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Bioavailability of Sugar-Coated Tablets of Thiamine Disulfide in Humans. II. Correlation with Bioavailability in Beagle Dogs

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The bioavailabilities of six brands of thiamine disulfide (TDS) sugar-coated tablets were investigated in male beagle dogs and the correlations with the bioavailabilities in humans and the *in vitro* dissolution rates were investigated. The volume of water coadministered with the drug did not affect the bioavailability. The bioavailabilities of slow-dissolving products at pH 3—7.2 under the weakly destructive conditions of the paddle and rotating basket methods were relatively higher in dogs than in humans, which suggested a stronger disintegration force in dogs. Gastric fluid pH seemed to affect the *in vivo* dissolution of TDS itself. The *in vivo* parameters in dogs correlated significantly with the dissolution rate, disintegration time and *in vivo* parameters in high gastric acidity humans, with the exception of one product.

Keywords——thiamine disulfide; sugar-coated tablet; bioavailability; human; dog; dissolution; disintegration; gastric fluid pH

Beagle dogs have often been used for bioavailability studies, but the relation between bioavailabilities in humans and dogs has not been clarified well. Thus, successive studies have been carried out on the human-dog bioavailability relation, using chemically different drugs: diazepam,[1] griseofulvin,[2] flufenamic acid,[3] nalidixic acid,[4] indomethacin[5] and metronidazole.[6] These studies revealed some discrepancies between the *in vivo* results in humans and dogs, which seemed to be attributable to the faster transition of drugs through the gastrointestinal tract of dogs[1,2] and a stronger disintegration force in the digestive tract of dogs,[2,4,5] as compared with humans.

Previous studies described the bioavailabilities of sugar-coated tablets of thiamine disulfide (TDS) in humans having high and low gastric acidity and the correlation with the dissolution rate and disintegration time.[7] The present study was undertaken to investigate the bioavailabilities of those tablets in dogs and to clarify the human–dog bioavailability relation.

Experimental

Formulation——Six brands of TDS sugar-coated tablets (10 mg TDS) which had different dissolution characteristics and were employed in a previous human study[7] were used. The dissolution rates of those products were determined at 37 °C in 900 ml of media of pH 1.2 (HCl) and pH 3—7.2 (0.1 M sodium phosphate–1 N HCl) by the JP X rotating basket and paddle methods (120 rpm), and in 950 ml by the oscillating basket method (30 strokes/min) with JP X disintegration apparatus and test conditions. Table I shows the mean times required for 50% of the drug to dissolve (T_{50}) from the tablets, determined after three dissolution runs.

Gastric Fluid pH of Beagle Dogs——Unanesthetized beagle dogs were given 200 ml of water through a stomach tube, and 2—3 min later their gastric fluid was collected with a stomach tube. The pH of the gastric fluid was

TABLE I. Time (min) Required for 50% of TDS to Dissolve from Sugar-Coated Tablets

Method	pH	Tablet					
		A	B	C	D	E	F
Paddle	1.2	5.8	12.5	8.8	30.4	58.5	23.6
	3	6.7	12.3	32.5	81.6	126.0	117.0
	5	8.6	15.3	106.7	61.1	86.4	>480
	7.2	9.9	17.2	>480	49.4	163.3	176.0
Rotating basket	1.2	6.1	11.9	10.2	32.2	52.9	25.2
	3	5.4	8.4	17.0	49.2	82.3	53.1
	5	7.9	10.8	77.7	54.8	53.8	92.0
	7.2	10.1	25.0	>480	75.6	103.6	81.3
Oscillating basket	1.2	4.2	7.9	7.4	24.1	29.1	14.8
	3	6.1	10.0	40.6	51.9	44.7	20.9
	5	6.9	13.0	36.8	51.2	38.3	28.5
	7.2	9.8	21.7	>480	35.5	11.7	22.8

TABLE II. Age, Weight and pH of Gastric Fluid of Male Beagle Dogs

No.	Age (month)	Weight (kg)	Gastric fluid pH	No.	Age (month)	Weight (kg)	Gastric fluid pH
1	14	13	4.0±2.1[a]	7	13	12	4.8±2.5
2	14	13	4.4±0.9	8	14	12	5.1±1.8
3	16	12	4.4±1.0	9	14	11	3.3±0.5
4	14	12	5.5±1.6	10	14	13	5.5±1.5
5	13	15	4.2±1.9	11	16	13	3.4±1.2
6	14	13	4.4±1.3	12	14	13	4.3±2.0

a) Mean±S.D. ($n=4$).

determined with a pH meter and the mean gastric fluid pH was calculated from 4 measurements at intervals of 4—20 d during about two months. Table II shows the age, weight and mean gastric fluid pH of twelve male dogs used for the bioavailability study.

