Effect of Complex Formation on Drug Absorption VIII

Intestinal Transfer Characteristics of the Salicylamide–Caffeine System

By RICHARD H. REUNING* and GERHARD LEVY†

Salicylamide and caffeine form a complex in aqueous solution which has an apparent lipoid–aqueous partition coefficient intermediate between the partition coefficients of free salicylamide and free caffeine. The transfer rate of salicylamide across the cannulated everted intestine of the rat is decreased in the presence of caffeine at a concentration sufficient to complex an appreciable fraction of the salicylamide. On the other hand, complex formation with salicylamide has no measurable effect on the intestinal transfer rate of caffeine. These observations suggest that the complex can be transferred as such and that diffusion of the drugs through the bulk solution to the membrane surface is not transfer rate limiting. A theoretical analysis of the simultaneous equilibria involved in the partitioning between an organic and an aqueous phase of two interacting drugs in the presence of one another shows that the apparent partition coefficient of a drug complex does not necessarily represent the ratio of transfer rate constants of the complex itself across the interface. For this reason, it should not be assumed that there will always be a correlation between the apparent partition coefficient and the intestinal transfer rate constant of drug complexes. The results of the reported study support this conclusion.

IN A PREVIOUS STUDY (1) salicylamide and caffeine were found to form a complex in aqueous solution. The stoichiometry and stability constant of the complex were determined at pH 5 where both drugs are essentially undissociated. The purpose of the present study was to investigate the effect of this complex formation on the overall[1] intestinal transfer of both salicylamide and caffeine and to compare the effects of complex formation on the intestinal transfer rates and on the apparent partition coefficients of salicylamide and caffeine, respectively.

The salicylamide–caffeine system was chosen as a model for a study of the effect of complex formation on drug absorption since extensive complexation of either drug is possible under suitable experimental conditions. Such extensive complexation, made possible by the adequate solubility of both drugs in water and by the relatively high stability constant of the complex (41 l./mole at 37°), is desirable because any pronounced effect of complex formation on intestinal transfer will then be reflected by a measurable change in the overall intestinal transfer rates of the two drugs.

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[1] In the context used in this paper, "overall" refers to the combined effect or behavior of free and complexed drug.

EXPERIMENTAL

Intestinal Transfer Rate Determinations—The cannulated everted intestine method developed by Crane and Wilson (2) was used with the modifications described previously (3). Each everted intestinal segment, 10 cm. in length, from a male Sprague-Dawley rat (290–380 g.), was suspended in a large test tube containing about 50 ml. of mucosal solution at 37°. Krebs-Henseleit-Acetate Ringer[2] solution (KHAR), pH 5.0, containing caffeine, salicylamide, or both in varying concentrations, was used as the mucosal solution. Two intestinal segments were obtained from each rat and an equal number of first and second segments were used for each experiment. Two milliliters of the serosal solution, Krebs-Henseleit-Bicarbonate Ringer solution (KHBR), pH 7.4 (4), was introduced into the everted intestinal segment. The entire serosal solution was withdrawn every 10 min. After each withdrawal the serosa was rinsed with KHBR and the rinse was combined with the previously withdrawn solution for subsequent assay. Immediately after completion of the rinsing procedure, another 2 ml. of KHBR was placed into the intestinal segment. This frequent replacement of the serosal solution prevented appreciable build-up of drug concentration on the serosal side. In one set of experiments involving the measurement of caffeine transfer rates in the presence of salicylamide in the mucosal solution, salicylamide was also included in the serosal solution.

The serosal samples were diluted with KHAR, pH 5.0, and the amount of caffeine or salicylamide transferred was determined by spectrophotometry at 273 or 299 mμ, respectively. When both drugs were present together, two-component spectrophotometry was used at these wavelengths. The absorbances of the two compounds were additive in the concentration range of the assay procedure. A small correction for blank was necessary only in the first two 10-min. samples, as determined in

[2] This is similar to Krebs-Henseleit-Bicarbonate Ringer solution (4) except that an equimolar amount of acetate buffer was substituted for the bicarbonate buffer.

experiments in which drug-free solutions were used. When known amounts of both drugs were added to KHBR solution which had been incubated for 10 min. with an intestinal segment (*i.e.*, using the cannulated everted intestine method as described above, but without drugs), an average of 101% of added caffeine and 100% of added salicylamide was recovered.

