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In Vitro Dissolution Test and Absorption Study of a Per Oral Controlled Release Dosage Form Containing Pyridoxine Hydrochloride or Sodium Riboflavin Phosphate with Hydroxypropyl Cellulose[1]

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In vitro dissolution tests by the U.S.P. rotating basket method were carried out on double layer tablets of pyridoxine hydrochloride (PDH) or sodium riboflavin phosphate (SRP) held in a simple powder mixture (PM) and a spray-dried mixture (SDM) of hydroxypropyl cellulose and lactose according to four different formulae. The dissolution properties of these tablets could be controlled by adjusting the amounts of drug combined into the PM layer and SDM layer and the volumes of the layers. In order to confirm the practical utility of the present dosage form, an absorption study was carried out in healthy male volunteers, using tablets containing PDH or SRP. The drug powder, mixed powder of the components of the tablet, or the same tablets used in the dissolution tests were administered orally and the urinary excretion of total VB_6 or VB_2 was determined.

The maximum excretion time was delayed quite markedly in the slowest releasing PDH and SRP tablets. The long half-life observed after the administration of the slowest releasing tablet of SRP appeared to be due to the low value of maximum excretion and the continuance of slow nonspecific drug absorption from the tablet, which retained about 60% of the initial drug content after passing the major absorption site. Plots of cumulative excretion amount against the amount dissolved *in vitro* at various periods indicated approximately constant absorption of VB_6 over a wide region of the intestine and were consistent with specific transport of VB_2 at the proximal region of the small intestine, as reported by Levy *et al.*

Keywords——hydroxypropyl cellulose; controlled release dosage form; dissolution test; pyridoxine hydrochloride; sodium riboflavin phosphate; urinary excretion; double layer tablet

It was reported in the previous paper[3] that directly compressed tablets of *dl*-isoproterenol hydrochloride held in a simple powder mixture (PM) or a spray-dried mixture (SDM) of hydroxypropyl cellulose (HPC) and lactose showed controlled dissolution properties. A double layer tablet of *dl*-isoproterenol hydrochloride held in 200 mg of SDM as the initial phase with 300 mg of PM as the depot phase afforded a suitable sustained release profile without a major change of the dissolution curve upon changing the dissolution medium from J.P. IX No. 1 medium (acidic) to No. 2 (neutral). On the other hand, a commercially available prolonged action preparation showed a sharp rise in the dissolution curve upon such a change of dissolution medium.

Generally, the differences in *in vivo* dissolution properties between different formulae, lots or preparations may be predicted from the results of *in vitro* dissolution tests. However, the conditions of *in vitro* dissolution tests differ somewhat from those in the gastro-intestinal tract, and thus *in vitro* results cannot directly reflect the *in vivo* dissolution properties of a preparation. In particular, the sustained release properties shown in an *in vitro* dissolution test are not always coincident with those obtained in practical administration.

1) This paper forms Part XVI of "Pharmaceutical Interaction in Dosage Forms and Processing." The preceding paper, Part XV: T. Ohkura, H. Ueda, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **28**, 612 (1980).
2) Location: *Ebara-2-4-41, Shinagawa-ku, Tokyo 142, Japan.*
3) Y. Machida and T. Nagai, *Chem. Pharm. Bull.*, **26**, 1652 (1978).

Therefore, the present study was carried out to confirm the practical usefulness of the present controlled release dosage form containing HPC and also to consider the validity of *in vitro* dissolution tests (U.S.P. XIX) for the evaluation of a controlled release dosage form. Experiments were carried out on both the *in vitro* dissolution profile and the urinary excretion pattern in healthy voluntary subjects after oral administration, using directly compressed tablets of pyridoxine hydrochloride (PDH) or sodium riboflavin phosphate (SRP) held in HPC and lactose (PM and SDM).

