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PERMEABILITY ASSESSMENT

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7.1 INTRODUCTION

Drug discovery is a lengthy and costly process with a very low probability of success. Approximately 50% of the investigational new drugs (INDs) fail in preclinical and

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clinical phases of the drug development process. The major reasons for such a high proportion of failures are poor biopharmaceutical properties, including low aqueous solubility, inadequate intestinal permeability, chemical instability, intestinal or hepatic metabolism, and systemic clearance. The advent of combinatorial chemistry and high throughput screening (HTS) has allowed the synthesis of thousands of compounds a day, leading to the rapid identification of therapeutically active compounds (i.e., hits and leads). Despite this dramatic increase in the speed of synthesis and screening, the number of drugs reaching the market has not increased in similar proportions.

However, the success rate is beginning to rise with the increase in availability of many *in vitro* screening techniques, which can be used for early prediction of new chemical entity (NCE) permeability through biological barriers and other physicochemical properties. It helps medicinal chemists to design compounds that exhibit the right pharmaceutical characteristics and avoid wasting valuable resources on developing molecules that are not likely to become successful drugs.

Drug permeability is considered an important parameter in drug discovery and lead optimisation; it is essential to have reliable methods of predicting the *in vivo* permeability by thoughtful use of *in vitro* permeability models.

7.2 NATURE AND PERMEABILITY OF SOME PHYSIOLOGICAL BARRIERS

The main physiological permeation barriers to be crossed by drugs are epithelia and endothelia. Epithelia cover the surface of the body and line various cavities. Endothelia line the blood capillaries so as to regulate the distribution of compounds between the blood and the interstitial fluids. Despite their extensive biochemical differences, they serve as highly selective permeability barriers, separating internal and external environments. Oral bioavailability is a highly desirable property for molecules under investigation in drug discovery, because approximately 90% of all marketed drugs are administered orally. The principal physiological barrier that drugs have to cross to enter the systemic circulation is the gastrointestinal mucosa. Alternatively, drugs can also be absorbed through the skin, cornea, and buccal, sublingual, nasal, vaginal, or rectal mucosa. Some of these routes have been explored as alternative paths for the delivery of drugs that are inactivated by first-pass metabolism. Another important physiological barrier is the blood–brain barrier (BBB), which separates the blood from the central nervous system. Due the difference in anatomical structure of the epithelia throughout the body, different drug application routes are impeded by different barriers.

7.3 DRUG TRANSFER PROCESS THROUGH A PHYSIOLOGICAL MEMBRANE

According to the *fluid mosaic model*, the structure of the cellular membrane is described as an interrupted phospholipid bilayer capable of both hydrophilic and hydrophobic interactions [1]. The membrane model for the understanding of relevant drug transport processes across the biological membranes is shown in Fig. 7.1.

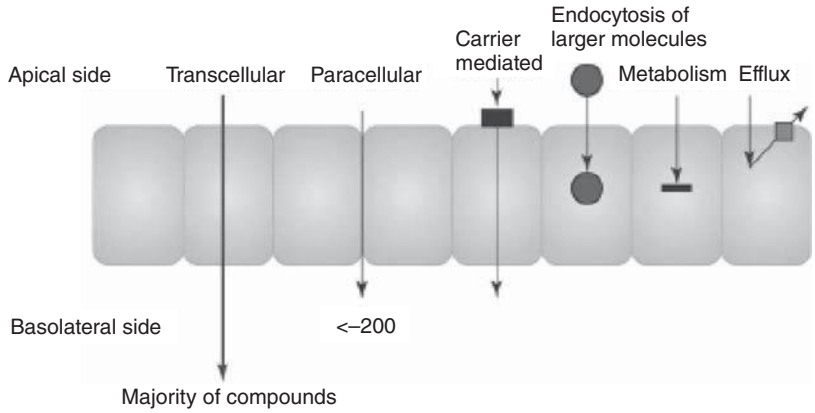

FIGURE 7.1 Different routes of drug entry from the intestine into the bloodstream. (From Ref. 52, with permission from Elsevier.)

1. The majority of the lipophilic drugs are absorbed via passive diffusion across the cellular membrane—transcellular transport [2].
2. Transcellular passage is the main route for both intestinal epithelium and BBB.
3. The hydrophilic molecules (molecular weight or MW < 200) passes through the water-filled tight junctions formed by fusion of adjacent cells—paracellular transport [3].
4. The MW cutoff for paracellular transport may be 400–500 [4]. However, paracellular permeation is negligible in brain endothelium.
5. Drug molecules transported against concentration gradient by active energy consuming transporter proteins, P-glycoprotein (P-gp)—carrier mediated transport [5].
6. Some carriers can permeate in the direction of the concentration gradient without consumption of energy—facilitated transport.
7. The high MW compounds (e.g., peptides and proteins) usually take another transcellular route—endocytosis or transcytosis [6].

7.4 PHYSICOCHEMICAL PROPERTIES DETERMINING MEMBRANE PERMEATION

The tenets of molecular properties influence drug permeability through various physiological membranes. The relevant physicochemical descriptors for membrane permeability, and their interrelationships, are presented in Fig. 7.2 [7].

7.4.1 Lipophilicity

Because of the lipid nature of cell membranes, a molecule's lipophilicity has long been considered an important factor in drug design. The most common expression of lipophilicity is the logarithm of the partition coefficient (log P). Since the

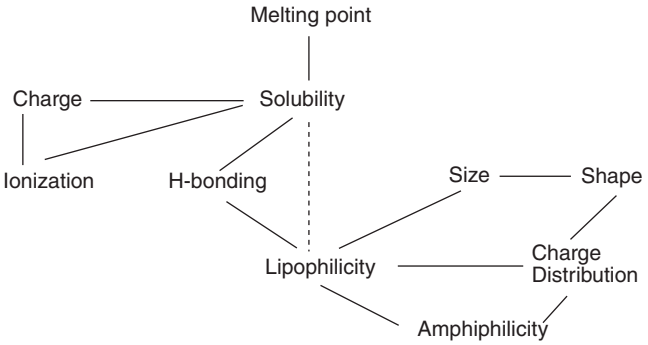

FIGURE 7.2 Physicochemical properties influencing drug permeability. (From van de Waterbeemd H. *Eur J Pharm Sci* 1993; 7, with permission from Elsevier.)

membranes are primarily lipophilic in nature and with the lipid domain playing an important role in their barrier function, the relationship between log *P* and permeability is nonlinear, with a decrease in permeability at both low and high log *P* values. These nonlinearities are theorized to be due to the limited diffusion of poorly lipophilic molecules into the lipophilic cell membrane, or the preferential partitioning of highly lipophilic molecules into the phospholipid cell membrane, preventing passage through the aqueous portion of the membrane [8, 9].

7.4.2 Molecular Size

Molecular size is believed to play a distinct role in permeation processes and can be a further limiting factor in oral drug absorption. The paracellular transport highly depends on the molecular size due to the *sieving effect*, the molecular weight limit for the paracellular permeation being approximately 400–500 [4]. The bigger the molecule is, the harder it is to diffuse. Diffusion coefficients across biological membranes have been shown to be highly dependent on molecular mass. It has been concluded that the permeation of drugs with MW < 300 Da is not significantly influenced by the physicochemical properties of the drug, which will mostly permeate through aqueous channels of the membrane. By contrast, the rate of permeation is highly sensitive to molecular size for compounds with MW > 300 Da. The Lipinski rule of five proposes an upper MW limit of 500 as being the limit for orally absorbed compounds [10].

7.4.3 Hydrogen Bonding Property

The hydrogen bonding ability (an estimate of hydrophilicity) of a molecule is an important property for cellular membrane permeability [11]. Conradi et al. [3] believe that the relative contributions of both lipophilicity and hydrogen bonding must be considered, as opposed to hydrophobicity alone. Lipids within membranes contain hydrophilic parts that have hydrogen bonding acceptor groups. These groups bond to the hydrogen bond donating solutes, preventing the solutes from penetrating the membranes and slowing down the diffusion process [12].

7.4.4 pH Partition (pK_a)

The majority of drugs in use are either weak acids or bases and, depending on the pH, exist in an ionized or un-ionized form. Membranes are more permeable to un-ionized forms of drugs than to ionized species due to the greater lipid solubility of the un-ionized forms and the highly charged nature of the cell membranes. However, Palm et al. [13] reported that the molecules with an un-ionized fraction of <10%, a common state for many drugs in the intestinal pH range, were permeable across Caco-2 cell membranes. As discussed by these authors, a variety of examples exist where ionized molecules are permeable, deviating from classical pH partition theory.

7.4.5 Solubility

Solubility is a major factor in determining drug absorption through physiological membranes. Poor aqueous solubility is likely to result in poor absorption, since the drug diffusing through intestinal membrane is proportional to its concentration gradient between the lumen of the intestine and blood. Therefore, even drugs with high permeation rate show a low absorption. Conversely, the highly aqueous soluble drugs are well absorbed despite low or moderate permeation.

7.5 BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS) IN DRUG PRODUCT OPTIMIZATION

The biopharmaceutics characteristics like solubility and permeability are vital in drug discovery and lead optimization due to the dependence of drug absorption on these two properties. The BCS is derived based on these two main properties [14–17]. By knowing a drug solubility and intestinal permeability, the BCS classifies drugs into one of the four categories (Fig. 7.3).

Class I drugs are highly soluble and highly permeable. Drug discovery programs are targeted to achieve Class I drugs, which are ideal candidates for oral delivery. Class II drugs are highly permeable across the gut by virtue of their high lipophilic-

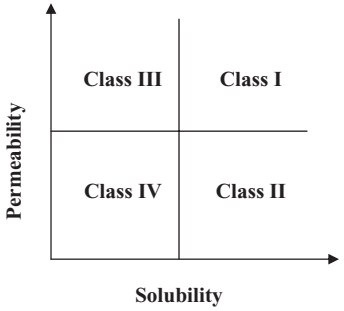

FIGURE 7.3 Model depicting BCS. Drugs with high solubility and high permeability are grouped into Class I; low solubility and high permeability into Class II; high solubility and low permeability into Class III; and low solubility and low permeability into Class IV.

ity. The bioavailability of products containing these compounds is likely to be dissolution rate limited due to low solubility. However, as drug dissolution is the rate-limiting step, the *in vitro* permeability data obtained from different experimental models may not accurately predict the *in vivo* absorption. Class III drugs are highly soluble but have less intrinsic permeability due to their physicochemical properties. For this reason dissolution is very rapid but absorption is permeability rate limited. Class IV drugs exhibit low solubility and low permeability. Low and variable oral bioavailability for these drugs is anticipated because of the combined limitation of solubility and permeability. Strategies to improve both solubility and permeability should be established for these molecules.

The BCS is considerated by the U.S. Food and Drug Administration (FDA) as a fundamental principle to define the rate and extent of absorption of a drug product. It has published several guidance based on the BCS covering scale-up and postapproval changes [18] and has allowed waiver of *in vivo* bioequivalence studies [19]. However, accurate use of permeability methods to measure the drug permeability is a critical component for this classification. The scientific aspects of the BCS have been extensively discussed and have been the subject of numerous studies [20, 21].

7.6 EXPERIMENTAL MODELS FOR THE EVALUATION OF DRUG PERMEATION

The need to screen drugs for their absorption characteristics has increased significantly in recent years due to use of techniques like combinatorial chemistry. An ideal screening tool should be fast and easy to use, require small amounts of the compound, be relatively inexpensive, and give reliable predictions. In practice, such ideal testing methods are not easy to develop. While evaluating the usefulness of these different methods in drug discovery, it is important to know exactly what each technique measures and how this can help identify potentially successful or unsuccessful compounds. In the following section, we look at key techniques for evaluating and predicting the drug permeability and critically assess their advantages and limitations in drug discovery and development.

7.6.1 Predicting Drug Permeability from Physicochemical Parameters

During drug development, laboratories might use computational approaches to predict the absorption potential of new chemical entities. These computational methods are based on lipophilicity, hydrogen bonding, and molecular size. It has long been recognized that these physicochemical descriptors are related to membrane permeability [22], although this relationship is often obscured when structurally different compounds are studied [23].

***Lipophilicity: log* P *and log* D** Drug lipophilicity is commonly used as a predictor of membrane permeability because transcellular permeation is dependent on the lipophilicity of the compound. The molecule requires a certain affinity for the phospholipid structure in order to enter the cell membrane [24]. Fick's first law of diffusion describes the passive transport of the drug across the membrane as being

TABLE 7.1 Computer Programs for Log *P* Calculations

Computer Program	Software Package/Vendor
PrologP and Prolog P	PALLAS/CompuDrug Chemistry Ltd.
ACD/logP and logD	Version 3.0/Advanced Chemistry Development Inc.
ALOGP	Tsar 3.1/Oxford Molecular Group
CLIP/logP	University of Lausanne
CLOGP	Pcmodels/Daylight CIS, CLOGP/Biobyte
HINT/logP	Edusoft
KLOGP	Multicase Inc.
LISP	O. Raevsky
LOGKOW	Syracuse Research Corp.
SCILOGP	SCILOGP/Scivision
TLOGP	TLOGP 1.0/Upstream Solutions
XLOGP	XLOGP V 2.0/Institute of Physical Chemistry, Peking University
ALOGPS	ALOGPS 2.1/Virtual Computational Chemistry Laboratory
MLOGP	DRAGON 3.0

proportional to the membrane–water partition coefficient. These coefficients are conventionally expressed as the *n*-octanol–water partition coefficient (log *P*), which is the concentration ratio of the compound between *n*-octanol and aqueous phase at equilibrium. The distribution coefficient *D* (log *D*) is pH dependent in the case of ionizable compounds.

The log *P* and log *D* are traditionally determined by the shake flask method using an *n*-octanol–water biphasic system. This has been one of the most suitable models of the lipidic biological membranes, due to the resemblance of *n*-octanol to lipids—with its long alkyl chain and the polar hydroxyl group. However, the log *P* and log *D* values for a single compound can vary as a result of different experimental conditions [25]. Both log *P* and log *D* are used for prediction of drug absorption, which can subsequently be measured by more sophisticated and standardized techniques like potentiometer titration of the compound in the biphasic system [26].

In the drug development program, an early estimate of log *P* and log *D* values can be determined by computational approaches. Many computational approaches have been developed to estimate log *P* [27, 28], including the popular *C* log *P* [29] and *A* log *P* [30] algorithms (Table 7.1). These approaches can calculate log *P* values using software programs and are based on data for either atomic contributions or molecular fragments, or on molecular properties such as the molecular lipophilicity potential (MLP) [31], molar volume, and hydrogen bonding.

Liposomal Partitioning Liposomes are lipid bilayer vesicles that can be used as membrane models in partition studies [32] with the potentiometric titration method [33]. The phosphatidylcholine liposomes seem to provide a more accurate partition system for absorption prediction than the *n*-octanol–buffer system. Beigi et al. [34] demonstrated a chromatographic technique for partition studies in liposomal systems. Liposomes of different lipid composition were immobilized in small agarose–dextran gel beads and packed into chromatography columns. The capacity factor of 12 compounds related well to their fraction absorbed in humans.

Chromatographic Methods The elution times in chromatography represent the partitioning behavior of the compound between stationary and mobile phases. Chong et al. [35] introduced immobilized artificial membrane (IAM) columns containing phosphatidylcholine or similar lipid-like ligands covalently bonded to silica particles. This lipid monolayer is similar to the lipid membrane in partition studies. This high performance liquid chromatography (HPLC) technique can be used to study the partition of a solute. In spite of the end capping, the solutes also interact with uncapped silanol groups, in addition to the lipid ligands. Once this problem is solved, IAM columns are likely to provide a good tool for absorption prediction.

Hydrogen Bonding Hydrogen bonding can be measured experimentally by allowing a compound to distribute between two solvents, of which only one can form hydrogen bonds. This is expressed as $\Delta \log P$, which is the difference between the *n*-octanol–water $\log P$ and an alkane–water $\log P$ [36], where *n*-octanol is the solvent that permits hydrogen bonding. $\Delta \log P$ has shown good correlation with membrane permeability in numerous studies. El Tayar et al. [37] found correlation of $\Delta \log P$ with skin absorption. $\Delta \log P$ was also compared with brain permeability. Optimal permeability was predicted for $\log D$ between 0 and 3 and $\Delta \log P < 2$ [38]. The main shortcoming of the $\Delta \log P$ method is that it requires two experimental determinations for each compound, since *n*-octanol is miscible with alkane and it is not possible to measure $\Delta \log P$ directly.

For reasonable prediction of membrane permeability, the hydrogen bonding capacity is calculated by counting the number of hydrogen bond donor atoms (hydrogens) attached to oxygen and nitrogen and hydrogen bond acceptor atoms (oxygens and nitrogens) [10], or counting the number of lone pair electrons [39]. Recently, more accurate methods of calculating hydrogen bonding capacity have been introduced. These include MolSurf [40], which calculates hydrogen bonding properties based on quantum mechanics, and Hybot [41], which is based on experimental results for the formation of complexes between hydrogen bond donors and acceptors.

Molecular Size As a molecular size descriptor, often the molecular weight is used in predicting the drug permeability. The paracellular permeability of drug compounds was successfully modeled based on MW as discussed earlier. Molecular size is not only a component of lipophilicity, but also of the diffusion coefficient ($\log D$) in physiological membranes. A rather strong dependence on molecular size has been observed for transcellular diffusion in physiological membranes. Camenisch et al. [42] reported that the physiological membrane permeability of high MW compounds is overestimated when using $\log D$ as a predictor. This effect is important for compounds with MW > 500. For compounds that have similar $\log D$ values, Caco-2 permeabilities were lower for high MW compounds as compared with compounds of lower MW. Camenisch et al. [2] reviewed suggestions for the correction of partition coefficients with MW functions that were made by several authors. In the case of BBB penetration, a correlation between logarithm of the BBB permeation and logarithm of MW also existed [43].

Polar Surface Area (PSA) The polar surface area (PSA) is the molecular surface area associated with hydrogen bonding acceptor atoms (i.e., oxygen and nitrogen)

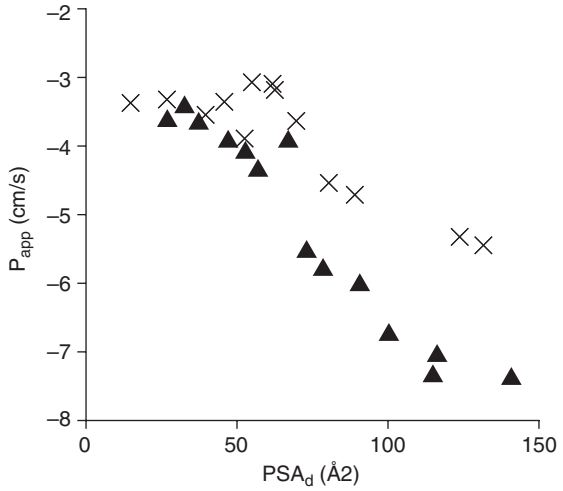

FIGURE 7.4 Relationship between dynamic polar surface area and drug permeability in perfused human jejunum (×) and in Caco-2 cell monolayers (▲). (From Ref. 46, with permission from Elsevier.)

plus the area of the hydrogen atoms attached to these heteroatoms—which are known as hydrogen donors. This model has been introduced to account for the neglected intramolecular hydrogen bond formation [44]. The dynamic polar surface area (PSAd) is the statistical average of the PSA of the low energy conformations of a molecule. The areas were calculated for an aqueous environment using atomic van der Waals radii. The smaller the PSAd of a compound, the better it permeates the biological barrier. Good inverse linear correlation was observed between the permeability across a Caco-2 model and the water-accessible PSA of six β-blockers and their ester prodrugs. Figure 7.4 shows the PSAd correlated to permeability in the human jejunum for 13 structurally different drugs investigated by Winiwarter et al. [45]. A comparison is also seen from the Caco-2 cell monolayer permeabilities for 14 model compounds presented by Stenberg et al. [46].

The long calculation time for the low energy conformations of large and flexible molecules is a limitation with this model. Stenberg et al. [46] noted that the relationship may break if the data for drugs that undergo intestinal metabolism or active transport, or have solubility problems, is included in the calculations. Therefore, integration of all these events into a computational model is vital.

Lipinski's Rule of Five Approach In a common computational approach for absorption and permeability, Lipinski et al. [10] introduced the simple "rule of five" approach for the quick evaluation of new chemical entities. This model states that absorption or permeation of a compound is more likely to be poor when the calculated MW > 500, log P > 5, hydrogen bond donors > 5, and hydrogen bond acceptors > 10. The numbers of hydrogen bond donors and acceptors are simply calculated by the counting method described. If any two of these criteria are met, poor absorption or permeability is to be anticipated. This rule is based on a variety of calculated properties among several thousand drugs belonging to different therapeutic

categories; therefore, certain drugs falling outside the rule, for example, orally active drugs like antibiotics, antifungals, vitamins, and cardiac glycosides, violate the rule of five. These drugs have structural similarities that allow them to act as substrates for biological transporters; therefore, they are outside the rule of five approach.

7.6.2 Cell Culture Based Models for Permeability Study

Caco-2 Cell Model The Caco-2 cell model is a well characterized and widely used *in vitro* model in the field of drug permeability studies [6, 47]. It has been considered the gold standard technique and has been used to standardize other absorption assessment methods [48, 49]. Caco-2 cells are grown on a filter support in a multiple well format and permeability is measured by the movement of molecules from one side of the cell monolayer to the other (Fig. 7.5).

Caco-2 cells derived from a human colon adenocarcinoma exhibit many *in vivo* intestinal cell characteristics by having tight intercellular junctions and microvilli and expressing intestinal enzymes and transporters (e.g., P-gp). Due to these similarities, the permeation characteristics of drugs across Caco-2 cell lines correlate with their human intestinal permeation characteristics; therefore, it has been recommended that the Caco-2 model can be used to predict the absorption of drugs in humans. An essential component of studies using Caco-2 cells is the culture of intact monolayer on permeable supports and the assessment of their integrity or cell damage either by measuring transepithelial electrical resistance or by using a paracellular flux marker such as mannitol [49]. Since this model is quicker to use, more convenient, and produces more reproducible data than animal studies, its use in the early stages of drug development can lead to savings in time and money and avoid the unnecessary use of experimental animals. Moreover, Caco-2 assays are relatively accessible and easier for higher throughput than the excised tissue assays.

Many drugs are absorbed by passive diffusion; therefore, physiochemical descriptors of the drugs are expected to play a vital role in this process. Caco-2 cell permeability data have frequently been plotted against log P or log D, molecular weight, or some descriptor for H bonding [44]. The sigmoidal relationship has been identified between the hydrogen bonding properties of the solutes and permeability coefficients through Caco-2 cell monolayers [50].

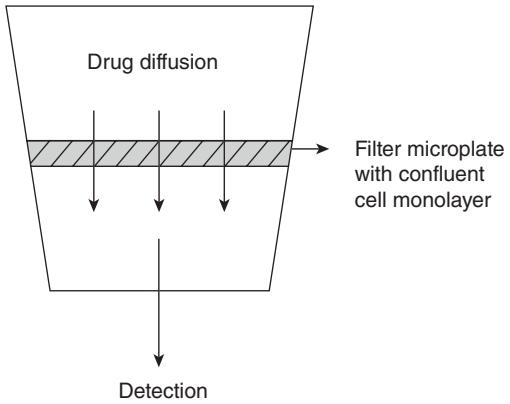

FIGURE 7.5 Caco-2 cell line model.

Although, Caco-2 cells can give evidence that a compound will be well absorbed, it is difficult to predict the compounds showing low or modest permeability. Other complications with Caco-2 cells include (1) variable expression of P-gp, metabolizing enzymes, and active transport systems [35, 51]; (2) interexperimental and interlaboratory variability, such as genetic change and sensitivity to differing culture conditions and protocols [49]; and (3) Caco-2 culture is laborious and costly—around 3–21 days are required to generate a confluent layer of cells [52].

Madin–Darby Canine Kidney (MDCK) Cell Model Apart from Caco-2 cells, the other models that are most frequently used for permeability studies are MDCK and LLC-PK1 cells. When the MDCK cells are cultured under standard conditions, they differentiate into polarized columnar epithelial cells and form tight cellular junctions. The main advantage of MDCK cells is shorter culture times, which can be equal to 24 hours. A good correlation was reported between permeation of passively absorbed drugs in Caco-2 and MDCK cells [53]. The permeability coefficients of hydrophilic compounds are usually lower in Caco-2 cells than in MDCK cells. Whereas Caco-2 cells originate from human colon adenocarcinoma cells, MDCK cells are from dog kidney cells, and thus the expression levels of intestinal transporters would be different in these two cell lines [54]. Hence, the MDCK model has to be extensively characterized to confirm the correlation of transport mediated drug permeability to human absorption.

Lewis Lung Carcinoma–Porcine Kidney (LLC-PK1) Cell Model LLC-PK1 cells are derived from normal pig kidneys. When cultured, these cells rapidly develop into a well formed monolayer with bush boarders and microvilli on their apical cell surface [55]. Several investigators have reported the use of these cells in passive cellular diffusion [56, 57]. As with MDCK cells, these cells also possess different transporters on their surface. A study has shown that uracil was transported by sodium-dependent and -independent pathways in LLC-PK1 cells; on the other hand, in Caco-2 cells uracil was transported independently of sodium pathway [18]. These differences in the transport mechanism can be attributed to differences in the origin of the cells types and the transporters that are expressed on these cells. Thus, further studies are warranted to determine the correlation between the LLC-PK1 cells and *in vivo* human absorption.

HT29 Cell Model The role of mucus on drug permeability is largely ignored, since Caco-2 cells are a widely used model in permeability studies and they are devoid of this property. Certain clones of HT29 cells produce mucus. The wild-type HT29 cells grown on media containing galactose lead to the selection of a subclone of HT29 cells that form polarized cell monolayers and secrete mucus. A study has shown that the presence of the mucus layer in HT29-H resulted in lower permeability coefficients than Caco-2 monolayers in a testosterone permeability study. This indicates that the mucus layer accounts for most of the permeability resistance to testosterone. An attempt has been made to coculture the Caco-2 cells with HT29-MTX or HT29-H cells [58] in order to provide representative characteristics of intestinal mucosa.

The major application of the cell culture based model is screening of chemical libraries for compounds that have favorable permeability characteristics. Caco-2

TABLE 7.2 Cell Culture Models Used for Permeability Assessment

Cells	Origin	Special Characteristics
Caco-2	Human colon adenocarcinoma	Well characterized and widely used Express some relevant efflux transporters; expression of influx transporters is variable
MDCK	Dog kidney epithelial cells	Polarized cells with low expression of ABC transporters, ideal for transfection
LLC-PK1	Pig kidney epithelial cells	Polarized cells with low intrinsic transport expression, ideal for transfection
HT-29	Human colon	Contains mucus-producing goblet cells
TC-7	Caco-2 subclone	Similar to Caco-2
IEC-18	Rat small intestine cells	Provides a size selective barrier for paracellularly transported compounds

cells grow very slowly in 3–21 days; this time is too long for the drug discovery industry. Other cell models (MDCK and LLC-PK1) can grow much faster and be available for studies in 5–7 days. However, recent improvements in culture media like the addition of 10% fetal bovine serum, manipulation of the seeding density, and use of BIOCOT® transwell plates can reduce the time required for cell growth. Various cell culture models used for permeability assessment are described in Table 7.2.

Data Analysis from the Cell Cultured Models The permeability coefficients (P_{eff}) through the cell monolayers are calculated from the following equation [48, 59]:

$$P_{eff} = \frac{V_A}{A(C_D - C_A)} \times \frac{dC_A}{dt} \tag{7.1}$$

where

- dC_A/dt (mg/s·mL) is the increase of drug concentration in the receiver chamber over the time period considered.
- A (cm^2) is the membrane surface area exposed to the compound.
- V_A (mL) is the solvent volume in the acceptor chamber.
- C_A and C_D (mg/mL) are the initial drug concentrations in the receiver and donor chambers, respectively.

Permeability rate (dC_A/dt) is calculated by plotting the amounts of drug measured over the linear diffusion range and determining the slope of the plot. P_{eff} from the equation is only valid under the experimental conditions, where a constant concentration gradient exists, so that backdiffusion is avoided. Under that condition, C_D is almost constant and C_A is negligible compared to C_D. In several findings, experimental conditions are usually accepted as sufficiently accurate for P_{eff} calculation, if the concentration difference between the donor and acceptor compartments does not diverge by more than 10% in the time interval studied [55].

7.6.3 Parallel Artificial Membrane Permeability Assay (PAMPA)

An alternative to the cell cultured models is the parallel artificial membrane permeability assay (PAMPA), which is a method for predicting passive permeability [60]. Recently, it has been suggested that artificial membrane methods such as PAMPA could soon replace high throughput screening Caco-2 assays [61], because PAMPA is an excellent biomimetic model, in terms of high throughput, reproducibility, and especially cost.

PAMPA is performed in a 96 micotiter well plate containing two parts. All the wells at the bottom part are filled with buffer solution. The top part contains a series of filter-immobilized artificial membranes composed of lipids, which match with the wells in the lower part. One-half of the filters on the top part are treated with an organic solvent, which supposedly acts as the cell membrane, and the other half are wetted with methanol/buffer. The test compound under investigation is applied to the top filters and the rate of appearance of the compound in the bottom wells should reflect the diffusion across the lipid layer. In a recent study by Lipinski et al. [62], a good correlation was found between diffusion in the PAMPA system and percentage absorption in humans for a selected series of compounds.

The filter immobilized artificial membranes are prepared by dispersing a phospholipid with the help of an organic solvent on a filter [60]. The various PAMPA models reported in the literature are presented in Table 7.3.

The problem of precipitation with low solubility compounds—when working at clinically relevant doses in cellular assays—is prevented in recent PAMPA models [63]. The limitations of the system include the lack of influx and efflux transporters, enzymes, and paracellular pathways.

7.6.4 Tissue Based Models for Permeability Study

Excised intestinal segments have been used to study intestinal drug absorption. The compound under investigation in a solution is applied to one side (mucosa or serosa) of the mucosa and the rate of drug absorption is determined by estimating

TABLE 7.3 Membrane Compositions of PAMPA Models

Assay	Model	Phospholipid Constituents[a]	Organic Solvent
Egg-PAMPA	Lecithin	10% Egg lecithin, cholesterol	*n*-Dodecane
BM-PAMPA	Biomimetic	0.8% PC, 0.8% PE, 0.2% PS, 0.2% PI, 1% cholesterol	1,7-Octadiene
HDM-PAMPA	Hexadecane	100% *n*-Hexadecane	*n*-hexadecane
DOPC-PAMPA	Synthetic phospholipid	2% Dioleylphosphatidyl choline	*n*-Dodecane
BBB-PAMPA	Blood–brain barrier	2% Porcine brain lipid extract (PC, PE, PS, PI, PA, cerebrosides)	*n*-Dodecane
DS-PAMPA	Double sink	20% Phospholipid mixture (PC, PE, PI, PA, triglycerides)	*n*-Dodecane

[a]PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PS, phosphatidyl serine; PI, phosphatidylinositol; PA, phosphatidic acid.

either the disappearance of drug from the drug solution or appearance of drug on the serosal side. These models preserve the characteristics of the biological membranes and also help in determining absorption across different gastrointestinal segments. Limited viability and versatility are the limitations with this type of preparation.

Everted Intestinal Segments Isolated intestinal segment is a simple technique used to measure the intestinal transport of a drug. In this model, the permeability assessment is carried out through a segment of the intestine, with the musculature intact. Commonly, rat intestine is used, although absorption in other animal models, such as monkey, also correlate well with human absorption [64]. To prepare the rings, a selected segment of the intestine is isolated immediately after euthanizing the animal, washed in ice-cold buffer to remove debris and digestive products, and tied at one end with a piece of suture. The closed end is carefully pushed through the intestine using a glass rod, resulting in eversion of the intestine, which is then cut into small rings, typically 2–4 mm wide [65]. Next, the rings are incubated in buffer at 37 °C containing the compound under investigation, and shaken well in a water bath. After a set time, the drug uptake is suppressed by rinsing the rings with ice-cold saline or buffer. The rings are then blotted dry, weighed, and then dissolved or processed for assay. Any segment of the intestine can be used to prepare a large number of rings. This model can be used to study both active and passive transport pathways especially that of amino acids and peptides [66]. A major disadvantage associated with this model is the lack of maintenance of viability in the isolated rings, due to absence of blood supply and nerves. Some other factors like drug metabolism and accumulation in the mucosa could lead to an overestimation of true drug absorption. For example, one such early study found that, for several beta-lactam antibiotics, absorption based on luminal disappearance was roughly twice the true amount transported across the tissue [67].

Everted Intestinal Sacs In this method, a 2–4 cm section of the intestine is tied off at one end and everted similar to the intestinal ring method. The sac is filled with buffer, tied off at the other end, and placed in a flask with oxygenated (95% O_2/5% CO_2) buffer solution as a test compound. Drug absorption is measured by sampling the solution inside and outside the sac. Unlike the *in vivo* situation, everted intestinal sacs only include the mucosa plus underlying muscle layers, which could lead to biased absorption values. Although this method is relatively simple and allows several experiments to be performed using tissue from just one intestine, it differs from the *in vivo* situation and drug accumulation in the muscle layer can lead to poor recovery of the drug.

