Studies on Complexes. XX.¹⁾ Effect of Complex Formation on Drug Absorption from Alimentary Tract. (7)

ISAO SUGIMOTO

Pharmaceutical Research Laboratory, Tanabe Seiyaku Co., Ltd.²⁾

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Absorption rates of carbazochrome and nicotinamide in the presence of each complexing agent (caffeine and hydroxyethyltheophylline, respectively) were determined in order to make sure that the increase or decrease of absorption rate in the presence of the complexing agent was due to the intra-luminal complex formation. The rate was determined by pretreatment, tied loop, and everted methods. In addition, the effect of buffer composition and the addition of the third additive to the complexing system were studied. These results suggest that the effect of complexing agent on the absorption rate of drug is apparently due to complex formation.

To demonstrate the specificity of the small intestine by comparing with the rat stomach, carbazochrome-caffeine complexing system was examined on the absorption rate from the stomach. It was found that the small intestine did not behave specifically to the absorption of carbazochrome in the presence of caffeine.

A theoretical equation was derived to obtain the stability constant at the surface of the absorption rate-limiting barrier. The constant did not differ appreciably from that determined in the bulk solution. A good fit of the absorption rate to the theoretical model suggested that the stability constant at the surface of the absorption rate-limiting barrier was similar to that in the bulk solution.

The effects of complexing agent on drug absorption are of interest from the pharmaceutical point of view to warrant the pharmacological efficacy of the drug itself and have been investigated extensively. Reuning and Levy³⁾ have suggested that the complex could be transferred as such and the stability constant of the complex at the transfer rate-limiting barrier of the intestine did not differ appreciably from that determined in the bulk solution. Recently, Goto and co-workers⁴⁾ investigated the effect of caffeine on gastric absorption of nonabsorbable drug, *p*-aminobenzoic acid (PABA), and found that the apparent absorption rate of PABA increased gradually with increasing addition of caffeine. They have concluded the absorption rate constants of complexes might be obtained as approximately intermediate values between those of caffeine and PABA, and this effect of complexation was consistent with the apparent partition coefficient of PABA and of the complex: the latter showed a higher apparent partition coefficient than the former.

Previous reports⁵⁻⁷⁾ from this laboratory have dealt with the apparent absorption rate of the drug when administered with its complexing agent, and certain physico-chemical properties of the complexing system. It was assumed that both the complexed fraction of the drug and the difference between the absorption rate constant of the drug and that of its complexing agent were large in order to result in a difference in the apparent absorption rate of the drug when administered with its complexing agent.⁷⁾

- 1) This is one of the series of "Studies on Complexes" (M. Samejima). Part XIX: M. Yoshida and M. Samejima, *Yakugaku Zasshi*, in preparation.
- 2) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka*.
- 3) R.H. Reuning and G. Levy, *J. Pharm. Sci.*, **58**, 79 (1969).
- 4) S. Goto, O. Tsuzuki, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **17**, 837 (1969).
- 5) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1527 (1968).
- 6) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 994 (1969).
- 7) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 1377 (1969).

The present investigation is designed mainly to elucidate more clearly that these changes of the absorption rate in the presence of the complexing agent are due to the complex formation in the luminal phase rather than other factors, namely, a) modification of membrane permeability characteristics in the presence of complexing agent, and b) pharmacological or toxicity effect of high concentration of the complexing agent.

In the previous reports complexation with hydroxyethyltheophylline (HET) at pH 6.0 decreased the apparent absorption rate of nicotinamide (NA),⁵⁾ and, on the other hand, complexation with caffeine at pH 3.0, 4.7, and 6.0 enhanced that of carbazochrome.^{6,7)} So, in this report these complexing systems were chosen as models to elucidate that the changes of the absorption rate were due to the intra-luminal complex formation. The absorption rate was determined by the pretreatment, tied loop, and everted gut methods (see Experimental for details). In addition, the effect of buffer composition and the addition of the third additive to the complexing system were studied. Further to demonstrate the specificity of the small intestine by comparing with the rat stomach, carbazochrome-caffeine complex was examined on the absorption rate from the stomach. Next, this report deals with whether the stability constant of the complex is the same at the surface of the absorption rate-limiting barrier as in the *in vitro* bulk solution. In order to reveal this clearly, the stability constants were assumed from the equation derived to calculate the absorption rate constant of a complex using the rate constant from the rat small intestine *in situ*.⁵⁾ The assumed stability constants were compared with those in the bulk solution.

