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Keyphrases

Biotransformation—salicylamide, sodium salicylate
Salicylamide effect—salicylic glucuronide formation
Salicylate, sodium, effect—salicylamide glucuronide formation
Salicylurate excretion—salicylate administration
UV spectrophotometry—analysis

Effect of Complex Formation on Drug Absorption VII

Effect of Complexation and Self-Association on the Absorption of Caffeine

By RICHARD H. REUNING* and GERHARD LEVY†

The effect of the formation of caffeine complexes having a higher apparent lipoid–aqueous partition coefficient than caffeine itself on the absorption of this drug from the rat stomach was investigated. In one such system, caffeine–salicylic acid, the salicylic acid actually decreased the absorption of caffeine due to an effect of the former on the gastric mucosa. In another such system, caffeine–*p*-hydroxybenzoic acid, the *p*-hydroxybenzoic acid did not increase the absorption of caffeine. The intestinal transfer of caffeine alone was studied at low concentrations (where caffeine exists mainly in monomeric form) and at high concentrations (where significant self-association occurs). The intestinal transfer rate constant of caffeine at high concentrations was significantly lower than at low concentrations. The mechanism of this effect was explored.

In the initial studies in this laboratory of the effect of complex formation on drug absorption, it was found that complexation with caffeine decreased significantly the overall[1] absorption of salicylic acid from the stomach of the rat (1). This effect was qualitatively consistent with the apparent lipoid–aqueous partition coefficient (PC) of salicylic acid and of the caffeine–salicylic acid complex; the latter showed an appreciably lower apparent PC than the former. It appeared reasonable to assume that the absorption of caffeine might be enhanced by complexation with salicylic acid, since this complex has a higher apparent PC than caffeine itself (1). Studies were therefore initiated to test this assumption. The investigation was then extended to include a study of the caffeine–salicylate ion complex and the caffeine–*p*-hydroxybenzoic acid complex. The latter was of considerable interest since it has a higher apparent PC than caffeine as well as an appreciably higher stability constant than the salicylic acid–caffeine complex (2). In the course of these studies it became apparent that the self-association of caffeine at high concentrations (3) can affect the absorption of this drug. Therefore, this effect was studied also.

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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

In Situ Gastric Absorption Rate Measurements—The procedure described previously by the authors (1) was followed with a few modifications. The

female Wistar rats, 150–270 g., were anesthetized with urethan (1.25 g./kg. i.p.). After the rat stomach had been exposed, it was washed once with 0.9% sodium chloride solution prior to injection of the drug solution (37°) into the ligated stomach through the pyloric sphincter. All injections were made with a blunt-tipped needle which was withdrawn while tightening the ligature around the pyloric sphincter. The absorption period was 1.5 hr. for the caffeine–salicylic acid solutions and 1.0 hr. for the caffeine–*p*-hydroxybenzoic acid solutions. At the end of the absorption period, the stomach was excised, homogenized,[2] and the homogenate was assayed for unabsorbed caffeine and, when present, for ethanol.

Assay for Caffeine in Stomach Homogenate—A modification of the method of Axelrod and Reichenthal (4) for the assay of caffeine in urine and biologic tissues was used. The stomach homogenate was adjusted to a volume of 50 ml. with water. To 15 ml. of this diluted homogenate was added 25 ml. of 0.1 *M* phosphate buffer, pH 7.5. A 3-ml. aliquot of this dilution (containing 3–5 mg.% caffeine) was placed in a 120-ml. glass-stoppered bottle along with 3 g. sodium chloride and 35 ml. washed benzene.[3] After shaking for 20 min., the phases were separated by centrifuging at about 900×*g* for 20 min. The benzene phase was transferred to another 120-ml. glass-stoppered bottle containing 3 g. sodium chloride and 3 ml. 0.1 *N* NaOH. After the bottle had been shaken for 5 min., the phases were separated as before. Fifteen or 20 ml. of the benzene phase was then pipeted into a 125-ml. separator containing 10 ml. of 5 *N* HCl. After shaking for 5 min., the separator was allowed to stand a few minutes until the phases had separated. The aqueous phase was removed and centrifuged at about 850× *g* for 30 min. to remove any remaining nonaqueous material. An aliquot of the aqueous phase was then assayed spectrophotometrically at 273 mμ. Blank values from tissues comprised about 7% of the usual absorbance and the average tissue recovery, after correction for blank values, was 96%. The amount of caffeine remaining unabsorbed was calculated from the concentration determined spectrophotometrically, the dilution factor of the assay procedure, and the corrections for blank and recovery. Each analysis was done in triplicate and the results were averaged.

