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Studies on Complexes. XII.[1] Effect of Complex Formation on Drug Absorption from Alimentary Tract. (3)[2]

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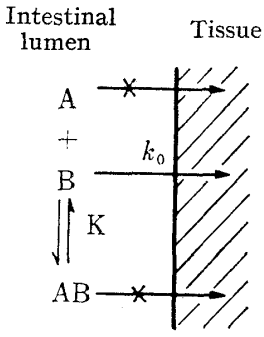
Effect of 1:1 complex formation with a nonabsorbable drug (A) and an absorbable one (B) on the absorption of B from rat small intestine according to the recirculating perfusion method *in situ* was described. The combination of drugs used as the model compounds was riboflavin phosphate (A)—sulpyrin (B), riboflavin phosphate (A)—caffeine (B), riboflavin phsophate (A)—4-aminoantipyrine (B), aminopropylon (A)—sulfamethizole (B), and aminopropylon (A)—sulfisomidine (B). The absorption rate constant of B was influenced by the incorporation of A and the rate was shown to be slowed and dependent on the concentration of A. The effect was rationalized and effectively quantitated by hypothesizing that the complex AB was absorbed little or not absorbed, and that only the noncomplexed form of the absorbable drug was observably absorbed. Complexation of B with A decreased the apparent partition coefficient of B. It is thought that this suggests one possible mechanism for the absorption of complex.

Complex formation of drugs has proved the useful way as a means of increasing the stability and solubility of drugs. As drugs may interact reversibly to form complexes with substances, such as gastric contents and mucosa occurring in the body, with other drugs that are administered simultaneously, and with pharmacologically inert compounds of pharmaceutical dosage forms, it is anticipated that unintended complex formation may occur between them. The complex will usually differ from the free drug with respect to its ability to penetrate biological membranes. Such differences are due to difference in physicochemical properties between the free drug and the complex.



A : nonabsorbable drug
B : absorbable drug
AB : complex
K : equilibrium constant
k_0 : absorption rate constant of free B

Fig. 1. Model of Absorption of Complex

It is of interest from the pharmaceutical point of view to study the effect of complex formation on the drug absorption to warrant the pharmacological efficacy of the drug itself.

Previous report[4] of a series of the effect of complex formation on drug absorption from the alimentary tract has shown that the absorption rate of drugs was modified by the formation of complex, using the rat intestinal recirculating perfusion method of Kakemi, *et al.*,[5,6] and that the increase or decrease of the absorption rate in the presence of complexing drug was illustrated by the pH–partition hypothesis. Further, the results[1] of the intestinal perfusion method *in situ* coincided with the results following oral administration in rabbits.

This report deals with a study of effect of complexation with an absorbable drug B and a nonabsorbable drug A on the absorption of the former from the small intestine of the rat as

1) Part XI: I. Sugimoto, M. Samejima, *Yakugaku Zasshi*, **88**, 698 (1968). This is one of the series of Studies on Complexes (M. Samejima).
2) Presented at the 22nd Annual Meeting of Pharmaceutical Society of Japan, Toyama, April 1966.
3) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka.*
4) M. Samejima, I. Sugimoto, and I. Utsumi, *Yakugaku Zasshi*, **88**, 618 (1968).
5) K. Kakemi, T. Arita, and S. Ohashi, *Yakugaku Zasshi*, **82**, 348 (1962).
6) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).

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illustrated in Fig. 1, according to the recirculating perfusion method and the specific object of the present study is to investigate whether the complex AB is absorbed or not.

Considering the transport to be a first order process the rate equation of B in the absence of A is

$$-\frac{d(B)_t}{dt}=k_0(B)_t \qquad (1)$$

where $(B)_t$ is molar concentration of B, k_0 is the first order absorption rate constant of the free drug B in the intestinal lumen.

If it is assumed that the complex AB is not absorbed in the presence of A in Fig. 1, the absorption rate of the drug B depends on concentration of the free drug, as equation shows:

$$-\frac{d(B)_t}{dt}=k_0(B)_f \qquad (2)$$

where $(B)_f$ is molar concentration of free B. 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 $(B)_f$ is expressed as equation (3), if concentration of the total A is much larger than that of B.

$$(B)_f=\frac{1}{K(A)_t+1}(B)_t \qquad (3)$$

Where $(A)_t$ is molar concentration of total A and K is the equilibrium constant for the complex.

