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Bioavailability of Sugar-Coated Tablets of Thiamine Disulfide in Humans. I. Effect of Gastric Acidity and *in Vivo–in Vitro* Correlation

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The dissolution of sugar-coated tablets of thiamine disulfide largely depended on the lag time, which was greatly affected by the pH of the medium, and was accelerated by mechanical destructive force. The disintegration time–pH profiles were very similar to the dissolution rate–pH profiles determined by using a disintegration test device. Markedly slow dissolution and disintegration of one of the tablets at pH 7.2 was attributed to the dissolving characteristics of polyvinylacetal-diethylaminoacetate (AEA®) applied to the tablet as a coating film, and the slow dissolution of another tablet at pH 3—5 was attributed to the use of 2-methyl-5-vinylpyridine-methylacrylate-methacrylic acid copolymer (MPM®). Six products were tested for bioavailability. Statistically significant differences were found in bioavailability among the products. Human gastric acidity greatly affected the bioavailability of the tablet that disintegrated poorly at pH 7.2, and the bioavailability was significantly lower in subjects with low gastric acidity than in those with high gastric acidity. The *in vivo* parameters of high and low acidity subjects correlated well with the *in vitro* parameters determined at pH 1.2—5 and pH 5—7.2, respectively.

Keywords——thiamine disulfide; sugar-coated tablet; bioavailability; human; gastric acidity; dissolution; disintegration; MPM; AEA

Sugar-coated formulations have often caused bioinequivalence problems, even when they contain comparatively water-soluble drugs.[1-6] One of the causes seems to be the physicochemical characteristics of coating and subcoating films applied to the formulations. On the other hand, the dissolution of drugs in the gastrointestinal tract is influenced by various physiological factors, and previous studies on diazepam tablets[7] and indomethacin capsules[8] indicated that human gastric acidity affects the *in vivo* dissolution of the drugs, which suggests that the gastric acidity also affects the release rate of drugs from sugar-coated formulations. There have been, however, few studies on the *in vitro* dissolution and disintegration behavior of sugar-coated formulations and on their bioavailability in relation to gastric acidity.

The present study was undertaken to investigate the dissolution and disintegration of sugar-coated formulations in the gastric pH range, and the effects of coating film characteristics on their dissolution. In addition, the bioavailabilities in humans having high and low gastric acidity and the correlation with the *in vitro* dissolution rate and disintegration time were also investigated. As a test formulation, sugar-coated tablets of thiamine disulfide (TDS) were used, because their dissolution is considered to depend upon the dissolving characteristics of the coating or subcoating films due to the relatively high solubility of TDS in water; this should make it easy to clarify the effect of the coating films on the dissolution. As many brands of TDS sugar-coated tablet are available in Japan, these studies should provide useful

information on the coating films currently being applied to commercial sugar-coated formulations.

Experimental

Materials——Twenty brands of sugar-coated tablets containing 10 mg of TDS marketed in Japan were used for the preliminary dissolution study and six of them (A–F) were further studied in detail. The drug contents (mg) per tablet determined fluorometrically by reducing TDS with cysteine,[9] were 9.6 ± 0.3 (mean $\pm$ SD, $n=10$), 9.8 ± 0.2, 9.2 ± 0.5, 10.8 ± 0.6, 9.4 ± 0.6 and 9.5 ± 0.2 for tablets A, B, C, D, E and F, respectively. Authentic samples of polyvinylacetal-diethylaminoacetate (AEA, Sankyo Co., Ltd., Tokyo) and 2-methyl-5-vinylpyridine-methylacrylate-methacrylic acid copolymer (MPM, Tanabe Seiyaku Co., Ltd., Osaka) were kindly provided by the manufacturers.

Solubility——The solubility of TDS at pH 1.2 (HCl), 3 and 5 (0.1 M sodium acetate buffer) and 7.2 (0.1 M sodium phosphate buffer) was determined at 37 °C. The drug concentration in the equilibrated solution was spectrophotometrically determined after filtration of the solution through a 0.5 μm membrane filter.

Dissolution Rate——The dissolution test was carried out at 37 °C using media of pH 1.2 (HCl), 2.5, 3, 4, 5 and 7.2 (0.1 M sodium phosphate–1 N HCl). The dissolution rate from a test tablet was determined in 900 ml of the medium by using the rotating basket and paddle methods (JP X) at 120 rpm and in 950 ml of the medium by the oscillating basket method (30 stroke/min) in which the JP X disintegration apparatus and test conditions were employed. The amount of the drug dissolved was determined spectrophotometrically by passing the solution through a glass filter (G-3) to a flow cell and expressed as a percentage of the labelled amount. The dissolution rates from twenty brands of tablets were determined after a single dissolution run and those from the selected six tablets after three runs. The dissolution rate is shown as the time required for 50% of the drug to dissolve (T_{50}).

Disintegration Time——The disintegration time of TDS tablets was determined using six of each product according to the JP X specification with the same solvents as used for the dissolution test.

