INTESTINAL DRUG ABSORPTION ENHANCEMENT: AN OVERVIEW

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1. INTRODUCTION

The oral route is the route of first choice for drug administration, and allows the attainment of systemic effects of a large variety of biologically active compounds. However, on oral administration various drugs exhibit relatively low bioavailability. This may be caused by precipitation or binding of the drug in the gastro-intestinal tract, degradation in the gastro-intestinal lumen or during absorption, by poor penetration of the intestinal mucosa or by extensive first-pass metabolism. For instance, the oral bioavailability of insulin is very low because of luminal degradation (Fujii et al., 1985) as well as poor mucosal passage (Aungst et al., 1988). As a consequence, insulin-dependent diabetes can only be treated by daily injections of insulin. Administration of several penicillins, cephalosporins and aminoglycosides also has to be performed by injection because of poor intestinal absorption of the orally delivered drug.

Because of the obvious drawbacks of drug delivery by injection, the development of alternative delivery routes with enhanced bioavailability is receiving much attention in pharmaceutical research. The current development of pharmacologically active compounds with a peptide structure, which exhibit poor bioavailability on oral or rectal administration because of hydrophylic properties, high molecular weight and poor resistance against proteolytic enzymes (Humphrey and Ringrose, 1986), has further stimulated research in this field. In recent studies non-oral administration has received a great deal of attention, e.g. nasal, buccal, rectal, transdermal and ocular delivery, and attempts have been made to promote drug uptake by co-administration of absorption promoting agents (Lee, 1985, 1986). Impressive effects on drug uptake have been obtained in laboratory animals, indicating that this approach may potentially lead towards the development of effective nasal, rectal or oral formulations of poorly absorbed drugs in man.

In the present overview general characteristics of intestinal drug absorption are presented, followed by a discussion of the effects of various absorption promoters on intestinal drug uptake and of the mechanisms which are supposed to be involved.

2. PHYSIOLOGY

The gastro-intestinal tract is composed of four principal layers: mucosal layer, submucosa, muscle layer and serous layer, and it presents common structural characteristics from stomach to anus (Smith, 1986). In man the terminal 15–19 cm part of the large intestine forms the rectum; in rats the terminal 4–5 cm segment is considered as the rectum (Fromm and Hegel, 1984). The luminal surface of the gastro-intestinal tract is covered by a lining of columnar epithelial cells, goblet cells and endocrine cells, the layer being one cell thick. Cells are interconnected by tight junctions. In the small intestine villi and crypts

are present, whereas the large intestine only contains crypts (Fig. 1). The apical surface of the absorptive cells of the entire intestine is provided with a brush-border consisting of microvilli. In man, a transition from columnar to stratified squamous epithelium occurs in the proximal part of the surgical anal canal, which corresponds with the terminal 4–5 cm section of the rectum (Netter, 1973). In rats, the squamous epithelium is keratinized (Iatropoulos, 1986).

The primary function of the small intestine is the uptake of nutrients (electrolytes, fat, monosaccharides, amino acids, di- and tripeptides and vitamins), whereas the major function of the colon comprises water and electrolyte absorption. In the colon of rat and man, absorption of water, sodium and chloride and secretion of potassium and bicarbonate occur; active glucose and amino acid absorption are lacking. In the human rectum sodium and water absorption are negligible (Binder and Sandle, 1987).

3. DRUG ABSORPTION

Principles of drug absorption have been reviewed previously (Aungst and Shen, 1986; Jackson, 1987; Morré, 1985; O'Hagan et al., 1987) and will only be summarized here. Drugs may be absorbed across the epithelial cell (transcellular) or between cells via the tight junctions and intercellular space (paracellular) (Fig. 2). Generally, transcellular drug uptake is mediated by passive diffusion. In the small intestine, drugs with structures similar to those of nutrients may be taken up by facilitated diffusion or by active transport, which both represent carrier-mediated transport systems. There are no indications for carrier-mediated drug transport in the large intestine (Muranishi, 1984).
Endocytosis may contribute to drug absorption by uptake of particles larger than 0.2 \( \mu m \) (phagocytosis) or by uptake of drug-containing luminal fluid (pinocytosis). Macromolecules like polyvinyl pyrrolidone (PVP, MW 40 kDa), bovine serum albumin (MW 60 kDa), IgG (MW 146–165 kDa), horseradish peroxydase (MW 40 kDa) and particles with a diameter up to 2 \( \mu m \) have been suggested to be absorbed by the latter process (Gruber et al., 1987; Beahon and Woodley, 1984; Jeurissen et al., 1985). Pinocytotic uptake may occur at the surface of columnar epithelial cells or by microfold cells ('M'-cells) of Peyer's patch tissue, formed by aggregated lymph nodules in the intestinal submucosa. In rats, pinocytotic activity has been reported to be higher in the small intestine than in the colon (Warshaw et al., 1977) and absent in the rectum (Hollmann, 1965).

Apart from the mechanisms described above, drug uptake may occur by transport of large particles via the paracellular route, through gaps caused by cells sloughed off (persorption). By this process, particles with a diameter up to 150 \( \mu m \) may be taken up (Volkheimer, 1974). Although by means of endocytosis and persorption macromolecules of impressive size may be absorbed, the contribution of these processes to drug absorption is considered to be small.

4. POTENTIAL ABSORPTION BARRIERS

The potential barriers for intestinal drug absorption have been reviewed extensively (Hayton, 1980; Aungst and Shen, 1986; Müller, 1986; Jackson, 1987). These may be located in the unstirred water layer, the mucous layer, the apical and basal cell membrane and cell contents, the tight junction, the basement membrane and the wall of lymph and blood capillaries (Fig. 2).

In addition to these physical barriers, the bioavailability of peptides and proteins is importantly reduced by a metabolic barrier of mucosal peptidases (Adibi and Kim, 1981; Stratford and Lee, 1986; Kashi and Lee, 1986).

4.1. MUCUS

The epithelial cells of the entire intestine are covered by a mucous layer, consisting of water, glycoproteins (mucins), electrolytes, proteins and nucleic acids. The layer is bound
to the apical cell surface by the glycocalyx, a 500 nm thick glycoprotein structure which is covalently linked to lipids and proteins of the brush border membrane. The mucous layer is a part of the unstirred water layer, and it is supposed that the minimal thickness of the unstirred water layer, 100–500 μm, corresponds with the mucous layer (Müller, 1986; Bugaut, 1987; Borgström et al., 1985). The mucous layer probably acts as a buffer, maintaining the epithelial surface at pH = 6.0, thus creating an acidic microclimate. Due to the presence of sialic acid residues and sulfate groups the mucins are negatively charged. Ca²⁺-binding to these sites produces a reduction in viscosity and formation of clumps of mucus (Allen, 1981).

Binding of drugs to mucus, which has been described for phenylbutazone and tetracycline (Barry and Braybrooks, 1975; Braybrooks et al., 1975), cephalosporins, penicillins and aminoglycosides (Nöbuchi et al., 1986), etoposide (Schurgers et al., 1985), ergot-alkaloid derivatives and pindolol (Nimmerfall and Rosenthaler, 1980), may decrease the extent of drug absorption. Because of its aqueous nature, the unstirred water layer is considered to form an absorption limiting barrier for compounds with a high lipid–water partition coefficient, e.g. aromatic hydrocarbons (Rahman et al., 1986) and long-chain fatty acids (Thomson and Dietschy, 1981).

4.2. APICAL CELL MEMBRANE

The apical cell membrane has the shape of a 1 μm thick brush border, and is formed by a 10 nm thick double layer of polar lipid molecules, containing a hydrophobic and a hydrophilic part (Bretscher, 1985; Curatolo, 1987; Thompson and Huang, 1985). The major lipid components are phosphatidylcholine, phosphatidylethanolamine and sphingomyelin (zwitterionic), phosphatidylserine, phosphatidylinositol and phosphatidic acid (anionic), cholesterol and glycolipids. Divalent metal ions may be necessary for the maintenance of membrane structure; Ca²⁺ chelates with negatively charged phospholipids, thus reducing membrane fluidity and permeability (Papp et al., 1985; Schachter and Shinitzky, 1977; Schlieper and Steiner, 1983; Brasitus and Dudeja, 1986; Otero and Carrasco, 1987).

Proteins are embedded in the lipid bilayer by their hydrophobic segments. Because optimal activities of membrane-bound enzymes require fluid-state membranes, cells maintain the membrane transition temperature ($T_m$), the temperature where transition from the stiff gel phase to the fluid liquid-crystalline phase occurs, below environmental temperature. Cholesterol exerts a regulating action on membrane structure, increasing fluidity of gel-state membranes and decreasing fluidity of liquid-crystalline membranes. Sphingomyelin has been suggested to enhance the ordering effect of cholesterol (Van Blitterswijk et al., 1987). Natural fatty acids also influence membrane order, their cis-double bonds disrupting phospholipid arrangement (Stubbs and Smith, 1984). For these reasons, fluidity of fluid-state membranes may increase with decreasing cholesterol/phospholipid molar ratio or increasing total-lipid/protein ratio and double-bond index (Brasitus et al., 1984), thus increasing permeability (McElmahey, 1986). In rat colonocytes lipid fluidity decreases from proximal to distal, transition temperatures amounting to 23–24°C and 26–27°C, respectively, corresponding with a higher enzyme activity in the proximal segment (Brasitus and Dudeja, 1985).

Generally, the transport of molecules across the phospholipid bilayer is correlated with their lipid–water coefficient (Diamond and Wright, 1969). Consequently, the lipid bilayer is absorption limiting for strongly hydrophilic substances, e.g. certain antibiotics and peptides. For this reason the transcellular transport of water, ions and polar solutes (e.g. monosaccharides) requires other mechanisms, e.g. diffusion through pores and carrier-mediated transport (Cooper et al., 1985; Bjarnason et al., 1986a; Bramhall, 1987).

