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# Pharmaceutical Approach to the Oral Dosage Form of Macromolecules: Effect of Bile Salts and Oil-in-Water Emulsions on the Intestinal Absorption of Urogastrone in the Rat[1)]

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The absorption of Urogastrone, glycoprotein with gastric antisecretory activity, from the rat intestine was studied using the Shild technique, in which the indirect assay of its appearance in the blood stream was facilitated by a concomitant lowering of gastric acid secretion. The intravenous or intraperitoneal administration of Urogastrone produced 40% inhibition of control levels of $H^+$ output. In the intrajejunal administration, it caused only a little inhibitory response. However, when Urogastrone was administered intrajejunally with various bile salts or oil-in-water emulsions, strong inhibitory response of gastric $H^+$ secretion was elicited. Trioctanoin emulsion was most effective on the activity of intrajejunally administered Urogastrone followed by olive oil, diethyl phthalate and liquid paraffin, respectively. The results suggested that orally active dosage forms for poorly absorbable macromolecules such as Urogastrone might be prepared by pharmaceutical modification.

**Keywords**——Intestinal absorption; macromolecules; Urogastrone; gastric antisecretory activity; oil-in-water emulsion; trioctanoin; bile salts; gastric glycoprotein; tetragastrin

The general impermeability of the mature gastrointestinal tract to the macromolecules such as protein and polysaccharide has long been recognized, and can be satisfactorily explained on the basis of the physical properties, namely, high molecular weight and low lipid solubility. It has been demonstrated that the small intestine of certain neonatal mammalian species could absorb large quantities of macromolecules by specialized transport systems as a transient phenomenon for acquiring passive immunity.[3-5)] However, there is now increasing evidence that macromolecules can cross the mucosal barrier of adult animals in biological active and immunogenic quantities.[6,7)] Using the everted gut sac technique or ligated intestinal loops, Isselbacher and his coworkers[6)] demonstrated that a small but significant fraction of intraluminal bovine serum albumin and horseradish peroxidase managed to traverse the mature mammalian gut and reach the intestinal lymph and portal blood intact. Engel, *et al.*[8)] showed that the intestinal absorption of insulin and heparin was enhanced by incorporating them into the form of emulsions. Patel and Ryman[9)] reported that liposome-entrap-

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2) Location: *Kasumi, 1-2-3, Hiroshima.*
3) J.P. Kraehenbuhl and M.A. Campiche, *J. Cell Biol.*, **42**, 345 (1969).
4) I.G. Morris, "Handbook of Physiology," Section 6, Alimentary Canal, Vol. 3, ed. by C. F. Code, Am. Physiol. Soc., Washington D.C., 1968, p. 1491.
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8) R.H. Engel, S.J. Riggi, and M.J. Fahrenbach, *Nature* (London), **291**, 856 (1968); R.H. Engel and M.J. Fahrenbach, *Proc. Soc. Exp. Biol. Med.*, **129**, 772 (1968); R.H. Engel and S.J. Riggi, *J. Pharm. Sci.*, **58**, 1372 (1969).
9) H.M. Patel and B.E. Ryman, *Febs Letters*, **62**, 60 (1976).

ped insulin, when administered orally, was able to reduce the blood-glucose level in diabetic rats, whereas the same amount of free insulin administered orally had no effect on blood-glucose level.

In this investigation, an *in vivo* model was utilized to show the absorption of Urogastrone, a known glycoprotein with gastric antisecretory activity,[10,11] since the indirect assay of its appearance in the blood stream was facilitated by a concomitant lowing of gastric acid secretion. Furthermore, in order to improve the bioavailability of poorly absorbable macromolecules such as Urogastrone, the effect of various dosage forms on the activity of Urogastrone was systematically studied.

## Experimental

**Materials**——Urogastrone extracted from pregnant horse by the method of Gray, *et al.*[10] was kindly supplied by Nippon Shinyaku Co., Ltd., Kyoto. This water soluble glycoprotein (isoelectric point: 4.2—4.5) is stable during the experiment. Gastric glycoprotein (G.G.P.) extracted from the mucosa of the third stomach of the finback whale was the gift from Nissui Pharmaceutical Co., Ltd., Tokyo[12]. Tetragastrin was purchased from San-a Pharmaceutical Co., Ltd., Tokyo. Sodium cholate, sodium deoxycholate, olive oil, diethyl phthalate (Nakarai Chemicals, Ltd.), trioctanoin, tween 80 (Tokyo Kasei Kogyo Co., Ltd.), and liquid paraffin (E. Merck Japan Ltd.) were used as supplied. Sodium taurocholate was synthesized by the method of Norman[13] and was chromatographycally pure. All other chemicals used were of analytical grade and were obtained commercially.

