CHAPTER 1
Hepatocytes

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KEY POINTS

- The liver is the largest metabolic organ of the human body with a multitude of physiological functions.
- Hepatocytes differentiate from cells of the anterior entoderm under the influence of fibroblast growth factors and bone morphogenic proteins.
- The hepatocytes are the central parenchymal cells, performing all important biochemical processes in close contact with the bloodstream, forming a network of bile canaliculi with their cell membranes that excrete biliary fluid and its various contents into the small bowel.
- The cell membranes of the hepatocyte show distinct domains equipped with specific transporters essential for the uptake of molecules from the bloodstream and on the opposite side of the cell for secretion into the bile.
- The hepatocyte is an important storage compartment for carbohydrates and lipids, the main site of amino acid metabolism and protein synthesis (i.e. serum albumin, transport proteins and many coagulation factors) as well as the site of the two stages of biotransformation facilitating excretion of many toxins and xenobiotics.

Introduction

The liver, located in the right upper abdomen, is the **largest metabolic organ** of the human body with an average weight of 1500 g. It is an organ with a multitude of physiological functions. The complex physiological tasks the liver fulfills in the organism can be divided into three basic categories.

- Resorption and storage
- Synthesis and secretion
- Detoxification and excretion

In the liver tissue that consists of a large array of diverse cells, the **hepatocyte** is the pivotal parenchymal cell for all physiological liver functions and by far the most abundant cell population, accounting for approximately 80% of the cytoplasmic mass of the liver [1].

The organ tissue architecture in many ways reflects its function. The liver receives blood inflow from **two circulations**: an arterial blood supply through the proper **hepatic artery** and as a distinctive feature, a second inflow of venous blood from most intraabominal organs via the **portal vein**. In particular, the venous blood from the gut, pancreas and spleen is drained into the portal circulation of the liver. It is this portal venous blood and its content of nutrients, toxins and xenobiotics that feeds into the various liver functions ranging from nutrient resorption and storage, detoxification and excretion of xenobiotics to biomolecule synthesis from resorbed nutrients and secretion of hormones into the bloodstream. The blood leaves the liver through the hepatic veins towards the heart. In addition to the vasculature, the liver possesses an important additional system of vessels, the arborescent system of **bile ducts** which transport the bile fluid produced by the hepatocytes out of the liver into the small bowel.

The further macro- and microarchitecture of the liver is determined by the aforementioned vessels and ducts with their surrounding hepatocytes and stromal cells.

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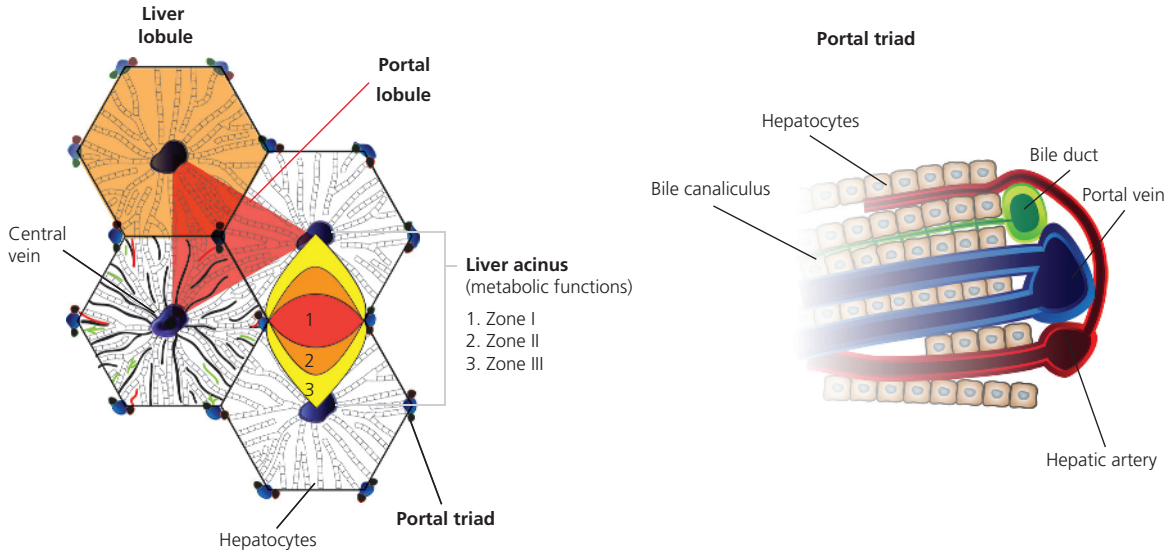

Figure 1.1 Functional units of the liver parenchyma. The liver lobule with its hexagonal outline (*orange*), the portal lobule (*red triangle*) centered on its portal triad and, the most metabolically active unit, the liver acinus, which expands between two portal triads and the neighboring two central veins (*diamond shape*) (*left*). Liver portal triad (*right*).

Along these anatomical structures the liver tissue can be divided into different functional units (Figure 1.1). The classic anatomical **liver lobule** has a hexagonal outline and is defined by a single terminal branch of the hepatic vein in its center with a bile duct, a terminal branch of the hepatic artery (arteriole) and a terminal branch of the portal vein (venule) on each of the six corners. This triad of portal venule, arteriole and bile duct is also called the "portal triad." In contrast to the classic lobule, the so-called **portal lobule** is centered around this portal triad and has a triangular shape with the corners defined by the adjacent three portal veins. Besides these two lobules, the third and most functionally relevant unit that can be defined is the "**liver acinus**." It expands between two portal triads and the neighboring two central veins and has a diamond shape. The liver acinus best reflects the metabolic functions. It is traditionally divided into **three zones** with the zone I or the periportal zone being closest to the arterial vascular supply of the portal triad and receiving the most oxygenation. Zones II and III are closer to the central vein and consequently less well oxygenated, making them more susceptible to hypoxic damage in situations of poor oxygen supply [2]. The zones of the liver acinus not only differ in oxygenation but contain hepatocyte populations that specialize in different metabolic functions. Oxidative energy metabolism, amino acid catabolism, cholesterol metabolism and fatty acid ß-oxidation take place primarily in the periportal zone I while lipogenesis, ketogenesis and metabolism of xenobiotics are localized pericentrally in zone III.