Experimental

Composition of Buffer—Compositions⁸⁾ of isotonic buffer solution are given in Table I.

TABLE I. Isotonic Buffer Compositions

pH	Citric acid	KH ₂ PO ₄	Na ₂ HPO ₄ ·12H ₂ O	H ₂ O
3.0	27.7		22.1	
4.7	11.1		35.3	to make
6.0		14.5	6.4	1000 ml
7.4		1.8	32.1	

unit: g

Absorption Rate by Rat Intestinal Recirculating Perfusion Method—The experimental technique employed was essentially the same as that reported already.⁹⁾

Pretreatment method was as follows. After the small intestine was cannulated for *in situ* recirculation pump, the intestine was first washed with about 30 ml of 0.9% NaCl solution (at 37°) and then 20 ml of the test solution of complexing agent (20 mM caffeine at pH 4.7 for carbazochrome-caffeine, or 40 mM HET at pH 6.0 for NA-HET) or 0.9% NaCl solution (control) was perfused. After 20 min, the complexing agent or control solution was washed out with 30 ml of 0.9% NaCl solution, and 20 ml of 0.5 mM carbazochrome (pH 4.7) or NA (pH 6.0) solution alone was perfused at a rate of 3 ml per min. After 40 min, the solution was collected completely in a 100 ml measuring flask by washing with 0.9% NaCl. The collected solution was then assayed for unabsorbed carbazochrome or NA, and the absorption rate constant was calculated from the amount of the drug before recirculation and that remaining in the recirculation fluid after 40 min.

Absorption from Tied Loop of Rat Intestine—The intestine of the rat was exposed as described in a previous report.⁹⁾ The tied loop, about 10 cm in length, was prepared from the jejunum of each rat intestine. A polyethylene tube was inserted into the lower portion of the loop. The loop was washed with 0.9% NaCl solution (at 37°) through the tube. Five ml of drug solution, previously warmed to 37°, was introduced into the loop through the tube and it was tied to prevent any leakage of the solution. After 1 hr, the drug

8) Nippon Kagakukai, "Jikken Kagaku Khoza," 24, Maruzen, Tokyo, 1958, p. 224.

9) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), 16, 1098 (1968).

solution was withdrawn completely and the loop was rinsed twice with 5 ml of 0.9% NaCl solution and this rinse was combined with the solution withdrawn initially. The collected solution was then assayed for unabsorbed carbazochrome or NA, and the absorption rate constant was calculated assuming the absorption to be a first order process.^{5,6)}

Gastric Absorption Measurements—Male Wistar rats, 200–250 g, were used. Food was withheld for 18–24 hr prior to the experiment. The rats were anesthetized with pentobarbital and remained under anesthesia throughout the absorption experiment. After the rat stomach had been exposed, the esophagus immediately adjacent to the cardiac sphincter was tightly ligated, and the polyethylene tube was inserted from the small intestine about 1 cm from the pyloric sphincter into the stomach. The stomach was washed twice with 0.9% NaCl solution at 37° through the tube. Two milliliters of the drug solution, previously warmed to 37°, was injected with a syringe through the tube and it was tied to prevent any leakage of the drug solution. The solution consisted of 0.5 mM carbazochrome at pH 3.0 (for control), or the solution with 20 mM caffeine (for complex absorption studies). At the end of 1.5 hr, the drug solution was withdrawn completely. Then the stomach was rinsed twice with 5 ml of 0.9% NaCl solution and this rinse was combined with the solution withdrawn initially. The combined solution was adjusted to a volume of 50 ml with water and assayed.

Intestinal Transfer Rate of Drug-Complexing Agent by Everted Intestine Method—The cannulated everted intestine method developed by Crane and Wilson¹⁰⁾ was used with a few modifications. Two everted intestinal segments, 10 cm in length, from a male Wistar rat (200–250 g) were obtained from the section

