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RELATIONSHIP BETWEEN IN VITRO INTESTINAL ABSORBABILITY AND PARTITION COEFFICIENTS OF XANTHENE DERIVATIVES

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ABSTRACT

Four xanthene derivatives, namely, 8-chlorotheophylline, caffeine, theophylline and 1-methyl-3-isobutylxanthene (SC-2964) were studied to ascertain their in vitro intestinal absorbability and partitioning property in an octyl alcohol/pH 7.4 buffer system. Absorbability in vitro was estimated as the cumulative transfer rate (μg/min.) of the test compounds transfering across everted rat intestine sacs. The results demonstrated a dependency of the cumulative transfer rates on the partition coefficients. A logarithmic correlation between cumulative transfer rates and partition coefficients of the test compounds was observed. It is concluded that this predictive methodology could be adopted to screen potentially active compounds and optimize the selection of compounds to be developed into orally bioavailable dosage forms.

INTRODUCTION

Several biopharmaceutical investigations (Crane and Wilson, 1958; Levy and Matsuzawa, 1965; Barr and Riegelman, 1970; Feldman and Gibaldi,1971; Kaplan and Cotler, 1972; Humphreys and Smy, 1975; Abruzzo et al., 1976) have shown that everted rat intestine sac preparations can be used in vitro for assessing the absorbability of nutrients and drugs. Conventionally, absorbability of a group of active compounds in a given series is estimated as the cumulative transfer rate of each compound traversing the intestinal wall to the serosal side. For development purposes, a higher cumulative transfer rate of a compound or its derivative may allow: (1) selection of a lead compound from a given series for additional pharmacological and toxicity testing; and (2) a prediction of the relative bioavailability of a selected compound and its derivatives in several oral dosage forms. Recent studies (C. Hansch. 1969; A. Leo et al., 1971; E. Lien, 1970; E. Lien et al., 1971) have shown correlations between a biological parameter such as drug--protein binding, pharmacological activity and partition coefficients of compounds in a given series. The purpose of this investigation was to ascertain whether any kind of relationship exists between the partition coefficients of a group of xanthene derivatives and their absorbability in vitro. By such an approach, the partition coefficient data might be used to predict the absorbability of potentially active compounds.

MATERIALS AND METHODS

Caffeine and theophylline were obtained from Sigma Laboratories (St. Louis, Mo.).1-Methyl-3-isobutylxanthene (SC-2964) and sodium salt of 8-chlorotheophylline were synthesized and released as pure compounds by Chemical Development group of the Development Department (Searle Laboratories, Chicago, Illinois). Octyl alcohol was purchased from Matheson Coleman & Bell Manufacturing Chemists (Norwood, Ohio). Sodium phosphate salts, mono and dibasic, were purchased from

Mallinkrodt Chemical Works (St. Louis, Mo.). pH measurements on all solutions were made with a portable pH meter (model 301, Orion Research Corporation, Cambridge, Mass.). All absorption spectra were recorded with a dual beam UV/VIS recording spectrophotometer (Coleman, model 124D, Perkin-Elmer Corporation, Maywood, Illinois).

A. Determination of Apparent Partion Coefficients (APC):

Solutions of each test compound at 10^{-3}M concentration were prepared in pH 7.4 isotonic phosphate buffer that was previously saturated with octyl alcohol. 10 ml of each solution was pipeted into 25 ml test tubes with screw caps (Pyrex glass, Corning Glass Works, Corning, N.Y.). Then, 10 ml of octyl alcohol, saturated previously with pH 7.4 buffer, was added to each test tube and the screw caps on the tubes were placed securely in position. Triplicate samples of each test compound were prepared and the two phases (octyl alcohol and pH 7.4 buffer) were mixed intimately for three hours with a rotating mixer (model 150V, Scientific Industries, Inc., Springfield, Mass.). The two phases were then allowed to separate and the octyl alcohol layer was discarded. The equilibrated buffer layer and control (non-equilibrated) solutions of the test compounds were diluted appropriately with pH 7.4 buffer and UV absorption spectra were recorded versus appropriate blanks. The apparent partition coefficient (APC) for each compound was estimated using the following expression:

$$APC = C_1 - C_2 / C_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where C_1 = original concentration of the test compound in buffer

C_2 = equilibrium concentration of the test compound in buffer.

B. Procedure for In Vitro Transfer Rate Studies:

The general procedure adopted to determine the cumulative transfer rate of xanthene derivatives across everted rat intestine sac preparations was similar to that reported previously (Levy and Matsuzawa, 1965; Feldman and Gibaldi, 1969; Tarazka, 1971).

