# Role of the Gallbladder in a Human

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Abstract:

The basic role of the gallbladder in a human is a protective. The gallbladder decreases the formation of the secondary hydrophobic toxic bile acids (deoxycholic and lithocholic acids) by accumulating the primary bile acids (cholic and chenodeoxycholic acids) in the gallbladder, thus reducing their concentration in gallbladder-independent enterohepatic circulation and protecting the liver, the mucosa of the stomach, the gallbladder, and the colon from their effect. In mammals the presence or absence of the gallbladder is determined by the synthesis of hydrophobic or hydrophilic bile acids, respectively. Since the gallbladder is contracted in 5-20 minutes after food is available in a stomach, and "the gastric chyme" moves from the stomach into the duodenum only 1-3 hours later, the role of the gallbladder bile in digestion may be insignificant.

*Key words:* gallbladder, absorption, cholesterol, bile acids, enterohepatic circulation, lipoproteins, gallstone disease, cholecystectomy, cancer.

*Abbreviations:* ACAT = acyl-coenzyme A:cholesterol-O-acyltransferase; BA = bile acids; CDCA = chenodeoxycholic acid; Ch = cholesterol; ChA = cholesterol anhydrous; ChE = cholesterol esters; CGD = cholesterol gallstone disease; ChM = cholesterol monohydrate; CSI = cholesterol saturation index; CA = cholic acid; CMR = chylomicrons remnants; DCA = deoxycholic acid; HA = hepatic artery; HV = hepatic vein; HDL = high density lipoprotein; HDL-Ch = high density lipoprotein cholesterol; HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A reductase; HCA = hyocholic acid; HDCA = hyodeoxycholic acid; LCA = lithocholic acid; LD = lymphatic duct; LDL = low density lipoprotein; LDL-Ch = low density lipoprotein cholesterol; MCA = muricholic acid; MDCA = murideoxycholic acid; PV = portal vein; TCh = total cholesterol; VLDL = very low density lipoprotein; VLDL-Ch = very low density lipoprotein cholesterol; UDCA = ursodeoxycholic acid.

## 1. The influence of the functions of the gallbladder on the process of gallbladder bile formation and enterohepatic circulation

### *Functions of the gallbladder*

The prevalent point of view is that gallbladder is not essential for life [1]. The gallbladder has the absorption, concentration, secretion, and evacuation functions [2, 3]. The absorption and concentration functions are interdependent. The absorption function of the gallbladder includes the absorption of water, $Na^+$, cholesterol, phospholipids, hydrophilic proteins, etc [4-14]. Since the absorption of the bile acids by the gallbladder mucosa is 2-6% of the total concentration in the gallbladder bile, the concentration function of the gallbladder consists in the accumulation of bile acids of hepatic bile in the gallbladder [10-12, 15, 16]. The secretion function of the gallbladder includes the secretion of glycoprotein mucin by the gallbladder mucosa, $H^+$ ions, $Cl^-$ and probably immunoglobulins and $Ca^{2+}$ [5, 17-23].

### *Conceptual model of gallbladder bile formation*

Considering the fact that the detailed structuring of the process of hepatic bile entering the gallbladder has not been worked out, we have introduced two new terms into practice: the "active" and "passive" passages of the hepatic bile. The "active" passage depends on the ejection volume of the gallbladder after meal or during the interdigestive period. The "passive" passage is connected with the rate of water absorption in the gallbladder. Hence the rate of the hepatic bile entering the gallbladder contains both the "active" and the "passive" passages. During the "active" passage only one volume (out of 6-9) of the hepatic bile enters versus 5-8 volumes during the "passive" passage. The rate of hepatic bile entering the gallbladder depends on the absorption rate of water by the gallbladder mucosa ($r=+0.99$, $p<0.001$) [24]. The absorption rate of water by the gallbladder mucosa is up to 100-250 μl/min; sometimes it may increases up to 500 μl/min [4]. The rate of hepatic bile entering the gallbladder is 75% of the basal secretion of hepatic bile [24]. It is indirectly confirmed by the fact that 78±10% of bile acids from their total pool is accumulated in the gallbladder [25]. The concentration of total bile acids in the gallbladder bile depends on the rate of

bile acids of hepatic bile entering the gallbladder (r=+0.87, p<0.001) [24]. Detailed structuring of the process of hepatic bile entering the gallbladder suggests that 83-89% of the bile acids, contained in gallbladder bile, enters during the "passive" passage, and only 11-17% of bile acids – during the "active" passage. Hence, the "passive" passage of hepatic bile into the gallbladder plays an important role in the mechanism of gallbladder bile formation (fig. 1.a).

