for 3 hr at 5°. The reaction mixture was filtered, washed repeatedly with acetonitrile and the combined filtrates were evaporated to give a crude product. Recrystallization from chloroform gave 1.04 g (57.5%) of pure N,O-dibenzoylhydroxylamine, mp 160—161°. A small amount of benzoic acid was obtained from the oxidant by dissolving in hydrochloric acid.

Oxidation of N-Benzoyl-N-Phenylhydroxylamine—To a solution of 6.39 g of N-benzoyl-N-phenylhydroxylamine in 400 ml of ether, 21.1 g of nickel peroxide was added at 10°. The mixture was stirred for about 5 hr at 10° until the unreacted material could not be detected by TLC. The reaction mixture was filtered and washed with ether and benzene, and the combined filtrates were evaporated to remove the solvent. The residue was recrystallized from benzene to give 1.73 g (29.2%) of benzanilide, mp 160—160.5°.

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Effect of Combination of Pharmaceuticals on Gastrointestinal Absorption. II.¹⁾ Combination of Caffeine with Non-absorbable Drugs

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In previous paper¹⁾ it has been shown that the gastric absorption rates on the relatively absorbable drugs were remarkably depressed by simultaneous administration with caffeine, and there was a proportional relationship between the apparent absorption rate constants for drugs and existing ratio of caffeine complexes in administered solution. The effect of caffeine on drug absorption was remarkable for more absorbable drugs but was so little for poorly absorbable drugs. And it was also conceivable that the apparent absorption rate constants for drugs approached to intermediate values between those of free drugs and caffeine with increasing of caffeine.

Accordingly, as the further extension of the previous study¹⁾ summarized above, it is followed that caffeine may act as a means of enhancing the gastric absorption for non-absorbable drugs which have the complexing tendency to caffeine. Since the gastric absorption rate usually increases with increasing lipid solubility, it is of interest to investigate the possibility of enhancing the absorption of certain drugs by formation of drug complexes which are more lipid-soluble than the drug itself.

The purpose of the present investigation is to explore the effect of caffeine on gastric absorption of non-absorbable drugs, sulfathiazole³⁾ (ST) and *p*-aminobenzoic acid³⁾ (PABA).

Experimental

Materials—Caffeine and PABA were recrystallized from distilled water. ST was recrystallized from ethanol. A diluted hydrochloric acid (0.1N) was used as the solvent for materials, and phenol red or ST was dissolved in the administered solution as the volume change indicator.

Analytical Procedures—Sulfathiazole: To 10 ml of sample solution added 0.5 ml of 0.1% sodium nitrite solution. After 3 minutes' shaking, 0.5 ml of 0.4% ammonium sulfamate was added to the sample

- 1) Part I: S. Goto, R. Takamatsu, M. Shibao, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 332 (1968).
- 2) Location: *Katakasu, Fukuoka*.
- 3) The result of our experiments using rabbit stomach showed that the gastric absorption of these compounds were almost zero at pH 1.0.

solution. And then the developed color in solution was read at 550 m μ against a reagent blank after 30 minutes' standing.

***p*-Aminobenzoic Acid:** Since ST was used as the volume change indicator, it was necessary to separate ST from sample solution for determination of PABA. Five-tenths ml of 0.2% sodium nitrite was added to 5 ml of sample solution diluted with distilled water. After 5 minutes' shaking, 2 ml of 2% β -naphthol in 20% sodium carbonate solution was added. Cooled in ice water bath and extracted with 8 ml of chloroform to separate the colored material of ST. The chloroform solution was filtered with filter paper, adequately diluted by chloroform and measured at 510 m μ for ST. A colored PABA which remained in water layer was analysed at 485 m μ .

Caffeine: It was done by the method described in the previous paper.¹⁾

Determination of Stability Constant for Complex—The modification of the method of Higuchi and co-worker^{1,4)} was applied.

