Drugs of the Future 1999, 24(11): 1221-1240

CCC: 0377-8282/99

**Review Article**

# The role of sorption promoters in increasing the bioavailability of drugs in oral preparations#

***P. Sharma[1], H.P.S Chawla[2] and R. Panchagnula[1]****

*Departments of [1]Pharmaceutics and [2]Pharmaceutical Technolog y, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, SAS Nagar - 160062 (Punjab), India .*Correspondence. #NIPER communication No. 37.*

## CONTENTS



## Summary

Rapid developments in biotechnology have resulted in new challenges for pharmaceutical scientists to deliver drugs into the systemic circulation with improved bioavailability when delivered orally. This review briefly discusses the poor permeation of drugs, anatomical and biochemical considerations of the gastrointestinal tract and various pathways of drug absorption upon oral administration. Detailed insight into the rationale for the concept of sorption promoters is provided, followed by definition, classification and different mechanisms of action, as well as toxicological implications of sorption promoters as pharmaceutical adjuvants. A fundamental understanding of the biochemistry and biophysics of the membranes constituting the epithelial cells which are exposed to the enhancers and the mechanisms by which peptides and proteins cross mucosal membranes is also described at the molecular level. Finally, the available opportunities and future challenges in this field are reviewed.

## Introduction

The most preferred route of drug administration is oral for various reasons, of which better patient compliance and the existence of highly developed pharmaceutical technology for production of oral dosage forms are the most important. The entire drug content of oral dosage forms, however, is generally not available to the patient. This is because drug absorption by this route is a multi-step phenomenon and at each step some quantity of drug is lost (Fig. 1). The amount and rate at which the drug is absorbed in its unchanged form and reaches blood circulation intact is referred to as bioavailability. Table I shows various factors which are responsible for decreased oral

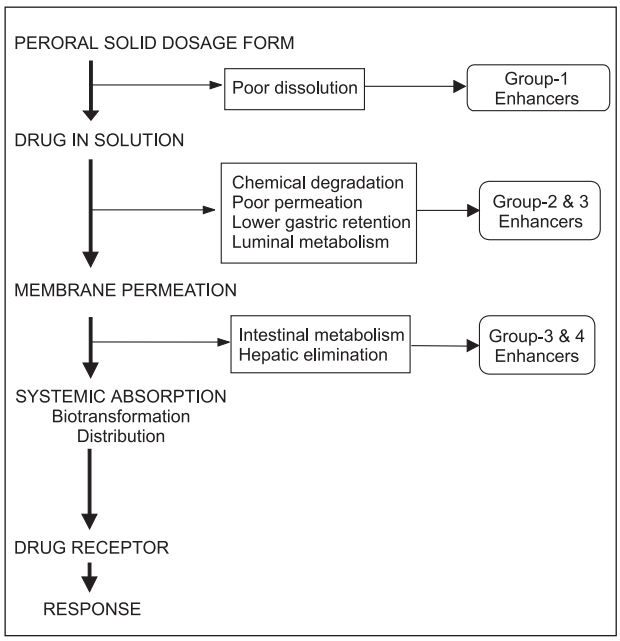


Fig. 1. Systematic representation of different steps involved in absorption of drugs upon oral administration along with problems associated with each step.

*Table I: Problems associated with oral drug delivery and approaches to overcome them.*

| Strategic group | Reasons for poor bioavailability | Absorption enchancing approach |
|---|---|---|
| Group 1 | Poor solubility or dissolution rate | Micronization<br>Use of solubilizing or wetting agents<br>Polymorphic forms<br>Solid solutions (solid dispersions, solvent deposition, *etc.*) |
| Group 2 | Degradation/metabolism in GIT lumen | Enteric coating<br>Encapsulation<br>Enzyme inhibitors<br>Lipid or surfactant vesicles (liposomes, microemulsion) |
| Group 3 | Poor membrane permeation | Sorption promoters/permeation enhancers<br>Ion-pairing<br>Complexation<br>Lipid or surfactant vesicles |
| Group 4 | Presystemic elimination of drugs | Metabolism inhibitors<br>Lipid or surfactant vehicles |
| Group 5 | Low gastric retention time | Novel drug delivery systems<br>Bioadhesive systems<br>Inflated systems |

Note: Prodrug approach can be used to solve problems of dissolution, metabolism and permeation.

*Table II: Drugs with poor permeation.*

| Drug class | Examples |
|---|---|
| Biopharmaceuticals (proteins, peptides and analogs) | Hormones, enzymes, antibodies, interferons |
| Antibiotics (aminoglycosides, macrolides, cephalosporins) | Streptomycin, gentamicin, dirithromycin, cefoxitin, cefotaxime, aztreonam |
| Immunosuppressants and antineoplastics | Cytarabine, cisplatin, doxorubicin, daunorubicin |
| Antivirals | Acyclovir, ganciclovir |

bioavailability of drugs along with the different approaches to overcome these problems.

Problems occurring in the gastrointestinal (GI) tract at various stages of drug dynamics have been successfully resolved with the exception of poor permeation of drugs through the absorptive epithelial surface. This last phase of drug absorption is critical and will be more so in the future due to the appearance of newer molecules whose physicochemical properties do not allow good permeation into cell membranes. Hence, the external use of excipients in oral dosage forms is required to facilitate entry into systemic circulation.

Recent developments in biotechnology have led to an increase in the availability of biopharmaceuticals which include large molecular weight and polar molecules like peptides, proteins, hormones, antibodies, vaccines, *etc.* There are approximately 36 recombinant protein drugs and over 26 peptide drugs approved by the FDA (1) and at least 450 products under development, with more than 120 in phase III clinical trials and beyond in the U.S. (2). These new compounds have the potential to become the most important group in therapeutics in the future. However, they have the disadvantage of very low bioavailability upon oral administration because of poor permeation through GI tract epithelia. To date, bioavailabilities achieved with oral peptide/protein delivery systems are 2-3% (3). For most drugs (*e.g.*, erythropoietin), a bioavailability of 2-3% is too low. The major therapeutic agents with poor permeation are shown in Table II.

When bioavailability is very low (*e.g.,* 20%), inter- and intrasubject variabilities are increased, and incomplete oral bioavailability can be a major concern. Low and variable bioavailability makes it difficult to control the pharmacological and toxic effects of a given dose (4). The high costs of using large doses of biopharmaceuticals can be curtailed by enhancing drug absorption.

Various approaches have been used to overcome problems associated with macromolecular drug delivery. The chemical modification of the parent compound to yield an absorbable drug molecule is one such approach (prodrug approach). However, alteration in structure may lead to changes in the tertiary structure of biomolecules with consequent partial or total loss of biological activity. The prodrug approach is time-consuming, expensive and may not always be successful. Transdermal drug delivery is another feasible alternative, although it has the disadvantage of poor penetration of macromolecules via the skin. The pulmonary route has the advantage of avoiding first-pass hepatic metabolism and passage through the adverse GI tract milieu. However, a basic problem with this route of administration is penetration into mucus pellicle and the underlying epithelial lining of respiratory tract

*Table III: The organizational hierarchy of the human intestine (13, 14).*

| Region of intestine | Absorbing surface area ($m^2$) | Structural features | Functional cell types | Absorption mechanism |
|---|---|---|---|---|
| *Small Intestine* | | | | |
| Duodenum | ~0.09 | Folds of Kerckring, villi, microvilli | Absorptive, goblet, endocrine, tuft and M-cells | Passive diffusion, ion pore transport, active transport, pinocytosis, facilitated transport, ion pair transport |
| Jejunum | ~60 | Folds of Kerckring, villi, microvilli | | Passive diffusion, ion pore transport, active transport, pinocytosis, facilitated transport, ion pair transport |
| Ileum | ~60 | | | Passive diffusion, ion pore transport, active transport, pinocytosis, facilitated transport, ion pair transport |
| *Large intestine* | | | | |
| Cecum | ~0.05 | Folds of Kerckring, less dense villi, microvilli | Absorptive and goblet cells | Passive diffusion, ion pore transport, active transport, pinocytosis |
| Colon (transverse, ascending, descending) | ~0.25 | | | Passive diffusion, ion pore transport, pinocytosis |

(1). Similarly, delivery of macromolecules through transmucosal routes (*e.g.*, buccal and vaginal) is limited due to the inability of hydrophilic large molecules to penetrate biological membranes. In all cases, the basic problem is the impermeability of macromolecular and polar compounds through biomembranes. Since these compounds do not have an intrinsic permeation profile, an external pharmacologically inert (nontoxic) excipient can be added to temporarily increase their permeation. Compounds which reversibly and specifically or nonspecifically increase the permeation of drugs via the GI tract are called (intestinal) permeation enhancers or sorption promoters (SPs). The development of safer and more effective permeation enhancers is currently an active area of research and if successful will stimulate oral pharmaceutical technology.

Other approaches for overcoming poor permeation of orally administered drugs are particulate delivery (liposomes and nanosomes) (5-7), microemulsion and multiple emulsion systems (5, 8), targeting to GI lymph (9) and receptor-mediated endocytosis systems (10) and bioadhesive drug carrier matrices (11). This review will focus only on the use of SPs for enhancing the oral bioavailability of biopharmaceuticals. Reviews on skin penetration enhancers have been published elsewhere (12).

This review describes the morphological, anatomical and biochemical features of the GI tract which are important in determining the oral absorption of drugs. Molecular aspects of biological membranes and cytoarchitecture of absorptive epithelia are discussed. Different physicochemical characteristics influencing the permeation of drugs through the GI tract are explained with examples. The problem of poor absorption of macromolecular therapeutic agents and consequently the evolution of SPs as novel excipients is described. SPs are defined and classified with examples, and their various mechanisms of action are described followed by toxicological implications. Future trends in SP research are mentioned in the conclusions.

## Absorptive surface of the GI tract

Knowledge of the morphological, anatomical, biochemical and physiological characteristics of the GI tract wall is essential in understanding the underlying absorption mechanisms and consequently poor oral bioavailabilities of drugs (9, 13-15). The GI tract consists of the buccal cavity, esophagus, stomach, intestine and rectum. The process of absorption of nutrients or drugs occurs throughout the GI tract but the extent of absorption varies greatly depending on the region. Since the greatest extent of absorption occurs in the intestine, this region will be used to illustrate mechanisms and barriers to absorption of biopharmaceuticals.

### *Anatomical considerations*

On the basis of anatomical considerations and physiological activities, the human intestine is divided into two regions, the small and large intestine. These are further subdivided into structurally distinct regions as shown in Table III. The intestine is organized into intestinal folds, villi and microvilli, which results in a 50,000-fold greater absorption capacity. The highly convoluted small intestine is invaginated, resulting in folds which are called plica circulares or Kerckring's folds. Present on the surface of these folds are very minute finger-like projections called macrovilli or villi. Each villi is lined with brush-bordered

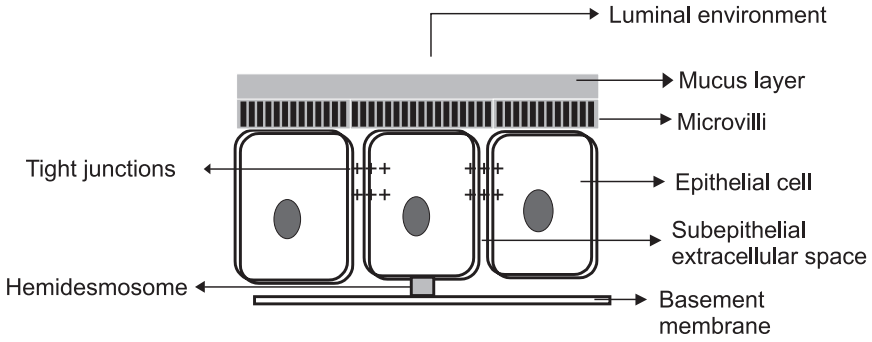


Fig. 2 Schematic diagram of intestinal epithelial cells.

cuboidal or columnar epithelial cells, as well as sparsely distributed goblet cells and tuft cells. The small filamentous projections on brush-bordered epithelial cells are known as microvilli and there can be more then 600 microvilli projecting from each cell (Fig. 2). Although the small intestine contains different types of epithelial cells, the absorptive cells are most important in terms of nutrient and drug absorption. The absorptive surface of the small intestine consists of monolayers of nonkeratinized brush-bordered columnar epithelial cells supported by underlying basement membrane and then the connective tissue lamina propria which consists of fibers scattered on the aqueous ground matrix. Absorptive epithelial cells are exposed to a harsh external environment at one side (luminal) and life-sustaining substratum at the other side. Exposure of a single cell to distinctly different environmental conditions leads to the intracellular redistribution of structural and physiological attributes. This adaptational change of different intracellular milieu at two opposite sides of the cell is called cellular polarization. Thus, the cell membrane of the absorptive enterocyte facing the lumen is called the apical pole, whereas the one facing the basement membrane is called the basolateral domain.