Assay for Ethanol in Stomach Homogenate—Ethanol was determined by the method of Hoult and Pawan (5) as modified previously (6). It was found that neither caffeine nor salicylic acid interferes with this assay.

Partition Coefficients—The partition coefficients of *p*-hydroxybenzoic acid and caffeine between 0.1 *N* HCl and isoamyl acetate were determined at room temperature as described previously (1). Assays of the aqueous phase were done spectrophotometrically at 255 and at 273 mμ, respectively, using 0.1 *N* HCl as the diluent. When both drugs were present together, two-component spectrophotometry was used at these wavelengths. In the concentration range of the spectrophotometric assay the absorbances of the two drugs were additive.

The partition coefficients of caffeine between distilled water or Ringer's solution and chloroform 10% (v/v) in isooctane were determined at 30° as described by Guttman and Higuchi (3). The concentration of caffeine was determined spectrophotometrically in each phase.

In Vitro Intestinal Transfer Rate Measurements (Caffeine)—The procedure outlined by Levy and Matsuzawa (7) was used. This is essentially the cannulated everted intestine method developed by Crane and Wilson (8). Male Long-Evans rats, weighing 330–470 g., were anesthetized with ether. The small intestine was removed, rinsed with Ringer's solution, and everted. Two segments, 10 cm. in length when stretched by an 8-g. weight, were obtained from the section of the small intestine just distal to the duodenum. The distal ends of the segments were tied and the proximal ends were attached to the cannula of the apparatus described by Crane and Wilson (8). An equal number of first (from the proximal end) and second intestinal segments was used in each part of the experiment. Each segment was suspended in 80 ml. of caffeine in Ringer's solution (at 30°) contained in a large test tube (mucosal solution). Two milliliters of Ringer's solution was introduced into the intestinal segment (serosal solution).[4] The serosal solution was withdrawn completely every 10 min. After each withdrawal the serosa was rinsed with 2 ml. of Ringer's solution and this rinse was combined with the solution withdrawn initially. Another 2 ml. of Ringer's solution was then placed into the intestine to serve as the serosal solution for the next 10-min. interval. The everted intestinal segment was suspended for 1 hr. in a solution containing a high caffeine concentration, for an additional hour in a solution containing a low concentration of caffeine, and then again for 1 hr. in the solution containing a high concentration of caffeine. A different sequence was used with other intestinal segments. This "pairing" minimizes the effect of variability between segments from different rats which may be due particularly to differences in diameter and surface area of the segments.

Assay for Caffeine in Serosal Solution from the Cannulated Everted Rat Intestine—Caffeine was determined by direct spectrophotometry without prior extraction. At the higher caffeine concentration of the mucosal solution (0.120 mole/l.) the serosal samples were simply diluted with water and their absorbance was determined at 273 mμ. At the 0.005 mole/l. concentration, however, it was necessary to correct for the interference from the blank[5] by two-component spectrophotometry at 273 and 299 mμ. This was done on the basis of a ratio of 1.5:1 for the absorbances of the blank at 273 and 299 mμ. The average recovery of caffeine in the concentration range where corrections were necessary was 101%.

Test for Transport Against a Concentration Gradient—The everted sac method described by Wilson and DeCarlo (9) was used. The intestine of the rat was removed, rinsed, and everted as described in a preceding section. One everted sac, about 6 cm. in length, was prepared from the proxi-

[2] Multi-mix Homogenizer, Lourdes Instrument Corp., Brooklyn, N. Y.

[3] Reagent grade benzene was washed successively with 0.2 vol. of 1 *N* NaOH, 1 *N* HCl, and twice with distilled water.

[4] The pH values of the serosal and mucosal solutions at the end of the absorption period ranged from 5.0 to 6.3. In this range, caffeine is nonionized.

