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Review

Does Fluid Flow Across the Intestinal Mucosa Affect Quantitative Oral Drug Absorption? Is It Time for a Reevaluation?

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Purpose. Hydrophilic and charged solutes have a lower membrane permeability which is due to a lower partition into the lipid membrane (low solubility in the membrane phase) and/or a slower transcellular diffusion coefficient. They are therefore anticipated to be absorbed through the paracellular route, which is a consequence of diffusion and a convective volume flow through the water-filled intercellular space.

Methods. Two approaches have been used to investigate the mechanisms underlying the paracellular drug transport across the intestinal mucosa: (a) including water transport by exposing the apical side of the epithelium with a hypotonic solution, and (b) stimulated paracellular transport by widening of tight junction and increased water absorption as a consequence of the sodium-coupled transport of nutrients.

Results. Among the first studies that recognized this fluid flux dependent transmucosal transport of drugs, was one published by Oschenfahrt & Winne in 1973 and the one by Kitazawa et al. in 1975. During the last two decades the importance of this paracellular route for drug delivery have been explored in vitro and in situ.

Conclusions. The limits concerning molecular weight, shape, ionization and the effect of physiological stimulants, such as luminal concentrations of nutrients, osmolality and motility, are currently under investigation. However, recently published in vivo human data by ourselves and others indicate that the promising results obtained in vitro and in situ for various hydrophilic compounds might not be valid in quantitative aspects in humans, especially not for drugs with a molecular weight over 200.

KEY WORDS: intestinal permeability; water absorption; solvent drag; tight junctions; paracellular absorption; human permeability.

I. INTRODUCTION

In general, drug absorption from the small and large intestine includes: dose/dissolution ratio, chemical degradation/metabolism in the lumen, complex binding in the lumen, intestinal transit, and effective permeability (P_{eff}) across the intestinal mucosa (Fig. 1). The transmucosal absorption of drugs in the human intestine is anticipated to occur by passive and/or carrier-mediated transcellular transport and/or passive paracellular transport by diffusion and/or convective transmucosal water flow (Fig. 2). In many cases the effective intestinal permeability (P_{eff}) is considered to be the rate-limiting step in the overall absorption process. Therefore is it of special interest to investigate those luminal factors and physiological conditions that might influence drug absorption. One major objective in optimizing oral drug delivery has been to find physiological mechanisms which could be subjected to modulation and thereby enhance drug absorption (1-3).

A suitable starting point for this review is to assume a model for passive transport across the intestinal mucosa as described in Figure 2, where sufficiently lipophilic drugs are transported across the cell membrane (transcellular route), and small hydrophilic compounds through the tight junctions in the water-filled space between the epithelial cells (paracellular route) (1-10). Fluid absorption across the intestinal mucosa has been demonstrated to influence the P_{eff} of drugs and other compounds *in vitro* and *in situ* (8-24). The suggested mechanism for fluid transport is that it to a certain extent occur by the paracellular route (8-10). The effect of transmucosal flow on membrane transport, a phenomenon called "solvent drag", was first reported by Andersson and Ussing in 1957 (4). The effect has been quantitatively estimated using a hydrodynamic hypothesis (24). However, the contribution of this effect to quantitative drug absorption in vivo in humans is controversial, and especially the nutrient-induced increase of intestinal permeability (25-30). Moreover, the fundamental transmucosal transport mechanism(s) of water molecules are still not fully understood, i.e. the proportion of water transported by transcellular and/or paracellular pathways, respectively (8-10, 25-27, 31-34, 51, 66). Another hypothesis for explaining increased intestinal P_{eff} values during fluid absorption status, concerns the increased

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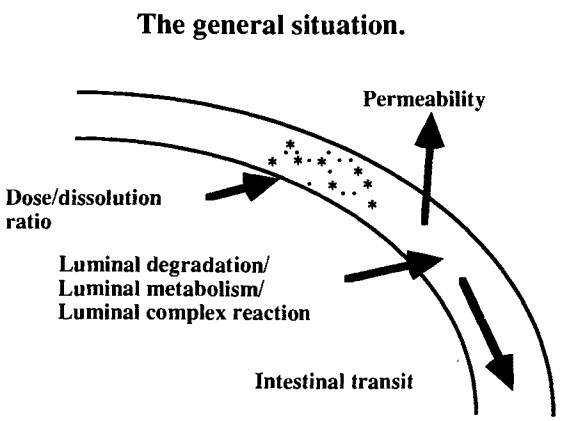

Fig. 1. Macro- and microaspects of oral drug absorption which includes: dose/solubility ratio, complex binding, chemical and enzymatic degradation, intestinal transit and effective intestinal permeability.

concentration gradient close to the intestinal wall, which is a consequence of water absorption and might work to increase the net passive diffusion across the cell membrane (14, 15, 21, 25-28, 35). This concentration gradient might also be substantiated by the wash-out flow of drug molecules within the enterocyte. This should then decrease the drug concentration in the cell cytosol, especially at the interior side of the apical cell membrane.

Beyond the scope of the present review is a detailed morphological description of the basic cellular structures involved in the physiological regulation of the paracellular transport of drugs in the different intestinal models. In that particular case, I refer the reader to recently published works by others (3, 37). Instead, I will focus on the effect of transmucosal fluid flux ("solvent drag"), as it concerns quantitative aspects of drug permeability by paracellular transport, and its implications for oral drug delivery in humans. Principally this involves the small intestine, which is the most leaky epithelium. Comparative data obtained in colon/rectum studies will be discussed where relevant.

