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Lipid-Containing Oral Dosage Form: Significance of the Intragastric Metabolism of Medium Chain Triglyceride in Relation to the Uniformity of Drug Absorption Rate

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The mechanism of the uniformity of diazepam absorption rate from medium chain triglyceride (MCT) solution was investigated in comparison wtih the absorption from an aqueous suspension.

The metabolism of MCT in the stomach was examined *in vitro* using the enzyme extract of the rat stomach. The content of a metabolite, medium chain monoglyceride (MCM), increased up to 1.5% at pH 3.6 during incubation for 30 min. Experiments using a newly devised gastric emptying simulator suggested that improved dispersibility of MCT solution, by virtue of the formed MCM, resulted in the uniform gastric emptying rate (GER) of this preparation.

In the case of diazepam aqueous suspension, both GER and the gastric absorption rate of the drug seemed to be significant factors in the variable absorption rate of diazepam, though GER was considered to be the major factor. In experiments with the gastric emptying simulator, the GER of the aqueous suspension seemed more variable than that of the MCT solution containing MCM.

Consequently, the apparent uniform absorption rate of diazepam-MCT preparation is considered to be attributable to the relatively uniform GER of the MCT digestion mixture which is formed in the stomach.

Keywords——medium chain triglyceride; diazepam; uniform absorption rate; rat; metabolism of MCT in the stomach; medium chain monoglyceride; gastric emptying simulator

In the preceding paper[2] it was shown that the reproducibility of plasma diazepam level with diazepam–medium chain triglyceride (MCT) soft capsules was superior to that of diazepam tablets in human subjects. Although various effects of lipids on the drug absorption have been reported elsewhere,[3] such uniformity of drug absorption rate attributable to MCT has not been reported before our reports of this series, for which the dose volume of the lipid vehicle was reduced in the rat to a level comparable to that of human clinical practice on a dose/body weight basis.[2,4–6]

The mechanism of the uniform absorption rate of diazepam was also investigated in our rat model employing 2 μl of drug preparations per rat.[2] Compared with the aqueous

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suspension, MCT solution gave relatively small variance in the gastric disappearance rate of diazepam. A contribution of intragastric metabolism of MCT to the uniform gastric emptying rate (GER) of the drug was suggested.

It was shown by Borgström *et al.* that lipolysis of fed triglyceride occurred to a considerable extent in the human stomach.[7] The presence of lipase, which is different from that of pancreatic origin, in the gastric aspirates of man was later reported by Cohen *et al.*, and a contribution of this gastric lipase to the digestion of milk triglyceride in infants was suggested.[8] Hamosh and Scow found a lingual lipase in rat, which was characterized by activity in the acidic pH range, with an optimum pH around 5.[9] In addition, a gastric lipase named the pharyngeal lipase has been found in man,[10] the major function of which is to hydrolyze long chain triglyceride (LCT) to diglyceride, monoglyceride and fatty acid.

It has been shown that MCT is absorbable from the intestinal lumen even in the absence of bile salts.[11] However, under identical experimental conditions the maximum absorption rate of MCT was found to be one-fourth less than that of MCT digestion mixture.[12] The hydrolysis of short and medium chain triglycerides in the stomach was reported by a few investigators.[8,13,14] Though the enzyme activity for MCT is higher than that for LCT,[8,14] the reported optimum pH of this enzyme is 5—7[8] and the hydrolysis of MCT is negligible at pH below 2.[8,13] Therefore, the extent to which gastric lipase hydrolyzes triglycerides was reported to be quite limited, because the pH of the gastric contents is quite acidic.[15]

However, the formation of medium chain monoglyceride (MCM) in the hydrolysate may facilitate the further emulsification and dispersion of MCT in the stomach, even if the amount of MCM is limited.

In the present work, the mechanism of uniform absorption of diazepam was further investigated in relation to MCT metabolism in the stomach.

Experimental

Materials——Diazepam was synthesized and purified by Sumitomo Chemical Co., Ltd. MCT, employed as a model vehicle, was obtained commercially (ODO®, Toshin Chemical Co., Japan). Medium chain monoglyceride (MCM) was derived from ODO® in the laboratory of Nikko Chemical Co. (Japan) and the glycerides contents of the MCT and MCM are shown in Table I. As standards for thin-layer chromatography (TLC),

Table I. Glycerides Contents of MCT and MCM Employed in This Investigation

Lipid	Glycerides contents (%)[a]		
	MG	DG	TG
MCT	0.4±0.2	4.0±0.9	95.6±0.8
MCM	70.8±0.1	29.2±0.1	0

Each value represents the mean±S.E. of 4 experiments.
a) expressed as glycerol equivalent.

