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Effects of Macromolecular Additives and Urea on the Intestinal Absorption of Acetaminophen in Rats

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The effects of sodium carboxymethylcellulose (CMC), sodium arginate (SA), methylcellulose (MC) and polyvinylpyrrolidone (PVP) on the intestinal absorption of acetaminophen *in situ* were investigated in rats. Among the macromolecular additives, only PVP exhibited an inhibitory effect on the absorption of the drug. Studies *in vitro* (solubility and dialysis experiments) showed that there is an interaction between acetaminophen and PVP. The inhibitory effect of PVP on the intestinal absorption of acetaminophen was suppressed by the addition of urea. Dialysis data showed that urea tends to reduce the binding between acetaminophen and PVP.

Keywords——intestinal absorption; polyvinylpyrrolidone; macromolecular additives; acetaminophen; interaction; urea; colorimetric assay; carboxymethylcellulose; methylcellulose; sodium arginate

Natural or synthetic macromolecular additives are generally used in the preparation of several dosage forms of drugs. When these preparations are disintegrated and dissolved, it is important to judge the interactions between these additives and drugs in solution, because such interactions often influence the absorption of the drug from the gastrointestinal tract following the administration of the preparations. The macromolecular additives used must be inert and nontoxic. There are many reports related to the interactions between macromolecular additives and organic compounds, *i.e.*, drugs[2] or dyes,[3] or inorganic compounds such as iodine.[4] Only a few reports, however, are available on changes in the bioavailability of drugs in the presence of such additives caused by the interaction between the additives and the drugs.[5]

The authors investigated the effect of some macromolecular additives on the absorption of acetaminophen, an antipyretic drug, in rats. The effect of urea, which can show strong hydrogen bonding to other compounds, on the absorption of acetaminophen in the presence of polyvinylpyrrolidone was also studied.

In addition to the *in situ* absorption studies, interaction of these macromolecular additives and acetaminophen was studied *in vitro* by the dialysis and solubility methods. The effect of urea on the interaction between the macromolecular additives and acetaminophen was also studied *in vitro*.

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2) *a*) G. Jürgensen E. and P. Speiser, *Acta Pharm. Suecica*, **4**, 185 (1967); *b*) S.J.A. Kazmi and A.G. Michell, *J. Pharm. Pharmacol.*, **23**, 482 (1971); *c*) J. Cohen and J.L. Lach, *J. Pharm. Sci.*, **52**, 132 (1963).

3) *a*) R.E. Phares, Jr., *J. Pharm. Sci.*, **57**, 53 (1968); *b*) J.C. Anderson and G.M. Boyce, *J. Pharm. Sci.*, **58**, 1425 (1969).

4) K. Takikawa, M. Nakano, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **26**, 1370 (1978).

5) *a*) P. Kahela, A. Perälä-Suominen, M. Ahomaa, and P. Voutilainen, *Farm. Aikakusl.*, **80**, 331 (1971); *b*) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 976 (1965).

Experimental

Materials——Acetaminophen, J.P. IX grade (Pyrinazin, Yamanouchi Pharmaceutical Co., Tokyo, lot WEW7) was recrystallized from 95% ethanol. The melting point of the acetaminophen sample was 169°. Macromolecular additives used were polyvinylpyrrolidone K-30 (PVP) (Daiichi Pure Chemicals Co., Tokyo), methylcellulose 500 cps (MC) (Wako Pure Chemical Ind., Osaka), sodium carboxymethylcellulose (CMC) and sodium arginate (SA) (Koso Chemicals Co., Tokyo). Urea and other reagents were of reagent grade.

***In Situ* Intestinal Absorption of Acetaminophen in Rats**——Male Wistar rats (180—220 g) fasted for 10—12 hr were anesthetized with Nembutal sodium solution (Abbott Laboratories, North Chicago) and an incision of about 5 cm was made in the abdominal area along the median line. Vinyl tubes were inserted at the upper side of the duodenum as an inlet for the sample solution, and at the lower side of the ileum as an outlet for the solution. Sample solution (450 μg/ml acetaminophen in pH 6.4 isotonic phosphate buffer, or in isotonic solution formed by mixing the urea solution and pH 6.4 phosphate buffer, 50 ml) was recirculated at a flow rate of 5 ml/min. After recirculating the solution for one hour, the rat was sacrificed, and the solution was rinsed out with saline. The solution was diluted appropriately prior to colorimetric assay.