[5] "Blank" refers to apparent caffeine values obtained in experiments in which caffeine-free solvents were used.

mal portion of each rat intestine. The everted sac was filled with 1 ml. of pH 7.4 Krebs-Henseleit Ringer solution (10) containing 0.005 *M* caffeine (serosal solution). The sacs were then placed in 15 ml. of the same solution (mucosal solution) contained in 50-ml. conical flasks. The flasks were flushed with a mixture of 95% oxygen and 5% carbon dioxide, sealed, placed in a 30° water bath, and agitated moderately by means of a reciprocating shaker. Aliquots of the mucosal and serosal solutions were obtained after 1 hr. of incubation and were assayed for caffeine using the two-component spectrophotometric analysis described in the preceding section.

In Vitro Intestinal Transfer Rate Measurements (Caffeine in the Presence of Salicylate)—The procedure was essentially the same as that outlined in a preceding paragraph for caffeine except for the use of female Wistar rats and a Krebs-Ringer buffer (11), adjusted with HCl to pH 4, as the solvent for the mucosal solution. The mucosal solution consisted of 0.1% caffeine, alone or together with 0.3% salicylic acid. Some of the solutions also contained 2% ethanol. The serosal solution consisted of pH 7.0 Krebs-Ringer solution (11). Each experiment was carried out for 60 min. at 37°, with sampling at 15-min. intervals. Caffeine was assayed by the method of Axelrod and Reichenthal (4) and ethanol by the method of Hoult and Pawan (5).

Calculation of Intestinal Transfer Rate Constants—The application of Fick's first law (12) to the transfer of substances across a biologic membrane yields the following equation (13):

$$R = kA \frac{C_1 - C_2}{x}$$

where R is the rate of diffusion across a barrier (*e.g.*, the everted intestine) which may be expressed in units of mg./hr., k is a constant whose units are then cm.²/hr., A is the cross-sectional area of the diffusion barrier in cm.², C_1 and C_2 are the concentrations of drug in the source (mucosal) and receiving (serosal) compartments, respectively, in mg./cm.³, and x is the thickness of the barrier in cm. The transfer rate constant used in this study, k_t, is defined as:

$$k_t = \frac{kA}{x} = \frac{R}{C_1 - C_2}$$

The units of k_t are then cm.³/hr. Total replacement of the serosal solution at short intervals (every 10 min.) prevented any pronounced buildup of C_2 ($<0.17\ C_1$). Also, C_1 remained essentially constant during the experiment due to the large volume of the mucosal solution. Thus, k_t approximates R/C_1 under steady-state conditions.

RESULTS AND DISCUSSION

Caffeine-Salicylic Acid Complex—There are three likely mechanisms by which salicylic acid can affect caffeine absorption from the rat stomach. First, the complex formed by these two drugs (2) may have an absorption rate constant different from that of caffeine itself. Since about 40% of the caffeine in the administered solution was in complexed form, a pronounced difference in the absorption rate constant of free and complexed caffeine would result in an appreciable difference in the overall absorption rate of caffeine when administered alone and together with salicylic acid, respectively. Second, salicylic acid may have a direct effect on the permeability characteristics of the gastric mucosa, and third, it may affect the *in vivo* absorption rate of caffeine by a systemic effect such as a change in the rate of gastric blood flow.

TABLE I—EFFECT OF SALICYLIC ACID ON THE *In Vivo* ABSORPTION OF CAFFEINE AND ETHANOL FROM THE STOMACH OF THE RAT

Initial Composition of Solution[a] % w/v	% Absorbed[b] in 1.5 hr. Caffeine	Ethanol	Ratio of % Absorbed Values, Without Salicylic Acid : With Salicylic Acid Caffeine	Ethanol
Caffeine, 0.2 Ethanol, 2.0	37.1 (4.1)	55.9 (3.8)	1.3	1.2
Caffeine, 0.2 Ethanol, 2.0 Salicylic Acid, 0.275	28.0 (4.0)	46.1 (6.1)		

[a] 0.1 *N* HCl was used as the solvent. [b] Mean of the values from 6 rats, standard deviations in parentheses.