Fig. 1. Everted Intestine Method

A,B: polyethylene tube
C: everted intestine
D: rubber stopper with three holes

of the small intestine just distal to the duodenum. As shown in Fig. 1 each end of the segments was tied to the polyethylene tube (A, B in Fig. 1), and the tubes were fixed with rubber stopper to an Erlenmeyer flask. The segment was suspended in 60 ml of drug solution or drug-complexing agent solution (mucosal solution), previously warmed to 37°, contained in the flask. Usually, the solution has been gassed continuously with a mixture of 95% oxygen and 5% carbon dioxide. But, there was no significant difference in the transfer rate of carbazochrome or nicotinamide between from gassed and non-gassed solution by the preliminary experiments, so the flask was not flushed with the mixture as the same as the method described by Reuning and Levy.³⁾ Two milliliters of isotonic phosphate buffer at pH 7.4 (Table I) was introduced into the intestinal segment from the tube A. This will be referred to as the serosal solution. All the solution in the intestinal sac was removed every 10 minutes by introducing 5 ml of fresh serosal solution into the tube A and the drained solution from the tube B was collected completely in a 20 ml measuring flask. The sac was then rinsed with 5 ml of fresh serosal solution through the tube A and the rinse was combined with the previously drained solution for subsequent assay. Experimental design described by Reuning and Levy^{3,11)} was followed with a few modifications. The everted intestinal

segment was suspended for 1 hr in a solution containing the drug only (0.5 mM carbazochrome or NA), then for an additional hour in a solution containing the drug plus complexing agent. An equal number of experiments was carried out in the sequence drug-complexing agent, drug. The transfer rate and its constant were obtained according to the method described by Reuning and Levy.¹²⁾

Transfer Rate Determinations of Caffeine and Hydroxyethyltheophylline—The cannulated everted intestine method developed by Crane and Wilson¹⁰⁾ was used with a few modifications described in a preceding section. The segment was suspended for 1 hr in 60 ml of 20 mM caffeine or 40 mM HET at pH 6.0 (at 37°), and 2 ml of pH 7.4 buffer solution was introduced into the segment. The solution in the intestinal segment was removed every 10 minutes as described in a preceding section. The transfer rate constant was obtained according to the method described by Reuning and Levy.¹²⁾

Determination of Stability Constants—The solubility method by Higuchi and Connors¹³⁾ was used at 37°. As each slope of the solubility diagrams was less than unity, apparent 1:1 stability constants were computed according to the phase-solubility technique.¹³⁾

Analytical Methods—Quantitative determination of carbazochrome except gastric absorption measurement was described in a previous paper.⁶⁾ Assay for carbazochrome in the stomach was as follows. The combined solution was filtered through a filter paper, Toyo Roshi No. 5c. To 5 ml of the filtrate was added 1 ml of 30% trichloroacetic acid. After shaking vigorously for a few minutes and centrifuging, 5 ml of the

10) R.K. Crane and T.H. Wilson, *J. Appl. Physiol.*, **12**, 145 (1958).

11) R.H. Reuning and G. Levy, *J. Pharm. Sci.*, **57**, 1342 (1968).

12) R.H. Reuning and G. Levy, *J. Pharm. Sci.*, **57**, 1335 (1968).

13) T. Higuchi and K.A. Connors, in "Advances in Analytical Chemistry and Instrumentation," Vol. 4, C.N. Reilly, ed., Interscience Publishers, Inc., New York, N.Y., 1965, pp. 144–148.

upper clear solution was removed and 1 ml of 1M phosphate buffer solution (pH 6.9) was added to the solution. This solution was then filtered through Millipore filter, type HA, and the absorbance of the solution was then determined at 355 m μ . Caffeine, NA, and HET were determined by the methods already reported.^{5,9} Sulfanilamide was diazotized following regular manner, coupled with 2-diethylaminoethyl-1-naphthylamine and the absorbance was determined at 550 m μ .

Results and Discussion

Effect of Pretreatment with Complexing Agent on the Intestinal Absorption of Drugs

In the previous report caffeine enhanced the apparent absorption rate of carbazochrome at pH 4.7⁷⁾ and HET decreased that of NA at pH 6.0⁵⁾ by the recirculating perfusion method *in situ*. The principal experimental approach used in this study was to compare the absorption rate of carbazochrome or NA in rat previously treated with each complexing agent (caffeine or HET, respectively) with that in rat recirculated only with 0.9% NaCl solution before recirculating carbazochrome or NA solution.

Recirculation of 20 mM caffeine at pH 4.7 for 20 min, followed by washing out with 0.9% NaCl, did not result in a significant change in the absorption rate of carbazochrome when the solution which did not contain the complexing agent (caffeine) was recirculated alone (Fig. 2). Results obtained in NA absorption experiment had similarly shown that the absorption rate of NA was not affected by HET-pretreatment (Fig. 3).