Infrared (IR) Analysis of Coating Agents——Sugar-coating films of tablets C and D were clearly separated and were placed in 10 ml of $CHCl_3$–methanol (1 : 1). After shaking for 5 min and centrifuging at 2000 rpm, 8 ml of the organic layer was taken and evaporated to dryness. The residue was dissolved in 0.5 ml of $CHCl_3$ and mixed with KBr, which was compressed to form a tablet for IR analysis.

Human Volunteers——Twelve male volunteers who participated in this study were confirmed to be healthy by clinical examinations. Their gastric acidity were estimated by using Gastrotest® (Chugai Pharmaceutical Co., Ltd.).[6] Gastrotest® employs two white tablets each containing 200 mg of caffeine-sodium benzoate, which stimulates gastric fluid secretion, and three yellow tablets each containing 50 mg of a protein-bound dye (3-phenylazo-2,6-diaminopyridine). The dye is liberated from the protein in the stomach at pH 3 or less, and is rapidly absorbed from the intestinal tract. Thus, the gastric acidity can be estimated from the amount of the dye excreted in urine after oral administration of the dye–protein complex.[10,11] All subjects were prohibited from taking beverages and food for 8 h before the gastric acidity test. They were given the white tablets, and urine samples were collected for 1.0 h after the

TABLE I. Age, Height, Weight, Gastric Acidity and Urinary Amount of Endogeneous Thiamine Excreted for 22 h in Human Subjects

No.	Age (year)	Height (cm)	Weight (kg)	Gastric acidity	Urinary[a] thiamine (μg)
1	22	179	65	high	33.0
2	30	163	55	low	62.2
3	23	170	63	high	37.0
4	50	168	68	low	41.1
5	34	168	63	high	164.3
6	37	170	55	low	29.8
7	22	166	57	high	131.3
8	23	172	56	high	41.7
9	22	175	60	high	41.5
10	51	160	54	low	49.6
11	37	165	60	high	71.4
12	32	172	60	low	41.5

a) Amounts of urinary thiamine excreted for 22 h in humans who took thiamine-deficient diets were expressed as amounts of thiamine hydrochloride (mean values of the two determinations).

administration (blank urine). Immediately thereafter, the subjects took the yellow tablets and urine samples were collected for 1.5 h (test urine). Both urine samples were diluted to 200 ml with water, and 2 ml of 25% HCl was added to 2 ml of each urine sample. The absorbances of the solutions were determined at 520 nm (cell length: 1.0 cm), and the absorbance due to urinary excretion of the dye was estimated by subtracting the absorbance of blank urine solution from that of the test solution. An absorbance value above or below 0.170 was considered to indicate high or low gastric acidity, respectively.[11] Table I shows the ages, heights, weights and gastric acidities of the subjects.

Basal Urinary Excretion of Thiamine——The effects of diet on the urinary excretion of thiamine were investigated in two subjects. In the first experiment, the subjects had meals of their choice at noon and in the evening and were allowed to ingest beverages freely. In the second experiment, they had a thiamine-deficient lunch at 1:00 p.m. and supper at 6:30 p.m. The lunch mainly consisted of 300 g of boiled rice, 250 g of boiled wheat noodles and a relish, while supper consisted of 300 g of boiled rice, 250 g of boiled buckwheat noodles and two fried prawns. The subjects were allowed to take water, coffee and green tea after 1:00 p.m., but no other food and beverages. Urine samples from the subjects were collected every 2 h from 9:00 a.m. to 11:00 p.m. and then when possible until 22 h. Each experiment was repeated twice at an interval of one week. Urine samples were stored at −15 °C until assay. Urinary excretion of endogenous thiamine by all twelve male volunteers taking a thiamine-deficient diet was also estimated for 22 h; this was carried out before and after the bioavailability test of TDS.

Dose–Bioavailability Relation——Four subjects were orally given 5, 10 or 20 mg of TDS (1 mg/ml JP X hydrochloric acid limonade) after fasting overnight together with 200 ml of water. They took 100 ml of water at 2 h after drug administration. Conditions for ingestion of thiamine-deficient diets and beverages were the same as described for the basal urinary excretion study. Urine samples were taken at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 11, 14 and 22 h after dosing and stored at −15 °C until assay. The amount of urinary thiamine excreted was determined by subtracting the mean basal amount of urinary thiamine.

Bioavailability——After an overnight fast, the twelve subjects ingested a test tablet with 200 ml of water at 9:00 a.m. and 2 h later, 100 ml of water. Drug administration was repeated every week according to a latin-square cross-over design. All subjects were also intravenously administered 1.0 mg of TDS (Fuso Yakuhin Kogyo Co., Ltd.) at 9:00 a.m. after fasting overnight; they immediately drank 200 ml of water and 2 h later 100 ml of water. Urine sampling time and other procedures were the same as described for the dose–bioavailability study. The bioavailability of each tablet was estimated from the observed maximum urinary excretion rate of thiamine (U_{max}), cumulative amount of urinary thiamine excreted in 22 h ($A_{e_{22}}$) and absorbed fraction (F), which was calculated from the equation:

$$F\ (\%) = 100 \times A_{e_{22}}\ (10\ \text{mg TDS},\ p.o.) / 10 \times A_{e_{22}}\ (1\ \text{mg TDS},\ i.v.)$$

The *in vivo* parameters were subjected to statistical analysis of variance (ANOVA)[12] and the differences among the treatments were examined by means of Tukey's multiple range test. On the other hand, four subjects (No. 4, 10, 11 and 12) orally took 10 mg of TDS dissolved in 10 ml of JP X hydrochloric acid limonade together with 200 ml of water. Urine sampling times and other procedures were the same as stated above.

