

Studies on Complexes. XIV.¹⁾ Effect of Complex Formation on Drug Absorption from Alimentary Tract. (5). Enhancing the Absorption of Certain Drugs by Complex Formation

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Attempts were made to enhance the absorption rate of drug by the complex formation. Following model complexes of rapidly absorbed drugs (A) with slowly absorbed drugs (B) were used: hydroxyethyltheophylline (A)-carbazochrome (B), caffeine (A)-carbazochrome (B), nicotinamide (A)-carbazochrome (B), and salicylic acid (A)-4-methylaminoantipyrine (B). Apparent absorption rate for a slowly absorbed drug with or without a rapidly absorbed one from rat small intestine were determined by the recirculating perfusion method at pH 6.0, and from these results and the equilibrium constant for the complex the absorption rate constants for the complexes were calculated by the equation shown in the previous report. It was found that the intestinal absorption of the drug B increased in the presence of the drug A in those complexes, and the calculated absorption rates for the complexes were higher than those of B and lower than those of A.

Previous report¹⁾ of a series of the effect of complex formation on drug absorption has shown the relationship between the intestinal absorption rate of a 1:1 complex and its equilibrium constant. The relationship is shown in the form Eq. (1),

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

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 drug A, and for the drug B alone, respectively, K is the equilibrium constant of the complex, and $(A)_t$ is the molar concentration of added A assuming that a) the concentration of drug A is much larger than that of drug B, b) drug A, B, and the complex AB are absorbed by an apparent first order process, and c) the complex is not dissociated at the surface of the intestinal membrane. From Eq. (1), it was possible for a few complexes to deduce the absorption rate constant of complex AB. The constant, k_{AB} , was smaller than that of the drug B alone, and almost constant under the various initial concentrations of drug A.

The present paper deals with further investigation of the relationship, shown in Eq. (1), with different series of complexes. Especially, the absorption rate of a slowly absorbed drug B complexed with a rapidly absorbed drug A were determined.

Experimental

Materials—Caffeine, nicotinamide, sodium salicylate, and carbazochrome were of JP VII grade. Hydroxyethyltheophylline was of commercially available product without further purification. 4-Methylaminoantipyrine was obtained by the method of Takahashi, *et al.*³⁾ Other materials were of JIS special grade.

1) Part XIII: I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1527 (1968). This is one of the series of "Studies of Complexes" (M. Samejima).

2) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka.*

3) T. Takahashi, J. Okada, M. Hori, A. Kato, K. Kanematsu, and Y. Yamamoto, *Yakugaku Zasshi*, **76**, 1180 (1956).

Determination of the Rate of Absorption from the Rat Small Intestine—The experimental technique employed was essentially the same as that reported already,⁴⁾ and each test solution which contained 0.5 mM of drug B was prepared by dissolving in the pH 6.0 isotonic phosphate buffer.

Analytical Methods—Quantitative determination of nicotinamide was carried out spectrophotometrically at 400 m μ by the cyanogen bromide method.⁵⁾ Salicylic acid,⁶⁾ and 4-methylaminoantipyrine⁷⁾ were determined by the methods already reported. Recirculation fluid, appropriately diluted by pH 6.9 phosphate buffer, was measured at 355 m μ for carbazochrome. Hydroxyethyltheophylline was determined by the method in previous paper.¹⁾ All additives did not interfere with these analytical methods under the concentrations used. All optical densities were measured using Hitachi Perkin-Elmer 139 UV-VIS Spectrophotometer.

Determination of Equilibrium Constant—The solubility method described by Higuchi and Connors⁸⁾ was used. Excess amount of carbazochrome were placed in 20 ml ampules together with isotonic buffer solution of nicotinamide, caffeine, or hydroxyethyltheophylline, and these ampules were agitated in a constant temperature bath (37°) until equilibrium. An aliquot of the supernatant liquid was then filtered through Millipore membrane, type HA, and assayed by the methods described above. Apparent 1:1 equilibrium constants were computed according to the phase-solubility technique.

