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Compared effects of synthetic and natural bile acid surfactants on xenobiotic absorption I. Studies with polysorbate and taurocholate in rat colon

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Summary

Some expected differences between synthetic and natural bile acid surfactants relative to their influences on xenobiotic absorption are briefly outlined on the basis of literature data. Then, experimental work is presented which shows that these differences exist and that they can be even more relevant than suspected. Absorption tests were developed in rat colon in situ with polysorbate (synthetic) and sodium taurocholate (natural) surfactants, using a homologous series of phenylalkylcarboxylic acids as test compounds. At the critical micelle concentration (CMC), the two previously reported actions of synthetic surfactants on xenobiotic absorption (i.e. the increase in absorbing membrane polarity, and the nullification of the resistance of the aqueous boundary layer adjacent to the membrane to solute penetration) were exerted ad libitum by polysorbate, while taurocholate completely lacked the latter action (it is even possible that the opposite exists, i.e., an increase of the aqueous layer resistance to solute diffusion), although it apparently produces an increase in membrane polarity. At supramicellar concentrations (SMC), the solubilising action of taurocholate was almost negligible as compared with that of polysorbate. It is concluded that, as far as colonic absorption is concerned, polysorbate and taurocholate behave as different biophysical species, which could lead to substantially dissimilar in vivo effects.

Introduction

In former papers (Plá-Delfina et al., 1987; Collado et al., 1988; Garrigues et al., 1989; Fabra-Campos et al., 1990; Garrigues et al., 1990), the effects of the synthetic surfactants on the gastrointestinal absorption of different xenobiotics were studied. On the basis of the correlations found between their in vitro lipophilicity indexes, P, and their in situ rat gut absorption rate constants obtained in free solution (denoted by k_a) as well as in the presence, in the perfusion fluids, of synthetic surfactants at the critical micelle concentration, CMC (symbolized as k_o) and at supramicellar concentrations, SMC (represented by k_s), equations were deduced which, accordingly interpreted, led to the following conclusions:

(1) While correlations between k_a and P were found to be hyperbolic, bihyperbolic or bilinear,

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depending on the type of absorption site used, correlations between k_o and P were always potential (i.e. linear and double-logarithmic) in nature, with a rather low slope. This latter feature would mean that surfactant molecules or ions are capable of increasing per se the polarity of the absorbing membrane, rendering it more permeable for highly hydrophilic substances. On the other hand, the potential nature of the correlation found would mean that the synthetic surfactant would, in some manner, nullify the limiting effect on solute diffusion which can be exerted by any covering layer at the luminal side of the membrane, either lipophilic (as occurs in stomach) or hydrophilic (as in the small intestine and colon), thus giving rise to an enhanced penetration of highly lipophilic compounds. Intermediate lipophilicity solutes would be less affected in their absorption behaviour by these two surfactant actions at CMC.

(2) When a synthetic surfactant is added at SMC to the perfusion fluid, the above effects became almost completely masked by the micellar solubilization of the xenobiotics. Correlations between k_s and P became bilinear as a result of the multiple-phase equilibrium arising for the solutes between the aqueous solution, the micellar cores and the absorbing membrane. This gives rise to a progressive decrease in k_s values relative to k_o as lipophilicity increases.

The above features can help to explain most of the results reported in the specialized literature, even those which were formerly considered as contradictory (Plá-Delfina et al., 1987; Collado et al., 1988). On the other hand, they could be also promising in the design of absorption-promoted pharmaceutical preparations with poorly absorbable drugs.

Notwithstanding, it would be desirable, in the opinion of the authors, to compare the effects of the natural bile salt surfactants with those of the synthetic ones with the aid of the general approaches outlined above. From literature data, it could be advanced, in principle, that some substantial differences are to be expected between these two groups of surfactants, which could lead to a different behaviour as their respective influences on xenobiotic absorption is concerned. Let us point out briefly some of these presumed dissimilarities.

(a) Whereas synthetic surfactants, practically without any exception, give rise to the formation of micelles from a given surfactant concentration (CMC) in the perfusion fluids, bile salts, in some instances, do not (Oehler et al., 1989). Although this is not the case of the most outstanding bile salts such as taurocholate and glycocholate, the aggregation number of molecules or ions per micelle is considerably lower than that found for most synthetic surfactants; this makes a great difference in micelle polarity. Phospholipids, fatty acids and other naturally occurring compounds, however, can give rise to the formation of mixed micelles with bile salts, which could lead to enhanced absorption of some drug compounds (Muranishi, 1985).

