4.06 Drug Classification and Drug Disposition Prediction

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For a drug to be effective, it must become available at the target site. However, target sites are often inaccessible and it is necessary to dose from a convenient location. The drug must therefore be absorbed from a dosing site, often from the gastrointestinal tract since many drugs are orally administered. Subsequently, the drug must distribute throughout the body until some drug reaches the target site. However, because drugs are xenobiotics, the body will attempt to protect itself by eliminating the drug, either by directly excreting it as the parent drug, usually in the urine or the bile, or by metabolizing it to something generally easier to excrete. The combination of these processes is given the acronym ADME and is regarded as drug disposition. Pharmaceutical scientists attempt to optimize each of these processes to ensure that drugs reach their targets safely and efficaciously, yet with few off-target effects, and can be dosed on a convenient schedule. This is affected by physiological factors and chemical properties of drugs. Pharmaceutical scientists have invested a lot of research into understanding these properties in order to predict disposition prior to developing a drug and testing it in humans.

ADME can be assessed with plasma concentrations over time. The area under this curve (AUC) represents drug exposure, which is generally correlated with therapeutic response. However, for some drugs, antibiotics, for example, the maximum concentration ($C_{\text{max}}$) may better predict therapeutic response. In other cases, a relatively immediate response is required, when using sleep-inducing hypnotics, for instance. Drugs of this nature must enter the systemic circulation and be able to access their target quickly, which means that the time to maximum concentration ($t_{\text{max}}$) must be short and that the drug must have properties that allow rapid distribution to the target tissue (e.g., the brain).

The primary goal of pharmacokinetics in therapeutics is to select an appropriate dose. AUC is the clinical output from which clearance, bioavailability, and volume of distribution, the primary pharmacokinetic parameters, can be calculated. Clearance and bioavailability contribute to dose and regimens selection. When conditions are nonnormal, pharmaceutical companies, physicians, or pharmacists must know how to adjust the dose. Changes in clearance and bioavailability are reflected in changes in AUC. By understanding how different factors can impact dispositional factors, we can deduce the mechanism of the observed exposure change and appropriately amend the treatment regimen.

Humans all have unique physiology, which is why some people can drink milk, eat wheat, or even smoke cigarettes for decades without a problem, while milk consumption can cause intestinal distress, wheat consumption can affect nutrient absorption, and cigarette smoke can lead to cancer in other people. Drugs also exhibit marked variability. Consider the chemotherapeutic drug cyclophosphamide, which is used to treat certain forms of leukemia: Some people experience extreme nausea, while others tolerate the treatment with no disturbance in their daily life. The dispositional profile of a drug also varies between people. The same dose can deliver poor systemic concentrations and lack of efficacy in some patients but overdose a second patient and leave them susceptible to toxicity. Pharmaceutical scientists and regulatory agencies recognize factors that increase this risk in variability and spend much time and money understanding the important contributing dispositional factors.

Much of the disposition is regulated by the active impact of metabolizing enzymes and drug transporters. Other factors such as blood flow, membrane permeability, pH, protein binding, and endogenous substances all play a significant role as well. There is marked variation in the expression and activity of metabolizing enzymes and drug transporters in healthy individuals. The impact of this variation is studied in a field called pharmacogenomics. In this article, we will discuss how pharmacists and physicians can garner this knowledge to predict how a drug will respond in an individual with a specific genetic profile. Even when a patient has a normal wild-type genetic variation of metabolizing enzymes and transporters, other drugs, supplements, and even food can alter the activity of metabolizing enzymes and transporters. Eating food also results in transient physiological changes that may impact how a drug is absorbed and disposed compared with when a drug is taken without food.

In renal disease, the ability to eliminate and clear drugs is directly impacted by decreased renal function. However, chronic kidney disease (CKD) can also impact drug disposition by affecting the concentrations of endogenous compounds, which can interact with drugs, or affecting physiological aspects like blood flow or protein binding. In celiac disease, structural and physiological changes and decreased CYP3A expression\(^1\) in patients may potentially alter drug absorption.\(^2\) Other diseases including, but certainly not limited to, diabetes, cystic fibrosis, cancer, and cardiac disease can significantly impact a drug’s disposition.

Understanding how physiological systems change with disease and how interindividual differences, either biological or due to external factors such as copharmacy, will impact drug disposition helps pharmaceutical companies develop safer, more efficacious dosing regimens with a clearer understanding of necessary alterations in dosing. In fact, pharmacokinetics was once a leading cause of drug failure during development, but is no longer a significant concern due to our improved understanding of drug disposition.\(^3,4\)

During development, it is advantageous to harness drug disposition predictions using preclinical models, including in silico, in vitro, or in vivo animal studies. Information can be gathered from predictive models more quickly and cheaply than from humans and mitigates unnecessary risk early in development. While evaluating some aspects of drug disposition, including the contribution of metabolizing enzymes or drug transporters, a negative result may obviate the need for additional studies in humans.

**4.06.2 Current Issues and Challenges in Pharmacokinetics**

Drugs can be administered through a myriad of routes. These include topical, optical, or oral administration or injections (including intramuscular, subcutaneous, or intravenous). Intravenously administered drugs are directly administered to the blood, and
therefore, the entire dose is available for distribution in the body. However, drugs administered by any other route must pass some barriers before entering the blood, in the process called absorption. Naturally, a percentage of the drug cannot pass through the barriers and is lost between the site of absorption and the blood. This loss depends on both the barrier that must be crossed and properties of the drug.

Some membranes are leakier than others, while some are perfused by higher blood flow. Other differences include higher or lower fat content, pH differences, and differences in the expression of transporters and metabolizing enzymes. This impacts how a drug can be administered. For instance, insulin is generally administered subcutaneously, but can also be inhaled because of the relatively high permeability of the alveolar epithelium. Insulin, however, cannot be given orally due to degradation by proteolytic enzymes and an inability to be transported in and through the gastrointestinal tract.

Metabolism dominated the understanding of drug disposition for a very long time. Eventually, scientists began to realize that drug transporters, initially called phase III metabolism, had an equally important role in drug absorption, distribution, and elimination. Hundreds of drug transporters have been identified in humans, but currently, at least seven are considered clinically important in regulating drug disposition. The FDA 2012 guidance on drug interactions recommends determining if a drug is a substrate, inhibitor, or inducer of P-gp and BCRP for all drugs, OATP1B1 and OATP1B3 if a drug is a substrate, and OAT1, OAT3, and OCT2 when a drug is renally eliminated. However, other transporters such as MATEs are considered clinically relevant by the International Transporter Consortium (ITC), who recommends prospectively studying MATE interactions. The ITC recommends that MRP2 and BSEP be evaluated in retrospective studies depending on clinical and preclinical observations. Other transporters such as OATP2B1, ENTs, and PEPTs are also considered clinically relevant. Other drugs, high-fat food or components in food, endogenous substrates, disease, and genetics can alter the function of these and other transporters. Since transporters often highly regulate drug exposure in the systemic circulation and tissues as well as play a role in drug elimination, when their function is altered, the safety and efficacy of a drug can be compromised. We will discuss when transport is relevant to the clinical outcome of a drug, as well as many cases when it is not.

4.06.2.1 Predicting Oral Absorption and Availability

Most drugs are preferably administered by the oral route to increase patient compliance and facilitate delivery and packaging. Before a drug can enter the systemic circulation after oral dosing, it must (a) be absorbed in the gut, where it may be affected by apical uptake and efflux transporters, (b) pass through gut epithelial cells (enterocytes) where it may be metabolized, and then (c) escape from metabolism or biliary elimination in hepatocytes. The combination of these processes determines the bioavailability of the drug or the fraction of the dose that enters systemic circulation.

In humans, bioavailability (F) can be readily measured by comparing exposure from intravenous and oral dosage forms: $F = \frac{AUC_{oral}}{AUC_{iv}}$, correcting for dose if necessary. However, it is very difficult to predict the fraction of the bioavailability due to absorption ($F_a$) and thus the extent of absorption. Hepatic bioavailability ($F_{H}$) can be estimated when an intravenous dose is given and total and renal clearances are measured ($F_{H} = 1 - \frac{CL_{H}}{Q_{H}}$, where hepatic blood clearance ($CL_{H}$) equals total blood clearance (CL) minus renal blood clearance ($CL_{R}$) and $Q_{H}$ is the estimate of hepatic blood flow rate). However, then, determining the fraction of the dose that is absorbed requires an estimate of the fraction of the dose that escapes gut metabolism since $F = F_a \times F_G \times F_{H}$. The gut bioavailability, requires invasive methods such as sampling from the portal vein. The rate and extent of absorption depend upon the physicochemical properties of the active component in a drug product, the formulation and release of the drug from the product, and physiological traits of the gastrointestinal system.

Additionally, microbiotic metabolism and luminal degradation of drugs can reduce the proportion of the parent drug that is available for absorption. Even after initial absorption, a drug can be effluxed by transporters in the enterocytes back into the gut lumen, effectively reducing absorption.

Drug absorption can be mediated through passive or active permeation across (primarily) enterocytes. Passively absorbed compounds can be transcellularly diffused (through the cell) or paracellularly diffused (between the cells). Active permeation requires the intercession of drug transporters, which can move a drug across either side of a polarized cell membrane. Active transporters are responsible for either bringing the drug into a cell or ejecting it from a cell.

4.06.2.1.1 Physicochemical determinants of absorption

It is generally presumed that lipophilicity correlates with cell permeability, within a reasonable boundary and when considering structurally similar compounds. (Lipophilicity is derived from the Greek “fat-loving.” Highly lipophilic molecules are often nonpolar and prefer to partition into nonpolar versus polar phases.) In 1997, Lipinski et al. developed a set of rules that aided in understanding the properties of drugs that are readily absorbed. Poor absorption is more likely when a drug has greater than 5 hydrogen bond donors (OH and NH), a molecular weight > 500 Da, $\log P_a > 5$, or greater than 10 hydrogen bond acceptors (oxygens and nitrogens). However, this rule does not apply when transporters mediate drug absorption.

4.06.2.1.1 Passive absorption

Passive absorption generally refers to permeation of compounds that have properties that allow them to cross through a cell (transcellular). Drugs can only pass transcellularly if they are small and relatively lipophilic. Compounds that instead pass between cells (paracellular permeation) may be small and hydrophilic.
4.06.2.1.2  Active absorption

Most compounds are known or presumed to be substrates of active transporters, even if they can also be passively absorbed. However, highly permeable, highly soluble compounds are not dependent upon active transporters for their absorption even if they are substrates and will not be impacted by disruptions to their function. This will be discussed in great detail in section "Biopharmaceutics Drug Disposition Classification System."

4.06.2.1.2  In vitro predictions

The Biopharmaceutics Classification System (BCS), as we will discuss later, opened the door to predicting absorption with surrogate in vitro systems, such as Caco-2 and Madin–Darby canine kidney (MDCK). The Biopharmaceutics Drug Disposition Classification System (BDDCS) allowed us to do the same with artificial membranes such as parallel artificial membrane permeability assay (PAMPA). Compounds with a high in vitro permeability rate are expected to be well absorbed.

Caco-2, an immortal cell line derived from colorectal adenocarcinoma cells, comes from human enterocytes. They confer the advantage of being human in nature with a microvillus surface. However, these cells take 2–3 weeks to culture and even then do not fully express transporters or metabolizing enzymes. Additionally, tight junctions predominate and resistance is high compared with in vivo morphology.11 This can lead to significant underprediction of permeability rate and absorption. The in vitro lack of expression of highly expressed transporters in humans can greatly underpredict the extent of absorption.12

MDCK cells are immortal cells that come from the kidney of dogs. These cells have a shorter culture time than Caco-2 and have lower resistance than Caco-2 cells, a condition more similar to the human gut. However, these cells are not human in nature and, similar to Caco-2, they poorly express CYP3A.

PAMPA is an artificial membrane that does not express transporters and has no cells to create tight junctions or cellular pores. It is representative of passive permeability through a lipid bilayer.

All in vitro models lack the flow of gut contents and blood on either side of enterocytes. Portal blood flow constantly removes drugs from the basolateral membrane of enterocytes, resulting in "sink conditions," a downhill concentration gradient that facilitates drug absorption in vivo. The fluidity of gut contents means that drugs in the gut lumen will be exposed to the different morphologies and expressions in different segments of the gut in vivo that are not simulated in in vitro cell studies.

While highly permeable compounds are almost all extensively absorbed, in vitro permeability rate predictions often underpredict absorption. This is because many compounds are actively absorbed, but have low passive permeability. Predicting extent of absorption is improved by including active drug transport. Larregieu and Benet12 showed that when transporter expression is decreased more than 10-fold in Caco-2 cells compared with humans, absorption of compounds that are substrates of highly expressed transporters such as PEPT1, amino acid transporters, and nucleoside transporters is poorly predicted.

Permeability rate as a surrogate for absorption will be extensively discussed in section "Biopharmaceutics Classification System."

