Part Five
Drug Development Issues

19
Application of the Biopharmaceutics Classification System Now and in the Future

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Abbreviations

ANDA	Abbreviated new drug application
BCRP	Breast cancer-resistant protein
BCS	Biopharmaceutics Classification System
CYP3A4	Cytochrome P450 3A4
EMEA	European Medical Evaluation Agency
ER	Extended release
FDA	Food and Drug Administration
GI	Gastrointestinal
HBD	Number of hydrogen-bond donors
hPepT1	Oligopeptide carrier for di- and tripeptides
ICH	International Committee of Harmonization
IR	Immediate release
IVIVC	*In vitro*/*in vivo* correlation
MCT	Monocarboxylic acid cotransporter
MRP	Multidrug-resistant protein family
NDA	New drug application
P-gp	P-Glycoprotein
PSA	Polar molecular surface area
SR	Solubilization ratio

Symbols

CL_{int}	Intrinsic clearance
Clog *P*	Logarithm of the calculated octanol/water partition coefficient (for neutral species)
E_G	Gut wall extraction
E_H	Hepatic extraction
F	Bioavailability

f_a	Fraction dose absorbed
f_u	Fraction drug unbound in plasma
log $D_{6.5}$	Logarithm of the distribution coefficient in octanol/water at pH 6.5
MW	Molecular weight
P_{eff}	Effective intestinal permeability
Q_h	Hepatic blood flow

19.1 Introduction

Almost all of the 50 most sold drug products in the US and European markets are administered orally (Figure 19.1). Significant drug absorption and appropriate drug delivery are prerequisites for successful oral treatment of diseases. Through retrospective analysis, the reasons behind failures in the development of oral drugs for the market have been poor pharmacokinetic properties, lack of efficacy, safety issues, and marketing, as shown in Figure 19.2 [1–3]. Among the pharmacokinetic aspects, a low and highly variable bioavailability, that is, the amount of drug that reaches the plasma compartment, is indeed considered to be the main reason for stopping the further development of the pharmaceutical product [3]. It is not surprising that pharmacokinetics is crucial for a successful drug development since the plasma drug levels are related in various ways to the effects at the sites of pharmacological and toxicological actions (pharmacodynamics) (Figure 19.3).

It is also well recognized that the design and composition of the pharmaceutical dosage form may have an important impact on the bioavailability and hence the therapeutic outcome of a drug product. This includes both intentional effects such as altered drug absorption rates by modified-release formulations or increased bioavailability for dosage forms including absorption-enhancing principles and undesirable effects such as reduction of the amount of drug reaching the systemic circulation as a result of poor product design. Consequently, bioavailability also reflects the pharmaceutical product quality and *in vivo* performance for oral dosage forms. This has to be considered in the development of generic products, which

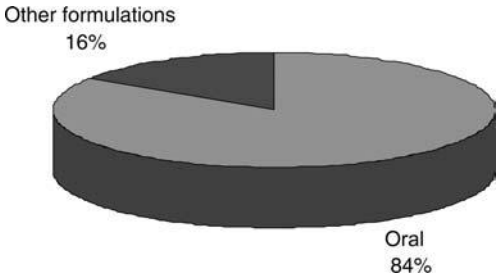

Figure 19.1 Percent sales of orally administered drugs for the 50 most sold products in the United States and Europe (from IMS Health 2001).

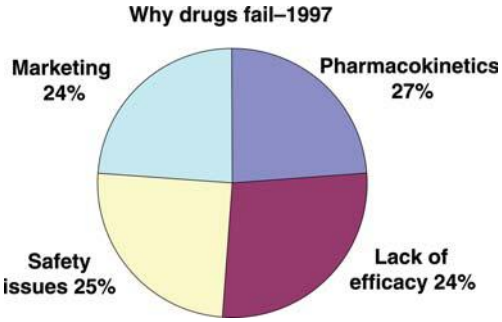

Figure 19.2 The reasons why clinical development of drugs are sometimes terminated and the drug does not reach the market are due to safety issues, marketing reasons, lack of efficacy, and/or pharmacokinetics/bioavailability (from Dr Lawrence Lesko, FDA Regulatory Standards: BA/BE & PK/PD; in Strategies for Oral Drug Delivery, Lake Tahoe, USA, March 6–10, 2000).

should be interchangeable with the original product and provide the same clinical outcome, or when formulations and manufacturing processes are changed during clinical development or for a marketed product. *In vivo* investigations comparing the bioavailability of two formulations of the same drug with the aim to verify sufficient similarity from a clinical perspective for a "new" versus an "old" formulation are called bioequivalence studies.

Successful development of pharmaceutical products for oral use requires identification of the rate-limiting step(s) of the intestinal absorption process of the drug. This will aid in the selection of suitable candidate molecules for drug development as well as in the design of a dosage form. Biopharmaceutical investigations are needed to obtain the necessary understanding of the intestinal absorption process. The rate and extent of drug absorption (f_a) from a solid dosage form during its transit through the

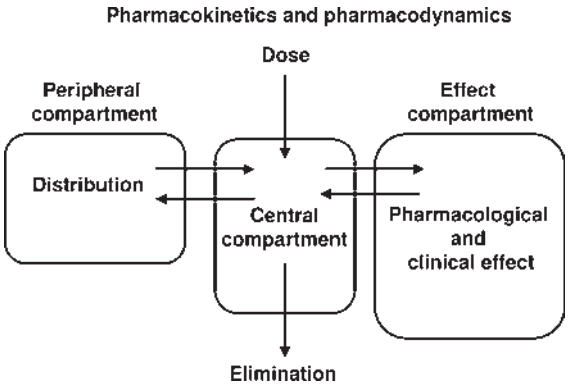

Figure 19.3 A schematic drawing of the relation between pharmacokinetics and pharmacodynamics to better understand the action of drugs.

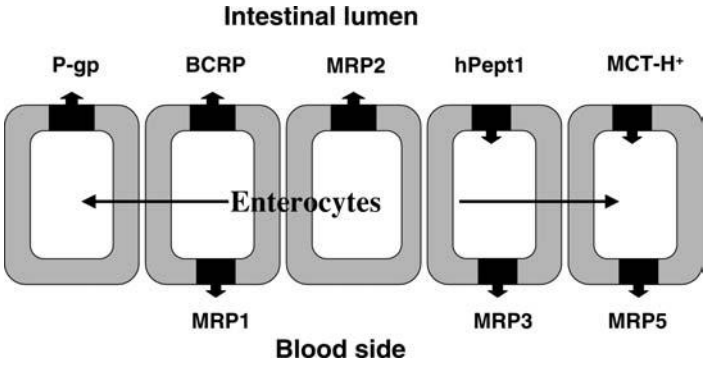

Figure 19.4 The intestinal permeability of drugs *in vivo* is the total transport parameter that may be affected by several parallel transport mechanisms in both absorptive and secretory directions. A few of the most important transport proteins that may be involved in the intestinal transport of drugs and their metabolites across intestinal epithelial membrane barriers in humans are displayed. P-gp, glycoprotein; BCRP, breast cancer-resistant protein; MRP1-5, multidrug-resistant protein family; hPepT1, oligopeptide carrier for di- and tripeptides; MCT-$H^{(+)}$, monocarboxylic acid cotransporter.

small and large intestines include several steps: drug release and dissolution, potential stability and binding issues in the lumen, transit time, and effective intestinal permeability (P_{eff}) [4, 5]. The transport of a drug across the intestinal barrier (P_{eff}) may be complex and involve multiple transport mechanisms as illustrated in Figure 19.4. For instance, the transport measured is a consequence of parallel processes in the absorptive directions such as passive diffusion and carrier-mediated uptake through proteins such as oligopeptide (PepT1), monocarboxylic cotransporter (MCT-H^+) amino acid transporters, and others [6–9]. Today, there is also evidence that transport in the secretory directions through various efflux proteins may restrict both the rate and extent of intestinal absorption. The following efflux proteins are located in the human intestine: P-glycoprotein, multidrug-resistant protein family (MRP-family 1–6), and breast cancer-resistant protein (BCRP) [10]. However, many efflux transport substrates show complete intestinal absorption, and the pharmacokinetics is superimposable with increasing dose [11–13].

In many cases, the intestinal P_{eff} is considered to be the rate-limiting step in the overall absorption process, and this poor intestinal permeability of drugs constitutes a major bottleneck in the successful development of candidate drugs [2, 5, 14–16]. However, in drug discovery today, several new pharmacological targets, for instance, intracellular receptors, and the use of high-throughput techniques, including permeability screens, have brought more lipophilic compounds into drug development [2, 14]. Novel candidate drugs will therefore often be poorly soluble in water [2, 14]. This could limit the bioavailability to an extent that endangers successful product development though poor permeability could be expected to be less of an issue for these molecules. However, several formulation principles are available that could be applied to increase dissolution and solubility. Thus, drug molecules with a favorable pharmacological profile but poor biopharmaceutical properties could thereby sometimes be

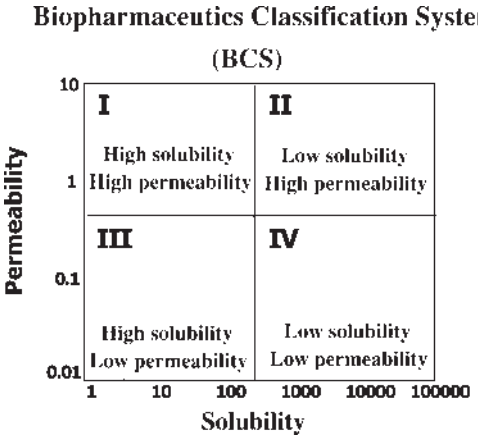

Figure 19.5 The Biopharmaceutics Classification System provides a scientific basis for predicting intestinal drug absorption and for identifying the rate-limiting step based on primary biopharmaceutical properties such as solubility and effective intestinal permeability (P_{eff}). The BCS divides drugs into four different classes based on their solubility and intestinal permeability. Drug regulation aspects related to *in vivo* performance of pharmaceutical dosage forms have been the driving force in the development of BCS. Guidance for industry based on BCS mainly clarifies when bioavailability/bioequivalence studies can be replaced by *in vitro* bioequivalence testing (www.fda.gov/cder/guidance/3618fnl.htm).

saved from development failures and turned into useful pharmaceutical products. Such a reliance on formulations that reduce the shortcomings of the pure active drug is more controversial for poor permeability drugs, for which the use of permeation enhancers to increase P_{eff} has been described, but this is still more of an explorative research area than an established tool in product development.

The Biopharmaceutics Classification System (BCS) provides a scientific basis for predicting intestinal drug absorption and identifying the rate-limiting step on the basis of primary biopharmaceutical properties such as solubility and effective intestinal permeability (P_{eff}) [4, 17, 18]. The BCS divides drugs into four different classes on the basis of their solubility and intestinal permeability (Figure 19.5). Drug regulation aspects related to *in vivo* performance of pharmaceutical dosage forms have been the driving force in the development of BCS. Guidance for industry based on BCS mainly clarifies when bioavailability/bioequivalence (BA/BE) studies can be replaced by *in vitro* bioequivalence testing [17, 19].

The aim of this chapter is to describe the BCS and the science behind BCS and to discuss its use in the development of oral pharmaceutical products from both regulatory and nonregulatory aspects.

19.2
Definition of Absorption and Bioavailability of Drugs Following Oral Administration

The general definition of the bioavailability (F) of a drug following oral administration is the rate at and extent to which a pharmacological active drug reaches the systemic

circulation. The bioavailability (F) of a compound is a consequence of several processes shown in Equation 19.1:

$$F = f_a \cdot (1-E_G) \cdot (1-E_H), \tag{19.1}$$

where the extent of absorption (f_a) includes all processes from dissolution of the solid dosage form to the intestinal transport of the drug into the intestinal tissue, that is, across the apical membrane of the enterocyte. This is the general definition of the extent of absorption and does not include the metabolic first-pass effect in the gut (E_G) and/or metabolism/biliary excretion in the liver (E_H) [4, 5].

The rate (mass/time) and fractional extent of drug absorption (mass/dose $= f_a$) from the intestinal lumen *in vivo* at any time t is schematically shown in Equation 19.2 [4]:

$$\frac{M(t)}{\text{dose}} = \int_0^t \iiint A \cdot P_{\text{eff}} \cdot C_{\text{lumen}} \cdot \mathrm{d}A\, \mathrm{d}t, \tag{19.2}$$

where A is the available intestinal surface area, P_{eff} is the average value of the effective intestinal permeability along the intestinal region where absorption occurs, and C_{lumen} is the free concentration of the drug in the lumen from the corresponding intestinal part [4, 20]. Several processes such as dissolution rate, degradation, metabolism, and binding in the gastrointestinal tract affect the free drug concentration in the lumen.

19.3 Dissolution and Solubility

Dissolution of a drug molecule into the GI fluids is a prerequisite for drug absorption since the permeability of particulate material over the GI mucosa is negligible in the context of oral drug bioavailability. The dissolution process could thereby affect both the rate and extent of oral drug absorption. The use of high-throughput techniques in the modern drug discovery process brings more lipophilic compounds into drug development [2, 14]. This means that drug dissolution in the gastrointestinal fluids has become increasingly important to be considered in the design, development, and optimization of a solid oral drug product.

Drug dissolution is the dynamic process by which solid material is dissolved in a solvent and is characterized by a rate (amount/time), whereas solubility describes an equilibrium state, where the maximal amount of drug per volume unit is dissolved.

The solubility, as well as the dissolution, in a water solution depends on factors such as pH and contents of salts and surfactants.

The solubility is most often experimentally determined from the drug concentration in the liquid phase after adding excessive amounts of a solid drug substance to the test medium. This apparent solubility is affected by the solid-state properties of the drug, for example, polymorphs, solvates, impurities, and amorphous content. An equilibrium with the thermodynamically most stable solid-state form, being the least

soluble, should eventually be reached. This could, however, be a very slow process requiring several days. More short-term supersaturation phenomena may also occur; that is, the measured solubility is much higher than the true saturation solubility during an initial phase before precipitation occurs from the supersaturated solution and an equilibrium can be reached. Thus, although solubility is a simple concept, it is far from being unproblematic to obtain robust data because of the indicated time dependence and effects of differences in solid-state properties as well as other sources of experimental variability.

The dissolution of drugs has been described by the Noyes–Whitney equation and later modified by Nernst and Brunner [21, 22]:

$$\frac{dX}{dt} = \frac{AD}{h(C_s - X_d/V)}, \tag{19.3}$$

where dX/dt is the dissolution rate in terms of mass X per unit time t, A is the available surface area of the solid drug, D is the drug diffusion coefficient, h is the effective diffusion boundary layer thickness, C_s is the saturation solubility of the drug in the test medium, X_d is the amount of drug already in solution, and V is the volume of fluid in the lumen available for dissolution. The dissolution rate is not an inherent property for a drug substance and will vary with the solid-state properties such as particles size, degree of crystallinity, and crystal form.

The drug dissolution rate could be determined by dispersing the powder in a test medium under suitable agitation or by studying the dissolution for a constant surface area by using the rotating-disk method (Figure 19.6). The latter method should be the technique of choice, except when studies of the effect of particle size are of special relevance. The rotating-disk method, which is described in the United State Pharmacopeia, has the advantage of providing very well-defined hydrodynamic test

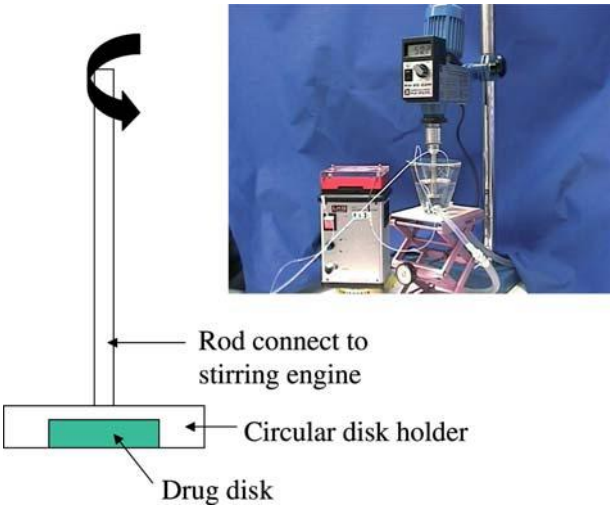

Figure 19.6 A schematic drawing and photo of the rotating-disk method.

conditions and a known drug surface area, which reduces the risk for artifacts and allows for accurate comparisons of data obtained for different drugs, in different labs. In addition, the test conditions in the rotating-disk method also allow for more mechanistic evaluations of the dissolution process, such as determination of the drug diffusion coefficient and estimation of an intrinsic dissolution rate, being independent of hydrodynamic conditions [23].

