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Intestinal Absorption of Drugs. The Influence of Mixed Micelles on on the Disappearance Kinetics of Drugs from the Small Intestine of the Rat

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Abstract—The solubilization of the hydrophilic drugs paracetamol and theophylline, and the lipophilic drugs dantrolene, griseofulvin and ketoconazole has been determined in mixed micellar aqueous dispersions composed of 10 mM taurocholate + 5 mM oleic acid. The solubilization of dantrolene and paracetamol has also been determined in aqueous (mixed) micellar dispersions of 1 g L^{-1} lysophosphatidylcholine (LPC), or taurocholate/LPC. The influence of these (mixed) micelles on the absorption of the model drugs from solution was studied in the rat chronically isolated internal loop. Absorption kinetics of the drugs were evaluated on the basis of the disappearance rate of the drug dissolved in the perfusion medium in this loop. Absorption experiments with taurocholate/oleic acid in the perfusate resulted in a reduction of the disappearance rate for the lipophilic drugs and the hydrophilic drug theophylline. This could partly be ascribed to the decreased fraction of drug free in solution as a result of its micellar solubilization for dantrolene, griseofulvin and ketoconazole, but the decrease in the disappearance rate of theophylline was unexpected. Taurocholate/oleic acid, LPC and taurocholate/LPC micelles had no effect on the disappearance of paracetamol. The disappearance rate of dantrolene in the presence of LPC alone was not altered, in spite of the decreased fraction of the drug free in solution owing to its micellar solubilization. In contrast, taurocholate/LPC micelles caused a reduction in the rate of disappearance of dantrolene, as expected according to the phase-separation model. In-vitro, taurocholate and taurocholate/LPC reduced the molecular cohesion of porcine intestinal mucus, whereas LPC alone did not exhibit an effect on the gel structure of mucus. It is concluded that the transfer of solutes across the pre-epithelial aqueous diffusion layer (including the mucus) and the epithelium is unaltered by (mixed) micelles at concentrations in the physiological range.

In previous studies we have investigated the influence of taurocholate on the disappearance of several hydrophilic and lipophilic drugs from solutions in segments of the rat intestinal tract (Poelma et al 1989, 1990a). The reduction of the disappearance rate of lipophilic compounds from the lumen, in the presence of a micellar solution of taurocholate, could partly be ascribed to a decreased thermodynamic activity of the drug in solution due to micellar solubilization and in some cases partly to an altered barrier function of the mucous layer by taurocholate (Poelma et al 1989, 1990a). However, the effects of bile salts alone on the absorption of drugs (Kakemi et al 1970; Yamaguchi et al 1986; Poelma et al 1989, 1990a) may not be relevant for the physiological situation, since in-vivo not only bile salts, but also phospholipids and fatty acids, occur in the small intestine. Borgström et al (1957) found that after a test meal the small intestine of man had bile salt concentrations of about 1–3 mg mL^{-1} and concentrations of phospholipids of 0·5–1 mg mL^{-1} with lysolecithin as the major component. In rat liver bile the concentration of phospholipids is similar to that reported for man (Coleman et al 1979). In combination with bile salts these lipoidal compounds can form mixed micelles; this combination might change the effect of bile salts on absorption. As reported previously (Martin & Marriott 1981) addition of phosphatidylcholine to the bile salt solution may provide protection against membrane damage caused by bile salts alone. The addition of unsaturated fatty acids or monoglycerides (e.g. oleic acid, monoolein) to bile salt solutions can also result in an increase in the absorption rate of hydrophilic drugs in the small intestine, as was reported for aminoglycosides (Muranishi et al 1979, Muranushi et al 1980) and heparin (Tokunaga et al 1978; Taniguchi et al 1980), the lipid component rather than the bile salt being responsible for the enhanced absorption rate of the drugs.

The present study has been performed to gain insight into the mechanism controlling the rate of transport of a drug from a luminal solution across the pre-epithelial diffusion barrier and the small intestinal wall of the rat in the presence of (mixed) micelles: micellar dispersions of taurocholate + oleic acid, lysophosphatidylcholine (LPC), and taurocholate + LPC. This approach eliminated the influence of the respective micellar systems on the dissolution rate of the drug. The micellar components, as well as their respective concentrations were selected on the basis of their occurrence in the rat small intestine, taurocholate being the major bile salt (Nakatomi et al 1985). The five model drugs were selected on the basis of their physicochemical nature, paracetamol and theophylline as hydrophilic compounds, and dantrolene, griseofulvin and ketoconazole because of their lipophilic character. To study the influence on the transport step the disappearance of the model compounds from a perfused intestinal loop under the influence of the (mixed) micelles was also studied. A chronically isolated internal loop (Poelma et al 1989) was used to permit a cross-over experimental scheme; in this scheme, the disappearance

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rate of the model compounds can be studied in the same rat/loop with and without additives. The extent of solubilization of the model compounds in the respective micellar solutions was derived from solubility measurements. The consequences of micellar solubilization for the absorption of the model drugs from solutions were evaluated on the basis of the phase-separation model (Stilbs 1982).

The mucous layer covering the intestinal surface may be an absorption barrier for lipophilic compounds. Therefore, the influence of taurocholate, LPC, and taurocholate + LPC on mucus has been studied by determining the effect of these (mixed) micellar solutions on the molecular cohesion ("viscosity") of porcine intestinal mucus in-vitro.