The effect of salicylic acid on caffeine absorption from the *in situ* ligated rat stomach is shown in Table I. It is evident that the absorption rate of caffeine was significantly decreased in the presence of salicylic acid ($p < 0.01$, t test). However, the absorption of ethanol, which was included in the drug solutions as a noncomplexed marker, was also decreased in the presence of this concentration of salicylic acid ($p < 0.02$, t test). For comparative purposes, the effect of salicylic acid on the absorption of caffeine and ethanol was expressed in terms of the ratios shown in Table I. It can be noted that salicylic acid decreased the absorption of both the complexed caffeine and the noncomplexed ethanol to a similar extent. This indicates that the decreased absorption of caffeine in the presence of salicylic acid was not due to complex formation. There was visual evidence of considerable gastric damage due to salicylic acid; the solutions containing caffeine, ethanol, and salicylic acid[6] frequently caused gastric bleeding and ulceration, while no such effects were noted with solutions containing only caffeine and ethanol. There appeared to be a rough inverse correlation between the absorption rates of caffeine and ethanol on one hand, and the extent of gastric bleeding on the other. It seems, therefore, that the decreased absorption of caffeine in the presence of a relatively high concentration of salicylic acid was due to an effect of salicylic acid on the gastric mucosa, and not to an effect of complex formation *per se*. These results demonstrate the need to distinguish between an effect of a complexing agent on the drug and a possible effect on the biologic membrane. They illustrate also the utility of a noncomplexed marker such as ethanol for this purpose.[7] It should be noted, however, that ethanol is not necessarily a suitable marker for the assessment of effects on biologic membranes involving mechanisms other than bleeding and erosions (14).

[6] The drug solution was essentially saturated with respect to free salicylic acid in order to complex as high a fraction of caffeine as possible.

[7] The absorption of ethanol is not decreased at a lower salicylic acid concentration (0.1%) which does not cause visible mucosal damage (1, 6).

TABLE II—EFFECT OF SALICYLATE ON THE TRANSFER OF CAFFEINE ACROSS THE CANNULATED EVERTED INTESTINE OF THE RAT

Composition of Mucosal Solution,[a] %		Transfer Rate[b] Caffeine, mg./hr.	Transfer Rate[b] Ethanol, mg./hr.
Caffeine, 0.1		2.38	67.6
Ethanol, 2.0		2.06	*c*
		1.95	63.8
	Mean	2.13	65.7
Caffeine, 0.1		2.06	69.2
Ethanol, 2.0		1.79	*c*
Salicylic acid, 0.3		1.86	62.0
	Mean	1.90	65.6

[a] The solvent was Krebs-Ringer buffer adjusted to pH 4. [b] Data from individual experiments, each based on 4 samplings at 15-min intervals. [c] Ethanol was not included in these experiments.

TABLE III—PARTITIONING BEHAVIOR OF *p*-HYDROXYBENZOIC ACID AND CAFFEINE, ALONE AND IN THE PRESENCE OF EACH OTHER

Initial Composition of Aqueous Phase, %	Apparent Partition Coefficient[a]
Parahydroxybenzoic Acid	
p-Hydroxybenzoic acid, 0.1	11
p-Hydroxybenzoic acid, 0.1 Caffeine, 2.2	1.6
Caffeine	
Caffeine, 0.194	0.40
Caffeine, 0.194 *p*-Hydroxybenzoic acid, 0.8	0.54
Caffeine, 0.194 *p*-Hydroxybenzoic acid, 4.0[b]	0.65

[a] Isoamyl acetate–0.1 *N* HCl, at 25 ± 2°. [b] In this instance, it was necessary to dissolve the *p*-hydroxybenzoic acid in the isoamyl acetate prior to equilibration because of the limited aqueous solubility of this compound.

TABLE IV—EFFECT OF *p*-HYDROXYBENZOIC ACID ON CAFFEINE ABSORPTION FROM THE STOMACH OF THE RAT

Initial Composition of Solution,[a] %	% Absorbed[b] in 1 hr.
Caffeine, 0.2	32.7 (3.1)
Caffeine, 0.2 *p*-Hydroxybenzoic acid, 0.83	27.7 (4.5)

[a] 0.1 *N* HCl was used as the solvent. [b] Mean of the values from 4 animals, standard deviations in parentheses.