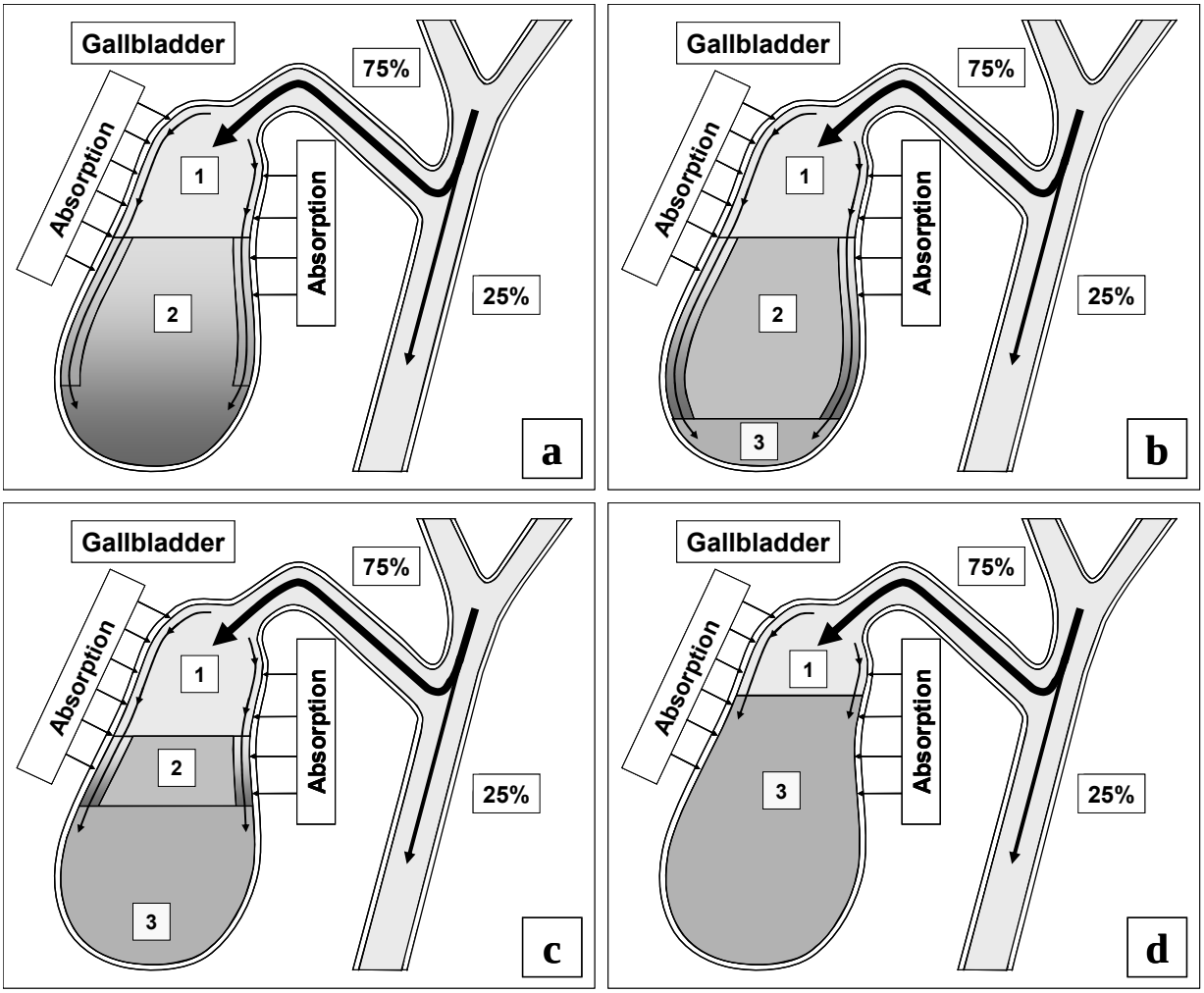


**Fig. 1.** Process of gallbladder bile formation in healthy humans on the data of dynamic intravenous cholecystography. **(a)** in 15-20 minutes after intravenous instillation of contrast; **(b)** in 30-40 minutes after intravenous instillation of contrast; **(c)** in 1.5-2.0 hours after intravenous instillation of contrast; **(d)** in 2.5-3.0 hours after intravenous instillation of contrast. **1** = Contrasting unconcentrated hepatic bile; **2** = uncontrasting concentrated gallbladder bile; **3** = contrasting concentrated gallbladder bile.

Normally the process of the gallbladder filling after the intravenous introduction of X-ray contrast is characterized by some regular features [26]. During the first 15-20 minutes the gallbladder bile has two layers: the upper contrasting and the low uncontrasting (fig. 1.a). The legible border between them is situated horizontally. During the 30-40th minute the upper layer contrasting bile near the wall thickens, its density grows because of the presence of iodine heavy atoms and exceeds the density of the uncontrasting concentrated bile. Besides, the "heavy" layers of the contrasting bile begins to trickle down along the walls, as if flowing round the uncontrasting concentrated bile, and accumulate at the fundus (fig. 1.b). The gallbladder shadow becomes three-layered: the contrasting, but unconcentrated bile above, the concentrated, but uncontrasting bile underneath and the contrasting and concentrated bile after the lower part of gallbladder. The boundary between them is legible and it does not change if a patient moves. The quantity of the concentrated contrasting bile at the fundus of the gallbladder increases gradually, and the upper boundary of the lower layer rises (fig. 1.c). The gallbladder shadow gains homogeneity 2.5-3.0 hours after the moment of the contrast introduction (fig. 1.d) [26].

Therefore, in a fasting state at night period or an interdigestive state the absorption of water by the infundibulum mucosa of the gallbladder plays the leading role in gallbladder bile formation (unpublished data).

*Mechanism of gallbladder bile formation*

Two points in the process of gallbladder bile formation should be distinguished: 1) in a fasting stomach; 2) after postprandial gallbladder emptying [24]. The absorbing and concentrating functions determine the mechanism of the gallbladder bile formation.

The rate of biliary cholesterol absorption by the gallbladder mucosa depends on the concentration of the cholesterol in the gallbladder bile ($r = +0.60$, $p < 0.001$) [7-9]. Taking into account the fact that the mixed (bile acids-phospholipid-cholesterol) micelles are not absorbed by the gallbladder mucosa, cholesterol can be absorbed as monomers or with phospholipid vesicles [7-12, 24, 27-29]. The solubility of anhydrous cholesterol monomers in water is 0.013 nmol/ml, in the intermicellar phase – 0.260 nmol/ml, while in phospholipid vesicles – 5.5 μmol/ml [30-37]. Therefore, according to the solubility of anhydrous cholesterol, it will be absorbed with the phospholipid vesicles to a greater degree (99.9%). The phospholipid vesicles can be absorbed by the gallbladder mucosa in different ways [7-12, 24, 27-29]. Therefore, the greater is the absorption of vesicular cholesterol by the gallbladder mucosa; the lower is the concentration of the cholesterol in the phospholipid vesicles of the gallbladder bile.

