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Review

Bile Acids: Chemistry, Pathochemistry, Biology, Pathobiology, and Therapeutics

A. F. Hofmann* **and L. R. Hagey**

Department of Medicine, University of California, San Diego, La Jolla, California 92093-0063 (USA),
Fax: 858 534-3338, email: ahofmann@ucsd.edu

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Abstract. Bile acids and bile alcohols in the form of their conjugates are amphipathic end products of cholesterol metabolism with multiple physiological functions. The great variety of bile acids and bile alcohols that are present in vertebrates are tabulated. Bile salts have an enterohepatic circulation resulting from efficient vectorial transport of bile salts through the hepatocyte and the ileal enterocyte; such transport leads to the accumulation of a pool of bile salts that cycles between the liver and intestine. Bile salt anions promote lipid absorption, enhance tryptic cleavage of dietary proteins, and have antimicrobial effects. Bile salts are signaling molecules, activating nuclear receptors in the hepatocyte and ileal enterocyte, as well as an increasing number of G-protein coupled receptors. Bile acids are used therapeutically to correct deficiency states, to decrease the cholesterol saturation of bile, or to decrease the cytotoxicity of retained bile acids in cholestatic liver disease.

Keywords. Enterohepatic circulation, bile acid metabolism.

Introduction

This review will attempt to give an overview of bile acid chemistry and biology in health and disease, as well as to discuss briefly the current therapeutic use of bile acid agonists and antagonists. The subject is large and many topics will be covered only superficially. Reviews will be cited rather than original papers because of space limitations.

Bile acid chemistry

Chemistry of primary bile acids and bile alcohols

Bile acid and bile alcohol chemistry is complex because of the great variety of chemical structures in naturally occurring compounds, as well as the complexity of nomenclature. The great number of bile acids and bile alcohols occurring in nature can be explained by the evolution of multiple biochemical pathways that serve to convert cholesterol, a poorly soluble membrane lipid, into conjugated bile acids or bile alcohols – highly water-soluble, amphipathic, membranolytic molecules. The complexity of bile acid and bile alcohol nomenclature arises from these compounds being named long before their structure was known. Bile acids were isolated from bile early in the 19th century, and the field of bile acid chemistry preceded the development of classical biochemistry [1].

There is no satisfactory collective term that includes all of the naturally occurring bile acids and bile alcohols. The term "cholanoid" should encompass all C_{24} compounds and "cholestanoids" all C_{27} compounds. The term "bile acid" denotes both C_{24} and

* Corresponding author.

C_{27} bile acids, but cannot include bile alcohols. To simplify the understanding of bile acid biology, we and others have suggested that it is useful to divide cholanoids and cholestanoids into three great classes, namely, the C_{27} bile alcohols, the C_{27} bile acids, and the C_{24} bile acids. These three classes encompass most of the naturally occurring cholanoids and cholestanoids. The C_{27} bile alcohols and C_{27} bile acids contain the C_8 side chain of cholesterol, whereas the C_{24} bile acids contain a C_5 side chain. There are frogs whose bile acids have chains containing C_7 or C_9 side chains, and we have found a fish that has a C_4 side chain. However, these examples are extremely rare exceptions to the rule that C_{27} cholestanoids and C_{24} cholanoids constitute the end products of cholesterol in vertebrates.

Cholesterol is a C_{27} sterol with a double bond at C-5 and, as noted, an isooctane side chain. All compounds in the three classes described above possess the C-3 hydroxy group of cholesterol and a C-7 hydroxy or oxo group, because the rate limiting step in their biosynthesis from cholesterol is cholesterol 7α-hydroxylase (CYP7A1). All three classes have a side chain with a carboxyl group (bile acids) or a primary alcoholic group (bile alcohols). Therefore, there are three default compounds: The first is the C_{27} alcohol with a hydroxyl group at C-27 and hydroxyl groups at C-3 and C-7 on the steroid nucleus (no trivial name proposed). The second is the C_{27} bile acid with a carboxyl group at C-27 and 3α and 7α-hydroxy substituents on the steroid nucleus (which might be termed cholestanoic acid). The third is the C_{24} bile acid with a carboxyl group at C-24 and again with 3α,7α-dihydroxy substituents (termed chenodeoxycholic acid, CDCA). During bile acid biosynthesis from cholesterol, the default compounds may undergo additional hydroxylations on the steroid nucleus, the side chain, or both to give the spectrum of naturally occurring cholestanoids and cholanoids. Two additional modifications occur in C_{24} bile acids. The first is epimerization of the 7α-hydroxy group, forming a 3α,7β-dihydroxy bile acid termed ursodeoxycholic acid (UDCA). UDCA is a major bile acid in nutrias, bears, and beavers. The second is oxidation of the C-7 hydroxy group to give a 3α-hydroxy, 7-oxo-bile acid as found in cavimorphs and the koala.

In "modern" mammals, C_{24} bile acids predominate. Cholic acid was discovered and named nearly a century before the isolation and naming of CDCA. The awkward name chenodeoxycholic acid was proposed to indicate that it was a major bile acid in the goose, and that it contained one less oxygen atom (by elemental analysis) than cholic acid. It was not known at that time that it would prove to be the building block for all C_{24} bile acids.

In most C_{24} and C_{27} bile acids, the cyclopentanophenanthrene nucleus, composed of rings A, B, C, and D, is saturated. During bile acid biosynthesis, there is isomerization of the $\Delta^{5,6}$ double bond of cholesterol to a $\Delta^{4,5}$ double bond which is then reduced to give 5β bile acids in which the A and B rings are in *cis* configuration (denoted by a 5β hydrogen atom). However, in some reptiles, and even to a very limited extent in mammals, the reduction differs and the resulting bile acid has an A/B *trans* configuration (denoted by a 5α hydrogen atom). Such 5α bile acids are described in trivial nomenclature as 'allo' bile acids. In the C_{27} bile alcohols, compounds with a 5α configuration are common. However, for historical reasons, the term 'allo' is not used.

Bile acids that are synthesized from cholesterol in the hepatocyte are termed *primary* bile acids. Bile acids that are formed by bacterial modification of primary bile acids are termed *secondary* bile acids. The most common primary C_{24} bile acids, in addition to CDCA, are cholic acid (3α,7α,12α-trihydroxy), avicholic acid (3α,7α,16α-trihydroxy), hyocholic acid (3α,6α,7α-trihydroxy) and beta-muricholic acid (3α,6β,7β-trihydroxy). The conversion of cholesterol to chenodeoxycholic acid, the root C_{24} bile acid is shown in Fig 1a which emphasizes the change in configuration of the A/B ring juncture.

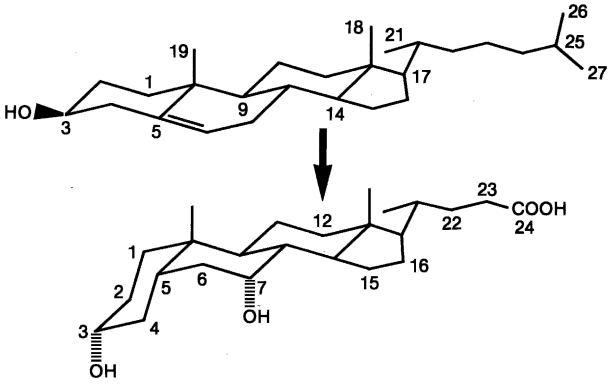

Figure 1. Conversion of cholesterol to CDCA, the root bile acid. Major changes are 1) hydroxylation at C-7; 2) modified β-oxidation of the side chain that results in shortening of the C_8 isooctane side chain to a C_5 isopentanoic side chain; 3) epimerization of the C-3 hydroxy group; and 4) reduction of the double bond to give a 5β-bile acid in which the A/B ring juncture is in the *cis* configuration.

Input of secondary bile acids

During their enterohepatic cycling, bile acids are exposed to bacterial enzymes. In the colon of animals with a cecum, anaerobic bacteria remove the hydroxy group at C-7 to form a new class of bile acids termed 7-deoxy bile acids. (When primary bile acids are modified by bacterial enzymes by removal, oxidation, or epimerization of the nuclear hydroxyl groups, the

resulting bile acid is termed a secondary bile acid.) The most common C_{24} 7-deoxy bile acids are lithocholic acid (3α-hydroxy, LCA) formed by bacterial 7-dehydroxylation of CDCA and deoxycholic acid (3α12α-dihydroxy, DCA) formed by bacterial 7-dehydroxylation of cholic acid. C_{27} bile acids also undergo bacterial modification of their hydroxy groups. The 7-deoxy bile acids and other minor, secondary bile acids are absorbed in part and return to the liver where they may or may not be structurally altered during hepatocyte transport (see below).

Biliary bile acids usually consist of mixtures of individual bile acids or bile alcohols; and more than one bile acid class may be present. A high pressure liquid chromatogram of biliary acids often consists of three to ten individual compounds. In most species with C_{24} or C_{27} bile acids, primary bile acids predominate in bile. In species that form 7-deoxy bile acids, these may or may not be present in biliary bile acids. Occasionally, secondary bile acids predominate in biliary bile acids. A notable example is the rabbit, whose major bile acid is DCA. Other species in which the proportion of DCA exceeds that of CA are the sloth, the sperm whale, and some primates. Table 1 shows the dominant bile acid class or classes present in different vertebrate orders, based on results from our own laboratory and that of many workers in the past, in particular G. A. D. Haslewood [2].

A list of most C_{24} bile acids occurring in adult mammals is given in Table 2.

Table 3 lists bile acids occurring in non-mammals, Table 4 lists C_{27} bile acids; these occur as major biliary acids in amphibians, reptiles, ancient birds, and two mammalian families (Equidae and Primates) and Table 5 lists C_{27} bile alcohol sulfates; these occur in cartilaginous as well as in early and cyprinid fish, amphibians, ancient birds, and ancient mammals.

Fig. 2 shows the conversion of cholesterol to the three main classes of bile salts. Hydroxy groups occurring in many vertebrate families are indicated by the broad arrows; those occurring in only a few species are indicated by the small arrows.

Reviews on bile acid chemistry and biology are available [3, 4]. Still older work is summarized in the Elsevier Encyclopedia of Organic Chemistry [5] and the classic textbook on Steroids by the Fiesers [6]. The laboratory of T. Iida has systematically prepared most of the natural bile acids and their epimers, as well as new, potential natural bile acids [7]. The Falk Foundation of Freiburg, Germany has sponsored a biennial symposium on bile acids for the past 38 years. The proceedings of the meeting serve to document the advances made in understanding these multifaceted molecules [8].

Table 1. Occurrence of major bile acid classes in vertebrates.

