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A modelistic approach showing the importance of the stagnant aqueous layers in in vitro diffusion studies, and in vitro-in vivo correlations

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Summary

The present study deals with the role of the aqueous diffusion layers on the in vitro penetration of xenobiotics across artificial lipoidal membranes, and their ability to reproduce biophysical absorption models when in vivo results are to be simulated from the in vitro tests. The aqueous boundary layers which are invariably formed on artificial lipoidal membranes can be optionally preserved or disrupted, according to the type of absorption site which should be simulated, a condition which could reasonably lead to a better correspondence between in vitro and in vivo results; in practice, disruption of water layers can be easily achieved by a synthetic surfactant solution at its critical micelle concentration, in contact with the desired membrane side. This approach is illustrated by using a dimethylpolysiloxane artificial membrane, 4-alkylanilines as permeant test compounds, and the nonionic polysorbate 80 as model surfactant, in a series of experiments developed with a Franz-type diffusion cell apparatus. Experimental designs simulating different types of biological absorbing membranes, merely by changing the number of aqueous boundary layers, are described and analyzed. Apart from the effects of the water layers, it is shown that perfect sink conditions at the receptor side must be strictly maintained in order to obtain reliable results.

Introduction

Passive drug penetration across absorbent membranes involves both the intrinsic resistance of the lipidic barriers themselves and that of the adjacent aqueous boundary layers, which can provide substantial additional strength against the passage of penetrants (Stehle and Higuchi, 1972; Lovering and Black, 1974). Since aqueous and lipoidal diffusion processes are consecutive, the ability of a given member of a homologous series of drugs or xenobiotics in general to reach any receptor phase, out of the membrane, will depend on its permeation capacity across both diffusion barriers. Thus, for highly lipophilic compounds of the series, which can readily cross the lipoidal barriers, diffusion across aqueous layers could become rate-limiting, whereas for highly hydrophilic, easily water-diffusible members of

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the series, diffusion in the lipoidal barrier could be a rate-limiting step for penetration (Stehle and Higuchi, 1972; Lovering and Black, 1974; Ho et al., 1977; Wagner, 1979).

When adjacent to the lipoidal membrane at its luminal side, aqueous boundary layer thickness can be modified by agitation (Ho et al., 1977) and, for in vitro experiments, the importance of suitable stirring conditions in order to obtain reproducible results has been recognized (Stehle and Higuchi, 1972; Barry, 1983). In comparative studies, the existence of the aqueous boundary layers, although acknowledged, has been sometimes virtually ignored because of its uniformity throughout a strictly designed and controlled steady-state experimentation work (Sato and Kim, 1984; Ackermann et al., 1985, 1987; Ackermann and Flynn, 1987). In the opinion of the present authors, however, it would be desirable to gain an insight into the general influence of the aqueous diffusion layers on in vitro solute penetration, particularly when homologous series of compounds, i.e. drug candidates, are handled, and particular penetration features, i.e. percutaneous or intestinal absorption, should be reproduced.

In the present paper, in vitro permeability studies using a series of weakly basic xenobiotics of long-term toxicological interest (4-alkylanilines) across a homogeneous lipoidal artificial membrane (dimethylpolysiloxane) have been carried out under several working conditions, including the presence and the partial or total absence of aqueous boundary layers. In order to remove these latter, a synthetic surfactant solution has been used instead of a vigorous stirring, whose effects seem to be rather incomplete (Stehle and Higuchi, 1972; Barry, 1983). Synthetic surfactants, either at the critical micelle concentration (CMC) or at supramicellar concentrations (SMC), have been reported to disrupt the in vivo aqueous stagnant diffusion layers adjacent to the membrane at its luminal side, as well as to increase the intrinsic membrane polarity through a direct penetration effect (Plá-Delfina, 1987; Collado et al., 1988; Garrigues et al., 1989, 1990), and it was thought to be desirable to evaluate these properties on in vitro artificial membranes. In order to achieve this, permeability-lipophilicity correlations were established and compared for each condition, according to previously reported discriminative equations (Stehle and Higuchi, 1972; Ho et al., 1977; Wagner, 1979; Plá-Delfina et al., 1987; Díez-Sales et al., 1991).