Dose–Bioavailability Relation——An aqueous solution of 10, 20 or 30 mg of TDS was orally administered to three dogs which had been fasted overnight. The TDS solution was prepared as follows: 300 mg of TDS was dissolved in the minimum amount of 1 N HCl, and diluted with 30 ml of water. After being adjusted to pH 5 with 1 N NaOH, the solution was diluted with water to a volume of 50 ml. For the dose of 30 mg, 5 ml of the dilution was used, and for 10 and 20 mg, solutions further diluted with water were administered, according to a latin-square cross-over design (3 × 3). Blood samples were taken at 0.5, 1, 2, 3, 4, 5 and 7 h after the oral administration and stored at −15 °C until assay. The dogs were kept in the fasting state until the final blood sampling time. The drug was administered at two-week intervals. Total thiamine concentration (thiamine plus its pyrophosphate) in the blood was determined by a thiochrome method after treatment with diastase[8] and expressed as the amount of thiamine hydrochloride. The blood concentration of thiamine resulting from the TDS administered was calculated by subtracting the thiamine concentration in the blood just before the drug administration.

Effect of Water Volume Coadministered——Three beagle dogs which had been fasted overnight were orally administered three tablets of tablet A with 30 or 60 ml of water according to a randomized block design. The time intervals between the experiments and other procedure were the same as described for dose–bioavailability relation study.

Bioavailability——Twelve beagle dogs which had been fasted overnight were given three tablets together with 30 ml of water; they were not given any food until the final blood sampling time. Test tablets were administered at two-week intervals according to a latin-square cross-over design. All of the dogs were also orally given 30 mg of TDS dissolved in pH 5 solution together with 30 ml of water. Blood sampling times and other procedures were the same as described for the dose–bioavailability relation study. The bioavailability was estimated from the observed maximum blood concentration (C_{max}), the areas under the blood drug concentration–time curves from zero to 7 h (AUC_7) and

zero to infinite time (AUC_∞) calculated by means of the trapezoidal rule and according to the method of Wagner,[9] respectively, and the ratio (F_a) of AUC_∞ of TDS tablets to AUC_∞ of TDS solution. The *in vivo* parameters were subjected to statistical analysis of variance (ANOVA).[10]

Results

Dose–Bioavailability

The relation of TDS dose with C_{max} and AUC_∞ are shown in Fig. 1. Although the mean C_{max} and AUC_∞ increased almost linearly in proportion to the dose, there seemed to be a slight threshold in dose–AUC_∞ and dose–C_{max}. This suggests that TDS orally administered may not completely reach the blood circulation due to first-pass elimination, although no first-pass effect on TDS has yet been reported.

Effect of Water Volume Coadministered

The effect of water volume given with the drug on the bioavailability of TDS was investigated using 30 and 60 ml of water. As shown in Fig. 2, the water volume did not significantly influence the blood level of thiamine after oral administration of tablet A.

Bioavailability

Figure 3 shows the mean blood concentration of thiamine after oral administration of

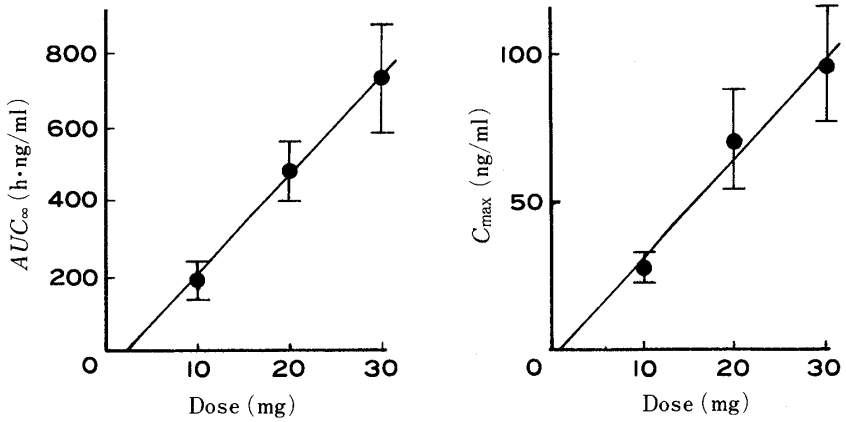


Fig. 1. Relations of TDS Dose with C_{max} and AUC_∞ after Oral Administration of TDS Solution to Dogs ($n=3$)

The vertical lines show the standard errors of mean values (●) and solid lines show the regression lines for C_{max} ($r=0.992$) and AUC_∞ ($r=0.899$).

