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Effect of Complexation with Caffeine on the *in Vitro* Transport of Drug Molecules through an Artificial Membrane Absorption Model[1]

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A three-compartment *in vitro* transport model, consisting of two aqueous compartments (buffered at pH 1.2 and 7.4) separated by a thin, olive oil-impregnated Millipore membrane, was employed to study the transport kinetics of four drugs in the absence and presence of the complexing agent, caffeine. The test drugs under investigation were salicylic acid, acetylsalicylic acid, dehydroacetic acid, and ethyl *p*-hydroxybenzoate. The apparent first-order transfer rate constants obtained for the drugs alone were found to correlate well with reported *in vivo* apparent first-order absorption rate constants.

In the presence of caffeine the rate of transfer of all of the test drugs was significantly reduced. Calculated *in vitro* transport rate constants were found to correlate well with reported *in vivo* absorption rate constants for the drug–caffeine complexes.

The gastrointestinal (G.I.) mucosa and other biological membranes are generally considered to be lipid in nature and possessing aqueous filled pores. The absorption of weakly basic and acidic drug molecules across these membranes generally occurs by means of a passive diffusion process. That is, absorption takes place from a region of high drug concentration (G.I. fluids) to a region of low drug concentration (plasma). The process usually obeys first-order kinetics.

According to the pH-partition hypothesis, it is the lipid soluble, undissociated drug species which is preferentially absorbed across the G.I. mucosa.[4–9] As a result, any physicochemical property which influences the degree of dissociation of a weakly acidic or basic drug substance will effectively reduce the concentration of the absorbable species in the fluids of the G.I. tract and thus influence the rate and/or extent of absorption. One finds therefore that weak acids are preferentially absorbed from the stomach and weak bases from the intestine.

Advances in the understanding of membrane function and structure has led to the construction of *in vitro* membrane models which have been employed to simulate the *in vivo* absorption process. It was, and is currently anticipated that the use of such model systems will provide a greater degree of insight into the complex process of drug absorption, as well as lead to a better understanding of the physicochemical factors influencing this process.

One of the first models employed to study the *in vitro* transport of drugs was the two-

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4) P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **119**, 361 (1957).
5) L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).
6) C.A.M. Hogben, L.S. Schanker, D.J. Tocco, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **120**, 540 (1957).
7) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958).
8) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).
9) J.T. Doluisio and J.V. Swintosky, *J. Pharm. Sci.*, **53**, 597 (1964).

phase partition model.[4-8] It consisted of an aqueous phase buffered to various pH values, which represented the G.I. fluids and a liquid lipid phase, used to represent the G.I. mucosa or membrane. The studies performed with this model essentially measured the equilibrium distribution of drugs between the two immiscible phases. Some qualitative rank order correlation was observed between the partition coefficient of the drug and *in vivo* absorption rate data. The model, however, suffers from the disadvantage that the partition coefficient of a drug is an equilibrium parameter, whereas *in vivo* absorption is a dynamic or kinetic process.

A three-phase liquid partition model, consisting of two aqueous phases separated by a lipid-containing phase has been introduced in an attempt to overcome some of the disadvantages of the two-phase system.[9-13] The additional aqueous compartment was buffered to a pH of 7.4 and served to represent the plasma. These studies were primarily concerned with determining the kinetics of drug transfer according to the following kinetic expression,

$$D_A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} D_B \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} D_C \qquad (1)$$

where D_A represents the concentration of a drug in the aqueous compartment used to simulate the G.I. fluids, D_B the concentration of drug in the membrane and D_C the concentration of drug present in the compartment used to simulate the plasma. The model is capable of simulating the *in vivo* condition of preferential transfer or absorption of the undissociated drug species. However, it has two major disadvantages. Firstly, emulsification can occur at the oil-water interface which can result in an increase in the interfacial area between the two immiscible, liquid phases and hence an increase in transport rates. Secondly, the thickness of the oil phase is many times greater than the natural membrane and consequently drug molecules can accumulate in this phase, even though such might not be the case *in vivo*.

