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**The physiological and molecular biochemical mechanisms of bile formation**

Reshetnyak VI. Mechanisms of bile formation

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**Abstract**

The review considers the physiological and molecular biochemical mechanisms of bile formation. The composition of bile and structure of a bile canaliculus, biosynthesis and conjugation of bile acids, bile phospholipids, formation of bile micelles structures, enterohepatic circulation of bile acids is described. In general, the review focuses on molecular physiology of the transporting systems of the hepatocyte sinusoidal and apical membranes. Knowledge of physiological and biochemical basis of bile formation has implications on the understanding of mechanisms of development of pathological processes, associated with diseases of the liver and biliary tract.

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**Key words:** Bile acids; Bile phospholipids; Bile micelle structures; Bile salt transporters

**Core tip:** over the past 50 years, significant progress has been made in understanding the mechanisms of bile formation and secretion. This became possible due to the advances of fundamental investigations in cell biology, molecular biology, genetics and biochemistry. Knowledge of physiological and biochemical basis of bile formation has implications on the understanding of mechanisms of development of pathological processes, associated with diseases of the liver and biliary tract.

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**INTRODUCTION**

Bile is lipid-rich hepatic secretion that is necessary for elimination of cholesterol and xenobiotics from the body and for dispersion and efficient absorption of digested dietary lipid in the upper small intestine[1]. Biliary function is a vital function of the liver, which results from the sequential vectorial transport of endogenous and exogenous substrates through three compartments: the vascular space, the cellular space and the biliary space. Canalicular bile is produced by polarized hepatocytes that hold transporters in their basolateral (sinusoidal) and apical (canalicular) plasma membrane[2]. The biliary function is responsible for the homeostasis of lipid metabolism in particular of cholesterol metabolism, the elimination of toxic endo- and xenobiotics such as bilirubin, lipid bacteria products (endotoxin) and several inflammatory mediators[3]. Bile coming into the canaliculi, is modified by cholangiocytes through secretion and absorption. The main determinant of bile formation is an osmotic filtration process resulting from active transport of bile acids and other osmotic solutes[3]. I.P. Pavlov and his colleagues established the basic mechanisms of secretion of bile, its entry into the duodenum, and the role of bile in digestion[4]. Bile is essential for the intestinal digestion and absorption of nutriments. Almost half a century had passed before the experimentally founded ideas on the mechanisms of bile formation appeared. Later on they were also clarified and in the late 1970s took shape as a number of hypotheses of the mechanisms of bile formation. Recent insights into the cellular and molecular mechanisms that control the function and regulation of hepatobiliary transport have led to a greater understanding of the physiological significance of bile secretion. Individual carriers for bile acids and other organic anions in both liver and intestine have now been obtained from several species. In addition, complex networks of signals that regulate key enzymes and membrane transporters located in cells that participate in the metabolism or transport of biliary constituents are being unraveled[5]. Most of the membrane transporters ensuring bile formation have now been identified. The expression of these membrane transporters is regulated through transcriptional and post-transductional mechanisms. Transcriptional regulation is under the control of nuclear receptors activated by ligands such as bile acids, which act as endogenous steroids synthesized from cholesterol in hepatocytes[3].

Bile is an isoosmotic electrolytic fluid that is formed in the liver and is a product of its secretory function. Bile primarily secreted by hepatocytes (*i.e.* the canalicular bile) subsequently is delivered to the intrahepatic bile ducts, where is modified by cholangiocytes (*i.e.* the ductal bile). Bile secretion by liver parenchymal cells is the result of vectorial transcellular transport of solutes and involves the coordinated action of transport proteins at the basolateral (sinusoidal) and apical (canalicular) membranes of the hepatocyte. A complex network of signals controls uptake and efflux transporters on a long- and a short-term timescale, including regulation at the level of gene transcription, protein translation and maturation, covalent modification, and dynamic localization of transporter proteins, as well as substrate availability[6].

**COMPOSITION OF BILE AND THE STRUCTURE OF A BILE CANALICULUS**

Bile contain almost all body components: proteins, lipids, carbohydrates, vitamins, mineral salts, and trace elements. The greater part of the bile proteins consists of globulins, the lesser one comprises albumins. Phospholipids, cholesterol and its esters, neutral fats, and fatty acids rank high among bile lipids. Lecithins (phosphatidylcholines, PC) are the major representatives of bile phospholipids. They are synthesized in the liver from the same components as plasma PC; however, they differ from the latter in the higher content of palmitic acid[7]. The human bile concentration of free fatty acids and α-monoglycerides is small. Human cystic bile shows small quantities of diglycerides and is virtually free of triglycerides.

The electrolyte content of bile is similar to that of plasma. The major cations are sodium, potassium, and calcium; the anions are chlorides and bicarbonates. The bile content of sodium is about 10 times higher than that of potassium. Excretion of sodium, potassium, and calcium into the bile is closely related to the rate of metabolic processes in the liver and depends on its functional state and on the content of salts in the body. The bile concentration of anions is 5-15 times smaller than that of cations.

A deficit of anions is compensated for by taurocholate (Figure 1). Bile contains a considerable quantity of phosphorus, magnesium, iodine, iron, and copper. The relative proportions of the major bile components are distributed in the following order: bile acids (67%), phospholipids (22%), proteins (4.5%), cholesterol (4%), and bilirubin (0.3%). Among the bile acids, the primary bile acids, cholic and chenodeoxycholic acids in a ratio of 1:1, account for approximately 50%. These are followed by the secondary bile acids, deoxycholic and lithocholic acids, as well as ursodeoxycholic and sulfolithocholic acids in the decreasing order.

The use of color cathode-luminescence scanning electron microscopy (CCL SEM) has made it possible to study first the qualitative composition of dehydrated human bile and its components in the native state. A combination of color cathode-luminescence and scanning electron microscopy provides video information not only on the structural pattern of bile, but also on its chemical composition. In 1998, Loginov *et al*[8] were the first to show the earlier unknown phenomenon of cathode autoluminescence of non-conjugated bilirubin, unesterified cholesterol, high-molecular-weight protein, and other organic compounds. The pictures (Figure 2) show that the amorphous powder mass of bilirubin is red-emitting (Figure 2A), crystal cholesterol luminesces blue (Figure 2B) and the green coloration tingled with red is predominant in the sample of protein (Figure 2C). Graphically, all three samples have chromaticity histograms in the form close to the Gaussian curve.

Examination of the dehydrated samples of normal bile by CCL SEM has ascertained the tree-type structure of precipitates (Figure 2D). The qualitative assessment of the samples suggests their balanced content of three major bile components: bilirubin, cholesterol, and protein. Hepatocytes are the major epithelial cells in the liver, and are polarized. The polarized surfaces of hepatocytes consist of a basolateral domain facing the circulation and an apical domain that forms the bile canaliculus, the smallest branch of the biliary tree[9]. A major function of the liver is biliary secretion, which requires hepatocyte polarization. The mechanisms controlling hepatocyte polarization are partially understood[10]. Structurally they include cytoskeletal, tight junction and intracellular trafficking components. Recently it was discovered that the major mammalian bile acid, taurocholate, accelerated polarity in primary rat hepatocytes. Taurocholate increased cellular cAMP and signals through an EPAC (Exchange Protein Activated by cAMP)-Rap1-MEK-LKB1 (Liver Kinase B1)-AMPK (AMP-activated Protein Kinase) pathway for its polarity effect. Review of Fu et al. discusses possible mechanisms for how taurocholate affects different cell polarity factors, particularly AMPK, and thereby regulates events that generate polarity[9].

