Clin Pharmacokinet 2002; 41 (10): 751-790
0312-5963/02/0010-0751/$25.00/0

Enterohepatic Circulation
Physiological, Pharmacokinetic and Clinical Implications

Michael S. Roberts,[1] *Beatrice M. Magnusson*,[1] *Frank J. Burczynski*[2] and *Michael Weiss*[3]

1 Department of Medicine, University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland, Australia
2 Faculty of Pharmacy and Department of Pharmacology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada
3 Section of Pharmacokinetics, Department of Pharmacology, Martin Luther University Halle-Wittenberg, Halle, Germany

Contents

Abstract

Enterohepatic recycling occurs by biliary excretion and intestinal reabsorption of a solute, sometimes with hepatic conjugation and intestinal deconjugation. Cycling is often associated with multiple peaks and a longer apparent half-life in a plasma concentration-time profile. Factors affecting biliary excretion include drug characteristics (chemical structure, polarity and molecular size), transport across sinusoidal plasma membrane and canniculae membranes, biotransformation and possible reabsorption from intrahepatic bile ductules. Intestinal reabsorption to complete the enterohepatic cycle may depend on hydrolysis of a drug conjugate by gut bacteria. Bioavailability is also affected by the extent of intestinal absorption, gut-wall P-glycoprotein efflux and gut-wall metabolism.

Recently, there has been a considerable increase in our understanding of the role of transporters, of gene expression of intestinal and hepatic enzymes, and of hepatic zonation. Drugs, disease and genetics may result in induced or inhibited activity of transporters and metabolising enzymes. Reduced expression of one transporter, for example hepatic canalicular multidrug resistance–associated protein (MRP) 2, is often associated with enhanced expression of others, for example the usually quiescent basolateral efflux MRP3, to limit hepatic toxicity. In addition, physiologically relevant pharmacokinetic models, which describe enterohepatic recirculation in terms of its determinants (such as sporadic gall bladder emptying), have been developed.

In general, enterohepatic recirculation may prolong the pharmacological effect of certain drugs and drug metabolites. Of particular importance is the potential amplifying effect of enterohepatic variability in defining differences in the bioavailability, apparent volume of distribution and clearance of a given compound. Genetic abnormalities, disease states, orally administered adsorbents and certain coadministered drugs all affect enterohepatic recycling.

In enterohepatic recycling, foreign chemicals entering the alimentary tract are absorbed into portal venous blood by enterocytes, removed from blood by uptake into hepatocytes, secreted into the bile, and then deposited back into the intestinal lumen where they may be reabsorbed by intestinal cells and available for recycling. Enterohepatic circulation may be significantly influenced by the formation of metabolites during absorption by the enterocytes and hepatocytes and during transit through the biliary system, intestinal tract or the systemic circulation (figure 1). For either the chemical or its metabolite, enterohepatic circulation can be terminated by elimination into the fae-

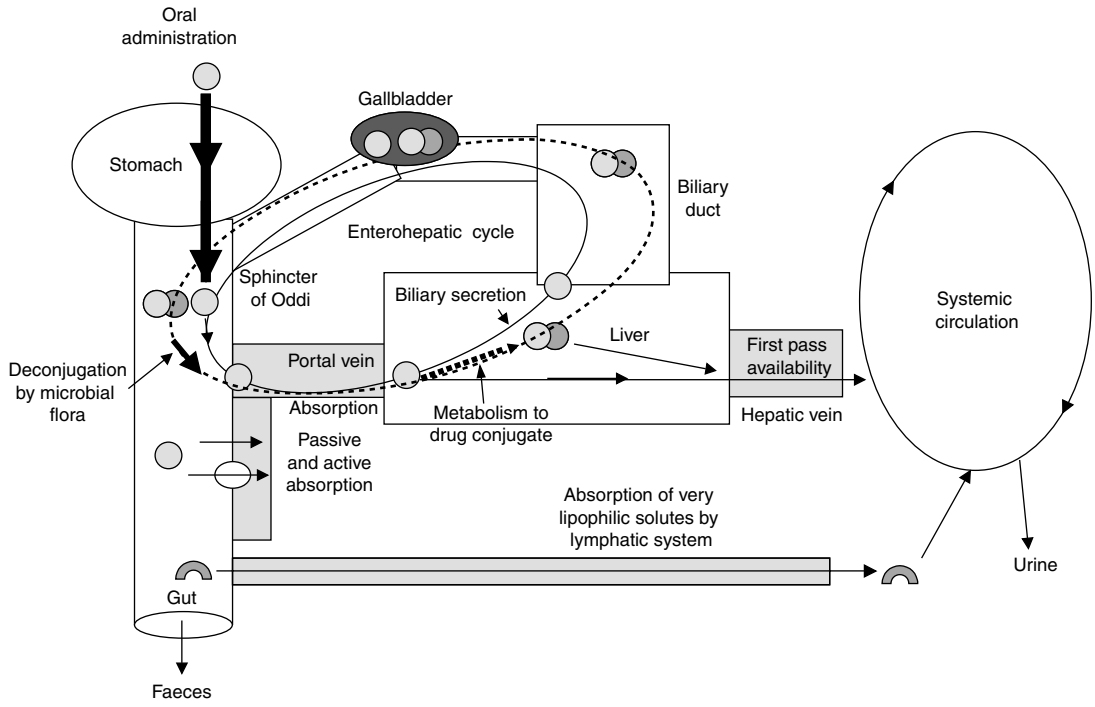

Fig. 1. Enterohepatic recirculation. Drugs entering the intestinal tract may be absorbed into the portal circulation where they can be removed from systemic circulation by hepatic uptake. The compound may then be excreted into the bile and pass back into the intestinal tract and become available for enterohepatic cycling. Biotransformation in enterocytes, hepatocytes, and the intestinal tract and throughout the body can convert the drug into metabolites, which may undergo enterohepatic cycling or escape into the urine and faeces. Some very lipophilic solutes may bypass the portal circulation and be absorbed into the systemic circulation via the lymphatic system.

ces or, if the compound has entered the systemic circulation, the urine.[1] Enterohepatic circulation can also be interrupted by reversible storage in various reservoirs. Therefore, an unequivocal understanding of the enterohepatic circulation of chemicals requires an appreciation of the influence of biotransformation and membrane transport at each potential site.

The liver is the major site in the body for xenobiotic metabolism. An important function of the liver is the clearance of drugs by either biotransformation or biliary excretion. It therefore also follows that hepatocytes are at risk for exposure to the toxic bioactivated metabolites that result from the metabolism of some toxicants.[2] The direct routing of blood to the liver from the gastrointestinal tract from which ingested xenobiotics are absorbed, as well as the tendency for such compounds to undergo enterohepatic cycling, also increase the vulnerability of liver cells to assault from toxicants.[3]

Various endogenous as well as exogenous compounds are known to undergo enterohepatic circulation, which may serve a physiological function, for example in the recycling of bile acids. For drugs that are excreted extensively into the bile, insight into the magnitude of enterohepatic circulation is of crucial importance, as it will significantly affect pharmacokinetic parameters such as plasma half-life and area under the plasma concentration-time curve (AUC) as well as estimates of bioavailability. The complete study of enterohep-

atic circulation requires determination of the rate of biliary excretion and re-excretion, intestinal absorption, and faecal and urinary elimination of parent compound and all metabolites.[4]

A major difficulty in reviewing this area is that much of the literature referring to enterohepatic recirculation has defined its contribution indirectly, often putting forward its contribution as a means to explain, for instance, the lack of excretion of certain compounds in urine. As an example, Jonsson et al.[5] suggested that the small amounts of active metabolites of glibenclamide (glyburide) [4-*trans*-hydroxy-glibenclamide and 3-*cis*-hydroxy-glibenclamide] excreted in the urine of diabetic patients with impaired renal function may indicate the involvement of complementary nonrenal elimination routes. These routes may include the shunting of metabolised glibenclamide to the biliary excretion route and/or enterohepatic recycling of both metabolites and unmetabolised glibenclamide.

Other studies have defined enterohepatic recirculation indirectly from urine studies following interruption of the enterohepatic cycle. For instance, Hellstern et al.[6] showed that although the fraction of lormetazepam and its glucuronide excreted in the urine during a 24-hour period was normally 9 to 35% of the dose, during interrupted enterohepatic recirculation this increased to 23 to 58% of the dose. However, the 24-hour post-dose bile fraction contained only 0.3 to 2.8% of the oral lormetazepam dose in the form of the drug and its glucuronide.

The use of nasobiliary catheters or biliary T-tubes to collect bile samples is an effective way to measure enterohepatic recirculation, but is often conducted using only small numbers of patients. Melnik et al.[7] showed that the mean bile/plasma ratios of trovafloxacin in three patients for peak plasma concentration and AUC were 13.9 and 14.9, respectively, with concentrations of the *N*-acetyl metabolite in the bile about one-tenth that of the parent. More information is available on the excretion of endogenous compounds such as the bile acids (for example, Lenzen et al.[8]). Given that the principles of enterohepatic recycling for these compounds are also likely to apply to drugs, some aspects of the kinetics of their enterohepatic recycling are considered in this review.

In this overview, we examine enterohepatic cycling using predominantly human data, but also data from animal studies as appropriate, to assist in our understanding of the clinical pharmacokinetics of enterohepatic recycling. Consideration is also given to the range of relatively recently discovered transport processes affecting the recycling of drugs.

1. Anatomy and Physiology of the Liver and Bile Formation

The liver has an important role in many metabolic and excretion processes. Nutrient-containing blood from the gastrointestinal tract travels first to the liver via the portal vein, where nutrients such as carbohydrates, lipids and vitamins can be removed and stored until needed.[9] The liver also synthesises and secretes bile, which provides a route for the excretion of endogenous and exogenous compounds such as bile acids, bilirubin, phospholipid, cholesterol, drugs and toxins.[9] Bile is an aqueous solution, and hence is more suitable for the excretion of water-soluble compounds. Many compounds are metabolised to more water-soluble products prior to excretion in bile. In addition, the presence of micelle-forming bile acids above their critical micellar concentration allows solubilisation of lipid-soluble compounds in bile. Thus, the liver can excrete water-soluble as well as lipid-soluble compounds.[10] Bile salts are later actively reabsorbed from the terminal ileum into the portal circulation to complete their enterohepatic circulation.

Hepatic bile acid secretion is a key determinant of the secretion of bile and biliary lipids. Bile acids are the major determinants of bile flow under physiological conditions (figure 2). The average bile flow in humans is 1.5 to 2.0 ml/min/kg bodyweight.[11] The ability of the liver to concentrate bile acids in bile is, therefore, an important aspect of bile formation. Bile also assists in fat digestion

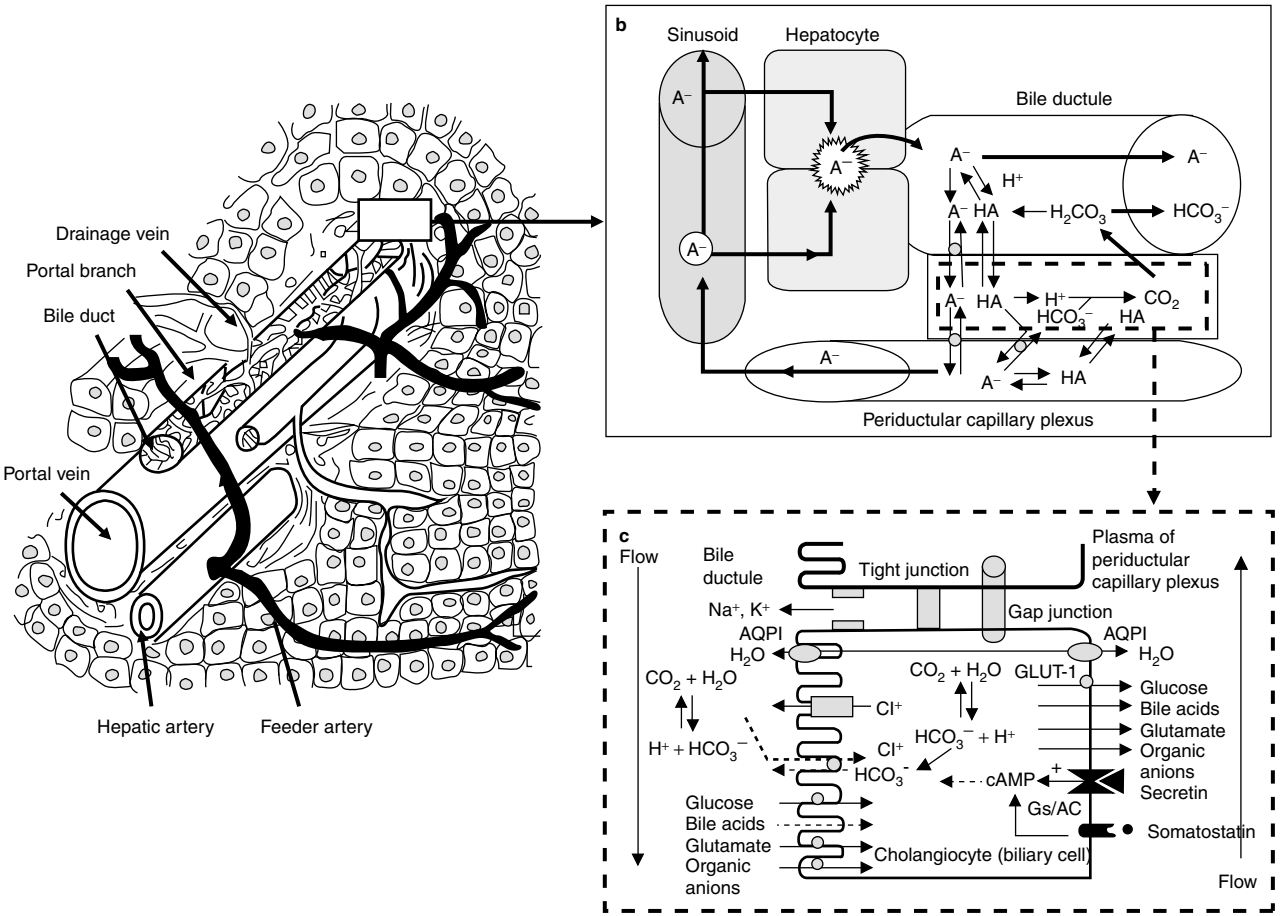

Fig 2. Schematic drawing of the bile duct and the peribiliary vascular plexus (reproduced from Roberts et al.,[12] with permission). (**b**) Cholehepatic circulation; and (**c**) Transport mechanisms in the cholangiocyte. **AQPI** = aquaporin I; **Gs/AC** = GTP-binding protein/adenylyl cyclase.

and absorption by providing bile acids and phospholipids to the duodenum, and it partially neutralises acidic chyme from the stomach and plays an immunological role by delivering IgA to the intestine. Because of its location and ability to efficiently extract a wide variety of compounds from the portal circulation, the liver plays a central role in removing toxic materials before their entry into the systemic circulation. Many of these agents are excreted into bile following detoxification and/or metabolism by the liver. Osmosis is considered to be the major mechanism of water movement during bile formation, although hydrostatic pressure may affect bile formation under experimental and pathological conditions. It is believed that the osmotic gradient is provided by organic and inorganic solutes secreted into bile. Bile formation is, thus, intimately related to hepatobiliary transport of biliary constituents.

The liver is composed of two types of epithelial cells: hepatocytes, which account for approximately 60% of the nuclear population, and cholangiocytes (epithelial cells that line intrahepatic bile ducts), which account for 3 to 5% of the liver cell population.[12,13] Hepatocytes have been characterised as 'polarised' cells in that they absorb substrates from the blood and secrete metabolites in the bile. They are the only cells in the body that convert cholesterol to bile acids, and their canalicular membrane secretes bile acids vectorially into bile. In addition, hepatocytes excrete endogenous metabolites and endogenous xenobiotics and degrade hormones. This variety of hepatocellular functions is at variance with the apparent morphological homogeneity of liver cells. The constantly changing metabolic demands on the liver are such that hepatocytes must coordinately express an unusually diverse array of structural, enzymatic and secretory proteins.[14] Cholangiocytes exhibit some morphological heterogeneity in that they tend to be cuboidal in small ducts and columnar in large ducts.[15,16]

Bile is made as the result of active transport of its constituents into the biliary space and it is formed primarily by hepatocytes and secreted at the bile canaliculus. The canalicular space is surrounded by two hepatocytes, and is separated from intercellular space by permselective tight junctions. The location of tight junctions also serves as a morphological and functional demarcation point between the apical (canalicular) and basolateral (sinusoidal and lateral plasma membrane) domains of hepatocytes. These two membrane domains differ in their lipid composition,[17] and tight junctions, which are believed to provide effective barriers against lateral movement of lipids and proteins, maintain these differences.[18] Tight junctions, along with desmosomes and gap junctions present at the lateral domain, are considered to represent important diffusional barriers between the interstitium and bile. In addition, microfilaments and microtubules in hepatocytes may be involved in canalicular bile formation.[11,19,20] Circumstantial evidence indicates that microfilament dysfunction may alter bile formation (see section 4.8.2). Cytochalasin B, an agent that destroys microfilaments, causes cholestasis in the isolated rat liver preparation.[21]

Bile formed at canaliculae is modified downstream in the bile ducts (ductular bile) by reabsorption and/or secretion of electrolytes and water. Although it has been suggested that water moves passively into bile,[22] recent studies with microperfused intrahepatic bile ductules suggest that that ducts both secrete and absorb water in response to osmotic gradients.[23] Bile entering the duodenum includes a canalicular secretion, elaborated by the hepatocytes, and a ductular/ductal secretion, elaborated in the biliary channels. Bile is concentrated by the gall bladder by reabsorption of electrolytes and water and secreted into the small intestine via the Sphincter of Oddie. The volume and composition of bile are modified within the lumen of intrahepatic bile ducts as a result of the absorption and secretion of water and solutes by cholangiocytes (figure 2). Cholangiocytes provide a large surface area for transport between blood and bile and play a significant role in bile formation.[24]

The fate of unconjugated bile acids entering the canaliculus depends on their lipophilicity. For example, ursodeoxycholate is secreted in the unconjugated form, protonated from H_2CO_3 into ursodeoxycholic acid with generation of HCO_{3-} (figure 2). The lipophilic acid is reabsorbed by the biliary epithelial cells into the periductule plexus and returned to the liver.[25] The cholehepatic cycling causes a bicarbonate choleresis, because intraluminal carbonic acid donates the proton, permitting passive bile acid absorption.[26] Other bile acids have similar bile secretory effects to ursodeoxycholate.[27]

2. Component Processes in Enterohepatic Circulation

2.1 Absorption from the Gastrointestinal Tract Into the Portal Blood Supply

Enterohepatic circulation occurs after the process of absorption of a solute from the gastrointestinal tract into the portal blood (figure 3). The amount of solute involved in enterohepatic circulation will be dependent on the availability of solutes to the liver and in turn on absorption from the gastrointestinal tract. The factors affecting oral absorption include the formulation and characteristics of the drug product as well as patient characteristics such as pH of lumen, gastric emptying time, intestinal transit time, surface area, gastrointestinal disease and mesenteric blood flow. Other factors are the presence of food and drugs in the gastrointestinal tract and the pharmacokinetic characteristics of the drug in terms of metabolism by the gut wall and gut microflora.[28] These factors will define the disintegration and dissolution properties of the drug formulation as well as the solubility of the drug molecule in the lumenal environment of the gastrointestinal tract.

The absorption of drugs across the gastrointestinal wall membrane may be affected by different rate-determining steps, and may be limited by the unstirred water layer, by the characteristics of the membrane or by portal blood flow.[29] The actual rate-determining step is defined by the physicochemical properties of the drug (pKa, water/lipid solubility, structural mimicry of endogenous substrates for transport proteins) and the physiology of the gastrointestinal tract. Some solutes such as di- and tri-peptides, amino acids, inorganic ions, glucose and galactose may be actively transported across the gut wall. The intestinal peptide transport system has broad substrate specificities for peptidomimetic drugs, including β-lactam antibiotics, ACE inhibitors, renin inhibitors, bestatin, thrombin inhibitors and thyrotropin-releasing hormone (protirelin) and its analogues.[30]

Drug absorption into the portal circulation can occur down the length of the gastrointestinal tract to the superior rectal vein. Absorption from the lower region of the rectum (middle and inferior rectal veins) bypasses the portal circulation.[31] Some highly lipophilic solutes, for example longer chain fatty acids (>12 carbon atoms), also bypass the portal blood supply by being absorbed via the intestinal lymph system and circulated via the thoracic duct back into the systemic circulation.[32] Examples of compounds transported by this pathway include cyclosporin, naftifine, probucol, mepitiostane, lipophilic vitamins, halofantrine, DDT and lipophilic prodrugs.[32]

2.2 Metabolism and Efflux by the Gut Wall

Transport across the wall is also affected by the susceptibility of the molecule to various plasma membrane transporters and intra- or extracellular biotransformation enzymes. The overall fraction of the dose reaching the liver (f_{liver}) will be a product of the net fractions of the dose absorbed across the apical membrane of the epithelial cell (f_{ab}) and escaping first-pass intestinal metabolism (f_{nm}), i.e. $f_{liver} = f_{ab} \times f_{nm}$.