(b) As pointed out above, all synthetic surfactants tested effectively cancelled the limiting action on solute diffusion which is exerted, in normal conditions (i.e. in the absence of surfactant) by the boundary layers adjacent to the membrane. This could not be the case of the natural bile salts since both their passive absorption rate constants in rat jejunum and the Michaelis K_m constants in rat ileum have been found to become progressively higher as the boundary layer thickness is mechanically reduced (Wilson and Dietschy, 1974; Wilson, 1981). Even more, it has been reported that some bile salts can increase the resistance of the intestinal boundary layers to the diffusion of some drugs, thus decreasing their absorption rate (Poelma et al., 1989).

(c) Whereas synthetic surfactants, under normal conditions, are not absorbed at all in the gastrointestinal tract, natural bile salts are, to a very limited extent, passively absorbed in some mean intestinal areas and in colon (Schiff et al., 1972), and very effectively absorbed by active transport in terminal zones of the small intestine (Wilson, 1981). This makes another fundamental difference when experimental conditions are to be designed. As will be pointed out later, through a careful selection of suitable proximal intestinal and colonic areas where bile salt absorption is practically negligible (and, at the same time, where their in vivo influences on xenobiotic absorption

are presumed to be maximal), most experimental design difficulties can be almost completely overcome.

From the above outlines, it becomes obvious that the effects elicited by the synthetic surfactants, either at CMC and SMC, on xenobiotic absorption could not be exerted ad libitum by the bile salt surfactants.

In this paper, some of these differences have been displayed and analyzed, from a functional point of view, through absorption studies in colon, as the ideal substrate to investigate any dissimilarity in behaviour relative to that found in free solution (Plá-Delfina et al., 1987). Tests were conducted in a rat gut in situ preparation by using a series of phenyl-alkylcarboxylic acids as test compounds. As type surfactants, polysorbate 80 (nonionic, synthetic) and taurocholate (natural bile salt) were selected. In two forthcoming papers, these features will be made extensive to the proximal small intestine and to the stomach as absorption sites.

Materials and Methods

Xenobiotics and surfactant

Six ω-phenylalkylcarboxylic acids reported in former papers (Fabra-Campos et al., 1990; Garrigues et al., 1990), from phenylpropionic to phenylcaprylic, were used as xenobiotics; they were true homologous compounds, practically completely ionized (96–99%) species at the pH of absorption tests. Perfusion concentrations ranging from 0.05 to 2 mg/ml were used, according to their own solubilities. Phenylacetic acid, which was formerly included in the series, was not finally included in correlations, as will be explained later.

Polysorbate 80 was selected as synthetic model surfactant, as it has been widely used as additive. Its effects on colonic mucosae have been clearly characterized in previous work using basic xenobiotics (Plá-Delfina et al., 1987; Garrigues et al., 1989). Two working concentrations have been used: (a) CMC, which was found to be 0.0022% w/v, and (b) an SMC of 5% w/v, in order to characterize separately molecular and micellar effects.

In another series of experiments, sodium taurocholate was employed as representative of the naturally occurring bile surfactants since it has been shown to be the major bile salt in the rat (Saunders, 1975). It was purchased from Sigma Chemical Co., with a purity of 98%. Concentrations in perfusion fluid of 4.0 mM (CMC) and 9.0 mM, a supramicellar concentration approaching that which sodium taurocholate reaches after dilution with duodenal contents (Dietschy, 1968), were used in absorption experiences.

Surfactant CMC values were determined by plotting surface tension against surfactant concentration in perfusion fluid, the first being measured by the ring method in a Lauda, Model 7201 tensiometer, at 20°C.

Colonic absorption studies

Male Wistar rats weighing 200–300 g, fasted for 20 h, were used (5 animals per compound and series except with taurocholate at CMC, where 8 animals were employed). The in situ rat gut absorption technique adapted as previously described to colon tests (Plá-Delfina et al., 1987) was performed. Previous experiences in the same conditions were developed in order to measure the extent and rate of absorption of taurocholic acid per se at SMC, as it has been established to be a passive diffusion process (Schiff et al., 1972); solutions containing 20 mM were used for this purpose.

