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Effect of Combination of Pharmaceuticals on Gastrointestinal Absorption. I. Combination of Caffeine with a Few Absorbable Drugs

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The effect of combination of caffeine with a few absorbable drugs (salicylic acid, dehydroacetic acid, aspirin, and ethyl *p*-hydroxybenzoate) on gastric absorption by rabbit was investigated. It is well-known that these drugs associate with caffeine to form soluble complexes. The experimental results at pH 1.3 showed that there was a proportional relationship between the apparent absorption rate constants for drugs and the existing ratio of complex in administered solution, and the effect of caffeine on drugs absorption was remarkable for more absorbable drug (*eg.* dehydroacetic acid) but was so little for poorly absorbable drug (*eg.* aspirin). On the other hand, it bacame clear that the absorption rate of salicylate ion was not affected with caffeine. This fact may be correlated with the different affinity between salicylate ion and caffeine as compared with that of undissociated salicylic acid.

The pharmaceutical preparations are composed of multiple components in many cases, therefore it is quite natural that the interaction between drugs have been especially noticed from various angles.

The extensive physicochemical studies on complexation of drugs have received considerable attention because of the pharmaceutical and biological importance of molecular complex formation, and it is well-known that the utilities of complexation in pharmaceutical field are potential for increasing the solubility and stability of drugs.

In recent years, it is appreciated that the simultaneous oral administration of drugs occur the unexpected complex formation between drugs in gastrointestinal tract. The several physicochemical properties of complexes of drugs can differ from those of the individual free drugs; that is, molecular size, electrical charge, oil–water partition coefficient and diffusion coefficient of molecule. Therefore, it is naturally considered that these different properties of complexes can have an appreciable effect on the rate of diffusion of the drugs across biologic membranes and the apparent first-order absorption rate constants of drugs from gastrointestinal tract can be different from that of single administration. Much has been already reported concerning the effect of drug complexation on gastrointestinal absorption, but the most of reports have been only described the apparent phenomena. Therefore, it is desirable to investigate and arrange its mechanisms with unified consideration.

On such a viewpoint, Levy and Reuning,[2] for example, have succeeded in combining the complexation effect on salicylic acid absorption with the kinetic analysis based on the stability constant of salicylic acid–caffeine complex. But they have taken no assay of caffeine in administered solutions in whole experimental time and also no investigation on combination of caffeine with another drugs except salicylic acid.

In our experiment, caffeine was also chosen because it forms easily the soluble complexes with many drugs and it is frequently used for the solubilizing agent in pharmaceutical pre-

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2) G. Levy and R.H. Reuning, *J. Pharm. Sci.*, **53**, 1471 (1964).

parations and moreover it has many chances to be mixed with another pharmaceuticals from the pharmacological point of view.

In the present investigation, it was made obvious that there is a proportional relationship between the apparent drug absorption rate constant and the existent ratio of complex in solution. And it will demonstrate the certainty of complexation effect on absorption of few drugs which are rapidly absrobed from rabbit stomach.

Experimental

Materials——Caffeine and salicylic acid were recrystallized from distilled water. Dehydroacetic acid, aspirin and etheyl *p*-hydroxybenzoate were recrystallized from ethanol. A diluted hydrochloric acid (0.1N) used as the solvent for materials and phenol red was dissolved in the above solution as the volume change indicator.

Experimental Procedure for Drug Absorption from Rabbit Stomach——Male rabbits weighing 2.0—2.5 kg fasted for about 24 hr prior to the operation. The rabbits were anesthetized with suitable amounts of barbiturate and thiobarbiturate solutions and were maintained under anesthesia during whole experimental time. The abdominal region was incised along the midline, and the stomach was exposed. The pyloric valve was also incised slightly and a small glass cannula was inserted through the pylorus into the lumen of the stomach. The incision portion of pylorus and the upper of small intestine were the tightly ligated. But the esohagen was intact because the solution did not flow backward to mouth under such a condition. The inside of rabbit stomach was washed and cleaned with 100ml distilled water and 50 ml 0.1N hydrochloric acid or isotonic buffer solution which are all previously warmed to 37°, through a glass cannula and a syringe with a needle. The greater portion of washing was withdrawn and 100 ml of drug solution, previously warmed to 37°, was injected from the syringe by inserting the needle. After completion of the procedure, the incision was closed with clips. And aliquots were withdrawn at regular intervals by another small syringe, and was then assayed for unabsorbed caffeine and drugs. Moreover, the blood pressure and respiratory rate were recorded for purpose of watching a physical change of rabbit, and if they were unusual, the all procedures were stopped immediately.