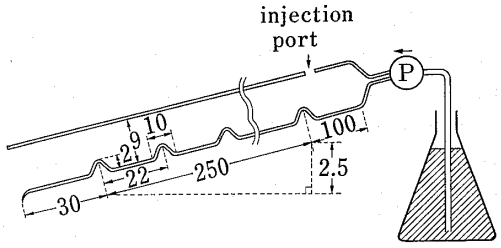

Fig. 1. Schematic Representation of an Apparatus employed for Simulating the Gastric Emptying of Diazepam

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8) M. Cohen, R.G.H. Morgan, and A.F. Hofmann, *Gastroent.*, **60**, 1 (1971).
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11) P.R. Holt, *J. Am. Dietet. A.*, **60**, 491 (1972).
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13) W.W. Tuttle and B.A. Schottelius, "Textbook of Physiology," 6th ed., C.V. Mosby, St. Louis, 1969, pp. 368—379.
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MCM, medium chain diglyceride (MCD), glyceryl dioleate (long chain diglyceride: LCD), glyceryl monooleate (long chain monoglyceride: LCM) and oleic acid were employed. They were purified and confirmed to be homogeneous by TLC employing the two different solvent systems described in the legend to Fig. 2. Other materials employed were of reagent grade.

Metabolism of MCT in the Rat Stomach——Male Wistar rats weighing 180—220 g were fasted for 20 hr prior to the experiments. After decapitation, the stomachs of five rats were removed and everted. An emulsion of MCT containing 2 mg of MCT and 0.5 mg of polysorbate 80 in 5 ml of distilled water was prepared with a homogenizer (Polytron® RT 10-35, Kinematica GmbH). An everted rat stomach was incubated with 5 ml of the MCT emulsion at 37° with shaking at 100 strokes/min. After 30 min the stomach was removed and washed with chloroform–methanol (4: 1). The washing was added to the incubation fluid and the fluid was extracted with 15 ml of chloroform–methanol (4: 1). The organic layer was pipetted off and dried under a gentle stream of nitrogen, and the residue was dissolved in 100 μl of acetone and subjected to TLC.

The enzyme extract of the rat gastric lumen was prepared as follows. The rat stomach was incubated with 2 ml of distilled water for 10 min, then centrifuged at $2000 \times g$ for 15 min. All these procedures were performed below 4°. An emulsion of MCT containing 4 mg of MCT and 1 mg of polysorbate 80 in 1 ml of buffer solution at pH 1.2 or 3.6 (double the isotonic level) was prepared by homogenization. An aliquot (0.5 ml) of this MCT emulsion was added to the same volume of the above enzyme extract and the mixture was incubated at 37° with shaking at 100 strokes/min. After 30 min, glycerides remaining in the incubation medium were extracted with chloroform–methanol (4: 1). The organic layer was dried and the residue was dissolved in 100 μl of acetone. Twenty μl of the acetone solution was subjected to TLC. The spot corresponding to each glyceride was scraped off and the glyceride was dissolved in acetone and filtered. The acetone solution was used for the assay. The amount of glycerides thus extracted was estimated by subtracting the value of the tissue blank.

Gastric Emptying Simulator——Gastric emptying of diazepam was simulated using the apparatus shown in Fig. 1. The model stomach made of hard glass was set at a fixed angle. By using a micro-tube pump (SJ-1210, Mitsumi Sci., Japan) buffer solution of pH 1.2 or 3.6, each containing 0.36% (w/v) pepsin, was injected into the upper part of the model stomach at a flow rate of 0.6 ml/min. The solution left the lower part of the model at the same rate in the steady state. When the elution rate became constant, 50 μl aliquots of various preparations of diazepam were injected through the injection port. The preparation was eluted from the model stomach together with the buffer solution flowing across obstructive mounds that simulated the gastric folds. The eluted fluid was collected at 5 min intervals for 20 min after the start of the experiment. The model stomach was then wahed with acetone and remaining diazepam was completely recovered. The eluted fluid and the washing were extracted with cyclohexane and assayed for diazepam.