Recently, Levy and Mroszczak[14] reported on the use of a three-compartment, *in vitro* transport model to study the kinetics of drug transport. The model, which was similar to that reported by Tobias and Agin,[15] consisted of a lipid-impregnated Millipore membrane separating two aqueous compartments. The thin, lipid-impregnated membrane employed in this model appears to essentially eliminate all of the disadvantages of the above-mentioned model systems, especially that of drug accumulation in the membrane.

The *in vivo* study of Goto and associates[16] on the rate of gastric absorption of a number of drugs in the absence and presence of the complexing agent, caffeine, prompted the present investigation, the purpose of which was to ascertain whether a correlation existed between their *in vivo* findings and the *in vitro* transport rates of the same drug and drug-caffeine systems. The importance of such *in vitro–in vivo* correlations is well recognized since they may permit the prediction of the gastrointestinal absorption rate characteristics of drugs from suitable *in vitro* transport rate studies.

Experimental

Materials——Caffeine, salicylic acid, acetylsalicylic acid, ethyl *p*-hydroxybenzoate, dehydroacetic acid, hydrochloric acid, sodium carbonate, sodium phosphate (monobasic), sodium hydroxide, and ferric nitrate were of reagent grade and were used without further purification.

Transport Studies——The *in vitro* absorption model was similar to that proposed by Levy[14] and Tobias[15]

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[10] J.T. Doluisio and J.V. Swintosky, *J. Pharm. Sci.*, **54**, 1594 (1965).
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[13] S.A. Khalil and A.N. Martin, *J. Pharm. Sci.*, **56**, 1225 (1967).
[14] G. Levy and E.J. Mroszczak, *J. Pharm. Sci.*, **57**, 238 (1968).
[15] J.M. Tobias and D.P. Agin, *J. Gen. Physiol.*, **45**, 989 (1962).
[16] S. Goto, R. Takamatsu, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 332 (1968).

and consisted of two—10 ml aqueous compartments (A and C) separated by a Millipore filter pad, type HA impregnated with olive oil (compartment B). The specially constructed plexiglass dialysis cells were maintained at room temperature (20—25°). The area of the membrane exposed to the aqueous compartments was 11.3 cm^2 and contained an average of 15 mg of olive oil per cm^2 following suitable impregnation procedures.

At time zero, 10 ml quantities of the following 0.1 N HCl solutions were introduced into compartment A, salicylic acid (3.0 mM), acetylsalicylic acid (3.0 mM), ethyl *p*-hydroxybenzoate (4.5 mM) and dehydroacetic acid (3.0 mM) alone or in the presence of a 60.0 mM concentration of caffeine. All of the test drugs were essentially present in the undissociated form at pH 1.2 (*i.e.*, 98—100%). A similar quantity of a pH 7.4 phosphate buffer was placed into compartment C, except in those studies involving the drug ethyl *p*-hydroxybenzoate where a pH 10.0 carbonate buffer was employed in order to maintain essentially uni-directional transport. The ionic strength of all solutions was 0.10. At appropriate time intervals the cells were rotated in order to obtain a homogeneous sample and 1.0 ml samples were simultaneously removed from compartments A and C. Each transport study was performed at least in duplicate.

Determination of Salicylic Acid——A 5.0 ml quantity of a 4.0% ferric nitrate reagent solution in 0.12 N HCl was added to a 1.0 ml sample of salicylic acid. The absorbance of the purple colored complex produced obeyed the Beer–Lambert law at a wave length of 540 mμ. Caffeine did not interfere with the assay procedure in the concentrations employed in this study.