Solubility Measuremcnt——About 2 gram of acetaminophen sample and about 20 ml of 0.17 M phosphate buffer (pH 6.4) were placed in the 50 ml stoppered glass flask in a shaking water-bath (Taiyo, M-1^{N}) maintained at a specified temperature, and shaken (100 rpm) for 2 days. After equilibration, sample solutions were removed with a syringe, and filtered quickly through a membrane filter (Toyo, TM-4, pore size 0.2 μm). The solutions were diluted appropriately with 0.1 N NaOH prior to assay for acetaminophen at 260 nm using a Hitachi-Perkin Elmer 139 spectrophotometer. No significant absorbance was found of the macromolecular additives over the range of concentrations used for the drug analysis.

Equilibrium Dialysis——Equilibrium dialysis was used to evaluate the interaction between acetaminophen and the macromolecular additives in aqueous solution. Possible preservatives in Visking cellulose membrane (18/32 type, Union Carbide Corp., Chicago) were leached out by frequently changing the water at about 60° for at least three days. Twenty ml of the macromolecular solution was pipetted into the dialysis sac. Both edges of the sac were tied with rubber bands. The sac and 20 ml of the drug solution (in pH 6.4 phosphate buffer, or in a mixture of the urea solution and pH 6.4 phosphate buffer) were placed in a 50 ml stoppered glass flask. The flask was set in a shaking water-bath maintained at 37.0±0.1°, and shaken (100 rpm) for five days. Sample solutions were pipetted out and diluted appropriately with 0.1 N NaOH then analyzed as described above. Drug bound (%) to the macromolecules was calculated as follows,

$$\text{Bound } (\%) = \frac{[C_{\text{I}}]-[C_{\text{II}}]}{[C_{\text{I}}]} \times 100$$

where $[C_{\text{I}}]$ and $[C_{\text{II}}]$ are the equilibrium drug concentrations in the macromolecular solution in the sac and the macromolecular free solution outside the sac, respectively. Cellulose membrane did not show any measurable degree of binding or interaction with acetaminophen.

Quantitative Determination of Acetaminophen in the Sample Solution Recirculated through the Intestine——Acetaminophen in the recirculated sample solution was analyzed colorimetrically following Brodie's method[6] with a few modifications. The method is shown in Chart 1.

3 ml of sample
- 5 g of NaCl
- 1 ml of 0.5 NHCl
- 30 ml of isoamyl alcohol-ethylene dichloride (10: 90 V/V)
- shake for 30 min
- centrifuge, 3000 rpm, 10 min

20 ml of organic layer
- 5 ml of 0.1 N NaOH
- shake for 20 min
- centrifuge, 2000 rpm, 10 min

2 ml of aqueous layer
- 2 ml of 5 N HCl
- keep at 100° for 40 min
- 2 ml of 5 N Na_2CO_3
- 2 ml of 3.5% (W/V) phenol in N NaOH
- 2 ml of 2 N Na_2CO_3 contg. small amt. of Br_2
- 10—80 min

measure absorbance at 630 nm

Chart 1. Analytical Method for Acetaminophen in Biological Samples

6) B.B. Brodie and L. Axelrod, *J. Pharmacol. Exp. Ther.*, **94**, 22 (1954).

The chief modifications of Brodie's method were in the selection of the extraction solvent and the color development method. The color development method using α-naphthol reported by Brodie *et al.* failed to give a stable color. Our method using indophenol formation did give a stable color.

Results and Discussion

Effect of Macromolecular Additives on the Intestinal Absorption of Acetaminophen in Rats

The effects of some macromolecular additives on the intestinal absorption of acetaminophen are shown in Fig. 1.

The percentage of acetaminophen unabsorbed after recirculating the acetaminophen solution without additives (control) was 41.1±1.3 (mean±SEM, n=11). CMC, MC or SA did not have any significant effect on the intestinal absorption of acetaminophen. PVP, on the other hand, inhibited the absorption of acetaminophen significantly. When the concentrations of PVP were 2% and 5%, the percentages of the drug unabsorbed were 51.7±2.7 and 54.3±2.3, respectively. The viscosity of 2% PVP solution was smaller than those of 0.5% solutions of CMC, MC or SA. It was not practical to study the effect of CMC, MC and SA at levels of more than 2% on the intestinal absorption because of their high viscosities. As the intestinal absorption of acetaminophen was inhibited in the presence of PVP, the effects of these additives on the solubility of acetaminophen was studied.