Test solutions were prepared by dissolving a fixed amount of the acid under study and the prescribed amount of surfactant in isotonic saline buffered to pH 7.5 by adding 1% Sørensen phosphate buffer made isotonic prior to use. The pH of the resultant solution was adjusted exactly to pH 7.5 by adding the necessary amount of 1 N NaOH, and tonicity was assessed — and corrected when necessary — with the aid of a Halb-Micro, Haver osmometer.

Sampling intervals of 5 min for a total time of 30 min were used for all experiments except for phenyloenanthic and phenylcaprylic acids in surfactant-free solution experiments, in which cases sampling was carried out every 3 min after the first 5 min, in order to overcome analytical problems derived from the low acid content of the

TABLE 1

Mobile phases used for the HPLC analysis of the tested acids and retention times found

Tested acids	Per cent phase volumes		Retention time (min)
	Acetonitrile	pH 3.0 buffer	
Phenylpropionic	25	75 [a]	8.48
Phenylbutyric	40	60	3.15
Phenylvaleric	50	50	2.67
Phenylcaproic	55	45	2.76
Phenyloenanthic	60	40	2.85
Phenylcaprylic	70	30	2.70

[a] pH 5.0 buffer.

final samples. The perfused volume was, in all cases, 5 ml.

Absorption rate constants were calculated as described previously (Plá-Delfina et al., 1987). Since very little reduction in volume was noted at 30 min (less than 6%) and since it was similar for all compounds and conditions, no correction for water reabsorption was made.

Analysis of the samples

Samples were analyzed by means of HPLC as previously reported (Garrigues et al., 1990) after centrifugation at 3000 rpm for 10 min, in order to remove particulate material.

Briefly, the equipment consisted of a Waters Model 590 pump, a variable-volume U6K Injector, a Lambda-Max detector, set at 258 nm, and a Model 730 Data Module. Analytical Novapak C18 columns (150 × 3.9 mm) with 5 mm Guardpak precolumns were used. Mobile phases were mixtures of acetonitrile and aqueous 0.1 N acetic acid (pH 3.0) in variable proportions, depending on the tested acid; they are listed in Table 1. Elution was carried out at room temperature, at a flow rate of 1 ml/min.

An excellent linearity between peak area and concentration was found along the entire range of concentrations assayed ($r > 0.999$) so that only a calibration line was developed with each series of determinations. The method showed variation coefficients ranging from 0.25 to 0.89%.

Sodium taurocholate samples derived from intrinsic absorption tests were analyzed as follows: mobile phase was methanol/phosphate buffer 14.3 mM (pH 2.5)/acetonitrile (65 : 70 : 20), at a flow rate of 1 ml/min, in a Spherisorb S5 ODS2 column (150 × 4.6 mm) and quantitated at 205 nm on the same equipment as described above.

Partition studies

Only tests in the absence of surfactant were carried out, as it has been established that the presence of surfactant in the partition systems does not significantly change the values obtained, as far as correlation purposes are concerned (Collado et al., 1988).

TLC partition constants In order to determine the chromatographic $(1/R_f) - 1$ values, Merck RP-8 plates were used as substrate and a mixture of Sørensen phosphate buffer 1/30 M (pH 7.5) and acetone (45 : 55, v/v) as mobile phase. Solutions of the individual acids in methanol (1% w/v) were prepared, and 1–2 μl of each were applied as discrete spots along the baseline, positioned 2 cm from the bottom edge of the plate. Development was carried out in conventional Desaga chambers at 22°C, to 10 cm above the starting line. The spots were detected under UV light (254 nm). The mean value of six chromatograms was used for correlation purposes.

HPLC capacity factors K' values were determined by reversed-phase HPLC, using the above equipment, on a Novapak C18 column (150 × 3.9 mm) with a mixture of acetonitrile and Sørensen phosphate buffer 1/30 M (pH 7.5) (25 : 75, v/v) as mobile phase. A volume of 40–100 μl of 1–2% solutions of the acids was separately injected into the column, and its retention time was determined. Dead time was measured by injecting a 0.1% solution of sodium nitrate. Four injections were made for every compound, and the means were used for further correlations.