Assay——Urinary thiamine was determined by a thiochrome method using BrCN after adsorption on Permutit T (E. Merck, Darmstadt) columns and eluted with 25% KCl in 0.1 N HCl, according to the method of Fujiwara and Matsui.[13,14] Urinary thiamine was expressed as the amount of thiamine hydrochloride.

Results

Dissolution

The solubility of TDS determined in the physiological pH range is shown in Table II. The solubility–pH profile indicates that the dissolution of TDS will decrease with increase of the medium pH.

Figure 1 shows the dissolution–time curves of TDS sugar-coated tablets obtained by the rotating basket method. The drug was released from the tablets after different lag times, which were greatly influenced by the medium pH. The lag time may reflect the time required for the sugar- or sub-coating films of the tablets to dissolve or rupture, indicating that the drug dissolution largely depended on the dissolving and physico-chemical characteristics of the coating films.

Figure 2 shows the T_{50} values of TDS tablets determined by the rotating and oscillating basket methods. The T_{50} of tablet A increased slightly with increase in the medium pH, which seemed to be attributable to the decrease in TDS solubility. On the other hand, the T_{50} of most of the other tablets did not increase in parallel with the medium pH. The pH–T_{50} profiles

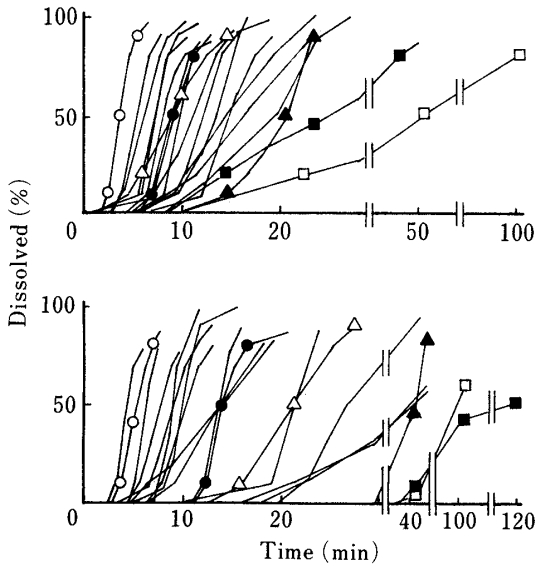

Fig. 1. Dissolution–Time Curves of Twenty Brands of TDS Sugar-Coated Tablets Using the Rotating Basket Method at pH 1.2 (Upper Figure) and 2.5 (Lower Figure)

A (○), B (●), C (△), D (▲), E (□) and F (■) represent the tablets used for further *in vitro* and *in vivo* studies.

Table II. Solubility of TDS at 37 °C

Medium (pH)	Solubility (mg/ml)
1.2	37.7
3	3.0
5	1.33
7.2	0.568

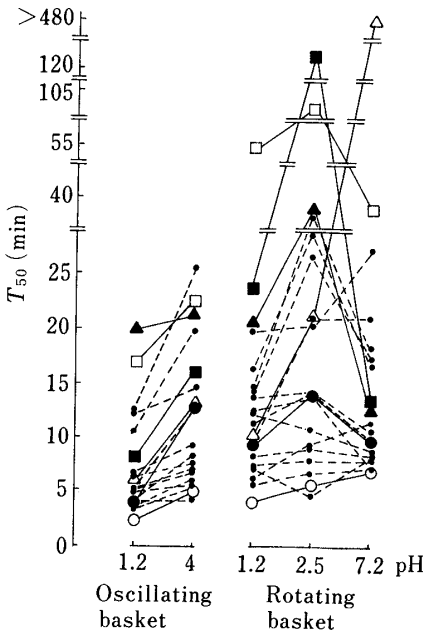

Fig. 2. Times (min) Required for 50% of the Drug to Dissolve from Twenty Brands of TDS Sugar-Coated Tablets in the Rotating and Oscillating Basket Methods in Different pH Media

The solid and dashed lines show the T_{50}–pH profiles of six tablets selected for further studies and the others, respectively.

Tablet A (○), B (●), C (△), D (▲), E (□) and F (■).