The solubility as well as the dissolution rate in the GI tract is affected by several physiological factors, which have to be taken into account when predicting the influence of drug dissolution on the oral bioavailability. These include physicochemical properties of the GI fluids, agitation provided by the GI motility, and available volumes of GI fluids. The *in vivo* situation is highly complex and depends, for example, on the nutritional status. The conditions also differ in different regions within the GI tract. Human GI juices have been characterized after both fasting and fed conditions by measuring aspiration through tubes [24, 25]. A summary of the most important variables for the fasting and fed states is given in Table 19.1. In addition to these main effects, the GI conditions of relevance for drug dissolution may also be affected by other cyclic variations, diurnal effects, diseases, age, and concomitant medications [22].

A summary of how physiological factors affect the dissolution rate is given in Table 19.2. More extensive characterizations of human gastrointestinal fluids, during both fasting and fed conditions, have been performed with respect to components and physicochemical parameters of relevance for drug dissolution [26, 27] The effective surface area will be affected by the wetting properties of the bile acids and other surface-active agents in the GI tract. The diffusivity of a drug molecule in the intestinal juice will be altered by changes in viscosity induced, for instance, by meal components. An increased dissolution rate could be obtained at more intense intestinal motility patterns or increased flow rates [22]. The effect on the dissolution of a low-solubility compound of different hydrodynamic conditions, being relevant

Table 19.1 Summary of physiological GI characteristics in fasting and fed state of importance for drug dissolution and solubility.

	Fasting	**Fed**
Stomach		
• Fluid volume (ml)	50–100	Up to 1000 ml
• pH	1–2	2–5
• Ionic strength	0.10	Varying
• Motility pattern/intensity	Cyclic/low–high	Continuous/high
• Surface tension (mN/m)	40	Often lower than fasting
• Osmolarity (mOsm)	hyposmotic	hyperosmotic
Upper small intestine		
• Flow rate (ml/min)	0.6–1.2	2.0–4.2
• pH	5.5–6.5	Similar
• Bile acids (mm)	4–6	10–40
• Ionic strength	0.16	0.16

Table 19.2 Physicochemical and physiological parameters important to drug dissolution in the gastrointestinal tract [22].

Factor	Physicochemical parameter	Physiological parameter
Surface area of drug (A)	Particle size, wettability	Surfactants in gastric juice and bile
Diffusivity of drug (D)	Molecular size	Viscosity of luminal contents
Boundary layer thickness (h)		Motility patterns and flow rate
Solubility (C_s)	Hydrophilicity, crystal structure, solubilization	pH, buffer capacity, bile, food components
Amount of drug already dissolved (C_t)		Permeability
Volume of solvent available (C_t)		Secretions, coadministered fluids

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for the fasting and fed states, was recently investigated in an *in vivo* study [28]. It was found that the hydrodynamic conditions significantly affected both rate and extent of bioavailability for slowly dissolving unmilled drug particles whereas for more rapidly dissolving micronized drug substances no effect was detected. The lack of influence of agitation on dissolution of small (radius $\sim$<5 μm) primary drug particles is in accordance with the theoretical work by Nielsen [29]. The lack of influence by different degrees of stirring on dissolution of small particles in contrast to the reverse for latter particles has also been observed in *in vitro* dissolution experiments [30]. The saturation solubility in the GI fluids could be affected by several factors such as pH, solubilization by bile acids, or dissolution in lipid food components [31]. The pH, which varies according to region as well as food intake, is a key factor for proteolytic drugs with pK_a values within or close to the physiological pH interval. The bile concentrations increase after food intake, and mixed micelles with nutritional lipids are formed. However, significantly enhanced solubility due to solubilization can be achieved already under fasting conditions. An example of the dramatic increase in solubility due to solubilization by bile components is given in Figure 19.7. The solubilization by bile acids increases by increased drug lipophilicity. An empirical algorithm for the solubilization ratio (SR) of drugs in bile acids has been developed (Equation 19.4), which indicates that for drugs with a log $P < 2$ no solubility improvement should be expected [32]. In a pharmacokinetic database of a total of 472 drugs, it was found that 235 drugs (50%) had a log P value higher than 2, which means that this process applies to a large part of the clinically used drugs [33].

$$\log \mathrm{SR} = 2.09 + 0.64 \log P. \tag{19.4}$$

The micellar solubilization of drugs in the small intestine is increased not only due to the higher bile acid secretion induced by a meal but also by lipid components of nutritional origin, such as fatty acids and monoglycerides. The solubility of six drugs

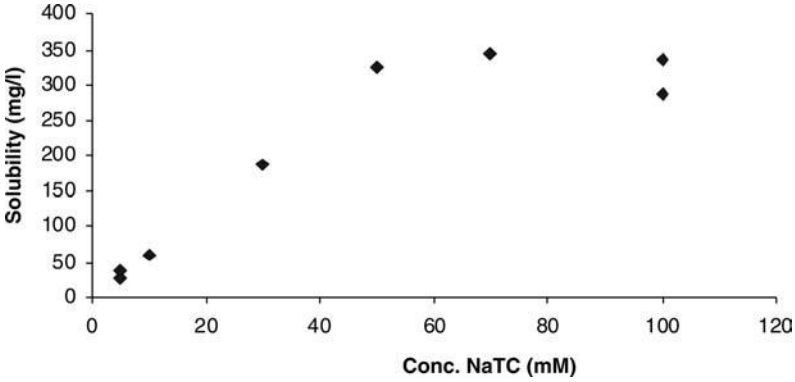

Figure 19.7 Solubility of a very poorly soluble drug, candersartan cilexitil, at different concentrations of bile acid/lecithin (2.5 : 1).

was increased 3–30 times in the fed compared to the fasting state in human intestinal fluid [34].

The amount of drug in solution, which will affect the drug dissolution rate at "nonsink conditions," is dependent on the available volume that is controlled by oral intake, secretions, and water flux over the GI wall. For instance, it has been approximated that the physiological volume of the small intestine varies from 50 to 1100 ml, with an average of 500 ml in the fasting state [4, 18]. A more recent study quantifying free water in the small intestine in man by the use of magnetic resonance imaging found liquid volumes of around only 100 ml [35]. It should also be realized that the small intestine is not a water-filled tube and that the fluids are irregularly located in pockets. The drug concentration in the intestinal lumen is not only a function of dissolution and available volumes but also dependent on the drug permeability, which will be of special importance when the drug levels in the intestine approach the saturation level.

It should, however, be noted that it is almost impossible to fully predict the *in vivo* dissolution rate because of the many factors involved, of which several have not yet been completely characterized. The introduction of new study techniques to directly follow drug dissolution *in vivo* in the human intestine should therefore be of importance [36, 37]. For example, *in vivo* dissolution studies, on the basis of the intestinal perfusate samples, discriminated between the dissolution rates of the two different particle sizes of spironolactone. In addition, dissolution rates of carbamazepine obtained *in vitro* were significantly slower than the direct *in vivo* measurements obtained from the perfusion method. The higher *in vivo* dissolution rate was probably due to the efficient sink conditions provided by the high permeability of carbamazepine [36, 37].

It is highly desirable in drug discovery and early drug development to predict the influence of the drug dissolution on oral absorption on the basis of rather simple measurements of dissolution or solubility [2, 14]. The primary variable for judgments of *in vivo* absorption is the dissolution rate rather than the solubility. Drug dissolution can be the rate-limiting step in the absorption process and thereby affect the rate of

Table 19.3 Proposed limits of drug dissolution on solubility to avoid absorption problems.

Factor	Limit	Reference
Solubility at pH 1–7	>10 mg/ml at all pH	[33]
Solubility at pH 1–8 and dose	Complete dose dissolved in 250 ml at all pH	[4]
Water solubility	>0.1 mg/ml	[32]
Dissolution rate at pH 1–7	>1 mg/min/cm^2 (0.1–1 mg/nm/cm^2 borderline) at all pH values	[33]

bioavailability, and, often more importantly, it can also limit the extent of bioavailability when the dissolution rate is too slow to provide complete dissolution within the absorptive region(s) of the GI tract. However, most often solubility data are more readily available than dissolution rates for a drug candidate, especially in early phases when only minute amounts of drug are available, preventing accurate dissolution rate determinations. Consequently, predictions of *in vivo* effects on absorption caused by poor dissolution must often be made on the basis of solubility data rather than dissolution rate. This can theoretically be justified by the direct proportionality between dissolution rate and solubility under "sink conditions" according to Equation 19.3. A list of proposed criteria to be used to avoid absorption problems caused by poor dissolution is given in Table 19.3 [4, 38, 39] and further discussed below. A solubility in water of $\geq$10 mg/ml in the pH range 1–7 has been proposed as an acceptance limit to avoid absorption problems, while another suggestion is that drugs with water solubilities less than 0.1 mg/ml often lead to dissolution limitations of absorption. It should be noted that these arbitrary limits may be conservative; that is, the bioavailability of drugs with even lower solubility may not always be limited by drug dissolution. For example, a drug with much lower solubility, such as felodipine (0.001 mg/ml), provides complete absorption when administered in an appropriate solid dosage form [40]. This may be explained by both successful application of dissolution-enhancing formulation principles and more favorable drug solubility *in vivo* owing to the presence of solubilizing agents such as bile acids.

The BCS provides another model for biopharmaceutical interpretation of solubility data. Two different classes of drugs have been identified on the basis of the drug solubility as outlined in Figure 19.5, that is, high and low solubilities. If the administered dose is completely dissolved in the fluids in the stomach, which is assumed to be 250 ml (50 ml basal level in stomach plus administration of the solid dose with 200 ml of water), the drug is classified as a "high-solubility drug" [4, 17]. Such a good solubility should be obtained within a range of pH 1–8 to cover all possible conditions in a patient and to exclude the risk of precipitation in the small intestine because of the generally higher pH there than in the stomach. Drug absorption is expected to be independent of drug dissolution for drugs that fulfill this requirement, since the total amount of the drug will be in solution before entering the major absorptive area in the small intestine and the rate of absorption will then be determined by the gastric emptying of fluids. Such "highly soluble drugs" are

advantageous in pharmaceutical development since no dissolution-enhancing principles are needed, and the process parameters that could affect drug particle form and size are generally not critical formulation factors. However, many low-solubility drugs according to BCS have been developed into clinically useful products, that is, this classification is hardly useful as a screening criterion in drug discovery.

A special case in dissolution-limited bioavailability occurs when the assumption of "sink condition" *in vivo* fails, that is, the drug concentration in the intestine is close to the saturation solubility. Class IV compounds, according to BCS, are most prone to this situation because of the combination of low solubility and low permeability, but it could also happen for class II compounds, depending primarily on the ratio between dose and solubility. Non "sink conditions" *in vivo* lead to less than proportional increases of bioavailability for increased doses. This is illustrated in Figure 19.8, where the fraction of drug absorbed has been simulated by the use of a compartmental absorption and intestinal transit model [41] for different doses and for different permeabilities of a low-solubility aprotic compound. Although the lowest permeability in this example is still within the range of high permeability according to the BCS, the fraction absorbed could vary between just a small percentage to complete uptake for a low-solubility compound (1 µg/ml) at a fixed dose just as a function of the permeability. Similarly, the dose level would also be a critical determinant of the amount absorbed for a given permeability and solubility. Thus, it is crucial in evaluating dissolution/solubility effects on bioavailability to consider these effects not in isolation but together with permeability and dose, as is the case in the BCS.

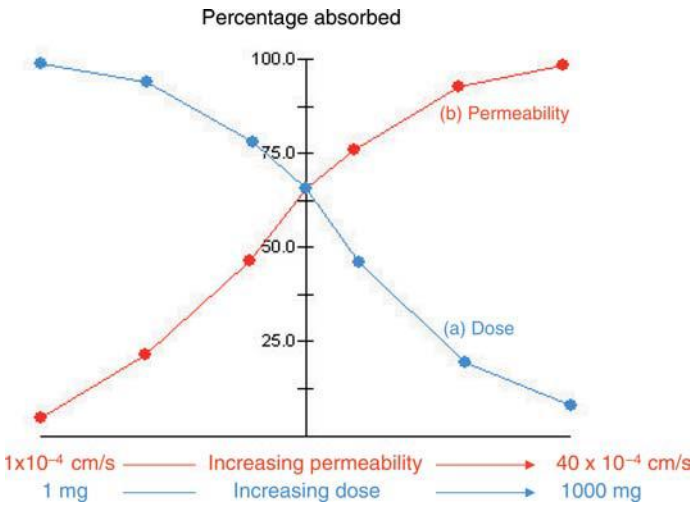

Figure 19.8 Fraction drug absorbed predicted by Gastroplus (SimulationPlus, Lancaster, CA, USA) for oral administrations of a poorly soluble (1 µg/ml), aprotic drug at (a) different doses with a constant, high permeability (4×10^{-4} cm/s) and (b) different permeabilities with a constant dose (100 mg).

19.4
The Effective Intestinal Permeability (P_{eff})

The intestinal permeability (P_{eff}) is a major determinant of fraction drug absorbed and quantitatively represents the principal membrane transport coefficient of the intestinal mucosa of a drug, which is possible to use regardless of the transport mechanism across mucosa [4, 5, 42]. The different transport mechanisms by which a drug may be transported across the intestinal barrier are displayed in Figure 19.4. There are different approaches to predicting and measuring intestinal permeability as summarized in Figure 19.9. Most *in vitro* models, such as cell monolayers (Caco-2 model) and excised tissue segment in a diffusion chamber (Ussing model), are based on the appearance of the drug on the serosal (basolateral) side. The measured *in vitro* P_{app} includes drug transport across the apical cell membrane, cytosol, and basolateral membrane for cell monolayers, as well as the interstitial fluid and connective tissue for the Ussing chamber model [20]. Consequently, such a definition also includes gut first-pass metabolism that may occur in the cytosol of the enterocyte (for instance, by CYP P450 isoenzymes and cytosolic localized peptidases). The activity of these intracellular enzymes will particularly influence the appearance rate of the drug on the basolateral side (i.e., in the portal vein *in vivo*). Thus, it may be useful to switch on and off genes coding for intestinal CYP3A4 in the Caco-2 model [43]. Another, and probably more accurate, definition suggests that the intestinal epithelial P_{eff} for most drugs reflects the transport across the apical membrane of the enterocyte [4, 5, 42]. This view is valid for most drugs that are absorbed by passive diffusion and/or carrier-mediated transport. Recently, it has also been reported that passive transcellular

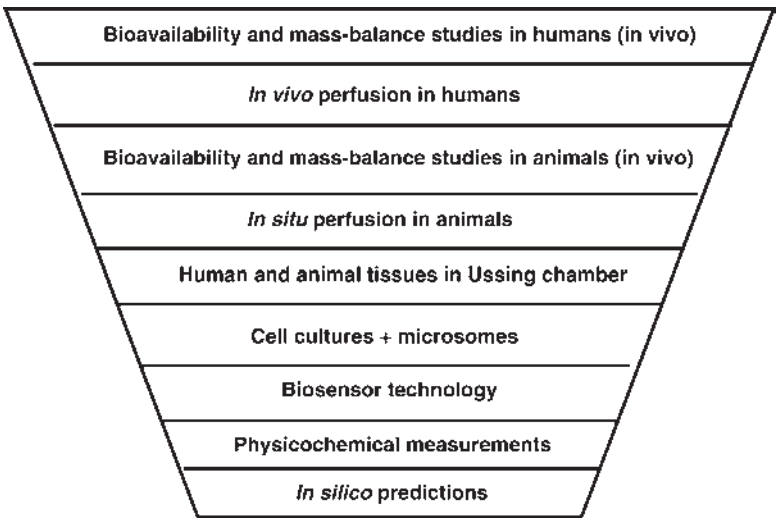

Figure 19.9 A short overview, from *in silico* to *in vivo* in humans, of the methods available to investigate and predict fraction dose absorbed and bioavailability following oral dosing.

permeability across epithelial cells is determined by the largest resistance, which is the apical epithelial membrane [43, 44]. Accordingly, intestinal permeability represents the transport of compounds into the enterocyte's cytosol [4, 5, 42]. This view is certainly the case for passive transcellular diffusion across the apical membrane, which is considered to be rate limiting. Intestinal perfusion models of the animal and human intestine are often based on the disappearance of the drug from the perfused gut lumen. Interestingly, results from single-pass perfusion of both rat and human small intestines have been shown to predict fraction dose absorbed in humans with high accuracy [5, 20, 45].