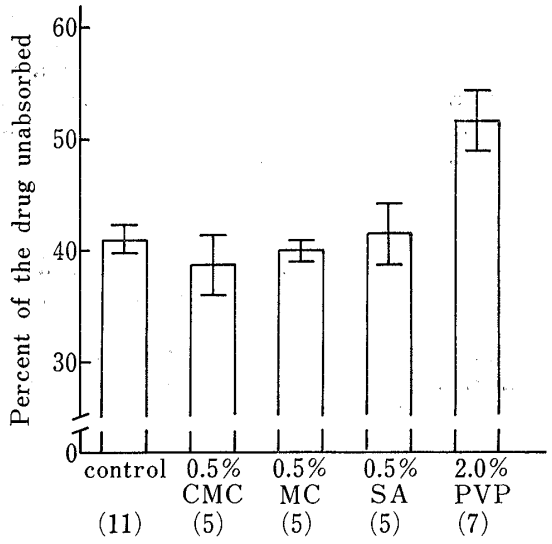

Fig. 1. Effect of Various Macromolecules on the Intestinal Absorption of Acetaminophen

Vertical bars represent standard errors of the mean for the number of experiments shown in parentheses.

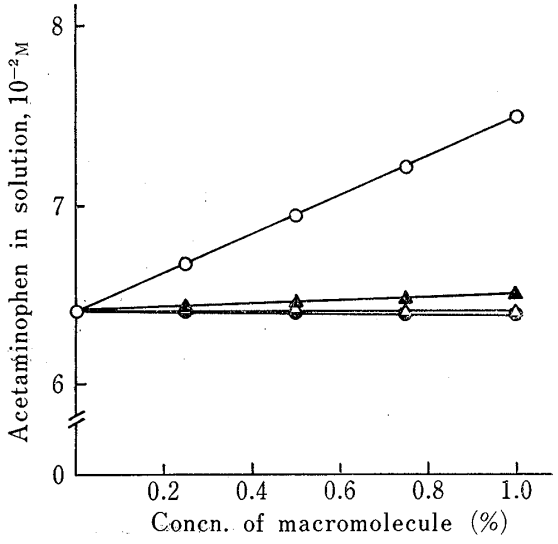

Fig. 2. Effects of Various Macromolecules on the Solubility of Acetaminophen

Key: ○, PVP; ▲, MC; △, CMC; ●, SA.
Each point represents the mean of three determinations.

Interaction of the Macromolecular Additives with Acetaminophen

The interaction between the macromolecular additives and acetaminophen in aqueous solution was studied by the solubility method. Hydrophilic colloids of natural or synthetic macromolecules have been reported to be coacervated by addition of some electrolytes[7] or organic compounds.[8] The authors have reported on the coacervation of PVP aqueous solution by the addition of some electrolytes or aromatic compounds.[9] The solubilities of acet-

7) H. Denel and J. Solm, *Kolloid-Z.*, **124**, 1 (1951).
8) *a*) B. Jirgensons, *J. Polymer Sci.*, **3**, 555 (1962); *b*) D. Guttman and T. Higuchi, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 659 (1956).
9) H. Sekikawa, R. Hori, T. Arita, K. Ito, and M. Nakano, *Chem. Pharm. Bull.* (Tokyo), **26**, 2489 (1978).

aminophen were studied at 15.0±0.1° to confirm that coacervation of the macromolecular solution did not occur. The results are shown in Fig. 2.

CMC, MC or SA hardly affected the solubility of acetaminophen, whereas PVP increased the solubility of the drug with increase in its concentration.

The dialysis data on the binding of acetaminophen to PVP were evaluated using a rearranged form of the Langmuir isotherm, as suggested by Klotz *et al.*,[10] as follows.

$$1/r = 1/nKC + 1/n$$

Where r is the number of moles of acetaminophen bound per base mole (monomer unit) of PVP polymer, n is the number of binding sites per base mole, C is the molar concentration of free acetaminophen at equilibrium and K is the binding constant (in liters/mole) of acetaminophen at the binding sites. The results are shown in Fig. 3, where $1/r$ is plotted against $1/C$; the slope of this plot is $1/nK$, and the intercept on the ordinate axis is $1/n$.

The binding constant K was 23 M^{-1} and $1/n$ was 18.5. These values agree reasonably well with the values derived for the interaction between benzoic acid or resorcinol and PVP reported by Molineux and Frank.[11]

The interaction between acetaminophen and PVP in aqueous solution may be considered to be one of the factors producing the inhibitory effect of PVP on the intestinal absorption of acecaminophen. Since only PVP exhibited an inhibitory effect among the macromolecular additives used, the acetaminophen-PVP system was studied.

