohepatic circulation of bile acids in mammals are reviewed. Inputs into the circulating bile acids are primary bile acids synthesized from cholesterol in the hepatocyte and secondary bile acids formed by bacterial modification of primary bile acids in the distal intestine. Intestinal conservation of bile acids generates pools of individual bile acids whose relative sizes determine biliary bile acid composition. Efficient hepatic clearance results in low plasma bile acid levels, and virtually no renal excretion. Methods for characterizing the enterohepatic circulation are summarized. Bile acids have numerous physiological functions in the liver, biliary tract, and intestine resulting from their signaling and physicochemical properties.

2. INTRODUCTION

The term "enterohepatic circulation (EHC) denotes the movement of bile acid molecules from the liver to the small intestine and back to the liver. If described in more detail, it is the movement of bile acid molecules from the hepatocyte into canalicular bile, flow through the biliary tract (with or without storage in the gallbladder) and into the duodenum. Bile acids then flow aborally in the intestinal content, as a result of propulsive small intestinal motility. They are actively absorbed from the distal Ileum (actively) and large intestine (passively), transported to the liver in portal venous blood, and then efficiently taken up by the hepatocyte. Bile acids traverse the hepatocyte and are actively secreted into canalicular bile, completing the enterohepatic cycle. A schematic depiction of the enterohepatic circulation is shown in Figure 1.

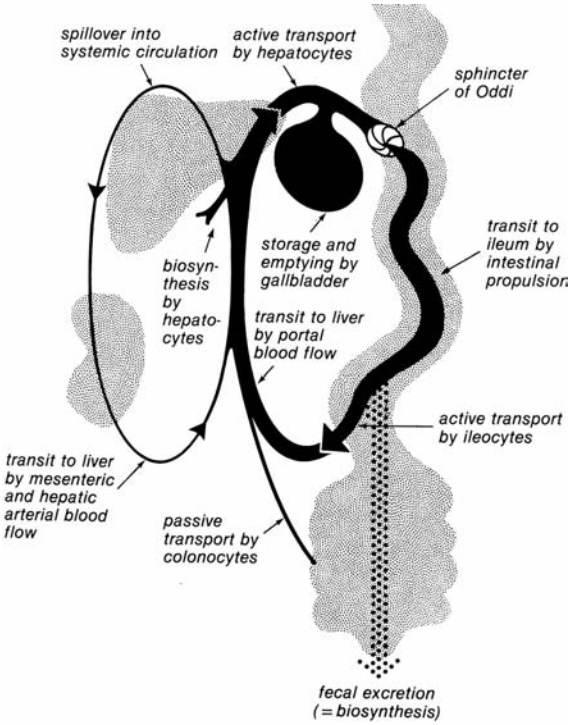

Figure 1. A schematic depiction of the enterohepatic circulation of bile acids in man. Gallbladder emptying with meals delivers bile acids to the small intestine where they promote digestion by forming mixed micelles with dietary lipids. Increased absorption of bile acids during meals is signaled by a rise in the plasma level of bile acids.

A number of older reviews are available on the enterohepatic circulation (1-4). The purpose of this brief review is to summarize our current knowledge as well as to provide a simple overview of this remarkable physiological process that should be helpful to someone beginning in this rather neglected area of physiology.

Intestinal conservation or salvage leads to the accumulation of a recycling mass of bile acid molecules, most of which is stored in the gallbladder during an overnight fast. As this mass was originally measured by an isotope dilution technique, it was termed a "pool" and this descriptor is still being used. Each biliary bile acid has its own pool and enterohepatic circulation. Biliary bile acid composition is determined by the pool sizes of individual bile acids.

The EHC has an input, which is the biosynthesis of bile acids from cholesterol. In the steady state, input is balanced by loss. So far as is known, renal excretion of bile acids is negligible compared to fecal excretion in all vertebrates. Therefore, input of bile acids occurs by biosynthesis in the hepatocyte, and the preponderant loss of bile acids occurs by fecal excretion. Because the steroid nucleus is considered to remain intact during colonic transit, fecal bile acid excretion is equivalent to hepatocyte bile acid biosynthesis.

Bile acids are divided into two great classes – C_{27} bile acids (with a C_8 (isooctanoic acid) side chain) and C_{24} bile acids (with a C_5 (isopentanoic acid) side chain). C_{27} bile acids (named cholestanoic acids) occur in reptiles, ancient birds, and some amphibians, but are only trace constituents of the circulating bile acids in healthy mammals. C_{24} bile acids (named cholanoic acids) occur in reptiles, birds, bony fish, and mammals. There are also C_{27} bile alcohols that are present in ancient mammals (elephant, manatee, hyrax, and rhinoceros), cartilaginous and herbivorous (bony) fish, and amphibians; they are present in bile as the C_{27} ester sulfate, the sulfate being on the first carbon atom (C-27) of the side chain. Little is known about the details of enterohepatic cycling of C_{27} bile acids and C_{27} bile alcohol sulfates. Even for C_{24} bile acids, little is known about the enterohepatic circulation of bile acids in reptiles, birds, and bony fish. Therefore this brief review will deal for the most part with C_{24} bile acids and their enterohepatic cycling in mammals. When the number of carbon atoms is not indicated, it should be understood that the text refers to C_{24} bile acids.

3. BILE ACIDS: CHEMISTRY AND METABOLISM

3.1. Overview

Bile acids are endobiotics, and their metabolism can be described in pharmacokinetic terms; at the same time, bile acids have multiple physiological functions that can be considered the pharmacodynamic properties of bile acids. This review will focus largely on the pharmacokinetic aspects of the enterohepatic circulation, although bile acid functions will be summarized briefly. Formation of bile acids may be considered a phase I step; bile acid conjugation (see below) may be considered a phase II step; and bile acid transport may be considered a phase III step.

Bile acid nomenclature is complex. Bile acids are named according to their substituents on the steroid nucleus or isopentanoic acid side chain (or both). Bile acids synthesized de novo from cholesterol are termed "primary" bile acids to distinguish such bile acids from "secondary" bile acids which are formed by bacterial modification of primary bile acids (5).

3.2 Bile Acid Conjugation

Bile acids are efficiently "conjugated" after their biosynthesis. Such conjugation is the linkage of the amino acid group of taurine or glycine to the carboxylic acid group of the bile acid. The taurine conjugate of cholic acid may be termed taurocholate (the name used in the 19th century before its structure was known) or cholyltaurine. Conjugation with glycine or taurine is termed N-acyl amidation, and taurine or glycine conjugated bile acids are referred to as "amidates" or N-acylamidates. In the older literature, the term "conjugation" denoted N-acyl amidation with taurine or glycine. With the discovery that bile acids could be sulfated or glucuronidated, it became necessary to distinguish the type of conjugation.