Gastric Absorption of Diazepam——Retention of Diazepam in the rat stomach was measured at 30 min after administering two aqueous preparations of diazepam. The concentrations of diazepam in these two preparations were 40 mg/ml and 80 μg/ml. The dose volume of the former was 2 μl/rat and that of the latter was 1 ml/rat, so that the amount administered was 80 μg/rat in both preparations. Isotonic buffer of pH 1.2 or 3.6 was used as the aqueous medium. Diazepam remaining in the stomach was extracted with ether.

Assay Procedure for Diazepam——The ether or cyclohexane extract of diazepam was analyzed by gas-liquid chromatography (GLC) as described in our preceding paper.[2]

Assay Procedure for Glycerides——Glycerides were determined as glycerol equivalents according to the method of Naito *et al.*[16]

Results and Discussion

Metabolism of MCT in the Rat Stomach

MCM is readily dispersible in water[17] and has greater activity for reducing the surface tension between cottonseed oil and water than LCM.[18] Therefore, an MCT preparation might well be smoothly dispersed in the stomach and delivered into the duodenum if a small, but significant, amount of MCM was formed by partial hydrolysis of MCT.

The formation of MCM by gastric metabolism of MCT was examined *in vitro* employing the everted rat stomach. Under the experimental conditions of this study the effect of adhering pancreatic enzymes does not seem to be eliminated.[14] However, we did not attempt to divert the pancreatic flow, taking the view that metabolism of MCT in the stomach should be examined under conditions as close to normal as possible.

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17) A.F. Hofmann, "Medium Chain Triglycerides," ed. by J.R. Senior, University of Pensylvania Press, Philadelphia, 1968, pp. 9—19.
18) A.T. Gros and R.O. Feuge, *J. Am. Oil Chem. Soc.*, **28**, 1 (1951).

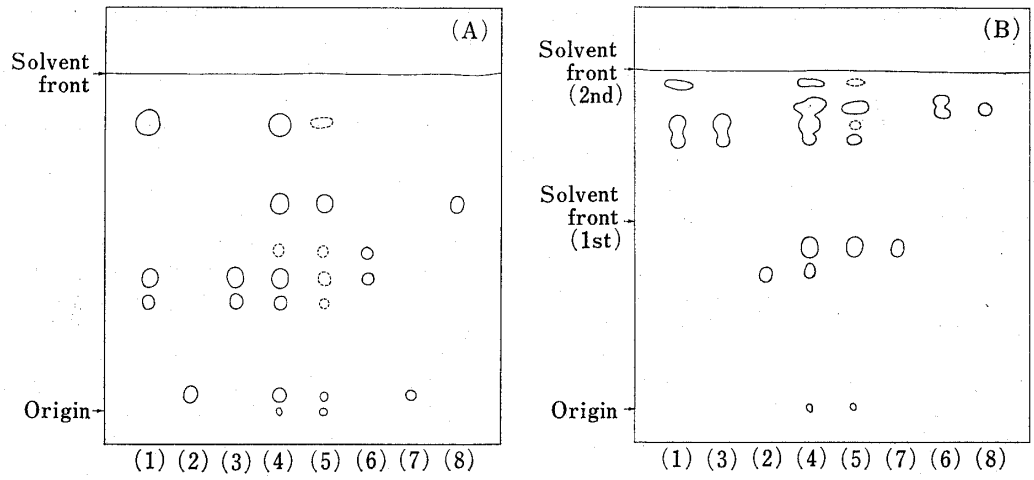

Fig. 2. Thin-Layer Chromatograms of MCT incubated with Everted Rat Stomach

Specimens subjected were (1) MCT in the initial medium, (2) MCM, (3) MCD, (4) MCT incubated with the everted stomach for 30 min, (5) tissue blank incubated for 30 min, (6) LCD, (7) LCM and (8) oleic acid. (A) developed with ether: petroleum ether: acetic acid (50: 50: 1), (B) developed first with the same solvent as (A) and then with isopropyl ether: acetic acid: methanol (90: 4: 6) in the same direction. Spots were detected with water spray.