Results and Discussion

In this report four model complexes in Table I were chosen by the following reasons: (a) preliminary experiment had shown carbazochrome was slowly absorbed (7% in 1 hour), and its solubility increased proportionally with caffeine, hydroxyethyltheophylline (HET), or nicotinamide concentration as shown later, and those solubilizers were absorbed more rapidly than carbazochrome,¹⁾ (b) the absorption of 4-methylaminoantipyrine increased in the presence of salicylic acid.⁹⁾

TABLE I. Model Complexes used in This Study and Equilibrium Constant (K) at 37° and pH 6.0

Rapidly absorbed drug A	Slowly absorbed drug B	K
Hydroxyethyltheophylline	carbazochrome	31
Caffeine	carbazochrome	42
Nicotinamide	carbazochrome	5.6
Salicylic acid	4-methylaminoantipyrine	15

Solubility diagrams of carbazochrome in the presence of nicotinamide, HET, or caffeine were recorded in Fig. 1. Each slope was less than unity, so it could be interpreted that a 1:1 complex was formed. Equilibrium constants computed by the phase-solubility technique⁸⁾ were shown in Table I. It was already reported that salicylic acid interacted with 4-methylaminoantipyrine.⁹⁾

Carbazochrome and 4-methylaminoantipyrine were absorbed according to a first order process in the rat intestinal perfusion experiment, to which phenol red was used for the volume change indicator¹⁰⁾ (Fig. 2). It has been found that HET, nicotinamide, salicylate, and caffeine in the previous paper¹⁾ are absorbed according to a first order process. Absorption rate constant, therefore, was calculated from the amount of the drug in perfusate before perfusion and that remaining in perfusion fluid⁴⁾ after circulation for a certain period of time.

- 4) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1098 (1968).
- 5) I. Utsumi and M. Samejima, *Yakugaku Kenkyu*, **29**, 980 (1957).
- 6) K. Kakemi, T. Arita, and H. Yamashina, *Yakuzaigaku*, **21**, 97 (1961).
- 7) K. Kato, M. Umeda, and S. Tsubota, *Yakuzaigaku*, **24**, 116 (1964).
- 8) T. Higuchi and K.A. Connors, in "Advances in Analytical Chemistry and Instrumentation," Vol. 4, C.N. Reilley, ed., Interscience Publishers, Inc., New York, N.Y., 1965, pp. 144—148.
- 9) M. Samejima, I. Sugimoto, and I. Utsumi, *Yakugaku Zasshi*, **88**, 618 (1968).
- 10) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).

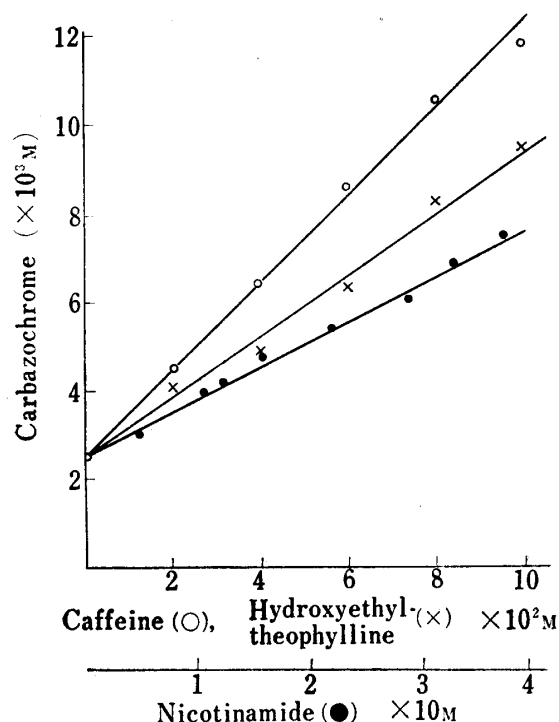


Fig. 1. Solubility of Carbazochrome in the Presence of Caffeine, Hydroxyethyltheophylline, and Nicotinamide at pH 6.0

