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Studies on Complexes. XIII.[1] Effect of Complex Formation on Drug Absorption from Alimentary Tract. (4)[2]

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The effect of complex formation on drug absorption from rat small intestine by recirculating perfusion method has been studied by following systems: sodium *p*-aminosalicylate (NaPAS)–sulfisoxazole, NaPAS–sulfisomidine, NaPAS–sulfamethoxypyridazine, sodium salicylate–caffeine, hydroxyethyltheophylline–nicotinamide, and its reverse combination, and the absorption rate of the latter of the each combinations was measured in the presence of the former. It was found that the absorption rate of each drug could be modified by the complex formation. Results of these studies were correlated with kinetic analysis based on the absorption rate constant in the absence and in the presence of complexing agent, and the equilibrium constant. It appears under the experimental conditions and used combinations of drugs that the complex itself may be absorbed, and that its absorption rate is slower than that of free drug. Complexation of the drug with complexing one decreased the apparent partition coefficient of the former. This suggests one possible mechanism for the absorption of complex.

Complex formation of drugs has been largely investigated as a means of improving pharmacological efficacy or the stability and solubility of the pharmaceutical preparations, and has been explored the potentialities of intentional complex formation as an approach to the modification of absorption characteristics of drugs.[4]

Evidence was presented in a previous report[1] which demonstrated that the absorption rate constant of the absorbable drug by the rat intestinal recirculating perfusion method was influenced by the incorporation of the nonabsorbable drug which complexed with the absorbable one. The rate was shown to be slowed and dependent on the concentration of the nonabsorbable drug. The effect was rationalized and effectively quantitated by hypothesizing that the complex was absorbed little or not absorbed, and that only the noncomplexed form of the absorbable drug was observably absorbed. Further the apparent partition coefficient of the absorbable drug in the presence of the nonabsorbable one was less than the former alone.

This present investigation is undertaken to provide more definitive information about the absorption of complex between both absorbable drugs according to the recirculating perfusion method and the specific object of the present study is to investigate whether the complex itself absorbed or not, when these drugs are absorbed from the rat small intestine, as illustrated in Fig. 1.

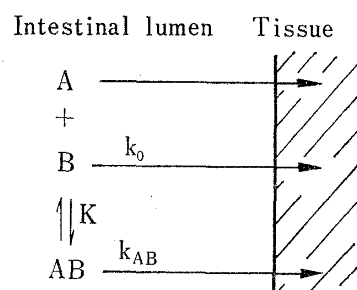

Fig. 1. Model of Absorption of Complex

k_0: absorption rate constant of free B
k_{AB}: absorption rate constant of complex AB

Considering the transfer to be a first order process the rate equations for the absorption of free drug B, complex AB, and total B are showed by equations (1), (2), and (3).

1) Part XII: I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1098 (1968). This is one of the series of Studies on Complexes (M. Samejima).
2) Presented at the 24th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1967.
3) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka.*
4) G. Levy and T. Matsuzawa, *J. Pharm. Sci.*, **54**, 1003 (1965).

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$$-\frac{d(\mathrm{B})_f}{dt}=k_o(\mathrm{B})_f \tag{1}$$

$$-\frac{d(\mathrm{AB})}{dt}=k_{\mathrm{AB}}(\mathrm{AB}) \tag{2}$$

$$-\frac{d(\mathrm{B})_t}{dt}=k(\mathrm{B})_t \tag{3}$$

where $(\mathrm{B})_f$, (AB), and $(\mathrm{B})_t$ are molar concentrations of noncomplexed B, complex AB and total B and where k_o, k_{AB}, k are the first order absorption rate constants of free B, complex AB, and total B.