The transfer rates were determined from the slope of a plot of cumulative amount of drug transferred *versus* time. The transfer rate constant was obtained by dividing the slope value by the mucosal drug concentration. This was possible because of frequent replacement of the serosal solution and because the volume of the mucosal solution was large enough so that the drug concentration in that solution remained essentially constant for the duration of the experiment. A derivation from Fick's law of this method of calculating the transfer rate constant has been presented previously (3).

Experimental Design—The study was designed to determine the effect of complex formation on drug absorption under conditions which also permitted an assessment of any possible alteration in the permeability characteristics of the everted intestine as a function of time or upon addition of the complexing agent. The everted intestinal segment was first suspended for 40 min. in a solution containing only the drug, then for 40 min. in a solution containing drug plus complexing agent, and then again for 40 min. in a solution containing only drug. An equal number of experiments was carried out in a different sequence. Comparison of the transfer rates observed in the first and third time periods permitted an evaluation of any possible changes in the permeability of the intestinal segment. Also, the comparison of transfer rates both in the absence and presence of complexing agent in the same intestinal segment eliminates any variability due to possible differences in the surface area, thickness, *etc.*, of different segments.

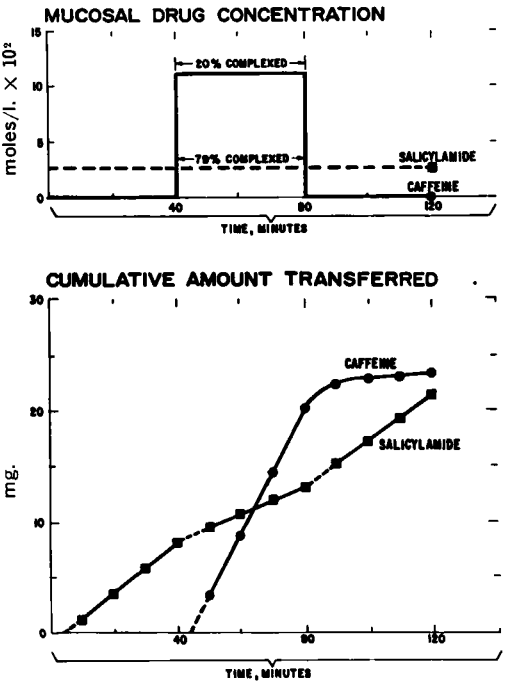

Fig. 1—Effect of complex formation with caffeine on the transfer of salicylamide across the cannulated everted intestine of the rat. In the upper portion of the figure the concentrations of salicylamide and caffeine in the mucosal solution are plotted as a function of time. In the lower portion of the figure, the cumulative amounts of salicylamide and caffeine transferred across the intestine are plotted as a function of time.

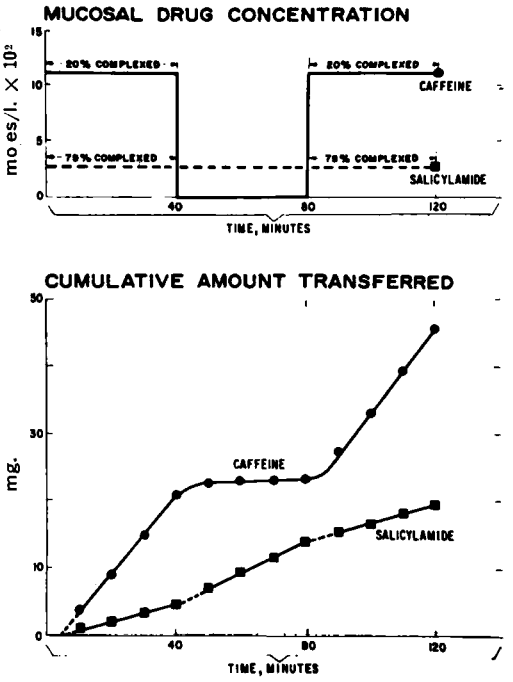

Fig. 2—Effect of complex formation with caffeine on the transfer of salicylamide across the cannulated everted intestine of the rat. Details as in Fig. 1 except that the order of addition of the complexing agent was changed.