Within these anatomical structures, the hepatocytes are arranged in **plates** that consist of double rows of hepatocytes each flanked by a small vessel, the liver sinusoid, which contains a mixture of portal and arterial blood (Figure 1.2). The gap between the discontinuous, fenestrated endothelial cell lining of the liver sinusoids and the hepatocytes is called the perisinusoidal space or **space of Disse**. Hepatocyte microvilli protrude into the space of Disse and hepatic stellate cells, sometimes called Ito cells, are located in this perisinusoidal space. The adjacent hepatocyte rows form a **bile channel** or canaliculus running in the middle of the plate with the bile flow in the opposite direction of the inflowing sinusoidal blood, towards the bile duct of the portal triad. This tissue architecture on the one hand provides for a maximized contact surface between the hepatocytes and the sinusoidal blood and on the other the secretory surface for bile secretion.

Liver and hepatocyte development

During organogenesis, hepatocytes derive from the anterior portion of the definitive endoderm after completion of the embryonic gastrulation [3]. Cell

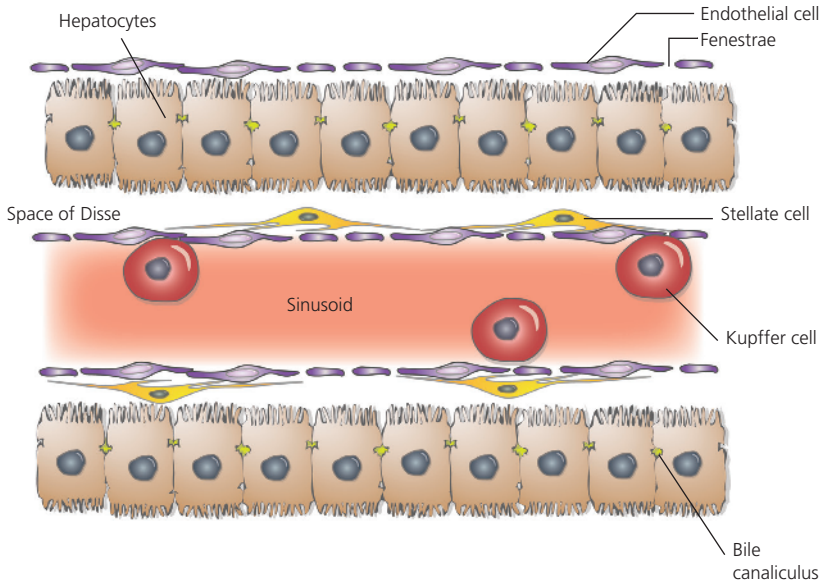

Figure 1.2 Hepatocytes and sinusoids. Hepatocytes are arranged in plates separated by liver blood sinusoids, which are lined by fenestrated endothelial cells. The extravascular space between the liver sinusoids and the hepatocytes is called the perisinusoidal space or space of Disse.

lineage tracing in mouse embryos shows three regions in the medial and bilateral foregut as sources of **hepatic progenitor cells** [4]. Upon closure of the foregut, progenitor cells from these regions come to lie in immediate proximity to the developing heart whose mesoderm has been shown to provide **fibroblast growth factors 1 and 2** (FGF1 and FGF2) crucial for the induction of the hepatic cell differentiation as determined by the initiation of albumin expression [5]. Besides FGF, **bone morphogenetic proteins** (BMP-2 and BMP-4), similarly derived from the septum transversum mesenchyme, were found to play an important role in hepatogenesis [6]. Both FGF and BMP signaling appear to be counterbalanced by **transforming growth factor-β** (TGF-β) signals that prevent inappropriate differentiation and as such represent a timer of embryonic liver development [7]. The **Wnt signaling pathway** has also been implicated in hepatogenesis during recent years but its function appears to be more complex than the clearly instructive function of the FGF and BMP signaling, with a time- and location-dependent Wnt-mediated differential regulation of the Hhex transcription factor.

Besides the central signaling molecules, pivotal **transcription factors** that have been described in hepatogenesis and the induction of hepatocyte-typical gene expression are Foxa2, GATA-4, C/EBPβ and HNF1β, which can form a transcription complex inducing transcription of the albumin gene [8]. Among the earliest genes expressed in the hepatic endoderm that indicated a hepatic cell fate are *Albumin*, *Afp*, *Ttr* (transthyretin), *Rbp* (retinol binding protein), and the transcription factor *Hnf4a* [9].

During the further development of the hepatic bud transcription factors Hhex and Prox1 have been identified as regulators of the dynamic cellular interaction and the associated matrix metalloprotease (MMP)-dependent remodeling of the extracellular matrix [10,11].

The hepatocyte precursor cells or hepatoblasts that form the hepatic bud appear to be **pluripotent** with the potential to differentiate into hepatocytes or cholangiocytes. Those hepatoblasts that differentiate into hepatocytes show a further embryonic and postnatal maturation during which they develop the typical gene expression profile and phenotype of adult hepatocytes [12]. This maturation of a hepatocyte gene expression profile depends on the complex interplay of a network of transcription factors with a set of six transcription factors in its center (HNF1a, HNF1β, FoxA2, HNF4a1, HNF6, and LRH-1). This highly and, with progressing differentiation, increasingly cross-regulated transcription factor network is thought to ensure correct terminal differentiation of hepatocytes [13].

Besides the differentiation into hepatocytes, a number of hepatoblasts differentiate into **cholangiocytes** which then line the intrahepatic bile ducts. Expression of the transcription factor **Sox9** is considered the earliest indicator of cholangiocyte differentiation. Sox9 has been demonstrated as regulator of the bile duct development and accordingly Sox9-expressing cells first appear in the vicinity of the portal venules and form the so-called ductal plate that encloses the periportal mesenchyme [14]. Ductal plate formation appears to depend on a **TGF-β gradient** with a critical required concentration for appropriate biliary differentiation. The **Notch** signaling pathway has been proposed as the second important pathway for cholangiocyte differentiation. Notch signaling seems to regulate the initial differentiation of cholangiocytes and also restricts the biliary differentiation to the periportal field, as expression of Jagged1 is less restrictive and can be found in the biliary cells as well as the periportal mesenchyme [15].

Phenotype and regeneration of the mature hepatocyte

Mature hepatocytes are polyhedral cubic cells with 15–20 μm sides and 5–12 facets. Typically 1–3 of the facets are facing the sinusoidal bloodstream while 4–9 facets are oriented towards the biliary capillaries and the neighboring hepatocytes. Hepatocytes are among the largest cells of the body although their cell size can vary substantially, depending on factors such as metabolic activity and blood flow as determined by the localization in the liver acinus. The eosinophilic cytoplasm of the hepatocyte is extremely rich in **organelles** with large numbers of mitochondria endoplasmic reticulum (ER) as well as free ribosomes causing a basophilic mottling.