Diffusion Cells Diffusion cells have been used to determine the transport of compound in living tissues, one of the first approaches being the so-called Ussing chamber. In this method, the long intestinal mucosal sheets are cut into mucosal strips of adequate size (~2 cm) and are clamped between two glass chambers filled with buffer. The compound under study is added to the donor compartment, and the accumulation of the compound at the other side of the membrane (acceptor side) is measured as a function of time. The permeability of the compound is calculated from the following equation:

$$P_{\text{eff}} = \frac{dc}{dt} \times \frac{V}{A \times C} \tag{7.2}$$

where dc/dt is the change in concentration in the receptor compartment per unit time, V is the receptor volume, and A is the area available for diffusion. P_{eff} is usually expressed in centimeters per second (cm/s). This method has been used to evaluate *in vitro* permeability with varying degrees of success. For example, intestinal mucosa of rat mounted in an Ussing chamber was useful in describing the regional variability in GIT absorption of a mixed series of compounds [68].

7.6.5 *In Situ* Model of Intestinal Drug Permeability

Single-Pass Perfusion Model Several different models of the single-pass perfusion system have been used [69–71]. In these *in situ* models the viability of the tissue is maintained, as the whole animal is used and the blood vessels and lymphatics are intact. This technique has been widely used to study both active and passive transport pathways [69, 71, 72]. In addition, the effect of various factors such as drug concentration, intestinal region, and flow rate can be studied. In the closed loop model, a solution containing the diluted drug is added to a segment of the intestine, and the intestine is closed. After a set time the intestine is excised and the drug content is analyzed [73]. Conversely, in the open loop model, the proximal and the distal end of a segment of the intestine are canulated with a glass tube. The drug is then pumped through the intestine and the ratio of drug in and out is measured.

The steady-state effective intestinal permeability coefficient (P_{eff}) is calculated according to the parallel-tube model [74]:

$$P_{\text{eff}} = \frac{-Q_{\text{in}} In(C_{\text{out}}/C_{\text{in}})}{2\pi r L} \tag{7.3}$$

where Q_{in} is the perfusion flow rate through the intestine segment (mL/s), and C_{in} and C_{out} correspond to the inlet and outlet concentrations (μg/mL), respectively. r is the radius of the intestinal loop (cm) and L is the length of the loop (cm). From the equation, it can be seen that by varying the length of the intestinal segment, the effect of transit time on drug absorption can also be studied [55, 66]. Even with these advantages, use of the single-pass perfusion model is limited because in this model the disappearance of the drug from the intestine is used to predict permeability. However, the rate of decrease in drug concentration does not always equal the rate of drug absorption; for example, drugs can interact with the lipid membrane [75]. Furthermore, the proximal and distal content of the lumen can enter the test segment of the intestine, making it difficult to control conditions such as flow rate [76].

Prediction of oral absorption in human beings is important in the early stage of the drug discovery process. *In vitro* assays in Caco-2 and MDCK models are frequently used for that purpose. However, an *in situ* technique might be more accurate in estimation of absorption. One such study [77] found that effective permeability coefficients determined in rats by single-pass intestinal perfusion for 14 compounds were correlated to the values obtained from humans.

Single-Pass Perfusion of the Dog Intestine (Loc-I-Gut) The Loc-I-Gut is a very effective model for investigating regional jejunum permeability. It is a perfusion instrument consisting of six channels and two inflatable latex balloons, which are set 10 cm apart [78]. The purpose of the balloons is to isolate the segment of the intestine that is being studied and to prevent the leakage of the proximal and distal luminal content into the selected area. It has been reported that less than 2% of the luminal content leaked into the jejunum when the balloons were inflated [76]. The tube is inserted into the proximal jejunum by using fluoroscopic techniques. Air is then pumped into the balloons, inflating them and creating a 10 cm separated jejunal segment. Insertion and setting up of the tube usually takes about an hour and the infusion rate is about 2–3 mL/min. A nonabsorbable marker such as ^{14}C-PEG 4000 is used in the perfusion solution to check the integrity of the balloons during the experiment [79]. In the dog model the drug permeability was calculated using the well-stirred model because it is assumed that the hydrodynamics are similar to the corresponding model in the human intestine. The P_{eff} can be calculated as [76]

$$P_{\text{eff}} = \frac{Q_{\text{in}}(C_{\text{in}} - C_{\text{out}})/C_{\text{out}}}{2\pi rL} \tag{7.4}$$

where Q_{in} is the perfusion flow rate, r is the radius of the tube, L is the length of the tube, C_{in} is the infused drug concentration entering the tube, and C_{out} is the diffusate concentration leaving the tube. The Loc-I-Gut model is also used for perfusion of suspended drug particles in human jejunum. In this model, the *in vivo* dissolution of the drug can be directly estimated by measuring the concentration of drug either in dissolved or in solid-state form [80]. The major limitation to this model is that it is invasive. Intubation may affect the normal physiology and function of the GI tract. Furthermore, the fluid that is used to flush out the drug from the tube may affect the absorption of the drug [81].

7.6.6 Whole Animal Models

InteliSite Capsule The InteliSite® capsule (Innovative Devices LLC, Raleight, NC) is a rapid and noninvasive technique for measuring site-specific permeability of a drug [82]. The permeability is assessed through measurement of the drug in the systematic circulation. When the capsule reaches the target site it is activated. Activation is accomplished by placing the magnetic field generator next to the subject's abdomen. This magnetic field is sensed by a receiver and is converted to heat. The heat will cause the shape memory alloys to straighten. This in turn results in the rotation of the interior sleeve in relation to the outer sleeve and the drug is released. Gamma scintigraphy and pharmacokinetics analysis are used to evaluate and measure drug absorption [81]. Pithavala et al. [81] evaluated the pharmacokinetic properties of ranitidine in eight volunteer subjects using the InteliSite capsule [81]. The result of the study correlated well with data available in the literature. Thus, it can be concluded that these capsules are a valuable tool for studying regional drug absorption.

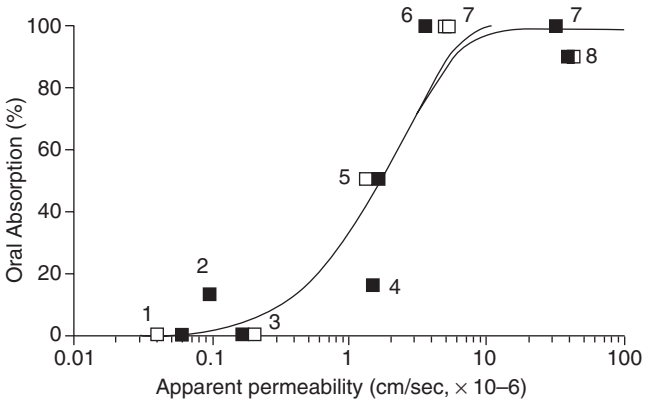

FIGURE 7.6 Correlation between the permeability coefficient (in cell culture) and fraction absorbed in human (From Ref. 83, with permission from Elsevier.)

7.7 *IN VITRO* AND *IN VIVO* CORRELATION (IVIV)

In order to use any experimental model for human permeability prediction, the drug permeability from the model has to correlate well with human absorption. To validate a permeability model, compounds with known absorption in humans are used and P_{eff} is calculated. Figure 7.6 [83] shows the general shape of the curve of fraction absorbed in human versus the *in vitro* P_{eff}. Any small change (such as the pH of the buffer or the intubation time) in the way that the techniques are carried out can effect the P_{eff} of the drug, and the curve may shift to left or right or alter its steepness. Therefore, well designed and validated techniques are to be used within laboratories to correlate the data collected.

Furthermore, in the case of animal models, there needs to be a correlation between the animal's P_{eff} and the fraction absorbed in human. It has been shown that rat and human jejunum P_{eff} of passively absorbed drugs correlate well [84]. Once the correlation is demonstrated for a series of compounds, then the experimental results can be used to make intelligent decisions about those compounds that can be further studied in animals.

7.8 CONCLUSION

Identifying appropriate models for permeation study of a new molecule is a very challenging task, but there are several options available to the pharmaceutical scientist. Careful review and selection of appropriate methods is crucial to a successful drug development program. The key feature that governs the process is *in vitro* and *in vivo* correlation in permeability studies. There is a potential to develop models that can provide good correlation.

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8

HOW AND WHERE ARE DRUGS ABSORBED?

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Contents

8.1 INTRODUCTION

A drug or a xenobiotic, in order to exert its pharmacological or toxic effects, must interact with its receptors at their action sites or therapeutic target. The essential step is therefore the absorption of the substance, that is, its entry from outside the body, unless the administration is done directly into the bloodstream. In this chapter, we review the biological barriers that the drug must cross in order to enter the body, which mechanisms are implicated in this process, and how the physiological characteristics of the administration route affect the absorption rate and extent.

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8.2 ABSORPTION IMPACT ON DRUG BIOAVAILABILITY

The terms absorption and bioavailability are often used interchangeably when there is actually a conceptual difference between them. *Bioavailability* is a measurement of the rate and extent of therapeutically active drug that reaches the systemic circulation and is available at the site of action [1, 2]. *Absorption* is the movement of drug molecules across biological barriers from the site of administration. In some cases absorption has been defined as the movement from the administration site into the systemic circulation, but this definition could be misleading if in the pathway to the blood circulation the drug crosses a metabolic organ or tissue. The systemic availability F_{sys} is the result of absorption and all possible losses before arriving at the systemic circulation:

$$F_{sys} = F_a \cdot F_m$$

where F_a is the fraction absorbed and F_m is the fraction escaping first-pass extraction. From the equation, it is easy to conclude that F_a, the fraction absorbed, represents the upper limit for systemic availability in the absence of any presystemic losses of the drug on its way to the systemic circulation. It is therefore clear, that the absorption process is relevant to the availability of the drug at the action sites.

On the other hand, it is necessary to be aware of the terminology classically applied to some administration routes: if the drug does not reach the systemic circulation, then it is considered that no absorption occurs, and therefore the administration is considered to be topical. This is the case, for instance, for ophthalmic administration or for the topical administration of drugs on the skin. The entry of drug into those tissues is called *penetration* or *permeation* but not absorption, because the drug is not intended to reach the systemic circulation. This nomenclature then leads to the conclusion that the drug is bioavailable (as it reaches its action sites) but not absorbed into the bloodstream (or does not present systemic absorption).

Throughout the chapter, the difference between absorption and bioavailability for those administration routes that involve a first pass through a metabolic organ (mainly liver) or tissue (i.e., intestinal tissue) is stressed, and the drug is considered absorbed once it is inside the body.

The next step is to analyze the factors affecting F_a by the different routes of drug administration. As described in this chapter, absorption is a transport phenomenon that can be described mechanistically, in order to identify the limiting steps. The complexity of the barriers—subcellular, cellular, or multicellular—are also addressed.

8.3 HOW: ABSORPTION MECHANISMS

The complexity and number of barriers that the drug has to cross before reaching the bloodstream differs from one route of administration to other, but in all the cases the barriers are integrated by semipermeable cell membranes. They are composed of a bimolecular lipid matrix, containing mostly cholesterol and phospholipids. The lipids provide stability to the membrane and determine its permeability

characteristics. Globular proteins, of various sizes and composition, embedded in the matrix are involved in transport and function as receptors for cellular regulation. Drugs may cross a biologic barrier by passive diffusion, facilitated passive diffusion, active transport, or endocytosis.

8.3.1 Passive Diffusion

Passive diffusion involves the movement of drug molecules down a concentration or electrochemical gradient without the expenditure of energy. Passive diffusion does not involve a carrier, is not saturable, and shows a low structural specificity [3]. Passive absorption of drug can be done by two pathways: paracellular and transcellular.

A paracellular pathway consists of the diffusion of xenobiotics across the intercellular spaces or tight junctions, aqueous pores, or fenestrae [4–6]. These junctions are more or less leaky, depending on the tissue, and allow the diffusion of water and small solutes. In general, the main restriction for using this route is the molecular size followed by the electric repulsion between the ionized groups of the molecules and the ionized groups in the constituents of the aqueous pathways [7–9].

From a mathematical point of view, paracellular diffusion follows the same diffusion law that is applied to transcellular diffusion. The passive movement of molecules down a concentration gradient is described by Fick's law. The process is depicted in Fig. 8.1.

$$J = \frac{1}{A} \cdot \frac{dM}{dt} = P \cdot \Delta C = \frac{D \cdot (K_1 \cdot C_{\text{aq-outside}} - K_2 \cdot C_{\text{aq-inside}})}{h}$$

where J represents the net flux, that is, the net rate of diffusion through the membrane surface (g/(cm^2·s)); P is the permeability coefficient (cm/s); D is the diffusion coefficient of the solute in the membrane (cm^2/s); ΔC is the concentration gradient across the membrane (g/cm^3); and K_1 and K_2 are the partition coefficients of the solute in the membrane (at both sides).

As can be seen from this equation, there are several factors that affect the diffusion of the molecules, such as lipophilicity, pH/pK_a, size, and area of the absorptive

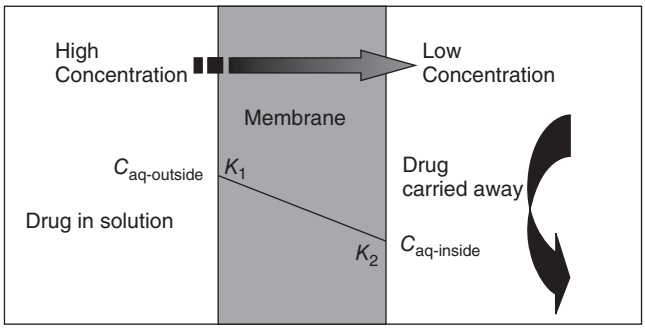

FIGURE 8.1 Scheme of the process of passive diffusion across biological membranes. The driving force for diffusion is the concentration gradient. The partition coefficient of the xenobiotic in the lipid bilayer is one of the main determinants of resistance to transport.

surface. Diffusion coefficients of drugs in the membrane increase with drug lipophilicity and decrease with molecular weight. On the other hand, it is the nonionized fraction of the molecules that mostly contributes to the overall transport, as the nonionized fraction has the highest partition coefficient.

The Henderson-Hasselbalch equation can be used to calculate the relative proportions of the ionized and nonionized forms of the drug [10]:

$$\text{For acids:} \quad \%\ \text{ionized} = \frac{100}{1+10^{(pK_a - pH)}}$$

$$\text{For bases:} \quad \%\ \text{ionized} = \frac{100}{1+10^{(pH - pK_a)}}$$

In other words, mass diffusion per unit area of a weakly acid drug will be higher from a more acid medium while mass diffusion per unit area of a weakly basic drug will be favored from a more alkaline medium.

A second consequence of the solute ionization in biological fluids is *ion trapping* phenomenon. When the pH of the fluids is different on both sides of the membrane, the solute will be trapped on the side favoring its ionization. A similar phenomenon is produced by the binding to macromolecules, for instance, proteins, as only the free drug is able to diffuse across the membrane.

8.3.2 Carrier Mediated

In addition to the passive diffusional processes over lipid membranes or between cells, substances can be transferred through the lipid phase of biological membranes through specialized systems, that is, active transport and facilitated diffusion [11].

Facilitated Diffusion Facilitated diffusion involves the participation of a carrier molecule, so this mechanism also shows selectivity and saturation, but the transport occurs always from a higher concentration to a lower concentration area and thus it does not require any energy source [12].

One theory is that a carrier component combines reversibly with the substrate molecule at the cell membrane exterior, and the carrier–substrate complex diffuses rapidly across the membrane, releasing the substrate at the interior surface. The carrier transports only substrates with a relatively specific molecular configuration, and the process is limited by the availability of carriers.

Active Transport Active transport of a xenobiotic is an energy requiring process that is saturable (i.e., is limited by the number of protein transporters present) and could proceed against a concentration gradient. Active transport can be affected by the presence of inhibitors [12]. There are two kinds of active transporters: primary active transporters use energy coming from the hydrolysis of ATP; while secondary active transporters use the energy coming from some electrochemical gradient generated by a primary transporter, for instance, a difference in H^+ or Na^+ ion concentration. Symporters transport the drug and the cotransported molecule or ion in the same direction, while antiporters transport the drug and the ion in opposite directions.

The active transport process can be described in mathematical terms using the Michaelis–Menten equation, as the binding of the solute to the carrier molecule and its translocation to the other side of the membrane have several similarities to the sustrate–enzyme interaction:

$$J_{\text{active}} = \frac{dM}{A \cdot dt} = \frac{V_{\max} \cdot C}{K_{\text{m}} + C}$$

where $V_{\max}$ is the maximal transport velocity (mass/(area·time)), C is the drug concentration, and K_{m} is the drug concentration at which velocity of transport is $\frac{1}{2} V_{\max}$.

When $C >> K_{\text{m}}$,

$$J \simeq \frac{V_{\max} \cdot C}{C} \simeq V_{\max}$$

and the rate of the transport process becomes independent of the concentration. But when the drug concentration is much lower than K_{m} (i.e., $K_{\text{m}} >> C$), then

$$J \simeq \frac{V_{\max}}{K_{\text{m}}} \cdot C$$

and the transport process follows an apparent first order kinetic.

When a drug is able to be absorbed by passive diffusion and active transport, the total flux is the sum of the passive and active fluxes, as can be seen in Fig. 8.2.

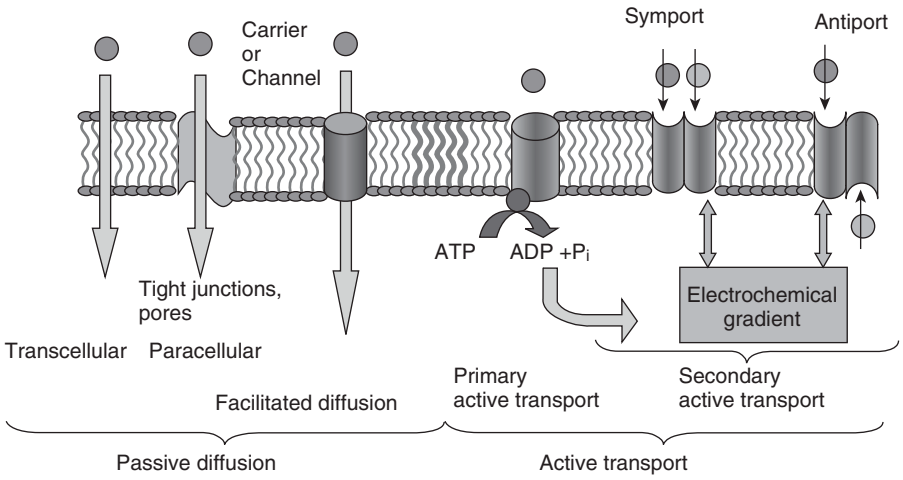

FIGURE 8.2 Transport flux as a function of drug concentration for a substance transported by passive diffusion, active transport, or a combination of both processes.

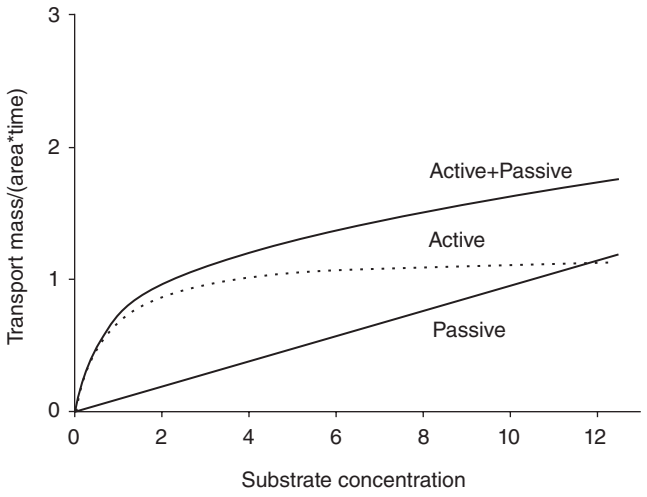

FIGURE 8.3 Summary of the transport mechanisms that xenobiotics use for crossing biological membranes (with the exception of endocytosis).

8.3.3 Endocytosis

Endocytosis is also called vesicle mediated transport. The molecules or particles are engulfed by a cell. The cell membrane invaginates, encloses the fluid or particles, then fuses again, forming a vesicle that later transports them across the membrane to the inside of the cell. This mechanism also requires energy expenditure. This diffusion is dependent on the size of the pore and the molecular weight of the chemical. Phagocytosis is the type of endocytosis where an entire cell is engulfed. Pinocytosis is when the external fluid is engulfed. Receptor-mediated endocytosis occurs when the material to be transported binds to certain specific molecules in the membrane. Examples include the transport of insulin and cholesterol into animal cells. Endocytosis probably plays a minor role in drug absorption, except for protein drugs.

In summary, chemicals can be introduced into the body by various mechanisms that are represented in Fig. 8.3. Once the drug is in the systemic circulation it has access to its target tissue or receptors by using some of the aforementioned mechanisms.

8.4 WHERE

8.4.1 Absorption Routes: Enteral Versus Parenteral

A tablet inside our stomach is, technically, outside the body. This image helps to define the two main groups of administration routes. Anything that is administered in the intestinal tract is using the enteral route (from *enteron* = intestine). By exclusion, all the other administration routes are defined as parenteral (*par* = outside). Enteral routes include buccal and sublingual, rectal, and oral. Among the parenteral routes we can distinguish those that require a needle to introduce the drug to the

depot site (e.g., subcutaneous or intramuscular routes) and those using other mucosal surfaces or epithelial barriers (e.g., nasal, ophthalmic, or transpulmonary).

Selection of the route of administration involves the evaluation of many different factors, from the physicochemical characteristics of the drug, which determine its ability to cross the barriers, to the pharmacological action, which could require a fast absorption rate to assure a quick onset of response. Chronic therapies require ease of use and patient friendly routes. Lastly, the existence of the appropriate technology could limit the use of an administration route if a suitable dosage form is not in place.

8.4.2 Routes of Drug Administration and Physiological Variables Affecting Xenobiotic Absorption

A prerequisite to absorption by any administration route is drug dissolution. Any solid drug products (e.g., tablets) have to disintegrate and deaggregate in order to release the drug particles and to allow drug dissolution. The solubility of the drug in biological fluids at the administration site determines the gradient for diffusion, while permeability in the membrane establishes the resistance to the transport. For most administration routes these two factors—drug solubility and drug membrane permeability—are the main factors governing drug absorption.

Enteral Routes Enteral absorption routes comprise all those involving any part of the gastrointestinal tract, that is, buccal and sublingual, rectal, and the so-called oral route that requires swallowing the substance, and its access to the intestine.

Oral The oral route of administration is the most used and convenient for patients. The gastrointestinal tract is a complex environment from the standpoint of drug absorption; therefore, a deep understanding of gastrointestinal physiology is essential to determine how and where drugs and xenobiotics are absorbed from the oral route [13, 14]. The main physiological factors that must be considered to understand nutrients and drug absorption are summarized in Table 8.1 and are discussed in this section.

The gastrointestinal system includes the gastrointestinal tract and all the organs that secrete substances into it. The main functions are digestion, secretion, absorption, and motility [15]. The gastrointestinal tract begins in the mouth and continues

TABLE 8.1 Physiological Factors Affecting Drug Absorption from the Gastrointestinal Tract [17]

Membrane permeability
Luminal pH
Intestinal secretions presence of food
Surface area
Transit time
Disease state
Water fluxes
Mucus and unstirred water layer (UWL)

with the esophagus, which is the pathway to the stomach. The stomach is a sac-like organ with a quite acidic environment (pH ranging from 2 in fasted state to 5 or 6 in fed state) thanks to the secretion of hydrochloric acid by parietal cells. This acidic environment constitutes the first line of immune protection of the gastrointestinal tract and, on the other hand, is the first barrier against absorption, as many substances are not stable in this environment.

Digestion's final stages and most absorption occur in the next section: the small intestine. The small intestine is divided into three segments. The initial short one, the duodenum, receives the secretions of the pancreas and liver through a common duct. The pH of this segment ranges from 5 near the stomach to 7 at the final portion. The jejunum has a thicker and more vascular wall and along with the duodenum is the site where most digestion and absorption occur, so actually the small intestine has a big functional reserve. The last portion, the ileum, is the longest segment, a little narrower in diameter and with a pH rather constant at 6.5–7. Only a small portion of water, salts, and undigested material reach the large intestine. The large intestine has three parts: cecum, colon, and rectum. Between the small and large intestines there is a valve that prevents the material from going back. The large intestine has some enzymes, which are able to metabolize some drugs, along with a big population of bacteria that contribute to the fermentation of undigested residues. The large intestine is responsible for absorbing water and electrolytes and for the formation, storage, and evacuation of feces [13].

There is extensive folding on the intestinal surface. There are folds extending from the surface of the geometrical cylinder. In the surface of the folds, there are finger-like projections called villi. Villi contain absorptive cells and goblet cells. The absorptive cells are characterized by the small projections of their apical membranes that are called brush border or microvilli. The apical membrane is highly specialized and contains enzymes, binding sites, and transporters. Other important features are the tight junctions, which play a major role in regulating paracellular permeability of water and solutes. Permeability of junctional complexes is higher in the upper intestine than in the ileum.

Figure 8.4 shows how the combination of folds, villi, and microvilli increases the surface available for absorption about 600 times [15]. Each villus is occupied by a blind-ended lymphatic vessel (called a lacteal) and by a capillary network, whose drainage leads to the hepatic portal vein. That means that any substance absorbed in the intestinal epithelium must cross the liver before arriving in the systemic circulation. During this first passage through the liver, the nutrients, xenobiotics, and drugs can be metabolized, reducing the fraction of substance that finally arrives in the systemic circulation. This is the *first-pass effect* whose effect on bioavailability is analyzed later. On the other hand, during the passage through the liver the drug or xenobiotic could be excreted into the bile and again reach the small intestine, where it is again absorbed. This process is called enterohepatic circulation and could affect the disposition of the compound.

The large intestine has folds but no villi. The smaller surface area available for absorption and the smaller amount of fluid present in the large intestine are reflected, in general, in a lower permeability for xenobiotics; but, on the other hand, the longer residence time in this area may compensate for the lower permeability and makes the large intestine a suitable target area for prolonged release drug formulations [16, 17].

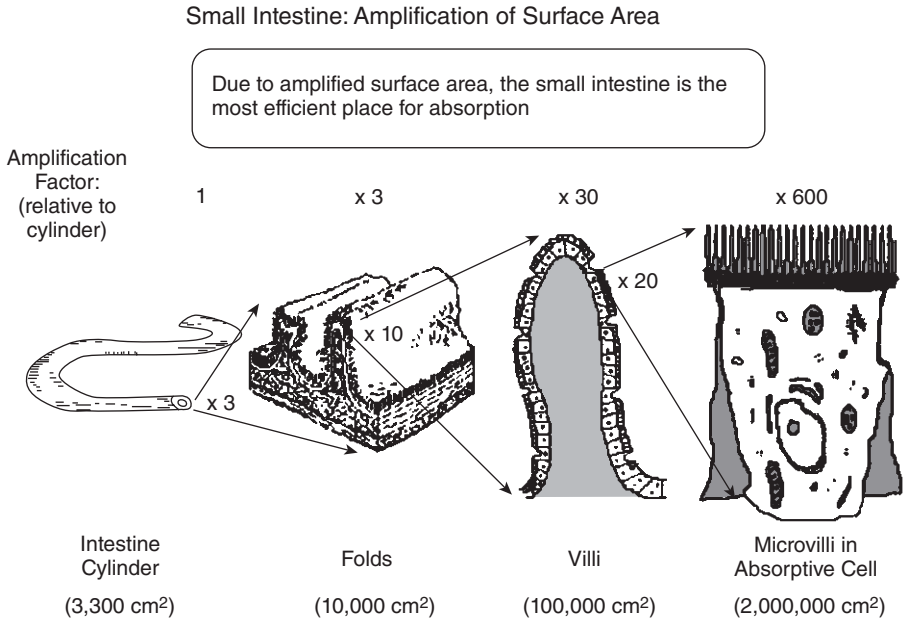

FIGURE 8.4 Anatomical modifications of the intestinal system that increase the available surface for absorption. (From Ref. 15, with permission from Modern Biopharmaceutics™.)

Gastrointestinal secretions contribute to the digestion and absorption of nutrients. The main gastrointestinal secretions, their main components, and the substances they contribute to digestion are summarized in Table 8.2 [14].

Carbohydrates are digested into monosaccharides, proteins are hydrolized into amino acids or small peptides, and fat droplets are transformed into free fatty acids and monoglycerides. The resulting compounds from digestion as well as fat-soluble and water-soluble vitamins are absorbed by some of the absorption mechanisms explained in the previous section. All essential nutrients including proteins, carbohydrates, fats, vitamins, and minerals are substrates of some active or facilitated carrier system. There are excellent reviews about the nature and diversity of intestinal transporters [12, 18–21].

Another process of particular relevance for fat absorption that may also influence drug absorption is emulsification. Emulsification of fat droplets, thanks to mechanical disruption and the presence of emulsifying agents (bile salts and phospholipids), increase the surface available for lipase action, thus increasing the rate of lipid digestion. The digestion products are incorporated into the core of aggregates known as micelles. Micelles consist of bile salts, fatty acids, monoglycerides, and phospholipids, all clustered together with the polar ends of each molecule oriented toward the surface. The micelles release their contents near the lipid bilayer and, in consequence, the released molecules can diffuse easily across the intestinal lining. Fat-soluble vitamins follow the pathway for fat absorption, and any other highly lipophilic molecule could also be incorporated in the micellar phase. Thus, for instance, any interference with the secretion of bile decreases the absorption of fat-soluble vitamins.

TABLE 8.2 Main Gastrointestinal Secretions, Their Components, and the Nutrients They Contribute to Digestion [14]

Secretion	Main Components	Digestion
Saliva	Water	
1–1.5 liters/day	Electrolytes	
pH ~ 8 at secretion rate	Mucus	
	Lysozime	
	α-Amylase	Disaccharides
Gastric fluid	Mucus	
1.5–2 liters/day	Hydrochloric acid	All
	Pepsinogen (pepsin precursor)	Proteins
	Intrinsic factor	Essential for vitamin B_{12} absorption
Pancreatic secretions	Bicarbonate	Neutralizes acid from stomach
1–1.5 liters/day		
	Trypsin, chymotrypsin, carboxypeptidases	Protein
	α-Amylase	Disaccharides
	Pancreatic lipase	Fatty acids
Bile	Bile salts	Fat digestion
0.5–1 liter/day	Cholesterol	
	Phospholipids	
	Bile pigments	
	Proteins	
	Bicarbonate, electrolytes	
Intestinal fluid	Electrolytes	
2 liters/day	Water	
	Mucus	

Water is the most abundant substance in chyme. Around 9 liters of ingested and secreted fluids enter the small intestine each day, but only 1.5 liters passes onto the large intestine, since 80% of the fluid is absorbed in the small intestine. Finally, only 150 grams of feces, consisting about 100 mL water and 50 g of solid material, is normally eliminated each day [14, 15]. The amount of water present in the gastrointestinal system is relevant for ensuring the complete dissolution of the administered dose. Some drugs present a dose-limited absorption when there is not enough water in the luminal fluids to allow the complete dissolution during transit time.

ABSORPTION OF DRUGS AND XENOBIOTICS The two main factors affecting rate and extent of absorption are drug permeability and solubility in intestinal fluids. Solubility of the drug determines the highest gradient for drug diffusion across the membrane. This concept is represented in Fig. 8.5.

Chemical stability or phenomena such as aggregation and complexation with luminal components may reduce the drug concentration in the lumen. Solubility and drug concentration in the luminal fluids are functions of the molecule pK_a for weak acids and bases and the pH of the luminal fluids. The presence of different intestinal secretions and the change in secretion rate because of the presence of food changes luminal pH, as summarized in Fig. 8.6.

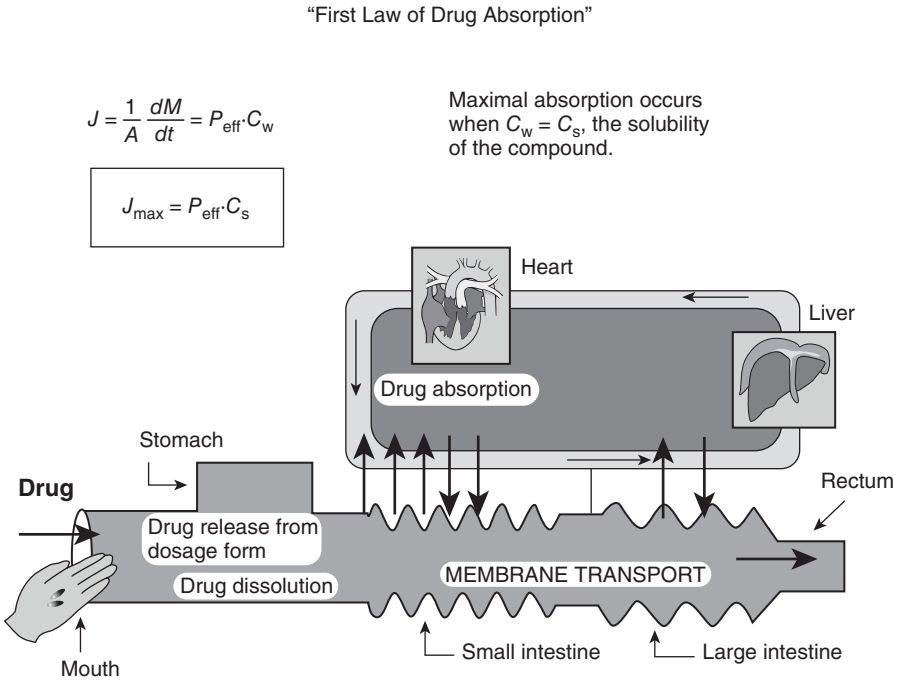

FIGURE 8.5 Factors determining rate and extent of absorption in the gastrointestinal tract.