Bile production starts in the intercellular bile canaliculi. The bile canalicular lumen is formed by the external hemileaflet of the apical part of the plasma membrane of the adjacent hepatocytes and the tight junctions located at the contact of hepatocytes. The hepatocyte tight junctions separate the bile canalicular lumen from the hepatic blood system[11]. Tight junction contains proteins, including occludin, claudin and ZO-1. The integrity of tight junctions depends on the presence of protein ZO-1 on the inner surface of the plasma membrane. The impaired integrity of tight junctions is accompanied by regurgitation of canalicular bile into the sinusoids. Three individual domains, such as sinusoidal, lateral, and canalicular parts of the membrane, are arbitrarily identified in the hepatocyte plasma membrane. The differences in the lipid and protein composition of the three domains of the hepatocyte plasma membrane determine its functional polarity. The sinusoidal membrane is enriched with receptors, enzymes, and transport proteins. The lateral membrane of the hepatocyte is involved in intercellular interaction, but it contains no transport systems. Transport systems for bile acids, organic anions and cations, as well as the enzymes γ-glutamyltransferase, Mg2+–ATPase, and alkaline phosphatase are located on the canalicular membrane. Cl–/HCO3– exchangers and selective Сl– channels are also identified[12]. The questions whether there are transport systems for glutathione and its conjugates in the apical membrane are still debated[13]. The release of glutathione S-conjugates from cells is an ATP-dependent process mediated by integral membrane glycoproteins[14].

The hepatocyte cytoplasm around the bile canaliculus contains cytoskeletal structures: microtubules, microfilaments, and intermediate filaments[15]. The microtubules take part in the mediated vesicular transport, secretion of lipids. The microfilaments determine canalicular contractility and motor activity. The intermediate filaments form a network between the plasma membranes, nucleus, intracellular organelles, and other cytoskeletal structures. The canaliculus is the least branch of a biliary tree and has a diameter of approximately 1 μmol/L.

**BIOSYNTHESIS OF BILE ACIDS**

In 1848 Strecker[16] discovered bile acids[17]. In chemical structure, they belong to a group of steroids and are cholanoic acid derivatives. Bile acids are the end metabolic product of cholesterol and one of the most important routes of its elimination from the body[18,19,20]. Bile acid synthesis occurs through two pathways: the classic (neutral) pathway or the alternative (acidic) pathway[21,22]. The liver is the only organ that has all 14 enzymes required for de novo synthesis of two primary bile acids in humans, cholic acid, (CA; 3α, 7α, 12α-trihydroxy-cholanoic acid) and chenodeoxycholic acid, (CDCA; 3α, 7α-dihydroxy-cholanoic acid)[23].

The biosynthetic pathway includes a number of successive enzymatic conversions associated with the oxidation of cholesterol in the smooth endoplasmic reticulum and the shortening of its side chain in mitochondria. The production of bile acids involves restoration of a double bond in cholesterol, С-3 inversion to give rise to a 3α-ОН-group, further α-hydroxylation of either only a 7-carbon atom or 7- and 12-carbon atoms, as well as β-oxidation of the side chain of cholesterol. Cytochrome Р450 is involved in all oxidative reactions. The classic bile acid biosynthetic pathway is initiated by enzyme cholesterol-7α-hydroxylase (CYP7A1) (Figure 3)[20]. The enzyme CYP7A1 of smooth endoplasmic reticulum is a limiting link in the synthesis of bile acids[20]. The activity of this enzyme is regulated by the quantity of intestinally absorbed bile acids, other than cholesterol[20,24]. Numerous studies have demonstrated that bile acids, steroid hormones, inflammatory cytokines, insulin, and growth factors inhibit *CYP7A1* transcription through the 5′-upstream region of the promoter[25-30]. Sterol 12 α-hydroxylase (CYP8B1) is required for synthesis of CA. Mitochondrial sterol 27 hydroxylase (CYP27A1) catalyzes sterol side chain oxidation, after which cleavage of a three-carbon unit in the peroxisomes leads to formation of a C24 bile acid. Cholesterol is converted to two primary bile acids in human liver, CA and CDCA. Key regulated enzymes, CYP7A1, CYP8B1, CYP27A1, and CYP7B1, in the pathways are indicated. CYP7A1 initiates the classic (neutral) bile acid biosynthetic pathway in the liver. CYP27A1 initiates the alternative (acidic) pathway in the liver and macrophages. CA and CDCA are conjugated to glycine (G) and taurine (T). BACS (bile acid: CoA synthase) and BAT (bile acid: amino acid transferase) are two key enzymes involved in amino conjugation of bile acids[20].

An alternative (acidic) pathway is initiated by CYP27A1, which in addition to the liver is expressed in macrophages and most other tissues, and may contribute significantly to total bile acid synthesis (Figure 3). Other minor pathways initiated by 25-hydroxylase in the liver and 24-hydroxylase in the brain also may contribute to bile acid synthesis. A nonspecific 7α -hydroxylase (CYP7B1) expressed in all tissues is involved in the generation of oxidized metabolites (oxysterols), which may be transported to the liver and converted to CDCA[20]. Farnesoid X receptor (FXR) plays a central role in the regulation of bile acid synthesis, excretion, and transport[31,32]. FXR inhibits *CYP7A1*[33], *CYP8B1*[34], and *CYP27A1*[35] transcription. It has been reported that peroxisome proliferator activated receptor α (PPARα) plays a role in the regulation of bile acid synthesis[36]. Bile acids induce *PPAR*α transcription via induction of FXR[37].

Di- and tri-hydroxycholanic primary bile acids are produced from cholesterol during biosynthesis of bile acids in man. Under normal conditions, approximately 200-600 mg of primary bile acids is formed daily[20,38]. Bile acid synthesis increases in the morning regardless of food intake[39]. In humans, bile acid synthesis exhibits a diurnal rhythm with two peaks around 3 and 9 PM[20]. Bile acids lost in the feces (0.2–0.6 g/d) are replenished by *de novo* synthesis in the liver to maintain a constant bile acid pool. Hepatic conversion of cholesterol to bile acid balances fecal bile acid excretion and this process represents a major route for elimination of cholesterol from the body[40,41].

# Bile acids are physiological detergents that generate bile flow and facilitate intestinal absorption and transport of lipids, nutrients and vitamins[20]. Bile acids, end products of the pathway for cholesterol elimination, are required for dietary lipid and fat-soluble vitamin absorption and maintain the balance between cholesterol synthesis in the liver and cholesterol excretion[21].

# CONJUGATION OF BILE ACIDS

# Under physiologic conditions, free bile acids are not virtually found in bile. Newly synthesized bile acids conjugate with glycine or taurine, by giving rise to bile salts: (chenodeoxy-)cholylglycine or (chenodeoxy-)cholyltaurine (Figure 3). The physiological significance of bile acids conjugations consists in that their salts are more polar compounds than free bile acids. Conjugation of bile acids to glycine or taurine decreases bile acid toxicity and increases their solubility that benefits the secretion into bile. Conjugated bile acids have smaller critical concentration for micellar formation[42]. The taurine conjugates of bile acids are more polar compounds than their glycine conjugates[43]. Taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid in humans and plays an important role in several essential biological processes such as bile acid conjugation, maintenance of calcium homeostasis, osmoregulation and membrane stabilization[44]. Taurine has the efficient action to reduce plasma and liver cholesterol concentrations. Cholesterol lowering effect of taurine is actually involved in the regulatory mechanism of cholesterol and bile acid homeostasis that mediated by CYP7A1, which has become a biomarker for cholesterol metabolism and itself is also regulated by several factors and nuclear receptors[45].