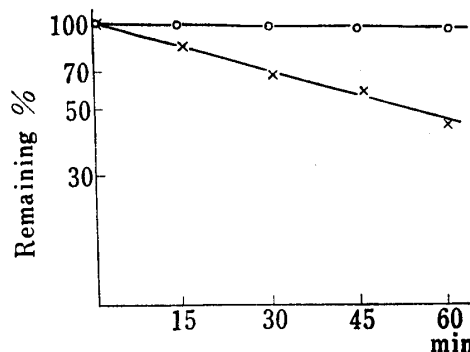


Fig. 2. Logarithmic Plots of Carbazochrome (O) and 4-Methylaminoantipyrine (x) in Recirculating Solution at pH 6.0

The absorption rate constant (k) of carbazochrome was increased from $0.069 \text{ (hr}^{-1}\text{)}$ to $0.108 \text{ (hr}^{-1}\text{)}$ in the presence of 40 mM HET (Table II). This difference was found to be statistically significant ($p < 0.05$). But k of carbazochrome in the presence of 20 mM HET was

TABLE II. k_{AB} for Carbazochrome-HET

Expt. No.	k_0^a	k^b	HET		k_{AB} from Eq. (1)		k_{AB} from Eq. (2)	
			% absorbed in 1 hr	k	Before perfusion	After 1 hr	Before perfusion	After 1 hr
1		0.108	40.0	0.51	0.14	0.16	0.14	0.16
2		0.095	38.3	0.48	0.12	0.13	0.12	0.13
3		0.128	31.1	0.37	0.18	0.27	0.17	0.20
4		0.095	29.9	0.36	0.12	0.13	0.14	0.19
5		0.115	42.7	0.56	0.15	0.18	0.15	0.18
Mean	0.069 ± 0.027	0.108 ± 0.013	36	0.45				

HET: hydroxyethyltheophylline

a) absorption rate constant of carbazochrome alone

b) Absorption rate constant of carbazochrome in the presence of 40 mM HET dimension of absorption rate was hr^{-1} .

0.075 hr^{-1} (average of two experiments) and there was no statistically significant difference from group without HET. The failure of 20 mM HET to enhance the absorption suggested that, as the ratios of complexed to total carbazochrome in the presence of 20 mM and 40 mM HET were 0.38 and 0.56 , respectively, it was necessary for the ratio to be large to enhance the absorption.

The theoretical absorption rate (k_{AB}) of the carbazochrome-HET complex in the presence of 40 mM HET was calculated by Eq. (1). These results were shown in Table II. For example in experiment No. 1, following information was used to solve Eq. (1): $k_0=0.069$, and $k=0.108$ (from Table II), $(A)_t=40$ mM, $K=31$ (from Table I. As K cannot be exactly estimated at the absorption site, the result by the phase-solubility method was used). From these data, k_{AB} was 0.14. HET was absorbed about 36% in 1 hour, so, strictly speaking, $(A)_t$ in Eq. (1) was not same as the initial concentration of drug A (in this case HET). Therefore, in each experiment, k_{AB} was calculated by assuming $(A)_t$ to be residual HET after 1 hour. But, from Eq. (1), there was not any significant difference among k_{AB} obtained from the added HET and residual HET after 1 hour as shown in Table II.