The BCS is based on a human *in vivo* permeability (P_{eff}) database of about 20 different drugs. This database was established by using the Loc-I-Gut technique, an *in vivo* single-pass perfusion technique, in the human proximal jejunum [46]. This region of the proximal small intestine is where the major absorption of most drugs takes place when they are given in immediate-release (IR) dosage form. These *in vivo* values of P_{eff} have been used to establish a correlation between measured *in vivo* permeability and fraction dose absorbed in humans for soluble drugs as shown in Figure 19.10. This fundamental *in vivo* correlation between permeability and fraction of dose absorbed has established *in vitro–in vivo* correlations (IVIVCs) between human *in vivo* jejunal P_{eff} and permeabilities from animal tissues or cell cultures [5, 20, 46–48]. Model correlations based on *in vivo* permeability data will be very useful when preclinical models are developed and validated regarding predictions of human intestinal absorption. They are also important for the development of theoretical models (*in silico*) where intestinal drug absorption is predicted from

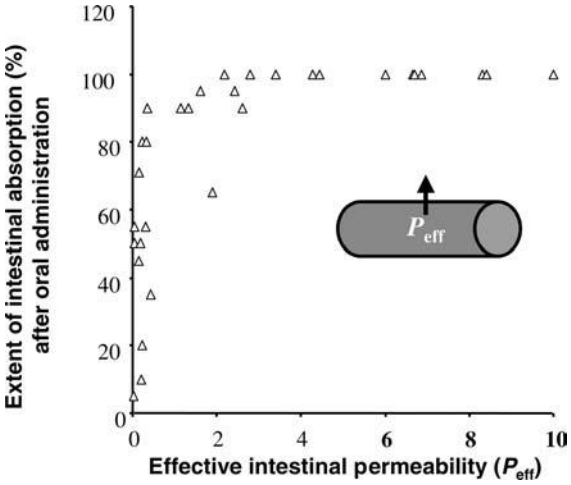

Figure 19.10 Human *in vivo* permeability values (P_{eff}) can be determined by the use of a single-pass perfusion technique (Loc-I-Gut) in humans. These human P_{eff} values have been excellently correlated to fraction dose absorbed (f_a) of oral doses for a large number of drugs from different pharmacological classes, which consequently are representing structural diversity.

molecular structure [2, 16, 49]. Altogether, they provide tools that might be very helpful in classifying drugs according to the BCS and consequently contribute to the regulatory evaluation of both bioavailability and bioequivalence [4, 18, 50].

According to the FDA BCS guidelines, measurements of the permeability and fraction dose absorbed of a drug can be made by mass balance, absolute bioavailability, or intestinal perfusion methods. The intestinal permeability of a drug can be determined by (1) *in vivo* intestinal perfusion in man [46]; (2) *in vivo* or *in situ* perfusion of a suitable animal model [45]; (3) *in vitro* transport across excised human or animal tissues [48, 51]; and (4) *in vitro* transport across epithelial cell monolayer [52] (www.fda.gov/cder/guidance/3618fnl.htm). When applying any of these models, it is crucial to understand the main transport mechanisms and metabolic route and characterization of the activity of the transporter/enzyme involved. It is well recognized that carrier-mediated processes in Caco-2 cells are considerably lower than those *in vivo* [20, 47, 53]. Therefore, it is crucial to extrapolate *in vitro* cell culture data to the *in vivo* situation with great care [18, 20, 47, 53]. This is especially important when carrier-mediated processes are involved, which has been made evident by a recent study showing significant differences in gene expression levels for transporters, channels, and metabolizing enzymes in Caco-2 cells compared to human duodenum [53]. If an animal model is used, potential species difference has to be considered [18, 20, 50].

The human *in vivo* permeability for various drugs is one of the cornerstones in the BCS, and their correlation with fraction dose absorbed and permeability values from other permeability models mentioned above would make it feasible to classify drugs according to the BCS and to define bioequivalence regulation for pharmaceutical product approval. These human *in vivo* P_{eff} were determined with a regional double-balloon perfusion approach (Loc-I-Gut), which is described shortly below (Figure 19.11). The tube was introduced through the mouth after an application of a local anesthetic (lidocaine) to the throat. The position of the tube was checked by fluoroscopy, and the perfused segment was located in the proximal part of the jejunum. Once the perfusion tube was in place, the two balloons were inflated with approximately 26–30 ml of air creating a 10 cm long segment. The jejunal segment was then rinsed with isotonic saline (37 °C) for at least 20 min, and a flow rate of 2.0 ml/min was most often applied. A more extensive description of this intestinal perfusion technique is published elsewhere [46, 54].

Jejunal P_{eff} and other variables were calculated from the steady-state level in the perfusate leaving the intestinal segment. We have reported earlier that a well-mixed model best describes the hydrodynamics within the perfused jejunal segment, and P_{eff} is calculated according to Equation 19.5:

$$P_{eff} = \frac{Q_{in} \cdot (C_{in} - C_{out})}{C_{out} \cdot 2\pi r L}, \tag{19.5}$$

where Q_{in} is the inlet perfusate rate, C_{in} and C_{out} are the inlet and outlet perfusate concentrations of the drug, respectively, r is the radius ($r = 1.75$ cm), and L is the length of the jejunal segment (10 cm) [46, 55].

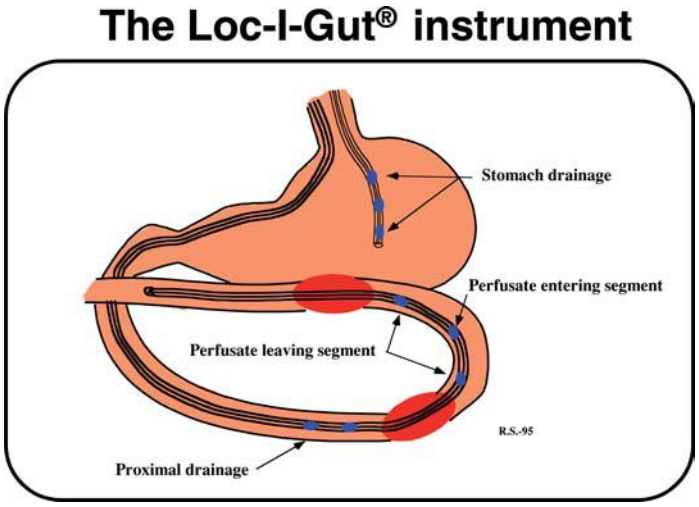

Figure 19.11 Loc-I-Gut is a perfusion technique for the proximal region of the human jejunum. The multichannel tube is 175 cm long and is made of polyvinyl chloride with an external diameter of 5.3 mm. It contains six channels and is provided distally with two 40 mm long, elongated latex balloons, placed 10 cm apart each separately connected to one of the smaller channels. The two wider channels in the center of the tube are for infusion and aspiration of perfusate. The two remaining peripheral smaller channels are used for administration of marker substances and/or for drainage. At the distal end of the tube is a tungsten weight attached in order to facilitate passage of the tube into the jejunum. The balloons are filled with air when the proximal balloon has passed the ligament of Treitz. Gastric suction is obtained by a separate tube. ^{14}C-PEG 4000 is used as a volume marker to detect water flux across the intestinal barrier.

The jejunal perfusion approach generates data, which may be used to predict absorption/bioavailability and to establish *in vivo–in vitro* correlation even for extended-release (ER) products. If a drug is transported mainly by passive diffusion and has a jejunal P_{eff} higher than that for metoprolol (1.5×10^{-4} cm/s = high-permeability compound), it can be expected to be completely absorbed throughout the small and large intestines [5, 51].

Predictions of human *in vivo* permeability can be made with a particularly high degree of accuracy in all preclinical models for drugs with passive diffusion as their main mechanism. It is only the dog model that seems to low-permeability (passively) drugs more efficiently than both humans and rats [5, 20]. Special care must be taken for drugs that are absorbed by a carrier-mediated transport mechanism as the main mechanism. It has been shown that absorptive carriers, such as the oligopeptide and amino acids carriers, have a low functional activity because of low protein expression in the Caco-2 model [7, 18, 47, 56, 57]. For these drugs, a scaling factor has to be developed and introduced, otherwise the *in vivo* permeability will be underestimated. This low expression is not surprising, since preliminary gene chip assays have reported that the Caco-2 cells have about 40% of the genes turned on compared to the normal gene expression in the human small intestine. Carrier-mediated absorption

by the oligopeptide carrier and the amino acid transport family in rats has not shown a significant species difference [7, 20, 45, 53, 56, 58–61, 90].

It is important to recognize that the *in vitro* permeability obtained in cell monolayers (such as Caco-2 models) should be considered as a qualitative rather than quantitative value. Especially poor are the predictions of fraction dose absorbed for carrier-mediated drugs with low Caco-2 permeability and predictions of high fraction dose absorbed in humans [7, 20, 47, 53, 56, 62]. However, it is possible to establish a reasonable good *in vitro–in vivo* correlation when passive diffusion is the dominating absorption mechanism.

19.5 Luminal Degradation and Binding

Degradation and formation of nonabsorbable drug complexes in the intestinal lumen is the third factor, in addition to dissolution and permeability, which could affect fraction absorption. Limitations of bioavailability due to these factors seem to be less frequent compared to the two other main factors. Regulatory guidelines for BCS-based biowaivers still ask for *in vitro* studies of luminal degradation in relevant test media whereas specific binding studies are not required [17].

The acidic environment in the stomach could degrade some substances. For example, the proton pump inhibitor omeprazol has a half-life of less than 5 min at pH 1, whereas it is practically stable in the intestinal pH range. Such limitations can be handled by the use of properly designed modified-release formulations with enteric coating, which protects the drug from the acid, as is the case for omeprazol [63]. Reverse forms of pH-dependent drug degradation could also occur, that is, the drug is stable at lower pH but has significant degradation at close to neutral pH.

Drugs may also undergo hydrolysis by intestinal esterases (hydrolases), more specifically carboxylesterases (EC 3.1.1.1) in the intestinal lumen and at the brush border membrane [64, 65]. It has been demonstrated that the intestinal hydrolase activity in humans was closer to that of the rat than the dog or Caco-2 cells [66]. They used six propranolol ester prodrugs and *p*-nitrophenylacetate as substrates and found that hydrolase activity was ranked in the order human > rat $\gg$ Caco-2 cells > dog for intestinal microsomes. The rank order in hydrolase activity for the intestinal cytosolic fraction was rat > Caco-2 cells = human > dog. The hydrolase activity toward *p*-nitrophenylacetate and tenofovir disoproxil has also been reported in various intestinal segments from rats, pigs, and humans. The enzyme activity in intestinal homogenates was found to be both site specific (duodenum $\geq$ jejunum > ileum > colon) and species dependent (rat > man > pig).

The bacteria in the intestinal tract exert is another well-known source for luminal drug degradation [67]. This is only important for the colon region since the luminal concentration of bacteria is 10^4–10^9 higher in the colon compared with that in the small intestine. Thus, this aspect is only relevant for drugs that reach this region as a result of, for example, poor permeability, slow dissolution, or delivery by modified-release formulations. It is predominantly hydrolytic and also includes other reductive

Table 19.4 Drug degradation reactions by intestinal bacteria.

Enzyme	Reaction	Representative substrate
Hydrolysis		
Amidase	Cleavage of amides of carboxylic acids	Methotrexate
Decarboxylase	Decarboxylation of amino acids and simple phenolic acids, primarily *p*-hydroxylated	L-Dopa, tyrosine
Dehydroxylase	Dehydroxylation of C- and N-hydroxy groups	Bile acids, *N*-hydroxyfluorenylacetamide
Esterase	Cleavage of esters of carboxylic acids	Acetyldigoxin
Glucuronidase	Cleavage of β-glucuronides of alcohols and phenols	Estradiol-3-glucuronides, morphine glucuronide
Glucosidase	Cleavage of β-glucosides of alcohols and phenols	Cycasin, rutin
Nitrase	Cleavage of nitrates	Pentaerythriol trinitrate
Sulfatase	Cleavage of *O*-sulfates and sulfamates	Cyclamate, amygdalin, estrone sulfate
Reduction		
Alcohol dehydrogenase	Reduction of aldehydes	Benzylaldehydes
Azoreductase	Reductive cleavage of azo compounds	Food dyes, sulfasalazine
Hydrogenase	Reduction of carbonyl groups, aliphatic double bonds	Unsaturated fatty acids, estrone
Nitroreductase	Reduction of aromatic and heterocyclic nitro compounds, nitrosamine formation	*p*-Nitrobenzoic acids, chloramphenic metronidazol, nitrazepam
N-oxide reductase	Removal of oxygen from N-oxides	4-Nitroquinolone-1-oxide, nicotine-*N*-oxide
Sulfoxide reductase	Removal of oxygen from sulfoxides	Sulfinpyrazone
Other reactions		
Aromatization	Quinic acid	
D-Demethylation	Biochanin A	
Dealkylation	3,4,5-Trimethoxycinnamic acid	
Deamination	Amino acids	
Dehalogenation	DDT	

reactions that are mediated by the bacterial enzymes. A list of the most prominent types of enzymes, reactions, and examples of substrates is given in Table 19.4 [61, 68]. The effectiveness of this process has been exemplified by the use of *in vitro* incubation studies showing rapid drug degradation [70].

Reduced absorption due to complex formation or other interactions between drugs and intestinal components leading to poor absorption has been described in a few cases. One example is the precipitation of cationic drugs as a result of very poorly soluble salts with bile acids, which has been reported for several compounds [31].

Another well-known example is the complex formation between tetracycline and calcium as a result of chelation after administration of the drug together with milk. It has also been shown that protease inhibitor drugs can bind very strongly to enzymes secreted by the pancreas [71].

Interactions between drugs in the lumen and intestinal components are generally a poorly studied area, and it is difficult to discriminate on the basis of *in vivo* data such effects from other factors affecting absorption. In addition, evidence for *in vivo* relevance of available *in vitro* methods is very sparse.

19.6
The Biopharmaceutics Classification System

19.6.1
Regulatory Aspects

19.6.1.1 Present Situation

BCS has been primarily developed for regulatory applications though its use has been extended beyond this area as discussed in more detail below. The aim of BCS in a regulatory context is to provide a basis for replacing certain bioequivalence studies by equally or more accurate *in vitro* dissolution tests. This could reduce costs and time in the development process as well as reduce unnecessary drug exposure in healthy volunteers, which is normally the study population in bioequivalence studies.

Numerous bioequivalence studies are presently being conducted for NDAs of new compounds, in supplementary NDAs for new indications and line extensions, in ANDAs of generic products, and in applications for scale-up and postapproval changes. For example, NDA bioequivalence studies may be required for comparing different clinical formulations in pivotal clinical trials and products aimed for market. The complexity and number of studies required are often boosted by the fact that several dose strengths might be included in the development process. In addition, bioequivalence documentation may also be needed for comparing blinded and original comparator products in clinical trials. Thus, an NDA typically contains a multitude of bioequivalence studies.

The BCS was first applied in a regulatory context in the US FDA guidelines for SUPAC's of oral immediate-release formulations [51]. More recently, guidelines for applying BCS in NDAs and ANDAs have been finalized by both FDA and the European agency, EMEA [17, 19]. In addition, the BCS principles are also included in ICH guidelines for requirements of *in vitro* dissolution testing as a quality control in manufacturing [73] and the recent WHO guidance on waiving *in vivo* BE studies for oral immediate-release formulations of essential medicines [74].

The BCS classes are defined as follows: Class I. High Solubility (S) – High P_{eff}; Class II. Low S – High P_{eff}; Class III. High S – Low P_{eff}; Class IV. Low S – Low P_{eff}. A drug is considered as highly permeable when the extent of absorption is complete in humans, defined by the US FDA as being more than 90%, whereas EMEA requires

"complete" absorption [17, 19]. This could be determined by any of these study methods:

- absolute bioavailability in humans (in case of no first-pass metabolism);
- mass balance studies in humans with the help of radiolabeled drug;
- determination of P_{eff} in humans by the "Loc-I-Gut method" and applying the correlation between P_{eff} and fraction absorbed presented in Figure 19.10;
- determination of P_{eff} in any animal *in vivo* perfusion or *in vitro* permeation method that provides a solid correlation to the fraction dose absorbed in humans for a predefined set of drug substances. Special consideration has to be given to indications of carrier-mediated transport across the intestinal membrane, in both the secretory and absorptive directions.

A compound can be classified as a high-solubility drug if the highest strength can be dissolved in 250 ml buffer at all pH values within range of pH 1–8. This criterion is applied by both the European and US guidelines [17, 19].

BCS is primarily used in this context to identify the substances that are suitable for *in vitro* bioequivalence testing, which in the United States is preceded by a request to the authority to gain a biowaiver, that is, an acceptance for replacing an *in vivo* study with *in vitro* dissolution testing. It is presently only oral IR formulations of class I compounds, that is, highly soluble/highly permeable drugs, for which such an option is available. Additional criteria for allowance of *in vitro* bioequivalence testing are that the drug stability in the GI fluids must be verified and it should also be a non-narrow therapeutic index drug. If these criteria are fulfilled, test and reference products can be compared by *in vitro* dissolution testing and deemed bioequivalent but achieved only when sufficiently similar results are obtained. The *in vitro* dissolution testing should be done at three different pH values within the physiological range, typically pH 1, 4, and 6.8. The product dissolution must be complete (>85%) within 30 min in order to utilize the *in vitro* bioequivalence route. The underlying rationale for this demand on product performance is to ascertain that drug dissolution is fast enough as not to become the rate-limiting step. It is assumed that gastric emptying will control the absorption rate for class I substances in products with such a fast dissolution and no effect on bioavailability will be obtained for different dissolution profile within acceptance limits. This has also been verified *in vivo* by studying metoprolol tablets with different *in vitro* release profiles [75].