4.06.2.2  Predicting Distribution

Drug distribution is determined by cardiac output, tissue blood flow and volume, and capillary permeability, as well as tissue permeability and drug transporters. Following drug dosing, well-perfused tissues such as the liver and kidney initially receive a high drug concentration. This initial distribution phase is apparent when considering the shape of a plasma concentration–time profile. A secondary distribution phase, characterized by slowly decreasing plasma concentrations, reflects drug distribution to the more poorly perfused tissues. Additionally, protein-bound drugs cannot traverse cellular membranes, and therefore, protein binding has an impact on drug distribution. In the plasma, drugs primarily bind to either albumin, if the drug is acidic, or α-1-acid glycoprotein, if the drug is basic. Protein binding can be modified by disease and drug–drug interactions. For instance, patients with cystic fibrosis often have hypoalbuminemia.13 However, Benet and Hoener14 have shown that changes in protein binding are only important for high-clearance, narrow therapeutic index drugs that are dosed intravenously, for example, lidocaine.

4.06.2.2.1  Volume of distribution

Distribution can be characterized by the theoretical pharmacokinetic term, the apparent volume of distribution. This term characterizes the apparent space in the body into which a drug distributes. That is, systemic concentration multiplied by the apparent volume of distribution is equal to the amount of drugs in the body. A large volume of distribution indicates that a compound is predominantly located outside of the systemic fluids flowing to the organs of elimination. Volume of distribution depends on the extent of drug binding to receptor sites, plasma proteins, and tissues, as well as the lipophilicity of a drug. Volume of distribution measures can be determined from plasma concentration–time curves. While the volume of distribution can be calculated a few ways, the volume at steady state or $V_{ss}$ is the most useful measure of the apparent space available in the body into which drugs may distribute, since it is not affected by elimination. $V_{ss}$ measures can be determined from plasma concentration–time curves using the following equation:

$$V_{ss} = \frac{AUC}{\left(\frac{AUMC}{AUC}\right) \times \text{CL}}$$

Here, AUMC is the area under the moment curve or the area under the curve of the product of concentration and time versus time.
Additionally, physiologically based PK models such as GastroPlus™ and Simcyp provide estimates of V. Age, percent of body fat, sex, and disease can all affect the volume of distribution. Accurately predicting volume of distribution is vital in predicting C<sub>max</sub> and can be important in defining clinically relevant half-life (t<sub>1/2</sub>) measures. Volume of distribution is also predicted in animal models, which include physiological features like blood flow and organ topology. The volume of distribution can be estimated by collecting plasma concentrations over time and using allometric scaling approaches to predict the volume of distribution in humans.

### 4.06.2.3 Predicting Metabolism and Elimination

Most drugs are eliminated by metabolism, renal elimination of unchanged drug, or biliary elimination of unchanged drug. To ensure safety, pharmacokinetic studies are conducted with mass balance or collection of the entirety of a dose in eliminated equivalents (parent drug or metabolites). Ideally, the entirety of the dose is eliminated in either the urine or the bile. This provides evidence that the compound is not sequestered and accumulating in a peripheral tissue, potentially resulting in unanticipated toxicity, and lends support to the validity of the calculated pharmacokinetic parameters. Incomplete recovery sometimes indicates that a drug is eliminated by another organ (e.g., the lungs). However, mass balance may not be as simple as it sounds. Realistically, the entirety of the dose often cannot be collected. Some drugs have very long half-lives, which makes collections in a clinical setting unrealistically arduous.

Many metabolites and some parent drugs are eliminated in the bile, which is a difficult fluid to accurately obtain and analyze. The bile drains into the lumen of the intestine and its contents are eventually eliminated as part of the feces. Fecal samples could be collected to estimate the fraction of the dose that is eliminated in the bile. However, the feces also contain orally administered material that was never absorbed from the lumen of the intestine. For this reason, it is impossible to differentiate between parent drug that is unabsorbed from an oral administration and parent drug that is eliminated in the bile in fecal samples. This means that, unless a drug was administered non-orally so that all of the drug in the feces must therefore come from biliary excretion, direct bile collection approaches such as collection from T-tubes or nasobiliary tubes are necessary to account for the elimination of unchanged drug in the bile. However, such approaches are rarely conducted and when done are predominantly done during surgeries. The patients often have hepatobiliary disease, so the donor samples do not necessarily represent healthy conditions. Other methods such as the bile string or duodenal collection studies are slightly less invasive and can be conducted with healthy volunteers. Duodenal collection studies are difficult to conduct, however, and are still invasive.

It is much easier to determine the extent of urinary elimination of unchanged drug or the extent of metabolism. Urine samples are almost always collected during pharmacokinetic studies to account for mass balance, and the parent and metabolites can be readily quantified. Parent drug collected in the urine represents absorbed drug only since the drug can only reach the kidneys after entering the systemic circulation. Metabolites can be quantified in the urine and may also be collected in feces. If the drug was not degraded or metabolized by bacteria in the gut, we can assume that the drug was absorbed since most metabolism occurs postabsorption. Degradation and presystemic metabolism can be confirmed with stability studies to be described in section “Determining permeability in humans.”

Prior to conducting trials in humans, pharmaceutical scientists try to predict what the major route of drug elimination will be. In silico, in vitro, and in vivo models of drug elimination have been developed to predict elimination routes and their potential liabilities. For instance, biliarily eliminated drugs may be subject to enterohepatic recycling, which exposes the drug to the intestine and liver multiple times and may result in several peaks. Metabolism may produce pharmacologically active or toxic metabolites that can alter pharmacodynamics or need to be evaluated for safety. A developer may want to avoid renal elimination if the drug is likely to be dosed to patients with failing kidneys. Alternatively, an eliminating organ may be the desired site of action and a developer may attempt to target that route.

Recently, there have been many efforts to associate the chemical properties of drugs with their major elimination routes. Certain trends have been noted for a long time, with properties such as lipophilicity, molecular weight, protein binding, and ionization state proposed as harbingers of elimination route. However, these relationships are sometimes considered too simple or were applied to small data sets of compounds that are often structurally similar. With the advent of “big-data” methodologies, more complicated and/or thorough analyses are possible.

### 4.06.2.3.1 Characteristics of metabolism

Most drugs are designed to be sufficiently lipophilic to cross biological membranes during absorption or distribution and to achieve biochemical potency by encouraging protein binding to a target site through hydrophobic interactions. Coincidentally, most drugs are metabolized, and indeed, lipophilicity is historically considered a prerequisite for drug metabolism.

There are at least a couple of reasons why lipophilicity and metabolism are associated with each other. The first is that relatively lipophilic drugs may be able to be passively reabsorbed across membrane barriers surrounding excretory fluids including the aqueous bile and urine. Metabolic enzymes generally convert a lipophilic substance into a more hydrophilic substance, which aids in accumulation in and thus elimination from the aqueous bile or urine. Secondly, lipophilicity is correlated with protein binding and may aid in binding to enzymatic proteins through hydrophobic interactions, which will convert a drug to a more hydrophilic molecule.

Many very lipophilic molecules are indeed metabolized, and in fact, we are unaware of any marketed poorly metabolized drugs with a measured or calculated LogP > 5. As always, it is important to note that these are trends and not rules. Despite the common assumption that metabolized compounds are lipophilic and vice versa, metabolized compounds cover a vast physicochemical
space, and not all lipophilic compounds are metabolized. In particular, drugs that are eliminated unchanged in the bile also exhibit relatively high LogP values, with no significant difference in this parameter from metabolized compounds as determined by the t-test (Fig. 1) and no rank-ordered differentiability as indicated by receiver operating characteristic curves. (The receiver operating characteristic is a plot that illustrates how well a continuous feature (e.g., LogP) classifies a binary outcome (e.g., biliary vs. metabolic elimination.) When the area under the ROC curve is greater than 0.8, the continuous feature is expected to differentiate between the classes well.) Furthermore, a large proportion of metabolized drugs have a low LogP. This may be because drugs eliminated unchanged in the bile require uptake and efflux transporters in the hepatocyte and therefore must be sufficiently lipophilic to bind to these transporters. Alternatively, almost all compounds eliminated as unchanged drug in the urine have a low LogP and are poorly bound to proteins.

However, given the somewhat ambiguous predictability of LogP, it is necessary to predict which compounds will be metabolized by other methods. Metabolism can be assessed using in vitro, in vivo, or in silico methodologies.

### 4.06.2.3.2 In vitro predictions

The extent, rate, and mechanisms of metabolism are often initially evaluated in vitro. Ideally, human hepatic and enterocytic tissues can be utilized to evaluate metabolism. However, these merely serve as predictive tools and are fraught with errors.

Microsomes are a subcellular fraction containing the contents of the endoplasmic reticulum including CYPs and UGTs. CYP metabolism accounts for about 70% of the metabolism of extensively metabolized drugs of the top 200 drugs, while UGT metabolism accounts for about 14% of the metabolism of the top 200 drugs prescribed in the United States.

In vitro microsomal experiments can determine the rate of microsomal metabolism, called the intrinsic clearance (CLint), representing the capacity of metabolizing enzymes to eliminate a compound in the absence of other factors like blood flow, membrane permeability, protein binding, and competing elimination mechanisms.

Intrinsic clearance can be expected to correlate with clinical clearance in humans, particularly for drugs that are primarily metabolized by CYP enzymes in the liver. Prior to the recognition of the contribution of transporters to drug disposition, human clearance was estimated from intrinsic clearance in microsomes generally using the well-stirred model. However, in the late 1990s, Miyauchi et al. proposed an extended clearance concept to include the effect of transporters on hepatic clearance.

Microsomes can provide a reliable estimate of metabolic kinetics (clearance). However, microsomes have been used to predict the extent of metabolism by measuring the percent of dosed drug that is unmetabolized after a set period of time. This may be an unreliable predictor of the extent of metabolism since presumably most drugs will be metabolized if they are left to incubate in
the presence of a variety of enzymes without interference. As we will discuss in detail later, in vitro permeability rate can predict the extent of metabolism in humans.

Supersomes, expressing only one enzyme, may be used to predict the metabolic intrinsic clearance by a single enzyme and to identify metabolites formed by a specific enzyme.

A major concern of predicting the extent and/or rate of metabolism in vitro, perhaps especially in microsomes, is that metabolism is assessed in isolation of competing processes. In vivo, transport-limited clearance into the bile or passive or transport-limited clearance into the urine may prevail. However, microsomal incubations cannot tell a researcher what is the major route of elimination, and metabolic clearance does not necessarily relate to extent of metabolism. Isolated or sandwich-cultured hepatocytes are more complex tools that incorporate transporters and may be used to predict metabolic or hepatic clearance.

4.06.2.3.3 In vivo predictions

In vivo approaches confer the advantage of including factors such as blood flow, sequestration due to transport, and membrane permeability. In vivo approaches include humanized animals and allometric predictions. Humanized animals, like supersomes, can help assess the impact of a single enzyme or transporter.

Allometry applies scaling factors based on body size to pharmacokinetics across species. Simple allometry from a single species is commonly used early in drug development, requiring only clearance data from preclinical species. Modifications of simple allometry have been proposed to improve the predictability of these models.23

These predictions require sampling plasma concentrations over time in preclinical animal models and may incorporate other physiological parameters such as plasma protein binding and the blood to plasma ratio. They can be supplemented with physicochemical drug properties such as molecular weight or LogP.

The most obvious disadvantage to using preclinical animal models is physiological differences. While allometry attempts to correct for differences in body weight, protein binding, blood flow, etc., animals often have different patterns of metabolizing enzyme and transporter expression and substrate specificity. While pharmacokinetics are frequently similar between species, marked differences can be seen in a variety of substrates. Consider, for instance, digoxin or zidovudine. Digoxin is extensively metabolized in humans, but is primarily eliminated unchanged in rats.24 Zidovudine is extensively metabolized in rats, but is primarily eliminated as unchanged drug in humans.25

4.06.2.3.4 In silico predictions

Several in silico methods predict aspects of metabolism, including understanding the affinity for a particular enzyme28 and the site of metabolism on the molecule, predicting metabolic clearance,29 identifying metabolites,30 and predicting metabolic stability.31 Unfortunately, many of the published methods rely on proprietary descriptors or are derived from small or structurally similar data sets.32 Since many compounds are metabolized by several enzymes and/or are sequentially metabolized, it is crucial to integrate many predictive models. Several reviews discuss the challenges and applications of in silico predictions of drug metabolism in depth and discuss available predictive software.28,30,32

Second to metabolism, renal elimination of unchanged drug is responsible for the elimination of most drugs.

4.06.2.3.4.1 Renal elimination of parent drug

Renal elimination of drugs is dependent upon three renal processes: glomerular filtration, renal secretion, and renal reabsorption. Glomerular filtration is a passive process where free (unbound) small-molecule compounds are drained from the blood in the afferent arteriole and collected in the filtrate. Large molecules, including drugs bound to proteins, cannot sieve through the glomerulus and they remain in circulation. While glomerular filtration rate \( = 120 \text{ mL.min}^{-1} \), urine is only formed at 1 mL min \(^{-1} \), so 119 mL of water is reabsorbed from the kidney tubules every minute. For this reason, many compounds, especially metabolized compounds, are passively reabsorbed from the filtrate as water is actively retained in the body. Several compounds are actively secreted into the filtrate directly from the blood via drug transporters expressed on the proximal tubule.

