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Gastric absorption of acidic xenobiotics in the rat: Biophysical interpretation of an apparently atypical behaviour

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Summary

In situ gastric absorption rate constants determined in rats for a series of phenylalkylcarboxylic acids in free solution were correlated with in vitro lipophilicity indexes. The correlations were bilinear in nature, in contrast with what occurs in other digestive absorption sites, where such correlations are always of the hyperbolic type. Bilinear correlations are found when heterogeneous barrier systems prevail at the absorption site; this conception seems to be in close agreement with the novel approaches describing the structure of the gastric mucosal surface, according to which a lipid lining of natural amphiphiles provides the mucosal surface with a physiological protection against its highly acid environment. This structure is not found in the intestinal mucosae, which act as homogeneous barrier systems. In the presence of surfactants in gastric perfusion fluids, the correlations became similar to those found in nonspecialized absorption sites such as colon. This would mean that the hydrophobic lining is disrupted by surfactants, thus leaving unprotected the absorbing membrane, which is then exposed to the aggressive surrounding medium. Physiological and pathological implications derived from these observations are discussed.

Introduction

Pioneer research work on passive absorption processes of drugs and other xenobiotics has included studies on every anatomical part of the gastrointestinal tract (Schanker et al., 1957, 1958; Schanker, 1959; Koizumi et al., 1964a,b; Kakemi et al., 1967a,b; Doluisio et al., 1969; Houston et al., 1974). However, since a rather irrelevant contribution of the gastric absorption was found for most compounds relative to their total absorption yield, these studies were virtually shelved, and attention was focused mainly on small intestinal and colonic absorption experiments. This led to the present situation, in which only a very limited amount of experimental data is available on absorption in the stomach.

In the present paper, the gastric absorption behaviour of several acidic xenobiotics with a wide range of lipophilicity is examined. Absorption-partition relationships are established for a true homologous series of these compounds according to a procedure which seems to be an outstanding approach to attaining a reliable interpretation of the data. The main aim of this research was to assess the absorption theories (Suzuki et al., 1970a,b; Wagner and Sedman, 1973; Kubinyi,

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1979; Plá-Delfina and Moreno, 1981) as the best tool for describing passive absorption mechanisms, and to elucidate some features associated with the physiological absorbing structures. It was also intended to assess the important question of the behaviour of these compounds in the presence of synthetic surfactants. The results not only confirmed previously established criteria for judging the influence of these types of additives on xenobiotic absorption (Plá-Delfina et al., 1987; Collado et al., 1988; Garrigues et al., 1989) but could also confirm, from a biophysical viewpoint, the unique structural features of the gastric mucosal surface, as reported by Hills and coworkers (1983); this structure could be substantially modified by synthetic surfactants, as it presumably is by other physiologically aggressive substances. If an extrapolation of the present conclusions to a general situation were possible, it would perhaps lead to a better insight into the mechanisms involved in some pathological processes such as peptic ulcerogenic activity of some xenobiotics and drugs.

Materials and Methods

Xenobiotics and surfactants

Seven ω-phenylalkylcarboxylic acids belonging to a true homologous series derived from phenylacetic acid, by extension of its alkyl chain with a $-CH_2-$ group, were used in the experiments (Table 1). They were used as practically unionized species, and were supplied as reactive grade products (Merck, A.G., Janssen Co. and Pfaltz and Bauer Co.); their purity was checked by HPLC. Perfusion concentrations ranging from 0.004 to 0.20% (w/v) were used, according to their own solubilities.

TABLE 1

Composition of the chromatographic phases and retention times for the tested acid compounds

Tested acid	Phase composition (%, v/v)		Retention time (min)
	Acetonitrile	pH 3.0 buffer	
Phenylacetic	30	70	2.77
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.

Polysorbate 80 and sodium lauryl sulfate were selected as model surfactants, which have been widely used as additives and which are entirely compatible with the tested acids. The concentration used was the critical micellar one (CMC; 0.0022% for polysorbate and 0.0173% for lauryl sulfate, both w/v), but a supramicellar concentration (SMC) was also employed for polysorbate (5.0%, w/v). The absorption rate constants found in the absence of surfactants are designated by the symbol k_a whereas those in the presence of surfactant at the CMC or SMC are represented by k_0 and k_s, respectively.