Materials and Methods

Xenobiotics and surfactant

Seven 4-alkyl anilines showing a perfect homology, from aniline to 4-*n*-hexylaniline, were used in the experiments. They are weakly basic compounds (pK_a values ranging from 4.4 to 4.9), and should be considered as highly lipophilic in nature, even at the working pH of 6.2 in the donor compartment (about 95–99% nonionized). The compounds were dissolved in a physiological solution buffered to pH 6.2 at the concentration of 0.2 mg/ml, except 4-*n*-butylaniline, 4-*n*-pentylaniline and 4-*n*-hexylaniline, which were prepared at 0.1, 0.03 and 0.0135 mg/ml, respectively, in order to avoid solubility problems during the in vitro experiments.

In order to disrupt aqueous boundary layers, the nonionic surfactant polysorbate 80 was added to donor or receptor solutions in several instances, as will be pointed out later.

Membrane and diffusion apparatus

Dimethylpolysiloxane was used as model membrane because of its homogeneous nature and structure, which make it quite suitable to interpret experimental results and to fit models to the data.

The working material was obtained from sheets of non-reinforced dimethylpolysiloxane (lot. HH 018873, Dow Corning Co., Midland, MI, U.S.A.; 0.05 cm thick). Sheets were washed in hot water, thoroughly rinsed with distilled water and allowed to equilibrate overnight into the receptor solution. Freshly treated membranes were used for each experiment.

A three-cell battery system based on the Franz-cell model (Franz, 1975), was used. The area available for diffusion was 3.14 cm^2, the receiver compartment capacity was 39 ml, and temperature was maintained at 37($\pm$0.5)°C by

means of a surrounding jacket. The receptor solution was in close contact with the membrane sheet during the whole experiment and continuously and homogeneously stirred (150 rpm) by a rotating teflon-coated magnet placed inside the cell. The donor solution was agitated with the aid of a three-blade stirrer motor shaft (150 rpm).

In vitro diffusion studies

Four series of experiments were carried out. In the first, the receptor cell was completely filled with saline solution buffered to pH 7.4, and donor vehicle was prepared in a physiological solution buffered to pH 6.2 (in order to reproduce with more or less approximation the pH which prevails at some absorption sites such as cutaneous surface or intestinal lumen), both without surfactant. In the second series, donor solution was the same as above, but the receptor solution was added with polysorbate 80 so that its CMC (0.022 mg/ml) was attained. In the third series, polysorbate at its CMC was used in both donor and receptor solutions. Finally, in the fourth series, donor solution contained polysorbate 80 at the CMC, whereas the receptor solution contained the surfactant at a clearly supramicellar concentration (1%, w/v).

The operating procedure was as follows: at zero time a 20 ml volume of the amine test solution was introduced into the donor cell and the stirrer shaft was connected. Samples of 1 ml were taken from the receptor compartment every 30 min. The volume withdrawn was always replaced with the same volume of receptor solution. In order to obtain steady-state permeation and to determine permeability coefficients, donor cell content was entirely replaced with fresh test solution every 15 min for all compounds tested.

Eqn 1 (Scheuplein, 1967; Crank, 1975) was used to fit experimental data.

$$Q(t) = AKhC\left[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2} \cdot \exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \quad (1)$$

where $Q(t)$ is the quantity which passes through the membrane and reaches the receptor solution at a given time (t), A represents the actual surface diffusion area (3.14 cm^2 in our particular case), K is the partition coefficient of the permeant between the membrane and donor vehicle, h denotes the membrane thickness (0.05 cm), D is the diffusion coefficient of the permeant across the membrane, and C represents the actual concentration of the permeant in the donor solution.

