Drug Permeation through Thin-Model Membranes III: Correlations between *In Vitro* Transfer, *In Vivo* Absorption, and Physicochemical Parameters of Substituted Benzoic Acids

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Abstract □ Comparative transfer-rate studies employing a series of benzoic acid analogs were conducted across a polymeric model biomembrane. Statistical correlations were sought between the determined *in vitro* transfer-rate constants and *in vivo* absorption data reported in the literature. Relationships with certain solubility-related parameters were also investigated. The *in vitro* transfer-rate constants were compared to *in vivo* absorption data reported in the literature for buccal and intestinal absorption. Significant correlations were obtained, indicating the strong association that exists between these two variables. The *in vitro* transfer rates of the benzoic acid derivatives were also shown to be statistically related to the reciprocal water solubility and the log partition coefficient of the substituent group on benzoic acid. No correlation was found between drug permeation, as expressed by the *in vitro* transfer-rate constant, and the molecular weight or volume of the compound, confirming the dominant role that solubility of the penetrating species plays in controlling permeation through this membrane system.

Keyphrases □ Benzoic acids, membrane permeation—correlations between *in vitro* transfer, *in vivo* absorption, physicochemical parameters □ Membrane permeation, benzoic acids—*in vitro* transfer, *in vivo* absorption, physicochemical parameters □ Biomembrane model, polymeric—*in vitro–in vivo* drug-absorption correlations □ Transfer rates, *in vitro*, benzoic acids—*in vivo* absorption and physicochemical correlations

Some correlations involving physicochemical parameters with either *in vitro* or *in vivo* absorption data have been reported in the literature. The absorption rates of barbituric acid derivatives from the rat stomach and small intestine were correlated with their respective partition coefficients (1, 2). Similar experiments were carried out using a series of sulfonamides (3,4). Correlations pertaining to *in vitro* transfer rates and partition coefficients appeared more recently. Thus, the apparent diffusion constants of various barbitals through silicone rubber sheeting were correlated to their partition coefficients between chloroform and pH 4.7 acetate buffer (5). These authors observed no significant correlation between the apparent diffusion constants and drug solubility, or its reciprocal, in pH 4.7 acetate buffer.

Recently, Bates *et al.* (6) examined the *in vitro* transport of both free and complexed drug species and qualitatively correlated it to the corresponding gastric absorption data derived by Goto *et al.* (7).

The present study was designed to demonstrate the utility of a polymeric biomembrane in establishing *in vitro–in vivo* correlations for passive drug absorption. This biomembrane, consisting of natural membrane components contained within an ethylcellulose polymer matrix, was described previously (8, 9). Accordingly, a comparative study of the *in vitro* transfer rates of various benzoic acid derivatives through the model biomembrane was undertaken. Subsequently, statistical correlations of these data were sought with *in vivo* absorption data reported in the literature and physicochemical parameters such as substituent partition coefficients, solubility, and molecular size.

Table I—Absorption Wavelength Used in Spectrophotometric Concentration Determinations of Benzoic Acid Derivatives

Compound[a]	Absorption Wavelength in pH 1.2 Buffer, nm.	Absorption Wavelength in Water, nm.	Absorption Wavelength in Water Saturated with 1-Octanol, nm.
BA[b]	274	—	270
2-Hydroxy-BA[c]	303	—	—
3-Hydroxy-BA	298	—	—
4-Hydroxy-BA	256	—	—
2,4-Dihydroxy-BA	258	248	250
2,5-Dihydroxy-BA	238	230	230
2-Methoxy-BA	238	284	280
2-Methyl-BA	233	—	224
3-Methyl-BA	236	—	—
4-Methyl-BA	242	—	—
2,4-Dimethyl-BA	244	238	—
2,5-Dimethyl-BA	238	280	—
2-Nitro-BA	264	—	268

[a] Eastman Chemicals, unless otherwise stated. [b] BA = benzoic acid. [c] Baker and Adamson.