The concentration function of the gallbladder consists in the accumulation of the bile acids of the hepatic bile in the gallbladder; it depends on the rate of bile acids of the hepatic bile entering the gallbladder and the rate of water absorption by the gallbladder mucosa, and it also determines the concentration of the total bile acids and the formation of mixed biliary micelles in the gallbladder bile. In hepatic bile 40-80% of biliary cholesterol is in phospholipid vesicles and 20-60% of it is in mixed biliary micelles [35-37]. The gallbladder, concentrating the bile acids, forms mixed biliary micelles and raises the level of biliary cholesterol in them up to 80-100% [35-37]. Therefore, the greater is the absorption of water by the gallbladder mucosa, the grater is the passage of bile acids of hepatic bile to the gallbladder and the higher is the concentration of total bile acids in the gallbladder bile.

Thus, the high concentration of the total bile acids and the low concentration of cholesterol in phospholipid vesicles result in the low cholesterol saturation index in the gallbladder bile (less than 1.0), which determines the stability of micellar carriers of biliary cholesterol and prevents the cholesterol monohydrate crystals from precipitating.

*Fate of the absorbed vesicular biliary cholesterol*

Part of the absorbed cholesterol may be esterified in the epithelial cells of gallbladder mucosa by means of ACAT. Normally the activity of ACAT makes up 92±23 pmol/min per mg protein and is 8-9 times as high as that of microsomes of the liver (11±2 pmol/min per mg protein) [38]. There is a positive correlation between the concentration of the cholesterol in the gallbladder bile and ACAT in microsomes of gallbladder mucosa ($r = +0.42$, $p < 0.05$). The cholesterol is also synthesized in the microsomes of gallbladder mucosa, and the activity of HMG-CoA reductase in them makes up 28±6 pmol/min per mg protein. But it is 4 times lower, than that in the microsomes of hepatocytes (120±40 pmol/min per mg protein). The concentration of free cholesterol in the microsomes of gallbladder mucosa (206±9 nmol/min per mg protein) is 4 times higher than that in microsomes of hepatocytes (55±3 nmol/min per mg protein), while the concentration of esterified cholesterol (34±5 nmol/min per mg protein) is 3.5 times higher (9±1 nmol/min per mg protein) [38]. Taking into account the low activity of HMG-CoA reductase and the high activity of ACAT in the microsomes of gallbladder mucosa, as well as the positive correlation between the level of cholesterol in the gallbladder bile and the microsomes of gallbladder mucosa ($r = +0.75$, $p < 0.01$), the higher concentration of free cholesterol in the microsomes of epithelial cells of gallbladder mucosa may result only from the excessive absorption of the biliary vesicular cholesterol. It testifies that the rate of the biliary vesicular cholesterol entering the epithelial cells of gallbladder mucosa 4 times exceeds that of hepatocytes.

By analogy with ileum, the removal of the absorbed vesicular cholesterol and phospholipids from the gallbladder wall may be realized by means of HDL and/or VLDL [39-43]. It was shown in

vitro that HDL are able to extract the excess of cholesterol out of cholesterol-saturated phospholipid vesicles and to dissolve the cholesterol crystals [44, 45]. It is possibly that the mechanism of the removal of the absorbed vesicular cholesterol and phospholipids by means of HDL may be prevalent and it may be determined by the concentration of HDL in serum and by the rate of the arterial bloodstream in the gallbladder wall [46, 47]. In the gallbladder wall HDL, interrelating with phospholipid vesicles, will extract biliary vesicular cholesterol and phospholipids and, with bloodstream, will first enter the gallbladder vein and then, through the portal vein, the liver. This supposition is indirectly corroborated by the negative correlative connection between CSI and the level of the total cholesterol (TCh) ($r = -0.65$, $p<0.05$) and Ch-HDL ($r = -0.62$, $p<0.05$) in the serum in practically healthy women [48]. In the gallbladder mucosa, small quantities of VLDL synthesize [49]. The number of apoproteins B, C-II and C-III absorbed by the gallbladder mucosa can make up 84-91% of total quantity of apoproteins B, C-II and C-III, entering the gallbladder with the hepatic bile [50]. Also, taking into account that the mucosa of the gallbladder absorbs phospholipid vesicles, apoproteins B, C-II and C-III, they can interrelate with serum HDL, LDL, and VLDL in the gallbladder wall below epithelium.

The absorbed vesicular cholesterol of gallbladder mucosa, interrelating with blood lipoproteins, can enter the liver or the peripheral blood stream through the portal vein (fig. 2.a).