As t approaches infinity the exponential terms in Eqn 1 become negligible, and therefore the linear steady-state expression is:

$$Q(t) = AKhC\left[D\frac{t}{h^2} - \frac{1}{6}\right] \quad (2)$$

Since K and D are unknown, the products kh and D/h^2 were replaced, in Eqn 2, by P_1 and P_2, respectively, and calculated through fitting the theoretical equation to individual in vitro permeation data using a computerized non-linear least-squares method (Multi) (Yamaoka et al., 1981). Then the permeability coefficients, K_p ($= P_1P_2$, i.e. kD/h; cm/h), were calculated and used as the main representative permeation parameters.

Structural or lipophilicity indexes

Since there was a perfect homology between the tested amines, the number of carbon atoms in the alkyl 4-chain, N, was used as structural index to replace the lipophilicity or partition constants usually recommended for correlation with biological parameters. It should be borne in mind that N is linearly related, in such cases, with the logarithm of any partition constant such as partition coefficient, P, or chromatographic capacity factor, K' (see, for example, Plá-Delfina and Moreno, 1981), with the advantage of being an error-free constant.

Analytical procedure

Amine concentration in samples was determined by gas chromatography after chloroform extraction of samples in alkaline medium, as previously reported (Díez-Sales et al., 1991). Aliquots

TABLE 1

Gas chromatography conditions using flame-ionization detection

Compound	Temperature (°C)		Internal standard
	Column	Detector	
Aniline	100	150	4-Methylaniline
4-Methylaniline	110	200	Aniline
4-Ethylaniline	120	200	4-Methylaniline
4-*n*-Propylaniline	130	200	4-Ethylaniline
4-*n*-Butylaniline	140	200	4-*n*-Propylaniline
4-*n*-Pentylaniline	150	200	4-*n*-Butylaniline
4-*n*-Hexylaniline	160	250	4-*n*-Pentylaniline

of 0.2–0.3 μl of the extracts of samples and reference solutions were injected onto a 2 m long, 2.4 mm diameter glass column, packed with OV-17 at 3% Supelcoport (80–100 mesh). A flow rate of 40 ml/min N_2 carrier gas and H_2 was used. The air-flow rate for detector was 275 ml/min. Other working conditions are summarized in Table 1. Detection was, in all cases, by flame ionization, and the internal standard method was employed.

Solubility determination

Assessment of the solubility (concentration at saturation) of each compound in the receptor solution became necessary in order to ensure that sink conditions were maintained as amine was accumulated in the receiver compartment during the experiment. In order to achieve this, a moderate excess of each particular compound was placed in an appropriately sized stoppered flask (of 1000 ml capacity in general), physiological solution buffered to pH 7.4 was added to the capacity of the flask and this latter was placed in a shaker bath and maintained at 37(±0.5)°C for 2 days, with gentle stirring. After filtration, an aliquot was taken and conveniently diluted for gas chromatographic analysis, which was developed as described above.

Fitting of models to data

Correlations between permeability coefficients and structural (lipophilicity) indexes for homologous series of xenobiotics were thought to be the most suitable source of information about the passive permeation mechanisms and the role of aqueous and lipoidal barriers in penetration. Consequently, K_p values and 4-alkyl chain lengths, N, were correlated through the three classical model equations: parabolic (as representative of the probabilistic approaches, together with bilinear ones), hyperbolic (as representative of the compartmental approaches), and potential (i.e. linear and logarithmic in nature as the most simple model approach), with the aid of the non-linear least-squares regression program, Multi:

$$\text{Parabolic:} \quad K_p = 10^{(aN^2+bN+c)} \tag{3}$$

$$\text{Hyperbolic:} \quad K_p = \frac{K_m \cdot 10^{aN}}{B + 10^{aN}} \tag{4}$$

$$\text{Potential:} \quad K_p = b \cdot 10^{aN} \tag{5}$$

In these equations, a, b, c and B are constants depending on the experimental technique, and are readily calculable by regression.