Drug-metabolising enzymes found in the intestine usually have concentrations much lower than have been found in the liver.[29] The variable and low oral bioavailability of cytochrome P450 3A (CYP3A) substrates such as cyclosporin and verapamil is caused to a considerable extent by the wide intra- and inter-individual variation in the activity of the intestinal enzymes.[33] Both phase I

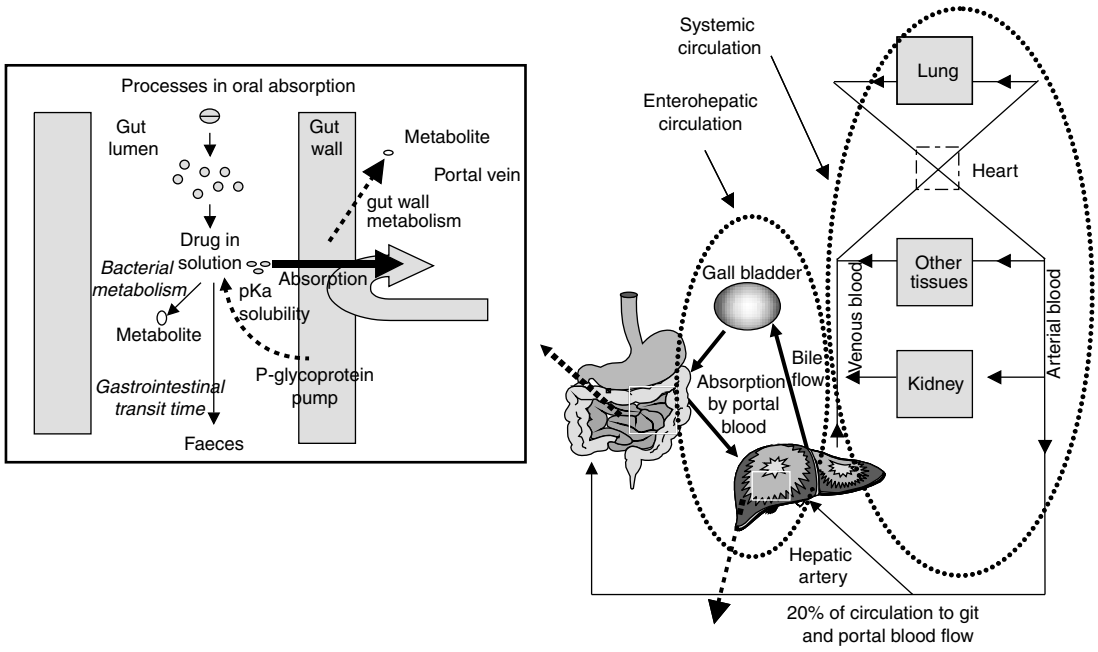

Fig. 3. Oral absorption into the enterohepatic and systemic circulation (see figure 4 for transport processes in the liver).

(oxidation and hydrolysis) and phase II (conjugation) metabolism exists in the gut wall, and the resulting metabolites may become involved in enterohepatic recirculation. The types of solutes metabolised in the gut wall have recently been reviewed by Ilett et al.[34] The phase I metabolic reactions occurring in the gut wall are oxidation (e.g. ethanol), *C*-hydroxylation (e.g. ethinylestradiol), *N*-dealkylation (e.g. flurazepam), *O*-dealkylation, deamination, *S*-oxidation, desulfuration, decarboxylation and hydroxamate reduction. The corresponding phase II reactions are esterification (e.g. hyodeoxycholic acid), ether formation (e.g. ethinylestradiol), sulfation (e.g. ethinylestradiol), glutathione conjugation (isosorbide dinitrate), glycine conjugation, *N*-acetylation, *O*-acetylation (e.g. *N*-hydroxy-aminofluorene), and *O*-methylation.[34]

P-glycoprotein within the brush border on the apical (luminal) surface of the intestinal epithelium reduces the absorption of a range of drugs (for example cyclosporin, tacrolimus, paclitaxel, diltiazem, dexamethasone, lidocaine, erythromycin and HIV protease inhibitors) by pumping the xenobiotic from the enterocyte back into the intestinal lumen.[35] One of the most abundant gut wall enzymes, CYP3A4, is located near the glycoprotein and has similar substrate specificity, leading to a more effective intestinal barrier to certain xenobiotics.[35]

2.3 Transport from the Portal Blood Across Hepatocyte Plasma (Sinusoidal) Membranes

Delivery of drugs from the circulation to the hepatocyte membrane surface has been the subject of intense investigation for many decades. The role of extracellular binding proteins on the uptake of highly protein-bound drugs also has been a controversial issue. Recent studies into this area have provided evidence that uptake may occur from both the unbound and protein-bound fractions.[35]

Once in the portal circulation, drugs are absorbed from the sinusoids by a transport process,

or they may diffuse across the hepatocyte plasma membranes (figure 4). For highly lipophilic drugs, traversing the membrane involves several steps. The first is reversible binding of the drug to the plasma side of the cell membrane leaflet. Traversing the lipid bilayer itself could occur by a flip-flop process of the hydrophilic region of the molecule. To aid in this process, the carboxyl group of fatty acids becomes protonated by hydrogen bonding, either with constituents in the membrane/water interface or constituents within the membrane itself.[35] The uptake process is completed by dissociation of the drug from the cytosolic side of the cell membrane leaflet. Any one of these barriers has the potential for being rate-limiting in the overall uptake process.

Transmembrane flux rate and the subsequent rate of metabolism are key determinants in the hepatic clearance of compounds. Accordingly, hepatic extraction is determined by hepatic blood flow,

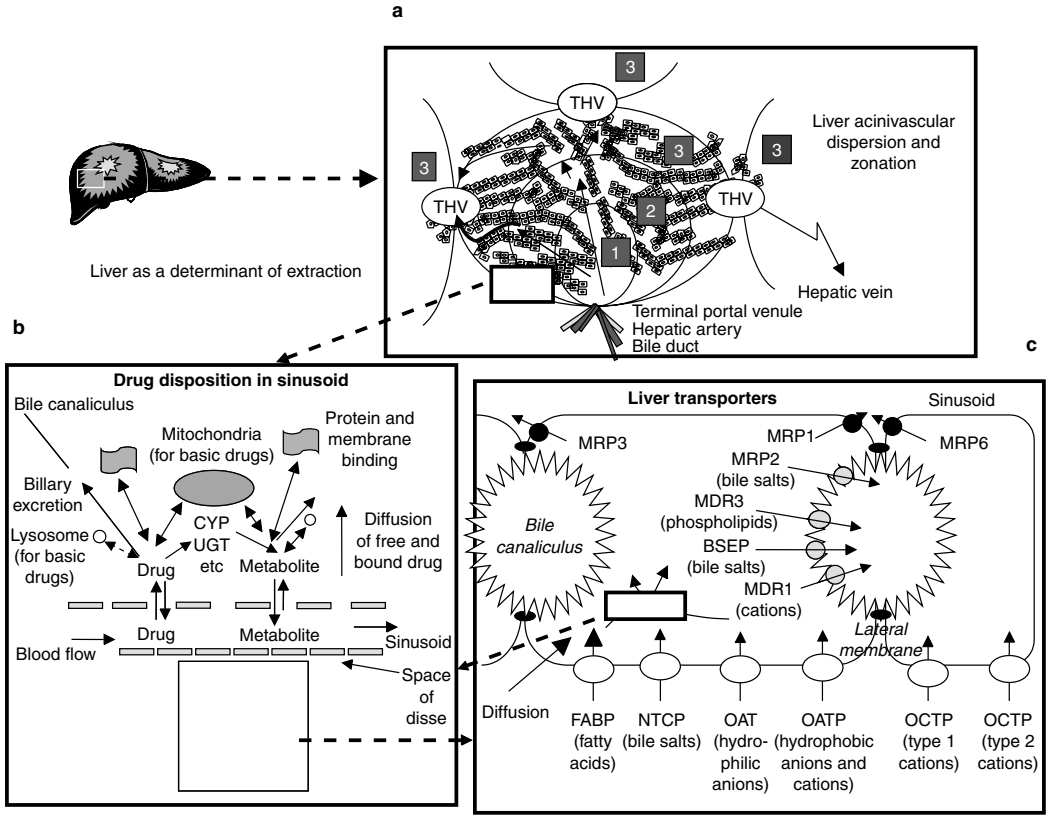

Fig. 4. Transport processes in the liver determining enterohepatic recirculation kinetics of drugs and their metabolites. (**a**) Uptake and clearance of drugs depends on the vascular dispersion in the acini (variation in times taken by blood cells form portal to hepatic venules; two pathlengths shown) and zonation of liver function (three zones shown). (**b**) Ion trapping, binding and transport in the hepatocyte. (**c**) Transporters into and from the hepatocyte into the bile (via biliary canniculus) and back into sinusoid. **BSEP** = bile salt export pump; **CYP** = cytochrome P450; **FABP** = fatty acid binding protein; **MDR** = multidrug resistance; **MRP** = multidrug resistance–associated protein; **NTCP** = Na^+-taurocholate cotransporting polypeptide; **OAT** = organic anion transporter; **OATP** = organic anion transporting polypeptide; **OCTP** = organic cation transporting polypeptide; **THV** = terminal hepatic venule; **UGT** = uridine 5′–diphosphate glucuronosyltransferase.

binding to blood constituents, hepatocyte permeability and biliary and/or metabolic elimination. Hepatic clearance is also a function of vascular dispersion caused by the heterogeneity in sinusoidal blood flows and interconnection between sinusoids (figure 4).[36,37] Vascular dispersion is used in the scaling of *in vitro* liver microsomal or hepatocyte data to predict *in vivo* availabilities.[38,39] Vascular dispersion is also being increasingly used in the modelling of hepatobiliary excretion.[40]

Compounds undergoing enterohepatic recirculation are usually transported as parent solutes through different carrier-mediated systems or by diffusion as uncharged solutes, which are later biotransformed in the liver to suitable polar solutes. Specific plasma membrane transporters exist, including Na^+-dependent and Na^+-independent anionic transporters as well as cationic transporters (figure 4). Many of the liver plasma membrane organic anion transporters into hepatocytes have only recently been cloned and their transport properties identified.[41,42] At this time, much more information is available on rat than human transporters, especially on drug uptake characteristics,[43] and so both are mentioned here. Human transporters include Na^+-taurocholate cotransporting polypeptide (NTCP), human organic anion transporting polypeptide (OATP1), human liver specific transporter (LST1)/OATP2, human organic anion transporter (hOAT1), human prostaglandin transporter (hPGT) and various organic cation transporters, including the organic cationic transporter associated with the uptake of hydrophilic aliphatic and certain aromatic cations (hOCT1). In addition, certain multidrug resistance–associated protein (MRP) transporters (MRP1, MRP3 and MRP6) exist at the plasma membrane but usually only show significant expression in certain diseased livers.

Proost et al.[44] have shown that the carrier-mediated uptake of the cationic aminosteroidal neuromuscular blockers is related to plasma protein binding and to the partition coefficient between octanol and aqueous buffer (log P). The cellular transmembrane electrical potential difference (membrane potential) also has been suggested to greatly affect cellular substrate availability. Membrane potential has been reported to influence the hepatic uptake of organic anions such as the fatty acids[45-47] and the bile acid taurocholate,[47,48] as well as other drugs into tissues other than liver.[49] Canalicular secretion of taurocholate also was shown to be dependent upon membrane potential.[47,48] Canalicular membrane hyperpolarisation resulted in the stimulation of taurocholate-dependent fluid secretion.

2.4 Transport from the Hepatocyte Membrane to Metabolising Sites and to the Bile Canniculae

There is some conjecture on the processes by which solutes traverse the hepatocyte to reach drug-metabolising sites such as the endoplasmic reticulum and the bile canniculae. In most pharmacokinetic studies, it has been traditionally assumed that most solutes move 'instantaneously' in the liver in the unbound form in accordance with the principles of a well-stirred tissue compartment in the radial direction to flow. More recent evidence suggests that transport in the liver may be limited by radial diffusion,[50,51] binding to proteins or membranes in transport through the liver,[52] or transport into pools.[53] Zucker et al.[54] have suggested that intrahepatocellular drug transport may occur through several processes. These include cytoplasmic diffusion, protein-mediated diffusion, cytoplasmic flow, vesicular transport and drug transfer from intracellular membranes to intracellular proteins.

Cytoplasmic flow has been postulated to result from the actions of intracellular motor proteins and filaments (myosin and actin) in directing flow (also known as cytocirculation). Although detailed studies have not been undertaken in hepatocytes, canalicular contractility may be expected to set up a cytocirculatory movement of drugs to the canalicular membrane. Although vesicular transport may play a minor role in the overall transport of drug molecules to the canalicular membrane, it is important in protein trafficking.

Cytoplasmic diffusion and protein-mediated diffusion of drugs have been suggested to play an important role in overall intracellular drug disposition. The transfer of bile acids from the plasma membrane to the canalicular membrane involves several different steps. These include lateral diffusion of the unbound ligand through the cytoplasm, lateral diffusion of the unbound ligand through membranes, vesicular transport and transport by cytosolic binding proteins. Cytosolic bile acid binding proteins include glutathione transferases, the Y′ bile acid binding protein and, to a lesser extent, fatty acid binding proteins (FABP).[55,56]

Vesicular transport of bile acids to the canalicular membrane was suggested over 30 years ago.[57] Using confocal scanning fluorescence microscopy, El-Seaidy et al.[58] reported a lack of evidence for microtubule-dependent trafficking of bile acids or the intracellular sequestration of bile acids prior to their canalicular secretion. The conclusions were based upon examining the effect of the microtubule inhibitor colchicine on the biliary secretion of the fluorescent bile salts cholyl-lysyl-fluorescein and chenodeoxycholyl-lysyl-fluorescein in hepatocyte couplets. The previously reported microtubule-dependent trafficking events were suggested to be important in bile salt–induced delivery of bile salt transporters to the membrane rather than have any direct role in the biliary secretion process of bile salts.

It is also possible that, although largely undefined at this time, transporters also facilitate the uptake of some solutes by intrahepatic organelles such as lysosomes and mitochondria. Lipophilic basic drugs appear to accumulate in the relatively acidic hepatic lysosomes as a result of the pH difference with the cytoplasm (pH-partition theory) but also possibly due to binding (partition or adsorption) to lipophilic substance(s) and/or aggregation within lysosomes.[59] Imipramine and other weak lipophilic bases such as chlorpromazine and propranolol increase intralysosomal pH, and it has been shown that the accumulation of imipramine in lysosomes can be inhibited by coadministration of lipophilic basic drugs.[59] However, it has been suggested that the uptake of psychotropic drugs by liver tissue may depend more on phospholipid binding than on lysosomal trapping.[60] We have recently shown that the sequestration of cationic drugs in the liver occurs by both slow and fast binding to protein and membrane sites as well as ion trapping into mitochondria (20% of hepatocyte, pH 6.67 in fasted state) and lysosomes (1% of hepatocyte and pH 4.70).[61] The extent of ion trapping is determined by the pH of the organelles, pKa of the drug and fraction of the hepatocyte volume occupied by the organelles.

The overall hepatic extraction ratio for cationic drugs is determined by perfusate flow rate, permeability surface area product and intrinsic clearance and, in turn, by the lipophilicity of the solute.[61] In liver disease, the extent of fibrosis and amount of metabolising protein present are other determinants affecting hepatic extraction ratio and other parameters.[62] The accumulation of cationic lipophilic drugs in the liver, probably due to trapping in mitochondria and lysosomes as well as binding, results in a slow elution and a low availability of cationic lipophilic drugs for excretion in the bile and enterohepatic transport.

2.5 Biotransformation in the Liver

Biotransformation is the cellular process that produces pharmacologically or toxicologically active metabolites, as well as inactive ones, by altering the structure of a xenobiotic or by conjugating it with an endogenous compound, thereby changing the aqueous solubility and eventual distribution of the chemical.[63] The ability to biotransform and eliminate potentially toxic chemicals from the body is a well-known protection mechanism[64] and is an important determinant of the rate of elimination of lipophilic chemicals, which can accumulate in the body.[65] Factors that influence biotransformation at each of several potential sites include species, age and development stage, gender, nutritional status and prior or concurrent exposure to various chemicals.

In general, blood-borne drugs and toxicants are most capable of crossing cell membranes and bind-

ing to protein targets if they are lipophilic, small and neutrally charged.[66] Subsequent detoxification is most efficient when the parent compound can be altered to a metabolite that is hydrophilic, large and charged,[67,68] so that it has the appropriate properties for biliary secretion (section 3). These changes typically occur in two stages. First, in what is often referred to as phase I reactions, the parent compound is typically hydrolysed or oxidised (or in some cases, reduced). This leads to formation of a metabolite that may be conjugated to a larger, much more hydrophilic, molecule, often containing a charged moiety. This second conjugation step is often referred to as a phase II reaction. Usually, this stepwise biotransformation is necessary due to the lack in the parent compound of a functional group for easy conjugation.

The phase I drug-metabolising enzymes are predominantly the CYP superfamily. Over 35 human CYPs identified have been classified into families, subfamilies and specific isoforms by amino acid sequence homology. Masimirembwa et al.[69] have suggested that their relative abundances in humans are CYP1A2 (13%), 2A6 (4%), 2B6 (<1%), 2C (20%), 2D6 (2%), 2E1 (7%), and 3A4 (30%), but can vary because of induction (CYP1A1/1A2, 2A6, 2E1, 2C and 3A4), inhibition (all CYPs) and genetic polymorphism (CYP2A6, 2C9, 2C19 and 2D6). The clinical pharmacokinetics associated with CYP3A have recently been reviewed.[70]

Probably the most common phase II (conjugation) reaction is glucuronidation, catalysed by the uridine 5′-diphosphate (UDP)-glucuronosyltransferases (UGTs), consisting of at least 10 different UGT isoforms.[71] Most drug [morphine, paracetamol (acetaminophen)] and endogenous (bilirubin, estradiol) glucuronides are excreted in the bile and undergo enterohepatic recirculation. Many drug conjugates formed by phenol sulfotransferase and cytosolic glutathione *S*-transferase (GST) can also undergo enterohepatic recirculation.

From a pharmacokinetic viewpoint, drug metabolites (especially conjugates) are usually more polar than the parent drugs and will have difficulty backdiffusing across the sinusoidal barrier.[53] Some of these metabolites, therefore, have longer hepatic disposition half-lives and there exists the potential for them to accumulate in the hepatocyte to a greater extent than the parent drug if diffusion is the main means of transport.[72] Larger polar metabolites are cleared in the bile (section 3) and others may be actively transported back into the sinusoid (figure 4). At this time, it is not possible to predict which metabolites will experience a diffusion barrier and accumulate or be actively transported out of the liver into the bile or into sinusoids.[73]

The major metabolic pathways in the liver show acinar gradients.[74] Hence, oxidative energy metabolism (for example fatty acid oxidation), amino acid catabolism, ureagenesis, gluconeogenesis, cholesterol metabolism and certain types of protective metabolism occur in the periportal region. Nonparenchymal cells such as Kupffer, stellate, endothelial and pit cells are generally also predominant in this region. In contrast, glycolysis, liponeogenesis, ketogenesis, glutamine formation and xenobiotic metabolism are preferentially located in the perivenous (also called centrilobular or zone 3) region. Accordingly, endoplasmic reticulum (microsomes) is more prevalent in the perivenous region. Zonation is determined by a range of gradients including oxygen tension, bile acids, hormones and cell expression.[74]

Most studies on the zonated expression of CYP genes have been in rat liver.[74] In general, the genes are mainly distributed in the perivenous region but there are some exceptions, such as CYP2C7 and FABP, which are mainly periportal (figure 5). Translation of such data to human livers is complicated by different CYP genes being present in rat and human livers (CYP2C7, CYP2C11 and CYP2C12 are common rat CYP2C genes whereas the more common human CYP2C genes are CYP2C9 and CYP2C19), and the limited amount of data on zonation of human liver metabolising enzymes. Palmer et al.[75] suggest that members of the CYP3A subfamily are preferentially expressed in hepatocytes in the perivenous region, whereas genes coding for CYP1A2,

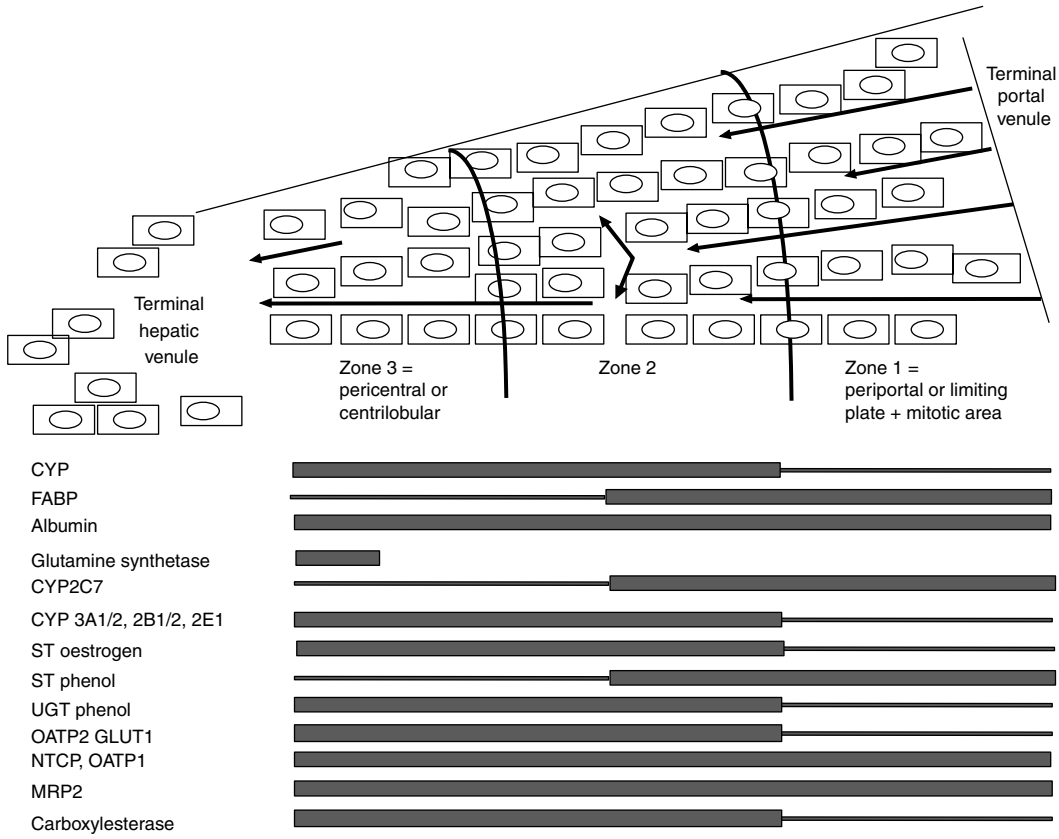

Fig. 5. Examples of heterogeneity of gene expression in the zones of the rat hepatic acinus.[74,81-100] A thick line indicates gene expression. **CYP** = cytochrome P450; **FABP** = fatty acid binding protein; **GLUT1** = glucose transporter protein 1; **MRP** = multidrug resistance–associated protein; **NTCP** = Na^+-taurocholate cotransporting polypeptide; **OATP** = organic anion transporting polypeptide; **ST** = sulfotransferase; **UGT** = uridine 5′-diphosphate glucuronosyltransferase.