II. THEORETICAL SECTION

II.1. Effective Passive Intestinal Permeability (P_{eff}), Transcellular and Paracellular Pathways

In order to discuss the quantitative importance of paracellular drug absorption, we have to consider what might limit the transcellular uptake. The effective rate of passive drug absorption (dM/dt, mass/time) across the intestinal wall, irrespective of the trans- or paracellular route, is determined by the available intestinal surface area (A), the effective permeability (P*eff*) and a reference concentration of the drug in the lumen (C_{lumen}). It has been shown that the reference concentration of a drug (antipyrine) in the lumen is about 100 times higher compared to the same drug concentration in the mesenteric vein, which is in accordance with the sink condition assumption (38). That situation is therefore assumed value throughout the theoretical analysis in this review (i.e. $C_{blood} \ll C_{lumen}$):

$$\left\langle \frac{dM}{dt} \right\rangle_{total} = A \cdot P_{eff} \cdot (C_{lumen} - C_{blood}) = A \cdot P_{eff} \cdot C_{lumen} \quad \text{(eq. 1)}$$

In order to better understand the relative contribution of the trans- versus paracellular pathways to the overall passive absorption rate of drugs, equation 1 can be divided into the respective transport routes;

$$\left\langle \frac{dM}{dt} \right\rangle_{total} = \left\langle \frac{dM}{dt} \right\rangle_{trans} + \left\langle \frac{dM}{dt} \right\rangle_{para} \quad \text{(eq. 2)}$$

and then further developed in equation 3;

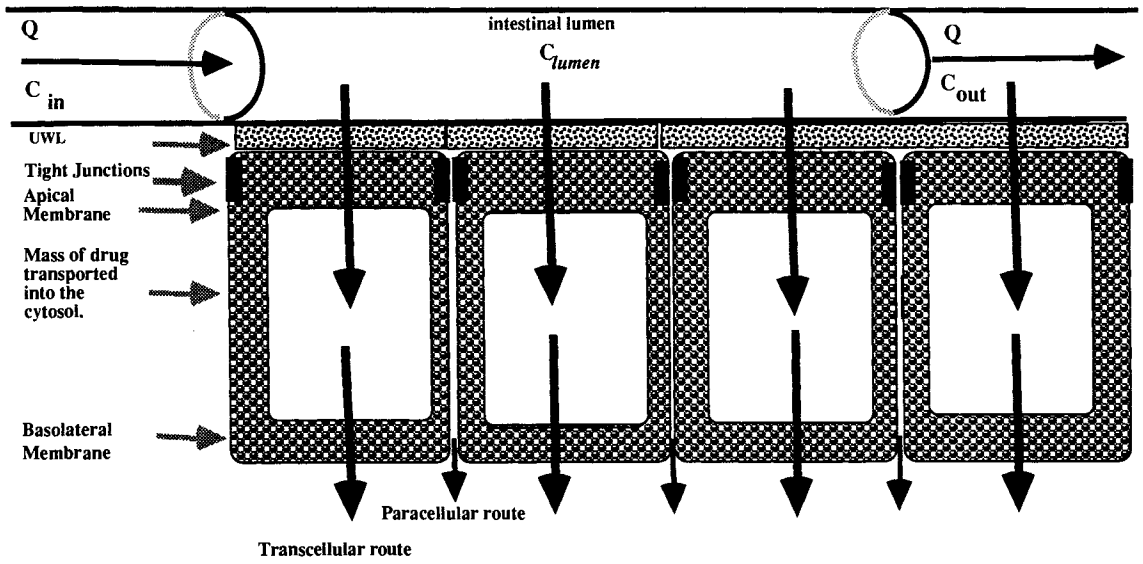

Fig. 2. A model for passive drug transport mechanisms of drugs across the intestinal mucosa. It includes both the trans- and paracellular routes, UWL = unstirred water layer.

$$\left\langle \frac{dM}{dt} \right\rangle_{total} = A_m \cdot P_{eff,trans} \cdot C_{lumen} + A_p \cdot P_{eff,para} \cdot C_{lumen} + J_{fluid} \cdot C_{lumen} \cdot (1 - \alpha) \quad \text{(eq. 3)}$$

where A_m and A_p are the available surface areas (cm^2) for the trans- and paracellular routes, respectively. A_p is determined by the width of the intercellular space, which according to the regulatory mechanism proposed for tight junctions, suggests that A_p is dynamic (8-10, 37, 39-40). Furthermore, $P_{eff,trans}$ and $P_{eff,para}$ are the effective permeability coefficients (cm/s) for the trans- and paracellular routes, respectively. J_{fluid} is the volumetric flow (cm^3/s) in the intercellular space which determines the direct fluid effect of the paracellular drug absorption mechanism (solvent drag). The reflection coefficient, α, is a measure of the sieving properties of a semipermeable membrane (32). According to the definition, α is the ratio of the flow of water relative to the solute flux compared with the total volume flow under the influence of a pressure gradient (32). The value of α is dependent on the molecular size, molecular volume, molecular charge and the hydration number (1-3, 6-22, 32, 42-43). In addition, the parameter (α) might also be influenced by the dynamic width of the tight junction (8-9, 39-43). The dimensions of these pores have been estimated to be in the range of 3-10 Å (1, 8-9, 37). However, the radii are not fixed and factors such as osmotic and hydrostatic pressure differences, as well as recently reported mechanistic control by nutrients (e.g. glucose and amino acids), might increase drug absorption by this route. This extracellular pathway has previously been localized at the intercellular junctions and intercellular spaces using electronmicroscopy and microelectrodes (3, 37). The intercellular space has been estimated to be approximately 0.01% of the total surface area of the intestinal epithelium (8-10, 37).