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

Absorption studies

Tests were performed on male Wistar rats weighing 200–280 g. Eight animals per compound were used for experiments carried out in free solution (i.e. in the absence of surfactant) and in the presence of surfactants at CMC, and five animals for the series with polysorbate at the SMC. The rat gut in situ preparation (Doluisio et al., 1969) was employed but only 4 ml of the test solution were perfused in order to avoid an excessive distension of the stomach.

The sampling intervals were 5 min for a total time of 30 min, except for ω-phenylcaproic, ω-phenyloenanthic and ω-phenylcaprylic acids in the experiments with surfactant at CMC, and for the last one in free solution. In these cases, sampling was carried out every 3 min after the first 5 min; the absorption kinetics have proven to be difficult to assess in any other way, at least in most animals, because of analytical problems derived from the low acid content of the final samples.

Absorption rate constants were calculated as

previously described (Martín-Villodre et al., 1986; Plá-Delfina et al., 1987). Since very little reduction in volume was noted at 30 min (less than 4%), no correction for water reabsorption was made (Doluisio et al., 1969).

Partition studies

Only tests in absence of surfactants were carried out, since it has been shown (Plá-Delfina et al., 1987) that there are no substantial differences between these results and those found in the presence of polysorbate as far as correlation with absorption constants is concerned.

Partition coefficients. Bulk-phase partition coefficients, P, between two selected solvents (chloroform and *n*-octanol, both of Merck analytical grade), and aqueous 0.082 M citrate-0.036 M phosphate buffer (pH 3.0) were determined according to the classical approaches (Leo et al., 1971; Curry and Whelpton, 1983). Six determinations for every acid were carried out and mean values were used for correlation.

TLC partition constants. Chromatographic $(1/R_f) - 1$ values were determined on Merck RP-8 chromatoplates. 1–2% solutions of the acids in methanol were prepared and 2 μl samples were applied to the plate, 2 cm from its lower edge. After evaporation of the solvent, an ascending, one-dimensional development (10 cm length) was carried out using a mixture of the above-mentioned citrate-phosphate buffer (pH 3.0) and acetone in volumetric proportions of 3 : 7, at 22 °C. The plates were then removed and, following solvent evaporation, the acids were detected under UV light at 254 nm. Six chromatograms were recorded and the mean values were used for absorption-partition correlations.

Capacity factors. Lipophilicity indexes, K', were determined on a Novapak C-18 column (150/39 mm) using as mobile phases several mixtures of 0.1 M acetic acid (pH 3.0) and acetonitrile in different volumetric proportions, from 65 : 35 to 35 : 65, with a step of 5 : 95. Solutions (1–2%) of the acids were injected into the column and their K' values were calculated using the classical formula ($K' = (t_r - t_0)/t_0$), where t_r represents the acid retention time, and t_0, the retention time of a nonretainable peak (sodium nitrate in our case). Four injections were performed for every compound and mobile phase, and the means were used for further calculations. Since the plots of log K' against proportion of acetonitrile in the solvent for the tested compounds showed a good relationship from the 45 : 55 mixture, log K'_0 values (i.e. values extrapolated to 0% acetonitrile in the mobile phase) were used for correlation purposes.

In addition to these lipophilicity indexes, the molecular weights of the compounds, M, were utilized for correlation with absorption rate constants. These values can be used when true homology exists between the compounds (Garrigues et al., 1989); their main advantage is that they are error-free constants.

Apparent internal partition coefficients

The partitioning behaviour of the tested acids between surfactant micelles and luminal gastric fluid was calculated. Internal partition coefficients, P_a, were determined from in situ absorption constants as previously described (Plá-Delfina et al., 1987). These values were correlated by means of the Collander equation (Leo et al., 1971) with the remaining lipophilicity indexes in order to ensure that micellar solubilization was a true partitioning process, as indeed proved to be the case.

Analysis of the samples

Both biological and partition samples were analysed for their acid content by HPLC, which provided an excellent separation and quantitation technique. A Waters Model 590 pump, a variable-volume U6K injector, a Lambda-Max detector set at 258 nm, and a Model 730 Data Module were utilized. Analytical Novapak C-18 columns (150/39 mm) with 5 mm Guardpak precolumns were employed.

Mixtures of acetonitrile and aqueous 0.1 M acetic acid (pH 3.0) in variable proportions, depending on the tested solutes, were used at a flow rate of 1 ml/min, at room temperature. The compositions of the mobile phases used for the different acids are listed in Table 1. Samples were injected into the column after centrifugation at 3000 rpm for 10 min, in order to remove par-

ticulate biological material or to achieve a complete phase separation.