Procedure for Drug Absorption from Rabbit Stomach—The procedure stated in the previous paper¹⁾ was adapted. However, ST was used as the volume change indicator for the assay of remaining amount of PABA in stomach.

Determination of Partition Coefficient—Ten ml of drug solution prepared at pH 1.0 was shaken with 10 ml of organic solvent, and the mixture was stood during about 24 hours at room temperature. The drug contents were determined in water layer and organic solvent, respectively. And the partition coefficients (P) were calculated by following equation:

$$P = \frac{\text{final concentration of drug in organic solvent}}{\text{final concentration of drug in water layer}}$$

Result and Discussion

Complex Formation between Caffeine and Drugs

Higuchi and his associate⁵⁾ have observed that caffeine had a solubilizing effect on ST and 1 g of caffeine per 100 ml of water increased the solubility of ST 50%. However the rather low stability constant, 11.3 M⁻¹ at 30°, may indicate that the interaction between them is not so strong. Since the pH value of complex solution was not measured in their experiment, the pH effect on the complex formation in solution was studied at first in our experiment. The stability constants evaluated by solubility method, assuming that a single complex species of one to one ratio is present, are tabulated in Table I. Although the stability constant

TABLE I. Effect of pH on the Apparent Stability Constants^{a)} of Caffeine-Sulfathiazole and Caffeine-*p*-Aminobenzoic Acid Complexes at 37°

pH	Apparent stability constant ^{a)}	
	Caffeine-ST	Caffeine-PABA
1.0	1.7	30
2.0	3.6	20
3.0	9.4	20
4.0	15	—
4.3	—	15

a) Calculated on an assumption that an interaction takes place 1:1 basis,
 $K = (\text{complex})/(\text{caffeine})(\text{drug})$

in the case of caffeine-ST complex was dependent with pH of the solution and proportionated to the existing degree of unionized form of ST calculated from its pK_a value ($pK_1=2.36$ at 37°),⁶⁾ the pH-independent stability constant for caffeine-PABA complex was observed in the weakly acidic region in spite of changing the presence of unionized form (an existence

4) T. Higuchi and D.A. Zuck, *J. Am. Pharm. Assoc.*, **42**, 138 (1953).

5) T. Higuchi and J.L. Lach, *J. Am. Pharm. Assoc.*, **43**, 349 (1954).

6) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).

of unionized PABA reaches maximum at pH 3.5⁷⁾). According to Higuchi and his associate,⁵⁾ the stability constant for caffeine-PABA complex was 48M^{-1} at 30° . It may be possible to detect the most electrical species of drugs related to the complex formation from the stability constant of complex *versus* pH profiles for drugs. For above-mentioned purpose, the more precise analysis than solubility method must be established for the decision of stability constant of complex.

Gastric Absorption Study

The gastric absorption of ST of the simultaneous administration with caffeine (ST: $5 \times 10^{-5}\text{M}$, caffeine: $5 \times 10^{-2}\text{M}$) was compared with single administration (ST: $5 \times 10^{-5}\text{M}$) at pH 1.0. And two procedures were used in this investigation. Namely the samples were withdrawn from rabbit stomach at regular intervals by small syringe, and then assayed for caffeine and ST. The another attempt consists in collecting perfectly all residual ST in rabbit stomach after 2.5 hours experiment. These results indicated that there was no measurable absorption at pH 1.0 during the experimental period. It is considered that the above result correspond with that the apparent stability constant for caffeine-ST complex at pH 1.0 is quite small as shown in Table I, that is, the presence of complex in solution is almost negligible. Therefore, it was concluded that when they were administered in to stomach simultaneously, they showed an original behavior in gastric absorption stage respectively.

On the other hand, the effect of complexation with caffeine on the gastric absorption of PABA was remarkable at pH 1.0. The apparent absorption rate of PABA increased gradually with increasing addition of caffeine. The typical results are shown in Table II.