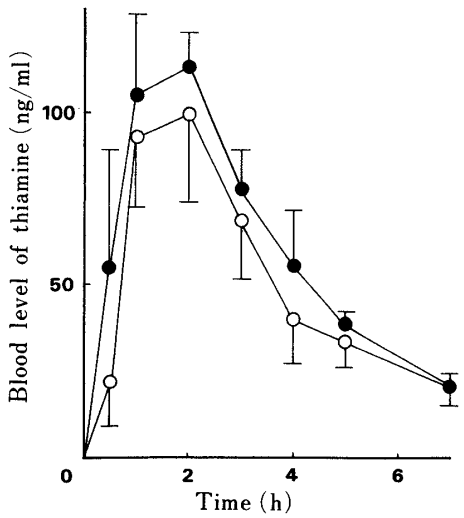


Fig. 2. Mean Blood Levels of Thiamine after Oral Administration of TDS Tablet A (30 mg) to Dogs ($n=3$) with 30 (○) and 60 ml (●) of Water

The vertical lines show standard errors.

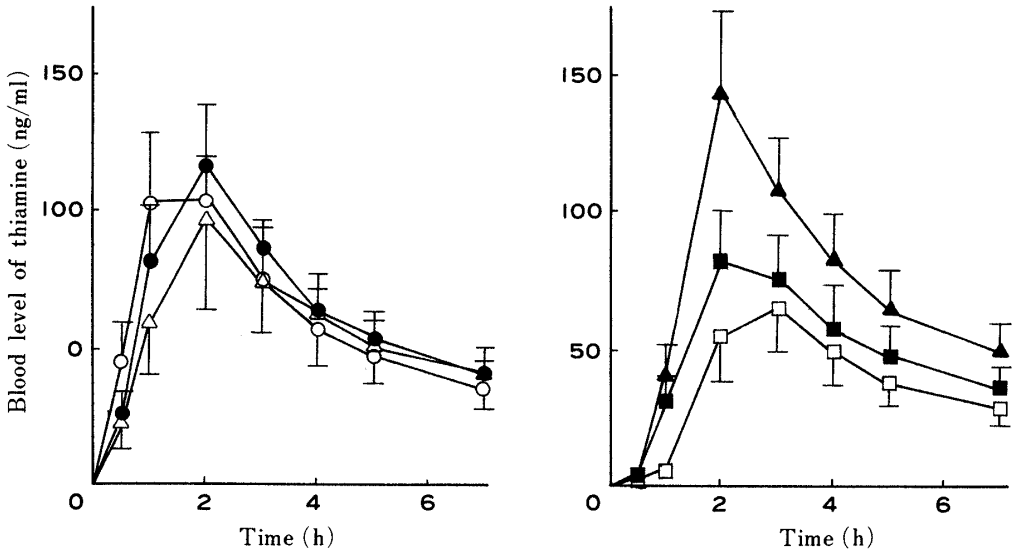


Fig. 3. Mean Blood Levels of Thiamine after Oral Administration of Six Brands of TDS Sugar-Coated Tablets (30 mg) to Dogs ($n=12$)

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

TABLE III. C_{max}, T_{max}, AUC and F_a after Oral Administration of Sugar-Coated Tablets of 30 mg TDS to Dogs ($n=12$)

	Tablet						ANOVA
	A	B	C	D	E	F	
C_{max} (ng/ml)	130±22[a)]	133±20	112±28	154±29	72±17	99±21	NS[b)]
T_{max} (h)	1.4±0.2	1.5±0.2	2.3±0.3[c)]	2.8±0.4	2.9±0.5[d)]	2.5±0.4	
AUC_7 (h·ng/ml)	444±61	469±72	411±96	524±94	263±58	349±58	NS
AUC_∞ (h·ng/ml)	615±89	633±104	587±139	706±132	397±81	495±95	NS
F_a[e)] (%)	73±10	72±11	67±17	80±16	50±11	58±11	NS

a) Mean±S.E. *b*) NS: not significant at $p<0.05$. *c*) $n=9$ (without No. 3, 4 and 10 dogs). *d*) $n=11$ (without No. 2 dog). *e*) $F_a=100\times AUC_\infty$ (TDS tablet)/AUC_∞ (TDS solution).

