Review Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2015, Volume 3, Issue 9
ISSN: 2321–3647(online)

A Rationalized Description on Study of Drug Permeability and Permeation Enhancers

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ABSTRACT

Hydrophilic drugs are one of the most challenges in biopharmaceutical research. Hydrophilic drugs show low bioavailability following oral administration because of their poor intestinal permeation. This review sets out to discuss about anatomy and physiology of the intestinal barrier, drug absorption from intestinal tract, mechanism of intestinal drug permeability, detail information about intestinal permeation enhancers and its mechanism of action, *in-vitro* methods for studying drug permeability, advantages and applications of intestinal permeation enhancers.

Keywords: Intestinal Permeation Enhancers, BCS, Tight Junctions, Oral Bioavailability.

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Received 21 August 2015, Accepted 09 September 2015

Please cite this article as: Sahu H *et al*., A Rationalized Description on Study of Drug Permeability and Permeation Enhancers. American Journal of Pharmacy & Health Research 2015.

INTRODUCTION

Oral administration is the most common method of drug delivery used today. Optimizing bioavailability of orally administrated drug is one of the most important aim for the pharmaceutical industry[1]. Transport across mucosal membranes is a fundamental step for oral absorption and systemic availability. The drugs which are small and lipophilic in nature are easily permeated through the intestinal barrier whereas oral administrations of macromolecules are restricted by the intestinal epithelial barrier which results in greatly reduced bioavailability[2]. A great number of currently available drugs fall under the class III of the biopharmaceutical classification system, possess high therapeutic potential but cannot be delivered by oral route because of its poor permeation across the GIT epithelia. 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 para-cellular pathway. The contribution of the latter to intestinal absorption is considered to be small, since this pathway occupies less than0.1% of the total surface area of the intestinal epithelium, and the presence of tight junctions(TJ) between the epithelial cells limits drug

WHO listed out the BCS class III drugs, they are shown in below Table-1[1].

Table 1: WHO listed out the BCS class III drugs

Drugs	Solubility	Permeability	Therapeutic activity
Abacavir	High	Low	Antiretroviral
Acyclocir	High	Low	Antiherpes
Amoxicillin	High	Low	Antibacterial
Atenolol	High	Low	Antianginal,
Benznidazole	High	Low	American tripanosomiasis
Chloramphenicol	High	Low	Antibacterial
ChlorpromazineHcl	High	Low	Psychotherapeutic
Codeine phosphate	High	Low	Opionid analgesic
Didanosine	High	Low	Antiretroviral
Enalapril	High	Low	Antihypertensive
Ergocalciferol	High	Low	Vitamin
Ethambutal Hcl	High	Low	Antituberculosis
Folic acid	High	Low	Antianaemia
HydralazineHcl	High	Low	Antihypertensive
Hydrochlorthiazide	High	Low	Diuretic
Levothyroxin Na salt	High	Low	Thyroid hormone
Mannitol	High	Low	Osmotic diuretic
α-Methyldopa	High	Low	Antihypertensive
Metoclopramide Hcl	High	Low	Anti emetic
Neostigmine bromide	High	Low	Muscle relaxant
Penicillamine	High	Low	Antibacterial

These drugs have low intrinsic membrane permeability, probably because of their low lipophilicity and zwitterionic character at physiological pH or act as a substrate to drug efflux pumps like p-glycoprotein, ionic charge and high molecular weight[1].

According to the Biopharmaceutics Classification System, drug substances are classified as follows:

- **Class I – high permeability, high solubility**
  - Example: metoprolol
  - Those compounds are well absorbed and their absorption rate is usually higher than excretion.
- **Class II - high permeability, low solubility**
  - Example: glibenclamide, bicalutamide, ezetimibe
  - The bioavailability of those products is limited by their solvation rate. A correlation between the *in vivo* bioavailability and the *in vitro* solvation can be found.
- **Class III - low permeability, high solubility**
  - Example: cimetidine.
  - The absorption is limited by the permeation rate but the drug is solvated very fast. If the formulation does not change the permeability or gastro-intestinal duration time, then class I criteria can be applied.
- **Class IV - low permeability, low solubility**
  - Example: hydrochlorothiazide, Bifonazole
  - Those compounds have a poor bioavailability. Usually they are not well absorbed over the intestinal mucosa and a high variability is expected[14].

Physiology of Barriers

The barrier is composed of a single layer of columnar epithelial cells, primarily enterocytes and goblet cells,

Tight junctions and epithelial barrier function

Tight junctions restrict epithelial cells immediately below the brush border forming a seal between neighboring epithelial cells. This seal acts as a gate to restrict passage of small molecules in a charge specific manner and completely occludes diffusion of molecules with molecular radii larger than 0.1nm. In addition, the tight junction acts as a fence that separates components of the apical and basolateral domains of the epithelial plasma membrane[12].

Biochemical composition of the tight junctions

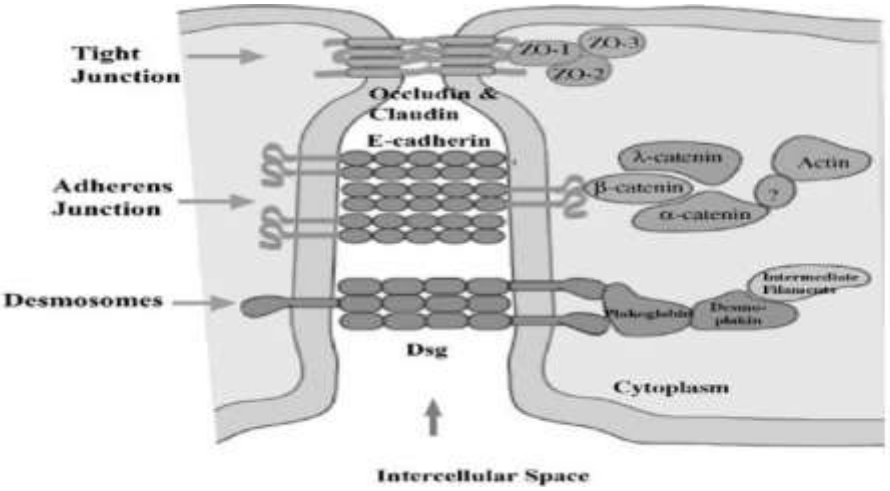


Figure 1: Biochemical composition of tight junction

Joined at their apical surfaces by tight junctions. The biochemical composition of tight junctions is still being elucidated, but many of the key components have been identified[11]. It is currently recognized that the tight junctions primarily are complex multicomponent protein structures. Identification of the principal transmembrane component has only recently come to light and it is now known that the tight junction is composed of a homotypic protein termed occludin. Other tight junctional complex proteins which have been identified are ZO-1[14], claudin. All of these proteins are oriented peripheral to the cytoplasmic surface of the tight junction complex and are thought to be involved in the stabilization and/or regulation of tight junction integrity[15].

Regulation of tight junction permeability

The tight junction complex is not a static structural component as once was thought, but slightly resembles a dynamic and elaborate protein signaling complex. Regulation of tight junction paracellular permeability by various physiological, pathological, and experimental agents has been extensively examined in a number of in situ and in vitro culture models, particularly Caco-2[15], brain endothelial, and MDCK cells. Peptide hormones, cytoskeleton perturbing agents, oxidants, Ca++ chelators and ionophores has all been shown to alter paracellular permeability by disrupting tight junctions. In addition, it has been determined that tight junction permeability is influenced by nearly all second messenger and signaling pathways, such as tyrosine kinases, Ca++, protein kinase C (PKC), protein kinase A (PKA), G proteins, calmodulin, CAMP, and phospholipase C.. Two factors which appear to play a prominent role in the regulation of paracellular permeability by absorption enhancers are contraction of the perijunctional actinmyosin ring, and protein kinase or phosphatase mediated changes in tight junction protein phosphorylation.