Determination of Acetylsalicylic Acid——To a 1.0 ml sample, contained in a 5 ml capacity culture tube, was added 0.5 ml of 0.5 N NaOH solution. The tube was sealed and placed in an oven at 100° for 40 minutes to hydrolyze the sample to salicylic acid. The tube was cooled and the solution acidified with 0.5 ml of 0.6 N HCl. A 1.0 ml sample was removed and assayed colorimetrically as outlined above.

Determination of Ethyl *p*-Hydroxybenzoate——A 1.0 ml sample was diluted with a 1% sodium carbonate solution and assayed spectrophotometrically at 294 mμ using a Beckman DB-G recording spectrophotometer. When caffeine was present in the sample it was removed by extracting the diluted sample with three—20 ml portions of chloroform.

Determination of Dehydroacetic Acid——A 1.0 ml sample was diluted with 0.1 N HCl and assayed spectrophotometrically at a wave length of 308 mμ. When caffeine was present in the sample it was removed by diluting the 1.0 ml sample with a 1% sodium carbonate solution and subsequently extracting the diluted sample with three—20 ml portions of chloroform. The resultant aqueous phase containing only the drug was assayed at a wave length of 292 mμ.

Determination of Caffeine——A 1.0 ml sample was diluted with 0.1 N HCl and assayed spectrophotometrically at 273 mμ.

Results and Discussion

The *in vitro* transport of drug from compartment A (pH 1.2) through the impregnated membrane into compartment C, initially containing no drug, was found to best fit a modification of the reaction scheme given by equation (1). That is,

$$D_A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} D_B \xrightarrow{k_2} D_C \tag{2}$$

The appropriate differential equations for this transport scheme are,

$$-dD_A/dt = k_1D_A - k_{-1}D_B \tag{3a}$$

$$dD_B/dt = k_1D_A - k_{-1}D_B - k_2D_B \tag{3b}$$

$$dD_C/dt = k_2D_B \tag{3c}$$

However, in all of the transport systems investigated, essentially all of the drug initially introduced into compartment A was eventually transferred to compartment C. Also, drug did not accumulate in compartment B. Therefore, steady state conditions were assumed for D_B (*i.e.*, D_B was constant and small in magnitude, and thus, $dD_B/dt=0$). Setting equation 3b equal to zero and solving for D_B results in,

$$D_B = k_1D_A/(k_{-1}+k_2) \tag{4}$$

Substitution of the relationship given by equation 4 into equations 3a and 3c, and simplifying the results yields,

$$-dD_A/dt = [k_1k_2/(k_{-1}+k_2)]D_A \tag{5a}$$

and $$dD_C/dt=[k_1k_2/(k_{-1}+k_2)]D_A \qquad (5b)$$

or $$-dD_A/dt=dD_C/dt=k_{app}D_A \qquad (6)$$

where k_{app}, is the apparent first-order rate constant governing the disappearance of drug from compartment A, and is equal to $k_1k_2/(k_{-1}+k_2)$. Finally, integration of equation 6 results in,

$$\log D_A=-k_{app}t/2.303+\log D_A^{\circ} \qquad (7)$$

where D_A° represents the concentration of drug in compartment A at time zero.

As indicated by equation 6, once steady state conditions have been established, measurement of the appearance of drug into compartment C as a function of time should provide a second means for determining the magnitude of k_{app}.

The overall transport scheme given by equation 2 was experimentally verified in the following manner. To establish the fact that the transport step $D_B \rightarrow D_C$ was uni-directional, drug solution was initially introduced into compartment C (pH 7.4 or 10.0) and its appearance in compartment A (pH 1.2) measured. Under these experimental conditions no drug appeared to be transported to compartment A. The existence of an equilibrium between drug in compartments A and B (*i.e.*, $D_A \rightleftarrows D_B$) was tested by impregnating the olive oil-containing membrane with drug and noting the appearance of drug in compartment A, in a transport cell devoid of a compartment C. The appearance of drug in compartment A confirmed the reversibility of this step in the over-all transport scheme.

