1 Introduction

1.1 New chemical entities (NCE-Discovery)

Drug research is a complex and time consuming process. A period of 8-10 years is often needed from the discovery of a new chemical entity to marketing authorization of a new drug. This process can be divided into two main phases (i) drug discovery, which includes target identification, hit discovery, and lead optimization; and (ii) drug development, which comprises preclinical and clinical studies. Inappropriate pharmacokinetic (PK) behavior has been recognized as one of the major factors leading to the rejection of NCEs during drug development. (Kennedy 1997)

In the 1990s the paradigm for finding NCEs changed drastically. New technologies like combinatorial chemistry, computational modeling, genomics and proteomics and high throughput screening (HTS) increased the possibility of finding new lead compounds within a much shorter time period than conventional medicinal chemistry. (Baum 1994), (Gordon 1996). It became feasible to screen hundreds of thousands of compounds across innovative assays to determine their *in vitro* receptor and enzyme affinity. Typical goals include enzyme inhibitors, receptor agonists or antagonists, and transporter inhibitors or activators. (Knowles & Gromo 2003)

The initial identification of candidate "lead" compounds usually involves high-throughput screening (HTS) of diverse small molecule collections or structurally selected compounds with known or theoretically predicted activity at a target site. (Dove 2003). Initial drug discovery thus requires a collection of compounds for testing and a robust assay of target activity. "Hits" from initial screening are evaluated on the basis of many criteria, including but not limited to potency, specificity, toxicity,

biopharmaceutical properties, and efficacy in cell culture models, to select lead compound(s) for optimization by synthetic chemistry and more extensive preclinical evaluation in animal models (Bleicher et al. 2003), (Kenakin 2003)). These preclinical data form the basis of an investigational new drug (IND) application which must be approved before clinical studies can be carried out. (Lipsky & Sharp 2001). The process of hit identification and lead optimization is depicted in Figure 1-1.