Materials and Methods

Materials

Dantrolene sodium and ketoconazole were gifts from Norwich Eaton Pharmaceuticals Inc., NY, USA and Janssen Pharmaceutica, Beerse, Belgium, respectively. Griseofulvin was supplied by Aldrich Chemical Co., Milwaukee, USA. Paracetamol and theophylline monohydrate were obtained from OPG, Utrecht, The Netherlands and from Brocacef, Maarssen, The Netherlands, respectively. Taurocholate, oleic acid and lysophosphatidylcholine (LPC) were supplied by Sigma Chemical Co., St. Louis, USA, and were used without further purification. All other chemicals were of analytical grade.

Mucus was collected from the small intestine of freshly slaughtered domestic pigs (from Encebe, Boxtel, The Netherlands), which had been starved for approximately 24 h. The mucus was gently scraped off the mucosal surface, divided into 5 mL samples and stored at −20°C until the viscosity determinations were made.

A pH meter, a constant temperature waterbath, a spectrophotometer, an HPLC-system (model 710B Waters Intelligent Sample Processor and model 440 UV Absorbance Detector, Waters Associates, Milford, MA, USA), and a peristaltic pump (VRX-22, Verder, Düsseldorf, FRG), a Ferranti–Shirley cone-plate viscometer (Epprecht, Rheomat 15, Zürich, Switzerland), a rotary evaporator (Büchi RE 111, Switzerland), and a sonicator (Sonicor Instrument Corporation, NY, USA) were used.

Distribution coefficient of the model compounds

For the aqueous phase, the same medium was applied as in the absorption experiments (perfusion solution). The aqueous phase was phosphate-buffered saline (PBS) (66 mM sodium phosphate, 88 mM sodium chloride, pH = 7·4 ($\approx pK_a$ of dantrolene, Product Information Norwich Eaton Pharmaceuticals Inc.); for ketoconazole the pH was adjusted to 6·5 (pK_a of ketoconazole, Product Information Janssen Pharmaceutica)). n-Octanol was used as the lipophilic phase. The distribution coefficients were determined at 21°C by shaking 2·0 mL of the octanol phase with 2·0 mL of the aqueous phase for 30 min. The concentration of the model drug in the aqueous phase before the addition of the octanol phase was similar to that used in the absorption experiments (Table 1) and was below the saturation concentration of the drugs in the aqueous phase. After centrifugation, the drug concentrations in the aqueous and the octanol phase were measured by HPLC. The experimental characteristics of the various HPLC methods are outlined in Table 2. The distribution coefficient (D) was calculated from:

$$D = C_{oc}/C_{aq} \quad (1)$$

where C_{oc} and C_{aq} are the concentrations of the drug in the octanol and aqueous phases, respectively.

Table 1. The concentrations of the model compounds in the aqueous phase for the log D determinations and in the perfusion solutions (in PBS).

Drug	Conc (10^{-5} M)	pH
Paracetamol	331	7·4
Theophylline	253	7·4
Dantrolene	1·25	7·4
Griseofulvin	1·42	7·4
Ketoconazole	0·94	6·5

Solubilization of the model compounds in the (mixed) micelles

The solubility of dantrolene, griseofulvin, paracetamol or theophylline was determined in PBS alone and with taurocholate + oleic acid, by shaking the suspensions for 24 h at 37°C. The solubility of ketoconazole was determined in PBS at pH = 6·5. The solubility of dantrolene and paracetamol was also determined in micellar solutions of LPC, or taurocholate + LPC. After centrifugation (30 min, 4000 rev min^{-1}), the concentration of the drug in the supernatant was determined by HPLC (Table 2). The composition of the different (mixed) micellar solutions was as follows: (1) 1 mg

Table 2. HPLC conditions for the determination of paracetamol, theophylline, dantrolene, griseofulvin and ketoconazole.

Drug	Stationary phase	Eluent	Wavelength of detection (nm)
Paracetamol/theophylline	LiChrosorb RP18	Methanol/water/acetic acid 15/85/1 (v/v/v)	254
Dantrolene	LiChrosorb RP 8	Acetonitrile/phosphate buffer, pH = 6·8, 45/55 (v/v)	405
Griseofulvin	LiChrosorb RP18	Methanol/water 3/2 (v/v)	254
Ketoconazole	Chrom-Sep Hypersil/ODS	Aacetonitrile/water/diethylamine 48/55/0·02 (v/v/v)	254

mL^{-1} (≈2 mM) LPC; (2) 10 mM taurocholate + 1 mg mL^{-1} LPC; (3) 10 mM taurocholate + 5 mM oleic acid. The concentrations of taurocholate and LPC were in the physiological range (Robinson 1961; Vonk et al 1978; Coleman et al 1979) and above the critical micelle concentration (CMC) of the respective surface active agents (CMC of taurocholate ≈5 mM (Ockner & Isselbacher 1974); CMC of LPC ≈25 μg mL^{-1} (Bates et al 1967)).

The (mixed) micellar solutions were prepared by dissolving the respective components in PBS, except for the taurocholate/oleic acid micellar system which was prepared by weighing the desired amount of oleic acid in a round-bottom flask, adding chloroform to dissolve the lipid, and then evaporating to dryness using a rotary evaporator under reduced pressure at 40–45°C for 30 min. Subsequently, glass beads and the aqueous medium (buffer solution with 10 mM taurocholate) were added. The oleic acid film was solubilized by shaking and by sonicating the solution for 30 min at 37°C.

From the solubility data of drug in micellar solutions an estimation can be made of the fraction solubilized, assuming that under those conditions the saturation concentration in the aqueous phase is identical with the solubility in the absence of micelles (eqn 2).