**Bioassay of Urogastrone and G.G.P.**——The assays for the gastric antisecretory activity of Urogastrone and G.G.P. were carried out on the procedure for the continuous recording of gastric secretion described by Ghosh and Shild.[14] Male Wistar albino rats, weighing 250—300 g, were fasted for 10 hr prior to the experiments, but water was allowed *ad libitum*. Under pentobarbital anesthesia, the stomach was canulated at the cardia by way of the esophageal tube, 2 mm o.d. and 20 cm long, and done at the pylorus in the same way. After the stomach washing with physiological saline, the stomach was perfused with pH 6.6 buffer ($6.16\times10^{-4}$ M $Na_2HPO_4$, $9.2\times10^{-5}$ M citric acid, $1.54\times10^{-1}$ M NaCl) at the rate of 1 ml/min. The pH of the perfusion solution was recorded periodically and the perfusate collected for 10 min was titrated against 0.01 N NaOH. Until a plateau of $H^+$ secretion was established, control collection were made for at least 60 min. Then, tetragastrin (2—6 μg/kg) used as the secretory stimulant was administered intravenously by continuous infusion or repeated single injection. In this way, a cumulative pH change was produced after each dose of stimulant and the amount of acid secreted above the basal concentration was measured. The activity of Urogastrone and G.G.P. from various routes of administration were assayed by their effect in reducing tetragastrin-induced gastric acid secretion. In the case of continuous infusion of tetragastrin, inhibitory solutions were given after stimulant and in the case of single injection of tetragastrin, inhibitory solutions were administered before stimulant. The inhibitory effect of Urogastrone and G.G.P. was estimated in terms of $H^+$ output, expressed as a ratio to the mean of $H^+$ output in control periods received tetragastrin alone.[15]

**Preparation of Drug Solutions**——Urogastrone and G.G.P. were dissolved in distilled water for injection, and administered to the Shild rats intravenously or intraperitoneally. For the intrajejunal administration of Urogastrone and G.G.P., they were prepared in the form of micellar solutions with various bile salts and oil-in-water emulsion containing oils and suitable surfactants.

**Stability of Urogastrone and G.G.P. in the Intestine**——Urogastrone (40 mg/4 ml) and G.G.P. (30 mg/4 ml) were injected to the intestinal loops of rats. The solutions were withdrawn after 10 min, and washed with physiological saline. The washings were combined to the perfusate and made up to 8 ml with physiological saline. The activity of their solutions was measured by the intraperitoneal administration to the Shild rats.

10) J.S. Gray, F. Wieczorowski, J.A. Wells, and S.C. Harris, *Endocrinol.*, 30, 129 (1942).
11) G. Lugaro, P. Pasta, M.M. Casellato, G. Mazzola, and G. Carrea, *Biochem. J.*, **153**, 641 (1976).
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15) R.A. Pederson and J.C. Brown, *Gastroenterol.*, **62**, 393 (1972).

## Results and Discussion

Figure 1 shows the titration curve of the buffered perfusion solution passing through the unstimulated stomach. In the pH values 3.8 to 6.6, there was an approximately linear relationship between the amount of HCl added and the pH change. The time integral of the pH deflexion could therefore be assumed to be proportional to the acid secreted. In this study, the values of $H^+$ output were expressed as ratio to the mean of plateau periods stimulated by administration of tetragastrine alone. This method was employed because of varying secretory capacities of different Shild rats to same dose of stimulant.

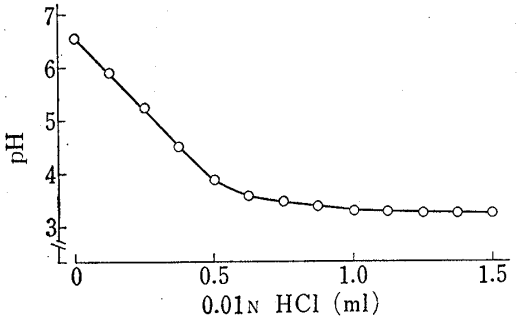


Fig. 1. Titration Curve of the Buffered Perfusion Solution

Figure 2 shows typically the effect of intravenous injection of Urogastrone and G.G.P. on $H^+$ secretion stimulated by continuous infusion of tetragastrin. As is evident from the figure, intravenous injection of Urogastrone (0.5 mg) and G.G.P. (0.3 mg) produced potent inhibition of gastric $H^+$ secretion stimulated by tetragastrin (6 μg/kg/hr). Their effects appeared in 1 hr after administration of inhibitory solutions, and continued for 3 hr. This procedure is useful for obtaining an outline of the pattern of pharmacological response.

