

## Dissolution Behaviors and Gastrointestinal Absorption of Tolbutamide in Tolbutamide-Polyvinylpyrrolidone Coprecipitate<sup>1)</sup>

HITOSHI SEKIKAWA, TSUTOMU NAGANUMA, JUN-EI FUJIWARA,  
MASAHIRO NAKANO,<sup>2a)</sup> and TAKAICHI ARITA<sup>2b)</sup>

*Faculty of Pharmaceutical Sciences, Hokkaido University<sup>2a)</sup> and  
Department of Pharmacy, Hokkaido University Hospital<sup>2b)</sup>*

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Coprecipitates were prepared in various ratio of tolbutamide-polyvinylpyrrolidone (PVP). The X-ray diffraction spectra indicated that tolbutamide in the coprecipitate did not exhibit its crystalline property. Comparative studies were made on the *in vitro* dissolution and the *in vivo* absorption of tolbutamide from the coprecipitate and the drug alone. The dissolution rate of tolbutamide was markedly increased in the tolbutamide-PVP coprecipitates in the pharmacopeial disintegration media at pH 1.2 and 7.5. *In vivo* absorption study of each preparation was carried out by determining the plasma level of tolbutamide following the oral administration to rabbits. Bioavailability of tolbutamide was improved in the coprecipitate 1.37 time as much as tolbutamide alone. It was shown that the drug was rapidly and completely absorbed following the oral administration of the coprecipitate. Quantitative analysis of PVP in the solution was also studied by the determination of the fluorescence of the complex of PVP and 8-anilino-1-naphthalenesulfonic acid sodium salt.

**Keywords**—bioavailability; tolbutamide; polyvinylpyrrolidone; coprecipitate; dissolution; gastrointestinal absorption; polyvinylpyrrolidone assay; blood levels; hypoglycemic agent; 8-anilino-1-naphthalenesulfonic acid

Tolbutamide is a member of the class of oral hypoglycemic agents designated as sulfon-ylureas and has been popular for the management of certain diabetic patients. Because of its poor water-solubility, dissolution process is considered to be a rate-determining step of its absorption from the gastrointestinal tract following the oral administration of tolbutamide.<sup>3)</sup> Nelson *et al.*<sup>4)</sup> reported that *in vitro* dissolution rate of the various salts of tolbutamide correlated with the extent of blood sugar lowering in humans. Nelson *et al.*<sup>5)</sup> showed the correlation of the available surface area of tolbutamide crystal powder with the maximum urinary excretion rate following the oral administration of several tolbutamide formulations. Dissolution rates of tolbutamide were markedly varied among the different brands.<sup>6)</sup> Wagner<sup>7)</sup> reported the correlation between the decrease in the blood sugar level and the dissolution rates of ten different commercial brand tablets.

Judging from lowering of blood glucose levels, dissolution rates of tolbutamide from the various salts were higher than that from its crystalline powder.<sup>4)</sup> Dissolved tolbutamide, however, may be considered to be partly recrystallized in the acidic gastric fluid prior to redissolution in intestine. Thus even if faster dissolution of the drug from salts may result

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- 2) Location: a) Kita 12-jo, Nishi 6-chome, Kita-ku, Sapporo, 060, Japan; b) Kita 14-jo, Nishi 5-chome, Kita-ku, Sapporo, 060, Japan.
- 3) J. Doluisio, D. Fedder, G. Manley, T. Mattei, C.H. Nightingale, and W. Barr, *J. Am. Pharm. Assoc.*, NS13, 278 (1973).
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in higher plasma level of the drug, recrystallization-redissolution processes of the poorly water-soluble drug may result in the variation in plasma levels among individuals.

Improvement in the dissolution characteristics of sulfisoxazole<sup>8)</sup> or phenytoin<sup>9)</sup> by coprecipitation with polyvinylpyrrolidone (PVP) has been made and better bioavailability with the coprecipitates than with the drugs alone has been obtained with less variations in absorption-excretion characteristics in humans. Similar observations have been made by others.<sup>10)</sup>

The authors therefore investigated the modification of the dissolution characteristics of tolbutamide by its coprecipitation with PVP to obtain better bioavailability with less variation among subjects. Gastrointestinal absorption of tolbutamide in tolbutamide-PVP coprecipitate was also evaluated in rabbits.