The important role of hepatocytes in protein synthesis is reflected by the amount of ribosome containing rough endoplasmic reticulum (RER). The similarly extensive meshwork of smooth endoplasmic reticulum (SER) contains large amounts of biotransformation enzymes. Other biotransformatory enzymes can be found in the cytosol. The Golgi complex of the hepatocyte consists of several stacks of interconnected cisternae that have a curved shape and are primarily located between the nucleus and the apical, biliary pole of the cell. The Golgi membranes carry associated vesicles and lysosomes. The convex surface, also called cis-Golgi, is oriented towards the ER and receives protein vesicles from the RER for further processing such as terminal glycosylation of secretory proteins. The concave trans-Golgi shows plenty of very low-density lipoprotein (VLDL)-containing vesicles which are secreted from the Golgi.

The cell nuclei are large, spherical and centrally located with clumps of chromatin and prominent nucleoli. Many hepatocytes contain two and more **nuclei**, and nuclei are often **polyploid**; more and more tetraploid nuclei appear with age. In the adult liver, up to 50% of hepatocytes contain two or more nuclei.

It is well known that cellular stress can induce polyploidy but the physiological role of more than one complete set of chromosomes is still unknown. Tetraploid human and mouse liver cells display an approximately two-fold increase in cell volume [16]. This doubling in cell volume distinctly changes the special relationships and interactions between subcellular structures in the hepatocyte as cell surface increases only 1.4-fold. Apart from the possibly reduced efficiency of intracellular trafficking, the polyploid nucleus has an altered geometry of the mitotic spindle apparatus, potentially resulting in increased genomic instability [17]. It has recently been demonstrated that polyploidy is a reversible state and that hepatocytes can reduce their DNA content in a process of mitotic cell division called **ploidy reversal** [18]. Polyploid hepatocytes can form bipolar or multipolar mitotic spindles due to their larger number of centrosomes. In the case of multipolar mitotic spindles, the ploidy of the resulting cells can be reduced by half, e.g. generating four diploid hepatocytes from one octoploid cell.

Although this process of multipolar mitosis comes at the cost of increased rates of missegregation and results in aneuploid hepatocytes, the fact that a majority (30–90%) of hepatocytes in the human liver are aneuploid indicates that ploidy reversal is most likely a common event [19]. Regarding a possible physiological advantage of polyploidy, more recent work proposes polyploidy and the resulting increased genetic diversity as an mechanism that facilitates adaptive reactions to hepatic injury [19]. In this study hepatocytes with an aneuploidy karyotype displayed increased resistance to toxic injury and toxin challenge led to selection of such aneuploid cells. This selective advantage might very well be similar for tumor cells, leading to increased growth and resistance to therapy. Besides polyploidization under healthy conditions, the doubling of chromosomal number is a known response to cellular stress. This is particularly true for hepatocytes given their continuous exposure to toxins and xenobiotics. Polyploidization similary increases in situations of reduced nutrient

supply, e.g. in the postnatal phase. It has been hypothesized that polyploidization might be a mechanism to conserve and direct resources into metabolic activity by avoiding cell division [20]. Genome-wide association studies of diploid versus polyploidy cells found very few differentially expressed genes, indicating that polyploidy does not result in an altered transcriptional program [21].

In the absence of liver damage, the hepatocyte turnover in the adult liver is rather low and hepatocytes are long-lived cells with an average lifespan of 6 months, accounting for the low mitotic index of 1 mitotic hepatocyte per 20,000 hepatocytes throughout the liver acinus [22]. In the healthy liver, the turnover of hepatocytes depends primarily on duplication of existing hepatocytes. Upon liver injury, hepatocytes display an unparalleled regenerative potential with reactive proliferation and compensatory hyperplasia. Depending on the damaging mechanism, the liver mass is replaced by replication of existing hepatocytes without activation of progenitor cells (e.g. partial hepatectomy and some chemical liver injuries) or proper activation and replication of progenitor cells takes place (e.g. galactosamine toxicity) [23]. In the normal liver, adult **liver progenitor cells** (LPC) have been identified based on their expression profile of cell surface markers such as epithelial cell adhesion molecule (EpCAM), CD13, and CD 133. However, the importance of LPC for liver cell turnover and regeneration is controversial [24].

Hepatocytes are organized into plates separated by vascular channels or sinusoids. This structure is important in directing the excretion of the products of biotransformation away from the hepatocytes into bile and blood. They are **polarized cells** with three very distinct types of cell surface carrying different sets of transporter molecules and channels. These three cell membrane domains are the basolateral or sinusoidal cell membrane domain, the canalicular domain, and the lateral domain.

The **basolateral or sinusoidal domain** faces the sinusoids and the perisinusoidal space of Disse. This domain forms the vascular pole of the hepatocyte and constitutes 70% of the total cell surface. It carries 25–50 microvilli/μm. The microvilli are not uniformly distributed as there are clusters of thinner and longer microvilli on concavities existing on the basolateral domain that face to concavities on the surface of the opposite hepatocyte. The microvilli span the space of Disse and protrude through the fenestrae of the sinusoidal endothelial cells into the sinusoids. They are thought to play a pivotal role in maintaining the integrity of the space of Disse [25]. Endo- and exocytosis is the major function of the basolateral domain. For this reason, the basolateral domain shows indentations or pits. Some result from exocytosis by secretory vesicles, others represent so-called coated pits which are involved in receptor-mediated endocytosis.

Each membrane compartment of the hepatocyte displays a distinct set of **transporter molecules**. For the basolateral or sinusoidal membrane, these are mainly the Na^+-dependent bile salt transporter (NTCP; SLC10), the Na^+-independent transporters for organic anions (OATPs; SLC21) and cations (OATs/OCTs; SLC22 family) and basolateral efflux pumps of the multidrug resistance protein family (MRP; ATP-binding cassette [ABC] transporter superfamily).