Role of the perijunctional actin-myosin ring

Adjacent to the tight junction in the cytoplasm is an actin-myosin ring which restricts the cell. This ring is associated with both the tight and intermediate junctional complexes and can contract exerting an inward force on the lateral plasma membrane. Such contractions are ATP-dependent and have been correlated with a loosening of the tight junctions indicating that contractions of the perijunctional ring pull on tight junction components and induce changes in paracellular permeability between neighboring cells[4]. Further evidence for a physical link between the perijunctional actin-myosin ring and tight junctions has been inferred by direct observation of tight junction-associated actin and by observations showing disruption in the structure and integrity of tight junctions by agents which disrupt actin filaments (e.g. cytochalasin D) possible to visualize the perijunctional actin-myosin ring by staining actin filaments with fluorescent-labeled phalloidin. Using this approach global changes in actin distribution have been documented with some tight junction disrupting agents including oxidants , protein kinase C activators , Ca++ depletion , and cytoskeleton disrupting agents while more subtle changes have been observed with other tight junction disrupting agents (i.e. interferon-y)[16].

Role of calcium

Extracellular calcium levels play a prominent role in the formation and regulation of tight junctions and paracellular permeability. Adhesion at the adherens junction is mediated by cadherins which are Ca’ ’ -dependent, cell-cell adhesion molecules that interact homotypically. Removal of Ca’ + has been known for many years to lead to an increase in tight-junction permeability and cause a redistributionof tight junction proteins. It appears that it is the disruption of cadherin adhesiveness by removal of Cat+ rather than a direct effect on the tight junction, which leads to the increase in paracellular permeability[18]. In addition, sensitivity of cadherin adhesiveness to Ca’ can be modulated by intracellular signaling events, such as. Tyrosine phosphorylation. Whereas extracellular Ca-- is required for formation and maintenance of tight junctions, intracellular Ca*’ may be involved in regulation of tight junction permeability. In isolated hepatocyte couplets (another cell model commonly used to investigate tight junction regulation), A calcium channel blocker has been shown to increase paracellular permeability with an accompanying inhibition of intracellular calcium[19].

Role of CAMP

Intracellular CAMP alters paracellular permeability by reducing NaCl diffusion potentials and increase passive permeability to Cl- as well as Cl: Na permeability ratios in intestinal and gall

bladder epithelium. CAMP may also decrease tight junction resistance, but this effect may be masked by the increased resistance that accompanies collapse of the lateral spaces. The exact role of CAMP in regulation of tight junction is not yet clear[1].

Role of ATP depletion

Under normal physiological conditions, the tight junction is maintained by an energy-dependent (ATP) process involving the actin cytoskeleton and tight junctions. Alteration in cellular energy status, a decrease in adenosine triphosphate (ATP) levels, has been shown to disrupt epithelial barrier function and increase permeability. Energy depletion results in net loss of phosphorylation of brush border, and possibly junctional, proteins[1].

Drug Absorption from the Gastro-Intestinal Tract

Drug absorption follo wing oral administration is a fairly complex Depending on the physico-chemical properties of the drug, either the dissolution rate or the transport rate across the intestinal epithelium may be the rate-limiting step for drugs to enter the systemic circulation. sequential series of events outlined in Figure shown below[3].

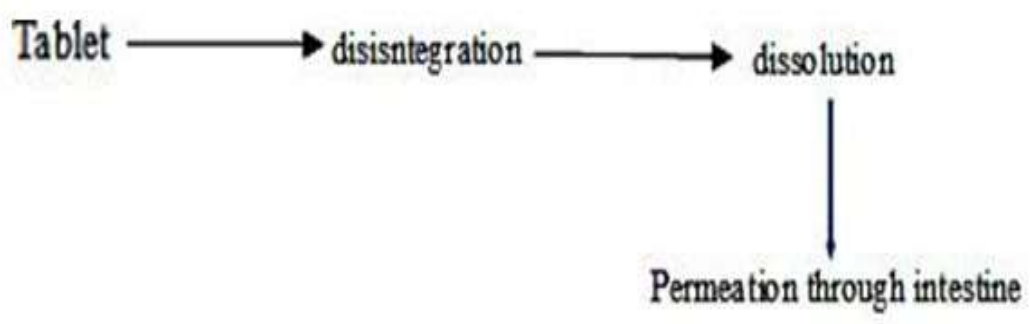


Figure 2: Drug absorption from the GI tract

Mechanisms of intestinal drug permeability

The intestinal mucosa can be considered as a system of sequential barriers to drug absorption, the outermost barrier being the mucus layer and the unstirred water layer. The gel-like structure of the mucus is thought to be a barrier to absorption of highly lipophilic drugs and some peptides be cause of the restricted diffusion in this matrix.

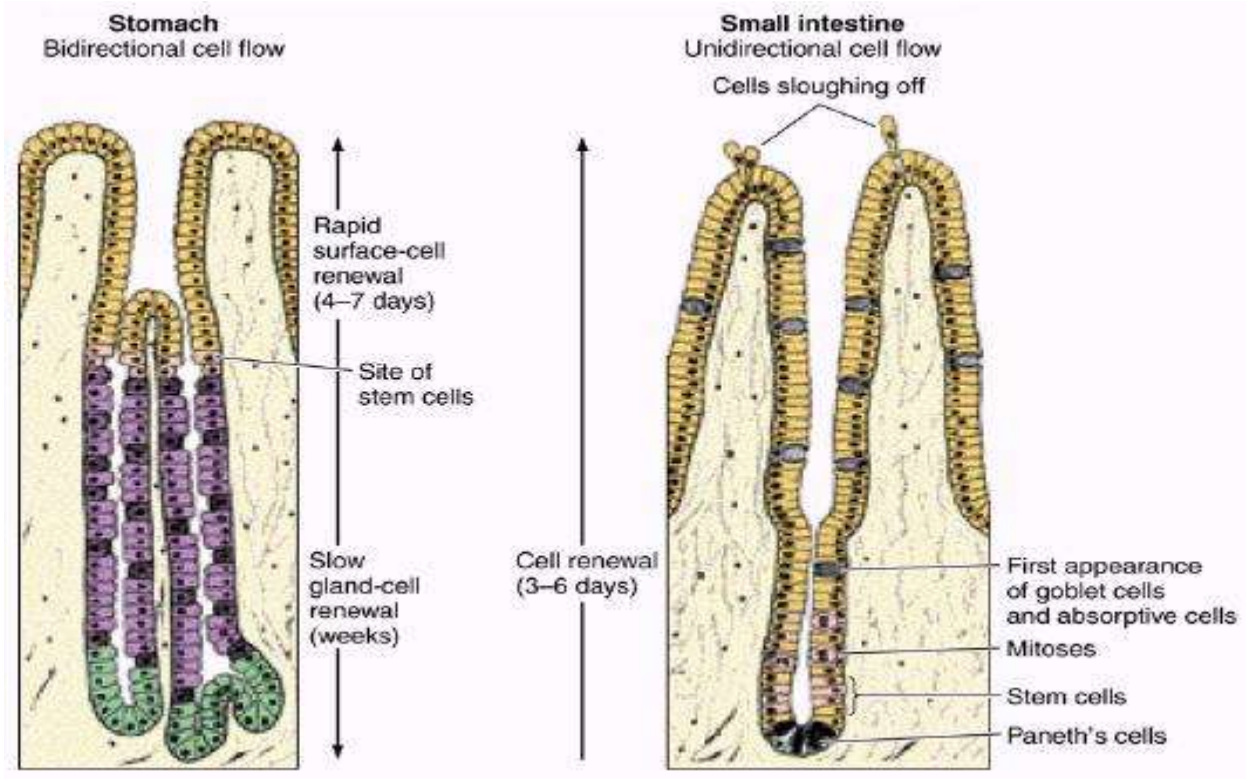


Figure 3: The absorptive epithelium lining of the GI tract

The absorptive epithelium lining the GI tract follows the folds and villi that increase the anatomical surface area of the mucosa several-fold in the small intestine. The villi are interspaced with crypts in which the regeneration of intestinal cells occurs. In between the crypts and the tips of the villi are the basal parts of the villi. The properties which are relevant for drug absorption differ between the cells along the crypt-villus axis. The main purpose of the intestinal epithelium is not only to restrict access and in this way protect the body from harmful agents, but also, to allow selective absorption of nutrients and secretion of waste products and xenobiotics.