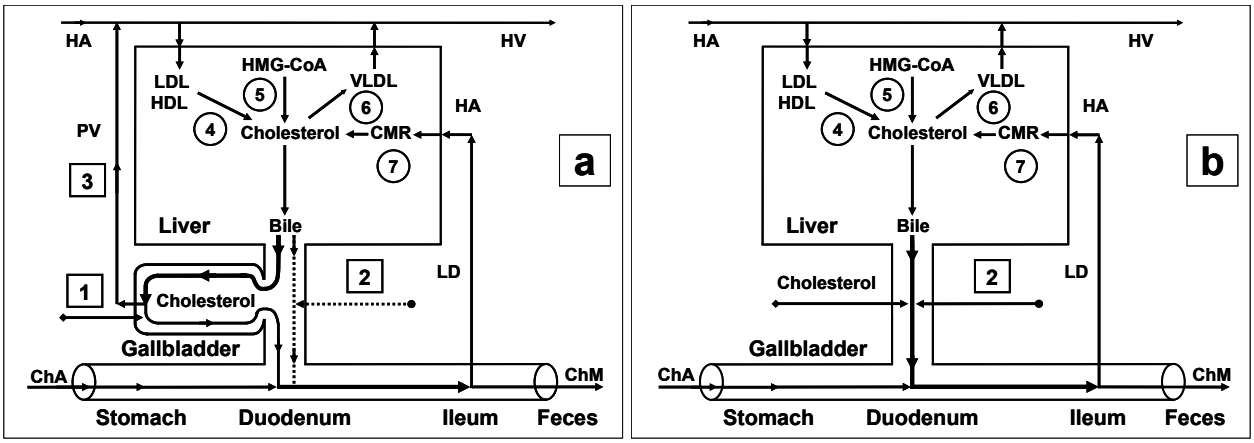


**Fig. 2.** Exchange of cholesterol in healthy humans **(a)** and patients after cholecystectomy **(b)**.
**1** = Gallbladder-dependent output of biliary cholesterol; **2** = gallbladder-independent output of biliary cholesterol; **3** = gallbladder-hepatic circulation of biliary cholesterol; **4** = hydrolysis of cholesterol esters entered the hepatocytes with HDL and LDL; **5** = synthesis of cholesterol; **6** = synthesis of cholesterol esters for VLDL; **7** = hydrolysis of cholesterol esters entered the hepatocytes with CMR.
**HMG-CoA** = 3-hydroxy-3-methylglutaryl coenzyme A reductase; **HDL** = high density lipoprotein; **LDL** = low density lipoprotein; **VLDL** = very low density lipoprotein; **CMR** = chylomicrons remnants; **Ch** = cholesterol; **ChA** = cholesterol anhydrous; **ChM** = cholesterol monohydrate; **HA** = hepatic artery; **HV** = hepatic vein; **PV** = portal vein; **LD** = lymphatic duct.

The way of biliary cholesterol [blood (lipoproteins) → liver (hepatic bile – phospholipid vesicles) → gallbladder (absorption of vesicular cholesterol) → portal vein (lipoproteins) → liver or blood] – was called by us "gallbladder-hepatic circulation of biliary cholesterol" (fig. 2.a).

The detailed structuring of these processes provides an opportunity to connect the excretory function of the liver and the absorption and evacuation functions of the gallbladder with the level of cholesterol in serum.

*Outflow of biliary cholesterol into duodenum*

For understanding the processes of the biliary cholesterol outflow into the duodenum, we have introduced two new terms, namely: the gallbladder-dependent and gallbladder-independent output of biliary cholesterol. The former depends on the ejection volume of the gallbladder and the concentration of biliary cholesterol in the gallbladder bile; the latter depends on the concentration of biliary cholesterol in the hepatic bile entering directly the duodenum (fig. 2.a). After cholecystectomy only gallbladder-independent output of biliary cholesterol to the duodenum is observed (fig. 2.b).

*Interdependence between the absorption of biliary cholesterol in the gallbladder and that of the ileum*

In the gallbladder, vesicular cholesterol absorbs effectively, but micellar cholesterol does not [7-12, 24, 27 28]. The absorption of micelles in the ileum is 100 times more effective than that of vesicles [51]. Hence, the greater is the absorption of vesicular cholesterol in the gallbladder, the higher is the concentration of micellar cholesterol in the gallbladder bile (CSI < 1.0) and the absorption of cholesterol in the ileum. Vice versa, the decrease of the vesicular cholesterol absorption in the gallbladder raises the vesicular cholesterol concentration in the gallbladder bile (CSI more than 1.0) and reduces the cholesterol absorption in the ileum. The ratio bile acids/cholesterol in the gallbladder bile may determine the ability of intestinal mixed micelles to solubilize dietary cholesterol. The rise of this ratio by more than 10-12:1 (CSI < 1.0) results in the increase of the solubilization, and its decrease by less than 7-10:1 (CSI > 1.0) results in the reduction of the solubilization.

*Effect of gallbladder functions on enterohepatic circulation*

Part of the bile acids of the hepatic bile enters the gallbladder and is accumulated in it; the other part enters the duodenum and participates in the enterohepatic circulation. To understand these processes, we have introduced two new terms: gallbladder-dependent and gallbladder-independent enterohepatic circulation of bile acids (fig. 3.a).

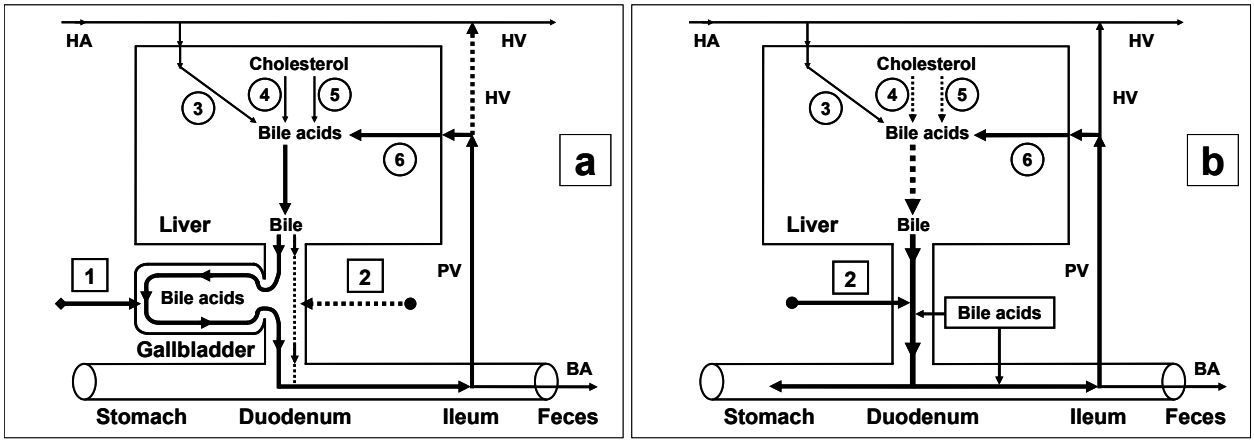


**Fig. 3.** Enterohepatic circulation of bile acids in healthy humans **(a)** and patients after cholecystectomy **(b)**. **1** = Gallbladder-dependent enterohepatic circulation of bile acids; **2** = gallbladder-independent enterohepatic circulation of bile acids; **3** = bile acids entering the liver through the hepatic artery; **4** = synthesis of cholic acid: cholesterol-7$\alpha$-hydroxylase; **5** = synthesis of chenodeoxycholic acid: cholesterol-27-hydroxylase; **6** = bile acids entering the liver through the portal vein. **BA** = bile acids; **HA** = hepatic artery; **HV** = hepatic vein; **PV** = portal vein.