The difference between a test (T) and a reference (R) product should be evaluated by use of the f_2-test (see Equation 19.6), where $f_2 > 50$ is the required limit for equivalence. This limit corresponds to an average difference in the amount dissolved at different times (t) of less than 10%. If the dissolution is very rapid, that is, complete dissolution within 15 min, the f_2-testing is not necessary.

$$f_2 = 50 \log \left[\left(1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right)^{-0.5} \times 100 \right]. \tag{19.6}$$

In addition to the *in vitro* testing, the test and reference products must not contain excipients that could modify drug absorption in any way except for dissolution effects. For example, the potential for permeability-enhancing effects by surface-active agents, sometimes included in solid formulations, has been identified as one potential concern. Furthermore, the effect on gastrointestinal transit by large amounts of sugars has been highlighted as another issue [18, 76].

19.6.1.2 Potential Future Extensions

The application of BCS in a regulatory context has gained acceptance and is now used in both NDAs and ANDAs [81]. However, one important limitation of the present application of BCS is that class I substances are quite rare in pharmaceutical development. It has also been recognized that the present application represents a deliberately conservative approach, and proposals for extensions have been discussed since the original publication of BCS. For example, it was suggested that the requirement of the highest pH for the solubility measurements could be changed from 7.5 to 6.8 since the latter one is more relevant for the pH in the stomach and upper small intestine [18]. This revision would thus somewhat relax the requirements for basic drugs. Another proposal in the paper by Yu to reduce the high-permeability definition from 90 to 85% fraction absorption-based observations that many drugs are considered completely absorbed provides experimental values below 90%, that is, 90% seems to be a too rigid a criterion considering the precision of the experimental methods. More radical developments are also fairly well supported like allowing biowaivers for very rapidly dissolving class III drugs, at least for those with intermediate permeability [81]. Since the majority of drugs in drug development today most probably are class II drugs, the greatest benefits to be gained should be sought in this area. Support has been expressed for allowing biowaivers especially for acidic class II drugs that have a high solubility at intestinal pH, thereby assuring a rapid and complete dissolution in the upper part of the small intestine [81]. An additional prerequisite for biowaivers of class II drugs would be to provide assurance of *in vivo* predictability of *in vitro* dissolution methods to be used as a surrogate for *in vivo* bioequivalence studies.

19.6.2 Drug Development Aspects

BCS has primarily been developed for regulatory applications. However, it has also several other implications in the drug development process and has gained a wide recognition within the research-based industry. The importance of drug dissolution in the GI tract and permeability over the gut wall in the oral absorption process has been well known since the 1960s, but the research carried out to constitute the BCS has provided new quantitative data of importance for drug development, so far especially within the area of drug permeability. Another merit of the BCS in a development context is that it provides very clear and easily applied rules to determine the rate-limiting factor in the drug

absorption process. Thereby, BCS has implications in the selection of candidate drugs for full development, predictions, and elucidations of food interactions, choice of formulation principle including suitability for oral ER administration, and the possibility for *in vitro*/*in vivo* correlations in dissolution testing of solid formulations. Most of these aspects will be discussed and exemplified in further detail below.

19.6.2.1 **Selection of Candidate Drugs**

Permeability and solubility are of key importance in the selection of candidate drugs for development [2]. Molecules with too low permeability and/or solubility will provide low and variable bioavailability, which increases the risk that a clinically useful product cannot be developed. Experimental methods and relevant acceptance criteria regarding permeability and solubility are needed in the early drug discovery process. Such procedures have also been introduced in the industry, including solubility screens using turbidimetric measurements and automatic permeability screens based on the Caco-2 cell model. Computational approaches have also been developed for permeability and solubility determinations [72, 77, 78]. If further refinement can be achieved for such methods leading to improved predictions, it may be possible in the future to displace cell-based permeability screens and early solubility estimates. It has even been suggested that BCS can be further developed toward consideration of true fundamental molecular properties for membrane permeability, as well as for drug solubility [91].

Selection of candidates that fulfill the BCS requirement of high permeability/high solubility (class I) almost guarantees the absence of failures due to poor absorption by the oral route. However, these limits are generally too conservative to use as an acceptance criterion since many useful drugs can be found in class II–III and even class IV. First, a class I drug is expected to provide complete absorption, whereas a certain reduction in bioavailability due to permeability or solubility, as well as due to other reasons (e.g., first-pass metabolism), is generally acceptable. A summary of the different factors that have to be taken into account when defining more relevant acceptance criteria is as follows:

- acceptability of a low and highly variable bioavailability depending on
  –medical need
  –width of therapeutic window
  –potency
  –substance manufacturing costs
- potential for poor *in vivo* predictability of early permeability and solubility characterizations as a result of, for example,
  –active transport across the gut wall
  –high paracellular transport through gut wall
  –*in vivo* solubilization by bile salt micelles
- possibilities to use formulation approaches that improve bioavailability, for example,

–dissolution and solubility enhancement
–permeation enhancers.

Thus, BCS points out some important variables in the screening of drug candidates though the proposed limits are less useful as acceptance criteria in a drug discovery context.

19.6.2.2 **Choice of Formulation Principle**

Oral dosage forms are often developed under time constraints and preferably by an efficient use of available resources. One way to reduce time and increase efficiency could be to minimize the number of different formulations included in different stages of clinical development. The BCS could be used as a framework to decide which types of formulation should be suitable for a certain compound.

If a drug is classified as having low solubility, it is obvious that bioavailability properties could be improved by the use of formulation principles that increase the dissolution rate and/or drug solubility. There are a number of different principles of varying complexity to achieve such improvements, ranging from selecting a suitable solid-state form or salt to the use of technologically more advanced formulation principles. Although their application could be limited by several practical factors such as poor drug stability, excessive size because of the need for large amounts of excipients in relation to the dose, technical manufacturing problems, and the high cost of goods, it is believed that many poorly soluble compounds with good pharmacological properties could be "saved" by such approaches. A list of different formulation principles for oral solid formulations including modifications of the drug substance form is given below.

Substance form:

- salt – choose most water soluble;
- crystal form – select the most soluble polymorph/anhydrate, if possible, from stability and technical points of view;
- amorphous form – provide the most rapid dissolution and the most often increased solubility by supersaturation, but practical usefulness is limited by stability issues including transformation of solid-state form;
- size reduction by milling/micronization – increase surface area in contact with dissolution medium.

Formulation approaches for solids:

- addition of wetting agents;
- solid solutions/eutectic mixtures;
- cyclodextrin complexes;
- lipid systems such as oils/emulsions/microemulsions/self-emulsifying systems in capsules;
- nanoparticles.

This list illustrates the numerous pharmaceutical possibilities to handle bioavailability problems due to low solubility. This is a continuously developing area

exemplified by more recent developments, such as self-emulsifying lipid systems and nanoparticles.

The optimal formulation should provide such a good dissolution and/or solubility that this step is no longer the rate-limiting step in the absorption process, that is, a situation comparable with the one for class I drugs. The obvious approach for reaching this goal is to formulate the drug as a solution that maintains the drug in solution even after mixing and dilution with the gastric fluids. However, such formulations are generally not feasible to use as drug products, where solid formulations such as tablets and capsules are strongly preferred. Absorption properties similar to a solution may, however, be obtained for a solid formulation of poorly soluble drugs in case of successful application of dissolution-enhancing principles.

Another important decision in oral formulation development where BCS could provide guidance is the start of an oral extended-release development. ER formulations could significantly improve the clinical usefulness of a drug substance by reducing the peak-to-trough ratio of drug levels in the body and the need for less frequent dosing. However, not all drugs that would benefit from ER delivery are suitable candidates because of unfavorable absorption properties. Solubility and permeability are crucial factors to consider before making a decision to embark on an ER development program.

The realization of the desirable clinical advantages most often requires a duration of drug release of 12–24 h. Thus, since the transit of formulations through the small intestine is 3–4 h, a significant part of the dose will be delivered to the colon, and the absorption of the drug over almost the entire GI tract is a prerequisite. Class I drugs, having both good solubility and good permeability, should therefore be the best candidates for ER development. Several well-documented ER formulations are also based on class I compounds such as metoprol but class II drugs are also frequently used. For example, felodipine ER tablets provide an example where a low-solubility compound is included in a useful ER product. Felodipine, which has water solubility in the physiological pH range of about 1 μg/ml, is possible to administer as a once-daily product in doses up to at least 10 mg without any reduced bioavailability compared to an oral solution [40]. Such a successful performance is indeed dependent on the use of a dissolution-enhancing formulation principle where the drug is given in a solubilized form.

A special consideration can be made regarding classification of low-solubility compounds in ER forms. The standard classification is based on the idea that the drug should be completely dissolved in the gastric fluids, which has been estimated to be 250 ml. However, this way of classifying drugs may be less relevant for ER formulations since only a very small part of the dose is made available for dissolution in the stomach. The dose is generally spread over the entire GI tract making the effective water volume available as a dissolution medium for the drug probably larger than the 250 ml used in the original BCS. Furthermore, the drug permeability of these compounds is often much faster than the drug release, further preventing solubility limitations for class II drugs in ER formulations. Thus, this gives a further explanation of the relative frequent abundance of class II drugs developed as ER

products, and if BCS is to be applied to ER formulations in the future, a different classification criterion regarding solubility may be needed.

The permeability classification of a drug according to BCS, based on theoretical considerations, should be very useful as a criterion for selecting a drug as an ER formulation. A classification of a drug as a low-permeability compound means that the drug is not completely absorbed after oral administration of a solution or an IR tablet. A certain amount of drug for such compounds is clearly delivered to the colon, and the permeability in the colon is so poor that a significant part of the dose passes through the entire colon without being absorbed. This implies that the permeability in the colon is very slow, preventing any significant drug absorption. Using rat intestinal and colonic tissues in an Ussing chamber, it has also been shown *in vitro* that the permeability of class III–IV drugs is even slower in the colon than in the small intestine, whereas class I–II drugs show a slightly higher permeability in the colon when passive diffusion is the dominating mechanism [52]. This permeability pattern has also been shown to be relevant for small and large intestinal specimens from humans when the Ussing chamber model [79] is applied. Consequently, it will not be possible to control the rate of absorption by an ER formulation for low-permeability drugs. In addition, a large part of the dose will not be absorbed, leading to a low and uneven variability. This is exemplified in ER tablets of amoxicillin. This high-solubility drug is classified as a low P_{eff}, even if it is transported across the intestinal barrier via the oligopeptide carrier (PepT1) [62]. Essentially no absorption occurred for an ER tablet when it entered the colon as determined by gamma scintigraphy [80].

19.6.2.3 *In Vitro/In Vivo* Correlation

In vitro dissolution testing is an important tool in the development of solid drug products as well as in batch quality controls. The aim of the test is to see that the drug is appropriately dissolved in the GI tract and made available for absorption. It is therefore highly desirable that the *in vitro* tests provide data that correlate to the *in vivo* situation. However, attainment of IVIVC has often failed, and the concept of IVIVC has been challenged.

The BCS could be used as a framework for predictions when IVIVC could be expected for solid immediate-release products as summarized in Table 19.5. It is

Table 19.5 Expectations for *in vitro/in vivo* correlations for IR products based on BCS.

Class	IVIVC expectations
I. High *S*/High P_{eff}	No IVIVC until product dissolution becomes slower than gastric emptying
II. Low *S*/High P_{eff}	IVIVC should be possible to establish provided that *in vitro* relevant dissolution test method is used and drug absorption is limited by dissolution rate rather than saturation solubility
III. High *S*/Low P_{eff}	No IVIVC until product dissolution becomes slower than intestinal permeability
IV. Low *S*/Low P_{eff}	Low chance for IVIVC

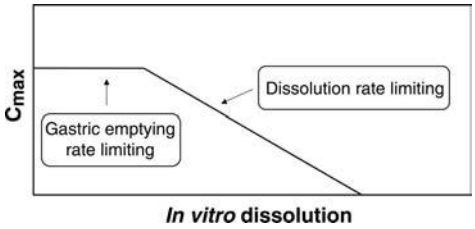

Figure 19.12 Principal level *C in vitro/in vivo* correlation for IR formulation of class I substance.

important to realize that the *in vitro* dissolution test only models the release and dissolution of the active drug substance from the formulation and it is only when these processes are rate limiting in the absorption process that IVIVC can be expected. In the case of class I drugs, the complete dose will be dissolved already in the stomach and, provided that the absorption over the gut wall is negligible, the gastric emptying of the dissolved drug will be the rate-limiting step. This is clearly not a factor that is included in the *in vitro* dissolution test. Thus, no IVIVC should be expected for class I drugs as long as the release of drug is faster than the gastric emptying. The half-life of gastric emptying of fluids in the fasting state is normally about 10 min though this could vary because of several factors such as the timing of drug administration in relation to gastric motility phase and fluid volume [84]. The relationship between *in vitro* dissolution, described as the time to dissolve half of the dose (t50%), and the peak plasma concentration (C_{max}) for a fictive class I drug is exemplified in Figure 19.12. This type of *in vitro/in vivo* relationship should only be expected for variables that are influenced by the absorption rate, whereas variables reflecting the extent of bioavailability, for example, AUC, should be independent of dissolution rate.

Class II drugs, that is, low-solubility/high-permeability compounds, are expected to have a dissolution-limited absorption. Thus, for these kinds of drugs, an IVIVC should be possible to establish by use of a well-designed *in vitro* dissolution test. One way to investigate and establish such a correlation is to study formulations containing drug particles with different surface areas. An example of such a study is given in Figure 19.13a and b, where *in vitro* dissolution and plasma concentration–time profiles are given for administration of tablets containing drug substance with two different mean particle sizes. The mean reduction of about 30% in C_{max} for the larger particles was predicted in this case by a somewhat slower dissolution *in vitro*.

Two cases could be identified for class II drugs when the establishment of simple IVIVCs is not feasible. First, there are a number of formulation principles that could enhance the dissolution rate and solubility of low-solubility compounds as discussed above. It may be possible to achieve such a rapid and complete dissolution of a class II drug that the gastric emptying becomes the rate-limiting step, that is, the bioavailability of the solid dosage forms equals that of an oral solution. Thus, in such a case, the prerequisites for IVIVC will be identical to the situation for class I drugs; that is, no correlation will be obtained as long as the dissolution rate is significantly faster than the gastric emptying.

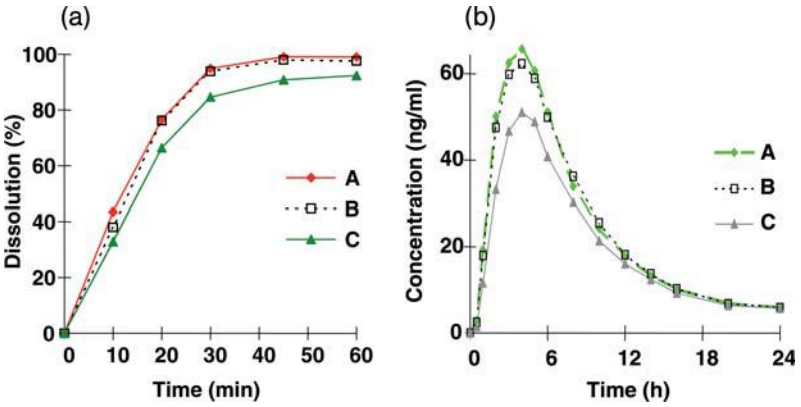

Figure 19.13 Mean (a) *in vitro* dissolution and (b) human plasma concentrations for candersartan cilexitil tablets containing drug particles with three different mean particle diameters (A, 3.9 μm; B, 5.7 μm; C, 9.1 μm).

The second case when IVIVC is not likely for class II drugs is the situation where the absorption is limited by the saturation solubility in the GI tract rather than the dissolution rate, as discussed in more detail above. In this situation, the drug concentration in the GI tract will be close to the saturation solubility and changes of the dissolution rate will not affect the plasma concentration profile and the *in vivo* bioavailability. Standard *in vitro* dissolution tests are carried out under "sink conditions," that is, at concentrations well below the saturation solubility. Thus, only effects related to dissolution rate can be predicted *in vitro*. If more physiologically relevant dissolution media are used, which not necessarily provides "sink conditions," the possibility for IVIVC could be improved as indicated by recent work using simulated intestinal medium [85].

The absorption of class III drugs is limited by their permeability over the intestinal wall. Thus, since this process is not at all modeled by the classical *in vitro* dissolution test, no IVIVC should be expected. When the drug dissolution becomes slower than the gastric emptying, a reduction of the extent of bioavailability will be found in slower dissolution rates because the time when the drug is available for permeation over the gut wall in the small intestine will then be reduced. Thus, the same type of relationship can be expected between bioavailability and *in vitro* dissolution as shown in Figure 19.12 for a class I drug.