Inserting equation (3) into (2), equation (4) is derived.

$$-\frac{d(B)_t}{dt}=k_0\frac{1}{K(A)_t+1}(B)_t \qquad (4)$$

And then equation (5) from (4) is obtained,

$$k=k_0\frac{1}{K(A)_t+1} \qquad (5)$$

where k is the apparent first order rate constant evaluated for the system containing the non-absorbable drug A, or more conveniently,

$$\frac{k_0}{k}=K(A)_t+1 \qquad (6)$$

Equation (6) predicts a linear relationship between the ratio k_0/k and the molarity of A, and its slope is K. Thus this equation serves as a means to check the validity of the assumption made.

Experimental

Material——Aminopropylon (4-Dimethylaminopropionylaminoantipyrine), commercial product, was used without further purification. Other drugs were of J.P. VII grade.

Spectrum Measurements——Visible absorption spectra were measured by a Shimadzu automatic recording spectrophotometer (Model SV-50A). The sample solutions were prepared with pH 6.1 isotonic phosphate buffer.[7]

Determination of the Rate of Absorption from the Rat——According to the recirculating perfusion method of Kakemi, *et al.*,[5,6] the decrease of amount of the drug remaining in the recirculation fluid was regarded as the amount absorbed.

7) Nippon Kagakukai; "Jikken Kagaku Khoza," **24**, Maruzen, Tokyo, 1958, p. 224.

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Phenol red which has been used for the volume change indicator of recirculation fluid was not used for fear of interacting the indicator with drugs in the test solution. Accordingly, the recirculation fluid was collected completely by washing with saline after recirculation.

Wister strain male rats weighting 180 to 220 g were fasten for about 24 hr prior to the experiments, but were allowed free access to water. The animals were anesthetized by the intraperitoneal injection of sodium pentobarbital in 5 mg per 100 g. The small intestine was exposed by a midline abdominal incision and cannulated at the duodenal and ileal ends with polyethylene cannulae. These cannulae were joined to a perfusion pump. This intestine was first cleared with saline by perfusion maintained at 37°, and then 20 ml of the test solution was perfused from duodenum to ileum at a rate of 3 ml per min. After one hour, the recirculation fluid was collected completely in 100 ml measuring flask by washing with saline.

Each test solution which contained 0.5 mM of drug B was prepared by dissolving in the pH 6.0 isotonic phosphate buffer solution.[7] But 5 mM formaldehyde sodium bisulfite was added to sulpyrin test solution, because sulpyrin aqueous solution was not stable at room temperature.[4,8] The experiments for each solution were repeated three times or more.

Analytical Methods——Riboflavin phosphate was determined fluorimetrically by the method of Yagi.[9] Aminopropylon was determined by the method of Ohata[10] with the following modifications to permit use of smaller volumes. One milliliter of approximately diluted recirculation solution, 3 ml of pH 7.4 phosphate buffer, and 8 ml of chloroform were placed in a 20 ml centrifuge tube, which was shaken vigorously for 10 min and centrifuged. Five milliliters of the chloroform phase were then transferred to another centrifuge tube, and 6 ml of 0.1N HCl was added. After vigorous shaking for 10 min and centrifuging, optical density of aqueous layer was measured at 260 mμ. Sulpyrin and 4-aminoantipyrine were determined by the method previously reported.[4] Caffeine was determined by UV absorption method. One milliliter of approximately diluted recirculation solution, 5 ml of pH 7.4 phosphate buffer, and 6 ml of chloroform were placed in a 20 ml centrifuge tube which was shaken vigorously for 20 min and centrifuged. Optical density of organic phase was measured at 273 mμ. Sulfamethizole and sulfisomidine were diazotized following regular manner, coupled with 2-diethylaminoethyl-1-naphthylamine and these optical densities were determined at 550 mμ. All additives did not interfere with these analytical methods under the concentration used. Hitachi Perkin-Elmer 139 UV-VIS Spectrophotometer was used for all of the determinations.