Excellent linearity between peak area and concentration was observed for every compound over the entire range of concentrations assayed, in both biological and partition samples, with a negligible ordinate-axis intercept. Nevertheless, a calibration line was employed with each determination or series of determinations in order to avoid miscalculations. Coefficients of variation ranging from 0.43 to 6.65 were found.

Fitting of models to data

Correlations found in the absence of surfactant. Since the stomach was considered to be, in principle, a nonspecialized absorption site, absorption-partition data were fitted, as in other instances, to the collapsed hyperbolic equation (Wagner and Sedman, 1973; Plá-Delfina and Moreno, 1981):

$$k_a = \frac{k_m \cdot P^a}{B + P^a} \tag{1}$$

Here, k_a represents the absorption rate constant of any compound belonging to the series; k_m denotes the limiting asymptotic value of k_a for the series, which would equal the diffusion rate constant of the compounds across the stagnant aqueous layer adjacent to the absorbing membrane; and a and B are constants for the technique. P represents the in vitro lipophilicity constant, and was substituted by the actual lipophilicity index used for every fitting, i.e., P_c and P_o for the chloroform and *n*-octanol bulk-phase partition coefficients, respectively, the value $(1/R_f) - 1$ for TLC partition constants, and K_0' for HPLC capacity factors. When molecular weight, M, was used and since a direct proportionality exists between log P and M for true homologous series of compounds (Plá-Delfina and Moreno, 1981), the figure 10^M was employed for correlation instead of P in Eqn 1 and in the subsequent expressions.

Since Eqn 1 did not yield, in any case, satisfactory results, the bilinear fit, proposed by some authors (Kubinyi, 1979), was also assayed through the following equation:

$$k_a = \frac{C \cdot P^d}{1 + E \cdot P^f} \tag{2}$$

where the parameters are constants depending on the technique.

The statistical criteria for assessing the quality of the fits were the same as those employed previously (Plá-Delfina and Moreno, 1981; Martín-Villodre et al., 1986; Casabó et al., 1987), i.e., the correlation coefficient found between experimentally observed and model-predicted k_a values, and the Akaike information criterion, AIC (Akaike, 1976).

Correlations found in the presence of surfactants. Absorption and partition data were fitted to previously established model equations (Plá-Delfina et al., 1987; Garrigues et al., 1989). Briefly, in the presence of surfactant at or below the CMC, a potential equation should be functional:

$$k_0 = G \cdot P^h \tag{3}$$

or, in logarithmic form:

$$\log k_0 = h \cdot \log P + g \tag{3a}$$

where k_0 is the absorption rate constant of any compound; P has the same meaning as above; and h, G and g ($= \log G$) are readily calculable constants, characteristic of the experimental technique.

In the presence of surfactant (polysorbate) at the SMC in the perfusion fluid, a bilinear-type equation should be applied:

$$k_s = \frac{G \cdot P^h}{1 + L \cdot P^m} \tag{4}$$

or, in logarithmic form:

$$\log k_s = h \cdot \log P - \log(L \cdot P^m + 1) + g \tag{4a}$$

where k_s represents the absorption rate constants characteristic of each compound within the series, and L and m are constants which can be readily calculated, like the others already mentioned, as described previously (Plá-Delfina et al., 1987).

For the series with polysorbate at the CMC and at the SMC, fitting was applied together (Eqns 3 and 4), as they are interdependent. To assess the goodness of fit, correlation coefficients between

experimentally found and model-predicted k_0 and k_s values were calculated. Since the correlations were excellent, no further fits were assayed.

Results

Absorption rate constants

The k_a, k_0 and k_s values found for the tested solutes under our experimental conditions are listed in Table 2. In all cases, they were clearly first-order constants and showed excellent correlation coefficients (> 0.990), for both individual and average values.

Partition constants

The bulk-phase partition coefficients determined in chloroform (P_c) or in *n*-octanol (P_o), chromatographic TLC ($(1/R_f) - 1$) and HPLC (K_0') constants, as well as molecular weights (M) are shown in Table 3. Internal partition coefficients (P_a) between micelles and free solution are also indicated; they were consistent with the in vitro lipophilicity constants already described (correlation coefficients ranging from 0.992 to 0.998), and, therefore confirm the theory on surfactant micellization.