N-acylamidation of a bile acid with taurine or glycine converts a weak acid ($pK_a \approx 5.0$) to a much stronger acid. The pK_a of glycine amidates is about 3.9, that of taurine amidates < 2. Conjugation alters the

physicochemical properties and metabolism of bile acids. From a physicochemical standpoint, conjugation increases solubility at acidic pH, and renders dihydroxy bile acids resistant to the formation of insoluble Ca^{2+} salts (6). From a metabolic standpoint, conjugation results in bile acids being fully ionized at the pH present in the biliary tract and small intestine. Passive absorption across the epithelial cells of the biliary tract and small intestine is prevented by the negative charge on the side chain of conjugated bile acids. Passive paracellular absorption is prevented by the size and negative charge of the bile acid molecules. The absence of membrane permeability and paracellular transport should be important for the maintenance of high intraluminal concentrations.

The usage of only two amino acids for conjugation – taurine and glycine – can be explained by the substrate specificity of pancreatic carboxypeptidases. If synthetic bile acid conjugates are prepared in which the conjugating moiety is an amino acid other than glycine or taurine, such conjugates are readily cleaved by pancreatic carboxypeptidases (7). Thus, to simplify, conjugation with taurine or glycine makes bile acids indigestible and unabsorbable in the proximal small intestine where most lipid absorption occurs.

Despite its resistance to pancreatic carboxypeptidases, the amide linkage of conjugated bile acids is readily cleaved by bacterial "deconjugases". In man, such bacterial deconjugation begins in the mid-ileum, and is completed in the cecum. In some rodents and the rabbit, deconjugation begins in the proximal small intestine.

Bile alcohol sulfates, like taurine amidates, have a pK_a below 2 and are thus present entirely in ionized form in the biliary tract and small intestine. Pancreatic juice, so far as is known, contains no sulfatase activity, and bile alcohol sulfates, like conjugated bile acids, are indigestible and unabsorbable in the proximal small intestine. From a structural point of view, a bile alcohol sulfate is quite similar in topology to the corresponding C_{24} taurine conjugate (8).

The gallbladder, which stores the bile acid pool, may be considered as evidence for an enterohepatic circulation. Absence of a gallbladder is rare in vertebrates including species such as cartilaginous fish in whom bile alcohol sulfates are the dominant biliary surfactant. Therefore, it is reasonable to assume that bile alcohol sulfates have an enterohepatic circulation. Indeed. evidence for an enterohepatic circulation of bile alcohol sulfates in the Asiatic carp (8) and skate (9) have been published.

Bile acids can be sulfated at C-3 in the hepatocyte, but in the healthy person or animal, the only bile acid that undergoes appreciable sulfation is lithocholic acid (LCA), the bacterially derived 7-deoxy metabolite of chenodeoxycholic acid (CDCA). When LCA enters the hepatocyte, it is amidated first, then sulfated. Accordingly, the “double” conjugates are termed sulfolithocholylglycine or sulfolithocholyltaurine (they are also referred to as glycolithocholate sulfate and taurolithocholate sulfate). As lithocholic acid is highly toxic when it accumulates in the circulating bile acid pool, such sulfation may be considered a detoxification step.

Bile acids are also capable of forming two types of glucuronides. The first type is an ester glucuronide, in which the C-1 carbon atom of glucuronic acid is coupled in ester linkage to the C-24 carboxyl group of an unconjugated bile acid. The second is an "ethereal" glucuronide in which the C-1 carbon atom of glucuronic acid is coupled in an ether linkage to the 3-hydroxy group of a bile acid. Glucuronide formation is a trace pathway of endogenous bile acid metabolism under physiological conditions (10).

4. COMPONENTS OF THE ENTEROHEPATIC CIRCULATION

4.1. Overview

At its simplest, the enterohepatic circulation involves input from biosynthesis of bile acids from cholesterol, conjugation with taurine or glycine, canalicular excretion of conjugated bile acids, active absorption of these conjugated bile acids in the terminal ileum, uptake by hepatocytes, and active transport across the canalicular membrane. For such a circulation, two hepatocyte transporters and two ileal enterocyte transporters are required. Ileal uptake of conjugated bile acids involves a sodium-dependent transporter (ASBT). Exit from the ileal enterocyte is considered to be mediated by an exchanger consisting of a heterodimer of two proteins. These are termed organic solute transporter α /organic solute transporter β (OSTα/ OSTβ) (11). Uptake of conjugated bile acids by the hepatocyte is largely mediated by a sodium-dependent cotransporter (NTCP) that shares homology with ASBT, the apical transporter of the ileal enterocyte. Uptake by sodium-independent transporters (OATP’s) may also occur. However the initial driving force for the enterohepatic secretion of conjugated bile acids is BSEP, the ATP-energized bile salt export pump, which mediates uphill canalicular secretion of bile acids (12, 13). Most substrate molecules for this transporter are bile acid molecules previously secreted into the intestine and not newly synthesized bile acids.

4.2. Structure of primary bile acids

The “root” bile primary bile acid is CDCA, a dihydroxy bile acid possessing 3α- and 7α- hydroxy groups. The 3-hydroxy substituent originates from the 3β-hydroxy group of cholesterol. A 7-hydroxy substituent and rarely a 7-oxo group is present on every primary bile acid and bile alcohol. The presence of a hydroxyl group at C-7 results from the enzymatic activity of cholesterol 7α-hydroxylase, firmly established as the rate limiting enzyme for bile acid (and presumably bile alcohol) biosynthesis from cholesterol. In general, C_{24} bile acids consist of modifications of CDCA.

The most common modification of CDCA is additional hydroxylation on the steroid nucleus or on the isopentanoic side chain (or rarely, on both the nucleus and

Table 1. Chemical structure of C_{24} bile acids occurring in healthy mammals[1]

Trivial name	A/B RJ, A ring substituents#	B ring substituents	C & D ring substitut-ents	C_5 Side chain substituents	Source	Occurrence
Cholic	5β,3αOH	7αOH	12αOH	None	L	Many species
Allocholic	5α,3αOH	7αOH	12αOH	None	L or I	Minor BA in newborn rabbit
Deoxycholic	5β,3αOH		12αOH	None	I	Many species
Allodeoxycholic	5α,3αOH		12αOH	None	L or I	Minor bile acid in rabbit
Chenodeoxycholic	5β,3αOH	7αOH		None	L	Many species
Ursodeoxycholic	5β,3αOH	7βOH		None	L or I	Major BA in nutria, bear, beaver
Lithocholic	5β,3αOH			None	I	Many species
(none proposed)	5β,3αOH		15αOH	None	I	Wombat
Lagodeoxoycholic	5β,3αOH		12βOH	None	I	Trace BA in rabbit
α-Muricholic	5β,3αOH	6βOH,7αOH		None	I	Rodents (minor bile acid)
β-Muricholic	5β,3αOH	6βOH, 7βOH		None	L	Rodents
ω-Muricholic	5β,3αOH	6αOH,7βOH		None	I	Rodents (minor bile acid)
Murideoxycholic	5β,3αOH	6βOH		None	I	Rodents
Hyocholic	5β,3αOH	6αOH,7αOH		None	L	Pigs
Hyodeoxycholic	5β,3αOH	6αOH		None	I	Pigs
Vulpecholic	5β,1αOH,3αOH	7αOH		None	L	Australian opossum
(none proposed)	5β,1βOH,3αOH	7αOH		None	L	Minor BA in infants, mice, sheep
(none proposed)	5β,3αOH	7-oxo		None	L	Caviomorphs, koala, sifaka)
(none proposed)	5β,3αOH	7-oxo	12αOH	None	L	Sloth
(none proposed)	5β,3αOH	7-oxo		None (Δ^{22E})	L	Paca (cavimorph)
(none proposed)	5β,3αOH	7βOH		None (Δ^{22E})	L	Agouti
(none proposed)	5α,3αOH	7αOH	12αOH	23-(R)-OH	L	Minor BA in Sea mammals
Phocaecholic	5β,3αOH	7αOH		23-(R)-OH	L	Major BA in sea mammals
(none proposed)	5β,3αOH		12OH	23-(R)-OH	L	Minor BA in sea mammals