The gallbladder-dependent enterohepatic circulation of bile acids depends on the ejection volume of the gallbladder and determines the concentration of bile acids of the gallbladder bile that participate in the enterohepatic circulation. The gallbladder-independent enterohepatic circulation includes the part of bile acids of the hepatic bile that enter directly the duodenum, but not the gallbladder. In healthy people 75-80% of bile acids participate in the gallbladder-dependent enterohepatic circulation, and only 20-25% of bile acids take part in the gallbladder-independent circulation (fig. 3a). Therefore, the concentration function of the gallbladder consists in the accumulation of bile acids of the hepatic bile and their exclusion from the enterohepatic circulation. The part of bile acids participating in the gallbladder-independent enterohepatic circulation after cholecystectomy increases up to 100% (fig. 3.b). Detailed structuring of these processes enables to connect the absorption, concentration and evacuation functions of the gallbladder with the enterohepatic circulation of bile acids. The rate of water absorption by the gallbladder mucosa determines the passive passage of the hepatic bile from the liver into the gallbladder and the gallbladder-independent enterohepatic circulation of the bile acids.

## 2. Characteristics of bile acids

According to the hydrophilic-hydrophobic index, the bile acids are divided into hydrophilic and hydrophobic ones (table 1) [3, 52, 53].

**Table 1. Hydrophilic-hydrophobic index (HHI) of bile acids in mammals [52].**

| Bile acids | HHI of BA | Mammals |
|---|---|---|
| **β-Hyocholic acid (β-HCA)** | –0.60 | rats |
| **α-Muricholic acid (α-MCA)** | –0.51 | rats |
| **β- Muricholic acid (β-MCA)** | –0.40 | rats |
| **Murideoxycholic acid (MDCA)** | –0.33 | rats |
| **Ursodeoxycholic acid (UDCA)** | –0.17 | bears |
| **α-Hyocholic acid (α-HCA)** | –0.03 | pigs |
| **Hyodeoxycholic acid (HDCA)** | +0.09 | pigs |
| **Cholic acid (CA)** | +0.23 | human |
| **Chenodeoxycholic acid (CDCA)** | +0.83 | human |
| **Deoxycholic acid (DCA)** | +0.98 | human, primates, rabbits |
| **Lithocholic acid (LCA)** | +1.23 | human |

If the hydrophobic index is less than that of cholic acid (CA), the bile acids are hydrophilic, if it is more than the hydrophobic index, they are hydrophobic [3, 52, 53]. The primary bile acids are more hydrophilic than the secondary ones, but the taurine conjugates of the bile acids are more hydrophilic than the glycine ones [3, 52, 53]. The hydrophilic bile acids have hepatoprotective properties [muricholic (MCA) > ursodeoxycholic (UDCA) > cholic (CA)] [54, 55]. The hydrophobic bile acids are hepatotoxic [lithocholic (LCA) > deoxycholic (DCA) > chenodeoxycholic (CDCA) > CA] [3, 52-57]. Depending on the concentration, the hydrophobic bile acids cause cholestasis (LCA > DCA), necrosis (LCA > DCA) or apoptosis of hepatocytes (LCA > DCA > CDCA) [52-57]. Furthermore, DCA is cancerogenic [58]. Experiments on animals showed that it causes cancer of the colon [59]. The hydrophilic bile acids prevent the development of cholestasis or necrosis/apoptosis of hepatocytes (UDCA, MCA), as well as cancer of the colon (UDCA) [54-57, 59].

In serum up to 40% of bile acids are transported with HDL, up to 15% – with LDL [2]. The mechanism of binding of bile acids with lipoproteins depends on their hydrophobic index (CDCA > DCA > UDCA > CA > 7-epicholic acid) [2]. In the liver, 60-80% of bile acids are uptake during one passage of portal blood [60]. In earlier experiments on hamsters, it was demonstrated that the hepatic LDL uptake could influence the bile flow rate, the biliary secretion of bile acids and cholesterol [61, 62]. The composition and concentration of bile acids participating in the enterohepatic circulation can modulate the LDL receptor activity and the receptor-dependent LDL uptake in the liver. More hydrophilic UDCA stimulates the receptor-dependent LDL uptake in the liver, but more hydrophobic CDCA decreases the LDL receptor activity [61, 62]. It was also shown that the addition of hydrophobic CDCA to the hypercholesterolemic diet reduces the decrease of HDL concentration in serum, but the addition of hydrophilic UDCA causes the opposite effect [63, 64]. In hepatocytes, the bile acids may inhibit the activity of HMG-CoA reductase and cholesterol-7α-hydroxylase, depending on their concentration and hydrophobic index (DCA > CDCA > CA > UDCA) [52, 65-67].

The hydrophilic bile acids stimulate the secretion of the hepatic bile (UDCA > CA), the hydrophobic ones lower it (LCA > DCA > CDCA) [68-70]. UDCA and CDCA reduce the secretion of biliary cholesterol in the hepatic bile, but CA and DCA raise it [3, 68-70]. In the gallbladder bile, the hydrophobic bile acids form mixed and simple micelles (DCA > CDCA > CA), but the hydrophilic bile acids form liquid crystalline lamellas (MCA > UDCA); that is, the lower the hydrophobic index of bile acids, the lower the ability to form micelles [71-73]. In the ileum, CA and CDCA raise the absorption of cholesterol, but UDCA and DCA reduce it [74-77].