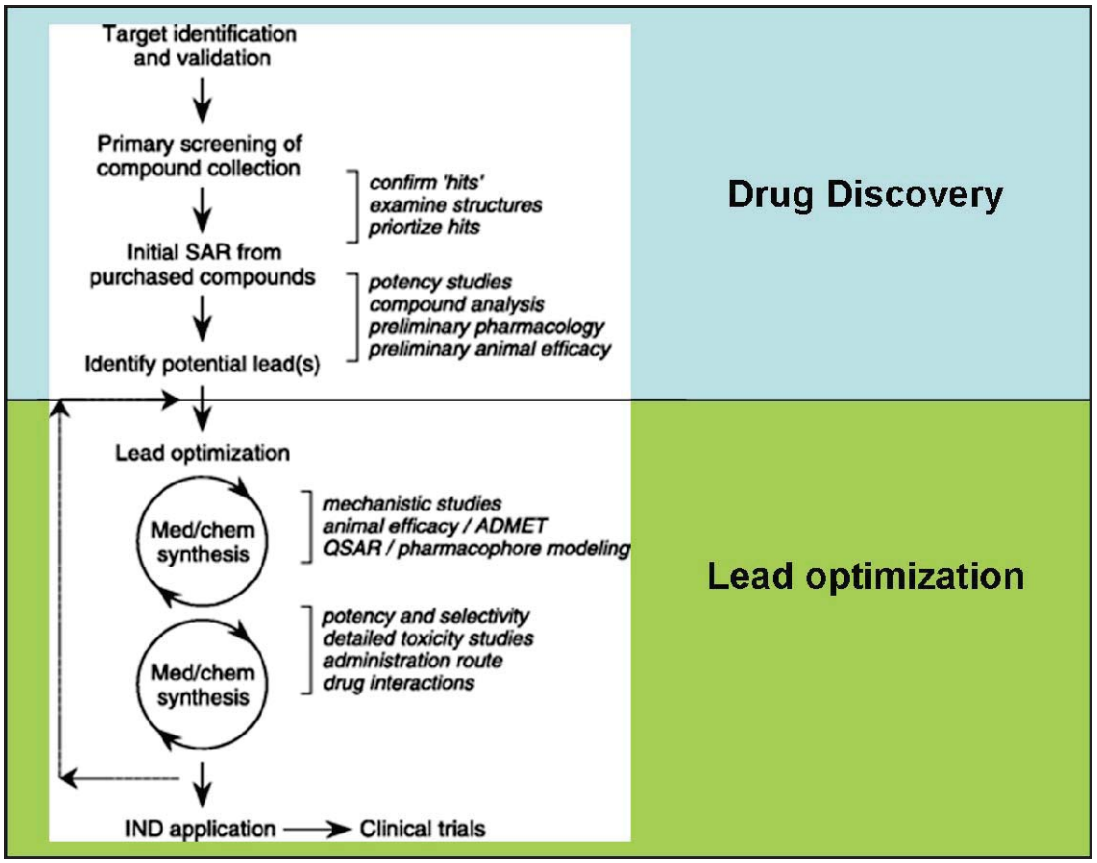

Figure 1-1 Strategy for preclinical drug discovery from (Verkman 2004)

By adapting these new techniques in the drug development process, the numbers of potential lead substances increases, but often seemingly promising compounds have failed at later stages of development because of unsatisfactory absorption, distribution, metabolism and excretion (ADME) properties and toxic effects. Hence

ADME and toxicological evaluation have increasingly been shifted into the early drug discovery phase. Nowadays lead identification and optimization occur in parallel to toxicological evaluation and ADME characterization.

Since in this early stage the amount of drug substance available is quite low (<10mg) and the toxicology of this substance has not evaluated, experiments in higher animals or humans are not yet possible. Thus, early ADME and toxicological studies using high throughput assays were established.

Concurrently it was identified that simple physicochemical properties (solubility, ionization and lipophilicity) can assist in predicting the ADME behavior of a compound (Smith et al. 1996) (Kerns 2001).

With time, HTS became an integral part of the discovery process and is now the preferred tools for identifying NCEs. (Bender A 2008) (Popa-Burke 2009)

As a result, drugs being developed are exhibiting an ever wider range of physical chemical characteristics. Substances identified by HTS show a trend to high lipophilicity and low solubility as well as to high molecular weight. (Lipinski 2000) The reason why the HTS technique leads to selection of highly lipophilic compounds is because hydrophobicity generally results in increased receptor affinity through non-specific binding to the target. Furthermore, candidates are often dissolved in organic solvents like dimethyl sulfoxide, N-Methyl pyrrolidone or dimethylformamide and then diluted with buffer in the HTS procedure. This circumvents the necessity for good aqueous solubility of the candidate.

To assess whether such compounds possess not only the desired pharmacological activity but also the properties necessary for adequate bioavailability following oral administration, additional tests are required.

Lack of efficacy, unexpected toxicity and poor pharmacokinetics are the major causes of drug failure in clinical trials. A key determinant of efficacy and pharmacokinetic properties is the ability of the drug to penetrate biological barriers such as the gastrointestinal wall, cell membrane, or blood brain barrier (BBB). (Kennedy 1997), (Tsaioun et al. 2009). Since about 2005 ADME and toxicity screening has been investigated earlier in the development program, increasing the attrition rate of poor drug candidates early in the discovery/development process, and thus decreasing the proportion of compounds that fail in clinical trials for these reasons. (Refsgaard et al. 2005)

As part of these screening tools, assays have been developed to specifically address the various ADME processes. For example, poor aqueous solubility often results in poor bioavailability after oral dosing. Screening tools for both solubility under gastrointestinal GI conditions and membrane uptake have been developed. But the gastrointestinal tract is only one of many barriers that the drug must pass in order to reach its site of action. For drugs that are intended to act in the central nervous system, the ability to cross the blood brain barrier (BBB) is a further, crucial prerequisite for success. Conversely, low BBB penetration may be desirable in order to minimize CNS-related side-effects for drugs that are designed to act at peripheral sites. Thus, the ability to cross the BBB has emerged as an issue of primary interest, even in the early stages of drug discovery. (Fu et al. 2001)

In conclusion, not only the pharmacological properties of new substances but also a fine balance between pharmacokinetic properties, safety and efficacy of substances is needed to successfully bring an NCE to market. Therefore, a great deal of effort has been made to screen for these characteristics as early as possible in the drug discovery process, preferably before the drug candidate enters the lead optimization phase. In this way, only compounds with high potency and suitable PK properties are selected for further development.