[1]Complex mixtures of C_{27} bile alcohol (sulfates) and C_{24} bile acids occur in the elephant, manatee, hyrax, rhinoceros, and some cavimorphs. The bile of horses contains a complex mixture of C_{27} alcohol sulfates, C_{27} bile acids, and C_{24} bile acids. Several primates contain a substantial proportion of C_{27} bile acids. #Abbreviations: A/B RJ, A/B ring juncture; L, liver; I, intestinal bacteria

the side chain). The most common bile acids are trihydroxy bile acids with hydroxyl groups at C-3, C-7, and one additional site. In mammals, the most common "third site" hydroxylation positions are C-6 (α or β) or C-12 (α). An Australian marsupial has its third site of hydroxylation at C-1 (α), and such 1α,3α,7α-trihydroxy bile acids have also been identified in infants. Whether still additional sites of hydroxylation on the steroid nucleus will be discovered in mammalian bile acids is not known, but seems unlikely.

The addition of a third hydroxyl group to the CDCA results in the formation of a trihydroxy bile acid with solubilizing properties that are less potent than those of CDCA. Therefore, it has been speculated that the evolution of trihydroxy bile acids was in response to the formation in the large intestine of LCA, which, as previously noted, is a highly toxic monohydroxy bile acid formed by bacterial 7α-dehydroxylation of CDCA. When a trihydroxy bile acid with the structure 3α,7α,X-trihydroxy undergoes bacterial 7-dehydroxylation, a 3α-X-dihydroxy bile acid is formed, and the current view is that such dihydroxy bile acids are much less toxic than LCA. Table 1 lists most C_{24} bile acids that have been reported to occur in vertebrates.

In some species, primary bile acids have a 7β- hydroxy group. This is present in ursodeoxycholic acid (3α,7β-dihydroxy) and two β-muricholic acid epimers (β-muricholic acid and ώ-muricholic acid. Details of the formation of these 7β-hydroxy bile acids are not known. In a few mammals (guinea pig, koala), the 7α-hydroxy group has been oxidized in part to a 7-oxo group.

In marine mammals, the side chain undergoes hydroxylation at C-23 (the α carbon atom) in the *R* configuration. The addition of an α-hydroxy group to CDCA causes a modest improvement in its biological properties (14). The side chain is saturated in most mammals, but in a few cavimorphs, a double bond is present at C-22.

4.3. Input of secondary bile acids

A second input of bile acids into the circulating bile acids originates in the distal intestine and consists of secondary bile acids formed by bacterial modification of primary bile acids. As noted above, bile acids are deconjugated, i.e. the amide bond of taurine or glycine conjugated bile acids is cleaved to form unconjugated bile acids. If the steroid moiety of the conjugated bile acid was a primary bile acid, then the liberated unconjugated bile acid is still a primary bile acid, as its pattern of hydroxylation has not been changed. However, in the colon, the hydroxy groups undergo two major biotransformations (15). The first of these is 7-dehydroxylation, and the resultant bile acids are termed 7-deoxy bile acids. In man, cholic acid (3α,7α,12α-trihydroxy) is converted to deoxycholic acid (3α,12α-dihydroxy) and CDCA (3α,7α-dihydroxy) is converted to LCA (3α-hydroxy). Dehydroxylation at C-7 is a complex, multienzyme process proceeding via a 3-oxo, Δ^4,Δ^6 intermediate.

The second major bacterial biotransformation is epimerization at C-3 to form 3β-hydroxy (so called "iso") bile acids. There are many other types of bacterial

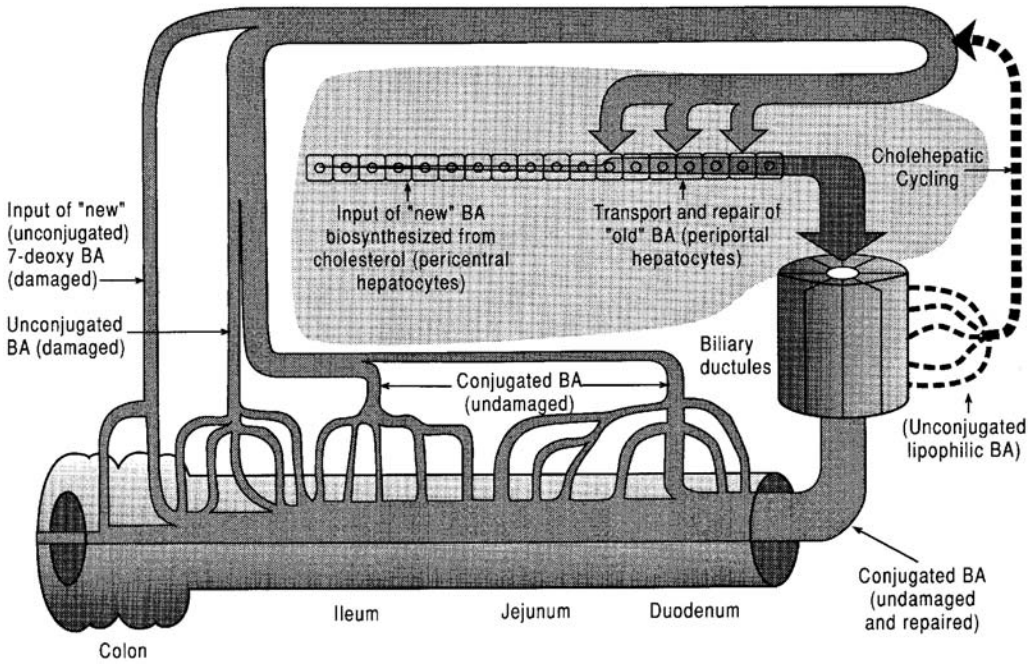

Figure 2. A more complex view of the enterohepatic circulation emphasizing synthesis of bile acids in the pericentral hepatocytes and transport of recycling bile acids by periportal hepatocytes. Reconjugation of unconjugated bile acids and epimerization of iso- bile acids returning from the intestine also occurs in the periportal hepatocytes. Cholehepatic shunting of unconjugated mono- or dihydroxy bile acids will occur if these are secreted in unconjugated form into canalicular bile. The figure suggests some proximal absorption of conjugated bile acids by mechanisms that are unclear. Bacteria in the distal small intestine deconjugate bile acids. In the colon, bile acids undergo 7-dehydroxylation and may also undergo epimerization of the 3α-hydroxy group to form iso- (3β-hydroxy) bile acids.

biotransformations that occur to a tiny extent. These include epimerization at C-7 (for example CDCA is converted to UDCA), as well as oxidation at C-3 or C-7 to form oxo or hydroxy-oxo bile acids. None of these minor pathways has any physiological significance so far as is known. A more complex depiction of the enterohepatic circulation indicating input of secondary bile acids as well as biosynthesis of primary bile acids is shown in Figure 2.