Experimental

Materials——Commercial pyridoxine hydrochloride J.P. IX, and sodium riboflavin phosphate J.P. IX were used without further treatment. HPC-M (48—100 mesh), lactose J.P. IX and SDM, *i.e.*, a spray-dried mixture of HPC-L and lactose (containing 20% HPC-L) were the same as in the previous paper.[3]

Preparation of the Tablet——The tablets were prepared as described in the previous paper,[3] according to the formulae given in Table I.

Table I. Formulae for Double Layer Tablets

Tablet	SDM layer		PM[b] layer	
	SDM (mg)[a]	Drug (mg)[a]	PM (mg)[a]	Drug (mg)[a]
A	190	10	280	20
B	195	5	275	25
C	90	10	380	20
D	95	5	375	25

a) Amount contained in 1 tablet.
b) Containing 90% HPC-M.

Dissolution Test——A Toyama Sangyo TR-5S3 U.S.P. type dissolution tester was used at 200 rpm, with 800 ml of the dissolution medium at 37°±2°. Dissolution media used are described in J.P. IX as disintegration media No. 1 (pH 1.2, for 120 min) and No. 2 (pH 7.5, successively for 22 hr). Aliquots (5 ml) of the solution were taken at intervals and the volume was made up to the original volume by adding the dissolution medium warmed to the same temperature. The amounts of PDH released in media No. 1 and No. 2 were determined spectrophotometrically at 292 nm and 220 nm, respectively. The amount of SRP released was determined similarly at 267 nm, in both media. A Hitachi 323 spectrophotometer was used in these determinations.

Absorption Study in Healthy Male Volunteers——The absorption study was done by a cross-over method at intervals of more than two days, using five or three healthy male volunteers aged 21 to 33, body weight 46—67 kg. One tablet was administered after breakfast and a urine sample was collected every hour from 0 to 7 hours after administration. In some experiments, the collection of urine was continued until the next morning. The urine collected was put in screw-capped polyethylene bottles and stored in a refrigerator at −20°. The total VB_6 and VB_2 were determined by the methods described below.

Determination of Total VB_6 in Urine——Total VB_6 in urine was determined spectrofluorometrically by a minor modification of the method reported by Fujita *et al.*[4] The frozen urine was kept standing at room temperature to thaw it. Five milliliters of urine, 3 ml of 1 N H_2SO_4 and 4 ml of ethanol were made up to 20 ml in total with purified water. An aliquot (5 ml) of this urine solution in a glass-stoppered test tube cooled in an ice bath was mixed with 2 ml of ice-cold 2% $KMnO_4$ solution, and the mixture was cooled immediately after shaking. Exactly 50 sec after shaking, 2 ml of 3% H_2O_2 was added and the test tube was taken out of the bath. Next, 3 ml of 6 N HCl was added and the mixture was heated in a boiling water bath. After heating for 8 min, the test tube was immersed directly in an ice bath, then 2 ml of 3 N NH_4OH and 5 ml of 3 N NaOH were added immediately and the solution was made up to 25 ml with purified water. This solution was centrifuged for 5 min at 3000 rpm, and the intensity of fluorescence of the supernatant solution was measured at 435 nm using a Hitachi 204 spectrofluorometer with excitation at 345 nm. The total VB_6 was determined from a calibration curve prepared in advance. The standard solution used was 5.5 μg/ml quinine sulfate solution in 0.1 N H_2SO_4.

4) *a*) A. Fujita, K. Matsuura, and K. Fujino, *J. Vitamin.*, **1**, 267 (1955); *b*) A. Fujita, D. Fujita, and K. Fujino, *ibid.*, **1**, 275 (1955); *c*) A. Fujita, D. Fujita, and K. Fujino, *ibid.*, **1**, 279 (1955); *d*) A. Fujita and K. Fujino, *ibid.*, **1**, 290 (1955).