As pointed out in previous work and in specialized literature data, the parabolic equation is indicative of the existence of a multiple-phase barrier system, constituted, at least, by three alternate barriers (hydrophilic/lipophilic/hydrophilic) (Stehle and Higuchi, 1972; Ho et al., 1977), whereas the hyperbolic equation indicates that the barrier is a single lipophilic phase with an adjacent aqueous boundary layer (Ho et al., 1977; Wagner, 1979; Plá-Delfina et al., 1987). The potential equation would indicate a single lipoidal barrier devoid of any water diffusion layer (Plá-Delfina et al., 1987).

Statistical analysis

A logarithmic transformation of the permeability coefficients, for the three series selected for comparison (I, II, and IV) was used in order to obtain homogeneous variability. Homogeneity was confirmed by Bartlett's test. One- and two-way ANOVA was used previously to Peritz's F test (Harper, 1984).

Akaike's information criterion (AIC) (Akaike, 1976), as well as the correlation coefficient between experimental and model-predicted K_p values, were used to assess the goodness of the fits.

Results and Discussion

Solubility and sink conditions

The solubilities of the tested amines in the saline solution buffered to pH 7.4 (receptor solution) are listed in Table 2, in which the concentrations of each amine in the receptor solution at the end of the experiences developed for each condition test are also reported as percentages of the saturation concentration, in order to ensure that sink conditions have been fulfilled during the experiment, and, consequently, that a perfect steady-state penetration rate has been maintained.

Although a general agreement about this question does not exist, it could be assumed that sink conditions are maintained when the final concentration does not surpass 10–20% of the concentration at saturation for a great majority of substances (Hanson, 1982; Barry, 1983; Guy and Hadgraft, 1989).

In vitro diffusion tests

Accumulated amounts as a function of time in the receptor compartment for the four series of tests were determined and permeability coefficients, k_p, calculated for each compound and condition; these are shown in Table 3. Values are the means of $n = 4$ determinations. Two-way ANOVA showed that there were highly significant differences between compounds, conditions and interactions.

Permeability / structure correlations

Plots relating permeability coefficients, k_p, and 4-alkyl chain -CH_2- groups, N, are reproduced in Fig. 1. Statistical figures found after fitting Eqns 2–4 to results obtained under the four selected conditions are shown in Table 4. Equation parameters found for the best fitting in each case are given in Table 5.

As can be observed, in tests developed in the absence of surfactant in both donor and receptor solutions (series I), the correlations are clearly parabolic in nature. Since dimethylpolysiloxane membrane is homogeneous and wholly lipoidal in nature and since the amine concentration in the receptor solution at the end of the experiment was, in all cases, below 3% relative to that of the saturated solution, and, consequently, sink conditions were completely fulfilled, the only possible explanation to this behaviour is that a heterogeneous barrier system is formed, which includes two aqueous stagnant water layers, on both the

TABLE 2

Solubility values in the receptor solution and concentration of amines at the end of experiments

Compound	Solubility (±SD) (mg/ml)	Concentration at the end of experiment (Cf, mg/ml × 10^3) and % of solubility					
		I		II		III	
		Cf	%	Cf	%	Cf	%
Aniline	83.57 (±1.17)	9.54	0.01	8.43	0.01	9.77	0.01
4-Methylaniline	19.97 (±0.42)	13.25	0.07	13.33	0.07	19.25	0.10
4-Ethylaniline	4.76 (±0.17)	21.84	0.45	19.74	0.41	37.87	0.79
4-*n*-Propylaniline	1.17 (±0.08)	27.02	2.31	28.33	2.42	52.00	4.44
4-*n*-Butylaniline	0.360 (±0.004)	9.59	2.66	16.69	4.63	30.46	8.46
4-*n*-Pentylaniline	0.087 (±0.008)	1.97	2.26	7.20	8.28	10.64	12.23
4-*n*-Hexylaniline	0.026 (±0.003)	0.36	1.39	3.59	13.80	4.97	19.13

TABLE 3

Permeability coefficients, k_p, found under the selected experimental conditions for the tested amines, model equations fitting each lot of data, and statistical significance of the differences between log k_p values of each compound as tested under different conditions