II.2. Transcellular Permeability by Passive and/or Carrier-Mediated Mechanisms

The effective transcellular permeability ($P_{eff,trans}$) might occur by passive and/or carrier-mediated transport, but irrespectively of the mechanism involved; includes the process of transport to the membrane (aqueous permeation, e.g. diffusion and convection to the membrane through the unstirred water layer, UWL, adjacent to the membrane); cell mucosa permeation including mucin and membrane translocation processes; and transport through the cytosol, basolateral membrane, interstitial fluid and capillary wall to the blood (Fig. 2). However, the passive transcellular permeability is determined by three in vivo determinants according to equation 4 (32):

$$P_{eff,trans} = K \cdot \frac{D_m}{\lambda} \quad \text{(eq. 4)}$$

where K is the membrane-aqueous partition coefficient of this compound, D_m is the diffusion coefficient (cm^2/s) of the compound within the membrane, and λ the thickness of the rate-limiting diffusion barrier (32). This equation describes the "solubility-diffusion" model and explains the dependence of lipophilicity, hydrogen bonding capacity, polar surface area of the molecule, molecular volume and shape on drug permeability and as well other crucial physicochemical functions and properties (32, 42-48). Therefore small and lipophilic drugs are generally absorbed rapidly across the intestinal epithelium because the partition into the lipid bilayer is high and the diffusion through the cell-membrane is not hindered by unfavourable molecular size and shape (6, 32, 42-48). The equation also includes factors such as membrane fluidity and composition, and the sieving properties and the mass dependence of the membrane diffusion coefficient are a consequence of that composition and structure of the hydrocarbon chains within the membrane phase (32).

The other transcellular mechanism, carrier-mediated transport, is determined by Michaelis-Menten kinetics which means that $P^*_{eff,trans}$ is described according to equation 5:

$$P^*_{eff,trans} = \frac{J_{max}}{K_m + C_{lumen}} \quad \text{(eq. 5)}$$

where J_{max} (mass/time/area) is the maximal transport rate (capacity) of the carrier-mediated process and K_m the substrate specificity of the transporter.

II.3. Paracellular Permeability

Hydrophilic and charged solutes have a lower membrane permeability which is due to a lower partition into the lipid membrane (low solubility in the membrane phase) and/or a slower transcellular diffusion coefficient. They are therefore anticipated to be absorbed through the paracellular route, which is a consequence of diffusion and a convective volume flow through the water-filled intercellular space (1-12, 20, 37, 39-43, 55-58), and together described in equation 6 (extracted from eq. 3) as the paracellular absorption rate (Fig. 2);

$$\left\langle \frac{dM}{dt} \right\rangle_{para} = A_p \cdot P_{eff,para} \cdot C_{lumen} + J_{fluid} \cdot C_{lumen} \cdot (1 - \alpha) \quad \text{(eq. 6)}$$

where J_{fluid} is the volumetric flow (cm^3/s) in the intercellular space which determines the direct fluid effect of the paracellular drug absorption mechanism (solvent drag). This flow is often referred to as occurring by convection across the intestinal mucosa through the narrow and winding intercellular spaces.

In order to study the total drug absorption rate equations 2, 4, 5 and 6 are combined into equation 7. The diffusion part of the paracellular absorption can be further described but the following assumptions must be made: (a) the total passive permeability through the paracellular route is determined by the diffusion coefficient of the compound in water (D_{aq}), the aqueous diffusion distance (λ_{aq}), the fluid flow (J_{fluid}) between epithelial cells, and the reflection coefficient (α), the partion coefficient (K) is 1, and (b) the transport of water molecules through the paracellular route has not been seen to incorporate any special assumptions regarding friction forces between water molecules and the walls of this intercellular space. Instead, it has been suggested that the interactions with the surroundings are similar to diffusion of water molecules in a lattice of bulk water molecules (32).

$$\left\langle \frac{dM}{dt} \right\rangle_{total} = Am \cdot K \cdot \frac{Dm}{\lambda} \cdot C_{lumen} + \frac{J_{max} \cdot C_{lumen}}{K_m + C_{lumen}} + A_p \cdot \frac{Daq}{\lambda aq} \cdot C_{lumen} + J_{fluid} \cdot C_{lumen} \cdot (1 - \alpha) \quad \text{(eq. 7)}$$

The partition coefficient (K) describes the relative tendency of a substance to dissolve in the membrane phase ($C_{membrane}$) compared with its tendency to dissolve in the surrounding aqueous phase (C_{lumen}). Therefore, the transcellular part of equation 7 might be simplified to;

$$\left\langle \frac{dM}{dt} \right\rangle_{total} = A_m \cdot \frac{D_m}{\lambda} \cdot C_{membrane} + \frac{J_{max}}{K_m + C_{lumen}} + A_p \cdot \frac{Daq}{\lambda_{aq}} \cdot C_{lumen} + J_{fluid} \cdot C_{lumen} \cdot (1 - a) \quad \text{(eq. 8)}$$

which demonstrates that the solubility of the drug in the membrane phase is crucial for the transcellular absorption route. The importance of lipophilicity was already shown at the end of the last century by Overton (47). This work was further extended in 1949 by the Finnish researcher, Collander, who studied the effect of both lipid solubility and molecular size on membrane transport (48). Furthermore, equation 8 also underlines the crucial role of the available surface area in the intestine, which have been demonstrated to be equally important for active and passive transcellular absorption (49).