It is convenient to discuss the two loops of the enterohepatic circulation individually – the hepatobiliary loop and the enteral loop. In addition, it is useful to consider the extrahepatic component of the enterohepatic circulation which encompasses the systemic blood compartment and renal excretion.

4.4. Hepatobiliary loop

4.4.1. Bile acid uptake by the hepatocyte

Bile acids return to the liver in portal venous blood. Here, bile acids are bound to plasma albumin at specific sites. Binding is structure dependent – dihydroxy bile acids, being more hydrophobic, are bound more than 90%. Trihydroxy bile acids are bound to a smaller extent – perhaps 60-80% (16). Thus the unbound concentration of trihydroxy bile acids is at least an order of magnitude higher than that of dihydroxy bile acids. Amidation is not believed to have any marked effect on albumin binding. Albumin molecules containing bound bile acids pass through the fenestrae of the sinusoidal endothelial cells of the liver, with the result that bile acids bound to albumin can collide with basolateral transporters

The hepatic lobule may be considered to possess at least two zones. Cells closest to the portal vein are termed periportal cells. It is these cells that mediate vectorial transport of bile acids returning from the intestine. Hepatic uptake of bile acids from sinusoidal blood is highly efficient -50 to 90%- depending on the bile acid. Bile acids that are taken up by the basolateral transporters are rapidly transported through the hepatocyte and pumped by the canalicular bile salt export pump (bsep) into the canalicular space. These "old" bile acids are mostly conjugated, but some are always in unconjugated form because of bacterial deconjugation in the distal intestine.

Conjugated bile acid uptake is mediated largely via NTCP, a Na^+/bile acid cotransporter. (17). Unconjugated monohydroxy and dihydroxy bile acids may be taken up passively or by carrier mediated transport. Monohydroxy and dihydroxy bile acids are membrane permeable, whereas trihydroxy bile acids permeate lipid bilayers slowly. Carrier mediated transport may involve several transporters. Uptake of unconjugated bile acids by a fatty acid transport protein (FATP5) has been shown. During uptake via FATP5, the unconjugated bile acid is acylated with Coenzyme A as soon as it crosses the basolateral membrane (18), Such CoA formation traps the bile acid in the hepatocyte (19) where it should

immediately be conjugated with glycine or taurine. Sodium-dependent uptake of unconjugated bile acids by NTCP has also been documented (20). Both conjugated and unconjugated bile acid uptake might also involve sodium-independent transporters as there is a large family of OATP molecules present on the basolateral membrane (21).

The basolateral transporters of the hepatocyte are not unidirectional. An ATP-energized transporter MRP4 mediates the co-transport of conjugated bile acids and reduced glutathione from hepatocyte cytosol to the space of Disse (22). Therefore, it has been proposed that there is continuous regurgitation of bile acids mediated by MRP4 and uptake mediated by NTCP. Such percolation could spread the site of conjugated bile acid uptake down the hepatic lobule and might also protect the hepatocyte from an excess of bile acids.

4.4.2. Bile acid biotransformation in the hepatocyte

The fate of the secondary bile acid amidates during hepatocyte transport is both structure and species-dependent. Deoxycholyl amidates are not biotransformed in man, but are simply excreted as such into bile. They are efficiently absorbed by the ileal bile acid transport system, leading to the accumulation of a recycling pool of deoxycholyl conjugates. The size of the pool in relation to the size of the cholic acid pool (precursor) depends on the input of deoxycholic acid and the efficiency of intestinal conservation. In other species (hamster, prairie dog, mouse), there is efficient 7-hydroxylation which converts deoxycholyl conjugates back to their precursor, cholyl conjugates. Lithocholic acid, a toxic bile acid, is efficiently amidated and then undergoes one or more detoxification steps during its transport through the hepatocyte. In rodents, there is hydroxylation at C-6, C-7, or both. In man and some primates, there is sulfation at C-3 to form sulfolithocholyl amidates (23). Such divalent sulfolithocholyl amidates are secreted into bile via BSEP (in man) or MRP2 (in animals). They are not substrates for the ileal bile acid transport system, so they are rapidly lost from the circulating bile acids (24). Sulfation during hepatocyte transport is incomplete; about half of lithocholyl amidates in human bile are sulfated.

Iso (3β-hydroxy) bile acids are efficiently epimerized to 3α-hydroxy bile acids (25), and in human biliary acids, there are no detectable iso bile acids. So far as is known, this process of bacterial epimerization followed by hepatocyte re-epimerization is of no pathophysiological significance.

The oxo group of hydroxy oxo bile acids, based on limited studies, is reduced to a hydroxy group (26). The orientation of the hydroxy group that is formed – i.e. whether it is α or β – is species dependent.

There are thus three and in some species four processes of "damage and repair" in the EHC. The first is deconjugation (bacterial) followed by reconjugation (hepatocytic). The second is epimerization (bacterial) followed by re-epimerization (hepatocytic); the third is dehydroxylation at C-7 (bacterial) followed by rehydroxylation at C-7 or possibly another site on the steroid nucleus (hepatocytic). In some species, LCA is not rehydroxylated, but is sulfated. The fourth is oxidation (dehydrogenation) at C-3 or C-7 (bacterial) followed by reduction to an α or β hydroxy group (hepatocytic).

4.4.3. Bile acid transport through the hepatocyte

Details of movement of bile acids across the hepatocyte are poorly understood. It is not known what fraction is monomeric, what fraction is protein bound, and what fraction is present in vesicles. The monomeric concentration is believed to be < 1 □M (27), but no satisfactory method for measuring the monomeric concentration of bile acids in the hepatocyte cytosol has been developed. As most unconjugated bile acids returning to the liver are conjugated efficiently on entering the hepatocyte and as bile acids are efficiently conjugated after their biosynthesis from cholesterol, most bile acids in the hepatocyte are conjugated before secretion into canalicular bile. Because bile acids are present as anions, they cannot partition into the smooth endoplasmic reticulum. However, hydroxylases are believed to be present on the outer face of the smooth endoplasmic reticulum, so that conjugated (amidated) bile acids can undergo hydroxylation on the steroid nucleus. Bile acids are not believed to interact with mitochondria or peroxisomes, except during steps occurring in the biosynthetic pathway.