The apical pole consists of hydrolases (*e.g.*, aminopeptidase A) and transport proteins (16). In the context of oral delivery of macromolecules, the apical pole acts as the degradation site for proteinaceous and peptide drugs (17). The basolateral pole is responsible for the normal housekeeping functions (*i.e.*, cellular metabolism). The cellular polarization of ion channels, pumps and enzymes together with the difference in apical and basolateral membrane composition are responsible for the directional nature of transepithelial transport (18).

The adjoining lateral sides of neighboring absorptive enterocytes is known as the “junctional complex”. This structure provides an intercellular sealing zone at the lateral sides of epithelial cells arranged in a monolayer, thus acting as a major barrier between the GI tract lumen and the subepithelial extracellular space (19). The junctional complex is comprised of a tight junction (zonulae occludens), an intermediate junction (belt desmosomes or zonulae adherens) and a spot desmosome (maculae adherens). The tight junction forms a continuous concentric belt around the apical pole of epithelial cell. The permeation of hydrophilic and macromolecular drug via the tight junction opening is an active area of research (20-23).

Another type of intestinal cells important for absorption of macromolecules are the M-cells which are specialized epithelial cells of gut-associated lymphoid tissue that transport antigens from the lumen to the cells of the immune system. M-cells are present in Peyer’s patches of the small intestine and are regarded as absorption windows for particulate carriers and macromolecules (6), although the extent of such absorption is very low and targeting of macromolecules to M-cells for absorption enhancement is still a controversial topic (6, 10).

Goblet cells are interspersed throughout the absorptive surface of villi and produce mucus which consists of glycoproteins, electrolytes and water along with suspended cell debris and microbes (24). For example, the dry weight of pig intestinal mucus contains the following components (in %w/w): mucin (5%), lipids (37%), proteins (39%), DNA (6%) and some unidentified matter (25). The highly viscous mucus forms a thin pellicle over the absorptive cell layer and its primary functions are lubrication and protection of the underlying layer from harsh extraneous conditions. Mucus, however, is a major permeation barrier for drugs before reaching the actual absorptive layer due to its high viscosity, binding capabilities and ability to modify the microenvironment (pH and ionic charge) in proximity to the absorption site.

### *Absorption barriers*

Different absorption barriers which evolved as a means of defense for the body against permeation of xenobiotics upon oral ingestion include, from the mucosal side, the unstirred water layer (UWL), thick viscous pellicle of mucus, superficial enzymes on apical pole of absorptive enterocytes, quasistatic biological membrane and tight junctions (14, 26, 27) as shown in Figure 3.

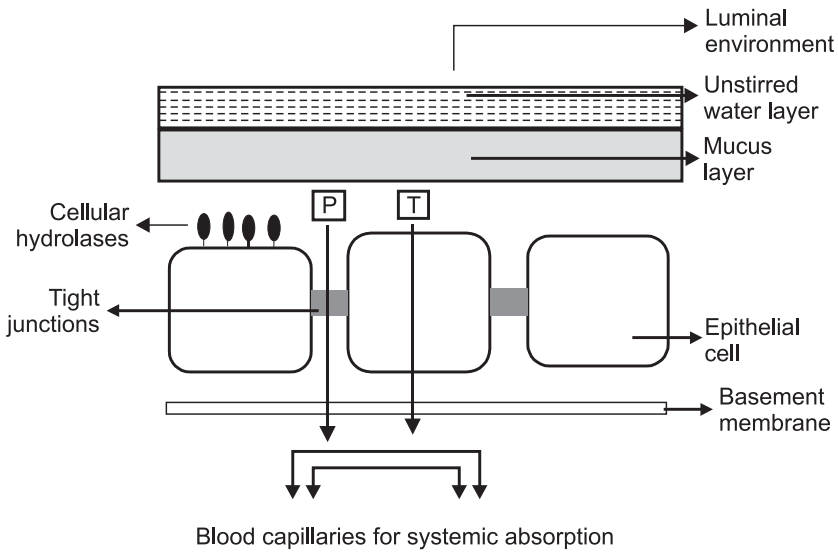


Fig. 3. Schematic diagram of different barriers to absorption from GIT. P = paracellular absorption, T = transcellular absorption.

The effect of the thickness of UWL adhered to the mucus layer of the absorptive cell on permeation of drugs into epithelial cell surface has been reported in *in situ* and *in vivo* studies (28, 29). Experimental data suggest that the thickness of UWL is not uniform throughout the GI tract; it is maximally 25 μm at the villous tips and approx. 600 μm at the intervillous aqueous space separating the lumen from crypts (30). Thus, the major diffusional limitation of UWL is in transport through the intervillous space (31). The second barrier is mucus which delays drug diffusion but does not abolish it (32-34). In a recent study, Bjork (25) using native pig intestinal mucus showed that lipids, rather than mucin glycoproteins, are a major component contributing to the reduced diffusion of lipophilic drugs in the native intestinal mucus. Mucin and DNA present in mucus do not react with hydrophilic and lipophilic low molecular weight drugs. However, these gel forming and viscosity increasing agents are more significant barriers to the diffusion of macromolecular drugs such as proteins and peptides (35). CaCo-2 cell lines obtained from human colon adenocarcinoma, are considered experimental models of the intestinal epithelium in drug absorption studies (36-38). Enzymatic assays revealed the presence of 8 cell surface peptidases on a CaCo-2 cell line (39-41), namely dipeptidyl peptidase IV, aminopeptidase N, peptidyl dipeptidase A (angiotensin-converting enzyme), aminopeptidase P, aminopeptidase W, endopeptidase-24.11, γ-glutamyl transpeptidase and membrane dipeptidase**.** Intestinal epithelial cells can process Phase II metabolic reactions – sulfation and glucuronidation. Phenol sulfotransferase isolated from CaCo-2 cells catalyzed the sulfation of *p*-nitrophenol, catecholamines (*e.g.,* dopamine) and catecholamine metabolites (42). The following sections describe the architecture of biological membranes and tight junctions and discuss how these models regulate drug absorption.

*Table IV: Chemical constituents of the biological membranes.*

| |
|---|
| Lipids |
| Phospholipids |
| Phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol |
| Glycolipids |
| Glycosylceramide, galactocylceramide |
| Sterols |
| Cholesterol |
| Proteins |
| Intrinsic or internal |
| Extrinsic or peripheral |

*Biochemistry of absorptive membrane and tight junctions*

The absorptive membrane of the GI tract is a typical fluid mosaic model (43) and is primarily composed of lipids and proteins. The lipids provide the basic infrastructure into which proteins are held. The different chemical constituents of biological membranes are shown in the Table IV. Membrane lipids occur in biological membranes as a bilayer and are amphiphilic, *i.e.,* their structure constitutes a polar hydrophilic head and a nonpolar hydrophobic tail. Due to this property, when dried phospholipids are suspended in water, they produce lipid bilayers separated by layers of water. When this mixture is subjected to sonication, formation of lipid vesicles known as liposomes occurs. These liposomes resemble naturally occurring biological membranes and hence are used as models in drug permeability studies (44-46).

The interior of biological membranes is fluid in nature due to noncovalent hydrophobic interactions of the fatty acyl chains and with the lipophilic amino acid moieties of integral proteins (47). This brings a high degree of

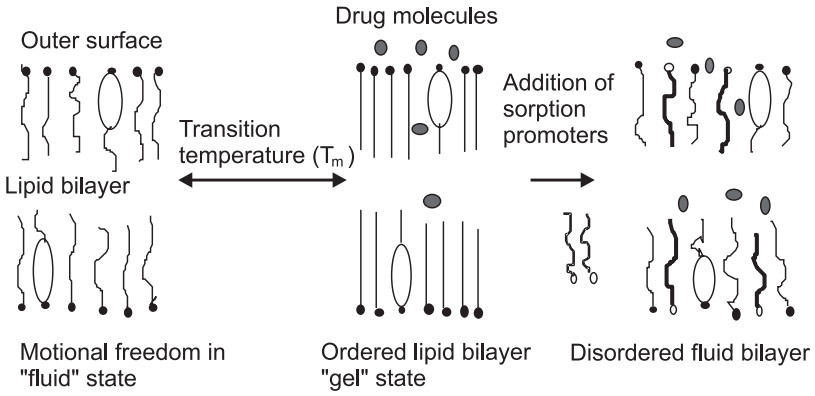


Fig. 4. Molecular dynamics in biomembranes. The change in temperature can convert membrane from one state to another. Increase in membrane disorder or fluidity occurs due to incorporation of sorption promoters (*e.g.*, oleic acid).

flexibility to the internal structure leading to three characteristic types of motion within biomembranes: (i) lateral diffusion of proteins and lipids traversing along the plane of membrane (2-dimensional motion); (ii) movement of individual hydrocarbon acyl chains of fatty acids produced by rotation about carbon-carbon (-C-C-) bonds (3-dimensional) in the interior of membrane; and (iii) a shift from one side of the bilayer to the opposite side which is referred to as a "flip-flop" movement which occurs less frequently due to thermodynamic hindrance.

The lipid bilayer *in vitro* can exist in a gel (paracrystalline) or fluid state. In the case of the gel state, lipids are packed tightly with a rigid acyl chain at the interior, whereas, in the fluid state, the fatty acyl chains have increased motion about their -C-C- bonds and have a more random configuration. The state of the bilayer depends on the chemical nature of constituent lipids, the presence of sterols and temperature. In general, fluidity is favored by short chain fatty acyl chains and the presence of unsaturated chains with *cis* double bonds. Both factors reduce the tight packing of lipid domains in the membrane allowing a less rigid structure to prevail. The sterols (cholesterol) possess a rigid steroidal nucleus. Insertion of this rigid nucleus in lipid bilayer prevents intermolecular interactions and highly ordered packing of fatty acid acyl chains in the gel state, thus favoring the fluid state. When present in the fluid state, the same rigid structure hinders flexible movements of fatty acyl chains reducing fluidity of the core bilayer. Sterols tend to buffer or moderate extremes of solidity or fluidity in membranes.

One state of the bilayer can be converted to another by changing the temperature and the characteristic temperature at which this happens is called transition temperature ($T_m$) of the phospholipid constituting membrane (Fig. 4). Natural membranes are made up of a wide variety of lipids and hence, their $T_m$ is not precise but is spread over a broad range. At body temperature, natural membranes are maintained above $T_m$ so that they exist in a fluid state, a property which is essential for physiological functioning of carrier proteins and pumps embedded inside the membrane (48-50). The permeability of inorganic ions is maximum at $T_m$ of the membrane but decreases at values above and below $T_m$ (51-53).

The intestinal absorptive biomembrane is rich in glycolipids and cholesterol (15, 54, 55). The glycolipids have a high $T_m$ and hence tend to rigidify GI tract epithelium so that it can face the harsh conditions of the GI tract. Cholesterol, on the other hand, moderates this rigidification so that an optimum level of fluidity is maintained and physiological functioning of membrane components is facilitated. The microvillus membrane is a major physical barrier to drug absorption (56, 57). This barrier can be disrupted transiently by SPs which can increase the fluidity of membranes resulting in increased permeation of impermeable drugs (58-60).

Various experimental techniques have been adopted to study the motion of lipids and proteins in the membrane which not only allows one to understand the nature of membranes but also the influence of membrane perturbants (*i.e.,* SPs) on the membrane at the molecular level (61, 62). These techniques include fluorescence polarization (62-66), differential scanning calorimetry (62, 67, 88), electron spin resonance (69, 70) and nuclear magnetic resonance (71, 72).

Three types of junctions exist in the intestinal epithelium: desmosomes (zonulae adherens), tight junctions (zonulae occludens) and gap junctions (73) (Fig. 5). All of these differ in structural and functional properties in that desmosomes hold cells together, tight junctions provide mechanical strength, cellular polarization and seal intercellular zone to inhibit the passage of small molecules, and gap junctions allow intercellular transport of low molecular weight compounds and ions. Of all these junctions, tight junctions are a major barrier to drug permeation and will be discussed in detail.