1.2 ADME-Properties

Pharmacokinetics is defined as the study of the absorption, distribution, metabolism, and excretion (ADME) of a drug introduced into a biological system, such as the human body. (Scherrmann 2009) The molecular properties for absorption, distribution, metabolism, and excretion (ADME) are crucial for drug design. Following oral administration, a drug must pass through the intestinal mucosa to reach the systemic circulation, as described in Figure 1-2. (van de Waterbeemd 2003)

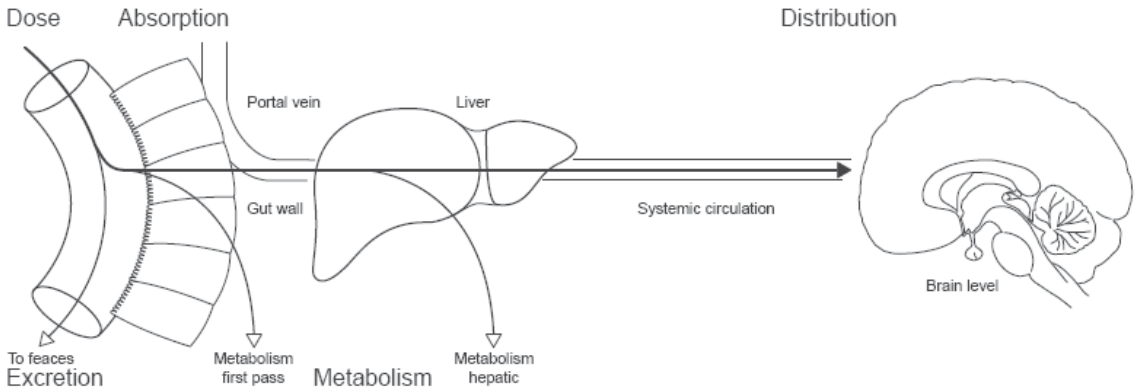

Figure 1-2 Absorption-Distribution-Metabolism-Excretion (ADME)-processes following oral absorption

1.2.1 Absorption

Absorption is defined as the process by which the dissolved drug proceeds across the intestinal mucosa. In the ideal case, the active ingredient is released from the dosage form, dissolves in the GI fluids before it reaches the site of absorption, gets absorbed and becomes 100% available in the systemic circulation, as shown in figure 1-3.

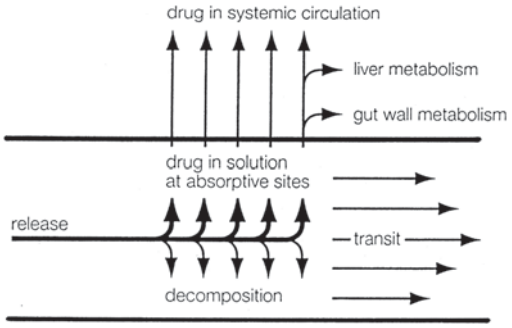

Figure 1-3 Events in the GI tract following the oral administration route taken von (Dressman & Reppas 2000)

For absorption via transcellular passive diffusion, the molecules have to distribute into the apical membrane of the enterocytes. The partitioning is a result of physical interactions between the membrane surface and the molecule. The absorption through the intestinal wall is elaborated in Chapter 1.4.1.

1.2.2 Distribution

Distribution is defined as a process of reversible transfer of a drug between the blood plasma and the peripheral tissues. In the majority of cases the concentration of a drug is measured in plasma, serum or whole blood but often the site of action is in other tissues. In these cases the drug needs to be carried to its site of action via the bloodstream. After a drug enters the systemic circulation, either by intravascular injection or by absorption from any of the various extra vascular sites, the drug is

subjected to numerous distribution processes that tend to lower its plasma concentration. Distribution is generally uneven because of differences in blood perfusion, tissue binding (e.g. because of lipid content or protein binding), regional pH, and permeability of cell membranes. Some factors affecting drug distribution include regional blood flow rates, molecular size, polarity and binding to proteins, either in the plasma or extra vascular tissues.