Molecular weights Molecular weights, M, can be used as lipophilicity indexes since a linear correlation exists between the logarithms of experimental lipophilicity constants and M, as will be shown. It occurs whenever a true homology exists between the tested compounds, and they have the advantage of being error-free constants.

Apparent internal partition coefficients These values, designated as P_a, are indexes of micellar

solubilization of the acids, which has been shown to be a partitioning process between the surfactant micelles and the aqueous luminal fluid (Tomida et al., 1978). They can be calculated through in vivo absorption rate constants as $P_a = (k_s/k_o) - 1$ (Plá-Delfina et al., 1987). They show a good correlation with dialysis data (Pérez-Buendía et al., 1989) and with lipophilicity constants. On the other hand, they can be compared with P_a values found at pH 3.0 for the same series of xenobiotics, to infer some features about the micellar solubilization process of the acids.

Fitting models to data and statistical methods

In order to correlate the absorption rate constants found under the different conditions tested, with lipophilicity indexes, the usual models previously reported (Plá-Delfina et al., 1987) were utilized. In short, three types of model equations were assayed:

(1) The collapsed bihyperbolic equation, which is theoretically applicable to correlate absorption rate constants found in colon in the absence of surfactant, but which are also suitable for series with sodium taurocholate at CMC, with lipophilicity constants:

$$k_a, k_o = \frac{k_m \cdot P^a}{B + P^a} \qquad (1)$$

where P represents any in vitro lipophilicity index, and a and B are constants for the technique.

(2) The potential equation, which, in the experiments with synthetic surfactants at CMC, shows good correlation of absorption rate constants with lipophilicities:

$$k_o = C \cdot P^d \qquad (2)$$

in which, d and C are constants depending on the technique.

(3) The bilinear equation, which should be applicable to correlate colonic absorption rate constants obtained in the presence of surfactants at SMC, and lipophilicity:

$$k_s = \frac{k_o}{1 + E \cdot P^f} \qquad (3)$$

where k_o had to be replaced by the suitable expression, that is, Eqn 1, if taurocholate experiments are fitted, or Eqn 2 when synthetic surfactants are employed; E and f are constants depending on the technique employed.

When correlations are established with respect to the molecular weight, M, the term P in the preceding equations, should be substituted by 10^M.

In all cases, parameter estimates were found through a nonlinear least-squares procedure based on the SIMPLEX algorithm (Yamaoka et al., 1985). Simultaneous fits were performed for the interdependent equations accounting for k_o and k_s estimates (i.e. Eqns 1 and 3 for taurocholate, and Eqns 2 and 3 for polysorbate).

The statistical criteria for assessing the goodness of the fits were the same as those used previously (Plá-Delfina et al., 1987): the correlation coefficient found between experimental and model-predicted absorption constants, provided that the slope was near 1 and the intercept was near 0, as well as the AIC value (Akaike, 1976) and the squared sum of residuals (SS). Peritz F-test (Harper, 1984) was used to assess differences between absorption rate constants found under the different assay conditions.

Results

Intrinsic absorption tests for sodium taurocholate in rat colon showed that it can be neglected for practical purposes. An average k_a value of 0.028 ($\pm$0.07) h^{-1} was found ($r = 0.999$). Water reabsorption studies demonstrated that less than 8% water loss existed ($V_0 = 5.03$ ml; $b = -0.0125$ ml/min; $r = 0.986$). These data account for an absorption half-life of about 24 h, so that, at the end of the experiments developed with taurocholate, about 98% of the initial amount would remain in the colonic cavity. Thus, it was assumed that taurocholic acid absorption was negligible and no correction for taurocholate content in luminal fluids was made.

Absorption rate constants found in free solution (k_a values), as well as in the presence of the tested surfactants, at CMC (k_o values) and at SMC (k_s values), are listed in Table 2, in which

TABLE 2

Absorption rate constants (± S.D.) found in rat colon for the tested xenobiotics in the absence and in the presence of surfactants (natural and synthetic), and significance of the differences assessed through the Peritz F-test (S, significant; NS, not significant); absorption rate constants are expressed in reciprocal time (h^{-1})