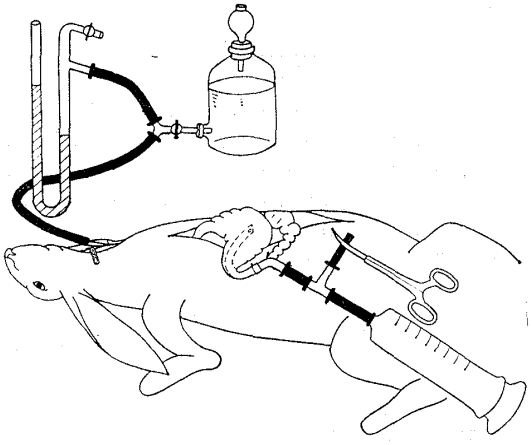

Fig. 1. Experimental Procedure for Drug Absorption from Rabbit Stomach

Determination of Salicylic Acid——For the determination of salicylic acid in rabbit stomach, a modification of the method of Brodie[3] was used. One milliliter sample solution was acidified with 0.5 ml concentrated hydrochloric acid, and extracted with 30 ml of ethylenedichloride for one hour using a mechanical shaker. The ethylene dichloride layer was separated by centrifuging. Twenty milliliters of layer was transferred to another separator and extracted for 30 minutes with 10 ml of aqueous solution which contained ferric nitrate (1%) and nitric acid (0.07N). The aqueous layer was separated and assayed by a Hitachi photoelectric spectrophotometer of the EPU 2A type.

Determination of Caffeine——Ten milliliters distilled water was added to a sample solution. The diluted sample solution was acidified with 0.5 ml concentrated hydrochloric acid and heated on a water bath. Two milliliters phosphomoribdic acid (20%) was added to the above solution. After few minutes heating, a yellow precipitate was collected on glass filter and washed with 5 ml portions 10% hydrochloric acid. Twenty milliliters acetone was used for dissolution of the precipitate. The acetone solution was filtered with filter paper and adequately diluted by acetone and measured at 440 mμ by the spectrophotometric method.

Determination of Aspirin——A sample solution (1ml) was boiled in alkaline medium. The hydrolyzed sample was acidified with 0.5 ml concentrated hydrochloric acid and the assay for salicylic acid was adapted.

Determination of Dehydroacetic Acid——A sample solution was acidified by diluted hydrochloric acid, and then was measured at 310 mμ.

Determination of Ethyl *p*-Hydroxybenzoate——An assay was carried out spectrophotometrically at 300 mμ in alkaline medium. Sodium carbonate solution (1%) was used to convert the ethyl *p*-hydroxy-

3) B.B. Brodie, S. Udenfriend, and A.F. Coburn, *J. Pharmacol. Exptl. Therap.*, **80**, 114 (1944).

benzoate to the sodium salt of its enolic form. Thus its absorption peak shifted to the right of the caffeine absorption curve.

Determination of the Stability Constants for Caffeine Complex——1) Solubility method: It was done by the modification of the method of Higuchi and co-workers.[4] Excess quantities of drugs were placed in glass stoppered tubes together with varying but accurately weighed amounts of caffeine. A quantity of 10 ml of 0.1N hydrochloric acid was added to each tube. The tubes were placed in a mechanical shaker in a constant temperature water bath and were shaken for suitable hours. Aliquot portions were diluted by 0.1N hydrochloric acid or aqueous sodium carbonate (1%) and then assayed by the spectrophotometric method.

2) Kinetic method: The kinetic method was adapted for aspirin–caffeine and dehydroacetic acid–caffeine complexes. In the case of aspirin–caffeine complex, varying amounts of caffeine (0, 5×10^{-2}, 8×10^{-2}, and 1×10^{-1}M), accurately weighed, were added to an aspirin solution (5×10^{-3}M), and the mixture was heated to reaction temperature (37°, 45°, and 60°). The aliquots were pipetted out at the regular time intervals and diluted with 0.1N hydrochloric acid. The diluted solutions were measured at 298.5 mμ which is the isosbestic point for absorbance curve of salicylic acid. The concentration of salicylic acid which is equal to the amount of degraded aspirin was estimated from the obtained absorbance. Under the experimental condition in which caffeine is largely in excess of aspirin, the stability constant for the aspirin–caffeine complex was calculated from the following equation.

$$K=\frac{(k-k_{obs})}{(k_{obs}-k')\ (\text{Caffeine})} \qquad (1)$$

where K is the stability constant of the complex, (Caffeine) is the molar concentration of caffeine, k_{obs} is the degradation rate constant of aspirin in the presence of caffeine, k is the degradation rate constant of aspirin, and k' is the degradation rate constant of the complex. When the values for k_{obs} in varying amounts of caffeine are adapted to the equation (1) and then the obtained equations are paired in all possible combinations, the stability constants can be calculated from the above simultaneous equations each pair. The mean values of stability constants calculated are listed in Table I.