Tested amines	Permeability coefficients (k_p) (cm/h) (± S.D.)				Statistical differences ($p <$)		
	I	II	III	IV	I–II	II–IV	I–IV
	No surfactant	Surfactant at CMC (receptor)	Surfactant at CMC (donor) CMC (receptor)	Surfactant at CMC (donor) SMC (receptor)			
Aniline	0.179 ± 0.009	0.176 ± 0.006	0.179 ± 0.011	0.163 ± 0.006	NS	[b]	[b]
Methylaniline	0.263 ± 0.009	0.252 ± 0.009	0.381 ± 0.026	0.330 ± 0.015	NS	[c]	[c]
Ethylaniline	0.394 ± 0.006	0.368 ± 0.012	0.693 ± 0.026	0.611 ± 0.020	[b]	[c]	[c]
Propylaniline	0.533 ± 0.017	0.550 ± 0.023	0.990 ± 0.091	1.068 ± 0.039	NS	[c]	[c]
Butylaniline	0.428 ± 0.009	0.691 ± 0.019	1.237 ± 0.163	2.041 ± 0.058	[c]	[c]	[c]
Pentylaniline	0.242 ± 0.018	0.821 ± 0.014	1.380 ± 0.093	3.150 ± 0.098	[c]	[c]	[c]
Hexylaniline	0.088 ± 0.006	0.977 ± 0.016	1.564 ± 0.060	5.828 ± 0.380	[c]	[c]	[c]
Selected equation	Parabolic	Parabolic Hyperbolic	Hyperbolic	Potential			

[a] NS, not significantly different.
[b] $p < 0.05$.
[c] $p < 0.01$.

donor and receptor sides of the membrane, and the membrane itself as central lipoidal phase. Conclusive features of the curve are observed in Fig. 1 (triangles).

In view of these results, two subsequent series of experiments were developed in order to disrupt the aqueous diffusion layer at one side of the membrane (receptor side, series II), and on both membrane sides (donor and receptor, series III).

In test series II, polysorbate 80 was added at the CMC to the receptor solution. The results showed that the k_p values of the hydrophilic members of the series, which were placed on the ascending branch of the parabola in series I tests, do not change appreciably relative to those found in the absence of surfactant, but the remaining compounds showed progressively higher K_p values as lipophilicity increases (Table 3 and Fig. 1, filled circles). Statistical figures found for parabolic and hyperbolic model equations (Tables 4 and 5) make it difficult to select the best model for data fitting; the absence of elements on the descending branch of the parabola, as well as on the asymptote of the hyperbola, did not aid in discriminating the model to be applied. Be that

TABLE 4

Statistical figures found for data fittings corresponding to the four test series according to Eqns 2–4

Model equations	Statistical figures	I	II	III	IV
Parabolic	r [a]	0.977	0.998	0.996	0.999
	AIC	−29.26	−40.57	−24.94	−13.92
Hyperbolic	r [a]	0.366	0.999	0.998	0.999
	AIC	−7.61	−41.27	−30.72	−13.76
Potential	r [a]	0.121	0.985	0.955	0.999
	AIC	−9.70	−24.76	−9.30	−15.77

[a] Between experimental and model-predicted K_p values.

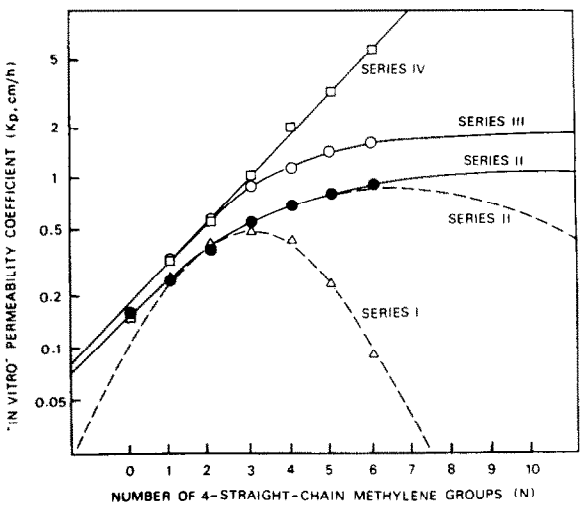

Fig. 1. Plots of permeability coefficients, K_p, found for the tested amines in the different test series, and structural constants, N, according to the best fitting model equation (parabolic for series I, hyperbolic or parabolic for series II, hyperbolic for series III, and potential for series IV). Test series differed in the number of water boundary layers, but perfect sink conditions were achieved only for series I and IV.