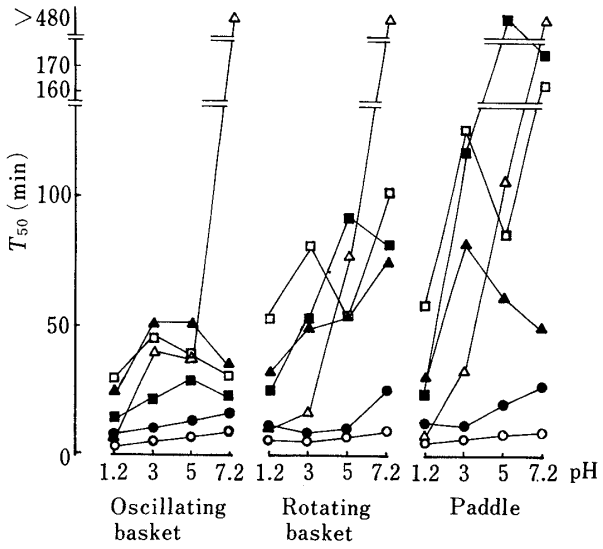

Fig. 3. T_{50}–pH Profiles of Six Sugar-Coated Tablets of TDS Determined Using the Paddle, Rotating Basket and Oscillating Basket Methods

Tablets A (○), B (●), C (△), D (▲), E (□) and F (■).

were very specific to each tablet, suggesting that the drug release was dependent upon the formulation characteristics, particularly the dissolving and rupturing properties of the sugar- or sub-coating films rather than upon TDS solubility. Based on these preliminary dissolution data, six tablets (A—F) showing different dissolution rates and dissolution characteristics were selected for further *in vitro* and *in vivo* studies. Figure 3 shows the T_{50} of the six tablets determined by the oscillating basket, rotating basket and paddle methods at pH 1.2—7.2. The

dissolutions of tablets A and B were faster than those of the other tablets and less dependent on the medium pH and methods. However, the dissolutions of the other tablets varied greatly depending on the medium pH, especially when determined by the rotating basket and paddle methods. The dissolution of tablet C was as fast as those of tablets A and B at pH 1.2 but was the slowest at pH 7.2, due to negligible disintegration over 8 h. The dissolution rates of TDS tablets, especially slow-dissolving ones (C, D, E and F), were accelerated by the oscillating basket method, which suggests that the plastic disk used in the device mechanically promoted the disintegration of the tablet, especially degradation of its coating film, as previously shown in the case of chloramphenicol.[15] The mechanical destructive force as well as medium pH seems to be important for the disintegration and dissolution of TDS tablets.

Disintegration Time

Figure 4 shows the mean disintegration times of TDS tablets A—F determined at pH 1.2—7.2 according to the JP X specification. The disintegration time–pH profiles of all tablets were very similar to the T_{50}–pH profiles determined by the oscillating basket method with the same device as in the JP X disintegration test.

Types of Coating Agents

It is of interest to investigate the nature of the coating agents applied to the tablets that showed slow and pH-dependent dissolution. The IR spectrum of the extract from the coating film of tablet C coincided with that of AEA® (2940, 2850, 1730, 1410, 1370, 1130 and 945 cm^{-1}), and the IR spectrum from tablet D coincided with that of MPM® (2910, 1720, 1600, 1490, 1440, 1160, 1030, 820 and 730 cm^{-1}), which indicates that AEA and MPM had been applied to those tablets, respectively. AEA and MPM are slightly soluble above pH 5.8[16] and in the pH range of 4—7,[17] respectively. This implies that the use of AEA and MPM for the coatings of those products was responsible for the slow dissolution of tablets C and D at pH 7.2 and pH 3—5, respectively. Specific coating agents responsible for the characteristic

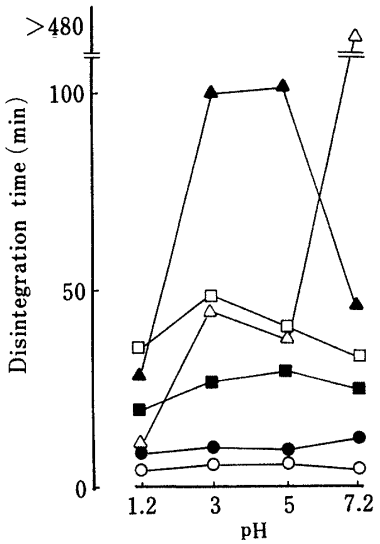

Fig. 4. Disintegration Time–pH Profiles of Six Sugar-Coated Tablets of TDS

Tablets A (○), B (●), C (△), D (▲), E (□) and F (■).

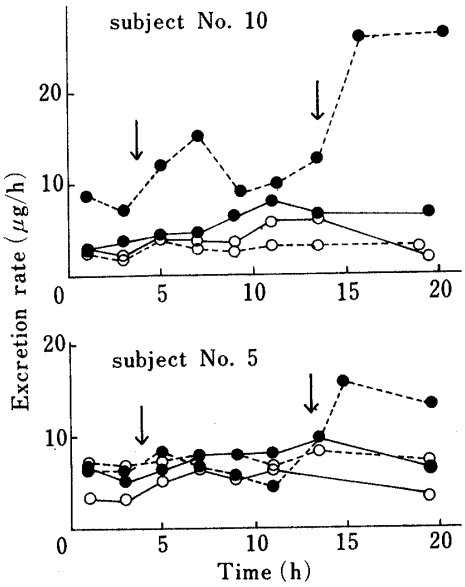

Fig. 5. Urinary Excretion Rate of Thiamine in Two Subjects Taking Meals of Their Choice (●) or Thiamine-Deficient Diets (○)

The experiments were repeated at an interval of a week. Experiment No. 1 (——) and No. 2 (------). The arrows show the time when subjects took meals of their choice. Thiamine-deficient diets were taken at 4 and 9.5 h.

disintegration and dissolution of other sugar-coated tablets which showed slow and pH-dependent dissolution could not be identified by IR analysis.