Vertebrate order		C_{27} bile alcohols	C_{27} bile acids	C_{24} bile acids
Fish				
	Ancient	x		
	Cartilaginous	x		
	Cyprinids	x		
	Transitional	x		x
	Bony			x
Amphibia		x (and higher homologues)	x	x
Reptiles				
	Varanids		x	
	Lizards			x
	Turtles		x	
	Crocodiles		x	x
	Snakes			x
Birds				
	Ancient	x	x	x
	Modern			x
Mammals				
	Ancient	x	x	x
	Modern			x

Bile Acid Conjugation

After their biosynthesis from cholesterol, bile acids and bile alcohols are conjugated. C_{27} bile alcohols are conjugated with sulfate. Esterification with sulfate occurs at C-27, the primary alcoholic group at the end of the C_8 side chain. C_{27} bile acids are conjugated exclusively with taurine. In such taurine conjugation, the carboxyl group of the bile acid is linked to the amino group of taurine in amide linkage. C_{24} bile acids are conjugated with either glycine or taurine. LCA, the toxic 7-deoxy derivative of CDCA, undergoes "double" conjugation during hepatocyte transport. It is amidated at C-24 and esterified with sulfate at C-3. The presence of a sulfate group at C-3 prevents intestinal absorption, and as a result, the compound is rapidly eliminated. Two types of names are used for the common natural C_{24} conjugated bile acids. The older name was taurocholate; a more chemically correct name would be cholyltaurine [9].

The term "conjugation" was used throughout the 20th century to denote N-acyl amidation with glycine or taurine. When it was shown that bile acids could also be sulfated or glucuronidated, it became necessary to use the term N-acyl amidation or just "amidation" to distinguish amidates from other conjugates of bile acids. Presently, five types of conjugation of C-24 bile acids are recognized. The first is N-acyl amidation

Table 2. C24 Bile Acids of Mammals.*

Trivial name	A/B RJ, A ring substituents#	B ring substituents	C & D ring substituents	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 BA in rabbit
Chenodeoxycholic	5β,3αOH	7αOH		None	L	Many species
Ursocholic	5β,3αOH	7βOH	12αOH	None	L or I	Trace BA in man
Ursodeoxycholic	5β,3αOH	7βOH		None	L or I	Major BA: nutria, bear, beaver
Lithocholic	5β,3αOH			None	I	Many species
(none proposed)	5β,3αOH		15αOH	None	I	Wombat
Lagodeoxycholic	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,3αOH,1αOH	7αOH		None	L	Australian opossum
(none proposed)	5β,3αOH,1βOH	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^{22})	L	Paca (caviomorph)
(none proposed)	5β,3αOH	7βOH		None (Δ^{22})	L	Agouti
(none proposed)	5β,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
Bitocholic	5β,3αOH		12OH	23-(*R*)-OH	L	Minor BA in sea mammals
Isolithocholic	5β,3βOH				I	Major fecal BA in man
Isodeoxycholic	5β,3βOH		12αOH		I	Major fecal BA in man
Isochenodeoxycholic	5β,3βOH	7αOH			I	Minor fecal BA in man
Isoursodeoxycholic	5β,3βOH	7βOH			I	Minor fecal BA in man
Isocholic	5β,3βOH	7αOH	12αOH		I	Minor fecal BA in man

* Many hydroxy-oxo bile acids as well as other epimers of DCA and CDCA, trace BA in both biliary and fecal BA are not listed. Complex mixtures of C_{27} bile alcohol (sulfates) and C_{24} bile acids occur in the elephant, manatee, hyrax, rhinoceros, and some caviomorphs. 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: BA, bile acid; A/B RJ, A/B ring juncture; L, liver; I, intestinal bacteria.

with glycine or taurine; the second is sulfation; the third is ester glucuronidation at C-24; the fourth is ethereal conjugation at C-3 (and possibly at other nuclear sites); and the fifth is N-acetylglucosamination at C-7 in bile acids such as UDCA with a β-hydroxy group. Bile acids, as noted, may undergo conjugation on both the steroid nucleus and the side chain. As yet, glutathione, glucose, or xylose conjugates have not been identified. Fig. 3 shows the major bile acids present in human bile.

Table 3. Chemical structure of most C_{24} bile acids identified in healthy non-mammals.*

Trivial name	A ring	B ring	C & D ring	Side chain	Source	Occurrence
5α (allo) bile acids						
Allochenodeoxycholic	3αOH	7αOH			L	Major BA in reptiles, birds
Alloavicholic	3αOH	7αOH	16αOH		L	Major BA in reptiles
Allocholic	3αOH	7αOH	12αOH		L	Loon, minor BA in reptiles
Allodeoxycholic	3αOH		12αOH		I	Major BA in reptiles
5β bile acids						
Chenodeoxycholic	3αOH	7αOH			L	Many species
Cholic	3αOH	7αOH	12αOH		L	Many species
	3αOH	7oxo	12αOH		L or I	Birds
	3αOH	7αOH	12oxo		L or I	Eagle
Cygnocholic	3αOH	7αOH	15αOH		L	Geese, plovers, swans
Avicholic	3αOH	7αOH	16αOH		L	Birds
Avideoxycholic	3αOH		16αOH		L or I	Snakes, herons
	3αOH	7αOH	12αOH,16αOH		L	Snakes
	3αOH		12αOH, 16αOH		L or I	Snakes
	1βOH,3αOH	7αOH			L	Pheasants, pigeons
	4βOH,3αOH	7αOH			L	Pheasants
	5βOH,3αOH	7αOH			L	Pheasants
	3αOH	7αOH	12αOH	Δ^{22}	L or I	Snakes
Haemulcholic	3αOH	7αOH		22*S*OH	L	Fish
Phocaecholic	3αOH	7αOH		23*R*OH	L	Snakes, birds
	3αOH	7αOH	12αOH	23*R*OH	L	Snakes, birds
Bitocholic	3αOH		12αOH	23*R*OH	I	Snakes

* Abbreviations: L, liver; I, intestinal bacteria.

For C_{27} bile alcohols, conjugation with sulfate converts a water insoluble uncharged molecule into a highly water soluble molecule that is present in anionic form in body fluids. For a C_{27} or C_{24} bile acid, conjugation with taurine results in the formation of a molecule that is fully ionized and highly soluble at small intestinal pH during digestion (pH 6–7). In addition, and perhaps most importantly, conjugation results in a molecule that is always negatively charged and is therefore impermeable to cell membranes. Moreover, the bile salt molecule is too large to diffuse through the paracellular junctions of the biliary tract and small intestine. Impermeability to the apical membrane of cholangiocytes and enterocytes and the paracellular junctions between these cells is a key factor in promoting the high intraluminal concentration of conjugated bile acids in the biliary tract and small intestine.

For C_{24} bile acids, conjugation with glycine converts a weak acid (pK_a of 5) to a slightly stronger acid (pK_a of 4). The result is increased solubility and ionization at the pH conditions prevailing during digestion. In man, intubation studies have shown that most glycine conjugated bile acids are in solution during digestion.

Bile acids as amphipathic molecules

Natural primary bile acids are planar amphipathic molecules having a hydrophobic side (the β face of the bile acid molecule) containing no substituents and a hydrophilic side (the α face) containing the hydroxy groups. Bile acid anions self associate over a fairly narrow concentration range to form micelles. It is convenient to name the approximate midpoint of this range the critical micellization concentration (CMC), based on a vast literature on the self-association of surfactants. The CMC of individual C_{24} bile acids is best measured by changes in surface tension with concentration, using a maximum bubble pressure device [10]. It is also possible to measure the CMC by many other techniques such as dye solubilization, light scattering or changes in the fluorescence of a probe molecule [11]. The CMC is lowered by sodium ion concentration, so that measurements of the CMC are commonly made in 0.15 M Na^+ (total Na^+ concentration) to simulate *in vivo* conditions. Such

Table 4. Chemical structure of C_{27} bile alcohols.*

A ring substituents	B ring substituents	C & D ring substituents	Side chain substituents	Occurrence
5α (allo) (A/B *trans*) bile alcohols				
3βOH	**7αOH**		**27OH**	Hagfish
3βOH	**7αOH**	12αOH	26OH,**27OH**	Coelacanth
3βOH	**7αOH**	16αOH	**27OH**	Hagfish
3oxo	**7αOH**	12αOH	**27OH**	Lamprey
3αOH	**7αOH**		**27OH**	Hagfish
3αOH	**7αOH**	12αOH	**27OH**	Fish
3αOH	**7αOH**	12αOH	24OH,**27OH**	Cartilaginous fish
3αOH	**7αOH**	12αOH	25OH,**27OH**	Lungfish, amphibians
3αOH	**7αOH**	12αOH	26OH,**27OH**	Fish
3αOH	**7αOH**	16αOH	**27OH**	Hagfish
3αOH	**7αOH**	12αOH	25OH, 26OH,**27OH**	Amphibians
5β (*A/B cis*) bile alcohols				
3βOH	**7αOH**		**27OH**	Hagfish
3βOH	**7αOH**	12αOH	26OH,**27OH**	Fish
3βOH	**7αOH**	16αOH	**27OH**	Hagfish
3αOH	**7αOH**		**27OH**	Amphibians, rhinoceros
3αOH	**7αOH**		25OH, **27OH**	Ancient mammals
3αOH	**7αOH**	12αOH	24OH,**27OH**	Chimaera
3αOH	**7αOH**	12αOH	25OH,**27OH**	Amphibians
3αOH	**7αOH**	12αOH	24OH, 26OH,**27OH**	Cartilaginous fish
3αOH	**7αOH**	12αOH	25OH, 26OH,**27OH**	Amphibians
3αOH	6αOH**, 7αOH**	12αOH	25OH,**27OH**	Manatee
3αOH	6αOH**, 7βOH**	12αOH	25OH,**27OH**	Manatee
3αOH	6αOH, **7αOH**	12αOH	25OH,**27OH**	Manatee
3αOH	6βOH, **7αOH**	12αOH	25OH,**27OH**	Manatee
3αOH	6βOH**, 7βOH**	12αOH	25OH,**27OH**	Manatee
2βOH**,3αOH**	**7αOH**	12αOH	**27OH**	Arapaima
2βOH**,3αOH**	**7αOH**	12αOH	26OH,**27OH**	Arapaima

* Default substituents are shown in bold font. Trivial names are not given, as these are complex and do not provide information on the chemical structure. They are available in the literature.

measurements show that dihydroxy bile acids have lower CMC values than those of trihydroxy bile acids. The CMC correlates inversely with the area of the hydrophobic face of the bile acid molecule. It is possible to synthesize an epimer of cholic acid in which all hydroxy groups are in the β configuration, i.e. 3β,7β,12β-trihydroxy. In such a bile acid, the hydrophobic area is much smaller, and there is no micelle formation. Conjugation with glycine or taurine results in a slight lowering of the CMC [10]. Fig. 4 shows a space-filling model of taurocholate viewed from the side, so that its hydrophobic and hydrophilic faces are readily seen.