Determination of Total VB_2 in Urine——Total VB_2 in urine was determined spectrofluorometrically by a minor modification of the method reported by Fujita.[5] An aliquot (5 ml) of urine in a test tube was mixed with 5 ml of 1 N NaOH. After centrifuging for 5 min at 3000 rpm, 6 ml of the supernatant was transferred to another test tube for photolysis. The photolysis was carried out for exactly 1 hr at a temperature below 30°, with two 100 watt incandescent bulbs at a distance of 15 cm. After photolysis, 5 ml of the solution was pipetted into a cetrifuge tube, and mixed with 0.4 ml of glacial acetic acid and 1 ml of 4% $KMnO_4$ solution. Exactly 50 sec after addition of the $KMnO_4$ solution, 2 ml of 3% H_2O_2 was mixed in, and then 5 ml of $CHCl_3$ was added. After vigorous shaking for 5 min followed by centrifugation for 5 min at 3000 rpm, 4 ml of the $CHCl_3$ phase was used to measure the intensity of fluorescence at 505 nm, and total VB_2 was determined in the same way as VB_6. Excitation was at 360 nm, and 15 μg/ml quinine sulfate solution in 0.1 N H_2SO_4 was used as a standard solution.

Results and Discussion

Dissolution Properties of Pyridoxine Hydrochloride Tablets

Figure 1 shows the dissolution profiles of PDH tablets prepared according to the formulae given in Table I. The times for 50% dissolution of the drug (T_{50}) obtained from the dissolution curves of tablets A, B, C, and D were 60, 80, 72, and 135 min, respectively. At earlier periods, *e. g.*, 20, 30, and 40 min, tablets A and C showed slightly more rapid dissolution than tablets B and D, because of the larger amount of drug combined in the SDM layer, *i.e.*, the initial phase of the present double layer dosage form. On the other hand, in later periods, the dissolution curves can be roughly divided into two groups, *i. e.*, A/B and C/D. This can be attributed to the larger volume of the PM layer, *i. e.*, the depot phase, in the latter tablets. These results suggest that the dissolution profile of the present prolonged release dosage form is adjustable by controlling the amount of drug combined in each layer. This property could be a significant advantage of the present dosage form, as already described in the previous paper.[3]

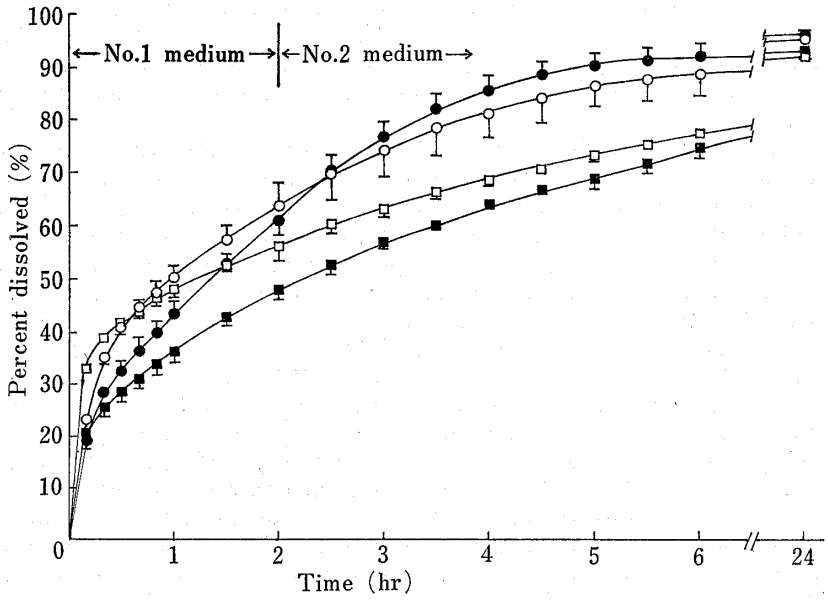

Fig. 1. Dissolution Profiles of PDH Tablets A, B, C, and D Obtained by the U.S.P. Method

Each symbol represents the mean and S. D. of 3 determinations.
○: tablet A, ●: tablet B, □: tablet C, ■: tablet D.

5) A. Fujita, *Bitamin*, **2**, 254 (1950).