Tested acids	Without surfactants (k_a)	Polysorbate 80		Peritz F			Sodium taurocholate		Peritz F		
		CMC (k_o)	SMC (k_s)	k_a/k_o	k_a/k_s	k_o/k_s	CMC (k_o)	SMC (k_s)	k_a/k_o	k_a/k_s	k_o/k_s
Phenylacetic [a]	0.730 ± 0.06	0.947 ± 0.07	1.068 ± 0.08	S	S	S	0.856 ± 0.17	0.993 ± 0.15	NS	S	NS
Phenylpropionic	2.045 ± 0.16	1.809 ± 0.10	1.387 ± 0.05	S	S	S	1.899 ± 0.17	1.859 ± 0.07	NS	NS	NS
Phenylbutyric	2.541 ± 0.14	2.252 ± 0.18	1.477 ± 0.05	S	S	S	2.273 ± 0.30	2.282 ± 0.41	NS	NS	NS
Phenylvaleric	3.245 ± 0.22	2.786 ± 0.28	1.652 ± 0.16	S	S	S	2.822 ± 0.42	2.631 ± 0.27	NS	S	NS
Phenylcaproic	3.629 ± 0.15	3.429 ± 0.17	1.497 ± 0.04	NS	S	S	3.295 ± 0.05	2.951 ± 0.22	S	S	NS
Phenyloenanthic	4.141 ± 0.32	4.088 ± 0.14	1.349 ± 0.06	NS	S	S	3.257 ± 0.50	2.973 ± 0.17	S	S	NS
Phenylcaprylic	4.339 ± 0.19	4.917 ± 0.30	0.885 ± 0.08	S	S	S	3.424 ± 0.39	2.884 ± 0.25	S	S	S

[a] Not included in absorption-partition correlations.

the statistical differences between rate constants are also indicated. In Table 3, partition constants found at pH 7.5 are given.

Equation parameters of the correlations between absorption and partition constants in all conditions are shown in Table 4, with indication of the statistical figures found for each fit. In Figs 1 and 2, graphical plots representing the correlations found in the presence of polysorbate and taurocholate, as well as those obtained in free solution, are shown. Note the different shape they acquire for the two surfactant types, especially when surfactant at CMC has been utilized.

TABLE 3

Partition constants found for the tested acids by thin-layer chromatography ($(1/R_f)-1$) and by liquid chromatography (K' values), and molecular weights, used as error-free lipophilicity indexes

Tested acid	$(\frac{1}{R_f}-1)$	K'	M
Phenylacetic [a]	1.035 ± 0.07	0.087 ± 0.00	136.15
Phenylpropionic	1.487 ± 0.07	0.165 ± 0.00	150.18
Phenylbutyric	2.009 ± 0.10	0.437 ± 0.00	164.20
Phenylvaleric	2.715 ± 0.14	0.976 ± 0.01	178.23
Phenylcaproic	3.883 ± 0.27	2.102 ± 0.01	192.26
Phenyloenanthic	5.513 ± 0.39	4.813 ± 0.02	206.29
Phenylcaprylic	7.816 ± 0.70	10.939 ± 0.03	220.31

[a] Not included in absorption/partition correlations.

In Table 5, internal partition behaviour of the solutes between micelles and luminal fluid is shown.

Discussion

Correlations in the absence of surfactants

Absorption/partition correlations obtained with k_a values versus partition constants are al-

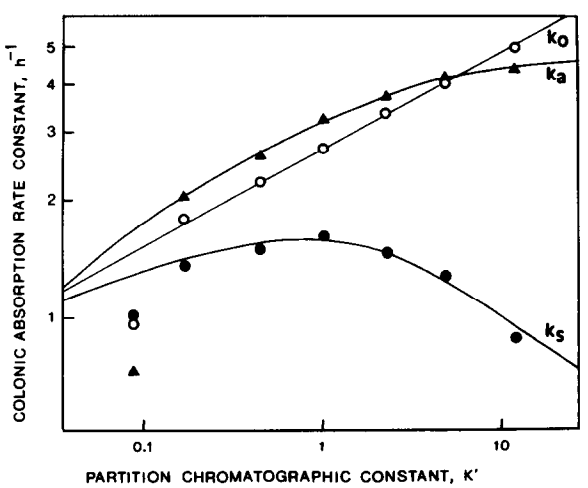

Fig. 1. Graphical plots representative of the absorption/partition correlations found for the tested acids in free solution (k_a), as well as in the presence of polysorbate, at CMC (k_o) and at SMC (k_s).