Site	fasted	fed
Stomach	1.4 - 2.1	3.0 - 7.0
Duodenum	4.9 - 6.4	5.1 - 5.2
Jejunum	4.4 - 6.5	5.2 - 6.2
Ileum	6.5 - 8.0	6.8 - 8.0

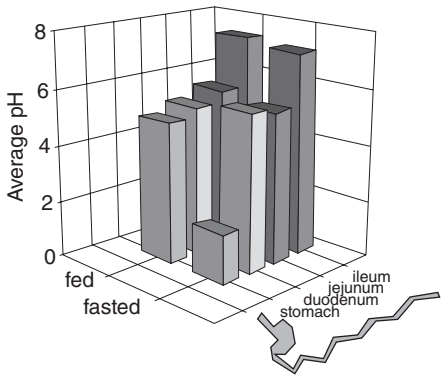

FIGURE 8.6 Average intestinal pH values in the gastrointestinal tract in the fasted and fed states. Data from Dressman et al. [85].

Finally, if the drug is administered in a solid form, any factor affecting its dissolution rate will have an impact on the rate and extent of absorption, so particle size may be relevant, in particular, for those substances of low solubility.

Permeability of the drug, as well as its permeation mechanism, depends on several molecular properties. Some of them are summarized in Table 8.3 [22]. Drugs administered by the oral route may cross the intestinal membrane by all the

TABLE 8.3 Molecular Descriptors Related to Drug Permeability in the Intestinal Epithelium

Lipophilicity	As drug partitioning into the cell membrane is one of the steps in membrane transport, lipophilicity is widely used as a predictor of drug permeability. Lipophilicity has two principal components, molecular size and hydrogen bonding potential [25, 87–90].
Molecular Weight (MW)	This is a component of lipophilicity as well as diffusion coefficient in biological membranes and fluids. A rather strong dependence between transcellular diffusion and molecular size has been observed. Compounds with MW < 200 are able to pass through the intestinal membrane by the paracellular pathway along with diffusion through the transcellular route. Compounds with MW > 250 use the transcellular route but further increases of the molecular weight (MW > 500) consequently lead to a decrease in membrane diffusion [91].
Hydrogen Bonding	The absorption ability of a molecule depends on the number and the strength of the hydrogen bonds that the molecule is able to form with water molecules, because the first step to enter into the membrane is the desolvation of the molecule. Hydrogen bonding capacity is detrimental for transport into the nonpolar environment of the cell membrane. Thus, these properties along with lipophilicity are good descriptors of drug permeation [92].
Polar Surface Area (*PSA*)	The PSA of a molecule is defined as the area of its van der Waals surface that arises from oxygen or nitrogen atoms plus the area of the hydrogen atoms attached to these heteroatoms. As such, PSA is clearly related to the capacity to form hydrogen bonds. Drugs with PSAd < 60 $Å^2$ would be completely absorbed (fraction absorbed, FA > 90%). Drugs with PSAd > 140 $Å^2$ would be absorbed less than 10%.
Nonpolar Surface Area	Nonpolar substituents facilitate membrane transport and hydrophobic compounds generally have higher permeabilities than hydrophilic ones (with similar hydrogen bonding properties). Nonpolar surface area can also correlate with membrane permeability. In general, this parameter is included in correlations along with PSA [88, 93–95].

Source: Adapted from Ref. 22.

previously mentioned mechanisms: passive diffusion, facilitated passive diffusion, active transport, or pinocytosis.

The paracellular pathway, between the epithelial cells, is both size (MW, volume) and charge dependent [6, 23, 24]; cations seem to penetrate the negatively charged tight junctional system much more easily than anions [23]. On the other hand, the available surface area for paracellular intestinal absorption has been estimated to be about 0.01% of the total surface area of the small intestine [4, 6].

Due to the lipidic nature of the intestinal membrane, the ability of the molecules to diffuse across it is dependent on their lipophilicity [25, 26]. In particular, one of the main factors affecting drug permeability is the distribution coefficient, which depends on the pH of the luminal fluids. As has been described, the pH of the luminal contents changes along the gastrointestinal tract and it is different in the fasted versus the fed state. That affects the solubility of ionizable compounds and their distribution coefficient; so, in general, permeability also varies along the gastrointestinal tract for a given solute [27].

The unstirred water layer (UWL) adjacent to the intestinal lining was considered to be the rate-limiting step for intestinal permeability of high permeability compounds [28]. However, several *in vivo* studies have clearly reported that the thick-

ness of this UWL is significantly thinner than was previously assumed, thanks to the motility of the gastrointestinal tract [29, 30].

There is now enough evidence about the involvement of intestinal transporters in the absorption of drugs [31]. Targeting intestinal transporters by means of development of prodrugs has been a successful strategy for improving oral absorption. For example, the intestinal peptide transporter is utilized in order to increase the bioavailability of several classes of peptidomimetic drugs, especially ACE inhibitors and beta-lactam antibiotics [19]. The bioavailability of poorly absorbed drugs can be improved by utilization of the transporters responsible for the intestinal absorption of various solutes and/or by inhibiting the transporter involved in the efflux system [32–35].

The last relevant physiological factor that determines drug absorption is intestinal motility, as this function limits the intestinal transit time of any solute. Since the small intestine is the main site for drug absorption, gastric emptying is the first limiting step for the access of the drug to the intestine. The average gastric emptying time is around 30 minutes, transit time in the small intestine is around 3 hours, and transit time in the large intestine is much more variable but an average of 3 days has been reported [15, 36]. Some factors affecting the gastric emptying rates of liquids are described in Table 8.4.

Solid emptying is slower than liquid emptying; also, emptying depends on the caloric content of the meal (as for liquids), and particles of size bigger than 2 mm remain in the stomach until the next "housekeeping wave" that completely empties the stomach [15].

Intestinal peristalsis is stimulated by voluminous meals and hypertonic contents. Anticholinergic drugs slow intestinal movements while cholinergic drugs stimulate transit, so simultaneous administration of these drugs with food may affect absorption.

To complete the description of this route of absorption, it is necessary to remember again the difference between absorption and oral bioavailability. Win L. Chiou [1] defined absorption as the movement of drug across the outer mucosal membranes of the gastrointestinal tract; while, in general, the systemic availability (F_{sys}) is defined as the fraction of the amount (dose) of the xenobiotic (drug) that reaches the systemic circulation. As described previously, during first passage through the liver and also during passage through the intestinal cells, the drug could be biotransformed by the intestinal or hepatic enzymes. In mathematical terms, this process is expressed as follows:

$$F_{sys} = F_a \cdot (1 - E_g) \cdot (1 - E_h) \tag{8.1}$$

TABLE 8.4 Factors Affecting Gastric Emptying Rates of Liquids [15]

Gastric Emptying	Comment
Volume	Larger volume, faster emptying rate
Osmotic pressure	Iso-osmotic content empties faster than hyper- or hypotonic solutions
pH	
Caloric content	Higher caloric content slows emptying
Viscosity	Higher viscosity delays emptying

where F_a is the fraction absorbed, E_g is the gut extraction ratio, and E_h is the hepatic extraction ratio. $(1 - E_g)$ is the fraction escaping intestinal metabolism and $(1 - E_h)$ represents the fraction escaping liver metabolism. In the absence of any presystemic losses, systemic bioavailability equals the fraction absorbed.

Buccal and Sublingual Administration of drugs using the oral cavity is an option well accepted by patients. Oral mucosa is relatively permeable with a rich blood supply [37–39]. A second advantage is the avoidance of first-pass effect and presystemic elimination in the GI tract. These factors make the oral mucosal cavity a potential alternative for systemic drug delivery [40].

Within the oral cavity, delivery of drugs can be made through the sublingual mucosa or by placing the dosage form in the mucosa lining the internal face of the cheeks, which is called the buccal route.

Oral mucosa is a stratified epithelium with a turnover time around 5–6 days [41]. It is estimated that the permeability of the buccal mucosa is 4–4000 times greater than that of the skin [42]. The buccal area has 40–50 cell layers, whereas the sublingual zone is slightly thinner. Oral mucosa is keratinized and relatively impermeable in all the areas that suffer mechanical stress, but the sublingual and buccal areas are not keratinized, rendering them more permeable [41, 43, 44]. In general, the permeabilities of the oral mucosa decrease in the order of sublingual > buccal > palatal [41].

For significant drug absorption, the drug must have a prolonged exposure to the mucosal surface so the drug taste must be acceptable to the patient [45].

The absorption potential of the buccal mucosa is influenced by the lipid solubility and therefore the permeability of the solution (osmosis), the ionization (pH), and the molecular weight of the substances. Because the pH of saliva is usually 6.5–6.9, absorption is favored for drugs with a high pK_a [41, 46].

The basic drug transport mechanism for buccal epithelium is passive diffusion, as for other epithelia in the body. There are two major routes involved: transcellular (intracellular) and paracellular (intercellular) [47]. The intercellular spaces are the major barrier to permeation of lipophilic compounds and the cell membrane acts as the major transport barrier for hydrophilic compounds. The route that predominates, however, is generally the one that provides the least amount of hindrance to passage [40]. Some authors have suggested the presence of a specialized transport system for cephadroxyl in the human buccal membrane [48].

One of the major disadvantages associated with buccal drug delivery is the low flux, which results in low drug bioavailability and the lack of dosage form retention at the site of absorption. Consequently, bioadhesive polymers have extensively been employed in buccal drug delivery systems.

To sum up, the buccal mucosa offers several advantages for controlled drug delivery for extended periods of time and is a promising area for systemic delivery of orally inefficient drugs (such as potent peptide and protein drug molecules). However, the need for safe and effective buccal permeation/absorption enhancers is a crucial component for a prospective future in the area of buccal drug delivery.

Sublingual literally means “under the tongue.” Sublingual mucosa is more permeable than buccal mucosa. Because of the high permeability and the rich blood supply, the sublingual route is capable of producing a rapid onset of action, but the area is continuously washed by a high amount of saliva, making the buccal area

more suitable for placement of retentive dosage forms. In clinical practice, sublingual drug administration is applied in the field of cardiovascular drugs, steroids, some barbiturates, and enzymes and it has been explored as an alternative for vitamins and minerals, which are found to be readily absorbed by this method. It could be especially useful for those who experience difficulty in swallowing tablets.

Rectal Absorption through the rectal mucosa is, in general, irregular and not complete so it can not be considered a first delivery option. The acceptance of the rectal route varies among countries but rectal delivery could be a convenient, alternative route when other routes are not available. The following situations represent opportunities for rectal administration:

- Patients having difficulty swallowing, nausea, vomiting, or gastric pain.
- Uncooperative or nonconscious patients.
- When access to the intravenous route is difficult.
- For drugs unstable in the gastrointestinal tract.
- For drugs with high first-pass effect.
- When a slow and prolonged input could have therapeutic interest.

For instance, rectal administration is used for anticonvulsants [49], nonnarcotic and narcotic analgesics, theophylline, antiemetics, and antibacterial agents and for inducing anesthesia in children [50, 51].

Its principal advantage is that it is independent of gastrointestinal tract motility and rate of gastric emptying [52].

However, this route should be avoided in the following situations:

- Painful anal conditions (fissures or inflamed hemorrhoids).
- Immunosuppressed patients in whom even minimal trauma could lead to formation of an abscess [53].
- Elderly patients with diarrhea.
- Patients physically unable to place the suppository.

The rectal route also has other disadvantages. There may be a great deal of variation among individuals regarding the necessary dose. Moreover, the rectal dose generally must be higher than the dose administered intravenously or orally, as absorption is slower and incomplete.

In humans, the rectum comprises the last 12–19 cm of the large intestine and the rectal epithelium is formed by a single layer of columnar or cuboidal cells and goblet cells; its surface area is about 200–400 cm^2. The absorbing surface area of the rectum is considerably smaller than that of the small intestine, as the former lacks villi and microvilli. However, the epithelia in the rectum and the upper intestinal tract are histologically similar, giving them comparable abilities to absorb drugs. The rectal mucosa is richly vascularized.

Drugs absorbed via the inferior and middle rectal veins can in part avoid first-pass metabolism because the blood flow from these veins bypasses the portal system and empties into the vena cava. First-pass effects are not completely avoided,

however, as there are anastomoses among inferior and middle veins with the superior one. The drugs absorbed via the superior rectal veins are transported to the liver via the portal system [54–56].

For a number of drugs the extent of rectal absorption has been reported to exceed oral values, which may reflect partial avoidance of hepatic first-pass metabolism after rectal delivery. This phenomenon has been reported for morphine, metoclopramide, ergotamine, lidocaine (lignocaine), and propranolol [54, 57].

Passive diffusion is the predominant mechanism of absorption and it is mainly dependent on the molecular weight, liposolubility, and degree of ionization of molecules. The contribution of other mechanisms, such as carrier mediated transport or pinocytosis, can be considered negligible. Paracellular diffusion is more restricted than in the upper zone of the gastrointestinal tract due to the lower permeability of the tight junctions in this area. The rate of rectal transmucosal absorption is affected by different factors: for example, the surface area available for absorption is small, particularly for drugs in solid dosage forms, which provide less extensibility than liquid forms. The volume present in the rectal cavity is also variable among patients but generally the volume is small and the viscosity is high. Diluent volume is also an important determinant of rectal drug uptake, as demonstrated with methohexital administered rectally for preprocedure sedation [58].

Lipophilic drugs are better absorbed than hydrophilic ones. Rectal pH may also influence drug uptake by altering the amount of drug that is ionised [59]. The pH of the rectal vault in children ranges from 7.2 to 12.2 [60]. This pH range favors absorption of barbiturates that will remain in a nonionized state because their pK_a is near the physiologic range (~7.6). The greater lipid solubility of nonionized drugs enhances their movement across the membrane. Finally, in a similar way to the small intestine where transit time limits absorption, rectal retention of the drug determines the absorption. Retention time, in many cases, is affected by the nature of the drug excipients. Hydrophilic excipients are more irritating for the rectal mucosa and increase peristaltic movements, reducing retention time.

Parenteral Routes (Noninjectables)

Transpulmonary The respiratory system consists of two main areas—the conducting and the respiratory regions. The first one is responsible for filtering and humidifying the air entering the respiratory region, where gas interchange takes place. The conducting region is formed by the nasal cavity, nasopharynx, bronchi, and bronchioles. Airways distal to the bronchioles and the alveoli constitute the respiratory region.

The transpulmonary route has classically been used for drugs intended to exert a local action in the lungs and more recently for systemic administration. In the first case, it allows the delivery of high drug concentration directly to the action site. In this way, the same therapeutic effect can be obtained with a fraction of the dose administered by any other systemic route, and simultaneously systemic and adverse effects are minimized. In general, a rapid onset of action can be achieved. For systemic action, the transpulmonary route offers the advantages of being a friendly needle-free option, with a huge absorptive surface area (100 m^2) of a highly permeable membrane in the alveolar region (0.2–0.7 μm). Compared to the gastrointestinal tract, the possibility of enzymatic degradation is reduced, and large molecules

can be absorbed in part thanks to a prolonged residence time, as the mucociliary clearance is less marked in the lung periphery.

Nevertheless, there are also barriers against absorption through this route. The respiratory system is designed to prevent the entry of particles. The airway geometry, the humidification mechanisms, and the mucociliary clearance contribute to this process and then constitute barriers for transpulmonary absorption. Once in the lower parts of the respiratory tract, the drug has to deal with the lung surfactant, surface lining fluid, the epithelium and basement membranes, and the capillary endothelium before arriving at the blood flow.

The main factors affecting the amount of drug that is finally deposited in the deep lung are the particle size and density. The combination of both factors defines the aerodynamic diameter, which is actually the factor governing the deposition site. The relation between particle diameter and aerodynamic diameter of a spherical particle is defined by the following equation:

$$d_a = \sqrt{\frac{\rho}{\rho_a}} \cdot d$$

where d_a is the aerodynamic diameter, d is the particle diameter, ρ is the particle density, and $\rho_a = 1\,g/cm^3$.

Particles of mean aerodynamic diameter of 1–3 μm deposit minimally in the mouth and throat and maximally in the lung's parenchymal (i.e., alveolar or "deep lung") region. Tracheobronchial deposition is maximal for particles with aerodynamic diameters between 8 and 10 μm. Particles possessing an aerodynamic diameter smaller than 1 μm (although greater than several hundred nanometers) are mostly exhaled, and particles larger than 10 μm have little chance of making it beyond the mouth.

Once deposited in the lungs, inhaled drugs are (1) cleared from the lungs, (2) absorbed into the systemic or lymphatic circulation, or (3) degraded via drug metabolism.

Mucociliary Clearance. Mucus is secreted by goblet cells and submucosal glands forming a double sol-gel layer covering the ciliated epithelium. Mucociliary clearance can be impaired in some pathological conditions either by impairment or ciliary movement or by the production of thicker mucus.

Absorption. Lipophilic molecules pass easily through the airway epithelium via passive transport. Hydrophilic molecules cross via paracellular pathways, such as tight junctions, or by active transport via endocytosis and exocytosis. The rate of protein absorption from the alveoli is size dependent. Effros and Mason [61] demonstrated an inverse relationship between alveolar permeability and molecular weight.

Metabolism. The lung is the only organ through which the entire cardiac output passes. The liver first-pass effect is thus avoided through this route, but metabolism during the passage through the epithelium can still happen even if the relevance of metabolic clearance in the lung for systemic availability of inhaled drug is not well understood. All metabolizing enzymes found in the liver are found to a lesser extent in the lung: phase 1 cytochrome P450 (CYP450)

enzymes, flavin-containing monooxygenases (FMOs), monoamine oxidase (MAO), aldehyde dehyrogenase, NADPH-CYP450 reductase, esterases, and proteases are all present in the lung. The monooxygenase system metabolizes fatty acids, steroids, and lipophilic xenobiotics. Esterase presents in high concentrations in alveolar macrophages, and to a lesser degree in alveolar type I and II cells. Proteins and peptides are subject to hydrolysis by proteases. However, for most proteins degradation in the alveoli is not a major clearance mechanism, with >95% of proteins, including insulin, being absorbed intact from the lung periphery.

Nasal The nasal mucosa is a promising site for the delivery of many drugs, including proteins and other large biomolecules that do not have good absorption through other routes. The nasal cavity is easily accessible and extensively vascularized, and compounds administered via this route avoid the hepatic first-pass effect. In this respect, it should be an ideal route for noninvasive delivery. In addition, absorption of drug at the olfactory region of the nose provides a potential pathway for a pharmaceutical compound to be available to the central nervous system. The nasal delivery of vaccines is another very attractive application in terms of efficacy and patient acceptance. However, there are a number of factors that limit its utility. The physiology of the nasal cavity presents the most significant barrier to drug absorption. Limiting factors include rapid mucociliary clearance, enzymatic degradation in the mucus layer, and low permeability of the nasal epithelium [62–66].

The nasal vestibule and atrium are the less permeable areas in the nasal cavity, whereas the respiratory region (turbinate) is the most permeable thanks to the increased surface area and the rich vasculature. This latter area has a large surface area ~150–160 cm^2 because of the presence of microvilli. The pH of the zone varies between 5.5 and 6.5 in adults and between 5.0 and 7.0 in children.

The mechanisms for drug absorption in this area include passive diffusion (para- and transcellular), carrier mediated transport, and transcytosis. Lipophilic drugs are well absorbed with bioavailabilities reaching 100%; but permeability is low for polar molecules (including low molecular weight drugs and large peptides and proteins). In general, absorption is restricted for drugs with molecular weights higher than 1000 daltons.

Deposition of the formulation in the anterior portion of the nose provides a longer nasal residence time but permeability is lower in this area; whereas deposition in the posterior zone, where the permeability is higher, provides shorter residence times. This shorter residence time is due to the mucociliary clearance, which must be maintained for normal physiological functions, such as the removal of dust, allergens, and bacteria. A prolonged residence time in the nasal cavity may also be achieved by using bioadhesive polymers, microspheres, and chitosan or by increasing the viscosity of the formulation. Nasal mucociliary clearance can also be stimulated or inhibited by drugs, excipients, preservatives, and/or absorption enhancers and thus affect drug delivery to the absorption site.

Several proteases and amino peptidases present in the nasal mucosa might affect the stability of proteins and peptides; nevertheless, the level of proteolytic activity is much lower than that in the gastrointestinal tract. Peptides may also form complexes with immunoglobulins (Igs) in the nasal cavity, leading to an increase in the molecular weight and a reduction of permeability.

A linear inverse correlation has been reported between the absorption of drugs and molecular weight up to 300 daltons. Absorption decreases significantly if the molecular weight is greater than 1000 daltons, except with the use of absorption enhancers.

These enhancers work by a variety of mechanisms, such as by modifying the phospholipid bilayer, leaching out protein from the membrane, or even stripping off the outer layer of the mucosa. Some of these enhancers also have an effect on the tight junctions and/or work as enzymatic inhibitors. The main problem associated with enhancers is that most of them cause significant mucosal damage at the concentrations required to enhance nasal absorption. Some of the enhancers that have been investigated include bile salts, dihydrofusidates, surfactants, and fatty acid derivatives.

Chitosan, a bioadhesive polymer, has been shown to improve the absorption by increasing the contact time between the drug and the nasal membrane and, hence, its retention on the nasal mucosa. It also had an effect on paracellular transport. Other bioadhesive materials are carbopol, cellulose agents, starch, and dextran. The use of cyclodextrins as a means of enhancing solubility and/or absorption also looks promising [67, 68].

Ophthalmic Systemic absorption of drugs from the ocular route is considered, in general, a nondesired effect. The main objective of the application of a drug on the front of the eye is to exert some effect in the eye and the annex structures. There are five potential targets for ophthalmic drugs: the precorneal structures (conjunctiva and eyelids), the cornea, the posterior and anterior chambers, the vitreous cavity, and the retina (Fig. 8.7). Preocular structures, cornea, and the anterior and

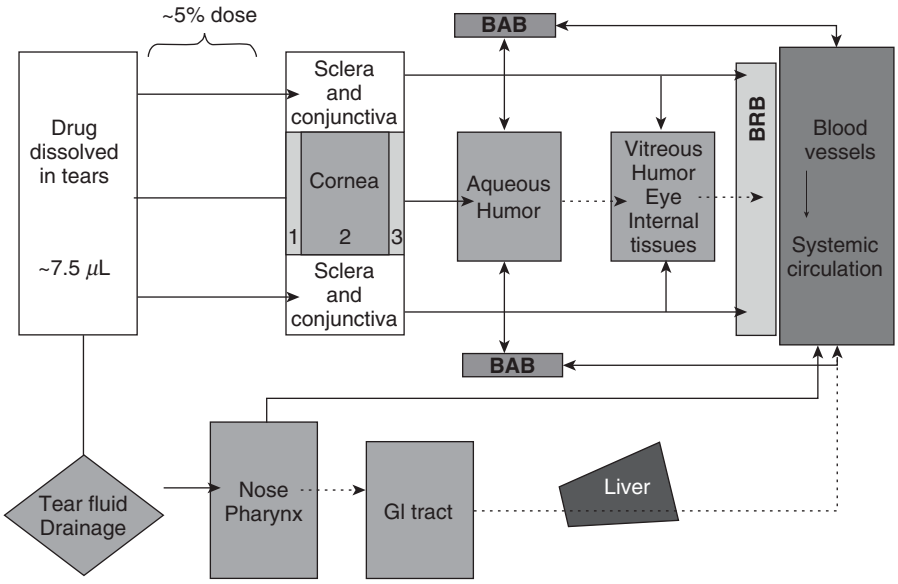

FIGURE 8.7 Absorption route for drugs applied on the eye surface. BAB, blood aqueous barrier; BRB, blood retinal barrier; 1, epithelium; 2, stroma; 3, endothelium. (Adapted from Ref. 86.)

posterior chambers can be accessed by topical application but the posterior side of the eye is difficult to reach from the eye surface, and, in general, systemic administration by another route or intraocular injection is used to deliver drugs to the vitreous cavity. The main pathway for drugs to enter the anterior chamber is via the cornea. Some large and hydrophilic drugs prefer the conjunctival and scleral route, and then diffuse into the ciliary's body [69], while small lipophilic compounds permeate through the corneal pathway.

On the other hand, reaching the anterior part of the eye from the systemic circulation is difficult due to the existence of the blood–ocular (blood–aqueous and blood–retinal) barrier, similar to the hematoencephalic barrier. The tight junctions of the capillary endothelial cells restrict the entry of substances from the blood into the aqueos humor and/or into the retina [70].

Once the drug is dissolved in the tear fluid, its residence time in the conjunctival sac is short (between 2 and 5 minutes) because of the drainage of the instillation fluid with tears and the tear turnover. Drainage rates decrease with increased viscosity of the instilled fluid but increase with increased volume of the instilled fluid because the eye tends to maintain the normal tear volume around 7.5 μL. If the drug is irritating to the eye or the conjunctiva, it will induce lacrimation and decrease residence time. The drug from the lacrimal duct arrives at the nose and then pharynx and can be swallowed and reach the gastrointestinal tract. From all these routes the drug can be absorbed, and potentially it could cause adverse effects. The drug in the tears is either absorbed by the conjunctiva, sclera, or cornea. The conjunctiva is a richly irrigated mucosa covering the inside of the eyelids and sclera. Its surface is higher than the corneal surface as well as its permeability, so this absorption pathway competes with the corneal route, reducing intraocular bioavailability.

The corneal barrier is formed by a series of three layers without blood supply: epithelium, stroma, and endothelium. The outer layer is a multistratified epithelium representing 10% of the total thickness. Between the epithelium and the stroma lies Bowman's membrane, containing strong collagen fibers, which help the cornea maintain its shape. Ninety percent of the thickness corresponds to the stroma, which is constituted by a net of parallel collagen fibrils and high water content. The endothelium pumps water from the cornea, keeping it clear. Epithelium and endothelium are lipophilic, while the stroma is a barrier for hydrophobic compounds. Lipophilicity, molecular weight, and ionization degree are the main factors affecting corneal permeability as well as the degree of binding to the protein content of the lacrimal fluid [71]. Lacrimal pH oscillates around 7.0–7.4 and has a low buffering capacity; thus, the pH and buffering capacity of the instillation solution could affect ophthalmic absorption. The optimum apparent partition coefficient (octanol/pH 7.4 buffer) for corneal absorption is in the range of 100–1000. It was shown that increasing molecular size of the permeating substance decreases the rate of paracellular permeation, as the pore size does not allow permeation of big molecules. Nevertheless, transcellular diffusion is the main pathway for most drugs used in the clinic [69].

Transdermal The application of drug substances over the skin could have two different purposes: acting locally over the skin surface or providing drug absorption into the systemic circulation. Cutaneous administration refers, in general, to the first purpose while transdermal absorption defines the incorporation of a drug inside the body, crossing the skin in order to reach the blood vessels and exert systemic effects.

The advantage of the transdermal route over other routes is its large accessible surface area (average surface 1.8 m^2) and the avoidance of drug degradation in the gastrointestinal tract. Nevertheless, the skin by itself has homeostatic and protective functions, and it is a formidable barrier membrane, thanks mainly to the contribution of the stratum corneum (SC).

SKIN PHYSIOLOGY There are two important layers to human skin: the epidermis, and the dermis, which contains blood vessels. The skin contains annex structures that include sweat and sebaceous glands and hair follicles. These structures cross the skin from the dermis and open on the epidermis surface.

The stratum corneum (or horny layer) is the top layer of the skin and varies in thickness from 10 to 20 μm depending on the region of the body. It is composed of 15–20 layers of dead, flat, and keratinized epidermal cells (keratinocytes) surrounded by a lipid matrix, which renders this structure as the most significant barrier to diffusion and drug transport. The viable epidermis lies below the stratum corneum. Its cells have a greater degree of hydration, so diffusion is faster through this area. Viable epidermis contains melanocytes, which provide skin with pigmentation and Langerhans cells (antigen-presenting cells to the immune system). Finally, the dermis is the layer containing the blood vessels, sensory neurons, and a lymphatic network. The thickness of the dermis (1 mm) is approximately 100 times the thickness of the stratum corneum.

DRUG PERMEATION Generally, drug absorption into the skin occurs by passive diffusion according to Fick's law. The transport rate is directly proportional to the surface area of the skin and inversely proportional to the thickness of the stratum corneum. Regarding the physicochemical characteristics of the compound, drug hydrosolubility, lipophilicity, and molecular weight, as well as charge, are the main factors affecting permeation, as in other routes where passive diffusion is the main mechanism. There is in most cases a parabolic relationship between the drug lipophilicity (expressed as octanol–water partition coefficient) and the permeation rate [72, 73]. This kind of correlation appears because compounds with low partition coefficient (log P) present little partitioning into the skin and therefore low permeability, while compounds with high partition coefficient also give low permeability due to their inability to partition out of the stratum corneum. The generally accepted range of log P for maximum permeation is between 1 and 3 [74]. For hydrophilic or charged molecules, resistance to transport is higher due to the lipid-rich nature of the stratum corneum and its low water content. Transport of lipophilic drug molecules is facilitated by their dissolution into intercellular lipids around the cells of the stratum corneum.

There are two main pathways by which drugs can cross the skin and reach the systemic circulation (see Fig. 8.8): the transfollicular and transepidermal pathways.

The *transfollicular route* is through the hair follicles and sebaceous glands. Hair follicles and sebaceous glands penetrate through the stratum corneum, allowing more direct access to the dermal microcirculation. Some hydrophilic substances can diffuse through this pathway. However, due to the low relative surface area (around 1%), very little drug actually crosses the skin via this route.

The *transepidermal pathway* consists of two paths. The *transcellular* represents the shortest path. However, the drug must cross environments of hydrophilic

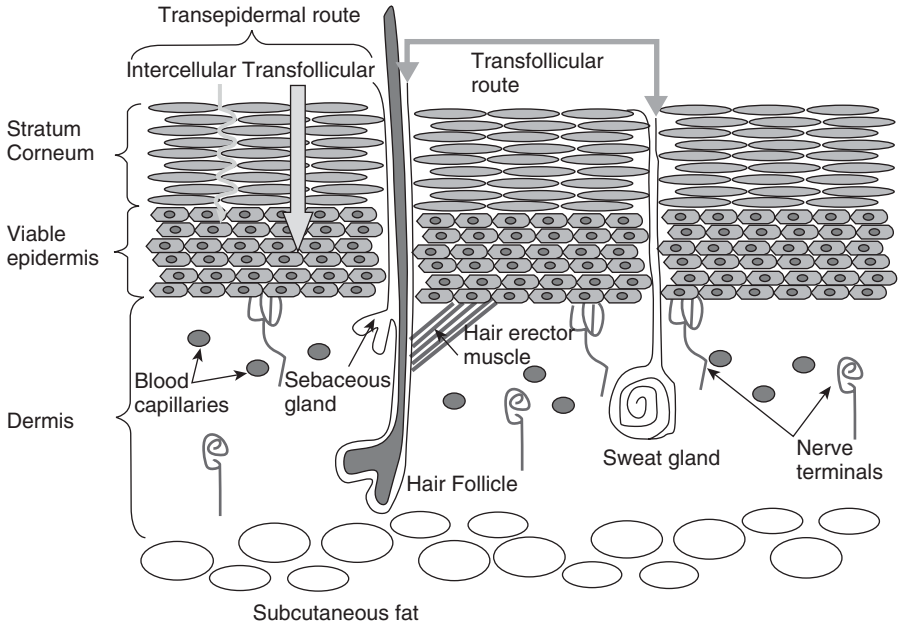

FIGURE 8.8 Skin structure and main pathways for transdermal absorption.

TABLE 8.5 Physiological Factors Relevant for Drug Penetration Through the Skin

Age	Neonates, elderly People: higher permeability [96]
Ethnicity	Controversial results in the literature: skin more permeable in Caucasians and Asians versus blacks, or no difference detected [97–99]
Body areas	Most permeable areas: mucous membranes, scrotal skin, eyelids Intermediate permeability: face, chest/back, buttocks, abdomen, upper arms/legs Less permeable zones: palms, feet soles, nails
Skin condition	Hydration degree: hydrated skin is more permeable [100] Irritation: if stratum corneum is broken, permeability is increased [100] Temperature: warmer skin is more permeable Eczema: increased permeability Psoriasis: thicker skin but disrupted barrier function; reported increase in permeability for low molecular drugs and for some high molecular entities [101]

(keratinocytes) and liphophilic nature (extracellular lipids) and that means that the substance needs a balanced hydrophilic–liphophilic nature to be able to follow this route and in general most substances find high resistance to permeation. The *intercellular* pathway is the more common pathway. Nevertheless, even if the thickness of the SC is very small, the tortuosity of the diffusion pathway around the cells increases the resistance to drug penetration.