No data are available on the CMC values of C_{27} bile acids, but their CMC values should be lower than those of the corresponding C_{24} homologues because of their longer side chain. The CMC of one bile alcohol sulfate (5α-cyprinol sulfate) has been measured and found to be similar to that of the taurine conjugate of cholic acid [12].

Solutions of typical anionic detergents, e.g. dodecyl sulfate, exhibit a narrow temperature range over which the excess of molecules suddenly changes from a crystalline state to a micellar state. The temperature at which this occurs is when the concentration of the monomer reaches the CMC. This narrow temperature range has been termed the critical micellization temperature (CMT) or Krafft point, and for most natural C_{24} bile acids it is well below the freezing point of water. Two notable exceptions are lithocholate for

Table 5. Chemical structure of C_{27} bile acids.*

A ring substituents	B ring substituents	C & D ring substituents	Side chain conformation	Side chain substituents	Occurrence
5α (allo) (A/B trans) bile acids					
3oxo	**7αOH**	12αOH	**?**	**27COOH**	Lamprey
3αOH	**7αOH**		25*R*	**27COOH**	Reptiles
3αOH	**7αOH**	12αOH	25*R*	24*R*OH,**27COOH**	Reptiles
3αOH		12αOH	25*R*	24*R*OH, **27COOH**	Reptiles
5β (*A/B cis*) bile acids					
3αOH	**7αOH**		**?**	**27COOH**	Reptiles, birds
3αOH	**7αOH**	12αOH	?	**27COOH**	Reptiles, birds, platypus, loris
3αOH	**7-oxo**	12αOH	25*S*	**27COOH**	Birds
3αOH	**7αOH**	16αOH	25*R*	**27COOH**	Birds
3αOH	**7αOH**	16αOH	25*S*	**27COOH**	Birds
3αOH	**7αOH**		?	22*S*OH, **27COOH**	Turtles
3αOH	**7αOH**	12αOH	?	22*S*OH, **27COOH**	Turtles
3αOH		12αOH	?	22*S*OH, **27COOH**	Turtles
3αOH	**7αOH**		25*R*	24*R*OH, **27COOH**	Reptiles
3αOH	**7αOH**		25*S*	24*R*OH, **27COOH**	Reptiles, birds
3αOH	**7αOH**	12αOH	25*R*	24*R*OH, **27COOH**	Frogs, reptiles
3αOH	**7αOH**	12αOH	25*S*	24*R*OH, **27COOH**	Frogs, reptiles birds
3αOH		12αOH	25*R*	24*R*OH, **27COOH**	Reptiles
3αOH		12αOH	25*S*	24*R*OH, **27COOH**	Reptiles
3αOH		12αOH	25*R*	24*R*OH, **27COOH**	Frogs
3αOH		12αOH	25*S*	24*R*OH, **27COOH**	Frogs

* Default substituents are shown in bold font. 7-deoxy bile acids are likely to be secondary bile acids generated by bacterial 7-dehydroxylation.

which the CMT of its sodium salt is above 75 °C and murideoxycholate (3α,6β-dihydroxy), whose CMT is > 90 °C [13]. No information is available on the CMT of C_{27} bile acids, but the CMT of a given C_{27} bile acid should be higher than that of its corresponding C_{24} homologue based on the properties of homologous alkyl sulfonate detergents.

Conjugated bile acids are exposed to relatively high concentrations of Ca^{2+} ions in the biliary tract and small intestine, as the paracellular junctions are permeable to Ca^{2+} ions. Resistance to precipitation by Ca^{2+} ions is an essential physicochemical property for digestive surfactants. Solutions of the taurine conjugates of natural bile acids are resistant to precipitation by added Ca^{2+} ions. Indeed, if calcium salts of taurine conjugated bile acids are prepared synthetically, they are water soluble. The solubility products of calcium salts of glycine conjugated bile acids are quite low, but supersaturated solutions are metastable and do not precipitate insoluble Ca^{2+} salts from solution for weeks to months. Solutions of dihydroxy and monohydroxy unconjugated bile acids are highly sensitive to the addition of Ca^{2+} ion, and their calcium salts quickly precipitate from solution [13]. No information is available on the solubility products of Ca^{2+} salts of C_{27} bile acids. The solubility product of the calcium salt of one C_{27} bile alcohol sulfate (5α-cyprinol sulfate) has been measured. It was sufficiently high that precipitation in the biliary tree should not occur [12].

Micelles composed of only bile acid anions (and their accompanying counterions) are called simple micelles. The most remarkable property of simple bile acid micelles is their ability to convert lipid bilayers into mixed micelles [11]. In bile, the major component of the mixed micelles is phosphatidylcholine. In small intestinal content, the major components of the mixed micelles are partially ionized fatty acids and 2-monoacyl glycerol. Approximately 1 molecule of phosphatidylcholine or 2 molecules of fatty acid-monoglyceride are dissolved per molecule of micellar bile acid. Because there is cooperative association between membrane lipids and bile acids, the concentration at which mixed micelle formation begins is

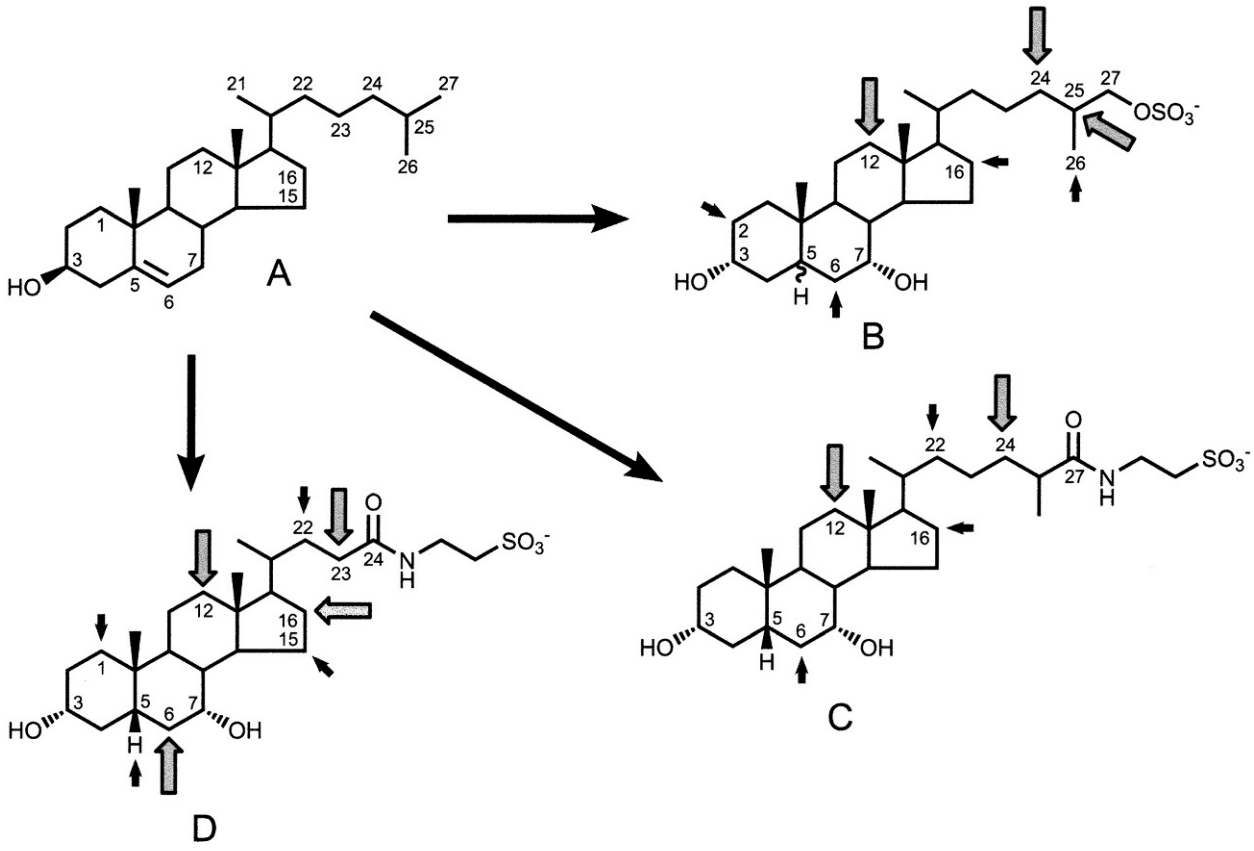

Figure 2. Sites of hydroxylation of the three main classes of bile salts. A, cholesterol; B, C_{27} bile alcohol (sulfates); C, C_{27} bile acids (shown as their taurine amidates); and D, C_{24} bile acids (also shown as their taurine amidates). The default structure is shown with 3α and 7α hydroxy groups for all three classes. Additional hydroxylation sites that occur in multiple species are indicated by the larger arrow; those occurring in only a few species by the smaller arrow. For C_{24} bile acids (D) , hydroxy groups at C-1 and C-6 may be in the α or β configuration. The orientation of the hydrogen atom at C-5 (which denotes the A/B ring juncture) in C_{27} bile alcohols is not indicated, as 5α bile alcohols occur as frequently as 5β bile alcohols. Bile salts with chain lengths other than C_8 (B,C) or C_5 (D) also occur by in only very few species. For C_{24} bile acids, 7β-hydroxy and 7-oxo bile acids also occur, but are not shown.

well below that of the CMC for simple micelle formation.

The molecular arrangements of the mixed micelles present in bile (containing phosphatidylcholine as a solubilizate) and those of small intestinal content (containing fatty acid-monoglyceride) are identical, based on small angle neutron scattering [14]. The current view is that the mixed micelle is spherical or cylindrical, with the bile acid molecules resting their hydrophobic backs between the polar groups of the solubilized molecules. The ionized head groups of the bile acid molecules repel each other, so that the mixed micelle is a polyanion. The ability of bile acids to solubilize membrane lipids in mixed micelles is essential for rapid digestion and absorption of fatty acids and monoglycerides. The mixed micelles can in turn solubilize additional lipids, such as fat soluble vitamins; indeed, such micellar solubilization of fat soluble vitamins is essential for their absorption [15]. Fig. 5 illustrates the conversion of lipid vesicles or bilayers to cylindrical mixed micelles by bile acids.

The ability of C_{27} bile acids to solubilize membrane lipids has not been examined. 5α-cyprinol sulfate solubilizes two molecules of fatty acid per molecule of micellar bile alcohol sulfate, indicating that its solubilizing properties are similar to those of C_{24} bile acids [12].

Simple micelles do not occur *in vivo* in health, although in the bile of many vertebrates, the ratio of phosphatidylcholine to bile salts is quite low [16]. In mice, knockout of the *MDR2* gene (a flippase for phosphatidylcholine that is present in the biliary canaliculus) results in phospholipid being absent from bile. In such animals, simple bile acid micelles are present. These micelles attack the membranes of the biliary tract, causing inflammation and fibrosis [17, 18].