Determination of Rat Body Temperature——This determination was carried out by a continuous temperature recorder with thermistor probe inserted into the abdomen of rat under the conditions similar to those of the absorption rate experiments except that, instead of test solution, pH 6.0 isotonic phosphate buffer was prefused. Toa Electrolytic Polyrecorder Model EPR-2TC was used as the recorder.

Determination of the Equilibrium Constant——The spectrophotometric method described by Benesi and Hildebrand[11] was used to determine the equilibrium constant of riboflavin phosphate-sulpyrin, caffeine, or 4-aminoantipyrine at 30° and pH 6.1. The equilibrium constant measurement for aminopropylon-sulfamethizole or -sulfisomidine complex was carried out by the solubility method described by Higuchi, *et al.*,[12] at 30° and pH 6.0. The buffer (pH 6.0) used in this solubility experiment contained KH_2PO_4 58.0 g and $Na_2HPO_4 \cdot 12H_2O$ 25.6 g per liter of solution (approximately four times of isotonic buffered solution). The pH of aminopropylon solution of this buffer was almost constant in the solubility experiments until 0.1 M aminopropylon.

Determination of the Apparent Partition Coefficient——Apparent partition coefficients were determined using the pH 6.0 isotonic phosphate buffer saturated with the organic solvent and the organic solvent saturated with the pH 6.0 isotonic phosphate buffer as the aqueous and organic phase, respectively. Drugs were dissolved in 0.5 mM in aqueous phase. Each 5 ml of the aqueous solution and organic solvent were placed in a 20 ml glass ampule. After vigorous shaking of this ampule for 24 hr at 30°, the drug in the aqueous phase was determined and the partition coefficients were calculated by the equation (7).[6]

$$\text{Partition coefficient} = \frac{\text{Initial concn. of aqueous layer} - \text{Equild. concn. of aqueous layer}}{\text{Equild. concn. of aqueous layer}} \quad (7)$$

Results and Discussion

As the absorption of riboflavin phosphate and aminopropylon was negligible under the conditions of the recirculating perfusion method of rat small intestine for one hour at pH

8) M. Samejima, I. Sugimoto, and I. Utsumi; *Arch. Pract. Pharm.*, **26**, 23 (1966).
9) K. Yagi and T. Arakawa, *Vitamins* (Kyoto), **6**, 523 (1953).
10) K. Ohata; *Nippon Yakurigaku Zasshi*, **54**, 124 (1958).
11) H.A. Benesi and J.H. Hildebrand; *J. Am. Chem. Soc.*, **71**, 2703 (1949).
12) T. Higuchi and J.L. Lach, *J. Am. Pharm. Assoc.*, Sci. Ed., **43**, 525 (1954).

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6.0 *in situ* as shown in Table I, riboflavin phosphate and aminopropylon were used as the model compounds of the nonabsorbable drug A. These data from present experiments *in situ* must be interpreted in the light of the conditions, such as anesthetization[13,14] and intestinal contents, under which they were performed. The apparent partition coefficient of aminopropylon was the smallest in the pyrazolon derivatives used,[4] so it was assumed that its poor absorbability was due to the small partition coefficient. As the absorbable drug B, sulpyrin, caffeine, and 4-aminoantipyrine in the case of riboflavin phosphate, and sulfamethizole and sulfisomidine in the case of aminopropylon were chosen for the following reasons.

Table I. Absorption Rate of Riboflavin Phosphate and Aminopropylon

	Initial concentration (μg/ml)	% Absorbed in one hour
Riboflavin phosphate	10	−0.2, −2.5
	20	2.0
	50	2.8
Aminopropylon	302	2.2, 0.1
	3024	0.5