# The C-24 conjugation of free cholanic acids with glycine or taurine is accomplished by hepatocyte acyltransferases. The reaction proceeds in two steps with the participation of ATP and in the presence of Mg2+. Bile acid:CoA synthase (BACS) and bile acid:amino acid transferase (BAT) are involved in amino acid conjugation of bile acids[20]. FXR stimulates bile acid conjugation by inducing expression of genes encoding BACS and BAT, which also are induced by hepatocyte nuclear factor 4α (HNF4α)[46]. Thus, FXR and HNF4α may coordinately regulate bile acid synthesis and conjugation.

# The normal human ratio of the glycine to taurine conjugates of bile acids is 3:1. The ratio of the glycine/taurine conjugates may be altered under the influence of alimentary and hormonal factors, in some liver diseases. The presence of unconjugated bile acids in the bile most frequently is a result of hepatic disease.

# In the intestine, glyco- and tauro-conjugated CA and CDCA are deconjugated, and 7α -dehydroxylase activity in bacteria flora removes a 7α-hydroxy group to form secondary bile acids deoxycholic acid (DCA; 3α,12α-dihydroxy) and lithocholic acid (LCA; 3α-monohydroxy), respectively[20].

# Bile acids may be also exposed to glucuronidation or sulfation, which results in a reduction of their toxic properties and promotes their urinary and fecal excretion[43,47-49]. A small amount of bile acids circulated to the liver is sulfoconjugated at the 3-hydroxy position by sulfotransferase (SULT2A1) and rapidly secreted into bile. Sulfation is the major pathway for detoxification of extremely hydrophobic bile acids in humans[50]. Details of bile acid chemistry, biology, physiology, and synthesis have been reviewed recently[51,52].

# BILE PHOSPHOLIPIDS

# Phospholipids are a heterogeneous group of substances. They contain fatty acids with a varying length of a carbon chain. Phospholipids are an important component of cell membranes, plasma, and bile. In all mammalian species, including man, bile phospholipids are represented solely by a mixture of phosphatidylcholine (PC, lecithin) molecules. The fatty acids of bile PC molecules greatly differ from those of lecithin of plasma and liver tissue. Depending on fatty-acid residues at 1 and 2 carbon atoms of the glycerine molecule that is part of PC, there are a great variety of lecithins. Human bile predominantly contains 1-palmitoyl, 2-linoleyl, and 1-palmitoyl, 2-oleoyl phosphatidylcholines. This lecithin subpool called dynamic represents nearly 5% of the total pool of hepatic phosphotidylcholines. The lecithins destined for bile secretion have been shown to be apparently completely synthesized *de novo*[53]. Selection of certain types of PC seems to occur during bile formation[7]. Bile salts and primarily phosphatidylcholine are the main organic solutes in bile, and play a crucial role in cholesterol and dietary lipid solubilization. Bile salts and phospholipids in concert increase the biliary solubility of cholesterol over a million-fold, thereby permitting entry of hepatocellular cholesterol into bile[54]. Hepatic PC biosynthesis is likely to be regulated by bile salts. Biliary lipid secretion is driven by bile salts, the primary metabolites of cholesterol. There is multiple data showing that the biliary secretion of PC (as cholesterol secretion) depends on that of bile acids[55,56]. It has been also shown that the secretion of bile phospholipids is not only associated with that of bile acids, but is regulated by the amount and type of bile salts passing through the hepatocyte[7,57].

# The use of electron microscopy has made to demonstrate that phospholipids are secreted as vesicles into bile, and that vesicles secretion is almost wholly dependent upon MDR3 P-glycoprotein function[58]. Biliary-specific phosphatidylcholine molecules are recruited initially from intracellular sources, predominantly the endoplasmic reticulum[59]. A proposed mechanism for phosphatidylcholine delivery to the canalicular plasma membrane involves monomeric transfer via binding to cytosolic phosphatidylcholine transfer protein (PC-TP[60]), a process that is stimulated by low concentrations of bile salts typical of the hepatocyte's cytosol. Alternatively, biliary lipid may arrive at the canalicular membrane via intracellular vesicle transport[1].

# Biliary phosphatidylcholine molecules are translocated from the endoplasmic to the exoplasmic hemileaflet by the action of a transmembrane translocator[61].

MDR3 P-glycoprotein is thought to translocate phosphatidylcholines from the internal to the external hemileaflet of the hepatocyte canalicular plasma membrane, possibly generating microdomains of the more fluid biliary-type phosphatidylcholines. Luminal bile salts, secreted as monomers through the action of bile salt transporters (cBAT), will interact preferentially with these microdomains to promote vesiculation of the membrane external hemileaflet, possibly involving nonlamellar phase transitions. Cholesterol, which can freely flip between the internal and external hemileaflets, may be released into bile either by diffusing laterally into nascent vesicles, or by bile salt–mediated transfer through the aqueous phase. The model is based on data from the study[59] and published work[1,62,63].

[Crawford](http://www.ncbi.nlm.nih.gov/pubmed/?term=Crawford%20JM%5Bauth%5D) *et al*[58] proposed the concept that biliary phosphatidylcholine molecules are flipped from the internal to the external hemileaflet of the hepatocyte canalicular membrane by the action of MDR3 P-glycoprotein, followed by release of phosphatidylcholine vesicles from the external hemileaflet into bile (Figure 4). Formation and detachment of unilamellar vesicles from the canalicular membrane, presumably mediated by the detergent action of luminal bile salts.

Vesiculation of the external hemileaflet provides an explanation of how luminal bile salts can extract large quantities of phospholipid on the basis of their detergent action, without disrupting the integrity of the detergent-resistant canalicular plasma membrane. This remarkable mechanism for selective secretion of membrane phospholipids appears to be uniquely adapted to the detergent environment of the bile canaliculus.

**BILE MICELLES**

Bile lecithins, bile acid salts, and cholesterol are amphiphiles molecules (Figure 5). In this connection, in the water medium, such as is bile, these compounds cannot exist in the monomolecular form and they generate micellar or lamellar structures.

According to the inclusions into the lipid complex, simple micelles (PC + cholesterol), up to 3 nm in size, mixed (PC + cholesterol + bile acids), 3-6 nm in diameter, and vesicles (PC + cholesterol + bile acids), 25-130 nm in diameter, are identified (Figure 6).

They all can contain on their surface amphiphilic proteins. Lipid molecular inclusions into the micelles of bile acids and the formation of mixed micelles are the main form of interaction of bile acids and lipids in the bile. By forming mixed micelles, bile acids along with lecithin provide cholesterol solubilization. Bile salts as detergents form micelles when the critical concentration of micellar formation (CCM) is achieved. CCM depends on the type of bile salts and their hydrophobicity. Based on their hydrophobic properties, bile acids may be arranged in the following order: Deoxycholic > Chenodeoxycholic > Cholic > Ursodeoxycholic[64]. Hydrophobic, but not hydrophilic, bile acids are efficacious endogenous ligands of the nuclear receptors FXR (NR1H4), pregnane X receptor (PXR; NR1I2), and vitamin D receptor (VDR; NR1I1) that play critical roles in the regulation of bile acid synthesis and metabolism[20].

A mixture of bile acids, lecithin, and cholesterol at certain molecular ratios is able to form lamellar liquid-crystalline structures[64]. Vesicles are monolamellar globules that consist of phospholipids (PL) and bile acids. At a certain concentration (approximately 0.1 mmol/L), PLs as amphiphiles have been found to produce enclosed globules, in which the polar heads are directed outward, and the nonpolar “tails” are inside. This particle is capable of comprising many substances, including protein, and cholesterol, by providing solubilization of the latter by bile acids. Vesiculation is largely determined by the hydrophilic-hydrophobic balance of bile acids and the content of cholesterol[65]. The proportion of bile mixed micelles and vesicles depends on the concentration and composition of bile acids. The most important feature of phospholipid masses is their ability to change their consistency from crystalline gel to liquid-crystalline state depending on environmental conditions. The liquid-crystalline states are typified by the structural order peculiar to true crystals with the preserved mobility of the molecules observed in solutions. The fusion of monolamellar vesicles may give rise to multilamellar vesicles. The vesicles are a rather stable formation and together with micelles play a pivotal role in the solubilization and transport of cholesterol into the bile[24].