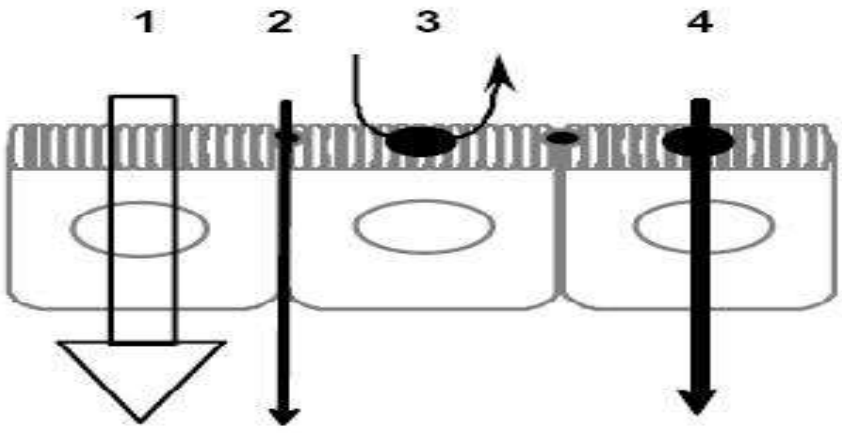


Figure 4: Mechanisms of intestinal drug permeability

Passive transcellular transport

Drug transport via the passive transcellular route requires that the solute permeates the apical cell membrane. Cell membranes are made up of phospholipids arranged in bilayers that are intermingled with membrane proteins. The composition of phospholipids and proteins varies from cell type to cell type and may theoretically give rise to different permeability properties depending on the cell type. In addition, the intestinal enterocytes have a polarized cell membrane with distinct differences in membrane composition in the apical and the basolateral membrane. It is generally believed that the apical membrane has a lower permeability than the basolateral membrane and the former is therefore considered to be the rate-limiting barrier to passive transcellular drug transport.

Active transport

The cellular membrane is a lipid bilayer composed of various amphiphilic phospholipids, cholesterol and proteins. It forms a thin, (approximately 5 nm) hydrophobic barrier which separates the cell interior from the extracellular aqueous environment. Passive diffusion is a concentration gradient-driven mass transport of compound across the cell membrane which is not consuming energy (ATP). Passive diffusion follows Fick's law, whereby the absorption rate is proportional to the drug concentration and the surface area. Passive diffusion can be divided into transcellular or paracellular processes, as shown in Figure 5.

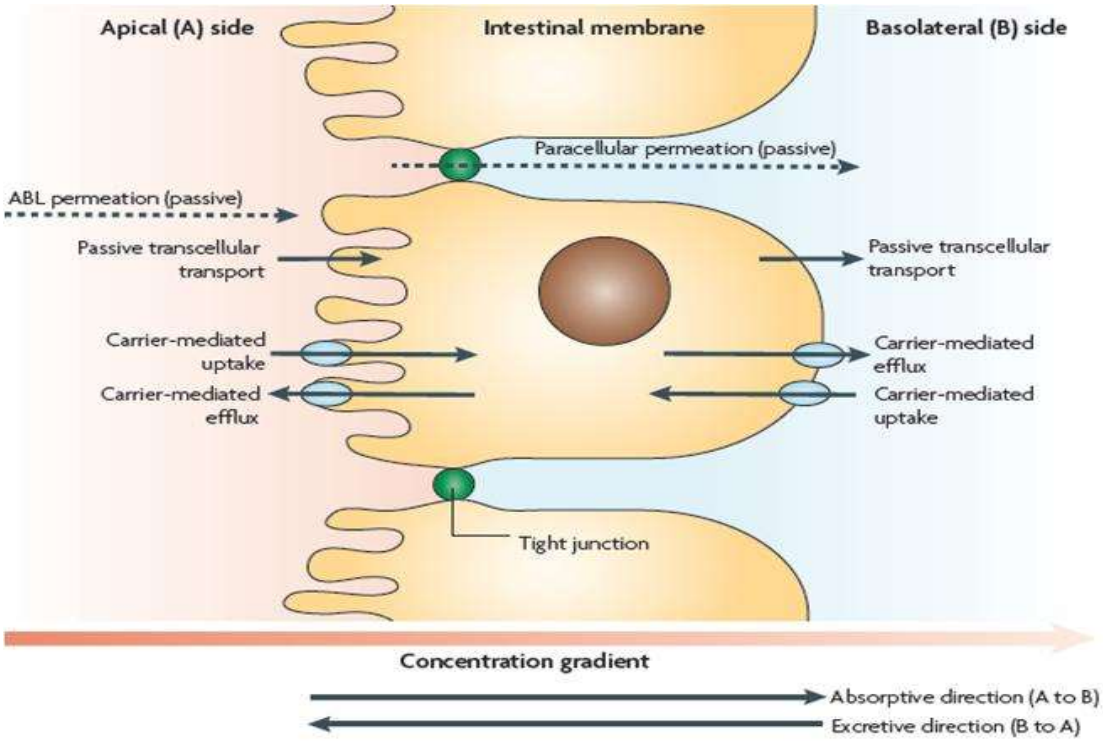


Figure 5: Transport mechanisms through the intestinal membrane

Transcellular diffusion through a membrane occurs across the lipid bilayer of enterocytes. The center of the lipid bilayer is highly hydrophobic; therefore a compound diffuses across lipid bilayer mainly as an uncharged molecule. Transcellular transport generally depends on the uncharged fraction of the compound defined by the pK_a of the molecule and the physicochemical properties of the molecule, such as lipophilicity (log P/log D), polar surface area, hydrogen bond number and molecular size[20].Passive paracellular diffusion in the intestine occurs via the tight junctions between the cells (Figure 5). The molecules with molecular weight approximately <200 g/mol may use this transport route. For larger molecules, it is considered as a minor transport route because of the limited intercellular space. The available surface area for paracellular intestinal absorption has been estimated to be approximately as low as 0.01% of the total surface area of the small intestine. However, paracellular transport may be an important absorption mechanism for hydrophilic drugs that have poor membrane permeability and that are not transported via intestinal uptake carriers[2].

Paracellular transport

Drugs of small to moderate molecular weights (MWs) can permeate the intestinal epithelium through the water-filled pores between the cells. This process is known as paracellular transport, and is generally considered to be a passive process, even if this pathway appears to be selective for cationic rather than anionic and neutral drugs. The paracellular pathway has also been shown to be saturable, by at least two separate mechanisms, one of which involves an intracellular process. The paracellular permeability is dynamically regulated and varies both along the path of the intestine and along the crypt-villus axis. The average pore radius of the human small intestine is 8–13 Å, which will limit the paracellular permeability of drugs >4 Å and restrict those >15 Å. The colon is even more size-discriminating since the paracellular pathway restricts drugs <3.5 Å.

Carrier-mediated transport

Carrier-mediated transport of drugs occurs through specific cells that express the transporter and the binding is stereo specific, and often enantio selective. When the transport process directly or indirectly consumes energy (ATP), the transport process is active and does not require any concentration gradient of the compound. Carrier-mediated transport occurs via transporters, which can transport drugs into (influx) a cell or out of a cell (efflux). In the case of primary active transport, transporters require binding and the hydrolysis of ATP to operate. While in the case of secondary active transport, transporters are driven by ion gradients created by ATP-dependent primary transporters, such as Na^+/K^+-ATPase. However, carrier-mediated transport can also occur passively and is then driven by the concentration of the substrate (facilitated

transport). The carrier-mediated transport is saturable. The saturation occurs when the total number of molecules exceeds the number of carrier protein binding sites available for transport. There are more than 400 membrane transporters in humans[4].