Bile acids have a range of polarity, and a hydrophobic index value (HI) can be derived from relative retention time during high pressure liquid chromatography (HPLC) with a C_{18} octadecylsilane stationary phase as described by Heuman [19]. The ranking of

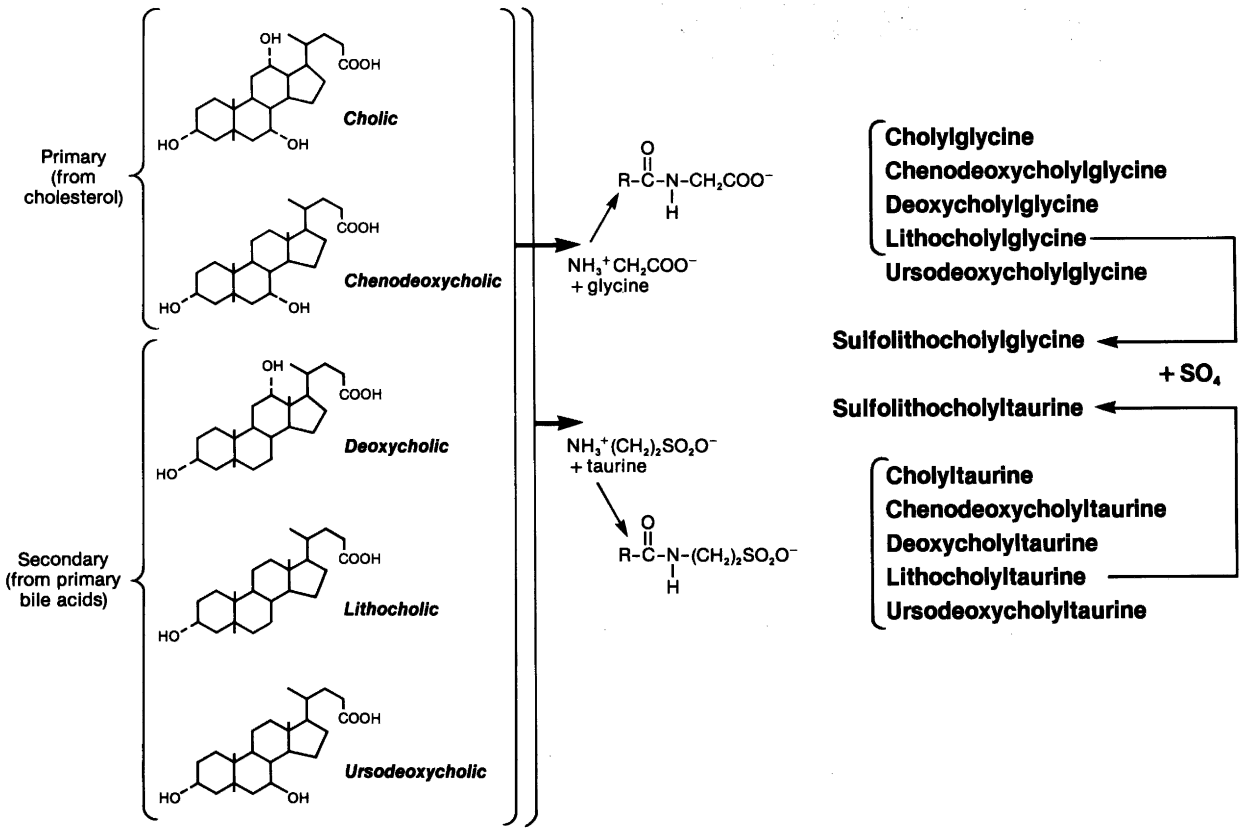

Figure 3. Chemical structure of the major bile acids present in human bile. Traces of the 7β- epimer of cholic acid (conjugated with glycine or taurine) are also present in some individuals. This bile acid (ursocholic") and ursodeoxycholic acid may also be formed as primary bile acids in some individuals.

bile acids by this method is not perfect, as UDCA is extremely hydrophilic by HPLC, yet by *n*-octanol/water partition coefficients and albumin binding, UDCA is much more hydrophobic [20]. HI values of the common taurine conjugated bile acids have been tabulated [16], as well as aqueous solubility of the protonated acids, CMC, critical micellization pH, *n*-octanol/water partition coefficients, and logarithms of the relative retention time [20, 21].

Bile acid pathochemistry

Bile acid pathochemistry occurs in inborn errors of bile acid biosynthesis and conjugation. As around 16 enzymes are required to convert cholesterol to C_{24} bile acids [22, 23], inborn errors of bile acid biosynthesis are not uncommon [24]. Because of lack of feedback by the end product of bile acid biosynthesis, there may be a striking increase in the formation of intermediates. If such intermediates are not substrates for the canalicular bile salt export pump (BSEP), they may accumulate in the hepatocyte inducing necrosis and/or apoptosis.

Defects in bile acid conjugation are extremely rare. Absence of conjugation should result in unconjugated bile acids being secreted into bile either as such or in the form of their glucuronides. In the biliary tract and small intestine, unconjugated bile acids may be absorbed passively, leading to a bile acid deficiency and malabsorption of lipids [25].

In some experimental models, bile acids may induce biliary tract injury. For example, the feeding of LCA causes cholangitis in rodents [26]. In other experimental models, in which unusual bile acids accumulate in the circulating bile acids, insoluble calcium salts of such bile acids may form in the gallbladder and aggregate to form calcium bile salt gallstones. In rats, the addition of LCA to the diet leads to the accumulation of both it and murideoxycholic acid (MDC), its 6-hydroxy metabolite, in the circulating bile acids. The increased demand for conjugation of the administered bile acid causes taurine depletion, and, as a result, LCA and MDC are conjugated instead with glycine. The two glycine conjugates precipitate in bile as insoluble calcium salts [13]. In prairie dogs, administration of MDC leads to accumulation of its taurine conjugate in bile

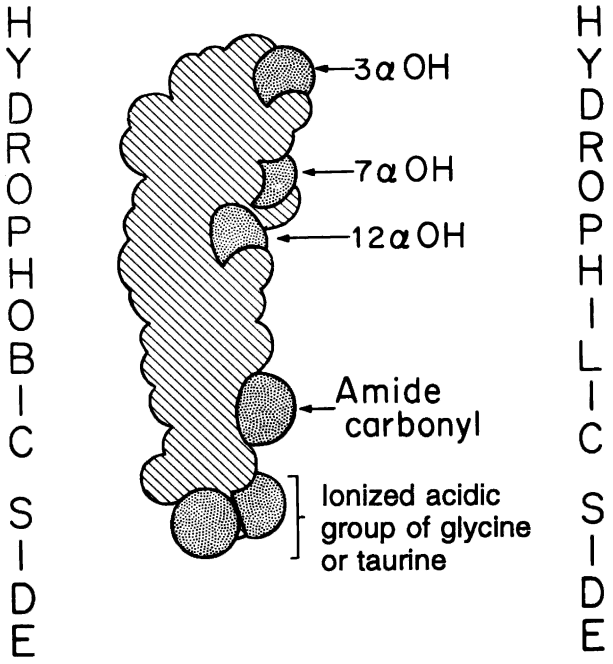

Figure 4. Space filling model of the taurine conjugate of cholic acid (viewed from the side) showing its planar amphipathic structure with a hydrophilic side and a hydrophobic side. So far as is known, virtually all bile amidates and bile alcohol sulfates have the same planar amphipathic character.

which, in time, precipitates as its insoluble calcium salt [13].

In rabbits, administered cholestanol (a 5α-saturated derivative of cholesterol), is converted to allocholic acid that is conjugated with glycine. During enterohepatic cycling, the allocholylglycine undergoes bacterial deconjugation and 7-dehydroxylation to form alloDCA. AlloDCA is absorbed from the intestine, conjugated with glycine, and then forms gallstones composed of the insoluble calcium salt of the glycine conjugate of alloDCA [13].

Bile acid biology: Enterohepatic cycling of bile acids

Enterohepatic cycling of bile acids and bile alcohol sulfates

Conjugated bile acids are secreted into the canalicular space between hepatocytes. Bile then flows distally into the bile ducts. In man, during overnight fasting, about half of the secreted bile acids pass into the gallbladder; the remainder enter the small intestine. Those bile acids entering the small intestine are absorbed both actively and passively and return to the liver where they are once again secreted into bile. With time, most of the circulating bile acids become stored in the gallbladder. When a meal is ingested, gradual gallbladder emptying occurs, delivering bile acids to the small intestinal lumen. These are efficiently absorbed by active and passive mechanisms, returned to the liver, and resecreted into the canaliculi. This movement of bile acid molecules between the liver and the intestine is termed the enterohepatic circulation and has been reviewed [27, 28]. The mass of circulating bile acids is termed the bile acid pool and can be measured by the technique of isotope dilution.

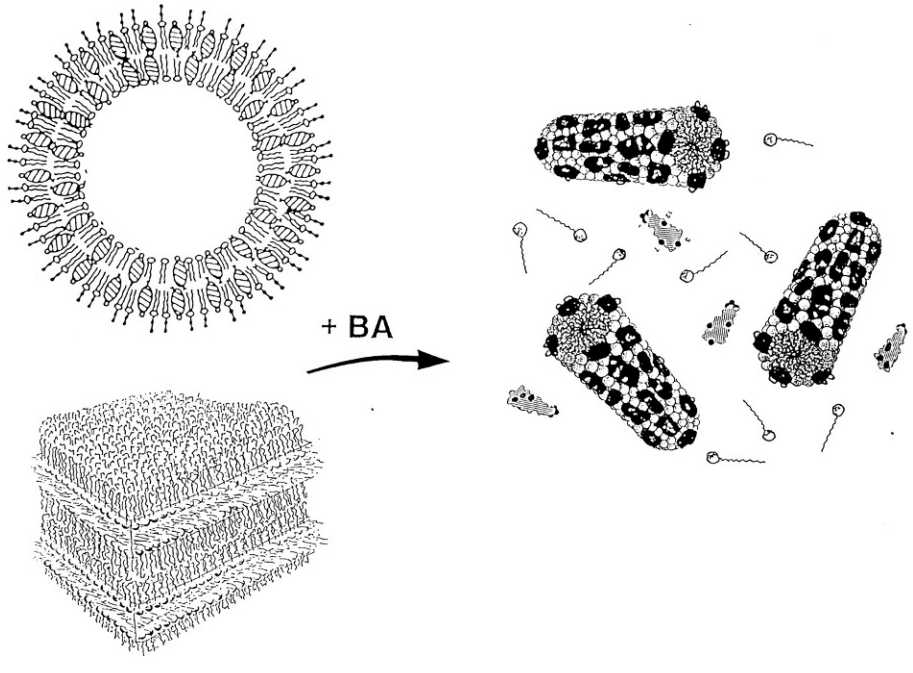

Figure 5. Conversion by bile acids of lipid vesicles or lipid bilayers to mixed cylindrical micelles. Micelles may also be spherical. Vesicles containing phosphatidylcholine-cholesterol bud out from the outer face of the apical membrane of the biliary canaliculus. Bilayers of fatty acids (partly ionized) and 2-monoacyl glycerides are generated on the surface of triglyceride droplets by the action of pancreatic lipase in small intestinal content during digestion.