One crucial point in oral drug delivery research is to establish how a change in permeability in the experimental model system will affect the fraction absorbed *in vivo* quantitative aspects. In equation 9, based on that the drug concentration in the intestinal lumen is best described according to a parallel tube model, is it possible from human jejunal permeability values (P_{eff}), an intestinal residence time of 3 hours (t_{res}), considered as the duration time for that P*eff*-value, and a intestinal radius (R) of 1.75 cm, to estimate the fraction absorbed (f_a) in humans (69):

$$fa = 1 - e^{-2\frac{Peff}{R}t_{res}} \quad \text{(eq. 9)}$$

In Figure 3 it is demonstrated what an effect a change in human effective intestinal permeability might have on the fraction absorbed (f_a) in vivo when the duration time for that particular permeability value is 3 hours. Furthermore, this model assumes that the hydrodynamics along the intestinal tract is best described by the parallel tube model and that no chemical degradation, metabolism, no dissolution problem and complex-formation occur in the intestinal lumen.

III. THE MICROORGANIZATION AND THE DEMANDS OF THE INTESTINAL MUCOSA

The two major functions of the intestinal epithelium are to perform vectorial transport (absorption and secretion of a broad structural diversity of compounds) and to act as a barrier for the uptake of noxious compounds and particles (2-3, 37). As earlier discussed, there exist two major pathways by which solutes can penetrate the mucosa: the intercellular and paracellular pathways as illustrated in Fig. 2. The transport characteristics of different compounds by this route are regulated by the junctional complex at the apical side of the cells, especially at the tight junctions. In parallel, the tight junctions are the maintenance of the apical/basolateral polarity in cell layers (50). Tight junctions appear as dark strands in the area closest to the surrounding cells (Fig. 2). Along the crypt-villus axis it has been found that more strands are present adjacent to the villus cells compared to crypt cells, making the villus region less permeable to water and electrolytes (since the paracellular absorbing area, A_p, decreases). However, the absorption and secretion of water is considered to occur in different regions along the villus axis; it has been suggested that the more mature villus cells are responsible for the absorption despite lower paracellular permeability, whereas crypt cells are the predominant region for the secretory process (37, 56-57, 66).

IV. THE ABSORPTION ROUTES OF FLUID FLUX ACROSS THE INTESTINAL MUCOSA

Water flux across membranes in mammals is a passive process closely related to the transmucosal movement of other compounds. The driving force is probably an osmotic gradient across the intestinal mucosa, and might be a consequence of either active transport mechanisms which thereby induces local osmotic forces and a widening of the tight junctions (8-10, 16-17, 31-33, 37, 39, 66), and/or a countercurrent multiplier of Na^+ between the vascular hairpin loops in the intestinal villi (51). The mechanism(s) underlying fluid flux in the intestinal tract is also influenced by the enteric nervous system and hormones and/or neurotransmitters (52-53). However, the fundamental mechanism(s) of the transmucosal transport of fluid is still unclear, even though it is well-known to occur by either the transcellular and/or paracellular transport. The intercellular space has been reported to be of major importance for the transepithelial flow of water and electrolytes. In animal studies it has been suggested that as much as 85% of the transmucosal electrolyte flow is via these aqueous pathways between the intestinal epithelial cells (8-10, 37, 66). The paracellular route is also assumed to be utilized by oral rehydration solutions in the treatment of dehydration (54, 60). However, other researchgroups have suggested that the transport (mucosal-serosal direction) might occur through specialized narrow channels present in the epithelial cell membrane (31-34). This is further emphasised by Stein, discussing the importance of the transcellular route for water and even urea (31-34, 51). Recently, the molecular structure of such a specialized transport channel have been identified, and therefore supports the transcellular hypothesis (34). These two different hypotheses concerning the absorption route for the quantitative water absorption is important to recognize when these kind of studies are interpreted.

It has been suggested that the upper part of the villus is the region exclusively involved in the fluid absorption (66). Conversely, secretion is thought to occur in the crypt region along the villus axis. The major purpose of water secretion is to provide a suitable aqueous medium for proper digestion in the intestinal lumen. In addition, the secretory process assists in the delivery of secretory IgAs and helps flushing the crypts of infectious agents and noxious stimuli (66).

V. THE EFFECT OF TRANSMUCOSAL FLUID FLUX ON DRUG ABSORPTION IN SITU, IN VITRO AND IN VIVO

Among the first studies that recognized this fluid flux dependent transmucosal transport of drugs, was one published by Oschenfahrt & Winne 1973 and one by Kitazawa et al, 1975 (13, 16). During the last two decades the importance

of this paracellular route for drug delivery have been explored *in vitro* and *in situ*. The limits concerning molecular weight, shape, ionization and the effect of physiological stimulants, such as luminal concentrations of nutrients, osmolality and motility, are currently under investigation (1-31, 37-43, 55-59). However, recently published *in vivo* human data by ourselves and others indicate that the promising results obtained *in vitro* and *in situ* for various hydrophilic compounds might not be valid in quantitative aspects in humans, especially not for drugs with a molecular weight over 200 (26-28).

Two approaches have been used to investigate the mechanisms underlying the paracellular drug transport across the intestinal mucosa: (a) inducing water transport by exposing the apical side of the epithelium with a hypotonic solution, and (b) stimulated paracellular transport by widening of tight junction and increased water absorption as a consequence of the sodium-coupled transport of nutrients.

As earlier mentioned it is of major interest to scale a change in permeability in the experimental model system to an estimate of the fraction absorbed *in vivo* quantitative aspects. In Figure 3 it is clearly demonstrated that the human effective permeability has to increase 25 times for a low permeable compound (P_{eff} = 0.01 * 10^{-4} cm/s) in order to obtain an fraction absorbed of approximately 25%. This value is important to have in mind during further reading.