Renal clearance tends to decrease with increasing lipophilicity.15 This is intuitive, since highly lipophilic compounds are often susceptible to reabsorption. Additionally, lipophilic compounds are more likely to be protein-bound.15 Unsurprisingly, compounds that are primarily eliminated as unchanged drug in the urine are expected to be small and polar, having low protein binding. However, Hosey et al. demonstrated that while this holds true for orally dosed compounds, many non-orally dosed (generally intravenously administered) compounds could be renally eliminated even if the molecular weight is high. Alternatively, protein binding of compounds primarily eliminated in the urine was low for both orally and non-orally administered medications.27 This is likely because all small molecules (<10,000 Da)14 can be filtered through the glomerulus, but compounds bound to proteins are not. Therefore, highly protein-bound drugs must be eliminated by other routes and protein binding is a determinant of renal elimination.

While charge does not determine if renal elimination is the primary route of elimination,22 it does trend with renal clearance. Anions and cations are primarily secreted, whereas neutral compounds are primarily reabsorbed. Additionally, renal clearance tends to decrease with increasing compound lipophilicity, while renal secretion increases with polar descriptors.33
of the primary endogenous substrates: bile salts. Millburn et al. suggested that drugs with a molecular weight and that biliary excretion was selective for these properties. This was likely derived by considering the weight and molecular species the hepatic apical membrane facing the bile canaliculi.

Greater hydrogen bond interactions have been associated with increased biliary excretion. Some studies indicate that biliarily eliminated compounds are primarily anions, while some indicate that cations are also eliminated in the bile. Lipophilicity has varying indications between studies, with some indicating that biliarily eliminated drugs are hydrophilic and some indicating they are lipophilic and in some cases have contesting associations with biliary excretion. Historically, it was hypothesized that high-molecular-weight (>500 Da) anions would preferentially be eliminated in the bile and that bile excretion was selective for these properties. This was likely derived by considering the weight and molecular species of the primary endogenous substrates: bile salts. Millburn et al. suggested that drugs with a molecular weight <500–600 g mol\(^{-1}\) were less susceptible to biliary elimination. More recently, Yang et al. predicted that when anions have molecular weights >475 Da, 10% or more of the dose is likely to be eliminated in the bile. Hosey et al. demonstrated that drugs whose major route of elimination is unchanged drug in the bile were poorly permeable and had a high polarizability, which is highly correlated with molecular weight, and a low predicted metabolic stability. That study also points out that high molecular weight is descriptive of biliarily eliminated drugs, but that high molecular weight does not qualify biliary elimination. In other words, almost all drugs that are predominantly eliminated as unchanged drug in the bile are heavy, but the primary route of elimination is not biliary excretion for the majority of high-molecular-weight drugs (Table 1).

Other properties associated with biliary elimination have been less clearly defined and in some cases have contesting associations between studies. Greater hydrogen bond interactions have been associated with increased biliary excretion. Some studies indicate that biliarily eliminated compounds are primarily anions, while some indicate that cations are also eliminated in the bile, and yet, others suggest that ionization is not an important characteristic. Greater dipole moments and the presence of a carboxylic acid group, and more rotatable bonds have also been associated with increased biliary excretion. Lipophilicity has varying indications between studies, with some indicating that biliarily eliminated drugs are hydrophilic and some indicating they are lipophilic and others discussing both lipophilic and polar regions, and some studies finding no relationship between lipophilicity and extent of biliary elimination. While the bile is a hydrophilic medium, compounds likely require a degree of lipophilicity to bind to and be transported by drug transporters such as P-gp. This may be especially true as the most widely accepted mechanism of P-gp transport relies on an initial partition into the membrane. These relationships likely depend on how “major” biliary elimination was defined and which drugs were included in the study.

As we mentioned earlier, biliary elimination data are difficult to gather and quantify in humans. However, in vitro, in vivo, and in silico approaches may provide a reasonable quantitative or qualitative understanding of biliary elimination.

Perhaps the most widely accepted in vitro approach to predict biliary clearance is the use of sandwich-cultured hepatocytes, isolated from humans or rats, which maintain the polarity of cell membranes, a crucial condition to determine canalicular efflux. These hepatocytes are plated on a collagen platform and maintained in the presence of calcium. After several days, transporter expression is optimized for vectorial transport. After introducing the test drug, the tight junctions are ruptured by removal of calcium, and differences in accumulated intracellular concentrations can be measured.

In vivo, one of the most common approaches to estimate the contribution of biliary elimination is with bile duct cannulation in an isolated perfused rat liver. Unfortunately, rats have a higher bile flow (relative to body weight), greater efflux transporter expression, and increased rate of efflux compared with humans, so more compounds are eliminated in the bile at greater concentrations and rats are not altogether reliable models.

Ghibellini et al. have published a comprehensive review of methods of evaluating biliary excretion in humans.

### Table 1: Molecular weight distribution of orally and non-orally administered drugs by major elimination route

<table>
<thead>
<tr>
<th>Major elimination route</th>
<th>Oral administration</th>
<th>Non-oral administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 380</td>
<td>&lt; 380</td>
</tr>
<tr>
<td>Biliary</td>
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<td>5</td>
</tr>
<tr>
<td>Renal</td>
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<td>65</td>
</tr>
<tr>
<td>Metabolism</td>
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<td>345</td>
</tr>
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<td></td>
<td>&gt; 475</td>
<td>&lt; 475</td>
</tr>
<tr>
<td>Biliary</td>
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<td>1</td>
</tr>
<tr>
<td>Renal</td>
<td>42</td>
<td>21</td>
</tr>
<tr>
<td>Metabolism</td>
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<td>50</td>
</tr>
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</tr>
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<tr>
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<td>21</td>
</tr>
<tr>
<td>Metabolism</td>
<td>42</td>
<td>29</td>
</tr>
</tbody>
</table>

#### 4.06.3 Biopharmaceutics Classification System

Small changes in drug product composition may alter pharmacokinetics, potentially resulting in toxicity or lack of efficacy. For this reason, a drug must be evaluated for pharmacokinetic differences any time a company uses a new manufacturing site, new synthesis, or a new formulation. A drug product that demonstrates very similar pharmacokinetics to the original product is said to be bioequivalent. A bioequivalence study generally consists of a crossover study of a single dose of the highest dose strength of the
For a drug to be considered bioequivalent, the 90% confidence interval of $C_{\text{max}}$, $\text{AUC}_0 \rightarrow \infty$, and $\text{AUC}_0 \rightarrow t_{\text{last}}$ must be between 80% and 125% of the reference product.

While many non-orally administered compounds and compounds administered as oral solutions that meet certain requirements (minimally including dosage as an aqueous solution with the same concentration of active drug as the reference product and the same or similar excipients at comparable concentrations as the reference product) are exempt from bioequivalence testing, the complexity of oral absorption requires bioequivalence testing for products administered orally outside of solutions. This is a large burden since the majority of compounds are administered orally and not in solution.

To determine bioequivalence in a clinical study, a drug is given to volunteers with 250 mL of water (about 8 oz.) on an empty stomach. Per the FDA, for a drug to be considered highly soluble, the highest dosage strength of the drug must be able to completely dissolve in this volume of water or less. The drug also needs to remain soluble over the entire pH range of the stomach and small intestine. The FDA recommends that solubility be tested at pH 1, pH 6.8, and a variety of pH in between, specifically at the $pK_a$ of the compound and above and below the $pK_a$ by one unit. This pH range represents the range of pH from the stomach to the jejunum under fasting conditions.

Highly soluble drugs are defined by a dose number ($D_o$) \(\leq 1\), which can be calculated from the minimum solubility of a drug over the pH range 1–6.8, at body temperature, 37°C:

\[
D_o = \frac{\text{Highest dose strength (mg)}}{250\text{mL} \times \text{minimum solubility (mg mL}^{-1})}
\]

In 1995, Amidon et al. developed the BCS on the basis of a good correlation between human jejunal permeability in single-pass perfusion studies and the fraction of the dose absorbed across the gut wall. They concluded that highly "permeable," highly soluble compounds were well absorbed and not susceptible to minor changes in the drug product. If 85% or more of the drug is dissolved within 30 min, it is considered rapidly dissolved, and if 85% or more is dissolved in 15 min, it is very quickly dissolved. The goal of this system is to use in vitro dissolution methodology to predict in vivo behavior.

The rate and extent of absorption for an immediate-release dosage form depend upon effective permeability, solubility, dissolution rate, and gastrointestinal transit time. In order for a compound to be absorbed, it must be in solution. Therefore, the compound must be completely in solution prior to reaching the absorption site, most commonly the intestine. This is dictated by a dissolution rate. The compound must also remain in solution at the pH of the absorption site, which is ensured by measuring the minimum solubility throughout the pH range of the absorbable regions.

The BCS classifies drugs on the basis of solubility and permeability (Fig. 2). The BCS predicts that highly permeable, highly soluble class 1 drugs are well absorbed and rate-limited by gastric emptying if the compound dissolves rapidly. If the compound is contained in an immediate-release dosage form and rapidly dissolved, bioequivalence can be ensured. Class 2 drugs are well absorbed, but absorption is rate-limited by dissolution, and there may be variability in absorption due to the drug formulation or in vivo factors. Class 3 and 4 drugs are limited by permeability.

This system provides a major advantage to pharmaceutical companies because in vitro methodology may qualify drugs for a waiver of in vivo bioequivalence studies. The principles of the BCS were adopted by the FDA, EMA, and WHO in terms of bio waivers. Biowaivers grant regulatory approval based on dissolution studies to certain immediate-release drug products for drugs already on the market, but are being manufactured differently or by a different company (generics), or having formulation changes, without requiring clinical evaluation of bioequivalence. Prior to the development of this system, drug products undergoing any production changes (e.g., manufacturing location or equipment) required additional clinical bioequivalence trials to be proved.
safe and efficacious. However, there are small differences in the regulations for biowaiver approval. As indicated in the 2015 draft guidance for biowaivers, the FDA now allows biowaivers for certain immediate-release, orally administered BCS class 1 and BCS class 3 drugs that have an acceptable therapeutic index, as well as some BDDCS class 1 drugs, which will be discussed in section "Biopharmaceutics Drug Disposition Classification System." Biowaivers are not available to narrow therapeutic index drugs or drugs absorbed in the oral cavity.

The EMA now requires a stricter definition of solubility, using the highest dose administered in a single setting, as opposed to the highest dose strength. This impacts only a few drugs, but does shift the classification in some instances. For example, the highest formulated dosage strength of verapamil is 12 mg, but two tablets are often given in one setting, so the highest dose administered in a single setting is 24 mg. By the FDA definition, verapamil is considered a class 1 drug and is eligible for a biowaiver, but is ineligible for a biowaiver under the new EMA guidance.

4.06.3.1 Biowaivers Granted by the US Food and Drug Administration

4.06.3.1.1 BCS class 1

These highly permeable, highly soluble compounds may be issued biowaivers if the compound is

- dosed as an immediate-release product,
- rapidly dissolved,
- an excipient in the formulation that does not interfere with the rate or extent of absorption,
- stable in the gastrointestinal tract.

Immediate-release products can be partially or completely dissolved in the stomach. These drugs are often rapidly absorbed, while extended-release products dissolve slowly in the small intestine by design. Using a highly soluble drug that is also rapidly dissolved ensures that solubility is not the rate-limiting step of absorption. Gastric emptying time is about 30 min to 1 h on a fasted stomach. Therefore, when 85% or more of a drug is dissolved within 30 min, the compound is likely to be dissolved prior to entering the intestine and is considered rapidly dissolving. In other words, the drug is ready to be absorbed once it reaches the small intestine. Additionally, the high permeability rate of class 1 drugs predicts little absorptive barrier. These BCS class 1 compounds do not have permeability barriers that would prevent the drug from being absorbed during the limited transit time through the gastrointestinal tract. Excipients that have been FDA-approved for immediate-release products will generally not interfere with drug absorption.

4.06.3.1.2 BCS class 3

These highly soluble but poorly permeable compounds may be issued biowaivers if the compound is

- dosed as an immediate-release product,
- very rapidly dissolved,
- composed of the same ingredients, in similar quantities, as the reference compound.

Class 3 drugs have slightly stricter requirements than class 1 drugs, being limited by permeability rate. Additionally, since the absorption of class 3 drugs can be site-dependent, gastrointestinal transit time is a limiting factor in its absorption. Some excipients alter gastrointestinal transit time and membrane permeability, therefore, there must be no alterations in the formulation including excipients. Class 3 drugs are required to be very rapidly dissolved (85% within 15 min).

4.06.3.1.3 BCS class 2a

In addition, the WHO also recommends that an additional category of BCS class 2 drugs, "2a," be granted biowaivers. This subset is composed of weakly acidic class 2 drugs. They must be rapidly dissolving and highly soluble at pH 6.8. These drugs have a low solubility at the low pH in the stomach, but are well absorbed due to high solubility in the more basic intestine.

4.06.3.2 Determining Permeability

Per the FDA, compounds are considered highly permeable for the application of biowaivers when the extent of absorption is ≥85% of the dose. The FDA permits a variety of methods of determining if a compound is highly permeable. Permeability can be assessed in humans, in vivo, or in vitro. When a pharmaceutical company intends to use a permeability-determining method for bioequivalence purposes, it should be validated by comparing permeability values generated in the assay with extents of absorption and confirming that they share a rank order.