The apparent stability constants (K_s) for the four drug–caffeine complexes in 0.1 N HCl at 20° were estimated from van't Hoff-type plots (Fig. 1) of the log K_s versus $1/T$ (°K), the data for which was obtained from the work of Goto and associates.[16] The magnitude of the K_s values at 20° were: salicylic acid–caffeine (58.8 M^{-1}); dehydroacetic acid–caffeine (13.1 M^{-1}); ethyl *p*-hydroxybenzoate–caffeine (50.7 M^{-1}); and acetylsalicylic acid–caffeine (20.6 M^{-1}). Employing these calculated stability constants for the complexes, the ratio of the concentration of uncomplexed drug (D_f) to the total concentration of drug in the system (D_t) was calculated. The results are listed in Table I (column 2).

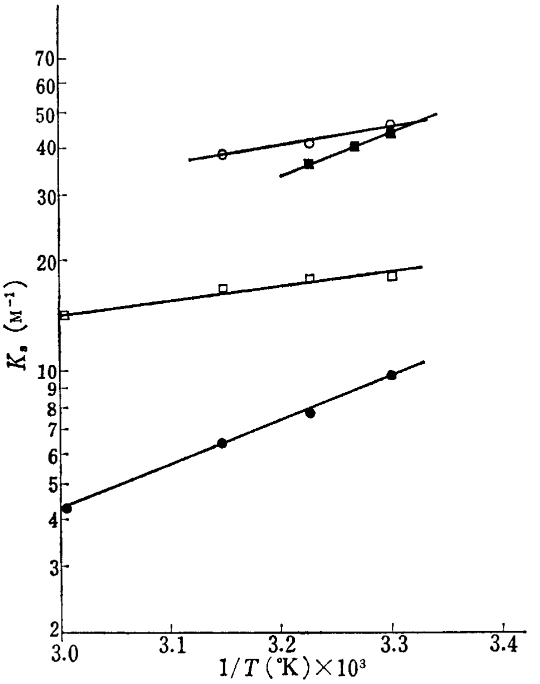

Fig. 1. Van't Hoff-Type Plots for the Drug Caffeine Complexes

key: dehydroacetic acid (●); salicylic acid (■); ethyl *p*-hydroxybenzoate (○); acetylsalicylic acid (□)

Figure 2 is a representative plot, in accordance with the proposed kinetic transport model, of the ***in vitro*** transfer of ethyl *p*-hydroxybenzoate from compartment A to compartment C. The lower curve in this figure was constructed with the aid of equation 7. The upper curve represents a plot of log $(D_C^{\infty}-D_C)$ *versus* time (where D_C^{∞}, which signifies the concentration of drug in compartment C at the end of the transport process, was experimentally determined to be essentially equal to D_A°). After steady state conditions have been established with respect to the concentration of drug in the lipid impregnated membrane, the rate of disappearance of ethyl *p*-hydroxybenzoate from compartment A becomes equal to its rate of appearance in compartment C, and the two curves become paralleled to one another. Hence, the magnitude of k_{app}, determined by sampling either compartment A or C is virtually identical

Table I. *In Vitro* Transport Kinetics of Drug Molecules through a Lipid-impregnated Membrane Transport Model, Alone and in the Presence of Caffeine[a]