Some physiological factors affecting drug penetration across the skin are summarized in Table 8.5.

Examples of drugs that are administered via the transdermal route include clonidine, estradiol, fentanyl, nicotine, nitroglycerin, scopolamine, testosterone, oxybutynin, and the combination products norelgestromin/ethinyl estradiol and estradiol/norethindrone acetate. The common characteristics is that they are all molecules of low molecular weight but with enough potency to be active at low blood concentrations (few ng/mL or less) [75].

Vaginal

VAGINAL PHYSIOLOGY The human vagina is a slightly S-shaped tube that communicates the cervix of the uterus with the external body surface. The tube is collapsed with the anterior and posterior walls in contact with each other. The average length of the vagina is 8–12 cm [76].

The vaginal wall is comprised of an epithelial layer, a muscular layer, and the tunica adventitia. During the menstrual cycle, the thickness of the vaginal epithelial cell layer changes by approximately 200–300 μm. The surface of the vagina is composed of numerous folds or rugae. The rugae provide distensibility, support, and an increased surface area for the vaginal wall [76].

The primary venous drainage occurs via the pudendal veins. The vaginal, uterine, vesical, and rectosigmoid veins from the middle and upper vagina lead the blood flow to the inferior vena cava, thus bypassing the hepatic portal system.

Age, hormone status, and pregnancy cause changes in vaginal physiology as well as the pH changes due to several factors including semen, menstruation, estrogen status, and bacterial colonization. Reproductive hormones control the thickness of the vaginal epithelium, with estradiol 17-β (E2) thickening the epithelium and hypoestrogenism resulting in atrophy [77]. The vaginal fluid ranges mostly a transudate from vaginal and cervical cells but also contains vulvar secretions, cervical mucus, endometrial and oviductal fluids, and microorganisms and their metabolic products.

The normal average vaginal pH in healthy women of reproductive age ranges from 3.8 to 4.2. This acidic environment is maintained by the production of lactic acid by the vaginal microflora and it constitutes part of the defensive mechanism of the vaginal mucosa. The amount and composition of the vaginal fluid also changes throughout the menstrual cycle. Women of reproductive age produce fluid at a rate of 3–4 g/4 h, while this amount decreases by 50% in postmenopausal women. The human vaginal fluid may contain enzymes, enzyme inhibitors, proteins, carbohydrates, amino acids, alcohols, hydroxylketones, and aromatic compounds.

VAGINAL ABSORPTION Vaginal delivery is a potential option for both local and systemic delivery. Vaginal mucosa possess several advantages when other routes of administration fail, such as avoidance of first-pass metabolism. On the other hand, this route of administration could be used for particular purposes, such as providing prolonged absorption from sustained release formulations or achieving local therapeutic effects while avoiding systemic administration and potential adverse effects of the drug. In general, the vaginal route provides good systemic absorption mostly for low molecular weight drugs [78].

Enzymatic activity of vaginal epithelium and vaginal fluids are two physiological factors that have shown to have influence on vaginal absorption. The external cell

layers and the basal cell layers of the vagina retain most of the enzyme activity. Among the enzymes present, proteases are likely to be the prominent barrier for the absorption of intact peptide and protein drugs into the systemic circulation [79]. The absorption of a drug that is poorly water soluble may be increased when the fluid volume is higher. However, the presence of overly viscous cervical mucus may present a barrier to drug absorption and increased fluid volume may remove the drug from the vaginal cavity and subsequently reduce absorption [80].

Since many drugs are weak electrolytes, the pH may change their degree of ionization and affect the absorption of drug. As in other membranes, the ionization decreases the permeability of the drug as the un-ionized form has the higher permeability [80].

Physicochemical properties such as molecular weight, lipophilicity, ionization, and surface charge can influence vaginal drug absorption [79]. Lipophilicity, in general, increases drug permeability but low molecular weight lipophilic drugs are likely to be absorbed more than large molecular weight lipophilic or hydrophilic drugs. On the other hand, the molecular weight cutoff above which compounds are not absorbed may be higher for the vagina than other mucosal surfaces, such as the small intestine or colon [81]. Drugs with molecular weight >300 Da have shown a higher permeability in *in vitro* models of vaginal mucosa compared to small intestine and colon [81].

In conclusion, knowledge about the relationship between physicochemical properties and human vaginal permeability is still very limited; much work needs to be done in this area.

Parenteral Routes (Injectables) Parenteral drug administration comprises all the nonenteral routes. Some of the parenteral routes require a needle for placing the drug at the absorption site. The most used extravascular parenteral routes are intradermal, subcutaneous, and intramuscular, which are represented in Fig. 8.9. Drug has to cross different barriers to be absorbed from the depot compartment.

Intradermal The dermis is a layer underneath the epidermis, which contains the blood capillaries and nerve terminations. In general, the body area used to administer intradermal injections is the upper part of the arm and/or the back. It is used mainly for diagnostic agents, vaccines, or desensitization agents (a method to reduce or eliminate an organism's negative reaction to a substance or stimulus). The maximum volume to be administered by this route is 0.1 mL. Intradermal drugs diffuse slowly from the injection site into local capillaries, and the process is a little faster with drugs administered subcutaneously.

Subcutaneous This route of administration can be used in either short-length or chronic therapies. The drug is injected, or the delivery system is placed in the interstitial tissue beneath the dermis. The most used zones for subcutaneous injection are the upper arm, the upper thigh, the lower part of the abdomen, or the upper part of the back. Maximal volume is around 2 mL. The blood supply to the subcutaneous tissue is less than the blood supply to the underlying muscular tissue; thus, subcutaneous absorption can be slower than intramuscular absorption. Nevertheless, compared to the oral route, subcutaneous absorption can be more rapid and predictable. The following factors can alter the absorption rate through this route:

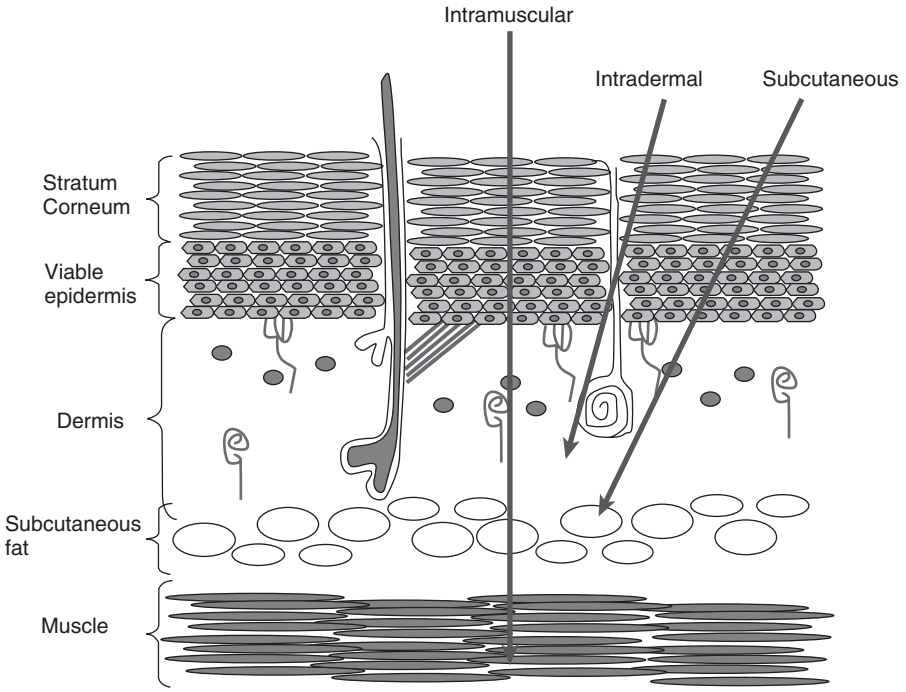

FIGURE 8.9 Parenteral routes of drug administration: intradermal, subcutaneous, and intramuscular. The injection depth determines where the drug depot is formed.

heating or massaging the injection zone (to increase absorption), coadministering vasodilators or hyaluronidase (to increase diffusion rate), or administering epinephrine to cause vasoconstriction (and thus decrease absorption rate).

Intramuscular This route is easier and less risky to use than the intravenous one, but it is more painful and the action onset is slower as it requires an absorption step. The injection is done in the muscular layer right under the subcutaneous tissue, avoiding blood vessels and nerves. The main sites of absorption are: deltoid muscle (up to 2 mL), gluteus muscle (5 mL), vastus lateral is muscle (upper thigh 15 mL). The absorption rate through this route depends on physiological factors, such as blood flow, exercise, and injection depth. The physicochemical characteristics of the drugs can also affect absorption rate (see Table 8.6) and in some cases the drug precipitates in the intramuscular depot further delaying the absorption.

Absorption Routes Once the drug is injected, a depot is formed in contact with the surrounding tissue. From the depot the drug can reach blood capillaries or lymphatic capillaries. Absorption proceeds through the blood or lymph, depending mainly on the drug molecular weight. Drugs with molecular weight higher than 2000 daltons preferentially use lymphatic vessels, while drugs with lower molecular weight are absorbed in the blood vessels. Blood capillaries have a thin wall formed by endothelial cells constituting a lipid barrier with aqueous pores and intercellular spaces called fenestrations. Pores and fenestrations represent only 0.1–0.2% of the capillaries' surface but the flow of water and soluble substances through them can

TABLE 8.6 Factors Affecting Parenteral Absorption by the Intradermal, Subcutaneous, and Intramuscular Routes

Physiological Factors	Physicochemical Factors
Blood flow	Lipophilicity
Muscular activity	Solubility
Vasoconstriction degree	Dissolution rate
Medium viscosity	pK_a

be very fast. Parenteral drug absorption occurs by passive diffusion through the lipid barrier or convective diffusion through the pores and fenestrations. Both processes can be described using Fick's diffusion law:

$$\frac{dQ_a}{dt} = \left(\frac{D \cdot P \cdot S}{L \cdot V_a} + \frac{D' \cdot S'}{\eta \cdot L' \cdot V_a} \right) \cdot Q_a$$

where dQ_a/dt is absorption rate from the depot compartment; Q_a, the amount of drug in the depot compartment; D, diffusion coefficient in the lipid membrane; P, partition coefficient; S, membrane surface; L, membrane thickness; V_a, volume of parenteral deposit; D', aqueous diffusion coefficient; S', aqueous pores and fenestration surface; L', average pore length; and η, medium viscosity.

Considering all the terms inside the parentheses as constants, the process is described as first order kinetics. In consequence, the absorption rate is directly proportional to the diffusion coefficient, available surface, and partition coefficient and inversely proportional to the membrane thickness, pore length, and medium viscosity. Absorption rate can be increased by including vasodilators or diffusion agents (hyaluronidase) in the drug formulation or decreased by using vasoconstrictors and thickening agents.

The drug's properties that exhibit more influence on drug absorption rate are those affecting dissolution rate and lipophilicity. Dissolution rate is conditioned by the particle size, crystalline structure, and polymorphs. Lipophilicity influences the drug's partitioning into the membrane and this factor depends on the drug's pK_a and the pH of the fluid, as the un-ionized form is more lipophilic. All these factors are summarized in Table 8.6.

In the last decades new needle-free technologies have been developed for injectable delivery. Needle-free injectors are devices that do not use a needle to place the drug in its depot site. The mechanism involves high pressure to push the drug formulation through the skin to the desired site. Pressure is produced by using either a gas (carbon dioxide or nitrogen) or a spring device. The pressure forces the medication through a small opening in the device while it is held against the skin. This creates a fine stream of the medication that penetrates the skin [82, 83].

Other Parenteral Routes Intraspinal (epidural and intrathecal) delivery of drugs is an alternative route used mainly for anesthesia and pain-killer medications, in particular, when patients experience intolerable side effects from systemic drugs. Intraspinal delivery can be given into the epidural space or into the intrathecal space [84].

Drugs delivered epidurally also circulate systemically. By contrast, drugs delivered intrathecally circulate only in the cerebropsinal fluid (CSF). In both cases, the drugs administered by the intraspinal route bypass the blood–brain barrier. However, epidurally administered drugs must first cross the dura (the protective outer layer of the spinal cord) before entering the CSF. Therefore, when the epidural route of delivery is used, more time and higher doses are required for the drugs to exert the same effect compared with intrathecal delivery.

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9

ABSORPTION OF DRUGS AFTER ORAL ADMINISTRATION

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Contents

Preclinical Development Handbook: ADME and Biopharmaceutical Properties,
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9.1 INTRODUCTION

Drugs are most commonly given orally and the oral route is the preferred one for the administration of new therapeutic agents. After oral administration, drugs must be absorbed through the gastrointestinal tract to achieve the systemic circulation and exert their pharmacological effects. The successful formulation of an optimized oral drug delivery system requires a detailed consideration and a good understanding of the intestinal absorption process, its possibilities and limitations. In fact, peroral delivery of new hydrophilic drugs, which include macromolecules frequently with null or scarce capacity of absorption through the intestinal barrier, is one of the greatest challenges in biopharmaceutical research.

Recent years have seen rapid developments in the field of intestinal absorption: more detailed insights into the structure and organization of intestinal mucosa (e.g., increased knowledge of tight junction physiology and regulation), new approaches to overcoming the problems associated with poor intestinal drug absorption (e.g., the use of paracellular enhancers), and novel methods for assessing the permeability of intestinal mucosa.

The goal of the present chapter is to give an overview and update on the concepts, possibilities, and limitations of drug absorption after oral administration. First, we introduce the anatomical and physiological aspects of the gastrointestinal tract, since they are decisive to understanding the absorption processes, and analyze the possible pathways of drug intestinal absorption. Second, the primary factors that influence oral drug absorption (e.g., physiological, physicochemical, and technological variables) are covered. Third, we analyze the use of permeation enhancers as a way to increase the rate and extent of drugs across the intestinal barrier. Fourth, we review the different methodologies (*in vitro, in situ*, and *in vivo*) that allow an investigator to establish the suitability of a drug candidate and to solve problems associated with drug intestinal absorption.

9.2 ANATOMY AND PHYSIOLOGY OF THE HUMAN GASTROINTESTINAL TRACT

The gastrointestinal tract (GIT) consists of a hollow muscular tube starting from the oral cavity, where food enters the mouth, continuing through the pharynx, esophagus, stomach, and intestines, to the rectum and anus, where food is expelled. There are various accessory organs that assist the tract by secreting enzymes to help break down food into its component nutrients. Thus, the salivary glands, liver, pancreas, and gallbladder have important functions in the digestive system. Food is propelled along the length of the GIT by peristaltic movements of the muscular walls.

The primary purpose of the GIT is to break down food into nutrients, which can be absorbed into the body to provide energy. First, food must be ingested into the mouth to be mechanically processed and moistened. Second, digestion occurs mainly in the stomach and small intestine, where proteins, fats, and carbohydrates are chemically broken down into their basic building blocks. Smaller molecules are then absorbed across the epithelium of the small intestine and subsequently enter the circulation. The large intestine plays a key role in reabsorbing excess water. Finally,

undigested material and secreted waste products are excreted from the body via defecation (passing of feces).

9.2.1 Basic Structure

The GIT is a muscular tube lined by a special layer of cells, called epithelium. The contents of the tube are considered external to the body and are in continuity with the outside world at the mouth and the anus. Although each section of the tract has specialized functions, the entire tract has a similar basic structure with regional variations. The wall is divided into four layers as follows (beginning with the luminal surface): (1) mucosa, (2) submucosa, (3) muscularis externa, and (4) serosa (mesentery). The three outer layers are similar throughout most of the tract; however, the mucosa has distinctive structural and functional characteristics.

1. *Mucosa.* The innermost layer of the digestive tract has specialized epithelial cells supported by an underlying connective tissue layer called the lamina propria. The lamina propria contains blood vessels, nerves, lymphoid tissue, and glands that support the mucosa. Depending on its function, the epithelium may be simple (a single layer) or stratified (multiple layers). Areas such as the mouth and esophagus are covered by a stratified squamous (flat) epithelium so they can survive the wear and tear of passing food. Simple columnar (tall) or glandular epithelium lines the stomach and intestines to aid secretion and absorption. The inner lining is constantly shed and replaced, making it one of the most rapidly dividing areas of the body. Beneath the lamina propria is the muscularis mucosa. This comprises layers of smooth muscle, which can contract to change the shape of the lumen.

2. *Submucosa.* The submucosa surrounds the muscularis mucosa and consists of fat, fibrous connective tissue, and larger vessels and nerves. At its outer margin there is a specialized nerve plexus called the submucosal plexus or Meissner plexus. This supplies the mucosa and submucosa.

3. *Muscularis Externa.* This smooth muscle layer has inner circular and outer longitudinal layers of muscle fibers separated by the myenteric plexus or Auerbach plexus. Neural innervations control the contraction of these muscles and hence the mechanical breakdown and peristalsis of the food within the lumen.

4. *Serosa (Mesentery).* The outer layer of the GIT is formed by fat and another layer of epithelial cells called mesothelium.

9.2.2 Stomach

The stomach is a J-shaped expanded bag, located just left of the midline between the esophagus and small intestine. It is divided into four main regions and has two borders called the greater and lesser curvatures. The first section is the *cardia*, which surrounds the cardial orifice where the esophagus enters the stomach. The *fundus* is the superior, dilated portion of the stomach that has contact with the left dome of the diaphragm. The *body* is the largest section between the fundus and the curved portion of the J. This is where most gastric glands are located and where most mixing

of the food occurs. Finally, the *pylorus* is the curved base of the stomach. Gastric contents are expelled into the proximal duodenum via the pyloric sphincter. The inner surface of the stomach is contracted into numerous longitudinal folds called *rugae*. These allow the stomach to stretch and expand when food enters. The stomach can hold up to 1.5 liters of material.

The functions of the stomach include (1) the short-term storage of ingested food, (2) mechanical breakdown of food by churning and mixing motions, (3) chemical digestion of proteins by acids and enzymes, and (4) killing bugs and germs by acidification. Most of these functions are achieved with the aid of the secretion of stomach juices by gastric glands in the body and fundus. Some cells are responsible for secreting acid and others secrete enzymes to break down proteins.

9.2.3 Small Intestine

The small intestine is composed of the duodenum, jejunum, and ileum. It averages approximately 6 m in length, extending from the pyloric sphincter of the stomach to the ileocecal valve separating the ileum from the cecum. The small intestine is compressed into numerous folds and occupies a large proportion of the abdominal cavity. The duodenum is the proximal C-shaped section that curves around the head of the pancreas. The duodenum serves a mixing function as it combines digestive secretions from the pancreas and liver with the contents expelled from the stomach. The start of the jejunum is marked by a sharp bend, the duodenojejunal flexure. It is in the jejunum where the majority of digestion and absorption occurs. The final portion, the ileum, is the longest segment and empties into the cecum at the ileocecal junction.

The small intestine performs the majority of digestion and absorption of nutrients. Partly digested food from the stomach is further broken down by enzymes from the pancreas and bile salts from the liver and gallbladder. These secretions enter the duodenum at the ampulla of Vater. After further digestion, food constituents such as proteins, fats, and carbohydrates are broken down to small building blocks and absorbed into the body.

The lining of the small intestine (Fig. 9.1) is made up of numerous permanent folds called plicae circulares or folds of Kerckring. Each plica has numerous villi (folds of mucosa) and each villus is covered by epithelium containing several specialized cells; some are responsible for absorption, the enterocytes, while others secrete digestive enzymes and mucus to protect the intestinal lining from digestive actions. Enterocytes in the small intestine are cells clearly specialized in absorption, having in their luminal pole the so-called microvilli (brush border). This anatomical structure is designed to increase the surface area for absorption by a factor of several hundred.

All the structure in the small intestine is conceived to increase effective surface for absorption (Fig. 9.2). The initial increase in surface area is due to the projection within the lumen of folds of mucosa. As indicated earlier, lining the entire epithelial surface are finger-like projections, the villi. These villi range in length from 0.5 to 1.5 m, and it has been estimated that there are about 10–40 villi/mm^2 of mucosal surface. Projecting from the villi surface are the microvilli, which represent the final large increase in the surface area of the small intestine. There are approximately 600 microvilli protruding from each enterocyte lining the villi. Relative to the initial

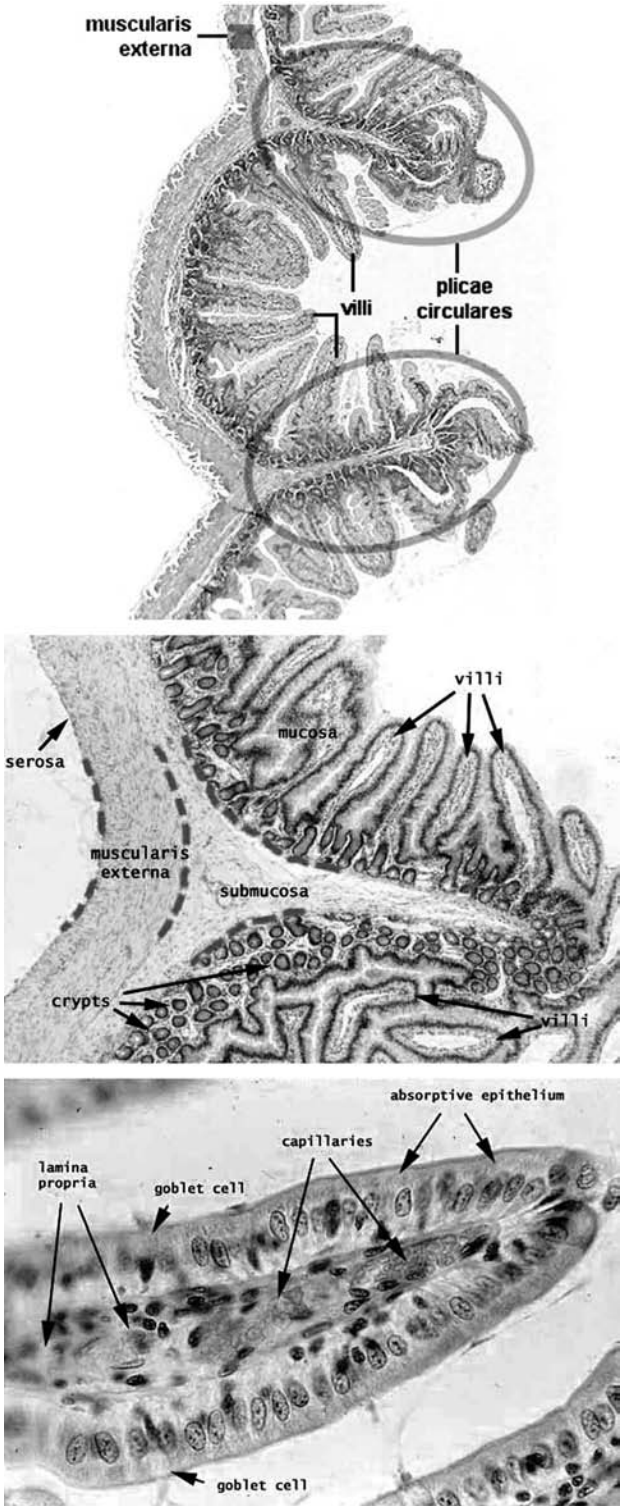

FIGURE 9.1 Photomicrographs showing the structure of the small intestine (jejunum). Magnification increases from the upper to the lower panel.

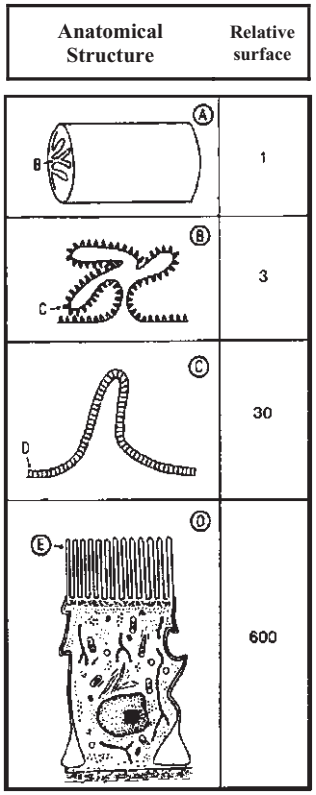

FIGURE 9.2 Schematic representation of the increase in the effective surface of the small intestine mucosa, relative to a simple cylinder, as a consequence of its particular structural features: Ⓐ simple cylinder, Ⓑ plicae circulares, Ⓒ villi, Ⓓ enterocyte, and Ⓔ microvilli.

surface of a smooth cylinder, the folds, villi, and microvilli increase the effective surface area by factors of 3, 30, and 600, respectively.

9.2.4 Large Intestine

The large intestine is horseshoe shaped and extends around the small intestine like a frame. It consists of the appendix, cecum, ascending, transverse, descending, and sigmoid colon, and the rectum. It has a length of approximately 1.5 m and a width of 7.5 cm. The cecum is the expanded pouch that receives material from the ileum and starts to compress food products into fecal material. Food then travels along the colon. The wall of the colon is made up of several pouches (haustra) that are held under tension by three thick bands of muscle (taenia coli). The rectum is the final 15 cm of the large intestine. It expands to hold fecal matter before it passes through the anorectal canal to the anus. Thick bands of muscle, known as sphincters, control the passage of feces.

The mucosa of the large intestine lacks villi seen in the small intestine. Thus, the effective surface area for absorption is clearly lower than that existing in the small intestine. The mucosal surface is flat with several deep intestinal glands. Numerous

goblet cells line the glands that secrete mucus to lubricate fecal matter as it solidifies.

The functions of the large intestine can be summarized as (1) the accumulation of unabsorbed material to form feces, (2) some digestion by bacteria the bacteria responsible for the formation of intestinal gas, and (3) reabsorption of water, salts, sugar, and vitamins.

9.2.5 Liver, Gallbladder, and Pancreas

The liver, gallbladder, and pancreas, although not part of the gut, have been included, since these organs secrete materials vital to the digestive and certain absorptive functions of the gut.

The liver is a large, reddish-brown organ situated in the right upper quadrant of the abdomen. It is surrounded by a strong capsule and divided into four lobes, namely, the right, left, caudate, and quadrate lobes. The liver has several important functions. It acts as a mechanical filter by filtering blood that travels from the intestinal system. It detoxifies several drugs and endogenous metabolites including the breakdown of bilirubin and estrogen. In addition, the liver has synthetic functions, producing albumin and blood clotting factors. However, its main roles in digestion are in the production of bile and metabolism of nutrients. All drugs and nutrients absorbed by the intestines pass through the liver and are processed before traveling to the rest of the body. The bile produced by cells of the liver enters the intestines at the duodenum. Here, bile salts break down lipids into smaller particles so there is a greater surface area for digestive enzymes to act.

The gallbladder is a hollow, pear-shaped organ that sits in a depression on the posterior surface of the liver's right lobe. It consists of a fundus, body, and neck. It empties via the cystic duct into the biliary duct system. The main functions of the gallbladder are storage and concentration of bile. Bile is a thick fluid that contains enzymes to help dissolve fat in the intestines. Bile is produced by the liver but stored in the gallbladder until it is needed. Bile is released from the gallbladder by contraction of its muscular walls in response to hormone signals from the duodenum in the presence of food.

Finally, the pancreas is a lobular, pinkish-grey organ that lies behind the stomach. Its head communicates with the duodenum and its tail extends to the spleen. The organ is approximately 15 cm in length with a long, slender body connecting the head and tail segments. The pancreas has both exocrine and endocrine functions. Endocrine refers to production of hormones, which occurs in the islets of Langerhans. The islets produce insulin, glucagon, and other substances and these are the areas damaged in diabetes mellitus.

The exocrine (secretory) portion makes up 80–85% of the pancreas and is the area relevant to the gastrointestinal tract. It is made up of numerous acini (small glands) that secrete contents into ducts, which eventually lead to the duodenum. The pancreas secretes fluid rich in carbohydrates and inactive enzymes. Secretion is triggered by the hormones released by the duodenum in the presence of food. Pancreatic enzymes include carbohydrases, lipases, nucleases, and proteolytic enzymes that can break down different drugs and components of food. These are secreted in an inactive form to prevent digestion of the pancreas itself. The enzymes become active once they reach the duodenum.

9.2.6 Structure and Composition of Intestinal Membrane

Drug absorption is ultimately the penetration of the drug across the intestinal membrane and its appearance unchanged in the blood draining the GIT. The term intestinal membrane is misleading since, as we commented earlier, this membrane is not a unicellular structure, but really a number of unicellular membranes parallel to one another and separated by aqueous fluid regions bounded by these membranes. Whatever the case, drug absorption implies a movement of drug molecules across biological membranes and, therefore, it could be useful to remember their basic structure and composition.

The biological membranes are bilipid layers. In a real cell the membrane phospholipids create a spherical three-dimensional lipid bilayer shell around the cell. The hydrophobic hydrocarbon chains of the phospholipids orient toward each other, creating a hydrophobic environment within the membrane. This leaves the charged phosphate groups facing out into the hydrophilic environment. The membrane is approximately 5 nm thick. This bilipid layer is semipermeable, meaning that some molecules are allowed to pass freely (diffuse) through the membrane. Molecules can diffuse through the membrane at differing rates depending on their ability to enter the hydrophobic interior of the membrane bilayer. The most accepted biological membrane model is referred to as the *fluid mosaic model* [1]. In this model lipid bilayers are fluid, and individual phospholipids diffuse rapidly throughout the two-dimensional surface of the membrane. These fluid bilayers include proteins, cholesterol, and other types of molecules besides phospholipids. Membrane proteins diffuse throughout the membrane in the same fashion, although at a slower pace because of their massive size (a phospholipid may be 650 daltons, and a medium sized protein can be 100,000 daltons). From time to time a given phospholipid will "flip-flop" through the membrane to the opposite side, but this is uncommon. To do so required the hydrophilic head of the phospholipid to pass fully through the highly hydrophobic interior of the membrane, and for the hydrophobic tails to be exposed to the aqueous environment.

There are also molecules of cholesterol embedded in the membrane. Cholesterol is a necessary component of biological membranes. Cholesterol breaks up the van der Waals interactions and close packing of the phospholipid tails. This disruption makes the membrane more fluid. Therefore, one way for a cell to control the fluidity of its membrane is by regulating its level of cholesterol in the cell membrane. Another way is to regulate the ratio of saturated to unsaturated hydrocarbon chains of the phospholipids. A group of phospholipids with saturated hydrocarbon chains can pack close together and form numerous van der Waals bonds that hold the phospholipids to each other. Phospholipids with unsaturated hydrocarbon side chains break up those van der Waals bonds and the tight packing by preventing the phospholipids from getting close together.

The cell membrane plays host to a large amount of protein, which is responsible for its various activities. The amount of protein differs between species and according to function; however, the typical amount in a cell membrane is 50%. These proteins are undoubtedly important to a cell. Three groups of membrane proteins can be identified:

1. *Integral Proteins.* They are located spanning the membrane and thus have a hydrophilic cytosolic domain that interacts with internal molecules, a hydrophobic membrane-spanning domain that anchors it within the cell membrane, and a hydro-

philic extracellular domain that interacts with external molecules. Examples of these integral proteins, also known as transmembrane proteins, are ion channels, proton pumps, transport proteins and efflux transporters, such as P-glycoprotein, and G protein-coupled receptors.

2. *Lipid Anchored Proteins.* They are located covalently bound to single or multiple lipid molecules, which hydrophobically insert into the cell membrane and anchor the protein. The protein itself is not in contact with the membrane. Examples are G proteins.

3. *Peripheral Proteins.* They are attached to integral proteins, or associated with the head groups of membrane lipids. As such, these proteins are not in contact with the hydrophobic membrane core and therefore associate only at the cytosolic and extracellular faces. They tend to have only temporary interactions with permanent membrane proteins, and once reacted, the molecule dissociates to carry on its work in the cytoplasm. Examples are some enzymes and some hormones.

It is also important to remember that the cell membrane, being exposed to the outside environment, is an important site of cell communication. As such, a large variety of protein receptors and identification proteins, such as antigens, are present on the surface of the membrane.

9.3 PATHWAYS OF DRUG ABSORPTION

For oral drugs to be therapeutically effective, they have to possess favorable characteristics to cross the biological membrane into the systemic circulation and reach the site of action: that is, they must be absorbed. Once a drug molecule is in solution, it has the possibility to be absorbed. At the intestinal epithelial cell surface, there are two pathways that are potentially available to molecules. Transepithelial transport of drugs can be achieved either across the cell (*transcellular* or *intracellular pathway*) or through the junctions that hold the cells together (*paracellular* or *intercellular pathway*) (Fig. 9.3). Historically, the transcellular pathway has received more

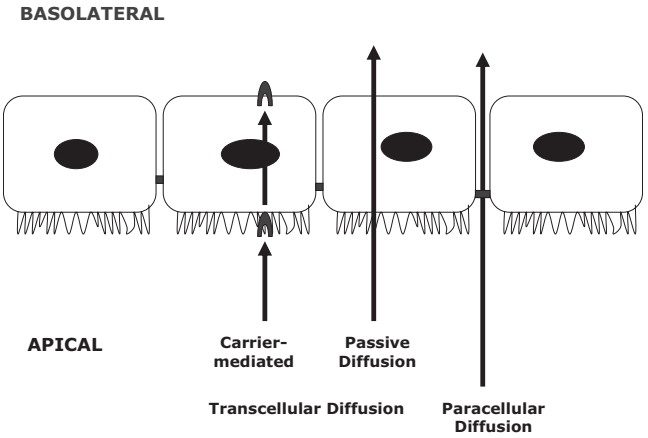

FIGURE 9.3 Schematic representation of the potential transcellular and paracellular pathways of drug intestinal absorption.

attention. However, the increasing understanding of tight junction physiology and regulation has shed light on the paracellular route.