CYP2A, 2B and 2C are expressed uniformly throughout the liver acinus. In contrast, McKinnon et al.[76] reported higher levels of CYP1A1 and CYP1A2 in the perivenous region. CYP2E1 is normally localised within the perivenous region.[77] In human liver disease, the acinar distribution of CYP2E1 and CYP2A follows that of fatty deposits and is most abundant in zone 3.[78] Zonation of drug-metabolising enzymes in the direction of flow from the periportal to perivenous regions of the hepatic acinus (figure 5) may affect the pharmacokinetics of generated metabolites, but has been studied only to a limited extent to date.[72,79] Zonation of CYP drug-metabolising enzymes is absent from the fetal liver.[80]

The higher expression of CYPs in the perivenous region, especially after xenobiotic induction, together with reduced 'mopping-up' mechanisms for reactive metabolites in this region, may account for increased perivenous xenobiotic toxicity.[74] Gumucio and Miller[101] have reported preferential pericentral toxicity for paracetamol, halothane and carbon tetrachloride, among others. The toxicokinetics associated with the production

and clearance of reactive metabolites in terms of zonation appears to have been studied only to a limited extent.

2.6 Transport from the Hepatocytes Across the Canalicular Membrane Into the Bile

Active transport of solutes across the canalicular membrane into the bile by several membrane proteins has been demonstrated. Evidence from the microperfusion of intrahepatic bile duct units suggests that the bile ducts both secrete and absorb water in response to osmotic gradients, actively absorb bile acid through a sodium-dependent mechanism, and transport HCO_{3-}.[23]

Mechanistically, biliary elimination of anionic compounds, including glutathione *S*-conjugates, is mediated by MRP2, whereas bile salts are excreted by a bile salt export pump (BSEP)[102] (figure 4). MRP2, previously called canalicular anion transporter, uses ATP to transport a number of drug conjugates that undergo enterohepatic recycling, including glucuronide, sulfate and glutathione conjugates. In humans, MRP2 function is defective in Dubin-Johnson syndrome. It has been suggested that MRP2 expressed on the canalicular membrane is homogenously distributed within the acinus,[103] as shown in figure 5.

P-glycoprotein, the multidrug resistance (MDR) gene product, is exclusively located on the canalicular membrane of hepatocytes.[104] Class 1 P-glycoprotein expression from the MDR1 gene mediates the excretion of hydrophobic, mostly cationic, metabolites, whereas class 2 P-glycoprotein expression from the MDR3 gene is involved in phospholipid transport.[102] Acridine orange, doxorubicin, verapamil, propranolol, phosphatidylcholine, estradiol glucuronide and daunomycin have been shown to inhibit the uptake of daunomycin by P-glycoprotein in isolated rat canalicular liver plasma membranes.[105]

As discussed later in this review (section 2.7), the expression of hepatic transporters and their activity are regulated in various situations, such as ontogenesis, carcinogenesis, cholestasis, cellular stress and after treatment by hormones and xenobiotics. Interestingly, the defective transport of many solutes directly correlates with the absence of BSEP, MDR3, P-glycoprotein and MRP2 transporters in specific liver diseases. Alkaline phosphatase plays an active role in downregulating the secretory activities of the intrahepatic biliary epithelium.[106] From a clinical pharmacokinetic perspective, as discussed later, various compounds and diseases affect transporter activity and the resulting enterohepatic recycling of drugs. Hence, the enterohepatic recycling of indomethacin glucuronide, which is mediated by canalicular multispecific organic anion transporter/multidrug resistance associated protein 2 (cMOAT/MRP2) is reduced in livers where cMOAT/MRP2 function is hereditarily defective.[42] Recently, inducible MRP3 on the central membrane has been shown to play a significant role in the cholehepatic circulation of bile salts and in the transport of organic anions such as 17β-estradiol 17β-D-glucuronide in rats.[107] It is clear that there is much more to learn about transporters, which will greatly increase our understanding of enterohepatic recirculation and modifications of drug kinetics as a result of drug structure, drug interactions and patient disease.

2.7 Active Transport from the Hepatocytes Across the Lateral Membrane Into the Sinusoid

As shown in figure 4, the active transporters MRP1, MRP3 and MRP6 also exist on the lateral membrane and can transport certain drugs and metabolites from hepatocytes back into the sinusoids. In some cases, as has been shown for intracellularly formed morphine glucuronides,[108] there is preferential excretion of the solutes back into the sinusoids rather than into the bile, thus minimising potential enterohepatic recycling. Other drug anion conjugates are also preferentially excreted into the sinusoids by saturable transport mechanism.[43] In general, transporters such as MRP1 are present in the lateral membrane only at very low levels in quiescent cells.[109] However, as discussed later (section 4.8.2), particular conditions such as cholestasis may induce the expression of the MRP3

transporter on the basolateral membrane and facilitate the cellular efflux of drug or drug metabolite anions back into the sinusoids.[43]

2.8 Bile Transport Into Duodenum

A significant fraction of bile may flow into the duodenum during fasting. Generally, bile is stored in the gall bladder and released into the small intestine via the Sphincter of Oddi on the sight, smell or ingestion of food. As a consequence, gallbladder emptying is sporadic, leading to complex pharmacokinetics when enterohepatic recycling is substantial. Patrick et al.,[110] in recognising that the multiple peaks in the plasma concentration-versus-time profiles of ezetimibe, conjugated ezetimibe and total ezetimibe were suggestive of enterohepatic recycling, commented that the multiple secondary peaks corresponded to the approximate time of meals. This timing was consistent with food intake stimulating the emptying of the gallbladder.

2.9 Gut Flora

The enterohepatic circulation of many solutes depends on the gut flora metabolism of the solutes and their metabolites excreted in bile (table I). Various enzymes, such as the gut flora cysteine conjugate β-lyase associated with the metabolism of cysteine conjugates, have been identified in the last decade.[111] Furthermore, drug metabolism by the intestinal microflora contributes to the pharmacological profile of various drugs. The metabolism of orally administered drugs can result in pharmacodynamic changes, and the hydrolysis of biliary drug conjugates is responsible for the enterohepatic circulation of drugs.[34] The β-glucuronidase activities in humans for the proximal and distal regions of small intestine are 0.02 and 0.9 μmol of substrate degraded/h/g content, respectively.[34] Activity of bacterial enzymes varies markedly among different host species.

3. Properties of Compounds Undergoing Enterohepatic Recycling

3.1 Size of Drug or Metabolite

The biliary route of excretion plays a major role in the elimination of anions, cations and non-ionised molecules containing both polar and lipophilic groups above a threshold molecular weight. The molecular weight threshold for humans is estimated to be 500 to 600, but it should be emphasised that this is a generalisation. Dyes such as bromosulfophthalein (BSP), indocyanine green (ICG) and various iodinated cholecystographic media and their respective metabolites, which are known to be excreted in human bile, have molecular weights greater than 600. Compounds with molecular weights less than this threshold are excreted primarily in the urine. It is important to recognise in scaling up that the molecular weight threshold differs between species. A minimum molecular weight of between 200[112] and 325 is required for significant biliary excretion in the rat, and 400 and 475 are the approximate values in the guinea pig and rabbit, respectively.[113]

Many exogenous substances are secreted into bile and undergo some degree of enterohepatic cycling. These include commonly used drugs (morphine, warfarin, indomethacin, cardiac glycosides), several antimicrobial agents (clindamycin, rifampicin, erythromycin, metronidazole, ampicillin, ceftriaxone and doxycycline), radiocontrast media and potentially toxic heavy metals. Table II lists examples of compounds reported to be excreted in the bile and therefore possibly available for enterohepatic recycling.

3.2 Biliary Excretion of Bile Acids

Bile contains a wide spectrum of anionic organic compounds, among which bile acids are the most abundant. Bile acids undergoing enterohepatic circulation are efficiently extracted from portal blood by liver and are concentrated in bile. Active solute transport into canaliculi is primary responsible for canalicular bile formation. Of all the compounds in bile, bile acids are the most con-

Table I. Examples of processes, drugs and enzymes involved in enterohepatic recirculation (adapted from Ilett et al.,[34] with permission)

Processes	Drug and compound examples	Enzyme	Sample tested and microflora containing enzymes
Hydrolysis			
Glucuronides	Chloramphenicol glucuronide	β-Glucuronidase	Human faeces, *Escherichia coli*
	Estradiol-3-glucuronide		
Glycosides	Cycasin	β-Glucosidase	Human intestinal content, *Streptococcus faecalis*, *Eubacterium rectale*
	Sennosoides		
Amides	Sulfonamides	Amide hydrolase	*Clostridium sphenoides*, *E. coli*, *Proteus vulgaris*, *Bacillus subtilis*, *B. mycoides*
	Penicillin		
	Chloramphenicol		
Sulfamates	Estrone sulfate	Arylsulfotransferase	Human faeces, *Streptococci*
Reductions			
Ketones	Estrone	Hydrogenase	Human faeces
Elimination reactions			
Demethylation	Methylmercury	Demethylase	Human faeces, *Streptococci, Lactobacilli, Clostridium*
N-Demethylation	Methylamphetamine	*N*-Demethylase	Human intestinal content, *Clostridium*, *Bacteroides*
	Imipramine		
	Choline		
Deamination	Putrescine	Deaminase	*Bifidobacteria, E. coli*, *Bacteroides*, *Clostridium*
	Cadaverine		
	Amino acids		
Decarboxylation	Caffeic acid	Decarboxylase	Human faeces, *S. faecalis*
	Phenolic acids		
	Amino acids		
Dehydrogenation	Bile acids	Hydroxysteroid dehydrogenase	Human faeces, *Clostridium welchii*
	Cholanic acids		
Other reactions			
Ring	*O*-Heterocyclic flavones	Tryptophase	Rat faeces
R-C-S-R′	Propachlor-cysteine	C-S lyase	Pig cecal content, *Eubacterium aerofaciens*, *Fusobacterium necrophorum*

centrated. Hepatic bile acid secretion involves uptake at the sinusoidal pole of the hepatocyte, intracellular transport to the canalicular pole and secretion into the canalicular lumen. At least three different transport mechanisms are involved in the uptake of bile acids:[141-143] (i) Na^+-dependent bile salt uptake system; (ii) Na^+-independent carrier-mediated transport; and (iii) nonionic diffusion followed by intercellular binding to cytosolic proteins and amidation with taurine or glycine or conjugation with sulfate or glucuronide. The first two mechanisms are responsible for concentrative uptake.[144]

Most studies on biliary excretion of anions have been reported with bile acids. In general, conjugated bile acids are transported by the Na^+-bile

acid cotransport mechanism, whereas unconjugated bile acids are transported via the Na^+-independent mechanism and/or by passive diffusion. Biliary secretion across the canalicular membrane is also a concentrative process mediated by an electrogenic, carrier-mediated transport mechanism.[142,145] This is consistent with the finding that only negatively charged bile acids are efficiently secreted into bile.[146] Uptake of conjugated bile acids is Na^+-dependent and thought to be mediated by a Na^+-bile acid co-transport (or symport) system, energised by the transmembrane Na^+ gradient maintained by the Na^+, K^+-ATPase. Bile acid binding proteins have been identified but their role in transport has not been established. There is a lobular gradient in bile acid transport, periportal hepatocytes removing most of the bile acid load at physiological concentrations. For some unconjugated bile acids, conjugation may be the limiting factor in overall transport from blood to bile. Canalicular secretion occurs via carrier-mediated diffusion sensitive to the membrane potential. Hepatic extraction of bile acids and bile salts from portal blood plasma is an extremely efficient process in animals as well as in humans, and the extraction efficiency appears to be inversely related to the extent of bile acid binding to serum albumin.[147,148] Hence, while the latter increases with increasing lipophilicity, the hepatic extraction efficiency is higher for the relatively hydrophilic cholates (taurocholate > glycocholate > cholate) than for the more hydrophobic dihydroxy (e.g. chenodeoxy- and deoxycholate) and monohydroxy (e.g. lithocholate) derivatives.[147,149,150] Furthermore, conjugation with taurine or glycine significantly increases the maximal uptake velocity (V_{max}) via a saturable and Na^+-dependent transport process.[146,151] In contrast, the relatively hydrophobic unconjugated dihydroxy bile acids are largely taken up into hepatocytes by nonsaturable (probably nonionic) diffusion.[149,152,153] Finally, a Na^+-independent saturable uptake process has been repeatedly suggested to be involved in overall

Table II. Examples of drugs, other foreign chemicals and their metabolites excreted in human bile

Amiodarone[114]	Estrone[115]	Phenol red[113]
Ampicillin[113,116]	Ezetimibe[110]	Phenolphthalein[117]
Benzylpenicillin[118]	2-Fluoro-β-alanine[119]	Phenytoin[117]
Bilirubin[120]	Gentamycin[118]	Pivampicillin[118]
Bromocresol green[121]	Glibenclamide (glyburide)[5]	Rifamide[113]
Bromosulfophthalein[122]	Gliclazide[123]	Rifamycin[113]
Cefixime[124]	Imipramine[125]	Roquinimex [126]
Ceftriaxone[127]	Indocyanine green[122]	Rose bengal[121]
Cefatzidime[128]	Indomethacin[125]	Spironolactone[125]
Cephaloridine[113]	Irinotecan[129]	Sulfamethoxazole[113]
Cephamandole[118]	Lanatocide C[118]	Sulindac[125]
Cephazolin[118]	Lorazepam[130]	Sulbactam[116]
Chenodeoxycholic acid[122,125]	Lormetazepam[6]	Temafloxacin[131]
Chloramphenicol[118]	Methotrexate[113]	Testosterone[125]
Chlortetracycline[118]	Metronidazole[117]	Tetracycline[113,118]
Clindamycin[117]	Mezlocillin[132]	Thiamphenicol[113]
Demethylchlortetracycline[118]	Morphine[133]	Tolfenamic acid[134]
Diazepam[113]	Mycophenolic acid [135]	Toremifene [136]
Digitoxin[113]	Mycophenolate mofetil[137,138]	Troglitazone[139]
Digoxin[113]	Nortriptyline[113]	Trovafloxacin[7]
Doxycycline[118]	Novobiocin[113]	Ursodeoxycholic acid[122,125]
Erythromycin[113]	Oltipraz[140]	Valproic acid[125]
Estradiol[125]	Pethidine (meperidine)[113]	Warfarin[133]

bile acid uptake, especially for the unconjugated trihydroxy bile salt cholate.[141,153]

The processes responsible for vectorial transport of organic anions from the sinusoidal side to the canalicular pole of the hepatocyte are still poorly understood. Part of the transfer may be directed by intracellular protein binding (see section 5.2). Within the cell, organic anions are largely bound to the cytosolic protein ligandin, also known as Y protein or glutathione *S*-transferase B,[154,155] FABP or Z protein,[156] and Y′binders. The last group of identified proteins, which includes among others 3α-hydroxysteroid dehydrogenase and a steroid sulfotransferase, has been claimed to play a role in the translocation of bile acids.[55]

3.3 Bilirubin

Bilirubin is the main degradation product of haem catabolism in mammals and has ideal properties for biliary excretion. The major source is haemoglobin, which is degraded in the reticuloendothelial system of the spleen, bone marrow and liver. The molecule is an open-chain tetrapyrrole (molecular weight 585) with two vinyl, four methyl and two propionic acid side chains. Because the pKa value is around 4.4,[157] the carboxylic acid groups would be expected to ionise almost completely at physiological pH in aqueous medium, so an unconjugated bilirubin would have polar properties. Surprisingly, the pigment is almost insoluble at pH 7.4.[158] This unexpected finding can be explained by intramolecular hydrogen bonding, which involves all polar groups of the molecule.[159] The formed bilirubin is reversibly bound by plasma albumin. The liver takes up bilirubin circulating in the plasma rapidly and almost exclusively.[120] The uptake is consistent with a carrier-mediated membrane transport.[160] Albumin is important in restricting bilirubin to the plasma compartment and directing it to the liver for its metabolic clearance. In analbuminetic rats, the plasma disappearance of an injected bolus of radiolabelled bilirubin is higher than in normal rats, but the recovery in bile is slower.[161] This demonstrates that in the absence of albumin the pigment repartitions indiscriminately in all body tissues and less is available for hepatic uptake.

Early work provided strong evidence that a characterised BSP- and bilirubin-binding protein in liver plasma membranes was responsible for the uptake of these anions.[162] A monospecific antibody against this membrane protein reduced the uptake of bilirubin and BSP, in contrast to free fatty acids and bile acids.[163] Bilirubin, BSP and ICG share a common transport system, which appears to be distinct from that for bile acids.[164-166] Specific high-affinity binding sites in the plasma membrane maintain a plasma/cell concentration gradient of the ligands. The cytosolic binding protein ligandin also serves to maintain a concentration gradient within the cytosol from the plasma membrane to canalicular membrane.[167] At the present time, although bilirubin monoglucuronide is known to be transported by OATP1 and human liver-specific transporter (HLST), the transporter of unconjugated bilirubin in the sinusoidal membrane has not as yet been identified.[41] Kamisako [41] note that unconjugated bilirubin may also cross the hepatocyte sinusoidal membrane by a diffusion process.

In the liver cell, bilirubin is largely bound to the two cytosolic binding proteins with high affinity, ligandin (Y-protein) and Z-protein.[155] Ligandin (molecular weight 46 000), identical with glutathione *S*-transferase B,[154] binds a variety of compounds including bilirubin, haem, steroids, BSP, ICG and carcinogens.[168] Approximately 30% of the bilirubin present in cytosol is bound with low affinity to Z-protein,[155] which seems mainly to be involved in the transport of fatty acids.[160] Both binding proteins are supposed to play a role in the transport of bilirubin from the liver cell membrane to the conjugating enzyme system in the endoplasmic reticulum.

In the endoplasmic reticulum, bilirubin is conjugated by bilirubin UGT (UGT1A1) to form mono- and diglucuronides of bilirubin.[41] The bilirubin conjugates formed in the endoplasmic reticulum are secreted in bile. Virtually all bilirubin excreted in bile is in the form of glucosidic conju-

gates. Bilirubin conjugates are secreted across the liver canalicular membrane by MRP2, a member of the ATP-binding cassette transporter family.[41] Finally, there is regurgitation of bilirubin glucuronides into blood under conditions of impaired biliary bilirubin excretion. MRP3, which is located in the lateral membrane, transports bilirubin glucuronides into blood.[41]

3.4 Other Anions and Anionic Drugs

Beside bile acids and bilirubin, bile contains varying amounts of endogenous organic anions, such as steroid hormones. In addition, anionic xenobiotics, including dyes like ICG and BSP, cholecystographic contrast agents and certain antibacterials, are readily excreted into bile.[122] Biliary excretion of organic anions is the result of a complex sequence of events, involving uptake by hepatocytes at the sinusoidal side of the cell, intracellular trafficking with or without biotransformation, and secretion at the canalicular pole of the cells, as discussed in details in section 2.