4.06.3.2.1 Determining permeability in humans

Evaluating permeability rate by direct intestinal perfusion in humans is the most preferable method. However, acquiring such measurements is difficult and invasive. Alternative methods that are routinely assessed include evaluating the mass balance of a drug or evaluating its bioavailability.
If a compound is a passively diffused drug, pharmaceutical companies may employ in vitro or in vivo methodology to determine permeability. However, while animal studies provide a physiological model, animal physiology significantly differs from humans. Animal models are less expensive and less time-consuming than clinical experiments in humans. In an acceptable animal model, such as bile salts, which can impact drug solubility and absorption. Animal models may be advantageous because experiments are less invasive in animals than clinical experiments in humans. The FDA defines a passively permeable drug as one with an efflux ratio < 2, when linear pharmacokinetics exist between dose changes and bioavailability, and when in vivo or in vitro permeability does not depend upon the initial concentration. This specification exists because many animals or cell lines have low or nonexistent efflux compared with a well-expressed and active homolog in humans. An animal or cell line with negligible efflux can artificially boost permeability and overpredict human permeability. An efflux ratio is defined as the basal to apical intestinal permeability divided by the apical to basal intestinal permeability.

### 4.06.3.2.2 Determining permeability in vivo or in situ

Animal models provide physiological conditions analogous, but not necessarily similar, to humans, including gastric and enteric dissolution, blood flow on the basolateral membrane of enterocytes, and the presence of endogenous components such as bile salts, which can impact drug solubility and absorption. Animal models may be advantageous because experiments in animals are less expensive and less time-consuming than clinical experiments in humans. In an acceptable animal model such as a rat, intestinal perfusion studies can be carried out to determine the rate and extent of drug absorption. Keep in mind, however, that while animal studies provide a physiological model, animal physiology significantly differs from humans. Animal models have different expressions, activities, and substrate specificity of transporters and metabolizing enzymes as well as different blood flows, membrane permeabilities, and compositions of endogenous fluids (e.g., stomach acid and bile).

Single-pass intestinal perfusion studies examine the continuous perfusion of drug from the duodenum to the ileum of anesthetized animals, generally rats. Drugs containing fluid are introduced and removed from cannulas at either end. These models tend to correlate better with human effective permeability and human absorption than cell-based models like Caco-2.

### 4.06.3.2.3 Determining permeability in vitro

Epithelial cell monolayers such as colorectal adenocarcinoma cells (Caco-2) or MDCK cells can be used to evaluate the permeability of the compound in question. The permeability of test drugs is assessed and compared with an acceptable standard compound to determine if the test compound has a relatively high or low permeability. As opposed to a quantitative value, a reference compound is an important control because permeability measurements are extremely variable between laboratories and are very sensitive to experimental conditions.

Excised intestinal tissues can also be used. Excised intestinal segments from animals (most commonly rats) or humans can be mounted on an Ussing chamber, and effective permeability can be calculated. Rat tissues are more commonly used than human tissues because human tissues are scarce. This approach is beneficial because it incorporates flow as well as an intact membrane and maintains transport polarity. The effective permeability rates calculated from rat tissues correlate very well with human jejunal permeability values. It is, however, a laborious approach.

When the BCS was first introduced, cellular permeability of a test drug was compared with that of metoprolol. As experiments progressed, however, it became readily apparent that metoprolol is an extremely permeable compound, and its usage resulted in many false negatives. The FDA now includes a list of potential model drugs representing high absorption, though pharmaceutical companies may select other drugs provided the mechanism of absorption is passive permeability and reliable human absorption data are available. To confirm the reliability of an in vitro permeability method, the sponsor should demonstrate a rank order between the permeability rate values determined via the method and human absorption values using high-, intermediate-, low-, and zero-permeability/absorption model drugs. A full list is provided in the FDA guidance.
4.06.3.3 Approval of BCS Class 1 Classification

The FDA Center for Drug Evaluation and Research (CDER) has created a BCS committee that approves submissions for drugs to be officially given a class 1 assignment. Solubility criteria are well standardized, but permeability can be confirmed in a variety of manners, as listed in the preceding text. The most commonly submitted permeability evidence comes from mass balance studies, followed by Caco-2, absolute bioavailability, RLD label, and then rat intestinal perfusion.

The BCS was developed and is successfully used to provide biowaivers for some highly soluble compounds. Since its development, similar classification systems have been created to predict aspects of drug disposition (BDDCS) and clearance (ECCS, ECCS, and CPathPred).

4.06.4 Biopharmaceutics Drug Disposition Classification System

The BDDCS was initially proposed when Wu and Benet recognized that compounds with a high passive intestinal permeability rate in the BCS were extensively metabolized, while drugs eliminated in an unchanged form in the urine or bile were primarily poorly permeable in the BCS. In this seminal publication, they suggested that extent of metabolism might serve as an appropriate surrogate for absorption and/or intestinal permeability when those data are unavailable, since the extent of metabolism is easier to assess than intestinal permeability/absorption, thus expanding the number of class 1 drugs available for a biowaiver. Importantly, the modified system was also used to predict drug disposition, especially when predicting when transport or metabolizing enzymes are clinically relevant, for which it is most appreciated today.

4.06.4.1 Introduction to BDDCS and Comparison With BCS

4.06.4.1.1 Metabolism as a surrogate for absorption

The extent of drug metabolism is often quantified during phase I pharmacokinetic/mass balance studies. Tabulating absorption, on the other hand, requires invasive intestinal perfusion studies in man or portal blood sampling. Absorption is a prerequisite to enzymatic metabolism, which occurs intracellularly in the endoplasmic reticulum or cytosol. Therefore, we can assume that enzymatically metabolized drugs are absorbed. Since metabolism is easier to quantify than absorption/intestinal permeability rate, Wu and Benet proposed that metabolism be used as an alternative measurement to predict absorption. The EMA and recently the FDA have incorporated this suggestion into the guidance recommendations for granting biowaivers, and highly soluble compounds with >85% metabolism are eligible for biowaivers.

The BDDCS differs from the BCS in two major aspects: (1) the primary goals of the systems and (2) the definition, interpretation, and relationship of "highly permeable drugs."

4.06.4.1.2 Primary goals of the classification systems

The primary goal of the BCS is to grant biowaivers using in vitro methodology to predict drug absorption and its limiting steps. Alternatively, while the BDDCS provides the basis for recommending biowaiver extension to extensively metabolized compounds, the primary goal of the BDDCS is to predict drug disposition.

4.06.4.1.3 Differences in defining permeability

Via application of the BCS, biowaivers are granted based on extent of absorption, which may not always correlate well with intestinal permeability rate. A high permeability rate necessitates drug metabolism as we will discuss later. While high permeability rate also predicts a high extent of absorption, the opposite is not necessarily true. There are many examples of highly absorbed drugs that have a poor passive permeability rate, not reflecting their high absorption extent. For example, sotalol, a BDDCS class 3 drug, is poorly permeable in Caco-2 cells, but has an absolute bioavailability of 98%, and thus is highly absorbed. Its high absorption is likely mediated by transport since it has a poor in vitro permeability rate, but is a substrate for the gut uptake transporter OATP1A2. While some drugs are considered highly permeable in the BCS because of their high absorption, they are not, in fact, highly permeable in terms of rate measures. This is, in fact, the basis of a major difference between the BCS and BDDCS. Specifically, BCS class 1 and 2 compounds may be class 3 or 4 in the BDDCS, since drugs are classified by metabolism extent, and not absorptive extent. These differences are crucial in predicting drug disposition.

4.06.4.1.4 Development of and dispositional predictions by BDDCS

The BDDCS predicts when disposition is driven by metabolizing enzymes versus drug transport or when both are important, especially when transport regulates metabolism in transporter–enzyme interplay. When the BDDCS was initially developed, predictions were made for the gut and the liver. It has since been extended to transporter effects in noneliminating organs such as the brain.

Wu and Benet serendipitously recognized that high-permeability rate drugs were extensively metabolized, while low-permeability rate drugs were primarily eliminated as unchanged drug in the bile and the urine. They were simultaneously carrying out studies involving the transport of metabolized drugs and noticed that they did not recognize any clinically significant transporter interactions for BCS class 1 drugs. They therefore proposed that extent of metabolism and solubility might reflect other aspects of drug disposition, including the clinical relevance of drug transport. The BDDCS therefore classified drugs by their extent
of metabolism and solubility (Fig. 3), where each class is associated with a series of predictions that will be thoroughly discussed. Initial applications included predicting when transporters, metabolizing enzymes, or the interplay between the two had clinically meaningful impacts on systemic concentrations and presystemic drug concentrations (after oral dosing), major routes of elimination and therefore drug–drug interactions, pharmacogenomics, and food effects in the intestine and liver. Since then, the BDDCS has been expanded to understanding the effect of end-stage renal disease on hepatic elimination and distribution across the blood–brain barrier. Hypotheses regarding toxicity, drug-induced liver injury (DILI), and clinical relevance in other tissues including the kidney will be discussed.

4.06.4.1.5 Value and appropriate uses of BDDCS
The BDDCS is invaluable during drug development because understanding the effect of transport and metabolizing enzymes is now essential for drug approval. Specifically, new drug applications must include the major routes of drug elimination, the quantitative contributions of enzymes and transporters, and drug–drug interaction studies. The BDDCS can alert developers to which enzymes and transporters are likely important and may even justify negating some studies.

The BDDCS does not predict quantitative values of drug disposition. It can, however, provide qualitative information about the absorption of some compounds, the extent of metabolism, the extent of biliary or renal elimination of unchanged drugs, and distribution. More accurately, it predicts what processes, that is, transport at specific membranes and/or metabolism, will affect each aspect of disposition and the direction of the effect.

4.06.4.1.6 How are interactions defined?
Defining which enzymes and transporters are important in the disposition of a drug can be a cumbersome, though necessary, process. The FDA provides guidance to drug companies regarding appropriate methods to determine substrate, inhibitor, or inducer potential that may influence the safety of a drug prior to approval. It initially consists of determining if a drug is a substrate, inhibitor, or inducer of a transporter or enzyme in vitro. For those drugs with positive identification in vitro, follow-up in vivo studies are conducted. Interactions are considered meaningful if they are "sufficiently large to necessitate a dosage adjustment …" Full drug–drug interaction predictions are outside of the scope of this article.

4.06.4.1.6.1 Pillars of BDDCS
The BDDCS predicts when a transporter or a metabolizing enzyme can clinically regulate the disposition of a drug, whether or not the drug is a substrate. When the BDDCS predicts that drug transport at a membrane is not clinically relevant for a particular drug, it does not presume that the drug is not a substrate for a transporter. In fact, it is likely that almost all drugs are substrates for transporters. Instead, the BDDCS predicts if a transporter significantly contributes to the disposition of a drug compared with passive diffusion. These effects are perhaps most obvious in clinical studies examining the effect of transport inhibition on drug absorption, distribution, metabolism, and elimination. In cases where a transporter or metabolizing enzyme is important in a drug’s disposition, affecting one, for example, by inhibiting transport, can cause clinically significant pharmacokinetic changes to elimination, bioavailability, or distribution, observed as changes in the plasma concentration versus time curve (AUC, \(C_{max}\), \(t_{max}\)) and altering the parameters CL, V, and F that define dose. These changes may impact the safety or efficacy of the drug, resulting in a dose change. If inhibition of transport does not cause dispositional changes severe enough to necessitate a dosage change, the transporter is not considered clinically significant in the drug’s disposition. The BDDCS predicts that extensively metabolized/highly soluble class 1 drugs are not clinically relevant substrates of drug transporters, even if in vitro evidence shows an affinity. In other words, while these drugs may have a biochemical affinity to transporters, the contribution of the active transporter to permeation across a membrane is minor compared with passive permeability, and any functional discrepancy of the transporter will not result in a significant change.
that requires dose adjustment to achieve safety or efficacy. For class 1 drugs, in vitro studies will provide a false-positive predicting interaction, and studies in vivo or in humans are unlikely to replicate these results.77 In vivo or clinical interaction studies are costly and time-restrictive. The FDA guidance recommends that P-gp and BCRP be evaluated as transporters for every drug, yet acknowledges that it may not be necessary for BCS class 1 drugs and sponsors may submit class 1 drugs without transporter data.6 This would more appropriately be acknowledged for BDDCS class 1 drugs, since some BCS class 1 drugs (e.g., sotalol) may be subject to transporter interactions.

Furthermore, the BDDCS does not presume to predict that there will be an interaction for every drug in a class, but rather that an interaction could exist and should be tested during development. Finally, the BDDCS makes no predictions regarding inhibitor or inducer status.

### 4.06.4.1 Processes affecting absorption

#### 4.06.4.1.6.2 Drugs as victims and perpetrators

Importantly, even if a transporter or metabolizing enzyme does not clinically affect the disposition of a drug, it does not mean that the drug cannot affect those transporters or metabolizing enzymes. In other words, the BDDCS predicts if a drug can be a victim of a drug interaction, but not whether or not it can be a perpetrator. Consider verapamil. Verapamil is a BDDCS class 1 drug and a P-gp substrate in vitro. As you will see throughout this article, inhibition of transport does not affect the disposition of class 1 drugs. Therefore, when P-gp is inhibited by quinidine, for example, the dispositional change is not severe enough to warrant a dosage change of verapamil. However, verapamil is a moderate inhibitor of P-gp. When verapamil is coadministered with digoxin, a BDDCS class 3 P-gp substrate, it inhibits P-gp, causing the plasma AUC of digoxin to spike into toxic concentrations. Digoxin then requires a lower dose.