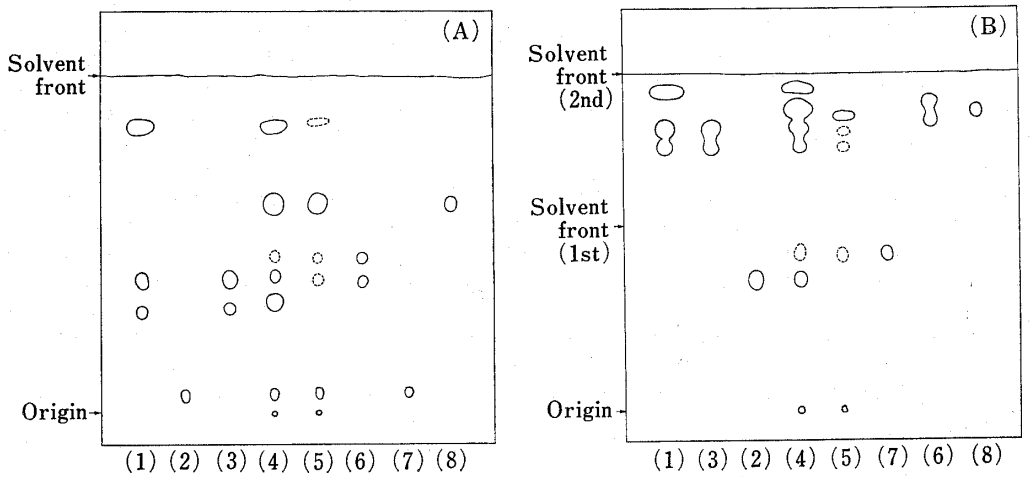

Fig. 3. Thin-Layer Chromatograms of MCT incubated at pH 3.6 with Rat Stomach Enzyme Extract

Details and developing solvents are the same as in Fig. 2 except for (4) and (5). (4) MCT incubated with the stomach enzyme extract, (5) blank of the incubated enzyme extract.

In Fig. 2, the results of TLC of MCT incubated with the everted stomach are shown. Though long chain lipids affected the tissue blank in this experiment, MCD was partly separated from LCD (Fig. 2-A) and MCM was clearly separated from LCM (Fig. 2-B). The formation of MCD was not clearly demonstrated, due to a large and variable tissue blank. In Fig. 2-B, the formation of MCM as an MCT hydrolysis product seems likely.

Next, MCT was incubated with rat gastric enzyme extract instead of the everted stomach, and similar experiments were performed at pH 1.2 and 3.6. The resulting thin layer chromatograms at pH 3.6 are shown in Fig. 3. Fig. 3-B shows the formation of MCM. The MCD and MCT spots from the plate of Fig. 3-A, and the MCM spot from the plate of Fig. 3-B were scraped off and the glycerols of these glycerides were assayed. Similar assay was also carried out after incubation at pH 1.2, and the results are shown for both pH 1.2 and 3.6 in Table II. The decrease of the sum of triglyceride (TG), diglyceride (DG) and monoglyceride (MG) from the initial value suggests the formation of medium chain fatty acids and glycerol, which

Table II. Metabolism of MCT by Rat Stomach Enzyme Extract

Medium pH	Glyceride remaining in the medium (%)[a]		
	MG	DG	TG
(Initial)	0.4±0.2	4.0±0.9	95.6±0.8
1.2	0.7±0.3	1.5±0.2	79.8±1.0
3.6	1.9±0.6	1.3±0.7	71.0±1.1

Each value represents the mean ±S.E. of 4 experiments.
a) expressed as glycerol equivalent.

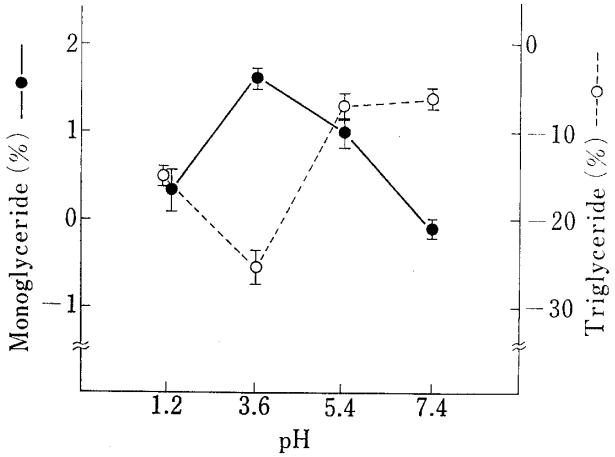

Fig. 4. pH-Profile of Glycerides Contents Following the Metabolism of MCT by Rat Stomach Enzyme Extract

The same stomach enzyme extract was used at every pH. Data are expressed as the percentage increases compared with the initial incubation medium as glycerol equivalents. Each point represents the mean ±S.E. of 4 experiments.

are hydrophilic and would not have been extracted from the incubation medium. An increase of MCM was observed on incubation, which suggested the possibility of MCM formation in the normal rat stomach.