All cholanoids and cholestanoids are believed to undergo enterohepatic cycling. In those few species without a gallbladder (rat, deer, elephant, horse, among others) and in patients who have undergone surgical removal of the gallbladder, bile acids also have an enterohepatic circulation, but presumably the bile acid pool is stored in the small intestine during overnight fasting. The enterohepatic circulation of C_{24} bile acids is shown in Fig. 6. It is likely that C_{27} bile acids have a similar enterohepatic circulation. For C_{27} bile alcohol sulfates colonic absorption of the (unconjugated) bile alcohol, were it to be formed, is unlikely because of its polarity.

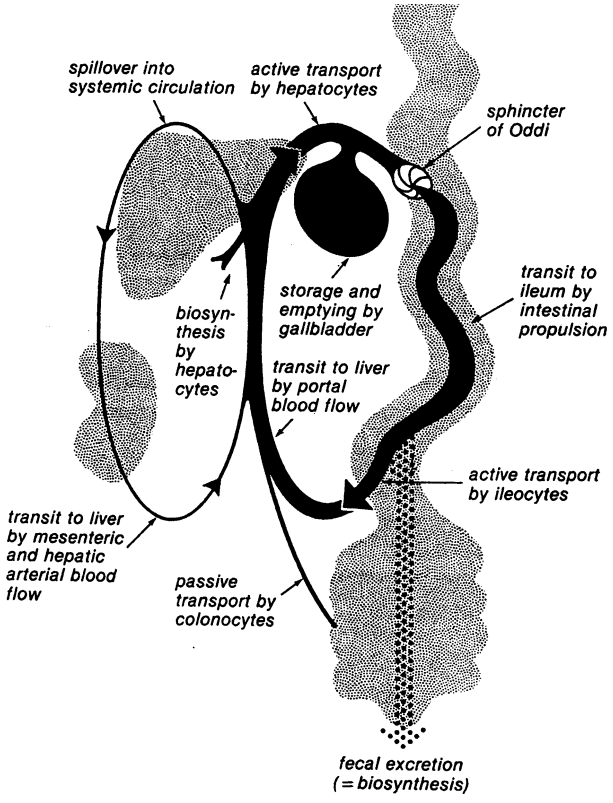

Figure 6. Schematic depiction of the enterohepatic circulation of C_{24} bile acids in man. Bile acids and bile alcohol sulfates are believed to have a similar enterohepatic circulation in vertebrates, but details are lacking. There is likely to be little absorption of (unconjugated) bile alcohols from the colon were they to be formed by bacterial sulfatases.

Bile acid Transporters and bile acid transport

For enterohepatic cycling of bile acids, both the hepatocyte and the enterocyte must efficiently transport bile acids. Thus, four transporters are required – two apical and two basolateral. Properties of these transporters have been reviewed [29–32]. The major site of bile acid absorption from the small intestine is in the terminal ileum. Ileal enterocytes have an apical bile salt transporter (ASBT) that mediates sodium-dependent cotransport of conjugated bile acids. Basolateral transport out of the ileal enterocyte into portal venous blood is mediated by a heterodimer of two proteins, OSTα/OSTβ. This transporter is thought to be an anion exchanger, but the solute exchanged for conjugated bile acids has not been identified. Within the ileal enterocyte is a bile acid binding protein whose synthesis is modulated by the nuclear receptor FXR. The function of this binding protein in bile acid transport has not yet been clarified [33].

Passive uptake of conjugated bile acids may also occur in the small intestine. In the duodenum, passive uptake of glycine conjugated C_{24} dihydroxy bile acids in protonated form occurs if luminal pH becomes sufficiently acidic [34]. Conjugated bile acids might also be absorbed paracellularly in patients with increased paracellular permeability, as is known to occur in a variety of gastrointestinal diseases [35].

Bile acids are transported by portal venous blood to the liver. In portal venous blood, bile acids are partly bound to albumin. In man, cholyl conjugates are 60–80% bound, and CDCA conjugates are more tightly bound (>95%). Hepatocyte uptake is mediated by Na^+-taurocholate cotransporting polypeptide (NTCP), a basolateral sodium-dependent cotransporter that shares homology with ASBT. Uptake may also be mediated by one or more of the multiple sodium-independent anion transporters belonging to the organic anion transporter (OATP) family, as at least one of these has been shown to mediate sodium-independent transport of conjugated bile acids.

Uptake of conjugated bile acids from sinusoidal blood occurs in periportal cells, as these are exposed to the highest concentration of bile acids. Conjugated bile acids also are transported from the hepatocyte back into sinusoidal blood by MRP4, a cotransporter of bile acids and glutathione [36]. There is thus uptake, regurgitation, and further uptake of bile acids as bile acids move down the sinusoid. Bile acids traverse the hepatocyte in monomeric form, although there is also evidence for vesicular transport. The details of the intracellular transport of bile acids remain to be clarified. Extrusion of bile acids into the canaliculus is mediated by the ATP-energized pump, BSEP (ABCB11).

These four transporters – two in the ileocytes and two in the hepatocyte – are sufficient to explain the enterohepatic cycling of conjugated C_{24} bile acids. Presumably similar carriers are involved in the enterohepatic cycling of C_{27} bile acids and C_{27} bile alcohol sulfates. However, additional transporters participating in the enterohepatic cycling of bile acids may be discovered in the future.

C_{24} bile acids undergo bacterial deconjugation in the distal small intestine and colon. The resulting uncon-

jugated bile acids are membrane permeable and can be absorbed passively. Whether exiting at the basolateral membrane of the enterocytes involves OSTα/OSTβ or another transporter, or indeed, whether any transporter is required for basolateral exiting of unconjugated bile acids is not known.

The mechanisms by which unconjugated bile acids enter the hepatocyte has not been clarified. Four possible mechanisms are likely. First, unconjugated C_{24} bile acids have been shown to be substrates for a fatty acid transport protein, FATP5 [37]. Translocation of unconjugated bile acids across the basolateral membrane of the hepatocyte is associated with simultaneous coenzyme A (CoA) formation. Second, at least one unconjugated bile acid (UDCA) is a substrate for NTCP [38]. Third, unconjugated bile acids might also be transported by the multiple (sodium-independent) OATP carriers. Finally, unconjugated mono- and dihydroxy bile acids might flipflop passively across the basolateral membrane, based on their behavior in model systems [39].

At any rate, unconjugated bile acids, presumably in the form of their CoA thio esters, travel to the peroxisomes, where they are amidated with glycine or taurine [40]. They are transported out of the peroxisomes by an as yet unidentified transporter, and then secreted into bile across the canalicular membrane by BSEP.

Experimentally, using the biliary fistula animal or isolated perfused liver, unconjugated bile acids may be infused at high doses and taken up by hepatocytes at a rate exceeding their amidation capacity. The fate of such unconjugated bile acids depends on bile acid structure. Trihydroxy bile acids are secreted as such into bile. Mono- and dihydroxy bile acids are in part partitioned into the smooth endoplasmic reticulum and undergo ethereal or ester glucuronidation; the glucuronides are then transported out of the smooth endoplasmic reticulum and secreted into bile. If unconjugated mono- or dihydroxy bile acids are secreted into bile, they may be absorbed in the biliary ductules and travel back to sinusoidal blood via the periductular capillary plexus. They are then resecreted into bile, once again inducing bile flow. Such short-circuiting of the enterohepatic circulation is called cholehepatic shunting, and is evidenced by a bicarbonate rich enhancement in bile flow (termed hypercholeresis) [41–43].

Damage and repair during enterohepatic cycling

During enterohepatic cycling, C_{24} primary bile acids are "damaged" by bacterial enzymes in the intestine and after their return to the liver, they are "repaired" partly or completely by hepatocyte enzymes. Major intestinal changes are deconjugation and 7-dehydroxylation. Minor changes include oxidation (dehydrogenation) of the hydroxy groups, epimerization of 3α- or 7α- hydroxy groups to their corresponding β-hydroxy epimers, isomerization of the A/B ring juncture and, in some species, desaturation of the side chain [7, 44].

Repair of unconjugated bile acids begins with reconjugation with glycine or taurine. Repair of 7-deoxy bile acids is species-dependent. In man and some other species, DCA does not undergo 7-rehydroxylation, but is secreted as such into bile after conjugation. In other species, such as the mouse and rat, rehydroxylation at C-7 occurs, with the result that DCA is converted back to cholic acid. LCA is either sulfated at C-3, or undergoes hydroxylation, most commonly at C-6 or C-7. Rarely, rehydroxylation of DCA or LCA at sites other than C-6 or C-7 occurs. In the snake, DCA is hydroxylated at C-16 [2]; in the wombat, LCA is hydroxylated at C-15 [45]. Bile acids with 3β-hydroxy groups ("iso"- bile acids) are re-epimerized to 3α-hydroxy bile acids [46]. Oxo groups are reduced partly or completely to α- or β- hydroxy groups (or both) in a species dependent manner. The 3-oxo, $\Delta^{4,6}$ resonating intermediate that is formed during the dehydroxylation process in C_{24} bile acids may be reduced to a 5α- configuration either in the intestine or possibly the hepatocyte, resulting in the formation of allo bile acids.

The only known side chain biotransformation of unconjugated C_{24} bile acids that is known to be mediated by intestinal bacteria is desaturation of the side chain. This has been shown for the C_{24} bile acid β-muricholic acid (3α,6β,7β-trihydroxy) which is converted to its Δ^{22} derivative (reviewed in [7]). Whether bacterial desaturation of the side chain occurs in C_{27} bile alcohols or C_{27} bile acids is not known.

C_{27} bile acids undergo bacterial deconjugation and reconjugation in all probability. They also undergo 7-dehydroxylation as evidenced by the presence of 7-deoxy bile acids in bile, at least in the alligator.

For C_{24} and presumably C_{27} bile acids, the end result of repair in the hepatocyte is that bile acids in bile are mostly in amidated form and only trace amounts of iso bile acids or hydroxy-oxo bile acids are present.

Spillover of bile acids into the systemic circulation

Hepatocyte uptake of bile acids returning from the intestine is not 100% efficient. The efficiency of hepatic uptake depends on bile acid structure: trihydroxy > dihydroxy, and conjugated bile acids > unconjugated bile acids [21]. Bile acids not taken up by the hepatocyte spill over into the systemic circulation, which is enriched in those bile acids having less hepatic uptake. However, bile acids spilling over into the systemic circulation are again presented to the

liver in hepatic arterial blood as well as by splanchnic venous blood so that the residence time of any bile acid in the systemic circulation is only a few minutes [47]. Therefore the level of bile acids at any moment is determined by input from the intestine via OSTα/OSTβ and hepatocyte uptake mediated by NTCP and possibly other basolateral bile acid transporters.