4.3. BASAL CELL MEMBRANE

The basal cell membrane consists of a 9 nm thick phospholipid bilayer, containing proteins. The lipid fluidity of basolateral membranes exceeds apical membrane fluidity
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4.4. Tight Junctions

Tight junctions (zonulae occludentes) are regions of close contact between apical ends of epithelial cells. They are constructed of a meshwork of strands, the tight junction permeability increasing with decreasing strand number, thus determining the 'leakiness' of the epithelium. The small intestine contains leaky epithelium, and intestinal permeability decreases in the distal direction, running parallel with apical cell membrane permeability; the proximal colon is moderately leaky, the distal colon moderately tight (Madara et al., 1980; Luciano et al., 1984). In leaky epithelia the tight junctions are permeable for medium-sized solutes (e.g. disaccharides, $^{56}$Cr-EDTA), ions and water, thus establishing the major route for passive ion permeation (Bentzel and Hainau, 1979; Erlij and Martinez-Palomo, 1978; Bjarnason et al., 1986a). However, even in leaky epithelia water transport is supposed to occur primarily transcellularly (Worman et al., 1986). Tight junctions are cation selective and they have been suggested to be impermeable for cations with a diameter exceeding 0.8 nm or with a molecular weight higher than 350 (Erlij and Martinez-Palomo, 1978; Aungst and Shen, 1986). Alternatively, it is conceivable that a distribution of pore sizes exists, with a large number of small pores and a few large ones (Aungst and Shen, 1986). The tight junctional structure is destabilized by exposition to hypertonic solutions (Erlij and Martinez-Palomo, 1978) and by $Ca^{2+}$ depletion (Gumbiner, 1987; Martinez-Palomo et al., 1980). In hamster small intestine Na-coupled solute transport has been suggested to increase junctional permeability towards small peptides, sugars and amino acids (Madara and Pappenheimer, 1987).

4.5. Basement Membrane

The epithelial cells rest upon the basement membrane, which is 40–60 nm thick in the rat rectum and 120 nm thick in the human rectum (Hollman, 1965). The basement membrane is composed of glycoproteins and proteoglycans and is provided with cationic sites with a diameter of 10–20 nm, repelling plasma proteins (Charonis and Wissig, 1983). In the rat jejunum this structure contains richly distributed fenestrations of 0.5 to 5 μm in diameter (Komuro, 1985). It has not been established to what extent drug absorption may be limited by the basement membrane.

4.6. Capillary Wall

At a distance of 500 nm below the basal cell membrane the blood capillary wall is located. The endothelial cell membrane contains small perforations of 0.4–1 nm radius and the blood capillary wall is fenestrated, fenestrae radius amounting to 20–30 nm. On the other hand, lymphatic capillaries are provided with intercellular junctions of larger size, allowing passage of particles with a radius up to 300 nm. The basement membrane surrounding fenestrated capillaries does not retain tracer particles with a radius smaller than 6 nm (Granger et al., 1987).

Because of the presence of relatively large pores, the intestinal blood and lymph capillaries are not considered to impose an important barrier for drug absorption. However, it is conceivable that strongly hydrophilic drugs will be transported slowly across the capillary wall, compared with hydrophobic compounds, as their absorption site will be limited to the pore area.

5. Absorption Enhancers

In order to lower the physical barrier function of structural elements of the intestinal mucosa towards poorly absorbed drugs, the potentials of co-administration of absorption enhancing agents have been investigated extensively in recent years. In most of such studies antibiotics, peptides and proteins have been used as model compounds. Because of their
TABLE 1. Classes of Enhancers of Intestinal Drug Absorption and some of their Representatives

<table>
<thead>
<tr>
<th>Classes of Enhancers</th>
<th>Representatives</th>
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<tbody>
<tr>
<td>Non-steroidal anti-inflammatory drugs and derivatives</td>
<td>sodium salicylate, sodium 5-methoxysalicylate, indomethacin, diclofenac</td>
</tr>
<tr>
<td>Surfactants</td>
<td>nonionic: polyoxyethylene ethers, anionic: sodium laurylsulfate, cationic: quaternary ammonium compounds</td>
</tr>
<tr>
<td>Bile salts</td>
<td>dihydroxy bile salts: sodium deoxycholate, trihydroxy bile salts: sodium cholate, Sodium tauro-24,25-dihydrofusidate (STDHF)</td>
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<tr>
<td>Medium-chain fatty acids</td>
<td>octanoic acid, decanoic acid</td>
</tr>
<tr>
<td>Medium-chain glycerides</td>
<td>glyceryl-1-monoctanoate, glyceryl-1-mondecanoate</td>
</tr>
<tr>
<td>Enamines</td>
<td>DL-phenylalanine ethylacetacetate enamine</td>
</tr>
<tr>
<td>Mixed micelles</td>
<td>glyceryl monooleate + sodium taurocholate</td>
</tr>
<tr>
<td>Calcium binding agents</td>
<td>EDTA</td>
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<tr>
<td>Phenothiazines</td>
<td>chlorpromazine</td>
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<tr>
<td>Liposomes</td>
<td>Azone</td>
</tr>
<tr>
<td>Fatty acid derivatives of carnitine and peptides</td>
<td>palmitoyl-DL-carnitine, N-myristoyl-L-propyl-L-prolyl-glycinate</td>
</tr>
<tr>
<td>Saponins</td>
<td>Concanavalin A</td>
</tr>
<tr>
<td>Phosphate and phosphonate derivatives</td>
<td>DL-α-glycerophosphate, 3-amino-1-hydroxypropylidene-1,1-diphosphonate (APD)</td>
</tr>
<tr>
<td>Polyacrylic acid</td>
<td>Ethyl maleate (DEM) and diethylethoxymethylene malonate (DEEMM)</td>
</tr>
</tbody>
</table>

hydrophilic properties, ionic charge and high molecular weight, the absorption-limiting barriers of these drugs are likely to be located in the mucous layer, the apical cell membrane and the tight junction. The absorption enhancers used belong to widely differing chemical entities, their absorption promoting properties appearing to be their only common characteristic. For the mere convenience of this discussion, enhancers have been assigned to groups (Table 1). However, this classification does not implicate the existence of fundamental differences in absorption promoting action between such groups. On the contrary, it is likely that the mechanism of action of some groups, e.g. surfactants and saponins, is similar.

Reduction of the metabolic barrier of mucosal peptidases is also supposed to be an important issue with respect to enhancement of peptide and protein bioavailability. It is conceivable that co-administration of peptidase inhibitors may offer interesting possibilities for peptide and protein delivery (Lee, 1988).

The choice of the absorption enhancers to be developed into formulations for human use is not only determined by their effectiveness in laboratory animals. The safety profile of the agent, e.g. the absence of serious damaging effects on the intestinal mucosa, is of primary importance in this respect. Hence, compounds which are already used in man for other indications (NSAIDs, medium-chain glycerides) are to be considered as potentially applicable as absorption promoters in humans. Nevertheless, the evaluation of the applicability of promoters in human pharmacotherapy should comprise a thorough assessment of their safety in animal experiments, including a study of the effects on mucosal structure.
The absorption enhancing properties of non-steroidal anti-inflammatory drugs (NSAIDs) have received much attention, as it is reasonable to hypothesize that many years of experience with oral and rectal NSAID-therapy indicate that these compounds may be applicable as absorption promoters in man.

In rats, 0.06 to 0.36% w/v of indomethacin, diclofenac, and phenylbutazone, and 3.6% w/v of acetyl salicylic acid proved to enhance rectal absorption of sulfanilic acid and of macromolecular drugs like insulin, inulin, PVP and albumin, indomethacin exerting the strongest effects (Nakanishi et al., 1984a; Nakanishi et al., 1986; Yamashita et al., 1985). The enhancing effect of these NSAIDs on drug uptake, however, proved to be accompanied by loss of goblet cells and protein release. Rectal absorption of ampicillin and cephalothin from fatty suppositories has been reported to be promoted by 1.5–10% of indomethacin sodium, diclofenac sodium, mepirazole, phenylbutazone and sodium salicylate in rats, whereas in dogs smaller effects were observed (Yaginuma et al., 1981; Yaginuma et al., 1982). Diclofenac proved to enhance rectal pepsleomyacin uptake in rats (Nishihata et al., 1984g). The effects of diclofenac salts on drug absorption correlated with the extent of histological damage, scored as epithelial cell loss, hyperemia, bleeding, inflammatory cell infiltration and submucosal edema (Yaginuma et al., 1982). Hence, it was concluded that the effect of diclofenac salts on drug absorption is mediated by irritation of the intestinal mucosa.

In the majority of studies on absorption enhancement with NSAIDs sodium salicylate, sodium 5-methoxysalicylate and closely related derivatives have been used. In rats, sodium salicylate in concentrations up to 20% w/v proved to promote rectal absorption of insulin (Caldwell et al., 1984), gentamicin (Fix et al., 1983a,b), [Asu$^{17}$]-eel calcitonin (Miyake et al., 1985), penicillins and cephalosporins (Suzuka et al., 1987b; Nishihata et al., 1982a,b; Nishihata and Higuchi, 1984a; Nishihata et al., 1984a,d; Nishihata et al., 1985d), $^{99m}$Tc-EDTA (Verhaeren et al., 1981), 1-$\beta$-d-arabinofuranosylecitosine (Nishihata et al., 1986a) and dextran (West, 1982). The effect appeared to depend on ionic strength (Nishihata et al., 1982b; Nishihata et al., 1984g; Fix et al., 1983a; Suzuka et al., 1985) and on the presence of lipids (Nishihata et al., 1986a). Also rectal bioavailability of methionyl-human growth hormone was enhanced from 0.2 to 10% by 0.4–4% w/v of sodium salicylate, the effect in mineral oil exceeding the effect in water (Moore et al., 1986a). In that study salicylate induced stronger effects in the large intestine, compared with the small intestine, indicating that this enhancer may be preferentially suitable for rectal absorption enhancement. In rats, the effect of salicylate on rectal cefoxitin absorption proved to be dependent on delivery rate; bolus delivery exhibited an absorption promoting effect, whereas rectal infusion proved to be ineffective (Van Hoogdalem et al., 1988e). Depending on the ionic strength used, sodium salicylate in absorption enhancing concentrations may cause epithelial cell loss (Nishihata et al., 1984a). The authors suggested that facilitated salicylate absorption by sodium ions may be involved in this phenomenon.