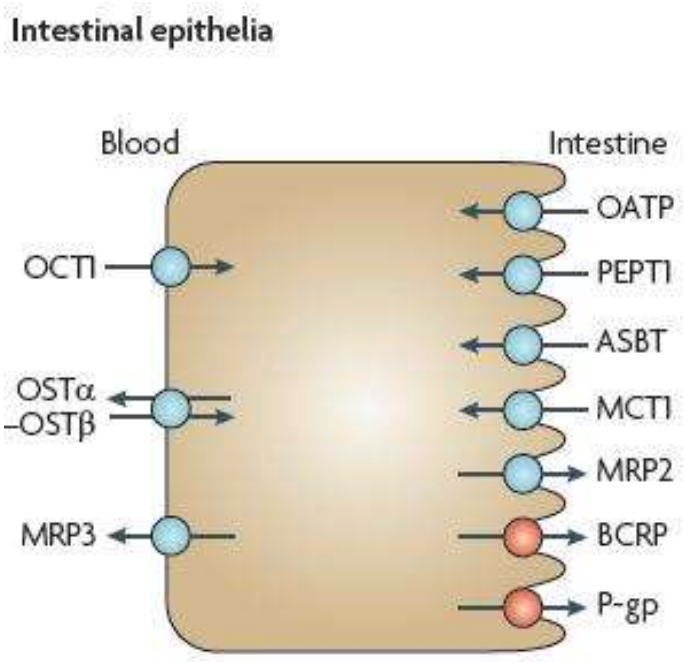


Figure 6: Most important transporters in the intestinal epithelia

Two major super families are the ATP-binding cassette (ABC) and the solute carrier(SLC) families. The clinically significant uptake and efflux transporters of the drugs in the intestinal membrane are shown in Figure 6. If the drug is a substrate of a transporter, it is translocated across a membrane by the transporter. An inhibitor of a drug transporter can decrease the influx and/or efflux of the drug competitively or non competitively, whereas an inductor of a drug transporter can increase the transport. The carrier-mediated transport via uptake transporters is a crucial absorption mechanism for some drugs, but it can also cause clinically significant interactions between drugs[8].There are several uptake receptors in the apical membrane of the enterocyte including transporters of the organic anion transporting polypeptide (OATP) family, peptide transporter 1 (PEPT1), ileal apical sodium/bile acid co-transporter (ASBT) and monocarboxylic acid transporter 1 (MCT1). The efflux transporters include multidrug resistance protein 2 (MRP2), breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp). The basolateral membrane contains organic cation transporter 1 (OCT1), heteromeric organic solute transporter (OST -OST) and MRP3[9].

Intestinal Permeation Enhancers

These are the exepients which increases the intestinal permeability of poorly absorbed drugs in

the small intestine and improve the oral bioavailability. These substances promote the permeability of poorly permeable drugs mainly by opening the tight junctions, leading to the increased paracellular permeability[21].

Table 2: Classification of intestinal permeation enhancers[5]

Surfactants	1.Ionic:
	Sodium lauryl sulphate
	Sodium dodecylsulphate
	Dioctyl sodium sulfosuccinate
	2.Nonionic:
	Polysorbitate
	Tween80
Bilesalts & its derivative	Sodium deoxycholate
	Sodium glycholate
	Sodium taurocholate
	Sodium dihydrofusidate
Fattyacids & its derivatives	Oleic acid
	Caprylic acid
	Lauric acids
	Sodium caprate
Chelating agents	EDTA
	Citric acid
	Salicylates
Chitosans & derivatives	N-sulfanto-N,O-carboxymethylchitosan
	N-trimethylated chloride(TMC)
	Chitosan glutamate
Other enhancers	Zonula occludens toxin (Zot)
	polycarbophyl-cysteine conjugate(PCP-Cys)

Mechanism of permeability enhancers

Surfactants

Disruption of intestinal epithelial cell membrane leads to increase in the permeability of drugs that cross the intestinal barrier through trans-cellular mechanism.

Fatty acids & its derivatives

Based on the research conducted in the last decade it has become clear that several sodium salts of medium chain fatty acids are able to enhance the paracellular permeability of hydrophilic compounds 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. In Vitro and In Situ Studies of Sodium Caprate produced information regarding its mechanism which is shown below.

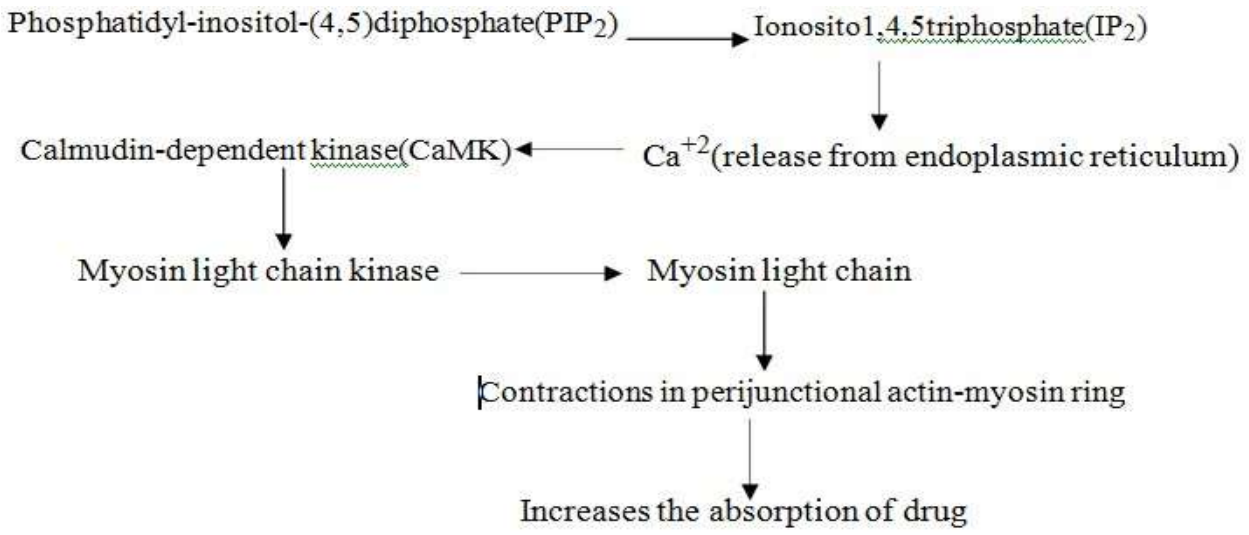


Figure 6: Mechanism of sodium caprate

Sodium caprate is able to modulate paracellular permeability by increasing intracellular calcium levels through the activation of phospholipase C in the plasma membrane, as represented in the above Figure. The increase in calcium levels is considered to induce the contraction of calmodulin-dependent actin microfilaments, resulting in increased paracellular permeability[1].

Bile salts & its derivatives

Bile salt shows the increases the permeability of intestinal barrier mainly by the following mechanisms.

-Denaturation of proteins

-Decrease of mucus viscosity

-Decrease of peptidase activity

-Solubilization of peptides

-Formation of reversed micelles

-Phospholipid acylchain disruption

Chelating agents

Chelating agent forms complexation of calcium and magnesium ions present in between intestinal epithelial cells and ultimately leads to opening of tight junctions and thereby increasing permeability for exogenous substances[2].

Chitosans and derivatives

Chitosan[11] is a cationic polysaccharide obtained by partial alkaline N-deactivation of chitin. Chitin is insoluble in alkaline P^H and neutral values where as its derivatives are soluble at these P^H. 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[20].These polymers were able to bind tightly to the epithelium and to induce redistribution of cytoskeleton F-actin and the TJ protein ZO-1, this being followed by enhanced transport via the paracellular pathway. Chitosan and its salts also act on tight junction and reduces its integrity and increases intestinal permeability. Chitosan derivatives are especially effective in enhancing the transport of small hydrophilic compounds (e.g., mannitol) though they also improve the transport of large molecules (drugs) such as buserelin, insulin, DGAVP and octreotide acetate[1].