TABLE 4

Equation parameters found for absorption/lipophilicity correlations and their statistical figures; symbols are those of Eqns 1–3: fittings for k_o and k_s were simultaneously developed and global statistical figures are shown

Condition	Partition constant	Equation parameters	r	AIC	SS
Free solution	$(\frac{1}{R_f}-1)$	$k_m = 4.783$ $a = 1.584$ $B = 2.321$	0.998	−19.81	0.0135
	K'	$k_m = 5.094$ $a = 0.541$ $B = 0.589$	0.997	−15.90	0.0260
	M	$k_m = 4.921$ $a = 0.0151$ $B = 264.3$	0.998	−18.56	0.0167
Polysorbate	$(\frac{1}{R_f}-1)$	$C = 1.544$ $d = 0.588$ $E = 0.153$ $f = 1.627$	0.998	−22.54	0.0785
	K'	$C = 2.786$ $d = 0.242$ $E = 0.775$ $f = 0.679$	0.998	−25.29	0.0624
	M	$C = 0.220$ $d = 0.00610$ $E = 0.00070$ $f = 0.0171$	0.998	−24.85	0.0647
Taurocholate	$(\frac{1}{R_f}-1)$	$k_m = 3.563$ $a = 1.949$ $B = 1.759$ $E = 0.0123$ $f = 1.377$	0.995	−32.97	0.0279
	K'	$k_m = 3.689$ $a = 0.654$ $B = 0.305$ $E = 0.0595$ $f = 0.483$	0.991	−23.46	0.0615
	M	$k_m = 3.843$ $a = 0.0144$ $B = 150.0$ $E = 2.2 \cdot 10^{-6}$ $f = 0.0227$	0.991	−25.01	0.0541

ways monohyperbolic in nature, regardless of the technique used to characterize lipophilicity. This is in agreement with all the experimental data reported in the literature about colonic absorption

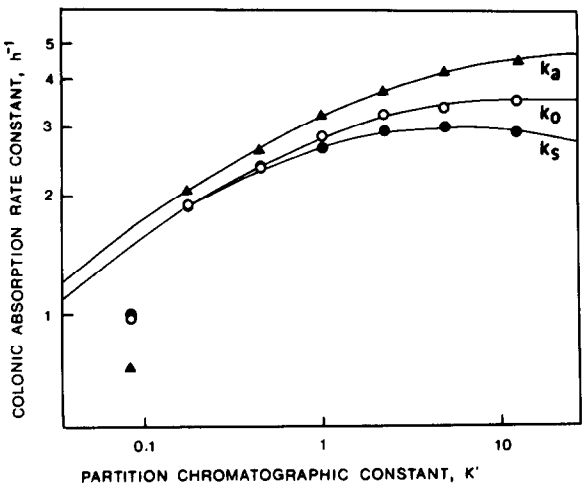

Fig. 2. Same as Fig. 1 but with the use of sodium taurocholate as surfactant instead of polysorbate.

and fits very well the model predictions (Plá-Delfina et al., 1987).

On the other hand, absolute values of k_a are much greater than expected in view of the actual ionization degree of the tested acids (96–99%); the asymptotic value ($k_m = 4.5–5.0\ h^{-1}$) is as high as has been found for lipophilic, much less ionized basic compounds using the same experimental technique (Martín-Villodre et al., 1986). This could be indicative of some absorption extent of the ionic species through the colonic membrane, as occurs with other organic substances such as

TABLE 5

Internal partition coefficients, P_a, between micelles and free solution, calculated for the acids in the presence of the two tested surfactants, at colonic pH (7.5): for comparative purposes, P_a values previously found with polysorbate at pH 3.0 are also indicated

Tested acid	Polysorbate (pH 7.5)	Polysorbate (pH 3.0)	Taurocholate (pH 7.5)
Phenylacetic [a]	(−0.113)	0.298	(−0.138)
Phenylpropionic	0.334	0.631	0.022
Phenylbutyric	0.525	1.475	0
Phenylvaleric	0.686	2.714	0.073
Phenylcaproic	1.290	4.472	0.098
Phenyloenanthic	2.030	9.647	0.102
Phenylcaprylic	4.556	23.701	0.187

[a] Not used for correlation.

sulfonamides (Plá-Delfina et al., 1980) or phenylalkylamines (Casabó et al., 1987), and should be considered in predicting the potentialities of the colonic mucosae to provide a lucrative absorption of drugs.