### Experimental

**Materials**—Tolbutamide (J.P.IX) was obtained from Yamanouchi Pharmaceutical Co., Tokyo (Artosin, Lot 508625). The mean particle size of tolbutamide, as measured by optical microscope, was 61.7  $\mu\text{m}$  (Green diameter). Melting point of a tolbutamide sample was 128°. Chlorpropamide, mp 128°, was obtained from Pfizer Taito Co., Tokyo. PVP K-15, average molecular weight of 10000, was obtained from Daiichi Pure Chemicals Co., Tokyo. The sodium salt of 8-anilino-1-naphthalenesulfonic acid (ANS) was obtained from Eastman Kodak Co., Rochester, N.Y. All other chemicals were of reagent grade.

**Preparation of the Coprecipitate**—Tolbutamide-PVP coprecipitate was prepared as follows: After dissolving both tolbutamide and PVP in a suitable weight ratio, in ethanol, the solvent was removed *in vacuo* using rotary evaporator at about 40°. Then the preparation was dried *in vacuo* at about 50° for 24 hr. The preparation was ground in a mortar and stored in a desiccator. A physical mixture was prepared by blending tolbutamide and PVP in a mortar with a spatula.

**Dissolution Rate Studies**—Dissolution rates of tolbutamide from the preparations in 11 of J.P.IX disintegration medium No. 1 (pH 1.2) or No. 2 (pH 7.5) were measured at  $37.0 \pm 0.1^\circ$  in a constant temperature water bath (Julabo, Model Exatherm U3). The beaker had a 1l capacity and 105 mm in diameter. A stainless steel three-braded propeller (40 mm in diameter and about 2 cm<sup>2</sup> in area of each brade) was immersed into the beaker at a depth of 30 mm from the bottom, and was rotated by the stirrer (MS-Stirrer, Tokyo Rikakikai Co.) at 60 rpm. The rate of rotation was checked occasionally using a Hand Tachometer (Teclock Co.). Each preparation was transferred directly into the dissolution medium. A suitable aliquot was removed at the specified time intervals with a syringe, then filtrated quickly through the membrane filter (Toyo, TM-4, pore size 0.2  $\mu\text{m}$ ) and analyzed. The same volume of fresh medium was added into the beaker.

**Quantitative Analysis for Tolbutamide**—Tolbutamide in the solution was analyzed by ultraviolet (UV) spectrophotometry. The sample solution diluted by the same medium used in the dissolution studies was analyzed at 227 nm using Hitachi 200-20 Spectrophotometer. As PVP in the sample solution interfered with the UV assay of the drug at this wave length, correction of the absorbance was necessary for the analysis of tolbutamide in the solution. The concentration of PVP was determined in the following manner, and calculated UV absorbance due to PVP was subtracted to obtain the tolbutamide concentration.

**Quantitative Analysis of PVP in the Solution**—PVP in the solution was determined using fluorescence of the PVP-ANS complex.<sup>11)</sup> Fluorescence of the solution containing 10  $\mu\text{g/ml}$  ANS and various concentration of PVP was determined. Fluorescence intensity was measured by Hitachi Spectrofluorometer 203, equipped with Xenon lamp. Fluorescence intensity was measured at excitation wavelength of 363 nm and emission wavelength of 450 nm in J.P.IX disintegration medium No. 1, and at excitation wavelength of 365 nm and emission wavelength of 460 nm in the medium No. 2. Tolbutamide in the solution did not interfere with the intensity of the fluorescence within the experimental concentrations.

**X-Ray Diffraction Patterns**—X-Ray powder diffraction patterns were obtained with Rigaku Denki D-9C X-Ray Diffractometer.

**Thin-Layer Chromatographic Procedure**—TLC method of Said *et al.*<sup>12)</sup> was used. Ethanolic solution of tolbutamide, PVP, 1:5 tolbutamide-to-PVP coprecipitate and the physical mixture were spotted on a

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silica gel plate (DC-Fertigplatten Kieselgel 60 F<sub>254</sub>, E. Merck), previously saturated with water vapor. Developing solvent system was a mixture of *n*-butanol, chloroform and diethylamine=9:9:1. Development was allowed to proceed to 10 cm, after that the plate was removed and dried in an oven at 120° for 10 min. Visualization of the spots was made by iodine vapor.