Urinary Excretion of VB_6 after the Administration of Tablets A, B, C, and D

Figure 2 shows the urinary excretion curves of total VB_6 after administration of tablets A, B, C, D, PDH powder (30 mg), and a mixed powder of the components of tablet D. In some experiments the urine was collected for 24 hr, but the excretion of VB_6 apparently due to the administration of PDH was only observed to occur for 7 hours after administration. Figure 2, therefore, shows the data obtained up to 7 hr. The urinary excretion of total VB_6 when PDH was not administered was also measured for 7 hr and the excretion rate was obtained as 0.55±0.17 mg/hr (mean±S.D.); no increase was observed after meals.

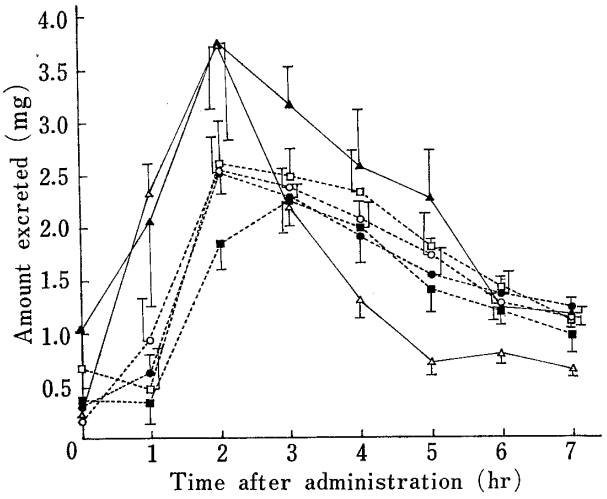

Fig. 2. Mean Urinary Excretion, with Standard Error, of Total VB_6 after Oral Administration of PDH as a Powder (30 mg), Mixed Powder of the Components of Tablet D, and Tablets A, B, C, and D to Five Healthy Male Volunteers

—△—: PDH powder,
—▲—: mixed powder,
---○---: tablet A,
---●---: tablet, B,
---□---: tablet C,
---■---: tablet D.

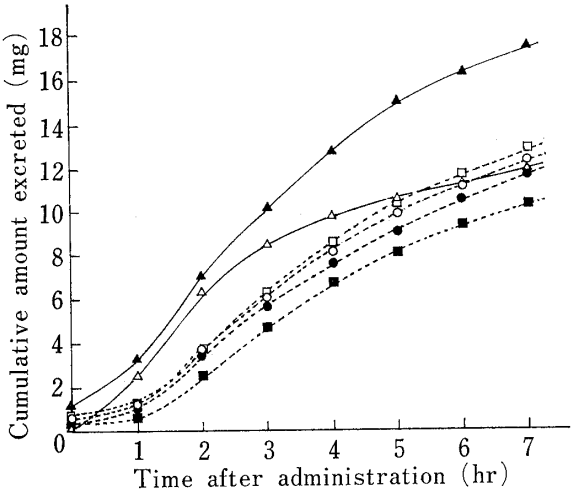

Fig. 3. Cumulative Urinary Excretion Patterns of Total VB_6 after Administration of PDH as a Powder (30 mg), Mixed Powder of the Components of Tablet D, and Tablets A, B, C, and D to Five Healthy Male Volunteers

—△—: PDH powder,
—▲—: mixed powder,
---○---: tablet A,
---●---: tablet B,
---□---: tablet C,
---■---: tablet D.

The maximum excretion time observed (T_{max}) for tablet D was somewhat slower than the others. No statistically significant difference was observed among the four types of tablet, but apparently high excretion was observed for PDH powder compared with the tablets at 1 and 2 hr.

Cumulative urinary excretion patterns obtained from the data shown in Fig. 2 are shown in Fig. 3. Generally, the initial rise of the excretion curve was slow in the case of the tablets compared with the powders. The cumulative excretion amount was not very different among the tablets and appeared to have no relation to the *in vitro* dissolution properties of the tablets. However, tablet D was shown to have comparatively better sustained release with low excretion.