19.6.2.4 **Food–Drug Interactions**

Alterations of bioavailability due to a concomitant food intake can have serious implications for the clinical usefulness of a drug, and it is therefore beneficial to predict such effects at an early stage. However, this is not easily done because of the multitude of factors involved in food–drug interactions including physicochemical effects such as increased solubility and binding to secretory or food components; physiological effects in the GI tract such as altered flow rates and gastric emptying;

Table 19.6 Most likely food effects on bioavailability for BCS class I–IV drugs.

BCS class	Absorption effect by food	Possible mechanism
I	Reduced rate but same extent	Slower gastric emptying
II	Increased extent	Increased solubility and first-pass metabolism
III	Reduced extent	Reduced intestinal drug concentrations
IV	Increased extent	See class II

mechanical effects on formulations due to different motility patterns; permeability effects due to interactions with active transporters; or effects on the membrane and altered first-pass metabolism. A more extensive review of different mechanisms for food–drug interactions in the absorption step including formulation factors can be found elsewhere [86].

The BCS can be used as a framework for predictions and to set up hypotheses for mechanisms of food effects related to permeability, solubility, and gastric emptying as outlined in Table 19.6. This was supported by a review of food interactions including more than 90 drugs, which showed a clear relationship between BCS class and direction of food interactions [82]. The prediction of food effects was possible to further improve by grouping drugs regarding maximal absorbable dose in relation to clinical dose, dose in relation to solubility (dose number), and lipophilicity (log *D*) [82]. The most severe cases of food interactions due to factors considered in BCS are generally found in the group of poorly soluble compounds given in high doses, that is, those that approach the saturation solubility in the GI tract. For such compounds, such as griseofulvin, the extent of bioavailability has been reported to increase up to five times by food and dosing recommendations requiring concomitant intake of the drug with a meal [31]. The saturation solubility will be significantly improved by food as a result of solubilization in mixed micelles, including bile acids, lecithin, and monoglycerides obtained from the dietary fat intake, and dissolution into emulsified nutritional lipids. Thus, the amount of drug available for absorption will significantly increase. A further contributing effect to an increased bioavailability could be the increased fluid volume in the stomach after a meal, that is, allowing an increased amount of drug that could be dissolved in comparison with the fasting situation. The increase in bioavailability for class II drugs after intake together with food that should be attributed not only to dissolution effects but also to reduction in first-pass metabolism, for example, because of the increased blood flow.

For highly permeable, poorly soluble drugs given in lower doses, the dissolution rate rather than the saturation solubility is the limiting factor. An increase in dissolution rate as a result of *in vivo* solubilization mediated by food intake could theoretically be obtained, but this is not always found *in vivo*. For example, food does not affect the rate and extent of bioavailability for candersartan cilexitil, a very poorly soluble compound [87]. An *in vitro* dissolution and solubility study of this compound

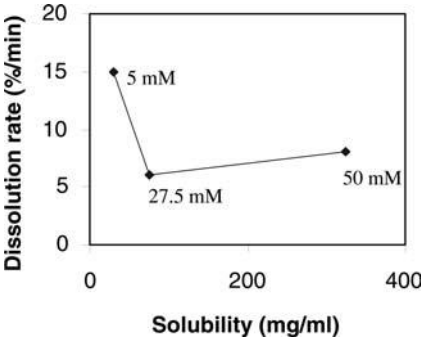

Figure 19.14 *In vitro* dissolution rate versus saturation solubility for candersartan cilexitil in different concentrations of sodium taurocholate:lecithin ratio (2.5 : 1).

in simulated intestinal media provided a potential explanation. It was revealed that the solubility was increased as a function of bile concentration as expected whereas the dissolution rate was not increased by the higher bile concentrations being representative for the fed state (see Figure 19.14). Thus, although intestinal solubility most often will be increased in the fed state for class II drugs, this will not always lead to a more rapid dissolution.

For class I drugs, a slower rate of absorption could be expected after concomitant intake with food as a result of the decreased gastric emptying rate induced by a meal. The gastric emptying in the fed state varies significantly depending on the meal composition, including factors such as energy content, osmolality, and pH. A gastric emptying half-life of about 45 min has been reported for fluids when measured under nonfasting conditions [88]. In addition to the meal composition, the extent of the reduction and delay in peak plasma concentration induced by food for a class I drug will also be influenced by the plasma concentration half-life; that is, food effects will be more pronounced for drugs with a shorter half-life.

Class III drugs will generally be less susceptible to both gastric emptying effects caused by slow permeability as well as solubility effects caused by the high solubility already under fasting conditions. However, the extent of bioavailability is often reduced for class III drug. Mechanisms behind this effect have not yet been clearly verified. One explanation could be the dilution of dissolved drug due to fluid intake and secretion decreasing the driving force for passive intestinal permeability. Another potential source for interactions between food components and low-permeability drugs could occur for drugs that are actively transported in the intestine, especially if nutritional carriers are involved. The two most important nutrient absorption carriers for drugs are the oligopeptide carrier (hPepT1) and the amino acid transport family. These carrier proteins have a high transport capacity in the human small intestine, and they seem less likely to be involved in direct food–drug interactions, unless high doses are given together with a protein rich meal. The nutritional status could also cause transcriptional activation of the PepT1 gene by selective amino acids and dipeptides in the diet [90]. It has also been reported that the

Table 19.7 Proposed role of BCS in assuring clinical performance by dissolution testing within QbD.

BCS class	Dissolution testing requirements
I	Dissolution within 30 min at most discriminating conditions
II	Dissolution acceptance limits validated by bioavailability data of formulation variants
III	Dissolution within 15 min at most discriminating conditions
IV	As II or III if shown to behave *in vivo* as class II or III drug, respectively, otherwise *in vivo* BE

integrated response to a certain stimuli may increase PepT1 activity by translocation from a preformed cytoplasm pool [58].

19.6.2.5 Quality by Design

Quality by design (QbD) has recently been introduced in pharmaceutical product development in a regulatory context [83], and the process for implementing such concepts in the drug development and approval process is presently ongoing. This has the potential to allow for a more flexible regulatory approach where, for example, postapproval changes can be introduced without prior approval and end product batch testing can be replaced by in-process monitoring. This is based on understanding and optimization of how design of a product and its manufacturing process are affecting product quality. Good pharmaceutical quality represents an acceptable low risk of failing to achieve the desired clinical attributes. Thus, adding restrictions to manufacturing beyond what can be motivated by clinical quality brings no benefits but only additional costs.

QbD therefore brings the need to link clinical product performance to critical manufacturing attributes. It is not desirable to do *in vivo* bioavailability studies to evaluate all pharmaceutical factors potentially influencing drug absorption, but *in vitro* dissolution must be used as a surrogate. BCS could provide a platform for establishing clinical relevance by *in vitro* dissolution. However, the application of BCS might need to be further developed in context of QbD compared to the bioequivalence area to take benefit of the higher level of understanding that is implied by utilizing QbD concepts. A proposal for using BCS in context of QbD is outlined in Table 19.7 [89].

19.7 Conclusions

In this chapter, we have discussed and emphasized the importance of the fundamental factors in BCS, solubility, and intestinal permeability for oral drug absorption. The main regulatory impact today is the use of BCS as a framework for identifying drugs for which *in vitro* dissolution testing could replace *in vivo* studies to determine bioequivalence. Extensions of this approach to cases other than IR formulations of

the rather rare class I drugs would significantly enhance the impact of BCS [18]. However, product quality assurance must not be jeopardized, and a brief discussion illustrated the possible difficulties involved if BCS were extended, for example, to oral ER products. Finally, we emphasize the great use of BCS as a simple tool in early drug development to determine the rate-limiting step in the oral absorption process.

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20
Prodrugs

Bernard Testa

Abbreviations

CYP	Cytochrome P450
DEPT	Directed enzyme-prodrug therapies
5-FU	5-Fluorouracil
PEG	Polyethylene glycol

20.1
Introduction

The concepts and examples presented here are meant to clarify the objectives of a prodrug strategy, to exemplify the major chemical moieties used in prodrug design, and to illustrate the biochemical pathways involved in prodrug activation (i.e., hydrolysis, oxidation, or reduction). More detailed information can be found in a number of reviews [1–15].

What makes prodrugs different from other drugs is the fact that they are devoid of intrinsic pharmacological activity. Thus, the simplest and clearest definition is the one given in 1958 by Adrien Albert [16], who coined the term. In a modified form, the definition reads

> *Prodrugs are chemicals with little or no pharmacological activity, undergoing biotransformation to a therapeutically active metabolite.*

The complete opposites of prodrugs are thus drugs whose metabolites make no contribution to the desired therapeutic effects, for example, oxazepam. However, prodrugs should not be confused (as is too often the case) with drugs that are intrinsically active, though they are transformed into one or more active metabolites. In this case, two or more active agents will contribute to the observed clinical response in proportions that depend on differences in pharmacological activities, in compartmentalization, and in time profiles. Examples include cisplatin (which is chemically transformed to the monoaqua and diaqua species), morphine and its

6-*O*-glucuronide, diazepam (which is N-demethylated to nordiazepam), and codeine (which is O-demethylated to morphine).

It is also important to distinguish prodrugs from soft drugs [17, 18] defined as "biologically active compounds (drugs) characterized by a predictable and fast *in vivo* metabolism to inactive and nontoxic moieties, after they have achieved their therapeutic role." An example is afforded by the short-acting β-blocker esmolol, whose half-life of hydrolysis in human blood at pH 7.4 and 37 °C is 23 min [19].

20.2 Why Prodrugs?

Although many prodrug studies in the literature bring useful information on activation mechanisms and structure–metabolism relation, they do not appear to address a genuine clinical need. In contrast, many other studies do attempt to improve some properties of a marketed drug or a drug candidate under development. As detailed below, the target properties to be improved (i.e., the objectives) may be pharmaceutical, pharmacokinetic, or pharmacodynamic. A summary of these objectives is presented in Table 20.1.

20.2.1 Pharmaceutical Objectives

Pharmaceutical scientists are often faced with serious formulation problems resulting from poor solubility, insufficient chemical stability, or poor organoleptic properties such as bitterness that affect patients' compliance. While pharmaceutical technology can sometimes solve such problems, success may be difficult and time

Table 20.1 Objectives of a prodrug strategy.

A. Pharmaceutical objectives		**Relation with**
A1. Improved solubility		B1
A2. Improved chemical stability		B5 and C2
A3. Improved taste and odor	Improved	
A4. Decreased pain and irritation	acceptability	B5
B. Pharmacokinetic objectives		
B1. Improved oral absorption	Improved	A1, B3, and B4
B2. Decreased presystemic metabolism	bioavailability	B4 and C1
B3. Improved absorption by nonoral routes		B1
B4. Improved time profile (often increased duration of action)		B1, B2, and C1
B5. Tissue-selective delivery		A2, A4, C1, and C2
C. Pharmacodynamic objectives		
C1. Masking of a reactive agent to improve its therapeutic index		B2, B4, B5, and C2
C2. *In situ* activation of a cytotoxic agent		A2, B5, and C1

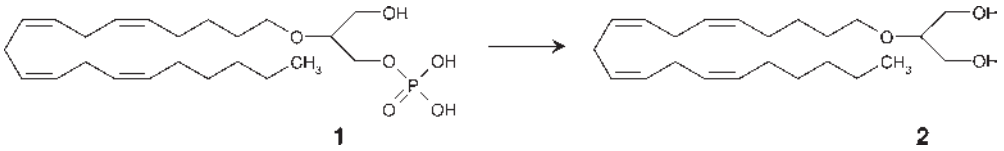


Figure 20.1 Noladin ether (**2**), a cannabinoid CB1 receptor agonist, and its monophosphate prodrug (**1**) [22, 23].

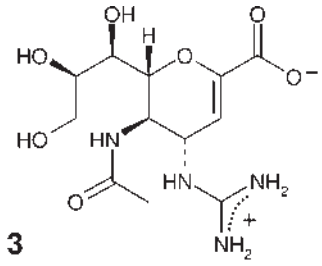
consuming to achieve. Project leaders may thus prefer to take advantage of a prodrug strategy and hope on an early solution. For example, medicinal chemists may consider the derivatization of phenols or alcohols by phosphate esterification to achieve greater solubility, although precipitation or absorption problems may result from premature hydrolysis [20], or the phosphate prodrug may even be too polar to allow good bioavailability [21]. A promising example of phosphorylation in prodrug design involves the endocannabinoid noladin ether (**2** in Figure 20.1). This cannabinoid CB1 receptor agonist reduces intraocular pressure, but its pharmacological profiling and pharmaceutical development are hindered by a poor aqueous solubility. Its monophosphate (**1** in Figure 20.1) and diphosphate esters increased the water solubility of noladin ether more than 40 000-fold. They showed high stability against chemical hydrolysis, yet were rapidly hydrolyzed by alkaline phosphatase and liver homogenates to the parent drug [22, 23]. Hydrolysis in 4% cornea homogenates was also fast. When tested *in vivo* in rabbits, the monophosphate ester (**2**) was very effective in reducing intraocular pressure.

Interestingly, increasing solubility is a pharmacokinetic as well as a pharmaceutical objective. Indeed, and as made explicit in the Biopharmaceutics Classification Scheme (BCS) [24], solubility is one of the main factors influencing oral absorption and hence oral bioavailability.

20.2.2 Pharmacokinetic Objectives

The need to improve the oral bioavailability of a drug or a candidate is one of the pharmacokinetic objectives in prodrug research, and it is currently the most important one. This can be achieved by improving the oral absorption of the drug and/or by decreasing its presystemic metabolism. Other pharmacokinetic objectives are to improve absorption by parenteral (nonenteral, e.g., dermal, ocular) routes, to lengthen the duration of action of the drug by slow metabolic release, and finally achieve the organ/tissue-selective delivery of an active agent. Some of these objectives are exemplified below with clinically successful prodrugs.

Achieving an improved oral absorption by a prodrug strategy is a frequent rationale in marketed prodrugs [5], as aptly illustrated with neuraminidase inhibitors of therapeutic value against type A and B influenza in humans [25]. Here, target-oriented rational design has led to highly hydrophilic agents that are not absorbed orally. One of the two current drugs is zanamivir (**3** in Figure 20.2), a highly hydrophilic drug administered in aerosol form. The other active agent is Ro-64-0802 (**5**), which also shows very high *in vitro* inhibitory efficacy toward the enzyme but low oral



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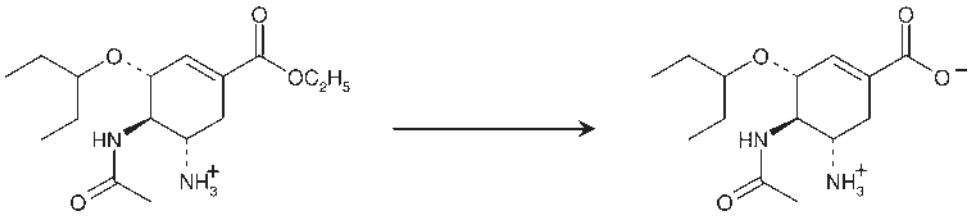

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Figure 20.2 Structure of neuraminidase inhibitors used against type A and B influenza in humans, namely, the drug zanamivir (**3**) and the prodrug oseltamivir (**4**) whose active agent is Ro-64-0802 (**5**) [25, 26].

bioavailability because of its high polarity [26]. To circumvent this problem, Ro-64-0802 has been developed and marketed as oseltamivir, its ethyl ester prodrug (**4**). Following intestinal absorption, the prodrug undergoes rapid enzymatic hydrolysis and produces high and sustained plasma levels of the active agent. As demonstrated by this example, the prodrug concept may thus be a valuable alternative to disentangle pharmacokinetic and pharmacodynamic optimization.

A slow-release pharmaceutical formulation is the most frequent method used when the objective is to prolong the duration of action of a given drug. However, there are examples of a prodrug strategy complementing a slow-release formulation, as exemplified by injectable depot formulations of esters of steroid hormones. A conceptually different and particularly elegant approach to slow metabolic release has been achieved with bambuterol (**6** in Figure 20.3), a prodrug of the β_2-adrenoreceptor agonist terbutaline (**7**) [27, 28]. Bambuterol is activated to terbutaline by hydrolysis in blood serum and by monooxygenase-catalyzed oxidation in the liver, lung, and other tissues. The hydrolysis reaction is catalyzed by cholinesterase (butyrylcholinesterase, EC 3.1.1.8). Following a first burst of terbutaline release, the enzyme is inhibited by covalent attachment of the dimethylcarbamate moiety (Me_2N-CO-), resulting in a potent and slowly reversible inhibition of cholinesterase. In clinical terms and when compared with terbutaline 5 mg taken three times

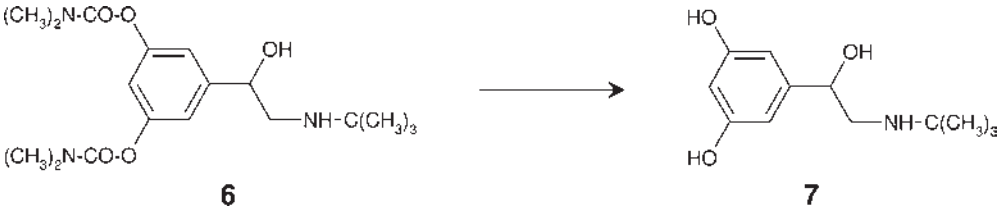

Figure 20.3 Structure of terbutaline (**7**) and its prodrug bambuterol (**6**) [27, 28].

daily, bambuterol 20 mg taken once daily provided smooth and sustained plasma levels of terbutaline and a greater symptomatic relief of asthma with a lower incidence of side effects.