Fig. 2. Absorption of Carbazochrome after Pretreatment with Caffeine

Vertical bars indicated standard deviation.
control: pretreatment with 0.9% NaCl
pretreatment: pretreatment with 20 mM caffeine

Fig. 3. Absorption of NA after Pretreatment with HET. Details as in Fig. 2

It was found that the pretreatment of the complexing agent and subsequent washing did not affect the absorption of carbazochrome or NA in these complexing systems.

Effect of Buffer Composition on the Absorption of Carbazochrome and Nicotinamide

Aoki and co-workers¹⁴⁾ have reported that the absorption rate of sulfonamides was affected by the isotonic buffer compositions used in the perfusion study, that is, citrate-phosphate

14) M. Aoki, A. Kamada, N. Yada, T. Morishita, and A. Ohtani, Presented at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.

buffer decreased the absorption rate more significantly than 0.9% NaCl solution. Therefore, to obtain further information on the drug absorption in the presence of its complexing agent, the absorption rate of carbazochrome or NA with or without each complexing agent from the buffer solution listed in Table I was compared with the rate from 5% sorbitol solution (isotonic).

The stability constants for those complexes in 5% sorbitol solution by the phase-solubility technique are summarized in Table II. Extents of molecular interaction for carbazochrome-

TABLE II. Stability Constant in 5% Sorbitol Solution at 37°

Complex		Stability constant (M^{-1})
Carbazochrome-Caffeine	5% sorbitol	32
	pH 4.7	43 ^{a)}
	pH 6.0	42 ^{b)}
NA-HET	5% sorbitol	5.5
	pH 6.0	11

a) from Part XVI¹⁵⁾ of this series

b) from Part XIV¹⁶⁾ of this series

caffeine and NA-HET systems in 5% sorbitol solution were found to be less than those in the buffer solution. It is assumed that this less extent of molecular interaction is due to the presence of the poly-alcohol, *i.e.*, sorbitol, since poly-alcohol has a tendency to interfere with the interaction between the preservatives and plastics.¹⁵⁾ In addition, it is known that ionization of the compositions in the complexing systems affects much an extent of complex formation,

Fig. 4. Absorption Rate Constant (hr^{-1}) of Carbazochrome and Nicotinamide with or without Complexing Agent from 5% Sorbitol Solution

that is, an extent of molecular interaction of protonated form of compositions in the complexing system, even if one of them is in protonated form, is much less than that in both non-protonated forms.¹⁶⁾ pH of 5% sorbitol solution is lower (about pH 5.2) than the phosphate buffer (pH 6.0) used in the NA absorption experiment, but NA is almost in non-protonated form in the sorbitol solution, as the ionization constant of NA is 3.80×10^{-4} .¹⁷⁾ It is assumed, therefore, much more likely that the less extent of molecular interaction is due to the interference by poly-alcohol rather than the fraction of non-protonated form.

Fig. 4 shows the effect of the complexing agent on the absorption of 0.5 mM carbazochrome and NA from 5% sorbitol solution from the rat small intestine by the recirculating perfusion method. In the solution caffeine enhanced the absorption of carbazo-

chrome as well as in pH 4.7 and 6.0 isotonic buffer,⁷⁾ and this difference was statistically significant. This effect may be due to the larger complexed fraction in the 5% sorbitol solution.⁷⁾ On the other hand, the absorption rate of NA in the presence of HET was not decreased in

15) K. Kakemi, "Khosei Kagaku Kenkyu Hokoku," 1968, p. 37.

16) K. Kakemi, H. Sezaki, T. Mitsunaga, and M. Nakano, *Chem. Pharm. Bull.* (Tokyo), **16**, 2018 (1968).

17) P. Finholt and T. Higuchi, *J. Pharm. Sci.*, **51**, 655 (1962).

the 5% sorbitol solution, though that of NA with HET in pH 6.0 phosphate buffer decreased as the concentration of HET was increased.⁵⁾ The failure of HET to decrease the absorption of NA in 5% sorbitol solution may be due to the lower stability constant than that in pH 6.0 buffer.