TABLE II. Remaining Percentage of *p*-Aminobenzoic Acid^{a)} in Rabbit Stomach after Administration with and without Caffeine

Time (min)	With caffeine		Without caffeine
	$5 \times 10^{-2}\text{M}$	$1 \times 10^{-1}\text{M}$	
0	100	100	100
30	96.8	96.3	100
60	94.4	93.2	102
90	93.6	90.2	102
120	89.6	85.6	101
150	—	81.9	100

a) initial concentration: $5 \times 10^{-3}\text{M}$

Since the absorptive behavior of most drugs in stomach proceeds by apparent first-order kinetics, the apparent absorption rate constant, k , for PABA can be calculated from the result of experiment. The calculated rate constants are shown in Table III. From these facts,

TABLE III. Absorption Rate Constants^{a)} for *p*-Aminobenzoic Acid^{b)} in Rabbit Stomach after Administration with and without Caffeine

Concentration of caffeine, 10^2M	Absorption rate constant for PABA, 10^2 hr^{-1}
0.0	0.0
1.0	2.0
5.0	5.6
10.0	7.2

a) Each value is expressed as the mean of three rabbits. b) Initial concentration : $5 \times 10^{-3}\text{M}$

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

it is appreciated that caffeine-PABA complex is able to penetrate from stomach, and it may be due to the change of several physicochemical properties, lipid-solubility and diffusion coefficient of drug molecule.

Despite of recognition of complex formation between caffeine and aspirin, a negligible effect of caffeine on the apparent rate of aspirin absorption was observed in the previous experiment.¹⁾ The significance of this phenomena may be explained as follows. Caffeine and aspirin resemble each other in the point of gastric absorption rate and then, it may be considered that the absorption rate of complex between caffeine and aspirin maintains also same degree with each component.

Lipid-aqueous Phase Partition Coefficient

Chloroform and isoamyl acetate were selected as organic solvents. The lipid-aqueous phase partition coefficients for ST and PABA were compared with the both cases of the presence and absence of caffeine. The results of experiments are summarized in Table IV. A

TABLE IV. Lipid-Water Partition Coefficients of Sulfathiazole and *p*-Aminobenzoic Acid with or without Caffeine at pH 1.0

Initial composition of aqueous phase, %		Partition coefficient	
		Chloroform	Isoamyl acetate
ST	0.013	0.07	0.07
{ST	0.013	0.07	0.07
{Caffeine	1.05		
PABA	0.068	0.09	0.13
{PABA	0.068	0.24	0.33
{Caffeine	1.05		
{PABA	0.068	0.26	0.36
{Caffeine	2.1		

constant partition coefficient was obtained for ST, but the partition coefficient of PABA increased with the presence of caffeine, thereby showing that the lipid soluble complex was formed and resulting that PABA in the presence of caffeine was more rapidly absorbed across gastric membrane than the free drug.

Fig. 1. Relationship between Apparent Absorption Rate Constant (k , hr^{-1}) and Ratio of Free to Total Drug Concentration in Administered Solution at 0 Hour

---●--- caffeine-salicylic acid
 ---▲--- caffeine-aspirin
 ---■--- caffeine-PABA

} These data were shown in previous paper.¹⁾

Conclusion

The potentiality of complex formation as a means of enhancing the gastric absorption of drug is demonstrated by caffeine-PABA complex (Fig. 1). And it may be explained that caffeine does not have the effect on the rate of absorption of ST on a view point of negligible complex formation of ST with caffeine. Since aspirin has a same degree with caffeine in the point of gastric absorption rate, it will not reflect the effect of complexation between them. It is concluded that the gastric absorption rate constants of their complexes may be obtained as approximately intermediate values between those of caffeine and drugs. If the complexation of caffeine with drug which differ from caffeine remarkably in the gastric absorption rate is recognized in rabbit stomach, the apparent absorption rate of drug will be affected by co-existing caffeine significantly.