4.4.4. Canalicular secretion of bile acids and bile acid induced bile flow

The flux of bile acids through the hepatocyte and across the canalicular membrane has important physiological effects that will be summarized briefly. Canalicular secretion of conjugated bile acids induces bile flow by the osmotic effects of the secreted bile acids (28, 29). Each mole of secreted bile acid induces about 6-10 µl of water. This value, the amount of water (or bile) flow induced per umol bile acid recovered in ductal bile has been defined as the "apparent choleretic activity'. The adjective "apparent' is used because neither the amount of bile acid nor the amount of water secreted into the canalicular space is known, as only ductal bile is collected. Bile acid molecules may be absorbed in the biliary ductules, and water may be either absorbed or secreted. (In isotonic saline, there are 3 µl/µmol of solute, or 6 µl/µmol of chloride ion).

Canalicular secretion of bile acids induces water flow through the paracellular junctions and this water is accompanied by filterable solutes such as electrolytes, glucose, and amino acids (solvent drag). As this paracellular filtrate is likely to be isotonic, canalicular bile is transiently hypertonic before becoming isotonic in the biliary ductules. Water might also flow across the canalicular membrane if aquaporins are present.

Canalicular bile flow is also induced by canalicular secretion of non-bile acid anions, and in many species, the fraction of canalicular bile flow induced by such non-bile acid anions (bile acid independent bile flow) is greater than that induced by bile acids. Details of bile formation are available in a number of reviews (28,29).

4.4.5. Bile acid biosynthesis

Bile acid biosynthesis occurs in the pericentral hepatocytes (30). The flux of newly synthesized bile acids is < 5% of the flux of circulating bile acids in healthy man. Bile acid biosynthesis is a complex multi-enzyme, multi-organelle process that converts cholesterol, a hydrophobic, integral membrane constituent to a bile acid molecule, which is water soluble, amphipathic, and when present in micellar concentrations, solubilizes membranes. There are considered at least two pathways for bile acid biosynthesis – a "neutral" pathway where biosynthesis begins with hydroxylation at C-7 (on the steroid nucleus) and an acidic pathway, where hydroxylation begins at C-27 (the terminal methylene group of the side chain. These two pathways may not occur at the same rate in all hepatocytes. The final steps of conjugation occur in peroxisomes, after which the newly synthesized conjugated bile acid molecules travel to the canalicular membrane for export. Details of bile acid biosynthesis are beyond the scope of this brief review, but are available elsewhere (31). Details of bile acid biosynthesis have become clinically relevant as inborn defects cause fatal cholestatic liver disease which may be cured by exogenous bile acid administration (32).

4.4.6. Postcanalicular events: cholangiocyte modification of bile

The biliary canaliculus has a blind end. Newly synthesized bile acids are secreted into its pericentral region. Canaliculi are squeezed by the pericanalicular myosin filaments (33). Bile flow carrying newly synthesized molecules flows toward portal area, counter current to sinusoidal blood flow. In the periportal area, the newly synthesized bile acids join the recycling bile acids and together enter the biliary ductules via the canals of Hering.

The physiology of the epithelium of the biliary ductules is an area of active investigation. Differences in gene expression between small and large cholangiocytes are being elucidated by microarray approaches (34). Some work on the proteome of large cholangiocytes has been reported (35). It is convenient to think that small cholangiocytes act to restore bile to isotonicity (as the tight junctions are believed to be permeable to water) and may also absorb filtered solutes such as glucose and amino acids. Large cholangiocytes monitor bile flow by their cilia, and promote bile flow by bicarbonate secretion via the CFTR chloride channel. ATP is also secreted by canaliculi. The ATP undergoes hydrolysis to form adenosine which acts on apical adenosine receptors to also stimulate bile flow. Still other mechanisms may contribute to ductular bile flow (36, 37).

The ileal apical bile acid transporter, ASBT, is also present in large cholangiocytes, and conjugated bile acids have a small flux through large cholangiocytes and back to the liver via the periductular capillary plexus. Bile acids are considered to promote CFTR-induced chloride secretion, and if this view is correct, when bile acid concentration increases (because of excessive water absorption) ductular bile flow is stimulated. Bile acids exit the cholangiocyte via OSTα/OSTβ (38).

4.4.7. Cholehepatic shunting of bile acids

In man, biliary bile acids are virtually all in conjugated form. In some species, for example bovids, there is always a small fraction -perhaps 10%- of unconjugated cholic acid in bile. It is known that cholic acid can be transported by bsep. Unconjugated dihydroxy bile acids, if secreted into bile, would be absorbed by cholangiocytes, and return to the sinusoid via the periductular capillary plexus. If unconjugated bile acids, in particular ursodeoxycholic acid are presented to the liver at a rate exceeding its conjugation capacity, they are secreted into canaliculus in unconjugated form, absorbed by cholangiocytes, and returned to the liver. It is believed that they are absorbed passively in protonated form. Such absorption removes a proton from ductular bile, thereby generating a bicarbonate anion. When these molecules return to the liver, they may be conjugated. Alternatively, they may be resecreted into the canalicular space in unconjugated form and once again induce osmotic bile flow. Therefore a single unconjugated dihydroxy bile acid may cycle repeatedly from ductular bile back to the hepatocyte, and so forth. Each cycle induces bile flow. The circulation is termed cholehepatic shunting and is signaled by a bicarbonate rich hypercholeresis (39). Bile acids with a C_4 side chain may be prepared synthetically. Such C_{23} *nor* bile acids are resistant to conjugation in the hepatocyte and recycle repeatedly by this cholehepatic shunt mechanism. However, there is no evidence that this process occurs physiologically in any species to date. Nonetheless, there are species such as the guinea pig in which bile flow is driven by bicarbonate secretion; the origin of this biliary bicarbonate secretion is not known.

Bile flows into the hepatic duct and either enters the gallbladder or bypasses the gallbladder, depending on the pressure relationships present in the biliary tree. In man, about half of secreted bile enters the gallbladder (40). As water and electrolytes are removed by the gallbladder mucosa, gallbladder volume remains rather constant as a progressively greater fraction of the circulating bile acids are stored in it. ASBT is present in the gallbladder, so there is likely to be some absorption of bile acids (41).

With meals, cholecystokinin is released from the I cells of the proximal small intestine and induces gradual gallbladder contraction and relaxation of the sphincter of Oddi that acts as a valve between the end of the common bile duct and the duodenum. The result is the gradual addition of concentrated bile to small intestinal content during digestion.

4.5. The enteral loop

4.5.1. Ileal transport of bile acids

As noted above, conjugated bile acids transit through the small intestine to the terminal ileum where they are absorbed. Such absorption is signaled by the postprandial elevation in the plasma levels of conjugates of cholic acid that occurs about two hours after the ingestion of a meal. However, in man, there is an earlier rise in the level of conjugates of CDCA (42). The mechanism responsible for this early peak is not known. Animal

studies suggest that glycine dihydroxy bile acids can be absorbed in protonated form when intestinal content is sufficiently acidic (43), and such might occur in the duodenum However, this peak seems to occur too late to be explained by duodenal absorption, and the most likely interpretation, is that a conjugated bile acid transporter is present in the human mid small intestine that has yet to be identified.