From equations (1), (2), and (3), equation (4) is derived

$$k(\mathrm{B})_t=k_o(\mathrm{B})_f+k_{\mathrm{AB}}(\mathrm{AB}) \tag{4}$$

When, as indicated by the postulated model in Fig. 1, a rapid reversible 1:1 complex is formed between A and B, then it can be shown that $(\mathrm{B})_f$ is expressed as equation (5), if concentration of the total A is much larger than that of B.

$$(\mathrm{B})_f=\frac{1}{K(\mathrm{A})_t+1}(\mathrm{B})_t \tag{5}$$

Since $(\mathrm{AB})=(\mathrm{B})_t-(\mathrm{B})_f$, equation (6) from (4) and (5) is derived.

$$k_{\mathrm{AB}}=\frac{kK(\mathrm{A})_t+k-k_o}{K(\mathrm{A})_t} \tag{6}$$

Measuring the equilibrium constant for the complex and the absorption rate constant of B, in the absence of A, and in the presence of varying concentrations of A, it is possible to deduce from these measurements the absorption rate constant k_{AB}, provided that the rates of formation and dissociation of AB are both very rapid with respect to the absorption rate of B, and provided that the absorption of A is slow. That the equilibrium between A, B and AB is usually very rapid and reversible is clearly indicated by the work of Hammick and Yule,[5] who found that the usual donor, acceptor, molecular compound systems were completely and rapidly reversible even at very low temperature. The validity of the assumption with absorption of A is subject to test by the constancy of the deduced k_{AB}.

Experimental

Determination of the Rate of Absorption from the Rat——Procedure of the absorption experiments have been described previously.[1] When phenol red was used for the volume change indicator of recirculation fluid, the absorption rate was measured accordingly to the recirculating perfusion method of Kakemi, *et al.*[6,7]

Analytical Methods——Phenol red was determined spectrophotometrically at 560 mμ in aqueous solution alkalized by addition of 0.1 N sodium hydroxide. Quantitative determination of nicotinamide was carried out spectrophotometrically at 400 mμ by the cyanogen bromide method.[8] Sulfisoxazole was determined spectrophotometrically at 550 mμ after removing *p*-aminosalicylic acid. One milliliter of sample was placed in a glass-stoppered test tube, and 1 ml of 1 N hydrochloric acid and 5 ml of isoamyl acetate were added. After shaking vigorously for 10 min and centrifuging, the aqueous phase was diazotized following regular manners.[1] Sulfisomidine and sulfamethoxypyridazine were determined after removing *p*-aminosalicylic acid by ion exchange resin. Five milliliters of sample were passed through Amberlite IR-4B (Cl-type) to remove *p*-aminosalicylic acid. After the solution had passed below the upper level of the resin, the column was washed with distilled water to a volume of 50 ml. This solution was diazotized following

5) D.L. Hammick and R.B.M. Yule, *J. Chem. Soc.*, **1940**, 1539.
6) K. Kakemi, T. Arita, and S. Ohashi, *Yakugaku Zasshi*, **82**, 384 (1962).
7) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).
8) I. Utsumi and M. Samejima, *Yakugaku Kenkyu*, **29**, 980 (1957).

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regular manners.[1] *p*–Aminosalicylic acid was determined by the method of Kakemi, *et al.*[9] Hydroxyethyltheophylline was determined by the similar method with caffeine previously reported.[1] All additives did not interfere with these analytical methods under the conditions used. All optical densities were measured using Hitachi Perkin–Elmer 139 UV–VIS Spectrophotometer.

Determination of Equilibrium Constant——The solubility method described by Higuchi, *et al.*[10] was used. Excess quantities of sulfonamides, caffeine, or hydroxyethyltheophylline were placed in glass–stoppered test tube together with isotonic pH 6.0 solution of sodium *p*–aminosalicylate, sodium salicylate, or nictotinamide in various concentrations, and the tubes were agitated in a constant temperature bath (30°) until equilibrium was attained. Aliquot portions of the supernatant were removed and analyzed as described above. As the solubility of the sulfonamides, caffeine, or hydroxyethyltheophylline increased proportionally with solubilizer concentration and the slope was less than unity,[11] it would be interpreted that a 1:1 complex was formed.