In electron microscopy, tight junctions appear as a region where membranes of 2 adjoining cells come in close contact giving rise to complementary templates fitting into each other like finger-like projections (74-76). A tight junction has a length of approximately 80 nm (77)

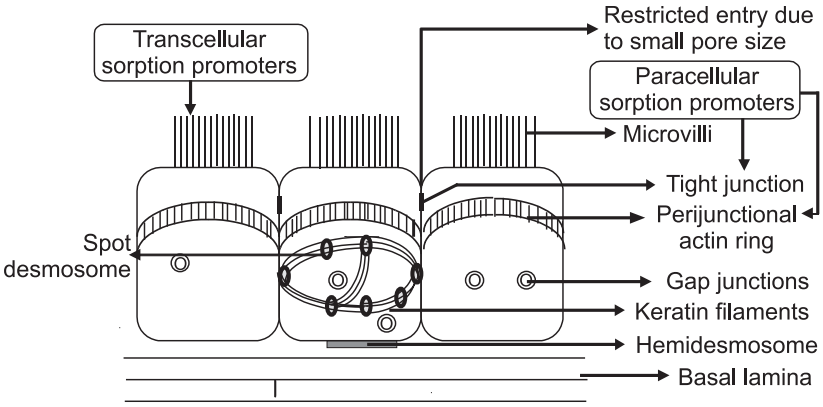


Fig. 5. Different types of cell junctions found in absorptive intestinal epithelial cells.

and occludes diffusion of molecules with molecular radii larger then 11 Angstrom (78). In the case of drugs which are predominantly absorbed by the paracellular route, the experimental data suggest that tight junctions at the villi are smaller and less permeable than those present in crypts (79). By preventing lateral diffusion of proteins and lipids, tight junctions bring about cellular polarization and are accountable for unidirectional absorption of nutrients and drugs.

To define the composition of tight junctions, two theories are proposed. One suggests that the tight junction is the result of fusion of lipid components of adjacent cell membranes (73, 80). Another view which is more prevalent, advocates the multicomponent protein structure as the building block of tight junctions. Various immunological techniques have confirmed the presence of 4 proteinaceous components in tight junctions: ZO-1 (81, 82), ZO-2 (83, 84), cingulin (85) and 7H6 (86). Recently, two more proteins have been identified and include a small GTP binding protein Rab 13 (87) and an uncharacterized 130 kDa protein (ZO-3) (88). Many of the protein subunits remain to be identified and can probably serve as target sites for SPs to regulate paracellular channel opening for macromolecule absorption.

## Pathways of drug absorption

In general, there are 2 main routes of absorption, transcellular and paracellular (see Fig. 3). When a drug or nutrient molecule traverses through the epithelial cell and reaches the serosal side whereby it is carried away into the systemic circulation, it has undergone transcellular absorption. To suit the biological needs of the human body, diverse types of transcellular absorption have developed, as shown in Table V. Regional variability for a particular absorption type in different parts of the GI tract is observed. Most of hydrophilic and small sized molecules tend to migrate through tight junctions into subepithelial extracelluar space and then into systemic circulation. This is known as paracellular absorption.

## Physicochemical characteristics of drugs that affect membrane permeation

The process of drug absorption is regulated by a variety of factors which include the physicochemical characteristics of drugs (solubility, polymorphism, $pK_a$), dosage form design parameters (surface area, disintegration time, dissolution time, manufacturing variables) and physiological factors (age, gastric emptying, intestinal transit time, disease state, GI tract contents and metabolism) (105). In the context of drug permeation through membrane together with physicochemical properties, 3 main variables are noteworthy, molecular weight, lipophilicity and conformation of molecule.

As the molecular weight of a drug increases, the extent of transcellular passive diffusion decreases because the rate of diffusion of the drug is inversely proportional to the $(\text{molecular weight})^{1/3}$ and paracellular uptake is generally limited in CaCo-2 cell monolayers to molecular weight < 400-500 (106). Mitscher (106) analyzed the relationship between molecular weight and oral activity of 286 marketed drugs and their findings are consistent with the above facts and further advance the belief that values between 500-750, in general, should be considered the practical upper limit of molecular weight for orally active compounds.

Lipophilic drugs are transported mainly by the transcellular route and hydrophilic drugs are absorbed through tight junctions. In both cases, if a drug is very lipophilic or hydrophilic it shows poor absorption. For a drug to permeate the membrane, it must be lipophilic so that it can diffuse through the lipoidal barrier. However, in order for the drug to be dissolved in the GI tract, it needs to be adequately hydrophilic. In the best case, a drug molecule should posses an optimized hydrophilic-lipophilic balance. The partition coefficient is a good indicator of passive diffusion of drugs (90, 107). For a drug to be passively absorbed, logP should be between –1 and +4. However, attraction of positively charged molecules (*e.g.*, bases with $pK_a$ values above physiological pH or

*Table V: Various absorption mechanisms in GIT.*

| Mode of absorption | Definition | Characteristics | Examples of substrates/drugs absorbed by this mechanism |
|---|---|---|---|
| Transcellular absorption | Drug molecule traverses through epithelial cells and reaches systemic circulation | | |
| Passive diffusion | Transport of drug from high concentration in GIT to low concentration in blood stream | No energy expendidure, small mol. wt. and lipophilic molecules transported primarily by this route | Sodium cromolyn (89), foscarnet, atenolol (90), aminopyrine, cardiac glycosides (91) |
| Carried-mediated transport | Specialized carrier molecule complexes with solute, traverses across the membrane to other side, dissociates and discharges solute molecule, carrier returns to original position | Transport is structure-specific, prone to competitive inhibition and saturation, absorption occurs at specific sites in GIT (absorption window), two subtypes are active transport and facilitated diffusion (92) | |
| Active transport | Carrier-mediated transport on energy expenditure | Transport occurs along and against concentration gradient, can be inhibited by metabolic poisons like fluorides and cyanides | Niacin, pyridoxin, ascorbic acid, 5-fluorouracil (93), 5-bromouracil, enalapril, captopril (94) |
| Facilitated diffusion | Carrier-mediated transport without energy consumption | Cannot be inhibited by metabolic poisons | Vitamin $B_1$, $B_2$, $B_{12}$ |
| Ion-pair transport | Formation of neutral reversible complexes of ionized drugs with endogenous ions of GIT (*e.g.*, mucin) | Complexation with counter-ions gives neutral molecule with high lipophilicity and acceptable aqueous solubility. neutral complexes are transported by passive diffusion (95, 96) | Paraquat, suxamethonium, phenothiazines and some quaternary ammonium compounds |
| Ion/electrochemical diffusion | Transport of ionized drugs down the electrochemical gradient | Membrane potential affects absorption of ions (97-99), cations need high kinetic energy to permeate via positively charged outer surface of membrane | |
| Endocytosis | Transport of macromolecules by incorporation into membrane vesicles | Internalization of particulates (phagocytosis) (100) and liquid droplets (pinocytosis) in form of vesicles | Vitamin A, D, E, K, insulin |
| Receptor-mediated endocytosis and potocytosis | Ligands bind to cell surface receptors and are subsequently internalized and trafficked within the cell | Clathrin mediates receptor internalization, non-clathrin mediated RME is known as potocytosis (10) | Calcitonin, insulin, interferon, prolactin, thryoid hormone, cholera toxin, IgE (10) |
| Paracellular absorption | Absorption through tight juctions of epithelial cells | Hydrophilic and small mol. wt. (<400-500) drugs are primarily absorbed by this route, major route of peptide and protein absorption (101) | Insulin, cefmetazole, bisphosphonates (102), ddAVP (103), ranitidine (104) |

quaternary amines) towards negatively charged polar lipids in polar membranes cannot be explained by the partition coefficient. Similarly, the logP *versus* the oral availability relationship often breaks down when structural diversity is introduced (108) or when compounds have elevated lipophilicity (109). In recent works (90, 110), theoretical models are constructed based on the dynamic polar molecular surface ($PSA_d$) properties which are responsible for the 3-dimensional shape of the molecule and its polarity. The major impediment in absorption of peptides across the CaCo-2 cell monolayers (111) or across the intestinal wall (112) are water- peptide hydrogen bond forming potential and desolvation energy. Miyazaki (113) employed electrostatic interactions between drugs and biological membranes to predict the passive diffusion of drugs by considering hydrogen bond formation and charges on the drugs. Lipinsiki (114), after studying 2500 entries from USAN and the world drug index, formulated the popular “rule 5” which asserts that poor absorption will be observed in agents having more than 5 hydrogen bond donating functions, 10 hydrogen bond acceptors, a molecular weight in excess of 500 and a calculated logP of +5. The drugs which are absorbed by active uptake mechanisms (polypeptides, quaternary ammonium salts) were excluded from the study and, hence, do not follow this rule.

*Table VI: Classification of sorption promoters according to chemical structure.*

| Category/Class | Examples |
|---|---|
| Surfactants | |
| Endogenous | Bile salts (sodium cholate, sodium deoxycholate) |
| Exogenous | Ionic surfactants (sodium lauryl sulfate, dioctyl sodium sulfosuccinate) |
| | Nonionic surfactants (polyoxyethylene alkyl ethers, polysorbates) |
| Carboxylic acids and derivatives | Fatty acids (sodium laurate, oleic acid, sodium caprate, sodium caprylate, citric acid) |
| | Salicylates (sodium salicylate, sodium methoxy salicylate) |
| Glycerides | Phospholipids (lysophosphatidylcholine) |
| | Natural oils (hydrogenated castor oil) |
| | Medium chain glycerides |
| | Polyoxyethylene glyceryl esters |
| | Acyl carnitines and cholines (palmitoyl carnitine, lauroyl choline) |
| Chelating agents | EDTA |
| | Bisphosphonates (tiludronate) |
| Swellable polymers and derivatives | *N*-Trimethyl chitosan, chitosan glutamate, starch, polycarbophil |
| Effervescent or dissolved gases | $CO_2$, NO donors (SNAP, NOR4, NOR1) |
| Saponins | Quillaja saponins (DS-1) |
| Bacterial toxins | Zonula occluden toxin |
| Cyclodextrins | Hydroxypropyl β-cyclodextrin, dimethyl β-cyclodextrin |
| Drugs | Antiinflammatory agents (indomethacin, phenylbutazone) |
| | Immunosuppressive agents (tacrolimus) |
| | Vitamin D and its derivatives |
| Alkaloids | Piperine |
| Oxidants | Hydrogen peroxide |

It has long been recognized that conformation of biomacromolecules is an important determinant in passive diffusion (115). However, the extent of and manner to which conformational change contributes to oral protein delivery has only been recently investigated (116). Tertiary structure of the peptide and the protein has been shown to play a significant role in the *in vitro* permeation of various cyclic peptides across a monolayer of CaCo-2 cells (117). Proteins cannot be translocated through a membrane in a tightly folded state. Using unfolded basic fibroblast growth factor (bFGF), Johnston demonstrated that the linear conformation of the macromolecules are more favorable to absorption than their nonlinear counterparts (118). Partial unfolding of proteins exposes the hydrophobic portions to the outside making the molecule more hydrophobic as compared to the native protein but it can pass through the ordered fluid lipid bilayer with less resistance (119). Unfolded proteins, however, may be more labile substrates to enzymatic degradation than native conformers.

## Sorption promoters

SPs or permeation enhancers constitute a diverse type of compounds that, when coadministered with drug(s) with an intrinsic poor permeation profile, augment their permeation and consequently increase their systemic bioavailability. SPs are novel excipients, which unlike conventional excipients, possess a definite mechanism of action on biological system and are important from the pharmacokinetic point of view. An ideal SP is one that: (i) reversibly and specifically increases drug permeation; (ii) is nontoxic; (iii) is reproducible in absorption enhancing activity; (iv) has a well-defined mechanism of action; (v) possesses suitable physicochemical properties so that it can be easily formulated; and (vi) is easy to commercially obtain and is cheap and effective in a low to moderate concentration. SPs are classified in Table VI on the basis of chemical structure. Recent findings on SPs are given in Table VII.

## Mechanism of action of sorption promoters

The mechanism whereby intestinal absorption of drugs is improved by SPs is an issue of major importance because it can be correlated to its toxicity, thus determining its applicability, reproducibility in activity and dosage regimen.