While unconjugated, uncharged bile salts such as cholate cross the plasma membrane by passive diffusion, the uptake of taurin- or glycine-conjugated bile salts, such as taurocholate, from the sinusoidal blood into the hepatocyte is an energy-consuming step and requires a specific carrier protein. The main uptake protein is the Na^+-taurocholate cotransporting polypeptide (NTCP). This is exclusively expressed on the basolateral membrane of the hepatocyte. Besides bile salts, NTCP transports few steroid conjugates such as dehydroepiandrosterone sulfate (DHEA). Altered NTCP expression has been linked to cholestatic diseases such as intrahepatic cholestasis of pregnancy (ICP) or primary biliary cirrhosis (PBC). NTCP gene expression is downregulated by high levels of bile acids as well as proinflammatory cytokines and upregulated by glucocorticoids, amongst others [26,27].

For nonbile salt anions, the organic anion transporting polypeptide family (OATP; SLC21/SLCO family) is the primary carrier into the hepatocyte. Many of the compounds and xenobiotics that enter the hepatocyte via the OATP then undergo biotransformation in two phases: a cytochrome P450-dependent addition of polar groups followed by phase II, the conjugation with glucuronate, sulfate, glycine or methyl group which results in detoxification and facilitates excretion into the bile or urine.

So far, 12 human OATP transporter molecules have been identified. Some of them, such as OATP1A2 (OATP-A), 1B1 (OATP-C, LST-1), 1B3 (LST-2), 2A1 (PGT) and 2B1 (OATP-B), are functionally well characterized. OATP1B1 and 1B3 are only expressed in liver tissue and these transporters contribute to hepatic uptake of xenobiotics from the sinusoidal blood across the basolateral hepatocyte membrane [28]. Substrates

of these OATPs include angiotensin II receptor blockers, angiotensin converting enzyme inhibitors, statins, cephalosporins, penicillins, fluoroquinolones, antihistamines, β-adrenergic blocking agents and others [29]. This broad substrate specificity underlines the importance of OATPs in pharmacokinetics.

Taken together, the OATP family of transporters play a central role in organic anion and drug clearance of the hepatocyte [30,31]. Although so far no specific diseases are thought to result from an impaired function of the OATP transporters, alterations of their activity might interfere with the biotransformation or catabolism of certain drugs, modifying their therapeutic effects. The genetic control of these transporters has been shown to depend on the activity of the hepatic nuclear factors HNF-1α and HNF-4α [32].

In addition to the NCTP (SLC10) and the OATPs (SLC21), the organic anion transporter family (OATs/OCTs; SLC22 family) represents a third group of transport molecules that mediate basal substrate uptake. The family comprises 13 human plasma membrane proteins that are functionally characterized and each contains 12 predicted α-helical transmembrane domains. Among the family members are organic cation transporters (OCTs), organic zwitterion/cation transporters (OCTNs), and organic anion transporters (OATs). The transporters can operate as uniporters, facilitating diffusion (OCTs, OCTNs), or as anion exchangers (OATs), and Na^+/zwitterion cotransporters (OCTNs) [33]. Of the 13 known transporters, OCT 1 and 3 and OAT 2 are the most prominent in the basolateral hepatocyte membrane.

The basolateral membrane of the hepatocyte also contains members of the multidrug resistance protein family (MRP) belonging to the superfamily of ABC transporters. MRP1 mediates ATP-dependent efflux of glutathione S conjugates, leukotriene C4, steroid conjugates or bile salt conjugates. MRP1 is normally expressed at low levels in hepatocytes. MRP3 mediates the basal efflux of the organic anions estradiol-17-β-D-glucuronide and S(2,4-dinitrophenyl) glutathione, the anticancer drugs methotrexate and etoposide and even monovalent bile salts [34].

The **canalicular or apical domain** is also called the biliary pole of the hepatocyte. It constitutes 15% of the total hepatocyte surface and is basically a trench in the apical hepatocyte cell membrane. Together with the canalicular domain of the opposite hepatocyte, it forms the bile canaliculus. This canaliculus wraps around the whole hepatocyte, thereby forming a "chicken wire"-like network of bile channels. The sides of the canalicular domain are limited by a smooth cell surface with junctional complexes. The canalicular diameter depends on the location within the acinus. In acinar zone 3 it is 0.5–1 μm and in acinar zone 1 it is 1–2.5 μm. The canaliculus contains microvilli which are primarily localized at the sides of the canaliculi. The hepatocyte cytoplasm around the canaliculus shows a network of contractile microfilaments modulating the diameter of the canaliculus and thereby regulating the bile flow.

In the canalicular domain the apical bile acid transporter, organic ion transporters and P-glycoproteins are located, being responsible for the primary triphosphate (ATP)-dependent transport of organic components [35].

The members of the **ABC transporter superfamily** represent the primary, ATP-dependent excretion pumps in the canalicular membrane. This is first the **bile salt export pump** (BSEP; ABCB11), which is exclusively found in the canalicular membrane and is highly substrate specific, transporting almost exclusively monoanionic conjugated bile acids. Its affinity is highest for taurochenodeoxycholate followed by taurocholate. Only a few drugs have been shown to be transported by the BSEP, such as pravastatin. In contrast to BSEP, **MDR1** (P-gylcoprotein; ABCB1) exhibits a wide range of substrates and has been among the first transporters identified in drug elimination [36]. Among the many substrates of MDR1 are amphiphilic cations, steroid hormones, glycolipids and hydrophobic peptides [37]. MDR1 is a widely but interindividually variably expressed transporter that besides the canalicular membrane can be found in the intestine, the blood–brain barrier, the placenta and kidney, indicating a fundamental importance for the transport of endogenous and xenobiotic substances [38]. **MRP2** (ABCC2) is an important transporter for the elimination of sulfate conjugates and divalent bile acids as well as glucuronide into bile [39]. Importantly, MRP2 functions as a transporter for glutathione and thereby supports the approximately 100-fold concentration gradient of glutathione between the blood and bile. This high biliary glutathione concentration by osmotic effects assures bile salt-independent bile flow. MPR2 defects are involved in the Dubin–Johnson syndrome where it leads to deficient excretion of bilirubin glucuronide [40]. **Breast cancer resistance protein** (BCRP; ABCG2), a transporter molecule with broad specificity first described in chemotherapy-resistant cell lines, is also found in the canalicular membrane where it excretes drugs such as methotrexate and most likely environmental carcinogens [41].

In contrast to the aforementioned, the **multidrug and toxin extrusion exchanger 1** (MATE 1) that was more recently identified as an efflux transporter for organic cations in the apical hepatocyte membrane [42] belongs to the solute carrier family 47 (SLC47). MATE functions as a secondary carrier system utilizing the electrochemical membrane gradient (H^+/organic cation) for its bidirectional transport. Substrates of MATE 1 are guanidine and thiamine as well as drugs such as oxaliplatin and metformin.