Theoretical

Drug solutions in micellar media can be considered to consist of two separate phases (Stilbs 1982): (i) an aqueous phase with a fraction of the drug free in solution, and (ii) a micellar phase with the remaining fraction of the drug solubilized in micelles.

The fraction of the drug solubilized in micelles (s) and the fraction of the drug free in solution (f) is calculated from the solubility data using equations 2 and 3:

$$s = (C^+ - C^-)/C^+ \quad (2)$$

$$f = 1 - s \quad (3)$$

where C^+ is the solubility of the drug in the solution with micelles and C^- is the solubility in the same medium without micelles. It is assumed that s is constant and independent of the drug concentration for a particular concentration of micelles and that the distribution of the solute between the aqueous and the micellar phase is instantaneous; it is also assumed that only one type of (mixed) micelles exists in the micellar solutions.

If the concentration of the drug free in solution is considered as the only driving force for absorption, it is expected that during absorption experiments with drug solutions the reduced concentration of the free drug due to micellar incorporation will result in a proportional decrease in the disappearance rate of the drug, provided that the absorption barrier is not affected by the micelles.

Another approach is to take into account the contribution of the fraction of the drug solubilized in micelles to the overall diffusion of the solute across the aqueous boundary layer adjacent to the intestinal wall (Stilbs 1982). Thus, according to that phase-separation model (Stilbs 1982), the diffusion of a solute in a micellar solution can be described by a combination of the diffusion coefficient of the fraction solubilized (s) and free (f) drug. The apparent diffusion coefficient of a drug in a micellar solution (D_{app}) is defined by the following expression:

$$D_{app} = s \cdot D_{mic} + f \cdot D_{free} \quad (4)$$

where D_{mic} and D_{free} are the diffusion coefficients of the drug in micelles and the free drug molecules in the aqueous phase, respectively.

Absorption experiments

Chronically isolated internal loop. A chronically isolated intestinal loop in the rat (Poelma & Tukker 1987) has been used to study the absorption kinetics of the model compounds. This model offers the opportunity to perfuse an isolated segment of the intestine of the rat with a constant volumetric flow of drug solution under well defined conditions in a conscious animal; an additional advantage of this approach is that the experiment can be performed in a cross-over mode over an extended period of time.

Briefly, an intestinal segment of approximately 8 cm (approximately 15 cm proximal to the ileo-caecal junction) was isolated with intact blood supply. The loop remained in the peritoneal cavity. The perfusion solution could enter and leave the segment via two Delrin cannulas in the abdominal wall. The head-tail connection of the remaining intestine was restored by end-to-end anastomosis. After surgery the rat was placed into a restriction cage, with water and food available. After recovery from the operation (2–4 days) the rat was ready for use in perfusion experiments. The absorption kinetics of the drugs were evaluated on the basis of disappearance rates of the drug from the perfusion solution. The disappearance of the drugs in the presence of micelles was compared with the disappearance of the drug from the same medium without micelles in the same intestinal segment.

Experimental set-up. Before starting the perfusion, the intestinal loop was cleaned by rinsing it for 30 min with saline at a flow rate of 1·0 mL min^{-1}. During perfusion experiments, the perfusion solution was pumped through a heat exchange device to bring the solution to body temperature just before entering the rat. Perfusions were performed in a recirculating mode (perfusion volume: 60 mL) at a rate of 1·0 mL min^{-1}. The pH of the perfusion solution remained within narrow limits (±0·1) during the perfusion experiments. The perfusions were performed at a pH of 7·4, except for ketoconazole when the pH of the perfusion solution was 6·5. The concentration of the drugs in the perfusion solutions (Table 1) was below the saturation concentration of the respective drugs in the buffer solution without micelles. All perfusions with and without micelles in the medium were performed with the same concentration of the particular model compound. The perfusion solutions were freshly prepared shortly before starting the experiments. During the perfusion, samples were taken from the perfusion solution at 0, 0·5, 1, 1·5, 2, 2·5 and 3 h and immediately analysed by HPLC.

The disappearance of the model compounds from the perfusion solution can be described by first order kinetics. The time dependence of the concentration in the perfusion solution, C, can be written as:

$$\ln (C_t/C_o) = k_{dis} \cdot t \quad (5)$$

where C_o and C_t are the concentrations of the model compound in the perfusion solution at time 0 and t, respectively. The first order disappearance rate constant, k_{dis} (h^{-1}) was calculated from plots of ln (C_t/C_o) vs t by linear regression.

Evaluation of the absorption data. The disappearance rate constants are determined in a cross-over experimental scheme; therefore the effect of the (mixed) micelles on the disappearance of the model drug can be described by the ratio (r_{obs}):

$$r_{obs} = k_{dis \text{ with micelles}}/k_{dis \text{ without micelles}} \quad (6)$$

The 95% confidence interval (CI) of the mean ratio r_{obs} was calculated (two-sided *t*-test for one sample at the 5% level (Bolton 1982)).

Influence of taurocholate, LPC, and taurocholate + LPC on the structure of mucus. The rate limiting step in the absorption for lipophilic compounds like dantrolene, griseofulvin and ketoconazole is considered to be the diffusion through the aqueous environment adjacent to the intestinal wall. This consideration was based on the work of Komiya et al (1980), who used steroids varying in lipophilicity to assess the major absorption barrier of these compounds. For hydrophilic compounds, on the other hand, the cellular membranes of the intestinal wall provide the highest resistance in the overall absorption process.