To investigate MCT metabolism further, the pH profile was examined. The results are shown in Fig. 4. Both the increase of MCM and the decrease of MCT were optimum at pH 3.6, which was rather lower than that previously reported.[8] The diglyceride content was constant at about 40% of that contained in the initial incubation medium throughout the whole pH range. The reasons for the difference in optimum pH and the small amount of diglycerides formation are not clear, but are probably related to the absence of bile salts in our experiments. For instance, formation of MCM was increased by adding 20 mM sodium taurocholate to the incubation medium, in which MCT was dispersed with 0.5% polysorbate 80.[14] However, the amount of bile salts in the normal stomach and the effect on lipid metabolism were not examined in this work.

Gastric Emptying Simulator

As diazepam is a weak base, having a p*K*a value of 3.3,[19] it is expected that diazepam will dissolve to some extent in an acidic aqueous medium. However, it has been estimated that the partition of diazepam from MCT to the aqueous phase in the rat stomach is limited, and that diazepam is emptied from the stomach mostly while retained in the lipid.[2] On the other hand, in the case of solid formulations, fluctuation of the gastric pH seems to result in a variable dissolution rate of diazepam particles, producing the variation of GER.

It has been reported recently that variability of the GER of solid formulations could be analyzed by means of an *in vitro* sedimentation apparatus.[20] In order to investigate the gastric emptying properties of the diazepam lipid formulation, a new gastric emptying simulator was devised. A schematic representation of this simulator is shown in Fig. 1. Since the ingested fluid usually flows along the lesser curvature,[21] a liquid preparation is

19) T. Barrett, W.F. Smith, and I.E. Davidson, *J. Pharm. Pharmac.*, **25**, 387 (1972).
20) V. Tamassia, L. Simioni, and R. Tomasini, *Bol. Chim. Pharm.*, **117**, 135 (1978).
21) W.W. Tuttle and B.A. Schottelius, "Textbook of Physiology," 6th ed., C.V. Mosby, St. Louis, 1969, pp. 352—367.

probably excreted from the stomach following its dispersibility on the lesser curvature. A drug in a solid formulation is excreted from the stomach as a solution or a suspension of smaller particles after disintegration. Thus, the dissolution of the drug into the aqueous phase and/or the liberation of small particles from the gastric folds were considered to be the rate-determining process of gastric emptying. The simulator was designed on this basis.

In order to confirm the validity of the simulator, the elution rates of various preparations of 2% amaranth solution, with viscosities in the range of 1—500 cps (adjusted with methylcellulose), were determined. The 50% elution time of amaranth was 5, 8 and 14 min at viscosities of 1, 10 and 500 cps, respectively. These results are consistent with previous reports on the effect of viscosity on the GER,[4,22] suggesting the usefulness of the gastric emptying simulator.

Using four diazepam preparations and two buffer systems of different pH, the time course of diazepam elution from the simulated stomach was investigated. The results are shown