Since caffeine forms a complex with salicylate ion having a stability constant similar to that of the caffeine–salicylic acid complex (2), the study was repeated at pH 4 by means of the everted intestine procedure (8). The results of these experiments are shown in Table II and indicate that complexation with salicylate ion had no appreciable effect on the intestinal transfer of caffeine and certainly did not increase the transfer rate of this drug. Since about 90% of the salicylate was in ionized form, it had no measurable effect on the permeability characteristics of the intestinal membrane, as reflected by the lack of any effect on the intestinal transfer of ethanol. It has recently been observed that caffeine has no effect on the intestinal transfer rate of salicylic acid at pH 5, but decreases the rate of transfer at pH 2 (15).

Caffeine–p-Hydroxybenzoic Acid Complex—Higuchi and Zuck (2) have estimated the stability constant of the caffeine–*p*-hydroxybenzoic acid complex to be at least 100 l./mole. This stability constant and the intrinsic solubility of *p*-hydroxybenzoic acid are considerably higher than the corresponding values for the caffeine–salicylic acid complex and salicylic acid, respectively, and thus permit more extensive complexation of caffeine (at least 83% in this experiment) than was possible with salicylic acid. It was observed also that *p*-hydroxybenzoic acid, unlike salicylic acid, did not visibly damage the gastric mucosa. Complexation of caffeine with *p*-hydroxybenzoic acid increases the apparent partition coefficient of caffeine to a value between that of free caffeine and free *p*-hydroxybenzoic acid, respectively (Table III). Contrary to expectations, this increase in apparent partition coefficient was not accompanied by an increased gastric absorption rate (Table IV). This study was terminated when it was found that caffeine is definitely not more rapidly absorbed in the presence of *p*-hydroxybenzoic acid.[8] The number of experiments was insufficient to determine if the apparent decrease in the absorption of caffeine in the presence of the complexing agent was real ($0.2 > p > 0.1$, t test). A discussion of possible reasons for the observed results will be deferred to a following report which deals with another complexing system and includes data which permit a more definitive interpretation of the type of effects observed with the caffeine–*p*-hydroxybenzoic acid system.

Effect of Self-Association on Intestinal Transfer of Caffeine—It is known that caffeine undergoes considerable self-association except at very low concentrations. Guttman and Higuchi (3) have made an extensive investigation of the equilibria involved in this system. Their data, which show a decrease in the apparent lipoid–aqueous partition coefficient with increasing concentration of caffeine, suggest that caffeine exists only in monomeric form in the organic phase used in their study (chloroform 10% v/v in isooctane), but that it also forms dimers and tetramers in water. These physicochemical characteristics made a study of the effect of concentration on the absorption of caffeine across biologic membranes of interest. The everted small intestine of the rat was used in this study because of the relative simplicity and sensitivity of this procedure.[9]

The results of two experiments, which involved the measurement of caffeine transfer rates from high, low, and high concentrations of caffeine (in that order) across an everted intestinal segment of the rat, are shown in Figs. 1 and 2. The data are plotted in terms of the ratio, transfer rate/mucosal concentration (to be referred to as the transfer ratio), which, in the steady state and under sink conditions, is equivalent to the intestinal transfer rate constant as defined under *Experimental*. At the high concentration of caffeine (0.12 mole/l.), the transfer ratio increased with time until steady state was

[8] This is particularly remarkable since, as is shown in the subsequent section, a reduction in the concentration of uncomplexed caffeine should, by decreasing the degree of caffeine self-association, result in more rapid absorption of the uncomplexed drug.

[9] The absorption studies of the caffeine–salicylic acid and caffeine–*p*-hydroxybenzoic acid complexing systems had to be carried out on the ligated rat stomach rather than the everted rat intestine because of the need to use solutions of very low pH to prevent ionization of the two weak acids.