Recently, Goto, *et al.*,¹¹⁾ developed Eq. (2) for calculating k_{AB} of caffeine—a few absorbable drug complexes,

$$k = (k_0 - k_{AB}) \frac{(D)_F}{(D)_T} + k_{AB} \quad (2)$$

where $(D)_F$ and $(D)_T$ were the concentration of free, and that of total drug B. Eq. (2) is essentially same as Eq. (1). As Eq. (1) was derived under the condition of the concentration of drug A to be much larger than that of B, Eq. (1) must be used under this condition. But, it is thought that Eq. (1) is a simple one to obtain k_{AB} under that condition as there is no necessity for calculating the fraction of free drug B, namely $(D)_F/(D)_T$. Using Eq. (2), k_{AB} of carbazochrome-HET complex was calculated from the concentration of carbazochrome and HET before and after 1 hour perfusion, and shown in Table II. As the results obtained from four ways were almost constant, it was evident that Eq. (1) could be satisfactorily used for estimating k_{AB} of complexes. So, for other complexes, Eq. (1) was used to estimate k_{AB} , and initial concentration of drug A was used as $(A)_t$ in Eq. (1). From Table II, absorption rate constant for carbazochrome-HET complex was lower than that of HET, and higher than that of carbazochrome.

Results of other complexes were shown in Table III and IV. As equilibrium constant of carbazochrome-nicotinamide complex was smaller than those of other complexes (Table I), and the complexed fraction of carbazochrome in the test solution was small, it was supposed that the absorption of carbazochrome was not measurably affected. Fortunately, nicotinamide was low toxic,¹⁾ so the added nicotinamide concentration was 100 mM and in this case the complexed fraction of carbazochrome was 0.36. The absorption rate of carbazochrome increased with an added nicotinamide or caffeine concentration (Table III), and that of 4-methylaminoantipyrine increased with an added salicylic acid (Table IV).

TABLE III. Absorption Rate Constant (k) of Carbazochrome in the Presence of Drug A and k_{AB}

A ^{a)}	k_0	k	Absorption rate of A alone ^{b)}	k_{AB} ^{c)}
Nicotinamide	0.069 ± 0.027	0.112 ± 0.018	1.14	0.19
	$p < 0.05$			
Caffeine	0.069 ± 0.027	0.118 ± 0.014	1.10	0.17
	$p < 0.05$			

Dimension of absorption rate was hr^{-1} .

a) Initial concentrations of nicotinamide and caffeine were 100 mM and 20 mM, respectively.

b) from previous report

c) Obtained from Eq. (1) before perfusion

11) S. Goto, R. Takamatsu, M. Shibao, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 332 (1968).

TABLE IV. Absorption Rate Constant (k) of 4-Methylaminoantipyrine in the Presence of Salicylic Acid and k_{AB}

k_0	k	Absorption rate of salicylic acid alone	$k_{AB}^{a)}$
0.585 ± 0.059	0.922 ± 0.142	1.43	1.3
\uparrow $p < 0.05$			

Dimension of absorption rate was hr^{-1} .

Initial concentration of salicylic acid was 60 mM as sodium salicylate.

a) Obtained from Eq. (1) before perfusion

As was mentioned above, absorption rate of carbazochrome-HET complex was intermediate between that of slowly absorbed carbazochrome and of rapidly absorbed HET. In a similar fashion, absorption rate of other complexes was lower than that of rapidly absorbed nicotinamide, caffeine or salicylic acid and higher than that of slowly absorbed carbazochrome or 4-methylaminoantipyrine.

HET, nicotinamide or salicylic acid as shown in the previous paper,¹⁾ and caffeine,^{11,12)} even at high concentration, did not exert any effect on the absorption membranes such as damage, membrane blocking, or toxicity under the concentrations used in this study during the 1 hour absorption period. The increased absorption could not be ascribed to a pH shift of test solution during experiment, since the pH of the solution was at first 6.0 and it was known that this pH was almost constant during circulation.¹⁰⁾ These changes of the absorption rates might be due solely (or at least predominantly) to complex formation.

From these results it was thought under the experimental conditions and the used model complexes: that (a) these complexes might be absorbed as such, (b) absorption rate enhancing of slowly absorbed drug was due to complex formation, and (c) absorption rate for complex was lower than that of a rapidly absorbed drug, and higher than that of a slowly absorbed one, as if the former might carry the latter or its complex from intestinal fluid to the membrane.

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