Concentration of drug solutions (pH 1.2) initially introduced into comparment A	$\left(\frac{D_f}{D_t}\right)$[b]	k_{app} or k'_{app} (hr^{-1})[c]	*In Vivo* absorption $k_f(hr^{-1})$[d]	*In Vitro* transport $k'_{com}(hr^{-1})$[e]	*In Vivo* absorption $k_{com}(hr^{-1})$[f]
Dehydroacetic acid, 3.0 mM	1.0	0.44	0.98	—	—
Dehydroacetic acid, 3.0 mM Caffeine, 60.0 mM	0.56	0.30	—	0.11	0.40
Salicylic acid, 3.0 mM	1.0	0.41	0.75	—	—
Salicylic acid, 7.2 mM Caffeine, 40.0 mM	0.45	0.26	—	—	—
Salicylic acid, 3.0 mM Caffeine,60.0 mM	0.22	0.19	—	0.12	0.34
Ethyl *p*-hydroxybenzoate, 4.5mM[g]	1.0	0.40	0.69	—	—
Ethyl *p*-hydroxybenzoate,4.5 mM[g] Caffeine, 60.0 mM	0.26	0.15	—	0.062	0.26
Acetylsalicylic acid, 3.0 mM	1.0	0.053	0.32	—	—
Acetylsalicylic acid, 3.0 mM Caffeine, 60.0 mM	0.45	0.037	—	0.024	0.23
Caffeine, 60.0 mM	—	0.0025	0.20	—	—

[a] Compartment A buffered to pH 1.2; compartment C buffered to pH 7.4.
[b] Ratio of the molar concentration of uncomplexed drug (D_f) to the total drug concentration initially introduced into compartment A with caffeine.
[c] Determined by measuring disappearance of drug from compartment A. Represents the average of at least two experimental runs.
[d] *In vivo* absorption rate constant for the drug alone; data obtained from reference 16.
[e] *In vitro* transport rate constant for the drug-caffeine complex (k'_{com}).
[f] *In vivo* absorption rate constant for the drug-caffeine complex (k_{com}) using data obtained from reference 16.
[g] Compartment C was buffered to pH 10.0.

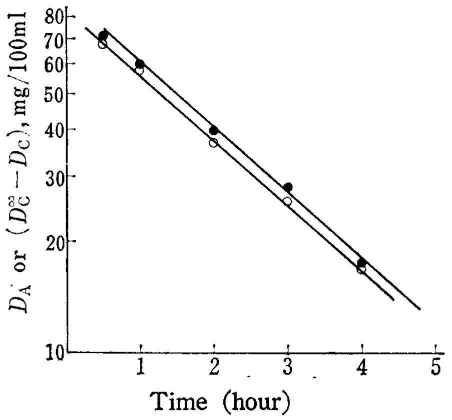

Fig. 2. Apparent First-Order Disappearance of Ethyl *p*-Hydroxybenzoate from Compartment A (○) and Its Appearance in Compartment C (●)

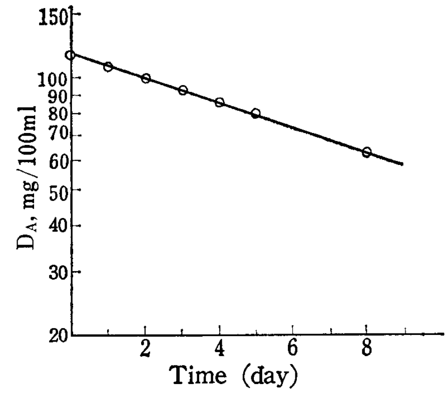

Fig. 3. Apparent First-Order Disappearance of Caffeine from Compartment A (pH 1.2)

(*e.g.*, compartment A, $k_{app}=0.40\ hr^{-1}$; compartment C, $k_{app}=0.39\ hr^{-1}$). A similar parallelism was obtained for the *in vitro* transport of the other three drugs studied, both in the absence and presence of caffeine. In accordance with equation 7, the apparent first-order rate constants, k_{app}, for the four pure drug systems were calculated from the least square slopes of their respective semilogarithmic plots and are listed in Table I (column 3). For the basis of comparison the transfer rate constant for pure caffeine from pH 1.2 to pH 7.4 is also included in this table and graphically represented in Figure 3. It is readily apparent from the magnitude of these rate constants that the transfer rate of the pure drugs decreases in the following order,

$$\text{dehydroacetic acid} > \text{salicylic acid} \simeq \text{ethyl } p\text{-hydroxybenzoate}$$
$$\gg \text{acetylsalicylic acid} \gg \text{caffeine}$$

This *in vitro* order qualitatively agrees with the order found for the *in vivo* rate constants governing the gastric absorption of these drugs (see Table I, column 4).