Effect of the Third Additive on the Absorption

In order to get some informations on the mechanism of the complex absorption from the rat small intestine, the absorption rate of carbazochrome (pH 4.7) or NA (pH 6.0) in the complexing system was measured in the presence of the third additive, 1 mM sulfanilamide (SF).

The molecular interaction between these agents was studied by the solubility technique, and the apparent 1:1 stability constants calculated from the phase-solubility diagrams are summarized in Table III.

TABLE III. Stability Constant of Sulfanilamide Complex at 37°

Solubilize	Solubilizer	pH	Stability constant (M^{-1})
SF	caffeine	4.7	12
Carbazochrome	SF	4.7	5.8
Carbazochrome	caffeine	4.7	43
SF	HET	6.0	8.8
SF	NA	6.0	1.6
HET	NA	6.0	11

SF: sulfanilamide

Fig. 5. Effect of the Third Additive (SF) on the Absorption of Carbazochrome and NA

The results of the absorption rate constants of carbazochrome and NA with each complexing agent in the presence of the third additive, SF, are shown in Fig. 5. As the absorption rate constant of 0.5 mM carbazochrome alone at pH 4.7 was 0.079 ± 0.016 (hr⁻¹),⁷⁾ 1 mM SF did not affect the absorption of carbazochrome as shown in Fig. 5. It is meaningless for reason of the difference of the experimental conditions to compare the absorption rate constant of carbazochrome in this section with that in Fig. 2. As the absorption rate constant of 0.5 mM carbazochrome with 20 mM caffeine at pH 4.7 was increased even in the presence of the third additive, it was found that SF might affect little the absorption of carbazochrome with caffeine. It may be assumed that this minor effect of SF is attributed to the less interaction between carbazochrome and SF than that between carbazochrome and caffeine as shown in Table III. Further, there may exist SF-caffeine complex in these components, so the complexed fraction of carbazochrome with SF must be smaller than that in the absence of caffeine. Consequently, this smaller complexed fraction of carbazochrome with SF in the presence of caffeine may be one of the reasons that SF did not affect the absorption of carbazochrome with caffeine.

Although complexation of NA with HET resulted in a decrease in the absorption of NA at pH 6.0 as the extent of complexation of this drug with HET was increased, 40 mM HET in the presence of the third additive (SF) had no measurable effect on the apparent absorption rate of 0.5 mM NA (Fig. 5). NA exhibited the almost same binding tendency with HET as SF in the interaction with HET as shown in Table III. So, there may exist many complex

forms in the three components; the plausible complexes are NA-HET and SF-HET, among others. Consequently, the complexed fraction of NA with HET in the presence of SF must be smaller than that in NA-HET system. Therefore, it may be due to the small complexed fraction of NA in the presence of the third additive that HET had no decreasing effect on the absorption rate of NA with SF.

Absorption from Tied Loop of Rat Intestine

In order to elucidate more clearly the changes of the absorption rate in the presence of the complexing agent by the perfusion method to be due to the intra-luminal complex formation, the effect of complexing agent on the drug absorption was examined by using a tied loop

Fig. 6. Absorption of 0.5 mM Carbazochrome in the Presence of Caffeine by Tied Loop Method at pH 4.7

Fig. 7. Absorption of 0.5 mM NA in the Presence of HET by the Tied Loop Method at pH 6.0

Fig. 8. Gastric Absorption of 0.5 mM Carbazochrome in the Presence of Caffeine at pH 3.0

method. The absorption rate constants of carbazochrome and NA with or without each complexing agent are shown in Fig. 6 and 7, respectively. The results of the tied loop method indicated that the absorption rate constant of carbazochrome increased in the presence of caffeine (Fig. 6) and that of NA decreased in the presence of HET. From these results it was found that good correlation has been noted between the results of drug absorption studies with the recirculating perfusion method and the tied loop method.

Gastric Absorption of Carbazochrome

In the previous report, caffeine enhanced the absorption of carbazochrome at pH 3.0 from rat small intestine by the recirculating perfusion method *in situ*.⁷⁾ In order to demonstrate

clearly the specificity of the small intestine by comparing with the rat stomach, carbazochrome-caffeine complex was examined on the absorption rate from the stomach. In Fig. 8, the results are shown. Complexation with caffeine enhanced the absorption rate of carbazochrome and the difference between the absorption rate of carbazochrome with or without caffeine was statistically significant. From the results, in respect of the absorption of carbazochrome in the presence of the complexing agent (caffeine), it was found that the small intestine did not behave specifically.