Compared with the action in rats, absorption promotion by salicylate appears to be less pronounced in larger mammals, e.g. dogs (Nishihata et al., 1984d; Yaginuma et al., 1981) and in man (Davis et al., 1985). In the latter study 2.3% w/w of polyoxyethylene-23-lauryl ether was co-administered with 4.5 to 15% w/w of sodium salicylate, presumably to potentiate the effect of salicylate (Fig. 3). Rectal cefoxitin bioavailability was increased from 3% to maximally 20%. In another study in man, the enhancing effect of salicylate on rectal cefoxitin absorption proved to be delivery-rate dependent, rectal infusion with 20% w/v of sodium salicylate increasing the mean cefoxitin bioavailability from 5.0 to 9.2%, whereas bolus delivery did not result in a statistically significant effect (Van Hoogdalem et al., 1988f). In this study the enhancer was well tolerated by the volunteers, as judged by scoring of the side effects on visual analog scales. In rabbits, sodium salicylate concentrations of 20–40% w/v were employed to enhance rectal ampicillin absorption (Nishihata et al., 1984e).
Sodium 5-methoxysalicylate is the most extensively studied absorption promoting salicylate derivative. In rats, rectal bioavailability of arginine vasopressin and 1-deamino-8-D-arginine vasopressin (DDAVP) was variably enhanced by 0.7–3% w/v of sodium 5-methoxysalicylate (Saffran et al., 1988). In the same species, 5% w/v of this agent enhanced rectal absorption of pentagastrin from 6 to 33% and gastrin absorption from negligible to 18%, estimated by pharmacological effect (Yoshioka et al., 1982). Rectal bioavailability of cefmetazole was enhanced by sodium 5-methoxysalicylate (0.7–3.5% w/v) to maximally 77% (Nishihata et al., 1982a). In dogs, the effect of sodium 5-methoxysalicylate (15–30% w/w) on rectal absorption of β-lactam antibiotics and gentamicin was superior to the action of salicylate (Nishihata et al., 1984d). In Fig. 4 the effect of 5-methoxysalicylate on rectal cefmetazole absorption is shown. Although sodium 5-methoxysalicylate was reported to enhance lymphatic cefoxitin uptake, enhancement of lymphatic absorption appears to play a minor role in total drug absorption on delivery.
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with enhancer, considering the low lymphatic recoveries of drug (Nishihata et al., 1985a; Nishihata et al., 1984g). Sodium 5-methoxysalicylate (5-15% w/v) was reported not to exert any damaging effects on rectal epithelial integrity (Caldwell et al., 1984). However, a solution of 12% w/v of this enhancer introduced into the small intestine of rats proved to cause extensive disruption of the epithelial cell layer (Peters et al., 1987).

Other salicylate derivatives with absorption promoting properties are epinephrine metabolites (Nishihata et al., 1984f), 3-methoxysalicylate, dihydroxybenzoates and homovanillic acid (Nishihata et al., 1982a) and sodium 5-bromosalicylate (Fix et al., 1983b).

Enhancement of intestinal insulin absorption by salicylates has been the objective of several studies. In rats, insulin bioavailability was increased from 2% to maximally 10% by 5-10% w/v of sodium 5-methoxysalicylate (Nishihata et al., 1981b), when administered into the small intestine, whereas Peters et al. (1987) reported a less pronounced effect of 12% w/v of this enhancer. Rectal insulin absorption was enhanced by 2-5% w/v of sodium salicylate in rats (Nishihata et al., 1981a; Nishihata et al., 1983c; Aungst and Rogers, 1988).

In larger species, considerably higher adjuvant concentrations are necessary to elicit a promoting effect on insulin absorption; in dogs 30% of sodium 5-methoxysalicylate or 30-66% of sodium salicylate promoted rectal insulin bioavailability to 25%, relative to i.m. delivery (Nishihata et al., 1983d; Nishihata et al., 1987b; Liversidge et al., 1986). In man, rectal insulin absorption from suppositories containing 2 U/kg of insulin was enhanced by 30% w/w of sodium salicylate, resulting in a hypoglycemic response (Nishihata et al., 1986d). However, for lack of i.v. data a quantitative interpretation of the insulin bioavailability could not be obtained. In rats the absorption promoting effect of salicylate, estimated by hypoglycemic response, was more pronounced for the rectal route, compared with the nasal and buccal route (Aungst and Rogers, 1988). This site-dependency was explained in terms of differences in susceptibility of the various membranes for the absorption enhancer.

Generally, the effect of salicylates on intestinal insulin absorption increases if spreading of the delivered preparation over the mucosa is reduced (Liversidge et al., 1986; Nishihata et al., 1981b; Nishihata et al., 1983d), which could be explained by a larger amount of enhancer available per unit of mucosal area. The same mechanism probably underlies the observation that 20-40% w/v of sodium salicylate is more effective in enhancing rectal ampicillin absorption in rabbits on delivery in suppositories than in microenemas (Nishihata et al., 1984e).

Various mechanisms have been postulated for the absorption enhancing action of NSAIDs. Sodium salicylate strongly reduced the length of the glycocalyx in the rectum, which possibly reduced the barrier function of the mucus layer (Sithigorngul et al., 1983). On the other hand, salicylate proved to increase lipid bilayer fluidity, either by interacting with phospholipid head groups or with membrane proteins, causing membrane perturbation of small intestinal epithelial cells (Kajii et al., 1985; Kajii et al., 1986a; Casal et al., 1987), which could result in enhanced transcellular transport. As chemical modification of membrane proteins reduced the absorption promoting effect of salicylate and indomethacin, it was speculated that this effect is mediated by an interaction with membrane proteins (Nakanishi et al., 1984b; Nishihata and Higuchi, 1984a; Nishihata et al., 1985d; Nishihata et al., 1987a). However, it cannot be excluded that reversal of the promoting effect of NSAIDs in these studies is caused by a reduction of membrane fluidity as a consequence of protein modification.

The absorption enhancing action of sodium salicylate was reported to be associated with reduced glutathione levels of the mucosal tissue, the mucosal barrier and non-protein thiol levels being restored by cysteamine suppletion (Nishihata et al., 1985c; Nishihata et al., 1986c). Although enhanced permeability and the reduced glutathione levels may be phenomena which occur in parallel or as successive processes, the authors suggested that reduced cellular non-protein thiol levels evoke an increase of membrane permeability and transcellular absorption, for instance by lipid peroxidation (Gibson et al., 1985).

Enhancement of paracellular transport of $^{31}$Cr-EDTA was observed to correlate with cyclooxygenase inhibition after oral NSAID therapy (Bjarnason et al., 1986b; Auer et al.,...
1987). On the other hand, sodium salicylate, which is not an inhibitor of cyclooxygenase, is also capable of enhancing colonic absorption of 99mTc-EDTA, suggesting that other mechanisms than cyclooxygenase inhibition are involved as well (Verhaeren et al., 1981).

In several studies co-administration of Ca$^{2+}$ was shown to abolish the absorption enhancing action of sodium salicylate (Nishihata et al., 1984f) and of various other enhancers. This was considered to be an important indication that absorption enhancement may be mediated by binding of mucosal Ca$^{2+}$ by the enhancer. On the other hand, the reversal of absorption enhancement by Ca$^{2+}$ may also be brought about by simple chemical inactivation of the enhancer by Ca$^{2+}$ or by precipitation of mucins by Ca$^{2+}$. In this light, Ca$^{2+}$ binding by the promoter may not necessarily be involved in the absorption enhancing mechanism.

A different action of salicylate, possibly contributing to enhancement of drug uptake, has been proposed by Touitou and Fisher (1986) and Touitou et al. (1987). In these studies it was reported that salicylate may reduce self-association of insulin and methylene blue molecules, possibly resulting in promoted absorption of the drug as free monomer.

The studies discussed above clearly indicate that sodium salicylate is capable of enhancing uptake of poorly absorbed drugs. For the development of a drug formulation with enhanced bioavailability in man, a concentration of 20% w/v may be used (Van Hoogdalem et al., 1988f). As discussed, the rate of delivery influences the salicylate effect, but this is possibly also dependent on the properties of the poorly absorbed drug. Consequently, concentration of enhancer and delivery rate should be optimized for each drug individually. Literature data indicate the occurrence of adverse effects of NSAIDs on the intestinal mucosa. Hence, an unambiguous assessment of the effects of salicylates on mucosal integrity is still required.

5.2. SURFACTANTS

Surfactants are widely used as additives in the preparation of pharmaceuticals. The effects of surfactants on drug absorption have been reviewed previously (Florence, 1981; Gibaldi and Feldman, 1970), and Möller et al. (1986) recently discussed the effects of detergents on membrane structure.

The enhancement of intestinal absorption of heparin and related glycosaminoglycan sulfates has been the objective of several experiments; in those studies 0.5% of sulfated and sulfonated surfactants (Engel and Riggi, 1969), 80% w/v of polyethylene glycol 1000 monocetyl ether with 20% w/v of polyethylene glycol 400 (Kidron et al., 1979; Touitou et al., 1978) and 30% of sodium laurylsarcosinate (Stanzani et al., 1981) were shown to increase drug absorption. Polyoxyethylene ethers were reported to enhance gastric or rectal absorption of lincomycin (Brookes and Marshall, 1981), penicillins and cephalosporins (Davis et al., 1970), sulfonamides (Kaneda et al., 1974), sulfanilic acid (Nakanishi et al., 1983a), [Asu$^{13}$]-eel calcitonin (Morimoto et al., 1985), Gly$^{1-18}$ ACTH amide (Hirata et al., 1978), fosfomycin (Ishizawa et al., 1987) and ergot alkaloids (Urbančič Smerkolj et al., 1987) in concentrations of 0.1 to 10%, in rats or rabbits (Fig. 5). In rats the combination of polyethylene glycol 1000 monocetyl ether with polyethylene glycol 400 enhanced rectal phenol red bioavailability to 77%, rectal insulin bioavailability to 14% and oral gentamicin bioavailability from 0 to 25% (Touitou et al., 1978; Rubinstein et al., 1981). In rats colonic absorption of interferon-α was increased from 3 to 8% relative to s.c. injection by a mixture of polyoxyethylene esters of oleic acid and oleic acid glycerides (Bocci et al., 1986). In the same species rectal LH–RH bioavailability was considerably increased by polyoxyethylene-9-lauryl ether from 6 to 38%, as estimated by ovulation-inducing activity (Yamazaki, 1984). Polyoxyethylene ethers have also been used in order to potentiate the effects of other enhancers, e.g. the effects of salicylate on rectal cefoxitin absorption in man (Davis et al., 1985) and the effect of medium chain glycerides on rectal cephalosporin absorption in dogs (Sekine et al., 1985b). Using suppositories, the effect of polyoxyethylene ethers proved to depend on the hydrophobicity of the suppository base (Hirata et al., 1978).
Various studies focused on the intestinal absorption enhancement of insulin. In rabbits, rectal insulin absorption was promoted by 3% of anionic surfactants, e.g. sodium laurylsulfate and sodium N-lauroyl-L-glutamate or cationic surfactants, e.g. polyoxyethylene-5-oleyl amine (Ichikawa et al., 1980). Jejunal bioavailability was promoted from 0.4% to 3 and 5%, relative to i.m. delivery, by 0.8% of sodium lauryl sulfate in water and in a W/O/W emulsion, respectively (Shichiri et al., 1978a).