**Plasma Level of Tolbutamide in Rabbits**—Six male albino rabbits weighing 2.5–3.3 kg were used in this study following stomach-emptying-time-controlling treatment,<sup>13)</sup> as used in the previous studies.<sup>9)</sup> Tolbutamide (30 mg/kg) or coprecipitate (weight ratio of tolbutamide:PVP=1:5, 180 mg/kg) in capsules (J.P.IX, No. 2) was orally administered to three rabbits in each group with about 50 g of special soft diet (Nihon Clea Co., Tokyo) and water was allowed *ad libitum*. Blood samples were taken from the rabbits at 0, 2, 4, 6, 8, 10 and 24 hr postadministration by the heparinized syringe. The blood samples were centrifuged (3500 rpm, 20 min), and the plasma was stored in the refrigerator until assayed. Rabbits were prevented from the coprophagy by wearing the muzzle during the night.

**Gas Chromatography (GC) Method for the Determination of Tolbutamide in Plasma Samples**—The concentration of tolbutamide in the plasma samples were assayed by the GC method of Midha *et al.* and Sabih and Sabih<sup>14)</sup> with some modifications. To 1 ml plasma samples in the glass stoppered test tube (20 ml) were added 6 ml of internal standard (35 µg/ml chlorpropamide toluene solution) and 1 ml of 1 N HCl. The samples were shaken for 20 min at 250 rpm, followed by centrifugation at 3000 rpm for 10 min. Organic layers were transferred into the other glass stoppered test tubes (20 ml) containing 5 ml of 10% (w/v) potassium carbonate. The tubes were shaken for 20 min, centrifuged for 10 min, and then the organic layer was discarded. Aqueous layer was transferred into the glass stoppered test tube. Five ml of methanol and 0.5 ml of dimethyl sulfate were added to the solution and the solution was kept at 60° for 20 min. After cooling it to room temperature, 5 ml of 1 M acetate buffer, pH 5.6, and 6 ml of *n*-hexane were added to the solution and the liquid layers were shaken for 20 min. After centrifugation, organic layer was transferred into the test tube (10 ml) and evaporated in the vacuum desiccator. Fifty micro-liter of chloroform was added to the residue and an aliquot (1–3 µl) was injected into a gas chromatograph equipped with a flame-ionization detector (Hitachi 063). The column was 2.0 m long with 3.0 mm i.d., packed with 5% phenylmethylsilicone fluid (OV-25) on acid washed, dimethyldichlorosilane-treated, Chromosorb W support (80–100 mesh). Operating conditions were; injection port temperature, 200°; column temperature, 220°; and detector temperature, 285°. The flow rate of nitrogen was 60 ml/min. Hydrogen and compressed air flow rates were adjusted to give the maximum response. Calibration curves of tolbutamide covered concentrations over the 0–200 µg/ml range.

## Results and Discussion

### The Properties of Tolbutamide-PVP Coprecipitate

The X-ray diffraction patterns of tolbutamide, tolbutamide-PVP coprecipitate and the physical mixture are shown in Fig. 1.

In coprecipitate with PVP, sharp diffraction peaks attributed to tolbutamide crystal disappeared, and only the halo, similar to that of PVP powder alone, was observed in the X-ray diffraction pattern. Sharp diffraction peaks still remained in the simple physical mixture. This observation was also made in sulfisoxazole-PVP coprecipitate,<sup>8)</sup> or phenytoin-PVP coprecipitate.<sup>9)</sup>

In the measurement with differential scanning calorimeter, endothermic peak accompanied by the melting of tolbutamide crystal (128°) disappeared in tolbutamide-PVP coprecipitate. Well-defined coprecipitate<sup>8)</sup> were formed in coprecipitates of 1:1, 1:3, 1:5 and 1:10 tolbutamide-to-PVP ratio.

Figure 2 shows the thin-layer chromatogram of tolbutamide, PVP, 1:3 coprecipitate and the physical mixture.

The spots at  $R_f=0.6$  was tolbutamide, and the spots on the base line was PVP. Said *et al.*<sup>12)</sup> reported the thin layer chromatogram of tolbutamide, PVP and the coprecipitate. They found two spots in the chromatogram of the coprecipitate, but,  $R_f$  value of the spots did not coincide with the  $R_f$  value of tolbutamide. They evaporated the solvent by heating the solution containing both tolbutamide and PVP to prepare the coprecipitate.