Effect of Complex Formation on Intestinal Transfer

Reuning and Levy^{3,11)} have reported the effect of complex formation on the intestinal transfer of both salicylamide and caffeine using the cannulated everted intestine method. In this section, the everted method with modifications described in Fig. 1 was used to determine the intestinal transfer rate constants of carbazochrome or NA in the presence of each complexing agent. The results were compared with those obtained by the recirculating perfusion method *in situ*.

The results of experiments, which involved the measurement of carbazochrome transfer rate from carbazochrome solution and the drug plus its complexing agent, caffeine, across an everted intestinal segment of the rat at pH 6.0, are shown in Fig. 9. The data are plotted in terms of the ratio, amount transferred/time (to be referred to as the transfer rate) and

Fig. 9. Effect of Caffeine on the Transfer of Carbazochrome across the Everted Intestine

first 1 hr: 0.5 mM carbazochrome
second 1 hr: 0.5 mM carbazochrome + 20 mM caffeine

Fig. 10 Effect of Caffeine on the Transfer of Carbazochrome across the Everted Intestine

first 1 hr: 0.5 mM carbazochrome + 20 mM caffeine
second 1 hr: 0.5 mM carbazochrome

plotted at the midpoint of each 10 min sampling period. Ordinarily, absorption proceeds at a constant rate after an initial short equilibration period. But, the transfer rate increased with time until steady state was reached. It is assumed this is due to a relative long equilibration period. At carbazochrome plus its complexing agent following the carbazochrome alone, amount of carbazochrome transferred increased gradually, until a new steady state was attained (Fig. 9). It is evident that transfer rate at the apparent steady state was considerably lower at carbazochrome alone than at the drug-complexing agent. Fig. 10 shows the results of experiments in which the sequence of the drug and the drug-complexing agent was reversed, *i.e.*, the intestinal segment was suspended for one hour in carbazochrome-caffeine and for a second hour in carbazochrome alone. At a steady state the intestinal transfer rate of carbazochrome was again lower at carbazochrome alone than at carbazochrome-caffeine. The effect of complexing agent on the intestinal transfer rate of carbazochrome by the cannulated everted intestine method shown in Fig. 9 and 10 consisted well with the results observed by the recirculating perfusion method.⁶⁾

The effect of HET on the intestinal transfer rate of NA at pH 6.0 is shown in Fig. 11 and 12. At a steady state, it is evident likewise carbazochrome-caffeine complexing system that

Fig. 11. Effect of HET on the Transfer of NA across the Everted Intestine

first 1 hr: 0.5 mM NA
second 1 hr: 0.5 mM NA + 40 mM HET

Fig. 12. Effect of HET on the Transfer of NA across the Everted Intestine

first 1 hr: 0.5 mM NA + 40 mM HET
second 1 hr: 0.5 mM NA

a pronounced decrease in the transfer rate of NA occurred when this drug complexed extensively with HET, and these results consisted also with those of the recirculating perfusion method.⁵⁾

In the previous report,⁵⁾ Eq. (1) was derived to calculate the absorption rate constant of a complex between drug A and B,

$$k_{AB} = \frac{kK(A)_t + k - k_0}{K(A)_t} \quad (1)$$

where k_{AB} , k , and k_0 are the absorption rate constants for the complex AB, for the drug B in the presence of complexing agent A, and for the drug B alone, respectively, K is the stability constant of the complex, and $(A)_t$ is the molar concentration of added complexing agent A. Using Eq. (1), the intestinal transfer rate constant, k_{AB} , for the carbazochrome-caffeine complex was calculated from the results of everted method in Fig. 9. The transfer rate constant of drug with or without the complexing agent was obtained by dividing the transfer rate by the mucosal drug concentration. Further, the intestinal transfer rate constant, k_{caffeine} , for the complexing agent (caffeine) was determined by the method that the intestinal segment was suspended in 20 mM caffeine solution at pH 6.0 and the serosal solution was exchanged every 10 min. These constants are summarized in Table IV. It was found that the intestinal trans-

TABLE IV. Intestinal Transfer Rate Constant for Carbazochrome-Caffeine Complex (liter/min $\times 10^5$)