These results indicate that the present dosage form afforded prolonged release *in vivo*. The absence of any significant difference among the four types of tablets may be attributable to the lack of site specificity for the absorption of PDH. In other words, the absorption of PDH seemed to occur over a comparatively wide region of the intestine, so that the differences in dissolution properties among these tablets had little effect on the absorption.

Dissolution Properties of Sodium Riboflavin Phosphate Tablets

The above experiments showed that the present dosage form did provide *in vivo* sustained release. The following experiments were carried out to investigate the extent of dissolution

by the time the tablet reached the proximal region of the small intestine. For this purpose, riboflavin was chosen as an active ingredient because it is absorbed from the proximal region of the small intestine by an active transport process.[6]

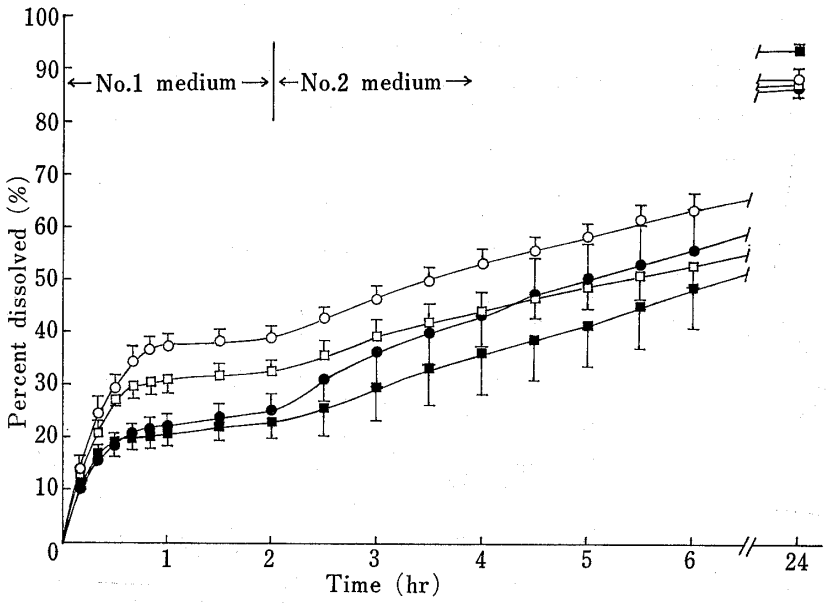

Fig. 4. Dissolution Profiles of SRP Tablets A, B, C, and D Obtained by the U.S.P. Method

Each symbol represents the mean and S.D. of 3 determinations.
○: tablet A, ●: tablet B, □: tablet C, ■: tablet D.

Figure 4 shows the dissolution profiles of SRP[7] tablets prepared according to the formulae shown in Table I. T_{50} values estimated for tablets A, B, C, and D were 214, 300, 315, and 372 min, respectively. At the initial stage of dissolution (up to 1 hr), the dissolution curves could be divided roughly in two groups, in the same way as for the tablets containing PDH. However, in the period between 1 hr and 2 hr, only a slight increase of dissolution was observed for each tablet, as occurred in the dissolution of SRP in the acidic dissolution medium (pH 1.2). In other words, SRP combined in the SDM layer dissolved with the rapidly soluble SDM layer, but SRP combined in the PM layer was entrapped in the swollen gel-like HPC matrix, in which the drug changes to free riboflavin. Therefore, rapid dissolution was observed again after 2 hr upon changing the dissolution medium to the neutral No. 2 medium. The standard deviations (S. D.) of the dissolution data were large in the cases of tablets B and D, both of which contained a larger amount of the drug in the depot phase than did tablets A and C. This tendency was not observed in the PDH tablets. This difference seemed to be related to the drug solubility, but further investigations are required to confirm this.