Determination of the Apparent Partition Coefficient——Procedures of the determination of the partition coefficient have been described previously.[1]

Results and Discussion

The model drugs used in this study were shown in Table I. Absorption rate of drug B was determined by perfusion method in the absence and in the presence of much larger concentration of drug A. These drugs were absorbed from intestinal tract mainly by passive diffusion, and a straight line obtained in Figs. 2 and 3 showed that the absorption of sodium

Table I. Model Drugs Used and Equilibrium Constant (K) of Complex

A	B	$K^{a)}$
Na *p*–aminosalicylate (NaPAS)	sulfisoxazole	74.7
NaPAS	sulfisomidine	33.1
NaPAS	sulfamethoxypyridazine	14.2
Na salicylate	caffeine	24.0
Hydroxyethyltheophylline	nicotinamide	11.6
Nicotinamide	hydroxyethyltheophylline	11.6

a) pH 6.0, 30°

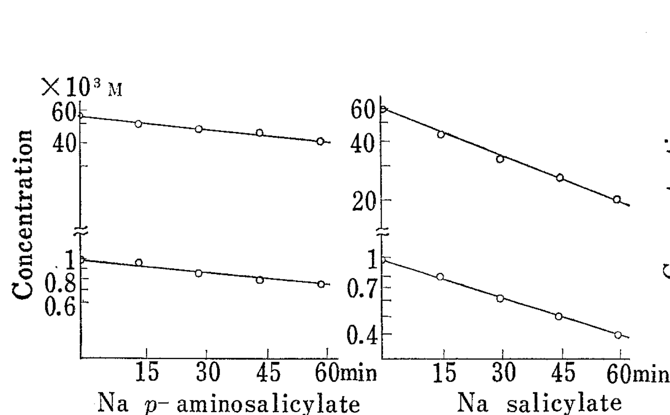

Fig. 2. Logarithmic Plots of Na *p*–Aminosalicylate and Na Salicylate in Recirculating Solution and Effect of Initial Concentration on the Absorption Rate, pH 6.0

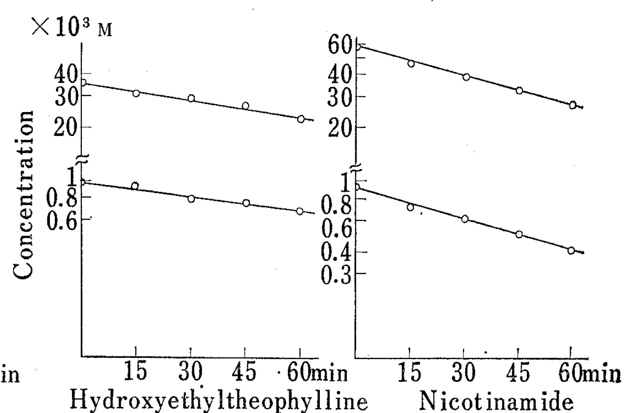

Fig. 3. Logarithmic Plots of Hydroxyethyltheophylline and Nicotinamide in Recirculating Solution and Effect of Initial Concentration on the Absorption Rate, pH 6.0

9) K. Kakemi, T. Arita, and H. Yamashina, *Arch. Pract. Pharm.*, 21, 97 (1961).
10) T. Higuchi and J.L. Lach, *J. Am. Pharm. Assoc.*, Sci. Ed., 43, 525 (1954).
11) T. Higuchi and K.A. Connors, "Advances in Analytical Chemistry and Instrumentation," Vol. 4, C.N. Reilley, ed., Interscience Publishers, New York, N.Y., 1965, p. 117.

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p-aminosalicylate, sodium salicylate, hydroxyethyltheophylline and nicotinamide from the rat small intestine using phenol red was a first order process, and absoption of sulfonamides was absorbed according to a first order process as reported already.[7] Therefore, absorption rate was calculated from the amount of the drug added and that remaining in the perfusion fluid after 1 hour assuming absorption to be a first order process and phenol red was not used by the reason of a previous report.[1]

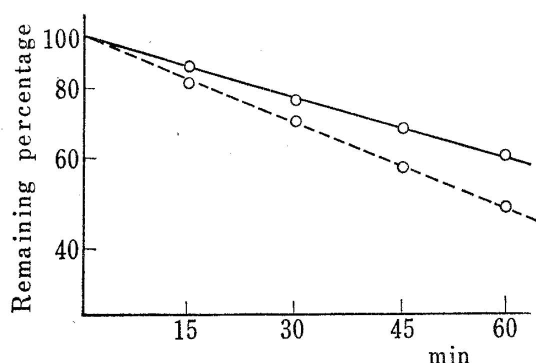

Fig. 4. Logarithmic Plots of Nicotinamide Remaining in Recirculating Solution, pH 6.0