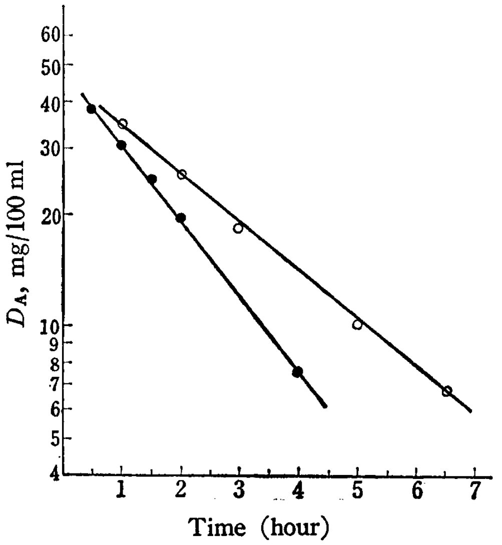

Fig. 4. Apparent First-Order Disappearance of Dehydroacetic Acid from Compartment A (pH 1.2)

key: alone (●); in the presence of caffeine (○)

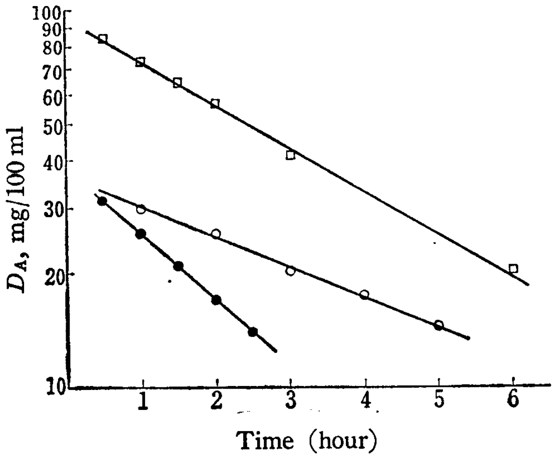

Fig. 5. Apparent First-Order Disappearance of Salicylic Acid from Compartment A (pH 1.2)

key: alone (●); at a concentration of caffeine sufficient to complex 78% (○) and 55% (□) of the drug

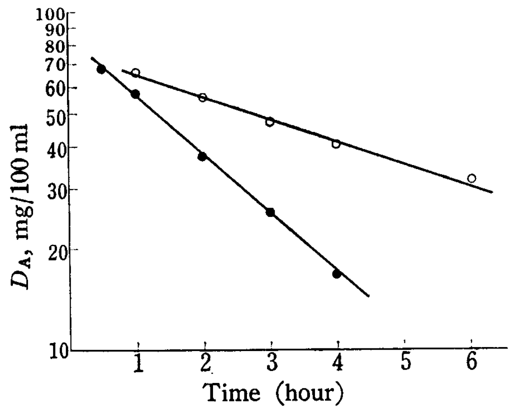

Fig. 6. Apparent First-Order Disappearance of Ethyl *p*-Hydroxybenzoate from Compartment A (pH 1.2)

key: alone (●); in the presence of caffeine (○)

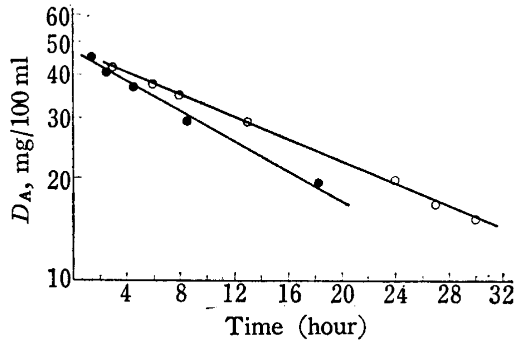

Fig. 7. Apparent First-Order Disappearance of Acetylsalicylic Acid from Compartment A (pH 1.2)

key: alone (●); in the presence of caffeine (○)