Finally, the **lateral domain** of the hepatocyte ranges from the edge of the canalicular domain to the edge of the sinusoidal domain. The lateral domain shows a panel of different cell adhesion molecules and junctional complexes. The canalicular domain is sealed off from the remaining intercellular space by tight junctions which are belt-like zones made up of 3–5 parallel strands with the number of strands determining the adhesiveness of the junction. On the remaining lateral domain gap junctions allow for cell–cell exchange through embedded transmembrane proteins with a central pore forming a channel for intercellular communication [43]. The lateral surface also shows "snap-fastener" types of intercellular junctions that consist of membrane protrusions that interact with membrane indentations on the opposite cell.

Metabolic functions of the liver

Resorption, storage, and synthesis

Carbohydrates

The liver is a pivotal regulator of blood glucose levels. To this end, it resorbs, stores, synthesizes, and redistributes carbohydrates. Up to 90% of intestinally absorbed glucose in the bloodstream is taken up by hepatocytes via integral membrane glucose transporters (GLUTs). Of the 14 known members of the GLUT family (solute carrier 2A family; SLC2A), the hepatocyte carries GLUT-1, GLUT-2, GLUT-9, and GLUT-10 [44]. Intracellular glucose is then channeled into the hepatocyte metabolism through phosphorylation by either hexokinase or glucokinase. The latter is found only in hepatocytes, is substrate specific for glucose and shows maximal enzymatic activity at higher glucose levels than hexokinase, making it the central enzyme for postprandial glucose metabolism. To achieve glycogen as the final hepatic storage form, the resulting glucose-6-phosphate (G-6-P) undergoes further modifications by phosphoglucomutase to glucose-1-phosphate (G-1-P) before metabolization to uridine-diphosphate-glucose by glucose-1-phosphouridyltransferase. Up to 50,000 glucose molecules are then organized around a central glycogenin protein core to form glycogen, a highly condensed form of glucose storage that remains accessible for almost immediate release of glucose in situations of demand. After carbohydrate loading, the liver can contain 100–200 g of glycogen. The initial G-6-P may alternatively enter the pentose phosphate pathway through G-6-P dehydrogenase and feed into the generation of nicotinamide adenine dinucleotide phosphate (NADPH) as well as nucleotide precursors. Nonglucose carbohydrates such as fructose and galactose are channeled into glycolysis after enzymatic modification.

In the event of low glucose blood levels, systemic release of glucagon and adrenalin results in cyclic adenosine monophosphate (cAMP)-mediated activation of α-glycanphosphorylase that releases G-1-P from glycogen which is further reverted to G-6-P by phosphoglucomutase and finally to glucose by G-6-P. After depletion of liver glycogen, the glucose demand has to be satisfied by gluconeogenesis. In a situation of intense exercise or metabolic demand, 60% of glucose is generated from lactate in anaerobic metabolism primarily in the skeletal muscles. Alternatively, glucose is produced by metabolization of glycerol from triacylglycerine or amino acids. As mentioned earlier, depending on their localization within the acinus, hepatocytes display different metabolic features resulting in a zonation of metabolic processes. In the case of glucose metabolism, periportal hepatocytes display more gluconeogenesis while pericentral cells are the primary site of glycolysis [45]. Hepatic gluconeogenesis is blocked by insulin and failure of this inhibition is a key element of hepatic insulin resistance.

Lipids

The hepatocyte receives lipids as free fatty acids (FFA) originating from adipocytes after lipolysis of stored triglycerides (TG) or from dietary fat bound to albumin and lipoproteins. While some of the lipoproteins undergo receptor-mediated endocytosis, others are processed by hepatic lipases to obtain FFA from the triglycerides [46]. Nonesterified fatty acids enter the hepatocyte through fatty acid transport protein (FATP), fatty acid translocase (FAT) or diffusion (so-called "flip-flop") [47]. In the hepatocyte, the FFA are converted to triglycerides for storage in lipid droplets, undergo β-oxidation providing energy and ketone bodies or enter synthesis pathways for phospholipids, prostaglandins, and leukotrienes. The β-oxidation of

fatty acids takes place in different cellular compartments. While very long chain fatty acids undergo peroxisomal β-oxidation facilitated by microsomal cytochrome P450 CYP4A, short and medium chain fatty acids are metabolized by mitochondrial β-oxidation [48].

Since FFA upon uptake into the hepatocyte are either covalently bound and activated by fatty acid binding protein (FABP) or rapidly modified by fatty acyl-CoA synthetases, efflux of FFA out of the hepatocyte is minimal. Several isoforms of fatty acyl-CoA synthetases have been described which are located on the outer mitochondrial membrane and in microsomes. The different isoforms direct the acyl-CoA towards storage, lipid synthesis or β-oxidation [49].

While intracellular transport is organized to keep the cytoplasmic concentration of FFA and acyl-CoA as low as possible, the overall hepatocyte content of triglycerides results from the regulation of facilitated uptake, the synthesis of FFA as well as FFA oxidation and TG export in the form of VLDLs, stabilized by the Apo-B lipoprotein. Apo-B expression itself is stimulated by triglycerides. The regulation of lipid metabolism at the gene expression level through interaction of FFA with nuclear receptors has been a focus of interest in recent years.

As for carbohydrates, lipid metabolism shows zonation within the liver acinus. Periportal hepatocytes display a higher rate of fatty acid oxidation while lipogenesis is more prominent in pericentral cells. This is reflected by the activity of the involved enzymes, such as the lipogenic acetyl-CoA carboxylase which shows increased periportal activity.

Glycerophosphate acyltransferase (GPAT), the rate-limiting enzyme of liposynthesis by esterification of free fatty acids via acetyl-CoA and glycerol, is activated depending on nutritional status and insulin while inhibited by glucagon. The *de novo* synthesis of free fatty acids from acetyl-CoA is regulated at the transcriptional level by the sterol regulatory element-binding protein-1c (SREBP-1c). The transcription factor SREBP-1c is activated by insulin and regulates the expression of lipogenic enzymes like free fatty acid synthetase.

By promoting the conversion of carboxyl-CoA to malonyl-CoA, insulin also directs free fatty acids towards either oxidation or esterification. In the absence of insulin, lower levels of malonyl-CoA shift free fatty acids towards carnitine palmitoyltransferase-1 (CPT-1) on the mitochondrial outer membrane and subsequently ß-oxidation. High levels of malonyl-CoA as induced by insulin inhibit CPT-1 and free fatty acids become esterified into triglycerides. At the same time, insulin inhibits the export of triglycerides from the hepatocyte by suppressing the expression of VLDL lipoprotein Apo-B.