Briefly, everted intestinal segments approximately 11 cm long were ligated at one end with surgical grade silk thread. The other (open) end was mounted and tied on a 1 3/8 inch Teflon tube that protruded through a neoprene rubber stopper of suitable dimensions. The ligated end was then tied to the end of a 14 inch "L" shaped blunt-edged hypodermic needle (Beckton-Dickinson Company, Rutherford, New Jersey). The modified needle, which was also fitted into the rubber stopper, was used to bubble a gaseous mixture of oxygen and CO_2 (95:5 % v/v, Matheson Gas Products, Jolliet, Illinois). A small hole was drilled into the rubber stopper to serve as the outl for the escaping gas. The everted intestinal sac was restrained parallel to the needle, thus facilitating access to fill and empty it as desired. Four of these intestinal preparations were immersed in 85 ml of 10^{-3} M solution of a test compound in pH 7.4 Krebs phosphate buffer without calcium and magnesium chlorides. The solution of the test compound and the intestine preparation were contained in a large 4 6/8 inch long and 1 1/2inch wide glass test tube that was modified to hold the intestinal sac assembly. The rubber stoppers of each preparation were fitted on the test tube mouths and the gaseous mixture of oxygen and carbon dioxide was bubbled at approximately 10 ml per minute through the mucosal solution via the needle inlet. At the start of the experiment, approximately 0.8 ml of Krebs buffer was placed in the intestinal sac and was then withdrawn and discarded. This procedure was repeated a second time and the withdrawn sample was collected to serve as a blank to quantify each test compound by UV spectrophotometry. The intestinal sac was filled again and fifteen minutes later, the solution was withdrawn followed by a quick rinse with Krebs buffer. This procedure was repeated every fifteen minutes for 90 minutes of incubation of the intestinal sacs maintained at 37° C.

The samples and the rinse withdrawn at each time interval were mixed and diluted to 10 ml with Krebs buffer and subsequently filtered through sintered glass funnels (Corning Glass Works, Corning, N.Y.). Upon appropriate dilution with Krebs buffer, UV absorption spectra on all samples were recorded with a dual beam

recording UV/VIS spectrophotometer. Absorbances of blanks were subtracted from absorbances (at $\lambda_{max.}$) of each test compound to quantitate its concentration in the serosal solutions. A calibration curve of absorbance (at $\lambda_{max.}$) versus concentration in ug./ml of each test compound was prepared to assist in the quantitation by the following expression:

$$\text{Amount Transfered per Unit Time} = 10\,[\text{corrected absorbance}]\left[\text{dilution factor}\right][1/m] \quad \ldots(2)$$

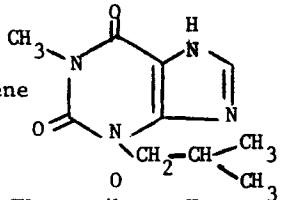
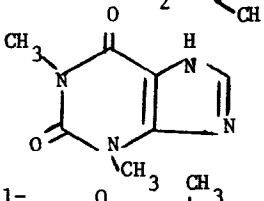
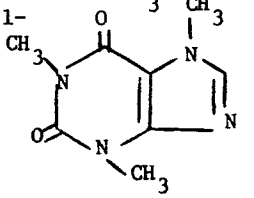
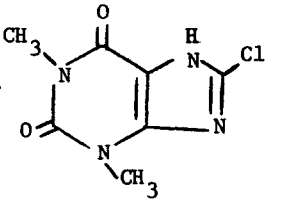
where 'm' is the slope of the calibration curve for each test compound in pH 7.4 Krebs buffer.

RESULTS AND DISCUSSION

Table I lists the test compounds, their structures, molecular weights and partition coefficient data. Clearly, the addition of an isopropyl group at 3 - position on theophylline increases the partition coefficient by as much as 24 times. In contrast, substitution of hydrogen by chlorine at position 8 of theophylline reduces the partition coefficient almost seven fold. Since the presence of chlorine, a polar substituent , provides a lability to the dissociable hydrogen on the adjacent nitrogen (in position 7), it is reasonable to expect such differences in partitioning properties of these compounds on the basis of substituent effects. Loehry et al. (1970) studied the in situ intestinal permeability of varous water soluble molecules in the 60 - 33000 molecular weight range. They reported that an inverse relationship exists between intestinal permeability and molecular weights. The xanthene derivatives were chosen for the present study because molecular weight differences between the compounds are insignificant and will not exert any contributory effect on their partitioning properties or intestinal absorbability in vitro.