9.3.1 Transcellular Pathway

In the transcellular or intracellular route, drugs are absorbed by passive diffusion, facilitated diffusion, or an active transport system (Fig. 9.3). Since these absorption mechanisms are discussed in Chapter 8, we will not dwell upon it in this chapter.

Passive diffusion (linear or first-order kinetics) is the predominant pathway taken by most oral drugs. Passive diffusion indicates that the transfer of a compound from an aqueous phase (intestinal lumen) through a membrane may be described by physicochemical laws and by the properties of the membrane. The driving force for diffusion across the membrane is the gradient concentration of the drug across the membrane. It is essential for a molecule to have characteristics with low molecular size and relatively high lipophilicity in order to pass across the intestinal membrane by this pathway. Factors influencing transcellular passive diffusion in the GIT are well studied and are reviewed here.

Facilitated diffusion is a process in which a molecule specifically and reversibly binds to its carrier protein in the enterocyte membrane. It crosses the membrane associated with the carrier and is then released to the cytoplasm of the cell. As with passive diffusion, facilitated diffusion can only occur down a gradient of chemical potential, since no energy source is required. The process follows the kinetic of enzymes, being saturable and subject to inhibition by competitive inhibitors. Actually, there are not many unequivocal examples of facilitated diffusion for drug molecules.

Active transport of drugs requires both membrane associated carrier proteins and an energy source, in order that the drug molecules could be transported up a gradient of concentration. Active transport satisfies the following criteria: (1) it is saturable, (2) metabolic inhibitors inhibit transport, and (3) substrate analogues compete with the drug for the active site on the carrier [2]. Absorption by specialized carrier mechanism from the small intestine has been shown to exist for several drugs having chemical structures that are very similar to essential nutrients for which the intestine has a specialized transport mechanism [3].

In direct contrast with specialized transport of a drug into epithelial cells is a process refered to as *efflux transport*, which implies the facilitated movement of a drug out of the cell. Today, it is well demonstrated that this mechanism exists in epithelial cells of the gastrointestinal tract. There is a cell surface glycoprotein (P-glycoprotein, P-gp) that is responsible for this mechanism. P-gp is believed to be responsible for poor intestinal uptake of several classes of compounds. The P-gp protein could be inhibited and the process may be saturated. The former is the basis for many drug–drug and drug–nutrients interactions, and the latter limits the efficacy of the transporter. Both situations may have repercussions in the intestinal absorption of drugs.

9.3.2 Paracellular Pathway

The paracellular route can be defined as the aqueous pathway along the intercellular space between adjacent cells, which is restricted by tight junctions (TJs) at the most apical part of the cells (Fig. 9.3). The aqueous nature of this route makes it favorable

for the absorption (through passive diffusion) of small hydrophilic solutes (nutrients, ions, etc.). TJs are dynamic structures, which normally regulate the trafficking of nutrients, medium sized compounds (≤15 Å), and relatively large amounts of fluids between the intestinal lumen and the submucosa [4]. Tight junction permeability differs according to the type of permeant involved, its size, and its charge. The molecular size cutoff for this route is approximately 500 Da [5].

Paracellular transport was belittled for much of the last century and was not considered an effective pathway until the 1960s, when it was discovered that molecules of different sizes can cross the epithelium via the paracellular route [6]. Today, it is well known that the flux across the TJs can be considerable, and that the permeability and selectivity of the junctions can be regulated [7]. Thus, in the last decades many efforts have been made to increase paracellular transport of less absorbable drugs—the use of enhancers being the most promising and studied strategy [8]. This topic is covered in Section 9.5, including an overview of the structure and function of this pathway.

9.4 FACTORS AFFECTING DRUG INTESTINAL ABSORPTION

Factors affecting gastrointestinal drug absorption have been classified into three broad categories. These have been summarized in Table 9.1. In the following, we analyze in a brief manner their influence on intestinal drug absorption.

9.4.1 Physicochemical Factors Affecting Gastrointestinal Drug Absorption

The primary physicochemical properties of a drug influencing absorption across gastrointestinal membrane are solubility, particle size, crystal form, lipophilicity, dissociation constant, and molecular weight.

Solubility Low aqueous solubility is a frequently encountered reason for poor oral absorption. Absorption requires that drug molecules be in solution at the absorption site. For that, drug molecules contained in the dosage forms must be delivered and then they must dissolve in the gastrointestinal fluids at the absorption site. Dissolution depends in part on the solubility of the drug. Together with solubility, the rate of dissolution is another variable to consider. In fact, there are cases of compounds with very low aqueous solubility, which have fully adequate oral bioavailability. This can occur because the rate of dissolution being a rapid phenomenon in spite of the overall solubility being low.

TABLE 9.1 Factor Affecting Gastrointestinal Drug Absorption

Physicochemical Factors	Physiological Factors	Technological Factors
Solubility	Gastric emptying and GIT motility	Pharmaceutical dosage form
Particle size	Degradation and excretion processes in the GIT	Manufacturing processes
Crystal form		
Lipophilicity		
Dissociation constant		

Polar solutes are more soluble in water than in organic media, whereas the opposite is true for nonpolar solutes. When the drug is ionizable, ionized species are more soluble in water than their un-ionized counterparts. On the other hand, solubility of weak acids or bases in aqueous medium is dependent on pH. For these compounds with acidic or basic functional groups, both solubility and rate of dissolution can be improved by suitable choice of salt forms.

The presence of charged groups can aid in solubility. However, charge, particularly high charge density, can have a negative impact on drug absorption since charged species do not readily penetrate the lipoidal membrane (*vide infra*). In such cases the reduction in charge by chemical modification can be a useful strategy. In other cases, charge also can have a negative impact on drug absorption by forming water-insoluble complexes or salts with the gastrointestinal contents.

Particle Size Surface area of drug particles influences the rate of drug dissolution and, therefore, drug absorption. Particle size determines the surface area of the solid. Small particles have greater surface area than larger particles and, consequently, they dissolve more rapidly. One important exception is constituted by the hydrophobic drugs. In this case, some examples have shown that the dissolution rate can increase with increasing particle size. For example, the rate of dissolution of the hydrophobic drug phenacetin increases as particle size increases from 0.11–0.15 mm to 0.50–0.71 mm [9]. It is probable that decreasing particle size of a hydrophobic drug actually decreases its effective surface area (i.e., the portion of surface without adsorbed air and actually in contact with dissolving fluid). In these cases, the addition of a surface-active agent significantly increases the effective surface area, improving the rate of dissolution and therefore the rate and extent of absorption.

Particle size appears to have little influence on the absorption of high aqueous solubility drug molecules. However, absorption of drugs such as griseofulvin, a molecule with low solubility in water, is highly dependent on the particle size. In these cases, strategies that increase particle surface area will result in improvement of drug absorption. The effective surface-area may be increased by physically reducing the particle size or by adding surface-active agents to the dosage form.

Crystal Form The physicochemical properties of crystal forms are influenced by the intermolecular forces present. Crystals with weak attractive forces between molecules exhibit greater solubility than those with strong attractive forces. In this sense, solubility could be dependent on the crystal form. There are a number of drugs with different crystal forms (polymorphs) and, in these cases, they can show differences in dissolution rates and, consequently, in absorption. For example, the rate of absorption of chloramphenicol appears to be directly related to the solubility of the different polymorphs of its palmitate ester [10].

Lipophilicity Gastrointestinal membranes are lipoidal in nature; therefore, they are more permeable to lipid-soluble drug substances. Lipid solubility of the diffusing species will influence, in part, passive diffusion across biological membranes. Lipid solubility of a drug is determined by the presence of nonpolar groups in the structure of the drug molecule, as well as by the presence of ionizable groups that are affected by local pH. It is logical to suppose that when an ionizable group exists in the drug molecule, it is very important, in order to improve lipophilicity, that this

group would be in the un-ionized state. High lipid solubility values must be accompanied, however, by adequate water solubility. When the water solubility is too low, a significant concentration of the drug molecule cannot be achieved at the membrane surface and absorption may be inefficient in spite of favorable lipid solubility.

The relative lipophilic to hydrophilic balance of the entire molecule can be described by the oil/water partition coefficient ($K_{o/w}$). In general, this parameter can be used as a predictor of the ability of a drug molecule to be absorbed by passive diffusion across lipoidal membranes. As $K_{o/w}$ increases, the rate of absorption increases. However, there are several exceptions to this general rule. Highly branched compounds are absorbed more slowly and small polar molecules more readily than would be expected based on their $K_{o/w}$ values.

The absorption of a hydrophilic drug may often be enhanced through appropriate structural modifications that increase $K_{o/w}$ of the compound (e.g., esterification). For example, esterification of one of the carboxylic groups of enalaprilat results in a significant increase in oral bioavailability [11].

Dissociation Constant The presence of ionizable groups in the drug molecule can condition the absorption across biological membranes. The importance of ionization in drug absorption is based on the observation that the nonionized form of the drug has a greater $K_{o/w}$ than the ionized form; therefore, the nonionized form of the drug in solution penetrates lipoidal membranes of the gastrointestinal tract more rapidly than the ionized species. The rate of absorption of an ionizable drug is therefore dependent on the concentration of its nonionized species at the absorption site, which is, as predicted by the Henderson–Hasselbalch equation, a function of the pK_a of the compound and the pH of the medium.

$$\text{For acidic drugs:} \quad f = \frac{1}{1+10^{(\text{pH}-pK_a)}} \tag{9.1}$$

$$\text{For basic drugs:} \quad f = \frac{1}{1+10^{(pK_a-\text{pH})}} \tag{9.2}$$

where f is the nonionized fraction.

The pH of the gastrointestinal tract ranges from 1.2 to 3.5 in the stomach, 5.0 to 6.0 in the duodenum, and 6.5 to 8.0 in the jejunum and large intestine. For acidic drugs with pK_a values between 2.5 and 7.5, the un-ionized fraction f decreases with increases in pH. The same is true for bases with pK_a values between 5 and 11.

A number of studies have related and quantified the influence of pH and pK_a on drug absorption in the gastrointestinal tract. These studies resulted in the so-called pH-partition theory. Briefly, this theory states that only the nonionized form of an ionizable drug is able to penetrate biological membranes because only this form has an adequate $K_{o/w}$. As a result, acidic drugs should best be absorbed from media with $\text{pH} < pK_a$, whereas basic compounds would best be absorbed from media with $\text{pH} > pK_a$. This does not mean that acidic drugs are best absorbed in the stomach and basic drugs best absorbed in small intestine. Even though the pH-partition theory provides a useful guide in predicting general trends in passive drug absorption, some

inconsistencies have been observed. The primary limitation of this theory derives from the assumption that only nonionized drug is absorbed, when in fact the ionized form of low to medium molecular weight drugs can be absorbed by passive diffusion through the pores (actually known as the paracellular route, *vide supra*), albeit at a slower rate. In fact, some models of intestinal drug absorption [12] propose that for ionized compounds, with low to medium molecular weight (below 250), diffusion through this route is the major contributor of absorption. For extremely low molecular weight, highly hydrophilic compounds, this route may be the only one. As the molecular weight and, consequently, the $K_{o/w}$ increase, passage through the transcellular route (i.e., through the lipoidal membrane) will predominate. For intermediate molecular weight drugs, diffusion using both routes will be possible.

9.4.2 Physiological Factors Affecting Gastrointestinal Drug Absorption

Once a drug molecule is in solution, it has the potential to be absorbed. However, there are a number of variables, other than the physicochemical ones, that can condition the absorption process. In the following sections we review the main physiological factors related to drug absorption.

Gastric Emptying and GIT Motility When an oral dosage form is swallowed by a patient, it travels through the GIT, starting this travel in the stomach. At this point, the dosage form must break down into small granules and particles and release the drug in order to facilitate its dissolution in the gastrointestinal fluids. Because the stomach has a reduced membrane surface area relative to small intestine, very frequently, the rate of drug absorption in this area is very low. So the rate at which the drug gets to the small intestine can condition the rate of absorption. Gastric emptying is the normal physiological process that controls the progression of the gastric contents toward the duodenum. Knowledge of the factors controlling gastric emptying is important because this process can control the rate of absorption in the small intestine.

There is a general consensus that gastric emptying (measured from the remaining volumes in the stomach after ingestion) follows first-order kinetics [13] especially when liquid or small volumes of semisolids are ingested. The half-life of the process ranges from 10 to 60 min in the case of fluids or semisolids, whereas this time increases up to 4 hours when nondisintegrating solids are ingested. Since, for the majority of the drugs, absorption occurs in the small intestine, the start of the absorption process will be conditioned by gastric emptying. When the intrinsic absorbability of the drug is elevated, gastric emptying become a rate-determining step of drug absorption. For example, paracetamol has good absorbability in the small intestine. Clements et al. [14] showed that, when this drug is orally administered, the absorption rate constant is highly correlated with the rate of gastric emptying.

Gastric emptying rate can be modified by several factors. For example, light physical activity stimulates gastric emptying, whereas strenuous exercise delays it. The volume of the ingested meal is another important factor conditioning gastric emptying. So Hunt and Macdonald [15] found that the half-life of gastric emptying increased from 7 min for a 50 mL standard meal to 50 min for a 1250 mL ingestion. The viscosity of the meal also conditions the rate of gastric emptying. Liquids abandon the stomach faster than semisolids. Nondigestible solids with size ≥ 2 mm

in diameter are handled by the stomach quite differently from liquids and will remain in the stomach for a long period (up to 12 hours). Concurrent drug therapy may also affect the rate of gastric emptying. Thus, cholinergic drugs increase the rate of emptying [16].

Delays in stomach emptying may decrease the rate of availability of a drug. The extent of availability, however, may be increased, decreased, or unaffected. For poorly water-soluble drugs, the extent of availability can be significantly reduced when the drug is ingested under fasting conditions. So Welling et al. [17] showed that erythromycin availability was reduced when 250 mg tablets were taken on a fasting stomach relative to that obtained when tablets were ingested after high fat, high protein, and high carbohydrate meals.

Concurrent drug therapy may increase or decrease intestinal motility. Such changes may increase, decrease, or have no effect on the extent of availability of the drug. So the concomitant use of metoclopramide, a drug that increases the gastrointestinal motility, can reduce the extent of availability of drugs with low values of rate of dissolution. For example, digoxin bioavailability is reduced in the presence of metoclopramide probably due to the increase in intestinal motility, so there is insufficient time for the release of digoxin from its dosage form and the subsequent dissolution in the gastrointestinal fluids before it abandons the GIT [18]. However, digoxin bioavailability increases in the presence of propantheline, a drug that slows gastrointestinal motility [18].

Degradation and Excretion Processes in the Gastrointestinal Tract Several processes in the GIT can reduce the extent of availability of a drug administered by the oral route. The main processes that can reduce the extent of availability are given in Table 9.2.

Some drugs can interact with endogenous or exogenous substances present in the gastrointestinal fluids, forming insoluble complexes. For example, biliary salts can interact with drugs such as neomycin and kanamycin, forming insoluble and nonabsorbable complexes.

Chemical degradation, especially pH-dependent reactions, can occur in the gastrointestinal fluids. Several polypeptides, nucleotides, or fatty acids may be susceptible to enzymatic degradation by several enzymes present in the gastrointestinal fluids. The role of the gastrointestinal microflora on the metabolism of drugs has long been recognized. In humans less than 1000 organisms/mL are usually found in the gastric juice. Only if the pH is high (>4) are relatively large numbers of bacteria found. The bacterial flora in the proximal jejunum is sparse, with increasing number in the distal small intestine. The number of organisms increases markedly in the

TABLE 9.2 Factors Reducing the Extent of Drug Absorption

Factors Acting in the Lumen of the GIT (Before Membrane Penetration)	Factors Acting After Membrane Penetration
Adsorption and nonsoluble complex formation	Gut wall metabolism
Chemical degradation	Gut secretion by efflux transporters
Enzymatic degradation	
Bacterial degradation	

large intestine. Microorganisms are capable of carrying out a multitude of reactions, and some of these reactions may have toxicological significance. Most of the metabolic transformations mediated by the microflora fall into the category of hydrolytic reactions, reactions involving the removal of various groups (e.g., dehydroxylations, descarboxylations, dealkylations) and reductive reactions.

Many drugs and toxic compounds that enter the body via the GIT suffer metabolism in the mucosal cells. In fact, enterocytes in the upper segment of the small intestine express, at high concentrations, two forms of cytochrome P450 (CYP3A4 and CYP3A5) that can potentially limit the availability of drugs [19]. Oxidation reactions mediated by CYP and also conjugation reactions play an important role in the metabolism of drugs in the small intestine [20]. For example, drugs such as midazolam or felodipine are subjected to an intense first-pass effect at the intestinal membrane [21, 22]. However, although it would seem that drug metabolism within the mucosal cells would serve only to reduce its extent of availability, in some cases, this process may enhance bioavailability. For example, clindamycin palmitate is a more stable form than the clindamycin HCl solution. The palmitate ester is rapidly hydrolyzed into the parent drug and its use increases the availability of clindamycin after oral administration [23]. Molecules such as clindamycin palmitate are chemical derivatives of drugs usually made to enhance the pharmaceutical properties (e.g., lipophilicity) of the parent molecule. These derivatives are called prodrugs.

Another recently described process that reduces the extent of bioavailability is the excretion process by efflux transporters in the small intestine. In this process, the drug—once it penetrates the intestinal membrane and is in the cytoplasm of the enterocyte—is subjected to a secretion process mediated by a transporter and is translocated, again, into the intestinal lumen. This process is mediated by an ATP-binding transmembrane transporter called P-glycoprotein. P-glycoprotein is a protein that belongs to the ATP-binding cassette (ABC) family. P-glycoprotein functions as a transmembrane efflux pump that translocates its substrates from its intracellular domain to its extracellular domain. P-glycoprotein is assumed to be one of the most important ABC transporters for drug disposition in humans. It is now established that P-glycoprotein is expressed constitutively in small intestine and in other organs. As a result of its anatomical localization, P-glycoprotein limits drug entry into the body after oral drug administration as a result of its expression in the luminal (apical) membrane of enterocytes [24].

Recently, it has been shown that enterocytes simultaneously express the major drug-metabolizing enzymes CYP3A and the efflux transporter P-glycoprotein [19]. This leads to a drug efflux–metabolism alliance, which increases the access of drug to metabolism by CYP3A4 through repeated cycles of absorption and efflux, further reducing the possibility of the drug reaching the systemic circulation unaltered.

9.4.3 Technological Factors Affecting Gastrointestinal Drug Absorption

As well as the physicochemical and physiological factors affecting intestinal drug absorption, there are technological variables that could affect the rate and extent of drug absorption. We have classified these variables into two subcategories: factors depending on pharmaceutical dosage form and manufacturing factors.

Pharmaceutical Dosage Form In considering, in a general manner, the availability of drugs from various classes of dosage forms, drugs administered in solution usually produce the most available drug product, assuming that the drug does not precipitate in the stomach and is not deactivated there. The second most available dosage form would be drug dispersed in a fine suspension, followed by micronized drug in a capsule, uncoated tablets, and finally by the coated tablets. In formulating and designing drug products, this ranking should be kept in mind.

The reason for this different availability is the difference in processes involved in drug release from the dosage form. Once the dosage form reaches the stomach, it must break down (if it is a solid dosage form) and release the therapeutic agent. Disintegration and dissolution are the key processes that precede the absorption process from a solid dosage form. As mentioned earlier, dissolution of the drug in the gastrointestinal fluid is the first step for drug absorption. The disintegration process increases the surface area of the dosage form and, in general, will increase the rate of dissolution. Disintegration is not a prerequisite when a pharmaceutical suspension or solution is employed.

Release of the therapeutic agent can be affected by the nonactive ingredients included in the dosage form. For example, for drugs formulated as tablets, diluents, disintegrants, binders, lubricants, surfactants, and even colorants can affect the release of the drug from the pharmaceutical dosage form as measured by the rate of dissolution in *in vitro* tests. Their proper choice becomes more critical when formulating water-insoluble drugs and when the total concentration of the drug in the dosage form is small.

Manufacturing Processes In addition to the above-mentioned variables, the characteristics and processing of the dosage form could have great influence on drug availability. Variables such as process of granulation method or compressional force in production of tablets can significantly affect the bioavailability of the drug [25].

9.5 INTESTINAL PERMEABILITY ENHANCEMENT: POSSIBILITIES AND LIMITATIONS

In the last decade incredible advances have been made in the application of molecular biology and biotechnology, which have led to a revolution in the development of new therapeutic compounds. These new drugs must be properly delivered in the body to have the desired pharmacological effect. In this challenging task, it is essential to consider the biopharmaceutical and pharmacokinetic properties of the drug, particularly those concerning intestinal absorption and bioavailability (BA) after oral administration. The oral route is the preferred administration route, and it must be taken into account that the newer promising drugs, especially peptides and proteins, cannot be developed as oral products because of their null or scarce bioavailability. In general, these are hydrophilic compounds, of medium to high molecular weight, and sometimes containing strongly charged functional groups—implying that transport across the intestinal barrier occurs essentially via the paracellular pathway [26]. The contribution of this pathway to intestinal absorption is considered to be small, since this pathway occupies less than 0.1% of the total surface area of

the intestinal epithelium [27], and the presence of tight junctions (TJs) between the epithelial cells limits drug absorption. Therefore, for the above-mentioned drugs, the main cause of low bioavailability is their poor intestinal permeability. Thus, considerable attention has been directed at finding ways to increase the paracellular transport of these compounds—the use of enhancers being the most promising and studied strategy [8, 28]. In recent years, knowledge of tight junction physiology and regulation has increased [29], which has facilitated the search for compounds capable of enhancing absorption via the paracellular pathway. To obtain maximum benefit from such enhancing compounds, it is necessary to confirm that reduced membrane permeability is the cause (or at least the main cause) of poor drug bioavailability. Moreover, the activity of an absorption enhancer should be inmediate and should coincide with the presence of the drug at the absorption site [8, 30]. In these cases, an increase in intestinal absorption would be achieved, leading to an enhancement in drug bioavailability, which in turn would allow oral administration of the drug and also a reduction in inter- and intrasubject variability in plasma concentrations and therefore in therapeutic effects.

In this section, we analyze the possibilities and limitations of the most promising intestinal paracellular enhancers. The basic anatomic and physiological properties of the paracellular route are first examined in order to better understand the absorption process, which could help in the selection of the appropriate enhancer. Interest has focused on medium chain fatty acids and chitosan and its derivatives, since these are the most studied and effective enhancers. In fact, the medium chain fatty acid sodium caprate is being used as an absorption enhancer in the clinical setting in Japan, Denmark, and Sweden. There have been no reports of serious side effects [31–33]. Additionally, the results obtained with the more recent paracellular enhancers are presented. Previous reviews are recommended for in-depth insight into this topic [4, 8, 28, 34].

9.5.1 Structure and Function of the Intestinal Paracellular Pathway

The junctional complex found in the apical portion of adjacent cells is composed of three distinct regions: tight junctions or zonula occludens, zonula adherens, and macula adherens or desmosome (Fig. 9.4). Paracellular permeability is primarily regulated by the TJs, because this is the rate-limiting barrier of the transport pathway. TJs have been described as gates (selectively allowing the passage of small hydrophilic compounds) and as fences (forming an intermembrane diffusion barrier maintaining enterocyte polarity and excluding potentially toxic molecules). These two functions are not separate unrelated phenomena [29]. It is becoming increasingly clear that TJs in themselves constitute the product of a global polarizing process; their role therefore does not seem to correspond to that of a simple fence [35].

In the last years some light has been shed on the architecture and regulation of TJs. Because a complete review of TJ structure, function, and molecular regulation is beyond the scope of this chapter, and has previously been done [35–38], only a brief summary of the more relevant aspects of the subject and its repercussions on paracellular permeability are presented here.

Under the transmission electron microscope, tight junctions appear as a series of focal contacts between the plasma membrane of two adjacent cells. In early freeze-fracture electron microscopy sections, the tight junctional complex appeared as a

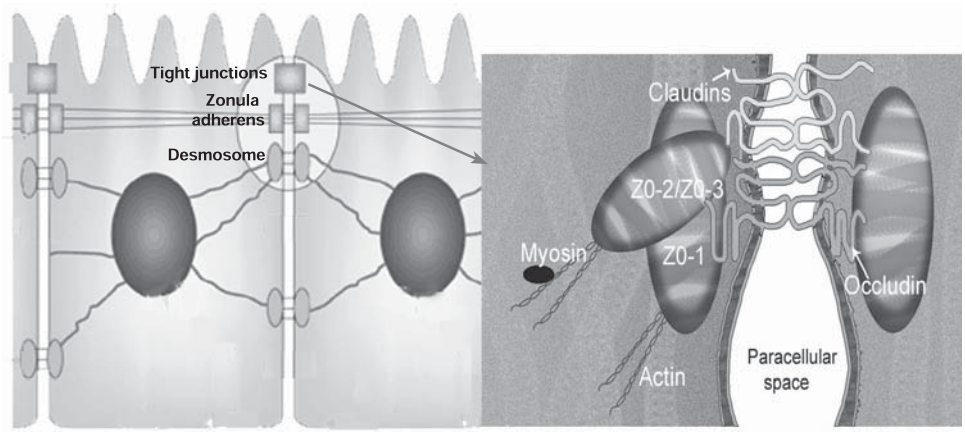

FIGURE 9.4 (Left) The functional complex located in the apical part of adjacent enterocytes (tight junctions, zonula adherens, and desmosomes) is depicted. (Right) Schematic representation of the protein interactions at tight junctions. The interaction is only represented in one part of the illustration. Other proteins have been localized to the cytoplasmic surface of tight junctions, although they are not represented. For more information see the text. (From Ref. 34, with permission.)

dense network of interdigitating strands or fibrils in the plane of the plasma membrane [39]. At some points the strands showed discontinuities, which might correspond to "pores" [40], and which would imply channel-like permeability (i.e., fluctuating aqueous pores embedded in the fibrils would account for diffusion through TJs). This early concept has progressed little and it is presently also postulated that fibrils on one cell interact with fibrils on an adjacent cell to seal the paracellular space and define the permeability characteristics [36]. To date, the commonly held view is that increases in paracellular permeability under perturbed or pathological conditions result from the dilatation of existing tight junction pores [41]. In contrast, the composition of the fibrils is now well known. Fibrils are formed by at least two types of tetra-spanning transmembrane proteins: occludin and claudin (Fig. 9.4). Occludin is a 65 kDa phosphoprotein [42–44]. The findings reported to date are consistent with a functional role for occludin in defining the barrier [45, 46]. Claudins are a family of proteins, named from the Latin *claudere*, "to close." They appear to represent the major structural components of tight junction strands [47]. Recent studies have proposed that claudins are the pore-forming structures in TJs [48, 49]—thus strongly supporting the idea that claudins confer specific selectivity to paracellular transport. Furthermore, a dense cytoplasmic network of proteins has been described at the TJs. These are referred to as tight junction associated proteins (TJAPs), and are designated ZO-1, ZO-2, and ZO-3. As can be seen in Fig. 9.4, these proteins interact among each other (ZO-1 and ZO-2 are bound to each other and to 130 kDa protein, ZO-3) and also serve as a link between occludin and the actin filaments of the cytoskeleton [28]. The association of TJs with the apical perijunctional actomyosin ring seems to regulate global TJ permeability [38]. It is also known that the barrier assembly and permeability characteristics of TJs are influenced by many cellular signaling mechanisms—although these remain largely

undefined [50]. Therefore, despite rapid progress in knowledge of TJ structure and molecular physiology, their function in the context of paracellular permeability is still far from fully clear.

In summary, evidence now exists to suggest that the regulation of paracellular permeability by the TJ is a complex process, illustrated by the diversity in functional reactions of this structure. At present, apart from conventional permeability studies, several probes have become available for detecting changes in activation of intracellular proteins, such as the protein kinase C isotypes. Moreover, *in situ* imaging techniques are being used [37]. Studies in the field of diseases originated by paracellular permeability alterations (food allergies, malabsorption syndromes, and inflammatory bowel diseases such as Crohn's disease and ulcerative colitis) also contribute to our understanding of paracellular transport. In future, all such intense research can contribute to secure in-depth knowledge of the control mechanisms implicated in paracellular transport and its modulation, which may have great repercussions for drug delivery.

9.5.2 Intestinal Paracellular Enhancers

As already outlined, attempts to reduce the absorption barrier are mainly based on the use of paracellular permeation enhancers as auxiliary agents in oral drug delivery systems [8]. Before presenting the potential compounds that could be used as enhancers, several general aspects are considered.

General Considerations According to Aungst [8] there are several critical issues to consider in the selection of a compound as a potential absorption enhancer for use in drug delivery: the degree of bioavailability enhancement achieved, the influence of formulation and physiological variables, the possible toxicity originated by the enhancing action, and the mechanism of permeation enhancement.

On the other hand, once the enhancer has been selected it should be highlighted that the efficacy of absorption enhancer, in terms of intensity and duration of its effect, depends on the concentration at its site of action. Therefore, it is necessary to have the means to control this concentration or at least to be able to regulate it within a certain effective concentration range. This, nevertheless, might be difficult to achieve in some cases, since many variables, as outlined before, influence the actual concentration of drug and/or enhancer at a specific site in the GIT. Alternatively, the application of controlled release dosage forms could be more effective in maintaining concentrations of enhancer in the effective range [51].

The vast majority of data published on the use of intestinal paracellular enhancers have been obtained using *in vitro* and *in situ* methodologies. The possibilities and limitations of these techniques are evaluated in the following section. The effectiveness of any compound for enhancing intestinal permeation must also be assessed by *in vivo* studies. The degree of bioavailability enhancement, as well as the effect on C_{max} and the area under the curve (AUC), are the indicators most commonly used in studies of this kind. The *in vivo* setting clearly constitutes a more complex and dynamic environment, which hampers assessment of the promoting effect. Furthermore, the formulation used to administer the drug and promoter (solution, capsules, microcapsules, enteric-coated capsules, suppositories) could influence the efficacy of an absorption enhancing excipient. Similarly, the route of

administration used (peroral, intrajejunal, intracolonic, rectal) is directly related to the success of oral drug bioavailability enhancement. For more information on the factors influencing the *in vivo* performance of permeation enhancers, readers are referred to the reviews by Aungst [8] and Ward et al. [28].

Medium Chain Fatty Acids (MCFAs) Based on research conducted in the last decade, it has become clear that several sodium salts of medium chain fatty acids (caprylate C8 (CH_3—$(CH_2)_6$—COOH), caprate C10 (CH_3—$(CH_2)_8$—COOH), and laurate C12 (CH_3—$(CH_2)_{10}$—COOH)) are able to enhance the paracellular permeability of hydrophilic compounds. Lindmark et al. [52] carried out a comparative study with these three fatty acids and sodium caproate (C6). They showed that C8, C10, and C12 (but not C6) exhibit dose-dependent enhancing effects on mannitol transport across cell monolayers—with C12 being the most effective enhancer. Interestingly, the lowest concentrations to enhance transport of the marker molecule were in the vicinity of their critical micellar concentration (CMC), which in turn differs considerably for each.

Among these MCFAs, sodium caprate is the most extensively studied and the only absorption-enhancing agent included in a marketed drug product. It is added in a suppository formulation intended for human use in Sweden and Japan [33]. Since this fatty acid has a low molecular weight, it could be absorbed from the intestine even more quickly than the drug itself [8]. The numerous studies conducted with C10 have addressed different aspects such as its enhancing effect on the permeability of compounds with different molecular weights (MWs), its concentration and time-dependent effects, and its toxicity and mechanism of action.

Most published data on *sodium caprate* as an absorption enhancer have been obtained using *in vitro* and *in situ* techniques. Collectively, the results obtained using these methodologies indicate that C10 can enhance intestinal permeability of low (MW 180–400) and high (MW 4000–19,000) molecular weight *marker molecules* [31, 52–56]. However, the authors point out that the effect would be significant only for substances of MW ≤ 1200 g/mol; that is, in the case of larger molecules, permeability enhancement would not result in a significant increase in the dose fraction absorbed [57]. Regarding the effects on *drug* permeability, sodium caprate enhances intestinal permeability of cefmetazole [58], ebitaride (a pentapeptide ACTH analogue) [57], peptide drugs [53, 59], and epirubicin, an anticancer drug [60]. Nevertheless, it is worth pointing out that Raiman et al. [61] have recently found that C10 (10 mM) does not affect the permeability of the bisphosphonate drug clodronate across Caco-2 cell monolayers.