Other enhancers: Zonula occludens toxin (Zot)

Zonula occludens toxin (Zot), a protein elaborated by *Vibrio cholera* that is able to reversibly regulate tight junction permeability. This toxin interacts with a specific intestinal epithelial surface receptor, with subsequent activation of a complex intracellular cascade of events that regulate tight junction permeability. It was also shown that the *in vitro* permeability's of drugs with low oral bioavailability such as paclitaxel, acyclovir, and cyclosporine and enamione anticonvulsants were increased with Zot.

Polycarbophyl-cysteine conjugate (PCP-Cys)

It is a class of permeation enhancers is represented by thiolated polymers[12] 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. This property is significantly improved as a result of the covalent attachment of cysteine (Cys) to this polymer (PCP-Cys).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. The thiol groups, covalently attached to the polymer, seem to be responsible for the improved permeation-enhancing properties of these conjugates. These compounds exert their permeation enhancing effects via glutathione. It seems that PCP-cys can transform oxidized glutathione (GSSG) to 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 leads to more phosphorylated occludin and to more open TJ as shown in the below figure.

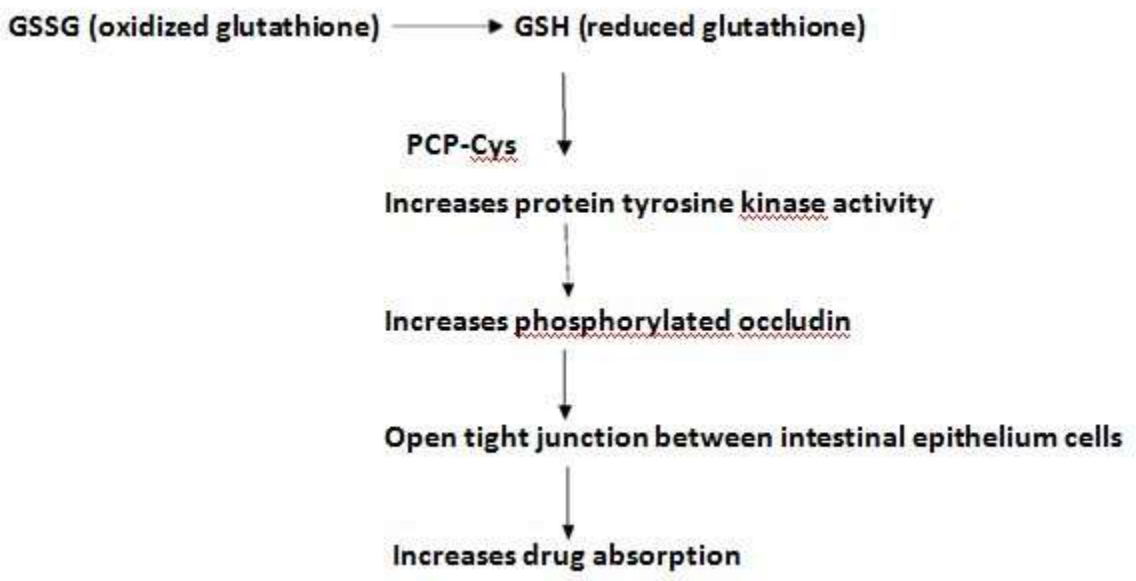


Figure 7: Mechanism action of polycarbophyl-cysteine conjugates (PCP-Cys)[1]

Permeability enhancer safety

The safety of absorption enhancers depends on the mechanism of action. Some enhancers may reversibly 'loosen' tight junctions, or transiently increase membrane permeability - without damage - under 'very controlled' conditions.

Major issues regarding safety are given below:-

- Ulceration
- immunological issues
- pin-point membrane 'erosions'
- Creating a provision for rapid access of bacteria, virus or even endotoxins to the general circulation.

Several marketed products containing proven absorption enhancers. Yet none have reported in an increased incidence of systemic toxicity. In theory, hyper absorption of a co administered poorly bioavailable drug would be possible during chronic use of an absorption enhancer.

Permeation Testing

Currently, no tests comprehensively examine the barrier function of the gut in entirety, and most methods test mainly the epithelial barrier. A noninvasive method that has been used in assessing the integrity of the epithelial barrier is the oral administration of test substances and the subsequent measurement of these substances in the urine. Probes used differ in molecular size and sometimes shape but have in common a relatively poor uptake in normal bowel and are poorly metabolized. Thus, significant uptake of a probe occurs only when there is an actual or

relative breech in the mucosal barrier. Probes, taken up by the bowel, ultimately are filtered and recovered in the urine. The urine usually is collected for 5 hours, and these probes are measured in the collected specimen. An increased amount of these probes in the urine implies that the integrity of the gut as a barrier to these probes has been compromised[22].

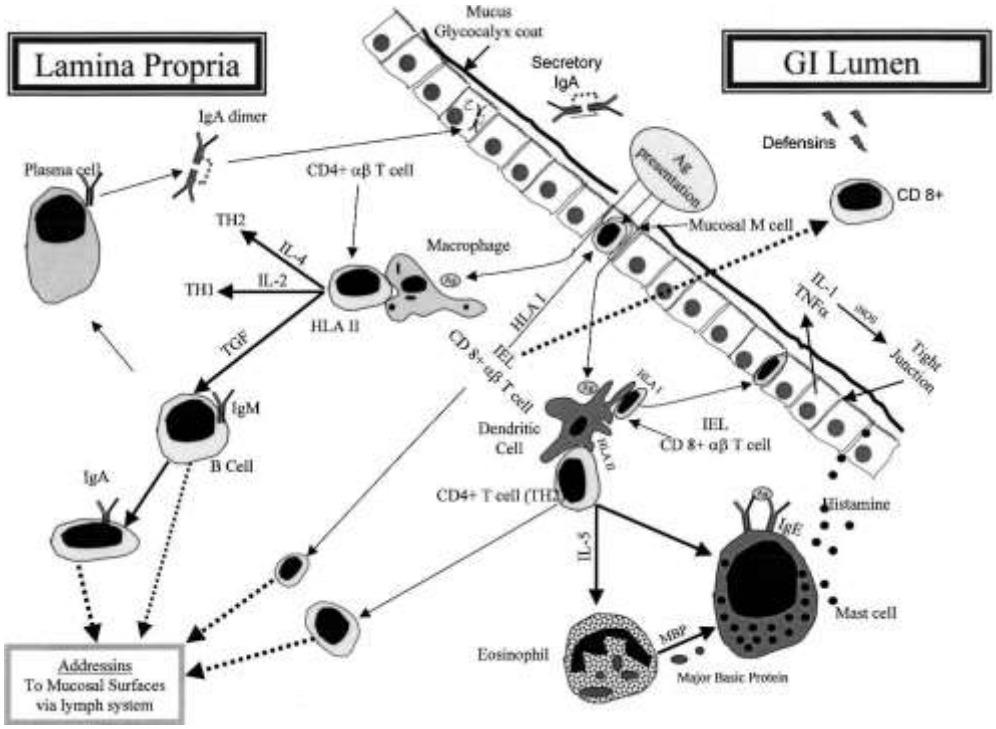


Figure 8:- Gut barrier

(The innate protection of the gut barrier is facilitated by the glycocalyx, tight junctions, secretory IgA, antimicrobial defensing (from Paneth cells and neutrophils), gastric acid, and digestive enzymes. These luminal forces allow)