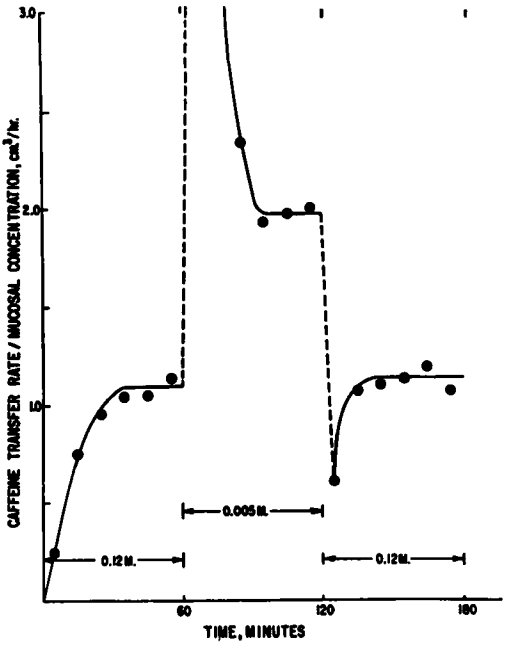

Fig. 1—Effect of concentration on intestinal transfer of caffeine. Transfer rate/mucosal concentration as a function of time, Exp. 1. The data were plotted at the midpoint of each 10-min. sampling period. The concentrations shown in the figure refer to caffeine concentration in the mucosal solution during the various time periods of the experiment. Values which were beyond the scale of the figure were: 17.1 cm.³/hr. at 65 min. and 3.9 cm.³/hr. at 75 min.

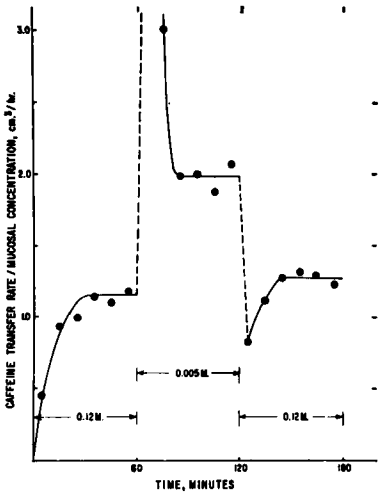

Fig. 2—Effect of concentration on intestinal transfer of caffeine, Exp. 2. Details as in Fig. 1. The value which was beyond the scale of the figure was: 12.4 cm.³/hr. at 65 min.

reached. This is due to a gradual build-up of drug concentration within the intestinal wall (16). At the low caffeine concentration (0.005 mole/l.) following the high concentration, drug which had been retained in the intestinal wall from the preceding period gradually diffused out of the wall until a new apparent steady-state concentration was attained. It is evident that the transfer ratio at the apparent steady state was considerably lower at the high concentration of caffeine than at the lower concentration. The similarity of the apparent steady-state transfer ratios in the first and third time periods indicates that intestinal permeability remained essentially constant during the 3-hr. experiment. The desirability of repeating the conditions of the first time period in a third time period to assess any possible effect of caffeine on the integrity of the intestinal mucosa made it necessary to limit each period to 60 min. Another indication that the intestinal membrane permeability remained essentially unchanged for the duration of the experiments in this study was provided by the results of preliminary experiments in which the plateau transfer ratio attained at about 30 min. (using only 0.005 mole/l. caffeine in the mucosal solution) did not change significantly ($<\pm 7\%$) in the following 3.5 hr.

Figures 3 and 4 show the results of experiments in which the sequence of caffeine concentration changes was reversed, *i.e.*, the segments were suspended for 1 hr. in a 0.005 mole/l. caffeine solution, for a second hour in a 0.12 mole/l. caffeine solution, and finally once more in a 0.005 mole/l. caffeine solution. Again, the apparent intestinal transfer rate constant of caffeine was higher at low concentrations than at high concentrations of the drug. However, the difference between the plateau transfer ratios in the first two time periods was not as pronounced in this series of experiments as in the series shown in Figs. 1 and 2. This may be due to two reasons: the initial exposure of the intestine to a high concentration of caffeine may cause an immediate increase in intestinal permeability which then remains constant throughout the experiment, or, the apparent plateau transfer ratio for caffeine

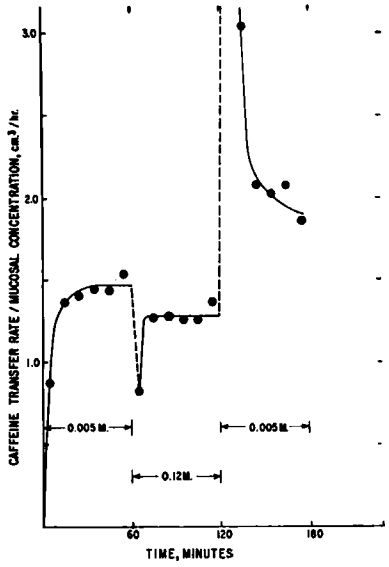

Fig. 3—Effect of concentration on intestinal transfer of caffeine, Exp. 3. Details as in Fig. 1. The value which was beyond the scale of the figure was: 12.7 cm.³/hr. at 125 min.

