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Drug Partitioning II

In Vitro Model for Drug Absorption

By JAMES T. DOLUISIO and JOSEPH V. SWINTOSKY

An *in vitro* model to simulate some factors involved in the absorption process is described. It consists of a tube containing two aqueous phases separated by an immiscible phase. A rocking apparatus agitates the fluids while causing the liquid interfaces to expand and contract. Rates of drug transfer and equilibrium drug distribution were determined under conditions where one aqueous phase was maintained at pH 7.4 and the other buffered at various pH values. Salicylic acid, barbital, antipyrine, aminopyrine, and tetracycline were studied in this manner. The initial drug transfer simulated a first-order rate process. Results of the equilibrium studies are in general agreement with predictions of the pH-partition theory. Tetracycline did not undergo transfer from one aqueous phase to the other at any pH condition of the study.

PREVIOUS INVESTIGATIONS (1–5) have demonstrated that the gastrointestinal absorption of drugs is often dependent upon their ability to penetrate a lipoidal barrier, and that for some compounds absorption is accomplished by passive diffusion of the unionized moiety. The following equations derived by Shore, *et al.* (1), give the theoretical ratios, R, of drug concentrations, C, in aqueous solutions of differing pH separated by a barrier which is selectively permeable to the unionized moiety:

for a base,

$$R = \frac{C_{gut}}{C_{plasma}} = \frac{1 + 10^{(pKa - pH_{gut})}}{1 + 10^{(pKa - pH_{plasma})}} \quad \text{(Eq. 1)}$$

and for an acid,

$$R = \frac{C_{gut}}{C_{plasma}} = \frac{1 + 10^{(pH_{gut} - pKa)}}{1 + 10^{(pH_{plasma} - pKa)}} \quad \text{(Eq. 2)}$$

In situ experiments (1–5) have shown that the distribution of some drugs approximates these equations. However, it is possible that an ionized

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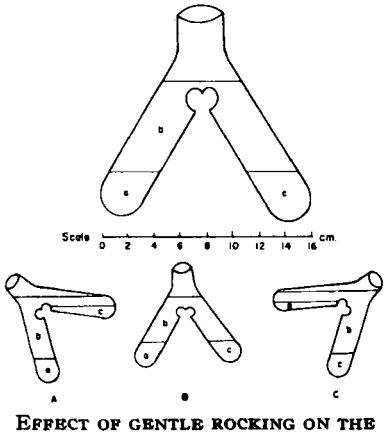
drug moiety might be sufficiently lipid-soluble to penetrate the gastrointestinal barrier, or that an unionized drug moiety might not be sufficiently lipid-soluble to penetrate the gastrointestinal barrier, or that an active transport process is involved. In these cases the above equations may be inadequate to predict gastrointestinal absorption.

It is suggested that the model depicted in Fig. 1 might be employed to simulate some factors involved in the absorption process—namely, the partitioning of drug between the gastrointestinal fluid and lipoidal phase and between lipoidal phase and plasma. Aqueous buffer systems of pH 2.0, 3.0, 5.0, and 7.4, cyclohexane, and an aqueous pH 7.4 buffer may be utilized to represent the various acidities of the gastrointestinal tract, the lipoidal barrier, and the acidity of the plasma, respectively. By use of this *in vitro* model the "absorption" process, *i.e.*, the passage of drug from one aqueous phase to the other, can be studied kinetically and as an equilibrium process.

EXPERIMENTAL

Apparatus and Reagents.—All chemicals were reagent grade unless specified otherwise. Salicylic acid, barbital N.F., antipyrine N.F., aminopyrine N.F.X, tetracycline HCl (Lederle Laboratories), potassium chloride, monobasic sodium phosphate, potassium acid phthalate, boric acid, sodium hydroxide, hydrochloric acid, cyclohexane, *n*-octyl alcohol, Leeds and Northrup model 7401 pH meter, and a Beckman model DB spectrophotometer were employed.