pH 6.0

The addition of caffeine to solutions of riboflavin phosphate at pH 6.1 resulted in the instantaneous generation of a faint red color and Benesi-Hildebrand plots were linear (Fig. 2), so it was found that caffeine and riboflavin phosphate reversibly interacted to form a 1:1 complex. Similar straight lines were observed between sulpyrin and riboflavin phosphate, or 4-aminoantipyrine and the vitamine. As the solubility of sulfamethizole or sulfisomidine increased proportionally with aminopropylon concentration and the slope was less than unity,[15] it would be interpreted that a 1:1 complex was formed between sulfamethizole and aminopropylon, or sulfisomidine and aminopropylon. It was found that sulpyrin, 4-aminoantipyrine, and caffeine were absorbed according to a first order process using the rat intestinal recirculating perfusion method to which phenol red was used[6] (absorption rate constant; sulpyrin: 0.16 hr^{-1}, 4-aminoantipyrine: 0.66 hr^{-1}, caffeine: 0.94 hr^{-1}). Sulfamethizole and sulfisomidine were also absorbed according to a first order process as reported already.[6] So absorption rate constant was calculated from the amount of the drug before perfusion and that remaining in the perfusion fluid after 1 hour.

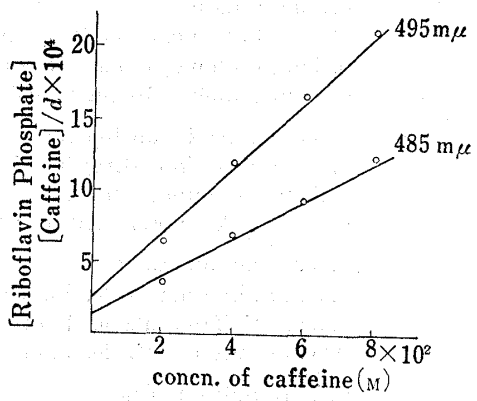

Fig. 2. Benesi-Hildebrand Plots for the Interaction of Caffeine and Riboflavin Phosphate at pH 6.1

cell: 1.0 cm d: absorbance

Continuous temperature recording in the abdomen of rats under conditions similar to those of actual absorption experiments indicated an average temperature of 32° initially, which fallen to 29° toward the end of one hour (Fig. 3). Consequently, all physicochemical measurements were made at 30°. This value compares favorably with that reported by Levy and Reuning[16] for the average intragastric temperature of rats, namely 33°. A fall of rats temperature is to be expected under the anesthetized experiments.

13) R.P. Spencer and N. Zamcheck, *Gastroenterology*, **40**, 794 (1961).
14) S. Kojima, H. Ichibagase, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **14**, 965 (1966).
15) T. Higuchi, K.A. Connors, "Advances in Analytical Chemistry and Instrumentation," Vol.4, ed. by C.N. Reilley, Interscience Publishers, New York, N.Y., 1965, p. 117.
16) G. Levy and R.H. Reuning, *J. Pharm. Sci.*, **53**, 1471 (1964).

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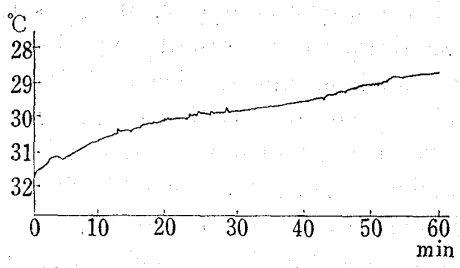

Fig. 3. Variation of Rat Body Temperature

Absorption rate constant of the drug B was shown in Table II and the ratios of the absorption rate constant of the drug B in the absence and presence of the drug A were plotted against the concentration of A, and Figs. 4 and 5 were obtained.

It is seen that, at least, under the conditions of present experiments, a linear relationship existed. In each case, the slope (equilibrium constant, K_{obs}) was determined by the method of least squares.

Table II. Effect of Complex Formation on Absorption of Sulpyrin, Caffeine, 4-Aminoantipyrine, Sulfamethizole, and Sulfisomidine