1.2.3 Metabolism

The principal site of drug metabolism is in the liver, after extraction of the drug from the general circulation. Drugs metabolism occurs in two phases.

Phase I reactions involve formation of a new or modified functional group or cleavage of an existing functional group (typically by oxidation, reduction or hydrolysis). Phase II reactions involve conjugation with an endogenous substance (e.g. glucuronic acid, activated sulfate, activated glycine). Metabolites formed in Phase II reactions are usually more polar and therefore more readily excreted by the kidneys (in urine) than those formed in Phase I reactions.

The enzymes involved in metabolism are present in many tissues but are generally more concentrated in the liver. The most important enzyme system of Phase I metabolism is cytochrome P-450 (CYP-450), a microsomal superfamily of isoenzymes that catalyze the oxidation of many drugs. The electrons are supplied by NADPH (CYP-450 reductase), a flavoprotein that transfers electrons from NADPH (the reduced form of nicotinamide adenosine dinucleotide phosphate) to CYP-450. CYP-450 enzymes can be induced or inhibited by many drugs and xenobiotica, helping explain many drug interactions in which one drug enhances the toxicity or reduces the therapeutic effect of another drug.

1.2.4 Elimination

Elimination is defined as the irreversible transfer of a drug from the site of measurement and includes drug metabolism, renal excretion and biliary excretion as well as several other minor routes. Drug metabolism is included in elimination since, if a drug is chemically modified, it is no longer the original compound and, unless the metabolism is reversible, has been irreversibly removed from the site of measurement. It is therefore important, that bioanalysis of the drug is specific. Only then can a true understanding of the pharmacokinetics of the drug be obtained. If information is required on a metabolite because it is pharmacologically or toxicologically active, it must be analyzed independently, since non-specific assays will invariably lead to erroneous conclusions.

The kidneys, which excrete water-soluble substances, are the principal organs of excretion. The biliary system contributes to excretion to the degree that drug is not subsequently reabsorbed from the GI tract. Generally, the contribution of intestine, saliva, sweat, breast milk, and lungs to excretion is small, except for the exhalation of volatile anesthetics.

1.3 Solubility

Since the paradigm shift in drug discovery, lead candidates have increasingly exhibited poor aqueous solubility characteristics. In the last decade, several methods to improve the solubility *in vivo* have appeared.

Most of these techniques rely on the modified Noyes-Whitney equations (Withney 1897) (Nernst 1904) to provide some hints as to how solubility might be improved in order to minimize the limitation to oral availability:

$$\frac{dC}{dt} = \frac{AD(C_s - C)}{V \cdot h} \qquad \text{1-1}$$

where dC/dt is the dissolution rate, A is the surface area available for dissolution; D is the diffusion coefficient of the compound, Cs the solubility of the compound in the dissolution medium and C the concentration of the drug in the medium at time t. The volume V and the thickness h of the diffusion boundary layer adjacent to the surface of the dissolving drug complete the parameters in this equation.

Many of the established methods to increase oral bioavailability are aimed at increasing the drug solubility, increasing the surface area available for dissolution by decreasing the particle size of the solid compound or optimizing the wetting characteristics of the compound surface.

These include:

- Micronization / nanosizing

The reduction of the particle size increases the specific surface area of the drug and may also, in the case of nanosizing, result in some increase in solubility due to the higher energy of the particle surface. (Keck & Muller 2006)

- Salt formation

The salt form can improve the aqueous solubility of a compound by presenting the drug in its ionized and thus better soluble form. (Paulekuhn et al. 2007)

- Lipid based formulations

Lipid based formulations include carrier systems such as oils, surfactants, liposomes and emulsions. Incorporation into emulsions is preferred for the formulation of highly

lipophilic compounds. (Pouton 2000) If the drug is present in solution, the dissolution step is replaced by a partitioning step, which may be aided and abetted by digestion of the oily vehicle.

