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Studies on Complexes. XVI.[1] Effect of Complex Formation on Drug Absorption from Alimentary Tract. (6). Some Factors Affecting Complex Absorption

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Effect of complex formation on the drug absorption from the rat small intestine by the perfusion method *in situ* was investigated. Apparent absorption rate of carbazochrome or nicotinamide alone was compared with that together its complexing drug. As the complexing drugs of carbazochrome, the following drugs were used: nicotinamide, 7-(2-hydroxyethyl) theophylline (HET), caffeine, 7-(2,3-dihydroxypropyl) theophylline sodium (DHPT), and *p*-aminosalicylate (NaPAS). HET was used as the complexing drug of nicotinamide.

It was found that the apparent absorption rate of carbazochrome was enhanced by complex formation with HET and caffeine at pH 3.0 and 4.7, and with NaPAS at pH 6.0, and that there was no significant difference in the apparent absorption rate between alone and together the complexing drug in nicotinamide–HET at pH 3.0 and 3.9, in carbazochrome–nicotinamide at pH 3.0 and 3.9, and in carbazochrome–DHPT at pH 6.0. From these absorption experiments and physico-chemical property for the complex, it is assumed that an extent of molecular interaction and the difference between the absorption rate of the drug and that of its complexing drug affect the absorption of the complex.

In the previous studies in this laboratory of a series of the effect of complex formation on drug absorption from rat small intestine, it was found that, a) complex formation of an absorbable drug with a nonabsorbable one decreased significantly the apparent absorption rate of the former,[3] b) the Eq. (1) was derived to calculate the absorption rate constant of a complex between absorbable drug A and B,[4]

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

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 association constant of the complex, and $(A)_t$ is the molar concentration of added complexing drug A, and the constant, k_{AB}, was smaller than that of the drug B alone, and c) complexation with a rapidly absorbed drug enhanced the absorption rate of a slowly absorbed drug.[5]

Levy and Reuning[6] have studied that complex formation of salicylic acid with caffeine decreased significantly the apparent absorption rate of salicylic acid from rat stomach and this effect of complexation was consistent with the apparent partition coefficient of salicylic acid and of the complex: the latter showed a lower apparent partition coefficient than the former. Recently, Goto and co-workers[7] investigated the effect of combination of caffeine with a few absorbable drugs on gastric absorption, and derived an equation to obtain the absorption rate constant, k_{AB}, for the complex.

1) Part XV: M. Samejima and M. Yoshida, presented at the Meeting of Kinki Branch, Pharmaceutical Society of Japan, Kyoto, Nov. 1968. This is one of the series of Studies on Complexes (M. Samejima).
2) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka.*
3) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1098 (1968).
4) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1527 (1968).
5) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 994 (1969).
6) G. Levy and R.H. Reuning, *J. Pharm. Sci.*, **53**, 1471 (1964).
7) S. Goto, R. Takamatsu, M. Shibao, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 332 (1968).

The present paper deals with further investigation about a decreased or enhanced case of the apparent absorption rate by complex formation from the rat small intestine *in situ*. And factors which affect the absorption rate for the complex across the intestine are explored by focussing on the association constant for the complex, on the complexed fraction, and on the absorption rate constants of the drug and its complexing drug. Nicotinamide—7-(2-hydroxyethyl)theophylline (HET) and carbazochrome—its complexing drugs (nicotinamide, HET, caffeine, 7-(2,3-dihydroxypropyl)theophylline (DHPT), and sodium *p*-aminosalicylate (NaPAS)) systems were chosen as model complexes for this study by the following reasons. First, in the previous reports it was found that complexation with HET at pH 6.0 decreased the apparent absorption rate of nicotinamide,[4] and that complexation with nicotinamide, HET, or caffeine at pH 6.0 enhanced the apparent absorption rate of carbazochrome,[5] so those systems were chosen to explore the effect of pH on the absorption of complex. Second, DHPT, one of the xanthine derivatives, and carbazochrome were found to form a 1:1 complex in aqueous solution and the association constant was almost the same as that for carbazochrome–caffeine or carbazochrome–HET complex. Third, NaPAS and carbazochrome also formed a 1:1 complex and the degree of complexation was found to be relatively high as shown later.

Experimental

Materials——Nicotinamide, carbazochrome, caffeine, and NaPAS were of JPVII grade. HET (Sankyo Co., Ltd.) and DHPT (Shionogi & Co., Ltd.) were used as received. All other chemicals used were of reagent grade.