### *Absorption enhancement by the transcellular route*

1) Alteration in mucus rheology

The barrier properties of the mucus coat on absorption by epithelial cells can be reduced by curtailing its viscosity and tenacity (147). Sodium deoxycholate, sodium taurodeoxycholate, sodium glycocholate and lysophosphatidylcholine reduce viscosity and elasticity of

*Table VII: Recent work on permeation enhancers.*

| Sorption promotor | Drug | Absorption model | Ref. |
|---|---|---|---|
| Chitosan and its derivatives | Mannitol | Monolayers of CaCo-2 culture cells | 120, 121 |
| *N*-Trimethylchitosan chloride | Mannitol, FITC-dextran, buserelin | Monolayers of CaCo-2 culture cells | 122 |
| Sodium caprate, $Na_2EDTA$, sodium glycocholate | Insulin | *In situ* loop method in different parts of rat intestine | 123 |
| Palmitoyl carnitine | Cefoxitin | *In vitro* study in rat intestine and stomach and bioavailability study in dogs | 124 |
| $CO_2$ effervescence | Tetracycline, caffeine, diazepam, PEG-400, PEG-900, mannitol, benzoic acid | *In vivo* single-pass intestinal perfusion in rabbits | 125 |
| Lipid bile salt mixed micelles (sodium taurocholate, oleic acid, monoolein) | Amphotericin B | Rat gut perfusion method | 126 |
| Phospholipids (lysophosphatidylcholines) | Desmopressin (DDAVP) | Monolayers of CaCo-2 culture cells | 127 |
| Surfactants (sodium dodecyl sulphate, sodium taurocholate, polysorbate-80, *etc.*) | Phenol red | Rat single-pass intestinal perfusion | 128 |
| Bisphosphonates (tiludronate) | | Monolayers of CaCo-2 culture cells | 129 |
| Soybean phosphatidylcholine and medium chain monoacyl glycerol | Hexarelin | Intestinal single-pass perfusion method in rats | 130 |
| Hydroxypropyl β-cyclodextrin, dimethyl β-cyclodextrin | Diphenylhydramine | Single-pass perfusion and recirculating perfusion methods in rat intestine | 131 |
| Sodium caprate | Polysucrose | *In vitro* studies in ileum in Ussing chamber | 132 |
| Sodium caprate, sodium laurate, sodium oleate and sodium taurocholate | Cefmetazole | | 101 |
| Sodium caprate | Cyclic peptide fibrinogen antagonist (DMP728) | *In vitro* intestinal perfusion in rat jejunum and bioavailability in dogs | 133 |
| Semisynthetic quijalla saponin (DS-1) | D-decapeptide and mannitol | Monolayers of CaCo-2 culture cells | 134 |
| Zonula occludents toxin | | | 135 |
| Vitamin D and derivatives | | Monolayers of CaCo-2 culture cells | 136 |
| Piperine | | Intestinal everted sacs in rats | 137 |
| Hydrogen peroxide | Mannitol | CaCo-2 and T84 cell monolayers | 138 |
| Nitric oxide | | Mast cell deficient and mast cell replete mice | 139 |
| Nitric oxide donors (SNAP, NOR1, NOR4) | Insulin (suppositories) | Bioavailability studies in rabbits after rectal administration | 140 |
| Sodium salicylate | Insulin | Bioavailability studies in diabetic rats | 141 |
| Sodium glycocholate | Insulin | Bioavailability studies in rabbits after p.o. administration | 142 |
| Hydroxypropyl β-cyclodextrin dimethyl β-cyclodextrin | Insulin zinc | Bioavailability studies in rats | 143 |
| EDTA, sodium caprate, decanoyl carnitine | | Monolayers of CaCo-2 culture cells | 144 |
| Oleic acid and sodium oleate | Captopril | *In vitro* study in rat jejunum and colon | 145 |
| Glyceryl monooleate, sodium taurocholate | Human calcitonin, horse radish peroxidase and PEG-400 | *In situ* study in rat colon | 146 |

mucus in a concentration range of 0.2-20 mM (148). The effectiveness of bile salts in causing structural breakdown of mucus is in the order of deoxycholate > taurocholate > glycocholate (149). Since divalent cations $Ca^{2+}$ and $Mg^{2+}$ are important in maintaining mucus layer structure (150), chelating agents such as *N*-acyl collagen peptides (151), bile acids (152) and saponins (151) may alter mucus viscosity and hence permeability of a drug through it. O'Hagen *et al.* (153) used *N*-acetyl-L-cysteine, a potent mucolytic agent, to increase the nasal bioavailability of human growth hormone from 7.5% to 12.5% in anesthetized rat.

2) Increase in membrane fluidity

The lattice theory of diffusion of small uncharged molecules through biological membrane states that diffusion across the membrane can occur by movement of a molecule into vacancies in the lattice structure of the lipid bilayers. Such vacancies arise from random lateral diffusion of lipid molecules in the bilayer. Conditions which increase lateral diffusion of lipids and therefore, membrane fluidity, are rises in temperature that result in an increased rate of diffusion of permeating molecules (Fig. 3). Muranishi (154) studied the effect of monoglycerides and fatty acids on lecithin bilayers as a model membrane using 5-nitroxide stearic acid (spin-label). Membrane disorder was observed by interaction of fatty acids and structure-membrane disorder relationships were deduced. Fatty acids with lower melting points and/or *cis*-unsaturated bond and short carbon chain fatty acids were found to be more effective in inducing disorder in membrane. Flexibility of the acyl chain of fatty acid was found to be a main determinant of its membrane disorder-inducing property. Similarly, Karnowsky (155) showed through a fluorescence polarization technique that the *cis*-unsaturated fatty acids disorder the lipid interior while *trans*-unsaturated and saturated fatty acids do not alter the bilayer interior.

Cullis (156) observed that the incorporation of oleic acid into erythrocyte ghost membranes broadened the characteristic $^{31}P$-NMR spectrum of the bilayer structure in the absence of $Ca^{2+}$. The $H^1$-NMR showed that incorporation of fatty acids and monoolein into egg phosphatidylcholine dispersed in pD 6.5 buffer solution ($D_2O$) caused the peaks of the phosphate and olefine protons to disappear and broadened the choline methyl resonance. These findings reveal an interaction of polar lipids (monoolein and fatty acids) with lecithin phosphate. Phase transition temperature of the biomembrane can be influenced by the nature of the head group (157). Any alteration in the polar head region can change the state of the inner hydrocarbon core of a membrane. Higaki (158) has shown that medium chain glycerides interact with the polar head regions of lipids in a manner similar to monoolein as proposed by Muranishi (154). These investigations suggest that the mechanism of increase in membrane permeability caused by fusogenic lipids is associated with the disorder in the membrane's interior and the interaction of the incorporated lipid with the polar head group of phospholipid.

A recent study conducted by Turunen (65) compared the effect of 5 penetration enhancers (azone, oleic acid, 1-dodecanol, 2-dodecyl [*N,N*-dimethylamino]isopropionate, dodecyl *N,N*-dimethyl aminoacetate) on epithelial lipid domains of the buccal membrane using the fluorescence polarization technique. All the enhancers examined were found to cause disorder in lipids in human buccal epithelial cell membranes. Oleic acid, in particular, strongly reduced lipid packing in both the hydrophobic core and the polar head group region of the bilayer.

3) Solubilization of membrane components

Membrane components are liable to be solubilized by surfactants by a mechanism of micellar solubilization resulting in perturbation of membranes. Bile salts have a high capacity for solubilization of phospholipids (159). They can extract phospholipid from the brush border membrane resulting in its disruption and loss of barrier properties. At low concentrations (below critical micelle concentration or CMC), bile salts penetrate and intercalate lipid molecules in the bilayer and at high concentrations (above CMC), extensive bile salt-bile salt contact occurs in the bilayer plane, ultimately resulting in fragmentation of the bilayer and formation of mixed micelles. Cholic acid and ursodeoxycholic acid increased the release rate of the 6-carboxyfluorescein (6-CF) from egg phosphatidylcholine liposomes in a concentration-dependent manner, reaching 50% release of 6-CF near CMC values of surfactants (45).

Swenson (128) studied absorption enhancement using diverse kinds of surfactants – sodium dodecyl sulphate (SDS), sodium taurocholate (TC), sodium taurodeoxycholate (TDC), polysorbate-80 (PS-80) and nonylphenoxypolyoxyethylene (NP-POE) with an average polar group size of 10.5 POE units. Their work demonstrated good correlation between absorption enhancement of the model drug, phenol red and intestinal wall damage in single-pass intestinal perfusion in rats. Release of lactate dehydrogenase into the lumen of the small intestine was observed during a 1-h coperfusion of phenol red with TC, TDC and NP-POE-10.5. When 1% SDS was coperfused with phenol red through rat jejunum, and the perfusate thus obtained subjected to lipid extraction and thin layer chromatography, the presence of the membrane lipids phosphatidylcholine, phosphatidylethanolamine, cardiolipin, cholesterol and monoglycosyl ceramide, was confirmed in the SDS perfusate. The glycolipid monoglycosyl ceramide is a major component of the apical membrane of intestinal epithelial cells and its presence in SDS perfusate is positive evidence of disruption of brush border membrane. Similarly, Erickson (160) reported damaged villi after perfusion of chenodeoxycholic acid through intestine. Gordon (161) substantiated evidence for the hypothesis that bile acids penetrate the

nonpolar interior of membranes before increasing the permeability or dissolving the membrane into bile acid/lipid mixed micelles. Cyclodextrins also act partly by solubilization of cell membrane components (162, 163). Dimethyl beta-cyclodextrin has multiple effects on epithelium, the primary one being extraction of membrane components (cholesterol) (164).

4) Interaction with membrane proteins

Permeation enhancers can act on protein domains within membranes (165) causing denaturation or even extraction of proteins. Sodium deoxycholate (0.5%) and sodium lauryl sulphate (0.1%) increased salicylic acid flux across rabbit buccal mucosa (166). Differential scanning calorimetery suggests that these penetration enhancers affect the protein domain of the tissue. It was proposed that mode of action is tissue swelling and an increase in the intercellular space causing uncoiling and extension of protein helices, thereby opening up the polar pathway. Nonprotein sulfhydryl groups (-SH) in the membrane are other potential targets for absorption enhancers like oleic acid and salicylate. Nishihata (167) showed a decrease in levels of nonprotein thiols by salicylate-enhanced transport of cefmetazole in the rat intestine.

5) Ion-pair formation

Lipophilic neutral species or an ion-pair are formed as a result of the electrostatic attraction between two oppositely charged species. Lee (147) regards ion-pair formation between enhancer and the peptide and protein drug as a method to increase the thermodynamic activity of a drug. Piperine is a pungent alkaloid present in *Piper nigrum* and is shown to enhance the bioavailability of structurally diverse kinds of drugs (137). It has been suggested that piperine might form an apolar complex with drugs and modulate membrane dynamics. Zhou (168) postulated that cholate and its analogs form an ion-pair with insulin enhancing its nasal absorption and simultaneously inhibiting enzymatic degradation. However, the validity of the ion-pair concept in penetration enhancement needs to be substantiated by more studies.

*Absorption enhancement by the paracellular route*

1) ATP depletion in absorptive epithelial cells

Several studies suggest that a decrease in intracellular ATP levels in absorptive epithelial cells increases paracellular permeability (169, 170). Reversible ATP depletion achieved by antimycin A treatment induced a reversible increase in epithelial permeability in the kidney cell line LLC-PK1. Ruthenium red, which is an electron dense marker, penetrated the tight junction during ATP depletion in a time-dependent manner, indicating that the effect was localized to the tight junction. Treatment of CaCo-2 cells with sodium dodecyl sulfate, which causes 50% decrease in the activity of intracellular mitochondrial dehydrogenase activity and therefore a reduction in ATP production, resulted in enhancement of epithelial permeability to hydrophilic marker molecules and DDAVP (vasopressin analog) (171). Madsen (172) showed that an approximately 50% reduction in ATP levels was required to increase the permeability of rat ileal mucosa *in vitro.*

2) Phospholipase C-mediated tight junction regulation

The cascade of intracellular events occurring with activation of phospholipase C (PLC) are depicted in Figure 6. Activated PLC converts phosphatidylinositol-4,5-bisphosphate ($PIP_2$) into two intracellular mediators, inositol-1,4,5-triphosphate ($IP_3$) and 1,2-diacylglycerol (DAG). $IP_3$ induces release of $Ca^{2+}$ from intracellular stores, thus increasing the intracellular $Ca^{2+}$ concentration, which could result in increased paracellular permeability across the epithelium (173). Excess intracellular $Ca^{2+}$ levels bring about the phosphorylation of regulatory light chain myosin by $Ca^{2+}$/calmodulin-activated myosin light chain kinase (MLCK) (174, 175). This causes contraction of the cytoskeletal structure adjacent to the tight junction and adherence junction, resulting in an increase in paracellular permeability (176, 177). Contraction of this structure is driven by ATP-dependent interactions of myosin with actin filaments that form the core structure of the terminal web (174, 175). Lindmark (178) has shown that medium chain fatty acids operate by PLC-dependent $IP_3$/DAG pathways.