Table II—Accumulated Data on Methyl-Substituted Benzoic Acids

Acid	*In Vitro* Transfer-Rate Constant, k_d, hr.$^{-1}$	*In Vivo* Buccal Absorption-Rate Constant, ml. min.$^{-1}$[a]	Log k_{pc} of Substituent Group[b]	Solubility at 25°, g. 100 ml.$^{-1}$
BA[c]	0.093	6.5	0.0	0.35[d]
4-Methyl-BA	0.148	9.6	0.32	0.035[d]
2-Methyl-BA	0.154	9.3	0.36	0.12[d]
3-Methyl-BA	0.182	8.9	0.45	0.098[d]
2,5-Dimethyl-BA	0.248	12.7	0.76	0.018[e]
2,4-Dimethyl-BA	0.288	10.6	0.90	0.016[e]

[a] From Beckett and Moffatt (11). [b] Derived from data of Beckett and Moffat (11) using *n*-heptane–0.1 *N* HCl partition coefficients. [c] BA = benzoic acid. [d] From Seidell (12). [e] Determined in this study.

EXPERIMENTAL

Materials—The materials used were reported previously (9), with the exception of the benzoic acid derivatives, which were used as received from the manufacturers (Table I).

General Experimental Protocol—The procedures for membrane formation and permeation studies using dialysis cells were published previously (8).

Comparative Transfer Studies—Benzoic acid and 12 mono- or disubstituent derivatives were used (Table I). In all cases, transfer of these compounds took place from 0.1 *N* HCl (pH 1.2) into pH

Table III—Accumulated Data on Hydroxy- and Methoxy-Substituted Benzoic Acids

Acid	*In Vitro* Transfer-Rate Constants ($hr.^{-1}$) $\times 10^4$, pH 6.0	*In Vivo* Intestinal Absorption Rate Constant, $min.^{-1}$ [a]	Log k_{pc} of Substituent Group	Solubility at 25°, g. 100 $ml.^{-1}$
BA[b]	14.4	0.92	0.0[c]	0.35[d]
2,5-Dihydroxy-BA	0.088	0.01	−0.13[c]	2.2[e]
2,4-Dihydroxy-BA	0.212	0.07	0.19[c]	0.60[e]
3-Hydroxy-BA	0.963	0.10	−0.38[f]	1.1[d]
4-Hydroxy-BA	1.23	0.19	−0.30[f]	0.64[d]
2-Hydroxy-BA	1.39	0.66	0.41[g]	0.22[d]
2-Methoxy-BA	1.44	0.56	−0.28[c]	0.42[e]

[a] Data from Nogami *et al.* (14). [b] BA = benzoic acid. [c] Octanol–water partition coefficients determined in this study. [d] From Seidell (12). [e] Determined in this study. [f] Octanol–water partition coefficients from Fujita *et al.* (10). [g] Octanol–water partition coefficients from Lien (15).

7.4 buffer. Two-millimolar concentrations were used, except where the aqueous solubility was less than this amount. The concentrations of benzoic acid derivative remaining in the pH 1.2 phase were determined at known time intervals, using predetermined calibration curves, at the wavelengths shown in Table I. In most cases, the duration of the experimental run exceeded one half-life of the test substance; all runs were carried out in triplicate. In all cases, except one, the experimentally observed rate constants were regarded as the true first-order disappearance-rate constants, since 98% or greater of the compound was undissociated. The determined rate constant for the exception, 2-nitrobenzoic acid, was corrected accordingly.

Partition Studies—Mutually saturated solutions of octanol and distilled water were used. Five milliliters of equilibrated octanol was added to 5 ml. of equilibrated water containing the drug at a concentration of 100 mg./l. The mixture was agitated at 25° for 36 hr., a time found to be adequate for establishing equilibrium conditions. The extent of partitioning was established by spectrophotometric analysis (Table I) of the aqueous phase, using the equilibrated water as the reference. The drug in the octanol phase was determined by difference, ignoring association. The true partition coefficient was computed, taking into account the degree of dissociation of the compounds, using the method of Fujita *et al.* (10). The pH of the aqueous phase was determined at equilibrium. The true partition coefficient was shown not to be significantly influenced by concentration. All determinations were carried out in duplicate; the results were averaged and expressed in terms of the partition coefficient of the substituent group so as to conform to the literature values used.

Equilibrium Solubility Studies—Equilibrium aqueous solubilities of five benzoic acid derivatives were determined at 25°. An excess of drug, together with approximately 100 ml. of distilled water, was placed in a flask. The sealed flasks were continuously agitated in a water bath, and samples were removed by means of a pipet fitted with a filter plug. In all cases, equilibrium was attained within 48 hr. Appropriate volumetric dilutions were performed on the equilibrated samples, and the concentrations were determined by spectrophotometric analysis. Duplicates were run, and the results were averaged.