In all of the studies just mentioned, the sodium caprate concentration used was in the vicinity of its CMC (i.e., 13 mM). Several research groups, using Caco-2 cells, have shown the C10 enhancement effect on membrane permeability to be concentration dependent [31, 52, 53]. We have also recently observed this *concentration-dependent effect in vitro* (Caco-2 cell) using acamprosate, an alcoholic antirelapse drug (unpublished data). However, when we performed the experiments *in situ* [62] and *in vivo* with rats, this effect disappeared. Our results agree with those obtained *in vivo* by Raoof et al. [63] in pigs. According to their data, the enhancing capacity of C10 is dose independent.

Regarding the *time-dependent effect* of C10 on intestinal permeability, studies carried out by Anderberg et al. [54], using Caco-2 cells, showed significant time-

dependent effects at 13 mM and higher concentrations (16 mM). These experiments were performed after long incubations (approximately 1 h) with C10. Since the immediate effect of the enhancer is presumably more relevant than the long-term effect *in vivo*, Lindmark et al. [32], in Caco-2 cell monolayers, determined both the long- and short-term effects. According to their results, the time-dependent effect of sodium caprate 13 mM upon permeability can be separated into two phases: an initial phase (10–20 min) in which a rapid increase in permeability was observed, and a later phase characterized by a slow but more prolonged enhancement in permeability. Kamm et al. [59] have also shown C10 absorption enhancement to be markedly dependent on incubation time in Caco-2 cells.

The local *toxicity* of sodium caprate in the small intestine is one of the main concerns with use of this fatty acid in pharmaceutical products. The toxicity of C10 has been extensively studied *in vitro*. Considering that cytotoxicity depends on the concentration and duration of exposure, comparisons among results obtained in experiments using different protocols may not always be valid. Cell damage can be assessed by several methods (morphological observations, the release of biological markers, and the recovery of transepithelial electrical resistance (TEER))—a fact that further complicates the drawing of firm conclusions. A closer examination of the reported data suggests that C10 at effective concentrations (around 13 mM) does not affect epithelial viability [64] and does not cause serious cytotoxicity—its effects moreover being reversible [53, 55, 65, 66].

The studies performed *in vivo* with sodium caprate are not as numerous as those using *in vitro* and *in situ* techniques. The drugs involved in these investigations have been peptides, antibiotics, and polar, high MW *drugs* such as antisense oligonucleotides or glycyrrhizin. When the drug and enhancer are administered as a solution, the effective C10 doses in the reported studies are comparable. Closer examination shows that all these doses are in the range of 0.1–0.5 mmol/kg. Curiously, when these amounts are transformed into concentrations, the values obtained are in some cases quite different and clearly higher (25–1000 mM) than those tested in the *in vitro* models [34]. Most investigations have been performed in rats, although dogs, pigs, rabbits, and even humans have also been used [34]. As a whole, it can be concluded that this medium chain fatty acid is capable of improving oral drug bioavailability. However, it must be stressed that only two studies have been carried out to date in humans, and no effect was reported in one of them [67].

It is worth mentioning that two studies have compared the *in vivo* enhancing effect of several medium chain fatty acids [68, 69], concluding that the strength of this effect is in the following order: caprate > laurate > caprylate. Hence, sodium caprate seems to be the most potent promoter among the MCFAs tested.

In vivo studies analyzing the toxicity of C10 are scarce but in concordance. This fatty acid, at the doses tested, was well tolerated by the intestinal mucosa, and no membrane damage was observed [53, 63, 68].

In conclusion, sodium caprate can be considered a promising agent for use as an enhancing excipient in drug delivery. The C10 effect is dependent on its permeability in the tissue upon which it is required to act. Further studies are needed to confirm its null or low toxicity, and to evaluate its enhancing efficacy for each particular drug substance.

Chitosan and Its Derivatives High MW polymers such as chitosan and its derivatives have gained considerable attention as permeation enhancers. Because of their

high MW, these polymers are supposedly not absorbed from the gut, and systemic side effects are thus excluded. In addition, prolonged localization in the mucosa is ensured, which in turn would prolong the promoting effect. Chitosan and its derivatives are also of great interest as excipients and drug carriers in the pharmaceutical field, due to their biodegradability, biocompatibility, and recent FDA application [34].

Chitosan is a partially deacetylated form of chitin, which is present in crustacean shells, insects, and fungi, as well as in some microorganisms (Fig. 9.5). Chitosan is actually a denomination describing a series of polymers with different molecular weights (from 50 to 2000 kDa) and degrees of deacetylation. These two factors are very important for the physicochemical properties of chitosan and thus exert a major impact on its promoting effects (as discussed later).

The effect of chitosan on intestinal permeability across tight junctions was first reported by Artursson et al. [70]. These authors found chitosan glutamate to enhance ^{14}C-mannitol transport *in vitro* (Caco-2 cell monolayers). Posteriorly, it was

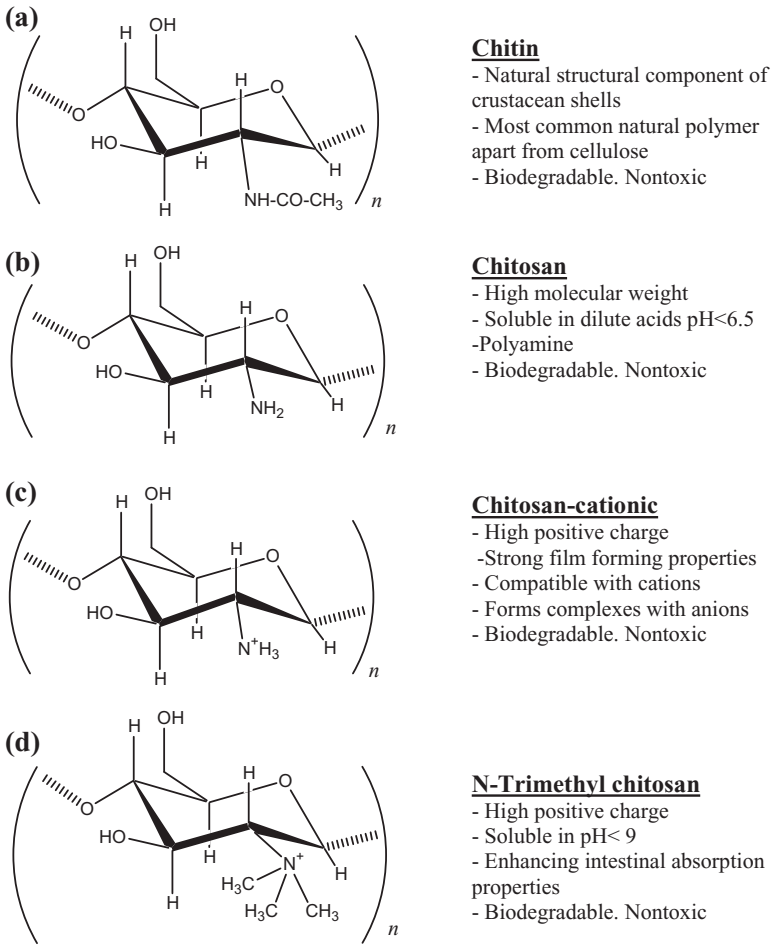

FIGURE 9.5 Chemical structures and properties of chitosan and its derivatives. (From Ref. 34, with permission.)

confirmed that chitosan hydrochloride and chitosan glutamate, in a slightly acidic environment, increase the permeation of low molecular weight *markers* as well as of large hydrophilic compounds (PEG-4000 and fluorescein dextrans) in intestinal epithelial cells. It was also found that both salts are insoluble at pH 7.4 and prove to be ineffective as permeation enhancers [71, 72].

Regarding the effect of chitosan salts on *drug* intestinal permeability *in vitro*, there is general agreement that these polymers are potent absorption enhancers for poorly absorbed drugs such as atenolol and peptide drugs. Studies with buserelin, 9-deglycinamide 8-arginine vasopressin (DGAVP), and insulin have evidenced a strong increase in the transport of these drugs in the presence of chitosan glutamate and chitosan hydrochloride (acidic environment) [34].

Schipper et al. [73] reported that the molecular weight and degree of deacetylation of chitosans influence their effects on Caco-2 permeability and cytotoxicity. Accordingly, one chitosan with an intermediate degree of deacetylation and high molecular weight had good enhancing characteristics and low cytotoxicity. Chitosan toxicity has been further evaluated by other researchers based on trypan blue exclusion studies and confocal laser scanning microscopy [71]. No deleterious effects on the cells were demonstrated. Thus, the general opinion regarding chitosan salt toxicity *in vitro* appears to be that it offers a very safe toxicity profile.

In vivo studies of the enhancing effects of chitosan on intestinal absorption are scarce but concordant. Chitosan hydrochloride has been observed to increase drug (buserelin, octreotide acetate) bioavalibility in rats and pigs in an acidic environment [34]. Hence, the chitosan pH-dependent effect observed *in vitro* was also confirmed *in vivo*. The explanation for this phenomenon is based on the fact that chitosan, being a weak base, loses its positive charge in neutral and basic media—thus proving to be ineffective as an absorption enhancer [74].

To overcome the solubility limitations of chitosans at neutral and basic pH, *N-trimethyl chitosan chloride* (*TMC*) *derivatives* (Fig. 9.5d) are obtained. These derivatives display much greater aqueous solubility than chitosan at neutral and alkaline pH values [75]. Consequently, a great volume of data have been published on the intestinal enhancing properties of these polymers.

In vitro, TMC derivatives are especially effective in enhancing the transport of small hydrophilic compounds (e.g., mannitol), although they also improve the transport of large molecules (drugs) such as buserelin, insulin, DGAVP, and octreotide acetate [34]. Throughout the multiple studies performed *in vitro*, the researchers have abundantly shown the degree of quaternization to play an important role in the absorption-enhancing properties of these polymers, especially in neutral and basic environments. There is general agreement that the promoting effect of TMC derivatives increases with an increase in their degree of quaternization [34]. Discrepancies exist regarding the optimum degree of quaternization. Thus, maximum absorption enhancement of mannitol and buserelin was recorded with TMC 60 (degree of quaternization 60%) [31, 76]. However, it has recently been reported that the best and maximum permeation-enhancing results are achieved with TMC 49 (degree of quaternization 48.8%) [77, 78]. The few *in vivo* studies reported to date are relatively recent and have been conducted using TMC derivatives with a high degree of quaternization (TMC 40 and TMC 60), and involving the same *drugs* tested *in vitro*—that is, buserelin and octreotide acetate [31, 79]. All these data reveal increased drug bioavailability when the drug is administered intraduodenally in rats

and pigs, with TMC 40 and TMC 60 at neutral pH values. Moreover, a concentration-dependent effect of these polymers was observed [79]. No toxicity studies have been presented to date.

TMC derivatives are only suitable for improving the intestinal absorption of macromolecular therapeutic agents with neutral or basic properties due to its positive charge. Hence, when chitosan salts and TMC were evaluated for their compatibility with low molecular weight heparin (LMWH), a highly anionic polysaccharide, strong aggregation was observed with subsequent fiber formation and precipitation. To overcome this problem, another chitosan derivative has been synthesized: *mono-N-carboxymethyl chitosan* (*MCC*) [80]. This polymer is perfectly soluble in aqueous environments at neutral and alkaline pH values and is compatible with anionic and neutral compounds. MCC was evaluated as a potential absorption enhancer of LMWH *in vitro* (Caco-2 cell monolayers) and *in vivo* (rats) [80]. Both the *in vitro* and *in vivo* results indicated that MCC derivatives are capable of significantly increasing the intestinal absorption of LMWH. Moreover, these polymers were seen to present nontoxic characteristics. The authors concluded that carboxymethyl modifications of chitosan may be suitable absorption enhancers for the peroral delivery of anionic macromolecules.

In summary, it can be concluded that chitosan and its derivatives are promising excipients for use as enhancers in the peroral delivery of poorly absorbed drugs.

Zonula Occludens Toxin (Zot) The great interest in the peroral administration of poorly absorbing therapeutic agents has led to the development of innovative strategies. This is the case, for instance, of *zonula occludens toxin* (Zot), a 45 kDa protein elaborated by *Vibrio cholerae* that is able to reversibly regulate tight junction permeability [81]. This toxin interacts with a specific intestinal epithelial surface receptor, with subsequent activation of a complex cascade of intracellular events that lead to a protein kinase C-dependent polymerization of actin microfilaments strategically localized to regulate the paracellular pathway, and consequently leading to opening of the TJs at a toxin concentration as low as 1.1×10^{-13} M [82, 83]. The potential of Zot to enhance the paracellular transport of marker compounds and drugs was first investigated by Fasano et al. [84]. These authors showed that Zot reversibly enhances rabbit intestinal permeability to insulin *in vitro* in the jejunum and ileum, although no substantial changes were detected in the colon. Posteriorly, Cox et al. [85] demonstrated that Zot enhances transport across Caco-2 cell monolayers of low and *high molecular weight markers*. It was also shown that the *in vitro* permeabilities of drugs with low oral bioavailability such as paclitaxel, acyclovir, and cyclosporine and enamione anticonvulsants were increased with Zot. Furthermore, the enhancing properties of Zot were found to be reversible and nontoxic [81–85]. Recent studies have identified a smaller 12 kDa fragment of Zot, referred to as ΔG [86]—this fragment being responsible for the intrinsic tight junction modulation activity. In 2003, Salama et al. [87] reported that this biologically active fragment (ΔG) is able to increase mannitol permeability across Caco-2 cell monolayers. ΔG was found to be noncytotoxic at the concentration tested. These authors also examined the *in vivo* effect of ΔG using the rat as animal model. When mannitol was administered intraduodenally with ΔG only, no significant differences were observed in terms of the pharmacokinetic parameters. However, when the active fragment was used in the presence of protease inhibitors (PIs), significant increases were

obtained for C_{max} and AUC of mannitol. Thus, protease inhibitors are necessary to minimize ΔG enzymatic degradation secondary to proteases/peptidases. Furthemore, ΔG was also able to increase the AUC and C_{max} for macromolecules [82]. Recently, it has been reported that ΔG significantly increased the *in vivo* oral absorption of some low bioavailable *drugs* (cyclosporin A, ritonavir, saquinavir, and acyclovir) in the presence of protease inhibitors [88]. In the opinion of the authors, these studies illustrate the potential usefulness of ΔG in enhancing oral drug delivery.

Thiolated Polymers An alternative class of permeation enhancers is represented by *thiolated polymers*—also called thiomers. These are polymers in which the thiol groups are covalently bound. It has been shown that polycarbophyl polymers (PCP) display permeation-enhancing effects [89]. This property, however, could be significantly improved as a result of the covalent attachment of cysteine (Cys) to this polymer (*PCP-Cys*) (Fig 9.6a), as has recently been shown by Clausen and Bernkop-

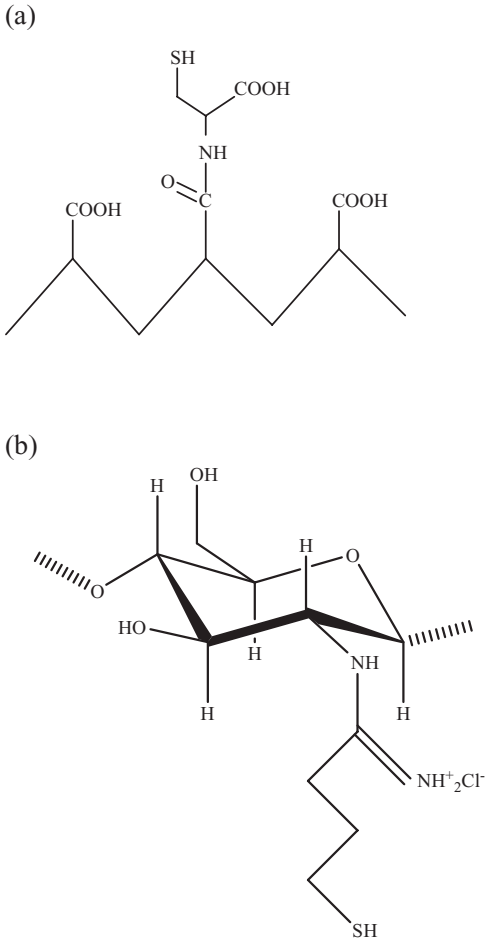

FIGURE 9.6 Chemical structure of (a) polycarbophyl-cysteine conjugate and (b) chitosan-4-thio-butylamidine. (From Ref. 34, with permission.)

Schnürch [90]. Accordingly, this thiolated polymer (PCP-Cys) is able to significantly increase the transport of *marker compounds* (sodium fluorescein) and *peptide drugs* (bacitracin-fluorescein isothiocyanate and insulin-fluorescein isothiocyanate) across the intestinal mucosa of guinea pigs (*in vitro* studies). The thiol groups, covalently attached to the polymer, seem to be responsible for the improved permeation-enhancing properties of these conjugates. Furthermore, the improved mucoadhesive properties of these polymers provide a somewhat prolonged residence time, which in some cases can lead to additional improvement in drug bioavailability. They are not absorbed from the GIT due to the high molecular mass. Based on all these features, the authors concluded that the thiolated polymers could constitute a promising excipient for the development of peptide drug delivery systems. Posteriorly, this same research group explored the mechanism of action of thiolated PCP-Cys in depth [91], showing that these compounds exert their permeation-enhancing effects via glutathione. It seems that PCP-Cys can transform oxidized glutathione (GSSG) into reduced glutathione (GSH), prolonging GSH concentration at the apical membrane. GSH is reportedly capable of inhibiting protein tyrosine phosphatase (PTP) activity by almost 100%, which results in a higher extent of phosphorylated tyrosine groups on the extracellular loops of the membrane protein occludin, leading to the opennig of the TJs [92].

Other thiolated polymers such as sodium carboxymethylcellulose-cysteine, chitosan-cysteine and chitosan-4-thio-butylamidine (chitosan-TBA) (Fig. 9.6b) [34] have also displayed permeation-enhancing properties for hydrophilic compounds and drugs when evaluated using *in vitro* techniques. The addition of the permeation mediator GSH improves this effect. This association is referred to as a *thiomer/GSH system* [93]. Different thiomer/glutathione systems have been used depending on the hydrophilic macromolecular drugs tested. Hence, for salmon calcitonin, a peptide drug of net cationic charge, chitosan-TBA was used. In contrast, insulin and low molecular weight heparin are anionic drugs—thus being incompatible with chitosan. In these cases, an anionic thiolated polymer—poly(acrylic acid)—cysteine conjugate—was used. When *in vivo* experiments were performed, a significantly improved pharmacological efficacy/bioavailability was achieved by using these systems. These results are in good agreement with those obtained *in vitro*. The authors concluded that due to their high efficacy and minimal toxicological risks, the thiomer/GSH systems represent a promising new generation of oral permeation-enhancing delivery systems for hydrophilic macromolecules [93]. Recently, the same research group has shown that the combination of thiomer/GSH system with bromelain, a proteolytic enzyme, represents a promising strategy in order to raise the *in vivo* efficacy of orally administered hydrophilic macromolecular drugs [92].

Nitric Oxide (NO) Donors In 1995, Salzman et al. [94] showed that sodium nitroprusside (SNP), an NO donor, induced a concentration-dependent increase in fluorescein sulfonic acid transport in Caco-2 cell monolayers. Similarly, Utoguchi et al. [95] reported that *S*-nitroso-*N*-acetyl-penicillamine (SNAP), another NO donor, was able to greatly enhance the rectal absorption of insulin—this effect being concentration dependent. Posteriorly, Yamamoto et al. [96] also observed this absorption-enhancing effect on 5(6)-carboxyfluorescein transport with other NO donors such as NO5 (3-(2-hydroxy-1-methylethyl)-2-nitrosohydrazino)-1-propanamine) and NO12 (*N*-ethyl-2-(1-ethyl-hydroxy-2-nitrosohydrazino)-ethanamine), using an *in vitro*

Ussing chamber method and the rat jejunum and colon. Regional differences in the promoting effect of NO12 were found (colon > jejunum). Their findings also demonstrated NO12 action to be mediated by nitric oxide, with partial inclusion of dilatation of tight junctions in the epithelium. As these agents exhibit low toxicity, the authors suggested that NO donors may be useful for enhancing the intestinal absorption of poorly absorbing drugs.

9.5.3 Technologies in Development

Gastrointestinal Permeation Enhancement Technology™ (GIPET™) is a proprietary solid-dose/microemulsion-based medium-chain fatty acid technology by Merrion Pharmaceuticals. In the first format of GIPET (GIPET I), enteric-coated tablets, comprising a pH-sensitive coating and a drug, were synthesized. The second variation of the technology (GIPET II) consisted of microemulsions of mono- and diglyceride mixtures of C8 and C10 entrapped with the drug in an enteric-coated soft gel capsule. GIPET I and II have been tested orally in rats, dogs, and humans, primarily to establish safety profiles but also to demonstrate efficay. Afterwards, these formulations have shown efficacy in human Phase I oral delivery studies of drugs comprising both single- and repeat-dosing regimes. Oral bioavailability of alendronate, desmopressin, and low molecular weight heparin in humans was increased using GIPET formulations compared with unformulated controls. Importantly, Phase I trials revealed no toxicity of concern and this was also observed in subjects receiving multiples doses of GIPET. The absorption-promoting effects of GIPET are transient and complete in less than 1 hour as shown in additional human studies [97]. According to the researchers involved in these studies, GIPET formulations have genuine potential as platform technology for safe and effective oral drug delivery of a wide range of poorly permeable drugs.

9.5.4 Limitations of Intestinal Promotion of Drugs

In 1995, Amidon et al. [98] reported the biopharmaceutical classification system (BCS) for oral delivery of inmediate release products [98], which was adopted by the FDA in 2000. The major outcome of this classification was to group major drug classes according to whether they had oral delivery issues related to solubility or permeability issues, neither of these issues, or both. So drugs that are soluble but poorly absorbed, including most peptides and newer drugs (Class III), are amenable to epithelial permeation enhancement. As outlined earlier, over the past fifteen years many attempts to promote oral absorption of poorly absorbed drugs (Class III) have been carried out but, unfortunately, some of them have failed. It must be highlighted that achieving a sucessful oral formulation with enhancers for a poorly absorbed drug implies that there is access to the appropiate intestinal region for a sufficient amount of time, release of intact soluble drug and enhancer, and an acceptable but reversible degree of epithelial cell permeability enhacement. Bearing this in mind, the limitations for paracellular permeation enhancement by oral formulation include the inability to deliver therapeutic levels over a sustained period, the requirement for massive amounts of material, and safety aspects regarding the long-term integrity of the intestinal epithelium. Additional limitations include the inability to follow through with practical and reproducible solid dose formulations in

scale-up manufacturing. Once the targeted pharmacokinetic and pharmacodynamic profile is achieved in humans, the formulation must have a safety profile to allow it to be given to patients on a repeated basis.

9.6 METHODOLOGIES FOR STUDYING INTESTINAL ABSORPTION

Assessments of rate, extent, site, and mechanism of intestinal absorption have been performed by a variety of experimental techniques in humans and animals with *in vitro*, *in situ*, and *in vivo* preparations. Details of the most common methodologies currently in use in experimental animals are given next, along with a discussion of the utility and limitations of each. It is the judicious use of these techniques that can help identify drug candidates that will be absorbed in humans. It is well recognized that human intestinal permeability cannot be accurately predicted based on a single methodology (*in vitro*, *in situ*, or *in vivo*) [99, 100] since each kind of method covers only some of the factors involved in the intestinal absorption and does not take into account other factors that may be important. Moreover, it must be highlighted that although animals provide the opportunity to gain much valuable information through screening, investigators must remain aware that the results cannot be extrapolated directly and exactly to humans.

9.6.1 *In Vitro* Techniques

Different *in vitro* methods utilizing tissues from rodents and other species have been developed to screen compounds for oral absorption and elucidate the mechanism involved in the intestinal transport, which could be decisive in obtaining maximum bioavailability. Cell cultures have also been shown to constitute a highly valuable tool in the decision making process to select candidates for *in vivo* clinical studies at early stage drug discovery and development [101].

Two *in vitro* biological methods are discussed. These are diffusion chambers equipped with intestinal tissues from animal origin and cultured cells.

Diffusion Cells Using Tissues At the end of the 1960s Schultz et al. [102] developed a method for the direct measurement of unidirectional influx of amino acids from the mucosal bathing solution into the intestinal epithelium. This method was a modification of the so-called Ussing chamber widely used to evaluate drug absorption. Posteriorly, the Shultz method was slightly modified by B.G. Munck, who performed a lot of experiments in order to study the intestinal absorption of amino acids from a mechanistic point of view [103, 104]. Other researchers adopted this technique to gain insight into the absorption mechanism of some drugs (e.g., acamprosate) [105] and trace elements such as Zn [106].

In this method, a chosen segment of the intestine of the animal (rat, rabbit, or guinea pig) is excised, opened along the mesenteric attachment, and rinsed in ice-cold buffer. Each segment is mounted on a Lucite plate with the mucosal surface facing upward and a Lucite block is clamped on top of the plate. The Lucite plate is depicted in Fig. 9.7. In this way, four mucosal areas, each of approximately 0.35 cm^2, can be exposed at the bottom of the wells, where the solution is oxygenated and stirred with high rates of O_2 flow. The use of two blocks allows eight measurements

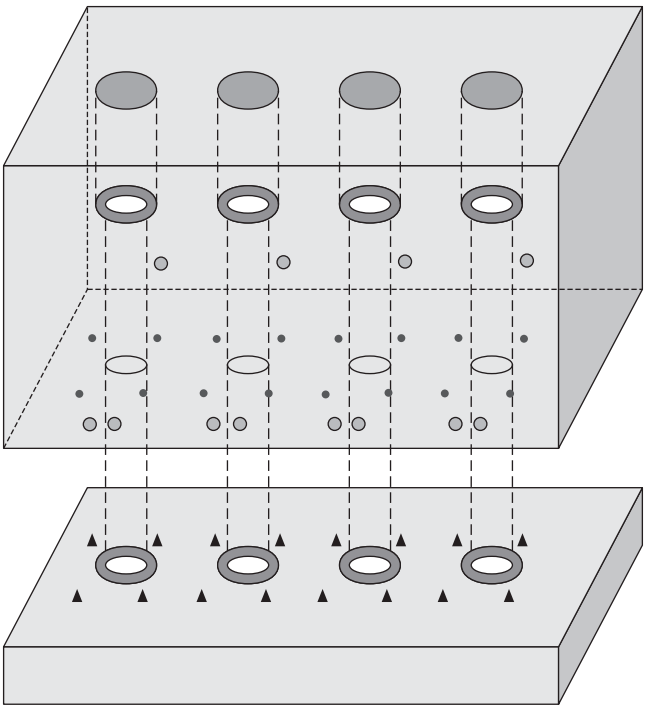

FIGURE 9.7 Schematic representation of a Lucite plate, where the segment of the intestine is mounted, and the Lucite block that is clamped on top of the plate.

from each rat. With guinea pigs and rabbits, 4 blocks can be used, allowing 16 measurements from each animal. The serosal surface of the tissue rests on moistened filter paper and is not exposed to the mucosal solution. After mounting, the tissues are preincubated at 37 °C, in a properly designed chamber (Fig. 9.8). The tissues are incubated for 15 min with a drug-free solution containing 5 mM glucose, which was then withdrawn. The well and mucosal surface are gently wiped with soft paper to remove the adhered incubation fluid before the test solution (containing the radiolabeled drug to test and radiolabeled PEG-4000) is injected. The 0.5 min incubation period is terminated by aspiration of the incubation fluid and flushing of the well with an ice-cold 300 mM mannitol solution. The mannitol wash serves to terminate the exposure both by diluting the remaining test solution and by suddenly cooling the tissues. The exposed tissues are then punched out, briefly rinsed in ice-cold mannitol solution, blotted on hard filter paper, and extracted for 18 h in 0.1 M HNO_3. Aliquots of tissue extract and test solution are assayed for drug content. Usually radiolabeled drugs are used since, as outlined before, the exposition time is very short so the amount of transported compound is really low. So the extract and tissue solution are analyzed by liquid scintillation spectrometry. The amount of radiolabeled PEG-4000 was used to correct for extracellular contamination; thus corrected, the radiolabeled drug activity is used to calculate the rate of influx across the brush border membrane. The unidirectional drug influx, J, is expressed in concentration/cm^2·h.

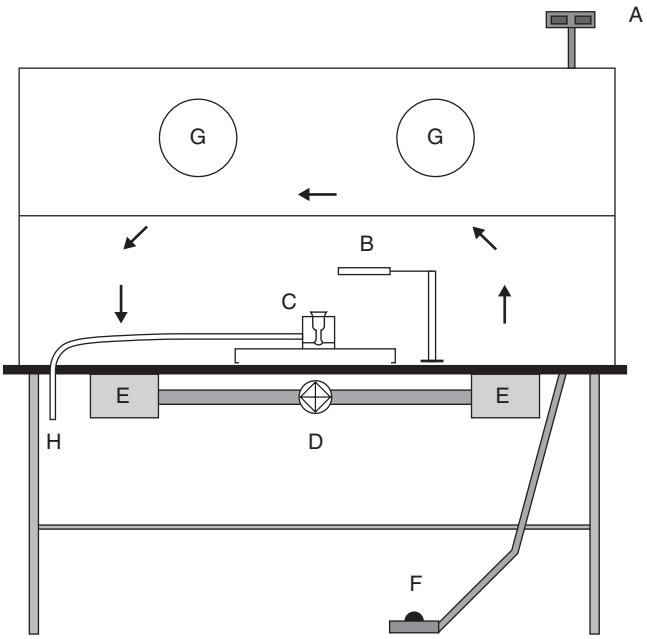

FIGURE 9.8 Schematic representation of the chamber used to incubate and oxygenate the tissue mounted in the Lucite plate: A, digital time and temperature controller; B, thermic sound; C, Lucite plate; D, air turbine; E, air inlet and outlet; F, chronometer pulsator; G, access to chamber inside; H, oxygen inlet.

This technique is simple and rapid, and a reduced number of animals, are used in comparison with the *in situ* and *in vivo* methods. Conditions of temperature, oxygenation, and availability of nutrients and energy sources can be controlled more closely for mechanistic studies than is possible *in situ* or *in vivo*. Moreover, researchers can study differences in regional absorption of drugs, which is not possible in cell cultures. The limitations include the problems associated with the use of radiolabeled compounds and the lack of mesenteric circulation, which is not a physiological condition. Another drawback of any *in vitro* system is the intrinsic variability that can be seen in the permeation data.

Intestinal Cell Cultures Within the past decades the use of monolayers of human intestinal epithelial cells for evaluating drug absorption has become possible. Actually, it was impossible to culture normal, mature enterocytes so colon adenocarcinoma cell lines (Caco-2 cells, HT29, T84) are used. Although isolated from a colon tumor, these cells all show an enterocyte-like differentiation; however, the degree of differentiation is variable (Table 9.3). These cells are relatively well defined while there is also no interference by feedback and/or other mechanism, which are present in intact organs or animals.

The most used cell line from human origin is the Caco-2 cell line. Even though these cells display colon-like properties regarding transepithelial electrical resistance (TEER) and passive permeability to drugs, their morphology, expression of brush border enzymes, and carriers for nutrients resemble more closely small

TABLE 9.3 Degree of Differentation of Epithelial Cell Lines Originating from Human Colon Carcinoma

	Differentiation		
Cell Line	Tight Junctions	Apical Brush Border (Microvilli + Enzyme)	Mucus Secretion
Caco-2 cells	+	+	0
HT29	+	+/–	+/–
T84	+	0	0

Source: Adapted from Ref. 120.

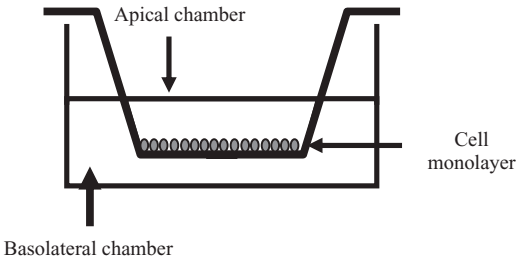

FIGURE 9.9 Schematic setup for transport studies in culture cell lines. Sampling can be carried out from the apical and/or basolateral chamber.

intestinal enterocytes [107]. Several studies have demonstrated the usefulness of these cells as a model for intestinal drug transport. According to published results, the properties of this cell line are the same as normal intestinal epithelium in *in vivo* model systems and are useful for studying transport of hydrophilic as well as lipophilic compounds. A vast amount of work has been done in characterizing the Caco-2 cell line. Several carrier and receptor systems have been identified. Moreover, in some studies, a correlation between human intestinal drug absorption after oral administration and permeability in Caco-2 cells was established [108].

Figure 9.9 illustrates the culturing system whereby the Caco-2 cells are seeded, usually onto polycarbonate filter support on which a polarized cell monolayer is formed within a few days and differentiates into absorptive intestinal cells. The microporous support allows access to drug to both the apical and basolateral sides of the epithelium, which means that drug transport could be studied in both the apical to basolateral and the basolateral to apical. The result of transport experiments can be expressed as the permeability, P_{app}, in cm/s.