In the majority of studies with insulin, however, polyoxyethylene ethers have been used. In man, oral application of polyoxyethylene-20-oleyl ether resulted in poor and variable effects on insulin absorption (Galloway and Root, 1972). In rats, polyoxyethylene-20-cetyl ether in a concentration of 0.1% w/v enhanced rectal insulin bioavailability to 3% (Bar-On et al., 1981), whereas a considerably higher concentration of 18% resulted in a jejunal bioavailability of 11% (Touitou et al., 1980). These figures were estimated by hypoglycemic response (Fig. 6). In rats and rabbits various polyoxyethylene ethers enhanced rectal absorption of insulin, with polyoxyethylene-9-lauryl ether demonstrating the strongest effects (Ichikawa et al., 1980; Touitou and Donbrov, 1983). Using this adjuvant in concentrations of 2-4% in dogs, rectal insulin bioavailabilities of 7-19% were reached,
relative to i.m. administration (Shichiri et al., 1978b; Yagi et al., 1983), whereas in rats 5% of the enhancer resulted in a relative efficacy of 32% (Aungst and Rogers, 1988).

Surfactants may exert their effects on drug absorption by modification of mucosal permeability or interaction with the drug (Florence, 1981). Because the surfactant monomer is the permeability enhancing entity, surfactant concentrations below the critical micelle concentration (CMC) may enhance intestinal drug uptake. On the other hand, concentrations exceeding the CMC may reduce drug bioavailability, because drug in micellar phase is unavailable for absorption. These opposing effects of surfactants have been demonstrated in several studies (Kaneda et al., 1974; Whitmore et al., 1979; Morimoto et al., 1985; Plá-Delfina et al., 1987).

The promoting effect of surfactant monomers on intestinal drug absorption may be mediated by reduction of the resistance of the unstirred water layer (Plá-Delfina et al., 1987) or of the mucus barrier (Whitmore et al., 1979; Martin et al., 1978; Sakai et al., 1986). Furthermore, increased permeability of the paracellular route may be involved, as described for sodium laurylsulfate (Kakemi et al., 1969). With regard to the transcellular route, interaction of surfactant with membrane proteins as well as membrane lipids appears to be involved (Engel and Riggi, 1969; Möller et al., 1986; Morrow and Anderson, 1986), resulting in increased lipid acyl chain mobility.

The effect of surfactants on drug uptake is not correlated with the value of the hydrophilic–lipophilic balance (HLB) or lowering of the surface tension; on the other hand, correlations with hemolytic effects or Ca2+-binding properties have been observed (Davis et al., 1970; Walters et al., 1982; Sakai et al., 1986). Optimal effects were reached using surfactants with a C12 hydrocarbon chain, probably corresponding with optimal size and shape (Florence et al., 1978; Florence, 1981; Walters et al., 1981; Walters et al., 1982; Ichikawa et al., 1980).

In general, the non-ionic surfactants of the polyoxyethylene-type exert benign effects on membrane structure, in comparison with cationic surfactants (tertiary and quaternary ammonium salts) and anionic surfactants, e.g. sodium laurylsulfate (Siegel and Gordon, 1985; Möller et al., 1986; Kakemi et al., 1969; Nakamura et al., 1985). The latter compound caused reversible epithelial cell loss and persistent loss of goblet cells after rectal delivery in a concentration of 0.1% w/v (Nakanishi et al., 1983b). Within the group of polyoxyethylene-derivatives, the ethers proved to induce stronger absorption enhancing effects than fatty acid esters and sorbitan derivatives (Touitou and Dombror, 1983; Sakai et al., 1986; Miyamoto et al., 1983; Ichikawa et al., 1980).

The effects of surfactants on drug absorption appeared to be correlated with the occurrence of adverse effects on mucosal integrity. This has been described in terms of epithelial cell loss (Walters et al., 1981; Yamasaki et al., 1981b), release of proteins and lipids (Whitmore et al., 1979; Sakai et al., 1986; Nishihata et al., 1985d) and unspecified mucosal damage (Siegel and Gordon, 1985; Nakanishi et al., 1983a). These observations probably explain the relatively scarce application of surfactants as absorption enhancers as published in recent literature and the predominant attention to polyoxyethylene-derivatives. However, polyoxyethylene-9-lauryl ether in a concentration range of 3–5% w/w induced epithelial cell loss of the rectal mucosa (Yamasaki et al., 1981b). These damaging effects, together with abdominal discomfort and defecation urge reported in human subjects (Yamasaki et al., 1981a), discourage the incorporation of this type of absorption promoting surfactant into formulations for long-term treatment.

5.3. BILE SALTS

Bile, which contains glycine- and taurine-conjugates of cholic acid and chenodeoxycholic acid, emulsifies dietary fat and facilitates lipolysis and transport of lipid products through the unstirred water layer of the intestinal mucosa by micellar solubilization. Bile salts which escape from active reabsorption in the ileum are metabolized by the bacterial flora to the secondary bile salts deoxycholic acid and lithocholic acid (Borgström et al., 1985; Breuer and Goebell, 1985). Hydrophilicity of bile salts decreases in the order taurine conjugates > glycine conjugates > free bile salts, polarity increasing with the number of hydroxyl
Intestinal drug absorption enhancement

Bile salts are able to bind calcium, their binding properties decreasing with increasing hydrophilicity (Carey, 1985).

The natural occurrence of bile salts in the intestinal tract has triggered various studies to investigate the applicability of bile salts as potentially safe absorption promoters. In rats, bile salts proved to enhance intestinal drug absorption in a concentration range of 0.02 to 2% w/v. These absorption enhancing properties were assessed for hexamethonium chloride (Bhuta et al., 1980), phenol red, insulin and polyvinyl pyrrolidone (Ecknauer et al., 1983), urogastrone (Hori et al., 1977), methionyl-human growth hormone (Moore et al., 1986b), fosfomycin (Ishizawa et al., 1987) and sulfaguanidine (Kakemi et al., 1970) in small intestinal loops, for cefmetazole in the colonic loop (Shiga et al., 1987), for ampicillin in the rectal loop (Murakami et al., 1984) and for horseradish peroxidase (Fagundes Neto et al., 1981) and sulfanilic acid (Kimura et al., 1972) in the perfused small intestine (Fig. 5). Relatively high concentrations of sodium deoxycholate (4–5% w/v) proved to enhance absorption of orally administered phenol red to 9% (Feldman et al., 1970) and also of heparin (Guarini and Ferrari, 1985) in rats. Rectal absorption of albumin (Nakanishi et al., 1986), interferon-α (Bocci et al., 1985) and heparin (Ziv et al., 1983) was promoted by bile salts in rats; however, in these studies reported bioavailabilities did not exceed 5%.

Much attention has been focussed on insulin. In the majority of studies, the hypoglycemic response has been used to estimate the extent of insulin absorption. As for an accurate assessment a dose–response profile is required (Aungst et al., 1988); measurement of blood insulin offers a more direct approach. In rabbits, rectal insulin absorption from suppositories was promoted by 2% of conjugated or unconjugated sodium cholate (Ichikawa et al., 1980), and jejunal bioavailability was enhanced from 0.4 to 3%, relative to i.m. administration, by 3% of sodium taurocholate (Shichiri et al., 1978a). In rats, 5% w/v of sodium glycocholate increased the relative bioavailability of rectally delivered insulin from 3 to 40%, as estimated by hypoglycemic response, using i.m. injection as a reference (Aungst et al., 1988). Sodium deoxycholate (0.1–1%) was reported to enhance ileal and rectal insulin absorption; higher concentrations reduced the effect, probably by inclusion of insulin into micelles (Kidron et al., 1982; Ziv et al., 1981). Sodium cholate (2% w/v) proved to enhance rectal insulin uptake in rats (Ziv et al., 1981) and in humans, resulting in a strong hypoglycemic response, as is illustrated in Fig. 7 (Raz et al., 1984).

![Fig. 7. Effect of cholate on rectal insulin absorption in man. Hypoglycemic effect (●) and insulin serum levels (○) after administration of 150 U of insulin in a microenema of 2.5 ml with (A) and without (B) sodium cholate (2% w/v) in non-insulin dependent diabetic volunteers, the arrow indicating time of administration (redrawn from Raz et al., 1984).](image-url)
Co-administration of enzyme inhibitors potentiated the effect of bile salts on ileal insulin uptake, presumably by inhibition of proteolysis (Kidron et al., 1982; Ziv et al., 1987).

The absorption enhancing potency of bile salts increased with increasing lipophilicity, which may be explained by increasing affinity for the lipoidal bilayer (Fagundes Neto et al., 1981; Guarini and Ferrari, 1984; Hori et al., 1977). Murakami et al. (1984) reported a good correlation with hemolytic effects. However, mucosa damaging properties appear to correlate with the effect on drug absorption, and increase with increasing lipophilicity as well. This was observed for loss of epithelial cells and goblet cells (Bhuta et al., 1980; Gaginella et al., 1977; Teem and Phillips, 1972), release of mucosal lipids and proteins (Feldman et al., 1973; Graham and Northfield, 1987) and damage of microvilli (Karlqvist et al., 1986; Saunders et al., 1975). Relatively low concentrations of some bile salts, e.g. 0.2% of sodium deoxycholate, may seriously affect mucosal structure (Gaginella et al., 1977; Nadai et al., 1975; Nakanishi et al., 1983b), whereas comparable concentrations of sodium taurocholate or sodium cholate did not affect small intestinal mucosal structure (Miyamoto et al., 1983). On the other hand, light and electron microscopic studies did not reveal any epithelial damage on enhancement of ileal insulin absorption by 1% w/v of sodium cholate (Ziv et al., 1987).

No unambiguous data are available on the mechanism of absorption enhancement by bile salts. It may be brought about by effects on the mucous layer and on paracellular and transcellular absorption routes. They have been reported to affect the intestinal glyocalyx structure (Guarini et al., 1986; Rafter et al., 1986) and to deplete gastric and intestinal mucus (Slomiany et al., 1984; Martin et al., 1978; Whitmore et al., 1979). A transcellular absorption enhancing effect is suggested by the phospholipid disordering action of unconjugated and conjugated bile salts (Walde et al., 1987; Schubert et al., 1986; Carey, 1985; O’Conner et al., 1985); both membrane proteins and phospholipids may be solubilized by bile salts (Moller et al., 1986; Billington and Coleman, 1978; Feldman et al., 1973). Colonic tight junction structure seems to be influenced by relatively low bile salt concentrations (5 mM and lower) in rabbits (Freel et al., 1983) and rats (Rafter et al., 1986). This paracellular absorption promoting effect is suggested to be mediated by binding of Ca^{2+} (Hunt, 1983).