A pharmacokinetic objective of great current interest is the organ- or tissue-selective delivery of a given drug, in other words, the search for the "magic bullet." A clinically significant example is that of capecitabine (**8** in Figure 20.4), a multistep, orally active prodrug of the antitumor drug 5-fluorouracil [29, 30]. Capecitabine is well absorbed orally and undergoes three activation steps resulting in high tumor concentrations of the active drug. It is first hydrolyzed by liver carboxylesterase (reaction **a**), the resulting metabolite being a carbamic acid that spontaneously decarboxylates (reaction **b**) to 5′-deoxy-5-fluorocytidine (**9**). The enzyme cytidine

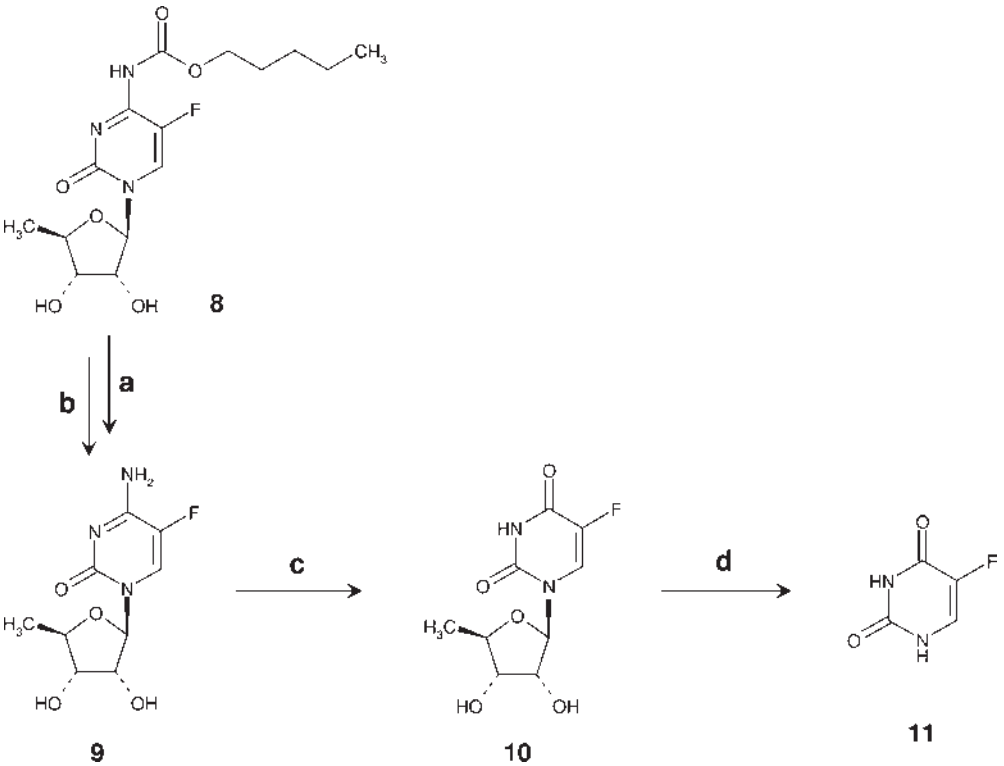

Figure 20.4 Stepwise activation of capecitabine (**8**) to the antitumor drug 5-fluorouracil (**11**) [29, 30].

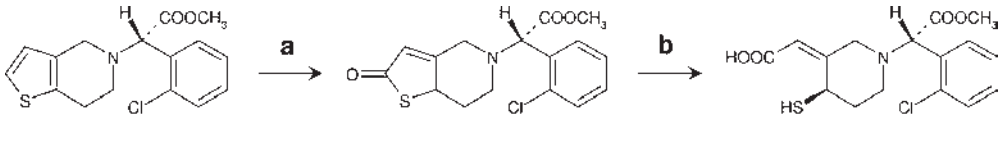

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Figure 20.5 Metabolic activation of clopidogrel (**12**) in humans. A small part of a dose is activated by CYP3A to 2-oxo-clopidogrel (**13**), followed by hydrolytic ring opening to the active agent, a highly reactive thiol metabolite (**14**) that irreversibly antagonizes platelet ADP receptors via a covalent S–S bridge [31, 32].

deaminase, which is present in the liver and tumors, then transforms 5′-deoxy-5-fluorocytidine into 5′-deoxy-5-fluorouridine (reaction **c** and compound **10**). Transformation into 5-fluorouracil (reaction **d** and compound **11**) is catalyzed by thymidine phosphorylase and occurs selectively in tumor cells. Capecitabine is of great interest in the present context, since it affords an impressive gain in therapeutic benefits compared to 5-FU as a result of its oral bioavailability and a relatively selective activation in and delivery to tumors.

20.2.3 Pharmacodynamic Objectives

In simple terms, pharmacodynamic objectives are synonymous with decreasing systemic toxicity. Two such cases are mentioned in Table 20.1 and are illustrated here. The masking of a reactive agent to improve its therapeutic index is aptly exemplified by the successful antiaggregating agent clopidogrel (**12** in Figure 20.5). This compound was known to be inactive without activation, but its metabolism and molecular mechanism of action remained poorly understood for years. In other words, clopidogrel should be considered as a fortuitous prodrug. The compound is of further interest among prodrugs in that its major metabolic route in humans (about 85% of a dose) is indeed one of hydrolysis, but this reaction leads to an inactive acid. In contrast, clopidogrel is activated by cytochromes P450 3A in a two-step sequence. The CYP-catalyzed reaction first oxidizes clopidogrel to 2-oxo-clopidogrel (**13**). This is followed by a rapid cleavage of the cyclic thioester to a highly reactive thiol metabolite (**14**) that irreversibly antagonizes platelet ADP receptors via a covalent S–S bridge [31, 32]. Interestingly, the same activation mechanism appears to account for the potent and irreversible inhibition of human CYP2B6 by clopidogrel [33], again demonstrating the high reactivity of the thiol metabolite.

In situ activation to a cytotoxic agent is part of the well-known mechanism of action of the antibacterial and antiparasitic nitroarenes such as metronidazol. This concept is now intensively investigated in the search for more selective antitumor agents [34–36]. Given that tumor cells have a greater reductive capacity than normal cells, various chemical strategies are being explored to design hypoxia-activated prodrugs of cytotoxic agents. Thus, the bioreductive antitumor agent tirapazamine (**15** in Figure 20.6) is seemingly the best studied drug candidate in this class [37, 38].

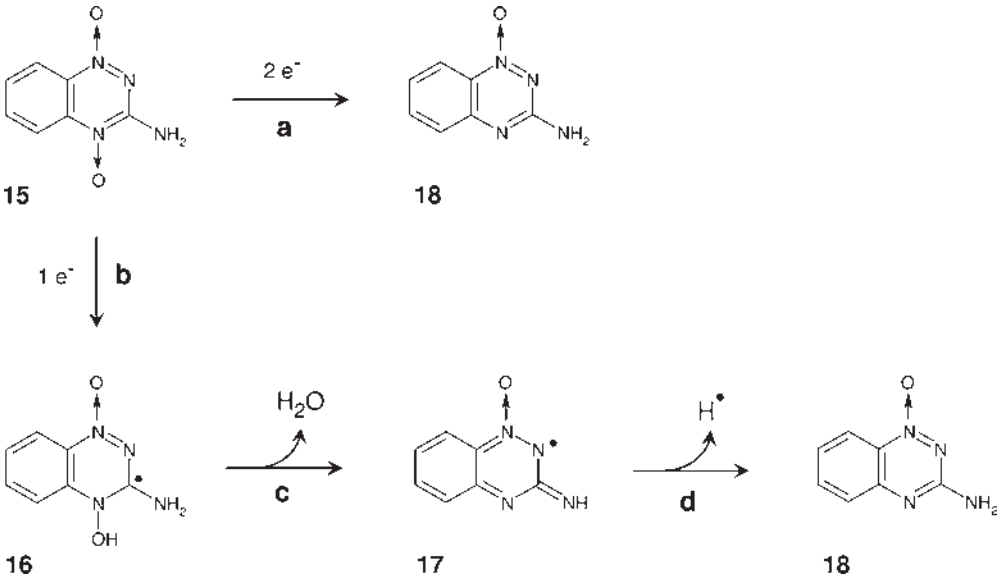

Figure 20.6 The hypoxia-selective antitumor agent tirapazamine **15**. Reaction **a**: reductive inactivation by two-electron steps catalyzed by quinone reductase (the first two-electron step being shown here). Reaction **b**: reductive activation (one-electron step catalyzed by cytochrome P450 reductase). Reaction **c**: dehydration to yield the reactive radical **17**, which abstracts a hydrogen radical from DNA [37, 38].

Tirapazamine is inactivated by two-electron reduction steps catalyzed by quinone reductase, yielding first the mono-*N*-oxide (reaction **a** and compound **18**). In contrast, it is activated to a cytotoxic nitroxide (**16**) by a one-electron reduction catalyzed by NADPH-cytochrome P450 reductase (reaction **b**). This delocalized radical loses one molecule of water to yield a reactive radical (reaction **c** and compound **17**). Radical **17** can then abstract one hydrogen radical from DNA (reaction **d** and compound **18**), leading to DNA breaks and cytotoxicity. In summary, both inactivation and activation involve reduction reactions, but cytotoxicity will depend on the relative levels of quinone reductase and CYP reductase in hypoxic cells.

20.3 How Prodrugs?

20.3.1 Types of Prodrugs

A useful way to classify prodrugs is the one based on chemical arguments. Thus, medicinal chemists find it useful to distinguish between four major classes of prodrugs, namely,

Table 20.2 Examples of common and less common carrier groups used in prodrug design.

Prodrug types	Generic structure(s)
Prodrugs of active carboxylic acids	
Simple alkyl or aryl esters	R-CO-O-R′
Alkyl esters containing an amino or amido group, or another functionality	For example, R-CO-O-(CH2)$_n$-NR′R″ or R-CO-O-(CH$_2$)$_n$-CO-NR′R″
(Acyloxy)methyl or (acyloxy)ethyl esters	For example, R-CO-O-CH$_2$-O-CO-R″
Esters of diacylaminopropan-2-ols	For example, R-CO-O-CH(CH$_2$-NH-COR′)$_2$
N,*N*-Dialkyl hydroxylamino derivatives	R-CO-O-NR′R″
Amides of amino acids	R-CO-NH-CH(R′)-COOH
Prodrugs of active alcohols or phenols	
Esters of carboxylic acids	R-O-CO-R′
Esters of ring-substituted aromatic acids	R-O-CO-aryl
Monoesters of diacids	For example, R-O-CO-(CH$_2$)$_n$-COOH
Esters of α-amino acids or other amino acids	For example, R-O-CO-CH(NH$_2$)R′
Carbonates or carbamates	For example, R-O-CO-O-R′ or R-O-CO-NR′R″
(Acyloxy)methyl or (acyloxy)ethyl ethers	For example, R-O-CH$_2$-O-CO-R′
(Alkoxycarbonyloxy)methyl or (alkoxycarbonyloxy)ethyl ethers	For example, R-O-CH$_2$-O-CO-O-R′ or R-O-CH(CH$_3$)-O-CO-O-R′
O-Glycosides	R-O-sugar
Phosphates or sulfates	R-O-PO(OR′)(OR″) or R-O-SO$_3$H
Prodrugs of active amines or amides	
Amides formed from acyl groups	RR′N-CO-R″
Amides of amino acids or peptides	RR′N-CO-CHR″-NHR″
Simple or structurally complex carbamates	RR′N-CO-O-R″ or for example, RR′N-CO-O-CH(R″)-O-CO-R″
N-Mannich bases	For example, RR′N-CH$_2$-NR″R″
O-Mannich bases	For example, RR′N-CH$_2$-O-CO-R″
Cyclic Mannich bases	For example, oxazolidines
Imines (Schiff bases)	RN=CH-NR′R″
Azo conjugates	R-N=N-R′

- Carrier-linked prodrugs, in which the active agent (the drug) is linked to a carrier (also known as a promoiety) and whose activation in most cases occurs by hydrolysis (esters, amides, imines, etc.). These are the most frequently encountered prodrugs and examples can be found in Figures 20.1–20.4. There also exist a limited number of carrier-linked prodrugs whose activation occurs by oxidation or reduction. A list of prodrug types derivatized with selected promoieties can be found in Table 20.2.
- Bioprecursors are distinguished from prodrugs by the lack of a promoiety, yet can be activated by oxidation (see Figure 20.5), reduction (see Figure 20.6), or hydrolysis (e.g., lactone opening) [14].
- Macromolecular prodrugs, where the carrier is a macromolecule such as a PEG (polyethylene glycol) [39].

- Directed enzyme-prodrug therapies (DEPTs), which are prodrugs derived from biotechnology [1, 4, 40–44]. These are highly specialized biochemical strategies that would require separate treatment. They include

  -drug–antibody conjugates where the carrier is an antibody raised against tumor cells, as in antibody-directed enzyme prodrug therapies (ADEPTs);

  -gene-directed enzyme prodrug therapies (GDEPTs).

20.3.2
Hurdles in Prodrug Research

This chapter has focused on carrier-linked prodrugs and bioprecursors, which remain by far the largest groups of prodrugs in use. Indeed, of the 1562 different active substances marketed in Germany in 2002, 6.9% were prodrugs, with one-half of these being activated by hydrolytic cleavage of a promoiety, and one-quarter being bioprecursors [3].

After discussing the objectives of prodrug research, one cannot ignore the difficulties involved. Indeed, developing prodrugs involves additional work in synthesis, physicochemical profiling, pharmacokinetic profiling, and toxicological assessment [3]. Two major challenges are biological variability and toxicity potential. The challenge of biological variety results principally from the huge number and evolutionary diversity of enzymes involved in xenobiotic metabolism. Inter- and intraspecies differences in the nature of these enzymes, as well as many other differences such as the nature and level of transporters, may render prodrug optimization difficult to predict and achieve. The high level of carboxylesterases in the plasma of rodents but not in the plasma of other mammals is but one example of a biological difference that may affect the rate and site of activation of some prodrug esters. A chemical strategy developed by medicinal chemists to overcome the problem of biological variety is the development of prodrugs activated by nonenzymatic hydrolysis, for example, imines, Mannich bases, (2-oxo-1,3-dioxol-4-yl)methyl esters, or oxazolidines. A more promising approach appears to be the two-step activation of carrier-linked prodrugs, involving first a relative facile enzymatic hydrolysis to unmask a nucleophilic group, followed by a nonenzymatic, intramolecular nucleophilic substitution and cyclization [1–4, 8, 45].

A second challenge is the toxicity potential of some prodrugs, namely, a toxic metabolite formed from the promoiety or a reactive metabolic intermediate generated during the activation of some bioprecursors. The former case is illustrated by the liberation of formaldehyde, as seen with Mannich bases or some double esters [1, 4]. The latter case involves a very few known examples of failed bioprecursors whose activation was via a reactive and toxic intermediate. Thus, arylacetylenes were examined as potential bioprecursors of nonsteroidal anti-inflammatory agents [1]. Although the nature of the final (and stable) metabolite (an arylacetic acid) was known, researchers at the time were not aware that the metabolic pathway involved an intermediate and highly reactive ketene.

20.4 Conclusions

The prodrug concept has allowed some apparently intractable pharmaceutical, pharmacokinetic, or pharmacodynamic problems to be overcome [4]. But there is more, since the objectives discussed above are often intertwined. Thus, an improved solubility can greatly facilitate oral absorption, while improving the chemical stability of an active agent can allow tissue-selective delivery and even lead to its *in situ* activation. As a result, medicinal chemists and biochemists in prodrug research should be aware that the behavior of their prodrug candidates may differ from that of the parent drug in ways that go beyond the original pharmaceutical, pharmacokinetic, or pharmacodynamic objectives being pursued.

A large number of prodrug examples published in the literature are clear cases of *post hoc* research that never advanced to development. In contrast, the examples presented above illustrate how well-designed or even fortuitous prodrugs allow to achieve medicinal objectives that remain out of reach of the active drug. Indeed, a prodrug approach is most fruitful when a traditional hit or lead optimization fails because the structural conditions for activity (i.e., the pharmacophore) are incompatible with the target pharmaceutical, pharmacokinetic, or pharmacodynamic properties. In other words, the gap between activity and other drug-like properties may be of such a nature that only a prodrug strategy can bridge it [1, 2].