TABLE 5

Equation parameters describing the best correlations between permeability coefficients (K_p) found under different conditions and lipophilicity (N)

Model equations	Equation parameters	
	Symbol	Value (±SD)
Parabolic (I)	a	−0.069770 ± 0.009930
	b	0.408154 ± 0.060589
	c	−0.896910 ± 0.087509
Hyperbolic (II)	K_m	1.249509 ± 0.083666
	b	0.223468 ± 0.017114
	a	6.384332 ± 0.413578
Parabolic (II)	a	−0.012915 ± 0.001980
	b	0.204323 ± 0.015856
	c	−0.774499 ± 0.030112
Hyperbolic (III)	K_m	1.618504 ± 0.054489
	b	0.341727 ± 0.027135
	a	6.911213 ± 0.767405
Potential (IV)	b	0.244491 ± 0.008089
	a	0.197348 ± 0.020528

as it may, it became evident from the reported results that, with a great deal of probability, the aqueous stagnant diffusion layer at the receptor side of the membrane has been more or less disrupted by the surfactant, so that the parabolic correlation found in the absence of polysorbate clearly tended to become hyperbolic under the new conditions used for series II tests.

In series III tests, polysorbate at the CMC was used in both donor and receptor solutions. In this case, the permeability coefficients, K_p, were higher for all amines tested excepting aniline, and the correlation found was decidedly hyperbolic, as shown in Tables 3–5 and in Fig. 1 (open circles).

Critical analysis of the above data showed that, under the described working conditions, the provisions were not strictly fulfilled. For series II, a true hyperbolic correlation should have been found, with a degree of probability much higher than that observed, relative to parabolic. On the other hand, for series III, a potential rather than hyperbolic relationship should have been generated by the experimental data. This could be due to the incomplete disruption of the water diffusion layers, as well as to losses of sink conditions in the receptor solution for some compounds. In view of the data shown in the last two columns of Table 2, this latter alternative appeared as more reasonable, since concentrations of higher elements of the group at the end of the experiments ranged from 8 to 20% of their saturation concentrations in the receptor solution. Although literature references about the subject are inconclusive (Hanson, 1982; Barry, 1983; Guy and Hadgraft, 1989) this was supposed to be a critical range of percentage concentrations where the existence of sink conditions could be called into question, and, consequently, some slowing down in membrane penetration rate for these highly lipophilic compounds could be expected.

Accordingly, a further series of experiments (series IV) was developed, with polysorbate at CMC in the donor solution but at a clearly supramicellar concentration, SMC (1%, w/v), in the receptor side, in order to provide a micellar reservoir for the uptake of the compounds, thus leading to a free amine concentration as low as

possible and, therefore, to perfect sink conditions. As can be seen in Fig. 1 (squares), as well as in Tables 3 to 5, a clearly potential (i.e. linear and logarithmic, as shown in Fig. 1) correlation was obtained between k_p and N values under these conditions, thus demonstrating that:

(1) In the absence of surfactant, two stagnant layers are formed on both sides of the membrane.

(2) Synthetic surfactant at the CMC will effectively remove the water layer on the side where it is dissolved.

(3) The presence of the synthetic surfactant at SMC in the receptor solution will provide the sink conditions necessary to achieve homogeneous steady-state penetration, independent, to a certain extent, of the water solubility of the compounds of a given series.