Lipid vesicles approximately 60 to 80 nm in diameter has been documented repeatedly by electron microscopy and quasielastic light scattering of freshly secreted hepatic bile[1,58]. Microscope laser light scattering of bile canaliculi in isolated rat hepatocyte couplets also detects intraluminal vesicles of similar size, the numbers of which increase upon cellular incubation with bile salts[66].

“Acid-dependent” and “acid-independent” bile fractions are identified[67]. The former is associated with the synthesis and active transport of bile acids in the hepatocyte. The volume of the resultant bile is linear with the concentration of bile acids, caused by the osmotic effect of bile acid anions, and I. Sperbet first argued it in 1959[68]. The bile composition greatly varies in the bile canaliculi due to the increased constant volume of water and bicarbonates irrespective of the secretion of bile acids. This “acid-independent” bile fraction is regulated by an active Na+ transport. Its physiological role is to dilute and excrete an acid-dependent fraction[69]. Further bile formation occurs in the bile ducts as a fluid enriched with bicarbonates and chlorides is produced in the latter. This process is under the control of secretin (80%) and other gastrointestinal hormones. The volume of the water secreted in the ducts depends to greater degree on the active transport of bicarbonates[70].

**ENTEROHEPATIC CIRCULATION OF BILE ACIDS**

The bile acids synthesized in the hepatocyte participate in the human body, in the so-called enterohepatic circulation. Conjugated bile acids are secreted from the hepatocyte into the bile canaliculus by canalicular bile salt export pump (BSEP, ABCB11). Some bile acids secreted in the bile duct are reabsorbed in the cholangiocytes and recycled back to hepatocytes (the cholangiohepatic shunt)[20]. The bulk of bile acids secreted to enter and stored in the gallbladder. After each meal, gallbladder contraction empties bile acids into the intestinal tract. Bile acids are mixed up with food masses (together with other digestive secretions), and take an active part in the processes of fat and fat-soluble vitamins metabolism and absorption.

Fat acids and monoglycérides formed from neutral fats with participation of bile acids and of lipases in the upper parts of the small intestine are absorbed by enterocytes as lipoids-biliary complexes. In enterocyte complex disintegrate. Released monoglycérides and fat acids can are used by the cell as an energy and building materials or are transported to basolateral part of the membrane of enterocyte and further enter into the system of portal vein. Bile acids may again go back into the intestine and continue to participate in the processes metabolism and absorption of fat and fat-soluble vitamins. As, according as bile acids are moving through the intestine they may 4-6 times to participate in the transportation of monoglycerides and fat acids in enterocyte.

In the small intestine, bile acids are absorbed by both passive and active mechanisms[71]. Whereas passive absorption occurs down the length of the intestine, active absorption of bile acids is restricted to the ileum[72,73]. Passing through the intestinal tract, some bile acids are reabsorbed in the upper intestine by passive diffusion, but most bile acids (95%) are reabsorbed in the distal ileum by apical sodium-dependent bile acid transporter (ASBT) located in the brush border membrane[74]. In man and all other vertebrates examined to date, the ileal epithelium has developed an efficient transport system for the active reclamation of bile acids[51]. Approximately 95% of the bile acids secreted into bile are derived from the recirculating pool[74]. Bile acids are transdiffused across the enterocyte to the basolateral membrane where the organic solute transporter and heterodimer (OST/OST) effluxes bile acids into portal blood circulation[75,76], transported to the sinusoid, and taken up by Na+-dependent taurocholate cotransport peptide (NTCP) into hepatocytes[20]. The bulk of bile acids are absorbed by hepatocytes into the liver, and again excreted into the bile. Later on, this enterohepatic cycle is recurred. Hepatocytes are well known to secret as high as 90% of the bile acids that have returned into the cells during enterohepatic circulation, and about 10% of the newly synthesized bile acids[20]. A bile acid pool of about 3 g is recycled 4–12 times a day[74]. At a fundamental level, the bile acid enterohepatic circulation can be viewed as a series of storage chambers (gallbladder, small intestine), valves (sphincter of Oddi, ileocecal valve), mechanical pumps (canaliculi, biliary tract, small intestine), and chemical pumps (hepatocyte and enterocyte transporters)[74].

The enterohepatic circulation of bile acids serves as an important physiological route not only for recycling of bile acids and absorption of nutrients but also for regulation of whole-body lipid metabolism[20]. Enterohepatic circulation ensures a continuous supply of bile acids to be used repeatedly for lipid absorption during the digestion of a single meal or multiple meals throughout the day[77]. Efficient intestinal reabsorption and hepatic extraction of bile acids also enables a very effective recycling and conservation mechanism that largely restricts these potentially cytotoxic detergents to the intestinal and hepatobiliary compartments.

FXR plays a key role in control of enterohepatic circulation of bile acids. FXR induces the expression of BSEP in the canalicular membrane, which is the driving force for bile formation. FXR inhibits *Ntcp* transcription by SHP-dependent inhibition of retinoid X receptor/RAR induction of *Ntcp*[78]. Thus, FXR plays a critical role in the coordination of bile acid synthesis, biliary bile acid secretion, intestinal bile acid reabsorption and secretion, and bile acid uptake into hepatocytes.

Enterohepatic circulation of bile acids is example of perfect principle of economical and efficient use substances synthesized by human organism derived in evolution process (as development).

Hepatocytes hold a central position in the enterohepatic circulation of bile acids, with which their highest (as compared to other cells) content of cholanic acids is associated.

***Molecular mechanisms of hepatocytic bile secretion***

The hepatocyte secretion of bile is the passive transhepatocellular filtration of water and electrolytes (Na+, K+, Cl–) from blood into the bile canalicular cavity, which caused by the osmotic gradient between the bile and blood, which results from an active transport of organic and inorganic anions from blood and hepatocytes to the bile canaliculi. The ionic transport systems that govern ionic and electrical gradients onto the membrane surface (Na+/K+, Na+/H+, Na+/HCO3–, Cl–/HCO3–, Na+/Ca2+, *etc.*) are the basis for the mechanism of targeted bile secretion[79]. The transport of bile salts, organic anions and cations, bilirubin and other substances from the portal blood into the biliary system is accomplished through the action of an array of transporter proteins in the hepatocyte[80].

The hepatocyte is a polar cell in which the transport of osmotically active bile components has a strict direction from the sinusoidal (basal) surface to its canalicular (apical) part. There is evidence on the importance of taurocholic acid in the directed transport of substances in the liver cells[44]. This vectorial trans-hepatocellular movement of bile acids is a remarkably concentrative transport process that is driven by a distinct set of primary (ATP-dependent), secondary (Na+ gradient-dependent), and tertiary (OH− or HCO3−-dependent anion exchange) transport systems at the sinusoidal and canalicular plasma membranes[81,82]. Bile secretion is a highly regulated process. To maintain this process, liver cells must transport bile acids efficiently from the portal blood into bile. ATP Binding Cassette (ABC) transporters[57] mediate transport of bile salts. Expression of these transporters is regulated in a coordinate fashion by a set of nuclear hormone receptors explaining the old observation of coupling between bile salt secretion and biliary lipid secretion. Over the past two decades, there has been significant progress toward identifying the individual membrane transporters and unraveling their complex regulation[74]. The hepatocyte is a polarized cell that expresses differential transport systems in its plasma membrane domains. Molecular cloning has identified most of these transport proteins, and their transport properties characterized by functional studies. The NTCP, the bile salt export pump (BSEP), the ASBT, and the organic solute transporter OSTα-OSTβ, the major bile acid transporters that control the fate of bile acids, either absorption and enterohepatic cycling or excretion and elimination from the body.