Basal Urinary Excretion of Thiamine

Figure 5 shows the effects of diet on the urinary excretion of thiamine in two subjects not administered TDS. In the subjects taking a regular diet, the urinary excretion of thiamine sometimes increased sharply just after eating, probably due to thiamine contained in the food. However, when the subjects took thiamine-deficient diets, their urinary excretion rate of thiamine was almost constant. Thus, the basal urinary excretions of thiamine in the twelve subjects were determined by using thiamine-deficient diets. Table I shows the basal thiamine excretion in urine over 22 h.

Dose–Bioavailability Relation

Gastrointestinal absorption of thiamine[18,19] and TDS derivatives[20] in humans has been estimated from the urinary excretion of thiamine, and there was a high correlation between plasma levels of thiamine and its urinary excretion after TDS administration.[21] Thus, the bioavailability of TDS can be estimated from the urinary amounts of thiamine derived from TDS. Before the bioavailability test of TDS tablets, the relations of TDS dose with $A_{e_{22}}$ and U_{max} were investigated. As shown in Fig. 6, convex relations were observed, which indicates

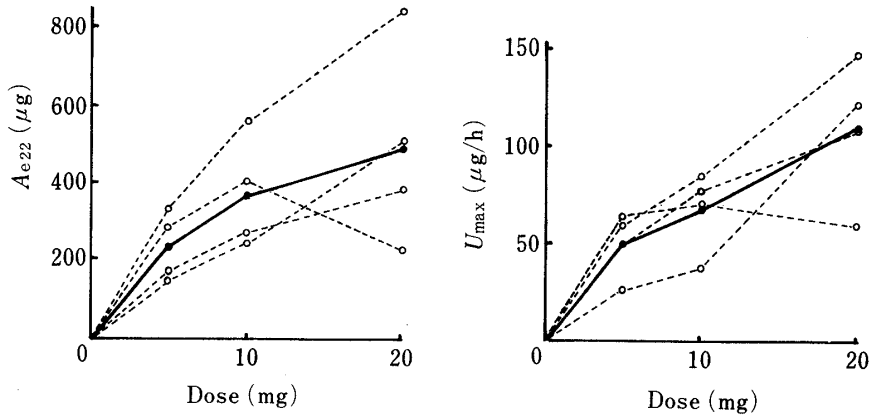

Fig. 6. Relations of TDS Dose with $A_{e_{22}}$ and U_{max}

The dotted lines show individual values in four subjects orally administered 5, 10 or 20 mg of TDS solution, and solid lines represent the mean values.

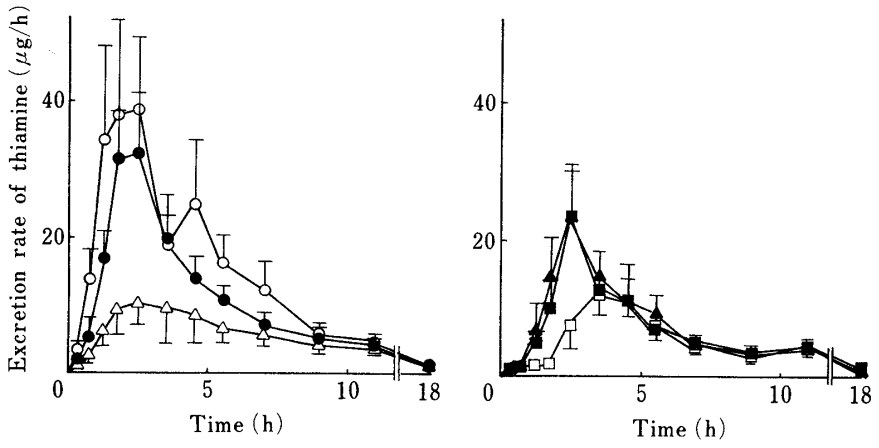

Fig. 7. Mean Urinary Excretion Rate of Thiamine after Oral Administration of Six Sugar-Coated Tablets of 10 mg of TDS to Humans ($n=12$)

Tablets A (○), B (●), C (△), D (▲), E (□) and F (■). The vertical lines show standard errors.