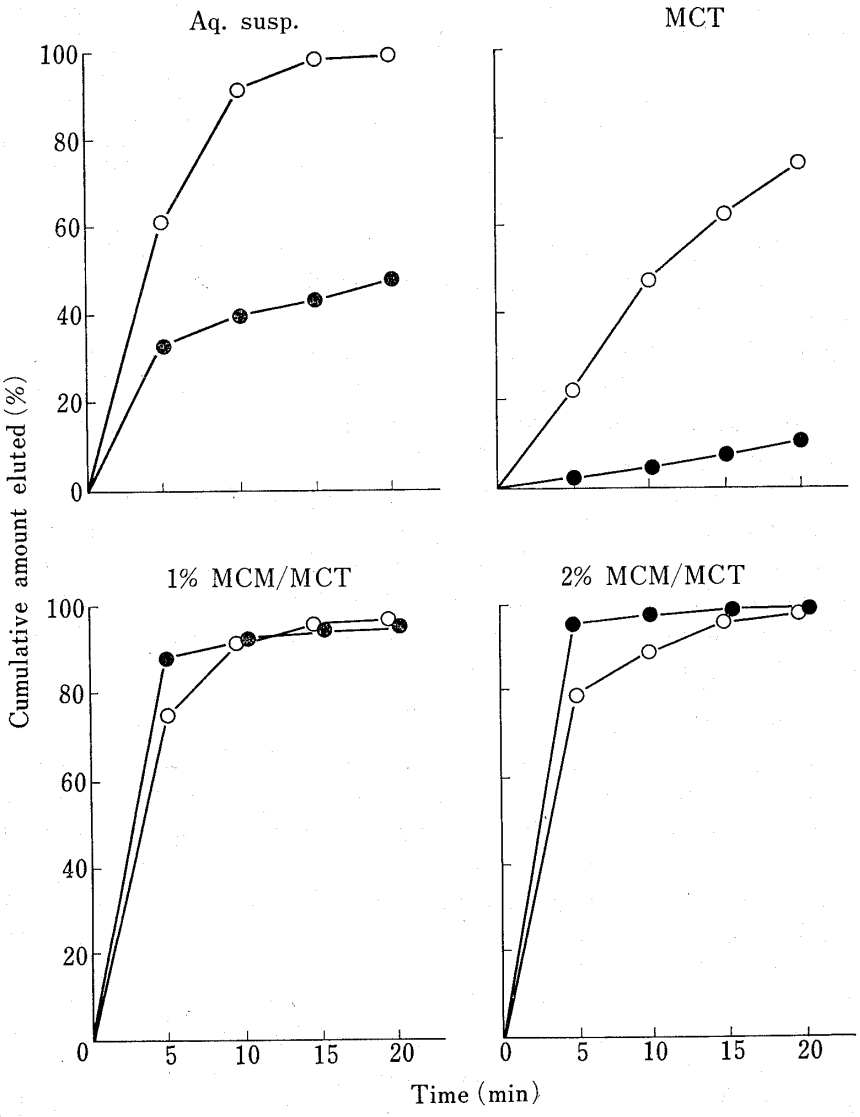

Fig. 5. Cumulative Amount of Diazepam eluted from the Simulated Stomach

○, pH 1.2; ●, pH 3.6.
Each point represents the mean of 3 experiments.

22) G. Levy and W.J. Jusko, *J. Pharm. Sci.*, 54, 219 (1965).

in Fig. 5. In the case of the aqueous suspension, like the MCT solution, the elution profile of diazepam differed considerably between pH 1.2 and 3.6. The elution rate of diazepam at pH 3.6 was very slow, probably due to the slow dissolution rate of the drug into the aqueous phase and to entrapment of the crystalline drug or drug-MCT droplets by the glass mounds.

On the other hand, in the case of diazepam-MCT solution containing 1 or 2% MCM in the lipid, the elution profile was independent of the pH of the medium. The blending percentage of MCM was compatible with the results of the *in vitro* hydrolysis experiments shown in Fig. 4. In the presence of small amounts of MCM, the elution rate was markedly accelerated, showing excellent dispersion in the apparatus. In view of the design basis of the simulator, it seems very likely that this improved dispersibility contributes to the uniform *in vivo* GER of diazepam.