TDS tablets, and Table III lists the *in vivo* parameters. The highest ratio (F_a) of AUC_∞ of TDS tablet (D) to that of TDS solution was 80%. On the other hand, in humans, $A_{e_{22}}$ (the ratio of the amount of thiamine excreted in urine during 22 h) of tablet A (showing the highest availability) to $A_{e_{22}}$ of TDS solution was also approximately 80%.[7] This indicates insufficient dissolution of the drug from the tablets during passage through the absorption site in both humans and dogs. Tablets A and B, showing faster dissolution rates than the others, gave higher C_{max}, AUC_7 and AUC_∞ than tablets E and F: However, tablets C and D (especially D) gave much higher bioavailability than expected from their *in vitro* dissolution rates. On the other hand, AUC_∞ of tablet C in three dogs (No. 3, 4 and 10) and tablet E in one dog (No. 2) were negligible (below 4 h·ng/ml), suggesting the passage of those tablets through the gastrointestinal tract in an intact state. The negligible drug absorption made it difficult to estimate T_{max} in those dogs administered tablets C and E. Therefore, mean T_{max} values of tablets C and E were obtained from the T_{max} values of the other dogs, and these T_{max} data were not subjected to ANOVA. Due to the large variation of the *in vivo* parameters, statistically significant differences were not found among the treatments by ANOVA.

In order to compare the difference in bioavailability between the fastest-dissolving

Table IV. Comparison of Relative Bioavailabilities of Six TDS Products between Dogs and Humans Having High and Low Gastric Acidities

		Tablet					
		A	B	C	D	E	F
Dog	Ratio of AUC_∞	1.00[a]	1.03	0.95	1.15	0.65	0.80
Human (high)[b]	Ratio of $A_{e_{22}}$	1.00	0.90	0.71	0.44	0.51	0.56
Human (low)[c]	Ratio of $A_{e_{22}}$	1.00	0.74	0.12	0.69	0.30	0.50

a) Ratio of mean AUC_∞ or $A_{e_{22}}$ of each product determined against that of tablet A. *b*) High gastric acidity humans. *c*) Low gastric acidity humans. Human data are from a previous study.[7]

Table V. Correlation Coefficients between Gastric pHs of Dogs and C_{max} or AUC_7 of Six TDS Products

Tablet	C_{max}	AUC_7
A	−0.469	−0.477
B	−0.065	−0.069
C	−0.486	−0.505
D	−0.270	−0.433
E	−0.615[a]	−0.648[a]
F	−0.463	−0.437

a) $p<0.05$.

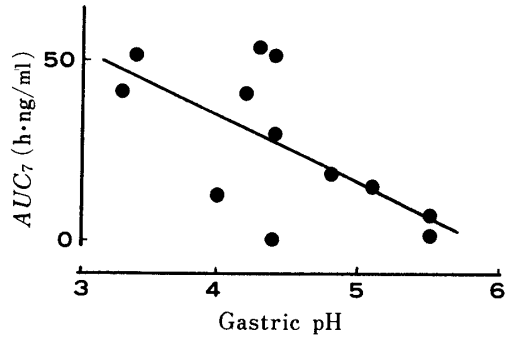


Fig. 4. Relation between the Gastric Fluid pH of Individual Dogs and AUC_7 of Tablet E

The solid line shows the regression line ($r=-0.648$).

product (A) and others in dogs with that in humans,[7] the ratios of mean AUC_∞ and $A_{e_{22}}$ (amount of thiamine excreted in urine during 22 h) of each tablet to AUC_∞ and $A_{e_{22}}$ of tablet A, designated as relative bioavailability, were calculated (Table IV). The relative bioavailabilities of slow-dissolving products (C, D and F) tended to be higher in dogs than in either group of humans (*i.e.*, those having high or low gastric acidity).