To investigate the influence of taurocholate, LPC, and taurocholate + LPC on the mucus, the effect of the respective (mixed) micelles on the "viscosity" (gel structure) of native mucus of porcine small intestine was studied in-vitro.

Mucus possesses both viscous and elastic properties. The characterization of the "viscosity" of mucus has no real physical meaning, but is indicative for the forces which are responsible for the gel structure of the mucus. The viscosity of the mucus was assessed by determining the relation between shear stress and shear rate by means of a cone-plate viscometer. The mucus samples were defrosted for 15 min at 37°C and gently stirred before use without further purification. A mucus sample (2·60 g) was gently mixed with 150 μL of saline (control) or a concentrated solution (in saline, pH = 7·4) of the respective micelles so that in the mucus sample the required final concentration of micelles resulted (10 mM taurocholate, 1 mg mL^{-1} LPC, or 10 mM taurocholate + 1 mg mL^{-1} LPC). After mixing, the sample was transferred between cone and plate of the viscometer and allowed to equilibrate in a saturated atmosphere at 37°C for 30 min. After adjustment of the shear rate, the shear stress was read after exactly 1 min. The procedure was followed with both increasing and, subsequently, decreasing shear rates. The viscous component of the mucus was assessed by determining the slope of the arbitrarily chosen linear region of the rheogram at decreasing shear rates, as described elsewhere (Poelma et al 1990b). The effect of the (mixed) micelles on the viscosity of the mucus was compared with a control (saline) based on the same mucus batch and performed on the same day.

Results

Physicochemical characteristics of the model drugs

To define the lipophilic nature of the model drugs, both the n-octanol/water distribution coefficient (D) and the extent of solubilization in (mixed) micelles were determined. The solubility data of the model drugs in buffer solutions and taurocholate/oleic acid micellar solutions, together with the log D value of the various drugs are given in Table 3.

The log D values for paracetamol and theophylline were 0·2 and 0·1, respectively (Table 3). This indicates that these drugs are more hydrophilic than dantrolene, griseofulvin and ketoconazole, with log D values of 1·4, 1·3 and 1·5, respectively.

Based on the solubility data given in Table 3 the fraction of the drug free in solution (f) and the fraction of the drug solubilized in taurocholate/oleic acid micelles (s) were calculated according to equations 2, 3. The hydrophilic drugs paracetamol and theophylline were not solubilized in taurocholate/oleic acid micelles (for paracetamol: s = 0; for theophylline the solubility in the solution with and without taurocholate/oleic acid micelles was not significantly different: s = 0; see Table 3), whereas the lipophilic drugs dantrolene, griseofulvin and ketoconazole were solubilized. The highest fraction of drug solubilized in taurocholate/oleic acid micelles was found for ketoconazole (s = 0·84), whereas griseofulvin and dantrolene were solubilized to a lesser extent

Table 3. Physicochemical characteristics of the model compounds and solubilization in taurocholate/oleic acid mixed micelles.

				Solubility (10^{-5} M) ±s.d.			
Drug	Mol. wt	pK_a*	log D (n = 3) ±s.d.	Buffer (n = 5–42)	+Taurocholate/oleic acid (n = 5–8)	s	f
Paracetamol	151	9·5	0·2 ± 0·2	117 ± 13**	117 ± 5**	0·00	1·00
Theophylline	180	8·6	0·1 ± 0·1	54 ± 5**	51 ± 4**	≈0·00	1·00
Dantrolene	314	≈7·5	1·4 ± 0·1	2·3 ± 0·3	5·4 ± 1·4	0·57	0·43
Griseofulvin	353	>9	1·3 ± 0·1	2·8 ± 0·4	5·6 ± 0·7	0·50	0·50
Ketoconazole	531	6·5	1·5 ± 0·2	1·3 ± 0·2	8·1 ± 0·8	0·84	0·16

Key: Mol. wt is the molecular weight; * pK_a of dantrolene (Product Information Norwich Eaton Pharmaceuticals), pK_a of ketoconazole (Product Information Janssen Pharmaceutica), pK_a of paracetomol, theophylline and griseofulvin (Martindale 1982); log D is the logarithm of the distribution coefficient of the compound between the n-octanol phase and the aqueous phase under the conditions described in the text; ** solubility in 10^{-3} M: taurocholate/oleic acid refers to the solubility in the taurocholate oleic acid micellar solution; s indicates the fraction of the drug solubilized in taurocholate/oleic acid micelles; f indicates the fraction of the drug free in solution.

Table 4. Solubility of paracetamol and dantrolene in LPC micellar solutions.

Drug	Solubility (10^{-5} M) ±s.d. Buffer (n=5–42)	+LPC (n=3)	s	f
Paracetamol	117±13*	110±5*	≈0·00	1·00
Dantrolene	2·3±0·3	3·1±0·5	0·26	0·74

Key: +LPC gives the solubility in 1 g L^{-1} lysophosphatidylcholine; * solubility in 10^{-3} M: s indicates the fraction of the drug solubilized in micelles; f indicates the fraction of the drug free in solution.

Table 5. Solubility of paracetamol and dantrolene in taurocholate LPC mixed micellar solutions.