- Solid dispersions or solutions

The poorly soluble substance is incorporated as a solid dispersion or preferably a solid solution in a carrier which dissolves readily in the GI fluids. (Kanzer et al.) In the case of solid solutions the drug automatically goes into solution as the carrier is dissolved.

- Co-solvents

The addition of co-solvents follows the approach of '*similia similibus solventur*' by decreasing the polarity of water via the addition of less polar additives. (Strickley 2004) Here too, the drug is presented as a solution, circumventing the dissolution step. A caveat to co-solvent solutions and solid solutions is that a supersaturated solution is often formed *in vivo*, from which precipitation of the drug may occur.

- Complexation

Using complexing agents like cyclodextrin or their derivates, the poorly water soluble drug is incorporated in a hydrophilic agent. (Loftsson et al. 2007) The complex then dissolves faster than would be possible for the drug by itself.

- Surfactants or micelles

Using physiologically well-tolerated surfactants, the wetting of the drug is improved and, if micelles are formed, solubilization of the drug may also occur.

The human GI-tract contains natural solubility enhancers, bile and lecithin, which solubilize and emulsify fats and lipophilic, poorly soluble compounds and therefore facilitate their absorption. Bile is composed of water (80%), bile salts (12%) and cholesterol, phospholipids and electrolytes (<5%). (Klein 2005) Both the bile salts and lecithin are surface active substances which lower the surface tension and tend to form micelles. The CMC level for the bile salts ranges from 13-20mM (Coello et al. 1996). Addition of lecithin lowers the CMC to below 1mM. (Sewell et al. 1980) Due to its surface activity, the bile salt lowers the surface tension between the substance and water by breaking up the internal structure of water. The open structure is able to better accommodate the solute, including highly lipophilic, poorly water soluble substances like fats.

Lecithin constitutes about 3-4% of the bile and forms mixed micelles with bile salts. These mixed micelles have a much lower CMC (<1mM) than the pure bile salts. The micelle diameter increases by a factor of about 100. (Naylor et al. 1993) Figure 1-4 is a schematic representation of the mixed micelle structure, with incorporation of lipophilic substrates.

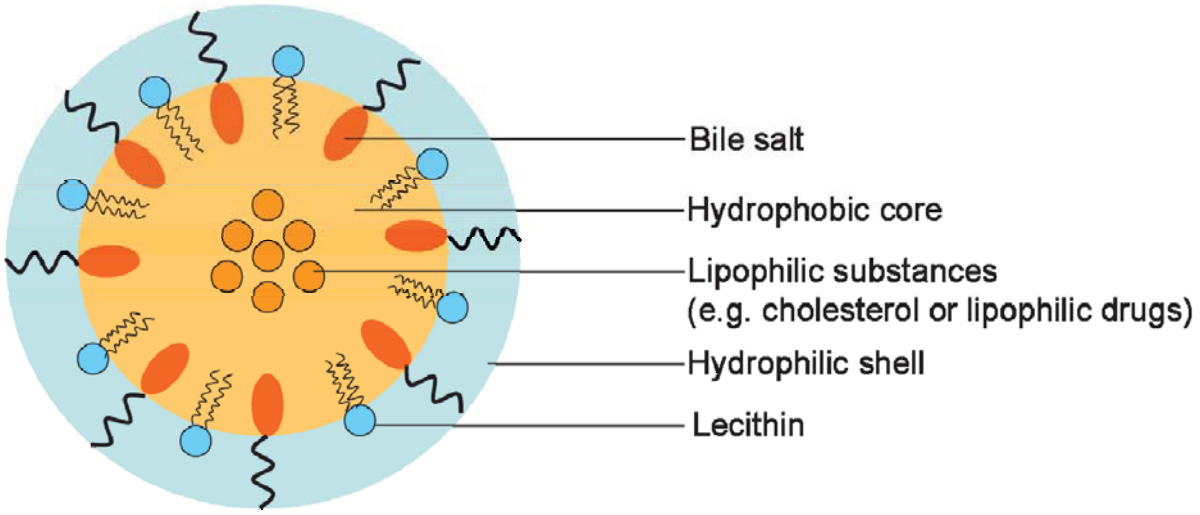

Figure 1-4 Composition of a mixed bile salt/lecithin micelle modified from Lindenberg 2007