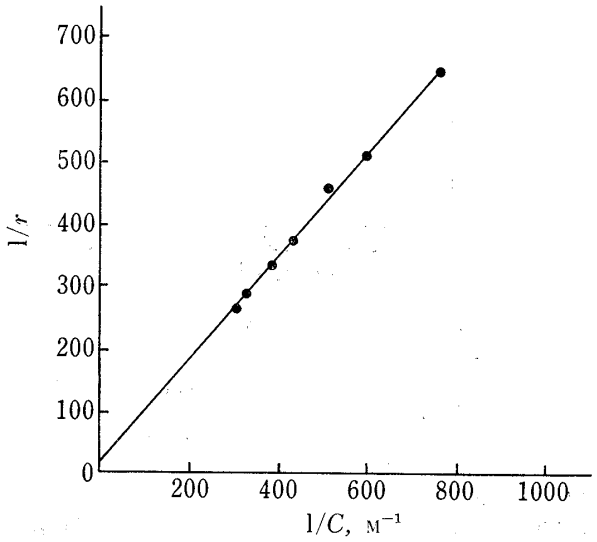

Fig. 3. Moles of PVP per Mole of Bound Acetaminophen versus Reciprocal Molar Concentration of Free Acetaminophen at 37°

Each point represents the mean of three determinations. The concentration of PVP was 5.0%.

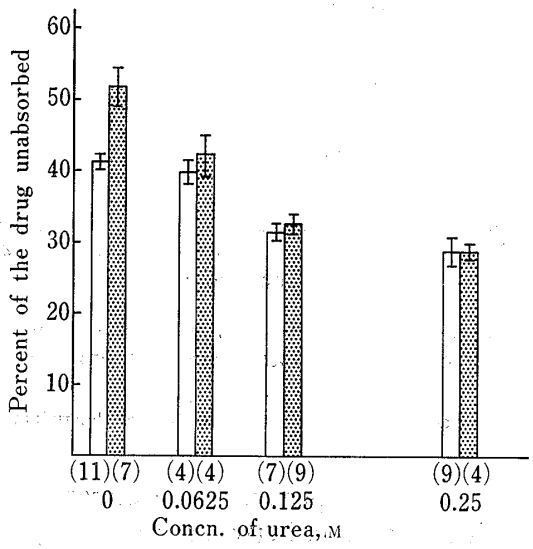

Fig. 4. Effects of PVP and Urea on the Intestinal Absorption of Acetaminophen in Rats

Key: ☐, control; ▨, with 2% PVP. Vertical bars represent standard errors of the mean for the number of experiments shown in parentheses.

Effect of Urea on the Intestinal Absorption of Acetaminophen in the Presence of PVP

Klotz and Shikama[12] reported on the inhibitory effect of urea on the interaction between PVP and methyl orange in aqueous solution. Emerson and Holtzer[13] reported on the effect of urea on the critical micelle concentration of some surfactant solutions.

10) I.M. Klotz, F. Walker, and R. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946).
11) P. Molineux and H.P. Frank, *J. Am. Chem. Soc.*, **83**, 3169 (1961).
12) I.M. Klotz and K. Shikama, *Arch. Biochem. Biophys.*, **123**, 551 (1968).
13) M.F. Emerson and A. Holtzer, *J. Phys. Chem.*, **71**, 3320 (1967).

The authors anticipated that the inhibitory effect of PVP on the intestinal absorption of the drug might be overcome by the addition of urea. Figure 4 shows the effect of urea on the intestinal absorption of acetaminophen in the presence and absence of PVP.

The inhibitory effect of PVP on the intestinal absorption of acetaminophen was suppressed by the addition of urea. When the urea concentration was 0.25 M, there was no significant difference of absorption rate between the two cases (presence and absence of PVP).

The percentage of acetaminophen absorbed increased with increase in the urea concentration. An accelerating effect of urea on intestinal absorption has been reported when aminopyrine was administered with urea in rabbits.[14]

Effect of Urea on the Interaction between Acetaminophen and PVP.

The mechanism of the inhibitory effect of urea on the interaction between acetaminophen and PVP may involve direct interaction of urea with acetaminophen, which also interacts with PVP. Fig. 5 shows the effect of urea on the solubility of acetaminophen.