To prevent the aqueous phases from mixing, a glass tube and rocking apparatus were designed and constructed. (See Figs. 1 and 2.) The gentle rocking of the tubes accelerated drug transfer by causing the interfaces to expand and contract as shown in Fig. 1. The phases were sampled as necessary by inserting a pipet through the neck of the tube. The rocking apparatus[1] is of aluminum construction and possesses two pivot bars mounted on ball bearings. The pivot bars are tilted in one direction and then the other through attachment to a constant speed electric motor of 2 r.p.m., as pictured in Fig. 2. The degrees of tilt are controlled by a variable adjustment connecting-link attached to the motor. Spring clips are fastened to the framework of the pivot bars to accommodate the special glass tubes. The rocking apparatus is somewhat similar in design and application to one used in two-phase partition coefficient studies (6). The special tubes for these studies bear some similarity to tubes used for other purposes and previously reported (7).



EFFECT OF GENTLE ROCKING ON THE INTERFACES

Fig. 1.—Model used to simulate some factors involved in the absorption process. Phase *a* represents the various acidities of the gastrointestinal tract, phase *b* the lipoidal barrier, and phase *c* the pH of the plasma.

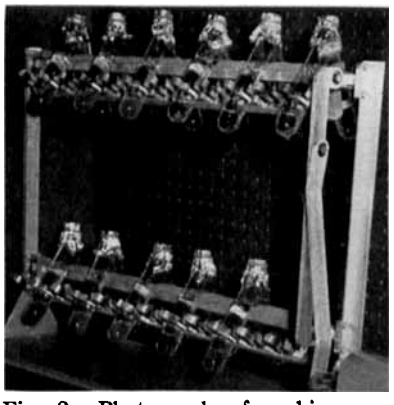


Fig. 2.—Photograph of rocking apparatus and glass tubes.

Procedure.—Aqueous buffered solutions of drug (15.0 ml.) were placed in one arm of the tube, 15.0 ml. of a pH 7.4 phosphate buffer solution was placed in the remaining arm, and 80.0 ml. of cyclohexane added. The system was gently rocked and the drug concentration determined at various time intervals. Salicylic acid, antipyrine, aminopyrine, and tetracycline concentrations were determined spectrophotometrically. Barbital solutions were adjusted to pH 9.4 with a borate buffer, then determined spectrophotometrically.

The pH 2.0 buffer was prepared by dissolving 3.0 Gm. of KCl in 1 L. of distilled water and adjusting the pH with concentrated HCl. The pH 3.0 buffer was prepared by dissolving 8.0 Gm. of potassium acid phthalate in 1 L. of distilled water and adjusting the pH with concentrated HCl. The pH 5.0 and pH 7.4 buffers were prepared by dissolving 5.7 Gm. of monobasic sodium phosphate in 1 L. of distilled water and adjusting the pH with 5 *N* NaOH. The pH 9.4 borate buffer was prepared by dissolving 6.0 Gm. of boric acid and 7.5 Gm. of KCl in 1 L. of distilled water and adjusting the pH with 5 *N* NaOH. All solutions used in the tetracycline experiments were prepared with deionized distilled water having a specific conductance of 2.5×10^{-6} ohm^{-1} cm.$^{-1}$ or less.

RESULTS AND DISCUSSION

Results are summarized in Table I. The initial disappearance of drug from phase *a* was approxi-

[1] The authors appreciate the assistance of Mr. Edward Bates and Mr. Allen Cook, R & D Mechanical Shop, and Mr. Andrew Airey, R & D Glass Shop, Smith Kline & French Laboratories, for assistance in constructing the equipment shown in Fig. 2.

TABLE I.—RESULTS OBTAINED FOR THE TRANSFER OF VARIOUS DRUGS IN THE *In Vitro* ABSORPTION MODEL

Drug	pH Phase *a* When Phase *c* is 7.4	$\frac{U}{U+I}k$, (hr.$^{-1}$)	k, (hr.$^{-1}$)	R, Theoret.	R, Exptl.
Salicylic acid	2.0	0.13	0.14	0	0
	3.0	0.12	0.24	0	0
	5.0	0.0046	0.46	0	0
	7.4	[a]	[a]	1.0	[a]
Barbital	2.0	0.0022	0.0022	0.72	0.74
	5.2	0.0022	0.0022	0.72	0.74
	7.4	0.0016	0.0021	1.0	1.2
Antipyrine	2.0	0.0059	0.0074	1.3	1.4
	5.0	0.0084	0.0084	1.0	1.1
	7.4	0.0090	0.0090	1.0	1.0
Aminopyrine	2.0	[a]	[a]	1000	[a]
	5.1	0.14	0.25	1.8	1.8
	7.4	0.26	0.26	1.0	1.0
Tetracycline HCl	2.0	[a]	[a]	...	[a]
	5.1	[a]	[a]	...	[a]
	7.4	[a]	[a]	...	[a]

[a] No drug transfer evident in 80 hours.