	Exp. No. in Fig. 9 1	2	Mean
k_0	1.52 ^{a)}	1.71 ^{a)}	1.61 $\pm$ 0.11 ^{b)}
k	2.15 ^{c)}	2.19 ^{c)}	
k_{AB}	2.9	2.8	2.9
k_{caffeine}			3.90 $\pm$ 0.59 ^{b)}

k_0 , k , k_{AB} : transfer rate constant of drug alone, that of drug in the presence of complexing agent, and that of complex, respectively.

a), c): mean of the values from 3 or 4 points, respectively, at the steady state portion

b): mean of the values from two segments

fer rate constant, k_{AB} , of carbazochrome-caffeine was higher than that of carbazochrome and lower than that of caffeine. Similarly, the rate constant, k_{AB} , of NA-HET complex was calculated, and that of HET was determined by the similar method as k_{caffeine} . These results are summarized in Table V. It was found that the intestinal transfer rate constant of the

TABLE V. Intestinal Transfer Rate Constant for NA-HET Complex
(liter/min $\times 10^5$)

	Exp. No. in Fig. 11		Mean
	1	2	
k_0	3.14 ^{a)}	3.18 ^{a)}	3.15 $\pm$ 0.45 ^{b)}
k	2.68 ^{a)}	2.96 ^{a)}	
k_{AB}	1.6	2.5	2.1
k_{HET}			2.13 $\pm$ 0.17 ^{b)}

k_0 , k , k_{AB} : see Table IV.

a): mean of the values from 4 points at the steady state portion

b): mean of the values from two segments

complex was approximately intermediate values between those of drug (NA) and its complexing agent (HET).

Assumption of Stability Constant from Absorption Data

It is of interest to assume whether the stability constant of the complex is the same at the surface of the absorption rate-limiting biological barrier as in the bulk solution. Reuning and Levy³⁾ assumed that the stability constant of the complex at the barrier was similar to that in the bulk solution from the good fit of the transfer data to the theoretical model. In order to reveal this more definitively, the stability constants were assumed from the absorption data.

Eq. (1) can be rearranged to yield:

$$\frac{1}{k-k_0} = \frac{1}{k_{AB}-k_0} + \frac{1}{K(A)_t(k_{AB}-k_0)} \quad (2)$$

where k , k_0 , and $(A)_t$ are experimentally determinable. Eq. (2) is the same as that of Colter and his collaborators,¹⁸⁾ which was derived to obtain the rate constant for acetolysis of com-

Fig. 13. Plot of $1/(k-k_0)$ vs. $1/(A)_t$ of Carbazochrome-Caffeine at pH 6.0

TABLE VI. Stability Constants from Absorption Data
(K_a) and Phase Solubility Technique (K_b)

Drug-Complexing agent	K_a	K_b
Sulfisoxazole-NaPAS	47	75 ^{a)}
Sulfisomidine-NaPAS	9	33 ^{a)}
Sulfamethoxypyridazine-NaPAS	16	14 ^{a)}
Caffeine-Na salicylate	22	24 ^{a)}
NA-HET	14	12 ^{a)}
Carbazochrome-Caffeine pH 6	20	42 ^{b)}
pH 4.7	13	43 ^{c)}

NaPAS: sodium *p*-aminosalicylate
unit: M^{-1}

a), b), c): from Part XIII,⁵⁾ XIV⁶⁾ and VI,⁷⁾ respectively, of this series

18) A.K. Colter, S.S. Wang, G.H. Megerle, and P.S. Ossip, *J. Am. Chem. Soc.*, **86**, 3106 (1964).

plex substrate. It is possible to obtain k_{AB} and K by plotting $1/(k-k_0)$ vs. $1/(A)_t$. The intercept of such a plot gives k_{AB} and, knowing this constant, K may be calculated from the slope. Fig. 13 shows such a graph of carbazochrome-caffeine complexing system. The linearity of the other plots using the data from the previous report⁵⁾ is excellent. The results of analysis of absorption data with the data from physico-chemical methods (phase-solubility technique) are shown in Table VI. Stability constants from absorption and physico-chemical data show a fair agreement with each other. This agreement suggests that the stability constant of the complex may be the same at the surface of the absorption rate-limiting barrier as in the *in vitro* solution.

General Discussion

It is evident that complexing agent plays an important role in modifying the absorption rate of drug from the intestine in the recirculating perfusion method.