1.4 Transport across lipid bilayers (intestinal/brain)

Biological membranes are amphiphilic structures composed of a hydrophobic interior and hydrophilic surfaces facing the aqueous environment on both sides of the membrane. These membranes consist, to a large extent, of lipids.(Parsons 1975) Many drug substances also exhibit amphiphilic properties, facilitating accumulation at the interface, which favors partitioning into the hydrophobic interior of the membrane. For the overall penetration of drug through the membrane, structural and physicochemical properties like molecular size, ionization, hydrogen bonding potential and lipophilicity are considered to be important. (Pauletti et al. 1997)

1.4.1 Morphological aspects of intestinal drug absorption

One essential process driving *in vivo* performance for oral administered drugs is the absorption of the compound from the GI tract into the general circulation. Oral absorption is defined as fraction dose absorbed (FA [%]). (Keldenich 2009)

The oral route of drugs administration is the most frequently used route, since it is a non-invasive and convenient method of delivering drugs to the body. Generally, absorption takes place in the small intestine, due to its special structure. The small intestine possesses a complex morphology, characterized by a high effective surface area with a large regional vasculature (Davenport 1982; Despopoulos 2007). Functionally, the small intestine is divided in three parts: duodenum, jejunum and ileum. Together, the jejunum and the ileum account for more than 99% of the total intestinal surface area of the gastrointestinal tract. As shown in Figure 1-5 the surface area in the intestine is increased about three-fold by circular folds (a) and a further 4-10 fold by the villi (b). Each villus consists of a monolayer border of

epithelial cells (c), which are characterized by microvilli-brush border at the apical side. This structure increases the absorption surface in the intestine to approximate 180m². (Lennernäs 2007) (Avdeef 2001)