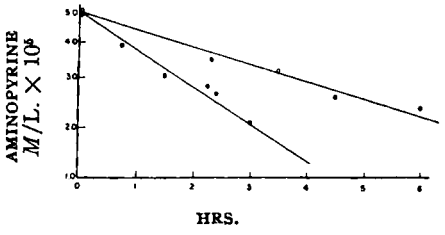


Fig. 3.—The effect of phase *a* pH on the disappearance of aminopyrine from an aqueous phase, through a cyclohexane phase, and into an aqueous pH 7.4 phase. Key: O, pH 5.1 phase *a*; ●, pH 7.4 phase *a*.

mately first order. Figure 3 illustrates that a change of pH in phase *a* affects the slope depicting disappearance of drug. This change in slope may be explained by the following equations. When the pH of phase *a* is such that the drug is completely unionized, transfer is represented by

$$-\frac{dC}{dt} = kC \qquad \text{(Eq. 3)}$$

and

$$\ln C = -kt + \ln C_0 \qquad \text{(Eq. 4)}$$

where C is drug concentration, t is time, and k is a constant. However, when the pH is such that the drug is partly ionized, transfer is represented by

$$-\frac{dC}{dt} = \frac{U}{U+I}kC \qquad \text{(Eq. 5)}$$

and

$$\ln C = -\frac{U}{U+I}kt + \ln C_0 \qquad \text{(Eq. 6)}$$

where U and I are unionized and ionized drug, and where $U/(U+I)$ is the fraction of drug unionized. According to these equations the "true" rate constant, k, is independent of pH. However, the slopes obtained by plotting $\ln C$ *versus* t yield apparent rate constants, *i.e.*, $[U/(U+I)]k$, which are a function of pH.

From the data in Table I, k is observed to be a constant at the different pH's at which barbital and aminopyrine were studied. In the case of antipyrine, some variability was observed for k. For salicylic acid k varied two to threefold. This greater variability in the value of k for salicylic acid is perhaps to be expected, since for a given concentration of this drug the concentrations of U and I vary approximately ninetyfold from pH 2 to 5. In this wide range of concentrations of U, the substantial change in I may influence the transfer rate of U between the immiscible phases.

In general, the results obtained are consistent with the hypothesis that for these drugs transfer is dependent upon the migration of the unionized drug moiety. The equilibrium distribution of drug between the two aqueous phases as indicated by R in Table I obeyed either Eqs. 1 or 2, depending on whether the drug was a base or an acid.

Table II lists the pKa and the fraction of drug unionized at various acidities for each drug studied.

Salicylic Acid.—Figure 4 illustrates the transfer of salicylic acid from a pH 2.0 aqueous phase to a pH 7.4 aqueous phase. In this case, as in most cases, the concentrations of drug in the cyclohexane phase were so low that they could not be accurately determined. Figure 5 is a first-order plot of the disappearance of salicylic acid from the pH 2.0

TABLE II.—FRACTION OF DRUG UNIONIZED AT VARIOUS ACIDITIES

Drug	pKa	pH	$\frac{U}{U+I}$
Salicylic acid	3.0	2.0	0.91
		3.0	0.50
		5.0	0.01
		7.4	[a]
Barbital	7.9	2.0	1.0
		5.2	1.0
		7.4	0.76
Antipyrine	1.4	2.0	0.80
		5.0	1.0
		7.4	1.0
Aminopyrine	5.0	2.0	0.00099
		5.1	0.56
		7.4	1.0
Tetracycline HCl	3.3,7.7,9.7	2.0	[a]
		5.1	[a]
		7.4	[a]

[a] Values less than 0.0001.