Initially, a single test substance was used for permeation testing; however, this approach was not ideal because pre-mucosal (i.e., gastric emptying, bacterial degradation) and "washing away" of potential pathogens. The epithelial cell through it cytoskeletal structure maintains intracellular junctions, which can be altered by proinflammatory cytokines produced locally by epithelial cells (interleukin [IL]-1, tumor necrosis factor [TNF]-alfa) themselves or from a distant focus. The cytokines may mediate changes in permeation via nitric oxide. If antigen penetrates the epithelium into the lamina propria, mast cells can recognize and respond via mediator and cytokine release. The antigen processing of the "M" cell facilitates the adaptive immunologic response. The M cell itself, dendritic cell, or macrophage present peptides to naïve T cells, which evolve to aB CD4+ T cells, or CD8+ T cells. The CD4+ cells via cytokines (IL-2,IL-4) influence the expression of Th1 or Th2 subpopulations, which can be altered in disease states. CD4+ cells

also assist in the maturation of B cells to produce antibody. CD8+ T cells play an important regulatory role as intraepithelial lymphocytes (IEL). These lymphocytes are then directed via addressins to other mucosal sites or return to the lamina propria. Postmucosal (i.e., renal disease, volume of distribution) fac-tors, as well as intestinal permeability, influenced urinary recovery of the test substance. To address this shortcoming, the urinary ratio of two probes was used (usually, a disaccharide and a monosaccharide) as an index of intestinal permeability. The ratio of administered test probes was a more accurate indicator of permeation because the premucosal and postmucosal factors should influence the probes equally, and, therefore, the urinary excretion ratio should not be affected.[15,16] The four major classes of probes include the ethylene glycol polymers (of varying molecular weights but most commonly PEG 400), oligo-saccharides (most commonly lactulose), monosaccharides (either L-rhamnose or mannitol), and radiolabeled chelates (chromium–ethylenediaminetetraacetic acid [Cr-EDTA])[5].

***In Vitro* Methods for Studying Drug Permeability**

For reasons of safety and cost, drug absorption studies in humans are only carried out for a limited number of well-characterized drugs. Studies of drug absorption in the intestine traditionally been carried out in experimental animals. However, the introduction of combinatorial chemistry and high throughput pharmacological screening in drug discovery has significantly increased the number of compounds entering the pre-clinical phase, and this has made it impossible to assess the absorption properties of all these com pounds in experimental animals. This fact has spurred the development and use of *in vitro* methods to assess drug permeability properties in most drug discovery settings. Also, the insight that drug absorption across biological barriers is a complex process involving several path ways that cannot easily be delineated in experimental animals has resulted in the large interest in academic and industrial institutions in these methods .The methods are, cultured cells and artificial membranes[20].

Cultured cells

The human adeno carcinoma cell line Caco-2 model suitable for screening intestinal drug permeability and predicting the oral absorption potential of new drug substances. The Caco-2 cells were grown on permeable supports and spontaneously formed polarized monolayers that resembled that of the intestinal epithelium as shown in below figure.

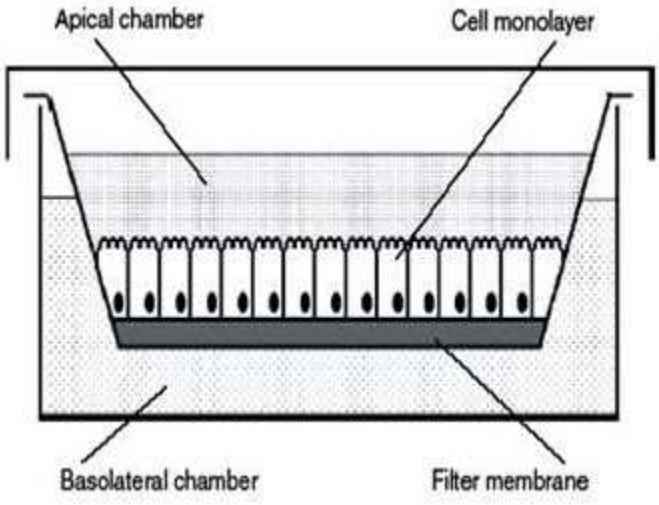


Figure 9: Caco-2 cell

In many respects the Caco-2 cells are therefore functionally similar to the human small intestinal enterocyte, despite the fact that they originate from a human colorectal carcinoma. The methods that are based on cultured cells such as Caco-2 cells are, however, not only useful for drug absorption screening. It is al so possible to extract information about specific transport processes that would be difficult to obtain in more complex models such as those based on whole tissues from experimental animals. For instance, the methods enable us to investigate the relative contribution of passive transcellular and paracellular transport, the effect of charge on paracellular transport and the effect of solvent drug[23].

Brush Border Membrane Vesicles

In this approach, cell homogenates or intestinal scrapings are treated with the $CaCl_2$ precipitation method using centrifugation. The final pellet contains the luminal wall-bound proteins and phospholipids, which contain most of the brush border enzymatic and carrier activity. Resuspension of the pellet in buffer results in the formation of vesicles. These vesicles are mixed with the permeant in buffer and filtered after a fixed time; the amount of permeant taken up by the vesicles is then determined. Because the precipitation–centrifugation procedure results in isolation of only the brush border components, typically only the apical transcellular transport is measured by this system.

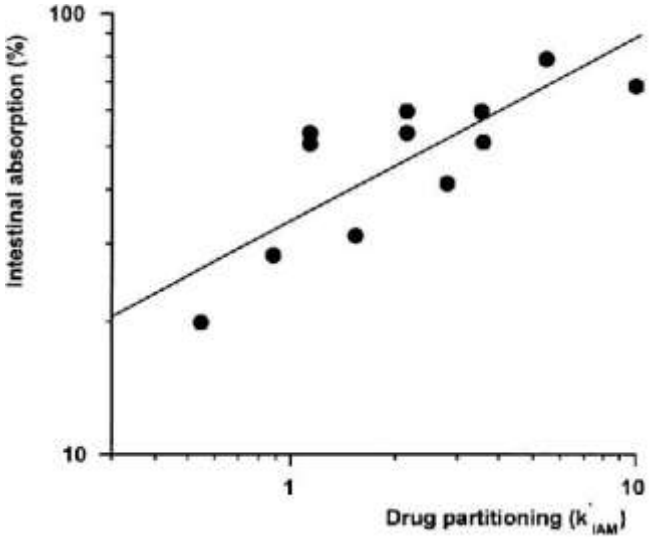


Figure 10: Correlation of rat intestinal absorption with drug partitioning to an IAM-column

Despite drawbacks, such as the need for a radio labeled compound and day-to-day variation in preparation, this method is useful for mechanistic studies of the drug absorption process. An interesting modification of this procedure has been proposed by Pidgeon et al., who isolated brush border components and immobilized the components on a chromatographic column, leading to immobilized artificial membranes (IAM). The drug(s) under investigation are eluted with an aqueous eluent, thus enabling the estimate of permeation capacity factors. Despite some of the limitations associated with this approach, the prediction of bioavailability for a series of compounds was comparable with the more-complicated permeability measurement procedure using Caco-2 cells. In Figure 10, partitioning behavior of a series of 12 structurally unrelated drugs is compared with in vivo absorption in rats, indicating that the intestinal transport of the compounds is well predicted by IAM-chromatography; it should be emphasized, however, that all of the compounds shown in the correlation are transported passively. This approach may be especially suitable for high-throughput and large-volume- screening of experimental compounds at an early developmental stage[7].

Isolated Intestinal Cells

Isolated cells from the intestine of animal or human origin can be used as uptake systems in the assessment of oral bioavailability and have been de-scribed in several papers. The procedures used to isolate mucosal cells can be divided fundamentally in two categories: an in situ procedure, in which the intestine is perfused with enzyme solutions that release the cells; and an *ex vivo* approach, in which the cells are treated by chelating agents or by enzymatic means. The freshly isolated cells are immediately suspended in Krebs–Henseleit buffer with 10 mM glucose

added and kept on ice for 15 min, during which they are bubbled with carbogen (95% O_2 /5% CO_2). The exposure to glucose increases the viability of the cells, even after the media have been replaced by glucose-free media. In a typical experiment, the cells are separated from the primary buffer by centrifugation, resuspended in buffer under O_2 /CO_2 in the presence of the permeant, and shaken well. After a designated time, the cells are separated by gradient-centrifugation or rapid filtration, and extracted. Because of the low volume of the cells, the assay is mostly based on radiolabel counting, which as previously mentioned, can be disadvantageous in the early stage of development when a radio labeled compound is unlikely to be available[6].