Although bile salts have been demonstrated to enhance drug uptake to a significant extent, applicability of these compounds as safe absorption promoters in man is not without problems, because mucosal damage seems to be correlated with their effect on drug uptake. On the other hand, 2 year therapy with oral chenodeoxycholic acid (350-750 mg/day) for dissolution of gallstones was associated with mild side effects (increase of serum levels of aminotransferase and cholesterol, diarrhea) (Schoenfield and Lachim, 1981). This observation indicates that long-term therapy with bile salt containing formulations may be feasible in man. However, the suggested co-carcinogenic and co-mutagenic effects of secondary bile salts (Breuer and Goebell, 1985; Rainey et al., 1986) discourage the development of bile salt containing pharmaceutical formulations.

### 5.4. Sodium Tauro-24,25-Dihydrofusidate

The potential toxicity of bile salts has led to the development of the bile salt derivative sodium tauro-24,25-dihydrofusidate (STDHF). In a concentration range of 0.15 to 2% w/v this agent proved to enhance nasal insulin absorption to maximally 16% in sheep (Longenecker et al., 1987). In man a nasal insulin bioavailability of 11% was reached on co-administration with 1% of STDHF (Moses, 1988). No damaging effects on nasal mucosal integrity were observed on acute and subchronic exposure in rats and dogs (Moses, 1988). In rats STDHF (0.15-8% w/v) proved to enhance the rectal bioavailability of cefoxitin, desglycinamide arginine vasopressin (DGAVP) and insulin from negligible to maximally 114, 47 and 7%, respectively (Van Hoogdalem et al., in preparation). The effect of STDHF on rectal DGAVP bioavailability proved to be delivery-rate dependent, rectal infusion resulting in a significantly higher mean bioavailability (47 ± 12%), compared with bolus delivery (27 ± 6%) (Fig. 8). This delivery-rate dependence may be based on a more extensive degradation of DGAVP by mucosal peptidases on bolus delivery.
Intestinal drug absorption enhancement

Fig. 8. Rate-controlled rectal absorption enhancement of desglycinamide arginine vasopressin (DGAVP) by sodium tauro-24,25-dihydrofusidate (STDHF). Histogram of the mean AUC(0-inf.) ± SD of DGAVP after i.v. infusion and after rectal administration of 30 µg of DGAVP as infusion (open bars) and as bolus (hatched bars) with various concentrations of STDHF in rats; *: significantly different from delivery without enhancer (p < 0.05, Wilcoxon rank sum test); **: significantly different from bolus (p < 0.05, Wilcoxon rank sum test) (Van Hoogdalem et al., in preparation).

No unambiguous data are available on the mechanisms involved in the absorption promoting action of STDHF. Longenecker et al. (1987) proposed that the formation of STDHF-insulin mixed micelles may be relevant for the effect on insulin uptake. On the other hand, the structural relationship with bile salts suggests that the absorption promoting mechanism of STDHF could be comparable with the action of bile salts.

Considering the effective nasal and rectal absorption promoting action of STDHF and the absence of nasal toxicity, STDHF is a promising enhancer of intestinal drug absorption. Further studies are necessary to provide further insight into the effectiveness and safety aspects of this enhancer and of its mechanism of action.

5.5. MEDIUM-CHAIN FATTY ACIDS

The natural occurrence of medium-chain fatty acids (carboxylic acids with a chain length varying from 6 to 12 carbon atoms) in food products has stimulated research concerning the applicability of these compounds as absorption promoters. In an elaborate study Nishimura et al. (1985) demonstrated the enhancing effects of sodium salts of medium-chain fatty acids, N-acyl-L-phenylalanine derivatives, p-substituted benzoic acid derivatives, α-bromo fatty acids and N-acyl-N-methylglycine derivatives on rectal ampicillin absorption from suppositories in rats. In a concentration of 5%, sodium decanoate proved to be the most effective medium-chain fatty acid in this regard (Fig. 9). This agent promoted the uptake of other penicillins and of cephalosporins as well. Rectal absorption of phenol red and sodium p-aminobenzoate was reported to be promoted by octanoic acid and decanoic acid (Yata et al., 1983), and 1% of C8 to C12 fatty acids appeared to enhance fosfomycin sodium absorption from rat jejunum and colon (Ishizawa et al., 1987) and cefmetazole absorption from rat colon (Tomita et al., 1988). Oral insulin bioavailability, estimated by hypoglycemic effect, was increased to 9–13%, relative to i.m. administration, by delivery in 20% w/v of a mixture of sodium dodecanoate and cetyl alcohol in gelatin capsules (Toutou and Rubinstein, 1986). In rats, bioavailability of rectally infused cefoxitin and ampicillin proved to be considerably improved by sodium salts of medium-chain fatty acids (Van Hoogdalem et al., 1988a; Van Hoogdalem et al., 1988c). In man, 10% w/v of octanoic acid as sodium salt increased rectal cefoxitin bioavailability from 7 to 17% on bolus delivery, rectal infusion exerting a less pronounced effect (Van Hoogdalem et al., 1988f). This enhancer proved to be well tolerated.

The effect of medium-chain fatty acids on drug absorption was not found to be correlated with the fatty acid chain length. Transdermal drug absorption studies (Aungst
et al., 1986) as well as intestinal absorption experiments (Ishizawa et al., 1987; Nishimura et al., 1985; Palin et al., 1986; Van Hoogdalem et al., 1988c) indicated an optimum in the length of the acyl chain between C8 and C12. The occurrence of an optimal chain length may result from increasing intrinsic absorption enhancing properties and decreasing aqueous solubility of fatty acids with increasing chain length (Van Hoogdalem et al., 1988c). On the other hand, this optimal range may also reflect a configuration which is favorable for bilayer destabilization, as has been suggested for surfactants containing a C12 hydrocarbon chain (Florence, 1981).

As medium-chain fatty acids have surface tension lowering and Ca$^{2+}$-binding properties, the mechanisms of their absorption promoting effects may be related to those of other anionic surfactants and bile salts. Effects on mucous layer and on transcellular and paracellular transport may be involved. Medium-chain fatty acids proved to disorder phospholipid bilayers (Mizuno et al., 1985; Inoue et al., 1988) and synaptosomal membranes (Perlman and Goldstein, 1984), probably by perturbation of membrane lipids and proteins (Kajii et al., 1986b; Muranushi et al., 1981). They were also shown to induce membrane fusion (Ahkong et al., 1973; Maggio and Lucy, 1976). The Ca$^{2+}$-binding properties of medium-chain fatty acids may affect tight junction permeability (Hunt, 1983; Mishima et al., 1987).

As has also been mentioned previously for other surfactants and for bile salts, the absorption mediating effects of medium chain fatty acids also may be related to damage of the intestinal mucosa. Relatively low concentrations of sodium decanoate and sodium hexanoate (0.04 M) proved to cause focal inflammatory reactions in the perfused dog colon (Pihl et al., 1966), and comparable effects were reported for dodecanoic acid in the mouse colon (Wargovich et al., 1984). These effects on mucosal integrity need further research in order to evaluate the applicability of medium-chain fatty acids in man.

5.6. MEDIUM-CHAIN GLYCERIDES

As is the case with medium-chain fatty acids, medium-chain glycerides are present in natural food stuffs and are therefore investigated for their drug absorption enhancing properties. The medium-chain glyceride preparations used in such studies, e.g. fractionated coconut oil, mainly contain triglycerides of medium-chain saturated fatty acids. On the other hand, a number of studies have been performed using MGK, a preparation containing glyceryl-l-monoocctanoate as the predominant component (55–57% w/v), along
with glyceryl-1,2-dioctanoate (9% w/v), glyceryl-1,3-dioctanoate (20% w/v), glyceryl trioctanoate (3% w/v), octanoic acid (3% w/v) and glycerol (8% w/v).

In rats, oral cefoxitin bioavailability was reported to be promoted from 4 to 12% by 10% v/v of fractionated coconut oil (Palin et al., 1986). In the same species, oral cefotaxime bioavailability was augmented from 10 to 38% on delivery in fractionated coconut oil. On oral administration of cefotaxime in fractionated coconut oil in man, the effect of the enhancer was less pronounced. Urinary excretion data indicated a bioavailability of maximally 10% (Ueda et al., 1983). Beskid et al. (1988) demonstrated an enhancing effect of glyceryl-1-monooctanoate on ceftriaxone absorption after oral, intraduodenal and rectal delivery in various animal species. In a series of studies, Sekine et al. demonstrated the strong effects of MGK on rectal absorption of cephalosporins in rats and rabbits (Fig. 10), whereas smaller effects were observed in the dog (1984a,b; 1985b). Higaki et al. reported moderate enhancing effects of MGK with HCO60, a polyoxyethylated hydrogenated castor oil derivative, on small intestinal absorption of phenol red (Higaki et al., 1987) and bromothymol blue (Higaki et al., 1986), but the influence on the absorption of the latter dye proved to be disappointing. The effect of MGK on rectal cefazolin absorption in rats appeared to be delivery-rate dependent, rectal infusion resulting in higher bioavailabilities as compared with bolus delivery (Van Hoogdalem et al., 1988d).

As medium-chain glycerides increased the permeability of liposomes (Higaki et al., 1988), a transcellular absorption promoting effect is conceivable. The monoglyceride component of MGK, glyceryl-1-monooctanoate, has been indicated to be the major factor determining the effect of MGK on drug absorption (Van Hoogdalem et al., 1988b). This component efficiently dissolves cholesterol (Leuschner and Baumgärtel, 1982; Mulligan and Corrigan, 1986), suggesting a transcellular absorption enhancing effect by membrane destabilization as a consequence of extraction of cholesterol out of the mucosal membrane. Both glyceryl-1-monooctanoate and glyceryl-1-monodecanoate proved to enhance rectal cefoxitin bioavailability in rats, the longer monoglyceride being more potent in terms of effective concentration (Watanabe et al., 1988). Diglycerides, which are minor components of MGK, might also contribute to this effect, as they have been reported to disorder phospholipid bilayers (Das and Rand, 1984). Triglycerides in fractionated coconut oil are not promoting absorption as such, but they exert their action after hydrolysis to the corresponding medium-chain fatty acids, e.g. by lipase in the duodenum (Palin et al., 1986;
Yoshitomi et al., 1987). As hydrolysis of triglycerides not only results in the formation of fatty acids, but of mono- and diglycerides as well, their overall effects probably involve those of free acids and of glycerides.