Determination of the Extent of Metabolism of Salicylamide by the Everted Rat Intestine—Three 10-cm. segments of everted intestine from a male Sprague-Dawley rat (280 g.) were tied on one end with suture and filled with 2.5 ml. of serosal solution (KHBR, pH 7.4, was the solvent) through the open end which was then also tied off. Each everted sac was placed in a 50-ml. flask containing 15 ml. of mucosal solution (KHAR, pH 5.0, was the solvent). The flasks were flushed with 95% O_2–5% CO_2 and incubated for 1 hr. The mucosal and serosal solutions contained the same concentration of the drug(s), namely either salicylamide, salicylamide and caffeine, or solvent alone. The latter was used as a control to determine the recovery of salicylamide by adding known amounts of this drug, similar to the amounts used in the other two experiments, to the serosal solution, the mucosal solution, and to a homogenate of the intestinal segment. Salicylamide in the mucosal and serosal solutions and in a homogenate of the everted intestinal segment was assayed by a method which is specific for free drug and

excludes glucuronide or sulfate conjugates, if present. This assay, which involves extraction of the drug from a pH 7 buffer into ethylene dichloride and subsequent reextraction into a ferric nitrate reagent solution, has been described elsewhere (5).

Determination of Apparent Partition Coefficients—Apparent partition coefficients were determined (at least in duplicate) with isoamyl acetate as the organic phase and KHAR, pH 5.0, as the aqueous phase. Equilibration was carried out at room temperature for at least 12 hr. using an automatic shaking apparatus. In the determination of salicylamide partition coefficients, the drug in the aqueous phase was assayed colorimetrically as previously described (1). In the determination of caffeine partition coefficients, caffeine alone in the aqueous phase was assayed spectrophotometrically at 273 mμ. When salicylamide was present, both drugs were assayed in the aqueous phase using two-component spectrophotometry at 273 and 299 mμ.

RESULTS AND DISCUSSION

Effect of Complex Formation of Intestinal Transfer of Salicylamide and Caffeine—The effect of caffeine (2.2%) on the overall intestinal transfer rate of salicylamide (0.384%) is shown in Figs. 1 and 2. It is evident that a pronounced decrease in the overall transfer rate of salicylamide occurred when this drug was complexed extensively with caffeine.[3] The figures also show the transfer of the complexing agent, caffeine, and the degree to which it was complexed. The intestinal transfer rate constant for salicylamide alone (0.384% in the mucosal solution) was 3.5 cm.³/hr. as compared to 2.0 cm.³/hr. in the presence of 2.2% caffeine (means of the paired observations from four segments). Subsequent experiments, to be reported in a following paper (6), yielded essentially the same values for the transfer rate constants (3.4 and 2.0 cm.³/hr., respectively) when the serosal solution contained caffeine and was exchanged every 6 min. rather than every 10 min. This shows that practically sink conditions were maintained in the present study.

The transfer rates of salicylamide and caffeine were essentially the same in the first and third time periods (Figs. 1 and 2). This indicates that the permeability of the everted intestine did not change measurably during the time of the experiment, regardless of the presence or absence of a high concentration of caffeine. These results suggest that the effect of caffeine on the intestinal transfer of salicylamide is not due to a modification of intestinal permeability characteristics.

The possibility that the transfer rate data are affected by the formation of salicylamide conjugates in the intestinal mucosa was examined. When salicylamide in a relatively low concentration (0.024% as compared to 0.384% used in the transfer study) was incubated with intestinal tissue for 1 hr., no significant metabolism of salicylamide was found, regardless of the presence or absence of caffeine (Table I). These results are in agreement with those of other workers who have found significant metabolism of salicylamide in rabbit intestinal tissue, but not in that of the rat (7).

[3] The calculations of the extent of complexation in this paper are based on a stability constant of 41.1 l./mole for the salicylamide–caffeine complex (1:1) and are not corrected for caffeine dimerization (1). These calculations indicated 79% complexation of salicylamide in the experiments shown in Figs. 1 and 2; direct experimental determination showed 73% complexation (1).