The enterohepatic circulation of each of the three major bile acids present in human bile (cholic, DCA, CDCA) has been modeled using a physiological pharmacokinetic model [48–50]. This model can also be used to categorize defects in the enterohepatic circulation of bile acids. No models have been proposed for the enterohepatic circulation of C_{24} bile acids in other species, or for the enterohepatic circulation of C_{27} bile acids or C_{27} bile alcohol sulfates.

Regulation of the enterohepatic circulation

The key components of the enterohepatic circulation are the synthesis of primary bile acids and efficient intestinal conservation. The synthesis of primary bile acids replaces the small amount of bile acids not absorbed by the intestine. Intestinal conservation results in a recycling pool of bile acids, permitting each bile acid to serve its function multiple times. Both the synthesis (input) of primary bile acids and intestinal absorption (conservation) are highly regulated. Details of this regulation are under active investigation.

Bile acid biosynthesis is downregulated in health. The rate limiting enzyme is cholesterol 7α-hydroxylase (CYP7A1) and the downregulation of this enzyme is mediated by several different pathways, all of which converge on CYP7A1. One pathway involves bile acid activation of the FXR nuclear receptor, which in association with another nuclear receptor, RXR, acts to induce the synthesis of a suppressor protein termed SHP. SHP displaces Hepatocyte Nuclear Factor 4 (HNF4) from the promoter of *CYP7A1,* thereby decreasing its transcription and thus bile acid biosynthesis [51]. This feedback mechanism requires the participation of a fibroblast growth factor, FGF15, a peptide hormone released by the ileal enterocyte that acts on a hepatocyte receptor FGFR4. This activation leads to Jnk activation that in turn suppresses expression of CYP7A1 [52]. Another player in the Jnk pathway has been shown to be a protein named β-Klotho [53].

There are also cell signaling pathways induced by inflammatory cytokines such as TNFα that act ultimately to induce the phosphorylation of cJun, that in turn phosphorylates HNF4α [54]. Phosphorylation of HNF4α reduces its affinity for the promoter site of *CYP7A1,* and therefore downregulates *CYP7A1.* Glucagon has also been shown to inhibit *CYP7A1* gene expression possibly by activating protein kinase A which in turn phosphorylates HNF4α, which, as noted, decreases its affinity for the *CYP7A1* promotor [55].

In man, the feeding of individual natural bile acids such as cholic acid, CDCA, or DCA downregulates bile acid synthesis by a factor of two [56]. Suppression of bile acid biosynthesis by cholic acid feeding may be mediated by its metabolite DCA. In contrast, ileal resection, which causes profound bile acid malabsorption and also abolishes FGF15 release, causes bile acid synthesis to increase ten fold [57]. The increase in bile acid biosynthesis is attributed to the decreased hepatocyte concentration of bile acids that diminishes FXR activation. In addition, the necessary co-inhibition of *CYP7A1* by the FGF15 pathway is absent. In the mouse, knockout of ASBT (the apical conjugated bile acid sodium dependent cotransporter) results in a nearly twenty fold increase in bile acid biosynthesis [58]. In contrast, knockout of basolateral OSTα/OSTβ results in bile acid malabsorption without a compensatory increase in bile acid biosynthesis [59]. The lack of compensatory increase is attributed to the high concentration of bile acids within the ileal enterocyte that in turn causes the release of FGF15. FGF15 acts to inhibit bile acid biosynthesis in the hepatocyte despite the presence of bile acid malabsorption [52]. Bile acid biosynthesis is believed to occur largely in pericentral hepatocytes, with biosynthesis in the periportal hepatocytes being downregulated by both the large bile acid flux through the periportal cells as well as by FGF15 from the intestine. When there is a compensatory increase in bile acid biosynthesis, it is likely that additional hepatocytes are recruited, moving in the direction of the portal triad [60].

Conjugated bile acid transport by the ileal enterocyte is also downregulated by the flux of bile acids through the cell. The bile acids activate FXR that leads to increased synthesis of SHP. The increase in SHP is thought to inactivate RAR (a retinoic acid receptor), leading in turn to downregulation of ASBT [61].

In some animals, bile duct ligation [62] or parenteral alimentation [63] leads to paradoxical downregulation of ileal bile acid transport. Thus, it is clear that there are multiple mechanisms for the regulation of intestinal bile acid transport, and much more work needs to be done to elucidate them.

The net effect of these negative feedback pathways is that bile acid feeding downregulates not only bile acid biosynthesis but also ileal conservation. The end result is that bile acid feeding of primary bile acids results in only a modest increase in the secretion of bile acids [56]. With bile acid malabsorption, there is increased bile acid biosynthesis which may partly restore bile acid secretion. Thus, although bile acids are the final

end product of cholesterol catabolism, the homeostatic mechanisms appear to be more concerned with maintaining the enterohepatic circulation than with eliminating cholesterol.

Methods for characterizing the enterohepatic circulation:

The bile acid pool size and the synthesis rate of a given bile acid may be estimated by an isotope dilution procedure. In this approach, a bile acid tagged with a radioactive isotope is administered and the time course of the decline in specific activity is measured. Satisfactory isotopes are tagged with ^{14}C at C-24 or ^{3}H at C-22 and C-23 [64]. The same technique can be performed using bile acids tagged with stable isotopes (^{13}C or ^{2}H) and measuring the decline in atom-percent excess in the bile acid isolated from plasma using mass spectrometry [65]. Input of secondary bile acids can be measured by isotope dilution.

Bile acid synthesis may also be measured indirectly by measuring fecal bile acids, a complex biochemical procedure. Bile acid secretion is a flux, and can only be measured by duodenal intubation using an indicator dilution technique [56]. Biliary bile acid composition can be measured by sampling bile by duodenal intubation and inducing gallbladder contraction by the intravenous administration of a cholecystokinin derivative. A test in which a string is swallowed, allowed to enter the duodenum, and then pulled out has also been used to measure biliary bile acid composition [66].

Functions of the enterohepatic circulation of bile acids

The enterohepatic circulation of bile acids has multiple functions in the liver, biliary tract, and intestine. Table 6 shows the micellar and non-micellar functions of bile acids in mammals. In the liver, bile acids activate TGR5, a G-protein coupled receptor present on sinusoidal endothelial cells. Its activation leads to nitric oxide release and, presumably, vasodilatation leading in turn to increased sinusoidal blood flow [67]. The flux of bile acids through the hepatocyte promotes insertion of the bile acid export pump (BSEP, ABCB11) as well as the phospholipid flippase, MDR2/3. When bile acids are secreted into the canalicular space they induce bile flow by their osmotic effects. They also adsorb to and detach the hemivesicles of phosphatidylcholine and solubilize them in mixed micelles [17, 68].

In the biliary tract, bile acids solubilize lipophilic xenobiotics that are secreted into bile and also bind heavy metal cations as counterions for the mixed micelles. Bile acids also modulate ductular secretion of bicarbonate and, in addition, have antimicrobial effects. When bile ducts are obstructed, bile acids may contribute directly or indirectly to ductular hyperplasia [69]. In the gallbladder, bile acids appear to modulate cAMP-mediated secretion [70], as occurs during gallbladder contraction [71].

In the small intestine, bile acids solubilize fatty acids and monoglycerides in mixed micelles; the mixed

Table 6. 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
    - Stimulation of synthesis and secretion of FGF-15
  - 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 epithelium
    - 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
    - Enhancement of tryptic hydrolysis of dietary proteins
  - Ileal enterocyte
    - Regulation of gene expression via nuclear receptors
    - Release of FGF-15
  - 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

micelles, in turn, solubilize fat soluble vitamins and other lipophilic molecules. Micelle formation greatly accelerates diffusion of fatty acids and monoglycerides to the intestinal epithelium [15]. Bile acids also bind to dietary proteins in small intestinal content during digestion. Such binding denatures the protein promoting more rapid proteolysis by pancreatic proteases [72]. Conjugated bile acids have antimicrobial effects in the lumen, and also stimulate the ileal enterocyte to secrete undefined antimicrobial agents [73]. Bile acids also stimulate release of the peptide hormone FGF15 from the ileal enterocyte.

In the large intestine, CDCA and DCA act as osmosensors, stimulating colonic secretion of water and electrolytes when their local concentration is elevated. LCA and cholic acid, as well as UDCA, isoCDCA, isoDCA and disubstituted oxo-hydroxy bile acids are devoid of secretory activity, based on *in vitro* studies using polarized monolayers of T-84 cells [74]. Under some circumstances, bile acids also induce colonic motility. How important the secretory and motility effects of bile acids are in colonic physiology in the healthy person remains to be established.

Bile acids have also been shown to act on the G protein-coupled receptor, TGR5 in brown adipose tissue, leading ultimately to triiodothyronine generation and increased thermogenesis [75]. Whether plasma levels are sufficient to mediate this process *in vivo* is uncertain. The multiple functions of bile acids that are currently recognized are summarized in Table 6.

A note of caution seems in order when attributing bile acid functions to effects observed *in vitro*. Unconjugated mono- and dihydroxy bile acids readily enter cells passively, and some of their effects in cell culture systems may be pharmacological, rather than physiological. In the hepatocyte, bile acids are likely to be present almost entirely in amidated (conjugated) form. In plasma, the majority of bile acids are also present in conjugated form, and unconjugated bile acids are present in concentrations < 1 μM. Even at this low concentration, the preponderance of unconjugated mono- and dihydroxy bile acids are bound to albumin. Therefore effects observed using unconjugated bile acids in concentrations above 20 μM may be pharmacological, rather than physiological.

Pathobiology: Defective enterohepatic cycling of bile acids

Impaired canalicular secretion

Bile acids are pumped uphill into the canalicular space by the ABC transporter BSEP (ABCB11). Absence of this transporter occurs as an inborn genetic defect in children who then present with cholestasis. Genotyping is used to establish a defect in the *ABCB11* gene, and the condition is termed Primary Familial Infantile Cholestasis, type 2 [76]. The absence of BSEP leads to bile acid retention in the hepatocyte which causes necrosis and apoptosis, as well as inflammation, explaining why such children present with severe jaundice. Recent work suggests that bile acids activate a transcription factor named early growth response factor-1 (ERF1), whose activation leads to upregulation of proinflammatory mediators. These proinflammatory mediators cause activated neutrophils to accumulate in the liver which in turn induce further hepatic damage [77]. Defective hepatocyte transport leads to accumulation of bile acids in plasma, and this directly or indirectly induces pruritus.