The second product of $PIP_2$ cleavage is DAG, which activates protein kinase C (PKC) and the release of intracellular $Ca^{2+}$, which in turn modulates tight junction permeability (179). Activation of PKC will increase or decrease tight junction permeability depending on the experimental environment (179). In the case of sodium caprate and sodium laurate, PKC activation downregulates tight junction permeability (178).

3) Contraction of actinomyosin ring

An actin myosin ring which circumscribes the cell is present near the tight junction in the cytoplasm of epithelial cells (175) (Fig. 5). This perijunctional ring is intimately associated with the plasma membrane and can contract, exerting an inward force on the lateral plasma membrane. Such contractions of the ring pull on the components of the tight junctions, inducing separations in the lateral walls of epithelial cells (176, 180).

4) Chelation of extracellular $Ca^{2+}$ ions

The integrity of the tight junction depends on extracellular calcium ions (144, 170) which indirectly control

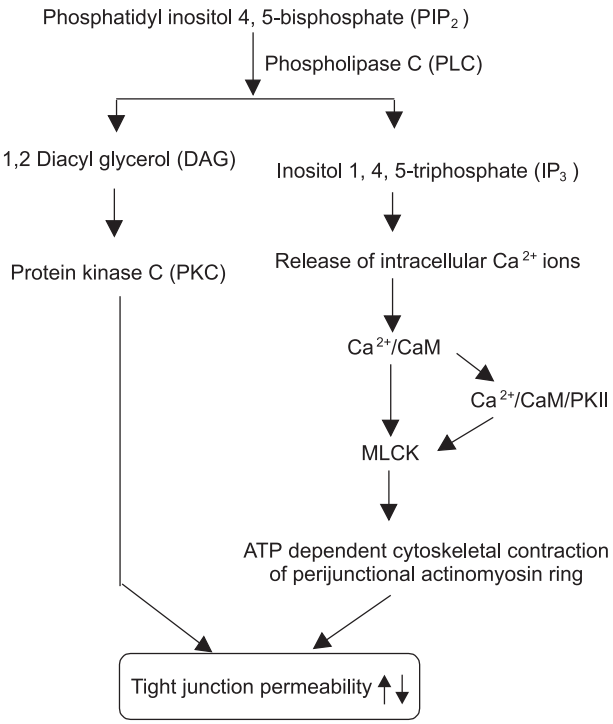


Fig. 6. Different intracellular pathways in tight junction regulation. CaM = calmodulin; PKII = protein kinase II; MLCK = myosin light chain kinase.

junctional elements through the cell adhesion molecule, uvomorulin (L-CAM). Tomita (144) demonstrated that EDTA depletes $Ca^{2+}$ in the extracellular space including the region of the tight junction and activates PKC resulting in the expansion of the paracellular route. However, due to the nonspecificity of calcium chelators, they can cause global changes in cells with undesirable effects. Moreover, $Ca^{2+}$ from gastric contents can bind to chelators and reduce their concentrations below the minimum required for penetration enhancement.

5) Increase in solvent drag

Glucose and amino acids were found to increase the diffusion flux of insulin and PEG-400 (181). It was postulated that active sodium transport, stimulated by addition of glucose, generates the driving force for water absorption through the paracellular pathway. The increased convective flow (solvent drag) increased the flux of 3 hydrophilic markers. It was shown by morphological and transepithelial resistance measurements (182) that glucose, alanine and leucine may be responsible for initiating contraction of the junctional actinomyosin leading to expanded geometry of the occluding junctions and, consequently, increased permeability. After activation of sodium-glucose cotransport, the permeability of intestinal tight junctions increases (183). Turner (183) developed a reductionist model consisting of CaCo-2 intestinal epithelial cells transfected with the intestinal $Na^+$-glucose cotransporter, SGLT1. They demonstrated that epithelial cells are mediators of physiological tight junction regulation subsequent to tight junction-activated SGLT1. The close relation between tight junction regulation and phosphorylation of myosin light chain indicates that a crucial step in regulation of epithelial tight junctions may be myosin ATPase-mediated contraction of the perijunctional actinomyosin ring and subsequent physical tension on the tight junction.

6) Recent mechanisms under study

Utoguchi (140) demonstrated an increase in insulin bioavailability from rectal suppositories using nitric oxide (NO) released from NO donors like *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP). Salzman (184) reported the mechanism of tight junction dilation by NO using cultured epithelial monolayers (CaCo-2). They also observed that the NO donor decreased cellular ATP levels, diminished staining of junctional actin and widened tight junctions. Although the actual mechanism remains to be elucidated, NO probably acts by means of a chemical mediator to dilate tight junctions. Komatsu (139) reported a correlation between NO synthetase activity in the jejunum of mast cell-deficient mice with enhanced mucosal permeability.

Robinson (125) studied the mechanistic aspects of effervescence-induced permeability enhancement by $CO_2$ bubbling over ileal epithelium mounted on a modified Ussing diffusion apparatus. $CO_2$ bubbling results in various effects which include (i) an alteration in tissue pH gradient; (ii) a buffering effect; (iii) solvent drag due to increased fluid flow; (iv) thinning or stripping of the mucus layer; (v) disruption of epithelial barriers; and/or (vi) increased membrane hydrophobicity. However, more studies are needed to determine the mode of action of effervescent preparations.

## Toxicity studies on sorption promoters

Historically, excipients have been considered inert additives. However, this belief is now no longer adequate particularly with the advent of controlled release and drug targeting systems (185). Search for nontoxic SPs which can be used repeatedly for long-term therapy or chronic therapy is an active area of research. Toxicity data for SPs is still scarce and needs to be supplemented by detailed studies on different classes of SPs. An updated description of the toxicity studies on some of the important class of SPs is provided below.

*Bile salts*

Bile salts are endogenous components of human bile and help in the solubilization, digestion and absorption of

fats. High local concentrations of bile acids near intestinal mucosa results in a varying degree of reversible damage to the absorptive cells. Erickson (160) showed recovery of damaged villi within 2 h of cessation of chenodeoxycholic acid (CDOC, chenodiol) perfusion through rat intestine. In fact, safe oral dosing of CDOC is well proven because of its long-lasting use in gallstone dissolution. In a study conducted to assess the safety of bile salts (128), it was found that biochemical and histological markers of intestinal wall damage due to sodium deoxytaurocholate and nonylphenoxypolyoxyethylene normalize rapidly after removal of the surfactant absorption enhancer from the intestinal lumen. Mucosal atrophy due to alterations in intestinal barrier function does not predispose translocation of enteric bacteria in gastroenterologic patients (186). Nakanishi (187) and Craven (188) observed that deoxycholic acid-induced damage to rat rectal epithelial cells and rat colonic epithelium, respectively, was rapidly repaired.

*Carboxylic acid and fatty acid derivatives*

Sodium caprate is a relatively nontoxic SP that is already in clinical use as an absorption enhancer in suppositories of sodium ampicillin (Ampirect™). Similarly, extract of naturally occurring cod liver oil containing 16 kinds of fatty acids (*e.g.,* oleic acid, linoleic acid, eicosapentaenoic acid, docosahexaenoic acid) has been used for buccal permeation of ergotamine tartarate (189). Two of its constituents, eicosapentaenoic acid and docosahexaenoic acid, increased insulin absorption from emulsion without any gross tissue damage after 6 h of application of the emulsion as observed microscopically (190). Hydrogenated castor oil is an effective SP (189) and castor oil, a commercial laxative, can be given in doses as high as 60 ml.

*Cyclodextrins*

Cyclodextrins (CDs) have been shown to impair the cytoplasmic membrane of CaCo-2 cells in monolayers (164). Significant rectal absorption enhancement of insulin by cyclodextrins was reported from hollow type suppositories in rabbits (191) within 24 h after treatment. As a matter of fact, some cyclodextrins are approved as food additives and are widely used in the food industry (163). Their overall cytotoxicity is lowered when used in conjunction with fatty acids, bile salts and a phospholipid (192), and toxic effects on nasal membranes have been shown to be largely reversible (193).

*Others*

Swenson (194) reported the structure-activity and structure-toxicity relationships for nonylphenoxypolyoxyethylene surfactant permeability enhancers in the rat intestinal perfusion model using phenol red as the model drug. A correlation between permeability enhancement and damage to intestinal wall was found for this class of surfactants.

NO induces permeation enhancement in CaCo-2 monolayers without loss of cell viability as confirmed by intact ultrastructure, unaltered release of the intracellular enzyme, lactate dehydrogenase and the ability to recover baseline permeability (140). Takeuchi (195) reported that unlike aspirin, the NO releasing derivative of aspirin, NCX-4016 (nitroxybutyl ester of aspirin), had neither a topical irritating action on the stomach nor exerted a worsening effect on gastric ulcerogenic response to stress. Instead it provided gastric protection against ethanol, retaining the antiinflammatory activity of aspirin and its cyclooxygenase inhibiting activity. It was suggested that NCX-4016, probably via NO release, exerted protective effects that counteracted the potential damaging effects of cyclooxygenase inhibition.

The effervescent delivery system shown to enhance absorption also causes stripping of the protective mucus layer and alters GI physiological processes such as stomach motility and the release of mucosal and gastric secretions (196). The disruptive effect of $CO_2$ could be reversed within a short period of 20 min. The rapid tissue restitution on effervescence discontinuation implies that carbonated drug delivery systems can be safely used (125) for permeation enhancement. In fact, numerous marketed products utilizing effervescent technology are already available (*e.g.*, Alka-Seltzer®) and in clinical use, implying the safety of their usage as SPs.

## Conclusions

Many of the new compounds emerging from the discovery processes based on combinatorial chemistry, high-throughput screening techniques and developments in biotechnological sciences have poor permeation profiles. This has prompted the search for novel excipients which would augment drug transport across biological membranes and aid in attaining minimum therapeutic levels in blood. Many SPs have shown promising results and many more are emerging due to the active research in this field. The ease with which these novel excipients can be incorporated in conventional oral dosage forms, thus circumventing the need for sophisticated and costly drug delivery systems of parenteral therapy, have made them a commercially important alternative.

## Acknowledgements

P. Sharma was supported by a research fellowship from CSIR, New Delhi, India.

## References

1. Mackellar, A., Osborne, N. *Breathing new life into drug delivery*. Manuf Chem 1998, 69: 31-3.

2. Anonymous. *Biopharmaceuticals increase their share of market*. Manuf Chem 1997, 68: 28-31.

3. Anonymous. *Drug delivery highlights from IDD-94.* Drug Market Dev 1994, 5: 59-64.

4. Aungst, B.J. *Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or presystemic metabolism*. J Pharm Sci 1993, 82: 979-87.

5. Muranishi, S. *Modification of intestinal absorption of drugs by lipoidal adjuvants*. Pharm Res 1985, 2: 108-18.

6. Florence, A.T. *The oral absorption of micro and nanoparticulates: Neither exceptional nor unusual.* Pharm Res 1997, 14: 259-66.

7. Florence, A.T., Hussain, N. *Utilizing bacterial mechanisms of cell entry: Invasion induced oral uptake of latex nanoparticles*. Pharm Res 1998, 15: 153-6.

8. Trenktrog, T., Muller, B.W., Seifert, J. *In vitro investigation into the enhancement of intestinal peptide absorption by emulsion systems*. Eur J Pharm Biopharm 1995, 41: 284-90.

9. Rubas, W., Grass, G.M. *Gastrointestinal lymphatic absorption of peptides and proteins*. Adv Drug Deliv Rev 1991, 7: 15-69.

10. Swaan, P.W. *Recent advances in macromolecular drug delivery via receptor mediated transport pathways*. Pharm Res 1998, 15: 826-34.

11. Bernkop-Schnurch, A., Pasta, M. *Intestinal peptide and protein delivery: Novel bioadhesive drug-carrier matrix shielding from enzymatic attack*. J Pharm Sci 1998, 87: 430-4.

12. Wiechers, J. *Excipients in topical drug formulations*. Manuf Chem 1998, 69: 17-21.

13. Ritschel, W.A. *Targeting in the gastrointestinal tract: New approaches*. Methods Find Exp Clin Pharmacol 1991, 13: 313-36.

14. Mackay, M., Williamson, I., Hastewell, J. *Cell biology of epithelia*. Adv Drug Deliv Rev 1991, 7: 313-38.

15. Swenson, E.S., Curatolo, W.J. *Means to enhance penetration. Intestinal permeability enhancement for proteins, peptides and other polar drugs: Mechanisms and potential toxicity*. Adv Drug Deliv Rev 1992, 8: 39-92.