Phenylacetic acid has been excluded from correlation ($r = 0.983$, AIC $= -1.65$ in front of $r = 0.997$, AIC $= -15.90$ when K' values are used). The atypical behaviour of this compound has been attributed to its ionization degree, proportionally greater than that of the remaining compounds of the series, which leads to a completely ionized species at the luminal pH, as well as, to the so-called 'first-element effect', a very usual phenomenon in partition processes (Plá-Delfina and Moreno-Dalmau, 1972).

Correlations in the presence of synthetic surfactant

The results obtained here (Table 4) fully confirm the model hypothesis predictions (Plá-Delfina et al., 1987; Garrigues et al. 1989). The CMC line (k_o, P data), corresponding to the potential Eqn 2, can be easily explained if one assumes the absorption modifications exerted by the synthetic surfactants at their CMC already described. In this case, as can be observed in Fig. 1, the CMC line intersects the free solution hyperbola, showing that, for most of these compounds, the aqueous diffusion in the stagnant layer does not properly act as a limiting step because of their hydrophilicity. Therefore, only the increase in membrane polarity exerts a significant influence. Nevertheless, the behaviour of phenylcaprylic acid and the type of correlation found allow one to predict an increase in absorption rate constant if more lipophilic xenobiotics are present in the tested series.

Concerning SMC data, a bilinear correlation was found, which means that a multiphase equilibrium has been created by surfactant micelles. The observed decrease in absorption rate constants is no more than a consequence of the acid solubilization in this new apolar phase through an actual partitioning process, i.e., the phenomenon is greater as more lipophilic compounds are considered. In fact, P_a values (Table 5) can be correlated with in vitro lipophilicity constants through the Collander equation ($r = 0.993$ for TLC constants; $r = 0.986$ for K' values; $r = 0.989$ for molecular weights). Ionization of the compounds should prevent this process to some extent, which is readily seen if one compares the P_a values obtained for the same series in the presence of polysorbate at pH 3.0 (Garrigues et al., 1990) with the results reported here; there is a good accordance between these two sets of results as a correlation through the Collander equation can be established ($r = 0.992$). Therefore, the Henderson-Hasselbach equation should be applied; when this is done, however, a deviation is observed, so that a partitioning process of the ionized species needs to be included, which is in good agreement with the free solution findings.

Correlations in the presence of natural surfactant

The correlation found when sodium taurocholate was added to the perfusion fluids at CMC was clearly hyperbolic. This would mean that the biophysical model used in the absence of surfactant is also functional in this particular case; that feature implies that the natural surfactant is not able to eliminate the limiting character of the aqueous diffusion layer adjacent to the luminal side of the membrane; on the other hand, the asymptotic value, k_m, obtained in the fittings is considerably lower than that found in free solution (Table 4), thus indicating that taurocholate does have an effect on the passive absorption process. Two possibilities have to be considered according to literature findings, to interpret such an effect: (a) the surfactant increases the resistance of the limiting aqueous layer, and (b) it only increases membrane polarity; in both cases, the absorption rate constants of the lipophilic compounds of the series should decrease. Experimental data supporting every reason exist. The work of Poelma et al. (1989) tends to substantiate the first possibility, since they have found an increase in the thickness of the mucous layer by enhanced mucus secretion, which leads to an increased diffusional barrier. On the other hand, a number of authors (Saunders et al., 1975; Kimura et al., 1985) have demonstrated a direct effect on membrane permeability by sodium taurocholate at the bilayer level. It can be, of course, possible, that both actions are elicited by the natural surfactant.

Concerning the SMC correlation, the model predictions are well fulfilled, as it can be described by adding a partitioning process to the CMC biophysical system. Despite the accuracy, the solubilization of xenobiotics cannot be completely appreciated because the surfactant micelles have a very small solubilization potential, probably due to specific geometrical requirements (Mukerjee et al., 1984) when they are as a single species. It has been clearly established that bile salts show their actual ability as solubilizers in natural conditions, that is, by forming mixed micelles with lecithin and other phospholipids (Lamabadusuriya et al., 1975; Carey, 1984; Watt and Simmonds, 1984; Poelma et al., 1989). This aspect of the problem has not been studied here and should be worthy of further investigation by using the homologous series reported here.