#### 4.06.4.2 Role in Predicting Absorption

##### 4.06.4.2.1 Processes affecting absorption

The stomach and enterocytes are the body’s initial security screening. The gastrointestinal environment is a harsh habitat. The pH in the stomach is so acidic that a mucosal layer protects the stomach lining. The stomach is responsible for initially grinding and dissolving oral tablets and regulating the release of its contents to the intestine. In the stomach and intestine, the acidic pH and an ecosystem of microorganisms contribute to the breakdown of foods, so that nutrients can be absorbed. Readers who are familiar with the concepts of probiotic diets will understand that bacteria significantly contribute to the breakdown and metabolism of foods. Bacteria are also capable of degrading or metabolizing drugs. To nourish the body, enterocytes must allow entry to many molecules. However, anything we eat may also contain substances toxic to our body. To reduce the harmful impact of xenobiotics, enterocytes are equipped with metabolizing enzymes, which can change a xenobiotic into a generally less toxic and easy to secrete substance, and transporters, which can help pump xenobiotics back into the gut. Transporters may also aid in absorption of nutrients and xenobiotics. One such example is PEPT1, which not only is responsible for bringing oligopeptides in but also helps to transport functionality results in decreased absorption and increased function with increased absorption. Uptake transport in the gut must therefore be evaluated for BDDCS class 3 and 4 compounds, which can exhibit highly variable extents of absorption.

##### 4.06.4.2.2 Requirements of absorption and predicting absorption

For an orally administered drug to be effective at a peripheral location, the drug must obviously be well absorbed. This is, in fact, one of the major barriers during drug development, since oral administration is preferred due to compliance, convenience, and stability reasons. Predicting absorption is therefore very important when selecting drug candidates. As an addendum to the prediction that high passive permeability rate predicts extensive absorption, the BDDCS also predicts that extensively metabolized class 1 and 2 drugs are well absorbed.

As we have just discussed, high-permeability rate compounds are both extensively metabolized and well absorbed from the gastrointestinal tract. However, not all well-absorbed compounds are highly permeable. In other words, the BDDCS predicts that extensively metabolized class 1 and 2 drugs must be well absorbed, but offers no prediction of the extent of absorption of poorly metabolized, poorly permeable class 3 and 4 compounds, which can exhibit highly variable extents of absorption.

##### 4.06.4.2.3 Transporter effects in the gut

#### 4.06.4.2.3.1 Apical uptake

BDDCS class 1 and 2 drugs can so readily penetrate enterocytes that gut apical uptake transporters have only a minor contribution to their absorption. Therefore, the BDDCS predicts no effect when uptake transporters are affected for highly permeable compounds since class 1 and 2 can enter enterocytes unaided by transporters.

Class 3 and 4 drugs are poorly permeable and require active uptake transporters to be absorbed, and therefore, alterations to their activity or expression will result in clinical differences in absorption and bioavailability. Specifically, decreased uptake transport functionality results in decreased absorption and increased function with increased absorption. Uptake transport in the gut must therefore be evaluated for BDDCS class 3 and 4 drugs.
4.06.4.2.3.2 Gut apical efflux and transporter–enzyme interplay

Apical efflux transporters counteract net xenobiotic absorption from the gut. Apical efflux transporters include P-gp, MRP2, and BCRP. After a drug is absorbed into an enterocyte, substrates for apical efflux transporters are pumped back into the gut, reducing the effective absorption. Not only can efflux transporters affect parent drug absorption, but also they can regulate the extent of metabolism of some drugs:

- BDDCS class 1 drugs are clinically unaffected by changes in transporter expression or activity in the gut, even if they are substrates. These drugs will be affected only by changes in metabolism, and the degree of metabolism is unaffected by transporters.
- BDDCS class 2: Apical efflux transporters can have a clinical impact on the absorption of class 2 drugs. When efflux is inhibited, an increase in absorption may be observed. BDDCS class 2 drugs are in a unique position because efflux transporters in the gut can impact both their parent drug availability and their intestinal metabolism. Wachter and coworkers discovered that inhibition of P-gp, even in the absence of CYP3A4 inhibition, decreases intestinal CYP3A4 metabolism, since CYP3A4 accounts for approximately 70% of CYP expression in the gut. One might expect that inhibiting efflux in the gut would increase metabolism by forcing a drug to interact with metabolizing enzymes for longer. One might also expect that metabolism would not be affected, but that inhibiting efflux would increase absorption and therefore bioavailability. However, because CYP3A4 and P-gp are coregulated and share many substrates, P-gp substrates are also likely to be metabolized by CYP3A4. Metabolizing enzymes are located just below the microvillus border in enterocytes. P-gp and CYP3A4 work in concert to eliminate the parent drug from the body. Efflux transporters recycle xenobiotics that have not yet been metabolized by CYP3A4, pumping them back into the gut lumen and allowing them to be absorbed multiple times, giving the drug not only multiple opportunities for drug exposure but also multiple opportunities for metabolism, a process called enzyme–transporter interplay. Therefore, when an enteric efflux transporter is inhibited, a class 2 drug may have decreased metabolism and increased bioavailability greater than would be expected by inhibiting absorption alone. This is specific to enterocytes, and inhibition of efflux transporters in hepatocytes leads to increased concentrations of parent drug and increased metabolism.
- BDDCS classes 3 and 4: Apical efflux transporters play a protective role against poorly permeable class 3 and 4 drugs by effectively limiting absorption of poorly permeable drugs that are substrates for an efflux transporter. Class 3 and 4 drugs that are substrates of apical efflux transporters may see an increase in drug absorption when these efflux transporters are inhibited.

4.06.4.2.3.3 Gut basolateral transporters

Little has been explored regarding basolateral transporters expressed on the enterocyte. It is unlikely that basolateral efflux is extremely important since concentrations in the portal vein will be very low compared with the cell, encouraging passive diffusion. Apical uptake transporters may be necessary for more hydrophilic class 3 and 4 drugs to enter the cell, but leaving the cell requires passage through the hydrophilic portion of the membrane and is likely not a limiting factor.

4.06.4.2.3.4 Effects on absorption rate: flip-flop kinetics

When a drug is given as an extended-release formulation, absorption rate is often slower than elimination. Alternatively, the absorption of an immediate-release drug is generally a relatively quick process compared with elimination. For most immediate-release drugs, elimination is the rate-limiting step. However, a very small number of immediate-release drugs exhibit flip-flop kinetics, where the rate of absorption is the rate-limiting step in the disposition of a drug, instead of elimination. Flip-flop kinetics may be a developmental concern when a compound is poorly permeable/poorly metabolized and also has a relatively short t1/2, specifically if it is shorter than gastrointestinal transit time. For drugs displaying flip-flop kinetics, the terminal slope of the systemic concentration–time curve actually reflects absorption processes because absorption rate is not limited for highly permeable/highly soluble drugs. We expect that only class 3 and 4 drugs would demonstrate flip-flop kinetics. Specifically, the absorption rate is probably limited by the affinity to and velocity of gut uptake transporters. Garrison et al. recently evaluated this hypothesis. For 19 drugs exhibiting flip-flop kinetics, 16 were indeed class 3 or 4. While the absorption of class 2 drugs is unlikely limited by uptake transporters, their absorption may be slow as a result of poor dissolution, and very slowly dissolving class 2 drugs may display flip-flop kinetics, but very few examples of this phenomenon have been found.

4.06.4.2.3.5 Pharmacogenomics affecting absorption

Genetics can directly impact a person’s ability to absorb a drug. For instance, patients with inflammatory bowel disease have increased MRP1 expression in the intestine, which can result in decreased absorption of class 2, 3, or 4 drugs. Genetic differences within healthy populations can also result in variation in absorption. The variant SLCO2B1*3, which codes for OATP2B1 and has decreased uptake activity, has an allele frequency of 30.9% in Japanese. When fexofenadine, a BDDCS class 3 drug, was dosed to a Japanese population, those with the allele had a 37% lower AUC than those without the allele, indicating that genetic differences can impact drug absorption. Alternatively, there was no significant difference observed in AUC when midazolam, a class 1 drug, was dosed. Genetic differences are sometimes highly related to race, highlighting the importance of selecting an appropriate population of healthy volunteers representing common genotypes for dosing in certain countries. Genetics, expression, and activity of metabolizing enzymes and transporters can directly impact absorption and other dispositional functions of a drug and increased or decreased functionality follow the predictions outlined for each class.
Complex considerations: food effects

Finally, concomitant food, drug, or supplement administration can potentially alter drug solubility. For instance, some tyrosine kinase inhibitors (TKIs), which are anticancer agents, or the malignancy itself, can cause gastric distress such as gastroesophageal reflux disease. To combat this unpleasant side effect, many patients take proton pump inhibitors, which increase gastric pH. Unfortunately, this can have the effect of decreasing the solubility of some of these weakly basic TKIs, thereby decreasing drug absorption.85 Yago et al.86 showed that absorption, presumably by improving drug solubility, could be improved in healthy volunteers with elevated gastric pH (hypochlorhydria) by predosing betaine hydrochloride, which acidified the stomach prior to dosing the TKI dasatinib. Solubility-based drug interactions are likely to affect poorly soluble BDDCS class 2 and 4 drugs only, since the solubility class is defined by the lowest solubility condition possible in the stomach and gut.

Food can have a significant impact on drug absorption by influencing drug solubility and active absorption. The complex interactions with food will be thoroughly covered in section “Complex considerations: food effects.”

An orally administered drug must be able to bypass a significant degree of gut and hepatic metabolism to be systemically available. Additionally, metabolism is also very important in mediating drug elimination once the drug has entered systemic circulation for both orally and non-orally administered drugs.

Role in Predicting Metabolism and Hepatic Elimination

Metabolism in the gut

The majority of drug metabolism occurs after drug absorption. While metabolizing enzymes are expressed to a significantly smaller extent than in the liver, the gut wall contains relatively high concentrations of metabolizing enzymes compared with other organs. Gut metabolism (Fg) is a component responsible for decreasing a drug’s bioavailability. Gut metabolizing enzymes are also frequently responsible for the bioactivation of produgs.

CYP3A is only expressed at 1.4% of the level found in hepatocytes,87,88 but accounts for 70–80% of CYP expression in the gut, and more than 50% of drugs eliminated by metabolism are substrates for CYP3A489 and may be presystemically metabolized. As a low-affinity, high-capacity enzyme, it may be particularly susceptible to drug concentration differences,90 influenced by permeability, transport, or solubility.

The BDDCS predicts that highly permeable BDDCS class 1 and 2 drugs will be metabolized and thus may be subject to intestinal metabolism, which may contribute to a possible reduction in bioavailability. Alterations in gut metabolism may significantly change the bioavailability of these compounds. When metabolism is inhibited, these compounds are predicted to be significantly more available to the systemic circulation and may require a smaller dose. Poorly metabolized class 3 and 4 drugs are unlikely to be metabolized in the gut. Metabolism may not significantly contribute to reduced bioavailability of these compounds. Therefore, they may not experience significant pharmacokinetic changes when intestinal metabolism is inhibited or induced and will not require dose adjustments.

Genetic differences in expression or activity of a metabolizing enzyme or transporter can significantly impact the metabolism or elimination of drugs. For example, a significant percentage of people are poor CYP2C19 metabolizers. Therefore, when a CYP2C19 substrate is prescribed, we would expect increased exposure in these patients. In fact, poor metabolizers have greater acid suppression and ulcer healing than extensive metabolizers when they take proton pump inhibitors such as omeprazole as a result of their increased exposure.91

When the BDDCS was developed, a primary observation was that there was a very dichotomous extent of metabolism. Drugs tend toward a primary route of elimination. Specifically, metabolism tends to contribute > 70% of total drug elimination for extensively metabolized drugs or < 30% for drugs that are eliminated as unchanged drug. There are few examples of drugs being eliminated with an intermediate extent of metabolism.

Hepatic basolateral uptake

After oral absorption, the liver is the first organ to process drugs and so hepatic transport is important. Hepatic drug exposure is often regulated by hepatic basolateral uptake. While at least seven major transporters (OATP1B1, OATP1B3, OATP2B1, NTCP, OCT1, OCT3, and OAT2) and the bidirectional transporters ENT1, ENT2, OAT7, OCTN2, and OSTα-OSTβ facilitate hepatic distribution, the FDA considers OATP1B1 and OATP1B3 to be the most clinically relevant and recommends evaluating hepatically eliminated drugs for their potential to interact with these transporters as substrates, inhibitors, or inducers.