Absorption-partition correlations

In the absence of surfactant in the perfusion fluid and through the statistical comparisons outlined above, the correlation found between k_a and partition constants was, surprisingly, clearly bilinear instead of hyperbolic in nature, irrespective of the type of partition constant used. This is in contradiction with the results obtained previously

TABLE 2

Absorption rate constants ($\pm$ SD) experimentally found for the tested acid xenobiotics in rat stomach under different conditions

Tested acids	No surfactant (k_a)	Polysorbate		Lauryl sulfate
		0.0022% (CMC) (k_0)	5.0% (SMC) (k_s)	0.0173% (CMC) (k_0)
Phenylacetic	0.957 ± 0.163	0.715 ± 0.122	0.553 ± 0.066	1.082 ± 0.258
Phenylpropionic	1.296 ± 0.164	0.902 ± 0.191	0.553 ± 0.082	1.359 ± 0.156
Phenylbutyric	1.366 ± 0.104	1.180 ± 0.118	0.477 ± 0.088	1.531 ± 0.223
Phenylvaleric	1.655 ± 0.151	1.513 ± 0.253	0.407 ± 0.070	1.718 ± 0.163
Phenylcaproic	1.905 ± 0.332	1.948 ± 0.477	0.356 ± 0.036	2.122 ± 0.292
Phenyloenanthic	2.085 ± 0.331	2.747 ± 0.190	0.258 ± 0.025	2.894 ± 0.297
Phenylcaprylic	1.870 ± 0.178	3.384 ± 0.490	0.137 ± 0.022	3.711 ± 0.726

TABLE 3

Partition constants ($\pm$ SD) and molecular weights of the tested solutes used for correlation with absorption rate constants (calculated micelle / solution 'internal' partition coefficients are also given)

Tested acids	P_c (Chloroform)	P_o (*n*-Octanol)	$(1/R_f) - 1$ (TLC)	K_0' [a] (HPLC)	M (Molecular weight)	P_a (internal)
Phenylacetic	3.72 ± 0.06	25.86 ± 0.11	0.444 ± 0.02	3.98	136.15	0.293
Phenylpropionic	20.56 ± 0.31	71.12 ± 0.62	0.569 ± 0.04	8.61	150.18	0.631
Phenylbutyric	65.45 ± 0.73	206.9 ± 9.4	0.701 ± 0.03	16.60	164.21	1.474
Phenylvaleric	244.9 ± 17.1	498.5 ± 43.4	0.867 ± 0.03	40.10	178.24	2.717
Phenylcaproic	949.0 ± 15.3	1866 ± 13.6	1.075 ± 0.07	96.74	192.27	4.472
Phenyloenanthic	3878 ± 51.6	4308 ± 85.9	1.318 ± 0.06	236.9	206.29	9.647
Phenylcaprylic	15976 [b]	12383 ± 601	1.692 ± 0.07	400.9	220.32	23.701

[a] Extrapolated, calculated through K' average values. [b] Theoretical value.

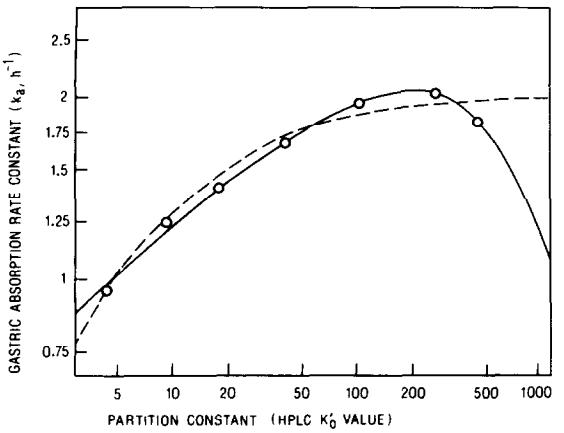

Fig. 1. Graphical plots representing hyperbolic (Eqn 1, broken line) and bilinear (Eqn 2, unbroken line) correlations between absorption rate constants determined in the absence of surfactant (k_a values), and chromatographic K'_0 constants. In light of statistical criteria, the bilinear correlation is clearly better (Table 4).