---○--- nicotinamide 0.5 mM
——○—— nicotinamide 0.5 mM + hydroxyethyltheophylline 10mM

Previous report[12] of a series on the effect of complex formation on drug absorption has shown that the absorption rate of drugs was modified by the complex formation. Further, it was found the absorption of nicotinamide in the presence of hydroxyethyltheophylline was slower than that of nicotinamide alone using phenol red (Fig. 4).

It is necessary to consider possible effects on the absorbing membranes such as damage to the gastrointestinal epithelium, a possible membrane blocking, and/or toxicity of high concentration of drug A. To investigate these possibilities, the absorption rate of A was compared at low concentration (0.5 mM) with that at high concentration (40 or 60 mM) of A. At a different concentration the straight lines with the same slope were obtained in Figs. 2 and 3. These agreement of the slopes indicates that absorption of A was first order process, and suggests, but does not prove, that damage, membrane blocking, or toxicity did not occur. It may be concluded therefore that change in nicotinamide absorption in the presence of hydroxyethyltheophylline (Fig. 4) are due solely (or at least predominantly) to complex formation.

The theoretical rate (k_{AB}) of absorption of complex was calculated by the equation (6). But these calculations had to be based on assumptions which, unfortunately, were rather speculative. The values of K are obtained *in vitro*, and the possible presence of (other than 1:1 complexes in the intestine makes it impossible to obtain a definitive conclusion with respect to the absorption of complex AB. Equation (6) applies in the strict sence only to systems where the complexing agent (drug A in this case) is not absorbed, but the equation can be used satisfactorily in the present case since drug A was used in high concentration.

The following information was used to solve absorption rate of sulfisoxazole in the presence of sodium p-aminosalicylate: $k_0=0.796$, $k=0.595$, $(A)_t=60$ mM (from data in Table II), and $K=74.7$ (from data in Table I). From these data k_{AB} was 0.55. Sodium p-aminosalicylate was absorbed 35% (average) in the sulfisoxazole 0.5 mM solution after perfusion for

Table II. Effect of Complex Formation on Sulfisoxazole Absorption from Rat Small Intestine

Compn. of solution × 10^3M				
Sulfisoxazole	0.5	0.5	0.5	0.5
NaPAS		20	40	60
k (hr^{-1})	0.796±0.095(4)	0.664±0.041(3)	0.622±0.020(3)	0.595±0.060(3)
k_{AB}		0.58	0.56	0.55

experiment No. in parentheses

12) M. Samejima, I. Sugimoto, and I. Utsumi, *Yakugaku Zasshi*, **88**, 618 (1968).

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Table III. Effect of Complex Formation on Sulfisomidine Absorption from Rat Small Intestine

Compn. of solution × 10^3M				
Sulfisomidine	0.5	0.5	0.5	0.5
NaPAS		20	40	60
k (hr^{-1})	0.444±0.025(4)	0.398±0.022(3)	0.363±0.040(3)	0.339±0.047(4)
k_{AB}		0.33	0.30	0.29

experiment No. in parentheses

Table IV. Effect of Complex Formation on Sulfamethoxypyridazine Absorption from Rat Small Intestine

Compn. of solution × 10^3M				
Sulfamethoxypyridazine	0.5	0.5	0.5	0.5
NaPAS		20	40	60
k (hr^{-1})	0.914±0.020(4)	0.766±0.041(4)	0.686±0.028(3)	0.608±0.043(4)
k_{AB}		0.25	0.28	0.25

experiment No. in parentheses

Table V. Effect of Complex Formation on Caffeine Absorption from Rat Small Intestine