16. Kenny, A.J., Marous, S. *Topology of microvillar membrane hydrolases of kidney and intestine*. Physiol Rev 1982, 62: 91-128.

17. Lee, V.H.L., Traver, R.D., Taub, M.E. *Enzymatic barriers to peptide and protein drug delivery*. In : Peptide and Protein Drug Delivery. Lee, V.H.L. (Ed.). Marcel Dekker: New York 1991, 303-58.

18. Simons, K., Ness, A.W. *Polarized sorting in epithelia*. Cell 1990, 62: 207-10.

19. Dragsten, P.R., Blumenthal, R., Handler, J.S. *Membrane asymmetry in epithelia: Is tight junction barrier to diffusion in plasma membrane*? Nature 1981, 294: 718-22.

20. Mandel, J.M., Bacallo, R., Zampighi, G. *Uncoupling of the molecular 'fence' and paracellular 'gate' functions in epithelial tight junctions*. Nature 1993, 361: 552-5.

21. Kotze, A.F., Luessen, A.F., De Leeuw, B.J. et al. *Comparison of the effect of different chitosan salts and N-trimethyl chitosan chloride on the permeability of intestinal epithelial cells*. J Controlled Release 1998, 51: 35-46.

22. Stenson, W.F., Eason, R.A., Riehl, T.E., Turk, J. *Regulation of paracellular permeability in CaCo-2 cell monolayers by protein kinase C*. Am J Physiol 1993, 265: G955-62.

23. Soderholm, J.D., Hedman, L., Olaison, G. *Tight junctional permeability in human ileal mucosa: Modulation with sodium caprate and cytochalasin B.* Gut 1995, 37 (Suppl. 2): A39.

24. Gandhi, R.B., Robinson, J.R. *Bioadhesion in drug delivery*. Indian J Pharm Sci 1988, 50: 145-56.

25. Bjork, E., Artursson, P., Larhed, W.A. *The influence of intestinal mucus components on the diffusion of drugs*. Pharm Res 1998, 15: 66-71.

26. Powell, D. *Barrier function in epithelia*. Am J Physiol 1981, 241: G275-88.

27. Artursson, P. *Cell cultures as model for drug absorption across the intestinal mucosa.* Crit Rev Ther Drug Carrier Syst 1991, 8: 305-30.

28. Hidalgo, I.J., Hillgren, K.M., Grass, G.M., Borchardt, R.T. *Characterization of the unstirred water layer in CaCo-2 cell monolayers using a novel diffusion apparatus*. Pharm Res 1991, 8: 222-6.

29. Karlsson, J., Artursson, P. *A method for the determination of cellular permeability coefficients and aqueous boundary layer thickness in monolayers of intestinal epithelial (CaCo-2) cells grown in permeable filter chambers*. Int J Pharm 1991, 71: 55-64.

30. Strocchi, A., Levitt, M.D. *Role of villous surface area in absorption. Science versus religion.* Dig Dis Sci 1993, 38: 385-7.

31. Oliver, R.E., Jones, A.F., Rowland, M. *What surface of the intestinal epithelium is effectively available to permeating drugs*? J Pharm Sci 1998, 87: 634-9.

32. Winne, D., Verheyen, W. *Diffusion coefficient in native mucus gel of rat small intestine*. J Pharm Pharmacol 1990, 42: 517-9.

33. Nimmerfall, F., Rosenthaler, J. *Significance of the goblet cell mucin layer, the outermost luminal barrier to passage through the gut wall*. Biochem Biophys Res Commun 1980, 94: 960-4.

34. Kearney, P., Marriott, C. *The effects of mucus glycoproteins on the bioavailability of tetracycline. III. Everted gut studies*. Int J Pharm 1987, 38: 211-20.

35. Larhed, A.W., Artursson, P., Grasjo, J., Bjork, E. *Diffusion of drugs in native and purified gastrointestinal mucus*. J Pharm Sci 1997, 86: 660-5.

36. Hillgren, K.M., Kato, A., Borchardt, R.T. *In vitro systems for studying intestinal drug absorption.* Med Res Rev 1995, 15: 83-109.

37. Camenisch, G., Folkers, G., Waterbeemd, H.V.D. *Comparison of passive drug transport through CaCo-2 cells and artificial membranes*. Int J Pharm 1997, 147: 61-70.

38. Artursson, P., Borchardt, R.T. *Intestinal drug absorption and metabolism in cell cultures: CaCo-2 and beyond*. Pharm Res 1997, 14: 1655-8.

39. Howell, S., Kenny, A.J., Turner, A.J. *A survey of membrane peptidases in two human colonic cell lines, CaCo-2 and HT-29*. Biochem J 1992, 284: 595-601.

40. Rousset, M., Laburthe, M., Pinto, M. et al. *Enterocytic differentiation and glucose utilization in the human colon tumor cell line CaCo-2 cells: Modulation by forskolin*. J Cell Physiol 1985, 123: 377-81.

41. Chantret, I., Barbat, A., Dussaulx, E. et al. *Epithelial polarity, villin expression, and enterocytic differentiation of cultured human colon carcinoma cells: A survey of twenty cell lines.* Cancer Res 1988, 48: 1936-41.

42. Baranczyk-Kuzma, A., Garren, J.A., Hidalgo, I.J., Borchardt, R.T. *Substrate specificity and some properties of phenol sulfotransferase from human intestinal CaCo-2 cells*. Life Sci 1991, 49: 1197-206.

43. Singer, S.J., Nicolson, G.L. *The fluid mosaic model of the structure of cell membranes*. Science 1972, 175: 720-31.

44. Lundhal, P. *Immobilized liposome chromatography of drugs for model analysis of drug membrane interactions*. Adv Drug Deliv Rev 1997, 23: 221-7.

45. O'Connor, C.J., Wallace, R.J., Iwamoto, K. et al. *Bile salt damage of egg phosphatidylcholine liposomes*. Biochim Biophys Acta 1985, 817: 95-102.

46. Ruiz, J., Goni, F.M., Alonso, A. *Surfactant induced release of liposomal contents. A survey of methods and results.* Biochim Biophys Acta 1988, 937: 127-34.

47. Lehninger, A.L., Nelson, D.L., Cox, M.M. (Eds.). Principles of Biochemistry. CBS Publishers: New Delhi 1993, 268-96.

48. Overath, P., Trauble, H. *Phase transitions in villi, membranes, and lipids of Escherichia coli. Detection by fluorescent probes, light scattering and dilatometry.* Biochemistry 1973, 12: 2625-34.

49. Shechter, E., Letellier, L., Gulik-Krzywicki, T. *Relation between structure and function in cytoplasmic membrane vesicles isolated from Escherichia coli fatty acid auxotroph*. Eur J Biochem 1974, 49: 61-76.

50. Grisham, C.M. Barnett, R.E. *The role of lipid phase transitions of the (sodium + potassium) adenosine triphosphate*. Biochemistry 1973, 12: 2635-7.

51. Papahadjopoulos, D., Jacobson, K., Nir., S., Isac, T. *Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measurements concerning the effect of temperature and cholesterol.* Biochim Biophys Acta 1973, 311: 330-48.

52. Blok, M.C., Neut-Kok, E.C.V.D., Deenen, V. et al. *The effect of chain length and lipid phase transitions on the selective permeability properties of liposomes*. Biochim Biophys Acta 1975, 406: 187-96.

53. Antunes-Madeira, M.C., Madeira, V.M. *Partition of malathoin in synthetic and native membranes*. Biochim Biophys Acta 1987, 901: 61-6.

54. Hauser, H., Howell, K., Dawson, R.M.C. et al. *Rabbit small intestinal brush border membrane: Preparation and lipid composition*. Biochim Biophys Acta 1980, 602: 567-77.

55. Forstner, G.G., Wherrett, J.R. *Plasma membrane and mucosal glycosphinolipids in the rat intestine*. Biochim Biophys Acta 1973, 306: 446-59.

56. Jackson, M.J. *Drug transport across gastrointestinal epithelia*. In: Physiology of Gastrointestinal Tract. Johnson, L.R. (Ed.). Raven Press: New York 1987, 1597-621.

57. Burton, P.S., Conradi, R.A., Hilgers, A.R. *Mechanisms of peptide and protein drug absorption (2). Transcellular mechanism of peptide and protein absorption: Passive aspects*. Adv Drug Deliv Rev 1991, 7: 365-86.

58. Muranushi, N., Kinugawa, M., Nakajima,Y. et al. *Mechanism for the inducement of the intestinal absorption of poorly absorbed drugs by mixed micelles. I. Effects of various lipid-bile salt mixed micelles on the intestinal absorption of streptomycin in rat*. Int J Pharm 1980, 4: 271-9.

59. Muranishi, S. *Absorption enhancers*. Crit Rev Ther Drug Carrier Syst 1990, 7: 1-33.

60. Ganem-Quintanar, A., Falson-Rieg, F., Buri, P. *Contribution of lipid components to the permeability barrier of oral mucosa.* Eur J Pharm Biopharm 1997, 44: 107-20.

61. Lee, V.H.L., Yamamoto, A., Kompella, U.B. *Mucosal penetration enhancers for facilitation of peptide and protein drug absorption*. Crit Rev Ther Drug Carrier Syst 1991, 8: 91-192.

62. Brasitus, T.A., Tall, A.R., Schachter, D. *Thermotropic transitions in rat intestinal plasma membranes studied by differential scanning calorimetry and fluorescence polarization.* Biochemistry 1980, 19: 1256-61.

63. Elson, E.L. *Membrane dynamics studied by fluorescence correlation spectroscopy and photobleaching recovery.* Soc Gen Physiol Ser 1986, 40: 367-83.

64. Cherry, R.J. *Keeping track of cell surface receptors*. Trends Cell Biol 1992, 2: 242-4.

65. Turunen, T.M., Urtti, A., Paronen, P. et al. *Effect of some penetration enhancers on epithelial membrane lipid domains: Evidence from fluorescence spectroscopy studies.* Pharm Res 1994, 11: 288-94.

66. Brasitus, T.A., Schachter, D. *Lipid dynamics and lipid-protein interactions in rat enterocyte basolateral and microvillus membranes*. Biochemistry 1980, 19: 2763-9.

67. Kimelberg, H.K. *Membrane fluidity and lipid composition*. In: Physical Methods on Biological Membranes and their Model Systems. Conti, F., Blumberg. W.E., de Gier, T., Pocchian, F. (Eds.). Plenum Press: New York 1982, 261-4.

68. Martini, A., Crivellente, M., De Ponte, R. *Use of thermal analysis to monitor the change in structure of a phospholipid bilayer due to absorption enhancers and cyclodextrins*. Eur J Pharm Biopharm 1996, 42: 67-73.

69. Subczynski, W.K., Wojas, J., Pezeshk, V., Pezeshk, A. *Partitioning and localization of spin labeled amantadine in lipid bilayers: An EPR study*. J Pharm Sci 1998, 87: 1249-54.

70. Gay, C.L., Murphy, T.M., Hadgraft, J. et al. *An electron spin resonance study of skin penetration enhancers*. Int J Pharm 1989, 49: 39-44.

71. Jain, A.K., Panchagnula, R., Gopalakrishnan, B. *Role of stratum corneum lipid in the permeation of water soluble drugs: $^{31}P$ NMR as probe.* J Pharm Pharmacol 1998, 50 (Suppl.): 135.

72. Sardon, S., Collier, S.W., Cortijo, M. et al. *Effects of ethanol and dexamethasone on epidermis examined by in vitro $^{31}P$ magnetic resonance spectroscopy*. J Pharm Sci 1998, 87: 249-55.

73. Smith, C.A., Wood, E.J. (Eds.). Cell Biology. Chapman and Hall: London 1997, 144-81.

74. Gumbiner, B. *Structure, biochemistry and assembly of epithelial tight junctions*. Am J Physiol 1987, 253: C749-58.

75. Madara, J.L. *Loosening tight junctions. Lessons from the intestine*. J Clin Invest 1989, 83: 1089-94.

76. Madara, J.L., Pappenheimer, J.R. *Structural basis for physiological regulations of paracellular pathways in intestinal epithelia.* J Membr Biol 1987, 100: 149-64.

77. Farquhar, M.G., Palade, G.E. *Junctional complexes in various epithelia*. J Cell Biol 1963, 17: 375-412.

78. Madara, J.L., Darmsathaphorn, K. *Occluding junction structure-function relationships in a cultured epithelial monolayer*. J Cell Biol 1985, 98: 1209-21.