All in all, the hepatocellular metabolism is adjusted to the current energy status of the organism. Important metabolic regulators are AMP-dependent protein kinase (AMPK) and the mammalian target of rapamycin (mTOR). Activation of AMPK, e.g. through starvation, hypoxia, adenosine triphosphate (ATP) depletion, oxidative stress, chronic alcohol consumption, signaling molecules such as adiponectin and leptin or drugs such as metformin or thiazolinediones, leads to a shift from anabolic metabolism and lipogenesis to catabolic pathways, resulting in ATP production [50,51].

AMPK reduces lipogenesis through acetyl-CoA-carboxylase 1, increases fat oxidation via acetyl-CoA-carboxylase 2, lowers cholesterol synthesis by HMG-CoA-reductase and inhibits mTOR, thereby lowering protein synthesis.

Hepatic lipid metabolism is also regulated at the gene expression level. Nuclear receptors that function as transcription factors are essential in this regulatory mechanism. The peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptor transcription factors that regulate cell differentiation and metabolism [52]. PPARs belong to the steroid nuclear receptor superfamily and include three isoforms: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3). As transcription factors, they form heterodimers with retinoid X receptors (RXRs) to regulate gene expression. When activated by endogenous fatty acids, PPARα is an essential regulator of fatty acid (FA) oxidation, supplying energy in situations of high demand or starvation [53]. In contrast, PPARγ becomes activated in a fed state and upregulates fatty acids synthesis as well as generation of associated lipids. PPARs have evolved as pharmacological targets with lipid-lowering thiofibrates activating PPARα and antidiabetic glitazones interacting with PPARγ.

Besides PPAR, the liver X receptors (LXR) represent another important group of nuclear receptors in the hepatocyte. Two subtypes have been identified and classified as LXRa (NR1H3) and LXRb (NR1H2) [54]. LXRa is strongly expressed in hepatocytes and is activated by oxysterols and metabolites of the cholesterol metabolism. Upon activation, LXR/RXR heterodimers regulate expression of gene products important for bile acid synthesis, thereby promoting cholesterol elimination. LXRs also induce CYP7a, FAS, acetyl-CoA carboxylase (ACC), and SREBP-1c expression, and inhibit expression of G-6-P while inducing glucokinase [55].

Amino acids and proteins

The hepatocyte takes the central position in the metabolism of amino acids and proteins as it facilitates the breakdown of amino acids for carbohydrate and fat metabolism while synthesizing the vast majority of circulating proteins for the organism. The liver ensures homeostasis of amino acid metabolism. Amino acid breakdown in the hepatocyte directly corresponds to the amino acid concentration of the portal venous blood. Amino acids are channeled into protein or glycogen synthesis. At the same time, increased amino acid concentrations inhibit proteolysis.

Breakdown of amino acids leads to the formation of keto acids, ammonia, and glutamine. Ammonia, which also shows high concentrations in portal venous blood, is converted to urea in the urea cycle.

Two of the central enzymes of amino acid metabolism are the cytosolic and mitochondria located aspartate aminotransferase (AST) and the strictly cytosolic alanine aminotransferase (ALT). These enzymes catalyze the bidirectional transfer of an amino group from donor to recipient molecule, in the case of AST conversion of aspartate and α-ketoglutarate to oxaloacetate, for ALT the conversion of alanine and α-ketoglutarate to pyruvate and glutamate. Both enzymes are released into the blood upon hepatocyte damage and are commonly measured as unspecific markers thereof.

Hepatic protein degradation occurs either through an autophagic-lysosomal mechanism or the ubiquitin-proteasomal degradation. During autophagy, parts of the cytoplasm become enclosed in vesicles which then fuse with lysosomes, resulting in the digestion of their content. For proteasomal degradation, proteins are tagged by enzymatic addition of ubiquitin residues which then trigger translocation of the labelled protein to the proteasome.

Hepatocytes produce the vast majority of circulating serum proteins including carrier proteins (e.g. albumin, transferrin, ceruloplasmin, haptoglobin, lipoproteins), immune-related proteins (proteins of the complement system, acute-phase proteins) and coagulation factors. **Albumin** as the predominant serum protein makes up approximately 15% of the hepatic protein synthesis. Albumin is crucial for the maintenance of normal oncotic pressure and functions as a carrier protein for various substances, making it an important carrier molecule. With a half-life of approximately 21 days, measurement of serum albumin does not reflect acute changes in liver synthesis function, e.g. due to acute liver injury.

Ceruloplasmin is the copper carrier molecule in the human organism synthesized in the liver. The liver is the central regulator of copper homeostasis with the biliary copper excretion being proportional to the hepatic copper pool, indicating a fine-tuned regulatory circle. Copper is an essential cofactor in many enzymes. Due to its cytotoxicity, copper uptake and transport are handled by specific transport and carrier molecules. Copper enters the hepatocyte through copper transporters hCTR1 and hCTR2. Biliary excretion of copper and heavy metals is a pivotal detoxification step. Copper transport across the cell membrane is mediated by copper-transporting ATPases (Cu-ATPases) ATP7A and ATP7B. While ATPL7A transports copper across the basolateral membrane of enterocytes, the second isoform ATP7B excretes copper into the bile at the apical hepatocyte membrane. ATP7B is also localized to the trans-Golgi network where it mediates incorporation of copper into cuproenzymes such as ceruloplasmin. Depending on the copper concentration, ATP7B is translocated from the trans-Golgi to the canalicular region and back, thereby regulating copper excretion [56]. Wilson's disease, a copper overload, is caused by ATP7B deficiency.

Acute-phase proteins (APPs) form a heterogeneous group of about 30 different biochemically and functionally unrelated immune-related plasma proteins produced by the hepatocyte. This includes α2-macroglobulin, complement factors, C-reactive protein, ceruloplasmin, ferritin, fibronectin, haptoglobin, and prothrombin, to name only a few. Approximately 90 min after the onset of a systemic inflammatory response (e.g. due to tissue injury or infection), APP serum levels increase in the case of positive APPs or drop in the case of negative APPs [57]. Most of the important APPs are glycoproteins except C-reactive protein (CRP) and serum amyloid A (SAA).