Determination of Rate of Absorption from Rat Small Intestine——The experimental technique employed was essentially the same as that reported already,[3] and each test solution which contained 0.5 mM of nicotinamide or carbazochrome alone or together its complexing drug was prepared by dissolving in the each pH isotonic buffer solution.[8] As pH of the test solution was slightly changed by the addition of nicotinamide as the complexing drug at pH 3.0 and 3.9, the solution was adjusted to the corresponding pH by addition of HCl.

Analytical Methods——DHPT was determined by the similar method to caffeine previously reported.[3] Others were the same as those reported already.[3,4,5]

Determination of Association Constants——The experimental procedures were essentially the same as that reported already.[5] Only a variation in method was as follows: When nicotinamide was dissolved in pH 3.0 or 3.9 isotonic buffer, pH of the solution was shifted to a higher value by the addition of nicotinamide. Therefore, the solution was adjusted to pH 3.0 or 3.9 by addition of HCl.

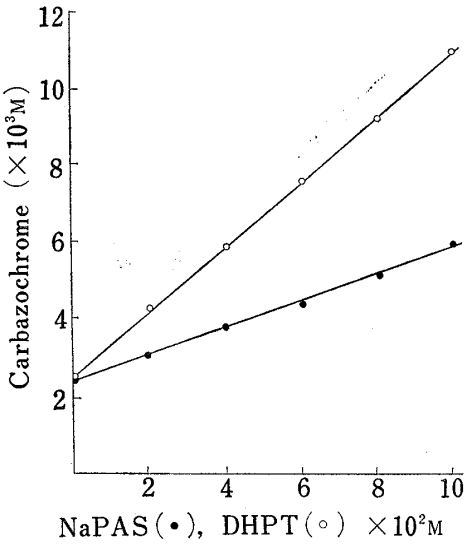

Fig. 1. Solubility of Carbazochrome in the Presence of NaPAS and DHPT at pH 6.0

Results and Discussion

Solubility diagrams of carbazochrome in the presence of NaPAS and DHPT at pH 6.0 are recorded in Fig. 1. Those in the presence of nicotinamide, HET, and caffeine at each pH, and those of HET in the presence of nicotinamide at pH 3.0 and 3.9 were linear similar to Fig. 1. Each slopes of those diagrams were less than unity, so it could be interpreted that a 1:1 complex was formed.[9] Association constants computed by the phase solubility technique[9] are shown in Table I.

8) Nippon Kagakukai, "Jikken Kagaku Khoza," 24, Maruzen, Tokyo, 1958, p. 224.
9) T. Higuchi and K.A. Connors, *Advances in Analytical Chemistry and Instrumentation*, 4, 117 (1965).

Table I. Association Constant (K) for Complexes

Complex		pH	K (liter/M)
HET	nicotinamide	3.0	2.5[a]
		3.9	5.4[a]
		6.0	12[a,b]
Carbazochrome	nicotinamide	3.0	2.4
		3.9	3.9
		6.0	5.6[c]
Carbazochrome	HET	3.0	22
		4.7	32
		6.0	31[c]
Carbazochrome	caffeine	3.0	34
		4.7	43
		6.0	42[c]
Carbazochrome	DHPT	6.0	36
Carbazochrome	NaPAS	6.0	14

a) at 30°. Others were obtained at 37°.
b) from Part XIII of this series[4]
c) from Part XIV of this series[5]

Since it is known that ionization of the components in the complexing system affect much an extent of complex formation,[10] an extent of molecular interaction was compared with the ionization constant. Ionization constant of HET[11] or caffeine[7] is about 1×10^{-1}, and that of nicotinamide is 3.80×10^{-4} [12] at 25°. As the structure of DHPT is analogous to HET or caffeine, its ionization constant is assumed to be the same order as that of HET or caffeine. The solubility of carbazochrome was almost independent on pH in this study (pH 3.0: 3.21×10^{-3}M, pH 4.7: 2.72×10^{-3}M, pH 6.0: 2.48×10^{-3}M, at 37°), it is, therefore, thought that carbazochrome is almost in nonprotonated form at those pH regions from the relationship between pH and its solubility or its UV absorption spectrum.[13] Extents of molecular interaction for HET–nicotinamide and carbazochrome–nicotinamide at pH 3.0, where nicotinamide was about 72% in protonated form, were found to be much less than those at pH 3.9 and 6.0 as shown in Table 1. Carbazochrome–HET and carbazochrome–caffeine systems in non-