Urinary Excretion of VB_2 after the Administration of Tablets A and D

Figure 5 shows the urinary excretion of total VB_2 up to 7 hr after the administration of SRP powder (30 mg), mixed powder of the components of tablet D, and tablets A and D. The data for 24 hr are not shown for the reason given in connection with the experiments on PDH tablets. The urinary excretion of total VB_2 when SRP was not administered was 0.07 ± 0.16 mg/hr (mean±S. D.). The values of T_{max} observed for the tablets appeared to be delayed. This delay observed in the absorption from the tablets seemed due to the sustained release

6) G. Levy and W.J. Jusko, *J. Pharm. Sci.*, **55**, 285 (1966).
7) SRP was used instead of riboflavin because it is more soluble than riboflavin.

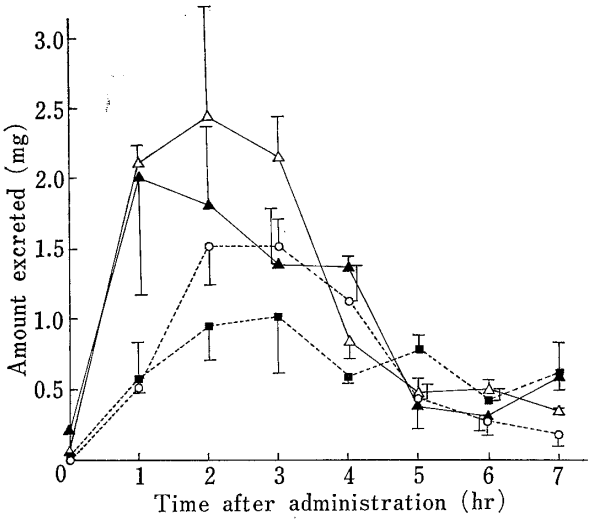

Fig. 5. Mean Urinary Excretion, with Standard Error, of Total VB_2 after Oral Administration of SRP as a Powder (30 mg), Mixed Powder of the Components of Tablet D, and Tablets A and D to Three Healthy Male Volunteers

—△—: SRP powder,
—▲—: mixed powder,
---○---: tablet A,
---■---: tablet D.

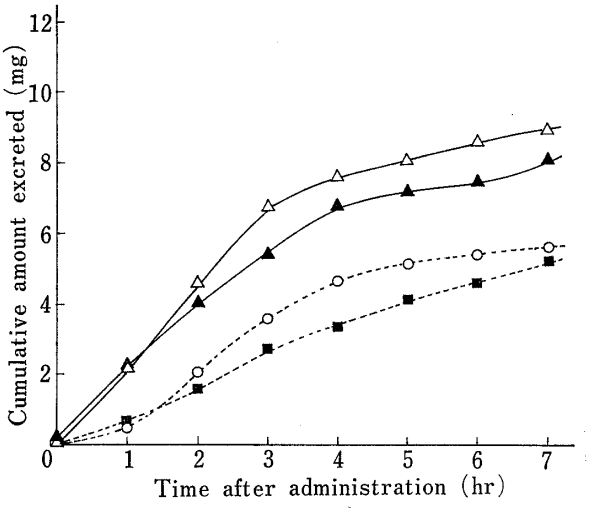

Fig. 6. Cumulative Urinary Excretion Patterns of Total VB_2 after Administration of SRP as a Powder (30 mg), Mixed Powder of the Components of Tablet D, and Tablets A and D to Three Healthy Male Volunteers

—△—: SRP powder,
—▲—: mixed powder,
---○---: tablet A,
---■---: tablet D.

characteristics of the tablets, because this tendency was not marked in the case of PDH tablets, which show more rapid dissolution than SRP tablets, as shown in Fig. 2.