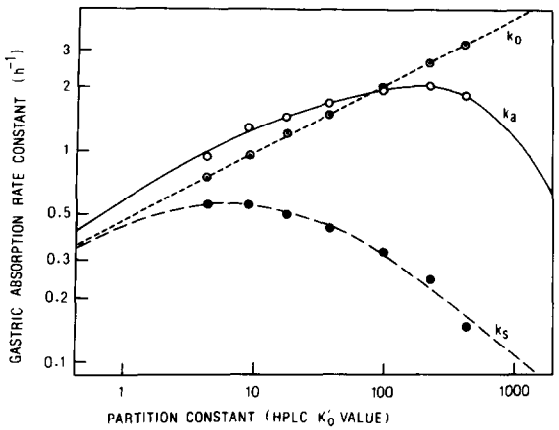

Fig. 2. Graphical plots showing the three types of correlation which are found between absorption rate constants determined in the absence of surfactant (k_a), and in the presence of polysorbate at CMC (k_0) or at SMC (k_s), and chromatographic K'_0 constants, for the acid compounds tested.

in nonspecialized absorption sites such as the colon (Schanker, 1959; Houston and Wood, 1980; Martín-Villodre et al., 1986; Casabó et al., 1987). The possible reasons for this apparently atypical behaviour will be discussed later. The fits and statistical data associated with each model equation are set out in Table 4. In Fig. 1 representative plots of k_a against capacity factors as partition constants (K'_0) are displayed in order to provide an indication of the quality of the two possible fits merely by visual inspection.

In the presence of surfactants, correlations were as presumed from previous work (Plá-Delfina et al., 1987; Garrigues et al., 1989). At the CMC, a clear potential (linear and double-logarithmic)

TABLE 4

Equation parameters and statistical figures associated to the hyperbolic and bilinear correlations between absorption rate constants of the solutes in the absence of surfactant in the perfusion solution, and lipophilicity indexes

Model equations	Equation parameters/ Statistical figures	P_c	P_o	$(1/R_f)-1$	K'_0	M
Hyperbolic (Eqn 1)	k_m	2.1201	2.1060	2.1118	2.1034	2.1041
	a	0.3768	0.5113	2.3795	0.6761	0.0160
	B	2.1031	6.3447	0.1808	3.0244	195.91
	r	0.968	0.967	0.966	0.976	0.967
	AIC	−13.57	−13.43	−13.04	−15.33	−13.37
Bilinear (Eqn 2)	C	0.8512	0.6615	1.8369	0.7651	0.2104
	d	0.1207	0.1537	0.7412	0.2113	0.0050
	E	1.58×10^{-5}	4.98×10^{-8}	0.0112	1.40×10^{-5}	1.10×10^{-11}
	f	1.0634	1.6882	7.0289	1.7286	0.0482
	r	0.996	0.990	0.993	0.992	0.992
	AIC	−25.47	−19.39	−23.02	−21.61	−21.35

TABLE 5

Equation parameters and statistical figures associated with the correlations between absorption rate constants found in the presence of lauryl sulfate at the CMC and SMC (Eqn 3) and in that of polysorbate at the CMC and SMC (Eqns 3 and 4, fitted together) in perfusion fluids, and lipophilicity indexes

Tested surfactant	Equation parameters/ Statistical figures	P_c	P_o	$(1/R_f)-1$	K_0'	M
Lauryl sulfate	G	0.7699	0.4877	2.1544	0.6678	0.1159
	h	0.1599	0.2117	0.9947	0.2759	0.0068
	r	0.992	0.987	0.991	0.983	0.990
Polysorbate	G	0.5286	0.3010	1.8401	0.4312	0.0529
	h	0.1935	0.2577	1.2000	0.3408	0.0082
	L	0.1572	0.0403	4.1132	0.1050	0.0004
	m	0.5074	0.6579	3.1071	0.8596	0.0215
	r	0.999	0.998	0.999	0.998	0.999

correlation was found between k_0 and the partition constants, for both the polysorbate and lauryl sulfate series. At the SMC, the correlation found was clearly bilinear for polysorbate, the only surfactant tested at this concentration. The goodness of the fits can be appreciated on consideration of Table 5 and Fig. 2, which shows representative plots of k_a, k_0 and k_s against K_0' as partition constants.

Discussion

Correlations found in the absence of surfactant

As deduced from the reported results (Table 4, Fig. 1), the correlation between gastric absorption rate constants and lipophilicity indexes is, undoubtedly, of the probabilistic type, i.e., bilinear, in contrast with those determined previously in the colon and small intestine, which were in every case of the compartmental type, i.e. hyperbolic or bihyperbolic (Ho et al., 1977; Plá-Delfina et al., 1980; Plá-Delfina and Moreno, 1981; Martín-Villodre et al., 1986; Casabó et al., 1987). Some apparently probabilistic correlations claimed to be functional in the small intestine have been demonstrated to be artifactual (Ho et al., 1977; Plá-Delfina et al., 1980; Plá-Delfina and Moreno, 1981). In fact, reports of true and reliable probabilistic correlations between absorption and partition constants in mucosal absorbing membranes are extremely rare in the literature; an outstanding example of these types of correlations is, however, that reported for steroid compounds in the corneal ocular epithelium by Schoenwald and Ward (1977), which will be discussed later.