Table II. Effect of HET on the Absorption of Nicotinamide

pH	k_0	k	k_{AB}	Fc
3.0	0.687 ± 0.152	0.778 ± 0.112		0.09
	N.S.			
3.9	1.04 ± 0.12	0.925 ± 0.169		0.18
	N.S.			
6.0[a]	1.14 ± 0.01	0.865 ± 0.039	0.27	0.32
	$p<0.05$			

k_0, k, k_{AB}: hr^{-1} Fc: complexed fraction of nicotinamide
Initial concns. of nicotinamide and HET were 0.5 mM and 40 mM, respectively. N.S. means the difference between k_0 and k was not statistically significant, even at the 5% level of significance using t-test, and $p<0.05$ means the difference was significant at that level.
a) from Part XIII of this series[4]

10) K. Kakemi, H. Sezaki, T. Mitsunaga, and M. Nakano, *Chem. Pharm. Bull.* (Tokyo), **16**, 2018 (1968).
11) K. Linek and C. Peciar, *Chem. Zvesti*, **16**, 692 (1962) [*C.A.*, **58**, 8409*h* (1963)].
12) P. Finholt and T. Higuchi, *J. Pharm. Sci.*, **51**, 655 (1962).
13) A.C. Alves, *Anais Fac. Farm. Porto.*, **14**, 37 (1954) [*C.A.*, **49**, 10058*h* (1955)].

Table III. Effect of Nicotinamide on the Absorption of Carbazochrome

pH	k_0	k	k_{AB}	Fc
3.0	0.077 ± 0.006	0.071 ± 0.023		0.19
	N.S.			
3.9	0.071 ± 0.09	0.091 ± 0.033		0.28
	N.S.			
6.0[a]	0.069 ± 0.027	0.112 ± 0.018	0.19	0.36
	$p<0.05$			

k_0, k, k_{AB}: hr^{-1} Fc: complexed fraction of carbazochrome
Initial concns. of carbazochrome and nicotinamide were 0.5 mM and 100 mM, respectively.
a) from Part XIV of this series[5]

protonated form at these pH regions, on the other hand, were independent on pH. This less extent of molecular interaction of protonated form can be rationalized by the reason that components in the complexing system, even if one of them is in protonated form, may not readily approach each other by the hydration of protonated form in aqueous solution.[10]

The effect of HET on the absorption of nicotinamide and that of nicotinamide on the absorption of carbazochrome from rat small intestine *in situ*, at pH 3.0 and 3.9, are shown in Table II and III. At these pH there was no statistically significant difference in the apparent absorption rate of nicotinamide or carbazochrome between alone and together the complexing drug in each systems. However, at pH 6.0, it was found that complexation with HET decreased the apparent absorption rate of nicotinamide,[4] and that complexation with nicotinamide enhanced that of carbazochrome.[5]

The effects of HET and caffeine on the absorption of carbazochrome are shown in Table IV and V, respectively.

Table IV. Effect of HET on the Absorption of Carbazochrome

pH	k_0	k	k_{AB}	Fc
3.0	0.077 ± 0.006	0.129 ± 0.011[b]	0.19	0.46
	$p<0.05$			
4.7	0.079 ± 0.016	0.141 ± 0.016[b]	0.19	0.56
	$p<0.05$			
6.0[a]	0.069 ± 0.027	0.108 ± 0.013[b]	0.14	0.56
	$p<0.05$			
		0.075 ± 0.007[c]		0.38
	N.S.			

k_0, k, k_{AB}: hr^{-1}
Initial concn. of carbazochrome was 0.5 mM.
a) from Part XIV of this series[5]
b) Initial concn. of HET was 40 mM.
c) Initial concn. of HET was 20 mM.

It is evident that a pronounced increase in the apparent absorption rate of carbazochrome occured at each pH when initial concentrations of HET and caffeine were 40 mM and 20 mM, respectively. And these differences were found to be statistically significant ($p<0.05$). The difference between the absorption rate constant, k, of the carbazochrome in the presence of caffeine at pH 3.0 and 6.0 in Table V was not statistically significant ($p>0.05$).

Table V. Effect of Caffeine on the Absorption of Carbazochrome

pH	k_0	k	k_{AB}	Fc
3.0	0.077 ± 0.006	0.169 ± 0.066	0.30	0.40
	$p<0.05$			
4.7	0.079 ± 0.016	0.138 ± 0.015	0.21	0.46
	$p<0.05$			
6.0[a)]	0.069 ± 0.027	0.118 ± 0.014	0.17	0.46
	$p<0.05$			

k_0, k, k_{AB}: hr^{-1}
Initial concns. of carbazochrome and caffeine were 0.5 mM and 20 mM, respectively.
a) from Part XIV of this series[5])

The effect of DHPT on the absorption of carbazochrome is shown in Table VI. It may be reasonable to assume that the absorption rate of carbazochrome may be enhanced by complexation with DHPT, since the association constant for this complex is almost the same as those for carbazochrome–HET and carbazochrome–caffeine. Contrary to expectation, the apparent absorption rate was not enhanced in the presence of DHPT. However, that of carbazochrome was enhanced by complexation with NaPAS (Table VII).