TABLE I—RECOVERY OF SALICYLAMIDE FROM EVERTED INTESTINAL SACS OF THE RAT AFTER 1 HR. OF INCUBATION[a]

Initial Composition of Mucosal and Serosal Solutions, %	% Recovery[b]
Salicylamide control (no incubation)	99
Salicylamide 0.024	98
Salicylamide 0.024 } Caffeine 2.0 }	99

[a] The everted sacs were incubated at 37° in an atmosphere of 5% CO_2 in O_2. Krebs-Henseleit-Bicarbonate Ringer solution, pH 7.4, was the solvent for the serosal solution and Krebs-Henseleit Acetate Ringer solution, pH 5.0, was the solvent for the mucosal solution. [b] One of each.

The decrease in the intestinal transfer rate of salicylamide in the presence of caffeine cannot be attributed to the decreased thermodynamic activity of salicylamide and an assumption that the complex itself is not absorbed since, in the presence of caffeine, the concentration of free salicylamide was about 21% of that when caffeine was absent while the transfer rate was 58% of that when caffeine was absent. It appears, therefore, that the salicylamide–caffeine complex itself is transferred across the everted intestine but at a rate different from that of free salicylamide.

In contrast to the results for salicylamide, the intestinal transfer rate of caffeine (0.388%) was not appreciably affected when complexed to the extent of about 54% due to the presence of a relatively high concentration of salicylamide (0.543%). The data from two of the four experiments that were carried out are shown in Figs. 3 and 4. The experi-

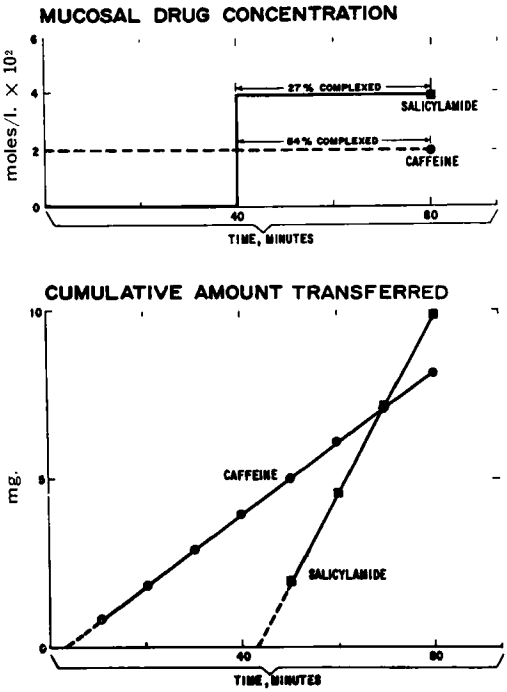

Fig. 3—Effect of complex formation with salicylamide on the transfer of caffeine across the cannulated everted intestine of the rat. Details as in Fig. 1.

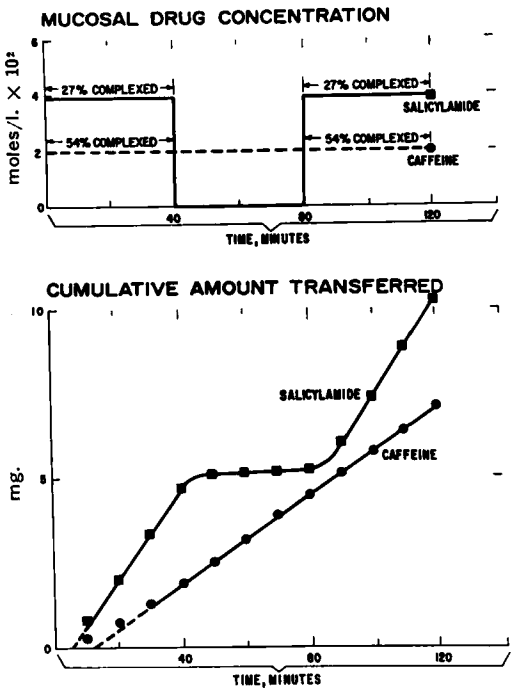

Fig. 4—Effect of complex formation with salicylamide on the transfer of caffeine across the cannulated everted intestine of the rat. Details as in Fig. 1. The order of addition of the complexing agent was different from that in Fig. 3.

ments were repeated under conditions where salicylamide was present in the serosal solution in a concentration equal to that of free salicylamide in the mucosal solution. These results, which are listed in Table II, also show that there was no appreciable difference in the intestinal transfer rate of caffeine alone and in the presence of salicylamide.