The systemic inflammatory response leads to release of cytokines including interleukin (IL)-6, IL-1, tumor necrosis factor (TNF)-α and interferon-γ which act as acute-phase mediators and together with glucocorticoids rapidly induce the hepatic synthesis of APPs. The magnitude of concentration change as well as effects of APPs have a wide spectrum ranging from protease inhibition to immunomodulation, direct host defense or the removal of xenobiotics [58,59].

Clotting factors and proteins involved in the fibrinolytic system are mostly synthesized in the hepatocyte. This comprises all the vitamin K-dependent coagulation factors (factors II, VII, IX, X, protein

C, protein S, and protein Z), as well as factor V, XIII, fibrinogen, antithrombin, α2-PI, and plasminogen. Exceptions are von Willebrand factor (VWF), tissue plasminogen activator (tPA), thrombomodulin, tissue pathway factor inhibitor (TPFI), and urokinase-like plasminogen activator (uPA). VWF, tPA, thrombomodulin, and TFPI are synthesized in endothelial cells, while uPA is expressed by endothelial cells, macrophages, renal epithelial cells, and some tumor cells [60]. The fat-soluble vitamin K is required for postribosomal modification of glutamic acid residues to γ-carboxyglutamic acid residues in the procoagulant factors (II, VII, IX, and X) and anticoagulant factors (proteins C, S, and Z) to allow for calcium binding and thus render them physiologically active.

Moreover, the reticuloendothelial system of the liver clears coagulation factors from the circulation, in particular tPA, thereby regulating coagulation and fibrinolysis.

Since coagulation factors have relatively short half-lives, acute liver damage becomes apparent in coagulation abnormalities rather quickly.

Detoxification and excretion

Hepatocytes are the site of biotransformation of both endogenous and exogenous lipophile compounds.

The steps of biotransformation can be separated into phase I and phase II reactions [61]. Phase I of biotransformation is centered around the cytochrome P450 (CYP) system. In the phase I reactions, lipophilic substances are conjugated with a reactive group to enhance the polarity of the molecule. The reactive groups added are -NH_2, -COOH, -OH or -SH groups. Depending on the group to be added, conjugation involves oxidation/hydroxylation, reduction or hydrolysis. During phase II of biotransformation, exogenous molecules are conjugated to endogenous cofactors.

Biotransformation is regulated by a set of nuclear receptors (PXR, CAR, PPAR, and AHR) that bind to and are induced by endogenous and exogenous molecules, thereby acting as xenosensors [62]. These regulatory mechanisms allow for adaption of the biotransformation system to changing metabolic requirements. CYP enzymes were identified in 1964 as a microsomal monooxygenase containing heme as its active group [63]. Today over 100 genes have been identified that encode for CYPs, including important isoforms like CYP3A4, a main enzyme in drug biotransformation. Reducing equivalents for biotransformation by CYPs are transferred from NADPH by the CYP reductase that colocalizes to the SER. Due to their broad substrate specificity and subsequent binding competition, CYPs are a main site for drug–drug interactions. Besides competing substrates, CYP induction by one inducer may alter biotransformation of other compounds. The genes encoding for CYPs display an enormous polymorphism with large numbers of gene variants resulting in enzymes with variable activity.

In addition to CYPs, there are several other enzymes taking part in phase I biotransformation reactions, some of them not located in the SER but in the cytoplasm. Flavin monooxygenases (FMOs) are one group of biotransformation enzymes that contain flavin as a cofactor and share many substrates with CYPs. FMOs also require NADPH and O_2 to oxidize nucelophilic nitrogen, sulfur and phosphorus groups in xenobiotics. Another cytosolic phase I enzyme in biotransformation is epoxide hydrolase (EH) that converts epoxides into dihydrodiols. The increase in polarity achieved by the phase I reaction can be sufficient to enable renal excretion of compounds.

In the phase II reaction, phase I products are conjugated with activated glucuronic acid, amino acids, activated sulfuric acid or mercapturic acid as a second, liver-derived substance. These conjugates show a further increase in polarity and are thus suitable for renal or intestinal excretion. The main enzymes of phase II of biotransformation are the uridine diphosphoglucuronyl transferase isoenzymes (UGT), which localize to the SER in close proximity to the CYPs and glucuronidize phase I products still in the SER. The enzyme family of glutathione S transferases (GST) that are localized in the cytosol, microsomes, mitochondria, and nucleus facilitate the important phase II glutathione conjugation of lipid peroxides, halogenated aromatics, and epoxides. Furthermore, sulfotransferases (SULT) transfer sulfonate groups to phase I products that contain alcohol or amine groups such as steroids, drugs, and many xenobiotics.

N-acetyltransferases (NAT) are a fourth group of phase II enzymes which catalyze N-acetylation of subtrates containing aromatic amines or hydrazines.

After the two phases of biotransformation, the now hydrophilic substrates require transmembrane transporters for excretion from the cell. These are the already described ABC transporters such as ABCC2 (MRP2), ABCG2, and ABCB1 (MDR1 P-glycoprotein) which effectively pump the hydrophilic conjugates across the canalicular membrane into the bile. Alternatively,

pumps such as ABCC3, ABCC4, and ABCC5 can transport conjugated compounds back into the bloodstream for renal filtration and excretion.

Detoxification of specific substances

Bilirubin

The human body produces about 300 mg bilirubin per day, mainly from the heme molecule of senescent erythrocytes. Albumin-bound unconjugated bilirubin is transported to the liver in the bloodstream where it is taken up into the hepatocyte by OATPs in exchange for glutathione. In the cytoplasm, the bilirubin is immediately bound to ligandin and Z-protein to prevent intracellular toxicity. This step is followed by conjugation to glucuronic acid by UGT in the SER, rendering the molecule nontoxic and soluble so that it can be easily secreted into bile by the ATP-dependent export pump MRP2. There is a minimal secretion of conjugated bilirubin into the plasma by MRP3. Once in the gut, bilirubin undergoes bacterial oxidation to stercobilin, excreted in the feces. Small amounts reentering the bloodstream are cleared by the kidneys after transformation to urobilirubin [40].