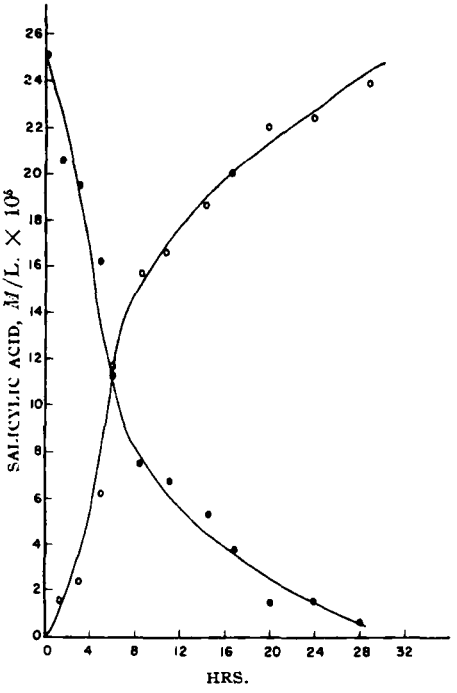


Fig. 4.—The transfer of salicylic acid from an aqueous pH 2.0 phase through a cyclohexane barrier to an aqueous pH 7.4 phase. The concentrations of salicylic acid in cyclohexane were low and could not be determined accurately (*e.g.*, less than 4×10^{-6} *M*/L.). Key; ●, pH 2.0 phase *a*; O, pH 7.4 phase *c*.

aqueous phase. It is evident from these data that at the pH of the stomach there is excellent drug transfer. As the pH of phase *a* rises and simulates that of the intestines, drug transfer falls off markedly. This is consistent with the statement that the absorption of weak acids occurs primarily from the stomach (2). It appears from Table I that at the pH of 7.4 in phase *a*, Eq. 2 is not obeyed. However, as shown in Table II, less than 0.01% of salicylic acid is unionized at pH 7.4. Since such a minimal amount of the migrating species is present, failure to detect drug transfer after 80 hours is perhaps a reasonable result.

Barbital.—The rates of barbital transfer were much slower than those of salicylic acid. It is also evident from the *R* values that salicylic acid should be absorbed more efficiently from the stomach than barbital. Barbital, which is largely undissociated in the pH range 2 to 7, should be absorbed throughout the entire gastrointestinal tract; whereas the rate of salicylic acid absorption by a passive mechanism would be expected to fall off markedly once the drug has reached regions of higher pH in the intestine.

Antipyrine.—The results illustrate that in the case of weak bases the higher the pH of phase *a* the better the drug transfer to phase *c*. From the data it would seem that antipyrine should be absorbed to some extent throughout the entire gastrointestinal tract. It is also evident that drug transfer should be identical when phase *a* is either pH 5.0 or 7.4. As shown in Table II, at these pH values antipyrine is essentially 100% unionized.

Aminopyrine.—Aminopyrine showed no transfer when the acidity of phase *a* simulated that of the stomach. At this pH aminopyrine is essentially completely ionized. At higher pH values the transfer increased and obeyed Eq. 1.

Tetracycline.—In the experiments involving tetracycline no transfer was evident when cyclohexane was employed as the lipoidal barrier. Pindell, *et al.* (8), have shown that tetracycline is absorbed from the stomach, duodenum, and ileum of the dog and have suggested that the absorption can be explained by the pH-partition hypothesis. It is interesting to note from their work that only 3% of an administered dose of tetracycline was absorbed in 1.5 hours. From the work of Stephens, *et al.* (9), it appears that at the pH of the stomach tetracycline would be cationic—as the pH rises the molecule would become a zwitterion—and then, at high pH values—an anion. In light of these findings it was then thought that perhaps cyclohexane was too nonpolar to simulate the intestinal barrier. In the case of tetracycline, for example, a more polar lipoidal barrier might allow transfer of a lipid-soluble charged species. It was found that *n*-octyl alcohol did not allow tetracycline transfer at pH 2.0 but did allow tetracycline transfer when the pH of phase *a* was 5.2 or 7.4. This transfer was also evident at these pH values in mixtures of *n*-octyl alcohol and cyclohexane up to a mixture of 80% cyclohexane. These studies, however, were complicated because buffer electrolytes also were able to pass very slowly through the *n*-octyl alcohol barrier, and to a lesser extent, through *n*-octyl alcohol–cyclohexane mixtures. It is of interest that a drug such as tetracycline which is ionized throughout the pH range of these studies may be soluble in lipid solvents. This suggests that some ionized drugs may probably show sufficient lipid solubility to be absorbed passively *in vivo* or that active transport processes may be involved. This idea and others will be subjects for some of our future studies of drug transfer and absorption.