TABLE III. U_{max}, T_{max}, A_{e22} and Absorbed Fractions after Oral Administration of Sugar-Coated Tablets of 10 mg of TDS to Humans ($n=12$)

	Tablet						ANOVA	Tukey's test ($p<0.05$)
	A	B	C	D	E	F		
U_{max} (μg/h)	55.4[a] ±14.4	40.4 ±9.9	17.2 ±3.9	24.5 ±7.4	14.5 ±7.5	24.9 ±5.5	$p<0.01$	A>B>F>D>C>E[b]
T_{max} (h)	2.2 ±0.3	2.0 ±0.2	3.2[c] ±0.9	2.6[d] ±0.2	4.2[e] ±0.3	3.3 ±0.5		
A_{e22} (μg)	214 ±46	177 ±37	98 ±21	116 ±29	89 ±29	113 ±24	$p<0.05$	A>B>D>F>C>E
F[f] (%)	8.4 ±1.7	7.1 ±1.5	3.9 ±0.9	4.6 ±1.0	3.9 ±1.4	4.1 ±0.6	$p<0.05$	A>B>D>F>C=E

a) Mean±S.E. *b*) The formulations underlined by a common line did not differ significantly at $p<0.05$. *c*) $n=10$ (without No. 2 and 6 subjects). *d*) $n=11$ (without No. 7 subject). *e*) $n=10$ (without No. 1 and 11 subjects). *f*) Absorbed fraction.

TABLE IV. A_{e22} (μg) and F (%) After Oral Administration of Aqueous Solution and Tablet A Containing 10 mg of TDS Humans ($n=4$)

Subject No.	Solution		Tablet A	
	A_{e22}	F	A_{e22}	F
4	562	13.8	530	13.0
10	245	7.7	139	4.3
11	403	19.0	334	15.8
12	271	10.5	240	9.3
Mean	370	12.8	311	10.6
SE	73	2.4	83	2.5

The differences in A_{e22} and F between TDS solution and tablet A were statistically significant at $p<0.05$ by the paired t-test.

that gastrointestinal absorption of TDS does not increase linearly in proportion to the dose; as in the absorption of thiamine.[18]

Bioavailability

Figure 7 shows the mean urinary excretion rate–time curves of thiamine after oral administration of TDS tablets and Table III lists the *in vivo* parameters. Tablets A and B, which showed faster and less pH-dependent dissolution than the other tablets, gave higher U_{max}, A_{e22} and absorbed fractions. The difference in the absorbed fraction between the two tablets and the others was statistically significant. Significant differences were also found in both U_{max} and A_{e22} between tablets A and C, and between tablets A and E. The absorbed fractions were rather low ($F<10\%$) even for the fastest-dissolving tablet (A). Table IV shows the values of A_{e22} and absorbed fraction of TDS solution and tablet A in four subjects. The absorbed fraction of TDS solution was also low, which indicates that TDS is poorly absorbed from the gastrointestine, as previously reported.[20] However, the absorbed fraction of tablet A was approximately 80% of that of TDS solution, and the difference was statistically significant, which suggests that the drug was not completely released from the product during its passage through the absorption site.

On the other hand, the absorbed fractions after administration of tablet C to No. 2 and 6 subjects were negligible ($F<0.04\%$), which suggests the passage of the tablets in an intact state

TABLE V. U_{max}, $A_{e_{22}}$ and Absorbed Fractions after Oral Administration of Sugar-Coated Tablets of TDS to Humans Having High ($n=7$) and Low Gastric Acidity ($n=5$)

	Gastric acidity	Tablet						ANOVA	Tukey's test ($p<0.05$)
		A	B	C	D	E	F		
U_{max} (μg/h)	High	55.2[a] ±23.2	45.7 ±16.1	26.7[b] ±3.1	16.3 ±10.5	18.5 ±12.9	26.4 ±6.8	NS[c]	
	Low	54.3 ±15.6	32.9 ±7.4	3.8[b] ±1.7	29.9 ±10.7	8.8 ±2.7	22.7 ±10.6	$p<0.01$	A>B>D>F>E>C[d]
$A_{e_{22}}$ (μg)	High	209 ±57	188 ±53	148[b] ±17	91 ±34	107 ±49	116 ±30	NS	
	Low	218 ±83	160 ±56	27[b] ±12	151 ±45	64 ±19	109 ±34	$p<0.01$	A>B>D>F>E>C
F[e] (%)	High	9.0 ±2.7	8.0 ±2.5	6.1[b] ±0.7	3.8 ±1.5	4.7 ±2.3	4.5 ±0.9	NS	
	Low	7.4 ±1.7	5.7 ±1.3	0.9[b] ±0.4	5.6 ±1.4	2.9 ±0.9	3.6 ±0.9	$p<0.01$	A>B>D>F>E>C

a) Mean ± S.E. *b*) Statistically significant difference between high and low gastric acidity subjects by *t*-test ($p<0.05$). *c*) NS: not significant at $p<0.05$. *d*) The formulations underlined by a common line did not differ significantly. *e*) Absorbed fraction.