During the process of enterohepatic circulation, in the ileum and the colon, anaerobic bacteria promote 7α-dehydroxylation of the primary bile acids (hyocholic (HCA), MCA, CA, CDCA) and the formation of the secondary bile acids (hyodeoxycholic (HDCA), murideoxycholic (MDCA), DCA, LCA) [3, 52, 78, 79]. The secondary bile acids are more hydrophobic than the primary ones (HDCA > HCA, MDCA > MCA, DCA > CA, LCA > CDCA) [3, 52, 53]. The secondary bile acids are usually

poorly absorbed in the ileum and the colon and are excreted with feces [3, 52, 53].

## 3. Protective role of the gallbladder

*Mammals*

In mammals (rats) that do not have the gallbladder, only the hydrophilic hepatoprotective bile acids are only synthesized; as for the secondary hydrophobic hepatotoxic bile acids, they are formed in small quantities or are poorly absorbed in the ileum and the colon (table 2).

**Table 2. Presence of gallbladder and type of bile acids.**

| Mammals | Presence of gallbladder | Type of bile acids or bile alcohols |
|---|---|---|
| **Rats** | No | Hydrophilic bile acids |
| **Camels** | No | ? |
| **Deers** | No | ? |
| **Elephants** | No | Hydrophilic bile alcohols |
| **Rhinoceros** | No | Hydrophilic bile alcohols |
| **Whales** | No | Hydrophilic bile alcohols |
| **Bears** | Yes | Hydrophilic bile acids |
| **Rabbits** | Yes | Hydrophobic bile acids |
| **Primates** | Yes | Hydrophilic and hydrophobic bile acids |
| **Human** | Yes | Hydrophilic and hydrophobic bile acids |

Since a long stagnation in the gallbladder may promote the formation of gallstones, the gallbladder may be absent in mammals which can manage without food and water for a long period of time (camels, deer) [4]. Since the size of the gallbladder must be proportional to the size of the liver, the gallbladder may be absent in big mammals (elephants, rhinoceros, whales) because of their anatomical peculiarities [4]. In these mammals there is a considerable synthesis of bile alcohols, which are poorly solubilize cholesterol [2]. In mammals that have the gallbladder (humans, monkeys, rabbits) both the hydrophilic and hydrophobic bile acids may be synthesized. The secondary hydrophobic hepatotoxic bile acids can be formed in large quantities, but they are poorly absorbed in the ileum and the colon [2-4]. Since a long stagnation in the gallbladder may promote the formation of gallstones, only the hydrophilic bile acids are synthesized in mammals that fall into long hibernation (bears), but the secondary bile acids are also hydrophilic [2-4].

Therefore, the basic role of the gallbladder in mammals in which hydrophobic hepatotoxic bile acids are synthesized or formed, is the protection of the liver from their effect by means of bile acids accumulation in the gallbladder and lowering the number of the cycles of enterohepatic circulation. The mammals in which the hydrophobic hepatotoxic bile acids are synthesized or formed must have the gallbladder. Those mammals in which the hydrophilic hepatoprotective bile acids are synthesized and the hydrophobic hepatotoxic bile acids are formed in small quantities, may manage without it. The mammals, in which bile alcohols are synthesized in large amounts, do not have the gallbladder.

*Human*

In a human, the formation of cholesterol gallstones is promoted by the decrease of absorption (the decrease of the water and phospholipid vesicles absorption), concentration (the decrease of total bile acids concentration in gallbladder bile) and evacuation (the decrease of the gallbladder-dependent output of biliary cholesterol) functions and by the increase of secretion (hypersecretion of glycoprotein mucin by the gallbladder mucosa) function of the gallbladder (fig. 4) [80]. The decrease of the water absorption rate of in the gallbladder wall limits the “passive” passage of the hepatic bile into the gallbladder and increases the hepatic bile passage into the duodenum (fig. 5) [6, 24, 80]. The decrease of the evacuation function of the gallbladder reduces the “active” passage of the hepatic bile into the gallbladder [81, 82]. This process is accompanied by the decrease of the total bile acids concentration and the increase of the biliary cholesterol concentration in phospholipid vesicles and it also promotes the increase of time for precipitation of cholesterol monohydrate crystals and the formation of cholesterol gallstones (fig. 6) [83-87]. The excessive hepatic bile passage from the liver into the duodenum increases the frequency of the gallbladder independent enterohepatic circulation of bile acids.

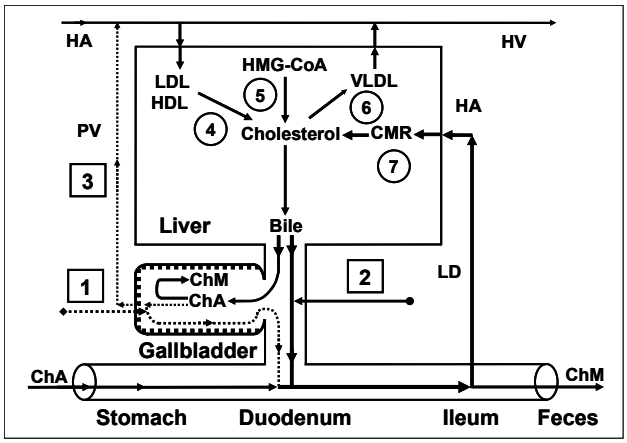


**Fig. 4.** Exchange of cholesterol in patients with chronic acalculous cholecystitis and chronic calculous cholecystitis. **1** = gallbladder-dependent output of biliary cholesterol; **2** = gallbladder-independent output of biliary cholesterol; **3** = gallbladder-hepatic circulation of biliary cholesterol; **4** = hydrolysis of cholesterol esters entered the hepatocytes with HDL and LDL; **5** = synthesis of cholesterol; **6** = synthesis of cholesterol esters for VLDL; **7** = hydrolysis of cholesterol esters entered the hepatocytes with CMR. **ChA** = cholesterol anhydrous; **ChM** = cholesterol monohydrate; **HA** = hepatic artery; **HV** = hepatic vein; **PV** = portal vein; **LD** = lymphatic duct.