Effect of Gastric Acidity

Our previous human study[7] revealed that the bioavailabilities of TDS tablets were affected by the gastric acidity, and the bioavailability of tablet C was poorer in low gastric acidity subjects than in high gastric acidity ones. In order to investigate the gastric acidity effect in dogs, the gastric fluid pHs of beagle dogs were determined, and were in the range of pH 3.3—5.5 (Table II). Human subjects had been classified into high and low gastric acidity groups by using the Gastro-test®, which distinguishes high gastric acidity from low with a boundary pH of 3.[11] Thus, the dogs used in this study all belonged to the low gastric acidity group, which made it impossible to estimate the bioavailability difference between high and low gastric activity groups. Therefore, the dependence of the bioavailability upon the gastric fluid pH was investigated by estimating the correlation between gastric fluid pHs of individual dogs and *in vivo* parameters (C_{max} and AUC_7) of each tablet. As shown in Table V, the correlation coefficients tended to be negative. Figure 4 shows the scattered plots of AUC_7 of tablet E of individual dogs *versus* their gastric fluid pHs, the correlation being statistically significant.

***In Vitro–in Vivo* Correlation**

In vitro–in vivo correlations were estimated using the time required for 50% of the drug to dissolve (T_{50}) as an *in vitro* parameter. Neither C_{max} nor AUC_7 correlated well with T_{50}

TABLE VI. Correlation Coefficients of C_{max} and AUC_7 in Dogs with Time for 50% of the Drug to Dissolve (T_{50}) Determined at Various pHs

In vitro test		C_{max}	AUC_7
Method	pH		
Paddle	1.2	−0.585 (−0.916[a])	−0.619 (−0.925[a])
	3	−0.593 (−0.924[a])	−0.629 (−0.935[a])
Rotating basket	1.2	−0.541 (−0.937[a])	−0.578 (−0.946[a])
	3	−0.600 (−0.973[b])	−0.638 (−0.983[b])
	5	−0.417 (−0.603)	−0.396 (−0.555)
Oscillating basket	1.2	−0.352 (−0.942[a])	−0.390 (−0.946[a])
	3	−0.134 (−0.798)	−0.122 (−0.728)
	5	−0.071 (−0.821)	−0.064 (−0.751)

The figures in parentheses show the correlation coefficients among five products excluding tablet D. *a*) $p<0.05$. *b*) $p<0.01$.

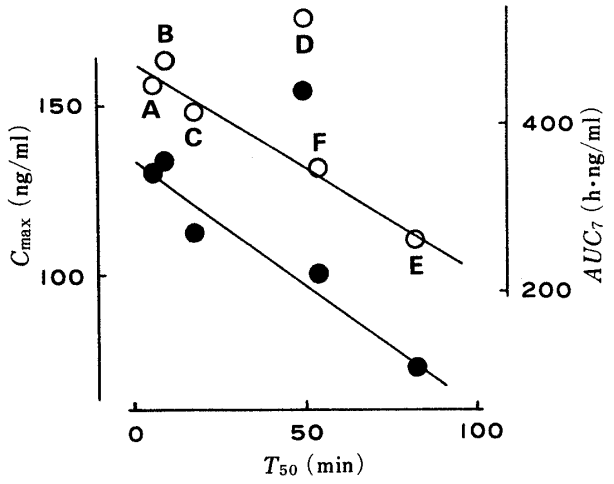
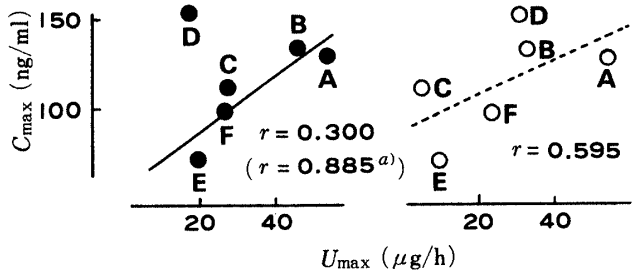


Fig. 5. Plots of C_{max} (●) and AUC_7 (○) *versus* T_{50} Determined by the Rotating Basket Method at pH 3

The solid lines represent the regression lines for C_{max} ($r=-0.973$) and AUC_7 ($r=-0.983$) among five products excluding tablet D.