RESULTS

General—As noted previously, pH 1.2 was chosen for Compartment A to facilitate transport, since only one of the weak acids examined was more than 2% ionized at this pH. Since the model membrane was shown previously (9) to comply with the pH-partition hypothesis, the *in vitro* rate constants obtained at pH 1.2 were corrected to the pH conditions used in the *in vivo* studies.

Methyl-Substituted Benzoic Acids—Beckett and Moffat (11) studied the buccal absorption of methyl-substituted benzoic acids. Although a pH of 4.0 was employed in their *in vivo* study, the reported rate constants were for the transfer of unionized drug; consequently, no correction of the observed *in vitro* rates are required. The true first-order *in vitro* disappearance-rate constants of the methyl-substituted benzoic acids are listed in Table II, together with the buccal absorption-rate constants reported by Beckett and Moffat (11). Table II also lists the aqueous solubilities of these compounds, together with the log partition coefficients of the substituent groups. The log partition coefficient of the substituent was used to draw attention to the effect of the various groups and is obtained by subtracting the log value of benzoic acid from that of the various derivatives. This approach was demonstrated by Fujita *et al.* (10) for octanol–water partition coefficients. Furthermore, Collander (13) showed that partition coefficients derived in one organic solvent may be related to those obtained from a second organic solvent.

Hydroxy- and Methoxy-Substituted Benzoic Acids—In the *in vivo* intestinal perfusion studies reported by Nogami *et al.* (14), the initial perfusion fluids were adjusted to pH 6.0. The apparent first-order *in vitro* rate constants at this pH are presented in Table III, together with the observed *in vivo* intestinal absorption coefficients. Table III also contains the aqueous solubilities of these compounds and the log values of the substituent group partition coefficients based on the octanol–water system. These latter are Hansch substituent constants (16).

DISCUSSION

***In Vitro–In Vivo* Correlations**—Tables II and III present the *in vitro* transfer data and the *in vivo* absorption data for the methyl-substituted and the hydroxy- and methoxy-substituted benzoic acids, respectively. Statistical *in vitro–in vivo* correlations were attempted with these groups of compounds. The results are contained in Table IV.

Correlation of the buccal absorption-rate constants, which reflect only unionized drug transfer, to the true first-order disappearance-rate constants at the 95% confidence level shows that all correlations are real. At the more stringent 99% confidence level, however, there is no real correlation. In the case of the intestinal absorption-rate coefficients determined by Nogami *et al.* (14), two of the four combinations used showed a real correlation at the 95% confidence level.

Table IV—Correlations between *In Vitro* Transfer-Rate Constants and *In Vivo* Absorption Data Reported in Literature

Type of Benzoic Acid Derivative	*Y versus X*	Regression Coefficient ± 95% Confidence Interval	Correlation Coefficient (Significance Level — Greater Than)
Methyl-substituted (Table II)	$\sqrt{k_{1.2}}$[a] *versus* $\sqrt{k_{buc}}$[b]	0.201 ± 0.153	0.88 (95%)
	$k_{1.2}$ *versus* $\sqrt{k_{buc}}$	0.166 ± 0.142	0.85 (95%)
	$\sqrt{k_{1.2}}$ *versus* k_{buc}	0.0347 ± 0.0307	0.84 (95%)
	$k_{1.2}$ *versus* k_{buc}	0.0287 ± 0.0279	0.82 (95%)
Hydroxy- and methoxy-substituted (Table III)	$\sqrt{k_{6.0}}$[c] *versus* k_{int}[d]	0.0274 ± 0.0209	0.83 (98%)
	$\sqrt{k_{6.0}}$ *versus* $\sqrt{k_{int}}$	0.0283 ± 0.0257	0.79 (95%)
	$k_{6.0}$ *versus* k_{int}	0.0011 ± 0.0011[e]	0.76 (95%)
	$k_{6.0}$ *versus* $\sqrt{k_{int}}$	0.0011 ± 0.0013[e]	0.68 (90%)

[a] True first-order disappearance-rate constant (pH 1.2). [b] Buccal absorption constant from Beckett and Moffat (11). [c] Apparent first-order disappearance-rate constant (pH 6.0). [d] Intestinal absorption coefficient from Nogami *et al.* (14). [e] Correlation is not real.