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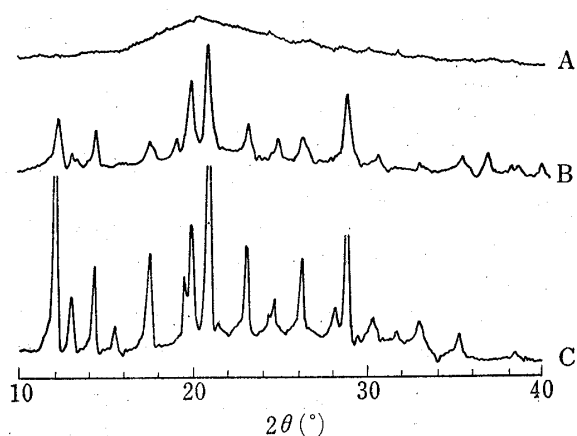


Fig. 1. Comparison of X-Ray Diffraction Spectra of Three Preparations

Key: A, tolbutamide: PVP=1:3 coprecipitate;  
B, tolbutamide: PVP=1:3 physical mixture;  
C, tolbutamide alone.

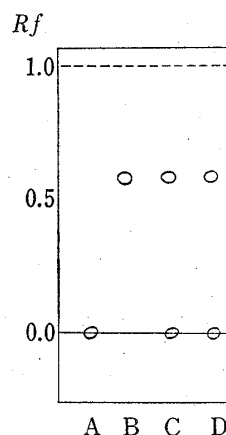


Fig. 2. Thin-Layer Chromatogram of PVP (A), Tolbutamide (B), 1:3 Coprecipitate (C) and 1:3 Physical Mixture (D)

Plate: silica gel (DC-Fertigplatten Kieselgel  
[ 60 F<sub>254</sub>, Merck).  
Developing solvent: *n*-butanol: chloroform:  
diethylamine=9:9:1.

Bottari *et al.*<sup>15)</sup> reported the thermal decomposition of tolbutamide in a series of aliphatic alcohol at 80°. They showed that 42% of tolbutamide was decomposed in 2 hr in ethanol at 80°. Tolbutamide in the coprecipitate prepared by Said *et al.*<sup>12)</sup> might have been degraded during the preparation in ethanolic solution at a high temperature. Tolbutamide in the coprecipitate prepared by the authors, on the other hand, was not degraded because the coprecipitate was prepared by removing the solvent *in vacuo* at lower temperature.

In PVP matrix, tolbutamide may be present without its crystalline structure. PVP in the solution might inhibit the crystallization of tolbutamide during the evaporation process of the solvent from the solution.<sup>16)</sup>

### Quantitative Determination of PVP

ANS does not fluoresce in water, but fluoresces strongly both in organic solvents and in water when bound to certain native protein.<sup>17)</sup> Kono *et al.*<sup>11)</sup> reported that ANS was fluorescent when bound to water soluble synthetic polymers such as polyethylene glycol and PVP. Figure 3 shows the calibration curves of PVP. Good straight lines were obtained over 20—220  $\mu\text{g/ml}$  of PVP in J.P.IX disintegration medium No. 2, and over 40—250  $\mu\text{g/ml}$  in the medium No. 1.

As the absorbance of PVP was found to follow Lambert-Beer rule<sup>18)</sup> at 227 nm, it is possible to correct for the absorbance due to PVP.

### Dissolution Studies

In our previous papers,<sup>8,9)</sup> PVP of the lower molecular weight has been shown to give faster dissolution among coprecipitates prepared from PVP of different molecular weight. PVP K-15 was therefore used to prepare the coprecipitate in this study. The dissolution behaviors of tolbutamide alone, coprecipitates and the physical mixture in J.P.IX disintegration medium No. 1 (pH 1.2) at 37° are shown in Fig. 4.

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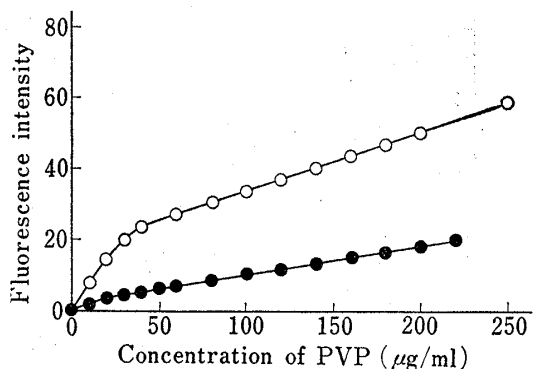


Fig. 3. Calibration Curves for PVP in J.P. IX Disintegration Medium No. 1 (●) and Medium No. 2 (○)

The concentration of ANS was 10  $\mu\text{g/ml}$ .  
Each point represents the mean of three determinations.