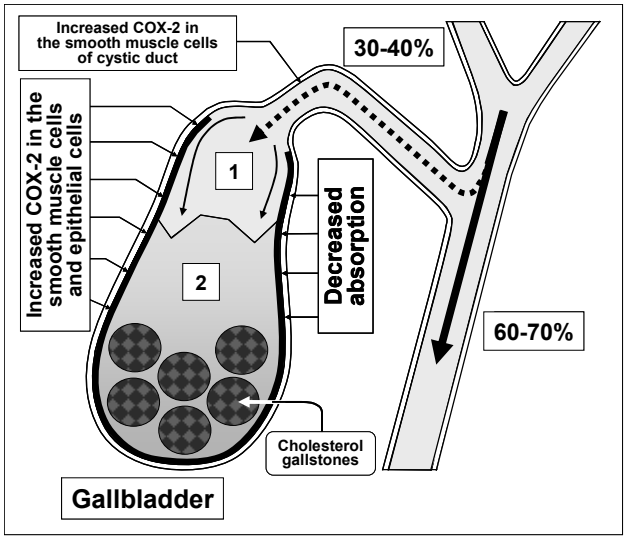


**Fig. 5.** **"**Active" and "passive" passage of hepatic bile into the gallbladder and into the duodenum in patients with chronic calculous cholecystitis.
**1** = unconcentrated hepatic bile;
**2** = low concentrated gallbladder bile with gallstones.

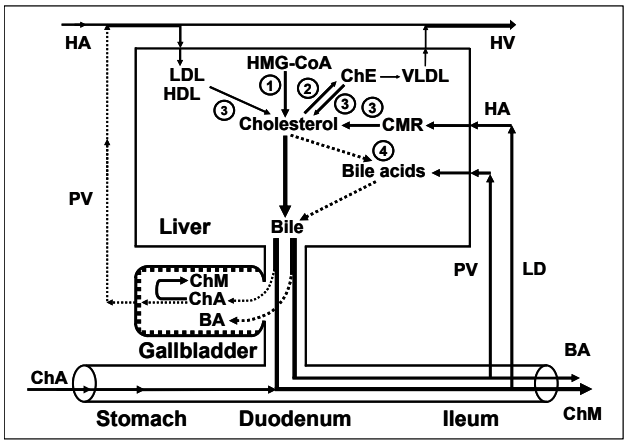


**Fig. 6.** Exchange of cholesterol and bile acids in patients with chronic acalculous cholecystitis and chronic calculous cholecystitis.
**1** = synthesis of cholesterol; **2** = synthesis of cholesterol esters for VLDL; **3** = hydrolysis of cholesterol esters entered the hepatocytes with HDL and LDL, and hydrolysis of cholesterol esters entered the hepatocytes with CMR; **4** = synthesis of bile acids. **ChE** = cholesterol esters; **ChA** = cholesterol anhydrous; **ChM** = cholesterol monohydrate; **BA** = bile acids; **HA** = hepatic artery; **HV** = hepatic vein; **PV** = portal vein; **LD** = lymphatic duct.

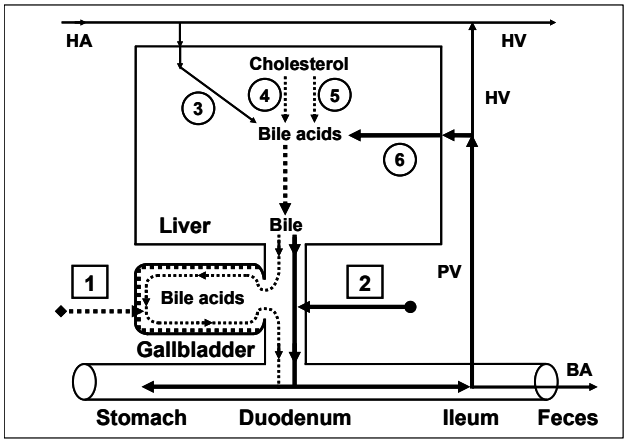


**Fig. 7.** Enterohepatic circulation of bile acids in patients with chronic acalculous cholecystitis and chronic calculous cholecystitis.
**1** = gallbladder-dependent enterohepatic circulation of bile acids; **2** = gallbladder-independent enterohepatic circulation of bile acids; **3** = bile acids entering the liver through the hepatic artery; **4** = synthesis of cholic acid: cholesterol-7$\alpha$-hydroxylase; **5** = synthesis of chenodeoxycholic acid: cholesterol-27-hydroxylase; **6** = bile acids entering the liver through the portal vein. **BA** = bile acids; **HA** = hepatic artery; **HV** = hepatic vein; **PV** = portal vein.

The gallbladder-independent enterohepatic circulation of bile acids in patients with the cholesterol gallstone disease (CGD) or after cholecystectomy is raised (fig. 7, fig. 3.b). It results in: 1) the increase of the hydrophobic hepatotoxic DCA formation and its accumulation in hepatocytes, 2) the formation of morphological changes in the liver (nonspecific reactive hepatitis) and 3) the appearance of cholestasis (table 3) [26, 88, 89].