Drug	Solubility (10^{-5} M) ±s.d. Buffer (n=5–42)	+Taurocholate/LPC (n=3)	s	f
Paracetamol	117±13*	113±3*	≈0·00	1·00
Dantrolene	2·3±0·3	3·7±0·4	0·38	0·62

Key: +Taurocholate LPC gives the solubility in 10 mM taurocholate+1 g L^{-1} LPC; * solubility in 10^{-3} M; s indicates the fraction of the drug solubilized in mixed micelles; f indicates the fraction of the drug free in solution.

(s=0·50 and s=0·57, respectively). For the lipophilic drugs tested in this study the following rule holds: the higher the log D value of the drug, the better the drug is solubilized in taurocholate/oleic acid micelles (compare the log D and s value in Table 3). In Tables 4 and 5 the solubility data of paracetamol and dantrolene in micellar solutions with 1 mg mL^{-1} LPC and in micellar solutions containing 10 mM taurocholate+1 mg mL^{-1} LPC, respectively, are shown. Paracetamol was not solubilized in LPC or taurocholate/LPC micelles (the values for s were negative, but not significantly different from zero: s≈0) which confirms the hydrophilic nature of the drug. Dantrolene was slightly solubilized in the LPC and taurocholate/LPC micellar solutions (s=0·25 and s=0·38, respectively) but in taurocholate/oleic acid micelles had a greater effect on the solubilization of dantrolene (s=0·57).

Effect of taurocholate/oleic acid micelles on the disappearance kinetics of the model compounds

Paracetamol and theophylline. The disappearance kinetics of the compounds in the isolated loop were studied both with and without micelles with the same overall concentration (below the saturation concentration) of the compound. In contrast to the solubilization data for these two hydrophilic drugs, a decrease in the disappearance rate was measured in the presence of taurocholate/oleic acid micelles: for paracetamol r_{obs}=0·69±0·21 (CI: 0·36–1·02) and for theophylline: r_{obs}=0·62±0·08 (CI: 0·49–0·75) (Table 6). Because of the scatter in the data, the reduction in the disappearance rate of paracetamol in the presence of taurocholate/oleic acid micelles was not statistically significant.

Dantrolene, griseofulvin and ketoconazole. Fig. 1 shows the disappearance of griseofulvin from the perfusion solution

Table 6. Influence of taurocholate/oleic acid mixed micelles on the absorption of the model compounds.

Drug	r_{obs} (±s.d.)	CI	f	r_{cal}
Paracetamol	0·69±0·21	0·36–1·02	1·00	1·00
Theophylline	0·62±0·08	0·49–0·75	1·00	1·00
Dantrolene	0·67±0·07	0·56–0·78	0·43	0·60
Griseofulvin	0·31±0·04	0·25–0·37	0·50	0·65
Ketoconazole	0·30±0·03	0·25–0·35	0·16	0·41

Key: $r_{obs}=k_{dis}$ +taurocholate/oleic acid/k_{dis} −taurocholate/oleic acid; n is the number of experiments; CI gives the 95% confidence interval of r_{obs}; f indicates the fraction of the drug free in solution; r_{cal} is calculated according to equation 7, assuming micelle-mediated transport of the model compounds (n=4).

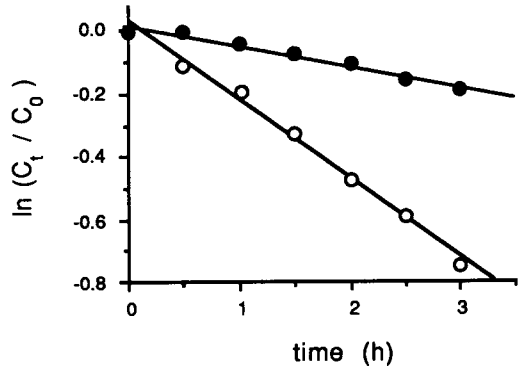

FIG. 1. Typical example of the disappearance of griseofulvin from the perfusion solution during a recirculating perfusion with [closed circles; k_{dis}=0·069 (a)] and without 10 mM taurocholate+5 mM oleic acid [open circles; k_{dis}=0·248 (b)] performed in one rat; r_{obs} (ratio a/b)=0·28.

with (k_{dis}=0·069 h^{-1}) and without 10 mM taurocholate+5 mM oleic acid (k_{dis}=0·248 h^{-1}) in the same rat. Fig. 1 illustrates that the disappearance of griseofulvin from the perfusion solution could be described by first-order kinetics. The disappearance of the lipophilic drugs, dantrolene and ketaconazole, was also markedly reduced in the presence of 10 mM taurocholate+5 mM oleic acid, as reflected in r_{obs} (Table 6).

When the disappearance data of dantrolene, griseofulvin and ketaconazole in the presence of (mixed) micelles are evaluated on the basis of the phase-separation model (eqn 4), it is expected that r_{obs} is similar to the calculated ratio (r_{cal}):

$$r_{cal} = D_{app}/D_{free} \quad (7)$$

D_{app} for griseofulvin in taurocholate/oleic acid solutions was calculated according to equation 4 based on the s value presented in Table 3 and based on data obtained by de Smidt et al (1990). They found diffusion coefficients for griseofulvin in saline (D_{free}) and in 10 mM taurocholate (D_{mic}), of 8·0 and $2{\cdot}4\times10^{-10}$ m^2 s^{-1}, respectively. The values of D_{app} for ketoconazole and dantrolene in taurocholate/oleic acid solutions and for dantrolene in LPC and taurocholate/LPC solutions were calculated in the same manner, assuming that the values of D_{free} and D_{mic} for dantrolene and ketoconazole are equal to the respective values for griseofulvin. As the molecular weights of dantrolene and ketoconazole are similar to the molecular weight for griseofulvin, this assumption can be justified. D_{app} for the (mixed) micellar solutions

Table 7. Influence of LPC and taurocholate LPC (mixed) micelles on the absorption of dantrolene and paracetamol.