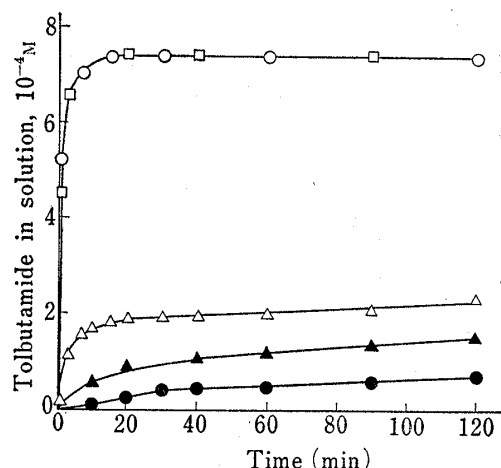


Fig. 4. Dissolution Profiles of Tolbutamide from Test Preparations in Disintegration Medium No. 1 (J.P.IX) at 37°

Key: ○, tolbutamide: PVP=1: 10 coprecipitate,  
□, 1: 5 coprecipitate,  
△, 1: 3 coprecipitate,  
▲, 1: 3 physical mixture,  
●, tolbutamide alone.

Each point represents the mean of three determinations.

These plots show the concentration attained in solution for each preparation containing 200 mg of tolbutamide. Since the drug solubility in this medium at 37° was  $5.64 \times 10^{-4} \text{ M}$ , 200 mg of the drug in 1 liter of the dissolution medium corresponded to about 1.3 times its solubility. The coprecipitates exhibited faster dissolution rates than tolbutamide alone or the physical mixture. The dissolution rate of tolbutamide in the coprecipitate was greater when the ratio of drug to PVP was smaller. Coprecipitate of 1: 5 or 1: 10 weight ratio, completely dissolved within 20 min. The solubility of tolbutamide in 0.1% and 0.2% PVP solution of this medium was  $5.71 \times 10^{-4} \text{ M}$  and  $5.78 \times 10^{-4} \text{ M}$ , respectively. The concentration following the dissolution of these coprecipitates exceeded the solubility of tolbutamide in this medium, indicating supersaturation. This supersaturated solution was stable, and recrystallization did not occur under the experimental conditions. Coprecipitate of 1: 3 weight ratio did not show as fast dissolution of tolbutamide as 1: 5 or 1: 10 coprecipitate, and the tolbutamide in the coprecipitate did not dissolve completely in 120 min. Tolbutamide in the physical mixture dissolved faster than tolbutamide alone. This may be explained by dispersion effect by powder<sup>19)</sup> and by possible lowering of the surface tension of the medium by PVP, resulting in better wetting of tolbutamide crystal surface.

Figure 5 shows the dissolution curves of the preparations in J.P.IX disintegration medium No. 2 (pH 7.5) at 37°.

Since the  $pK_a$  value of tolbutamide is 5.3 at 37.5°,<sup>20)</sup> most of the drug molecule is considered to be ionized in this medium (pH 7.5). The dissolution rate of tolbutamide alone was greater than that in the medium No. 1. Following the dissolution of 1: 5 or 1: 10 coprecipitate, tolbutamide in the preparation dissolved quickly and completely as in the medium No. 1.

### In Vivo Absorption Studies

Tolbutamide in the tolbutamide-PVP coprecipitate was expected to exhibit a better bioavailability because of its greater dissolution rate. Tolbutamide alone and tolbutamide-PVP coprecipitate were orally administered to rabbits to evaluate their absorption charac-

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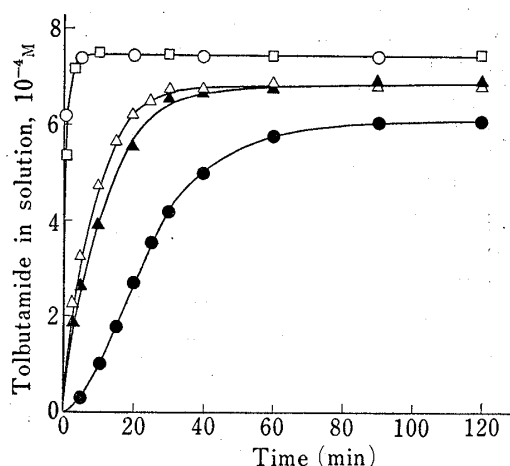


Fig. 5. Dissolution Profiles of Tolbutamide from Test Preparations in Disintegration Medium No. 2 (J.P. IX) at 37°

Key: ○, tolbutamide: PVP=1: 10 coprecipitate.  
 □, 1: 5 coprecipitate.  
 △, 1: 3 coprecipitate.  
 ▲, 1: 3 physical mixture.  
 ●, tolbutamide alone.