Result and Discussion

The Experiment at pH 1.3——The general tendency of caffeine to form soluble and insoluble complexes with a number of pharmaceuticals has been observed by many investigators. Higuchi and his associates[4] have proposed the mechanisms of the complexation of caffeine and have succeeded in a quantitative expression for complex formation by the solubility method. The solubility method is quite simple and was reported to be more applicable method for detecting small complexing tendencies. But it is considered that the application of more than one experimental technique[5] is advisable in complexation studies for determination of the stoichiometric ratios and the stability constants. Therefore, the kinetic method was also employed together with the solubility method as an analytical procedure for the observation of complexation of caffeine with the some investigated drugs in our study. It became evident that caffeine associated with these drugs to form soluble complexes. And there is a linear relationship between the molar concentration of caffeine and the molar solubility of drug in the solubility diagram. The stability constants evaluated by both solubility method and kinetic method, assuming that a single complex of one to one ratio is present, were completely identical in the cases of dehydroacetic acid–caffeine and aspirin–caffeine complexes. This result may show that only one to one complex between these substances is present. Since the ionization constants for drugs are 1.1×10^{-3} (19°) for salicylic acid, 5.3×10^{-6} (25°) for dehydroacetic acid, 3.3×10^{-4} (25°) for aspirin, and 3.4×10^{-9} (25°) for ethyl *p*-hydroxybenzoate, respectively, a considerable concentration of undissociated drugs will be present in 0.1N hydrochloric acid. On the other hand, the ionization constant for caffeine is 1.6×10^{-1} (25°). It is possible that both undissociated and protonated caffiene can associate with the drugs employed, therefore, the stability

4) T. Higuchi and D.A. Zuck, *J. Pharm. Sci.*, **55**, 138 (1953).
5) K.A. Connors and J.A. Mollica, JR., *J. Pharm. Sci.*, **55**, 772 (1966).

constants listed in Table I must be considered as an apparent stability constants. The apparent stability constants at 37° were used to estimate the molar concentrations of free drugs in the presence of caffeine.

Table I. Summary of Apparent Stability Constants for Caffeine–drug Complexes

Complex	Temp. (°C)	Stability constant (M^{-1})
salicylic acid–caffeine	30	44
	33	40
	37	36
dehydroacetic acid–caffeine	30	9.8
	37	7.7
	45	6.4
	60	4.3
aspirin–caffeine	30	18.0
	37	17.8
	45	16.7
	60	13.9
ethyl *p*–hydroxybenzoate–caffeine	30	45.3
	37	41.0
	45	38.4

$$K=(D\text{–}C)/(D)_f\,(C)_f \qquad (2)$$

$$=(D\text{–}C)/[(D)_T-(D\text{–}C)]\;[(C)_T-(D\text{–}C)] \qquad (3)$$

where (D–C) is the concentration of the drug–caffeine complex, $(D)_T$ and $(C)_T$ are the total concentration of drug and caffeine, respectively, and $(D)_f$ and $(C)_f$ are the concentration of

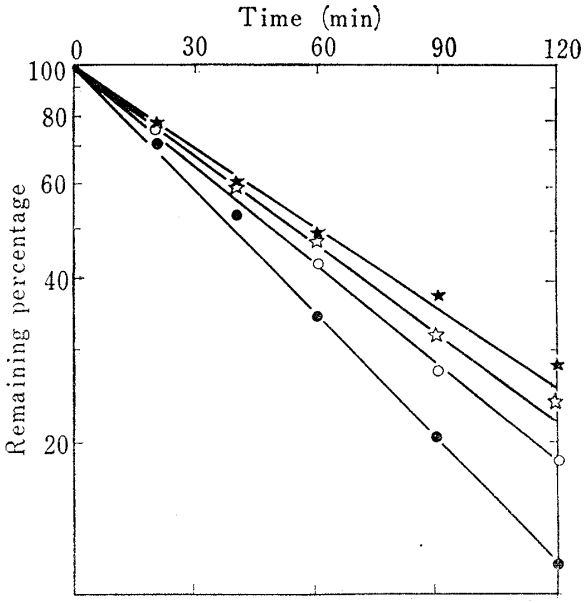

Fig. 2. First Order Plots for Dehydroacetic Acid remaining in Rabbit Stomach

—●— dehydroacetic acid 5×10^{-3}M
—○— {caffeine 2.2×10^{-2}M, dehydroacetic acid 4×10^{-3}M}
—☆— {caffeine 4.7×10^{-2}M, dehydroacetic acid 5.7×10^{-3}M}
—★— {caffeine 9.3×10^{-2}M, dehydrocaetic acid 9.1×10^{-3}M}