Tables II through V illustrate the transfer rate of these xanthene derivatives across 10 cm everted rat intestinal sac preparations maintained at 37° C. The cumulative transfer rate

Table I. Physicochemical parameters of xanthene derivatives

Compound	Structure	M.W.	Partition Coefficient
1-Methyl-3-isobytylxanthene (SC-2964)		222.17	22.82 ± 0.51
1,3-Dimethyl-xanthene (Theophylline)		180.17	0.946 ± 0.034
1,3,7-Trimethyl-xanthene (Caffeine)		194.19	0.801 ± 0.060
1,3-Dimethyl--8-chloro-xanthene (8-Chlorotheophylline)		214.63	0.141 ± 0.042

for each preparation was determined by least square linear regression analysis of the data performed with the aid of a programmable calculator (model SR-51 calculator, Texas Instruments, Inc., Fort Worth, Texas). The slope of the regression line provided a value for the cumulative transfer rate in µg / min for each test compound. The importance of the partition coefficient parameter in biopharmaceutical studies and dosage form design has been reviewed extensively by Schumacher (1971). For a series of biologically active compounds, an improvement in the partitioning property reflects an improvement in absorbability. Recently,

Table II. Transfer of 8-Chlorotheophylline across 10 cm. everted rat intestine sacs immersed in 10^{-3} M solutions of sodium 8-chlorotheophyllinate in pH 7.4 buffer maintained at 37°C.

Time, Min.	Cumulative amount transfered, µg.			
Segment #	1	2	3	4
0	0	0	0	0
15	76.2	96.0	66.0	91.0
30	142.2	184.4	152.0	162.0
45	218.0	290.0	233.0	243.0
60	295.0	300.0	324.0	350.0
75	370.0	407.0	440.0	459.0
90	452.0	542.0	556.0	575.0
Cumulative Transfer Rate: (ug / min.)	5.03	5.50	6.40	6.50

Mean ($\bar{x} \pm$ S.E.) 5.86 ± 0.36 µg/min.

Table III. Transfer of Caffeine across 10 cm. everted rat intestine sacs immeresed in 10^{-3} M solutions of caffeine in pH 7.4 buffer maintained at 37°C.

Time, Min.	Cumulative amount transfered, µg.			
Segment #	1	2	3	4
0	0	0	0	0
15	78.0	83.3	99.0	112.0
30	151.0	181.0	184.0	230.0
45	233.0	297.0	260.0	341.0
60	324.0	400.0	351.0	464.0
75	412.0	511.0	445.0	587.0
90	507.2	640.0	541.0	717.0
Cumulative Transfer Rate: (ug/min.)	5.0	7.4	5.9	8.0

Mean ($\bar{x} \pm$ S.E.) 6.58 ± 0.685 µg/min.

Table IV. Transfer of Theophylline across 10 cm. everted rat intestine sacs immersed in 10^{-3} M solutions of theophylline in pH 7.4 buffer maintained at 37°C.

Time, Min.	Cumulative amount transfered, µg.			
Segment #	1	2	3	4
0	0	0	0	0
15	68.0	78.0	80.0	90.0
30	132.0	157.0	174.0	202.0
45	186.0	249.0	263.0	312.0
60	285.0	340.0	354.0	414.0
75	358.0	437.0	464.0	563.0
90	438.0	524.0	553.0	677.0
Cumulative Transfer Rate: (ug / min.)	5.0	6.0	6.3	7.8
Mean ($\bar{x} \pm$ S.E.)	6.28 ± 0.58 µg/min.			

Table V. Transfer of 1-Methyl-3-isobutylxanthene (SC-2964) across 10 cm. everted rat intestine sacs immersed in 10^{-3} M solutions of SC-2964 in pH 7.4 buffer maintained at 37°C.

Time, Min.	Cumulative amount transfered, µg.			
Segment #	1	2	3	4
0	0	0	0	0
15	121.0	158.0	146.0	160.0
30	300.0	308.0	302.0	293.0
45	431.0	446.0	427.0	424.0
60	548.0	621.0	554.0	548.0
75	633.0	810.0	732.0	696.0
90	...	882.0	854.0	817.0
Cumulative Transfer Rate: (ug / min.)	8.48	10.1	9.44	8.79
Mean ($\bar{x} \pm$ S.E.)	9.20 ± 0.36 µg/min.			

it has been demonstrated (Houston et al., 1974) that the percent absorption of a series of carbamate derivatives was related to the partition coeffifient P by the expression:

$$\text{Log \% Absorbed} = m \text{ Log } P + b \quad \ldots\ldots\ldots\ldots(3)$$

where "m" and "b" are slope and intercept respectively of the above expression. Thus,

$$\text{\% Absorbed} = b\, P^{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Since absorbability in vitro refers to the cumulative transfer rate of a compound across everted rat intestine sacs, the following equation is applicable:

$$R = k (C_m - C_s)(P_1)^{m}1 \quad \ldots\ldots\ldots\ldots\ldots\ldots(5)$$

where "R" is the cumulative transfer rate of a compound, "k" is the transfer rate constant, "C_m" and "C_s" are pseudo-steady state concentrations of the compound in the mucosal and serosal (inside the sac) solutions respectively; and "P_1" is the partition coefficient of the compound with reference to lipoidal intestinal tissue and pH 7.4 buffer. In this study, $C_m >> C_s$; and since during the experimental period, C_m remains virtually constant, equation (5) becomes:

$$R = k_1(P_1)^{m}1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(6)$$

Assume $k_1 = kc_m$

It is generally recognized that octyl alcohol may be used as a lipid phase with the partitioning property resembling that of biological tissue, i.e., the biophase. Under this assumption then,

$$R = k_1(P_1)^{m}1 = k_2(P_2)^{m}2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(7)$$

where "P_2" is the partition coefficient and "m_2" is an exponential term referring to the octyl alcohol/buffer (pH 7.4) system. The logarithmic form of equation (7) is:

$$\text{Log } R = m_2 \text{ Log } P_2 + \text{Log } k_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(8)$$

By this equation, a plot of log cumulative transfer rate (Log R) versus log partition coefficient (Log P_2) of the xanthene derivatives should be linear with the slope equaling "m_2" and the intercept equaling "Log k_2", a constant. Figure 1 illustrates this relation-

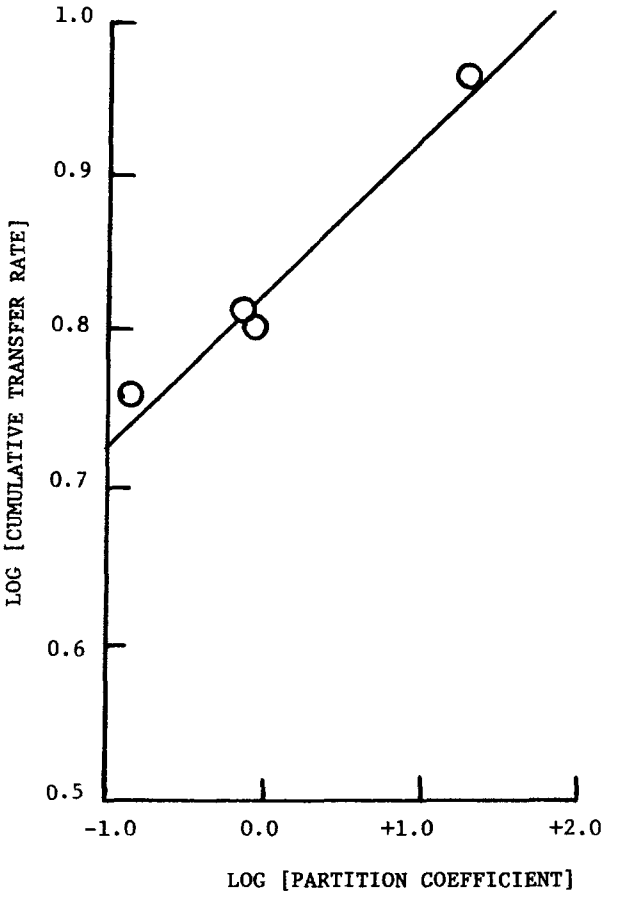


Figure 1. Linear relationship between log (partition coefficient) and log (cumulative transfer rate) of four xanthene derivatives. Each point in the figure represents the mean (± S.E.) of four observations. Standard errors are too small to be shown on th figure.

ship for the xanthene derivatives tested. A multifit linear regression analysis of the observed data yielded a correlation coefficient (r^2) value of 0.945. An equation that best describes the Log R - Log P_2 relationship for the xanthene derivatives tested is:

$$\text{Log } R = 0.0929 \text{ Log } P_2 + 0.735 \quad \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

The observed and the predicted values for the cumulative transfer rate of the xanthene derivatives tested are listed in Table VI. Equation (9) was used to predict the transfer rates of these compounds. The close agreement between the observed and the calculated values suggests that equation (9) can be used to predict the cumulative transfer rate (i.e., absorbability) of other xanthene derivatives on the basis of their partitioning data.

Thus, if at least 4-6 compounds in a series are tested in vitro, this relationship can be used to predict the absorbability of a new compound in the same series on the basis of their known partition coefficient data. It should be pointed out that for highly lipophilic and highly hydrophilic compounds in a given series, such a predictive approach may have limitations for applicability of this methodology.

Table VII. Observed and calculated values for the cumulative transfer rate of four xanthene derivatives.

Compound	Observed		Calculated
	Log P_2	Log R	Log R
8-Chloro-theophylline	-0.85	0.768	0.748
Caffeine	-0.094	0.818	0.818
Theophylline	-0.0241	0.798	0.825
SC-2964	+1.350	0.964	0.953

Determination of absorbability of a potential drug entity or its derivative such as a salt form or an ester form can provide meaningful results for the development of stable and bioavailable oral dosage forms.

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