TABLE VI. Correlation Coefficients of U_{max} and $A_{e_{22}}$ in Humans Having High and Low Gastric Acidity with T_{50} and $1/T_{50}$ Determined by the Rotating Basket Method in Different pH Media

	pH	U_{max}		$A_{e_{22}}$	
		High	Low	High	Low
T_{50}	1.2	0.755	0.470	0.791	0.379
	3	0.805	0.516	0.856[a]	0.443
	5	0.758	0.725	0.731	0.739
$1/T_{50}$	1.2	0.874[a]	0.599	0.906[a]	0.482
	3	0.968[b]	0.759	0.962[b]	0.685
	5	0.947[b]	0.838[a]	0.900[a]	0.801

a) $p<0.05$. *b*) $p<0.01$.

through the gastrointestinal tract. The absorbed fraction of tablet D in No. 7 subject and tablet E in No. 1 and 11 subjects were also minimal ($F<0.10\%$). These very poor absorptions make it difficult to determine the T_{max} values accurately. Thus, the T_{max} values of these tablets shown in Table III are the mean values from the other volunteers and were not subjected to ANOVA.

Effects of Gastric Acidity

Of the twelve participants, five were estimated to have low gastric acidity and seven to have high acidity (Table I). Table V shows the mean U_{max}, $A_{e_{22}}$ and absorbed fractions after oral administration of TDS tablets. Gastric acidity had the greatest influence on the bioavailability of tablet C, which did not disintegrate at pH 7.2. The mean U_{max} and $A_{e_{22}}$ after administration of tablet C in low gastric acidity subjects were approximately 1/7 and 1/5 those in high acidity subjects, respectively. In addition, tablet C was hardly available in two subjects having low gastric acidity (No. 2 and 6), while being available in all high gastric acidity subjects. The gastric acidity, however, did not significantly affect the bioavailabilities of the other tablets.

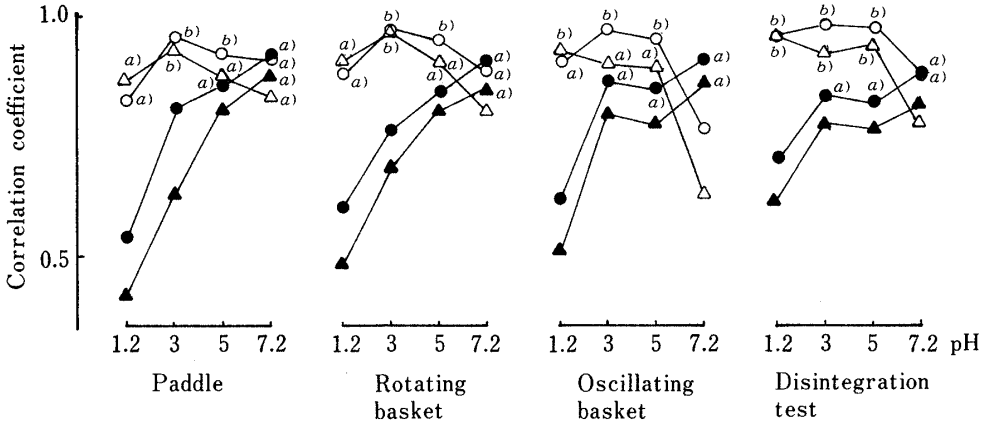

Fig. 8. Correlation Coefficients of the Reciprocals of T_{50} and Disintegration Time Determined at Various pHs with U_{max} (Circles) and $A_{e_{22}}$ (Triangles) in Subjects Having High (Open Symbols) and Low (Closed Symbols) Gastric Acidities

a) $p<0.05$. *b*) $p<0.01$.

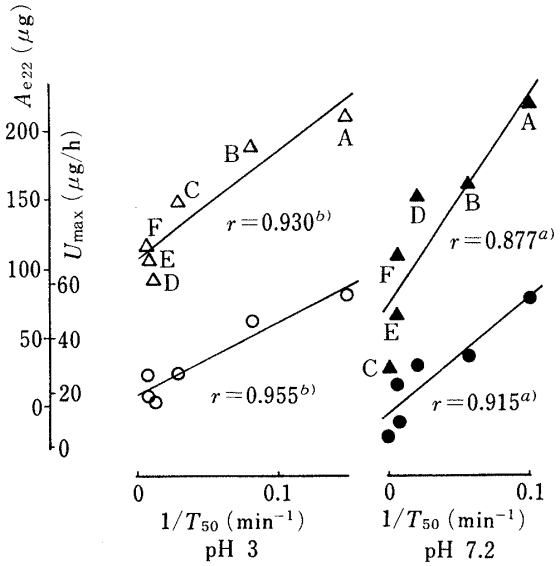

Fig. 9. Correlations of the Reciprocals of T_{50} Determined by the Paddle Method at pH 3 and 7.2 with U_{max} (Circles) and $A_{e_{22}}$ (Triangles) in High (Open Symbols) and Low (Closed Symbols) Gastric Acidity Subjects

The solid lines show the regression lines. *a*) $p<0.05$. *b*) $p<0.01$.

The *in vivo* results differed between the two acidity groups. The ranking among the products according to U_{max} and $A_{e_{22}}$ in high gastric acidity subjects was A > B > C > F > E > D, while that in the low acidity subjects was A > B > D > F > E > C. In addition, the differences of the *in vivo* parameters among the products were statistically significant in the low acidity subjects, but not in the high acidity subjects (Table V).