From the above results, it becomes evident also that the synthetic surfactant does not significantly increase the polarity of the artificial lipoidal membrane, as assessed from the slope of the straight line (in logarithmic paper) fitting series IV data relative to the hyperbola found for the data of series II: the ascending branch of this latter curve and the straight line show virtually a common origin, as shown in Fig. 1, in contrast to what occurs with the results found in the in vivo tests, in which the slope of the line found when surfactant is present at or just below its CMC in the luminal solution becomes much lower than expected due to the increment in membrane polarity exerted in situ by the surfactant (Plá-Delfina et al., 1987; Collado et al., 1988; Garrigues et al., 1989; Díez-Sales et al., 1991), a phenomenon which results in an increase in membrane permeation rate for highly hydrophilic members of the series and a reduced permeability rate for highly lipophilic ones. If any effect on the polarity of the artificial membrane does exist, it would indeed be practically negligible.

In vivo-in vitro correlations

One of the ultimate aims of the in vitro permeability tests on artificial lipophilic membranes is to approximate the in vivo absorption processes through specific membranes, and to reproduce absorption-lipophilicity correlations.

Some biological membranes behave like homogeneous barrier systems (including, for instance, a lipoidal absorbing phase and an adjacent aqueous boundary layer), as occurs with the intestinal and colonic absorbent mucosae, which generate hyperbolic-type absorption-lipophilicity correlations (Ho et al., 1977; Wagner, 1979; Plá-Delfina and Moreno, 1981; Plá-Delfina et al., 1987; Collado et al., 1988); this would mean that a stagnant water diffusion layer exists at the luminal side of the lipoidal membrane, whereas the serosal capillary side would act as a perfect sink (Ho et al., 1977; Plá-Delfina et al., 1987). In contrast, a number of other natural membranes behave as heterogeneous barrier systems (including for example, lipoidal and aqueous alternate phases), as occurs with ocular and percutaneous epithelia (Schoenwald and Ward, 1976; Barry, 1983, Díez-Sales et al., 1991) and, as recently shown, with gastric mucosa (Hills et al., 1983; Garrigues et al., 1990), which generate parabolic or bilinear-type absorption-lipophilicity correlations.

If one intends to reproduce in vivo absorption data from those found through in vitro penetration tests across artificial lipoidal membranes, care should be taken to mimic, in the in vitro experimental design, the fundamental characteristics of the biophysical system which prevail at each particular absorption site. This could lead, in the opinion of the authors, to the attainment of a better correspondence between in vitro and in vivo results.

Thus, if one intends to mimic intestinal absorption rate constants (or absorption-lipophilicity correlations) from in vitro permeability tests, donor solutions free of surfactants should be employed, whereas receptor solutions will contain a surfactant at a clear SMC or some other artifices leading to a disruption of the aqueous boundary layer at this membrane side, as well as to the attainment of perfect sink conditions. Contrarily, when ocular, percutaneous (whole skin penetration) or even gastric absorption mechanisms are to be simulated, very different in vitro features should be reproduced. In general, these types of biological barriers can be reproduced merely by a three-phase system such as that reported here for series I experiments or, of course, by more so-

phisticated heterogeneous barrier systems such as a battery of lipoidal membranes together with their water layers.

It should be emphasized that, although the above conditions are supposed to be fundamental, they are not the only conditions required. Many other experimental circumstances, which are, in general, modulable, should also be taken into account in order to simulate in vivo conditions as much as possible, such as fluid composition and volume, degree of agitation and, particularly, the type of artificial membrane to be used. This can readily be understood through the following examples.

Series I and IV tests, the only ones for which sink conditions were clearly fulfilled, could be used to simulate percutaneous penetration data through the whole skin (Díez-Sales et al., 1991) and intestinal absorption data through a lipoidal membrane route in the presence of polysorbate at the CMC in luminal fluid (Collado et al., 1988), respectively, both achieved at the same pH and using the same compounds tested here. As shown in Figs 2 and 3, there is a fairly good correspondence between in vivo and in vitro results.