Composition of solution	Animals No.	Absorption rate constant (hr^{-1})
0.5 mM Sulpyrin	4	0.180(0.021)[a]
0.5 mM Sulpyrin, 5 mM Riboflavin phosphate	3	0.159(0.009)
0.5 mM Sulpyrin, 7.5 mM Riboflavin phosphate	3	0.151(0.003)
0.5 mM Sulpyrin, 10 mM Riboflavin phosphate	3	0.143(0.006)
0.5 mM Caffeine	3	0.996(0.056)
0.5 mM Caffeine, 5 mM Riboflavin phosphate	3	0.876(0.010)
0.5 mM Caffeine, 7.5 mM Riboflavin phosphate	3	0.840(0.037)
0.5 mM Caffeine, 10 mM Riboflavin phosphate	3	0.824(0.035)
0.5 mM 4-Aminoantipyrine	3	0.594(0.042)
0.5 mM 4-Aminoantipyrine, 5 mM Riboflavin phosphate	3	0.555(0.039)
0.5 mM 4-Aminoantipyrine, 7.5 mM Riboflavin phosphate	3	0.527(0.016)
0.5 mM 4-Aminoantipyrine, 10 mM Riboflavin phosphate	3	0.492(0.045)
0.5 mM Sulfamethizole	4	0.309(0.026)
0.5 mM Sulfamethizole, 5 mM Aminopropylon	3	0.256(0.025)
0.5 mM Sulfamethizole, 10 mM Aminopropylon	3	0.209(0.020)
0.5 mM Sulfamethizole, 15 mM Aminopropylon	3	0.171(0.010)
0.5 mM Sulfisomidine	3	0.287(0.046)
0.5 mM Sulfisomidine, 5 mM Aminopropylon	3	0.276(0.009)
0.5 mM Sulfisomidine, 10 mM Aminopropylon	3	0.255(0.029)
0.5 mM Sulfisomidine, 20 mM Aminopropylon	3	0.244(0.026)

a) standard deviation in parentheses

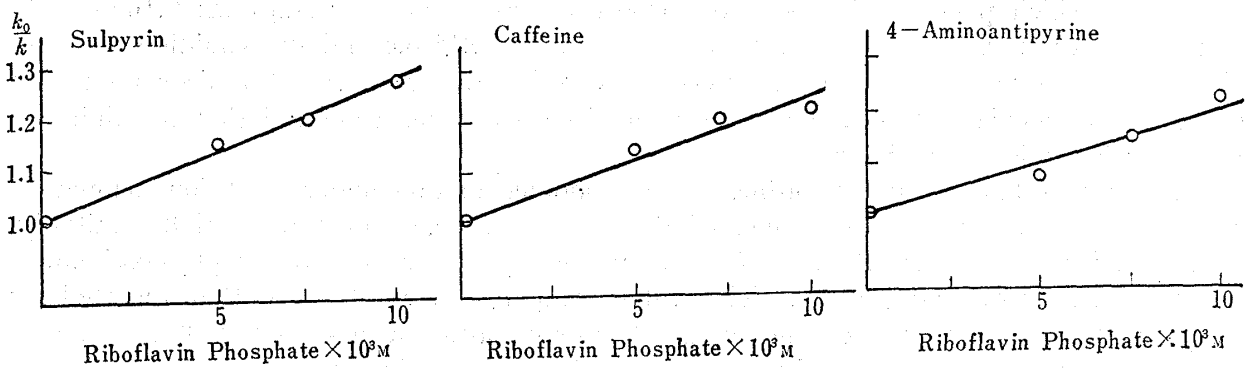

Fig. 4. Relationship between k_0/k and Riboflavion Phosphate Concentration (pH 6.0)

These observed values, K_{obs}, and those obtained from the physicochemical methods, K, are shown in Table III.

The close agreement obtained between the physicochemical methods and absorption experiments suggests one possible mechanism for the absorption of the complexing systems used