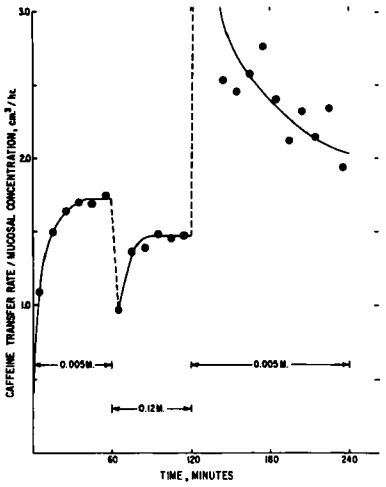

Fig. 4—Effect of concentration on intestinal transfer of caffeine, Exp. 4. Details as in Fig. 1. Values which were beyond the scale of the figure were: 14.2 cm.³/hr. at 125 min. and 3.3 cm.³/hr. at 135 min.

at low concentration following exposure of the intestine to a high concentration of caffeine (Figs. 1 and 2) may not actually reflect steady-state conditions, but rather be the result of a slow efflux of previously accumulated caffeine from the intestinal wall.

Experiments which will be reported in a subsequent publication have shown that the permeability of the everted rat intestine to salicylamide was not affected by exposure of the intestine to a high (0.113 mole/l.) caffeine concentration (17). This would tend to rule out the possibility of an effect of the high concentration of caffeine on intestinal permeability in the present study. In addition, the transfer ratio data for the third time period of the experiments shown in Fig. 3 and particularly in Fig. 4 (where the time period was extended to 240 min.) give a strong indication of a slow efflux following an initial rapid efflux of caffeine from the intestinal wall after the intestine had been exposed to a high concentration of this drug. It appears, therefore, that the transfer rate constants represented by the plateau transfer ratios in the first and second time periods of Figs. 3 and 4 are the most realistic and conservative index of the difference in the intestinal transfer rate constants of caffeine at low and high concentrations of the drug.

The study of Guttman and Higuchi (3) yields information concerning the degree of self-association of caffeine at the concentrations used in this study. It was found that the apparent aqueous–organic partition coefficients of caffeine under the conditions of their study, where distilled water was used as the aqueous phase, were essentially the same as when the aqueous system of the present study, Ringer's solution, was used (Table V). This shows that the self-association of caffeine was not measurably affected by the presence of the components of the electrolyte solution and justifies the direct use of the data from Guttman and Higuchi's study (Table VI).

The results of the intestinal transfer study are summarized in Table VI, which also shows the extent of self-association of caffeine at the two

Table V—Partition Coefficient of Caffeine Between Distilled Water or Ringer's Solution and an Organic Solvent

Initial Concn. of Caffeine in Aqueous Phase, %	Solvent, Aqueous Phase	Aqueous–Organic Apparent Partition Coefficient,[a] This Study	Literature[b]
0.107	Distilled water	26	25
0.107	Ringer's solution	24	
2.14	Distilled water	57	57
2.14	Ringer's solution	56	

[a] The organic phase was chloroform 10% (v/v) in isooctane and the temperature was 30°. [b] See *Reference 3.*

Table VI—Effect of Caffeine Association on the *In Vitro* Rate of Transfer of Caffeine Across the Small Intestine of the Rat

Caffeine Concn. moles/l.[a]	% Total Caffeine Present as a Particular Species[b] Mono-mer	Dimer	Tetra-mer	Transfer Rate Constant cm.³/hr.[c]
0.005	90	10	0	1.67 (0.29)
0.120	39	37	24	1.44[d] (0.23)