The different nature of the correlations found in gastric and intestinal absorption sites should be considered to be fundamental, since hyperbolic-type correlations are associated with homogeneous, single-lipophilic barrier systems (Suzuki et al., 1970a,b; Wagner and Sedman, 1973; Plá-Delfina and Moreno, 1981), while bilinear or parabolic relationships are found whenever a heterogeneous system (i.e. alternate lipophilic and hydrophilic coatings) acts as an absorption barrier (Lien, 1975; Plá-Delfina et al., 1975; Kubinyi, 1979). On this basis, an essential structural difference seems to exist between the mucosal gastric surface and the remaining digestive mucosae.

A further revision was, therefore, undertaken in order to gain an insight into the nature of the gastric epithelium. For several years, researchers' attention has been focused on the fact that gastric mucosa is extremely resistant to its highly acidic environment. A presently generalized view is that along the time course of evolution, the stomach of superior animals has developed, against its sur-

rounding aggressive medium, a protective barrier (Davenport, 1965), amphiphilic in nature (Hills et al., 1983), which perhaps may have replaced the single original aqueous stagnant layer which exists, for example, in the small intestine or the colon (Ho et al., 1977; Higuchi et al., 1981; Plá-Delfina et al., 1987). Such a structure would consist of a natural phospholipidic layer with its lipoidal ends directed towards the luminal cavity and its hydrophilic heads oriented to tissue cells and connected with the glycocalix stroma by moderate-energy electrovalent bonds (Hills et al., 1983). Since the glycocalix is, in turn, in intimate contact with the lipoidal absorbing membrane of