V.1. In Situ Perfusion of Intestinal Segments in Animals

A correlation between the intestinal absorption of water and drugs (as well other compounds) has been demonstrated by several groups (8-23, 39, 54-60). These reports have, however, presented different hypotheses regarding the underlying mechanisms. For instance, Ochsenfahrt & Winne in 1973 reported increased absorption of urea in parallel with water flux in the upper small intestine, which they suggested to be a consequence of altered blood flow in the subepithelial capillaries as well some interaction with the solutes within the cell membrane (13). Furthermore, later they found that fluid

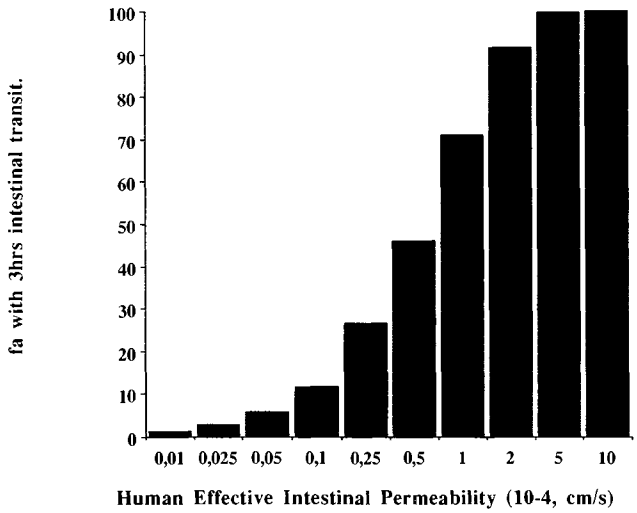

Fig. 3. Estimated fraction absorbed (fa) based on human effective permeability values (from references 25–28), when the duration-time of the intestinal enhancement, intestinal transit time, is 3 hours.

absorption and secretion across the rat jejunal mucosa (perfusion at 0.11 m/min) increased and decreased drug absorption, respectively, of two basic drugs (amidopyrine and antipyrine) and as well two acidic drugs (benzoic acid and salicylic acid) (14-15). The effect of fluid flux was mainly attributed to an interaction at the cell membrane level (transcellular route), which also was supported by the lack of charge selectivity. However, in the same reports they also suggested that small and hydrophilic drug molecules appear to permeate the cell membrane to a higher extent through special hydrophilic areas (14-15). In those animal studies the water flux across the mucosa was obtained by modulating the osmolality of the perfusion solution, which was reported to have no affect on the physiological function(s) of the intestinal epithelium (14-15). Kitazawa et al. reported as early as in 1975 that luminal concentrations of D-glucose (between 150-530 mM) in the small intestine of rats perfused at 5.0 ml/min had an enhancing effect (approximately 100%) on transmucosal absorption of both fluid and cationic drugs (such as metoclopramide, Quinine, ephedrine etc.) (16-17). The glucose effect was present throughout the small intestine, but was most pronouncedly expressed in the proximal region (16-17). These luminal concentrations of D-glucose are, however, much higher than the reported values of 0–30 mM which are considered as physiological relevant (62). The same studies also showed a clear charge selectivity of the transmucosal transport rate during nutrient induction, since the absorption of cation and anionic drugs was increased and decreased, respectively (16-17). The unionised compounds were unaffected by the volume flow across the intestinal mucosa (16-17). A possible explanation for the charge selectivity might be the ion partioning effect, which is due to the overexpressed number of negative charges at the membrane components closest to the paracellular pathway (32). This may lead to an increased concentration of the cationic compounds (C) adjacent to the entrance of the paracellular route, and thereby increased absorption rate ($dM/dt = A * P_{eff} * C$). This suggests that the charge selectivity is not due to an increased permeability for the cations, instead, a more pronounced concentration gradient is the most plausible hypothesis (32). The generality of the effect of transmucosal fluid movement on the charge selective drug transport was verified for 23 different compounds (16-17). Later the same group reported that differences in fluid movement along the gastrointestinal tract in rabbits might contribute to the observed regional intestinal variation in drug absorption (23). In contrast to earlier result they did not find any charge selectivity (acetylsalicylic acid and metoclopramide) in the drug transport as an effect of fluid transport (16-17, 23).

In 1987 a mechanistic hypothesis of this nutrient induced increase of the intestinal permeability was presented by Pappenheimer, Reiss and Madara based on animal experiments (8-9). They suggested that luminal concentration of D-glucose (D-glc) or amino acids (about 25 mM) might trigger an opening of the tight junctions between the epithelial cells at the apical side, and as well as volume flow via the tight junctional pathway due to local difference in osmolality at both sides of the membrane (8-9). The luminal nutrient concentration used in the experiments is representative for the physiologically found values, which are between 0-30 mM on average (62). The study was performed as a perfusion

experiment of 50–80 cm of the small intestine at a flow rate of 3.0 ± 1.0 ml/min. The widening of the paracellular pathway and stimulating effect on the water transport between the epithelial cells resulted in an increased uptake of compounds covering a wide range of molecular weights (MW 200-5500) (8-9).