Most nonsteroidal anti-inflammatory drugs (NSAIDs) are metabolised by the liver, with subsequent excretion into urine or bile. Enterohepatic recirculation occurs when a significant amount of an NSAID or its conjugated metabolites are excreted into the bile and then reabsorbed in the distal intestine.[169]

The clinical pharmacokinetics of therapeutic bile acids, chenodeoxycholic and ursodeoxycholic acids, have also been reviewed.[122] The absorption of chenodeoxycholic acid by the intestine is better than that of ursodeoxycholic acid. Both solutes are extracted efficiently by the liver, conjugated with glycerine and taurine, secreted in bile, and then undergo enterohepatic circulation with the endogenous bile acids. These therapeutic bile acids are metabolised by intestinal bacteria to lithocholic acid, which is mainly excreted with faeces.

A significant enterohepatic recirculation has been reported to delay the final elimination of a large fraction of tricyclic antidepressants taken in large quantities during suicide attempts.[170]

The compound calcitriol (1,25-dihydroxyvitamin D_3), critical in calcium absorption, is formed by the sequential hydroxylation of colecalciferol (vitamin D_3) in the liver and kidney, and excreted in the bile as polar metabolites (such as glucuronides and, possibly, sulfates and neutral polar steroids) which undergo enterohepatic recirculation in both humans and experimental animals.[171]

The topoisomerase I inhibitor SN-38, a minor metabolite of irinotecan, is glucuronidated in the liver, then excreted in the bile. Enterohepatic recirculation occurs after the glucuronide is deconjugated to SN-38 by β-glucuronidase produced by the intestinal flora, and subsequently reabsorbed.[129] The major metabolite of 5-fluorouracil, 2-fluoro-β-alanine, and its conjugates are also believed to undergo enterohepatic recycling.[119]

3.5 Organic Cations

Many drugs are compounds with one or more tertiary or quaternary amine functions. Quaternary ammonium compounds are permanently positively charged, whereas tertiary amines can be protonated and therefore become positively charged at physiological pH depending on the compound's pKa value. Among the monoquaternary drugs are anticholinergic, antineoplastic and antihelminthic drugs, whereas the biquaternary drugs represent predominantly neuromuscular blocking agents. Cationic drugs can pass membranes by carrier-mediated transport, and such processes have been characterised in kidney tubular cells,[172] in the intestine,[173] in the choroid plexus,[174] and in the liver.[10,143,175] Several routes for hepatic transport of organic cations have been described based on the structure-kinetic relationship of the compound. Factors involved in the extent of biliary excretion of organic cations are molecular size,[176] number of charged groups and lipophilicity of the agents. Protein binding, metabolism and the chemical structure may also influence the relative contribution of this excretion route.[177]

Organic cations of relatively low molecular weight (<200) tend to be poorly excreted in bile. Therefore a so-called 'molecular weight threshold' has been proposed, above which the biliary excretion of drugs becomes appreciable (>0% of the dose). For monovalent organic cations, this threshold is suggested to be 200 ± 50 in the rat.[112] Lipophilicity of the organic cations seems to be a good physicochemical parameter to predict the extent of biliary output. The biliary excretion rate of the type I cations (for example triethylmethylammonium, tripropylmethylammonium, procainamide ethobromide, thiazinamium, and *N*-methyldeptropine) generally increases with increasing lipophilicity.[176,178] A molecular weight of 500 to 600 has been proposed as a threshold for hepatobiliary excretion of most bivalent type II organic cations (for example D-tubocurarine, trimethyltubocurarine, stercuronium, vecuronium, *N*-propyldeoxyajmalinium, pancuronium and hexafluronium) in the rat, guinea pig and rabbit.[179] Similar to the type I cations, the balance between lipophilicity and hydrophilicity appears to be an important factor determining hepatic transport at the uptake and secretion level.[180]

Carrier-mediated transport of cationic drugs in uptake into and secretion from cells is, in principle, saturable, so that a nonlinear kinetic pattern is to be expected. Potential transport interactions between drugs can be anticipated based on chemical structure. However, many drugs can be present in various forms (dissociated, undissociated, complexed) and therefore transported by various carrier systems at the same time. Carrier-mediated transport is not restricted to the plasma membranes of the secretory cells; various organelles within the cells can accumulate drugs via similar translocation mechanisms, leading to persistent storage of such drugs, changes in organelle function or local toxicity.[180,181] Apart from carrier-mediated uptake processes, an alternative uptake route is proposed for some hydrophilic cations. For instance, stercuronium,[182] decamethonium and hexamethonium[179] might be solely accumulated by an endocytotic uptake mechanism. As discussed in section 2.4, intrahepatic binding is a major determinant in the hepatic disposition of the lipophilic cations.

3.6 Other Drug Examples – Hormones and Antimicrobial Agents

The extent of biliary excretion of endogenous and exogenous hormones (natural or synthetic) differs significantly depending upon the compound investigated. In general, it may be assumed that steroids are excreted into bile to different extents depending on their structure: metabolites of less polar steroids, e.g. progesterone, show a greater tendency to be excreted into bile than metabolites of polar steroids (e.g. hydrocortisone). In humans, biliary excretion of exogenously administrated estradiol, estrone or estriol was found to be 65, 48 or 23% of the dose per 12 hours, respectively.[183-185] With progesterone, approximately 30% of the applied dose was excreted in bile following intravenous injection, whereas 13% of an intravenous dose of testosterone and only 4% of intravenously administrated hydrocortisone were reported to be excreted into bile by patients with biliary fistula.[183,186,187] Biliary metabolites of hormones are at least partly reabsorbed from the intestine and may undergo extensive enterohepatic circulation. This results in a prolongation of their overall elimination half-lives. Intestinal metabolism, especially deconjugation reactions of glucuronide and sulfate adducts, plays a central role in this process.[188] The pharmacokinetics and enterohepatic cycling of estradiol have been recently reported after three oral single-dose administrations of equimolar doses of estradiol alone, estradiol plus desogestrel and estradiol valerate in a three-way cross-over mode in 18 healthy postmenopausal women.[115]

The mechanisms responsible for the biliary excretion of antimicrobials are poorly understood quantitatively. It cannot be predicted from the molecular weight or other physicochemical properties whether an antibacterial agent will be excreted to a significant extent by the hepatobiliary route. The molecular weights of the third generation cephalosporins are very similar: ceftazidime (molecular

weight 546) is almost exclusively excreted by the renal route in contrast to ceftriaxone (molecular weight 554), which is excreted about 40% by the hepatobiliary route.[127,132] Similar figures can be given for azlocillin (5%) and mezlocillin (25%). In humans, the biliary route is not the predominant route of elimination despite the fact that hepatic elimination plays an important role for many drugs. For new antibacterial agents, a detailed balance of urinary and faecal excretion, preferably after intravenous administration, should be determined. Data on concentrations in common duct bile and gallbladder bile should also be provided.

Amiodarone, a type III antiarrhythmic, is poorly bioavailable (20 to 80%) and undergoes extensive enterohepatic circulation before entry into a central compartment.[114]

3.7 Formation of Glutathione and Glutathione Adducts

The concentration of glutathione (GSH) in bile depends on the species studied, the assay conditions and the intrahepatic glutathione status. Most of the glutathione in bile is in form of GSH, and only a small fraction is oxidised glutathione (GSSG). The excretion of GSSG and glutathione *S*-conjugates is an important mechanism to eliminate potentially toxic compounds from the organism and the liver. The role of GSH in bile is less clear. It clearly plays an important role in the elimination of potentially toxic metals from the liver. The biliary excretion of many metals is closely coupled to the secretion of GSH.[189] Zinc, methylmercury, cadmium, chromium and lead are excreted as GSH complexes, and depletion of hepatic GSH decreases the biliary excretion of the compounds together with the excretion of GSH.[190-192] In species where substantial concentrations of GSH are preserved throughout the biliary tree, the tripeptide might play a role in the detoxification of potentially mutagenic or carcinogenic compounds generated in the upper gastrointestinal tract.[193]

3.8 Biliary Excretion of Glucuronide and Glutathione Conjugates

Biliary excretion of paracetamol glucuronide has been shown by using MRP2-deficient rats be facilitated by cMOAT/MRP2.[194] MRP2 is responsible for the biliary excretion of a variety of endogenous and exogenous organic anions, including many glucuronide conjugates, some glutathione conjugates and a few sulfate conjugates.[195] The majority of MRP2 substrates identified to date have at least two negative charges.[195] Glucuronide conjugates, depending on their reactivity, can covalently bind to proteins within hepatocytes. However, glucuronides are also likely to be secreted into the bile across the canalicular plasma membrane, where they could react with targets in the biliary tree. Hepatobiliary transport of anionic xenobiotics, including many types of glucuronide and glutathione conjugates, normally occurs by ATP-dependent transport, which is mediated by the multispecific conjugate export pump cMOAT.[195-197] Indomethacin glucuronide is an example of one metabolite excreted into the bile by MRP2.[42] As discussed in section 2.7, MRP3 is also involved in glucuronide excretion. A direct transport function of MRP2 for glutathione conjugates and leukotriene C_4 has recently been demonstrated by expression of the carrier in COS-7 cells and in *Xenopus laevis* oocytes.[198] Glutathione conjugates appear to be a poor substrate for MRP3.[107]

It should be noted that most of our information on transporters has come from rats. Overall, the primary active transport of organic anions across rat bile canalicular membrane is greater than in humans, although the difference between the species is less marked for glucuronides.[199]

4. Factors Affecting Biotransformation and Enterohepatic Circulation

The absorption, biotransformation and enterohepatic circulation of a xenobiotic can be influenced by factors including the role of bile acids; the biological influences of species variation, gen-

der, age and developmental stage; nutritional status; disease states; and pharmacological effects of other chemicals after intentional administration or inadvertent exposure.

4.1 Bile Acids

The primary metabolic pump for enterohepatic circulation is the active excretion of bile acids across the canalicular membrane into bile. The rate-limiting step is the transfer of bile acids derived either from blood or from synthesis into bile. Bile acid depletion, induced by either prolonged biliary diversion or administration of cholestyramine, markedly decreases the biliary excretion of digitoxin and troglitazone,[139,200] bilirubin,[201] ICG, bromocresol green, rose bengal, BSP and eosin.[202,203] In contrast, increasing the bile acid pool, as occurs with diabetes, increases the biliary excretion of bromocresol green, ICG, phenol red and rose bengal.[121,204]

4.2 Chemical Adsorbents

Chemical adsorbents may stimulate movement of endogenous compounds and xenobiotics through the intestine.[205] In rats and in humans, cholestyramine markedly increased the faecal excretion of the pesticide chlordecone.[206-208] This can only partly be attributed to interruption of enterohepatic circulation, because chlordecone is also excreted into the intestine by transmucosal transport.[209] In rhesus monkeys, dietary supplementation with 4% cholestyramine enhanced faecal excretion of pentachlorophenol from 3 to 54%.[210] Consequently the urinary excretion decreased from 35 to 5%, and thus the net effect of treatment was a 40% enhancement of the total excretion of pentachlorophenol.[211]

Cholestyramine, a nonabsorbable anion exchange resin, effectively binds bile acids and anionic drugs. Oral administration of cholestyramine was shown to decrease the absorption and biliary excretion of bile acids in rats.[202,212] Oral administration of cholestyramine or activated charcoal diminished the urinary recovery of an intravenous dose of paracetamol by 20 to 30%, indicating significant biliary excretion of paracetamol conjugates and almost complete reabsorption.[213] Moreover, cholestyramine treatment was effective in preventing paracetamol-induced hepato- and nephrotoxicity.[214] Activated charcoal adsorbs paracetamol,[213] carbamazepine, phenobarbital, and phenylbutazone[215] in the intestinal tract, preventing their absorption and future enterohepatic circulation.

Oral ingestion of activated charcoal also appears to inhibit enterohepatic recycling of estriol, as shown in figure 6. Recently, Elomaa[216] examined the extent to which oral activated charcoal affected the bioavailability of norethisterone acetate and gestodene during steady-state conditions by inhibiting their enterohepatic recirculation. They suggested that the enterohepatic circulation of the steroids was not of clinical importance and that women on oral contraceptives can take activated charcoal for the treatment of diarrhoea when administered 3 hours after and at least 12 hours before pill intake.

4.3 Species and Age Differences

Variations among species in the capacity for hepatobiliary transport[10] are primarily responsible for species differences in enterohepatic circulation.[218] Interspecies variations in hepatic and extrahepatic biotransformation are well known.[219-221] Moreover, species variations in gastrointestinal microflora may result in significant qualitative and/or quantitative differences in microbial biotransformation.[222] Studies in several species indicate that β-glucuronidase activity is higher in intestine from rats and mice than intestine from humans.[223]

In addition to metabolic differences, the anatomical, physiological and biochemical differences between the gastrointestinal tracts of humans and the common laboratory animals can cause significant variation in drug absorption from the oral route. Among the physiological factors, pH, bile, pancreatic juice, and mucus and fluid volume and content can modify dissolution rates, solubility, transit times, and membrane transport of drug mol-

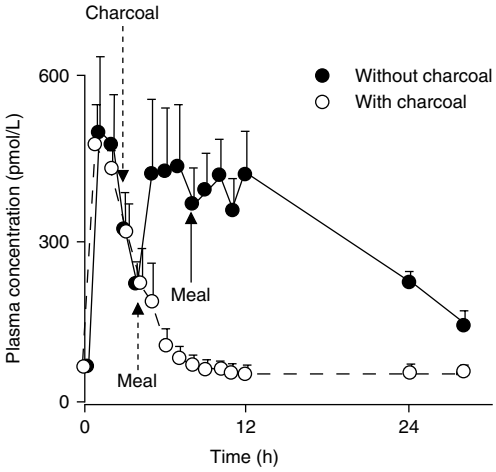

Fig. 6. Plasma levels of estriol after oral administration of estriol 12mg without and with 20g of activated charcoal. Values are means ± standard error for n = 8 (reproduced from Heimer and Englund,[217] with permission).

ecules.[224] Enterohepatic circulation is also subject to age-related effects in the development of, for example, hepatic uptake mechanisms for bile acids,[225] exogenous organic acids[226] and hepatic excretory function.[227] Aging also decreases the rates of phase I and phase II biotransformations in rat colon.[228]

4.4 Xenobiotics

Exposure to xenobiotics may alter the biotransformation, hepatobiliary transport and intestinal absorption of a given chemical. Exposure of animals and humans to microsomal enzyme–inducing agents may increase biliary excretion by augmenting biotransformation and increasing bile formation. A specific interaction is the increased conjugation with glucuronic acid induced after exposure to numerous drugs which facilitates the biliary excretion of paracetamol,[229] alfentanil and sufentanil,[230] bilirubin,[231] and diethylstilbestrol and phenolphthalein.[232]

4.5 Intravenous Colloidal Nanoparticles

Enterohepatic recirculation may also be facilitated by the intravenous administration of a drug (for example, indomethacin) formulated using colloidal nanoparticles, which are then taken up by liver Kuppfer cells to facilitate the drug's absorption into liver parenchymal cells.[233]

4.6 Coadministration of Drugs

Drug interactions can occur at any part of the enterohepatic cycle, through effects on absorption, distribution, transporters, metabolism or other mechanisms. Sections 4.2 and 4.4 examined the effects of adsorbents and certain xenobiotics on the enterohepatic recycling process. A number of studies have been carried out on inhibition of carrier-mediated plasma membrane transport. For instance, the hepatic membrane transport of 1-*O*-gemfibrozil-β-D-glucuronide is carrier-mediated and inhibited by the organic anion dibromosulfophthalein.[234] Clofibric acid increases the hepatic clearance but decreases the biliary clearance of 1-*O*-gemfibrozil-β-D-glucuronide.[234]

Kim et al.[235] have suggested that a number of drugs, including peptidomimetic renin inhibitors, propranolol, cyclosporin and progesterone, are potent inhibitors of the bile acid uptake transporter NTCP, responsible for the uptake of bile acids from the portal circulation into hepatocytes. Antiarrhythmic agents, including bupivicaine, lidocaine and quinidine, were found to enhance the polypeptide activity.

The modulator LY-335979 is among the most potent modulators of P-glycoprotein, and does not modulate resistance mediated by MRP1 or MRP2. LY-335979 significantly enhances the survival of mice implanted with P-glycoprotein-expressing murine leukaemia (P388/ADR) when administered in combination with anticancer agents (daunorubicin, doxorubicin or etoposide).[236]

Shipkova et al.[135] have recently suggested that cyclosporin attenuates the enterohepatic recirculation of mycophenolic acid glucuronide/mycophenolic acid in kidney transplant recipients. The reduction in the bioavailability of mycophenolate mofetil in liver transplant patients treated with antibacterials for selective bowel decontamination is

probably due to an interruption of enterohepatic cycling.[137]

4.7 Genetic Defects and Biliary Transport

Three (bile acids, phospholipids and bilirubin) of the four (the other is cholesterol) major substrates transported out of hepatocytes have their own transport mechanisms. Bile acids and phospholipids each have a specific transporter, and that for bilirubin is multispecific. In each of these transporters, isolated autosomal recessive defects have now been identified and have helped to confirm the physiological role of these proteins.[22] An abnormality in plasma membrane aminophospholipid distribution leading to a secondary defect in bile acid transport has also been identified.[22] Studies using isolated perfused livers from MRP2-deficient Wistar rats have shown that the hepatobiliary disposition of paracetamol is impaired relative to normal rats.[194] There was also upregulation of a basolateral organic anion transporter in the MRP2-deficient rat livers. The mutant strain Eisai-hyperbilirubinaemic rats (EHBR) used in certain pharmacokinetic studies by Kato et al.[197] have a hereditary defect in cMOAT/MRP2. Using this same model, Morikawa et al.[237] showed that the transport of 17β-estradiol 17β-D-glucuronide across the bile canalicular membrane is predominantly mediated by cMOAT/MRP2.

4.8 Effect of Disease

The biliary excretion of drugs is a unique route of elimination; enterohepatic circulation and intestinal metabolism may also determine the fate of a drug in the organism. Liver and kidney diseases might influence the biliary excretion of drugs by decreasing the uptake into hepatocytes and altering the distribution and metabolism within hepatocytes. The disease might also decrease the transfer mechanism into bile. In animals, cholestasis results in an inhibition of hepatic uptake[238,239] and drug metabolism,[240] whereas hepatocellular damage decreases the transfer from the hepatocyte into bile.[241] In rats with ureter ligation, the biliary excretion of total paracetamol was enhanced to 38% of the dose compared with 28% in normal rats, mainly due to an enhanced sulfation of paracetamol.[241]

Relevant to the clinical pharmacokinetics of enterohepatic recirculation is the coordinated regulation and function of sinusoidal and canalicular transporters to produce bile. In the diseased state, downregulation of the unidirectional sinusoidal uptake system NTCP will protect the hepatocyte from further intracellular accumulation of bile salts. In contrast, expression of the canalicular bile salt export pump maintains bile salt secretion even in complete biliary obstruction.[242] In cholestasis, there is also a strong downregulation of canalicular MRP2 and a converse upregulation of hepatocyte basolateral efflux systems such as MRP3 and MRP1.

Interruption of enterohepatic circulation can be used in patients with toxic drug concentrations.[125] α-Amanitin, a toxic substance from *Amanita phalloides*, which potently inhibits DNA-dependent RNA polymerase II,[243] undergoes extensive enterohepatic circulation.

4.8.1 Cirrhosis

Cirrhosis is known to be associated with alterations in both hepatic blood flow and cellular function, including biliary secretion. Thus, it is not surprising that the hepatic uptake rate of many substrates is reduced.[244,245]

Two hypotheses have been proposed to account for the decreased uptake.[244,246] The intact cell hypothesis attributes the reduced uptake to changes in the liver microcirculation. These changes include capillarisation of hepatic sinusoids, collagenisation of the space of Disse and intrahepatic shunting, resulting in reduced perfusion of liver cells that contain normal enzymatic activity.[247-250] The sick cell hypothesis attributes the decreased uptake to a reduction in cellular transport capacity. In cirrhosis, the lipid composition of the hepatocyte plasma membrane and membranes from intracellular organelles is altered.[251,252] As well, Na^+-K^+ ATPase activity has been reported to be decreased.[253,254] Recently, the effect of CCl_4-induced experimental hepatic failure on the sequen-

tial hepatobiliary transport of the model organic cations triethylmethylammonium and tributylmethylammonium in a rat model was reported.[255] It was suggested that the hepatic failure affected sinusoidal membrane transport systems (uptake and/or efflux) rather than those on the bile canalicular membrane (excretion). We have reported that transport can be related to the degree of fibrosis.[62]

4.8.2 Cholestasis

The term 'cholestasis' literally means stagnation of bile flow and can be due either to physical obstruction of the biliary tree, termed extrahepatic cholestasis, or a decrease in the secretion of bile by the hepatocyte, termed intrahepatic cholestasis. Extrahepatic cholestasis involves a mechanical obstruction to the bile duct that may be caused by duct stones, sclerosing cholangitis or pancreatic carcinoma.