Drug	r_{obs} (±s.d.)	n	CI	f	r_{cal}
LPC					
Dantrolene	1·03±0·12	8	0·93–1·13	0·74	0·83
Paracetamol	1·04±0·26	10	0·85–1·23	1·00	1·00
Taurocholate+LPC					
Dantrolene	0·83±0·10	4	0·67–0·99	0·62	0·73
Paracetamol	0·81±0·17	6	0·59–1·03	1·00	1·00

Key: LPC and taurocholate/LPC refer to perfusion experiments with 1 g L^{-1} lysophosphatidylcholine and 10 mM taurocholate + 1 g L^{-1} LPC, respectively; for further information: see the legends of Tables 5 and 6 and the text.

was calculated assuming that D_{mic} for these (mixed) micelles equals the value for D_{mic} of 10 mM taurocholate (for griseofulvin). The values for r_{cal} were calculated according to equation 7 and are given in Tables 6 and 7.

The reduction in the disappearance rate of dantrolene was in good agreement with the phase-separation model (eqns 4, 7): r_{obs} for dantrolene: 0·67±0·07 (CI: 0·56–0·78) vs r_{cal}=0·60 (Table 5). For griseofulvin, the disappearance rate in the presence of taurocholate/oleic acid micelles was reduced more than expected on the basis of the model, which considers only the fraction of free drug in solution (f) as the driving force for absorption or on the phase-separation model: r_{obs}=0·31±0·04 (CI: 0·25–0·37) vs f=0·50 (r_{cal}=0·65). For ketoconazole, the reduction in the disappearance rate in the presence of taurocholate/oleic acid micelles could not be fully explained on the basis of the phase-separation model or on the basis of the reduction of the fraction of drug free in solution (f): r_{obs}=0·30±0·03 (CI: 0·25–0·35) vs r_{cal}=0·41 (f=0·16).

Effect of LPC and taurocholate/LPC on the disappearance of paracetamol and dantrolene

The disappearance rate of paracetamol in the presence of LPC was not altered compared with its disappearance from solutions without micelles in the medium: r_{obs}=1·04±0·26 (Table 7). This was expected because the fraction of free paracetamol in the presence of LPC is unaltered (Table 4). Its disappearance rate from micellar solutions with taurocholate/LPC was reduced to a small extent: r_{obs}=0·81±0·17 (CI: 0·59–1·03) (Table 7); however, because of the scatter in the data the reduction in the disappearance rate was not statistically significant. These results suggest that the major absorption barrier for paracetamol (the cellular membranes of the intestinal wall) was not affected, or only to a limited extent, by micellar solutions of LPC and taurocholate/LPC.

The disappearance rate of dantrolene from solutions with LPC was unchanged compared with the solutions without LPC: r_{obs}=1·03±0·12 (CI: 0·93–1·13) (Table 7). If the driving force for transport is the fraction of free dantrolene in LPC solutions (f=0·75) alone, or based on the phase-separation model (r_{cal}=0·83), a slight decrease in the disappearance rate was expected.

Since dantrolene is solubilized by taurocholate/LPC micelles (Table 5), a reduction of the disappearance rate after the addition of taurocholate/LPC to the perfusion solution was expected. Because the scatter in the disappearance data was small compared with that obtained in the experiments with paracetamol, the decrease in the disappearance rate of dantrolene in the presence of taurocholate/LPC was statistically significant and in accordance with the phase-separation model: r_{obs}=0·83±0·10 (CI: 0·67–0·99) vs r_{cal}=0·73 ($P<0·05$).

Influence of taurocholate, LPC, and taurocholate+LPC on the structure of mucus

The determination of the viscosity of the mucus in the presence of the different test solutions revealed the following: (i) the viscosity of the mucus treated with 10 mM taurocholate was reduced to 68±11% (±s.d., n=13) of the value of the control sample; (ii) 1 mg mL^{-1} LPC did not affect the consistency of the mucus: a relative viscosity of 99±4% (±s.d. n=3) of the control value was measured; and (iii) addition of 10 mM taurocholate + 1 mg mL^{-1} LPC resulted in a similar reduction of the viscosity to that with 10 mM taurocholate: a relative viscosity of 73±10% (±s.d., n=3) of the control value was measured.

Discussion

The rate limiting step in the absorption for the lipophilic solutes dantrolene, griseofulvin and ketoconazole is considered to be their diffusion through the pre-epithelial aqueous diffusion layer (including the mucus) adjacent to the intestinal wall. For the hydrophilic compounds paracetamol and theophylline, the epithelial wall provides the highest resistance in the absorption.

The results presented in this report show that the hydrophilic drugs paracetamol and theophylline were not solubilized in mixed micellar solutions composed of 10 mM taurocholate+5 mM oleic acid. Because of the scatter in the data, the reduction in the disappearance rate of paracetamol in the presence of taurocholate/oleic acid micelles was not statistically significant. The observed reduction of the disappearance rate of theophylline would imply that taurocholate/oleic acid micelles affect the barrier function of the intestinal wall resulting in a decreased permeability to theophylline. This is in contrast with earlier observations with 10 mM taurocholate alone, which did not affect the epithelial wall, resulting in an altered disappearance rate of theophylline (Poelma et al 1990a). Feldman & Gibaldi (1969) reported an increased permeability to salicylate in the rat everted small intestine by 10 mM sodium taurodeoxycholate. After the addition of 5 mM phosphatidylcholine or 1 mM glyceryl monooleate+3 mM oleic acid into the bile salt solution, a pronounced decrease of the transfer rate of salicylate was observed. In contrast with these observations, Muranushi et al (1980) reported an enhanced absorption for the hydrophilic drug streptomycin in the presence of 40 mM taurocholate+40 mM oleic acid in a closed loop of rat small intestine. An explanation for this discrepancy could be the different experimental set-up, the different concentration of mixed micelles and the different physicochemical nature of the model drugs used in this study.