Based on these findings a more physiological way to increase the intestinal permeability emerged in the pharmaceutical sciences. Several laboratories tested the hypothesis to find a physiological, reversible and non-toxic absorption-enhancing mechanism by using drugs with different physico-chemical properties. Fleisher et al. demonstrated an increased permeability of phenytoin (a highly permeable compound) during perfusion (0.5 ml/min) of rat duodenum and jejunum. The permeability increased when D-glc was present in the lumen (20 and 100 mM), which also stimulated an increased water absorption. In addition, inclusion of 500 μM phlorizin (inhibits carrier-mediated transport of D-glc), resulted in a baseline permeability value of phenytoin (i.e. same as without nutrient induction) (21). Later the same group also found that the rat jejunal permeability increased by 133% and 38% for aceminophen and prednisolone, respectively. They therefore suggested that hydrophilic compounds might be more affected by transmucosal fluid transport than lipophilic compounds (11). See and Bass in 1993 reported that D-glucose must be in contact with the apical intestinal membrane in order to increase the mucosal permeability. They perfused a 10 cm jejunal segment at a perfusion rate of 3.0 ml/min and found that absorption of the small, hydrophilic and passively transported compound L-glucose increased in parallel with water absorption. The extent of absorption increased from approximately 15 ± 1.9% (sd) to 39 ± 2.2% (sd), which was significantly different ($p < 0.05$) compared to controls (22). A Canadian group reported that no absorption of their probes (PEG 400, dextran 4400 and Dextran 17 200) took place during a jejunal perfusion (3.0 ml/min) with no nutrients present. However, luminal concentration of L-alanine or D-glucose at 30 mM resulted in significantly increased permeability of the probe molecules (55). Recently, Ma et al. reported that the rat colon absorbs PEG 400 and water 4 times higher than the small intestine at a perfusion rate of 1 ml/min (56). In addition they also found a higher transmucosal transport of even inulin in colon compared to the rat small intestine. These results were attributed to less back flux, the higher crypt surface area and more extensive water absorption (higher solvent drag effect) in the colon (56). Previously, in a similar publication by the same group, they also found a linear relationship among osmolality, water absorption and permeability of PEG 400. They concluded that PEG 400 is absorbed by diffusion and convection through water pathways, and not across the lipid membrane. During isotonic conditions they reported that 43% of PEG 400 permeation was due to passive diffusion and 57% was explained by convection across the mucosa of the rat small intestine. Furthermore, increasing transmucosal water absorption by decreasing osmolarity resulted in a proportional increase of paracellular permeability due to convection (solvent drag) (20).

Based on these in situ animal perfusion studies of the intestine, it has been reported that absorption of fluid across the intestinal mucosa will lead to increased permeability of a wide range of molecular sizes (MW 60-5500) (8-23, 55-56). Furthermore, it seems likely that the presence of nutrients in the intestinal lumen during perfusion increases the permeability of hydrophilic compounds (8-12, 16-17-21-22, 39, 54-60). Transport through the paracellular route has been supported by the charge selectivity found in some reports (16-17, 57-58). However, all these results should be evaluated with some caution since most studies used a rather high perfusion flow (between 1.0 - 5.0 ml/min) (8-23, 55), which has been reported to distend the intestinal segment and therefore expose the crypt cells and as well increase the available surface area (65). The crypt cells are expected to be more permeable since they are less mature and have not fully developed the tight junction (66). However, even if solvent drag through the paracellular route is the dominating hypothesis, some investigators have proposed other mechanisms such as membrane effects, increased drug concentrations adjacent to the intestinal wall and increased wash-out of drugs in the cytosol/extracellular fluid compartment(s) (13-15, 21, 26-30, 35).

V.2. In Vitro Studies in Cell Culture Models

The relative importance of the trans- and paracellular routes was investigated for β-blockers with different physico-chemical properties in the Caco-2 model. It was shown that the absorption across the cell monolayer was increased for drugs with low effective permeability (atenolol, practolol) when the tight junctions were widened by adding a Ca^{2+} chelating agent (EGTA) at the apical side. However, the absorption of compounds with high permeability (propranolol, alprenolol, metoprolol) was unaffected (7). It was recently demonstrated that apical application of a hypotonic solution in the Caco-2 model increased the transepithelial transport of two large hydrophilic compounds approximately 10-fold, fluorescein-Na and fluorescein-isothiocyanate-labeled dextran (MW 4400), respectively (57). They also demonstrated by confocal laser scanning microscopy that the transport pathway occurred predominately via the paracellular route (3, 57). The Caco-2 model was used to best reflect the villus type cells, and therefore another cell model was applied in order to investigate crypt cells (HT-29.cl19A cell model). However, apical exposure of a hypotonic solution did not increase the permeability of the same compounds in this crypt cell model (3, 57). In a recently published thesis at Uppsala University, it was clearly demonstrated that the passive transmucosal transport of foscarnet, erythritol and creatinine across the intestinal epithelium was charge-selective in the Caco-2 model and excised intestinal segments. The largest relative increase in drug absorption, during influence of water transport, was also seen in epithelia with low baseline permeability of the paracellular route, such as in the large intestine (58).

Noach et al in 1994 concluded that further investigation is needed to determine whether these in vitro data have any relevance for quantitative drug absorption in vivo in humans (3, 57).

V.3. Does Quantitative Intestinal Absorption of Drugs and Nutrients by the Paracellular Route Exist in vivo in Humans?

Historically the importance of the paracellular route for

in vivo absorption in humans has been discussed for hydrophilic drugs. They are usually absorbed during approximately four hours following oral administration in man. Absorption is then ceased which probably occur when the drug passes into the colon (5-6, 43). This conclusion agrees with the general view that the small intestine has a leaky epithelia and colon/rectum has a tighter epithelia in vivo (5-6, 42-43, 67). As earlier discussed, one approach to investigate the importance of paracellular transport in vivo in humans is to study whether small hydrophilic molecules are influenced by the volume flow across the intestinal mucosa or not. If the permeability of the investigated compounds is increased, it is assumed that paracellular transport will increase proportionally with transmucosal water absorption. However, the effect of transmucosal water flux on quantitative absorption of drugs and other compounds is difficult to assess *in vivo* in humans as pointed out by Peeters et al. (59).