Rectal administration of 88–89% w/w of MGK to rats, rabbits and dogs demonstrated no important morphological changes of the rectal mucosa (Sekine et al., 1985a). However, infusion of 94% of glyceryl-1-monoctanoate into the bile duct at 5–10 ml/hr for 11 days caused duodenal ulceration in man (Cohen et al., 1984). Exposure of the stomach to 1–25% of glyceryl-1-monoctanoate for 1 hr disrupted gastric mucosal structure in dogs (Lillemoe et al., 1982). These observations demonstrate that safety may represent a problem, indicating the need for additional studies of the effects on intestinal mucosa.

5.7. ENAMINES

Enamines are compounds containing a C—C double bond which is directly attached to an amino group (chemical feature: R—C=C—NR). Although many agents fit this description, only a limited number has been demonstrated to exert an enhancing action on intestinal drug absorption. In rabbits and dogs, Kamada et al. (1981) observed a promoting effect of 0.5–20% of phenylglycine enamines of ethylacetoacetate, diethyl ethoxymethylenemalonate and ethoxyethyl acetoacetate on rectal absorption of insulin and inulin (Fig. 11). In rabbits, 5% w/w of DL-phenylalanine- and D-phenylglycine-enamines of ethylacetoacetate, ethyl acetoacetylglycolate, glyceryl-1,3-diactoacetate and 1,2-isopropylidene glyceryl-3-acetoacetate enhanced rectal absorption of lysozyme and heparin (Miyake et al., 1984). The latter two acetoacetic acid esters also enhanced rectal absorption of insulin in the same species (Nishihata et al., 1983b).

In the majority of studies, amino acid enamines of ethylacetoacetate were used, the DL-phenylalanine derivative being given primary attention. Of this enamine 3–10% w/w enhanced rectal insulin absorption in rats (Kim et al., 1984) and in dogs (Kim et al., 1983; Yagi et al., 1983; Nishihata et al., 1985b). In the latter experiments insulin bioavailabilities of 28 to 36%, relative to i.m. delivery, were achieved. Nishihata et al. (1986d) demonstrated that 10% w/w of this enamine is moderately effective as enhancer of rectal insulin absorption from suppositories in man. Rectal absorption of pepleomycin (Nishihata et al., 1986d)
Intestinal drug absorption enhancement

Intestinal drug absorption enhancement (1984g), \([\text{Asu}^{\text{L7}}\]-eel calcitonin (Miyake et al., 1985) and methionyl-human growth hormone (Moore et al., 1986b) proved to be considerably enhanced by this enamine promoter in rats. Rectal uptake of ampicillin and cephalosporins proved to be promoted by 10% w/w of \(\text{p-phenylglycine} \) enamine of ethylacetocacetate in rabbits (Murakami et al., 1981b). Maintenance of high concentrations of enhancer by reducing spread of the intestinally delivered mass increased the effect of enamines (Nishihata et al., 1985b).

Intestinal delivery of amino acids and ethylacetocacetate resulted in considerably enhanced cefmetazole absorption from the rat jejunum and colon (Nishihata et al., 1983a; Choh et al., 1985). As the extent of absorption enhancement correlated with the \textit{in vitro} formation constants of the corresponding enamines, it was suggested that \textit{in vivo} formation of enamines triggered the absorption promoting action.

In rabbits, the rectal absorption of amino-penicillins and cephalexin proved to be considerably increased by delivery as enamine prodrug, which is supposed to be caused by increased lipophilicity (Murakami et al., 1981a). Because these enamine derivatives have absorption promoting properties, these prodrugs are capable of enhancing uptake of co-administered parent drug. This prodrug approach offers interesting opportunities for the enhancement of intestinal absorption of peptide drugs containing free amino groups.

In various reports enamines have been suggested to exert their action on drug absorption by their \(\text{Ca}^{2+} \) chelating properties (Kamada et al., 1981; Murakami et al., 1982). As enamines also have surface tension lowering properties (Murakami et al., 1982), it is conceivable that their effect on drug absorption is mediated by various mechanisms.

Published data of the absorption promoting properties of enamines are promising and they warrant the performance of further studies, including a safety evaluation.

5.8. \textbf{Mixed Micelles}

Mixed micelles are composed of 0.2–2% w/v of a fusogenic lipid (linoleic acid, oleic acid, glyceryl monooleate or dodecanoic acid), solubilized by addition of a surfactant, e.g. polyoxyethylated hydrogenated castor oil (HCO60; 0.1–0.8% w/v) or 2% w/v of sodium taurocholate or sodium glycocholate. The effects of such solubilized lipids on intestinal drug absorption have been reviewed by Muranishi (1985). In the rat small intestine, mixed micelles of sodium taurocholate and fusogenic lipid promoted the absorption of carboxyfluorescein (Hashida et al., 1984), fosfomycin (Ishizawa et al., 1987), heparin (Muranishi et al., 1977) and streptomycin (Muranishi et al., 1980a). Using various bile salts and lipids, Muranishi et al. (1979a) observed an increase of gentamicin bioavailability from 0–4% to 45 and 58%, using 10 \( \text{mm} \) mixed micellar solutions and freeze-dried mixed micellar solutions, respectively. The stronger effect of the latter preparation is probably caused by the higher local concentrations of enhancer. In rabbits, jejunal insulin absorption was enhanced from 0.4 to 31%, relative to i.m. delivery, by mixed micelles containing sodium taurocholate, oleic acid and glyceryl monooleate (Shichiri et al., 1978a).

In rats, oral absorption of the nonapeptide leuprolide was moderately enhanced from 0.03 to 0.05%, estimated by ovulation-inducing effect, by mixed micelles of monoolein and bile salts (Okada et al., 1982).

In the rat large intestine, mixed micelles containing sodium taurocholate and glyceryl monooleate or oleic acid promoted the absorption of fosfomycin (Ishizawa et al., 1987), 5-fluorouracil (Muranishi et al., 1979b), cefmetazole (Tomita et al., 1988) and heparin (Taniguchi et al., 1980). Absorption of human interferon-\(\alpha\) and \(\beta\) (Yoshikawa et al., 1984; Yoshikawa et al., 1985b) and bleomycin (Yoshikawa et al., 1986a) proved to be promoted by mixed micelles of linoleic acid and HCO60, or by mixed micelles composed of oleic acid and polyoxyethylene stearil ether (Yoshikawa et al., 1985a) (Fig. 12). As pretreatment of the large intestinal mucosa with mixed micelles did not enhance drug absorption, the effect of mixed micelles was considered to be reversible (Yoshikawa et al., 1985b, 1986a). Compared with delivery in solution, mixed micelles in fatty suppositories proved to be considerably less effective, which may be caused by poor release of mixed micelles out of the fatty base (Yoshikawa et al., 1986b). Using colloidal gold particles, the upper size limit
of drug absorption enhancement by mixed micelles has been estimated at 40 nm (Fukui et al., 1987).

After delivery of interferon (MW 23 kDa) with mixed micelles, the interferon levels in lymph exceeded those in serum (Yoshikawa et al., 1984; Yoshikawa et al., 1985a; Yoshikawa et al., 1985b) (Fig. 12). It was suggested that the lymphotropic delivery of drugs with a high molecular weight might be a consequence of the large pore radius of the lymphatic capillary wall, as compared to the blood capillary. This phenomenon was also observed using other compounds of relatively high molecular weight, e.g. 1-hexylcarbamoyl-5-fluorouracil in cyclodextrin inclusion complex (MW 10 kDa) (Kaji et al., 1985) and bleomycin–dextran sulfate complex (MW 500 kDa) (Yoshikawa et al., 1981). On the other hand, lymphotropic delivery may possibly take place by an interference of mixed micelles with the lymphatic system (Takada et al., 1985). As a consequence, the contribution of lymph to drug absorption, relative to blood, is increased, as demonstrated for cyclosporin A (Takada et al., 1985). Although lymphatic uptake of certain drugs can be increased by mixed micelles, total amount absorbed into lymph is low, not exceeding 1% of the dose administered (Yoshikawa et al., 1981; Yoshikawa et al., 1984).

Generally, the effect of mixed micelles on intestinal drug absorption increases in distal direction, from small intestine to rectum, suggesting that this enhancer should be quite suitable for rectal absorption enhancement (Yoshikawa et al., 1981; Fukui et al., 1987; Muranishi et al., 1979a; Muranishi et al., 1979b; Taniguchi et al., 1980). This observation has been explained by differences in sensitivity of the mucosa for the action of mixed micelles. However, as the effect of mixed micelles increases when spread of the preparation is reduced (Muranishi et al., 1979a), it is conceivable that a reduction in spreading after delivery more distally contributes to this observation.

Various mechanisms may explain the absorption enhancing action of mixed micelles. Murakami et al. (1985) suggested a decrease of the barrier function of the mucous layer by HCO60-containing mixed micelles. A transcellular mechanism also may be involved, as caused by incorporation of solubilized fusogenic lipid into the epithelial membrane, resulting in an increase of membrane fluidity (Cullis et al., 1986; Maggio and Lucy, 1976; Muranushi et al., 1980a; Muranushii et al., 1981; Muranushi et al., 1980b; Hori et al., 1978). As protein-SH modifying reagents reduced the effect of unsaturated fatty acids on drug absorption, it was suggested that intact SH groups of membrane proteins mediate the enhancing action (Murakami et al., 1988). However, it cannot be excluded that thiol reagents decrease membrane permeability, thus directly reducing the effect of the fatty acid, not necessarily indicating a role of SH-groups in absorption enhancement. Furthermore, enhancement of the membrane-perturbing effect of the bile salt component by the
fusogenic lipid has been proposed (Vallet-Strouve et al., 1985). Interference with paracellular transport has also been suggested, although unambiguous indications have not been obtained (Muranishi et al., 1986; Masuda et al., 1986).

The majority of papers on absorption enhancement by mixed micelles claim that they are relatively safe to use. However, such statements are at variance with a study of Taniguchi et al. (1980), who reported a disordering effect of 10 mM glyceryl-monoooleate/sodium taurocholate mixed micelles on epithelial cells of the small intestine. In addition, Tokunaga et al. (1978) observed release of mucosal proteins by micelles of comparable composition. The use of bile salts as components of mixed micelles imposes the issue of applicability of these agents as discussed in a previous paragraph. On rectal administration fusogenic lipids appeared to cause inflammatory reactions in mouse colon (Wargowich et al., 1984). These reports clearly indicate that effects of mixed micelles on intestinal integrity after acute and chronic exposure remain to be assessed before their relative safety can be judged upon.