Intrahepatic cholestasis is defined as a syndrome with decreased bile flow and regurgitation of biliary constituents such as bile acids, conjugated bilirubin and lipids into plasma. Intrahepatic cholestasis is caused by an impairment of normal biochemical processes within the hepatocyte. Permeability of the canalicular membrane and tight junctions to bile acids are affected. Significant changes result in many subcellular membranes, including the actin-containing microfilaments (implicated in bile duct contraction) and the endoplasmic reticulum. Also affected are the intracellular bile salt transport proteins responsible for transporting bile salts from the plasma membrane to the canalicular membrane (intracellular trafficking) and bile salt metabolism. Interruption of bile flow results in elevated serum bile acid levels as well as cytosolic levels.[256] Cholestasis also affects the CYP system, possibly through bile salt retention. Bile salts have been shown to inhibit CYP *in vitro*, which may lead to hypertrophy of the smooth endoplasmic reticulum.[257]

The morphological features of cholestasis, both intra- and extrahepatic, include the presence of biliary material (primarily bile pigments) in hepatocytes and Kupffer cells, and bile thrombi in dilated bile canaliculi.[258] Electron microscopy shows a decreased number of canalicular microvilli, and distorted microvilli with a blunted or oedematous appearance. Hypertrophy of the Golgi apparatus and smooth endoplasmic reticulum is seen in addition to increased lysosomes and dilation and vesiculation of the rough endoplasmic reticulum.[259] It is not possible to distinguish extra- and intrahepatic cholestasis on the basis of morphological features alone, substantiating the idea that many of the morphological changes are the result of, rather than the cause of, cholestasis.

The alterations of hepatobiliary transport that occur in cholestasis can be divided into transporter defects (such as mutations of transporter genes or acquired dysfunctions of transport systems that cause defective canalicular or cholangiocellular secretion) and those that result from biliary obstruction.[242] Multiple alterations of hepatocellular transporter expression can be caused by the hepatocellular accumulation of toxic cholephilic compounds in progressive familial intrahepatic cholestasis or by the direct inhibition of transporter gene expression by cytokines.

α-Naphthylisothiocyanate (ANIT) produces cholestasis in a reproducible and dose-dependent manner following its acute administration, whereas chronic administration results in bile duct hyperplasia and biliary cirrhosis.[260] Plaa and coworkers[261,262] have shown that a single dose of ANIT to the rat causes retention of BSP, an organic anionic dye excreted primarily in bile, within 2 to 4 hours and hyperbilirubinaemia at 12 to 24 hours. Bile flow decreases within 16 to 24 hours and remains depressed for about 5 days.[263] A dose of ANIT greater than 150 mg/kg causes complete cholestasis in the rat, whereas the mouse is more sensitive, the hamster less sensitive, and the dog and rabbit resistant to ANIT-induced cholestasis.[262]

Phalloidin, cytochalasin and colchicine are thought to induce cholestasis by their toxic actions on microfilaments and microtubules in the hepatocyte. These organelles are thought to be important for maintenance of cell shape, motility and secre-

tion of various substances, including lipoproteins, proteins and bile. Phalloidin is taken up into the hepatocytes, apparently by the bile acid transport system and is thus concentrated in the liver.[264,265] Chronic treatment of rats with low doses of phalloidin (500 μg/kg/day for 3 or more days) causes a marked accumulation of actin microfilaments in the pericanalicular region of the hepatocyte[266] and a decrease in bile flow.[267] Many of the bile acids are hepatotoxic and will induce cholestasis if infused at rates that exceed the transport maximum for their excretion in bile. Thus, infusion of taurocholate at increasing concentrations increases bile flow to a maximum. Further infusion decreases bile flows rather than maintains it at the maximal rate.

Cholestasis has been shown to have a number of effects on transporters involved in the biliary excretion process. Overall, there appear to be adaptive processes whereby bile salts not able to exit the hepatocyte in cholestasis are effectively removed across the basolateral membrane.[268] Transcriptional and post-transcriptional mechanisms are involved in this high level of regulation, with experimental evidence supporting communication between hepatocytes and cholangiocytes, especially in primary biliary cirrhosis. In cholestatic rats, the multispecific organic anion transporter MRP2 has a decreased expression. The expression of the bile salt export pump BSEP and the phospholipid transporter MDR2 are less affected. Interestingly, as a balance to NTCP, the carrier protein for hepatic uptake of bile salts, being sharply downregulated, a basolateral ATP-dependent transporter for glucuronides and bile salts (MRP3) is upregulated.[43]

4.8.3 Diabetes

Diabetes mellitus is a commonly occurring disease, characterised by the inability of the body to produce or use insulin, and often accompanied by various pathological states including coronary heart disease, renal insufficiency, cerebrovascular disorders and neuropathy. In addition to these disorders, there is some evidence that the course of diabetes may also alter the biotransformation of pharmaceutical agents and increase the risk of drug toxicity and adverse effects. Early researchers found an increased incidence of gallstones at autopsy of patients with diabetes,[269,270] as well as an increased frequency of cholelithiasis among diabetic patients.[271] Other workers have shown changes in biotransformation due to diabetes, both in humans and in animal models,[272,273] as well as alterations in pharmacokinetic parameters for various drugs and xenobiotics.[274,275] Other studies have shown elevated mRNA expression of some plasma membrane proteins.[276] All these studies suggest that diabetes mellitus has a profound influence on hepatobiliary structure and function.

The influence of insulin on hepatic protein synthesis may impact on the protein carriers controlling hepatic uptake and canalicular secretion, and on the enzymes involved in hepatic biotransformation. For example, the rate of uptake of isoniazid into liver, lung, diaphragm and brain is enhanced in the presence of insulin.[277] Recent studies suggest that hepatic protein synthesis is also affected by insulin-dependent diabetes.[278]

4.8.4 Renal Disease

Renal disease has been suggested to affect biliary transport through the accumulation of some endogenous P-glycoprotein substrates/modulators in the plasma in disease states.[279] Using glycerol-induced acute renal failure, Huang et al.[279] showed that, associated with an increase in P-glycoprotein level in the kidney, P-glycoprotein function in the liver and brain was suppressed.

4.8.5 Impaired Biliary Bilirubin Excretion

Impaired bilirubin secretion in the bile will lead to active transport of bilirubin glucuronides back into blood via MRP3.[41] Interestingly, Kato et al.[197] found that the biliary excretion of telmisartan is relatively unaffected in Eisai-hyperbilirubinemic rats relative to normal rats, and suggested that telmisartan glucuronidation is enhanced in the Eisai-hyperbilirubinemic rats so that, overall, there is comparable excretion of glucuronide in both the mutant and normal animals.

4.8.6 Transporter Malfunction and Disease

In patients with inflammation-induced icteric cholestasis (mainly cholestatic alcoholic hepatitis), the mRNA levels of the basolateral transporters NTCP, OATP2 and BSEP are reduced by about 40% when compared with controls.[280] In addition, mRNA levels for the apical transporter MRP2 remained unchanged, but canalicular immunolabelling for MRP2 was also decreased. The mRNA expression of MRP3, MDR1, MDR3, and familial intrahepatic cholestasis gene 1 (FIC1) remained unchanged. Zollner et al.[280] suggest that reduced expression of hepatobiliary transporters for bile salts and other organic anions may contribute to inflammation-induced cholestasis in humans.

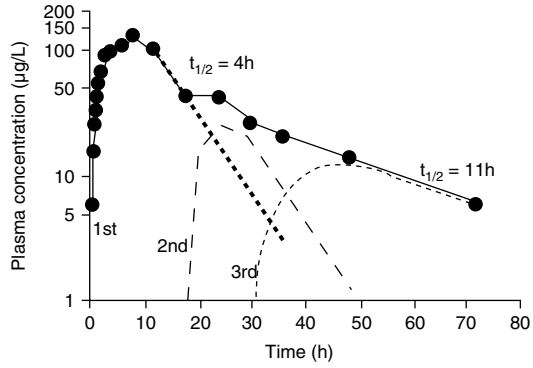

Fig. 7. Plasma concentration-time profile of estrone after oral administration of estradiol 1.5mg in a desogestrel combination to 18 healthy postmenopausal women. Also shown are the second and third enterohepatic recycling phases leading to secondary peaks and an increase in the half-life ($t_{½}$) of oestrone (reproduced from Vree and Timmer,[115] with permission).

5. Pharmacokinetics of Enterohepatic Recycling

Because of the recycling of solutes between the intestinal tract, portal circulation and liver via the biliary tract, drugs subject to enterohepatic recirculation are often characterised by a long half-life and multiple peaks. As an example of this process, the enterohepatic cycling of furosemide acyl glucuronide, followed by hydrolysis, results in a second and slow elimination phase with a half-life of 20 to 30 hours.[281] A similar increase in half-life is seen for estrone after administration of estradiol (figure 7). The pharmacokinetics of the immunosuppressant mycophenolate mofetil also show secondary peaks of mycophenolic acid, suggesting a significant enterohepatic cycling process.[282] Bullingham et al.[282] suggested that enterohepatic cycling must involve colonic bacterial deconjugation of phenolic glucuronides and, based on an oral cholestyramine interaction study, calculated that the mean contribution of enterohepatic cycling to the AUC_∞ of mycophenolic acid was around 40%. More recently, Funaki[283] used a population analysis approach to define the pharmacokinetics of mycophenolate mofetil.

5.1 Compartmental Models

The first models developed to describe enterohepatic cycling were classical compartmental models. Thus, two- (or three-) compartment models of drug disposition with first-order absorption after oral administration (gut as a well-mixed compartment) were extended by a chain of compartments describing the transport of the drug from the central compartment to the gut.[284-286] In its simplest form, this chain consists of only one compartment, the gallbladder (figure 8).

Recirculation loops of more than one compartment allowed a better simulation of the delay caused by the recirculation process. Such a model was successfully applied to describe enterohepatic cycling of morphine,[287] valproic acid[288] and morphine-3-glucuronide[289] in the rat. Although these models with a continuous delay time appear sufficient for animals without gallbladder (like the rat), they are not appropriate to describe the discontinuity in the enterohepatic cycling process caused by gallbladder emptying. Enterohepatic cycling models that account for the effect of gallbladder emptying (lag-time models) can further be classified in models where emptying is assumed to occur at regular intervals (τ, 2τ, …) and models with specific irregular emptying times (τ_1, τ_2,.. τ_n). Steimer et al.[290] showed that a simple two-compartment lag-time model already accounts for the occurrence of second peaks in the plasma concen-

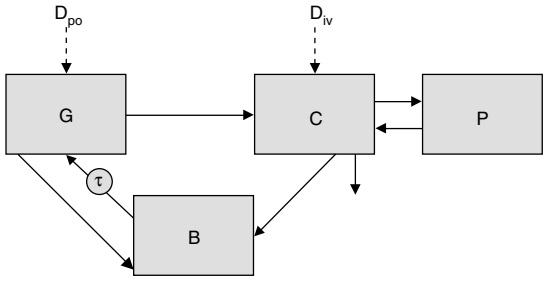

Fig. 8. Compartment model for enterohepatic cycling. **B** = gallbladder; **C** = central compartment; **D** = dose; **G** = gastrointestinal tract; **iv** = intravenous; **P** = peripheral compartment; **po** = oral; τ = lag-time.

tration-time profile. A general treatment of enterohepatic recirculation of drugs can be based on the fraction of drug in the systemic circulation that is excreted in the bile and the fraction of drug reabsorbed from the gut that reaches the systemic circulation in each enterohepatic cycle.[291] A four-compartment enterohepatic model for unchanged drug described by Wang and Reuning[292] contained central, peripheral, gallbladder and intestinal compartments and an intermittent gallbladder-emptying rate constant.

Models based on irregular lag-time intervals are more near to physiological reality since gallbladder emptying starts when food begins to be digested in the upper gastrointestinal tract. Furthermore, the extent of biliary emptying may depend on the quantity of fat in the meal. Models with regular biliary emptying have been studied extensively in the literature,[293,294] but models with irregular intervals are mathematically more complex. Although the latter were first developed for only one or two recirculations,[295] these models have been later formulated for any number of cycles occurring at various times.[296,297] However, their application through data analysis has been restricted to two recirculations, τ_1 and τ_2. As shown by Plusquellec et al.[297] for the pharmacokinetics of an antihypertensive drug in humans, this may not be a limitation for many drugs as only two (or one) recirculations could be detected (fitting the plasma concentration-time data by analytical functions derived from the model). In using compartmental models with regular (or continuous) gallbladder emptying, the resulting differential equations are usually solved numerically. For certain compartmental structures, equations for mean residence time in the body (MRT), mean absorption time (MAT), the area under the concentration-time curve (AUC), clearance (CL) and bioavailability (F) have been derived.[64,285,286,298]

5.2 System Dynamics Approach

A generalisation of these compartmental models is possible on the basis of a system theoretical approach using the method of numerical inverse Laplace transformation.[299] The integration of the physiological dispersion model description of vascular transit times in the liver[36] with enterohepatic recirculation has been described using this approach.[300] This approach uses subsystems to model the processes of intestinal absorption, systemic disposition and enterohepatic cycling, and is much more flexible. The basic structure of the recirculatory system describing enterohepatic cycling (figure 9) is analogous to that of pharmacokinetic models of circulatory drug transport, and the corresponding equation for the plasma concentration-time profile [C(t)] after intravenous bolus administration can readily be written in the Laplace domain[299,301] (equation 1):

$$C(s) = \frac{D_{iv}}{CL^1 F_B} \frac{f_D(s)}{1 - f_{EHC}(s) f_D(s)}$$

where D_{iv} is the intravenous dose, CL^1 is the total clearance without enterohepatic cycling and F_B denotes the biliary excretion ratio.

Any appropriate mathematical function can be assumed – only their Laplace transforms $f_D(s)$ and $f_{EHC}(s)$ must exist – for the systemic drug disposition function $f_D(t)$ [processes inside the body] and the transit time density describing enterohepatic cycling $f_{EHC}(t)$ [processes 'outside' the body, i.e. intestinal tract, portal vein, liver and bile duct]. Note that for a system without enterohepatic cy-

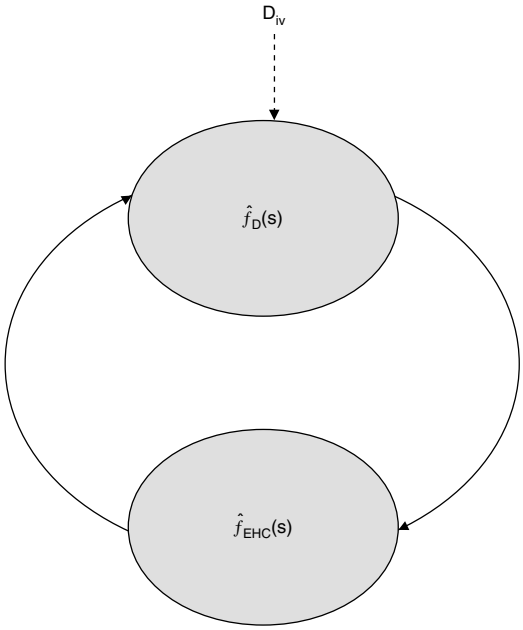

Fig. 9. Modelling of enterohepatic cycling (EHC) in pharmacokinetics by a circulatory arrangement of subsystems characterised by residence time density functions $\hat{f}_D(s)$ and $\hat{f}_{EHC}(s)$ describing drug disposition and EHC in the Laplace domain.[299]

cling (e.g. bile duct cannulation), equation 1 reduces to equation 2:

$$C^1(s) = \frac{D_{iv}}{CL^1 F_B} f_D(s)$$

Calculating the moments of C(t) from its Laplace transform C(s) [equation 1], equations for pharmacokinetic parameters such as MRT, AUC and F can be derived that are independent of the specific forms of the disposition and enterohepatic cycling functions. Thus, enterohepatic cycling causes AUC and MRT to increase (equation 3 and equation 4):[299]

$$AUC_{iv} = AUC^1 \frac{1}{1 - F_{EHC} F_B}$$

$$MRT_{iv} = MRT^1 + \frac{(MRT^1 + MTT_{EHC}) F_{EHC} F_B}{1 - F_{EHC} F_B}$$

where $AUC^1 = D_{iv}/CL^1$ and MRT^1 denote the measures without enterohepatic cycling, and F_{EHC} and MTT_{EHC} are the availability and mean transit time from the intestinal tract to the systemic circulation, respectively. In other words, $E_{EHC} = 1 - F_{EHC}$ is the extraction in a single enterohepatic circulation. Note that 'without enterohepatic cycling' means blockade by cholestyramine and antibacterials or biliary cannulation, situations where one can estimate the extent of enterohepatic recirculation from a comparison of the respective AUC values (figure 10).

This concept (equation 1) was used to study the disposition of cefixime[299] and diclofenac[302] in rats. The basic model equation can also be readily extended to the case of oral administration and/or multiple doses. When the enterohepatic cycling function contains a lag time, it accounts for discontinuous (but regular) gallbladder emptying and secondary peaks in the C(t) curve appear if the lag time increases. Recently, Yasui et al.[301] have applied the recirculatory concept to evaluate the stereoselective enterohepatic cycling and the unidirectional chiral inversion of ketoprofen enantiomers in the rat. Although the system approach proposed by Yamaoka et al.[299] was up to now only applied to analyse the pharmacokinetics of drugs after intravenous administration (equation 1), it can be extended to account for the first-pass effect after oral administration as suggested by Peris-Ribera et al.,[291] who developed more general equations for AUC, F and CL based on mass-balance considerations. This would allow, for example, the assumption of a more realistic absorption process where the fractional absorption rate is a function of time instead of a constant (as in the case of the first-order absorption process in compartmental modelling).

Equations that describe the effect of enterohepatic cycling on drug bioavailability, F, are more complex than equation 3. It has been shown that enterohepatic cycling increases F and that this effect can be pronounced for drugs with high biliary excretion, where small changes in the pharmaco-

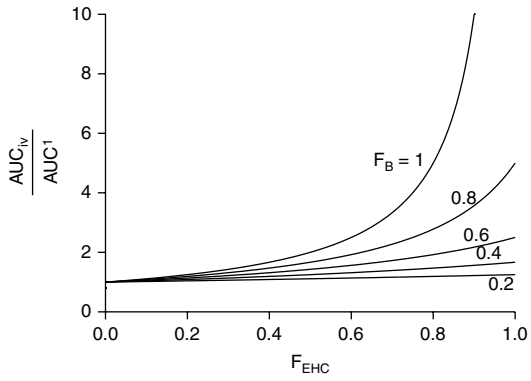

Fig. 10. Effect of the drug absorption ratio, F_{EHC}, on the ratio of areas under the concentration-time curve with (AUC_{iv}) and without (AUC^1) enterohepatic recycling for different values of the excretion ratio into the bile (F_B) [equation 3].

kinetic parameters of the drug can cause large changes in F.[64]

5.3 Parameter Estimation

The construction of a model that is sufficiently realistic from a pharmacokinetic (or physiological) point of view is one problem, the identifiability of the model, i.e. the reliability of parameter estimation based on available concentration-time data, is another. Experimental design strategies for quantifying pharmacokinetic models with enterohepatic cycling were studied by Wang and Reuning.[292] These authors also discussed the advantage of getting additional information from bile sampling (a necessary condition for the identification of more complex models). Recently, a simple three-compartment enterohepatic cycling model (based on a one-compartment disposition model) has been applied in a population pharmacokinetic study of mycophenolate mofetil.[283] Figure 11 is an illustration of that modelling, showing the secondary peak for mycophenolate mofetil after gallbladder emptying. Recently, Ploeger et al.[303] used scaling from a rat to a human physiologically-based pharmacokinetic model to describe the enterohepatic cycling of glycyrrhetic acid metabolites.

5.4 Enterohepatic Circulation, or Some Other Mechanism?

Multiple peaks in the concentration-time profile could be due to mechanisms other than enterohepatic circulation. A double-peak behaviour could, for instance, be due to biphasic gastric emptying behaviour. Wang et al.[304] attributed the double peak of alprazolam to a reduction in gastric motility caused by its muscle relaxant effect. They suggested that enterohepatic recycling be precluded from being the underlying mechanism as the presence of double peaks was not evident after an intravenous dose. Solutes undergoing enterohepatic recirculation are frequently characterised by a relatively high hepatic extraction and low plasma concentrations, as illustrated by the pharmacokinetics of chenodeoxycholic and ursodeoxycholic acids, where majority of bile acid is confined within the enterohepatic circulation. When the enterohepatic recirculation is efficient and dominant, the solutes may also be characterised by a high apparent volume of distribution and a low clearance.

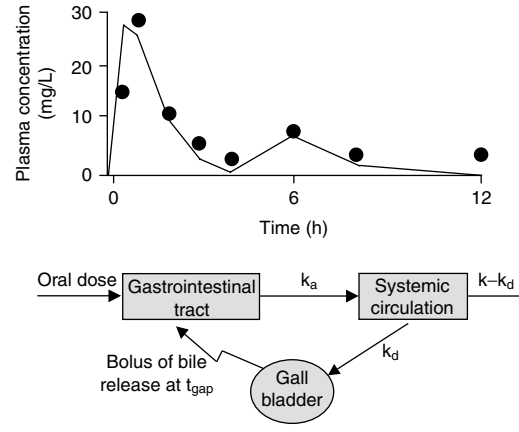

Fig. 11. NONMEM fit of mycophenolate mofetil plasma concentration-time profiles for a typical patient and the associated enterohepatic circulation model used (reproduced from Funaki,[283] with permission). **k** = elimination rate constant; $\mathbf{k_a}$ = absorption rate constant; $\mathbf{k_d}$ = excretion rate constant into gallbladder; $\mathbf{t_{gap}}$ = expulsion time of gallbladder.