The absorption of water and electrolytes from the jejunal lumen increases following the ingestion of a meal (41, 53-54). It has been suggested that this observed postprandial proabsorptive response is mediated by cholinergic, endogenous and not yet elucidated neurotransmitters and hormones (41). A study design using ingestion of a meal in human subjects is difficult to interpretate, since several other factors might contribute significantly to the overall absorption of the test compounds (59). The complexity is clearly demonstrated in a work by Riley et al. in 1992 where the urinary excretion of atenolol and hydrochlortiazide was increased 3 times when a hypoosmotic solution was ingested. However, the orocaecel transit time also increased 3 times making it difficult to conclude that the increased absorption of these two drugs was due to increased transmucosal water absorption (68). Furthermore, an orally taken hypotonic solution in all likelihood adjusts very rapidly to the isotonic conditions in the human duodenum, which means that the potential for solvent drag is probably short-lived. An argument favoring water absorption as an explanation in this particularly study is the fact that the urinary excretion of furosemide and salicylic acid was unchanged. However, since these two compounds were most rapidly absorbed they might not be particularly sensitive to variation in transit time (68). Gramateé et al. reported 1994 a study where an open perfusion technique was used and a 30 cm long segment of the small intestine in humans was perfused at flow rate of 10 ml/min. They found a weak correlation ($r^2 = 0.49$) between net water movement across the intestinal mucosa and the absorption rate of ranitidine (61). Sandle et al. reported that increased water absorption increased the absorption of hydrocortisone ($r^2 = 0.49$) from a 15 cm long jejunal segment in humans during an open perfusion at 15 ml/min. They suggested that the increased bulk flow of water may increase the transport of hydrocortisone towards the intestinal mucosa and thereby increase the drug concentration close to the intestinal wall ($dM/dt = A * P_{eff} * C$) (35).

Human drug permeabilities, measured by a regional perfusion technique between two balloons using a physiological flow rate of 2.0-3.0 ml/min, have been shown to be highly correlated to the fraction absorbed obtained from ordinary pharmacokinetic (PK) studies, when the drug was given as solution or immediate release (26-28, 38, 49, 64, 69). However, perfusions of a jejunal segment in humans with this approach did not lead to an induced net water absorption status at physiologically relevant luminal concentrations (20-40 mM) of D-glucose or L-leucine (Fig. 4) (26-28). Therefore, the hypothesis for induced absorption of water and solute ("solvent drag") by addition of nutrients to the lumen does not seem to be valid in vivo in humans. Similar results were recently published (29), which showed that carrier-mediated D-glc absorption does not increase passive permeability of the human jejunal mucosa to solutes with molecular radii between 2.6 (urea: MW 60) and 4.0 Å (mannitol: MW 182) (29-30). Later, the same group reported an almost identical investigation where luminal D-glc (120 mM) increased the permeability of L-xylose (MW 121, hydrophilic and passively transported) (36). Furthermore, another open perfusion study of the human small intestine at 15 ml/min by Gisolfi et al. in 1992 revealed that luminal D-glc concentrations between 40-180 mM increased water absorption in comparison with saline (60). They also reported that the transmucosal transport of D-glc increased linearly with the glucose levels in the intestinal lumen, which might be attributed to D-glc absorption by other mechanisms than the active glucose-carrier (60). However, these results are opposite to human data obtained by employing the regional perfusion technique. The P_{eff}-value decreased significantly from 12.0 ± 7.9 to 2.2 ± 2.4 * 10^{-4} cm/s ($p < 0.02$), when the D-glc concentration was increased from 10 to 80 mM (26-28).

We obtained net water absorption in human jejunum by using a hypotonic perfusion solution which also contained sodium (135 mM) and D-glc (40-80 mM) at a perfusion rate of 2.0 ml/min (Fig. 4) (26-28). However, no quantitative increased permeability of any of the following compounds were found (Fig. 5): antipyrine ($K_{octanol/water\ pH\ 7.4} = 0.4$, MW 188), atenolol (MW 266, $K_{octanol/water\ pH\ 7.4} = -1.8$), enalaprilat (MW 348, $K_{octanol/water\ pH\ 7.4} = -5.2$) and terbutaline (MW 225, $K_{chloroform/0.01M\ HCl} = 0.01$) (26-28). This clearly demonstrates that a net flux of water in the direction mucosa-serosa does not simultaneously increase the permeability of these slowly transported drugs. The recovery of the non-absorbable volume marker PEG 4000, was complete (95-105%) during human perfusion studies, which imply an intact barrier despite significant water absorption (26-31, 67). All together this strongly indicates that hydro-

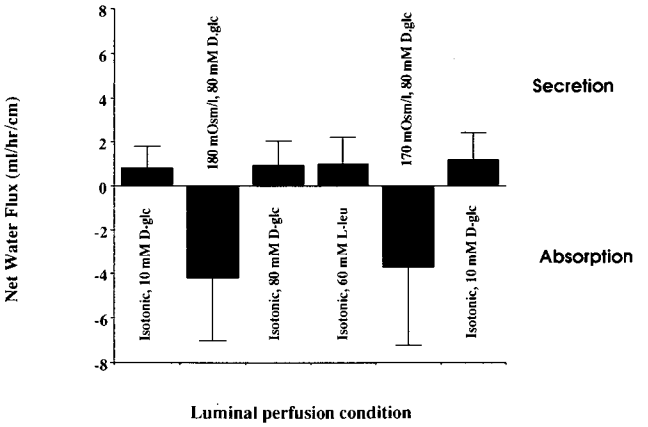

Fig. 4. The possible way to stimulate a net fluid flux across the human proximal jejunum *in vivo;* data given as mean ± SD (from references 26–28).