Table VI. Effect of DHPT on the Absorption of Carbazochrome

pH	k_0	k	Fc
6.0	0.069 ± 0.027	0.077 ± 0.007	0.59
	N.S.		

k_0, k: hr^{-1}
Initial concns. of carbazochrome and DHPT were 0.5 mM and 40 mM, respectively.

Table VII. Effect of NaPAS on the Absorption of Carbazochrome

pH	k_0	k	k_{AB}	Fc
6.0	0.069 ± 0.027	0.121 ± 0.015	0.16	0.58
	$p<0.05$			

k_0, k, k_{AB}: hr^{-1}
Initial concns. of carbazochrome and NaPAS were 0.5 mM and 100 mM, respectively.

It must be considered the possibility that these changes of the apparent absorption rate may be due to the other factors such as damage to the gastrointestinal epithelium, a membrane blocking, toxicity of high concentrations of complexing drugs, and/or a pH shift during the perfusion experiment rather than to complex formation. Nicotinamide, HET, or NaPAS as shown in the previous report,[4]) 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 experimental period. Also, it may be possible that caffeine causes such effects to the small intestine in the experiment. To investigate these possibilities, the absorption rate was compared at low concentration (0.5 mM) with that at high concentration (20 mM) at pH 4.7. The residual ratio of caffeine after perfusion for one hour at low and high concentration was 61.5% (average) and 69.5% (average), respectively. There was statistically no difference between them. Therefore, it seems that caffeine

has not caused such effects to the small intestine within the limits of these experiments. The changes of the apparent absorption rates cannot be ascribed to a pH shift during the experiment by the following reasons. First, as the pH of the test solution of carbazochrome–NaPAS complex was at first 6.0 and it is known that this pH is almost constant during experiment,[14] and second, in the carbazochrome–HET or carbazochrome–caffeine complexing system at pH 3.0 or 4.7, all compounds employed must be almost in nonprotonated form from their ionization constants as described early though the pH of those solutions may shift to a higher pH value.[14]

Eq. (1) was used to calculate the absorption rate constant, k_{AB}, for the complex where a pronounced enhancement in the apparent absorption rate occured in this study, from the concentration of the added complexing drug and the experimental measurements of K, k_0 and k. These calculated results are shown in Table IV, V, and VII. The absorption rate constant of each complexing drug alone are listed in Table VIII. It was found that the k_{AB} values of carbazochrome–its complexing systems at each pH were higher than that of carbazochrome alone, and considerably lower than that of each complexing drug from these results.

Table VIII. Absorption Rate Constants (hr^{-1}) for HET, Caffeine, DHPT, and NaPAS

pH	HET	Caffeine	DHPT	NaPAS
3.0	0.388±0.041	0.966±0.124		
4.7	0.437±0.045	1.02±0.07		
6.0	0.419±0.052[a)]	1.10±0.09[a)]	0.108±0.031	0.266±0.011

Initial concn. was 0.5 mM. a) from Part XIII of this series[4]

The absorption characteristics of the complexes in this study were compared with the apparent absorption rates of carbazochrome, nicotinamide and the complexing drugs, and with the physico-chemical property. The complexed fractions (Fc) of nicotinamide and carbazochrome at pH 3.0 and 3.9 as shown in Table II and III were considerably lower than those Fc values at pH 6.0. There was no significant difference in the apparent absorption rates of nicotinamide and carbazochrome between alone and together the complexing drug, HET and nicotinamide, respectively, at pH 3.0 and 3.9. But, at pH 6.0, complexation resulted in a difference in the apparent absorption rate in each complex. Therefore, these facts may be correlated with an extent of molecular interaction. First, it may be necessary for the complexed fraction to be large in order to result in a difference in the apparent absorption rate of the drug when administered with its complexing drug. Consequently, it is necessary for the association constant and the concentrations of the components in the complexing system to be large. However, the result in Table VI exceptionally indicates that complexation with DHPT had no appreciable effect on the apparent absorption rate of carbazochrome, although DHPT formed a 1:1 complex with carbazochrome having the association constant and complexed fraction similar to those of carbazochrome–HET and carbazochrome–caffeine systems. Apparent absorption rate of DHPT at pH 6.0 was considerably slower than that of caffeine, HET, or NaPAS as shown in Table VIII, and similar to that of carbazochrome. Second, for this reason, it may be necessary for the difference between the absorption rate constant of the drug and that of its complexing drug to be large in order to result in a difference in the apparent rate of the drug when administered with its complexing drug. Further studies of these factors are in progress.

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14) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).