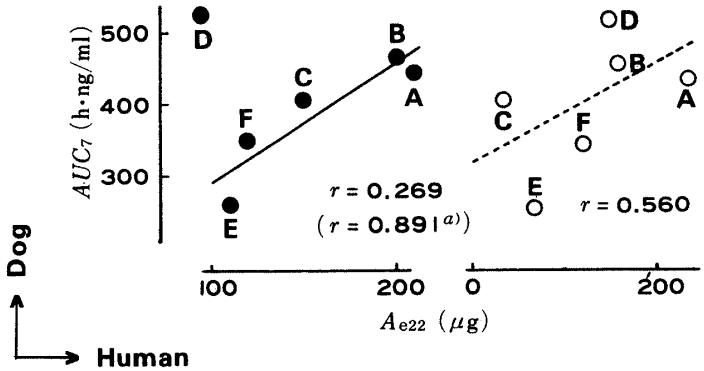


Fig. 6. Relations of C_{max} and AUC_7 in Dogs with U_{max} and $A_{e_{22}}$ in Humans Having High (●) and Low (○) Gastric Acidity, Respectively

Human data are from a previous study.[7] The values in parentheses and solid lines indicate the correlation coefficients and regression lines among five tablets excluding tablet D. Dotted lines show the regression lines among six tablets. *a*) $p<0.05$.

determined under various conditions (Table VI). However, when the data for tablet D were omitted from the estimation, the correlation coefficients were markedly increased. When the disintegration time was used as an *in vitro* parameter, similar correlation results were obtained (data not shown). Figure 5 shows the scattered plots of C_{max} and AUC_7 *versus* T_{50} determined by the rotating basket method at pH 3.

Correlation between Results in Humans and Dogs

Figure 6 shows the correlations of C_{max} and AUC_7 in dogs with the maximum urinary excretion rate (U_{max}) and the amount of urinary thiamine excreted for 22 h ($A_{e_{22}}$) in humans.[7] The correlation between the two species was poor for six brands, but when tablet D was omitted, significant correlations were observed between humans having high gastric acidity and dogs. The correlations between low gastric acidity humans and dogs were poor.

Discussion

The bioavailabilities of TDS from sugar-coated tablets in dogs were estimated and compared with those in humans. The relative bioavailabilities of tablets C—F were higher in dogs than in humans (Table IV). The *in vitro* dissolutions of these tablets were faster under the strongly destructive conditions of the oscillating basket method using plastic disks than under the mild conditions of the paddle and rotating basket methods (Table I),[7] which suggests that the disintegration of these products in the gastrointestinal tract was mechanically accelerated in dogs as compared with humans. The stronger destructive force in dogs was also found in previous studies on griseofulvin,[2] nalidixic acid[3] and indomethacin,[5] in which the products not disintegrated and/or not dispersed well under relatively mild *in vitro* conditions also had relatively higher bioavailability in dogs than in humans. The destructive force of the digestive tract in humans seems to be relatively weak in the fasting state compared with that in dogs.

In humans, gastric acidity significantly affected the bioavailability of tablet C (not disintegrated at pH 7.2), which was much poorer in low gastric acidity subjects than in high acidity ones (Table IV).[7] In order to investigate the gastric acidity effect on the bioavailability in dogs, the gastric fluid pHs of dogs were determined, but all were above pH 3, so that all of the dogs belonged to the low gastric acidity group. This made it impossible to compare the bioavailability of TDS tablets between high and low gastric acidity dogs, and therefore the correlations of gastric fluid pH of dogs with C_{max} and AUC_7 were investigated. They tended to be negative (Table V). Assuming that gastric fluid pH affected the dissolution and disintegration of the coating films of TDS tablets in the gastric pH range (3.3—5.5) of dogs, the correlation coefficient of tablet E (showing faster dissolution at pH 5 than at pH 3) should be positive, but actually it was negative. *In vitro*, the effects of medium pH on the dissolution of TDS tablets were less under the strongly destructive conditions of the oscillating basket method than under the weak conditions of the paddle and rotating basket methods (Table I).[7] This implies that under the strongly destructive gastric conditions of dogs, the degradation of the coating films of tablets might be mechanically promoted and the dissolution of TDS from tablets may depend on the solubility of TDS rather than on the dissolving characteristics of the coating films. On the other hand, judging from the low gastric acidity of dogs, the bioavailability of tablet C in the dogs was expected to be poor, as in the low gastric acidity subjects; however, it was much higher than in the low acidity subjects and even than in the high acidity ones (Table IV), again suggesting enhanced disintegration of the coating film of the tablet.