As noted, bile acid deconjugation begins in the distal small intestine, so that the terminal ileum is presented with a mixture of conjugated and unconjugated bile acid. Based on studies of human volunteers, this proportion is likely to vary widely between individuals (44). Conjugated bile acids are absorbed via the ASBT/OSTα/OSTβ system. Unconjugated mono- and dihydroxy bile acids can be absorbed passively, and cholic acid is a substrate for ASBT. Therefore it is not necessary to postulate additional transporters at present.

Very recently, a group of children with functional constipation have been identified in whom the major fecal bile acid was the 3-sulfate of CDCA (45). It has been proposed that in such children conjugates of CDCA undergo bacterial deconjugation in the small intestine forming unconjugated CDCA. The CDCA is then absorbed, sulfated in the enterocyte, and pumped back into the intestinal lumen. This pathway could operate in healthy individuals, so that an additional possible fate of unconjugated dihydroxy bile acids in the distal small intestine, is to be absorbed, sulfated and extruded back into the lumen. However, it is not known whether this hypothetical pathway is present in patients receiving ursodeoxycholic acid for therapy. Were it to be present, an explanation would be at hand for the failure of some patients to enrich their biliary bile acids when receiving UDCA for treatment of cholestatic liver disease.

4.5.2. Colonic absorption of bile acids

The chemical form of bile acids crossing the ileo-cecal valve has not been defined. In the cecum, based on samples obtained from subjects undergoing an unnatural death, bile acids are fully unconjugated. In addition, there is nearly complete 7-dehydroxylation, indicating that this multienzyme bacterial process is a rapid one. Bile acids are largely in solution, despite the acidic pH of cecal contents and insoluble food residues (46).

Bile acids are absorbed in part from the large intestine. The dominant colonic bile acids in man are DCA and LCA, and both can be absorbed passively (47). Iso bile acids (isolithocholic and isodeoxycholic acid) are present, and these are also presumably absorbed passively. Those bile acids not absorbed pass out of the organism in fecal bile acids.

4.6. The Extrahepatic (systemic) Loop

The majority of bile acids returned in the liver in portal venous blood are conjugated bile acids that were absorbed by the ileal bile acid transport system. The minority of bile acids returned to the liver consists of a complex mixture of unconjugated bile acids. The majority of these are unconjugated bile acids formed by bacterial deconjugation of primary bile acid conjugates. In addition, there are unconjugated bile acids entering from the colon. These include the 7-deoxy bile acids DCA and LCA as well as the 3β-hydroxy epimers of these bile acids. In animals such as rodents whose bile acids belong to the muricholic acid family, the 7-deoxy bile acids will be hyodeoxycholic (3α,6α-dihydroxy) and murideoxycholic (3α,6β-dihydroxy) acids. Other bacterial metabolites are likely to be present in trace proportions.

All of these bile acids are efficiently removed by the hepatocyte. Those not removed spill over into the systemic circulation.Thus, in healthy man, plasma bile acids are enriched in CDCA conjugates as these are extracted less efficiently than conjugates of cholic acid. Bile acids escaping first pass extraction are presented to the hepatocyte once again via hepatic arterial blood flow as well as by intestinal blood flow. As a result, the half life of any plasma bile acid is < 5 minutes.

The dominant source of plasma bile acids is intestinal absorption, not hepatocyte regurgitation. In animals with an external biliary fistula in whom bile acid synthesis is greatly increased, the level of plasma bile acids is too low to measure (48). In man, fasting state plasma bile acid levels average 2 μmol/liter and the concentration triples during digestion of a meal. Plasma bile acids are thus an "enterohepatic" quality, with the level fluctuating in direct proportion to the fluctuations of intestinal absorption. Because of the fluctuations in bile acid levels of peripheral venous plasma, measurement of bile acid levels has not proven to be superior valley to conventional tests (aminotransferase levels) for the detection of liver injury (49).

Bile acids not bound to albumin enter the glomerular filtrate. The amount of bile acids entering the glomerular filtrate is small because of albumin binding and the great efficiency of hepatic uptake. Those bile acids entering the glomerular filtrate are reabsorbed via ASBT in the proximal renal tubule. Urinary bile acid loss is less than 1 mg/day compared to the fecal loss of 200-600 mg/day.

5. MODELING THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

The extensive studies of the enterohepatic circulation of bile acids in health have permitted a physiological pharmacokinetic model to be developed for the three major bile acids in man – cholic acid (50), chenodeoxycholic acid (51), and deoxycholic acid (52). The model is a linear model and includes bile acid biotransformation, transport, and flow (between anatomical compartments). The model permitted simulation of the enterohepatic circulation of these bile acids in health and there was reasonable agreement between values obtained by simulation and those occurring *in vivo*. At present, there is not sufficient data on bile acid metabolism in any other species to permit such detailed modeling.

6. REGULATION OF THE ENTEROHEPATIC CIRCULATION

6.1. Regulation of input of primary bile acids

The enterohepatic circulation has at least two sites of regulation. The first is regulation of biosynthesis of bile acids in the hepatocyte. The second is regulation of intestinal transport in the ileal enterocyte. Both of these sites of regulation are under active investigation and will only be discussed in broad outline. Both may be classified as negative feedback mechanisms.

Regulation of bile acid biosynthesis appears to be controlled by the monomeric activity of conjugated bile acids in the hepatocyte. Such monomeric bile acid molecules enter the nucleus either as such or bound to the bile acid activated transcription factor FXR. The RXR-FXR heterodimer in association with additional coregulator molecules induce the synthesis of a repressor-like transcription factor (SHP). SHP, in turn, displaces a third transcription factor (HNF4) from the promoter of cyp7A1 (cholesterol 7α-hydroxylase) thereby downregulating bile acid biosynthesis (53). Also required for such downregulation is a protein. FGF-15, that is released by the ileal enterocyte (54). Normally, down regulation occurs in the pericentral hepatocytes of the liver. In health, bile acid biosynthesis is quite repressed, as bile acid feeding, at least in man, decreases bile acid synthesis by only 50%. When less bile acids return to the hepatocyte and the concentration inside the hepatocyte falls, bile acid biosynthesis increases. This occurs commonly in patients with ileal dysfunction or patients receiving bile acid sequestrants, as ileal dysfunction or sequestrant administration decreases not only the efficiency of bile acid absorption but also decreases the release of FGF-15.

In man, bile acid biosynthesis can increase ten fold. In the mouse, knockout of *Asbt,* results in a twenty-fold increase in bile acid biosynthesis (55). There is limited work suggesting that the increased bile acid biosynthesis occurs not only in pericentral hepatocytes, but there is progressive recruitment of hepatocytes toward the periportal zone of the hepatic lobule.