Comparative behaviour of polysorbate and taurocholate

Two principal features make the difference between polysorbate and taurocholate as their effects on xenobiotic absorption are concerned: their behaviour on the aqueous diffusion layer at CMC, and the completely different solubilization potential at SMC.

The ability of polysorbate and, in general, of the synthetic surfactants to eliminate the limiting character of the stagnant aqueous layer for diffusion of lipophilic compounds is completely lacking in taurocholate. Consequently, the effects of the latter on xenobiotic absorption in vivo should be quite different; taurocholate as a single ionic species would rather reduce the diffusion of lipophilic solutes in all instances, relative to that found in free solution. It should be pointed out that these features could perhaps be extended to more hydrophilic substances, since it has been shown that, for other compound series, a k_o regression line which does not intersect with the k_a hyperbola has been found, thus leading to higher absorption rate constants than those of the acids tested here in the presence of polysorbate at CMC (Plá-Delfina et al., 1987).

The dissimilar solubilization potential of taurocholate micelles in front of those of polysorbate (Table 5) can be also important in the context of this investigation. The drastic reduction in absorption characteristics of the synthetic surfactants at SMC would be much lower for taurocholate, so that its actual influence on absorption could be much lower. This effect, however, could be changed in vivo when mixed micelles with physiological lipids are formed; this is, indeed, a very interesting point which should be investigated through the use of homologous series of compounds already tested in the presence of synthetic surfactants. More will be said about the question in forthcoming papers.

The above differences, however, are, in the opinion of the authors, sufficiently important to state that, as a general rule, taurocholate and polysorbate behave, as far as xenobiotic absorption is concerned, as different biophysical species, so that their effects on drug and xenobiotic in vivo absorption could also be different.

Concluding remarks

The use of a homologous series of xenobiotics has undoubtedly been a decisive feature in order to interpret comparatively the behaviour of polysorbate and taurocholate on absorption; punctual studies including only a test compound could induce errors and mislead in the appreciation of the effects of these two types of surfactants.

For example, for four of the tested acids (from phenylacetic to phenylvaleric), no differences had been found between k_a and k_o when tested alone when taurocholate was used as test surfactant, as shown in Table 2. Similarly, if phenylcaproic or phenyloenanthic acids were assayed punctually with polysorbate at CMC, no differences had been assessed between k_a and k_o. In such instances, the punctual results could have led to the erroneous impression that the surfactants are devoid of any effect on xenobiotic absorption.

On the other hand, when each of the acids, from phenylacetic to phenylcaproic, were tested in the presence of taurocholate and in the presence of polysorbate at CMC, no significant differences had been found in k_o, thus leading to the impression that the actions of both surfactants on absorption could be exactly the same; only the assay of phenylcaprylic acid in both conditions could have revealed the drastic differences in behaviour

the surfactants actually show. Most of the literature reports about the subject deal with punctual compounds, often with intermediate lipophilicity characteristics, for which the comparative effects of surfactants can hardly be discerned.

The results presented here show that the sequence of the differences found between k_a and k_o values for polysorbate series is no more than a consequence of the tendency of the latter to increase logarithmically as lipophilicity increases, whereas, for the taurocholate series, the increase in k_o runs parallel to that of k_a and is governed by a similar equation, although the k_o asymptote is lower. This constitutes a fundamental difference between the two groups of surfactants tested; through the use of a homologous series, one should conclude that synthetic surfactants show clear potentialities to increase absorption of lipophilic compounds when they are maintained in luminal fluids at submicellar concentrations, while taurocholate completely lacks such potentialities.

As far as the results with surfactants at SMC are concerned, similar considerations can be made. If any of the acids (excepting perhaps phenylacetic) was punctually tested in the presence of polysorbate and of taurocholate at SM concentrations like those reported here, the results could lead to the erroneous impression that the latter would be much more suitable to promote xenobiotic absorption; actually, the in vivo dilution of the surfactant with the intestinal contents could lead to a drastic change in behaviour for polysorbate, with actual promoting absorption actions, whereas taurocholate is devoid of such actions.

Therefore, on the whole, the use of homologous series of xenobiotics together with a correct selection of surfactant test concentrations will allow one to gain a more complete knowledge of the behaviour of each group of compounds, leading to a more rational interpretation of their effects.

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