Diffusion Cells Using Tissue

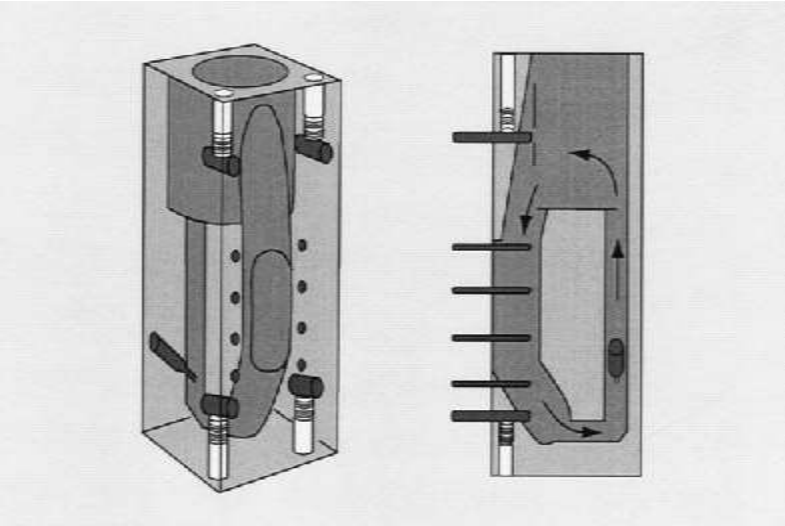


Figure 11: The Sweetana–Grass diffusion cell: this cell is designed for tissue that is mounted between two acrylic half-cells. Buffer is circulated by gas lift

Since the early 1950s, side-by-side diffusion cells have been used to determine the transport of compound in living tissue, one of the first approaches being the so-called Using chambers. In this method small sections of the intestine (2 cm) are clamped between two glass chambers filled with buffer and nutrients such as glucose, at a temperature of 37 degree C. The buffer solutions at both sides of the membrane are gased continuously with carbogen (95% O_2 /5% CO_2) to maintain the viability of the tissue and to ensure reproducible hydrody-namics (good mixing). A modification of the original method was published, using smaller acrylic chambers, allowing diffusion experiments with smaller amounts of compound; again in this method, carbogen bubbling is applied to increase viability and improve hydrodynamics. This cell is depicted in Figure 11, in this example it is adapted for the use of tissue. The temperature is maintained by clamping the closed half-cells in a heating block[9]. The compound under study is added to one of the compartments (the ‘‘donor’’ compartment), either the serosal or the mucosal side, and the

accumulation of the compound at the other side of the membrane (in the ‘‘receptor’’) is measured as a function of time. From the appearance in the receptor compartment, the permeability of the compound is calculated according to equation: P_{app} d*C*/d*t* (*V*/*A C*), where d*C*/d*t* is the change in concentration in the receptor compartment per unit time, *V* is the receptor volume and *A* the area available for diffusion or transport. The P_{app} is usually expressed in centimeters per second (cm/s). In many studies the investigators prepare the intestinal tissue before mounting by stripping the serosa and the outer musculature; for studies designed to determine the mechanistic and rate of transport stripped tissue is claimed to be preferable because this resembles more closely the in vivo situation: in vivo, drug permeation occurs through the enterocytes and the basal membrane to the blood capillaries, and permeation through the serosa and musculature contributes little or nothing to the bioavailability. In the original design of Using chambers (see Figure 12), the setup was equipped with electrodes and a voltage clamp for monitoring epithelial potential difference and short-circuit current (*PD* and I_{sc} , resp.). Alternatively, the potential difference or current could be clamped to any value to investigate the influence of electrical driving force on the transport characteristics of the solute. These electrical parameters can be used to verify the viability of the tissue during and at the end of the experiment: at the end of the experiment a small amount of glucose is added to the mucosal bathing solution, and this glucose will be transported to the serosal side by the glucose transporter (only in the small intestine): this glucose transport is accompanied by the trans-location of Na , which can be detected as a long-lasting increase in current. For colonic tissue, which does not have glucose transporters, prostaglandin E_1 (PGE_1) stimulates the Cl secretion which, in turn, also gives rise to an increase in I_{sc}. In our own laboratory we have observed viability of stripped and mounted tissue for at least 3–4 h using the same technique. In Figure 12, a typical example of the electrical parameters over the membrane of rat duodenum is given. A constant value for the calculated resistance *R* over a 3-hr experiment was achieved. The electrical parameters are now widely accepted for monitoring the tissue viability and integrity in Using chambers.

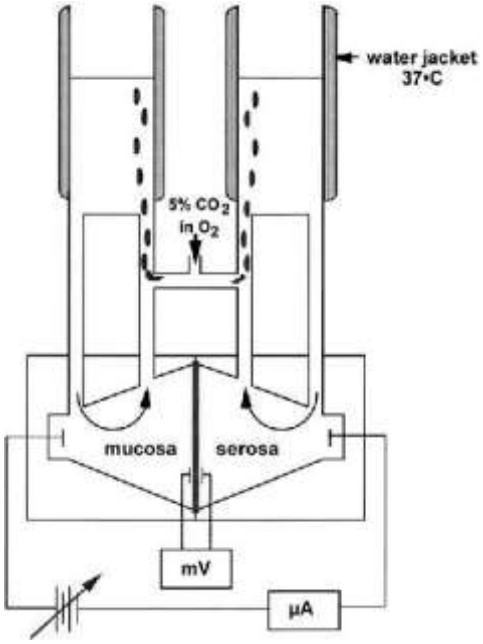
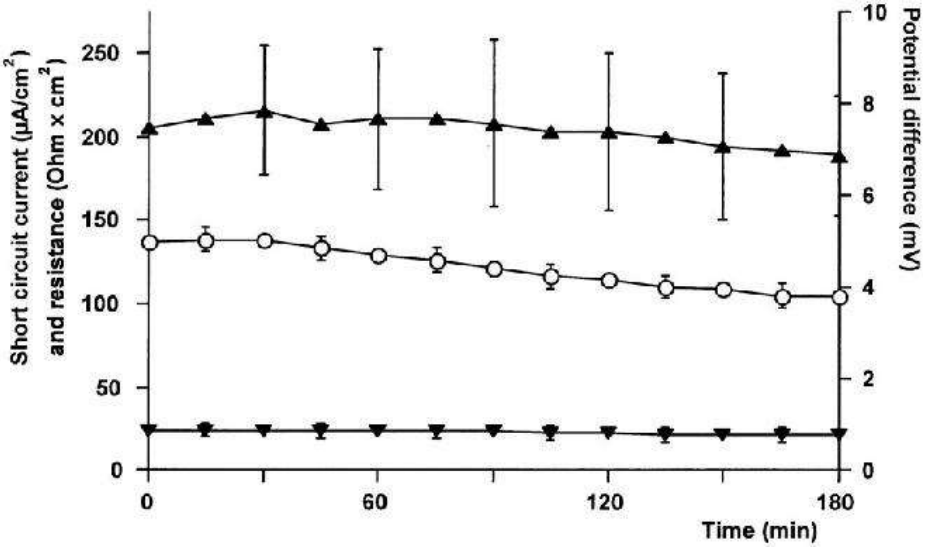


Figure 12: Schematic drawing of the original Using chamber, showing the half-cells with thermo stated water jackets, gas lifts, and potential difference and cur-rent electrodes.

The integrity of the tissue, especially after stripping, can also be followed using an integrity marker, in addition to and independent of monitoring the electrical parameters. Normally, a compound with low intrinsic permeability such mannitol, PEG_{4000}, inulin, or fluorescein is used. To overcome the environmental problems with radio labeled compounds (the first three are, for ease of detection, applied mostly as labeled compounds), we apply fluorescein in a donor concentration of 5 □M and measure the concentration in the receptor compartment with time using fluorescence spectrometry (after bringing the pH of the samples to 10 using NaOH).