Compn. of Solution × 10^3M				
Caffeine	0.5	0.5	0.5	0.5
Na Salicylate		20	40	60
k (hr^{-1})	1.10±0.09(3)	1.02±0.04(3)	0.973±0.040(3)	0.957±0.067(4)
k_{AB}		0.85	0.84	0.86

experiment No. in parentheses

Table VI. Effect of Complex Formation on Nicotinamide Absorption from Rat Small Intestine

Compn. of Solution × 10^3M			
Nicotinamide	0.5	0.5	0.5
Hydroxyethyltheophylline		20	40
k (hr^{-1})	1.14±0.01(3)	0.973±0.032(3)	0.865±0.039(3)
k_{AB}		0.25	0.27

experiment No. in parentheses

Table VII. Effect of Complex Formation on Hydroxyethyltheophylline Absorption from Rat Small Instestine

Compn. of Solution × 10^3M				
Hydroxyethyltheophylline	0.5	0.5	0.5	0.5
Nicotinamide		20	40	60
k (hr^{-1})	0.419±0.052(5)	0.395±0.025(4)	0.377±0.028(3)	0.348±0.021(4)
k_{AB}		0.29	0.29	0.25

experiment No. in parentheses

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1 hour. Going to the other extreme by assuming *p*-aminosalicylate concentration to be 35% lower than the beginning ($(A)_t$ is 39 mM), k_{AB} was 0.53. There is no significant difference between k_{AB}. So initial concentration was used as $((A)_t$. As shown in Table II, the experimentally obtained k_{AB} was same even when initial concentration of sodium *p*-aminosalicylate was varied. It appears that (a) the equation (6) can be used satisfactorily for estimating purposes, (b) from Table II, sulfisoxazole–sodium *p*-aminosalicylate complex may be absorbed, and (c) the absorption rate constant (k_{AB}) obtained from equation (6) is smaller than absorption rate constnat (k_o) of sulfisoxazole.

Results of other complexes were shown in Tables III, IV, V, VI, and VII. Similar tendency to sulfisoxazole–sodium *p*-aminosalicylate was observed in other combinations of drugs. Interestingly, the observed k_{AB} from the absorption rate of nicotinamide was coincide with that from hydroxyethyltheophylline, as shown in Tables VI and VII.

Complexation of-drug B with A decreased the apparent partition coefficient of the former (Table VIII). This suggests one possible mechanism for the decreased absorption of drug B in the presence of A as found in the present study.

Table VIII. Effect of Complex Formation on Apparent Partition Coefficient of Sulfisoxazole, Sulfisomidine, Sulfamethoxypyridazine, Caffeine, Nicotinamide, and Hydroxyethyltheophylline

Composition of solution	Organic phase	Partition coefficient
0.5 mM sulfisoxazole	chloroform	0.333
0.5 mM sulfisoxazole, 40 mM NaPAS	chloroform	0.021
0.5 mM sulfisoxazole, 60 mM NaPAS	chloroform	0.001
0.5 mM sulfisomidine	chloroform	0.230
0.5 mM sulfisomidine, 40 mM NaPAS	chloroform	0.117
0.5 mM sulfisomidine, 60 mM NaPAS	chloroform	0.089
0.5 mM sulfamethoxypyridazine	chloroform	2.55
0.5 mM sulfamethoxypyridazine, 40 mM NaPAS	chloroform	2.20
0.5 mM sulfamethoxypyridazine, 60 mM NaPAS	chloroform	2.02
0.5 mM caffeine	benzene	0.875
0.5 mM caffeine, 40 mM Na salicylate	benzene	0.521
0.5 mM caffeine, 60 mM Na salicylate	benzene	0.367
0.5 mM nicotinamide	benzene	0.068
0.5 mM nicotinamide, 20 mM HET	benzene	0.055
0.5 mM nicotinamide, 40 mM HET	benzene	0.019
0.5 mM HET	benzene	0.060
0.5 mM HET, 20 mM nicotinamide	benzene	0.042
0.5 mM HET, 40 mM nicotinamide	benzene	0.017

HET: hydroxyethyltheophylline pH 6.0, 30°

It is thought that the conclusions are as follows under the experimental conditions and the used combinations of drugs: (a) that the complex itself may be absorbed, and (b) that its absorption rate is slower than that of free drug itself.

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