In Vivo–in Vitro Correlation

Table VI shows the correlation coefficients of the *in vivo* parameters with the time required for 50% of the drug to dissolve (T_{50}) and $1/T_{50}$ determined by the rotating basket method, respectively. U_{max} and $A_{e_{22}}$ correlated better with $1/T_{50}$ than with T_{50}. Other dissolution methods (paddle and oscillating basket methods) also gave similar correlation results. Thus, $1/T_{50}$ was used as an *in vitro* parameter for the correlation estimation. Figure 8 shows the *in vivo–in vitro* correlation coefficients plotted against the medium pH in the *in vitro* methods. The correlation coefficient–pH profiles were similar among the *in vitro* methods. The *in vivo* parameters of high gastric acidity subjects correlated better with $1/T_{50}$ determined at pH 1.2—5, especially at pH 3, than at pH 7.2 and those of the low acidity subjects with $1/T_{50}$ at pH 3—7.2, especially at pH 7.2, than at pH 1.2.

The reciprocal of disintegration time also showed similar correlation profiles (Fig. 8). Figure 9 shows the correlations of U_{max} and $A_{e_{22}}$ in high gastric acidity humans with $1/T_{50}$ determined by the paddle method at pH 3 and those in low gastric acidity humans with $1/T_{50}$ at pH 7.2.

Discussion

There have been few detailed studies on the dissolution and disintegration behavior of sugar-coated formulations over a wide pH range. The present *in vitro* study on sugar-coated tablets of TDS carried out using media of pH 1.2—7.2, corresponding to the range of pH variation of gastric fluid, revealed that the dissolution rates of TDS tablets were greatly affected by medium pH, especially under the mildly destructive conditions of the paddle and rotating basket methods. The dissolution greatly depended on the lag time, namely the time required for the coating film to dissolve or rupture. This shows that the coating films characteristics are very important for drug release from sugar-coated formulations. All products showed relatively rapid dissolution and disintegration at pH 1.2 but not at pH 3—7.2, indicating that the sugar-coated tablets were formulated to meet the JP X disintegration requirement using the first fluid (pH 1.2) but without consideration of their dissolution and disintegration at other pHs. The pH-dependent dissolution of TDS tablets suggested that their bioavailabilities might be affected by gastric acidity. Thus, six products having different dissolution characteristics were subjected to bioavailability test in humans having high and low gastric acidities.

The bioavailability of TDS from fast-dissolving products (A and B) was higher than that from slow-dissolving ones (C, D and E), which suggests that the absorption of TDS from the products is dissolution-limited, although the absorption efficiency of TDS is very poor, as can be seen from low F values (Tables III and IV).

Gastric acidity markedly affected the bioavailability of tablet C, which did not disintegrate at pH 7.2; the bioavailability was much poorer in low gastric acidity humans than in high gastric acidity subjects. Tablet C had AEA® (polyvinylacetal-diethylaminoacetate), which shows low solubility above pH 5.8, as a coating agent, and the results indicate that AEA is inadequate as a coating agent for sugar-coated formulations. On the other hand, in a previous study on metronidazole sugar-coated tablets,[6] slow-dissolving products at pH 5—7.2 also gave poorer bioavailability in low gastric acidity humans than in high gastric acidity subjects, like TDS tablet C. These findings show that sugar-coated formulations showing slow dissolution and/or disintegration at pH 5—7.2 may be generally inferior in bioavailability in low gastric acidity humans to the fast-dissolving formulations.

The *in vivo–in vitro* correlation of TDS tablets varied greatly depending on the medium pH of the *in vitro* methods (Fig. 8), and the correlation–pH profiles differed between high and low gastric acidity subjects. The *in vivo* parameters of low gastric acidity subjects correlated better with the reciprocals of T_{50} and disintegration time determined at pH 3—7.2, especially at pH 7.2, than at pH 1.2, while those of high gastric acidity subjects correlated better with the *in vitro* parameters determined at pH 1.2—5, especially at pH 3. The highly correlated pHs or pH ranges seem to reflect the gastric pHs of humans having low and high gastric acidities, respectively. It appears that the *in vitro* dissolution and disintegration test for sugar-coated formulations should be carried out in at least two different media of pH 3 and 7.2 in order to predict bioavailability in high and low gastric acidity humans, respectively, although the JP X disintegration test for sugar-coated tablets is carried out only in the first fluid (pH 1.2).

To date, there have been few studies on the effects of gastric acidity on drug bioavailability. However, previous studies on diazepam[7] and indomethacin[8] and this study on TDS tablets have shown that gastric acidity significantly influences bioavailability. Thus,

gastric acidity effects must be taken into consideration for the *in vitro* and *in vivo* estimation of bioavailability of orally administered drug products, and it is necessary to design formulations less affected by gastric acidity in order to decrease inter-subject variation of clinical response. In addition, if possible, it seems desirable to investigate the gastric acidity effects with consideration of the degree of gastric acidity, although in the present study we did not determine the degree of acidity, since the Gastro test® used to classify subjects into high and low gastric acidity groups can not estimate accurately the degree of gastric acidity or the gastric pH.

The following report will describe the bioavailabilities of TDS tablets in beagle dogs and the correlation with those in humans.

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