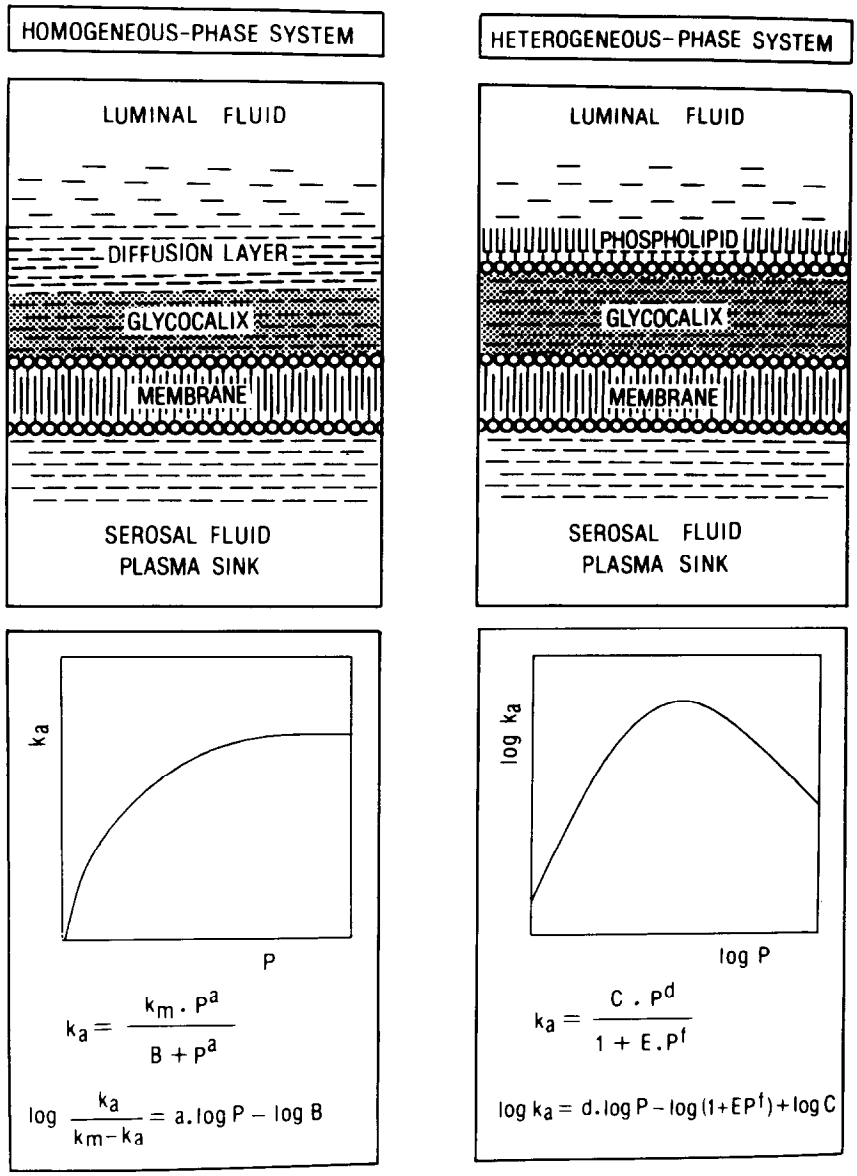

Fig. 3. Schematic representation showing the ideal features of absorption sites as barrier systems, as well as the correlations which can be expected in each case. (Left) Colon; (right) stomach, presumed case.

the columnar cells, the above structure must act, as far as xenobiotic diffusion is concerned, as a heterogeneous barrier system:

Phase system	Nature
Intraluminal xenobiotic solution	Hydrophilic
↓ ↑	
Phospholipidic layer	Lipophilic
↓ ↑	
Aqueous glycocalix system	Hydrophilic
↓ ↑	
Columnar cell membrane bilayer	Lipophilic
↓	
Serosal and plasma fluids (sink)	Plasma sink

As pointed out above, whenever such a system exists at the absorption site, correlations between absorption and lipophilicity constants for homologous series of compounds become probabilistic in nature, as occurs with pharmacological activity/lipophilicity correlations in a broader sense (Hansch and Clayton, 1973). In Fig. 3, the differences between the two types of biological substrates and the respective correlations as concerns absorption and lipophilicity are depicted schematically. This could effectively explain the bilinear correlation found in the stomach for the tested series of xenobiotics when absorption experiments are carried out in free solution.

The specialized literature contains criteria that lend support to the above assumptions. As pointed out earlier in this article, the only probabilistic correlation found in mucosal membranes between absorption and lipophilicity which can be considered reliable is that obtained for steroids in the rabbit cornea (Schoenwald and Ward, 1977). A detailed revision of the literature dealing with the features of the corneal epithelium revealed that the corneal connective tissue is covered by a water layer which, in turn, is covered by a thin layer of natural lipids (Holly et al., 1974). The stability of this structure is of great importance in vision physiology; from our viewpoint, however (i.e. as referred to transcorneal passage of xenobiotics), it is no more than a heterogeneous barrier system which must be traversed by the compounds in order to reach the systemic bloodstream beyond the cornea. The morphological similarities between corneal and gastric lining structures — if we momentarily forget the quite different functions that each biological substrate must fulfill — become evident.

Thus, we believe that the above assumptions are reasonable, although they should be confirmed for other homologous series of xenobiotics, since correlations which have been reported in the stomach up to now are not conclusive, mainly because of an insufficient range of lipophilicity of the compounds which have been assayed (Koizumi et al., 1964a; Kakemi et al., 1967a) but also because of other experimental shortcomings (Houston et al., 1974).

Correlations found in the presence of surfactants

The present results indicate that the correlations between absorption and lipophilicity found in the presence of surfactants are of the same type as those reported previously in other digestive mucosal surfaces, such as the colon or small intestine (Plá-Delfina et al., 1987; Collado et al., 1988; Garrigues et al., 1989). In other words, in the presence of surfactants at the CMC, correlations are potential (i.e. linear and double-logarithmic) in nature, whereas in the presence of polysorbate at the SMC they are bilinear (Table 5, Fig. 2). In view of the results obtained for the tested compounds in free solution, this observation was, in principle, somewhat surprising, since the only possible explanation for such behaviour is that this relationship arises as a consequence of the rupture or degradation of the phospholipidic protective layer, undoubtedly because of the action of a surfactant.

Confirmatory criteria for this assumption can be found again in the literature dealing with the features of the corneal epithelium. In order to ensure complete stability of the amphiphilic protection film, the eye must use natural surfactants at the two sides of the aqueous intermediate layer; these substances can interact with the lipids, thus enhancing the pressure contact of the film and contributing in this manner to correct visual function (Holly et al., 1977); the properties of these natural surfactants have been compared with those

of the synthetic forms, and it has been shown that their properties are completely different in fundamental aspects (García-Domínguez, 1980). For example, whereas the former effectively contribute to lipid film stability, synthetic surfactants break up and disintegrate the lipid layer, allowing the corneal stroma to be exposed directly to the external environment, with the consequent harmful effects. Unfortunately, permeability tests of xenobiotics across the cornea in the presence of surfactants, which could be taken as reference points for comparison, are not available.