79. Hollander, D. *The intestinal permeability barrier. A hypothesis as to its regulation and involvement in Crohn's disease*. Scand J Gastroenterol 1992, 27: 721-6.

80. Kachar, B., Reese, T.S. *Evidence for the lipidic nature and tight junction strands.* Nature 1982, 296: 464-6.

81. Stevenson, B.R., Siliciano, J.D., Mooseker, M.S., Goodenough, D.A. *Identification of ZO-1: A high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia*. J Cell Biol 1986, 103: 755-66.

82. Anderson, J.M., Stevenson, B.R., Jesaitis, D.A. et al. *Characterization of ZO-1: A protein component of the tight junction from mouse liver and Madin-Darby canine kidney cells*. J Cell Biol 1988, 106: 1141-9.

83. Gumbiner, B., Lowenkopf, T., Apatira, D. *Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1.* Proc Natl Acad Sci USA 1991, 88: 3460-4.

84. Citi, S., Sabanay, H., Jakes, R. et al. *Cingulin: A new peripheral component of tight junctions.* Nature 1988, 333: 272-6.

85. Citi, S., Sabanay, H., Kendrick-Jones, J., Geiger, B. *Cingulin: Characterization and localization.* J Cell Sci 1989, 93: 107-22.

86. Zhong, Y., Saitoh, T., Minase, T. et al. *Monoclonal antibody 7H6 reacts with a novel tight junction-associated protein distinct from ZO-1, cingulin and ZO-2*. J Cell Biol 1993, 120: 477-83.

87. Zahraoui, A., Joberty, G., Arpin, M. et al. *A small rab GTPase is distributed in cytoplasmic vesicles in non-polarized cells but co-localized with the tight junction marker ZO-1 in polarized epithelial cells*. J Cell Biol 1994, 124: 101-15.

88. Fasano, A. *Novel approaches for oral delivery of macromolecules.* J Pharm Sci 1998, 87: 1351-6.

89. Leone-Bay, A., Leipold, H., Sarubbi, D. et al. *Oral delivery of sodium cromolyn: Preliminary studies in vitro and in vivo*. Pharm Res 1996, 13: 222-6.

90. Palm, K., Stenberg, P., Luthman, K., Artursson, P. *Polar molecular surface properties predict the intestinal absorption of drugs in humans*. Pharm Res 1997, 14: 568-71.

91. Ritschel, W.A. (Ed.) Handbook of Basic Pharmacokinetics - Including Clinical Application. Drug Intelligence Publications, Inc.: Hamilton 1992, 19-41.

92. Muranishi, S., Yammamoto, A. *Mechanism of absorption enhancement through gastrointestinal epithelium.* In: Drug Absorption Enhancement: Concepts, Possibilities, Limitations and Trends. De Boer, A.G. (Ed.). Harwood: Switzerland 1994, 67-100.

93. Schanker, L.S., Jeffrey, J.J. *Active transport of foreign pyrimidines across the intestinal epithelium*. Nature 1961, 190: 727-8.

94. Hu, M., Amidon, G.L. *Passive and carrier mediated intestinal absorption components of captopril*. J Pharm Sci 1988, 77: 1007-11.

95. Guerrero, D.Q., Allemann, E., Fessi, H., Doelker, E. *Applications of ion-pair concept to hydrophilic substances with special emphasis on peptides*. Pharm Res 1997, 14: 119-27.

96. Schanker, L.S. *On the mechanism of absorption of drugs from the gastrointestinal tract*. J Med Pharm Chem 1960, 2: 343-59.

97. Kramer, S.D., Braun, A., Deiser, C.J., Allenspach, H.W. *Towards the predictability of drug membrane interactions: The pH dependent affinity of propranolol to phosphatidylinositol containing liposomes.* Pharm Res 1998, 15: 739-44.

98. Sugawara, M., Hashimoto, A., Toda, T. et al. *Changes in the permeation rate of organic anions through the intestinal brush border membranes with membrane surface potential*. Biochim Biophys Acta 1994, 1190: 85-90.

99. Iseki, K., Kaido, K., Kobayashi, M. et al. *The effect of membrane surface potential on the permeability of anionic compounds across the apical membrane in human intestinal epithelial (CaCo-2) cells.* Biol Pharm Bull 1997, 20: 794-9.

100. Lehr, C.M. *The transcytosis approach*. In: Drug Absorption Enhancement: Concepts, Possibilities, Limitations and Trends. De Boer, A.G. (Ed.). Harwood: Switzerland 1994, 325-65.

101. Tomita, M., Shiga, M., Hayashi, M., Awazu, S. *Enhancement of colonic drug absorption by the paracellular permeation route.* Pharm Res 1988, 5: 341-6.

102. Boulenc, X., Marti, E., Joyeux, H. et al. *Importance of paracellular pathway for the transport of a new bisphosphonate using the human CaCo-2 monolayers model*. Biochem Pharmacol 1993, 46: 1591-600.

103. Lundin, S., Artursson, P. *Absorption of vasopressin analogue 1-deamino-8-arginine ddAVP in a human intestinal epithelial cell line CaCo-2*. Int J Pharm 1990, 64: 181-6.

104. Gan, L-S., Hsyu, P-H., Pritchard, J.F., Thakker, D. *Mechanism of intestinal absorption of ranitidine and ondansetron: Transport across CaCo-2 cell monolayers*. Pharm Res 1993, 10: 1722-5.

105. Gibaldi, M. Biopharmaceutics and Clinical Pharmacokinetics. Lea & Febiger: Philadelphia 1991, 14-61.

106. Mitscher, L.A., Fecik, R.A., Menon, S.R. et al. *The search for orally active medications through combinatorial chemistry*. Med Res Rev 1998, 18: 149-85.

107. Charman, W.N., Stella, V.J. *Transport of lipophilic molecules by the intestinal lymphatic system*. Adv Drug Deliv Rev 1991, 7: 1-14.

108. Artursson, P., Palm, K., Luthman, K. *CaCo-2 monolayers in experimental and theoretical predictions of drug transport.* Adv Drug Deliv Rev 1996, 22: 67-84.

109. Palm, K., Luthman, K., Ungell, A.L. et al. *Correlation of absorption with molecular surface properties*. J Pharm Sci 1996, 85: 32-9.

110. Alcorn, C.J., Simpson, R.J., Leahy, D., Peters, T.J. *In vitro studies of intestinal drug absorption. Determination of partition and distribution coefficients with brush border membrane vesicles*. Biochem Pharmacol 1991, 42: 2259-64.

111. Conradi, R.A., Hilgers, A.R., Ho, N.F.H., Burton, P.S. *The influence of peptide structure on transport across CaCo-2 cells*. Pharm Res 1991, 8: 1453-60.

112. Karls, M.S., Rush, B.D., Wilkinson, K.F. et al. *Desolvation energy: A major determinant of absorption, but not clearance, of peptides in rats.* Pharm Res 1991, 8: 1477-81.

113. Sugawara, M., Miyazaki, K., Takekuma, Y. et al. *A general approach for the prediction of the intestinal absorption of drugs: Regression analysis using the physicochemical properties and drug membrane electrostatic interaction*. J Pharm Sci 1998, 87: 960-6.

114. Lipinski, C.A., Lonbardo, F., Dominy, B.W., Feeney, P.J. *Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings.* Adv Drug Deliv Rev 1997, 23: 3-25.

115. Verner, K., Schatz, G. *Protein translocation across membranes*. Science 1988, 241: 1307-13.

116. Milstein, S.J., Leipold, H., Sarubbi, D. et al. *Partially unfolded proteins efficiently penetrate cell membranes – Implications for oral drug delivery*. J Controlled Release 1998, 53: 259-67.

117. Gangwar, S., Jois, S.D.S, Borchardt, R.T. et al. *The effect of conformation on membrane permeability of an acyloxyalkoxy-linked cyclic prodrug of a model hexapeptide*. Pharm Res 1996, 13: 1657-62.

118. Johnston, T.P., Rahman, A., Alur, H. et al. *Permeation of unfolded basic fibroblast growth factor (bFGF) across rabbit buccal mucosa- Does unfolding of bFGF enhance transport?* Pharm Res 1998, 15: 246-53.

119. Schatz, G., Dobberstein, B. *Common principles of protein translocation across membranes*. Science 1996, 271: 1519-26.

120. Artursson, P., Lindmark. T., Daviss, S.S., Illum, L. *Effect of chitosan on the permeability of monolayer of intestinal epithelial cells (CaCo-2).* Pharm Res 1994, 11: 1358-61.

121. Schipper, N.G.M., Olsson, S., Hoogstraate, J.A. et al. *Chitosans as absorption enhancers for poorly absorbable drugs 2: Mechanism of absorption enhancement*. Pharm Res 1997, 14: 923-9.

122. Kotze, A.F., Verhoef, J.C., Junginger, H.E. et al. *N-Trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: In vitro evaluation in intestinal epithelial cells (CaCo-2).* Pharm Res 1997, 14: 1197-202.

123. Morishita, M., Morishita, I., Takayama, A. et al. *Site dependent effect of aprotinin, sodium caprate, $Na_2EDTA$ and sodium glycocholate on intestinal absorption of insulin.* Biol Pharm Bull 1993, 16: 68-72.

124. Sutton, S.C., LeCluyse, E.L., Engle, K. et al. *Enhanced bioavailability of cefoxitin using palmitoylcarnitine. II. Use of directly compressed tablet formulations in rat and dog.* Pharm Res 1993, 10: 1516-20.

125. Eichman, J.D., Robinson, J.R. *Mechanistic studies on effervescent-induced permeability enhancement*. Pharm Res 1998, 15: 925-30.

126. Dangi, J.S., Vyas, S.P., Dixit, V.K. *Effect of various lipid bile salt mixed micelles on the intestinal absorption of amphotericin-B in rat*. Drug Dev Ind Pharm 1998, 24: 631-5.

127. Hovgaard, L., Brondsted, H., Nielsen, H.M. *Drug delivery studies in CaCo-2 monolayers. II. Absorption enhancer effects of lysophosphatidylcholines*. Int J Pharm 1995, 114: 141-9.

128. Swenson, E.S., Milisen, W., Curatolo, W. *Intestinal permeability enhancement: Efficacy, acute local toxicity, and reversibility*. Pharm Res 1994, 11: 1132-42.

129. Boulenc, X., Roques, C., Joyeux, H. et al. *Bisphosphonates increase tight junction permeability in the human intestinal epithelia (CaCo-2) model*. Int J Pharm 1995, 123: 13-24.

130. Fagerholm, U., Sjostrom, B., Wijk, A. et al. *The effect of a drug delivery system consisting of soybean phosphatidylcholine and medium chain monoacylglycerol on the intestinal permeability of hexarelin in rat*. J Pharm Pharmacol 1998, 50: 467-73.

131. Corre, P.L., Dollo, G., Chevanne, F., Verge, R.L. *Influence of hydroxypropyl β-cyclodextrin and dimethyl beta-cyclodextrin on diphenyhydramine intestinal absorption in a rat in situ model.* Int J Pharm 1998, 169: 221-8.

132. Soderholm, J.D., Oman, H., Blomquist, L. et al. *Reversible increase in tight junction permeability to macromolecules to rat ileal mucosa in vitro by sodium caprate, a constituent of milk fat.* Dig Dis Sci 1998, 43: 1547-52.

133. Aungst, B.J., Saitoh, H., Burcham, D.L. et al. *Enhancement of the intestinal absorption of peptides and non-peptides.* J Controlled Release 1996, 41: 19-31.

134. Chao, A.C., Nguyen, J.V., Broughall, M. et al. *Enhancement of intestinal model compound transport by DS-1, a modified quillaja saponin*. J Pharm Sci 1998, 87: 1395-9.

135. Fasano, A. *Modulation of intestinal permeability: An innovative method of oral drug delivery for the treatment of inherited and acquired lumenal diseases*. Mol Genet Metab 1998, 64: 12-8.

136. Chirayath, M.V., Gajdzik, L., Hulla, W. et al. *Vitamin D increases tight junction conductance and paracellular $Ca^{2+}$ transport in CaCo-2 cell cultures.* Am J Physiol 1998, 274: G389-96.

137. Khajuria, A., Zutshi, U., Bedi, K.L. *Permeability characteristics of piperine on oral absorption – An active alkaloid from peppers and a bioavailability enhancer*. Indian J Exp Biol 1998, 36: 46-50.

138. Rao, K.R., Baker, R.D., Baker, S.S. et al. *Oxidant induced disruption of intestinal epithelial barrier function: Role of protein tyrosine phosphorylation*. Am J Physiol 1997, 273: G812-23.