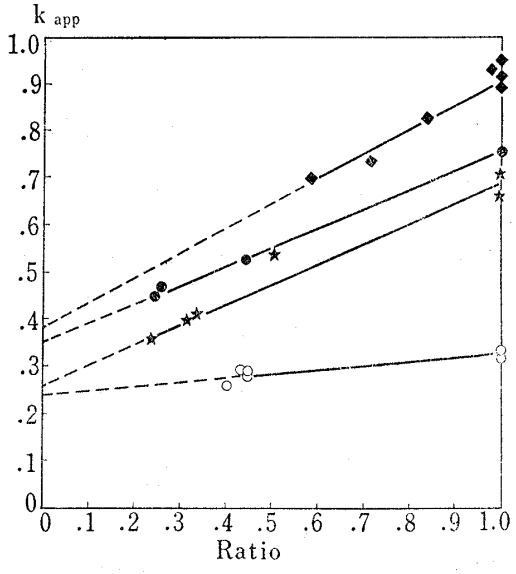

Fig. 3. Relationship between Apparent Absorption Rate Constant (hr^{-1}) and Ratio of Free to Total Drug Concentration in Administered Solution at 0 Hour

dehydroacetic acid–caffeine ···◆—
salicylic acid–caffeine ···●—
ethyl *p*-hydroxybenzoate–caffeine ···★—
aspirin–caffeine ···○—

free drug and free caffeine, respectively. The $(D)_T$ and $(C)_T$ were obtained by the spectrophotometric method, and (D–C) could be calculated from eq. 3.

The ratio of $(D)_f$ to $(D)_T$ was employed as an interaction magnitude of drug with caffeine in the administered solution.

In general, the gastric absorption of drugs in the absence of caffeine can be expressed by the first-order rate equation. When the drug were simultaneously administered with caffeine in stomach, it became evident that the drug absorption rate from stomach followed apparently first-order kinetic as the result of experiment and was decreased in proportion to an increased amount of caffeine added in solution. The combination of dehydroacetic acid and caffeine was adapted as the typical result in Fig. 2.

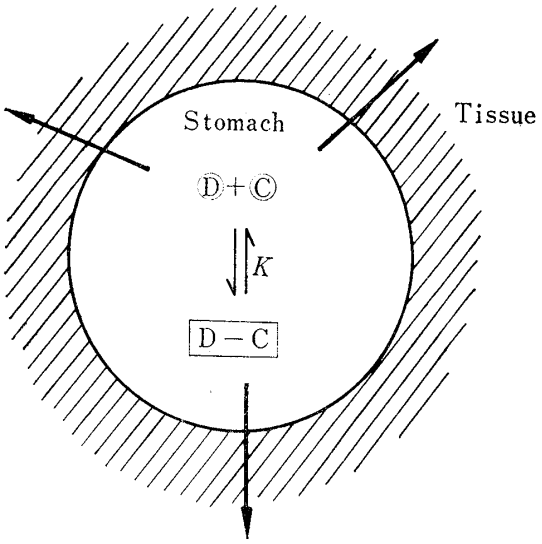

Fig. 4. Model of Drug Absorption from Rabbit Stomach

And the ratio of free drug concentration to total drug concentration was plotted against the apparent first-order absorption rate constant. There is a straight line relationship between them (Fig. 3).

Assuming that there are three components, that is, free drug, free caffeine and complex in the solution, that the equilibria between them are always formed, and that the absorption rates of drug, caffeine and complex are constant at all experimental time and in any combination between drug and caffeine, the model of gastric absorption of drug is expressed as in Fig. 4.

And the absorption rate of drug can be written:

$$-\frac{d(D)_T}{dt}=-\frac{d(D)_f}{dt}-\frac{d(D\text{–}C)}{dt} \tag{4}$$

$$=k_f(D)_f+k_c(D\text{–}C) \tag{5}$$

$$=k_{app}[(D)_f+(D\text{–}C)] \tag{6}$$

$$=k_{app}(D)_T \tag{7}$$

where k_f and k_c are the first-order absorption rate constants of drug and complex, respectively. k_{app} is the apparent first-order absorption rate constant of drug in the solution with caffeine.