Paracetamol is neither solubilized in 1 g L^{-1} lysophosphatidylcholine (LPC), nor in taurocholate+LPC (mixed) micellar solutions. Because of the scatter in the observed disappearance data, it was not possible to determine if, and

to what extent, these (mixed) micelles affect the disappearance of the drug. These results suggest that the barrier function of the intestinal wall, which is the major absorption barrier for paracetamol, is not affected by LPC or only to a limited extent by taurocholate/LPC micelles.

In order to examine the influence of taurocholate and taurocholate/LPC on the mucus layer, the effect of those micellar systems on the molecular cohesion ("viscosity") of porcine intestinal mucus was determined in-vitro. Taurocholate and taurocholate/LPC reduced the viscosity of mucus to the same extent, whereas LPC alone did not exhibit an effect on the gel structure of mucus. These results are in conflict with the results obtained by Martin et al (1978), who observed a decreased viscosity of mucus in the presence of LPC in a concentration range between 0·5 and 10 mM. However, those workers measured the viscosity of purified and homogenized bronchial mucus of man, which may possess different rheological properties compared with the unpurified porcine mucus samples used in this study. Moreover, the different sources of LPC (used without further purification) and the concentration of LPC ($\approx$2 mM) applied in this study may be the reason for the difference between our data and the results obtained by Martin et al (1978).

The effect of these micelles on the mucous structure may have consequences for the absorption of lipophilic drugs. As the disappearance rate of the lipophilic drug dantrolene in the presence of LPC micelles was not altered, in spite of the decreased fraction of the drug free in solution due to micellar solubilization, this finding suggests that LPC induced a small decrease in the barrier function of the absorption barrier for dantrolene. In contrast, taurocholate/LPC caused a reduction of the disappearance rate of this drug as expected according to the phase-separation model, suggesting that the absorption barrier is not affected by taurocholate/LPC. This result is in contrast with the effect of 10 mM taurocholate alone on the disappearance of dantrolene: perfusions with the drug and 10 mM taurocholate resulted in a larger reduction of the disappearance rate than expected on the basis of the phase-separation model: $r_{obs} = 0{\cdot}54 \pm 0{\cdot}07$ (CI: 0·45–0·63) vs $r_{cal} = 0{\cdot}83$. It has been suggested that taurocholate induced an increased barrier function of the mucus layer (Poelma et al 1989). One may speculate that taurocholate and taurocholate/LPC have different interactions with the pre-epithelial diffusion barrier (including the mucus) resulting in different transport rates for dantrolene across this diffusion barrier. The effect of taurocholate and taurocholate/LPC on the gel structure of mucus does not seem to have pronounced consequences for the transfer rate of dantrolene across the pre-epithelial diffusion barrier.

The lipophilic drugs dantrolene, griseofulvin and ketoconazole were solubilized in taurocholate/oleic acid micellar solutions, and their disappearance rate in the presence of these micelles was reduced. The reduction of the disappearance rate of dantrolene could be explained on the basis of the phase-separation model. In contrast with dantrolene, the reduction in the disappearance kinetics of ketoconazole and griseofulvin by taurocholate/oleic acid could neither fully be explained on the basis of the phase-separation model nor on the basis of the model which considers only the free fraction of the drug as the driving force for transport. It is possible to speculate about the reason for the different disappearance profiles of the lipophilic drugs in the presence of taurocholate/oleic acid micelles. It could be that the assumptions made concerning the diffusion coefficients for the free drug (in the presence and absence of taurocholate/oleic acid) and the solubilized drug are oversimplifications, and that will then lead to a deviation between the expected and the observed disappearance profile. For instance, the presence of ionic charges in the molecules may also play a role in the effects of micelles on the disappearance rate. Dantrolene is partly negatively charged, ketoconazole is partly positive.

It is concluded, that the major role of micelles in the absorption process of dissolved drugs is micellar incorporation especially of lipophilic drugs, which results in a decrease of the disappearance rate. Because of the scatter in the data, caused by biological variation, the results with the micellar systems used in this study could not always be quantitatively predicted by simple physicochemical models like the phase-separation model. However, such models predicted in qualitative terms the observed reductions in disappearance rates: the more the drug is solubilized, the higher the expected reduction. In general this rule held. Reductions of the disappearance rate constant up to 70% compared with the micelle free situation were observed. The micelles definitely do not have a pronounced beneficial effect on the transfer of solutes across the pre-epithelial aqueous diffusion layer (including the mucus) and the epithelium. However, it should be realised that in-vivo these micelles can promote the overall process of drug absorption of poorly water soluble lipophilic drugs by enhancing the dissolution rate, which in many cases is the rate-limiting step.