These transporters play a key role in the vectorial transfer of solutes and water from sinusoidal blood into the bile, thus contributing to bile formation and the biliary excretion of various xenobiotic. In liver and intestine, transporters play a critical role in maintaining the enterohepatic circulation and bile acid homeostasis[74,83].

Bile acids determine the secretion of an “acid-dependent” bile fraction, but inorganic ions and proteins that of an “acid-independent” fraction. The transport of bile acids from the sinusoidal space to the bile canaliculus comprises their penetration across the sinusoidal membranes, canalicular membranes and movement inside the hepatocyte. The molecular and metabolic processes occurring onto the hepatocyte sinusoidal membrane involve: (1) the hepatocyte absorption of bile acids and proteins from blood; and (2) the transmembrane transfer of inorganic ions. The principal intracellular molecular and metabolic processes determining the mechanisms of hepatocyte bile secretion are: (1)the biosynthesis and conjugation of bile acids; (2) the hydroxylation and conjugation of bile acids entering the hepatocyte from blood; and (3) the transfer of bile acids and proteins from the perisinusoidal area to pericanalicular on of a hepatocyte.

The molecular mechanisms of hepatocyte canalicular membrane function ensure the transmembrane transfer of bile acids, proteins, inorganic and organic cations and anions into the bile canalicular cavity.

**Molecular physiology of the transporting systems of the hepatocytic sinusoidal membrane:** The specific feature of transport of bile acids from blood to the hepatocyte across the sinusoidal membrane is to overcome the high gradient of their concentration and electrical potential. Hepatocytes absorb bile acids from blood by the bile acid carriers, integral hepatocyte sinusoidal membrane proteins[79] (Figure 7).

Liver sinusoids possess a specific architecture that allows passage of organic compounds bound to albumin through endothelial fenestrae into the space of Disse, from where they can be taken up by the sinusoidal transport systems of the hepatocytes[84,85].

Since the bulk (about 85%) of the bile acids present in plasma as a complex of plasma proteins (mainly, albumin)–bile acid, it has been shown that albumin performs its inherent transport function and contributes to the interaction of bile acids with specific sinusoidal membrane receptors in the mechanisms of hepatocyte absorption of bile acids. The bile acids in sinusoidal blood are efficiently taken up by hepatocytes from Disse’s space despite being highly albumin bound, due to the existence in the basolateral membrane of transporters[86,87]. The powerful techniques of molecular biology have enabled gene cloning of the transporters involved in biliary secretion and the enterohepatic circulation of bile acids. This has permitted elucidation of their function as well as their regulation by nuclear receptors[88]. The enterohepatic circulation results from efficient ileal absorption, and is highly regulated at two sites. In the hepatocyte, biosynthesis of bile acids is regulated in negative feedback manner by the nuclear receptor FXR as well as by cytokines and by a peptide (FGF-19) liberated by bile acids from the ileal enterocyte. In the ileal enterocyte, bile acid reclamation is regulated in negative feedback manner by FXR and other nuclear receptors. The bile salt export pump (BSEP) mediates uphill canalicular bile acid secretion[88]. The plasma membranes of hepatocytes have been found to contain the proteins that selectively bind bile acids[88]. This uptake is carried out against an electrochemical gradient, is saturable[89] and depends on the structure of the bile acids. It is more efficient for trihydroxy- than for dihydroxy-bile acids and for conjugated more than for unconjugated bile acids[90]. Transporters on the basolateral membrane, which faces the space of Disse, are responsible for the uptake of bile salts and organic anions[80]. The liver cells have an active transport system to transfer bile acids from blood to the hepatocyte. Basolateral uptake transporters can be divided into Na+-dependent and Na+-independent systems. The sodium taurocholate-cotransporting polypeptide (NTCP), the main Na+-dependent bile acids transporter[91-93] is only expressed in the basolateral membrane[94]. NTCP, (gene name SLC10A1) is the founding member of the SLC10 family of solute carrier proteins, which includes two bile acid carriers (SLC10A1/NTCP and SLC10A2/ASBT), one steroid sulfate transporter (SLC10A6/SOAT), and four orphan carriers (SLC10A3, SLC10A4, SLC10A5, SLC10A7)[95-97]. Na+-dependent uptake involves co-transport of solutes with Na+. Moreover, the driving force is the energy of ATP generated by sodium-potassium-activated adenosine triphosphatase (Na+,K+-АТPase). This enzyme is located on the sinusoidal and lateral membranes of hepatocytes[98,99] and undetectable on canalicular (apical) part[100,101]. The active transport of bile acids ensures their continuous hepatocyte “pumping” from blood to bile, thus causing their low level in the blood flowing from the liver and in plasma as a whole. The properties of NTCP satisfied all the functional criteria for hepatocyte Na+-coupled bile acid uptake[74,95]. NTCP's major physiological substrates include all the major glycine and taurine-conjugated bile acids[94,102-105]. Depending on the structure of the bile acid and NTCP, unconjugated bile acids are moderate or weak substrates[103,106,107] and sulfated bile acids appear to be only weakly transported[108]. The high level of NTCP expression at the sinusoidal membrane of hepatocytes and NTCP's high affinity for conjugated bile acids promotes their efficient extraction from portal blood. Thus, NTCP functions to maintain the enterohepatic circulation of bile acids and keeps plasma concentrations at a minimum.

The uptake of bile acids by Na+-independent mechanisms seems to be mediated by less specific transporters, known as organic anion-transporting polypeptides (OATPs), which exchange these molecules for other anions, such as HCO3-, glutathione (GSH) or even other bile acids[90,91,109].

Four OATPs have been cloned and characterized from human liver, namely: OATP1A2 (SLCO1A2/SLC21A3; formerly, OATP-A), OATP1B1 (SLC21A6; formerly, OATP-C or LST-1), OATP1B3 (SLC21A8; formerly, OATP-8) and OATP2B1 (SLC21A9; formerly, OATP-B). These transporters may take up bile acids (mainly non conjugated forms), endogenous OA- (thyroid hormones, monoconjugated bilirubin) and xenobiotic compounds (toxins, drugs, food components, *etc.*)[110]. In contrast to NTCP, members of the OATP family expressed on the hepatocyte sinusoidal membrane, such as Oatp1a1 (Slco1a1), efficiently transport unconjugated or sulfated bile acids, suggesting that these carriers participate in the hepatic uptake of those bile acid species *in vivo*[90,103,111]. The heterodimeric protein OSTalpha/OSTbeta is expressed at the basal membrane of hepatocytes and cholangiocytes[112]. This is a sodium-independent bile acid transporter that may play a role in bile acid efflux from hepatocytes toward blood when these compounds get accumulated in cholestatic conditions. Moreover, in cholangiocytes, in addition to play a similar role, this transporter may also be involved in the cholehepatic shunting of bile acids.