In view of the indications from the salicylamide transfer rate determinations that the salicylamide–caffeine complex is transferred as such, and considering the lack of a measurable difference in the overall intestinal transfer of free and complexed caffeine, there is strong indication that the salicylamide–caffeine complex is transferred across the intestine at the same rate as free caffeine. A consequence of this reasoning, which obtains strong support from the results of studies to be reported subsequently (6), is that diffusion of caffeine and the salicylamide–caffeine complex through the aqueous solution to the intestinal mucosa is not

Table II—Effect of Salicylamide in the Mucosal and Serosal Solutions on the Intestinal Transfer Rate Constant of Caffeine

Composition of Mucosal Solution, %	Transfer Rate Constant,[a] cm.³/hr.
Caffeine, 0.388	1.4 (0.05)
Caffeine, 0.388 Salicylamide,[b] 0.543	1.5 (0.32)[c]

[a] Mean of the values from 4 segments, standard deviation in parentheses. [b] Salicylamide was also present in the serosal solution at a concentration equal to the calculated concentration of free salicylamide in the mucosal solution. [c] The larger standard deviation in this set of experiments is due to one unusually high value (2.0 cm.³/hr.). The average transfer rate constant excluding this value is 1.4 cm.³/hr.

Table III—Apparent Partition Coefficients of Salicylamide, Caffeine, and Salicylamide–Caffeine Complex

Amount of Drug in the System,[a] mg. Caffeine	Salicylamide	% Complexation of Caffeine in Aqueous Phase at Equilibrium	Apparent Partition Coefficient
—	10	—	23
19	—	—	0.44
19	100	12	0.57[b]
19	300	27	0.52[b]
19	500	38	0.56[b]

[a] Isoamyl acetate–acetate buffer, pH 5.0, 10 ml. each at 25 ± 2°. [b] Apparent partition coefficient of the salicylamide-caffeine complex itself, calculated as shown in *Footnote 4.*

rate limiting in the intestinal transfer of these two species.

Effect of Complex Formation on the Apparent Partition Coefficients of Salicylamide, Caffeine, and Salicylamide–Caffeine Complex—It is well known that there is a positive relationship between the lipoid–aqueous partition coefficient and the gastrointestinal absorption rate of many drugs (8, 9). It was found that the partition coefficient of salicylamide is higher, and that of caffeine is lower, than the apparent partition coefficient of the salicylamide–caffeine complex itself (Table III). The apparent partition coefficient of the complex was determined[4] at three different salicylamide–caffeine concentration ratios, with essentially the same value being obtained in each case.

An analysis of the simultaneous equilibria which will determine the apparent partition coefficient of a drug complex is appropriate to assess the likely relationship between this property and the rate of transfer of complex across a biologic membrane. These simultaneous equilibria are shown in the following model:

$$\begin{array}{ccc} A_o & + & B_o \overset{K_5}{\rightleftharpoons} A\text{-}B_o \\ \uparrow\downarrow K_1 & & \uparrow\downarrow K_2 \qquad \uparrow\downarrow K_4 \\ A_a & + & B_a \underset{K_3}{\rightleftharpoons} A\text{-}B_a \end{array}$$

where A and B are the two individual components of the drug complex and A-B is the complex itself. The subscript *o* refers to the species in the organic phase and the subscript *a* to the species in the aqueous phase. Both A and B are assumed to partition between the organic and aqueous phases. The definitions of the various constants involved in these equilibria are as follows:

$$PC_{app.} = \frac{[B_o] + [A\text{-}B_o]}{[B_a] + [A\text{-}B_a]} \qquad K_1 = [A_o]/[A_a]$$

$$K_2 = [B_o]/[B_a] \qquad K_3 = [A\text{-}B_a]/[A_a][B_a]$$

$$K_4 = [A\text{-}B_o]/[A\text{-}B_a] \qquad K_5 = [A\text{-}B_o]/[A_o][B_o]$$

[4] The apparent partition coefficient of the complex was calculated as shown below, where A represents salicylamide, B represents caffeine, and where the subscripts and constants have the meanings defined subsequently for the model in the text. (*a*) $[A_a]$, $[B_a]$, and $[A\text{-}B_a]$ are calculated from K_3 and the experimentally determined total salicylamide and caffeine concentrations in the aqueous phase at equilibrium, according to the procedure described in Method I in the appendix of *Reference 1.* (*b*) $[B_o]$ is determined from $[B_a]$ and the partition coefficient, K_2. (*c*) $[A\text{-}B_o]$ = [(total amount B in both phases)-(amount of free B in both phases plus amount of A-B in aqueous phase)] ÷ volume of organic phase. (*d*) Apparent partition coefficient of complex = $[A\text{-}B_o]/[A\text{-}B_a]$.