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21
Modern Delivery Strategies: Physiological Considerations for Orally Administered Medications

Clive G. Wilson and Werner Weitschies

Abbreviations

BMI	Body mass index
[^{51}Cr]-EDTA	Chromium-51 labeled ethylenediamine-tetraacetic acid
CYP3A4	Cytochrome P450, 3A4 isozyme
GI	Gastrointestinal
IMMC	Interdigestive migrating motor complex
MMC	Migrating motor complex
PEG	Polyethylene glycol
q.d.s.	Four times a day
SITT	Small intestinal transit time (h)
t.d.s.	Three times a day
WGTT	Whole-gut transit time (h)

Symbols

AUC	Area under the plasma concentration–time profile (mg h/l)
Cmax	Maximum concentration reached in the concentration profile
[^{111}In]	Indium-111
t_{50}	Time for 50% emptying of the stomach (h)
t_{max}	Time for maximum concentration (h)
[^{99m}Tc]	Technetium-99m

21.1 Introduction

The control of physiological processes to achieve reliable plasma concentration–time profiles following oral drug administration is a key goal in therapy. At the investigational level, it would allow the scientist to use manageable numbers of volunteers in

appropriately powered tests to proceed with formulation development and at the medicinal level, it might allow more consistent outcomes. The weasel word "might" is important here, since disease and/or concomitant medication may alter transit, pH, disposition, metabolism, and clearance in a manner unforeseen in the original clinical trials. The opposite scenario can also happen, with patients showing consistent blood level–time curves and studies in *volunteer cohorts* yielding huge differences in AUC and C_{max}. More generally, the issue relates to erratic blood levels with patients or volunteers grouped into "poor" and "good" absorbers or those with troughs in the profile. At this stage, there is usually a frantic appeal to the formulator to come up with a strategy to solve the problem. This will result in futile endeavors if the phenomenon is related to a physiological process that was not controlled in the trial providing the anomalous results. This state of affairs occurs because the impact of some elements of physiological processes, particularly those relevant to drug absorption, are poorly appreciated and physicians and pharmaceutical scientists have failed to reach a common perspective.

In our view, physiological and pathophysiological factors have to be considered hand-in-hand with pharmaceutical and physicochemical issues in the exploitation of modern drug delivery strategies. In this chapter, attempts have been made to illustrate this point from the perspective of clinical pharmacology, drawing on examples largely from imaging, patient, and volunteer studies conducted by us and our colleagues. To make the task manageable, we will confine our analysis to the oral route, although similar consideration could be applied to any mode of delivery.

21.2 The Targets

For most drugs, the limitations to delivery are defined in terms of solubility and permeability. The solubility issues are addressed by various means including selection of an amorphous form where appropriate, reduction in particle size, and the use of cosolvents. For permeability problems, a mechanism to increase flux by altering the membrane/microenvironment conditions or even a simple increase in concentration gradient might achieve the goal. Examples of design strategies include the use of absorption enhancers and formulations designed to keep the drug in the upper gastrointestinal (GI) tract, which may achieve reduction of first-pass effect (buccal), increased solubility in acid (stomach), or avoidance of degradation in the colon. When the compound selected is problematic in terms of both solubility and permeability, the number of permutations becomes almost infinite, especially when juggling with the added dimensions of dose and plasma half-life. In general, the desired outcomes could be listed as in Table 21.1.

The gastrointestinal tract is conveniently divided into a number of areas, primarily on the basis of morphology and function. For most pharmaceutical purposes, we can group the target tissues into three regions: the upper, mid- and lower gastrointestinal tract.

Table 21.1 Desired outcomes of targeted treatments at various regions of the gut.

Region	Objective	Strategy	Issues
Buccal	Avoid first pass	Adhere to buccal mucosa (prophylaxis)	Taste
		OR	Saliva (some drugs cause dry mouth)
		Sublingual (immediate)	Potency (has to be high)
			Talking, eating, drinking
	Increase flux	Open tight junctions	Irritancy
	Increase convenience	Use fast-dissolving system	Will not have equivalent profile to simple IR systems
Esophagus	Ensure transit	Adjust tablet shape, coating	Surface area/weight ratio
	Avoid sticking	Stand up, take with water	(How much? When?) Age, previous Rx, posture
Stomach	Promote rapid absorption	Control intrinsic dissolution	*In vitro*/*in vivo* correlation
		Control emptying	Fed/fasting effects
	Prevent degradation	Enteric coat	Achlorhydria in elderly (especially Japanese)
	Extend absorption (in intestine)	Float, swell, adhere	Posture
			Rate of degradation after delivery, bezoars
			Food effects, gastric inhomogeneity
			Disease effects
Intestine	Extend exposure	Adhere to villus tip	Access?
		Promote Peyer's patch uptake	Wall–lumen mixing
	Increase absorption	Decrease P-glycoprotein efflux	Timing
		Block Cytochrome P450 3A	Concomitant administration
		Open tight junctions	Unselective
	Utilize lymphatic system	Increase absorption of lipophiles	Food effects persist for longer than 24 h
	Increase exposure	Target with coated preparation	Variability in transit and pH
		Utilize bacterial fermentation	Variability, disease effects?
		Increase surface area of preparation	Stirring, dispersion available water, gas, time of dosing

21.3 The Upper GI Tract: Mouth and Esophagus

The surface tissue of the mouth is squamous epithelium, and the cells lining the cheek are dead and enucleated. The effective permeability barrier is quoted as between 10^{-9} and 10^{-5} cm/s due in part to the activity of membrane coating granules

positioned near superficial tissues. These granules, after fusion with the plasma membrane, empty their contents into the intercellular space [1] like mortar around bricks, and the principal mechanism of drug entry is diffusion [2]. The liquid phase is provided by saliva and therefore any disease or treatment that affects production of saliva may alter distribution of drugs within the buccal cavity.

The three main areas of development over the last decade have been in smoking cessation, pain control, testosterone replacement, and to some less significant extent, novel formulations. A recent review by Smart [3] summarizes these latter directions in reformulation. Buccal delivery remains an area of interest for most companies, particularly for niche products, where conventional oral medication does not provide sufficiently faster onset of action or where treatment may be discontinuous.

Clearance of drugs from the buccal mucosa is generally slow, which may reflect high nonspecific binding in the tissue: plasma morphine concentrations, for example, decline more slowly than that of an intramuscular site and may be used with advantage to extend the period of analgesia [4, 5]. Oddly enough, Gordon [6] has concluded that morphine is not absorbed faster sublingually than when swallowed. Diffusion through the tissue can be improved for many drugs by manipulating lipophilicity (prodrug or pH control) to increase partitioning into the tissue. A simple application of this concept can be seen in the development of nicotine formulations where raising buccal pH increases the flux significantly, producing a rise in drug absorption from nicotine formulations held in the mouth [7, 8].

Utilizing absorption enhancers in this area is probably more benign than elsewhere in the gastrointestinal tract. The maximum size of a buccal patch that would be acceptable to a patient has been defined as >15 cm^2 (or more usually between 0.5 and 3 cm^2), and Hoogstraate and colleagues [9] have commented that an absorption enhancer would therefore probably be necessary to keep buccal patches to a desirable size. Commonly employed enhancers such as fatty acids, synthetic surfactants (sodium dodecyl sulfate), and natural surfactants (bile salts) have appeared in many experimental formulations and polymer salts. Chitosan glutamate, for example, enhances the solubility of nifedipine [10]. In addition, the small area for delivery dictates that the drug must be potent and although the buccal mucosa is less hostile with regard to peptidase and other enzymatic activity, it may be necessary to incorporate enzyme inhibitors as excipients in the formulation.

Although gels can be used to increase contact and promote absorption [11], the requirement for unidirectional delivery cannot be met by simple systems, and release of actives from erodible matrices are influenced by physiological abrasion from the cheek surfaces by talking. Novel systems for oral delivery have been extensively investigated by Hoogstraate, and others have reviewed developments in the field including melatonin delivery and low molecular weight heparin in the Cydot system [9]. In pain management, Gordon [12] has reviewed a number of novel transmucosal and transdermal systems including the effervescent fentanyl tablet designed for the control of breakthrough pain using the effervescent system to produce local pH shifts. The drug is rapidly absorbed with a peak concentration achieved 40 min after dosing with a

complex triexponential clearance at high doses [13]. Later investigations showed that t_{max} was not related to mouth dwell time [14].

Fast-dissolving formulations (flash dispersing) are not primarily intended for buccal delivery; the issue here is that they may be taken without water. This causes an important difference in performance relative to ordinary immediate-release products, especially if the drug is in suspension. If the material is swallowed dry, it may adhere to the fundus area of the stomach, where the amount of shear is low. This causes a significant fraction of the material to be retained resulting in tailing of the absorption phase and an apparently decreased AUC as the material is released over several hours.

21.3.1 Swallowing the Bitter Pill...

It is commonly assumed that swallowed dosage forms pass without hindrance into the stomach unless an underlying esophageal condition is present. However, it has been shown that esophageal transit of capsules or tablets is strongly influenced by the volume of the coswallowed water and the body position [15, 16].

Radiological studies of an asymptomatic group of 56 patients (mean age 83) showed that a normal pattern of deglutition was present in only 16% of the patients and 63% of this population experienced difficulty in swallowing [17]. To assist the swallowing of a tablet, patients are instructed to take a dosage form in an upright position with plenty of water. It might be expected that simple encouragement and education could encourage compliance. In practice, when patients are presented with a 240 ml glass of water and instructed to swallow a tablet "according to normal practice," they imbibe only two to three mouthfuls (between 50 and 100 ml). Radiological studies conducted back in the mid-1980s concluded that the effect of taking diazepam with either 10 or 50 ml water made little difference to absorption, although 20% of the study group showed delayed absorption irrespective of volume imbibed [18]. In our studies, the effects of small volumes of water (30 or 50 ml) on the esophageal clearance of either a small, uncoated circular tablet or film-coated oval tablet were compared [19]. Clearance of the oval tablet was significantly faster and stasis occurred in 5 instances with the uncoated tablet versus zero in the same 28 patients taking the film-coated oval tablet.

Deliberate attempts to adhere to the esophagus have been made to treat ulcer sites in esophagus and stomach, for example, with sucralfate [20]. These data suggested that acid-activated sucralfate showed high retention in man, and separate supporting data were obtained in the dog. Unfortunately, a later study failed to confirm this observation in the clinic [21]. The explanation for visible coating in the dog is probably related to the angle of the esophagus. The development of an esophageal bandage based on graft polymers that possess mucoadhesive and thermosetting properties was investigated in our laboratory and some limited success was achieved as shown in Figure 21.1 [22]. However, the issue of starting temperature and heat transfer during mouth-hold and deglutition proved to be problematic.

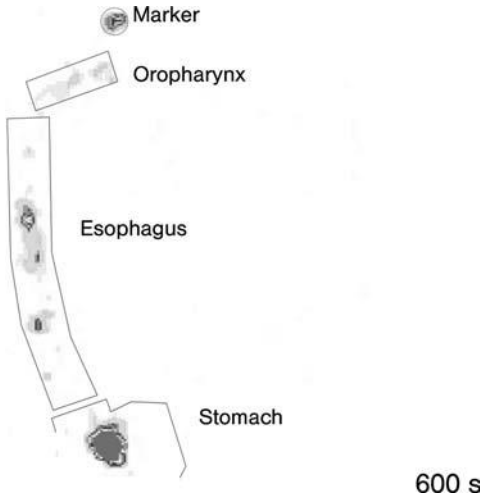

Figure 21.1 Scintiscan of [^{99m}Tc]-labeled "Smartgel," 10 min after administration showing material adhering in mid-esophagus.

21.4
Mid-GI Tract: Stomach and Intestine

The stomach provides a reservoir allowing food to be made available to the small intestine at a rate that is controlled by the complex system of the brain–gut axis. To do this, the food contents are churned with acid and enzymes, which begins the digestive process. There is essentially no absorption of food components from the stomach. The same holds true for drug substances. Even the highly permeable, small-molecule ethanol is only poorly absorbed from the stomach [23]. The early stage of digestion allows the duodenum to sample the contents and adjust the gastroduodenal pressure difference to regulate the supply of calories. These function impacts explain some of the characteristics of the first part of the intestine (i) that absorption will be extremely efficient and (ii) transit will be very rapid to allow modulation by further food intake.

21.4.1
Gastric Inhomogeneity

For the most part, the resting pH of the stomach is close to 2 than 1 and during feeding, the meal causes a transient rise to 4–5 depending on the volume and nature of the meal consumed. The fundus undergoes receptive relaxation to allow the proximal stomach to accommodate the food mass: in the distal stomach, the food is triturated to form chyme that is ejected into the duodenum in spurts of 2–5 ml. The division of function causes significant inhomogeneity in the conditions of the stomach, as the proximal stomach is more stagnant and the number of parietal cells is much less in the fundal area [24].

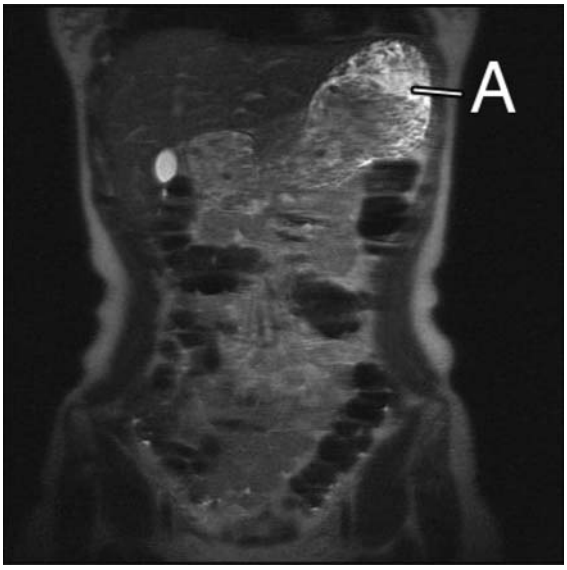

Figure 21.2 T2-weighted image showing water in the fundus when the subject is recumbent [47].

The stomach is not rigid and, as a predominately muscular structure, changes shape on filling. The upper part of the stomach (the fundus) undergoes receptive relaxation to accommodate the ingested food that is kneaded at the bottom of the stomach (the pyloric region). In the upright position when visualized with X-ray contrast, the stomach has classically a fishhook shape, but on lying down, the fundal area falls into the abdomen and becomes the lowest part. In the T2-weighted MRI image in Figure 21.1, the subject is lying on the back and the water appears brightest in the image. Thus, posture will influence the relative distribution in the stomach strongly (Figure 21.2).

Stratification of gastric contents is also evident when an upright posture is adopted. For example, if a raft-forming alginate-based antacid formulation is taken after a meal, with the subject upright or seated, the formulation may be retained in the upper stomach by a mixture of flotation and stratification provided there is sufficient gas generated to increase buoyancy. The timing of the dosing of the formulation relative to the meal is quite critical. When taken after a meal, an almagate-based preparation Flot-Coat was demonstrated to reside in the stomach on top of the meal and empty from the stomach more slowly than the food. The time for 50% of the Flot-Coat granulate to empty was more than 4.5 h compared to 2.3 h for the meal. The standard granulate mixed and emptied with the digestible solid phase of the meal, with 50% emptying in 1.7 h [25].

Mixing in the stomach and the intragastric distribution result from postural and motility effects, which are strongly influenced by the type of the meal. The effects of functional separation into an upper, low-motility reservoir and a lower grinding chamber can be shown by differences in absorption rates according to the sequence

of administration relative to meal intake. For water-soluble drugs given in liquid meal, the substance will be uniformly distributed within about 40 to 80 min; however, this is not the case for drug intake after solid meals [26, 27]. The currently unsolved problem of the inhomogeneity of intragastric distribution is, in our experience, a major source of unwanted food effects after administration of ER products (dose dumping), erratic blood levels with class II substances, and a mostly ignored critical issue of many current concepts for gastroretentive delivery systems [28]. The influence of this effect is nicely shown with data obtained following administration of extended release formulation of amoxicillin and clavulanate (Augmentin XR). In the prescribed information, it is advised that Augmentin XR has to be taken at the beginning of a meal. The formulation was labeled with small amounts of magnetite to permit imaging of the position and the rate of disintegration by magnetic moment imaging. Intake under fasting conditions led to a decreased amoxicillin absorption and intake after the meal resulted in a decreased clavanulate absorption. These differences were the result of early gastric emptying of the tablets in case of fasting administration and prolonged intragastric residence in case of administration after the meal. Early gastric emptying causes a reduced absorption of amoxicillin due to its absorption window in the upper GI tract and long intragastric residence results in a poor bioavailability of clavulanic acid due to acid-catalyzed hydrolysis [29].