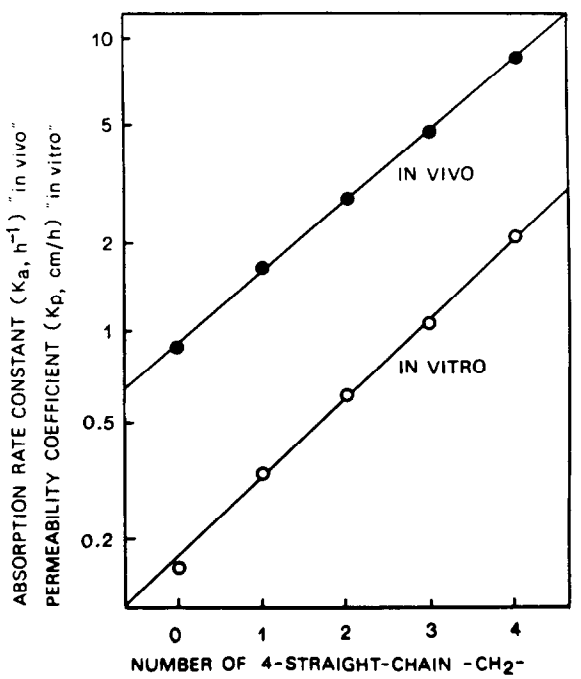

Fig. 2. Permeability coefficients, K_p, found in series IV tests, and intestinal absorption rate constants, K_a, obtained in vivo in the rat small intestine in the presence of polysorbate at CMC in the luminal fluid (Collado et al., 1988) for the same amines, both at pH 6.2.

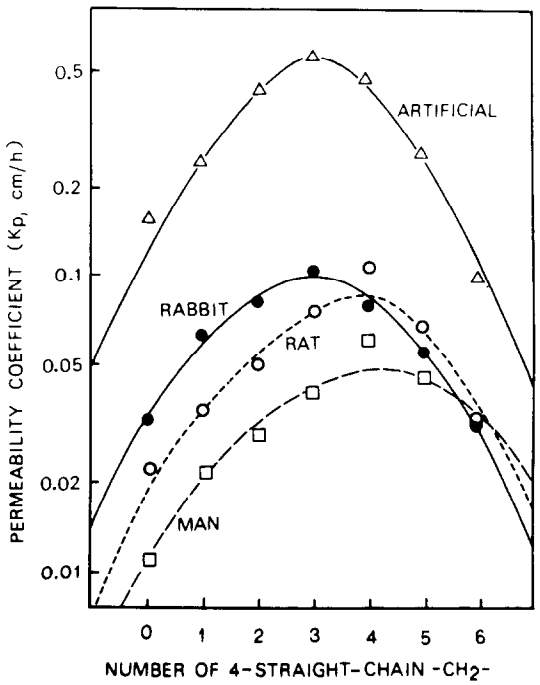

Fig. 3. Plots of permeability coefficients, K_p, found in series I tests, and K_p values previously obtained under similar experimental conditions through several biological membranes (Díez-Sales et al., 1991), according to parabolic equations.

However, the results shown in Fig. 2, corresponding to intestinal absorption rate constants through the lipoidal route in the presence of polysorbate at the CMC in luminal fluid, as reported by Collado et al. (1988), which show a very good correlation with those found here for series IV tests ($r = 0.998$), could even have been improved if an artificial membrane of somewhat enhanced intrinsic polarity properties had been employed, since this could have led to more similar slopes for the two lines.

Similarly, from Fig. 3, whose results correspond to permeability tests on excised skin sheets obtained from different species, as reported by Díez-Sales et al. (1991), it can be seen that the in vitro results obtained here simulate rather well

those found with rabbit excised skin ($r = 0.971$). In order to reproduce more accurately those found with rat and human skin sheets, artificial membranes with intrinsic polarity properties other than that used here would have been utilized.

Notwithstanding, we believe that basic experimental designs as recommended could help in obtaining more reliable in vitro-in vivo correlations and to dissipate some misleadings in the design and development of in vitro permeability tests.

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