In a previous study on sugar-coated tablets of metronidazole,[6] the gastric fluid pH range of dogs was found to be 1.7—7.8, wider than that of the dogs used in this study, and the dogs could be classified into two groups of high and low gastric acidities with a boundary pH 3.

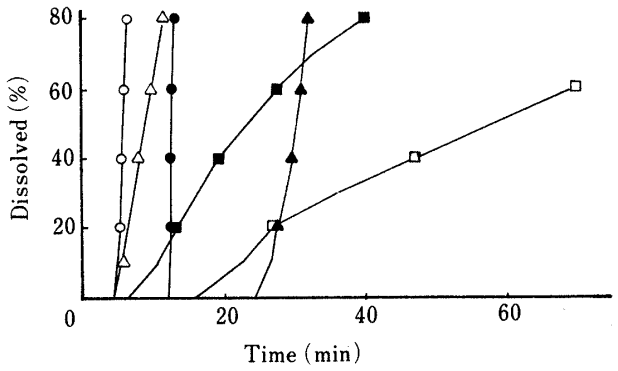


Fig. 7. Dissolution–Time Curves of Six Brands of TDS Sugar-Coated Tablets Using the Paddle Method at pH 1.2

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

Their *in vivo* data after administration of metronidazole tablets agreed well with those in humans having high and low gastric acidities. The difference of gastric fluid pH range between the two groups of dogs used for the metronidazole and TDS studies may be ascribed to intrinsic difference of their gastric fluid pHs, since large inter-individual variability of the gastric fluid pH in dogs was reported,[12,13] although the methods of determination of the gastric fluid pH may affect the data. In addition to the variability in the gastric fluid pH among individual dogs, large intra-individual variability was also observed,[12,13] indicating that it would be better to determine the gastric fluid pH when the drugs are administered, if possible, in order to obtain reliable results on the gastric fluid pH effects on drug bioavailability in dogs.

The correlations of the *in vivo* parameters of dogs with the *in vitro* dissolution rate, disintegration time and *in vivo* parameters in humans were very poor (Table VI, Fig. 6). One reason may be the unexpectedly high bioavailability of table D in dogs, since, when tablet D was omitted from the correlation estimations, the *in vivo* parameters of dogs correlated well with those in humans having high gastric acidity and with *in vitro* parameters. Although this could not be reasonably explained, one possibility is that the drug dissolved from tablet D might escape from the first-pass effect due to the burst-type disintegration and dissolution of the product under the strongly destructive gastric conditions of dogs, because the drug tended to be released rapidly from tablet D after a lag time which probably corresponded to the time required for the coating film to rupture or dissolve (Fig. 7). In addition, the dose–*AUC* relation (Fig. 1) suggests that TDS orally administered may be eliminated before reaching the blood circulation due to first-pass effects. The steep rise of the serum thiamine level from 30 min after administration of tablet D (Fig. 3) seems to indicate the burst-type dissolution of the drug after a lag phase in the gastrointestinal tract. On the other hand, one reason for the low correlations of the *in vivo* parameters between humans having low gastric acidity and dogs may be the poor bioavailability of tablet C in those humans. The complicated correlations between the results in humans and dogs seems to be ascribable to the complex dissolution behavior of TDS tablets, which is greatly affected by pH and mechanical destructive force (Table I). Consequently, their bioavailability would be greatly influenced by differences in physiological conditions (gastric emptying rate, gastrointestinal motility and pH, *etc.*) between humans and dogs, compared with drug formulations, the dissolution of which is less affected by pH and disintegration conditions.

From this study, it was shown that beagle dogs may be used for the estimation of bioavailability of TDS sugar-coated tablets in place of humans, although some products, the dissolution of which is largely influenced by pH and mechanical destructive force, may lie off the regression line of the human–dog correlation. However, in order to clarify the usefulness and limitations of dogs for bioavailability studies of sugar-coated formulations, further studies using other sugar-coated products containing drugs chemically different from TDS or metronidazole will be required. The present study confirmed strong gastric disintegration

conditions in dogs as found in previous studies,[2,3,5] but it seems desirable to investigate the gastric disintegration conditions in dogs and in humans by a more straightforward method, *e.g.* by the use of $BaSO_4$ tablet, *etc.*

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