6. Clinical Implications of Enterohepatic Recirculation

The impact that enterohepatic cycling has on the pharmacokinetics and pharmacodynamics of a drug depends on: (i) the importance of biliary excretion of the compound relative to renal and metabolic clearance processes; and (ii) the efficiency with which the compound is absorbed into the circulation from the gastrointestinal tract. Enterohepatic circulation influences drug pharmacokinetics by increasing drug bioavailability and influencing plasma concentration curves of the drug.[297,305] The absorption of a drug is usually more rapid than its elimination. If drug elimination includes enterohepatic circulation, drug secreted in bile can serve as a secondary source for drug absorption. Enterohepatic cycling may influence drug pharmacokinetics by prolonging elimination. This has been shown to be of clinical significance for spironolactone.[306] The clinical significance of the enterohepatic circulation depends on the pharmacological or toxicological properties of the biliary excretory products, their availability for absorption, and whether the absorbed products are re-extracted by the liver or pass into the general circulation. In some cases, the enterohepatic circulation of a drug may be a therapeutic advantage. For example, sulindac, an NSAID, is recycled through the biliary system to a greater degree than its active sulfide metabolite.[307] Thus, sustained plasma concentrations may be achieved by the enterohepatic circulation of the prodrug, minimising exposure of the intestine to active drug. On the other hand, it is also possible that enterohepatic cycling leading to secondary peaks in plasma concentration could result in toxicity.

A limited number of studies involving enterohepatic recirculation have considered pharmacodynamic outcomes. The slow elimination phase associated with the enterohepatic cycling of furosemide acyl glucuronide, for instance, coincides with a pharmacodynamic rebound phase of urine retention.[281] These investigators suggested that accumulation of furosemide and its acyl glucuronide in patients with end-stage renal failure results from infinite hepatic cycling and that dose increments are required to maintain a required level of diuresis. A pharmacodynamic model associated with enterohepatic recycling has been described.[308]

Most clinical studies for quantification of the biliary excretion of drugs have been performed in patients following cholecystectomy or other biliary tract surgery. Such studies should be interpreted with caution, as the patients generally have had some kind of hepatobiliary disease and may have experienced extrahepatic cholestasis. There is a possibility that their disease may have influenced some component of the enterohepatic recirculation of the drugs. Patients with cholestatic liver disease show a lower biliary excretion of drugs, increasing the risk of toxicity.[258,260] Although there are a number of studies on the kinetics of biliary excretion of drugs,[5-7,33] the results reflect the small number of patients in many studies, the methods of bile sampling and the influence of hepatobiliary diseases. Future research should obtain more information about biliary excretion of xenobiotics in humans based on improved methods of bile sampling and specific drug analyses.

A number of studies have used a T-tube inserted in the common bile duct of patients to study biliary pharmacokinetics. Most of these studies have been performed in post-surgical patients with T-tube drainage, and it should be recognised that this method of bile collection is not ideal because bile flow and composition are often severely altered during the period of study, not all bile is collected and enterohepatic circulation is partially interrupted.[118] Some examples of these studies are as follows. Serra et al.[309] studied the excretion of free and conjugated BSP in 19 adult patients with choledocholithiasis. Westphal et al.[124] studied the hepatobiliary extraction profile of cefixime, a dianionic cephalosporin antibacterial, in 10 patients after cholecystectomy. They suggested that cefixime biliary clearance was nonlinear, mostly in its initial phase, which is consistent with a concentrative uptake and intracellular protein binding. The biliary pharmacokinetics of sulbactam and

ampicillin have also been reported for 19 patients with normal liver function after surgery of the biliary tract.[116] The biliary excretion of chlorthalidone[310] and methadone and its metabolites[311] was low. In contrast, the biliary excretion of the glucuronide/sulfate conjugates of hydroxylated derivatives of tolfenamic acid was found to be significant.[134] High concentrations of ceftazidime in the bile have been reported, but with a low biliary excretion in terms of the dose used.[128] Biliary excretion of unchanged temafloxacin and temafloxacin glucuronide measured by T-tube only accounted for approximately 2.2 and 1% of the administered dose, respectively.[131]

The effect of antimicrobials on female hormones remains controversial, especially in terms of the effects on the enterohepatic recycling of oral contraceptive steroids. Substantial differences have been observed in plasma estriol levels in patients treated with ampicillin 250mg every 8 hours in the 48 hours before delivery (figure 12).[312] It is also suggested that there is probably a group of susceptible women who have lower plasma concentrations of oral contraceptive hormones and experience breakthrough bleeding or become pregnant when given broad-spectrum antibacterials. This may relate to interruption of the enterohepatic recirculation of ethinylestradiol (figure 13).[313,314] Weisberg[315] suggests that while the effectiveness of oral contraceptives may be impaired by concomitant treatment with antimicrobials, there are no scientific data to support the anecdotal evidence that concomitant use reduces contraceptive efficacy in the majority of women. It is thus apparent that a number of drugs currently in clinical use have the potential to interact to affect not only the liver plasma membrane transporter activities and hepatic bile acid uptake, but also enterohepatic recirculation.

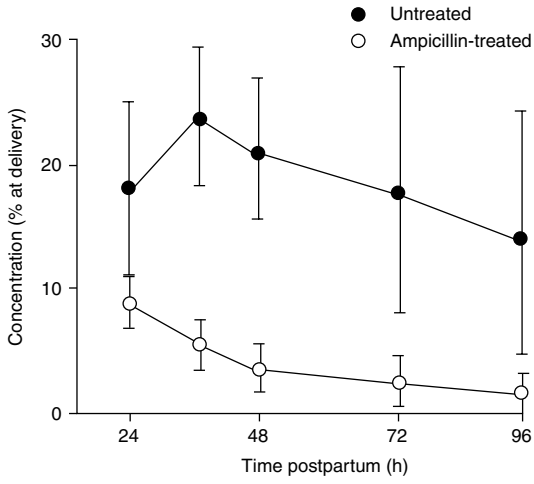

Fig. 12. Postpartum plasma total estriol concentrations (as a percentage of concentrations at delivery) in patients treated with ampicillin for 48 hours prior to delivery and untreated patients (reproduced from Buchan and Klopper,[312] with permission).

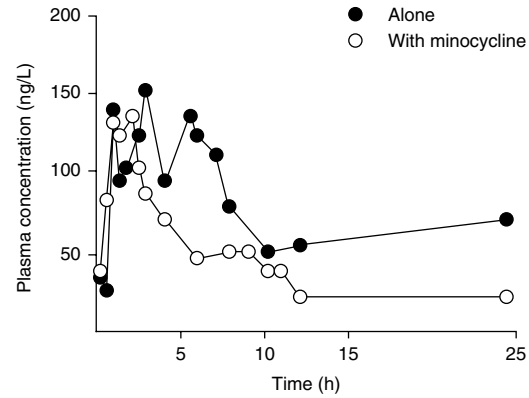

Fig. 13. Plasma concentration-time profiles of ethinylestradiol on day 15 of a pill cycle on two occasions in the same individual taking a triphasic contraceptive pill with and without minocycline 100mg twice daily.[313]

Also indirectly affecting enterohepatic recirculation are a multitude of drug interactions that affect the production of polar metabolites by CYP, conjugation, transporters, intestinal transit time and bioavailability, amongst others. Associated with such drug interactions is interindividual variability in the inhibition and induction of enzymes,[316] with varying degrees of clinical significance as a consequence.

7. Conclusions

Enterohepatic recirculation is dependent on a range of factors associated with gastrointestinal

absorption, hepatic uptake, metabolism and biliary excretion. Each of these processes is dependent on species, age, disease, drug, genetics, physiology and coadministered drugs. An effect on any one of these processes may interrupt or enhance the enterohepatic cycle. Many of the determinants affecting these processes are still being defined. Compartmental and noncompartmental models originally developed to analyse the pharmacokinetics of enterohepatic recycling in rats are only partly applicable to humans. Models that account for irregular biliary emptying are more realistic in clinical pharmacokinetics; however, based on drug plasma concentration data, reliable parameter estimation may be possible for one or two recirculations only.

Acknowledgements

We wish to acknowledge the support of the Australian National Health and Medical Research Council (M.S.R., B.M.M., M.W.), Medical Research Council of Canada Grant No. MT 13683 (F.J.B.), and the Princess Alexandra Hospital Research Foundation (F.J.B.).

References

1. Hofmann AF. The enterohepatic circulation of bile acids in man. Adv Intern Med 1976; 21: 501-34
2. Plaa GL. Toxic responses of the liver. In: Amdur MO, Doull J, Klaassen CD, editors. Casarett and Doull's toxicology. New York: Pergamon Press, 1991
3. Mehendale HM. Hepatotoxicity. In: Haley TJ, Berndt WO, editors. Handbook of toxicology. Washington, DC: Hemisphere, 1987
4. Laurent A. Analysis in the rat of 4-hydroxynonenal metabolites excreted in bile: evidence of enterohepatic circulation of these byproducts of lipid peroxidation. Chem Res Toxicol 1999; 12: 887-94
5. Jonsson A, Rydberg T, Sternber G, et al. Pharmacokinetics of glibenclamide and its metabolites in diabetic patients with impaired renal function. Eur J Clin Pharmacol 1998; 53 (6): 429-35
6. Hellstern A, Hildebrand M, Humpel M, et al. Minimal biliary excretion and enterohepatic recirculation of lormetazepam in man as investigated by a new nasobiliary drainage technique. Int J Clin Pharmacol Ther Toxicol 1990; 28 (6): 256-61
7. Melnik G, Schwesinger WH, Teng R, et al. Hepatobiliary elimination of trovafloxacin and metabolites following single oral doses in healthy volunteers. Eur J Clin Microbiol Infect Dis 1998; 17 (6): 424-6
8. Lenzen R, Bahr A, Eichstadt H, et al. In liver transplantation, T tube bile represents total bile flow: physiological and scintigraphic studies on biliary secretion of organic anions. Liver Transpl Surg 1999; 5 (1): 8-15
9. Erlinger S. Review article: new insights into the mechanisms of hepatic transport and bile secretion. J Gastroenterol Hepatol 1996; 11: 575-9
10. Klaassen CD, Watkins III JB. Mechanisms of bile formation, hepatic uptake, and biliary excretion. Pharmacol Rev 1984; 36: 1-67
11. Erlinger S. Bile flow. In: Arias IM, Boyer JL, Fausto N, et al., editors. Liver biology and pathobiology. New York: Raven Press, 1994: 769-86
12. Roberts S, Ludwig J, Larusso N. The pathobiology of biliary epithelia. Gastroenterology 1997; 112: 269-79
13. Alpini G, Phillips JO, LaRusso NF. The biology of biliary epithelia. In: Arias IM, Boyer JL, Fausto N, et al., editors. Liver biology and pathobiology. New York: Raven Press, 1994: 623-53
14. Seifter S, Englard S. Energy metabolism. In: Arias IM, Boyer JL, Fausto N, et al., editors. Liver biology and pathobiology. New York: Raven Press, 1994: 323-64
15. Schaffner F, Popper H. Electron microscopic studies of normal and proliferated bile ductules. Am J Pathol 1961; 38: 393-410
16. Steiner J, Carruthers J, Kalifat S. The ductular cell reaction of rat liver in extrahepatic cholestasis: I. Proliferated biliary epithelial cells. Exp Mol Pathol 1962; 1: 162-85
17. Meier PJ. Structural and functional polarity of canalicular and basolateral plasma membrane vesicles isolated in high yield from rat liver. J Cell Biol 1984; 98: 991-1000
18. Gumbiner B. Structure, biochemistry and assembly of epithelial tight junctions. Am J Physiol 1987; 253: 749-58
19. Coleman R. Biochemistry of bile secretion. Biochem J 1987; 244: 249-61
20. Phillips MJ, Poucell S, Oda M. Biology of disease: mechanisms of cholestasis. Lab Invest 1986; 54: 593-608
21. Phillips M. Intrahepatic cholestasis as a canalicular motility disorder: evidence using cytochalasin. Lab Invest 1983; 48: 205-11
22. Thompson R, Jansen P. Genetic defects in hepatocanalicular transport. Semin Liver Dis 2000; 20 (3): 365-72
23. Masyuk A. Perfused rat intrahepatic bile ducts secrete and absorb water, solute, and ions. Gastroenterology 2000; 119 (6): 1672-80
24. Marinelli R, Larusso N. Solute and water transport pathways in cholangiocytes. Semin Liver Dis 1996; 16 (2): 221-9
25. Dumont M, Uchman S, Erlinger S. Hypercholeresis induced by ursodeoxycholic acid and 7-ketolithocholic acid in the rat: possible role of bicarbonate transport. Gastroenterology 1980; 79: 82-9
26. Kitani K, Kanai S. Effect of ursodeoxycholate on the bile flow in the rat. Life Sci 1982; 31: 1973-85
27. Kanai S, Kitani K, Sato Y. Bile secretory characteristics of beta-muricholate and its taurine conjugate are similar to those of ursodeoxycholate in the rat. Life Sci 1991; 48: 949-57
28. Sjoqvist F. Fundamentals of clinical pharmacology. In: Speight T, Holford N, editors. Avery's drug treatment. Auckland: ADIS Press, 1997: 1-73
29. Doherty M, Pang K. First-pass effect: significance of the intestine for absorption and metabolism. Drug Chem Toxicol 1997; 20 (4): 329-44
30. Yang CY, Dantzig AH, Pidgeon C. Intestinal peptide transport systems and oral drug availability. Pharm Res 1999; 16 (9): 1331-43
31. Florence A, Attwood D. Physicochemical principles of pharmacy. 2nd ed. Wallington: Macmillan Press, 1988

32. Charman WN. Physiochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH. J Pharm Sci 1997; 86 (3): 269-82
33. Wu C. Differentiation of absorption and first-pass gut and hepatic metabolism in humans: studies with cyclosporin. Clin Pharmacol Ther 1995; 58: 492-7
34. Ilett K, Tee LB, Reeves PT, et al. Metabolism of drugs and other xenobiotics in the gut lumen and wall. Pharmacol Ther 1990; 46: 67-93
35. Hall SD. Molecular and physical mechanisms of first-pass extraction. Drug Metab Dispos 1999; 27 (2): 161-6
36. Roberts M, Rowland M. Hepatic elimination: dispersion model. J Pharm Sci 1985; 74: 585-7
37. Roberts MS, Anissimov YG, Weiss M. Commentary: using the convection-dispersion model and transit time density functions in the analysis of organ distribution kinetics. J Pharm Sci 2000; 89 (12): 1579-86
38. Iwatsubo T. Prediction of in vivo drug metabolism in the human liver from in vitro metabolism data. Pharmacol Ther 1997; 73 (2): 147-71
39. Izumi T. Pharmacokinetics of troglitazone, an antidiabetic agent: prediction of in vivo stereoselective sulfation and glucuronidation from in vitro data. J Pharmacol Exp Ther 1997; 280 (3): 1392-400
40. Ueda K. Enantioselective local disposition of semotiadil (R-enantiomer) and levosemotiadil (S-enantiomer) in perfused rat liver. Drug Metab Dispos 1997; 25 (3): 281-6
41. Kamisako T. Recent advances in bilirubin metabolism research: the molecular mechanism of hepatocyte bilirubin transport and its clinical relevance. J Gastroenterol 2000; 35 (9): 659-64
42. Kouzuki H, Suzuki H, Sugiyama Y. Pharmacokinetic study of the hepatobiliary transport of indomethacin. Pharm Res 2000; 17 (4): 432-8
43. Suzuki H, Sugiyama Y. Transport of drugs across the hepatic sinusoidal membrane: sinusoidal drug influx and efflux in the liver. Semin Liver Dis 2000; 20 (3): 251-63
44. Proost J, Roggeveld J, Wierda JM, et al. Relationship between chemical structure and physicochemical properties of series of bulky organic cations and their hepatic uptake and biliary excretion rates. J Pharmacol Exp Ther 1997; 282 (2): 715-26
45. Weisiger R, Fitz J, Scharschmidt B. Hepatic oleate uptake: electrochemical driving forces in the intact rat liver. J Clin Invest 1989; 83: 411-20
46. Fitz J, Bass N, Weisiger R. Hepatic transport of a fluorescent stearate derivative: electrochemical driving forces in intact rat liver. Am J Physiol 1991; 261: 83-91
47. Weinman S, Graf J, Boyer J. Voltage-driven, taurocholate-dependent secretion in isolated hepatocyte couplets. Am J Physiol 1989; 256: 826-32
48. Weinman S, Carruth M, Dawson P. Bile acid uptake via the human apical sodium-bile acid cotransporter is electrogenic. J Biol Chem 1998; 273: 34691-5
49. Smith P, Pritchard J, Miller D. Membrane potential drives organic cation transport into teleost renal proximal tubules. Am J Physiol 1988; 255: 492-9
50. Luxon B, Weisiger R. A new method for quantitating intracellular transport: application to the thyroid hormone 3,5,3′-triiodothyronine. Am J Physiol 1992; 263: 733-41
51. Luxon BA, Weisiger RA. Sex differences in intracellular fatty acid transport: role of cytoplasmic binding proteins. Am J Physiol 1993; 265: 831-41
52. Weiss M. Cytoplasmic binding and disposition kinetics of diclofenac in the isolated perfused rat liver. Br J Pharmacol 2000; 130 (6): 1331-8
53. Schwab AJ. Transfer of enalaprilat across rat liver cell membranes is barrier limited. Am J Physiol 1990; 258 (3 Pt 1): G461-75
54. Zucker S, Goessling W, Gollan J. Intracellular transport of small hydrophobic compounds by the hepatocyte. Semin Liv Dis 1996; 16: 159-67
55. Stolz A. The role of cytoplasmic proteins in hepatic bile acid transport. Annu Rev Physiol 1989; 51: 161-76
56. Erlinger S. Do intracellular organelles have any role in transport of bile acids by hepatocytes. J Hepatol 1996; 24 Suppl. 1: 88-93
57. Gregory D. Mechanism of secretion of biliary lipids: role of a microtubular system in hepatocellular transport of biliary lipids in the rat. Gastroenterology 1978; 74: 93-100
58. El-Seaidy A, Mills CO, Elias E, et al. Lack of evidence for vesicle trafficking of fluorescent bile salts in rat hepatocyte couplets. Am J Physiol 1997; 272: 298-309
59. Ishizaki J. Uptake of imipramine in rat liver lysosomes in vitro and its inhibition by basic drugs. J Pharmacol Exp Ther 2000; 294 (3): 1088-98
60. Daniel W, Wojcikowski J. Contribution of lysosomal trapping to the total tissue uptake of psychotropic drugs. Pharmacol Toxicol 1997; 80 (2): 62-8
61. Hung DY. Structure-hepatic disposition relationships for cationic drugs in isolated perfused rat livers: transmembrane exchange and cytoplasmic binding process. J Pharmacol Exp Ther 2001; 297 (2): 780-9
62. Hung D. Cationic drug pharmacokinetics in diseased livers determined by fibrosis index, hepatic protein content, microsomal activity and nature of drug. J Pharmacol Exp Ther 2002; 301 (3): 1079-87
63. Jakoby WB. Detoxication: conjugation and hydrolysis. In: Arias IM, Boyer JL, Fausto N, et al., editors. Liver biology and pathobiology. New York: Raven Press, 1994: 429-42
64. Horkovics-Kovats S. Efficiency of enterohepatic circulation, its determination and influence on drug bioavailability. Drug Res 1999; 49: 805-15
65. Caldwell J. Biological implications of xenobiotic metabolism. In: Arias IM, Jakoby WB, Popper H, editors. The liver: biology and pathobiology. New York: Raven Press, 1988: 355-62.
66. Mulder GJ, Meerman JHN. The role of extrahepatic and hepatic sulfation and glucuronidation in chemical carcinogenesis: an overview. In: Rydstrom J, Montelius J, Bengtsson M, editors. Extrahepatic drug metabolism and chemical carcinogenesis. Amsterdam: Elsevier Science, 1983 : 227-8
67. Hussain MD. Mechanisms of time-dependent kinetics of diltiazem in the isolated perfused rat liver. Drug Metab Dispos 1993; 22: 36-42
68. Seitz S, Boelsterli UA. Diclofenac acyl glucuronide, a major biliary metabolite, is directly involved in small intestinal injury in rats. Gastroenterology 1998; 115: 1476-82
69. Masimirembwa CM, Otter C, Berg M, et al. Heterologous expression and kinetic characterization of human cytochromes P-450: validation of a pharmaceutical tool for drug metabolism research. Drug Metab Dispos 1999; 27 (10): 1117-22
70. de Wildt SN. Cytochrome P450 3A: ontogeny and drug disposition. Clin Pharmacokinet 1999; 37 (6): 485-505
71. de Wildt SN. Glucuronidation in humans: pharmacogenetic and developmental aspects. Clin Pharmacokinet 1999; 36 (6): 439-52
72. Ballinger LN, Cross SE, Roberts MS. Availability and mean transit times of phenol and its metabolites in the isolated perfused rat liver: normal and retrograde studies using tracer con-