Hepatocellular uptake of organic cations is mediated by two separate transport systems, which depends on the substrate molecular size[113]. Thus, small (type I) organic cations are taken up by the organic cation transporter, OCT1/Oct1 (SLC22A1/Slc22a1), which is electrogenic in nature. On the other hand, human OATP-A mediate the uptake of bulky (type II) organic cations. In addition to conjugated and unconjugated bile salts, Oatps/OATPs accept other cholephilic compounds, including glucuronidated (and maybe unconjugated) bilirubin, exogenous organic anions (*e.g.,* sulphobromophthalein), leukotrienes, estrogen-conjugates (*e.g.,* estrone-3-sulfate or estradiol-17-β-d-glucuronide), thyroid hormones, mycotoxins, and numerous xenobiotics[93,114-116].

**Molecular physiology of the intracellular processes underlying the hepatocyte secretion of bile:** In order to explain the transit of bile acids from the sinusoidal membrane to the pericanalicular region, two different, not mutually excluding, mechanisms have been proposed: (1) simple diffusion of bile acids bound to intracellular proteins[117]; (2) and/or vesicular transport of bile acids driven by cytoskeleton contractile activity[118,119]. Two arguments have been raised against the role of the second mechanism. One is that hepatic transit of labeled bile acids is too fast[119]. The second one is that the baseline secretion of bile acids is not modified by microtubules disruption[118]. However, the overload of bile acids intensify the vesicular trafficking from the Golgi complex to the pericanalicular zone[120], and under these circumstances the alteration in the functional integrity of the cytoskeleton results in impaired bile acid secretion[121] and subsequently cholestasis[122].

The quantity of ABC transporters in the apical membrane is regulated by the amount of biliary components available for secretion[123,124]. The regulated intracellular vesicular traffic of canalicular ABC transporters[124,125] is crucial for normal bile secretion. The bile salt export pump (BSEP, formerly SPGE, a sister of P-glycoprotein) is the main, if not the only, canalicular bile acid transporter[126], and it is also located in subcanalicular vesicles that may act as an intracellular pool. It is therefore probable that the impaired secretion of bile acids observed in overloaded conditions would be an indirect result of the distortion of the increased vesicular traffic of transporters to the canalicular membrane[121]. These and other studies[127,128] have established not only the actual role of vesicular trafficking in hepatocytes, but also demonstrated that specific vesicle trafficking machinery is required for membrane polarity. The overall functions based on hepatocyte polarity are not attributable to the mere presence of transporters in both poles of these cells[129] but also to their intracellular trafficking and temporary anchorage to the different hepatocyte membranes (Figure 7).

**Molecular physiology of the transporting systems of the hepatocyte canalicular membrane:** After traversing the cell by Fick’s diffusion, cholephilic compounds mostly bound to high-affinity cytosolic proteins are excreted into the bile mainly by ATP-dependent pumps of the superfamily of ATP-binding cassette (ABC) transporters, in particular those belonging to the family of multidrug-resistance proteins, MDR/Mdr, or to the family of multidrug-resistance-associated proteins, MRP/Mrp[57,74].

MDRs/Mdrs are members of the ABC superfamily that were originally described in cancer cell lines, where they confer resistance to therapeutic agents. Three gene products were identified in rodents, Mdr1a (Abcb1a), Mdr1b (Abcb1b) and Mdr2 (Abcb4), and two in humans, MDR1 (ABCB1) and MDR 3 (ABCB4). MDR1/Mdr1 functions as an efflux pump for a wide range of amphiphilic, bulky type II cationic drugs, together with other hydrophobic compounds, such as endogenous and exogenous metabolites or toxins, steroid hormones, hydrophobic peptides and even glycolipids[114]. Two closely related but functionally distinct Mdr1 isoforms, mdr1a and mdr1b are present in the murine but not in the human phenotype[130]. MDR3/Mdr2 functions as a flippase, which translocate phosphatidylcholine (PC) from the inner to the outer hemileaflet of the canalicular membrane, followed by release of PC-containing vesicles from the outer hemileaflet into bile, a process facilitated by the detergent properties of luminal bile salts[58].

Monoanionic bile salts are excreted in the canalicular pole by the bile salt export pump (BSEP/Bsep; ABCB11/abcb11), another member of the MDR family[131]. In contrast, canalicular efflux of divalent, bipolar sulfated or glucuronidated bile salts is mediated by the multidrug-resistance-associated protein 2 (MRP2/Mrp2; ABCC2/Abcc2)[132,133]. This carrier is also engaged in the biliary excretion of many other organic anions, including glutathione-S-conjugates (*e.g.,* of leukotriene C4 or sulphobromophthalein, among others), glucuronides (*e.g.,* of bilirubin and estrogens), and reduced (GSH) and oxidized glutathione (GSSG), the former with low affinity[134,135]. Both GSSG and GSH are major determinants of the so-called “canalicular bile-salt-independent bile flow”[136].

The canalicular membrane domain also contains the electroneutral anion exchanger 2 (AE2/Ae2; SLC4A2/slc4a2), which extrudes HCO3- by exchanging the anion for biliary Cl-[137]. It functions to regulate intracellular pH when hepatocytes are exposed to an alkaline load[137]. In addition, AE2/Ae2 plays a role in bile flow generation, since HCO3- excretion is thought to be an additional primary driving force of the canalicular bile-salt-independent bile flow[137,138]. Both in humans and rats, three transcript variants of AE2/Ae2 have been described, namely the full-length transcript AE2a/Ae2a, expressed from the upstream promoter in most tissues, and the alternative transcripts AE2b1/Ae2b1 and AE2b2/Ae2b2, expressed in a more tissue-restricted fashion (mainly in liver and kidney). AE2b1/2/Ae2b1/2 transcription is driven from overlapping promoter sequences within intron 2, which result in AE2/Ae2 protein isoforms with short N-terminal differences[139,140].

Water transporters, for a solute to drive blood-to-bile vectorial water transport primarily, resultant osmotic forces need to be associated with aquaporin (AQP)-mediated transcellular movement of water molecules from plasma to the bile canaliculus[141]. Both immunochemical and functional studies have demonstrated the constitutive expression of the water channel AQP9 at the basolateral membrane of rat hepatocytes, and the regulated expression of the water channel AQP8 at the hepatocellular canalicular membrane domain[141-143]. AQP8 is suggested to play a role in bile formation, facilitating the osmotic movement of water under a choleretic stimulus[141,143]. AQP isoforms that mediate polarized water transport in human hepatocytes, if any, remain to be identified.

***Gallbladder***

The bile formed outside the digestive periods enters the gallbladder that performs two important functions: concentration of bile and its evacuation into the duodenum. Minor quantities of bile acids (approximately 1.3%) are absorbed in the gallbladder walls.Micelles and vesicles enter the gallbladder where in the concentration of lipids is increased by water absorption, which causes some physicochemical changes of bile (Figure 8).

If the gallbladder functions well due to its contraction, all agglomerated vesicles and micelles with a bile flow attain of the duodenum. The gallbladder mucosa actively absorbs amino acids (this has been established by 35S-labelled methionine) and an albumin bile protein fraction[144].

Thus, the gallbladder takes an active part in changing the composition of bile via reabsorption of its components into the blood and making the bile components circulating along the small circuit: liver-gallbladder-blood-liver.

The flow of bile is lowest during fasting, and a majority of that is diverted into the gallbladder for concentration. When chyme from an ingested meal enters the small intestine, acid and partially digested fats and proteins stimulate secretion of cholecystokinin and secretin. These enteric hormones have important effects on pancreatic exocrine secretion. They both are also important for secretion and flow of bile:

Cholecystokinin: The name of this hormone describes its effect on the biliary system-cholecysto = gallbladder and kinin = movement. The most potent stimulus for release of cholecystokinin is the presence of fat in the duodenum. Once released, it stimulates contractions of the gallbladder and common bile duct, resulting in delivery of bile into the gut.