6.2. Regulation of intestinal conservation

Regulation of intestinal transport of bile acids has not been adequately characterized in man. It is clear that bile acid feeding must down regulate ileal transport, as bile acid secretion may or not increase or at most doubles (56). Invariably, a new steady state is reached, indicating down regulation of ileal transport. In the guinea pig and mouse, but not in the rat, bile acid feeding decreases ileal bile acid transport, and bile acid sequestrant administration increases ileal bile acid transport (57). The negative feedback is considered to be regulated by FXR, as in the hepatocyte. Again, as in the liver, upregulation of intestinal transport may involve recruitment of more proximal enterocytes. There must be additional factors regulating ileal bile acid transport, as bile duct ligation (58) and parenteral feeding (59) both decrease ileal bile acid transport, yet the intracellular concentration of bile acids in the ileal enterocyte should decrease with these maneuvers. Regulation is considered to be mediated by ASBT, the apical bile acid transporter, rather than the basolateral transporters.

7. METHODS FOR QUANTIFYING THE ENTEROHEPATIC CIRCULATION

7.1. Measurement of bile acid synthesis (and loss)

The rate of synthesis of primary bile acids can be determined by isotope dilution, as primary bile acid metabolism follows first order kinetics (60). In man, this is commonly done by administering a bile acid tagged with the radioactive isotopes ^{14}C or ^{3}H or the stable isotopes ^{13}C or ^{2}H. If radioactive bile acids are given, bile is sampled. If stable isotopes are given, either bile or plasma can be sampled (61). Sampling is done daily for several days. In any event, bile acids are isolated from bile or plasma, and the natural logarithm of the decline in specific activity (or atoms per cent excess) is plotted against time. The intercept of the axis with zero time divided by the dose administered gives the functional pool size, and the slope of the curve gives the fractional turnover rate. The product of the pool size and the fractional turnover rate gives the daily synthesis rate. A similar procedure can be performed to measure the input of newly formed secondary bile acids. In man, the input of newly formed deoxycholic acid from the colon is 20 to 50% of cholic acid synthesis. Probably, the input of lithocholic acid is a still smaller fraction of CDCA synthesis.

Each bile acid has its own input and its own efficiency of intestinal conservation. For a given input, the size of the bile acid pool is determined by the efficiency of intestinal absorption. The sum of the individual bile acid pools gives the total bile acid pool. The proportion of each pool size in the total bile acid pool determines biliary bile acid composition.

The efficiency of intestinal conservation of a secondary bile acid may exceed greatly that of its primary bile acid precursor. In the rabbit, the pool size of deoxycholic acid is more than forty times that of its precursor, cholic acid (62). In man, the deoxycholic acid pool is usually smaller than that of cholic acid, although in some patients, the deoxycholic acid pool may exceed that of cholic acid. When bile acids such as CDCA or DCA are fed, their individual pool size expands greatly because of the increased input.

Bile acid biosynthesis may also be measured by determining fecal bile acid output, provided the fecal collection is complete and the analytical methodology is accurate. A simple enzymatic method for measuring 3α-hydroxy bile acids has been reported (63), but such a method will not measure 3-sulfated bile acids or 3β-hydroxy bile acids which may constitute up to 30% of fecal bile acids. The ideal method for measuring all fecal bile acids involves class separation by ion exchange chromatography followed by liquid chromatography- mass spectrometric analysis of each class (64).

The bile acid exchangeable pool size can be measured by isotope dilution as discussed above. The

Table 2. Functions (micellar and non-micellar) of bile acids in mammals

Whole organism
• Elimination of cholesterol
Liver
• Hepatocyte
• Insertion of canalicular bile acid and phospholipid transporters
• Induction of bile flow and biliary lipid secretion
• Promotion of mitosis during hepatic regeneration
• Regulation of gene expression by activation of FXR
• Endothelial cells
• Regulation of hepatic blood flow via activation of TGR5
Biliary Tract
• Lumen
• Solubilization and transport of cholesterol and organic anions
• Solubilization and transport of heavy metal cations
• Cholangiocyte
• Stimulation of bicarbonate secretion via CFTR and AE2
• Promotion of proliferation when obstruction to bile flow
• Gallbladder
• Modulation of cAMP-mediated secretion
• Promotion of mucin secretion
Small intestine
• Lumen
• Micellar solubilization of dietary lipids
• Cofactor for bile salt dependent lipase
• Antimicrobial effects
• Ileal enterocyte
• Regulation of gene expression via nuclear receptors
• Ileal epithelium
• Secretion of antimicrobial factors (FXR mediated)
Large Intestine
• Colonic epithelium and muscular layer
• Promotion of defecation by increasing propulsive motility
• Colonic enterocyte
• Modulation of fluid and electrolyte absorption
Brown adipose tissue
• Promotion of thermogenesis via TGR5

plasma level of "C4" -7α-hydroxy-cholest-4-ene-3-one, an intermediate in bile acid biosynthesis has been shown to rise in direct proportion to bile acid biosynthesis (65).

Biliary bile acid composition which is measured using GC-MS or LC-MS provides information on relative pool sizes. Bile acid secretion is a flux and can only be measured by an indicator dilution procedure (40). Plasma spillover of bile acids can be inferred by measuring plasma bile acids. The plasma bile acid level is determined by the instantaneous transport of bile acids into the plasma compartment from the intestinal epithelial cells and the instantaneous removal of bile acids by hepatocytes.

Bile acid deconjugation during enterohepatic cycling can be determined by labeling the steroid and amino acid (glycine or taurine) moieties separately and using the isotope dilution technique. The rate of deconjugation can also be assessed crudely by measuring $^{14}CO_2$ in breath after administration of cholyl-1-^{14}C-glycine (44).

8. ENTEROHEPATIC CYCLING OF DRUGS

No drug has been shown to have an enterohepatic circulation with the features of the enterohepatic circulation of bile acids. For a substance to enter canalicular or ductular bile, it must be a substrate for one of the apical transporters. Generally drugs will enter bile after having undergone a phase II biotransformation, i.e. they will be conjugated with sulfate, glucuronate, glutathione, or glycine. All such conjugates are membrane-impermeable. If a drug were to be secreted into bile in membrane permeable form, it would be absorbed by the bile duct epithelium and appreciable proportions would not reach the gallbladder. Drug conjugates are not hydrolyzed by pancreatic enzymes, and enterocyte transporters for drug conjugates have not yet been identified. As yet, no drug has been identified that is transported by ASBT. Drug conjugates can undergo bacterial hydrolysis in the distal small intestine. In this case, the unconjugated drug (aglycone) could be absorbed passively, if membrane permeable. The drug could be conjugated with sulfate or glucuronate in the ileal enterocyte and returned to the intestinal lumen. Or the drug could return to the liver, be reconjugated and excreted in bile. In principle, such a pseudo-enterohepatic circulation could lead to the accumulation of a recycling pool; but such has not been shown experimentally to the best of this reviewer's knowledge. There is some evidence for bilirubin glucuronide being hydrolyzed distally in some patients with absorption of unconjugated bilirubin (66).

9. FUNCTIONS OF THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

9.1. Hepatobiliary functions

Bile acids are the major chemical form in which cholesterol is eliminated in vertebrates. However, enterohepatic cycling is not required for this pathway. Enterohepatic cycling results in micellar concentrations of bile acids in bile and intestinal content, and the functions of the enterohepatic circulation may be considered to result from micelle formation. Table 2 summarizes all recognized functions of bile acids, both micellar and non-micellar.