The tight junctions in the Caco-2 cell monolayers resemble the junctions in the human colon, resulting in far higher TEER than typical for small intestine tissue. The higher TEER makes these cells a useful and widespread method for studying both pharmacological pretreatments and direct additives on cell integrity because the paracellular permeability of a typical nonpermeant such as PEG-4000 or mannitol can easily be monitored. The direct measurement of TEER is also used as a method to evaluate cell monolayer integrity. To measure TEER values, a four-electrode system, which measures the resistance of a cell monolayer on permeable support, could be used. The results of TEER measurements can be expressed as

absolute values or as percentages of the initial values, which is preferable when TEER values are measured after various treatments since variability in the starting values between monolayers exists and different measurement setups might result in different absolute values.

One of the major advantages of the use of intestinal cell cultures lies in its possible application to high throughput screening (HTS) strategies. This model has a high potential for HTS because the cells are rather simple to culture in high quantities and in a reproducible manner. Keep in mind that to time and cost-effectively develop a new drug application, the selection of candidates for clinical studies has to be made during an early-decision process made with *in vitro* data. So *in vitro* permeability and solubility screening has become a routine measurement in drug discovery and development. In some cases, the fraction of drug absorbed in humans could be predicted by *in vitro* Caco-2 cell permeability. In addition, *in vitro* permeability and solubility data can be used to classify compounds according to the BCS system and, subsequently, to direct formulation optimization strategies [101]. Another important advantage is that, as analyzed in the previous section, Caco-2 cells are also a valuable tool to investigate absorption-enhancement of poorly absorbable drugs. Furthermore, the mechanism of action of absorption-enhancing compounds may be elucidated and if combined with microscopical techniques, the paracellular enhancement could be visualized [108].

Apart from these advantages, Caco-2 cells also have striking disadvantages. First, these are cells with tighter tight junctions than those present in the small intestine. This might give rise to underpredictions of the permeabilities of hydrophilic compounds with a low *in vivo* uptake. Second, transporter substrates usually demonstrate lower permeability than their actual permeability *in vivo*, due to the low transporter expression levels in Caco-2 cells [101]. Third, between laboratories, or even batch-to-batch, variability can occur. These limitations should be taken into consideration when assessing the "developability" of a drug and during the decision making process for selecting candidates, before processing a drug to clinical studies.

9.6.2 *In Situ* Techniques

The isolation of segments of the gastrointestinal tract *in situ* in anesthetized animals with intact mesenteric blood flow is widely used to gain valuable information as to the site and mechanism of drug absorption and permeability assessment [2]. Different *in situ* preparations (single-pass perfusion, recirculating perfusion, oscillating perfusion, and static perfusion) have been used. One of the most employed techniques is the *in situ* rat gut preparation described by Doluisio et al. [109]. In short, after anesthetizing the rats, the abdomen is opened through a middle incision and a selected segment of intestine is cannulated at either end. After rinsing with physiological saline to eliminate fecal residue and debris, an isotonic drug solution properly buffered is perfused at 37 °C. At selected times (usually every 5 min for a total of 30 min), the solution is forced out of alternate ends of the segment and sampled. Drug concentration in the samples is quantified and used for calculations. The correspoding kinetic parameters (first-order absorption rate constant, k_a, or V_{max} and K_m) can be estimated. In some cases, it is necessary to correct for water reabsorption in order to obtain the actual concentration. A nonabsorbable water marker, such as

phenol red, or radiolabeled PEG-4000 could be used. Since our previous experience with these markers was not entirely satisfactory, an alternative method based on the direct measurement, at fixed times, of the remaining volumes of the test solutions perfused independently in selected animals was developed [110]. This technique has proved to be very useful in studying the correlation between gastrointestinal absorption and partition constants for homologous series of xenobiotics [110, 111], the influence of synthetic and natural bile acid surfactants on passive diffusion of xenobiotics [112, 113], and the mechanism of drug absorption. For example, intestinal transport of baclofen, cefadroxil, and cefuroxime axetil through carrier-mediated transport has been evidenced [114–116]. More recently, we have reported, by using this technique, that acamprosate, an anti-craving drug, is absorbed in the small intestine of the rat by passive diffusion [100]. All these investigations have helped to solve problems related with the low bioavailability of the drugs studied. The influence of the potential inhibitors and/or enhancers as well as the participation of the P-gp in the absorption of drugs could also be evaluated [100, 117, 118].

With the use of any of the *in situ* techniques, intact circulation is maintained in such a way that absorbed drugs are taken away from the basement membrane in a more normal physiological manner than in the *in vitro* techniques discussed above. Particularly, with the *in situ* rat gut preparation, absorption rates that are near to *in vivo* values are obtained [12], which allow one to fit absorption models more easily to experimental data. Recently, the *in situ* single-pass perfusion model has proved to be a robust means of assessing permeability for BCS classification [119]. The drawbacks inherent in these techniques are the effects of anesthesia and the short time that the preparations are viable.

9.6.3 *In Vivo* Techniques

The *in vivo* studies in laboratory animals are needed to validate the results obtained *in vitro* or *in situ* in order to avoid the risk of inaccurate predictions. Furthermore, it should be borne in mind that *in vivo* studies are also required during formulation development.

Usually, animals were placed under anesthesia and subjected to surgical cannulation of a vein (e.g., jugular vein) to facilitate blood sampling. Oral administration of the drug is performed usually by gastric intubation. If it is requiered, the animals can be housed in metabolic cages in order to collect urine and feces. Once the sampling period is over, the samples are properly processed to obtain plasma and urine concentrations, which are used for kinetic calculations (C_{max}, AUC, bioavailability). Whole animal studies are also useful for investigating specific parts of the absorption process. For example, chronic portal vein cannulae may be inserted surgically and maintained patent for sampling portal venous blood after oral administration. A portal-vein cannula also allows drug infusions to simulate presentation of drug to the liver by controlled-release devices.

One of the main disadvantages of *in vivo* animal studies is that they are impractical for the screening of a large number of compounds due to the intensive work, slow speed, high cost, and lack of automation. Additionally, *in vivo* oral bioavailability studies in animals are not always predictive of bioavailability in humans. Nevertheless, in spite of these limitations, *in vivo* studies provide some initial estimates of how well a compound is absorbed, the variables that affect absorption

(including enhancers), and the probability that the compound undergoes first-pass metabolism.

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10

DISTRIBUTION: MOVEMENT OF DRUGS THROUGH THE BODY

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Contents

10.1 INTRODUCTION

10.1.1 Drug Distribution

Drug distribution refers to the reversible movement of drug from blood to various tissues of the body [1]. Following entry into the systemic circulation, a drug

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distributes into different tissues, the rate and extent of which is dependent on the drug's physicochemical properties and the blood flow to the tissues. The rate and extent of distribution to different tissues, in turn, affects the duration and magnitude of therapeutic effect and toxicity [2]. This chapter explores the various factors that affect drug distribution and the different approaches available to modify drug distribution.

10.1.2 Effect of Distribution on Drug Action

The intensity and duration of therapeutic effects derived from drugs other than those exerting an immediate, irreversible action depend, theoretically, on maintaining adequate concentrations of the active form of the drug at receptor sites [3]. For the purpose of this chapter, receptor sites are considered to be target sites located anywhere in the body that combine with a drug or its active metabolite to produce a pharmacological effect. To elicit a therapeutic effect, a drug has to distribute from the systemic circulation to its site of action [4]. Therefore, it is important to consider the effect of distribution in selecting an appropriate dose, dosage form, dosage interval, and route of administration; appropriate modification of these parameters permits attainment of effective concentrations of the active form of a drug at receptor sites [5].

For blood levels of a drug to correlate with pharmacological effect, the concentration of the drug in blood must be in equilibrium with the concentration of drug at the receptor site through which it produces its effect [6]. Furthermore, the pharmacological response being investigated must bear a direct relationship to the drug's concentration at the receptor site [5, 6]. Certain drugs, such as monoamine oxidase and cholinesterase inhibitors, must accumulate at receptor sites to a certain concentration before pharmacological effects will ensue [7]. During this initial period of drug accumulation at receptor sites, no direct relationship between the drug level in blood and pharmacological effects may be seen. Thus, the distribution process could make a graded response (response proportional to drug concentration), giving the impression of a quantal response (response does not have a linear relationship with drug concentration) [8].

10.2 DRUG DISTRIBUTION INTO DIFFERENT COMPARTMENTS

10.2.1 Distribution Among Plasma/Blood, Tissues, Organs, and Body Fluids

After absorption into the bloodstream, drugs tend to distribute into all body water. The total body water can be divided into intravascular, extracellular, and intracellular water [9]. For a 70 kg person, the total body water is about 42 L. The intravascular water accounts for about 3 L of the total body water while the extracellular and intracellular waters account for 16 L and 23 L, respectively [2]. Drugs that permeate freely through cell membranes become distributed, in time, throughout the body water. Drugs that pass readily through or between capillary endothelial cells, but do not penetrate other cell membranes, are distributed into the extracellular fluid space [10]. Occasionally, the drug molecule may be so polar, large, or highly

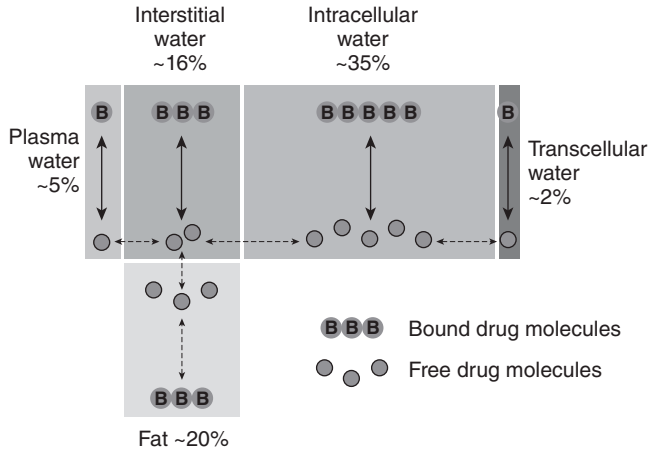

FIGURE 10.1 The main body fluid compartments, expressed as a percentage of body weight. Drug molecules exist in bound or free form in each compartment, but only the free drug is able to move between the compartments. (From Ref. 11, with permission.)

bound to plasma proteins that it remains in the intravascular space after IV administration [2].

In all these body fluid compartments, drug molecules are present in either protein-bound or unbound form (Fig. 10.1), with the equilibrium existing between the unbound form of the drug in different tissues [11]. Furthermore, drugs that are weak acids or bases will exist as an equilibrium mixture of ionized or un-ionized forms, the position of the equilibrium depending on the pH of the compartment. Un-ionized and lipophilic drug molecules can generally diffuse from interstitial space across the cell membrane into the cytoplasm of a cell, while ionized and highly polar drugs cannot readily diffuse across the cell membrane [12].

The effect of pH on diffusion of drugs across a cell membrane could be understood from the gastric pouch experiments [13]. It was shown that drugs cross the gastric epithelium in their nonionized form, and that the ionized form penetrates very slowly, if at all. In the experiments, various acidic and basic drugs were administered intravenously to dogs with gastric pouches, and the concentrations of drug in gastric juice and plasma were measured. At steady state, basic drugs appeared in gastric juice in concentrations ranging from 1 to 40 times that of plasma. In contrast, acidic drugs appeared in gastric juice in low concentrations, which ranged from 0 to 0.6 that of plasma. The results were explained in terms of a model system in which gastric juice is separated from plasma by a barrier permeable only to the nonionized form of a weak electrolyte (Fig. 10.2) [7]. At steady state, the concentrations of nonionized drug in plasma and gastric juice are the same (correcting for the degree of plasma binding), but the concentrations of the ionized form are unequal because of the difference in the pH of the two fluids. Accordingly, the total concentration of drug (ionized plus nonionized) on both sides of the gastric mucosa is a function of the pH of the two fluids and the dissociation constant of the drug. From this relationship, it can readily be calculated that a basic drug will be more concentrated in tissues with acidic pH than in plasma, and an acidic drug will be more concentrated in plasma than in tissues with acidic pH [7].

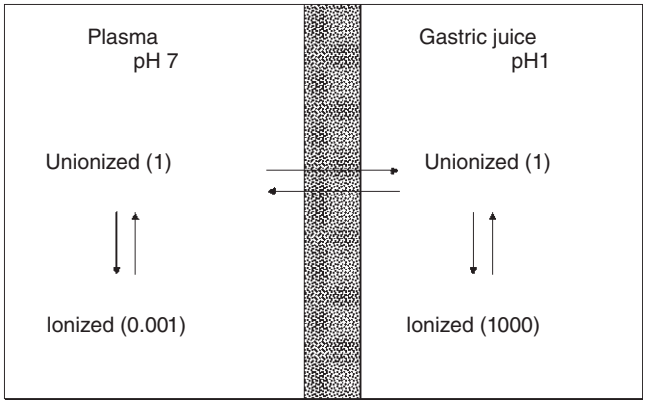

FIGURE 10.2 The theoretical distribution of an organic base, pK_a 4, between plasma and gastric juice, assuming that the fluids are separated by a boundary permeable only to the unionized drug molecule. (From Ref. 7, with permission.)

Drugs may also undergo redistribution in the body after initial high levels are achieved in tissues that have a rich vascular supply. As the plasma concentration falls, drug readily diffuses back from a tissue with initial high concentration into the circulation to be quickly redistributed to other tissues with high blood-flow rates, such as the muscles. Then, over time, the drug also becomes deposited in tissues with poor blood supply, such as the fat depots [14]. Some drug molecules are distributed to eliminating organs like liver and kidney. Many drugs are not distributed equally throughout the body but tend to accumulate in certain specific tissues or fluids [15, 16]. For example, chloroquine mainly accumulates in liver, tetracycline in bone and teeth, and iodine in thyroid glands.

10.2.2 Volume of Distribution (V_D)

Anatomically, the total volume of body water available for a drug to distribute into is about 42 L [2]. If all drugs were to distribute into all of body water equally, then the initial plasma concentration of all drugs for a given intravenous bolus dose of the drug would be similar. However, this is rarely the case. The plasma concentration of the drug appears to be as if the total body water was less than or greater than 42 L. The reason for the discrepancy is twofold. First, most drugs do not distribute evenly in all body water. Second, only the drug concentration in the intravascular compartment is measurable. Two extreme cases of distribution should be considered for a better understanding. Some molecules like Evan's blue are strictly localized within the intravascular compartment. When the concentration of Evan's blue in the plasma is measured, it appears as if the total volume in which the molecule is distributed is small. For example, following a 100 mg IV bolus dose, the initial plasma concentration measured would be about 100 mg/3 L = 33 mg/L. Now, if the dose (100 mg) and initial concentration (33 mg/L) were used to estimate the volume, this would be about 3 L. For the other extreme, consider a drug molecule like quinine, which accumulates extensively in intracellular water. When the concentration of such a drug molecule in the plasma is measured, it appears as if the total volume in

which the drug is distributed is large. For example, consider a drug molecule that shows 90% partitioning out of the intravascular water. Thus, following a 100 mg dose, 10 mg will stay in the intravascular water while 90 mg will be distributed into extravascular water. The initial concentration measured for this drug after an IV bolus dose would theoretically be about 10 mg/3 L = 3.3 mg/L. If the dose and initial concentration were used to estimate the volume, this would be about 33 L.

In order to account for these differences, the term *apparent volume of distribution* is used. Apparent volume of distribution (V_D) is the volume that must be considered in estimating the amount of drug in the body from the concentration of drug found in the sampling compartment [1]. Volume of distribution relates the concentration of the drug in plasma (C_P) and the amount of drug in the body (A), as in the following equation:

$$V_D = \frac{A}{C_P}$$

Some drugs have V_D as small as 5 L while others have a V_D of 50,000 L. Since V_D is not a true anatomic/physiologic space, it is called *apparent* volume of distribution. Volume of distribution is a measure of the tendency of a drug to move out of blood/plasma to other tissue sites. Thus, the larger the volume of distribution, the more extensively is the drug distributed outside the vasculature. Volume of distribution is useful in the determination of plasma drug concentration when a known amount of drug is in the body or conversely in the determination of dose required to achieve a particular plasma concentration. Examples of drugs with small and large volumes of distribution are shown in Table 10.1 [11]. It is important to note that volume of distribution is dependent on a patient's body weight and is often quoted with the units of L/kg [11].

10.3 FACTORS AFFECTING DRUG DISTRIBUTION

There are several factors that affect the rate and extent of drug distribution. Blood perfusion to a tissue and permeability of the drug across cell membrane play an important role in determining the rate of distribution. Extent of distribution is mainly affected by molecular weight, lipid solubility, pK_a, and protein binding of the drug. Presence of drug efflux transporters and genetic differences in transporter activity in different patient populations also significantly affect drug distribution.

10.3.1 Rate of Distribution

Distribution of drugs from blood to different tissues is a two-step process [11]. The first step involves the transport of drug by blood to different tissues. The second step is the transport of the drug from blood into the tissue across the cell membrane. For lipophilic, small molecular weight drugs that can cross cell membranes efficiently, transport to the tissue is the rate-limiting step, while for hydrophilic and large molecular weight drugs, transport across the cell membrane is the rate-limiting step. Thus, depending on the drug's characteristics, drug distribution could be either permeability limited or perfusion limited.

TABLE 10.1 Distribution Volumes for Some Drugs Compared with Volume of Body Fluid Compartments

Volume (L/kg body weight)	Compartment	Volume of Distribution (V_D; L/kg body weight)	
0.05	Plasma	0.05–0.1	Heparin
			Insulin
		0.1–0.2	Warfarin
			Sulfamethoxazole
			Glibenclamide
			Atenolol
0.2	Extracellular fluid	0.2–0.4	Tubocurarine
		0.4–0.7	Theophylline
0.55	Total body water		Ethanol
			Neostigmine
			Phenytoin
		1–2	Methotrexate
			Indomethacin
			Paracetamol
			Diazepam
			Lidocaine
		2–5	Glyceryl trinitrate
			Morphine
			Propranolol
			Digoxin
			Chlorpromazine
		>10	Nortriptyline

Permeability-Limited Distribution The cell membrane separates extra- and intracellular compartments. The cell membrane allows a bidirectional, dynamic, and selective exchange of organic molecules, ions, and gas molecules between the two compartments. Small, noncharged lipid molecules pass through the membrane freely. Small polar molecules (carbon dioxide, water) can also pass cell membranes easily following their concentration gradient. Most hydrophilic molecules and macromolecules cannot cross the cell membrane effectively [17].

Thus, cell permeability is the limiting factor in the tissue distribution of polar and high molecular weight drugs. For such drugs, equilibrium is reached faster in tissues without permeability constraints, even though these tissues may have a lower perfusion rate. For example, brain has a perfusion rate of 0.5 mL/min/mL while muscle has a perfusion rate of 0.025 mL/min/mL. However, the cell junctions in the brain capillary endothelium are tighter than those found in the muscle capillary endothelium. Lipophilic drugs that can cross the cell membrane efficiently reach equilibrium faster in brain than in muscle while polar molecules reach equilibrium faster in muscles [18].

Cell permeability varies in characteristics depending on the tissue [19]. The endothelial cells of the brain capillaries, which are more tightly joined to one another than those found in other capillaries, contribute to the slower diffusion of water-soluble drugs into brain. Another barrier to water-soluble drugs is the glial

connective tissue cells (astrocytes), which form an astrocytic sheath close to the basement membrane of the brain capillary endothelium [14]. The tight junctions between brain vascular endothelial cells lead to high endothelial electrical resistance, in the range of 1500–2000 $\Omega \cdot cm^2$ (pial vessels), as compared to 3–33 $\Omega \cdot cm^2$ in other tissues [20, 21]. Thus, polar compounds cannot enter the brain effectively but can enter the interstitial fluids of most other tissues [22].

Even within a tissue, the distribution of a drug may vary between the different regions of the tissue. For example, drugs penetrate into the brain cortex more rapidly than into white matter, probably because of the greater delivery rate of drug via the bloodstream to the tissue [23]. The consequences of the diverse rates of entry of different drugs into the CNS include the following: (1) water-soluble or ionized drugs will not enter the CNS very well; (2) low ionization, low plasma–protein binding, and a fairly high lipid–water partition coefficient confer ready penetration; and (3) direct injections into the CSF often produce unexpected effects [24, 25].

Perfusion-Limited Distribution For most lipophilic drugs with small molecular weight, cell membranes do not create any barrier to their distribution [26]. For such drugs, distribution to different tissues is mainly governed by the blood perfusion rate to that tissue. Perfusion rate is often expressed as milliliter of blood per minute per unit volume of tissue. Perfusion rate could be as much as 10 mL/min/mL for lungs to only 0.025 mL/min/mL for resting muscle (Table 10.2) [1].

TABLE 10.2 Blood Flow, Perfusion Rate, and Relative Size of Different Organs and Tissues Under Basal Conditions in a Standard 70 kg Human[a]

Organ[b]	Percent of Body Volume	Blood Flow (mL/min)	Percent of Cardiac Output	Perfusion Rate (mL/min/mL of Tissue)
Adrenal glands	0.03	25	0.2	1.2
Blood	7	(5000)[b]	(100)	—
Bone	16	250	5	0.02
Brain	2	700	14	0.5
Fat	20[c]	200	4	0.03
Heart	0.4	200	4	0.6
Kidneys	0.5	1100	22	4
Liver	2.3	1350	27	0.8
Portal	1.7 (gut)	(1050)	(21)	—
Arterial	—	(300)	(6)	—
Lungs	1.6	(5000)	(100)	10
Muscle (inactive)	43	750	15	0.025
Skin (cool weather)	11	300	6	0.04
Spleen	0.3	77	1.5	0.4
Thyroid gland	0.03	50	1	2.4
Total Body	*100*	*5000*	*100*	*0.071*

[a]Compiled and adapted from data in Guyton AC. *Texbook of Medical Physiology*, 7th ed. Philadelphia: Saunders; 1986, p 230; Lentner C, Ed. *Geigy Scientific Tables, Volume 1*. Edison, NJ: Ciba-Geigy; 1981; and Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993;10:1093–1095.

[b]Some organs (e.g., stomach, intestines, and pancreas) are not included.

[c]Includes fat within organs.

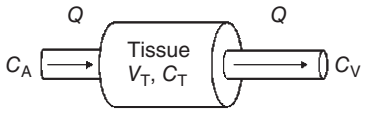

FIGURE 10.3 Perfusion-limited tissue distribution: Q = rate of blood flow; C_A = arterial concentration; C_V = venous concentration; V_T = tissue volume; and C_T = tissue concentration.

All other factors (membrane permeability, protein binding, pH, etc.) remaining equal, well-perfused tissues take up more drug than poorly perfused tissues. There is a direct correlation between tissue perfusion rate and time to distribute to a tissue. This can be explained as follows.

Consider a tissue T whose volume is V_T and the concentration of the drug in the tissue is C_T (Fig. 10.3). The rate of blood flow to the tissue is Q. If C_A is concentration of the drug in arterial blood and C_V is the concentration of the drug in venous blood, then the rate of presentation of the drug to the tissue is given by $Q \cdot C_A$, while the rate of leaving of drug from the tissue is given by $Q \cdot C_V$. The net rate of drug uptake by the tissue is $Q \cdot (C_A - C_V)$.

The amount of the drug in the tissue is obtained using the volume of the tissue and the concentration of the drug in the tissue: amount of drug in tissue = $V_T \cdot C_T$. The relative affinity of the drug for the tissue can be defined in terms of a partition coefficient K_P, where $K_P = C_T/C_V$. Rearranging this relationship, and substituting for C_T in the above equation, we get

$$\text{Amount of drug in tissue} = V_T \cdot K_P \cdot C_V$$

The exit of the drug from the tissue can be defined by the fractional rate of exit, which is given by

$$\text{Fractional rate of exit} = k_T = \text{Rate of exit/Amount in tissue}$$

Substituting for rate of exit and the amount of the drug in the tissue, we get

$$k_T = (Q \cdot C_V)/(V_T \cdot K_P \cdot C_V) = (Q/V_T)/K_P$$

where Q/V_T is perfusion rate of tissue and K_P is the partition coefficient of the drug for the tissue. The time to distribute into a tissue is given by tissue distribution half-life (time for 50% distribution):

$$\text{Tissue distribution half-life} = 0.693/k_T = 0.693K_P/(Q/V_T)$$

If each organ has the same ability to store the drug (K_P is equal), then the distribution half-life is governed by the blood flow Q and the volume (size) V_T of the organ. A large blood flow to the organ decreases the distribution time; whereas a large organ size increases the distribution time because a longer time is needed to fill a large organ volume with drug. Also, it is important to note that if a tissue has a long distribution half-life, a long time is needed for the drug to leave the tissue

when blood level decreases. If arterial concentration is maintained constant, tissue concentration goes up but rate of uptake decreases with time. Tissue concentration (C_T) at any time t is given by the following relationship:

$$C_T = K_P C_A (1 - e^{-k_T t})$$

Equilibrium in tissue concentration and the loss of drug from tissue take longer the poorer the perfusion and the greater the partitioning of drug into the tissue.

10.3.2 Extent of Distribution

Factors like molecular weight, lipid solubility, pH–pK_a, and protein binding play an important role in determining the extent of drug distribution. It is important to note that all the above factors also affect the rate of drug distribution into the tissue.

Molecular Weight The diffusion of a molecule across a cell membrane depends mainly on its molecular size, the diffusion coefficient for small molecules being inversely proportional to the square root of molecular weight. Consequently, while large molecules diffuse at a slower rate than small ones, the effect of molecular weight for most drug molecules is modest. Most drugs fall within the molecular weight range 200–1000, and variations in aqueous diffusion rate have only a small effect on their overall pharmacokinetic behavior. However, beyond this molecular weight range, diffusion of molecules across cell membranes is considerably limited. For compounds with molecular diameter above 100 Å, transfer across membranes is significantly slow [11].

Lipid Solubility Distribution of drugs is significantly influenced by lipid solubility. Lipid solubility affects the ability of the drug to bind to plasma proteins and to cross lipid membrane barriers [27]. A drug needs to be lipid soluble to penetrate membranes, unless there is an active transport system for the drug or the drug is so small that it can pass through the aqueous channels in the membrane. For weakly acidic and weakly basic drugs, ionization, and therefore lipid solubility, is pH dependent (see pH–pK_a discussion). As discussed earlier, very high lipid solubility can result in a drug initially partitioning preferentially into highly vascularized lipid-rich areas. Subsequently, these drugs slowly redistribute into body fat, where they may remain for long periods of time.

pH–pK_a Most drugs are weak acids or bases and therefore exist in both un-ionized and ionized forms, the ratio of the two forms varying with pH. For a weak base, the ionization reaction is

$$BH^+ \xleftrightarrow{K_a} B + H^+$$

The negative logarithm of the acid dissociation constant is designated by the symbol pK_a and is given by the Henderson–Hasselbalch equation:

$$pK_a = pH + \log_{10}([BH^+]/[B])$$

For a weak acid,

$$AH \xleftrightarrow{K_a} A^- + H^+$$

$$pK_a = pH + \log_{10}([AH]/[A^-])$$

Drug accumulates on the side of a membrane where pH favors greater ionization of drug. This is known as the pH-partition hypothesis [28]. Only un-ionized nonpolar drug can permeate through the lipid membrane, and at equilibrium, the concentration of the un-ionized species is the same on both sides, but there will be more total drug on the side on which the degree of ionization is greater. Basic drugs tend to accumulate in tissues with pH values lower than the pK_a of the drug; conversely, acidic drugs concentrate in regions of higher pH, provided that the free drug is sufficiently lipid soluble to be able to penetrate the membranes that separate the compartments. Even small differences in pH across membranes, such as those that exist between CSF (pH 7.3) and plasma (pH 7.4), milk (pH 6.5–6.8) and plasma, renal tubular fluid (pH 5.0–8.0) and plasma, and inflamed tissue (pH 6.0–7.0) and healthy tissue (pH 7.0–7.4), can lead to unequal distribution of drugs [11].

Figure 10.4 shows how a weak acid (e.g., aspirin, pK_a 3.5) and a weak base (e.g., pethidine, pK_a 8.6) would be distributed at equilibrium between three body compartments, namely, plasma (pH 7.4), alkaline urine (pH 8), and gastric juice (pH 3). Within each compartment, the ratio of ionized to un-ionized drug is governed by the pK_a of the drug and the pH of that compartment [11]. It is assumed that the un-ionized species can cross the membrane and therefore reaches an equal concentration in each compartment. The ionized species is assumed not to cross at all. The result is that, at equilibrium, the total (ionized + un-ionized) concentration of the drug will be different in the different compartments, with an acidic drug being concentrated in the compartment with high pH ("ion trapping"), and vice versa [29]. The concentration gradients produced by ion trapping can theoretically be very large, if there is a large pH difference between compartments. Thus, aspirin would be concentrated more than fourfold with respect to plasma in alkaline renal tubule, and about 6000-fold in plasma with respect to the acidic gastric contents. Such large gradients, however, are unlikely to be achieved in reality for two main reasons. First, the attribution of total impermeability to the charged species is not realistic, and even a small permeability will considerably attenuate the concentration difference that can be reached. Second, body compartments rarely approach equilibrium. Neither the gastric contents nor the renal tubular fluid stands still, and the resulting flux of drug molecules reduces the concentration gradients well below the theoretical equilibrium conditions. The pH-partition mechanism nonetheless correctly explains some of the qualitative effects of pH changes in different body compartments on the pharmacokinetics of weakly acidic or basic drugs, particularly in relation to penetration of the blood–organ barriers. Values of pK_a for some common drugs are shown in Fig. 10.5 [1].

Protein Binding Binding to proteins in tissue, dissolution in adipose tissue, formation of nondiffusible complexes in tissues such as bone, incorporation into specific storage granules, or binding to selective sites in tissues all impede movement of drugs in the body and account for differences in the cellular and organ distribution

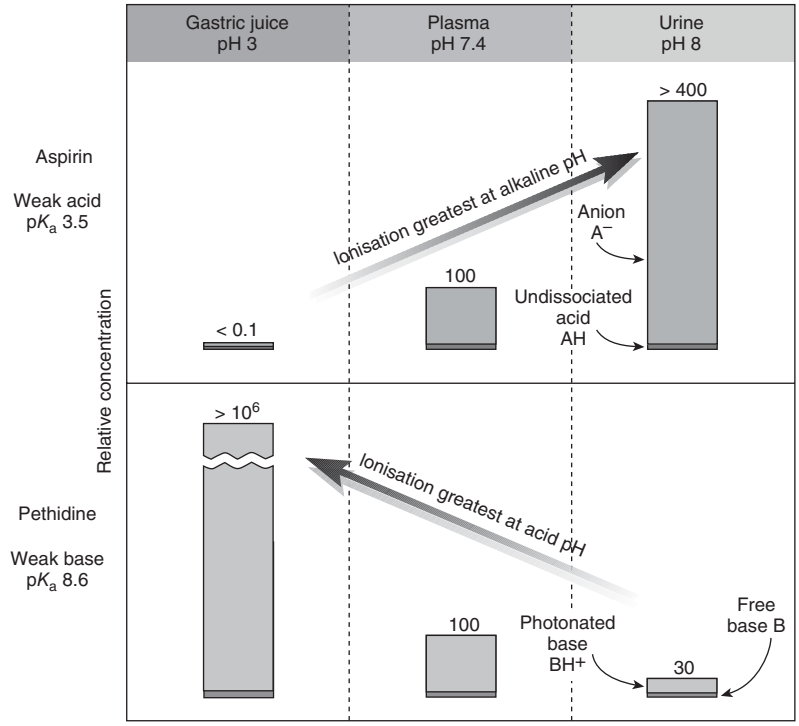

FIGURE 10.4 Theoretical partition of a weak acid (aspirin) and a weak base (pethidine) between aqueous compartments (urine, plasma, and gastric juice) according to the pH difference between them. Numbers represent relative concentrations (total plasma concentration = 100). It is assumed that the uncharged species in each case can permeate the cellular barrier separating the compartments and therefore reaches the same concentration in all three. Variations in the fractional ionization as a function of pH give rise to the large total concentration differences with respect to plasma. (From Ref. 11, with permission.)

of particular drugs [30, 31]. Extensive plasma–protein binding will cause more of the drug to stay in the central blood compartment, because only the unbound or free fraction of a drug can diffuse out of capillaries into tissues. Therefore, drugs that bind strongly to plasma proteins tend to have lower volumes of distribution. Similarly, drugs that bind extensively to tissue proteins (proteins outside the vascular compartment) tend to have large volumes of distribution [32].