Sulfation of amidates of CDCA occurs in the hepatocyte, and the amidated CDCA sulfates are regurgitated into plasma and excreted in urine [78, 79]. Sulfation also occurs in the renal tubular cells, and such sulfates are also eliminated in urine. Amidates of cholic acid are also eliminated in urine to a limited extent because of their weaker binding to albumin. However, the greatly increased regurgitation of bile acids from the hepatocyte back into plasma and the subsequent urinary excretion of bile acids are adaptations to cholestasis that are still insufficient to prevent hepatocyte destruction. If cholestasis is severe, DCA is absent from plasma bile acids because few bile acids enter the large intestine. The only satisfactory treatment for this fatal condition is liver transplantation. It is possible to ablate the *BSEP* gene in mice [80]. It is remarkable that the mice appear relatively healthy, although such mice are unable to transport an intravenously infused taurocholate load into bile. Mice adapt to the retention of trihydroxy bile acids by forming tetrahydroxy bile acids that are secreted from the hepatocyte by another canalicular transporter, probably MDR1.

Obstruction to bile flow

In primary biliary cirrhosis, there is autoimmune destruction of cholangiocytes causing obstruction to bile flow at the ductular level [81]. The most sensitive plasma marker of this condition is alkaline phosphatase whose synthesis increases in the hepatocyte when bile acids are retained. BSEP is not downregulated in cholestasis, suggesting that bile acids continue to be secreted into the canaliculus, but are then absorbed by cholangiocytes proximal to the site of obstruction, thus undergoing a cholehepatic circulation. Probably, conjugated bilirubin and phosphatidylcholine remain in the ductular lumen and, as bile is concentrated, form so called “bile plugs”. As in BSEP deficiency,

amidates of CDCA are sulfated, regurgitated into plasma and excreted in urine.

Plasma bile acids are markedly increased and are responsible, directly or indirectly, for the severe pruritus that occurs in this condition. Pruritus can be treated symptomatically by aspiration of bile [82] or by albumin dialysis which removes plasma bile acids [83]. It may be helped by administration of UDCA or bile acid sequestrants such as cholestyramine, colestipol, or colesevelam. A recent report has shown that sertraline, a serotonin reuptake inhibitor, also decreases pruritus [84].

The administration of UDCA to patients with primary biliary cirrhosis improves liver tests and pruritus, as well as extending considerably the interval between diagnosis and liver transplantation or death [81]. (See below).

Obstruction to bile flow may also occur because of gallstones or neoplasms in the bile ducts. However, such obstruction is usually treated immediately by a drainage procedure such as percutaneous transhepatic insertion of a drainage catheter into the obstructed bile duct.

Gastroesophageal reflux

In the healthy person, there is little reflux of duodenal content into the stomach or esophagus, because intragastric pressures are higher than intraduodenal pressures. However, in patients with disordered gastroduodenal motility or pyloric sphincter dysfunction, there may be gastroduodenal reflux that in turn leads to gastroesophageal reflux. Duodenal content is rich in bile acids, but also contains active pancreatic enzymes, as well as digestion products generated by pancreatic enzymes such as lysophosphatidylcholine and fatty acids. Bile acids will be exclusively in conjugated form unless there is abnormal bacterial deconjugation in the duodenum.

There is ongoing controversy as to the role of bile acids in the pathogenesis of esophageal inflammation or Barrett's esophagus, a condition in which the esophageal squamous epithelium become transformed into columnar epithelium [85]. One study found that refluxing duodenal content in patients with esophagitis contained unconjugated DCA, suggesting that the combination of bacterial deconjugation (which generates cell permeable DCA) together with bile acid reflux may play a causal role [86]. Other studies have suggested that bile reflux when acidic is particularly dangerous. Acidification of bile has two contrasting effects. If bile is sufficiently acid, glycine amidates may precipitate from solution. On the other hand, glycine conjugated dihydroxy bile acids (CDC-glycine and DC-glycine) could, in principle, remain in supersaturated solution and enter esophageal cells passively in the protonated form (non-ionic diffusion). After entry, such glycine conjugated bile acids would be trapped in the esophageal cells and might be cytotoxic or alter gene expression or both.

UDCA administration decreases the cytotoxicity of circulating bile acids. Clinical trials testing the efficacy of UDCA in the treatment of reflux esophagitis are in progress. A study many years ago from the laboratory of G. Salen reported a striking beneficial effect of UDCA on symptoms in patients with gastritis and gastroduodenal reflux. However, no change in gastric inflammation was observed in this one month study [87].

Biliary diversion

Complete biliary diversion was frequently done in the past when there were space occupying lesions causing bile duct obstruction. However, currently, obstructed bile ducts are often stented to permit bile flow to reach the small intestine. Historically, biliary diversion was done during the immediate postoperative period in patients undergoing open cholecystectomy in whom a T-tube was placed into the common bile duct. Today, most cholecystectomies are performed by the laparoscopic technique and T-tubes are no longer inserted. The loss of bile acids, phosphatidylcholine, and cholesterol, as well as other biliary constituents such as electrolytes and immunoglobulins, probably has no deleterious effect on health. The only effect of biliary drainage is to create a conjugated bile acid deficiency in the small intestine. This will cause malabsorption of saturated fatty acids and fat soluble vitamins because of the lack of mixed micelle formation. However, the clinical utility of conjugated bile acid replacement (see below) is not at all clear.

The discovery that bile acids have antimicrobial effects [73] suggests that bile acid deficiency in the small intestine could promote bacterial growth. As yet, there are no rigorous clinical studies testing this hypothesis. Bile acids also denature proteins, thereby enhancing their tryptic digestion [74]. In pigs, diversion of the common bile duct to the ileum causes an increase in urinary nitrogen. This finding can be explained by malabsorbed protein undergoing deamination in the colon. The liberated ammonia is then absorbed, converted to urea in the liver, and the urea excreted in urine [88]. Nonetheless, children with cholestasis who are receiving cholestyramine to treat pruritus should have very low intraluminal bile acid concentrations, and such children may have normal growth. At present, the physiological importance of the observation that conjugated bile acids enhance dietary protein digestion is unclear.

Cirrhosis

In cirrhosis, there is extensive fibrosis with regenerative nodules. Bile acid synthesis [89] and bile acid secretion [90] are markedly reduced. Bile acid metabolism in regenerative nodules has not been characterized.

Functional constipation

Multiple lines of evidence suggest that bile acids act as endogenous laxatives. The feeding of CDCA induces dose-related diarrhea [91], and CDCA administration can be used to treat constipation [92]. Bile acid malabsorption induces diarrhea that is treated effectively with bile acid sequestrants [57]. In a small subset of children with functional constipation, the dominant fecal bile acid was the 3 sulfate of (non-amidated) CDCA [93]. Sulfation of CDCA is known to abolish its secretory activity [94]. It was speculated that such children have bacterial overgrowth in the small intestine causing deconjugation of CDCA amidates. The liberated CDCA is absorbed, sulfated in the enterocyte, and extruded back into the intestinal lumen. If this speculation is valid, the constipation of such children might respond to antibiotics.

Defective ileal conservation

In patients with ileal resection, there is profound bile acid malabsorption and, as noted, a five to tenfold increase in bile acid biosynthesis [57]. With small ileal resections (< 100 cm), the marked increase in bile acid synthesis results in small intestinal bile acid concentrations being only minimally decreased, and this fact along with ample intestinal surface area results in fat malabsorption being mild. Bile acids pour into the colon, inducing colonic secretion. Malabsorption of electrolytes and water occurs because of the loss of ileal surface; this together with bile acid induced secretion, results in watery diarrhea. Administration of bile acid sequestrants abolishes the diarrhea.

With larger ileal resections (> 100 cm) the compensatory increase in bile acid synthesis is insufficient to maintain intraluminal bile acid levels. (Normal bile acid secretion is 12000 mg/day, whereas maximal bile acid synthesis is 2000–4000 mg/day). Bile acid concentrations in the jejunum fall progressively during the day. The decreased bile acid concentrations plus loss of intestinal mucosal surface area results in such patients having severe malabsorption of lipids as well as fluid and electrolytes, this manifesting clinically as diarrhea and steatorrhea. Colonic secretion appears to be induced by malabsorbed fatty acids rather than by bile acids, as replacement of long chain triglycerides by medium chain triglycerides ameliorates the diarrhea [57]. Bile acid sequestrants, in contrast, cause little improvement in diarrhea.

Short bowel syndrome

The term short bowel syndrome denotes loss of so much small intestinal surface that the patient has difficulty maintaining adequate fluid and calories by oral intake. Most patients with short bowel syndrome have lost their ileum and therefore have profound bile acid malabsorption.

Therapeutics: Bile acid agonists and antagonists

Definitions and rationales

Here we will use the term "agonists" to denote bile acids and their derivatives, and the term "antagonists" to refer to therapeutic approaches that remove bile acids or diminish their pharmacodynamic effect. Neither bile acid agonists nor bile acid antagonists have as yet become widely used agents for reasons that will be discussed.

For bile acid agonists, it is useful to distinguish four rationales of treatment. The first is "replacement therapy" where bile acids or bile acid derivatives are administered to correct a bile acid deficiency. The second is displacement therapy where the aim is to change the composition of the circulating bile acids without any great change in tissue concentrations or secretion. The third rationale is to activate FXR, the nuclear receptor for which bile acids are a potent ligand, and thus upregulate its target genes. The fourth rationale is ductular targeting using a membrane permeable bile acid that is secreted into bile as such and undergoes cholehepatic shunting. Bile acids based on the latter two rationales are still in the development stage, and it is quite unclear whether they will eventually reach the market.

Bile acid replacement

In patients with inborn errors of bile acid biosynthesis [24], intermediates are formed that are not substrates for transport by BSEP. They accumulate in the hepatocyte, inducing the biosynthesis of inflammatory mediators, as well as apoptosis/necrosis. In enzyme defects involving oxido-reduction modification of the C-3 hydroxy group as well as reduction of the $\Delta^{4,5}$ double bond, administration of primary bile acids is life saving (24).

In cerebrotendinous xanthomatosis (CTX), there is a deficiency of CYP27A1, resulting in decreased biosynthesis of CDCA and loss of downregulation of bile acid biosynthesis via FXR (because CDCA is the potent activator of FXR in man). Large amounts of polyhydroxylated alcohols, some of which undergo glucuronidation, are excreted in both bile and urine. Treatment with CDCA restores the CDCA deficiency and turns off the pathological increase in bile acid

synthesis by activating FXR, thereby downregulating CYP7A1 [24]. CDCA is no longer available in many countries, but efforts are being made to bring the compound back as a marketed pharmaceutical.

For the extremely rare condition of defective bile acid conjugation, conjugated bile acids should be therapeutic.

A bile acid deficiency is present is when intestinal conservation of bile acids is no longer present, either because of massive ileal resection or in short bowel syndrome. In these conditions, there are two defects – not enough epithelial surface and a bile acid deficiency. The latter can be corrected by oral conjugated bile acid feeding. Cholylsarcosine, a synthetic conjugated bile acid was prepared in our laboratory and shown to have the physicochemical properties of cholylglycine (glycocholate) [95], in addition to being resistant to bacterial deconjugation and dehydroxylation [96]. Cholylsarcosine was shown to be as effective as desiccated ox bile in improving fat malabsorption in a patient with short bowel syndrome who lacked a colon. With continued administration of cholylsarcosine, the patient gained weight [97].