References

Bates, T. R., Lin, S. L., Gibaldi, M. (1967) Solubilization and rate of dissolution of drugs in the presence of physiological concentrations of lysolecithin. J. Pharm. Sci. 56: 1492–1495

Bolton, S. (1982) Pharmaceutical statistics. In: Drugs and the Pharmaceutical Sciences. vol. 25, Marcel Dekker, Inc., New York, pp 138–142

Borgström, B., Dahlquist, A., Lundh, G., Sjövall, J. (1957) Intestinal digestion and absorption in the human. J. Clin. Invest. 36: 1521–1525

Coleman, R., Iqbal, S., Godfrey, P. P., Billington, D. (1979) Membranes and bile formation. Composition of several mammalian biles and their membrane damaging properties. Biochem. J. 178: 201–208

de Smidt, J. H., Offringa, J. C. A., Crommelin, D. J. A. (1990) Dissolution kinetics in bile salt solutions. J. Pharm. Sci. in press

Feldman, S., Gibaldi, M. (1969) Bile salt-induced permeability changes in the isolated rat intestine. Proc. Soc. Exptl. Biol. Med. 132: 1031–1033

Kakemi, K., Sezaki, H., Konishi, R., Kimura, T., Murakami, M. (1970) Effect of bile salts on the gastrointestinal absorption of drugs I. Chem. Pharm. Bull. 18: 275–280

Komiya, I., Park, J. Y., Kamani, A., Ho, N. F. H., Higuchi, W. I. (1980) Quantitative mechanistic studies in simultaneous fluid flow and intestinal absorption using steroids as model solutes. Int. J. Pharm. 4: 249–262

Martin, G. P., Marriott, C. (1981) Membrane damage by bile salts: the protective function of phospholipids. J. Pharm. Pharmacol. 31: 754–759

Martin, G. P., Marriott, C., Kellaway, I. W. (1978) Direct effect of bile salts and phospholipids on the physical properties of mucus. Gut 19: 103–107

Martindale (1982) The Extra Pharmacopoeia. 28th edn, Reynolds, J. F. (ed.) The Pharmaceutical Press, London

Muranishi, S., Muranushi, N., Sezaki, H. (1979) Improvement of

absolute bioavailability of normally poorly absorbed drugs: inducement of the intestinal absorption of streptomycin and gentamycin by lipid-bile salt mixed micelles in rat and rabbit. Int. J. Pharm. 2: 101–111

Muranushi, N., Kunugawa, M., Nakajima, Y., Muranishi, S., Sezaki, H. (1980) Mechanism for the inducement of the intestinal absorption of poorly absorbed drugs by mixed micelles 1. Effects of various lipid-bile salt mixed micelles on the intestinal absorption of streptomycin in rat. Ibid. 4: 271–279

Nakatomi, F., Kihira, K., Kuramoto, T., Hoshihata, T. (1985) Intestinal absorption and metabolism of norcholic acid in rats. J. Pharmacobio-Dyn. 8: 557–563

Ockner, R. K., Isselbacher, K. J. (1974) Recent concepts of intestinal fat absorption. Rev. Physiol. Biochem. Pharmacol. 71: 107–114

Poelma, F. G. J., Tukker, J. J. (1987) Evaluation of a chronically isolated internal loop in the rat for the study of drug absorption kinetics. J. Pharm. Sci. 76: 433–436

Poelma, F. G. J., Tukker, J. J., Crommelin, D. J. A. (1989) Intestinal absorption of drugs I The influence of taurocholate on the absorption of dantrolene in the small intestine of the rat. Ibid. 78: 285–289

Poelma, F. G. J., Breäs, R., Tukker, J. J. (1990a) Intestinal absorption of drugs. III. The influence of taurocholate on the disappearance kinetics of hydrophilic and lipophilic drugs from the small intestine of the rat. Pharm. Res. 7: 392–397

Poelma, F. G. J., Breäs, R., Tukker, J. J. (1990b) Intestinal absorption of drugs IV The influence of taurocholate and L-cysteine on the barrier function of mucus. Int. J. Pharm. 64: 161–169

Product Information of Janssen Pharmaceutica, Beerse, Belgium

Product Information Norwich Eaton Pharmaceuticals, New York, USA

Robinson, N. (1961) Review article. Lysolecithin. J. Pharm. Pharmacol. 13: 321–325

Stilbs, P. (1982) Fourier transform NMR pulsed-gradient spin-echo (FT-PGSE) self-diffusion measurements of solubilization equilibria in SDS solutions. J. Colloid Interface Sci. 87: 385–394

Taniguchi, K., Muranishi, S., Sezaki, H. (1980) Enhanced intestinal permeability to macromolecules II. Improvement of the large intestinal absorption of heparin by lipid-surfactant mixed micelles in rat. Int. J. Pharm. 4: 219–228

Tokunaga, Y., Muranishi, S., Sezaki, H. (1978) Enhanced intestinal permeability to macromolecules I. Effect of monoolein-bile salts mixed micelles on the small intestinal absorption of heparin. J. Pharm. Dyn. 1: 28–38

Vonk, R. J., van Doorn, A. B. D., Strubbe, J. H. (1978) Bile secretion and bile composition in the freely moving, unanaesthetized rat with a permanent biliary drainage: influence of food intake on bile flow. Clin. Sci. Mol. Med. 55: 253–259

Yamaguchi, T., Ikeda, C., Sekine, Y. (1986) Intestinal absorption of a β-adrenergic blocking agent nadolol. II. Mechanism of inhibitory effect on the intestinal absorption of nadolol by sodium cholate in rats. Chem. Pharm. Bull. 34: 3836–3843