5.9. CALCIUM-BINDING AGENTS

In many studies the enhancing effects of calcium-binding agents on intestinal drug absorption have been appreciated, disodium EDTA being the compound that has been given the greatest attention. In concentrations of 0.5 to 5% this agent enhanced colo-rectal absorption of gentamicin (Fix et al., 1983b), cefoxitin (Nishihata et al., 1985d), cefmetazole (Nishihata et al., 1986b; Shiga et al., 1987; Suzuki et al., 1985; Suzuki et al., 1987a), inulin (Suzuki et al., 1987a), fosfomycin (Ishizawa et al., 1987), phenol red, sodium p-amino-benzoate and sodium ampicillin (Yata et al., 1983; Murakami et al., 1982) and insulin (Aungst and Rogers, 1988) in rats (Figs 5 and 13). In the same species jejunal absorption of phenol red, insulin and polyvinyl pyrrolidone was increased by EDTA (Ecknauer et al., 1983). In dogs it promoted oral absorption of heparin (Windsor and Cronheim, 1961).

Ionic strength proved to be an important variable for the action of EDTA on drug absorption (Suzuki et al., 1985). Disruption of the diffusion barrier composed of mucus by sodium chloride has been suggested to be involved in this process.

EDTA is assumed to enhance paracellular drug absorption by widening of the intercellular space (Cassidy and Tidball, 1967; Martinez-Palomo et al., 1980). On the other hand, swelling of microvilli also has been observed (Cassidy and Tidball, 1967), suggesting that a transcellular absorption promoting mechanism may be involved as well (Otero and Carrasco, 1987).

Despite the strong absorption enhancing properties of EDTA, the applicability of this agent in human pharmacotherapy is questionable, considering its damaging effects on animal colon (Wargowich et al., 1984). These reports clearly indicate that effects of mixed micelles on intestinal integrity after acute and chronic exposure remain to be assessed before their relative safety can be judged upon.

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Despite the strong absorption enhancing properties of EDTA, the applicability of this agent in human pharmacotherapy is questionable, considering its damaging effects on

**Figure 13.** Influence of EDTA on colonic cefmetazole absorption. Cefmetazole plasma levels (+ SE) after administration of 47 mg of cefmetazole without (△) and with (◊) 2% of sodium EDTA in the rat colon (redrawn from Shiga et al., 1987).
mucosal integrity. Disodium EDTA 0.8% w/v induced a reversible loss of rectal epithelial cells, whereas goblet cell loss was not reversible in 24 hr (Nakanishi et al., 1983b). Blood loss was observed after bolus delivery of the same concentration in the dog jejunal loop (Tidball and Lipman, 1962). A concentration of 1% w/v induced severe damage of small intestinal epithelium in rats (Nadai et al., 1972). In their study, epithelial cell loss and opening of blood capillaries were demonstrated by light microscopy.

Organic acid salts like trisodium citrate represent a group of agents which have been reported to enhance drug absorption, probably by binding calcium ions, as shown for ampicillin in rats (Murakami et al., 1982). Okada et al. (1983) reported an increase of vaginal leuprolide bioavailability from 4% to maximally 39%, by 1-7% of various organic acids in rats. The influence of these agents on epithelial integrity remains to be investigated.

5.10. PHENOTHIAZINES

Fix et al. (1984) reported an enhancing action of several phenothiazines, e.g. chlorpromazine, triflupromazine and trifluoperazine, in a concentration of 2% w/v on rectal drug absorption in rats. Cefoxitin bioavailability increased from 3% to maximally 68%. Gentamicin bioavailability was shown to exceed 100%, which may indicate an effect of phenothiazines on gentamicin elimination kinetics or inaccuracy of the assay. Suzuka et al. (1987a) confirmed the enhancing effect of phenothiazines on intestinal drug uptake by using cefmetazole as poorly absorbed model drug. Maximal effects in the rat colonic sac model were observed with perphenazine, profenamine, pericyazine and trifluoperazine, in a concentration range of 20-75 μM.

Phenothiazines are used in human pharmacotherapy for their antipsychotic, antiemetic and antihistaminic properties. Part of their action is mediated by antagonizing calmodulin-dependent processes (Hait and Lee, 1985). As calmodulin is involved in the regulation of the activity of more than 30 proteins, e.g. adenylate cyclase, cyclic nucleotide phosphodiesterase, protein kinases and ATPases in a calcium-dependent manner (Johnson and Mills, 1986), it is not surprising that phenothiazines exhibit diverse pharmacological effects. Chlorpromazine and trifluoperazine in concentrations of 20-30 μM induce an ATP-dependent increase of membrane permeability (Kitagawa and Akamatsu, 1985) and increase erythrocyte membrane fluidity (Minetti and Di Stasi, 1987), which indicates a membrane destabilizing effect.

On the other hand, phenothiazines have non-specific membrane-destabilizing effects (Hait and Lee, 1985). Because of their hydrophobic properties, they readily partition into biological membranes or into phosphatidylcholine-cholesterol bilayers (Bereza et al., 1982). As a consequence chlorpromazine causes physical expansion of one bilayer leaflet (Morrow and Anderson, 1986) and interpenetration of phospholipid chains (McIntosh et al., 1983) and may cause membrane lysis at concentrations exceeding 30 μM (Luxnat and Galla, 1986). This perturbing effect of phenothiazines, in particular of chlorpromazine, on artificial and biological membranes has been recognized in various studies (Goldstein, 1984; Nakae and Asada, 1986; Brindley et al., 1975). In rats, perfusion of the small intestine with 0.07% of chlorpromazine induced protein and phospholipid release, indicating microvilli damage (Chung et al., 1983).

These reports strongly indicate a transcellular absorption promoting effect of phenothiazines. However, an effect on paracellular permeability cannot be excluded, as trifluoperazine has been reported to destabilize inter-Sertoli junction structure (Franchi and Camatani, 1985).

Despite the reported effectiveness of phenothiazines as intestinal absorption promoters, limited attention has been paid to this entity of absorption enhancers. It is conceivable that the strong pharmacological responses elicited by these drugs, which may give rise to many side effects in man, importantly contribute to this limited interest.

5.11. LIPOSOMES

Liposomes are vesicles composed of bilayers, containing phospholipids and cholesterol, surrounding aqueous compartments. Rowland and Woodley (1981) reported an increased
intestinal tissue uptake of $^{125}$I-labelled polyvinylpyrrolidone in the everted gut sac model of rats, on delivery in distearoyl phosphatidylcholine/cholesterol liposomes. However, a clear assessment of the effect of liposomes on drug absorption in that study is difficult, because of adsorption of drug-containing liposomes to tissue and deterioration of mucosal integrity. Kashiwagura et al. (1987) suggested that atropine methyl bromide absorption into frog intestinal cells was enhanced by fusion of lipid bilayers with the cell membrane.

In rats, orally delivered insulin, encapsulated into liposomes, proved to exert a considerably smaller hypoglycemic response than i.p. delivered free or encapsulated insulin (Patel and Ryman, 1976). Dapergolas and Gregoriadis (1976) reported an hypoglycemic response of intragastrically delivered insulin in dipalmitoyl phosphatidylcholine liposomes in rats. The effect of liposomes containing saturated and shorter lipids exceeded the effect of fluid phosphatidylcholine liposomes (Dapergolas and Gregoriadis, 1977). Recently, Chiang and Weiner (1987b) suggested that this effect was caused by protection of insulin from premature degradation, as orally delivered liposomes are efficiently degraded in the presence of bile salt and lipase (Chiang and Weiner, 1987a). Liposomal entrapment was shown not to facilitate jejunal absorption of glucose or carboxyfluorescein in rats, and liposomes proved not to be absorbed intact. This confirmed the results of previous studies on the absence of absorption of liposomes from the rabbit ileum (Patel et al., 1985) as well as of intragastrically delivered phosphatidylcholine–cholesterol liposomes in mice (Deshmukh et al., 1981). Entrapment into egg phosphatidylcholine–cholesterol liposomes strongly reduced carboxyfluorescein absorption from the rat everted jejunum and only marginally increased absorption of fluorescein isothiocyanate-conjugated dextran (Hashida et al., 1984; Kimura et al., 1984). Patel et al. (1982) reported an irreproducible increase of plasma immunoreactive insulin on administration of liposomal insulin in the dog duodenum. As the subsequent fall in plasma glucose was negligible, it was concluded that a very small amount of insulin of not more than 1% was absorbed intact.

Because of the unfavorable results of the majority of studies, it was concluded that liposomes do not appear to have any absorption promoting properties of practical importance (Muranishi, 1985; Chiang and Weiner, 1987b).

On the other hand, carboxyfluorescein absorption proved to be enhanced by entrapment into ufasomes, vesicles of bilayers formed by unsaturated long chain fatty acids (Murakami et al., 1986b). As with mixed micelles, the effect in the large intestine exceeded that in the small intestine. Results indicate that the fusogenic lipid, liberated on intestinal degradation of the ufasomes, promotes drug absorption. Future research will be necessary to decide on the applicability of this type of lipid vesicle as an intestinal absorption enhancer.

5.12. AZONE

Azone (laurocapram, 1-dodecylazacycloheptan-2-one) and related amides of cyclic amines have been studied as transdermal drug penetration enhancers (Mirejovsky and Takruri, 1986; Barry, 1987). Fukui et al. (1986) reported an increase of 6-carboxyfluorescein absorption from the rat large intestine by co-administration in micellar solution with 5–20 mM Azone and 0.5–2 mM HCO60, or in ufasomes of oleic acid (11–14 mM) and Azone (2–5 mM). The effect of Azone-containing preparations on drug absorption proved to be reversible. Murakami et al. (1986a) observed a stronger effect of Azone solubilized with HCO60 on drug absorption from the large intestine, compared with the effect in the small intestine.

As Azone has been shown to lower the phase transition temperature of phospholipid bilayers and to increase lipid fluidity (Beastall et al., 1988) a transcellular absorption enhancing action may be involved, whereas HCO60 is thought to facilitate transport across the mucus (Murakami et al., 1986a). On delivery without emulsifying agent, Azone exerted only a variable effect on rectal cefoxitin absorption in rats (Van Hoogdalem et al., 1989a). This observation confirms that the presence of an emulsifier is necessary for Azone to permeate the mucous layer and to reach the mucosal membrane in absorption promoting quantities. The absence of ‘gross morphological damage’ was reported after exposure of
the mucosa to Azone-containing formulations (Murakami et al., 1986a), which does not exclude the occurrence of minor damage to the mucosal structure.