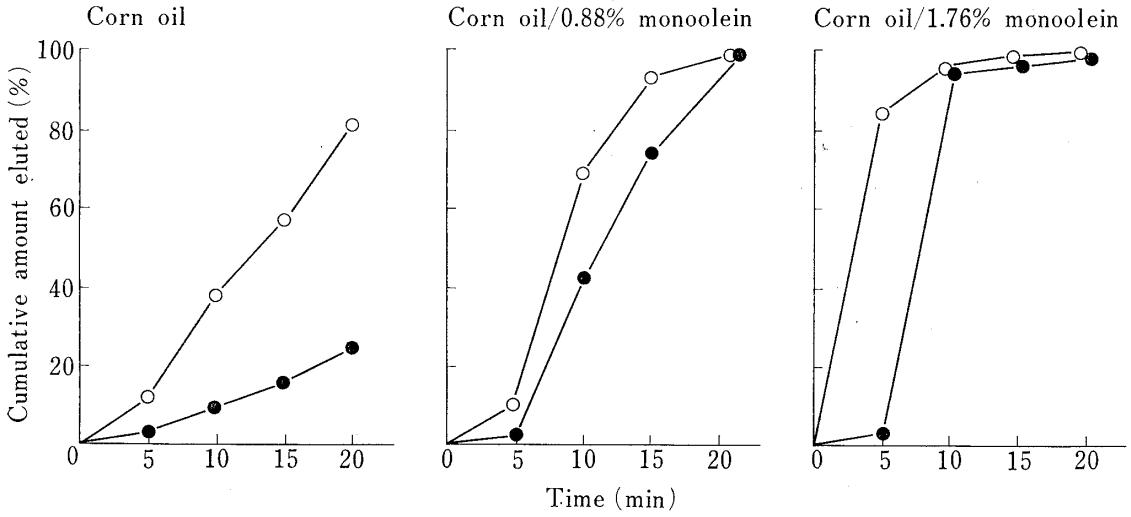

Fig. 6. Cumulative Amount of Diazepam eluted from the Simulated Stomach

○, pH 1.2; ●, pH 3.6.
Each point represents the mean of 3 experiments.

Similar experiments were done with diazepam-corn oil preparations, the vehicle of which was a typical vegetable oil which has been employed for usual soft capsule preparations. Monoolein was employed as a hydrolysis product of corn oil. In the case of corn oil preparations (Fig. 6), monoolein at the same blending percentage as MCM on a molar basis failed to increase the elution rate of diazepam from the simulated stomach as much as MCM/MCT preparations (Fig. 5). Moreover, in a rat with ligated pylorus it was reported that the rate of hydrolysis of LCT was slower than that of MCT.[14] Thus, it is suggested that this uniform GER was characteristic of MCT preparations in comparison with both solid formulations and LCT preparations.

pH Dependency of the Gastric Absorption of Diazepam

In the case of the MCT preparation, it was shown in the rat stomach that the rate of gastric absorption was limited and that the major part of the drug was retained in the lipid. In contrast, the absorption rate of diazepam *via* the aqueous phase seemed larger when an aqueous suspension of diazepam was ingested into the rat stomach.[2] The pH dependency of gastric absorption of diazepam was therefore examined employing the aqueous solution and suspension (Table III). When the solution of diazepam (80 μg/ml) was administered to rats, the gastric absorption of diazepam was independent of the pH of the medium. However, when the suspension of diazepam (80 μg/2 μl) was administered to rats, the absorption rate seemed faster at pH 1.2 than at pH 3.6. This seems to be attributable to the higher solubility

Table III. Effect of pH on the Gastric Absorption of Diazepam from Aqueous Suspensions in Rats

Medium pH	Conc. (μg/ml)	Volume (ml)	% of dose remaining after 30 min	
1.2	4×10^4	2×10^{-3}	80.4±3.9	*a)*
3.6	4×10^4	2×10^{-3}	91.5±4.3	
1.2	80	1	86.4±3.6	*b)*
3.6	80	1	82.4±2.3	

Significance by t-test: *a)* $0.05<p<0.1$, *b)* N.S.
Each value represents the mean ±S.E. of 4 animals.

of diazepam at pH 1.2 than at pH 3.6, suggesting that the dissolution of diazepam into the aqueous phase is the rate-determining step of gastric absorption of the drug.

As shown in experiments with the gastric emptying simulator (Fig. 5), the dissolution of diazepam into the aqueous phase is also the rate-determining step for gastric emptying of the diazepam aqueous suspension. The ratio of the GER to the gastric absorption rate was estimated to be about 3: 1, and the major portion of the ingested drug was absorbed from the small intestine after being emptied from the stomach.[2] Therefore, it was considered that compared with GER, gastric absorption rate was a minor contributor to the variable absorption rate of diazepam from the solid formulation.

Consequently, the uniform absorption rate of the diazepam-MCT preparation compared with the solid formulation is probably attributable to the improved dispersibility of the intragastric digestion mixture of MCT, which is delivered smoothly into the small intestine carrying the major part of the diazepam. In contrast, in the case of the solid formulation, both GER and the gastric absorption rate of diazepam seem to be dependent on the dissolution rate of drug particles, resulting in a variable absorption rate of diazepam.