An increase or a decrease in the apparent partition coefficient of drug B ($PC_{app.}$) when partially complexed with drug A indicates that the ratio $[A\text{-}B_o]/[A\text{-}B_a]$ is greater or less, respectively, than the ratio $[B_o]/[B_a]$, or K_2.[5]

However, the ratio $[A\text{-}B_o]/[A\text{-}B_a]$, the apparent partition coefficient of the complex, reflects only the equilibrium condition and does not give any indication of the relative contribution of each of the several pathways by which this equilibrium can be attained. In fact, it can be shown algebraically that the ratio $[A\text{-}B_o]/[A\text{-}B_a]$ is equal to $K_1K_2K_5/K_3$ whether or not the pathway represented by K_4 actually exists.

It is evident from the above discussion that, unlike the partition coefficient of a pure substance which reflects the ratio of its interfacial transfer rate constants in both directions, the apparent partition coefficient of a complex does not necessarily reflect the rates of transfer of the complex itself from one phase to the other. Yet, it is this direct transfer of the complex itself which would most likely bring about a change in the overall absorption rate of complexed drugs. Herein lies the limitation of the use of partitioning data, which are representative of an equilibrium situation, for correlation with intestinal transfer data, which represent a rate phenomenon. The lack of correlation between the transfer rate constants and partition coefficients of salicylamide, caffeine, and the salicylamide–caffeine complex is consistent with this reasoning.

[5] This statement can be substantiated mathematically, taking as an example the case where complexation results in an increase in the apparent partition coefficient ($PC_{app.} > K_2$). If $PC_{app.} > K_2$, then

$$\frac{[B_o] + [A\text{-}B_o]}{[B_a] + [A\text{-}B_a]} > \frac{[B_o]}{[B_a]}$$

Cross-multiplying yields

$$[B_o][B_a] + [A\text{-}B_o][B_a] > [B_o][B_a] + [B_o][A\text{-}B_a]$$
$$[A\text{-}B_o][B_a] > [B_o][A\text{-}B_a]$$

Therefore,

$$\frac{[A\text{-}B_o]}{[A\text{-}B_a]} > \frac{[B_o]}{[B_a]}$$

It appears that factors which determine the rate of transfer of drug complexes across biologic membranes are likely to be resolved only by a consideration of physicochemical effects at the biologic interface and not on the basis of *in vitro* apparent partition coefficient data or transfer experiments using simple nonbiologic membranes or barriers (10). It is therefore appropriate to direct further attention to the equilibria which exist at the surface of biologic membranes in systems consisting of two interacting drugs. This will be the subject of the following paper.

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Keyphrases

Drug absorption—complex formation effect
Salicylamide-caffeine complex—transfer rate
Caffeine retardation—salicylamide transfer
Everted intestine—experimental technique
Partition coefficients-transfer rates—comparison
UV spectrophotometry—analysis

Thiopental Pharmacokinetics

By K. B. BISCHOFF* and R. L. DEDRICK

A mathematical pharmacokinetic model, including flow limitations, lipid solubility, protein binding, and metabolism, is used to make *a priori* predictions of the distribution of thiopental in four body regions. Tissue binding is correlated by means of "effective" protein fractions and bovine serum albumin data. Metabolism is represented by a Michaelis-Menten rate equation. Close agreement with existing experimental data lends confidence in the model as a valuable tool for predictions in a variety of therapeutic situations.

MANY PRIOR studies of thiopental pharmacokinetics have been made, but there remains some question concerning the relative importance of metabolism and lean and adipose tissue uptakes. Some years ago, Brodie and co-workers (1, 2) made quantitative measurements of thiopental concentrations in various body regions and concluded that significant amounts were present

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