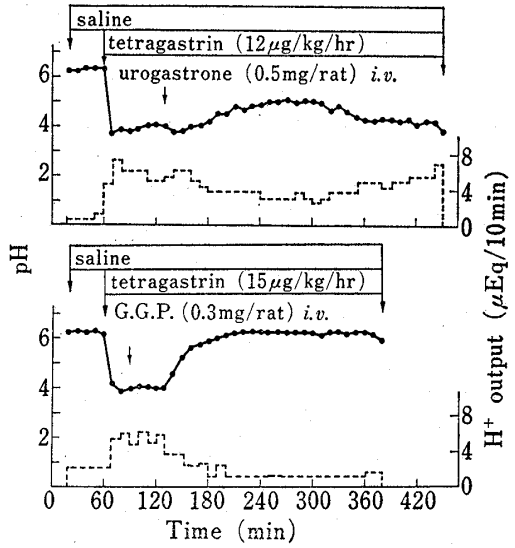


Fig. 2. Effect of Intravenous Injection of Urogastrone and G.G.P. on the $H^+$ Secretion Stimulated by Continuous Infusion of Tetragastrin

——: pH of the gastric perfusate.
------: $H^+$ output into the gastric perfusate.

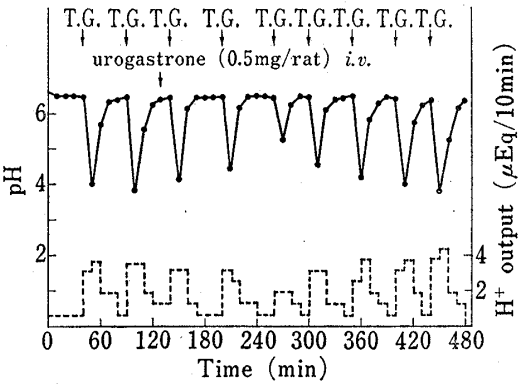


Fig. 3. Effect of Intravenous Injection of Urogastrone on the $H^+$ Secretion Stimulated by Repeated Single Injection of Tetragastrin

——: pH of the gastric perfusate.
------: $H^+$ output into the gastric perfusate.
Six μg/kg of tetragastrin (T.G.) was administered intravenously at each signal.

On the other hand, the effect of intravenous injection of Urogastrone (0.5 mg) on $H^+$ secretion stimulated by repeated single injection of tetragastrin (4 μg/kg) is shown in Fig. 3. The inhibitory effect, 2 hr after the administration of Urogastrone, was more sensitive and reproducible than that of the continuous method, and the effect was estimated quantitatively by comparing the $H^+$ output after each tetragastrin stimulation. In the following experiments, therefore, tetragastrin was administered intravenously by repeated single injection.

In Table I are summarized the effects of different administration routes of Urogastrone and G.G.P. on the $H^+$ output stimulated by tetragastrin. The intravenous(*i.v.*) administra-

Table I. Effect of Administration Routes of Urogastrone and G.G.P. on the $H^+$ Secretion Stimulated by Tetragastrin

| Drug | Administration route | Dose (mg/rat) | $H^+$ output[a] |
|---|---|---|---|
| Saline | *i.v.*[b] | | 1.01±0.01 |
| Urogastrone | *i.v.* | 0.5 | 0.55±0.03 |
| | *i.p.*[c] | 2.5 | 0.68±0.07 |
| | *i.j.*[d] | 20.0 | 0.93±0.05 |
| G.G.P. | *i.v.* | 0.3 | 0.62±0.03 |
| | *i.p.* | 1.3 | 1.13±0.09 |
| | *i.p.* | 2.5 | 0.74±0.03 |
| | *i.j.* | 15.0 | 0.99±0.01 |

*a*) $H^+$ output is expressed as a ratio to the mean of control periods received tetragastrin alone. Each value is the mean ± S.E. in at least 4 animals.
*b*) *i.v.*: intravenous.
*c*) *i.p.*: intraperitoneal.
*d*) *i.j.*: intrajejunal.

tion of Urogastrone and G.G.P. produced 40% inhibition of control levels of $H^+$ output. The degrees of inhibition of $H^+$ output observbed in the intraperitoneal (*i.p.*) administration of Urogastrone and G.G.P. were similar to that produced by intravenous administration. In the intrajejunal (*i.j.*) administration, however, Urogastrone caused only a little inhibitory response but G.G.P. did never. Their inactivity upon intrajejunal administration of Urogastrone and G.G.P. may be the result of poor absorption and/or rapid inactivation in the intrestine.