Studies were performed to determine the influence of the complexant, caffeine on the *in vitro* transport rate of the test drugs. The concentration of caffeine used was sufficient to complex approximately 75% of either salicylic acid or ethyl *p*-hydroxybenzoate, and approximately 50% of either acetylsalicylic acid, salicylic acid or dehydroacetic acid. The upper curves in Figures 4—7 represent the transport kinetics for the disappearance of total drug (free and complexed) from compartment A, under these experimental conditions. The transport kinetics for the pure drug systems are included in these figures (lower curves) for comparison purposes. The apparent rate constants for the drugs in the presence of caffeine (k'_{app}) are summarized in Table I (column 3). A comparison of these values with those for the drugs alone (k_{app}) shows that complexation with caffeine produces approximately a 60% reduction in the rate of transport of salicylic acid and ethyl *p*-hydroxybenzoate, and a 30% reduction for dehydroacetic and acetylsalicylic acids at an initial caffeine concentration of 60.0 mM.

The inhibitory effect of caffeine on the drug transport rate can be shown to be directly related to the extent of complexation, as is mathematically illustrated by equation 8.[16)]

$$k'_{\mathrm{app}}=(k_{\mathrm{app}}-k'_{\mathrm{com}})D_f/D_t+k'_{\mathrm{com}} \tag{8}$$

where k'_{app} is the apparent transport rate constant for the drug in the presence of caffeine, k_{app} the transport rate constant for the drug in the absence of caffeine, k'_{com} the rate constant for the drug–caffeine complex, and D_f/D_t the fraction of drug present in the free or uncomplexed form. Figure 8 represents a plot of k'_{app} *versus* D_f/D_t for the salicylic acid–caffeine system. The linearity observed demonstrates the adherance of this system to the assumptions made in the derivation of equation 8.[16)] The magnitude of the rate constant for the salicylic acid–caffeine complex, k'_{com}, was estimated from the intercept of the plot and is listed in Table I (column 5) together with the calculated values for the other three drug–caffeine complexes. Inspection of these values with the corresponding *in vivo* gastric absorption rate constants for the same drug–caffeine complexes,[16)] tabulated in Table I (column 6), indicates that a rank order *in vitro–in vivo* correlation exists between these two sets of data.

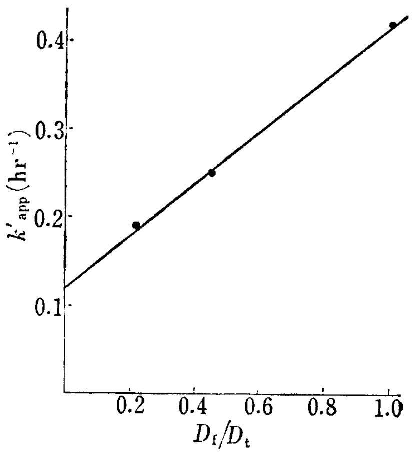

Fig. 8. Plot of k'_{app} *versus* D_f/D_t for the Salicylic Acid–Caffeine System

The results of the present investigation demonstrates the usefulness of an *in vitro*, physicochemical transport model consisting of two buffered aqueous compartments separated by a lipid-impregnated Millipore membrane to study the effect of complexation with caffeine on the transfer kinetics of various drug molecules. It has been shown that a good rank-order correlation exists between the *in vitro* transport and *in vivo* absorption rates of chemically diverse drug molecules and their caffeine complexes. The establishment of such a correlation suggests that the transport model employed in this investigation may be of value in studying the effects of other physicochemical factors on drug transport. In this connection, additional studies are in progress in our laboratories, utilizing this transport model, to determine if similar *in vitro–in vivo* correlations are realized for other drug and drug-complexant systems.