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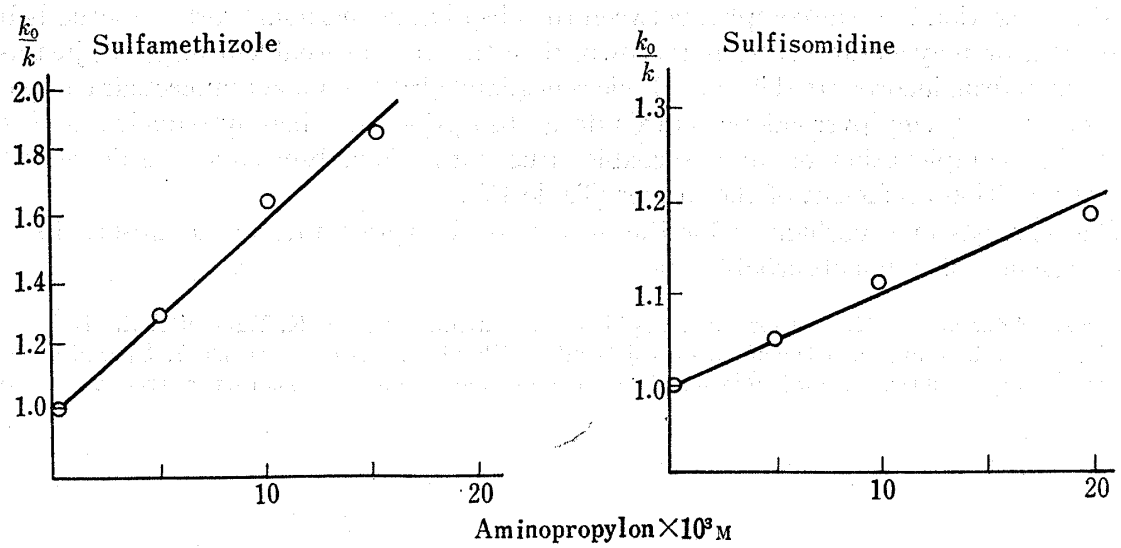

Fig. 5. Relationship between k_0/k and Aminopropylon Concentration (pH 6.0)

Table III. Equilibrium Constant (K) for the Complex

	K[a)]	K_{obs}[b)]
Riboflavin phosphate–Sulpyrin	40.0	29
Riboflavin phosphate–Caffeine	26.4	24
Riboflavin phosphate–4–Aminoantipyrine	14.8	18
Aminopropylon–Sulfamethizole	64.7	61
Aminopropylon–Sulfisomidine	9.1	10

a) obtained by spectral or solubility method at 30° *b*) observed by intestinal perfusion

Table IV. Effect of Complex Formation on Apparent Partition Coefficient of Sulpyrin, Caffeine, 4–Aminoantipyrine, Sulfamethizole, and Sulfisomidine

Composition of solution	Organic phase	Partition coefficient
0.5 mM Sulpyrin	Isoamyl acetate	0.127
0.5 mM Sulpyrin, 5 mM Riboflavin phosphate	Isoamyl acetate	0.085
0.5 mM Sulpyrin, 10 mM Riboflavin phosphate	Isoamyl acetate	0.039
0.5 mM Caffeine	Benzene	0.882
0.5 mM Caffeine, 5 mM Riboflavin phosphate	Benzene	0.711
0.5 mM Caffeine, 10 mM Riboflavin phosphate	Benzene	0.582
0.5 mM 4–Aminoantipyrine	Isoamyl acetate	0.148
0.5 mM 4–Aminoantipyrine, 5 mM Riboflavin phosphate	Isoamyl acetate	0.106
0.5 mM 4–Aminoantipyrine, 10 mM Riboflavin phosphate	Isoamyl acetate	0.077
0.5 mM Sulfamethizole	Chloroform	0.181
0.5 mM Sulfamethizole, 10 mM Aminopropylon	Chloroform	0.114
0.5 mM Sulfamethizole, 20 mM Aminopropylon	Chloroform	0.100
0.5 mM Sulfisomidine	Chloroform	0.297
0.5 mM Sulfisomidine, 10 mM Aminopropylon	Chloroform	0.277
0.5 mM Sulfisomidine, 20 mM Aminopropylon	Chloroform	0.219

pH 6.0, 30°.

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in this investigation that the complex between the absorbable and nonabsorbable drug is hardly absorbed, or may be absorbed more slowly than the free absorbable drug itself, just as the absorption of drug incorporated in the micelle is negligible,[16,17] or a macromolecular complexing agent such as polyvinylpyrrolidone retards drug absorption and thereby reducing toxicity.[18]

Further complexation of an absorbable drug with a nonabsorbable one decreased the apparent partition coefficient of the former (Table IV).

This suggests one mechanism for the decreased absorption rate of an absorbable drug in the presence of a nonabsorbable one.

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17) H. Yamada and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 1279 (1965).
18) G. Levy: in J.B. Sprowls, Jr., "Prescription Pharmacy," J.B. Lippincott Co., 1963, p. 64.

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