**Table 3. Percentage of hydrophobic DCA in bile of rabbits, primates and human.**

| Mammals | % of DCA |
|---|---|
| **Rabbits** | up to 90% |
| **Primates** | up to 50% |
| **Human (healthy)** | up to 10-20% |
| **Human (patient with gallstone disease)** | up to 30-40% |
| **Human (after cholecystectomy)** | up to 30-60% |

The risk of cancer of the liver, the pancreas, the small intestine, and the colon increases as well [58, 90-97]. The increases of DCA, participating in the enterohepatic circulation, and of other toxic agents in the hepatic bile can result in chronic pancreatitis and duodeno-gastral reflux [98-101].

Hence, the basic role of the gallbladder in a human is a protective. The gallbladder decrease the formation of the secondary hydrophobic hepatotoxic bile acids (DCA and LCA) by accumulating the primary bile acids (CA and CDCA) in the gallbladder and by reducing their concentration in the gallbladder-independent enterohepatic circulation, thereby protecting the liver, the mucosa of the stomach, the gallbladder, and the colon from their effect. Besides, the increase of the cycles of enterohepatic circulation in a human can determine the raised enterohepatic circulation of estrogens, progesterons and the formation of their active metabolites: 1. a) 16$\alpha$-hydroxy-estrone (it activates proliferation and induces breast cancer); b) 4-hydroxy-estrone (proliferation, cancer); c) 2-hydroxy-estrone (it stimulates fat accumulation in the body of a human); 2. a) pregnanolone (inflammation, cholestasis); b) pregnandiol (inflammation, cholestasis) [102-105].

Probably, the excessive formation of these “active” metabolites and DCA determine the increased risk of cancer of various sites [96, 97]. 9.4% of the patients with gallstones and cholecystectomized patients have cancer of various sites (cancer of the liver, the pancreas, the colon and the small intestine, breast cancer in women) [96, 97]. For women, who underwent cholecystectomy before the age of 50 (i.e. before menopause), the risk of colon cancer is higher than for women who underwent cholecystectomy at the age over 50 [90]. Estrogens intensify the cancer effect of the hydrophobic DCA [106]. The concentration of total bile acids in blood serum is 3 times higher and the risk of intrahepatic cholestasis is 2.5 times higher in cholecystectomized pregnant women (19%) than in non-cholecystectomized pregnant women [107]. Children with inborn absence of the gallbladder have infringements of the function of the liver and lag behind in physical development [26]. Probably, the evolution of a human lacking the gallbladder would have been extremely difficulty.

## 4. The role of the gallbladder and gallbladder bile in digestion

The evacuation volume of the gallbladder depends on the quality and quantity of accepted food. The gallbladder is emptied to a greater extent when the fat food is accepted [4]. Since the gallbladder is contracted in 5-20 minutes after food is available in a stomach, and “the gastric chyme” moves from the stomach into the duodenum only 1-3 hours later, the role of the gallbladder bile in digestion may be insignificant. The gallbladder bile, coming into the duodenum, stimulates the peristalsis of the intestine and promotes the cleaning of the intestine for “a new gastric chyme”.

The hepatic and gallbladder bile volumes and the bile acids concentration, participating in the first circle of gallbladder-dependent and gallbladder-independent enterohepatic circulation, determine the bile acids-stimulated secretion of the hepatic bile that in a greater extent participates in digestion.

### Conclusion

Thus, the basic role of the gallbladder in a human is the protection of the liver, the mucosa of the stomach, the gallbladder and the colon from the effect of hepatotoxic hydrophobic bile acids and the regulation of serum lipids level (fig. 8).

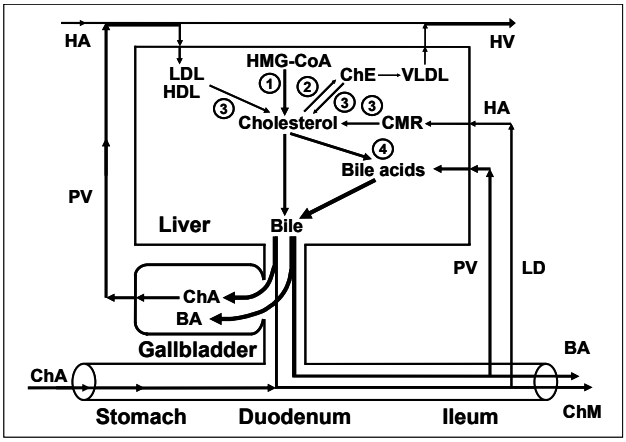


**Fig. 8.** Exchange of cholesterol and bile acids in healthy humans. **1** = synthesis of cholesterol; **2** = synthesis of cholesterol esters for VLDL; **3** = hydrolysis of cholesterol esters entered the hepatocytes with HDL and LDL, and hydrolysis of cholesterol esters entered the hepatocytes with CMR; **4** = synthesis of bile acids.
**ChE** = cholesterol esters;
**ChA** = cholesterol anhydrous;
**ChM** = cholesterol monohydrate;
**BA** = bile acids; **HA** = hepatic artery;
**HV** = hepatic vein; **PV** = portal vein;
**LD** = lymphatic duct.

If the genetics of the biosynthesis of bile acids in a human had evolved in a different way (by analogy with bears [presence of cholesterol-7β-hydroxylase instead of cholesterol-7α-hydroxylase] or rats [presence of cholesterol-6β-hydroxylase instead of cholesterol-12α-hydroxylase]), a human being would have probably never suffered from gallstone disease, some hepatic and colon diseases (liver cirrhosis, colorectal cancer) [1-3, 52-56, 59, 108, 109].

This model of the gallbladder bile formation that we have worked out provides a better understanding of the causes of the diseases of the hepatobiliary zone. It also allows to foresee various trends in their treatment and prevention and to make prognosis of the appearance of various disorders in hepato-biliary-pancreato-duodeno-gastral region after cholecystectomy.

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