139. Komatsu, S., Grisham, M.B., Russell, J.M., Granger, D.M. *Enhanced mucosal permeability and nitric oxide synthetase activity in jejunum of mast cell deficient mice.* Gut 1997, 41: 636-41.

140. Utoguchi, N., Watanabe, Y., Shida, T., Matsumoto, M. *Nitric oxide donors enhance rectal absorption of macromolecules in rabbits*. Pharm Res 1998, 15: 870-6.

141. Hosny, E.A., Khan-Ghilzai, N.M., Al-Dhawalie, A.H. *Effective intestinal absorption of insulin in diabetic rats using enteric coated capsules containing sodium salicylate*. Drug Dev Ind Pharm 1995, 21: 1583-9.

142. Mesiha, M., Plakogiannis, F., Vejosoth, S. *Enhanced oral absorption of insulin from desolvated fatty acid sodium glycocholate emulsions.* Int J Pharm 1994, 111: 213-6.

143. Shao, Z., Li, Y., Chermak, T., Mitra, A.K. *Cyclodextrins as mucosal absorption promoters of insulin. Part 2. Effects of β-cyclodextrin derivatives on α-chymotryptic degradation and enteral absorption of insulin in rats.* Pharm Res 1994, 11: 1174-9.

144. Tomita, M., Hayashi, M., Awazu, S. *Absorption enhancing mechanism of EDTA, caprate and decanoylcarnitine in CaCo-2 cells*. J Pharm Sci 1996, 85: 608-11.

145. Sintov, A., Simberg, M., Rubinstein, A. *Absorption enhancement of captopril in rat colon as a putative method for captopril delivery by extended release formulations.* Int J Pharm 1996, 143: 101-6.

146. Hastewell, J., Lynch, S., Fox, R. et al. *Enhancement of human calcitonin absorption across the rat colon in vivo.* Int J Pharm 1994, 101: 105-20.

147. Lee, V.H.L. *Protease inhibitors and penetration enhancers as approaches to modify peptide absorption*. J Controlled Release 1990, 13: 213-23.

148. Martin, G.P., Marriott, C., Kellaway, I.W. *Direct effect of bile salts and phospholipids on the physical properties of mucus.* Gut 1978, 19: 103-7.

149. Martin, G.P., Marriott, C., Kellaway, I.W. *The effect of natural surfactants on the rheological properties of mucus.* J Pharm Pharmacol 1976, 28 (Suppl.): 76P.

150. Pitelka, D.R., Taggart, B.N., Hamamoto, S.T. *Effect of extracellular calcium depletion on membrane topography and occluding junctions of mammary epithelial cells in culture.* J Cell Biol 1983, 96: 613-24.

151. Yata, N., Sugihara, N., Yamajo, R. et al. *Enhanced rectal absorption of β-lactam antibiotics in rat by monodesmosides isolated from pericarps of Sapindus mukurossi (enmei-hi).* J Pharmacobio-Dyn 1985, 8: 1041-7.

152. Murakami, T., Sasaki, Y., Yamajo, R., Yata, N. *Effect of bile salts on the rectal absorption of sodium ampicillin in rats.* Chem Pharm Bull 1984, 32: 1948-55.

153. O'Hagen, D.T., Critchley, H., Farraj, N.F. et al. *Nasal absorption enhancers for biosynthetic human growth hormone in rats.* Pharm Res 1990, 7: 772-6.

154. Muranushi, N., Takagi, N., Muranishi, S., Sezaki, H. *Effects of fatty acids and monoglycerides on permeability of lipid bilayer.* Chem Phys Lipids 1981, 28: 269-79.

155. Karnovsky, M.J. *Lipid domains in biological membranes. Their structural and functional perturbation by free fatty acids and the regulation of receptor mobility*. Am J Pathol 1979, 97: 211-22.

156. Cullis, P.R., Hope, M.J. *Hydrocarbon phase transitions, heterogeneous lipid distributions and lipid-protein interactions in erythrocyte membranes*. Nature 1975, 271: 672-4.

157. Esko, J.D., Gilmore, J.R., Glaser, M. *Use of a fluorescent probe to determine the viscosity of LM cell membranes with altered phospholipid compositions*. Biochemistry 1977, 16: 1881-90.

158. Higaki, K., Kato, M., Hashida, M., Sezaki, H. *Enhanced membrane permeability to phenol red by medium chain glycerides: Studies on the membrane permeability and microviscosity.* Pharm Res 1988, 5: 309-12.

159. Helenius, A., Simens, K. *Solubilization of membranes by detergents.* Biochim Biophys Acta 1975, 415: 29-79.

160. Erickson, R. *Effect of 16,16 dimethyl $PGE_2$ and indomethacin on bile acid induced intestinal injury and restitution in rats.* J Lab Clin Med 1988, 112: 735-44.

161. Gordon, G.S., Moses, A.C, Silver, R.D. et al. *Nasal absorption of insulin: Enhancement by hydrophobic bile salts.* Proc Natl Acad Sci USA 1985, 82: 7419-23.

162. Nakanishi, K., Nadai, T., Masada, M., Miyajima, K. *Effect of cyclodextrins on biological membranes. II. Mechanism of absorption enhancement of the intestinal absorption of non-absorbable drug by cyclodextrins.* Chem Pharm Bull 1990, 40: 1252-6.

163. Szejtli, J. *Medical applications of cyclodextrins.* Med Res Rev 1994, 14: 353-86.

164. Hovgaard, L., Brondsted, H. *Drug delivery studies in CaCo-2 monolayers. IV. Absorption enhancer effects of cyclodextrins.* Pharm Res 1995, 12: 1328-32.

165. Ganem-Quintanar, A., Kalia, Y.N., Falson-Rieg, F., Buri, P. *Mechanisms of oral permeation enhancement.* Int J Pharm 1997, 156: 127-42.

166. Gandhi, R., Robinson, J. *Mechanisms of penetration enhancement for transbuccal delivery of salicylic acid.* Int J Pharm 1992, 85: 129-40.

167. Nishihata, T., Nghiem, B.T., Yoshitomi, H. et al. *Changes in intestinal mucosal permeability caused by non-protein thiol loss in rats.* Pharm Res 1986, 3: 345-51.

168. Zhou, X.H., Po, A.L.W. *Effects of cholic acid and the other enhancers on the bioavailability of insulin from a subcutaneous site*. Int J Pharm 1991, 69: 29-41.

169. Canfield, P.E., Geerdes, A.M., Molitoris, B.A. *Effect of reversible ATP depletion on tight junction integrity in LLC-$PK_1$ cells.* Am J Physiol 1991, 261: F1038-45.

170. Unno, N., Baba, S., Fink, M.P. *Cytosolic ionized $Ca^{2+}$ modulates chemical hypoxia-induced hyperpermeability in intestinal epithelial monolayers.* Am J Physiol 1998, 274: G700-8.

171. Anderberg, E.K., Artursson, P. *Epithelial transport of drugs in cell culture. VIII. Effect of pharmaceutical surface excipient sodium dodecyl sulfate on cell membrane and tight junction permeability in human intestinal epithelial (CaCo-2) cells.* J Pharm Sci 1993, 82: 392-8.

172. Madsen, K.L., Yanchar, N.L., Sigalet, D.L. et al. *FK506 increases permeability in rat intestine by inhibiting mitochondrial function.* Gastroenterology 1995, 109: 107-14.

173. Tai, Y.H., Flick, J., Levine, S.A. et al. *Regulation of tight junction resistance in T84 monolayers by elevation in intracellular $Ca^{2+}$ levels: A protein kinase C effect.* J Membr Biol 1996, 149: 71-9.

174. Citi, S., Kendrick-Jones, J. *Regulation of non-muscle myosin structure and function*. BioEssays 1987, 7: 155-9.

175. Keller, T.C.S., Mooseker, M.S. *$Ca^{2+}$-calmodulin dependent phosphorylation of myosin, and its role on brush border contraction in vitro*. J Cell Biol 1982, 95: 943-59.

176. Madara, J.L., Barenberg, D., Carlson, S. *Effects of cytochalasin D on occluding junctions of intestinal absorptive cells: Further evidence that the cytoskeleton may influence paracellular permeability and junctional charge selectivity.* J Cell Biol 1986, 102: 2125-36.

177. Madara, J.L., Stafford, J., Barenberg, D., Carlson, S. *Functional coupling of tight junctions and microfilaments in T84 monolayers.* Am J Physiol 1988, 254: G416-23.

178. Lindmark, T., Kimura, Y., Artursson, P. *Absorption enhancement through intracellular regulation of TJ permeability by medium chain fatty acids in CaCo-2 cells.* J Pharmacol Exp Ther 1998, 284: 362-9.

179. Stuart, R.O., Nigam, S.K. *Regulated assembly of tight junctions by protein kinase C.* Proc Natl Acad Sci USA 1995, 92: 6072-6.

180. Hochman, J., Artursson, P. *Mechanism of absorption enhancement and tight junction regulation.* J Controlled Release 1994, 29: 253-67.

181. Pappenheimer, J.R., Reiss, K.Z. *Contribution of solvent drag through intercellular junctions to absorption of nutrients by small intestine of the rat.* J Membr Biol 1987, 100: 123-36.

182. Pappenheimer, J.R. *Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters.* J Membr Biol 1987, 100: 137-48.

183. Turner, J.R., Rill, B.K., Carlson, S.L. et al. *Physiological regulation of epithelial tight junction is associated with myosin light chain phosphorylation.* Am J Physiol 1997, 273: C1378-85.

184. Salzman, A.L., Menconi, M.J., Unno, N. et al. *Nitric oxide dilates tight junctions and depletes ATP in cultured CaCo-2 BBe intestinal epithelial monolayers*. Am J Physiol 1995, 268: G361-73.

185. Anonymous. *Excipients to the year 2000*. Pharm Manuf Rev 1995, 7: S6-8.

186. Dave, K., Sagar, P.S., Poon, P. et al. *Alterations in intestinal barrier function do not predispose to translocation of enteric bacteria in gastroenterologic patients.* Nutrition 1998, 14: 358-62.

187. Nakanishi, K., Masada, M., Nadai, T. *Effect of pharmaceutical adjuvants on the rectal permeability of drugs. III. Effect of repeated administration and recovery of the permeability.* Chem Pharm Bull 1983, 31: 4161-6.

188. Craven, P.A., Pfansteil, J., Saito, R., Derubertis, F.R. *Relationship between loss of rat colonic surface epithelium induced by deoxycholate and initiation of the subsequent proliferative response*. Cancer Res 1986, 46: 5754-9.

189. Tsutsumi, K., Obata, Y., Takayama, K. et al. *Effect of cod-liver oil extract on the buccal permeation of ergotamine tartarate.* Drug Dev Ind Pharm 1998, 24: 757-62.

190. Morishita, M., Suzuki, A., Kajita, M. et al. *Enhanced colonic and rectal absorption of insulin using a multiple emulsion containing eicosapentaenoic acid and docosahexaenoic acid.* J Pharm Sci 1998, 87: 1196-202.

191. Watanabe, Y., Matsumoto, Y., Seki, M. et al. *Absorption enhancement of polypeptide drugs by cyclodextrins. I. Enhanced rectal absorption of insulin from hollow type suppositories containing insulin and cylcodextrins in rabbits*. Chem Pharm Bull 1992, 40: 3042-7.

192. Gill, I.J., Fisher, N., Farraj, N. et al. *Cyclodextrins as protective agents against enhancer damage in nasal delivery systems. II. Effects on in vivo absorption of insulin and histopathology of nasal membrane.* Eur J Pharm Sci 1994, 1: 237-48.

193. Schipper, N.G.M., Verhoef, J.C., Merkus, F.W.H.M. et al. *Absorption enhancers in nasal drug delivery: Efficacy and safety.* J Controlled Release 1993, 24: 201-8.

194. Swenson, E.S., Curatolo, W., Milisen, W.B. *Intestinal permeability enhancement: Structure activity and structure toxicity relationships for nonylphenoxypolyoxyethylene surfactant permeability enhancers*. Pharm Res 1994, 11: 1501-4.

195. Sugawa, Y., Takeuchi, K., Ukawa, H. et al. *Effect of nitric oxide releasing aspirin derivative on gastric functional and ulcerogenic responses in rats: Comparison with plain aspirin.* J Pharmacol Exp Ther 1998, 286: 115-21.

196. Eichman, J.D., Bakery Yasin, A., Robinson, J.R. *The influence of in vivo carbonation on GI physiological process and drug permeability.* Eur J Pharm Biopharm 1997, 44: 33-8.