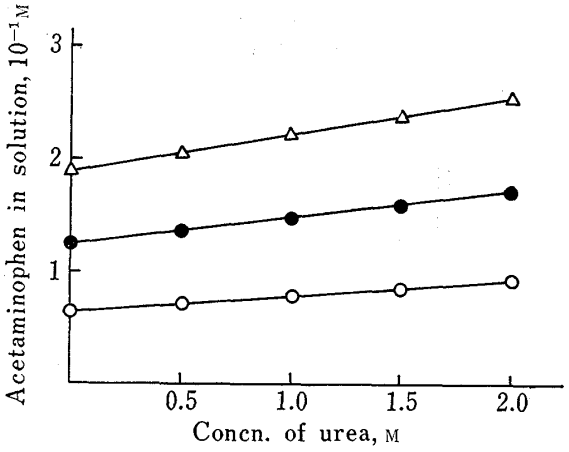

Fig. 5. Effect of Urea on the Solubility of Acetaminophen at Three Temperatures

Key: △, 48°; ●, 37°; ○, 15°.
Each point represents the mean of three determinations.

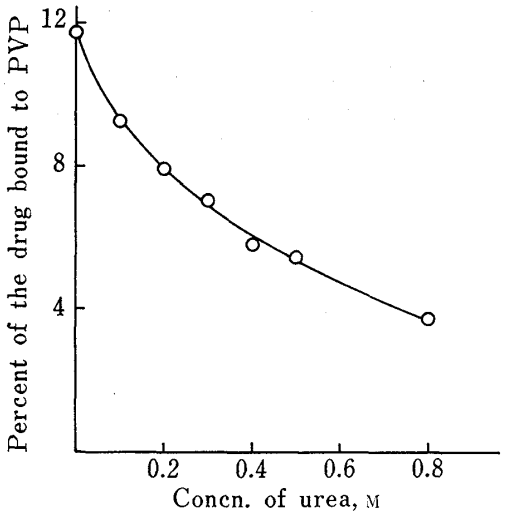

Fig. 6. Effect of Urea on the Binding of Acetaminophen to PVP at 37°

Each point represents the mean of three determinations.
The concentration of PVP was 5.0%.

At each temperature, the increase in the solubility of acetaminphen was so small that complex formation between acetaminophen and urea may not be a major factor affecting the interaction between acetaminophen and PVP. Feldman and Gibaldi[15] reported on the solubilizing effect of urea derivatives on benzoic acid and salicylic acid, and claimed that the solubilizing effect of the urea derivatives correlated with the destruction of water structure around the drug molecules.

Figure 6 shows the effect of urea on the binding between acetaminophen and PVP studied by the dialysis method. The decrease in the percentage of acetaminophen bound to PVP with increase in the urea concentration is shown.

The authors have reported on the effects of electrolytes, aromatic compounds and urea derivatives on the cloud point of PVP aqueous solution in a study of the interaction between these compounds and PVP.[9] Aromatic compounds lowered the cloud point of the solution, whereas urea derivatives elevated it. This phenomenon suggests that aromatic compounds which interact strongly with PVP might release water hydrating the PVP molecule. On

14) S. Naito, *Yakugaku Kenkyu*, **35**, 25 (1963).
15) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **56**, 370 (1967).

the other hand, urea derivatives might influence the structure of water in which the interaction between the other compounds and PVP takes place, and consequently reduce the binding tendency.[16]

One of the dosage forms containing PVP was studied to compare the bioavailability of sulfisoxazole-PVP physical mixture and sulfisoxazole alone in human subjects.[17] Following the oral administration of sulfisoxazole-PVP physical mixture, urinary excretion of total sulfisoxazole up to 24 hr was 86% of the urinary excretion following the administration of sulfisoxazole alone. Similar results were reported in oral toxicity studies of digitoxin; *i.e.*, when a digitoxin-PVP physical mixture was administered to rats, mortality was smaller than in the case when digitoxin alone was administered.[18] These results indicate that PVP in these cases might inhibit the absorption of the drug from the gastrointestinal tract.

The present investigation showed that it is necessary to examine the interaction between macromolecular additives and the drug when macromolecular additives are used in the dosage forms, because they may influence the intestinal absorption of the drug. From the results of addition of urea to acetaminophen solution in the presence of PVP, it is expected that the intestinal absorption of a drug inhibited by macromolecular additives may be restored by the addition of urea to the formulation.

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16) K. Kristiansen, M. Nakano, N.I. Nakano, and T. Higuchi, *J. Pharm. Sci.*, **59**, 1103 (1970).
17) H. Sekikawa, M. Nakano, and T. Arita, *Yakugaku Zasshi*, **98**, 62 (1978).
18) E.I. Stupak and T.R. Bates, *J. Pharm. Sci.*, **62**, 1806 (1973).