As in the gut, the BDDCS predicts that uptake transport will be clinically irrelevant for class 1 drugs. The BDDCS predicts that hepatic basolateral uptake transporters may play a significant role in class 2 drugs, which differs from predictions for the gut, and are necessary for hepatic exposure of poorly permeable class 3 and 4 drugs. The gut has “leakier” membranes (composed of epithelial cells) than the liver, which is composed of endothelial cells. This may possibly explain the difference in observed uptake transporter effects for class 2 drugs.88

Metabolism in hepatocytes is the major eliminating mechanism of class 1 and 2 drugs. Since class 1 and 2 drugs are extensively metabolized and a substantial portion of metabolism occurs hepatically, it follows that hepatic transporters (for class 2 drugs) and/or metabolizing enzymes (for class 1 and 2 drugs) will be significant determinants in their disposition.

Class 1: Class 1 drugs do not depend on uptake for their hepatic access and transporters will have no impact on their disposition. However, hepatic metabolizing enzymes mediate the majority of elimination of class 1 drugs and their function will affect the
disposition of class 1 drugs. If a hepatic metabolizing enzyme contributing to the clearance of a class 1 compound is inhibited, drug exposure (AUC) is expected to increase, and a lower dosage may be required to avoid toxicity. Alternatively, if the metabolizing enzyme is induced, clearance may be greater than expected, resulting in poor exposure and a potential for drug inefficacy.

Example: St. John’s wort is an herbal supplement sometimes taken as an antidepressant. It also acts as an inducer of CYP3A4 and CYP1A2. In numerous cases, drugs including the anticoagulant warfarin, the immunosuppressant cyclosporine, some oral contraceptives, and the HIV protease inhibitor indinavir have seen decreased plasma concentrations or reduced drug-efficacy outcomes when a patient is also taking St. John’s wort, presumably as a result of metabolizing enzyme induction. If a patient has a lower drug concentration than expected, it can lead to serious problems with drug efficacy. If, for instance, the efficacy of warfarin is compromised, INR levels can decrease, which indicates quick blood clotting and is potentially life-threatening. Inefficacy of immunosuppressants can instigate organ rejection, while inefficacy of HIV protease inhibitors could potentially see an increase in HIV viral load.

**Class 2**: The systemic and metabolic disposition of class 2 drugs can be affected by both transport and metabolism. For BDDCS class 2 drugs, the BDDCS predicts that decreased function of a hepatic basolateral uptake transporter may result in increased portal vein concentrations and decreased hepatocyte concentrations. Subsequently, when uptake is inhibited, metabolism in hepatocytes may be decreased, while induction may lead to increased metabolism. Obviously, when metabolism is inhibited, there may be increased plasma or hepatic concentrations of parent drug and decreased elimination.

**Classes 3 and 4**: Class 3 and 4 drugs are primarily eliminated by either renal or biliary elimination of unchanged drugs. We expect that the poorly permeable class 3 and 4 drugs require a transporter to enter hepatocytes, while biliarily eliminated drugs require active canalicular efflux into the highly concentrated bile. Thus, especially if a compound is biliarily eliminated, inhibition of uptake transporters in the liver may result in increased AUC and increased half-life as a result of decreased clearance. However, since inhibition of hepatic uptake transporters will usually also decrease volume of distribution for biliarily eliminated compounds, the increase in half-life will not be as great as the decrease in clearance. A lower dose may be required for biliarily eliminated compounds whose hepatic uptake has been inhibited.

For instance, rosuvastatin and pravastatin are primarily eliminated as unchanged drug in the bile. They are clinically relevant substrates of OATP1B1, and polymorphisms in the gene encoding OATP1B1, SLCO1B1, or drugs inhibiting 1B1 have been shown to increase plasma concentrations and decrease hepatic concentrations of these drugs. Not only does this decrease the efficacy of these statins, whose mechanism of action is in the liver, but also it increases the risk of rhabdomyolysis, a severe muscle toxicity.

### 4.06.4.3.3 Hepatic apical efflux

Apical efflux transporters regulate parent drug and metabolite entry into the bile. Apical efflux transporters in the liver can contribute to the disposition of some class 2, 3, and 4 drugs. Drugs that are eliminated as parent drug in the bile must be actively transported into the bile by canalicular efflux transporters and follow bile flow through the biliary tree until the biliary contents are dumped back into the duodenum. Some drugs, especially highly permeable class 1 or 2 drugs, may be reabsorbed through the gut. Sometimes, when this happens, the plasma concentration–time curve will show two or more peaks. This process is called enterohepatic recycling, which helps conserve bile salts and, in this case, drugs. Drugs that are not reabsorbed in the gut will be eliminated in the feces. Apical efflux can regulate biliary efflux as well as hepatic retention.

**Class 2**: When apical efflux is inhibited, concentrations in hepatocytes are increased. For class 2 drugs, this may result in increased metabolism. However, apical efflux inhibition in the gut results in the opposite effect: decreased metabolism. We hypothesize that this is because the drug is exposed to the apical transporter after metabolizing enzymes in the hepatocyte, while the drug interacts with efflux transporters prior to metabolizing enzymes in the gut.

**Classes 3 and 4**: It has been hypothesized that canalicular efflux does not contribute to the systemic clearance of poorly metabolized drugs. If this is true, there will not be increased exposure of class 3 and 4 drugs. In this case, decreasing the dose may not be necessary to reduce toxic systemic exposure. However, accumulation within hepatocytes sometimes mediates hepatotoxicity, and a decreased dose may be required for this mechanism. There is a paucity of data regarding the effect of apical efflux inhibition on systemic concentrations.

Many parent drugs and metabolites may be (initially) eliminated in the bile. Upon inhibition of biliary efflux, class 2, 3, and 4 drugs may potentially accumulate in hepatocytes. Accumulation of drugs in hepatocytes sometimes leads to DILI. This injury is generally mediated by metabolites. Fortunately, compensatory mechanisms such as increased expression or activity of basolateral efflux transporters can pump some drugs back into the circulation to reduce the chance of injury. Additionally, while it has been generally presumed that unbound concentrations were equivalent throughout the body, we now understand that transporters alter the unbound concentrations of drugs inside a cell. Therefore, since class 1 drugs are not clinically relevant substrates of transporters, pharmaceutical companies presumably have a better understanding of class 1 drug concentrations inside the liver. We therefore expect that DILI may be less of a problem for class 1 drugs or for poorly metabolized class 3 or 4 drugs, but may be a greater problem for class 2 drugs. This hypothesis is currently being investigated. Analogously, the BDDCS may potentially predict as to which drugs may be problematic when dosed to people with dysfunctional efflux transporters, such as in disorders such as Dubin–Johnson syndrome, where MRP2 is poorly functional.
4.06.4.3.4 Insights into metabolism from BDDCS

The BDDCS was pivotal in observing that drugs with a high intestinal passive permeability rate were also extensively metabolized. The rationale behind this observation is that highly permeable drugs, when excreted into hydrophilic secretions, that is, urine and bile, are rapidly reabsorbed due to the high concentration gradients. This gives the drug multiple chances at metabolism, until eventually the compound is changed to a generally more hydrophilic substance that resides in fluidic secretions. Data sets examining reabsorbed compounds support this hypothesis. In a data set published by Dave and Morris,106 82% of drugs that were reabsorbed from the kidney tubule were extensively metabolized drugs. In a data set published from Pfizer,19 slightly more than half of the extensively metabolized drugs were reabsorbed from the kidneys compared with <20% of class 3 and 4 drugs.17 Renal reabsorption is primarily a passive process driven by high tubular concentrations compared with the blood, though reabsorptive transporters are functional and can play a role. Analyzing this data set and using permeability rate values generated from the same group, higher permeability rate compounds tended to be reabsorbed, while lower permeability rate compounds were either passively filtered or secreted (ROC AUC = 0.80).

Highly permeable drugs might also be reabsorbed directly from the biliary tract. When phenolphthalein glucuronide was dosed directly into the bile and prevented from undergoing enterohepatic recycling, it was recovered in hepatocytes, indicating that reabsorption from the bile is possible.107 The BDDCS predicts that if highly permeable BDDCS class 1 and 2 drugs are initially secreted into the bile, they will be reabsorbed. This reabsorption process means that drugs will eventually be metabolized as a necessary elimination step. This is particularly important for some low-clearance compounds such as diazepam, which are too lipophilic to remain in secretions.

Passive permeability, not active transport or the extent of absorption, correlates with the extent of metabolism. In this way, extensive metabolism can be predicted with immortal cell lines such as Caco-2 and MDCK, which express low transport levels, or even artificial membranes such as PAMPA. Artificial membranes do not express transporters and accurately reflect passive permeability. This reduces the need for human tissue in evaluating metabolism with microsomes, supersomes, or hepatocytes. Permeability rate does not necessarily correlate with metabolic clearance, however.

Hosey and Benet showed that the extent of metabolism can be predicted with either in vitro or in silico tools.16 Extreme variability in permeability rate values persists between labs,60 and therefore, numerical permeability rate cutoffs are incapable of predicting the extent of metabolism or absorption. As a solution to this variability, metoprolol has generally been regarded as a standard to determine high permeability rate.62 Compounds exhibiting a permeability rate greater than metoprolol were predicted to be extensively metabolized and highly absorbed. However, metoprolol’s permeability rate is restrictively high when measured in MDCK or Caco-2.12,16,108 mispredicting many extensively metabolized drugs because they had lower permeability rates than metoprolol, and the alternative standards labetalol and zidovudine were assigned for Caco-2 and MDCK, respectively,16 and correctly predicted more compounds across several data sets. While metoprolol was far too conservative to be an effective standard compound in Caco-2 or MDCK, it performs well as a standard in PAMPA.16 Theophylline was selected as an optimal standard when permeability rate studies are conducted in PAMPA and is also too conservative in MDCK or Caco-2. While the BDDCS predicts that drugs that are highly permeable in vitro will be extensively metabolized clinically, and therefore subject to changes in metabolizing enzymes, in vitro systems are not interchangeable and unique protocols must be established for each.

From 20 data sets, 97 ± 5% of compounds with a permeability rate greater than metoprolol were extensively metabolized.16 While most compounds with a permeability rate greater than the selected standards labetalol, zidovudine, or theophylline are extensively metabolized, in many cases, 20–25% of the compounds with a permeability rate lower than these standards are also extensively metabolized. Therefore, while high-permeability rate compounds are almost always extensively metabolized, not all extensively metabolized compounds have a high permeability rate. For increased predictability, a very low-permeability rate marker, chlorothiazide, was established. Most compounds with a permeability rate less than chlorothiazide’s are poorly metabolized. The predictability of compounds with a permeability rate between chlorothiazide and the reference standard is only around 50%, however, and these compounds should be investigated in humans.16

4.06.4.4 Predicting Bioavailability

Bioavailability depends upon the extent of absorption, the extent of metabolism in the gut and the liver, and drug loss due to first-pass biliary elimination. In addition to understanding the metabolic component of bioavailability, all of these processes can potentially be affected by drug transport.

High absorption does not necessarily predict high bioavailability, especially since many highly absorbed drugs are also extensively metabolized. As such, the BDDCS predicts that highly permeable class 1 and 2 drugs will have good absorption, but not necessarily good bioavailability. There is a caveat to this prediction, however. The BDDCS assumes that metabolized compounds are absorbed compounds. However, there may be some compounds that are not metabolized by phase I or phase II metabolism, but by bacteria in the gut lumen. When over 900 drugs were classified into the BDDCS, extensively metabolized drugs were categorized regardless of the mechanism. However, this is a nascent field and few drugs are currently known to be metabolized in this manner.

Class 1: The bioavailability of class 1 compounds can be affected by metabolizing enzymes and inhibition of metabolism will increase the bioavailability.
After eating, some physiological changes occur that can result in dispositional changes to drugs. The predicted dispositional changes as a result of physiological response to food, and more specifically high-fat meals, are tabulated in Table 3.

### Biochemical inhibition

Components in high-fat meals (a high-fat meal contains 800–1000 calories with 50–65% from fat and 25–30% from carbohydrates and 15–20% protein) may inhibit intestinal transport. Lipids consumed, monoglycerides and fatty acids liberated during fat digestion...

**Table 2** Effects of gut apical transporters on pharmacokinetic parameters

<table>
<thead>
<tr>
<th></th>
<th>Decreased functionality of apical uptake</th>
<th>Decreased functionality of apical efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$k_a$</td>
</tr>
<tr>
<td>Class 1</td>
<td>$\uparrow$</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Class 2</td>
<td>$\uparrow$</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Class 3</td>
<td>$\uparrow$</td>
<td>$\downarrow$</td>
</tr>
<tr>
<td>Class 4</td>
<td>$\uparrow$</td>
<td>$\downarrow$</td>
</tr>
</tbody>
</table>

*Class 2:* Class 2 compounds can be affected by both transporters and metabolizing enzymes. Inhibition of efflux transporters in the gut can lead to increased absorption, decreased metabolism, and increased bioavailability. Inhibition of hepatic basolateral uptake may lead to decreased metabolism and increased bioavailability, while inhibition of hepatic basolateral efflux may lead to increased metabolism and decreased bioavailability. Inhibition of metabolizing enzymes may increase bioavailability.