In order to explain our results, let us consider the two cases separately, as are the correlations found in the presence of surfactant at CMC and at SMC.

Surfactants at CMC. The rupture of the phospholipidic layer would allow intimate contact of xenobiotics and surfactant molecules or ions with the absorbing membrane and would give rise to two surfactant effects, if one were to take the series of events occurring in the small intestine or the colon as being representative (Plá-Delfina et al., 1987; Collado et al., 1988; Garrigues et al., 1989). In these substrates one observes: (a) the elimination of the limiting character of any aqueous layer adjacent to the membrane; and (b) an increase in membrane polarity, due to the direct action of the surfactant on biological substrate.

Whereas the first effect was clearly evident from our results (i.e. Eqn 2 reduces to Eqn 3), the second phenomenon was difficult to assess, since the correlation found in free solution was not hyperbolic and cannot be taken strictu sensu as a comparison term. On the other hand, the lipophilicity range of the intermediate acids of the series makes these compounds not excessively sensitive to permeability-increasing phenomena (Plá-Delfina et al., 1987; Garrigues et al., 1989), so that it could be expected that they would reach k_0 values not very different from k_a; the fact that the k_0 line found in the presence of lauryl sulfate has a lower slope (h in Table 4) than that of polysorbate would appear to indicate, however, that some effect on membrane permeability does exist and that it differs between the two surfactants tested.

Be that as it may, one could summarize these observations by saying that a dramatic change in behaviour due to the disappearance of limiting factors for diffusion is clearly observed. The specific features of this change seem to indicate that the protective gastric phospholipid layer is removed by the tested surfactants at their CMC in the perfusion fluids.

Surfactants at SMC. The fact that synthetic surfactants give rise to micelles satisfactorily explains the bilinear correlation which has been found in the presence of polysorbate at a clearly SMC value, as is 5%. Such a phenomenon has already been described and interpreted (Plá-Delfina et al., 1987). Briefly, micelle cores will act as a nonpolar phase where xenobiotics are trapped to an extent which depends on their lipophilicity, since it is a partitioning process. By virtue of this phenomenon and taking into account that micelles cannot be absorbed (Levy et al., 1966; Gibaldi and Feldman, 1970), the tested xenobiotics will show a substantial decrease in their absorption constants relative to those found at the CMC according to a bilinear sequence due to the appearance of a multiple-phase heterogeneous equilibrium of the solutes (i.e. luminal fluid/micelles/luminal fluid/membrane/plasma), as shown in Fig. 2 (k_s values). Note that as lipophilicity decreases, the CMC and SMC lines tend to converge, since micellization becomes minimal or negligible.

Concluding remarks

The reported observations could transcend, in some aspects, the context of the present investigation and could have some implications in other fields, that are apparently not related with xenobiotic absorption, such as the mechanisms involved in the drug-mediated ulcerative processes.

On the basis of contact angle measurements on the gastric surface after incubation of the mucosa with solutions of deoxycholate or aspirin, it has been postulated that these xenobiotics are capable of disrupting the phospholipidic layer which lines the gastric luminal surface (Hills et al., 1983). In the present paper, the same evidence has been obtained for the synthetic surfactants polysorbate and lauryl sulfate through the study of the correlations between absorption and partition constants.

Since all these compounds (anti-inflammatory drugs, bile acids and surfactants) have been shown to be ulcerogenic in nature (Davenport, 1964, 1968; Black et al., 1971), one can wonder whether this effect might cause the unprotected gastric mucosa to become exposed to the highly acidic environmental medium, thus constituting the first step toward ulceration of large mucosal areas. It has been suggested that some indirect ulcerogenic mechanisms (i.e. via prostaglandins) may also be explained in light of the integrity of the lipid layer, since prostaglandins are known to stimulate phospholipid secretion, and any factor that inhibits their production such as anti-inflammatory drugs, would be potentially ulcerogenic (Hills et al., 1983); this would offer, as these authors point out, a single physicochemical explanation for the current theories about the cytoprotective action of the prostaglandin drugs (Robert, 1979).

Be that as it may, in the opinion of the present authors, the possible role of these phenomena in the context of the peptic ulcer etiology may be worthy of investigation through the establishment and interpretation of suitable absorption-partition correlations for xenobiotic series in the absence and presence of potential or suspected ulcerogenic substances.

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