In patients with short bowel syndrome who possess a colon, nutritional problems are usually less. Malabsorbed carbohydrate is converted in the colon to short chain fatty acids which are then absorbed and serve as a caloric source. In such patients, administration of desiccated bile improved steatorrhea, but greatly worsened diarrhea. Cholylsarcosine improved fat absorption but, in contrast to desiccated bile, induced little change in fecal weight [98].

Cholylsarcosine no longer has patent protection and may never be brought to market. Studies are needed to compare its efficacy for improving fat absorption with that of cholyltaurine. It may be that bile acid replacement therapy is not cost effective. The nutritional status of such patients with short bowel syndrome can be solved by administration of formula diets rich in medium chain triglycerides, whose fatty acids are water soluble and do not require bile acids for absorption. Fat soluble vitamins can be given parenterally.

A bile acid deficiency in the small intestine is likely to be present in patients with cirrhosis because bile acid secretion is greatly reduced. Such patients have bacterial overgrowth, bacterial translocation to lymph nodes, and endotoxemia. They often have ascites and are at risk for developing bacterial peritonitis. Cirrhosis can be induced in rats by carbon tetrachloride administration. Such animals resemble the cirrhotic patient in having bacterial overgrowth, bacterial translocation to lymph nodes, and endotoxemia. Administration of cholylsarcosine (or cholylglycine) to such animals decreased bacterial overgrowth, bacterial translocation, and endotoxemia [99]. It also improved survival. It would seem worthwhile to test whether cholylsarcosine administration to cirrhotic patients would have similar antimicrobial effects and reduce the risk of bacterial peritonitis, as has recently been shown for norfloxacin. Of course, it could be that the antimicrobial effects of conjugated bile acids prove to be weaker than those of conventional antibiotics.

Bile acid displacement

The only therapeutic use of bile acids in the first half of the 20th century was the administration of dehydrocholic acid (3,7,12-trioxo-) to patients with liver disease based on the belief that choleretics were efficacious [100]. Older studies had shown that dehydrocholic acid induced more bile flow than cholic acid, presumably because it did not form micelles. Therefore for a given rate of biliary secretion, its secretion resulted in a greater number of osmotically active particles for the induction of bile flow. However, dehydrocholic acid was shown to be metabolized with time to cholic acid, so that dehydrocholic acid did not persist in the circulating bile acids. Today, it is rarely used.

CDCA was shown in the 1970s to decrease the cholesterol saturation of bile and induce gradual dissolution of cholesterol gallstones [101]. It was soon widely used for this purpose throughout the world as moderate efficacy was shown in large studies [91]. Initially, it was proposed that its mechanism of action was an increase in bile acid secretion. However, intubation studies showed that it acted by decreasing cholesterol secretion rather than increasing bile acid secretion [56]. One mechanism of action of CDCA was considered to be its down regulation of HMG CoA reductase, the rate limiting enzyme in cholesterol biosynthesis. CDCA also decreased the efficiency of cholesterol absorption.

Within a decade of the discovery of medical dissolution of gallstones, UDCA was shown by Japanese workers also to induce cholesterol gallstone dissolution. Unlike CDCA which induced a dose related elevation in plasma transaminase levels, UDCA was devoid of hepatotoxicity. Gradually, UDCA replaced CDCA for cholesterol gallstone dissolution [102]. The mechanism of action of UDCA is totally different than that of CDCA, as UDCA causes increased bile acid biosynthesis and increased bile acid secretion [103]. Now it is known that UDCA does not activate FXR, and is, in fact, an FXR antagonist.

In the 1980s, ultrasonography replaced oral cholecystography for the detection of gallstones. Medical dissolution continued, although there was dissatisfaction with the length of treatment required for com-

plete stone dissolution, the resistance of some gallstones to dissolution, and stone recurrence after stones had dissolved. By 1990, laparoscopic cholecystectomy was shown to be safe and curative. Hospitalization was brief, and no subcostal incision was required. The practice of medical dissolution waned. Today, its use is largely restricted to patients with complex medical conditions that make them poor surgical risks.

The major use of bile acid displacement today is in primary biliary cirrhosis (PBC) [81] and cholestasis of pregnancy [104]. In PBC, treatment with UDCA improves liver tests, decreases pruritus, and increases the time interval to liver transplantation (or death). If treated sufficiently early with UDCA, PBC may not progress [105]. In cholestasis of pregnancy, UDCA has been shown to improve liver tests and fetal outcome.

The mechanisms of action of UDCA are complex and include a mild choleretic effect, a decrease in the cytotoxicity of the circulating bile acids, and possibly other mechanisms such as decreased periductular inflammation [106].

Activation of FXR by 6α-ethyl-CDCA

Of the bile acids present in human biliary bile acids, CDCA is the most potent activator of human FXR. Pellicciari *et al* showed that the synthetic 6α-ethyl-derivative of CDCA was two orders of magnitude more potent at activating human FXR than CDCA [107]. In animal studies, 6α-ethyl-CDCA was shown to have antifibrotic effects and to protect against acute cholestasis induced by LCA [108]. Clinical studies aimed at testing the efficacy of 6α-ethyl-CDCA in PBC are in the planning stage.

Ductular targeting using norUDCA

norUDCA has one less carbon atom in the side chain and therefore is a C-24-nor C_{23} bile acid; this shorter chain homologue of UDCA is prepared synthetically from UDCA. Unlike UDCA, the compound undergoes little amidation during hepatocyte transport as it is not a substrate for either the bile acid CoA ligase or the amino acid transferase. norUDCA is secreted into bile in the form of the unchanged compound as well as its glucuronide. The unconjugated compound is reabsorbed in the biliary ductules and undergoes cholehepatic shunting. Its absorption in the protonated form generates a bicarbonate anion. Each time the molecule is secreted into bile, it induces canalicular bile flow. As a result, norUDCA induces a bicarbonate rich hypercholeresis in rodents [42]. In man, the compound also undergoes little amidation during hepatocyte transport and induces a bicarbonate rich hypercholeresis, based on a case report [109]. The pharmacology of norUDCA differs between rodents and man. In rodents, the major metabolite is the C-3 ethereal glucuronide. In man, the major metabolite is the C-23 ester glucuronide [109].

norUDCA was shown to cure the peribiliary fibrosis that occurs in the *MDR2* knockout mouse, and to be far more efficacious than UDCA [18]. norUDCA is now undergoing preclinical toxicology studies with hopes that clinical trials in cholestatic liver diseases such as primary sclerosing cholangitis can be initiated. norUDCA should be useful in patients with defective MDR2 function [110], and might also be useful in cystic fibrosis, where there is impaired secretion of bicarbonate by the biliary ductules.

Bile acid sequestrants

Cholestyramine and colestipol were developed some decades ago. Both are resins consisting of a plastic skeleton covered with positively charged groups that bind bile acids both electrostatically and hydrophobically. Bile acid sequestrant ingestion increases bile acid biosynthesis by a factor of four to six. However, bile acid sequestrants are quite inefficient, as active ileal absorption desorbs bile acids from the resin [111]. Bile acid sequestrants were developed to treat hypercholesterolemia. Their administration increases the synthesis of cholesterol and thereby upregulates low density lipoprotein (LDL) receptors, which in turn lowers plasma LDL cholesterol. Sequestrants have had limited use as an adjunct to the statins because of their unpalatability and the side effect of constipation. With the development of ezetimibe, a potent inhibitor of cholesterol absorption, their use for this purpose is likely to wane.

In the past decade, a new bile acid sequestrant, colesevelam has been brought to market. Colesevelam is a hydrogel that binds bile acids electrostatically and hydrophobically with a much better binding isotherm than either cholestyramine or colestipol [112]. In anecdotal reports, colesevelam has been reported to be superior to cholestyramine in the treatment of diarrhea caused by bile acid malabsorption as well as pruritus caused by cholestatic liver disease. Colesevelam has recently also been shown to have insulin sensitizing effects [113], possibly mediated by ileal release of glucagon [114], and was recently approved by the United States Food and Drug Administration for treatment of type II diabetes.

Ileal bile acid transport (IBAT) inhibitors

Several companies have developed small molecules that inhibit ASBT, the apical transporter of the ileal enterocyte, and thereby induce bile acid malabsorption. A potent, nonabsorbable compound was developed by Monsanto-Searle [115] a company now

owned by Pfizer. Commercial development of the compound has been put on hold because of limited efficacy in treating hypercholesterolemia as well as dose-related diarrhea. In principle, diarrhea could be prevented by the simultaneous administration of a bile acid sequestrant. IBAT inhibitors are of potential value for the treatment of cholestatic pruritus because in cholestasis, there is evidence for inappropriate ileal conservation of bile acids [116].

Biliary drainage

In some cholestatic children in whom there is residual bile secretion, partial biliary diversion is done. A small bile duct is cannulated and drained to a collection bag worn by the patient. This procedure causes dramatic relief of pruritus [117]. Presumably, such a drainage procedure results in less bile acids being presented to the ileum. This decreases the amount of bile acids returned to the liver that in turn leads to more efficient hepatic uptake and less spillover of bile acids into the plasma compartment. Such children can also be treated by ileal bypass surgery [118], consistent with this interpretation.

Albumin dialysis to remove elevated plasma bile acid levels

Several devices are either on the market or are being tested that remove bile acids from plasma. In the MARS™device, blood passes through a multifilament membrane dialyzer; a solution of albumin flows countercurrent to the blood. The membrane permits bile acids (and conjugated bilirubin) to pass through it and bind to the albumin in the dialyzate. The dialyzate, containing albumin and its newly bound molecules, passes through both a charcoal column and an anion exchange column and is continuously stripped of its bound molecules. This technique of extracorporal albumin dialysis is quite effective for treating cholestatic pruritus, presumably because it removes plasma bile acids as well as some bile acids in tissue stores [82]. Other devices are based on plasma ultrafiltration through a large pore membrane, permitting albumin to pass through the membrane pores. The albumin is then exposed to an adsorbent which removes albumin bound molecules such as bile acids and conjugated bilirubin. Such devices should also be effective for treating cholestatic pruritus.

Epilogue

In the past two decades, new natural bile acids have been discovered and synthesized. The major apical and basolateral bile acid transporters have been cloned, characterized, and ablated in mice. A nuclear receptor for bile acids and its downstream genes have been identified. Bile acids have been shown to act on G-protein coupled receptors and may now be classified as hormones. New functions for bile acids in the intestine have been elucidated. Most importantly, new bile acid agonists and antagonists are being developed for therapeutic purposes. We would like to think that bile acid workers may take satisfaction for their achievements in translational science.

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