*Classes 3 and 4:* Metabolism is not a significant factor in the bioavailability of class 3 and 4 drugs. However, uptake and efflux transporters can potentially regulate bioavailability in both enterocytes and hepatocytes. One would expect decreased bioavailability if an enteric uptake transporter responsible for a drug’s uptake was inhibited due to decreased absorption. Conversely, inhibition of enteric apical efflux would result in increased absorption and bioavailability. However, first-pass biliary excretion may play a role. Hepatic uptake is generally considered the rate-limiting step of biliary elimination. Therefore, inhibited hepatic uptake of class 3 and 4 drugs would likely result in a decrease in biliary elimination and an increase in bioavailability. It has been suggested that hepatic canalicular efflux does not regulate systemic clearance, and therefore, inhibition of hepatic canalicular efflux would not likely have an effect on bioavailability; however, given the lack of clinical studies on biliary elimination, there are little data to confirm or deny this hypothesis.

In Table 2, we tabulate pharmacokinetic changes that may be expected when the function of an enterocytic drug transporter is decreased for any number of reasons including chemical inhibition from other drugs, food, or endogenous substrates and genetic mutations.

**4.06.4.4.1 Complex considerations: food effects**

#### Predicting food effects

Eating a meal can have a substantial impact on pharmacokinetics. In fact, many drug labels advise that the drug be taken either with a meal or separate from a meal. The act of eating causes a cascade of physiological changes in the gastrointestinal system. These can greatly affect the solubility and the transit time of the drug. In turn, the transit time can affect the exposure to intestinal fluids and membranes, as well as the location of drug-membrane exposure, where different segments of the intestine have different properties including membrane tightness, transporter, and metabolizing enzyme expression. Additionally, food, drinks, and supplements can have a biochemical impact on the drug, where components of each can serve as inhibitors of transport or metabolism. Obviously, the effect of food on pharmacokinetics is a multifaceted problem, which makes predicting food effects a priori quite difficult.

Because food effects are multifactorial, predicting them is a very difficult problem. For one, food chemistry is extremely complex. Even a single food can have multiple molecular components that inhibit uptake, efflux, metabolism, or any combination, forcing a complex interaction. For example, grapefruit juice, famous for its ability to inhibit CYP3A4, is composed of many compounds, including flavonoids and furanocoumarins. Because of the complexity of the composition of a food or drink such as grapefruit juice, the mechanism of inhibition has to be sifted from many compounds. While flavonoids were initially expected to be the perpetrators of this interaction, it was ascertained that certain furanocoumarins are the culprits. When taken with antihistamines such as terfenadine and astemizole, grapefruit juice increased drug exposure to dangerous concentrations that caused cardiotoxicity and, in some cases, death. Both drugs were eventually removed from the market. Many drugs metabolized by CYP3A4 are now labeled with cautions against consuming grapefruit juice. Additionally, components in grapefruit juice have been shown to inhibit uptake transporters and P-gp. Meanwhile, human gastrointestinal physiology is incredibly variable, as are the contents of the gastrointestinal tract. Baseline gastric efflux on a fasted or fed stomach and pH can vary markedly between humans. The microbiome, which can break down drugs, having a direct effect, or influence food digestion, causing an indirect effect, is signature to each person. Despite this complexity and variability, the BDDCS is able to predict the effects of food for approximately 70% of drugs.

#### Physiological responses to meals and their dispositional impact

After eating, some physiological changes occur that can result in dispositional changes to drugs. The predicted dispositional changes as a result of physiological response to food, and more specifically high-fat meals, are tabulated in Table 3.

#### Biochemical inhibition

Components in high-fat meals (a high-fat meal contains 800–1000 calories with 50–65% from fat and 25–30% from carbohydrates and 15–20% protein) may inhibit intestinal transport. Lipids consumed, monoglycerides and fatty acids liberated during fat digestion...
digestion, and bile salts released to aid in digestion have all been shown to inhibit transport, especially that of P-gp\textsuperscript{135,122–124,126} but also of uptake\textsuperscript{115}.

Components in any food, whether or not it is part of a high-fat meal, have the potential to serve as biochemical inhibitors of transport or metabolism and, if consumed daily, inducers. Certain fruit juices, teas, beer, and wine can cause biochemical inhibition of transporters and/or metabolites. For instance, orange, grapefruit, and apple juice have been shown to inhibit OATPs and P-gp in the gut\textsuperscript{110,111,127}.

The specific inhibitors in food can be quite difficult to identify since foods contain small concentrations of many compounds and compounds may have an additive effect.

### Table 3 Dispositional impacts due to physiological changes after a meal

<table>
<thead>
<tr>
<th>Physiological change</th>
<th>Effect on absorptive component</th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>Class 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric fluid increases from 300–500 to 800–900 mL, while intestinal fluid volume increases from 800 to 900–1000 mL\textsuperscript{113–115}</td>
<td>Increase volume for drug dissolution and solvent drag</td>
<td>F \uparrow</td>
<td></td>
<td>F \uparrow</td>
<td></td>
</tr>
<tr>
<td>Phospholipids and bile salts increase four- to fivefold\textsuperscript{116,117}</td>
<td>- Bile salts increase breakdown of fats into monoglycerides - Bile salts inhibit P-gp - Bile salts inhibit uptake - Bile salts increase solubilization - Monoglycerides and lipids liberated by bile salts can inhibit uptake and efflux as well</td>
<td>F \leftrightarrow</td>
<td>F \uparrow</td>
<td>F \leftrightarrow</td>
<td>F \leftrightarrow</td>
</tr>
<tr>
<td>pH decreases with some foods such as juices</td>
<td>Weakly acidic drugs have decreased solubility, while weakly basic drugs have increased solubility</td>
<td>F \leftrightarrow</td>
<td>F \downarrow for acidic drugs</td>
<td>F \leftrightarrow</td>
<td>F \leftrightarrow for acidic drugs</td>
</tr>
<tr>
<td>pH generally increases with food</td>
<td>Decreases solubility for weakly basic drugs\textsuperscript{115,118}</td>
<td>F \leftrightarrow</td>
<td>F \downarrow for basic drugs</td>
<td>F \leftrightarrow</td>
<td>F \leftrightarrow for basic drugs</td>
</tr>
<tr>
<td>Splanchnic blood flow is increased</td>
<td>Improve bioavailability\textsuperscript{115,119}</td>
<td>F \uparrow</td>
<td>F \uparrow</td>
<td>F \uparrow</td>
<td>F \uparrow</td>
</tr>
<tr>
<td>Gastric emptying is delayed</td>
<td>- Increases time before drug can be absorbed in the gut - Increases interactions with gastric acid, which may contribute to drug decomposition\textsuperscript{112,120}</td>
<td>F \uparrow</td>
<td>F \uparrow</td>
<td>F \uparrow</td>
<td>F \uparrow</td>
</tr>
<tr>
<td>Gastric secretions including bile are increased by up to fivefold\textsuperscript{117}</td>
<td>- Increased bile volume allows for increased micellar solubilization and increased drug wetting of poorly soluble drugs - Increased bile concentrations - Break down fats into lipids - Bile salts and lipids can interfere with drug transport\textsuperscript{112–124} - Aggregation into micelles that help with solubilization</td>
<td>F \uparrow</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.06.4.4.1.4 Chemical solubility effect
Drug solubility depends on the pH of fluid, temperature, volume, and contents of fluid. The lipophilicity of a drug is also correlated with water solubility. The rate and extent of absorption can be altered by food.

Factors that increase the amount of drug solubilized are particularly important for BDDCS class 2 and 4 drugs, whose absorption is limited by their poor solubility. Foods can increase solubility by increasing the volume into which a drug can be solubilized, changing the pH of the fluid, and increasing concentrations of bile salts four- to fivefold. After a meal, the volume of intestinal fluids increases two- to threefold, which can potentially increase the amount of drug that is solubilized, meaning more drugs may be available for absorption. Bile salts can improve the solubility of some drugs by acting as surfactants. Changes in the pH of gastrointestinal fluids can alter the solubility of drugs. Acidic drugs will be more poorly soluble in acidic media, while basic drugs will lose solubility in basic media and vice versa.

4.06.4.4.1.5 Overall predicted food effects after consuming high-fat meals for each BDDCS class

Class 1: The overall extent of bioavailability is unlikely to change for BDDCS class 1 drugs since increasing solubility will have no effect and class 1 drugs are not subject to transporter inhibition. Since gastric emptying will be delayed after eating, however, these drugs may be more slowly absorbed, and \( t_{\text{peak}} \) may be later.

Class 2: When class 2 drugs are administered with a high-fat meal, the bioavailability will likely increase, while time to reach peak concentrations may shift in either direction.

High-fat meals may inhibit P-gp, resulting in increased bioavailability. As we discussed earlier, P-gp inhibition can also limit intestinal metabolism of BDDCS class 2 drugs. Therefore, by decreasing both efflux and metabolism, BDDCS class 2 drugs are likely to be more bioavailable when P-gp is inhibited. Additionally, fatty food and the release of bile acids can form micelles, promoting drug solubilization.

The time to reach a maximum concentration for a class 2 drug can be affected by a multitude of factors, and no single trend is predicted. By delaying gastric emptying, a high-fat meal can increase the amount of time it takes for the drug to be absorbed in the intestine, increasing \( t_{\text{peak}} \). The time to reach a maximum concentration may also decrease due to the inhibition of efflux cycling by high-fat meals. Additionally, these processes may compete, causing no effective change in \( t_{\text{peak}} \).

Class 3: High-fat meals, bile salts, and chemical components in food can inhibit intestinal uptake transports, which class 3 drugs rely on to be absorbed. Patients taking a poorly permeable class 3 drug might experience decreased bioavailability and poor exposure when the drug is administered with a high-fat meal. Because their uptake is inhibited, the time to reach \( C_{\text{max}} \) may also increase. \( T_{\text{peak}} \) may also increase as a result of delayed gastric emptying after a high-fat meal.

Class 4: BDDCS class 4 drugs are very difficult to predict because of many interacting effects including increased solubility, increased gastric emptying time, and inhibited uptake. With so few class 4 drugs, it is difficult to predict a single trend.

4.06.4.5 Role in Predicting Distribution

Wu and Benet observed that the volumes of distribution were somewhat higher in the highly permeable class 1 and 2 drugs compared with those for class 3 and 4 drugs. Transporters can markedly affect the volume of distribution by concentrating drug in tissues. When certain major transporters in the liver or kidney are inhibited, Grover and Benet noticed certain trends in distribution.

The liver has a primary effect on the volume of distribution. In peripheral tissues, altered transporter function may have a pharmacodynamic effect and the compound may be attenuated in tissues, but the calculated volume of distribution does not appear to change.

When hepatic uptake is inhibited, there is an increase in plasma concentration coupled with a decrease in hepatic distribution, leading to a decrease in volume of distribution. When hepatic efflux is inhibited, there is also a decrease in the volume of distribution (or is not predictable). Inhibition of hepatic basolateral efflux results in an increase in the volume of distribution.

However, when renal uptake is inhibited, there is generally no effect on the volume of distribution. When renal efflux is inhibited, the volume of distribution often increases. Grover and Benet hypothesize that the discrepancy between changes in volume of distribution due to inhibition of uptake in the liver versus the kidney is likely a result of the larger mass of the liver, coupled with increased capacity for transporter expression and drug sequestration.

Finally, gut transporters will not have an effect on the volume of distribution because volume of distribution is a systemic parameter.

Since class 1 drugs have no clinically relevant transporter effects, we expect no changes in the volume of distribution of class 1 drugs when a transporter has increased or decreased function or expression. Alternatively, since uptake and efflux transporters can affect class 2, 3, and 4 drugs in the liver, we would expect changes to the volume of distribution as described in the preceding text.

4.06.4.5.1 Distribution into the brain

Distribution to various tissues can be predicted by the BDDCS. Specifically, we now understand the conditions necessary for central nervous system penetration. This is a particularly difficult problem during drug development of central nervous system...
(CNS)-targeted drugs, as the brain is well protected from xenobiotics with tight junctions and high efflux transporter expression. Understanding and predicting brain penetration are also important to avoid central side effects for a peripherally acting drug. P-gp, BCRP, and various MRPs are expressed on the apical membrane of brain capillary endothelial cells, poised to extrude drugs that gain entry across its membrane. In development, substrate specificity for efflux transporters is a cue that the drug will be unable to successfully penetrate the brain. When the brain is the intended site of action, lipophilic compounds with a low polar surface area are expected to be available to the CNS.125,130

The brain is also a particularly concerning tissue for drug resistance. Some diseases, including some cancers and epilepsy, are resistant to drug penetration in the brain as a result of overexpressed P-gp or other efflux transporters. This overexpression is sometimes innate to the disease and sometimes acquired, potentially due to drug treatment. To overcome drug resistance, some scientists have proposed codosing with efflux inhibitors. Alternatively, dosing class 1 drugs may be a more thorough and facile approach when brain concentrations are desired.