Therefore, in order to ascertain their stability in the intestine, solutions of G.G.P. and Urogastrone were kept in the intestine for a period of 10 min, then inhibitory response of their solutions was determined by intraperitoneal administration and the results are shown in Fig. 4. Urogastrone produced the inhibition of $H^+$ secretion stimulated by tetragastrin, whereas G.G.P. did not. Based on these results, it was elucidated that G.G.P. was inactivated in the intestine within 10 min, but Urogastrone seemed to be intact. Therefore, inability of intrajejunal administration of Urogastrone to the $H^+$ output inhibition would be due to its poor absorption characteristics.[10,16]

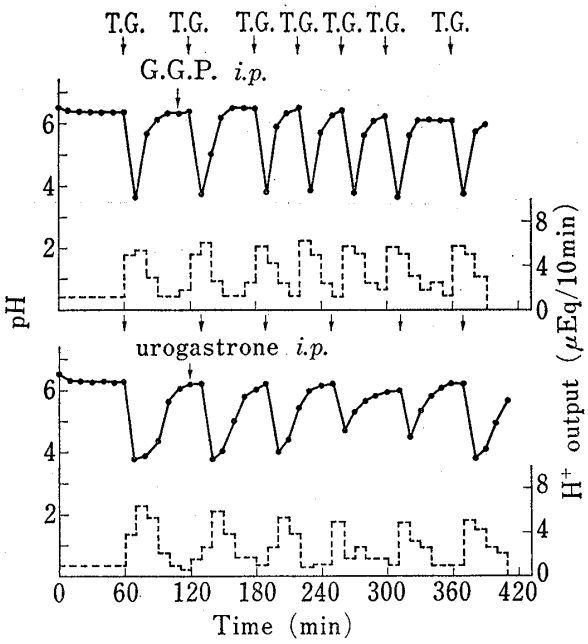


Fig. 4. Gastric Inhibitory Response of Intraperitoneal Administration of Urogastrone and G.G.P. Treated in the Intestine

——: pH of the gastric perfusate.
------: $H^+$ output into the gastric perfusate.
Four μg/kg of tetragastrin (T.G.) was administered intravenously at each signal.
Urogastrone (40 mg/4ml) and G.G.P. (30 mg/4ml) were kept in the intestine for 10 min.

As a means of facilitating intestinal absorption of poorly absorbable macromolecules, the useful dosage forms of Urogastrone were explored. The data obtained with Urogastrone and bile salt-containing preparations tested in Shild rat intestine are presented in Table II. Urogastrone alone or micellar solutions of bile salts alone failed to affect the inhibition of $H^+$ secretion. When Urogastrone was administered intrajejunally with various bile salts,

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Table II. Effect of Various Bile Salts on the Activity of Intrajejunally Administered Urogastrone

| Bile salt | | $H^+$ output[a] | |
|---|---|---|---|
| | | None | Urogastrone (20 mg/rat) |
| Sodium cholate | 20 mM | | 1.06±0.02 |
| Sodium cholate | 30 mM | 1.07±0.05 | 0.58±0.09 |
| Sodium deoxycholate | 20 mM | 1.07±0.03 | 0.68±0.07 |
| Sodium taurocholate | 30 mM | 1.03±0.02 | 0.79±0.03 |

a) $H^+$ output is expressed as a ratio to the mean of control periods received tetragastrin alone. Each value is the mean ± S.E. in at least 4 animals.

strong inhibitory response of gastric $H^+$ secretion was elicited. Sodium cholate and sodium deoxycholate appeared to be more effective than sodium taurocholate. Windsor and Cronheim[17] found that ethylenediaminetetraacetic acid (EDTA) facilitated intestinal absorption of heparin, presumably by chelation of intestinal calcium and magnesium. As bile salts possess the EDTA-like effect to intestinal membrane,[18,19] it may be considered that these effects of bile salts were caused by alteration in the permeability characteristics of the absorptive membrane.