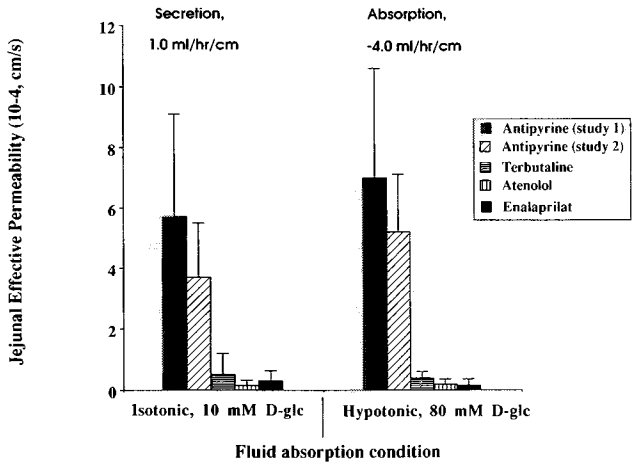

Fig. 5. The effect of net water absorption across the human jejunum on drug permeability on compounds with different physicochemical properties; data given as mean ± SD (from references 26–28).

philic drugs with a molecular weight over 200 Da are probably absorbed only to a minor extent by the paracellular route *in vivo* in humans, but only if we assume that water is absorbed to a great extent by the paracellular route. Additional support has been reported for L-dopa, a hydrophilic compound with a carrier-mediated transport, which exhibited a slightly decreased P_{eff} (from 1.1 to 0.85 * 10^{-4} cm/s, $p < 0.01$) during net water absorption conditions (~180 mOsm/l and L-leucine 50 mM) (25, 27). The result might be due to a higher degree of saturation of the transmucosal transport of L-dopa, since the drug concentration will increase adjacent to the intestinal wall and then approach the Km-value of the LNAA-transporter (Large Neutral Amino Acids) (25, 27). These data provide additional evidence that drug concentration close to the intestinal wall might increases locally as a consequence of increased fluid absorption as suggested for other drugs as well (13-15, 21, 26-30, 35-36). These results are in agreement with a discussion by Soergel, 1993, who suggested that the intestinal mucosa is nearly impermeable to paracellular transport of hexoses (30), which has been demonstrated in the human rectum (67). The fact that the paracellular pathway represents only fractions of a percent of the available intestinal epithelium indicates that uptake via this route can only play a minor role in the overall absorption of solutes in vivo (30). Recently it was suggested that the major absorption route for drugs and other compounds, regardless of physicochemical properties, is the transcellular route (26-28). Further evidence for that hypothesis was found in the Caco-2 model where various analogues of vasopressin were faster transported when the lipophilicity increased following pretreatment of the cell monolayer with palmitoyl lysophosphatidylcholine (63). If paracellular transport had been the only transport route affected by the added lipid compound, this clear relationship had not been possible to establish (63).

VI. CONCLUSIONS

A major conclusion in the present review is that neither fluid nor solute absorption in the human jejunum (*in vivo*) is stimulated by addition of high luminal concentrations of D-glucose or L-leucine (25-30). Therefore, this physiological approach to increase the paracellular diffusion/convective transport of hydrophilic compounds in quantitative amounts might not be feasible for optimising oral drug delivery of low permeability compounds in humans (Figures 3-5). Moreover, based on our own human and others perfusion studies we suggest that hydrophilic drugs with a molecular weight over 200 Da are only absorbed *in vivo* in small quantitative amounts by the paracellular route in humans (26-30). However, such a conclusion is only valid if we assume that water is absorbed to a major extent by the paracellular route (8-10, 37, 66). This might not be the case since water is suggested to be absorbed in specialised transport protein(s) present in the epithelial cell membrane, so called aquaporins (31-34). This emphasise the need to reappraise the physiological role in vivo of the nutrient induced "solvent drag" through the paracellular pathway, and as well to further investigate the general importance of drug transport in quantitative aspects by the paracellular route *in vivo* in man.

The other major issue of this review is the model difference concerning the effect of transmucosal water flux on the effective intestinal permeability of drugs. This is probably related to experimental conditions in the *in situ/in vitro* and *in vivo* perfusion models (8-23, 26-30, 35-37, 39, 55-58). The majority of these in situ results might be evaluated with some precautions as they used a rather high perfusion flow (between 1.0 - 5.0 ml/min), which have been reported to distend the intestinal segment and therefore expose the more permeable crypt cells and as well to increase the available surface area (65). Moreover, these in situ and in vitro models might be more sensitive to changes in the luminal (apical) conditions, and therefore develop tissue damages that might explain some of the deviations from the in vivo situation in man (26-30). Further support for the model discrepancy is found by Nellans which reported that luminal glucose did not stimulate paracellular solute flux in intact rats (in vivo) (1). The difference, between animal and humans results regarding effect of water absorption on drug permeability, can probably also be due to species variation. For instance, the absorption route of fluid across the jejunal epithelium might occur mainly by the paracellular route in rat and by transcellular route in man (aquaporins) (8-10, 37, 66, 31-34).

Finally, the available surface area of the apical membrane for both actively and passively transported drugs is the main diffusion barrier for drugs in vivo (38, 49, 70, 71). The surface area (A) might be variable due to non-specific regulation of the intestinal mucosa by hypertrophy and changes in microvillous and villous dimensions (71). This might be a rapid and dynamic mechanism explained by the villous contractility that have the potential to alter the functional absorptive area and therefore absorption rate ($dM/dt = A * P_{eff} * C$) (49, 71). Based on the hypothesis that the membrane is the rate-limiting barrier regardless of chemical properties, a deeper understanding of the relation between physicochemical properties of the drug-molecule and the *in vivo* transport mechanisms across the intestinal membrane (both passive and carrier-mediated) is required. Together with equivalent knowledge of the mechanisms underlying the presystemic metabolism in the gut lumen, intestinal epithelium and liver, a more rational optimization of the oral drug delivery of drugs will be possible in the discovery process.

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