From eq. (5) and eq. (7), the apparent absorption rate constant is expressed as:

$$k_{app}=k_f\frac{(D)_f}{(D)_T}+k_c\frac{(D\text{–}C)}{(D)_T} \tag{8}$$

$$=(k_f-k_c)\frac{(D)_f}{(D)_T}+k_c \tag{9}$$

The ratio of free drug concentration to total drug concentration was calculated from eq. (3) at regular time intervals. And the results of calculation showed that it was almost constant during 1.5—2 hours absorption period. The calculated values in the case of salicylic acid–caffeine combination were shown in Table II.

According to eq. (9), the relationship between k_{app} and $(D)_f/(D)_T$ will be linear, and a slope and an intersept of the line represent (k_f-k_c) and k_c, respectively. The values of k_f and k_c on each drug are summarized in Table III.

Table II. Change of Ratio of Free to Total Salicylic Acid Concentration in Rabbit Stomach during Experimental Period

Composition of solution (10^2M)		Ratio			
Salicylic acid	Caffeine	0	0.5	1.0	1.5 hr
0.724	4.0	0.45	0.46	0.48	0.48
0.724	8.0	0.27	0.28	0.29	0.29
0.326	8.0	0.27	0.28	0.33	0.33

Table III. Summary of Absorption Rate Constants for Drugs and Complexes

Drug	Absorption rate constant (hr^{-1})	Complex	Absorption rate constant (hr^{-1})
caffeine	0.20	salicylic acid–caffeine	0.34
salicylic acid	0.75	aspirin–caffeine	0.23
aspirin	0.32	dehydroacitic acid–caffeine	0.40
dehydroacetic acid	0.98	ethyl *p*–hydroxybenzoate–caffeine	0.26
ethyl *p*–hydroxybenzoate	0.69		

It might be tentatively concluded that the effect of caffeine on drug absorption is remarkable for more absorbable drug (*eg.* dehydroacetic acid) but is so little for poorly absorbable drug (*eg.* aspirin) from the slope of line in Fig. 3.

The Experiment at pH 5——The gastric absorption of salicylic acid was investigated at pH 5 (isotonic citric acid–sodium diphosphate buffer was used). The absorption rate of drug in the presence of caffeine at pH 5 coincided completely with that of single administration in the absence of caffeine. This result is different from that at pH 1. And the experimental result is shown in Fig. 5.

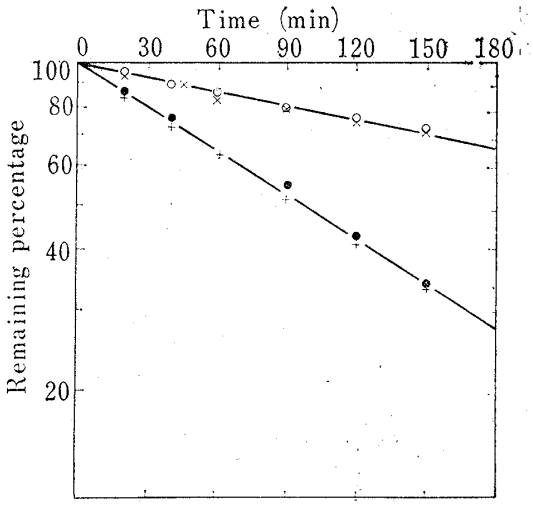

Fig. 5. First Order Plots for Drugs remaining in Rabbit Stomach (pH 5.0)

—●— {salicylic acid (7.24×10^{-3}M) caffeine (8.0×10^{-3}M) simultaneous administration
—○— {
—†— salicylic acid (7.24×10^{-3}M) single administration
—×— caffeine (8.0×10^{-2}M) single administration

Higuchi and Zuck[4] have proposed that there was some difference between the affinity of caffeine for salicylate ion and that for salicylic acid. Sekiguchi[6] has also reported that the solubilizing ability of substituted benzoic acid on caffeine was assumed to be not due to formation of complex of constant composition but due to the formation of more complicated association owing to hydrogen bond between them, with the water molecule acting as the hydrogen-bond donor, from the break-through curve using the ion exchange resin for solution of caffeine. A considerable concentration of salicylic acid is a dissociated form in pH 5, and the different affinity will be present between salicylate ion and caffeine as compared with the case of undissociated salicylic acid. And it was assumed that the salicylate ion in the presence of caffeine may act almost in the same manner as that of a single administration in the absence of caffeine.

6) K. Sekiguchi, *Yakugaku Zasshi*, **81**, 664 (1961).