Each point represents the mean of three determinations.

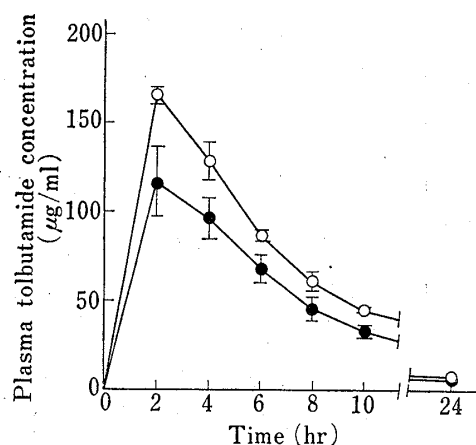


Fig. 6. Plasma Levels of Tolbutamide following Oral Administration of the Coprecipitate (○) and Tolbutamide Alone (●)

Each point represents the mean  $\pm$  S.E.M. of three rabbits.

TABLE I. Plasma Level of Tolbutamide compared by the Paired *t*-Test

Time hr	Plasma level, $\mu\text{g/ml}$								Significance level
	Coprecipitate Rabbit				Control (tolbutamide powder) Rabbit				
	A	B	C	Mean $\pm$ S.E.M.	D	E	F	Mean $\pm$ S.E.M.	
2	173.3	164.1	157.8	165.1 $\pm$ 4.5	150.1	116.2	83.9	116.7 $\pm$ 19.1	$p < 0.01$
4	150.9	124.6	112.7	129.4 $\pm$ 11.3	96.7	115.6	75.7	96.0 $\pm$ 11.5	$p < 0.025$
6	91.9	83.3	85.2	86.8 $\pm$ 2.6	76.0	75.4	52.2	67.9 $\pm$ 7.8	$p < 0.025$
8	61.8	69.2	52.4	61.1 $\pm$ 4.9	53.7	51.4	33.2	46.1 $\pm$ 6.5	$p < 0.05$
10	44.3	43.1	46.9	44.8 $\pm$ 1.1	37.4	35.5	25.6	32.8 $\pm$ 3.7	$p < 0.005$
24	8.5	5.6	4.5	6.2 $\pm$ 1.2	6.3	7.4	3.2	5.6 $\pm$ 1.3	not significant
AUC 0-24 hr ( $\mu\text{g hr/ml}$ )	1370	1266	1223	1286 $\pm$ 44	1096	1053	717	955 $\pm$ 120	$p < 0.01$

teristics. Tolbutamide-PVP coprecipitate (1: 5) was used in the absorption study. Figure 6 shows the mean plasma levels of tolbutamide following the oral administration of two preparations in three rabbits.

Plasma level of tolbutamide following administration of the coprecipitate was significantly higher than that of control (tolbutamide powder) up to 10 hr (Table I). The variation of the plasma levels of tolbutamide was considerably smaller when the coprecipitate was administered. Bioavailability of tolbutamide from tolbutamide-PVP coprecipitate up to 10 hr post-administration, was 1.37 times as much as that from tolbutamide alone.

From the *in vitro* dissolution behaviors of the coprecipitate and tolbutamide alone, and the *in vivo* absorption data, the following consideration may be made. After oral administration of the coprecipitate, tolbutamide dissolves rapidly in the gastrointestinal fluid. Tolbutamide in the fluid may be absorbed rapidly from the gastrointestinal tract without recrystallization. Following the administration of tolbutamide alone, however, the drug dissolved slowly, and this dissolution rate of tolbutamide may be rate-determining step in

the absorption process in individual animals with a slightly different physiological conditions, resulting in large variation in bioavailability.

The present investigation showed that improvement of the dissolution rate of tolbutamide by coprecipitation of the drug with PVP resulted in increase in the bioavailability of the drug with less variation. The authors already demonstrated improvement of the dissolution characteristics of some drugs<sup>8,9)</sup> by coprecipitation with PVP. The method of increasing the dissolution characteristics of poorly water-soluble drugs by coprecipitation with PVP may be widely applicable to other drugs which exhibit poor bioavailability because of slow dissolution.

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