centrations of phenol. J Pharm Pharmacol 1995; 47 (11): 949-56
73. Evans AM. Membrane transport as a determinant of the hepatic elimination of drugs and metabolites. Clin Exp Pharmacol Physiol 1996; 23 (10-11): 970-4
74. Oinonen T, Lindros K. Zonation of hepatic cytochrome P-450 expression and regulation. Biochem J 1998; 329: 17-35
75. Palmer C. Localization of cytochrome P-450 gene expression in normal and diseased human liver by in situ hybridization of wax-embedded archival material. Hepatology 1992; 16 (3): 682-7
76. McKinnon R, Hall PD, Quattrochi LC, et al. Localization of CYP1A1 and CYP1A2 messenger RNA in normal human liver and in hepatocellular carcinoma by in situ hybridization. Hepatology 1991; 14 (5): 848-56
77. Tsutsumi M. The intralobular distribution of ethanol-inducible P450IIE1 in rat and human liver. Hepatology 1989; 10 (4): 437-46
78. Niemela O. Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. J Hepatol 2000; 33 (6): 893-901
79. Schwab AJ, Pang KS. The multiple indicator-dilution method for the study of enzyme heterogeneity in liver: theoretical basis. Drug Metab Dispos 1999; 27 (6): 746-55
80. Ring JA. Fetal hepatic drug elimination. Pharmacol Ther 1999; 84 (3): 429-45
81. Kakyo M. Immunohistochemical distribution and functional characterization of an organic anion transporting polypeptide 2 (oatp2). FEBS Lett 1999; 445: 343-6
82. Burger H. Different capacities for amino acid transport in periportal and perivenous hepatocytes isolated by digitonin/collagenase perfusion. Hepatology 1989; 9: 22-8
83. Kool M. MRP3, an organic anion transporter able to transport anti-cancer drugs. Proc Natl Acad Sci U S A 1999; 96: 6914-9
84. Kwon Y, Morris ME. Membrane transport in hepatic clearance of drugs II: zonal distribution patterns of concentration-dependent transport and elimination processes. Pharm Res 1997; 14: 780-5
85. Mainwaring G. The distribution of theta-class glutathione S-transferases in the liver and lung of mouse, rat and human. Biochem J 1996; 318: 297-303
86. Meyer-Wentrup F. Membrane localization of the electrogenic cation transporter rOCT1 in rat liver. Biochem Biophys Res Commun 1998; 248: 673-8
87. Moseley R, Jarose S, Permoad P. Hepatic Na+-dicarboxylate cotransport: identification, characterization, and acinar localization. Am J Physiol 1992; 263: G871-9
88. Pang K. Acinar factors in drug processing: protein binding, futile cycling and cosubstrate. Drug Metab Rev 1995; 27: 325-68
89. Ploemen J. Ethacrynic acid and its glutathione conjugate as inhibitors of glutathione S-transferases. Xenobiotica 1993; 23: 913-23
90. Ploemen J, Ommen BV, Bladeren PV. Inhibition of rat and human glutathione S-transferase isoenzymes by ethacrynic acid and its glutathione conjugate. Biochem Pharmacol 1990; 40: 1631-5
91. Ploemen J. Reversible conjugation of ethacrynic acid with glutathione and human glutathione S-transferase P1-1. Cancer Res 1994; 54: 915-9
92. Redick J, Jakoby W, Baron J. Immunohistochemical localization of glutathione S-transferases in livers of untreated rats. J Biol Chem 1982; 257: 15200-3
93. Saiki H. Zonal distribution of cysteine uptake in the perfused rat liver. J Biol Chem 1992; 267: 192-6
94. Sippel H, Lindros K, Oinonen T. Distribution of glutathione S-transferase isoforms in rat liver after induction by naphthoflavone or 3-methylcholanthrene. Pharmacol Toxicol 1996; 79: 80-6
95. Stieger B. In situ localization of the hepatocyte Na+/taurocholate cotransporting polypeptide in rat liver. Gastroenterology 1994; 107: 1781-7
96. Tal M. Restricted expression of the erythroid/brain glucose transporter isoform to perivenous hepatocytes in rats: modulation by glucose. J Clin Invest 1990; 86: 986-92
97. Tan E, Tirona R, Pang K. Lack of zonal uptake of estrone sulfate in enriched periportal and perivenous isolated rat hepatocytes. Drug Metab Dispos 1999; 27: 336-41
98. Tirona R, Pang K. Bimolecular glutathione conjugation kinetics of ethacrynic acid in rat liver: in vitro and perfusion studies. J Pharmacol Exp Ther 1999; 290: 1230-41
99. Oinonen T. Growth hormone-regulated periportal expression of CYP2C7 in the liver. Biochem Pharmacol 2000; 59 (5): 583-9
100. Saarikoski S. Induction of UDP-glycosyltransferase family 1 genes in rat liver: different patterns of mRNA expression with two inducers, 3-methylcholanthrene and beta-naphthoflavone. Biochem Pharmacol 1998; 56 (5): 569-75
101. Gumucio J, Miller D. Functional implications of liver cell heterogeneity. Gastroenterology 1981; 80 (2): 393-403
102. Lecureur V. Expression and regulation of hepatic drug and bile acid transporters. Toxicology 2000; 153 (1-3): 203-19
103. Tirona R. Uptake and glutathione conjugation of ethacrynic acid and efflux of the glutathione adduct by periportal and perivenous rat hepatocytes. J Pharmacol Exp Ther 1999; 291 (3): 1210-9
104. Kwon Y, Kamath A, Morris M. Inhibitors of P-glycoprotein-mediated daunomycin transport in rat liver canalicular membrane vesicles. J Pharm Sci 1996; 85 (9): 935-9
105. Fardel O. Up-regulation of P-glycoprotein expression in rat liver cells by acute doxorubicin treatment. Eur J Biochem 1997; 246 (1): 186-92
106. Alvaro D. The function of alkaline phosphatase in the liver: regulation of intrahepatic biliary epithelium secretory activities in the rat. Hepatology 2000; 32 (2): 174-84
107. Hirohashi T. ATP-dependent transport of bile salts by rat multidrug resistance- associated protein 3 (Mrp3). J Biol Chem 2000; 275 (4): 2905-10
108. Milne RW. Comparison of the disposition of hepatically-generated morphine-3- glucuronide and morphine-6-glucuronide in isolated perfused liver from the guinea pig. Pharm Res 1997; 14 (8): 1014-8
109. Muller M, Roelofsen H, Jansen PL. Secretion of organic anions by hepatocytes: involvement of homologues of the multidrug resistance protein. Semin Liver Dis 1996; 16 (2): 211-20
110. Patrick J, Kosoglou T, Stauber KL, et al. Disposition of the selective cholesterol absorption inhibitor ezetimibe in healthy male subjects. Drug Metab Dispos 2002; 30 (4): 430-7
111. Mikov M. The metabolism of drugs by the gut flora. Eur J Drug Metab Pharmacokinet 1994; 19 (3): 201-7
112. Hughes RD, Millburn P, Williams RT. Molecular weight as a factor in the excretion of monoquaternary ammonium cations in the bile of the rat, rabbit and guinea pig. Biochem J 1973; 136: 967-78
113. Levine W. Biliary excretion of drugs and other xenobiotics. Ann Rev Pharmacol Toxicol 1978; 18: 81-96
114. Freedman M, Somberg J. Pharmacology and pharmacokinetics of amiodarone. J Clin Pharmacol 1991; 31 (11): 1061-9

115. Vree T, Timmer C. Enterohepatic cycling and pharmacokinetics of oestradiol in postmenopausal women. J Pharm Pharmacol 1998; 50 (8): 857-64
116. Morris D. Biliary pharmacokinetics of sulbactam plus ampicillin in humans. Rev Infect Dis 1986; 8: 589-92
117. Carey MC, Duane WC. Enterohepatic circulation. In: Arias IM, Boyer JL, Fausto N, et al., editors. Liver biology and pathobiology. New York: Raven Press, 1994: 719-67
118. Rollins D, Klaassen C. Biliary excretion of drugs in man. Clin Pharmacokinet 1979; 4 (5): 368-79
119. Zhang R. A mathematical model of the kinetics and tissue distribution of 2-fluoro-beta-alanine, the major catabolite of 5-fluorouracil. Biochem Pharmacol 1993; 45 (10): 2063-9
120. Brown WR, Grodsky GM, Carbone JV. Intracellular distribution of tritiated bilirubin during hepatic uptake and excretion. Am J Physiol 1964; 159: 23-7
121. Watkins JB, Dykstra TP. Alteration in biliary excretory function by streptozotocin-induced diabetes. Drug Metab Dispos 1987; 15: 177-83
122. Crosignani A. Clinical pharmacokinetics of therapeutic bile acids. Clin Pharmacokinet 1996; 30: 333-58
123. Davis T. Pharmacokinetics and pharmacodynamics of gliclazide in caucasians and australian aborigines with type 2 diabetes. Br J Clin Pharmacol 2000; 49: 223-30
124. Westphal J, Jehl F, Adloff M, et al. Role of intrahepatic protein binding in the hepatobiliary extraction profile of cefixime in humans. Clin Pharmacol Ther 1993; 54 (5): 476-84
125. Dobrinska M. Enterohepatic circulation of drugs. J Clin Pharmacol 1989; 29: 577-80
126. Strandgarden K. Absorption and disposition including enterohepatic circulation of [14C]roquinimex after oral administration to healthy volunteers. Biopharm Drug Dispos 2000; 21 (2): 53-67
127. Brogden RN, Ward A. Ceftriaxone: a reappraisal of its antibacterial activity and pharmacokinetic properties, and an update on its therapeutic use with particular reference on once-daily administration. Drugs 1988; 35: 604-45
128. Walstad R. Pharmacokinetics of ceftazidime in patients with biliary tract disease. Eur J Clin Pharmacol 1986; 31 (3): 327-31
129. Kuhn J. Pharmacology of irinotecan. Oncology (Huntingt) 1998; 12 (8): 39-42
130. Chaudhary A. Multiple-dose lorazapam kinetics: shuttling of lorazepam glucuronide between the circulation and the gut during day- and night-time dosing intervals in response to feeding. J Pharmacol Exp Ther 1992; 267: 1034-8
131. Sorgel F. Hepatobiliary elimination of temafloxacin. Clin Pharmacokinet 1992; 22: 33-42
132. Barriere SL, Flaherty JF. Third-generation cephalosporins: a critical evaluation. Clin Pharm 1984; 3: 351-73
133. Zeuzem S. Gut-liver axis. Int J Colorectal Dis 2000; 15 (2): 59-82
134. Pentikainen P. Pharmacokinetics of tolfenamic acid: disposition in bile, blood and urine after intravenous administration to man. Eur J Clin Pharmacol 1984; 27 (3): 349-54
135. Shipkova M, Armstrong VW, Kuypers D, et al. Effect of cyclosporine withdrawal on mycophenolic acid pharmacokinetics in kidney transplant recipients with deteriorating renal function: preliminary report. Ther Drug Monit 2001; 23 (6): 717-21
136. Taras T. Clinical pharmacokinetics of toremifene. Clin Pharmacokinet 2000; 39 (5): 327-34
137. Schmidt L. The effect of selective bowel decontamination on the pharmacokinetics of mycophenolate mofetil in liver transplant recipients. Liver Transpl 2001; 7 (8): 739-42
138. Johnson A. The kinetic of mycophenolic acid and its glucuronide metabolite in adult kidney transplant recipients. Clin Pharmacol Ther 1999; 66: 492-500
139. Young M, Lettis S, Eastmond R. Concomitant administration of cholestyramine influences the absorption of troglitazone. Br J Clin Pharmacol 1998; 45: 37-40
140. O'Dwyer P. Pharmacokinetics of the chemopreventive agent oltipraz and of its metabolite M3 in human subjects after a single oral dose. Clin Cancer Res 2000; 6 (12): 4692-6
141. Frimmer M, Ziegler K. The transport of bile acids in liver cells. Biochim Biophys Acta 1988; 947: 75-99
142. Meier PJ. Transport polarity of hepatocytes. Semin Liver Dis 1988; 8: 293-307
143. Meijer DKF. Current concepts on hepatic transport of drugs. Hepatology 1987; 4: 259-68
144. Anwer MS, Hegner D. Effect of Na+ on bile acid uptake by isolated rat hepatocytes. Hoppe-Seylers Z Physiol Chem 1978; 359: 181-92
145. Stravitz RT. Induction of sodium-dependent bile acid transporter messenger RNA, protein, and activity in rat ileum by cholic acid. Gastroenterology 1997; 113: 1599-608
146. Anwer MS. Influence of side-chain charge on hepatic transport of bile acids and bile acid analogues. Am J Physiol 1985; 249: 479-88
147. Aldini R. Hepatic bile acid uptake: effect of conjugation, hydroxyl and ketogroups, and albumin binding. J Lipid Res 1982; 23: 1167-73
148. Roda A. Quantitative aspects of the interaction of bile acids with human serum albumin. J Lipid Res 1982; 23: 490-5
149. Iga T, Klaassen CD. Hepatic extraction of bile acids in rats. Biochem Pharmacol 1982; 31: 205-9
150. O'Maille ER, Richards TG, Short AH. The influence of conjugation of cholic acid on its uptake and secretion: hepatic extraction of taurocholate and cholate in the dog. J Physiol 1987; 189: 337-50
151. Hoffman NE, Isler JH, Smallwood RA. Hepatic bile acid transport: effect of conjugation and position of hydroxyl groups. Am J Physiol 1975; 229: 298-302
152. Aldini R. Uptake of bile acids by perfused rat liver: evidence of a structure-activity relationship. Hepatology 1989; 10: 840-5
153. Dyke RWV, Stephens JE, Scharschmidt BF. Bile acid transport in cultured rat hepatocytes. Am J Physiol 1982; 243: 484-92
154. Habig WH. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proc Natl Acad Sci U S A 1974; 71: 3879-82
155. Levi A, Gatmaitan Z, Arias IM. Two hepatic cytoplasmic fractions, Y and Z, and their possible role in the hepatic uptake of bilirubin, sulfobromophthalein, and other anions. J Clin Invest 1969; 48: 2156-67
156. Mishkin S, Stein L, Gatmaitan Z. The binding of fatty acids to cytoplasmic proteins: binding to Z protein in liver and other tissues of the rat. Biochem Biophys Res Commun 1972; 47: 997-1003
157. Hansen P, Thiesen H, Brodersen R. Bilirubin activity: titrimetric and 13C NMR studies. Acta Chem Scand 1979; 33: 281-93
158. Brodersen R. Bilirubin: solubility and interaction with albumin and phospholipid. J Biol Chem 1979; 254: 2364-9
159. Bonnet R, Davies J, Hursthouse M. Structure of bilirubin. Nature 1976; 262: 326-8
160. Paumgartner G, Reichen J. Kinetics of hepatic uptake of unconjugated bilirubin. Clin Sci Mol Med 1976; 51: 169-76
161. Inoue M. Metabolism and transport of amphiphatic molecules in analbuminemic rats and human subjects. Hepatology 1985; 5: 892-8

162. Stremmel W. Physicochemical and immunohistological studies of a sulfobromophthalein- and bilirubin-binding protein from rat liver plasma membranes. J Clin Invest 1983; 71: 796-805
163. Stremmel W, Berk PD. Hepatocellular uptake of sulfobromophthalein and bilirubin is selectively inhibited by an antibody to the liver plasma membrane sulfobromophthalein/-bilirubin binding protein. J Clin Invest 1986; 78: 822-6
164. Scharschmidt BF, Waggoner JG, Berk PD. Hepatic inorganic anion uptake in the rat. J Clin Invest 1975; 56: 1280-92
165. Hunton DB, Bollman JL, Hoffman HN. The plasma removal of indocyanine green and sulfobromophthalein: II. effect of dosage and blocking agents. J Clin Invest 1961; 40: 1648-55
166. Goresky CA. Bilirubin and sulfobromophthalein uptake by the liver [abstract]. Gastroenterology 1971; 60: 194
167. Berk PD, Potter BJ, Stremmel W. Role of plasma membrane ligand-binding proteins in the hepatocellular uptake of albumin-bound organic anions. Hepatology 1987; 7: 165-76
168. Litwack G, Ketterer B, Arias IM. A hepatic protein which binds steroids, bilirubin, carcinogens, and a number of exogenous anions. Nature 1971; 234: 466-7
169. Davies N, Skjodt N. Choosing the right nonsteroidal anti-inflammatory drug for the right patient: a pharmacokinetic approach. Clin Pharmacokinet 2000; 38 (5): 377-92
170. Jarvis M. Clinical pharmacokinetics of tricyclic antidepressant overdose. Psychopharmacol Bull 1991; 27 (4): 541-50
171. Kumar R. Vitamin D metabolism and mechanisms of calcium transport. J Am Soc Nephrol 1990; 1 (1): 30-42
172. Weiner IM. Organic acids and bases and uric acid. In: Seldin DW, Giebisch GL, editors. The kidney, physiology and pathophysiology. New York: Raven Press, 1985: 1703-24
173. Lauterbach F. Intestinal secretion of organic ions and drugs. In: Kramer M, editor. Intestinal permeation. Amsterdam: Excerpta Medica, 1977: 173-95
174. Suzuki H. Transport of cimetidine by the rat choroid plexus in vitro. J Pharmacol Exp Ther 1986; 239: 927-35
175. Meijer DKF. Carrier-mediated transport of drugs in the hepatic distribution and elimination of drug, with special reference to the category of organic cations. J Pharmacokinet Biopharm 1990; 18: 35-70
176. Neef C, Meijer DKF. Structure-pharmacokinetics relationship of quaternary ammonium compounds: correlation of physicochemical and pharmacokinetic parameters. Naunyn Schmiedebergs Arch Pharmacol 1984; 328: 111-8
177. Feitsma KG. Unequal disposition of enantiomers of the organic cation oxyphenonium in the rat isolated perfused liver. J Pharm Pharmacol 1989; 41: 27-31
178. Neef C, Oosting R, Meijer DKF. Structure-pharmacokinetics relationship of quaternary ammonium compounds: elimination and distribution characteristics. Naunyn Schmiedebergs Arch Pharmacol 1984; 328: 103-10
179. Hughes RD, Millburn P, Williams RT. Biliary excretion of some diquaternary ammonium cations in the rat, guinea pig and rabbit. Biochem J 1973; 136: 979-84
180. Meijer DKF, Weitering JG, Vonk RJ. Hepatic uptake and biliary excretion of d-tubocurarine and trimethylcurarine in the rat in vivo and in isolated perfused rat livers. J Pharmacol Exp Ther 1976; 198: 229-39
181. Weitering JG. On the localization of d-tubocurarine in rat liver lysosomes in vivo by electron microscopy and subcellular fractionation. Naunyn-Schmiedebergs Arch Pharmacol 1975; 289: 251-6
182. Westra P. Mechanisms underlaying the prolonged duration of action of muscle relaxants caused by extrahepatic cholestasis. Br J Anaesth 1981; 58: 217-27
183. Dunkerly R. Gastric and biliary excretion of meperidine in man. Clin Pharmacol Ther 1977; 20: 546-51
184. Sandberg AA, Slaunwhite WR. Studies on phenolic steroids in human subjects: II. the metabolic fate and hepato-biliary-enteric circulation of 14C-estrone and 14C-estradiol in women. J Clin Invest 1957; 36: 1266-78
185. Sandberg AA, Slaunwhite WR. Studies on phenolic steroids in human subjects: VII. metabolic fate of estriol and its glucuronide. J Clin Invest 1965; 44: 694-702
186. Peterson RE. The physiological disposition and metabolic fate of hydrocortisone in man. J Clin Invest 1955; 34: 1779-94
187. Sandberg AA, Slaunwhite WR. Metabolism of 4-14C-testosterone in human subjects: I. Distribution in bile, blood, faeces and urine. J Clin Invest 1956; 35: 1331-9
188. Adlercreutz H. Biliary excretion and intestinal metabolism of progesterone and estrogens in man. J Steroid Biochem 1980; 13: 231-44
189. Ballatori N, Clarkson TW. Biliary secretion of glutathione and of glutathione-metal complexes. Fundam Appl Toxicol 1985; 5: 816-31
190. Alexander J, Aaseth J, Mikalsen A. Excretion of lead in rat bile - the role of glutathione. Acta Pharmacol Toxicol 1986; 59 (7): 486-9
191. Gregus Z, Varga F. Role of glutathione and hepatic glutathione S-transferase in the biliary excretion of methyl mercury, cadmium and zinc: a study with enzyme inducers and glutathione depletors. Acta Pharmacol Toxicol 1985; 56: 398-403
192. Norseth T. Biliary excretion of chromium in the rat: a role of glutathione. Acta Pharmacol Toxicol 1982; 51: 450-5
193. Chipman JK. Bile as a source of potential reactive metabolites. Toxicology 1982; 25: 99-111
194. Xiong H. Altered hepatobiliary disposition of acetaminophen glucuronide in isolated perfused livers from multidrug resistance-associated protein 2-deficient TR (-) rats. J Pharmacol Exp Ther 2000; 295 (2): 512-8
195. Oude-Elferink R. Hepatobiliary secretion of organic compounds: molecular mechanisms of membrane transport. Biochim Biophys Acta (Rev Biomembr) 1995; 1241: 215-68
196. Keppler D, Konig J. Expression and localization of the conjugate export pump encoded by the MRP2 (cMRP/cMOAT) gene in liver. FASEB J 1997; 11: 509-16
197. Kato Y, Igarashi T, Sugiyama Y. Both cMOAT/MRP2 and another unknown transporter(s) are responsible for the biliary excretion of glucuronide conjugate of the nonpeptide angiotensin II antagonist, telmisartan. Drug Metab Dispos 2000; 28 (10): 1146-8
198. Madon J. Functional expression of the rat liver canalicular isoform of the multidrug resistance-associated protein. FEBS Lett 1997; 406: 75-8
199. Niinuma K. Primary active transport of organic anions on bile canalicular membrane in humans. Am J Physiol 1999; 276 (5 Pt 1): G1153-64
200. Greenberger NJ, Thomas FB. Biliary excretion of 3H-digitoxin: modification by bile salts and phenobarbital. J Lab Clin Med 1973; 81: 241-51
201. Goresky CA. The enhancement of maximal bilirubin excretion with taurocholate-induced increments in bile flow. Can J Physiol Pharmacol 1974; 52: 389-403
202. Gregus Z, Fischer E, Varga F. Effect of cholestyramine-induced bile acid depletion on the hepatobiliary transport of cholephilic organic anions in rats. Arch Int Pharmacodyn Ther 1980; 245: 311-22
203. Gregus Z. Comparison of the effects of cholestyramine and aluminium hydroxide on the biliary bile acid excretion in rats:

an experimental model for the depletion of bile acids in bile. Acta Biol Med Ger 1980; 39: 705-9
204. Watkins JB, Noda H. Biliary excretion of organic anions in diabetic rats. J Pharmacol Exp Ther 1986. 239: 467-73
205. Rozman K. Hexadecane enhances non-biliary, intestinal excretion of stored hexachlorophenol by rats. Toxicology 1982; 24: 107-13
206. Cohn WJ. Treatment of chlordecone (Kepone) toxicity with cholestyramine. N Engl J Med 1978; 298: 243-8
207. Cohn WJ. Distribution and excretion of kepone in humans [abstract]. Gastroenterology 1976; 71: 901
208. Boyland JJ, Egle JL, Guzelian PS. Cholestyramine: use a new therapeutic approach for chlordecone (Kepone) poisoning. Science 1978; 199: 893-5
209. Guzelian PS. Comparative toxicology of chlordecone (Kepone) in humans and experimental animals. Annu Rev Pharmacol Toxicol 1982; 22: 89-113
210. Ballhorn L. Cholestyramine enhances fecal elimination of pentachlorophenol in rhesus monkey. Chemospere 1981; 10: 877-88
211. Rozman K. The effect of cholestyramine on the disposition of pentachlorophenol in Rhesus monkeys. J Toxicol Environ Health 1982; 10: 277-83
212. Billiau A, Bosch JVD. The influence of cholestyramine on the intestinal absorption of glycocholic acid. Arch Int Pharmacodyn Ther 1964; 150: 46-51
213. Siegers CP, Rozman K, Klaassen CD. Biliary excretion and enterohepatic circulation of paracetamol in the rat. Xenobiotica 1983; 13: 591-6
214. Siegers CP, Moller-Hartmann W. Cholestyramine as an antidote against paracetamol-induced hepato- and nephrotoxicity in the rat. Toxicol Lett 1989; 47: 179-84
215. Neuvonen PJ, Elonen E. Effect of activated charcoal on absorption and elimination of phenobarbitone, carbamazepine and phenylbutazone in man. Eur J Clin Pharmacol 1980; 17: 51-7
216. Elomaa K. The possible role of enterohepatic cycling on bioavailability of norethisterone and gestodene in women using combined oral contraceptives. Contraception 2001; 63 (1): 13-8
217. Heimer GM, Englund DE. Enterohepatic recirculation of oestriol: inhibition by activated charcoal. Acta Endocrinol (Copenh) 1986; 113 (1): 93-5
218. Gregus Z, Klaasen CD. Enterohepatic circulation of toxicants. In: Rozman K, Hanninen O, editors. Gastrointestinal toxicology. New York: Elsevier, 1986: 57-118
219. Caldwell J. Comparative aspects of detoxication in mammals. In: Jacoby WB, editor. Enzymatic basis of detoxication. New York: Academic Press, 1980: 85-114
220. Gregus Z. Hepatic phase I and phase II biotransformation in quail and trout: comparison to other species commonly used in toxicity testing. Toxicol Appl Pharmacol 1983; 67: 430-41
221. Laitinen M, Watkins III JB. Mucosal biotransformation. In: Rozman K, Hanninen O, editors. Gastrointestinal toxicology. Amsterdam: Elsevier, 1986: 169-92
222. Scheline RR. Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacol Rev 1973; 25: 451-523
223. Drasar BS, Hill MJ. Human intestine flora. New York: Academic Press, 1974
224. Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos 1995; 16 (5): 351-80
225. Suchy FJ, Heubi JE, Balistreri WF. The enterohepatic circulation of bile acids in suckling and weandling rats [abstract]. Gastroenterology 1981; 80: 1351
226. Klaassen CD. Hepatic excretory function in the newborn rat. J Pharmacol Exp Ther 1973; 184: 721-8
227. Kleeberg U. Effect of 3-methylcholanthrene on the biliary excretion of bromsulphthalein and eosine in newborn rats. Acta Physiol Acad Sci Hung 1979; 53: 363-7
228. McMahon TF, Beierschmitt WP, Weiner M. Changes in phase I and phase II biotransformation with age in male Fischer 344 rat colon: relationship to colon carcinogenesis. Cancer Lett 1987; 36: 273-82
229. Greenberger DJ, Engelkling LR. Enterohepatic circulation of lorazepam and acetaminophen conjugates in ponies. J Pharmacol Exp Ther 1988; 244: 674-9
230. Meuldermans W. Excretion and biotransformation of alfentanil and sufentanil in rats and dogs. Drug Metab Dispos 1987; 15: 905-13
231. Solymoss B, Zsigmond G. Effects of various steroids on the hepatic glucuronidation and biliary excretion of bilirubin. Can J Physiol Pharmacol 1973; 51: 319-23
232. Levine WG. The role of the hepatic endoplasmic reticulum in the biliary excretion of foreign compounds by the rat: the effect of phenobarbitone and SKF525-A (diethylaminoethyl diphenylpropylacetate). Biochem Pharmacol 1970; 19: 235-44
233. Fawaz F. Influence of poly (DL-lactide) nanocapsules on the biliary clearance and enterohepatic circulation of indomethacin in the rabbit. Pharm Res 1993; 10 (5): 750-6
234. Sabordo L. Hepatic disposition of the acyl glucuronide 1-O-gemfibrozil-beta-D-glucuronide: effects of clofibric acid, acetaminophen, and acetaminophen glucuronide. J Pharmacol Exp Ther 2000; 295 (1): 44-50
235. Kim R, Leake B, Cvetkovic M, et al. Modulation by drugs of human hepatic sodium-dependent bile acid transporter (sodium taurocholate cotransporting polypeptide) activity. J Pharmacol Exp Ther 1999; 291 (3): 1204-9
236. Dantzig A. Reversal of multidrug resistance by the P-glycoprotein modulator, LY335979, from the bench to the clinic. Curr Med Chem 2001; 8 (1): 39-50
237. Morikawa A. Biliary excretion of 17beta-estradiol 17beta-D-glucuronide is predominantly mediated by cMOAT/MRP2. Pharm Res 2000; 17 (5): 546-52
238. Yam J, Roberts RJ. Hepatic uptake of foreign compounds: influence of acute extrahepatic biliary obstruction. J Pharmacol Exp Ther 1977; 200: 425-33
239. Klaassen CD. Comparison of the effects of two-thirds hepatectomy and bile-duct ligation on hepatic excretory function. J Pharmacol Exp Ther 1974; 191: 25-31
240. Siegers CP. Effects of liver injury and cholestasis on microsomal enzyme activities and in vivo metabolism of halothane, enflurane and methoxyflurane. Xenobiotica 1981 11: 293-9
241. Siegers CP, Klaassen CD. Biliary excretion of acetaminophen in ureter-ligated rats. Pharmacology 1984; 29: 177-80
242. Kullak-Ublick G, Beuers U, Paumgartner G. Hepatobiliary transport. J Hepatol 2000; 32 (1): 3-18
243. Lindell T. Specific inhibition of nuclear RNA polymerase II by α-amanitin. Science 1970; 170: 447-9
244. Reichen J. Decreased uptake of taurocholate and ouabain by hepatocytes isolated from cirrhotic rat liver. Hepatology 1987; 7: 67-70
245. Beuers U. Tauroursodeoxycholic acid stimulates hepatocellular exocytosis and mobilizes extracellular Ca++ mechanisms defective in cholestasis. J Clin Invest 1993; 92: 2984-93
246. Wood A. Intact hepatocyte theory of impaired drug metabolism in experimental cirrhosis in the rat. Gastroenterology 1979; 76: 1358-62

247. Varin F, Huet PM. Hepatic microcirculation in the perfused cirrhotic rat liver. J Clin Invest 1991; 76: 1904-12
248. Sherman I, Pappas S, Fisher M. Hepatic microvascular changes associated with development of liver fibrosis and cirrhosis. Am J Physiol 1990; 258: 460-5
249. Milani S. Differential expression of matrix-metalloproteinase-1 and -2 genes in normal and fibrotic human liver. Am J Pathol 1994; 144: 528-37
250. Murawaki Y. Serum collagenase activity reflects the amount of liver collagenase in chronic carbon tetrachloride-treated rats. Res Commun Chem Pathol Pharmacol 1994; 84: 63-72
251. Krähenbühl S, Stucki J, Reichen J. Reduced activity of the electron transport chain in liver mitochondria isolated from rats with secondary biliary cirrhosis. Hepatology 1992; 15: 1160-6
252. Gebhart A, Brabec M. Carbon tetrachloride depresses hepatic phospholipid synthesis in rats. Toxicol Lett 1985; 24: 71-8
253. Muriel P, Favari L, Soto C. Erythrocyte alterations correlate with CCl4 and biliary obstruction-induced liver damage in the rat. Life Sci 1993; 52: 647-55
254. Yahuaca P. Cryptic adenosine triphosphatase activities in plasma membranes of CCl4-cirrhotic rats. Lab Invest 1985; 53: 541-5
255. Hong S, Chung S, Shim C. Functional impairment of sinusoidal membrane transport of organic cations in rats with CCl4-induced hepatic failure. Pharm Res 2000; 17 (7): 833-8
256. Feuer G, Fonzo CD. Intrahepatic cholestasis: a review of biochemical-pathological mechanisms. Drug Metabol Drug Interact 1992; 10: 1-161
257. Reichen J. Mechanisms of cholestasis. In: Tavoloni N, Berk P, editors. Hepatic transport and bile secretion: physiology and pathophysiology. New York: Raven Press, 1993: 665-72
258. Schaffner F, Popper H. Classification and mechanism of cholestasis. In: Wright R, editor. Liver and biliary disease: pathophysiology, diagnosis, management. London: WB Saunders Company Ltd, 1979: 296-323
259. Phillips M, Latham P, Poucell S. Electron microscopy of human liver disease. In: Schiff L, Schiff E, editors. Diseases of the liver. Philadelphia: JB Lippincott Co, 1987: 47-76
260. Plaa G, Priestly B. Intrahepatic cholestasis induced by drugs and chemicals. Pharmacol Rev 1976; 28: 207-73
261. Becker B, Plaa G. Quantitative and temporal delineation of various parameters of liver dysfunction due to alpha-naphthylisothiocyanate. Toxicol Appl Pharmacol 1965; 7: 708-18
262. Indacochea-Redmond N, Plaa G. Functional aspects of alpha-naphthylisothiocyanate in various species. Toxicol Appl Pharmacol 1971; 19: 71-80
263. Lock S, Lavigne J, Plaa G. The effect of alpha-naphthylisothiocyanate on bile secretion prior to and during the onset of cholestasis in the rat. Toxicol Lett 1982; 10: 427-35
264. Wieland T. Identity of hepatic membrane transport systems for bile salts, phalloidin, and antamanide by photoaffinity labelling. Proc Natl Acad Sci U S A 1984; 81: 5232-6
265. Kramer W. Bile-salt-binding polypeptides in plasma membranes of hepatocytes revealed by photoaffinity labelling. Eur J Biochem 1982; 129: 13-24
266. Gabbiani G. Phalloidin-induced hyperplasia of actin filaments in rat hepatocytes. Lab Invest 1975; 53: 562-9
267. Tuchweber B, Gabbiani G. Phalloidin-induced hyperplasia of actin microfilaments in rat hepatocytes. In: Preisig R, Bircher J, Paumgartner G, editors. The liver: quantitative aspects of structure and function. Basel: S Karger AG, 1976: 84-90
268. Jansen P. The pathophysiology of cholestasis with special reference to primary biliary cirrhosis. Baillieres Best Pract Res Clin Gastroenterol 2000; 14 (4): 571-83
269. Liber M. The incidence of gallstones and their correlation with other diseases. Am Surg 1952; 135: 394-405
270. Feldman M, Feldman M. The incidence of cholelithiasis, cholesterosis and liver disease in diabetes mellitus: an autopsy study. Diabetes 1954; 3: 3505-9
271. Goldstein M, Schein C. The significance of biliary tract disease in the diabetic: its unique features. Am J Gastroenterol 1963; 39: 630-4
272. Grant M, Duthie S. Conjugation reactions in hepatocytes isolated from streptozotocin-induced diabetic rats. Biochem Pharmacol 1987; 36: 3647-55
273. Salmela P, Sotaniemi E, Pelkonen R. The evaluation of the drug-metabolizing capacity in patients with diabetes mellitus. Diabetes 1980; 29: 788-94
274. Watkins J, Dykstra T. Alterations in biliary excretory function by streptozotocin-induced diabetes. Drug Metab Dispos 1987; 15: 177-83
275. Carnovale C, Marinelli R, Rodriguez-Garay E. Bile flow decrease and altered bile composition in streptozotocin-treated rats. Biochem Pharmacol 1986; 35: 2625-8
276. Berk P, Stump D. Mechanisms of cellular uptake of long chain free fatty acids. Mol Cell Biochem 1999; 192: 17-31
277. Danysz A, Wisniewski K. The influence of insulin on drug passage into the tissues. Arch Int Pharmacodyn Ther 1965; 158: 30-8
278. Jefferson L. Diabetes induced alterations in liver protein synthesis. J Biol Chem 1983; 258: 1369-75
279. Huang Z, Murakami T, Okochi A. Expression and function of P-glycoprotein in rats with glycerol-induced acute renal failure. Eur J Pharmacol 2000; 406 (3): 453-60
280. Zollner G, Fickert P, Zenz R. Hepatobiliary transporter expression in percutaneous liver biopsies of patients with cholestatic liver diseases. Hepatology 2001; 33 (3): 633-46
281. Vree T, Ven AVD. Clinical consequences of the biphasic elimination kinetics for the diuretic effect of furosemide and its acyl glucuronide in humans. J Pharm Pharmacol 1999; 51 (3): 239-48
282. Bullingham R, Nicholls A, Kamm B. Clinical pharmacokinetics of mycophenolate mofetil. Clin Pharmacokinet 1998; 34 (6): 429-55
283. Funaki T. Enterohepatic circulation model for population pharmacokinetic analysis. J Pharm Pharmacol 1999; 10: 1143-8
284. Shepard T, Reuning R, Aarons L. Interpretation of area under the curve measurements for drugs subject to enterohepatic cycling. J Pharm Sci 1985; 2: 227-8
285. Shepard T, Gannaway D, Lockwood G. Accumulation and time to steady state for drugs subject to enterohepatic cycling: a stimulation study. J Pharm Sci 1985; 12: 1331-3
286. Shepard T, Reuning R, Aarons L. Estimation of area under the curve for drugs subject to enterohepatic cycling. J Pharmacokinet Biopharm 1985; 6: 589-608
287. Dahlstrom B, Paalzow L. Pharmacokinetic interpretation of the enterohepatic recirculation and first-pass elimination of morphine in the rat. J Pharmacokinet Biopharm 1978; 6: 505-19
288. Pollack G, Brouwer K. Physiologic and metabolic influences on enterohepatic recirculation: simulations based upon the disposition of valproic acid in the rat. J Pharmacokinet Biopharm 1991; 2: 189-225
289. Ouellet D, Pollack G. Biliary excretion and enterohepatic recirculation of morphine-3-glucuronide in rats. Drug Metab Dispos 1995; 23: 478-784
290. Steimer J, Plusquellec Y, Guillaume A, et al. A time-lag model for pharmacokinetics of drugs subject to enterohepatic circulation. J Pharm Sci 1982; 3: 297-302

291. Peris-Ribera J, Kanba M, Toyoda Y, et al. General treatment of the enterohepatic recirculation of drugs and its influence on the area under the plasma level curves, bioavailability, and clearance. Pharm Res 1992; 10: 1306-13
292. Wang Y, Reuning R. An experimental design strategy for quantitating complex pharmacokinetic models: enterohepatic circulation with time-varying gallbladder emptying as an example. Pharm Res 1992; 2: 169-77
293. Colburn W, Lucek R. Noncompartmental area under the curve determinations for drugs that cycle in the bile. Biopharm Drug Dispos 1988; 9 (5): 465-75
294. Semmes R, Shen D. A reversible clearance model for the enterohepatic circulation of drug and conjugate metabolite pair. Drug Metab Dispos 1990; 18 (1): 80-7
295. Veng-Pedersen P, Miller R. Pharmacokinetics of doxycycline reabsorption. J Pharm Sci 1980; 69: 204-398
296. Plusquellec Y, Houin G. Drug recirculation model with multiple cycles occurring at unequal time intervals. J Biomed Eng 1992; 6: 521-6
297. Plusquellec Y, Arnaud R, Saivin S, et al. Enterohepatic recirculation of the new antihypertensive drug UP 269-6 in humans: a possible model to account for multiple plasma peaks. Arzneimittel Forschung 1998; 48: 138-44
298. Shepard T. Mean residence time for drugs subject to enterohepatic cycling. J Pharmacokinet Biopharm 1989; 3: 327-45
299. Yamaoka K, Kanba M, Toyoda Y, et al. Analysis of enterohepatic circulation of cefixime in rat by fast inverse Laplace transform (FILT). J Pharmacokinet Biopharm 1990; 6: 545-59
300. Yamaoka K. Disposition analysis by fast inverse Laplace transform. Yakugaku Zasshi 1992; 112 (8): 503-15
301. Yasui H, Yamaoka K, Nakagawa T. Moment analysis of stereoselective enterohepatic circulation and unidirectional chiral inversion of ketoprofen enantiomers in rat. J Pharm Sci 1996; 85: 580-5
302. Fukuyama T. A new analysis method for disposition kinetics of enterohepatic circulation of diclofenac in rats. Drug Metab Dispos 1994; 22: 479-85
303. Ploeger B, Mesinga T, Sips A, et al. A human physiologically-based model for glycyrrhzic acid, a compound subject to presystemic metabolism and enterohepatic cycling. Pharm Res 2000; 17 (12): 1516-25
304. Wang Y, Roy A, Sun L, et al. A double-peak phenomenon in the pharmacokinetics of alprazolam after oral administration. Drug Metab Dispos 1999; 27 (8): 855-9
305. Veng-Pedersen P, Miller R. Pharmacokinetics and bioavailability of cimetidine in humans. J Pharm Sci 1980; 69 (4): 394-8
306. Abshagen U. Effect of enterohepatic circulation on the pharmacokinetics of spironolactone in man. Naunyn Schmiedebergs Arch Pharmacol 1977; 300: 281-7
307. Duggan D. The disposition of sulindac. Clin Pharm Ther 1977; 21: 326-35
308. Hoglund P, Ohlin M. Effect modelling for drugs undergoing enterohepatic circulation. Eur J Drug Metab Pharmacokinet 1993; 18 (4): 333-8
309. Serra M, Caballero A, Del Olmo JA, et al. Maximal biliary transport of sulfobromophthalein in patients with a T-tube placed in the common bile duct. Eur J Drug Metab Pharmacokinet 1997; 22 (2): 135-9
310. Fleuren H, Wissen CV-V, Thien T. Biliary excretion of chlorthalidone in humans. Biopharm Drug Dispos 1980; 1 (3): 103-10
311. Kreek M. Biliary secretion of methadone and methadone metabolites in man. Res Commun Chem Pathol Pharmacol 1980; 29 (1): 67-78
312. Buchan PC, Klopper A. Enterohepatic circulation of oestriol: a study of the effects of ampicillin on plasma oestriol levels. Br J Obstet Gynaecol 1979; 86 (9): 713-6
313. Shenfield GM, Griffin JM. Clinical pharmacokinetics of contraceptive steroids: an update. Clin Pharmacokinet 1991; 20 (1): 15-37
314. Shenfield G. Oral contraceptives: are drug interactions of clinical significance? Drug Saf 1993; 9 (1): 21-37
315. Weisberg E. Interactions between oral contraceptives and antifungals/antibacterials: is contraceptive failure the result? Clin Pharmacokinet 1999; 36 (5): 309-13
316. Lin J, Lu A. Interindividual variability in inhibition and induction of cytochrome p450 enzymes. Annu Rev Pharmacol Toxicol 2001; 41: 535-67

Correspondence and offprints: Dr *Michael S. Roberts*, Department of Medicine, University of Queensland, Princess Alexandra Hospital, Brisbane, QLD 4102, Australia.
E-mail: mroberts@medicine.pa.uq.edu.au