Secretin: This hormone is secreted in response to acid in the duodenum. Its effect on the biliary system is very similar to what was seen in the pancreas-it simulates biliary duct cells to secrete bicarbonate and water, which expands the volume of bile and increases its flow out into the intestine.

***Sphincter of Oddi***

The sphincter of Oddi is located at the interface of the common bile duct and the main pancreatic duct at their confluence into the duodenum. Anatomic and immunohistochemical studies have indicated that the sphincter of Oddi is richly innervated with cholinergic, adrenergic, and peptidergic neurons.

The location of the sphincter of Oddi determines its function – to regulate the entry of bile and pancreatic juice into the duodenum and to prevent reflux of duodenal contents into the common bile duct and the main pancreatic duct. The sphincter coordinates the time and rate of secretion of about 3 liters of bile and pancreatic juice into the duodenum daily[145]. Normally, the sphincter is characterized by considerable phase contractions throughout the interdigestive period.

***Transport of bile acids along the portal venous system***

Bile acid salts come from the intestine into the portal venous system. Bile acids with venous blood enter again the liver where they are apparently completely (99%) absorbed by hepatocytes. And only a small quantity (about 1%) of bile acids enters peripheral blood. In this connection, in healthy individuals the blood concentration of circulating bile acid salts is very small.

In the hepatocytes, the secondary bile acids, deoxycholic and lithocholic, are subject to hydroxylation and they conjugate with glycine or taurine. Bile acids come as conjugates from the liver into bile again. The normal enterohepatic circulation of bile acids occurs 2-6 times daily depending upon the dietary regimen.

There are available data that the normal kidneys are not apparently involved in the excretion of bile acids from the body. Portal venous blood bile acids are bound by plasma proteins, mainly albumins[146] and, to a lesser extent, by α-and β-globulins. The data available suggest the binding of blood bile salts to lipoproteins[147,148]. The bile acids are loosely bound to lipoproteins. In healthy individuals, bile acids are detectable in all classes of lipoproteins[148].

**METABOLISM OF BILE PIGMENTS**

Bilirubin is a principal bile pigment. Bilirubin is formed in the cells of the reticuloendothelial system. Unconjugated bilirubin is a product of heme catabolism (Figure 9). Degradation of heme involves its conversion to biliverdin followed by reduction of biliverdin to bilirubin[149,150]. Two different enzyme systems are involved in the production of bilirubin from heme: (1) microsomal heme oxygenase[152,153]; (2) cytosolic biliverdin reductase[154,155].

The heme oxygenase activity is detectable in all body tissues. However, the highest activity of this enzyme is found in the spleen[151]. Heme oxygenase has an evident stereospecificity for the α-meso-bridge of a heme molecule, resulting in the formation of primarily IX-α isomers of biliverdin in man.

Cytosolic biliverdin reductase is also present in all body tissues; its activity is most pronounced in the spleen, liver, and kidney.

A gram of hemoglobin produces 36.2 mg of bilirubin[152]. In an adult, the daily bilirubin production determined by different methods is 3.9 ± 0.7 mg/kg (or 250-350 mg daily)–when determined with labeled bilirubin; 4.4 ± 0.7 mg/kg established from СО2 formation when heme is converted to bilirubin[150,155]. Normally, a human being generates bilirubin IX-α and a minor quantity of its structural isomers: bilirubins IX-β, IX-γ or IX-δ.

Then bilirubin is liberated from the endothelial tissue cells to plasma, binds to albumin and is delivered to the hepatocytes and up-taken by liver cells in a form dissociated from albumin. Bilirubin transport across the basolateral part of a membrane within the hepatocyte is accomplished by transport systems such as organic anions transport protein[156], *via* the flip-flop mechanism[157], or by simple diffusion due to chemical concentration gradient. The uptake of bilirubin is highly effective because of its rapid hepatic metabolism and excretion into the bile and of the presence of cytosolic binding proteins, such as glutathione-S-transferase. Unconjugated bilirubin is a nonpolar (fat-soluble) substance. In the endoplasmic reticulum of hepatocytes bilirubin, however, is converted by microsomal UDP-glucuronyl transferase to a polar conjugate with a glucuronic acid. Mono- and diglucuronidbilirubin is formed. Water-soluble conjugate of bilirubin is easily excreted into bile. Much smaller fractions of bilirubin are conjugated to sulfates, glucose, or xylose[158].

The physiologic significance of conjugation of bilirubin to sugars or glucuronic acid is diminishing its toxic properties through increasing molecular hydrophilicity, which improves bilirubin transport across the membrane structures and ensures secretion into the bile. However, IXβ-, IXγ-, and IXδ-isomers of bilirubin require no conjugation for bile secretion because of no rigid structure as compared to the molecule of bilirubin IXα. The latter has a rigid structure due to intramolecular hydrogen bonds. Interestingly, the destruction of the bonds can improve the biliary secretion of bilirubin IXα. It has been demonstrated, that ultraviolet blood irradiation results in destruction of intramolecular hydrogen bonds in bilirubin. This facilitates the diffusion of bilirubin IXα molecules across the membrane structures without conjugation with glucuronic acid or sugars.

A greater portion of conjugated bilirubin in the bile is present in the mixed micelles containing cholesterol, phospholipids, and bile acids. Conjugated bilirubin is hydrolyzed by bacterial glucuronidases under urobilinogens. Having a nonpolar molecule, urobilinogen is well absorbed in the small intestine, and, in minimal quantities, in the colon. The liver and kidneys reexcrete small quantities absorbed of urobilinogen. In hepatocyte dysfunction, hepatic urobilinogen reexcretion is impaired and renal excretion is increased[152,154,155].

**SECRETION OF WATER AND ELECTROLYTES INTO BILE**

Formation of bile and generation of bile flow are driven by the active secretion of bile salts, lipids and electrolytes into the canalicular and bile duct lumens followed by the osmotic movement of water[159]. Thus, water has to cross rapidly into and out of the cell interior driven by osmotic forces. Bile as a fluid, results from complicated interplay of hepatocyte and cholangiocyte uptake, secretion and concentration that involves various transporters of lipids, anions, cations, and water. Considering bile is composed of more than 95% water, the molecular basis and regulatory mechanisms of water transport in hepatocytes during bile formation are still under evaluated[160].

Theoretically, water can flow through the hepatocyte epithelial barrier either across tight junctions between adjacent hepatocytes (paracellular route) or across hepatocyte plasma membranes (transcellular route). The paracellular route was traditionally proposed as the major pathway for water movement.

The tight junctions play an important role in the paracellular secretion of water and the latter’s dissolved low-molecular-weight compounds. Water and electrolytes are assumed to pass from the intercellular space through the tight junctions into the bile canalicular lumen[100]. Moreover, the selectivity of electrolyte excretion is considered to be cause by the presence of a negative charge at the site of a tight junction. This charge serves also as a barrier to regurgitation of substances from the bile canaliculus into the sinusoidal space. The molecular mechanisms of paracellular permeability are currently associated with the functioning of the specific tight junction protein ZO-1. ZO-1 is phosphorylated with protein kinases, which is of great importance in the molecular mechanisms responsible for the regulation of paracellular permeability[161]. Tight junctions exhibited low water permeability but allowed for electrolyte permeation that enables canalicular spaces to shrink below van't Hoff equilibrium during the osmotic maneuver[162]. Nonetheless, the experimental data supporting this view remained limited and largely indirect[163].

The cloning and functional characterization of a family of proteins that works as membrane water channels, named aquaporins (AQPs)[164], challenged the former concepts of water transport and contributed to the better understanding of bile physiology. The discovery of the aquaporin water channels, has clarified the mechanisms by which water, the major component of bile, moves across the hepatobiliary epithelia[165].