[a] Ringer's solution was the solvent. [b] See *Reference 3.* [c] Mean of the values from 4 rat intestinal segments determined at 30°. Standard deviations in parentheses. [d] The difference between the two mean transfer rate constants is statistically significant ($p < 0.01$) as determined by the t test for paired observations.

concentrations used. The transfer rate constants were determined from the steady-state portion of the first two time periods of only the experiments in which the transfer at low (0.005 mole/l.) caffeine concentration was determined first. These transfer rate constants reflect not only the data in Figs. 3 and 4, but also the results of two additional experiments not represented by figures. The intestinal transfer rate constant at the low concentration of caffeine is significantly higher ($p < 0.01$, t test for paired observations) than that at a high concentration. This difference would have been even more pronounced if the experiments shown in Figs. 1 and 2 had been included in the data analysis.

Incubation of everted intestinal sacs containing 0.005 mole/l. caffeine for 1 hr. in solutions containing the same concentration of caffeine in an oxygenated medium yielded no evidence of uphill transport; the mean mucosal–serosal concentration ratio was 0.97 for four experiments. Thus, the concentration dependence of the intestinal transfer of caffeine does not appear to be due to the saturation of an active transport process in the small intestine of the rat. However, other modes of specialized transport cannot be ruled out on the basis of the experimental data. But, since the structural specificity of most specialized intestinal transport processes is quite exacting, it seems unlikely that a drug such as caffeine would be transferred by this type of process. The experimental results also cannot be explained by assuming that only the monomeric form of caffeine is transferred across the intestine; the decrease of the intestinal transfer rate constant of caffeine at the higher concentration is much smaller than the decrease in the fraction of total caffeine which exists in monomeric form (Table VI). The overall diffusivity of caffeine, determined on the basis of the fraction and molecular

TABLE VII—RATIO OF THE INTESTINAL TRANSFER RATE CONSTANTS OF CAFFEINE AT LOW AND HIGH CONCENTRATION COMPARED TO THE THEORETICAL RATIO OF THE OVERALL DIFFUSIVITIES

$k_{0.005\ M}/k_{0.12\ M}$[a]		Diffusivity$_{0.005\ M}$/ Diffusivity$_{0.12\ M}$ Thovert's Equation[b]	Stokes-Einstein Equation[c]
1.14 1.15 1.17 1.18	Mean: 1.16	1.38	1.24

[a] Ratio of intestinal transfer rate constants for caffeine in low and high concentration. [b] D = constant/$M^{1/2}$ (18). In this instance, $M = \Sigma M_i f_i$, where M_i is the apparent molecular weight of a particular species (monomer, dimer, or tetramer) and f_i is the fraction of total caffeine present existing as this species. [c] D = constant/$M^{1/3}$ (19), calculated as in *b*.

weight of each species (*i.e.*, monomer, dimer, and tetramer) is lower at higher concentrations of caffeine. This could affect transfer rate if diffusion of the drug to the membrane surface is transfer rate limiting or if the aggregates remain intact during transfer across the intestine. The ratio of the overall diffusivity of caffeine at low and high concentrations, as calculated by two different equations, is somewhat higher than the ratio of intestinal transfer rate constants determined experimentally (Table VII). It may be possible, however, that diffusion to the mucosal membrane surface affects the transfer rate constant of one or both of the associated species of caffeine but not that of the monomer. The experimental data may also be rationalized by assuming that each of the caffeine species is absorbed as such but that the transfer rate constant of each species is different. This may be complicated further by a possible change in the self-association equilibria at or adjacent to the rate-limiting biologic barrier (7).

The important conclusions of this study are that (*a*) drug complexes with a higher apparent partition coefficient than the free drug are not necessarily more rapidly absorbed, and (*b*) a concentration dependence in the apparent absorption rate constant of a drug does not necessarily reflect the presence of a specialized transport process, but may be due to the physicochemical characteristics of the drug as they affect passive diffusion to and across biologic membranes.

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Keyphrases

Drug absorption—complex formation effect
Caffeine complexes—increased partition coefficients
Salicylic acid retardation—caffeine transfer
Self-association—caffeine
Gastric absorption rate—*in situ* technique
Everted intestine—experimental technique
UV spectrophotometry—analysis