Figure 6 shows the cumulative urinary excretion patterns of total VB_2 obtained from the data shown in Fig. 5. Tablet D showed a low excretion amount. These data were obtained from three volunteers, and show a statistically significant difference between SRP powder and tablet D for all periods except 0 hr, as determined by the *t*-test at the 5 to 10% level. In the cases of SRP powder, the mixed powder of the components of tablet D, and tablet A, a comparatively rapid increase in the excretion of VB_2 was observed until 3—4 hr after administration, and the excretion then declined. However, in the case of tablet D, an approximately constant increase was observed for 7 hr. Levy *et al.* obtained an average half-life of 1.1 hr for riboflavin after oral administration of 10 mg of riboflavin from a semilogarithmic plot of the excretion amount (μg/min) *versus* time after administration, using the data from 1.5 hr to 5 hr or 5.5 hr.[6] On plotting the data between 3 hr to 7 hr obtained from the present experiments in a way similar to that of Levy *et al.*, the half-life values for SRP powder, mixed powder of the components of tablet D, tablets A and D were roughly 1.7, 1.2, 1.2, and 4.6 hr, respectively. Except in the case of tablet D, these values are compatible with the value reported by Levy *et al.* The apparently long half-life observed after the administration of tablet D is presumably due to the sustained release of the drug from this tablet. The cumulative urinary excretion amount of total VB_2 up to 3 hr after administration of tablet D was 2.7 mg. This corresponds to about 40% of that in the case of SRP powder (6.8 mg), suggesting that the dissolution of 40% of SRP in the tablet had occurred by this time, since rapid urinary excretion of excess VB_2 absorbed is known to occur. Therefore, about 60% of SRP appears to be still present in tablet D at 3 hr after administration, and is thus available for slow absorption by passive diffusion from the intestine, as distinct from any specific absorption mechanism for VB_2. The low maximum value of excretion and slow decline of the urinary excretion curve due to such slow absorption may account for the comparatively large half-life for tablet D.

As shown in Fig. 4, about 23% of the drug was released at 2 hr from tablet D in the *in vitro* experiment; this is rather low compared with the value estimated (40%) from the *in vivo*

experiment. This difference may be explicable in terms of the following two factors: the tablet received strong agitation *in vivo* compared with that *in vitro*, and high availability is expected for a low dose (*e. g.*, 10 mg) compared with a high dose (30 mg) because of saturation of the carrier for VB_2, as reported by Levy *et al.*[6]

In the case of PDH tablets, the difference in half-life was observed to be slight. This may be due to the comparatively rapid dissolution and the lack of specific absorption sites for VB_6. The half-life values for PDH tablets A, B, C, D, PDH powder, and mixed powder of the components of tablet D were 3.5, 4.4, 3.3, 3.2, 2.0, and 2.8 hr, respectively.

The above results confirm the practical usefulness of the present prolonged action tablets, especially tablet D. If active ingredients are readily soluble in water, an increase in the volume of the PM layer (depot phase) may be required for the attainment of more effective prolonged action. In this connection, a significant effect of food on the absorption of riboflavin, resulting from the delay of gastric emptying time and intestinal transit, was reported by Levy *et al.*[6] We are therefore carrying out further investigations on the effect of food on the prolonged action of the present dosage forms.

Relationship between *in Vitro* Dissolution and *in Vivo* Absorption of PDH and SRP from Controlled Release Tablets

Usually, VB_6 and VB_2 are supplied sufficiently from food, and excess amounts absorbed should be rapidly excreted in the urine. Thus, the urinary excretion amount should accurately reflect the amount absorbed.

From the results of *in vivo* experiments, it is clear that the excretion of drug absorbed from the tablets and other forms started within 0.5 hr after administration. Therefore, in order to discuss the relation between *in vitro* dissolution and *in vivo* absorption, the cumulative amounts excreted at 1, 2, 3, 4, 5, and 6 hr were plotted against the amounts dissolved at 0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 hr, respectively. In the case of PDH tablet, a good linear relationship was obtained between dissolution and excretion, as shown in Fig. 7. The correlation coefficients obtained for tablets A, B, C, and D were 0.989, 0.985, 0.994, and 0.992, respectively. The slopes of the regression lines obtained by the least-squares method for tablets A, B, C, and D were 0.71, 0.52, 1.08, and 0.69, respectively. These results indicate that approximately con-