The results of this investigation suggest that this apparatus and experimental method may have application in studying effects of additives on the transfer rates of absorptive processes. Also the methodology may be useful for studying drug binding. The substitution of a liquid membrane for a solid one in drug binding or other dialysis studies may overcome some of the experimental problems

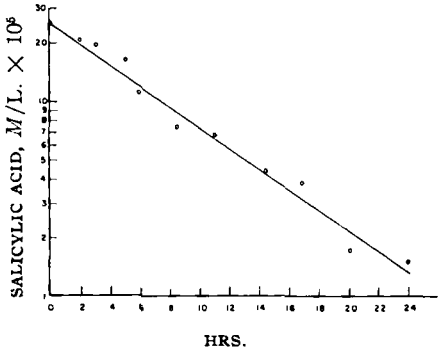


Fig. 5.—First-order plot of the disappearance of salicylic acid from an aqueous pH 2.0 phase, through a cyclohexane phase, and into an aqueous pH 7.4 phase.

arising when drugs or additives are adsorbed at a solid membrane interface, when impurities are solubilized from a solid membrane, or when pore size of a membrane influences diffusion through it.

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Distribution of Tritiated Derivatives of Fluorene in the Rat

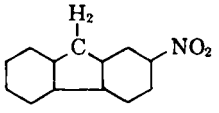
By F. E. RAY and O. O. WEJEBE

By the study of tritiated derivatives we have found that the liver carcinogen, 2-acetylaminofluorene, is concentrated to a greater extent in the liver of the rat than 2-acetylaminofluorenone, which produces only an occasional liver tumor. 2-Nitrofluorene, which also produces few, if any, liver tumors, gives a concentration that is intermediate. 2-Nitrofluorenone, which has not been tested for carcinogenicity, gives a concentration in the liver similar to 2-nitrofluorene. The highest concentrations in the liver result when the compounds are given by intraperitoneal injection.

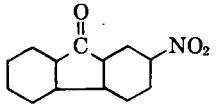
IN 1940, THE U. S. Department of Agriculture proposed the use of 2-acetylaminofluorene (AAF) as an insecticide; but before releasing it for field tests, it was sent to the Regional Laboratory in California for the determination of toxicity. After an extensive series of tests, Wilson, De Eds, and Cox (1) reported it to be carcinogenic to rats and mice. While this ended its career as an insecticide, it became of fundamental importance in the study of the etiology of cancer.

Whereas the carcinogenic hydrocarbons produce tumors almost exclusively at the site of application, acetylaminofluorene produces a wide variety of tumors distant from the site of application. The principal organ of attack, however, is the liver. It has been shown to be carcinogenic to mice, rats, rabbits, hamsters, dogs, and fowl. It is indeed fortunate that this compound was thoroughly tested *before* being released for general use.

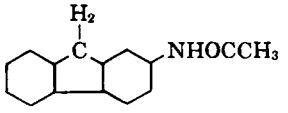
It seems reasonable to assume that a compound that has a specific effect on an organ must have an affinity for that tissue. In previous work we found this postulate to be true: a derivative causing gastric cancer localized in stomach tissue to a greater extent than a closely related nongastric carcinogen (2). Because 2-acetylaminofluorene (III) causes many liver tumors, but 2-acetylaminofluorenone (IV) does not, but is carcinogenic to other tissue (3, 5), one might expect a greater concentration of the former in the liver. To determine if this thesis is correct, radioactive derivatives of these compounds were administered and their distribution studied.



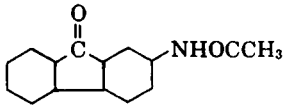
2-Nitrofluorene
(2-NF)
I



2-Nitrofluorenone
(2-NFone)
II



2-Acetylaminofluorene
(AAF)
III



2-Acetylaminofluorenone
(AAFone)
IV

Recent work by Ray, Cromer, Aycock, and Pitzer (2) has shown that it is practical to pre-

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