In the liver, bile acids induce canalicular bile flow by their osmotic properties as discussed above. In the biliary tract, bile acids induce the secretion of phospholipid resulting in the formation of mixed bile acid-phospholipid micelles. Phospholipid molecules flipflop across the canalicular membrane, a movement mediated by mdr2 (3); the result is the formation of hemivesicles that bud from the canalicular membrane. Bile acids adsorb to these hemivesicles and solubilize them in mixed micelles. In species with a relatively high phospholipid/bile acid ratio in biliary lipids, the phospholipid is predominantly phosphatidylcholine (67). The presence of phospholipid diminishes the monomeric activity of bile acids as well as the cytotoxicity of bile, thereby preventing injury to the biliary ductular epithelium, at least in those species whose bile acids are hydrophobic. Absence of biliary phospholipid in the mouse can be achieved by knockout of the mdr2 gene; such animals develop a severe cholangitis (68). The mixed micelle can also serve as a sink for xenobiotics secreted into bile, lowering their monomeric activity. In man, the mixed micelle serves to solubilize biliary cholesterol. However, in most species, biliary cholesterol concentrations (expressed as cholesterol/bile acid ratio) are quite low, and cholesterol solubilization is not an important function of the mixed bile acid-phospholipid micelle (67). If xenobiotics were secreted into bile in membrane-permeable form, then such micellar trapping would

diminish passive absorption by the biliary ductular epithelium. Bile is a route for the excretion of heavy metal cations that cannot be eliminated in urine because of their tight binding to albumin. The mixed micelle, is a polyanion and can therefore bind heavy metal cations electrostatically.

9.2. Intestinal functions

In the small intestine, the phosphatidylcholine of the mixed micelle is hydrolyzed by pancreatic phospholipase to lysophosphatidylcholine and fatty acid, both of which are absorbed. Pancreatic lipase generates fatty acid and monoacylglyceryl (monoglyceride) on the surface of the oil phase droplets. These lipolytic products form a liquid crystalline phase which is solubilized into mixed fatty acid-monoglyceride-bile acid micelles. Monoglycerides and saturated fatty acids C_{16} or longer have low aqueous solubility and these form mixed micelles with the conjugated bile acid anions. Such micelle formation increases their concentration in the aqueous phase by three orders of magnitude. At the same time, solubilized lipids diffuse more slowly in mixed micelles than as monomers. However, the effect of decreasing the diffusion constant is much smaller. Therefore the net effect of micellar solubilization is to enhance the diffusion of insoluble lipids through the unstirred water layer coating the small intestinal epithelial cells (69). Solubilization of fatty acids accelerates their absorption, and in health most fatty acid is absorbed by the mid-jejunum. In the absence of bile acids, insoluble fatty acids can still be absorbed to some extent. However, absorption is much slower, relying on diffusion alone and is spread throughout the small intestine. The most important function of mixed micelle formation in the small intestine is the solubilization of fat soluble vitamins (A,D,E,K) which is required for their absorption.

Bile acids have two additional functions in the small intestine. These are concentration-dependent, but it is not clear that micelle formation is required.

The first of these is denaturation of dietary proteins, thereby promoting their hydrolysis by pancreatic proteolytic enzymes (70). This is a recent *in vitro* finding, and physicochemical details have yet to be elucidated. In pigs, diversion of bile to the distal small intestine, caused no change in fecal nitrogen, but a marked increase in urinary nitrogen (71). This could have resulted from dumping of malabsorbed protein into the colon, liberation of ammonia by bacterial degradation of amino acids, absorption of the ammonia, and conversion by the liver to urea, which in turn is excreted in urine. Nonetheless, children with cholestatic liver disease who have low intraluminal bile acid concentrations had no apparent problems with protein absorption, even when they were ingesting cholestyramine, a bile acid binding resin that should lower intraluminal bile acid concentrations still further. Therefore, the biological significance of this effect of bile acids is unclear.

Bile acids also have an antimicrobial effect in the small intestine. There is a direct intraluminal effect, related to the detergent properties of the bile acid anion or to the solubilized fatty acid or both. In addition, there is an indirect effect. It has long been known that biliary diversion or bile duct ligation in animals causes bacterial overgrowth in the small intestine. Rats with cirrhosis induced by carbon tetrachloride administration develop impaired bile acid secretion, bacterial overgrowth in the small intestine, bacterial translocation to abdominal lymph nodes, and endotoxemia. The feeding of conjugated bile acids to such rats abolishes bacterial overgrowth, and decreases bacterial translocation and endotoxemia (72).

Bacterial overgrowth occurring in association with bile duct ligation is also abolished by the administration of an FXR agonist, an effect that does not occur in the FXR knockout mouse (73). The mechanism of this indirect antimicrobial effect of bile acids mediated by an FXR-dependent pathway has not been clarified.

There can be other functions of a micellar phase that have escaped scrutiny. As in the biliary tract, the mixed micelles should bind calcium as well as heavy metal cations. Decreased concentrations of bile acids apparently leads to increased monomeric activity of fatty acids. This results in calcium soap formation that lowers luminal Ca^{2+} activity and this in turn permits dietary oxalate to remain in solution. When such soluble oxalate anions pass into the colon, they are absorbed, causing hyperoxaluria that in turn can lead to nephrolithiasis (74).

In addition the presence of a micellar phase should also provide a sink for bubbles of carbon dioxide gas that might form when pancreatic bicarbonate is neutralized by acidic small intestinal content.

9.3. Hormonal functions

Very recently, bile acids have been shown to act on a G-coupled receptor TGR5 present in adipocytes. Activation of TGR5 leads to thyroxin generation and improved thermogenesis (75). However, it remains unclear whether the level of bile acids in systemic venous plasma is sufficiently high in health to act on this receptor. In the neonate, plasma bile acid levels are higher, raising the possibility that bile acids do promote thermogenesis. Bile acids also act on a G-coupled protein receptor in hepatic endothelial cells, and such activation may modulate sinusoidal blood flow (76).

It is beyond the scope of this review to discuss the use of bile acids as therapeutic agents. Bile acids are currently being used for inborn errors of bile acid biosynthesis, cholestatic liver disease; other uses are under investigation (76).

10. EPILOGUE

It has been some 4 centuries since Giovani Borelli discussed the enterohepatic circulation in his monumental treatise on biomechanics. The work of countless investigators has led to elucidation of the form and function of the enterohepatic circulation in man. Much remains to be done to define the form of the enterohepatic circulation in other vertebrates. In these days when

recycling has become official government policy in many countries, the enterohepatic circulation can be considered the way that evolution developed to recycle valuable functional molecules.

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Abbreviations: EHC, enterohepatic circulation; CDCA, chenodeoxycholic acid; LCA, lithocholic acid, MRP, multidrug resistance protein, ASBT, apical sodium-dependent bile acid transporter; OATP, organic anion transporting polypeptider; NTCP, sodium taurocholate cotransporting polypeptide, FGF, fibroblast growth factor

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