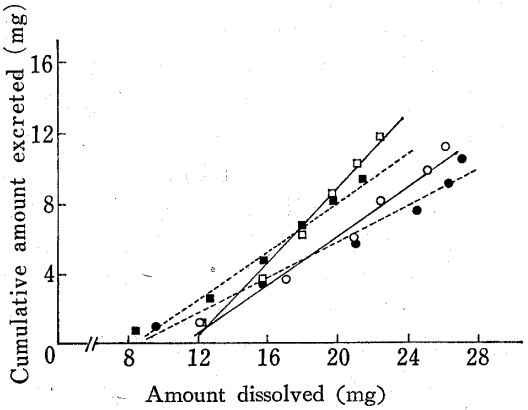

Fig. 7. Relationship between Cumulative Amount of Total VB_6 Excreted at 1, 2, 3, 4, 5, and 6 hr after Administration of a PDH Tablet and Amount of PDH Dissolved at 0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 hr in Dissolution Tests of PDH Tablets

Each line was obtained by the least-squares method.
—○—: tablet A,
---●---: tablet B,
—□—: tablet C,
---■---: tablet D.

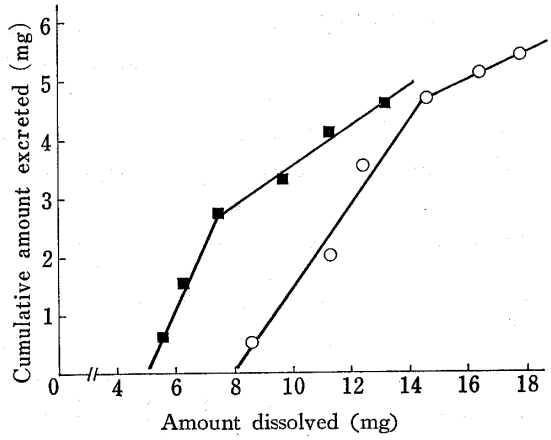

Fig. 8. Relationship between Cumulative Amount of Total VB_2 Excreted at 1, 2, 3, 4, 5, and 6 hr after Administration of an SPR Tablet and Amount of SRP Dissolved at 0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 hr in Dissolution Tests of SRP Tablets

Each line was obtained by the least-squares method.
○: tablet A,
■: tablet D.

stant absorption took place over a wide region of the intestine and that the dissolution profile of tablet C was most suitable for the PDH tablet, because the slope of the excretion–dissolution curve was close to 1.0.

On the other hand, the relationship between *in vitro* dissolution and excretion was biphasic in the case of SRP tablets, and a flexional point existed in the cases of tablets A and D at the 4 th and 3 rd data points, respectively, as shown in Fig. 8. These flexional points may correspond to a change of the absorption rate at around 3 or 4 hr and suggest the existence of a specific transport mechanism for VB_2[6] at the proximal region of the small intestine, because the average gastric emptying time was reported to be about 3 hr by Blythe *et al.*[8] The correlation coefficients before and after the flexional point for tablets A and D were 0.986, 0.998, 0.996, and 0.992, respectively. The slopes of the regression lines before and after the flexional point for tablets A and D were 0.72/0.23 and 1.11/0.34, respectively. The small increase after the flexional point suggests a slight absorption of VB_2 by passive diffusion after passing the specific absorption site. Usually, *in vitro* dissolution tests are done to provide a basis for estimating *in vivo* dissolution, local dissolution in the intestinal tract which affects the bioavailability, absorption rate, and other factors. However, the *in vitro* data do not always reflect the actual dissolution, especially in the case of a prolonged action dosage form. Therefore, a combination of both *in vivo* and *in vitro* experiments as described here may be preferable for monitoring the development of a new dosage form.

Acknowledgement The authors are very grateful to Messrs. Yasunobu Okada, Toshiharu Kakizaki, Takayuki Inoh, and Hirokazu Yamashita for their assistance in the experimental work.

8) R.H. Blythe, G.M. Grass, and D.R. MacDonnell, *Am. J. Pharm.*, **131**, 206 (1959).