Table V—Correlations between *In Vitro* Transfer-Rate Constants and Solubility-Related Parameters

Type of Benzoic Acid Derivative	X^a	Regression Coefficient ± 95% Confidence Interval	Correlation Coefficient (Significance Level — Greater Than)
Methyl-substituted (Table II)	Log k_{pc} of substituent	0.219 ± 0.030	0.99 (99%)
	Water solubility^{-1}	0.0025 ± 0.0017	0.90 (98%)
	Log water solubility^{-1}	0.119 ± 0.092	0.87 (95%)
Hydroxy- and methoxy-substituted (Table III)	Log k_{pc} of substituent	0.141 ± 0.140	0.76 (95%)
	Water solubility^{-1}	0.0353 ± 0.0210	0.89 (99%)
	Log water solubility^{-1}	0.117 ± 0.129[b]	0.72 (90%)
ortho-Substituted[c]	Log k_{pc} of substituent	0.195 ± 0.189	0.89 (95%)
	Water solubility^{-1}	0.0210 ± 0.0242[b]	0.85 (90%)
	Log water solubility^{-1}	0.208 ± 0.181	0.90 (95%)

[a] *Y*-axis in all cases was *in vitro* true first-order disappearance-rate constant. [b] Correlation is not real. [c] Data from benzoic acid, 2-hydroxybenzoic acid, 2-methoxybenzoic acid, 2-methylbenzoic acid, and 2-nitrobenzoic acid ($k_d' = 0.0155$; log $k_{pc} = 0.04$; $S = 0.74$ g. 100 ml.$^{-1}$).

***In Vitro* Physicochemical Parameter Correlations**—Certain physicochemical parameters of the compounds under investigation are contained in Tables II and III. Correlations between the true first-order rate constants and solubility expressions were sought by the use of three parameters: the log partition coefficient of the substituent group, the reciprocal water solubility, and the log of the reciprocal water solubility. The use of the first parameter places emphasis on the solubility properties of the substituent groups and was used before in relating to *in vivo* absorption data (11, 17). The reciprocal of the water solubility should be a reflection of the lipoidal solubility of the compound; the log value was used to parallel the log partition coefficient.

As may be seen from Table II, increased lipid solubility with increasing methylation is suggested by the log partition coefficients of the substituent groups and the aqueous solubility values. This increase in lipid solubility is reflected in the true first-order disappearance-rate constants. It also appears that the degree of substitution, and not the position of substitution, plays the dominant role. The hydroxy and methoxy analogs (Table III) produced substantially smaller true first-order disappearance-rate constants when compared to the methyl analogs. This was anticipated and verified by the higher water solubility exhibited upon the addition of these hydrophilic groups onto the parent molecule. The one exception was salicylic acid. Its unique lipid solubility may be in part attributed to its potential for intramolecular hydrogen-bond formation. The accelerated rate of transfer of the 2,4-dihydroxy analog may also be predicted from its lower water solubility and higher partition coefficient. The benzoic acid derivatives are assumed to undergo total dimerization in *n*-heptane (used with the methyl derivatives); dimerization will not occur in octanol (used with the hydroxy and methoxy derivatives).

The results of the statistical evaluations are contained in Table V. Seven of the nine correlations attempted were real at the 95% confidence level. In two of the three series, the reciprocal water solubility correlated better than the logarithm of this parameter, pointing to the utility of this simple property in predicting membrane permeation.

Attempts also were made to establish a correlation between the *in vitro* transfer rates and the reciprocal of the square root of the molecular weight and the molecular volume. The latter was calculated according to the method and data of Bondi (18). However, in no instance was a real correlation at the 95% confidence level found, indicating that the permeation of the compounds studied is independent of molecular size and that the real influence is that of solubility.

When the results of the statistical correlations presented in Tables IV and V are examined together, several points become apparent. First, solubility plays a dominant role in determining the *in vitro* transfer rates across this membrane. Second, significant relationships do exist between these *in vitro* transfer rates and the *in vivo* absorption data in the literature. This suggests that the thin polymeric model biomembrane, whose mechanism of transfer is based on solubility, adequately mimics the passive diffusion functionality of the biological membrane. Third, although it would be desirable to have but one form of data presentation result in correlations for all types of *in vivo* data, this is not the case in the present study. This may be a reflection of the route of absorption and may also arise from the diverse experimental protocols employed in the *in vivo* investigations whose data were used in this work.

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