Alcohol

The zinc-dependent alcohol dehydrogenase (ADH) is the primary enzyme for alcohol metabolism in the liver. In addition, the liver can metabolize alcohol in the ER by microsomal oxidative enzymes and by catalases in the peroxisomes. The interindividual differences in alcohol metabolism are easily explained by the vast number of ADH isoenzymes. There is no induction or upregulation of ADH by chronic exposure to alcohol. Alcohol metabolism by ADH is oxygen dependent and can consume up to 90% of the cellular oxygen uptake. The reaction product of ADH is the highly toxic aldehyde acetate which undergoes further microsomal transformation by aldehyde dehydrogenase to acetic acid. Alcohol flush reaction which affects certain individuals, especially in East Asia, is due to a polymorphism in the acetaldehyde dehydrogenase. Acetic acid is further metabolized to acyl-CoA and can enter the citric acid cycle, the lipid acid cycle or cholesterol synthesis. In the case of chronic alcohol metabolism, the microsomal P450 enzyme system is induced and takes over more of the metabolic load. The CYP2E1 is the alcohol-induced isoform. As mentioned above, a third route of alcohol detoxification in case of high blood alcohol levels is by peroxisomal catalases resulting in acetaldehyde [64].

Ammonium

Bacterial degradation of proteins in the colon is the primary source of urea and ammonium (NH_4^+). The hepatocyte metabolizes ammonium in the periportal located urea ornithine cycle and the pericentral active glutamate cycle. In the first, ammonia undergoes mitochondrial transformation to carbamylphosphate by conjugation with bicarbonate and is further metabolized to citrulline by addition of ornithine. Citrullin is then shifted to the cytoplasm and broken down into arginine and ornithine. In the glutamate cycle, α-ketoglutaric acid serves as a conjugate for ammonium, resulting in glutamine. Both of these metabolic pathways are bicarbonate dependent.

Excretion

Bile acids

The primary bile acids cholic and chenodeoxycholic acid are synthesized from cholesterol via the classic neutral or the alternative acidic pathway, with the classic pathway contributing two-thirds of the bile acids. After initiation by hydroxylation in position 7, cholesterol undergoes modification of the sterol ring, oxidation, shortening of the side chain and finally conjugation with glycine or taurine. Primary bile acids are then secreted into bile and reach the small intestine where the anaerobic flora dehydroxylates the primary bile acids into the secondary bile acids deoxycholic acid and lithocholic acid. The remaining primary and secondary bile acids are reabsorbed in the terminal ileum by the Na^+-dependent apical sodium bile acid transporter (ASBT) and enter the enterohepatic circulation, returning to the liver where they are taken up via the previously described basolateral transporters, reconjugated and again secreted into the bile. The enterohepatic circulation is highly effective, retaining more than 95% of bile acids, the so-called bile acid pool. A daily loss of approximately 500 mg is balanced by newly produced bile acids. The bile acid pool amounts to 1.5–2.5 g of bile acids which undergoes up to 10 intestinal passages per day. In the liver, small amounts of secondary bile acids are further modified to tertiary bile acids such as ursodeoxycholic acid. As cholesterol is the starting material for bile acid synthesis, bile acids are a method of cholesterol excretion thus maintaining cholesterol levels. Pharmacological absorption of bile acids can be used to deplete cholesterol from the body. Apart from cholesterol elimination, bile acids fulfill various functions in the intestine and liver. Conjugated bile acids are effectively detergents that are essential for digestion and absorption of nutritional fats by micelle formation. They also

activate and stabilize pancreatic digestive enzymes such as lipase, phospholipase, and cholesterolesterase and act as chemical stabilizers of the bile.

More recently, bile acids have been recognized as signaling molecules with important regulatory function in various metabolic processes [65]. Bile acids activate the membrane-bound G protein-coupled bile acid receptor TGR5 as well as the intracellular farnesoid X receptor (FXR). FXR belongs to the aforementioned family of nuclear hormone receptors that act as transcription factors when activated. As such, it not only regulates bile acid metabolism by upregulating bile acid conjugation and increasing bile flow to prevent bile acid toxicity; it also influences cholesterol, triglyceride, and glucose metabolism.

Bile

The liver produces about 600 mL of bile per 24 h, which consists of 400 mL canalicular bile that is secreted by the hepatocytes on their apical membrane and 200 mL of ductular bile that is secreted in the bile ducts. The transporter systems of the apical membrane described in detail above determine the composition of bile. BSEP actively excretes conjugated bile acids against a concentration gradient that is up to 1000-fold. This transport is the rate-limiting step in bile salt excretion. MRPs are essential for the excretion of nonbile salt organic anions. The ATP-dependent flippase MDR3 is highly expressed in the canalicular membrane of the hepatocytes [66] and allows for export of phospholipids into the bile.

MULTIPLE CHOICE QUESTIONS

1 The liver parenchyma can be structured into different functional units. The most metabolically important structure is:
- **A** the liver lobe
- **B** the classic liver lobule
- **C** the portal liver lobule
- **D** the liver acinus
- **E** the portal triad.

2 During embryonic hepatocyte development the earliest expressed genes indicating a hepatocyte differentiation are:
- **A** retinol binding protein (Rbp)
- **B** α-fetoprotein (AFP)
- **C** transthyretin (Ttr)
- **D** Hnf4a
- **E** albumin, AFP, Ttr, Rbp and Hnf4a.

3 The basolateral or sinusoidal cell membrane of the hepatocyte contains a specialized set of transporter molecules. Which of the following transporters is not found in the basolateral membrane?
- **A** Sodium taurocholate cotransporter (NTCP, SLC10)
- **B** Organic anion transporter proteins (OATP, SLC21)
- **C** Organic cation transporter 3 (OCT3, SLC22)
- **D** Bile salt export pump (BSEP, ABCB11)
- **E** Multidrug resistance protein 3 (MRP3)

4 The synthesis of glycogen from glucose in the hepatocyte requires several enzymatic steps. Which is the correct sequence of enzymes involved?
- **A** Glucokinase, phosphoglucomutase, glucose-1-phosphouridyltransferase
- **B** Phosphoglucomutase, glucokinase, glucose-1-phosphouridyltransferase
- **C** Glucose-1-phosphouridyltransferase, glucokinase, phosphoglucomutase
- **D** Phosphoglucomutase, glucose-1-phosphouridyltransferase, glucokinase
- **E** Glucokinase, glucose-1-phosphouridyltransferase, phosphoglucomutase

5 Biotransformation can be separated into phase I and phase II reactions. Which of the following is not a classic phase II enzyme of biotransformation?
- **A** Uridine diphosphoglucuronyl transferase (UGT)
- **B** Glutathione S transferases (GST)
- **C** N-acetyltransferases (NAT)
- **D** Epoxide hydrolase (EH)
- **E** Sulfotransferase (SULT)

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