There are four mechanisms by which complexing agent can affect drug absorption from the intestine. First, the complex formed between drug and its complexing agent may have an absorption rate constant different from that of drug itself. Because about half of the drug in the perfusion solution was in complexed form,⁷⁾ a pronounced difference in the absorption rate constant of free and complexed drug would result in an appreciable difference in the apparent absorption rate of drug when administered alone and together complexing agent. Second, the complexing agent may have an important factor in the accumulation of drug in the tissue or binding of drug to the mucosa. If the accumulation or binding of drug was large, the intrinsic absorption would differ from the drug alone. Third, the complexing agent may have a direct effect on the permeability characteristics of the mucosa, and fourth, it may affect the absorption rate of drug by a systemic, or pharmacological effect such as a change in the rate of gastric blood flow. Each of the mechanisms are discussed in detail.

The absorption by the perfusion method, tied loop, or gastric absorption was estimated solely from the disappearance amount from the test solution. Therefore, this apparent disappeared amount is assumed to contain the absorbed amount into the vascular system, the amount of accumulation in the gut, and/or the amount bound to the surface or the gut (mucosa).^{19,20)} If the accumulation in the tissue or binding to the mucosa was significantly large, the effect of complex formation observed in this and preceding publications in this series cannot be attributed exactly to the intrinsic absorption process. As the everted method determines directly the transferred amount of drug when administered alone and together with complexing agent, it is not necessary to consider the accumulation of drug in the tissue or binding of drug to mucosa by the everted method at the apparent steady state. The results at the steady state by the method were well in accord with those by the recirculating perfusion method (Table IV and V). So, the changes of the absorption rate of carbazochrome⁶⁾ or NA⁵⁾ in the presence of each complexing agent by the perfusion method may be due to the complex formation in the luminal phase more definitively.

The possibility that the effect of complexing agent on the absorption rate of drug may be due to a modification of membrane permeability characteristics, and/or systemic effect. Absorption experiments from the rat small intestine by pretreatment of the complexing agent were carried out with carbazochrome-caffeine and NA-HET complexing systems. The pretreatment of complexing agent and subsequent washing did not affect the absorption rate of carbazochrome or NA at all (Fig. 2 and 3). This indicates that there was no detectable effect of the complexing agent on the permeability characteristics of the intestinal membranes by pretreated complexing agent, or systemic effect by the complexing agent absorbed during

19) K. Kakemi, T. Arita, R. Hori, R. Konishi, and K. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 248 (1969).

20) K. Kakemi, T. Arita, R. Hori, R. Konishi, K. Nishimura, H. Matsui, and T. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 255 (1969).

the pretreatment. Further, the absorption rate constants of the complexing agent at high concentration were same as at low concentration under the experimental conditions.^{5,7)} In a further data, the absorption rate constant of complex, k_{AB} , was almost constant with various initial concentrations of complexing agent, even at high concentration.⁵⁾ These lead to the conclusion that the complexing agent did not exert any toxic effect under the concentration of the complexing agent used. All these results indicate that the effect of complexing agent cannot be ascribed to the alternation in membrane permeability characteristics, or systemic effect.

Further, the following four facts suggest strongly that the increase or decrease of the absorption rate in the presence of the complexing agent is due to the intra-luminal complex formation.

a) The results by the three absorption experiments (perfusion, tied loop, and everted sac method) showed the same tendency to change of the absorption rate in the presence of the complexing agent.

b) In respect to the absorption of carbazochrome, it was found that good correlation has been noted between the results of absorption studies by the gastric absorption and the perfusion method, and that the small intestine did not behave specifically.

c) In the isotonic sorbitol solution, caffeine enhanced the absorption of carbazochrome as well as in pH 4.7 (citrate-phosphate) or pH 6.0 (phosphate) isotonic buffer. But HET failed to decrease the absorption of NA in the isotonic sorbitol solution. This may be due to the less extent of molecular interaction in the sorbitol solution than in the isotonic buffer (Fig. 4).

d) The effect of the third additive on the absorption of carbazochrome or NA were also correlative to the extent of molecular interaction (Fig. 5).

Finally, the good fit of the intestinal absorption data to the theoretical model suggests that stability constant of the complex is the same at the site of absorption as that measured in the bulk solution (Table VI). It may be assumed that this leads to the conclusion that complex as such is absorbed across the rate-limiting barrier of the intestine.

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