Table III. Effect of Various Emulsions on the Activity of Intrajejunally Administered Urogastrone

| Dosage form | $H^+$ output[a] | |
|---|---|---|
| | None | Urogastrone (20 mg/rat) |
| Aqueous solution | | |
| Distilled water | | 0.93±0.05 |
| Tween 80 | | 0.98±0.06 |
| Emulsion | | |
| Trioctanoin–Tween 80 | 0.98±0.04 | 0.65±0.05 |
| Olive oil–Tween 80 | 1.02±0.01 | 0.79±0.01 |
| Diethylphthalate–Tween 80 | 0.98±0.06 | 0.87±0.01 |
| Liquid paraffin–Tween 80 | 1.04±0.03 | 0.94±0.01 |

Concentration of Tween 80: 0.2% (w/v).
Concentration of oil: 5% (v/v).
a) $H^+$ output is expressed as a ratio to the mean of control periods received tetragastrin alone. Each value is the mean ± S.E. in at least 4 animals.

Table III shows the effect of intrajejunal administration of emulsions containing Urogastrone on the gastric $H^+$ secretion. Neither Urogastrone alone nor Urogastrone in a micellar solution of 0.2% Tween 80 produced the inhibition of $H^+$ secretion significantly. However, when Urogastrone was administered in trioctanoin emulsion, stabilized with Tween 80, potent inhibition of $H^+$ secretion stimulated by tetragastrin was observed. Control emulsions prepared in the absence of Urogastrone gave no response. It is known that trioctanoin is absorbed easily without transformation of the molecule.[20] Olive oil emulsion containing Urogastrone caused significant inhibition of $H^+$ secretion in the rat. On the other hand, Urogastrone in unabsorbable oil emulsions (diethyl phthalate and liquid paraffin) caused

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18) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and A. Okita, *Chem. Pharm. Bull.* (Tokyo), **18**, 1034 (1970); T. Kimura, K. Inui, and H. Sezaki, *Yakuzaigaku*, **31**, 167 (1971).
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slight antisecretory activity. From these results, dietary lipids might play an important role on the intestinal absorption of macromolecules such as Urogastrone.

To reinforce the results which impress the usefulness of trioctanoin emulsion, intrajejunal administration study of emulsions containing Urogastrone was made at various concentrations of trioctanoin. As shown in Fig. 5, the inhibitory effect of Urogastrone was dependent on the emulsion composition, and maximum inhibitory activity was appeared in 5% trioctanoin emulsion.

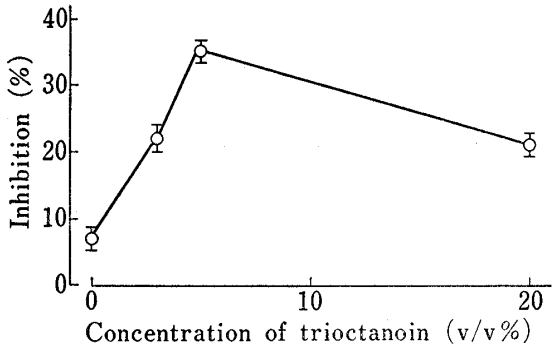


Fig. 5. Effect of Concentration of Trioctanoin on the Activity of Intrajejunally Administered Urogastrone

Each point is the mean ± S.E. in at least 4 animals.

Furthermore, the result of dose response is presented in Table IV. In the case of intrajejunal administration of emulsions containing Urogastrone, the inhibition of $H^+$ secretion was also related with Urogastrone dose. Urogastrone in distilled water produced a little or no inhibitory effect in this dose range.

Table IV. Dose Response of Intrajejunal Administration of Urogastrone

| Dosage form | $H^+$ output[a] Dose of Urogastrone (mg/rat) | | |
|---|---|---|---|
| | 5 | 10 | 20 |
| Distilled water | 1.01±0.02 | 1.03±0.09 | 0.93±0.05 |
| 5 % Trioctanoin–0.2 % Tween 80 emulsion | 0.95±0.07 | 0.79±0.01 | 0.65±0.05 |

a) $H^+$ output is expressed as a ratio to the mean of control periods received tetragastrin alone. Each value is the mean ± S.E. in at least 4 animals.

Engel, *et al.*[8] reported that the intestinal absorption of heparin and insulin could be increased by their intraduodenal administration in an emulsion form containing vegetable oil and a suitable surfactant. In this study, when Urogastrone was administered intrajejunally in an emulsion form, marked inhibition of $H^+$ secretion was observed although not so much as intravenous administration. It is therefore reasonable to assume that Urogastrone, in some association with oil phase, is absorbed in a manner incidental to oil absorption.

Thus, orally active dosage forms for poorly absorbable macromolecules such as Urogastrone may be prepared by pharmaceutical modification. The physicochemical and/or physiological mechanisms by which emulsion dosage forms markedly enhance the absorption characteristics of Urogastrone and other poorly absorbable drugs, is currently under intensive investigation in this laboratory.

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