Mahar Doan et al.129 suggested that highly permeable, non-P-gp substrates were likely to cross the blood–brain barrier, while poorly permeable and P-gp substrates are less likely to cross the blood–brain barrier. While this holds true for a majority of compounds, an analysis by Wager et al. revealed that 20% of CNS drugs were both poorly permeable and P-gp substrates.131 Broccatelli et al.132 incorporated BDDCS classifications, correctly predicting the CNS distribution of >90% of their data set. Ninety-eight percent of class 1 drugs in their data set were able to cross the CNS, whether or not they were a substrate for P-gp. In fact, after correcting for a misclassification, all of the BDDCS class 1 P-gp substrates were able to distribute into the CNS. Even when P-gp was able to partially efflux the drug, there was significant brain penetration. In contrast, 75% of P-gp substrates in classes 2, 3, and 4 were unable to traverse the blood–brain barrier. While presumably all of the class 1 drugs have CNS exposure, even if they are P-gp substrates, clearly, 25% of P-gp substrates in other classes were still able to access the brain, likely because they are good substrates for uptake transporters at the brain. While Broccatelli et al. only considered P-gp substrate specificity, other efflux transporters such as BCRP are expressed at the blood–brain barrier and are responsible for extruding drugs. The same principles should apply to substrates of other efflux transporters. Based on these findings, each class is predicted to behave as follows:

Class 1: Transporter effects are minimal and drugs are expected to penetrate the CNS.
Class 2: Efflux transporters at the blood–brain barrier may affect class 2 drugs.
Class 3: Uptake transporters at the blood–brain barrier (OATP1A2 and OATP2B1) are required for brain penetration, while efflux transporters can extrude drugs from the brain.
Class 4: Uptake transporters at the blood–brain barrier are required for brain penetration, while efflux transporters can extrude drugs from the brain.

Therefore, when developing a drug with a CNS indication, a class 1 drug may be preferable for candidate selection, since it will penetrate, regardless of transporter affinity. Class 2 drugs may be developable as long as they are not substrates for efflux transporters. Class 3 and 4 drugs have more stringent requirements. For a class 3 or 4 drug to be effective as a CNS agent, it must be a substrate for an uptake transporter in the gut (if it is orally administered) and at the blood–brain barrier and should not be a substrate for efflux transporters at the blood–brain barrier.

Alternatively, when developing peripherally acting drugs, class 1 drugs may have potential CNS side effects, even if they are substrates for efflux transporters. Class 2 drugs may have central effects if they are not substrates of efflux transporters. To avoid central effects for class 3 and 4 drugs, it is best to avoid substrates of uptake transporters at the blood–brain barrier. Non-class 1 drugs will need to be evaluated as substrates of CNS-expressed transporters to predict brain penetration. In Table 4, we show the predicted effects of apical transporters on CNS exposure.

### Table 4
Effect of apical transporters on central nervous system distribution

<table>
<thead>
<tr>
<th>BDDCS class</th>
<th>Apical uptake</th>
<th>Apical efflux</th>
<th>CNS exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>No effect</td>
<td>No effect</td>
<td>Yes</td>
</tr>
<tr>
<td>Class 2</td>
<td>• May be necessary for some class 2 drugs to penetrate the brain</td>
<td>• May decrease CNS exposure</td>
<td>If a nonsubstrate for efflux and may possibly require an uptake transporter</td>
</tr>
<tr>
<td></td>
<td>• Inhibition may lead to decreased CNS exposure</td>
<td>• Inhibition may lead to increased CNS exposure</td>
<td></td>
</tr>
<tr>
<td>Class 3</td>
<td>• Required for CNS exposure</td>
<td>• Will prevent exposure to the CNS</td>
<td>If a substrate for an uptake transporter and a nonsubstrate for an efflux transporter</td>
</tr>
<tr>
<td></td>
<td>• Inhibition will lead to decreased CNS exposure</td>
<td>• Inhibition may lead to increased CNS exposure</td>
<td></td>
</tr>
<tr>
<td>Class 4</td>
<td>• Required for CNS exposure</td>
<td>• Will prevent exposure to the CNS</td>
<td>If a substrate for an uptake transporter and a nonsubstrate for an efflux transporter</td>
</tr>
<tr>
<td></td>
<td>• Inhibition will lead to decreased CNS exposure</td>
<td>• Inhibition may lead to increased CNS exposure</td>
<td></td>
</tr>
</tbody>
</table>
Chronic kidney disease (CKD) is a serious condition, affecting more than 10% of adults in the United States. Since the kidney
4.06.4.5.2.2 Renal impairment
As discussed earlier, renal elimination is a combination of passive filtration processes, reabsorption, and active secretion. Reabsorption is primarily passive, and the vast majority of water and solutes are reabsorbed along the tubule, resulting in only 1 mL of urine production every minute. There are transporters responsible for active reabsorption expressed primarily in the proximal tubule, however. While a number of secretory transporters are expressed along the proximal tubule, OAT1, OAT3, and OCT2 are currently considered the most clinically significant, and the FDA recommends studying renally eliminated drugs for interactions with these transporters. All drugs should be evaluated as substrates of P-gp, as well. However, the ITC lists a number of renal transporters that they consider important to evaluate during drug development, including the bidirectional transporters ENT2, expressed on the basolateral membrane, and ENT1, OC1N1, and OAT4, expressed on the apical membrane. Secretory transporters including OAT2 and OATP4C1, which are expressed on the basolateral membrane, and MATE1, MATE2-K, MATE2, MR2, and MRP4, all of which are expressed on the apical membrane, are considered relevant by the ITC. They also include the absorptive transporter URAT1, which is expressed on the apical membrane.

Class 1 and 2 drugs are likely to be reabsorbed from the tubule, as we have discussed. We expect that class 2 drugs may interact with basolateral uptake and apical efflux transporters, similar to hepatic predictions. We expect that uptake and efflux transport will be required to contribute to net secretion of class 3 and 4 compounds. However, renal elimination can also be completely passive, and class 3 and 4 compounds are not necessarily substrates of renal transporters even if they are eliminated as unchanged drug in the urine.

4.06.4.5.2.2 Renal impairment
Chronic kidney disease (CKD) is a serious condition, affecting more than 10% of adults in the United States. Since the kidney eliminates many drugs and metabolites, impaired renal function can also seriously decrease renal clearance of these drugs, mandating dose adjustments in patients. One may understandably, but mistakenly, conclude that renal dysfunction should only affect renally eliminated drugs. In fact, metabolism can be dangerously altered in CKD patients, particularly as disease progresses. When the kidneys begin to lose their function, endogenous compounds that are eliminated by the kidneys in healthy people can accumulate to toxic concentrations. These compounds are called uremic toxins. Initially, it was hypothesized that uremic toxins inhibited metabolizing enzymes. Investigations showed that uremic toxins inhibited some, but not all, CYP metabolizing enzymes. As it became clear that drug transporters also played a role in controlling drug access to metabolizing enzymes, Reyes and Benet questioned if perhaps uremic toxins could also inhibit transporters, potentially reducing metabolic clearance in vivo. They concluded that uremic toxins could inhibit uptake transporters in hepatocytes. Since the disposition of a class 1 drug is unlikely to be affected by drug transporters, they tested whether uremic toxins inhibited hepatic exposure of propranolol, a class 1 drug. While uremic toxins did not inhibit the uptake of propranolol, some uremic toxins did inhibit the uptake of losartan, a class 2 drug, and eprosartan, a class 4 drug.

In this study, uremic toxins were unable to inhibit phase 1 metabolism when human uremic serum was incubated with microsomes dosed with propranolol or losartan, both of which are extensively metabolized. Given previous evidence, it would be unwise to suggest that metabolizing enzymes are not inhibited by uremic toxins. Therefore, decreased metabolism may be observed for class 1 and 2 drugs taken by patients with CKD. Alternatively, uptake transporters are likely inhibited by uremic toxins, which may decrease the metabolism of class 2 drugs and decrease hepatic clearance of class 3 and 4 drugs. All drugs should be tested for increased parent drug exposure, though the mechanism of inhibition will differ between classes.

This is a critical prediction that may (a) increase the safety of drugs in end stage renal disease (ESRD) patients, many of whom require several drugs, and (b) ease the developmental burden. The FDA now recommends that most new molecular entities be evaluated in ESRD patients, excepting drugs predominantly cleared by the lungs, monoclonal antibodies, and drugs intended for single-dose administration. However, generating these studies and recruiting patients are difficult, costly, and variable. Applying BDDCS concepts to pharmacokinetic studies in renal disease may help prioritize what studies are necessary and help understand if clinicians should be concerned about inhibition of transporters, metabolizing enzymes, or both in administering one or multiple drugs.
The BDDCS provides many valuable predictions that can be useful in guiding drug development decisions. It currently relies on the extent of metabolism, which cannot be assessed until phase I studies and solubility studies. It would be extremely useful to have accurate high-throughput methods to predict BDDCS class prior to phase I trials.

### 4.06.4.6 Predicting BDDCS Class

#### 4.06.4.6.1 In vitro

Based on the recognition of the ability of membrane permeability rate to differentiate extensively versus poorly metabolized compounds in the development of the BDDCS, Varma et al.\(^1\) have confirmed that BDDCS class can be predicted well using in vitro apparent permeability rate as measured in MDCK-LE (low eflux) cells at pH 6.5 for acids and pH 7.4 for bases and solubility measured at pH 1.2 in PBS for acidic compounds and in FaSSIF for all other compounds. They used an internally developed permeability rate cutoff of $5 \times 10^{-6}$ cm s$^{-1}$, above which compounds were predicted to be extensively metabolized and below which compounds were predicted as poorly metabolized. Dose strength is generally determined during phase II and III clinical trials. This makes it difficult to accurately predict the dose number of a drug. This group proposed a solubility cutoff of 200 µg mL$^{-1}$, which corresponds to a 50 mg dose being entirely soluble in 250 mL of water. This approach correctly predicted 84% of the compounds in their data set, specifically 83%, 83%, 88%, and 67% of class 1, 2, 3, and 4 drugs, respectively. Additionally, over 90% of the drugs predicted as class 1 or class 2 actually belonged to those classes, and over 80% of the drugs predicted to be class 3 were actually class 3, while 40% of the drugs predicted to be class 4 actually were class 4. Due to the small number of drugs that actually are class 4 and that are predicted to be class 4, the poor class 4 results should not dissuade the reader from realizing how well this in vitro approach predicted BDDCS class.

#### 4.06.4.6.2 In silico

To ease the time and cost of these predictions during development, an in silico approach is preferable. There have been at least two attempts to predict BDDCS class in silico. In 2007, Khandelwal et al.\(^1\) developed models using machine learning methods including recursive partitioning, random forest, and support vector machines. They used molecular features to assign drugs to one of the four BDDCS classes, predicting 33.3% correctly overall. In 2012, using the extended data set published by Benet et al.,\(^2\) Broccatelli et al.\(^3\) used a binary approach to predict the solubility and the extent of metabolism of the drugs before making a class prediction. Solubility was predicted using naive Bayes, k-nearest neighbor, and support vector machine models, where the solubility class was assigned using a consensus model, which predicted the class based on how it was predicted in a majority of the models. This model was 77% accurate. The extent of metabolism was predicted from a consensus model of a naive Bayes and two support vector machine models. This model was 79% accurate. When combining the solubility and extent of metabolism models to predict BDDCS class, however, this approach was only 55% accurate.

There are currently other efforts ongoing to improve in silico BDDCS class predictions. However, at this time, in vitro approaches provide more accurate predictions.

### 4.06.5 Conclusions

Predicting drug disposition is often difficult since there are many variables at play, which we do not yet fully understand. While quantitative predictive approaches from in vitro and in vivo models serve a crucial role in drug development, qualitative classification approaches such as the Biopharmaceutics Classification System and the BDDCS can help predict when such in vitro and in vivo studies may provide useful and interpretable results. These systems can potentially reduce both time and cost of drug development, which is ideal for both developers and consumers.

The BCS has been successfully implemented to grant biowaivers to at least 41 drugs,\(^4\) reducing time and costs, which can potentially be passed down to consumers.

The BDDCS makes a broad range of current and potential dispositional predictions. This system can be useful for drugs already on the market to predict potential transporter or metabolizing enzyme effects that were not tested during development, thereby ensuring that these drugs remain safe and efficacious. These predictions can also help guide the development of new drugs, where a complete understanding of transporters and enzymes is required. This system predicts when metabolizing enzymes and transporters will be important in the absorption, metabolism, and elimination of drugs and currently helps us understand where transporters are relevant in the distribution of drugs to the central nervous system, with potential applications to other organs such as the heart, kidneys, and placenta. In this way, important transporters and enzymes will certainly be evaluated during development, whereas transporters or enzymes that are predicted to be clinically irrelevant may be safely dismissed. This can help speed up the process of development and reduce unnecessary costs. The BDDCS has also been used to help understand disposition in special states, such as kidney failure, and is currently being investigated for its predictive utility in toxicology.

We know of no class 1 drugs for which a dose adjustment has been required due to a transporter alteration as a result of drug–drug interactions, food, endogenous substrates, or pharmacogenomics, among other possible alterations. Drug development is trending toward larger, more lipophilic, and less soluble drugs. Therefore, BDDCS class 2 drugs are rapidly becoming the most important class. While these drugs will likely have improved potency, we might also expect more dispositional issues since they...
can be affected by both metabolizing enzymes and transporters. Finally, class 3 and 4 drugs are not significant substrates of metabolizing enzymes, but are substrates of many transporters and require transporter investigation.

References


16. Hosey, C. M.; Benet, L. Z. Predicting the Extent of Metabolism Using In Vitro Permeability Rate Measurements and In Silico Permeability Rate Predictions. Mol. Pharm. 2015, 12, 1436–1446.