In response to food, the parietal cells secrete acid that will be moved upward by contractions of the pylorus. In an individual with an incompetent cardiac sphincter, this can result in a reflux of acid into the esophagus. To assess food and acid reflux, patients are provided with a refluxogenic meal, after which the reflux of technetium-99m-labeled food and acid appearance in the esophagus are monitored using ambulatory pH and radioisotope telemetry [30]. Using this technique, the reflux of acid and food was noted to be quite separate events and consistent with a mechanism by which acid can move around the food mass. This related to another observation during the reformulation of paracetamol (acetaminophen) into both fast-acting and sustained-acting preparations. Gamma scintigraphy was utilized to examine the emptying of a novel paracetamol formulation in the fed and fasting state. In the fasting state, we expected to be able to show faster dissolution of the formulation and a consequent increased rate of gastric emptying. This we were able to do; however, we also found that the novel formulation showed faster gastric emptying in the fed state. The scintiscans in Figure 21.3 indicate that the phenomenon is probably due to rapid dissolution and movement of the formulation around the food mass in the stomach [31]. It is known that liquid and solids empty the stomach at different rates, although the significance of the discrete boundary phase has been ignored.

It is well established that a meal containing sufficient fat will stratify. By using MRI, several investigators have been able to show the appearance of a fat layer on the fundal gastric contents after a fatty meal. In this situation, the inhibitory effects of fats are reduced, since the fat must be homogenized, emulsified, and presented to the duodenal receptors before an action is initiated. If a suitable substrate for dispersion of fat is provided (i.e., minced beef), the inhibitory effects of fats on the rate of gastric emptying are increased. This indicates that significant differences in intragastric distribution of fat occur after eating a meal [32].

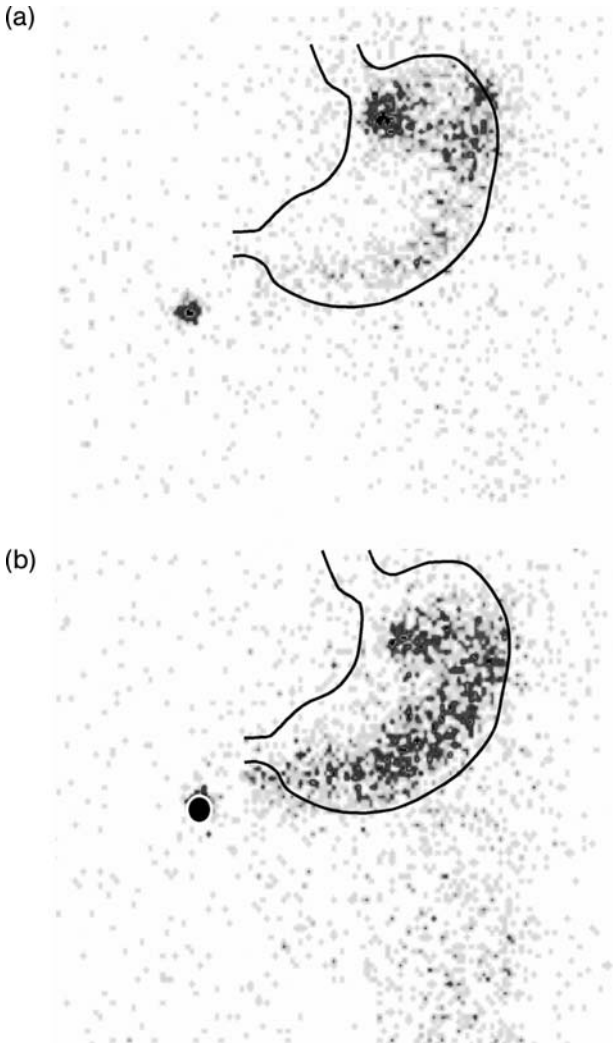

Figure 21.3 (a and b) The start of a sequence of static views of a radiolabeled paracetamol tablet. In frame (a), the tablet has started to dissolve and the released drug and radioactivity move along the greater curvature of the stomach. In frame (b), antral mixing has started to move the label and the drug into the food mass in the pyloric antrum.

21.4.2 Gastric Emptying

The rate of gastric emptying under fed conditions is controlled by the energy content of the meal, the energy requirement of the body, and feedback mechanisms including the ileal brake mechanism [33, 34]. Furthermore, the rate of gastric emptying of

nondisintegrating systems under fed conditions is also influenced by particle size. For food components, the mean emptying rate depends on the caloric content as described in the classical studies of Hunt and colleagues [35]. As a consequence, the emptying of drug substances from the stomach in the digestive phase depends on three main factors: the gastric emptying rate of the meal, the intragastric distribution of the drug, and the particle size of the formulation. In clinical food effect studies, a serving of a high-caloric (1000 kcal), high-fat (50% of calories) breakfast is administered before the drug formulation [36] resulting in very long gastric residence times. In our experience, this huge and unnatural food load for small individuals will cause large physiological effects, and typical volunteers with body weights less than 80 kg will not have completely emptied a test high-fat breakfast even when lunch is served 4–6 h later.

There has been an intensive effort to determine a distinct upper size limit with regard to particle emptying under fed conditions for years. This concept was mainly driven by the hypothesis that the pylorus acts under fed conditions as a filter with a fixed effective aperture of about 2 mm This concept is not valid. Gastric emptying is the result of a coordinative function of pyloric opening and antral contractions, producing what is known as gastric sieving [37]. Owing to the involvement of sedimentation processes, the particle size of withdrawn (retropulsed) material is a function of meal viscosity and particle density [38].

In the elderly, subtle changes in emptying are seen and distinct trends are hard to elucidate [39]. There is an increased incidence of reflux associated with slow esophageal clearing, probably associated with weaker peristaltic contractions and the presence of muscular abnormalities such as hiatus hernia. The MMC complex is maintained until the eighth or ninth decade of life with clear propagation of phase-III activity suggesting that short-range intrinsic intestinal nervous pathways are maintained [40]. Studies on aging laboratory animals suggest that neurons are lost from the myenteric plexus, particularly the nonadrenergic pathway of the proximal jejunum, but plasticity and adaptation of the human bowel compensate to preserve motility. Thus, diseases normally associated with abnormal motility such as bacterial overgrowth are not primarily a reflection of normal aging. The clearest evidence for aging effects are seen with regard to the emptying of mixed meals and in the slower gastric emptying of the liquid phase suggesting changes in the motility of the fundus [41], although fasting and postprandial antral motility remains normal [42].

Gender – or, more particularly, the menstrual cycle – has also been mentioned as a significant influence on the whole-gut transit. Madsen and colleagues carried out a study to elucidate the influences of gender, age, and body mass index (BMI) on gastrointestinal transit times using a meal containing [^{99m}Tc]-labeled cellulose as a fiber and 2–3 mm [^{111}In]-labeled plastic particles. Seventeen healthy young and sixteen healthy older subjects (eight men, eight women) were studied. All transit variables were unaffected by gender. The older subjects had a slower mean colonic transit time of radiolabeled plastic particles than the young subjects ($p < 0.05$), while BMI affected the gastric emptying of fiber but not other gastrointestinal variables [43]. In another study comparing young and elderly males, which employed ultrasonographic and radiographic techniques to measure transit, no age-related effect could be determined [44].

According to a survey of the literature conducted by Baron and colleagues between 1963 and 1992, there appears to be a reduction in the rate of small-bowel and colonic transit during pregnancy [45]. Gastrointestinal symptoms were reported predominantly as abdominal bloating and constipation. These effects are mediated by progesterone, with estrogen probably acting as a primer.

21.4.3
Small Intestinal Transit Patterns

Small intestinal transit of nondisintegrating dosage forms as well as chyme is extremely discontinuous and characterized by phases of rest and short episodes of transport. In Figure 21.4, the velocity profile of an enteric-coated tablet from ingestion until disintegration (141 min postadministration) is shown. The postulation that the small intestine is a tube filled with fluid allowing the transit dosage forms in a continuous movement is completely misleading [46]. Nonabsorbable materials including fibers or modified release dosage forms usually gather in the terminal small ileum together with remaining fluid. Generally, the contents by the end of the small intestine are a homogeneous mass unlike the very high heterogeneity of the stomach contents.

After intake of the next meal, motility is increased and the contents of the terminal ileum are transported into the ascending colon, a mechanism known as the gastroileocecal reflex [47, 48]. Small intestinal transit time (SITT) of nondissolving dosage forms in humans is reported to be usually quite constant with a mean transit time of about 3 h. This mean small intestinal transit time of solids depends on the interval between dosing of the medication and the serving of the next meal, illustrating the strong influence of the gastroileocecal reflex. Accordingly, the scheme of food administration after dosing of the medication is of major influence on small intestinal transit times.

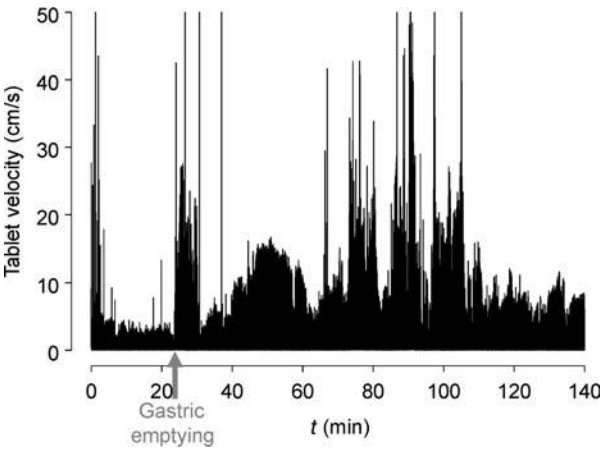

Figure 21.4 Velocity profile of an enteric-coated tablet from ingestion until disintegration in the small intestine (gastric emptying at 24 min).

21.4.4
Modulation of Transit to Prolong the Absorption Phase

Adhesion and slowing intestinal transit are two mechanisms that have been proposed to extend the absorption phase of drugs, particularly if colonic permeability is poor. Our real-time measurements of the transit of formulations along the GI tract elegantly demonstrate that during the initial phases of transit through the duodenum and jejunum, the formulation is swept forward in a series of pulsatile movements that would leave little opportunity for adhesion in the upper gut in the fasting state [28].

It is well appreciated that dietary fat retards proximal gastrointestinal transit. Since absorption of fats is generally complete in the healthy gut by the distal ileum, the colon epithelium is not equipped to process fats and excess load leads to steattorhea.

The role of lipids on absorption has been extensively reviewed by Porter and Charman [49, 50]. The influences are diverse and include effects on lumenal drug solubility, altering the metabolic and barrier function of the intestinal wall, stimulating lymphatic transport, and a reduction in gastric transit, thereby increasing the time available for dissolution.

21.4.5
Absorption Enhancement

As mentioned earlier, treatments that transiently open up intercellular gaps increase absorption significantly. In the villus, the loss of apical cells may cause large gaps at the tip, allowing drug to enter through the lymphatic spaces. Prolonged insult leads to an alteration of the goblet cell/enterocyte ratio and a histological phenomenon known as goblet cell capping. This effect can be seen after quite short exposures with high concentrations of low molecular weight polyethylene glycol [51] and is a response to osmotic stress.

As pointed out by Baluom and colleagues, absorption enhancers are efficient in small body cavities such as the nasal and the rectum [52]. In the fed state, the issues of dilution during gastric mixing would probably obviate the possibility of interaction between the dispersed phase and the small intestine. Using a perfused rat model, the authors showed that a synchronized administration of an absorption enhancer (sodium decanoate) is required for optimal absorption of a poorly absorbed drug (cefazoline) and that levels of the enhancer need to be sustained rather than high.

The finding that grapefruit juice can increase the bioavailability of certain drugs, by reducing presystemic intestinal metabolism, led to interest in the area of "food–drug interactions." It has been suggested that this could be exploited to increase bioavailability, especially for poorly soluble compounds. Interest focused particularly on the effects of the grapefruit flavonoid, naringin, and the furanocoumarin, 6′,7′- dihydroxybergamottin on the activity of intestinal CYP3A4. Given that P-gp and canalicular multispecific organic anion transporters are involved in the intestinal absorption and biliary excretion of a wide range of drugs and metabolites, it is reasonable to

suspect that furanocoumarins may alter drug disposition in humans [53]. Wagner and colleagues [54], in a review of food effects on intestinal drug efflux, have recently suggested that the effect of grapefruit juice is likely to be due to the inhibition of intestinal P-glycoprotein rather than metabolism. Recent reviews suggest that the influence of grapefruit juice is controversial with activation or inhibition of P-gp being noted, although the inhibitory action on intestinal rather than hepatic CYP3A4 is clearly established [55].

Animal data suggest that dietary salt modulates the expression of renal CYPs. Darbar and colleagues [56] extended this observation to suggest that intestinal CYP3A may be similarly modulated by dietary salt. They studied the effects of dietary salt on the kinetics of quinidine on normal volunteers, each given high-salt (400 mEq/day) and low-salt (10 mEq/day) diets for 7–10 days. They found that plasma concentrations after oral quinidine were significantly lower during the high-salt phase, with the difference between the two treatments attributable to changes within the first 1–4 h.

The possibility of exploiting excipient effects, particularly of the nonionic detergents, such as Tween 80 or Pluronic, has been of interest to several laboratories. The data suggest that a strategy based on bioavailability enhancement for drugs undergoing intestinal secretion might be valid with the caveats general to absorption enhancers. The effects are evident with model peptides and with cyclosporin, whose bioavailability is increased in normal subjects when the drug is coadministered with D-α-tocophenylpolyethylene glycol 1000 succinate [57].

21.5
The Lower GI Tract: The Colon

At the end of the small intestine, deposition is almost complete and there is no need for intestinal secretions to aid assimilation. The principal role of the colon is to resorb water and reclaim sodium, which it does very efficiently; for every 2 l of water entering the colon, the residual water in the stools will be less than 200 ml.

The material that arrives in the colon will contain cellulosic materials from the vegetables in the diet, which cannot be broken by the intestinal secretions. In the cecum, a bacterial ecosystem digests the soluble, fermentable carbohydrates to yield short-chain fatty acids that are assimilated into the systemic circulation by the colon, together with vitamin K released from the plant material. Carbon dioxide release is also a fermentation product, and if the redox potential is sufficiently low, bacteria can produce methane and hydrogen, which can be detected in the breath particularly after the ingestion of pulses. In the upright position, the gas will rise to the transverse colon: an adult produces approximately 2–3 l/day of which most is exchanged through the lungs. The accumulation is illustrated in Figure 21.5.

The average bacterial load of the colon has been estimated at just over 200 g (equivalent to approximately 35 g dry weight). Water available for dissolution is maximal in the ascending colon and 1.5–2 l of water enters from the terminal small intestine each day. The amount of water present varies, being maximal in the period

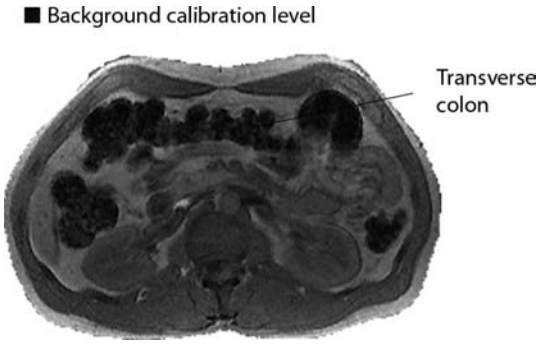

Figure 21.5 Accumulation of gas in the transverse colon illustrated by MRI.

4–8 h after ingestion of a meal. In the morning, the colon is often empty, and any material remaining in the ascending colon is slowly cleared. Thus, availability of water appears to be very small. In a recent MRI study conducted by one of the authors, the total water content of the colon was determined at about 10–15 ml [46]. These estimates have been recently confirmed by direct cecal sampling by a collaboration between Reppas, Dressman, and colleagues. The consequences of this poor availability of water or colon drug targeting or extended release systems must be carefully considered.

21.5.1 Colonic Transit

The data available concerning the movement through the gut were previously confined to measurements of whole-gut transit time until gamma scintigraphy became a routine tool in pharmaceutical research. Both the stomach and the colon can be identified unambiguously in planar images, allowing the contribution of colonic absorption in the plasma-concentration–time profile to be assessed in each individual. Table 21.2 shows colonic transit of single-unit dosage forms when dosed at different times of the day.

There is a large variability in the data, but in general, units administered prior to retiring for the night have a slower colonic transit than those dosed in the morning, which is in agreement with the pattern of electrical and contractile activity measured by

Table 21.2 Colonic transit times (h) of single unit dosage forms [37].

Colon region	Time of dosing			
	Morning fasting	Morning light breakfast	Morning heavy	Evening breakfast
Ascending	3.6 ± 1.2	2.5 ± 1.5	4.8 ± 3.9	8.9 ± 4.0
Transverse	5.6 ± 2.9	—	—	11.3 ± 3.2