It has been shown by several investigators that the tissue in Using chambers, as with everted

sacs, can deteriorate within 30–60 min. Addition of energy sources such as glucose or L-glutamine can improve this life span considerably; proper handling and gasing with carbogen further improves the viability. it reported that during an experiment in Using chambers using excised rat tissue, the morphology of the tissue changed, as did the permeation of the marker compounds investigated (propranolol and mannitol). Information on time-dependent structural changes would be necessary for a complete interpretation of the permeability data. However, ac-cording to this and other publications it seems quite possible to perform trans-port experiments in Using chambers for at least 2–3 h, provided that the experimental conditions are adequately designed and that the permeation of the marker compounds into the receptor compartment is linear over the study period.

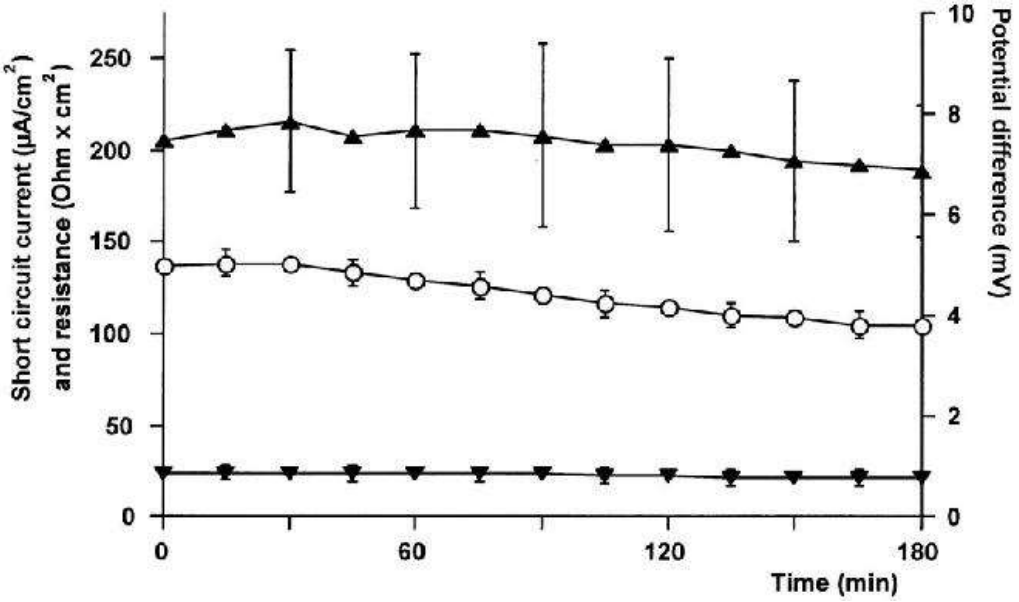


Figure 13:- Mean electrical parameters, potential difference (PD, in mV), short-circuit current (▲ SCC, in □A/cm^2), and resistance (▼ R, in Ohm cm^2) for rat intestinal tissue during 180-min experimental time

The Using diffusion chambers are now used by several groups, in some cases slightly modified, and have been useful tools for the determination of the permeability characteristics of drugs and for the selection of development candidates based on the *in vitro* permeability measurements. The same method can be used for tissue other than intestinal tissue (e.g., buccal, esophageal, gastric, rectal, nasal, lung, and skin tissue). With a slight modification, the diffusional method, as described, can also be applied for monolayers of cells cultured on membranes fixed in inserts, but one of the advantages of the original approach using segments of excised tissue, is the possibility of studying differences in regional absorption of drugs. More-over, the using diffusion

cell is especially applicable for mechanistic studies of paracellular transport, because the transport through the aqueous pores can be influenced rather easily[23].

In silico methods

Numerous computational structure-based models to predict passive intestinal absorption have been published (Hou et al. 2006). The attempt is to establish a relationship between different molecular properties and the absorption in humans. The most widely used computational approach to the coarse estimation of passive intestinal absorption is "rule of 5" proposed by Lipinski and coworkers in 1997 from the analysis of 2245 drugs from the World Drug Index. It presents that poor absorption is more probable for compounds with molecular weight > 500 Da, calculated LogP >5, a number of hydrogen donors >5 and number of hydrogen bond acceptors >10. The rule can only be used to rapidly distinguish between well-absorbed molecules and poorly-absorbed molecules, and therefore it has been necessary to develop prediction models for specific absorption properties. Most computational prediction methods applied in the estimation of drug absorption are using data. Data modeling can be applied with great efficiency to a large number of compounds, but it requires a significant quantity of high quality data to deduce a relationship between the structures and the modeled property. Therefore, the reliability and applicability of a computational model is highly dependent on the quality of the dataset used during model development. Typically, models applied to predict intestinal absorption, are quantitative structure-property relationship (QSPR) models which are based on appropriate descriptors. QSPR modeling is developed from simple multiple linear regression to modern multivariate analysis techniques or machine-leaning methods. Many import physicochemical descriptors are introduced into the prediction of drug absorption, such as polar surface area, lipophilicity parameters (log*P*, Log*D*), molecular weight, and the number of hydrogen bond acceptors and donors. However, it is not possible to determinate drug absorption by single defined descriptor, but rather by the combination of different physicochemical descriptors. Several QSAR approaches have been proposed to predict human intestinal absorption and Caco-2 cell permeability. Linnankoski et al. (2008) demonstrated that computationally derived mathematical models can predict with a reasonable accuracy human passive absorption, when models were compared to cell lines, artificial membrane models, and *in vivo* rat experiments. The results showed that three of seven models were found to be significantly more reliable in predicting human passive intestinal absorption than the artificial membrane models. Two of the models were found to be as reliable as the Caco-2 and 2/4/A1 cell lines. Moreover, one of the computational models even predicted the absorption of drugs nearly as well as absorption studies

on rats. Therefore, the simple computational models with high throughput are particularly useful tools in the early screening of drug candidates to predict intestinal passive absorption[23].

Advantages of Intestinal Permeation Enhancers

- Several types of intestinal permeation enhancers are available
- They have diverse mechanism of action
- They are cost-effective
- They are companionable with several drugs

Applications

- Enhances oral bioavailability of drug
- Increases pharmacological action of drug
- Complete absorption of poorly permeated drugs and no wastage

Ideal Characteristics of Intestinal Permeation Enhancers

- They should increase drug shipping across intestinal barrier
- They should be reversible in their action
- They should be non-hazardous to GIT epithelium[24.]

Future of Absorption Enhancers

Although many safe and effective permeability enhancers are currently available, the control of their exposure at the epithelium will require a measure of formulation sophistication. Fortunately, such formulation capability is within reach of most pharmaceutical companies. Emerging evidence suggests that multiple cellular signaling pathways are likely to be involved in the modulation of paracellular permeability by these absorption enhancers. As such, they may be directly or indirectly altering the actin microfilament network and/or its association with peripheral proteins of the tight junction complex. Future research on mechanisms should focus on what concentrations and exposure times result in,

1. Transcellular or paracellular enhancement
2. Disruption of ion flux, energy and/or tight junction integrity
3. Readily reversible, slowly reversible, and irreversible permeability
4. Foliation of the epithelium.

As the time- and concentration-dependent mechanisms of permeability enhancers become fully characterized, their marketing potential will become recognized.

CONCLUSION

It is most promising that absorption enhancers increase drug transport through the intestinal barrier and there by increases the drug bioavailability. Utilization of different intestinal permeation enhancers may increase drugs bioavailability significantly. Further more and more study and work is necessary on the field of permeability enhancing agent to improve and development of Pharmaceutical preparation. Fortunately apparently safe and effective intestinal permeability enhancers are currently available, so bioavailability can be increased of any Drug.

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