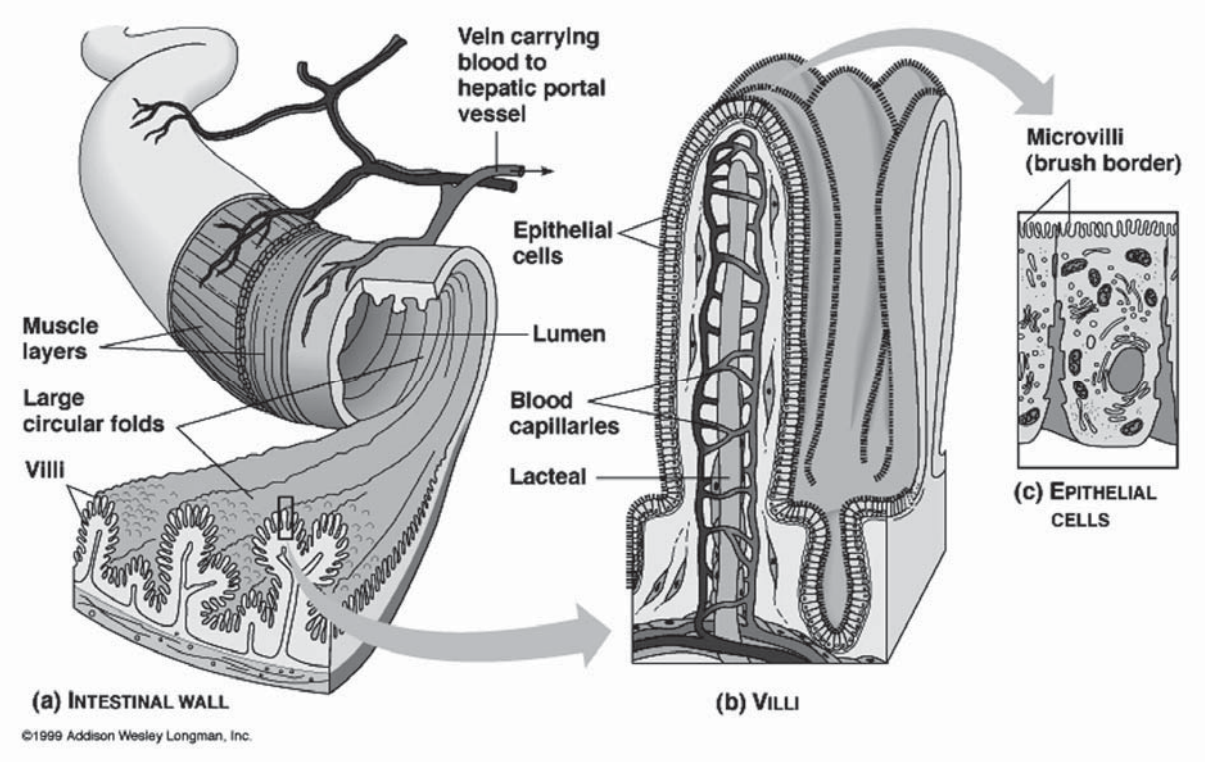

Figure 1-5 The intestinal mucosa- villi structure- taken from Addison Wesley Longman. Inc. (Avdeef 2001)

While the small intestine facilitates the absorption of nutrients like electrolytes, monosaccharides, amino acids, peptides and vitamins through specialized uptake mechanisms, it limits the absorption of xenobiotics like drugs, chemicals and bacteria. In order to distinguish between nutrients and potentially harmful substances, the intestinal mucosa contains cells with unique features.

The most important part for drug absorption are the epithelium cells of the intestinal mucosa. (H. Lennernäs 1996) This epithelial monolayer consists of cells with different characteristics on the apical side to those characteristics on the basolateral side. The

apical membrane of a polarized cell is the surface of the plasma membrane that faces the lumen, while the basolateral membrane forms its basal and lateral surfaces, facing towards the interstitium, and away from the lumen. The surface area of the apical membrane is enlarged by microvilli. Tight junctions, located at the apical end of neighboring epithelia cells, bind the cells together and limit access to the intercellular space.

1.4.2 Mechanisms of intestinal transport

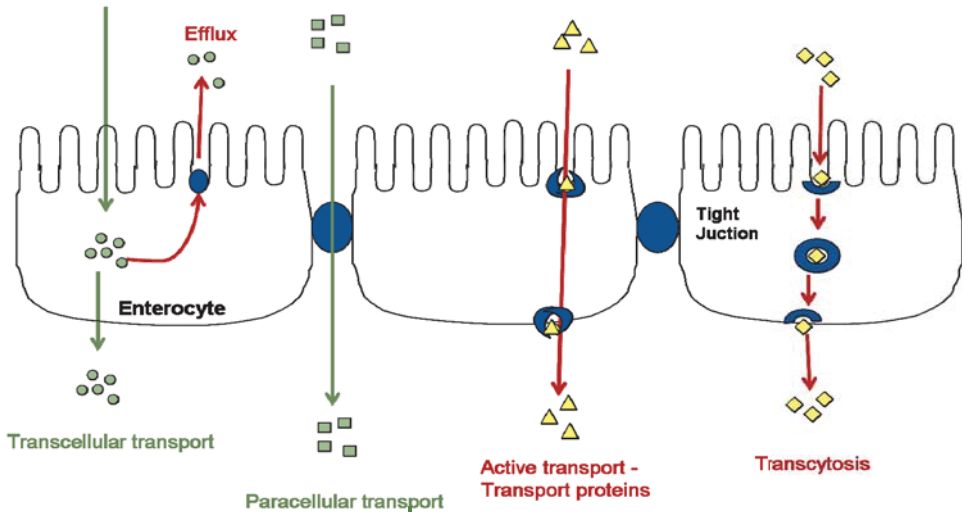

Figure 1-6 Scheme of paracellular, active, passive and vesicular transport through the intestinal epithelial Membrane

As shown in Figure 1-6, dissolved molecules gain access from the intestinal lumen to the blood via two major pathways:

1.) Passive diffusion processes

I. transcellular pathway through the gut membrane

II. paracellular pathway through the water-filled pores of the tight junctions between adjacent cells.