As the effects of Azone on mucosal integrity have not been assessed unambiguously, further safety studies are required. The moderate rectal absorption promoting properties of Azone on delivery without emulsifier indicate that Azone is less suitable as promoter of intestinal drug uptake, as compared with the effect on transdermal drug absorption.

5.13. FATTY ACID DERIVATIVES OF CARNITINE AND PEPTIDES

Long-chain carnitine esters are present in bile under physiological circumstances and, consequently, also in the intestinal contents (Hamilton and Hahn, 1987). In rats, esters of carnitine with fatty acids of 12 to 18 carbon chain atoms proved to enhance rectal drug absorption; 2% w/v of palmitoyl-DL-carnitine considerably promoted uptake of cefoxitin, gentamicin, cytarabine, α-methylldopa and of a cyclic hexapeptide analog of somatostatin (Fig. 14). In dogs, the effect was less pronounced, resulting in a maximal cefoxitin bioavailability of 13%. The absorption enhancing action of 0.5 to 1% w/v of palmitoyl-DL-carnitine was accompanied by moderate to complete loss of epithelial cells (Fix et al., 1986).

A study by Haeyaert et al. (1987) strongly indicated that the absorption promoting action of acylcarnitines is mediated by detergent-like properties, resulting in a disturbance of the packing of phospholipid bilayers, and consequently in facilitation of the transcellular absorption route.

Yata et al. (1983) observed an enhancing effect of N-acyl tripeptides on rectal absorption of phenol red, sodium p-aminobenzoate and ampicillin. In rabbits, rectal ampicillin absorption was increased by N-acyl derivatives of a collagen peptide, the effect increasing with chain length (Yata et al., 1985b). In the latter study no histological damage was observed. From the rat rectal loop, 0.1–0.4% w/v of N-acyl derivatives of amino acids enhanced ampicillin bioavailability from 0.5% to maximally 70% (Wu et al., 1987).

Because in these studies the effect of the N-acyl derivatives of peptides and amino acids correlated with their Ca$^{2+}$-binding properties, it is tempting to suppose that a paracellular absorption promoting mechanism is involved. On the other hand, Schott et al. (1988) reported a destabilizing effect of palmitoyl derivatives of amino acids on liposomal membranes. Structural similarity with N-acyl carnitines and correlation between absorption enhancing effect and surface tension lowering properties (Yata et al., 1985b) indicate a transcellular absorption promoting effect by destabilization of membrane structure.

Considering the data presently available, these N-acyl derivatives represent an interesting group of absorption promoting agents. Therefore, the performance of further studies, concerning both absorption promoting effects and relative safety, is desirable.

![Fig. 14. Effect of palmitoyl-DL-carnitine on rectal drug absorption. Mean bioavailabilities (± SE) of cefoxitin (A), gentamicin (B), cytarabine (C), theophylline (D), somatostatin analog (E) and α-methylldopa (F) after rectal administration without (open bars) and with (hatched bars) 2% w/v of palmitoyl-DL-carnitine in rats; *: significantly different from control without promoter (p < 0.01, Student's t-test) (redrawn from Fix et al., 1986).](image-url)
5.14. SAPONINS

Saponins are glycosides of vegetable origin with surface tension reducing properties and an hemolytic action. They are capable of precipitating sterols and exert intestinal and transdermal absorption promoting properties. The biological assay of saponins in fish is based on these permeability enhancing effects (Steinegger and Hänsel, 1972). Yata et al. (1985a) assessed a promoting effect of 0.05–0.08% of monodesmosides from pericarps of Sapindus mukurossi on rectal absorption of ampicillin and cephalosporins in rats; i.v. data and bioavailabilities were not presented in their study. Jejunal absorption of PEG 4000 was enhanced by triterpenoid saponins and steroidal amine saponins (Johnson et al., 1986). Ishizawa et al. (1987) reported an increase of fosfomycin absorption from rat jejunum and colon by co-administration of 1% of an unidentified saponin (Fig. 5).

It is conceivable that the absorption promoting properties of saponins are mediated by their surfactant-like properties. On the other hand, a transcellular promoting effect may also be caused by interaction with the membrane stabilizer cholesterol, as discussed previously for medium-chain glycerides.

These results show that saponins exhibit absorption promoting activity at relatively low concentrations. However, also for these compounds the issue of safety vs efficacy requires further investigation.

5.15. CONCANAVALIN A

Concanavalin A is a carbohydrate-binding protein which proved to enhance rectal cefoxitin absorption in rats in a concentration of 0.5% w/v (Nishihata and Higuchi, 1984b). However, as a lower concentration of 0.1% w/v affected ileal structure in rats in terms of shortened and irregular microvilli (Sjölander et al., 1984), this compound may not meet the safety requirements.

5.16. PHOSPHATE AND PHOSPHONATE DERIVATIVES

In rats, 6–11% w/v of disodium DL-α-glycerophosphate enhanced rectal cefoxitin bioavailability from 2% to maximally 43% (Nishihata et al., 1984b). This effect was potentiated by 0.5% w/v of sodium tripolyphosphate and 1% w/v of sodium phytate to a bioavailability of maximally 54%. As with various other absorption promoters, the effects were less pronounced in a larger animal species, viz. the dog. The authors speculated that glycerophosphate might interact with membrane proteins, whereas tripolyphosphate and phytate could bind calcium.

In man 3-amino-1-hydroxypropylidene-1,1-diphosphonate (APD) is used orally in the treatment of Paget's disease of bone. In rats, APD in a concentration range of 0.5 to 6% w/v has been demonstrated to enhance rectal cefoxitin bioavailability to maximally 85%, the effect on rectal infusion exceeding the effect on bolus delivery (Van Hoogdalem et al., 1989b). It was suggested that binding of calcium ions mediated the effect of APD on drug absorption.

As the effects of phosphate derivatives on cefoxitin bioavailability were only moderate in these experiments, the phosphate derivatives investigated do not appear to be promising absorption enhancers. The results obtained with APD warrant the performance of further experiments with phosphonates, including studies on absorption enhancement and safety evaluation.

5.17. POLYACRYLIC ACID

In rats and rabbits, rectal insulin absorption was increased by delivery as suspension in 0.1% w/v of polyacrylic acid (Morimoto et al., 1980). I.v. data were not presented in that study. In rats, this base promoted rectal absorption of [Asul^-]-eel calcitonin, but bioavailability was only as low as 0.8%, relative to i.m. delivery (Morimoto et al., 1984). In the same species rectal insulin bioavailability was estimated by hypoglycemic response to be enhanced from 0.3 to 10%, relative to i.m. administration, by co-administration of 1% w/v of oleic acid, linolic acid or linolenic acid in 1% w/v of polyacrylic acid gel (Morimoto et al., 1983).
Polyacrylic acid gel (0.01 to 0.1% w/v) proved to exert various effects on rectal mucosal integrity, including a reduction of the mucous layer, dilation of the intercellular spaces and shortening of the microvilli (Morimoto et al., 1987), and release of protein and Ca\(^{2+}\) (Morimoto et al., 1984). These observations on the one hand suggest potential transcellular as well as paracellular absorption promoting effects, but on the other hand indicate that safety may represent a problem.

Despite the presence of polyacrylic acid, the bioavailabilities of insulin and calcitonin derivative remained low, indicating that this agent is less suitable for peptide absorption enhancement.

5.18. Diethyl Maleate and Diethylethoxymethylene Malonate

Both diethyl maleate (DEM) and diethylethoxymethylene malonate (DEEMM), a chemical used in the preparation of absorption promoters with enamine structure, have been reported to enhance rectal cefmetazole absorption (Miyake et al., 1985; Nishihata et al., 1984c; Nishihata et al., 1985c) and [Asu\(^{125}\)]-eel calcitonin absorption (Nishihata et al., 1986b), which occurred concurrent with a reduction of glutathione levels in the rectal tissue. As discussed for sodium salicylate, the reduction of glutathione levels was speculated to induce transcellular absorption. High DEM levels decrease cefmetazole absorption and reduce cell viability (Nishihata et al., 1987c), again emphasizing that the safety issue of these promoters will be of major importance for further research.

6. CONCLUSIONS

Considerable research efforts have been devoted to the development of orally, rectally or nasally applicable formulations of poorly absorbed drugs with absorption enhancing agents. In recent years a large variety of compounds have clearly been demonstrated to exert an absorption promoting action. Of these compounds, sodium salicylate, sodium tauro-24,25-dihydrofusidate, enamines, mixed micelles, medium-chain fatty acids and fatty acid derivatives of carnitine and peptides seem to be candidates of first choice for further studies, considering their effectiveness and the preliminary data on their safety profile. Undoubtedly, in the near future many more compounds will be detected to exhibit absorption promoting effects.

As the enhancers discussed in this overview have widely divergent physico-chemical properties, absorption promoting agents appear not to have any characteristic in common, apart from their action on drug uptake. Paracellular absorption promoting effects by Ca\(^{2+}\)-binding and transcellular effects by interaction with membrane proteins or lipids are the most frequently suggested mechanisms of action. As no unambiguous conclusions can be drawn on this issue, further research is highly desirable to develop insight in the mechanisms involved in absorption enhancement. This should also rationalize the development of absorption promoting agents and formulations.

In several studies a quantitative evaluation of the effects of the enhancer in terms of absolute bioavailability is not possible for lack of i.v. data. In order to enable a clear evaluation of the effects of absorption promoters, the performance of i.v. experiments in future studies is highly desirable. While the effects of several agents on drug absorption have been investigated extensively, the influence on mucosal integrity has been studied considerably less thoroughly. This is a major shortcoming of the information available so far, because the safety profile will be decisive for the ultimate applicability of effective enhancers in man. Data available demonstrate that absorption enhancement is frequently associated with morphological changes of the intestinal epithelium, indicating the need for a close investigation of the effects of absorption promoters on mucosal integrity and of the consequences of affected mucosal integrity.

Only a limited number of agents have been investigated for their absorption promoting properties in man. As in larger species absorption promoters tend to be less effective, optimization of formulations with enhanced bioavailability in man will be necessary, once more information on the safety issue is available. The further development of optimized
drug delivery systems should not only include formulation parameters, e.g. volume, nature and concentration of enhancer and ionic strength, but also site of delivery and rate of release characteristics.

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Intestinal drug absorption enhancement


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