Direct osmotic water permeability assessment by stopped-flow spectrophotometry in canalicular and sinusoidal plasma membrane vesicles revealed the presence of both lipid (non-channel) and AQP-mediated pathways for sinusoidal and canalicular water movement[166]. The study demonstrated that the canalicular plasma membrane domain has lower water permeability than the sinusoidal membrane, and thus it is rate limiting for transcellular water transport in hepatocytes[160]. However, upon cAMP stimulus the intracellular AQP8 inserts to the canalicular domain and so this membrane becomes highly water permeable. Therefore, the transcellular pathway via water channels seems to account for most of the water entering the bile canaliculus. Hepatocytes express aquaporins, a family of membrane channel proteins that facilitate the osmotically-driven movement of water molecules. Aquaporin-8 (AQP8), localized to canalicular membranes, modulates membrane water permeability providing a molecular mechanism for the osmotically-coupled transport of solute and water during bile formation.

There is experimental evidence suggesting that defective hepatocyte AQP8 expression leads to alterations in normal bile physiology. Thus, AQP8 protein is downregulated (and canalicular water permeability decreased), in established rat models of cholestasis, such as sepsis-associated cholestasis, estrogen-induced cholestasis and extrahepatic obstructive cholestasis[167].

The entry of inorganic cations and anions into the hepatocyte is effected with the participation of specific ion channels and carriers, which include Na+,K+-АТPase, Na+/H+-exchanger, Na+/HCO3–-cotransporter, and Na+-independent carrier of SO42–[154]. It is suggested[168] that by analogy with the mechanisms of water reabsorption from the bile in the gallbladder, water is reabsorbed by the bile duct epithelial cells as a result of Na+-coupled cotransport of Cl-. Enhanced reabsorption after removal of the gallbladder is regarded as an adaptive mechanism that provides bile concentration.

The primary mechanism of biliary copper excretion involves ATP7B-mediated vesicular sequestration of copper rather than direct copper translocation across the canalicular membrane[169].

The microfilament system is of no small importance in the canalicular secretion of bile. Bile is transported to the ducts via contraction of a canaliculus, which is associated with the activity of microfilaments. The latter play a great role in the regulation of permeability of a tight junction for water and electrolytes.

**SECRETION OF PROTEINS INTO BILE**

Proteins, as bile acids, are referred to as the osmotically active components of bile, which determine the rate of its secretion[100]. Albumin and the other proteins that are close to its molecular weight make a major contribution to the creation of an osmotic gradient.

Tens of different proteins with a molecular weight of 6 to 220 kDa are detectable in the bile[100]. The greater part of them is blood proteins; the lesser is the proteins entering the bile directly from the hepatocytes and epithelial cells of bile ducts. The experimental data[100] suggest that many plasma proteins interact with the specific receptors available on the sinusoidal membrane of hepatocytes and form a plasma protein-receptor complex that enters the liver cells via endocytosis.

The intracellular transfer of proteins from the perisinusoidal area of a hepatocyte to its pericanalicular one is mainly accomplished by targeted vesicular transport. The large portion of proteins entering the hepatocyte from blood as a receptor-plasma protein complex generates a receptosome (endosome) and is transported in such a form to the Golgi apparatus, then to the canalicular membrane[100]. The newly synthesized proteins entering the bile canaliculi are also transported just from the hepatocytes by targeted vesicular transport. It is presumed that apolipoproteins perform in the bile the same function as lipoproteins in the serum, i.e. ensure lipid transport[170].

Apoproteins are taken up from the sinusoidal space by endocytosis, transported in the hepatocytes as vesicles and released into the bile across the canalicular membrane by exocytosis[171].

In conclusion, the cholesterol that is newly synthesized in the hepatocytes serves as a substrate for the synthesis of bile acids. The synthesis of bile acids is a key point in the formation of bile. Acid-dependent and acid-independent bile fractions are identified. The structure of a hepatocyte provides a targeted transport of bile constituents from the basolateral to apical membrane of a liver cell. The transport of bile acids across the sinusoidal membrane is associated with the overcoming of a high concentration gradient and an electrical potential and it is accomplished by an active transport with the participation of carrier proteins. The vesicles are the basic form of the transport of lipids in and out of the cell itself. Bile lipids are secreted into the bile canaliculi as monolamellar vesicles. Bile acids pass across the apical part of a hepatocytic membrane independently, by ATP Binding Cassette (ABC) transporters, solubilize the phospholipids and cholesterol from the membrane surface in the bile canalicular lumen.

There is a constant transformation of vesicles to and from micelles in the bile canaliculi and ducts as a result of absorptive and secretory processes with the participation of bile acids.All the aforesaid suggests considerable progress made in the understanding of the mechanisms of bile formation and excretion, which has made possible due to the advances of fundamental investigations in cell biology, molecular biology and biochemistry.

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**Figure 1 The bile content of anions, cations and taurocholic acid.**

**Figure 2 Color cathode-luminescence scanning electron microscopy micro images.**

CCL SEM micro images of unconjugated bilirubin (а), unesterified cholesterol (b), high molecular weight protein (c), and dehydrated sample of normal human cystic bile (d). CCL SEM: Color cathode-luminescence scanning electron microscopy.

**Figure 3 Bile acid synthesis.**

**Figure 4 Proposed model for lipid secretion into bile[58].**

**Figure 5 Chemical formulas and simple images of cholesterol, lecithins, and bile salts on a boundary of two phases–water: lipids (air, hydrocarbons).**

**Figuer 6 Formation of micelles structures in the system containing bile acids, lecithin, and cholesterol.**

BA: Bile acids; Ch: Cholesterol; L: Lecithin.

**Figure 7 Mechanisms of the transport of bile acids, water, and electrolytes through the hepatocyte (transcellular pathway) and intercellular space (paracellular pathway). Localization and function of sinusoidal and canalicular hepatocellular transporters.** The Na+-dependent sinusoidal uptake of bile salts (BS-) is mediated by Na+/taurocholate cotransporting polypeptide (NTCP). The Na+-independent hepatic uptake of organic anions (OA-), bile salts and type II organic cations (OC+) is mediated by members of the OATP family. Sinusoidal uptake of type I OC+ is mediated by OCT1. Transport across the canalicular membrane is driven mainly by ATP-dependent export pumps. Multidrug-resistance protein 1 (MDR1) mediates canalicular excretion of amphiphilic type II OC+ and other hydrophobic compounds. MDR3 functions as a phosphatidylcholine (PC) flippase. Bile salt export pump (BSEP) mediates apical excretion of BSs. Multidrugresistance-associated protein 2 (MRP2) transports non-bile-salt organic anions, such as bilirubin glucuronides (BGs), GSH, and sulfated/glucuronidated bile salts. Canalicular transport of HCO3- is mediated by the Cl-/HCO3- exchanger AE2. Aquaporins AQP9 and AQP8 are involved in the transport of water across the sinusoidal and the canalicular membrane, respectively. The nature of the water channels in human liver has been characterized[84]. GC: Golgi apparatus (complex); ER: Endoplasmic reticulum; N: Nucleus.

**Figure 8 31P-NMR spectroscopy hepatic (left part of figure) and gallbladder (right part of figure) bile.** Signals: 12,31 (standard); Pi (inorganic phosphate); PCh (phosphatidilcholine). PCh signal of gallbladder bile is increased with respect to such signals from hepatic bile. Signal of standard and inorganic phosphate of gallbladder bile are decreased with respect to such signals of hepatic bile. The concentration of lipids is increased by water absorption by gallbladder mucosa.

**Figure 9 Formation of IXα bilirubin from heme.**