The most important binding of drugs in circulation is to plasma albumin, which comprises about 50% of the total plasma proteins and binds the widest range of drugs [33]. Acidic drugs commonly bind to albumin, while basic drugs often bind to α_1-acid glycoproteins and lipoproteins (Table 10.3) [34]. Many endogenous substances, steroids, vitamins, and metal ions are bound to globulins. A drug may become bound to plasma proteins to a greater or lesser degree, depending on a number of factors, for example, plasma pH, concentration of plasma proteins, concentration of the drug, the presence of another agent with a greater affinity for the limited number of binding sites, and the presence of acute-phase proteins during active inflammatory conditions [9]. The degree of plasma–protein binding and the

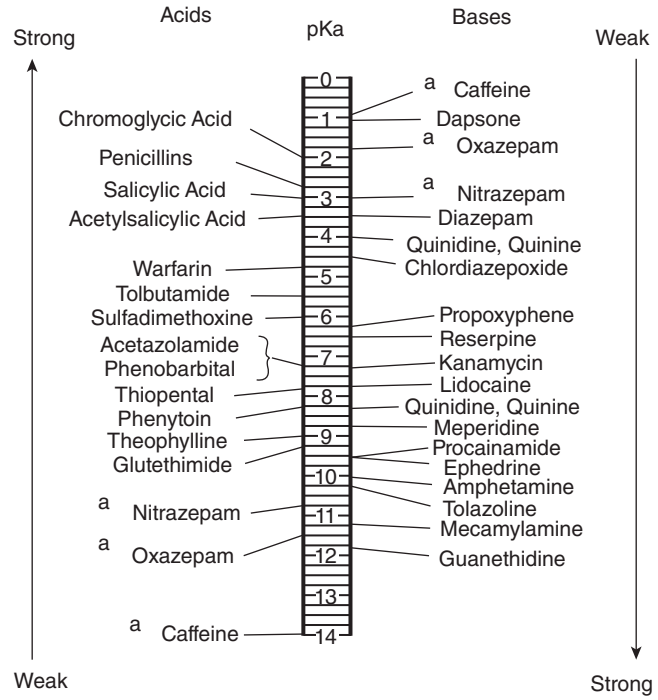

FIGURE 10.5 The pK_a values of acidic and basic drugs vary widely. Some of drugs are amphoteric (a): that is, they have both acidic and basic functional groups. (From Ref. 1, with permission.)

TABLE 10.3 Potential Binding Sites of Proteins for Various Drugs

Binding Sites	Drugs
For Acidic Agents:	
Albumins	Bilirubin, bile acids, fatty acids, vitamin C, salicylates, sulfonamides, barbiturates, phenylbutazone, penicillins, tetracyclines, probenecid
For Basic Agents:	
Globulins, α_1, α_2, β_1, β_2, γ	Adenosine, quinacrine, quinine, streptomycin, chloramphenicol, digoxin, ouabain, coumarin

affinity of a drug for the nonspecific protein-binding sites are of great clinical significance in some instances and much less so in others [35]. For example, a potentially toxic compound (such as dicumarol) may be 98% bound, but if for any reason it becomes only 96% bound, then the concentration of the free active drug that becomes available in the plasma is doubled, with potentially harmful consequences. The concentration of a drug administered in overdose may exceed the binding capacity of the plasma protein and lead to an excess of free drug, which can distribute into various target tissues and produce exaggerated effects. Of equal importance is the readiness with which drugs dissociate from plasma proteins. Those that are more tightly bound tend to have much longer elimination half-lives, because they

are released gradually from the plasma protein reservoir. The long-acting sulfonamides are good examples of this phenomenon.

10.3.3 Role of Transporters

Drug transporters, proteins that transport drugs across the cell membrane, either from or to the intravascular compartment, play an important role in determining drug disposition. Transporters have been shown to be important in the disposition of endogenous compounds, drugs, and other xenobiotics in many organs such as the intestine, liver, kidney, and brain [36–39]. This section focuses on the role of primary transport proteins like ATP-binding cassette transporters and secondary and tertiary transporters like organic cation and anion transporters in the distribution of drugs.

Primary Transporters (ATP-Binding Cassette Transporters) It is now well recognized that membrane efflux transporters, especially P-glycoprotein (P-gp), play an important role in determining the absorption, distribution, metabolism, excretion, and toxicology (ADMET) behaviors of many drugs and molecules in development. Many of the important mammalian efflux transporters are members of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily, where transport is driven by hydrolysis of ATP. The first member of this family to be discovered was P-gp. P-gp is a 170 kDa, integral membrane protein found in most organisms from bacteria to humans. It was originally identified as a key reason for the development of multidrug resistance in certain cancers [40]; however, constitutive expression of P-gp in many normal tissues such as intestinal epithelia, hepatocytes, kidney proximal tubules, blood–brain barrier endothelia, and placental trophoblast demonstrates its protective roles in limiting drug absorption and distribution, contributing to pharmacokinetics, and potentially impacting pharmacodynamics and toxicity [41, 42]. A list of P-gp substrates is shown in Table 10.4. The tissue localization and probable role of transport proteins in drug absorption, distribution, and excretion are depicted in Fig. 10.6.

P-gp is only one of 48 known transporters belonging to seven subfamilies (A–G) [43]. So the potential for other transporters to affect drug disposition is high. As the importance of transport proteins in drug disposition emerges, it is also clear that these transport proteins are saturable, inducible, can be inhibited, and display some degree of polymorphism—factors that need to be considered with respect to variability in drug disposition and response [44–47].

As discussed earlier, the extent of membrane permeability is largely governed by the physicochemical properties of drugs, and lipophilicity is generally considered a key determinant in extent of drug absorption, hepatic transport, and brain penetration. However, increased lipophilicity alone is not predictive of increased permeability when the transport proteins contribute to drug transport across the membrane. While determining the kinetics of drug movement across a barrier, contributions of both passive diffusion and active transport have to be considered. The flux of a drug across a membrane is linearly related to drug concentration, if only passive diffusion is present. Active transport (forward transport or efflux) is usually saturable at high concentrations. If both passive diffusion and active transport are present, net flux will depend on the relative contribution of the two processes and the concentration

TABLE 10.4 List of P-Glycoprotein Substrates

Anthracyclines (doxorubicin, daunomycin, epirubicin)
Acridines (m-AMSA)
Azatoxins (azatoxin)
Benxzoheptalene compounds (colchicine)
Benzothiazepines (diltiazem)
Dihydropyridines (azidopine, nicardipine)
Epipodophyllotoxins (etoposide)
Isoquinolines (cepharantine)
Macrolides (FK506)
Organometallic cations (99mTc-sestamibi)
Peptides (actinomycin D, bleomycin, cyclosporin A, valinomycin)
Phenothiazines (chlorpromazine)
Phenylalkylamines (verapamil, tiapamil)
Pyrroloindoles (mitomycin C)
Quinolines (chloroquine, quinidine)
Rhodamines (rhodamine 123)
Steroids (aldosterone, corticosterone, cortisol, dexamethasone, testosterone)
Taxanes (paclitaxel, docetaxel)
Vinca alkaloids (vincristine, vinblastine)
Other (digoxin, Hoechst 33342)

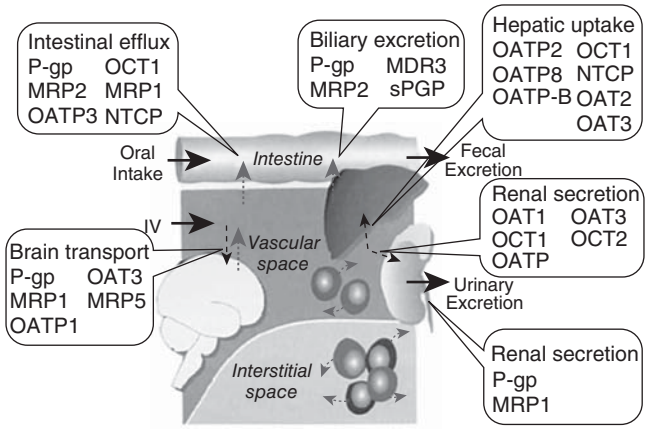

FIGURE 10.6 Tissue localization and role of transport proteins in drug disposition. (From Ref. 19, with permission.)

of drug. At very high concentrations, the contribution of active transport to the kinetics of drug flux across the membrane is minimized.

Secondary and Tertiary Transporters (Driven by an Exchange or Cotransport of Intracellular and/or Extracellular Ions) Besides the ATP family of transporters, other transporters, such as the organic anion transporters (OATs) and the organic cation transporters (OCTs) are present in the human body and significantly influence drug distribution [48].

Organic anion transporters (OATs) play an essential role in the disposition of clinically important anionic drugs, including antiviral drugs, antitumor drugs, antibiotics, antihypertensives, and anti-inflammatory agents. The activities of OATs are directly linked to drug toxicity and drug–drug interactions. So far, four members of the OAT family have been identified: OAT1, OAT2, OAT3, and OAT4 [49]. These transporters share several common structural features including 12 transmembrane domains, multiple glycosylation sites localized in the first extracellular loop between transmembrane domains 1 and 2, and multiple phosphorylation sites present in the intracellular loop between transmembrane domains 6 and 7 and in the carboxyl terminus. The impact of these structural features on the function of these transporters has just begun to be explored. OAT1 and OAT3 are predominantly expressed in the kidney and brain. OAT2 is predominantly expressed in liver. OAT4 is present mainly in placenta and kidney. These transporters are multispecific with a wide range of substrate recognition [49].

OAT1 has been shown to interact with a wide range of organic anion drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), β-lactam antibiotics, antiviral drugs, diuretics, antitumor drugs, and angiotensin-converting enzyme inhibitors. The prototype substrate for OAT1 is para-aminohippuric acid (PAH) [50]. OAT2 interacts with organic anion drugs, such as NSAIDs, and antibiotics. Unlike for OAT1, PAH is a low affinity substrate for OAT2. For OAT3, the prototype substrates are sulfate- and glucuronide-conjugated steroids. OAT3 also interacts with various drugs and endogenous substances such as NSAIDs, antitumor drugs, H2-receptor antagonists, prostaglandins, diuretics, angiotensin-converting enzyme inhibitors, β-lactam antibiotics, and various neurotransmitter metabolites. PAH is a low affinity substrate for OAT3. OAT4 interacts with sulfate-conjugated steroids, antibiotics, and ochratoxin A and shows very little transport of PAH. Compared with OAT1 and OAT3, OAT4 has much narrower substrate specificity [51].

Organic cation transporters (OCTs) are critical in drug absorption, targeting, and disposition. It has become increasingly clear that multiple mechanisms are involved in organic cation transport in the key tissues responsible for drug absorption and disposition: kidney, liver, and intestine. Drugs from a wide array of clinical classes—including antihistamines, skeletal muscle relaxants, antiarrhythmics, and adrenoceptor blocking agents—are organic cations. In addition, several endogenous bioactive amines—such as dopamine, choline, and *N*1-methylnicotinamide (NMN)—are organic cations. Since many of these molecules (pK_a 8–12) are polar and positively charged at physiologic pH, OCTs generally are involved in the absorption, distribution, and elimination of these compounds [52].

10.3.4 Pharmacogenetic Factors

Pharmacogenetics deals with inherited differences in response to drugs. It is well recognized that most medications exhibit wide interpatient variability in their efficacy and toxicity. For many drugs, these interindividual differences are due, in part, to polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters, and/or drug targets (e.g., receptors, enzymes). Pharmacogenomics is aimed at elucidating the genetic basis for differences in drug efficacy and toxicity, and it uses genome-wide approaches to identify the network of genes that govern an individual's response to drug therapy.

Polymorphism The best recognized examples are genetic polymorphisms of drug-metabolizing enzymes, which affect about 30% of all drugs [53]. Loss of function of thiopurine *S*-methyltransferase (TPMT) results in severe and life-threatening hematopoietic toxicity if patients receive standard doses of mercaptopurine and azathioprine. Gene duplication of cytochrome P4502D6 (CYP2D6), which metabolizes many antidepressants, has been identified as a mechanism of poor response in the treatment of depression. There is also a growing list of genetic polymorphisms in drug targets that have been shown to influence drug response. In this section, we discuss genetic polymorphisms in drug transporters that are involved in drug distribution.

As previously described, many drugs are substrates of active transporters, membrane proteins that maintain cellular homeostasis by importing and exporting endogenous compounds. Because of their localization in intestinal, hepatic, and renal epithelial cells, these transporters are important in the absorption, distribution, bioavailability, and elimination of many drugs. Moreover, they can be important in targeting drugs to organs because they are localized in blood–organ barriers.

Genetic polymorphisms affecting a transporter's expression or affinity for substrates can alter drug concentrations at the site of action despite similar blood concentrations. MDR1, MRPs, OATPs, OCTs, OATs, and nucleoside transporters are of particular interest because they transport exogenous substrates, including drugs, as well as endogenous compounds. P-gp, the product of *ABCB1* gene, has received much attention because its substrates include many important drugs. Systemic screening initially revealed 15 genetic variants of human *ABCB1*. A total of 28 SNPs have now been identified; those in exons 21 (G2677T) and 26 (C3435T) are of particular interest because they affect expression or function [47, 55]. Although several studies have addressed the association of these variants with disposition and effects of P-gp substrates, controversy remains about the influence of different variants on pharmacokinetics and pharmacodynamics. Tissues studied so far have shown an average eight- to tenfold difference in P-gp expression. The 3435CC and 3435TT genotypes show a two- to threefold difference in P-gp expression in duodenum, kidney, peripheral leukocytes, and placenta, with substantial overlap between genotypes. This modest difference suggests a moderate impact of the *ABCB1* genotypes on the disposition and effects of P-gp substrates; nongenetic factors probably play an important role in modifying P-gp expression. Differences of ~25–35% in the bioavailability and renal clearance of digoxin in relation to the exon 21 or exon 26 SNP have been reported [56–59]. Several studies [60, 61] have addressed the relevance of *ABCB1* polymorphism to dose requirements, blood concentrations, chronic rejection, and chronic nephrotoxicity in renal transplant patients receiving the calcineurin inhibitors cyclosporin A and tacrolimus.

P-gp expressed on the luminal side of endothelial cells of the brain capillaries significantly limits the transfer of many drugs from blood to brain, as evidenced by a huge increase in brain-to-blood concentration in the P-gp knockout mouse [54]. Because many CNS-active drugs are P-gp substrates, differences in *ABCB1* expression at the blood–brain barrier could help explain why patients with identical plasma drug concentrations respond differently and have different side effects. The consequences of genetic polymorphism have been assessed *in vivo* for another transporter of relevance for drug therapy, OATP-C (SCP1B1), which facilitates the uptake of drug substrates from the blood into the hepatocyte. The relatively common

TABLE 10.5 Genetic Polymorphisms of Human Transporters

BSEP	Conjugates	Not yet elucidated
MDR-1	Natural product anticancer drugs, CYP3A4 substrates, digoxin	Not yet elucidated
MRPs	Glutathione, glucuronide, and sulfate conjugates, nucleoside antivirals	Not yet elucidated

variant OATP-C*5 is associated with markedly reduced transporter function [62]. Carriers of the *5 allele have high plasma concentrations of the OATP-C substrate pravastatin [63–65], suggesting impaired uptake of pravastatin by hepatocytes. Indeed, pravastatin concentrations in hepatocytes are low, which results in less inhibition of cholesterol synthesis as assessed by decreased lathosterol concentration and lathosterol/cholesterol ratio [65]. Whether cholesterol-lowering efficacy is impaired in carriers of these variants during long-term treatment is yet unknown. A profound impact of OATP-C polymorphism was recently demonstrated for the antidiabetic drug repaglinide, for which AUC values were approximately three times higher in carriers of the variant *5 allele than in wild-type subjects. This effect was associated with a more pronounced reduction of blood glucose levels [65].

Table 10.5 provides a list of human drug transporters that exhibit functional genetic polymorphisms and their substrates [66].

10.4 MODIFYING DRUG DISTRIBUTION

A number of drugs often exhibit undesirable pharmacokinetic properties. Some of these include unfavorable distribution profiles such as lack of penetration into target tissues or binding to specific tissues that could result in toxicity. A number of approaches are available to modify the distribution profile of a drug. These include alterations in the chemical structure of the drug, use of another drug that alters the pharmacokinetics of the drug under investigation, and encapsulation of the drug in a delivery system.

10.4.1 Alterations in Chemical Structure: Prodrug Design

Prodrug design strategies have been employed to improve the efficacy of drugs with undesirable pharmacokinetic properties such as chemical instability and lack of specificity. Targeted prodrug design represents a strategy for site-directed and efficient drug delivery. Targeting of drugs to transporters and receptors to aid in site-specific carrier-mediated absorption is emerging as a novel and clinically significant approach. Various prodrugs have been successful in achieving the goals of enhanced availability and are therefore considered to be an important tool in biopharmaceutics.

Strategies in targeted prodrug design include antibody-directed enzyme prodrug therapy, gene-directed enzyme prodrug therapy, and peptide transporter-associated prodrug therapy. The term *prodrug* or *proagent* was first introduced to signify pharmacologically inactive chemical derivatives that could be used to alter the

physicochemical properties of drugs, in a temporary manner, to increase their usefulness and/or to decrease associated toxicity [67]. Often, use of the term prodrug implies a covalent link between a drug and a chemical moiety, although some authors also use it to characterize some forms of salts of the active drug molecule. Although there is no universal definition for a prodrug itself, and the definition may vary, generally prodrugs can be defined as pharmacologically inert chemical derivatives that can be converted *in vivo* to active drug molecules, enzymatically or nonenzymatically, to exert a therapeutic effect. Ideally, the prodrug should be converted to the original drug at the target site of action, followed by subsequent rapid elimination of the released derivatizing group [68, 69].

Prodrugs can be designed to target specific enzymes or carriers by considering enzyme–substrate specificity or carrier–substrate specificity in order to overcome various undesirable drug properties. This type of "targeted-prodrug" design requires considerable knowledge of particular enzymes or carrier systems, including their molecular and functional characteristics. Targeted prodrug designs are classified into two categories: (1) targeting specific enzymes and (2) targeting specific membrane transporters.

Prodrug Design Targeting Enzymes In prodrug design, enzymes can be recognized as presystemic metabolic sites or prodrug–drug *in vivo* reconversion sites. Usually, targeting enzymes to reduce the presystemic metabolism is more successfully achieved by irreversible chemical modification rather than by a prodrug approach. Therefore, our discussion focuses on the enzymes as *in vivo* reconversion targets for prodrugs. The enzyme-targeted prodrug approach can be used to improve oral drug absorption, as well as site-specific drug delivery. In the case of improving oral drug absorption, gastrointestinal enzymes may be the main targets for prodrug design, and the use of a nutrient moiety as a derivatizing group permits more specific targeting for gastrointestinal enzymes to improve oral drug absorption [70]. These prodrugs have the additional advantage of producing nontoxic nutrient by-products when they regenerate the active drugs *in vivo*.

Site-specific drug delivery can be obtained from tissue-specific activation of a prodrug, which is the result of metabolism by an enzyme that is either unique for the tissue or is present at a higher concentration (compared with other tissues); thus, it activates the prodrug more efficiently. This type of site-specific drug delivery has been of particular interest in cancer chemotherapy. Appropriately designed prodrugs have been found to be effective in the treatment of animal tumors possessing high levels of an activating enzyme [71, 72]. However, clinical results were disappointing when it was found that human tumors containing appropriately high levels of the activating enzymes were rare and that the high levels of activating enzymes were not associated with any particular type of tumor [73]. Recently, new therapies have been proposed to overcome this limitation of prodrug therapy. These new approaches are referred to as ADEPT (antibody-directed enzyme prodrug therapy) and GDEPT (gene-directed enzyme prodrug therapy), which attempt the localization of prodrug activation enzymes into specific cancer cells prior to prodrug administration.

Prodrug Design Targeting the Membrane Transporters Although the classical approach to improve membrane permeability of polar drugs uses lipophilic derivatives to increase passive membrane penetration, the targeted prodrug approach uses

transporters designed for facilitating membrane transport of polar nutrients such as amino acids and peptides. There is direct and indirect evidence for the participation of carrier-mediated membrane transport mechanisms, where several hydrophilic compounds seem to be absorbed efficiently via specific transporters [74]. Therefore, targeting specific membrane transporters is particularly important when prodrugs are polar or charged. Prodrugs can be designed to structurally resemble endogenous compounds and to be transported by specific carrier proteins. In this case, prodrugs may have the additional advantage of producing nontoxic by-products when prodrugs are converted to the parent drug molecules. The brain uptake of the potent glycine-NMDA receptor antagonists, such as 7-chlorokynurenic acid and 5,7-dichlorokynurenic acid, was significantly improved by their respective prodrugs, L-4-chlorokynurenine and L-4,6-dichlorokynurenine, which are amino acid derivatives [70]. L-4-chlorokynurenine was shown to be rapidly delivered into the brain by the large neutral amino acid transporter of the blood–brain barrier and to be converted intracellularly to its parent drug, 7-chlorokynurenic acid [70]. Furthermore, there have been some reports on prodrug design targeting peptide transporters, including peptidyl derivatives of methyldopa and alafosfalin and tripeptidyl prodrugs of foscarnet [75–77]. Developing prodrugs targeting specific membrane carriers requires considerable knowledge of the carrier proteins, including their distribution and substrate specificity.

10.4.2 Pegylation

Pegylation refers to the modification of a therapeutic agent by the attachment of poly(ethylene glycol) (PEG) molecules through covalent conjugation to the therapeutic agent. Pegylation may be an effective method of delivering therapeutic proteins and modifying their pharmacokinetic properties, in turn modifying pharmacodynamics, via a mechanism dependent on altered binding properties of the native protein [78]. PEG moieties are inert, long-chain amphiphilic molecules produced by linking repeating units of ethylene oxide [79]. A large number of potential PEG molecules are available, and they can be produced in different configurations, including linear or branched structures, and in different molecular weights (Fig. 10.7). Using pegylation to increase the size and molecular weight of a therapeutic protein

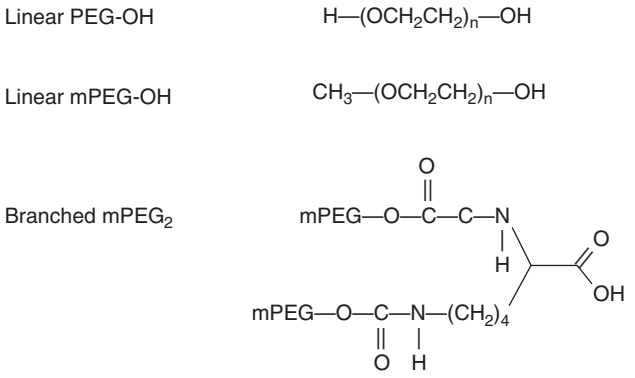

FIGURE 10.7 Structural formulas of poly(ethylene glycol) (PEG) molecules. mPEG = monomethoxypoly(ethylene glycol). (From Ref. 80, with permission.)

alters the immunological, pharmacokinetic, and pharmacodynamic properties of the protein in ways that can extend its potential uses [80, 81]. Goals for chemically coupling PEG to peptide and protein drugs include decreased renal clearance and, for some products, more sustained absorption after subcutaneous administration as well as restricted distribution [82]. These pharmacokinetic changes may result in more constant and sustained plasma concentrations, which can lead to increases in clinical effectiveness when the desired effects are concentration dependent. Maintaining drug concentrations at or near a target concentration for an extended period of time is often clinically advantageous and is particularly useful in antiviral therapy, since constant antiviral pressure should prevent replication and may thereby suppress the emergence of resistant variants [83]. Additionally, PEG modification may decrease adverse effects caused by the large variations in peak-to-trough plasma drug concentrations associated with frequent administration and by the immunogenicity of unmodified proteins. Pegylated proteins may have reduced immunogenicity because PEG-induced steric hindrance can prevent immune recognition [84]. Furthermore, pegylation can enhance targeting of peptides and proteins to tumor tissue through enhanced permeation and retention (EPR) effect [78]. Tumor tissues have a very porous vasculature, and large molecular weight compounds (like pegylated drugs) have enhanced permeation into tumor tissue. Once permeated into the tumor, they are retained in the tumor for a prolonged period of time due to poor drainage from tumor. This EPR effect associated with pegylation has also been used to improve the therapeutic effectiveness of a number of drug molecules like doxorubicin [85, 86]. Pegylation has also been used to improve the circulation times of nanometer-size delivery systems such as liposomes and nanoparticles [87]. Table 10.6 lists the potential types of PEG conjugates.

TABLE 10.6 Types of PEG Conjugates

Conjugate Type	Properties and Applications[a]
Small molecule drugs	Improved solubility, controlled permeability through biological barriers, longevity in bloodstream, controlled release
Affinity ligands and cofactors	Used in aqueous two-phase partitioning systems for purification and analysis of biological macromolecules and cells; enzymatic reactors
Peptides	Improved solubility, conformational analysis, biologically active conjugates
Proteins	Resistance to proteolysis, reduced immunogenicity and antigenicity, longevity in bloodstream, tolerance induction; uses include therapeutics, organic-soluble reagents, bioreactors
Saccharides	New biomaterials, drug carriers
Oligonucleotides	Improved solubility, resistance to nucleases, cell membrane permeability
Lipids	Used for preparation of PEG-grafted liposomes
Liposomes and particulates	Longevity in bloodstream, RES evasion
Biomaterials	Reduced thrombogenicity, reduced protein and cell adherence

[a]PEG, poly(ethylene glycol); RES, reticuloendothelial system.

10.4.3 Use of Another Drug to Modify Distribution

A drug molecule that affects the pharmacokinetics of a second drug can be used clinically to modify the disposition, and therefore the pharmacodynamics, of the second drug. This phenomenon has been used previously to modify the absorption and elimination of a number of drugs. Classic examples include the use of probenecid to decrease the urinary excretion of penicillin [88] and the use of vasoconstrictors to decrease the rate of absorption of local anesthetics from subcutaneous injection site [89]. Use of hyperosmotic mannitol to transiently open up the tight junctions of the blood–brain barrier represents one of the earliest attempts to use one agent to modify the distribution of another drug [90]. With increasing knowledge of the effects of transporters on drug distribution and pharmacokinetics, a number of attempts have been made to use the inhibitors of these transporters to improve the distribution profile of drugs. Because the use of inhibitors of P-gp have been the most studied, this is discussed further.

As discussed earlier, P-gp is overexpressed in many normal tissues such as the capillary endothelium of the blood–brain barrier (BBB), the intestinal epithelium, and also in tumor tissues. Overexpression of P-gp in the blood–brain barrier results in the reduced transport of P-gp substrates into the brain. The significance of this problem is highlighted by the estimations that up to 98% of the newly developed small molecules will not cross the BBB [91]. This limits the number of drugs available for treating diseases like epilepsy [92], stroke and brain injury [93], brain cancer [94], HIV infection of the brain [95], and amyotrophic lateral sclerosis [96]. Numerous preclinical studies and a few clinical studies have indicated the potential of P-gp inhibitors to enhance brain delivery of P-gp substrates [91, 94, 97–99]. P-gp inhibition was evaluated as a means of increasing the brain delivery of the peripherally acting opioid loperamide [100]. Healthy volunteers received a single oral dose of loperamide with or without the P-gp inhibitor quinidine. The central effect of loperamide (change in ventilatory response in response to carbon dioxide) was only observed in volunteers receiving both loperamide and quinidine, indicating the ability of the P-gp inhibitor to enhance the brain delivery of P-gp substrates. Other case studies have demonstrated the use of the P-gp inhibitor verapamil to enhance the brain delivery of anticonvulsant drugs in refractory epilepsy [101, 102].

Many important anticancer agents like doxorubicin and paclitaxel are substrates of P-gp. Overexpression of P-gp in tumor cells results in the development of drug resistance. Initial clinical trials with P-gp inhibitors to treat resistant tumors were performed with "first generation" P-gp inhibitors such as cyclosporin, which were already in use for other indications [103]. Absence of confirmation of P-gp expression in the tumors and P-gp inhibitor toxicity at doses administered to achieve serum concentrations comparable to those that were effective in animal models resulted in the failure of these drugs in clinical trials [104]. Second generation inhibitors (e.g., PSC 833) were developed solely for the purpose of overcoming drug resistance [105]. These agents were tested in clinical trials in various malignancies for which there was evidence that P-gp is expressed or associated with a poorer therapeutic outcome [106]. One major limitation of these trials, however, was the reduction in anticancer drug doses that was required with concurrent administration of inhibitor [107]. P-gp inhibitors increased the serum levels of the coadministered

chemotherapeutic agent. A number of studies found that reduction in the dose led to a number of patients being undertreated, which could have contributed to the failure of these combination treatments [107]. Pharmacokinetic interactions between the P-gp inhibitor and the drug could also result from the inhibitor's ability to inhibit other proteins involved in drug metabolism such as cytochrome P450 [108] P-gp inhibitors with fewer pharmacokinetic interactions are being developed [109], and functional assays to verify the role of P-gp in drug resistance, such as sestamibi imaging, are proving helpful in assessing the development of the newer inhibitors [110]. A number of other approaches to overcome P-gp that are currently in development include the use of monoclonal antibodies against P-gp [111, 112], antisense oligonucleotides [113], pH-sensitive polymeric micelles [114], Pluronic$^{(r)}$ copolymers [115], peptide-based transmembrane inhibitors [116], and inhibitors of signal transduction [117].

10.4.4 Encapsulation in Delivery Systems

A delivery system is often used to encapsulate a drug, because of the following advantages: (1) prolonged drug availability in the body because of sustained release of the drug from the delivery system; (2) enhanced availability of the drug at the target site—by choosing appropriate formulation parameters, a favorable tissue distribution profile could be obtained; and (3) enhanced stability of the drug due to protection from drug-metabolizing enzymes. Sustained (or continuous) release of a drug from a delivery system involves slow diffusion of the drug out of a polymeric matrix and/or slow degradation of the polymer over time. Pulsatile release is sometimes the preferred method of drug delivery, as it closely mimics the way in which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g., exposure to light, changes in pH or temperature) [118].

Choice of the delivery system often depends on the route of administration, nature of the drug, and nature of the disease. Applications involving intravenous administration require the use of nanometer-size delivery systems. This is necessary to reach distant target sites perfused by fine capillaries (diameter $\approx 1\,\mu m$) and to prevent embolism. Colloidal drug carrier systems such as micelles, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10–400 nm diameter have been found useful as intravenous drug delivery systems. Figure 10.8 demonstrates different types of drug carriers. When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life, and low toxicity [119].

Micelles formed by self-assembly of amphiphilic block copolymers (5–50 nm) in aqueous solutions are of great interest for drug delivery applications [120]. Drugs can be physically entrapped in the core of block copolymer micelles and transported at concentrations that can exceed their intrinsic water solubility. Moreover, the hydrophilic blocks can form hydrogen bonds with the aqueous surroundings and form a tight shell around the micellar core. As a result, the contents of the hydrophobic core are effectively protected against hydrolysis and enzymatic degradation. In addition, the corona may prevent recognition by the reticuloendothelial system and therefore preliminary elimination of the micelles from the bloodstream.

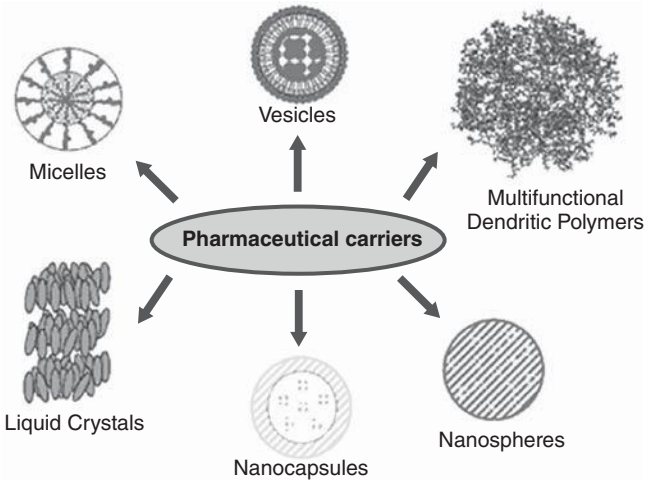

FIGURE 10.8 Schematic of various pharmaceutical carriers. (From Ref. 120, with permission.)

Functionalization of block copolymers with crosslinkable groups can increase the stability of the corresponding micelles and improve their temporal control. Substitution of block copolymer micelles with specific ligands is a very promising strategy to achieve targeted drug delivery.

Liposomes are a form of vesicles that consist either of many, a few, or just one phospholipid bilayer [121]. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity toward the phospholipids. Channel proteins can be incorporated without loss of their activity within the hydrophobic domain of vesicle membranes, acting as a size-selective filter, only allowing passive diffusion of small solutes such as ions, nutrients, and antibiotics. Thus, drugs that are encapsulated in a nanocage functionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes. The drug molecule, however, is able to diffuse through the channel, driven by the concentration difference between the interior and the exterior of the nanocage.

Dendrimers are nanometer-sized, highly branched, and monodisperse macromolecules with symmetrical architecture [122]. They consist of a central core, branching units, and terminal functional groups. The core, together with the internal units, determines the environment of the nanocavities and consequently their solubilizing properties, whereas the external groups determine the solubility and chemical behavior of these polymers. Targeting effectiveness is affected by attaching targeting ligands at the external surface of dendrimers, while their stability and protection from the phagocytosis is achieved by functionalization of the dendrimers with PEG chains.

Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed [123]. Biodegradable polymeric nanoparticles have many applications in the controlled release of drugs, in targeting

particular organs/tissues, as carriers of DNA in gene therapy, and in their ability to deliver proteins, peptides, and genes to the target tissue. Like for other nanocarriers, pharmacokinetics and the biodistribution profile of nanoparticles may be altered by varying the surface properties (e.g., addition of PEG chains) and attaching specific tissue-targeting ligands on the surface.

10.5 CONCLUSION

Distribution plays an important role in determining the magnitude and duration of a drug's therapeutic effect. Drug distribution is influenced by physical properties of the drug as well as physiologic factors such as blood perfusion, protein binding, and transporter activity. A number of physical and chemical methods are available to alter the distribution of the drug. These allow the therapeutic use of drug molecules that would otherwise have unfavorable distribution in the body.

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