sample of dihydroprotopine (II) by admixture and TLC (silica gel, $CHCl_3$: MeOH, 4: 1). spec. activity 2.48×10^6 dpm/mmole.

Tetrahydrocoptisine Methiodide (III B)——Dihydroprotopine (II) (37 mg) was dissolved in 20% HCl (5 ml) and refluxed over a free flame for 10 min. After addition of potassium iodide to the reaction mixture, the resulting precipitates were collected by centrifugation. Recrystallization of the precipitates (67 mg) from MeOH gave colorless needles, mp 260° (decomp.), spec. activity 2.37×10^6 dpm/mmole.

Pyrolysis of Tetrahydrocoptisine Methiodide (III B)——The iodide (III B) (60 mg) was heated on an oil bath at 270—280° in a stream of N_2 for 1 hr and the resulting methyl iodide was introduced into a solution of 30% trimethylamine. After removal of the solvent, the resulting white residue (12.5 mg) was recrystallized from MeOH, which was identified with an authentic sample of tetramethylammonium iodide by TLC (silica gel, $CHCl_3$: MeOH, 4: 1), spec. activity 2.44×10^6 dpm/mmole.

The residue in the reaction flask was chromatographed on silica gel with benzene: ether (1: 1) as eluent to give a non-radioactive light yellow residue (3.1 mg), which was identified with an authentic sample of tetrahydrocoptisine (IV) by TLC (silica gel, benzene: ether, 1: 1).

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Effect of Gastric Emptying on Absorption of Aminopyrine in Rat[1a,b]

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In the previous paper,[3] it was described that the initial plasma concentration of aminopyrine increased significantly by the simultaneous oral administration with barbital in rabbits as compared to the single administration. Furthermore, it was also reported[4] that the rate of gastric emptying of aminopyrine increased by barbital in spite of the inhibition of the gastric emptying by aminopyrine. As for gastrointestinal absorption of aminopyrine, it has been known that the delay of the time at which the peak level of plasma concentration of aminopyrine becomes the highest is proportional to the increasing dose.[5] It is not clear, however, why such a delay of it occurs. In consideration of the reason of the delay, the gastric emptying might be one of the important factors.

The present study was performed to clarify the relationship between the gastric emptying and the absorption rate of aminopyrine in rat.

Experimental

Procedure——Male Donryu rats weighing between 190—210 g were fasted for 14—16 hr. However, drinking water was permitted *ad libitum* until 2 hr before the experiment. One ml of 0.07% phenol red solution containing aminopyrine (20 mg/kg or 50 mg/kg) was introduced into the stomach by intubation.

1) *a*) This paper forms Part VI of a series entitled "Effect of Combination of Pharmaceuticals on Gastrointestinal Absorption"; *b*) Part V: S. Goto, T. Yamagata, O. Tsuzuki, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **21**, 2495 (1973).
2) Location: *3-1-1 Maedashi, Higashi-ku, Fukuoka.*
3) S. Goto, O. Tsuzuki, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **19**, 944 (1972).
4) S. Goto, O. Tsuzuki, and S. Iguchi, *J. Pharm. Sci.*, **61**, 945 (1972).
5) K. Fukumoto, *Nippon Hoigaku Zasshi*, **25**, 464 (1971).

Control rats received 1 ml of phenol red aqueous solution. At various times after intubation, the rats were killed to collect the whole blood from an artery on groin with a syringe wetting with heparin solution. Then, the stomach was immediately ligated at the cardia and pylorus and the small intestine was tied off at the ileocecal valve. The stomach and small intestine were exposed to assay for phenol red content. The blood sample was used for the determination of aminopyrine. In no experiment had any of the phenol red passed through the ileum into the cecum. The analytical procedure is a modification of Gibaldi's method.[6] The segments were homogenized with 5 g of sea sand (20—30 mesh) in a mortar. The homogenate was brought into a glass stoppered bottle containing 30 ml of 0.01% NaOH. After the mixture was shaken for 1 min, the alkaline solution was separated by centrifuge. This procedure was repeated three times. NaOH solution collected was adjusted to 100 ml. To 10 ml of the solution, was added 2 ml of 10% trichloroacetic acid solution to remove protein as precipitate. After centrifugation, 5 ml of the supernatant to which 2 ml of 20% NaOH was added was used to measure the absorbancy at 550 nm.

Intravenous Administration——Aminopyrine solution was administered into the tail vein by injection. The blood sample was collected from an artery on groin with a syringe wetting with heparin solution.

Methods of Stomach Ligation and Intestinal Recirculating Perfusion——Male Donryu rats weighing 160—210 g were used. The procedure was described in the previous paper.[3]

Determination of Aminopyrine——The colorimetric method developed by Ono[7] was used. To 2 ml of plasma were added 2 ml of ammonium chloride buffer (pH 8.0), 1 ml of 0.2% phenol and 2 ml of 1% $K_3Fe(CN)_6$. The mixture was kept at room temperature for 30 min, and was shaken violently with 8 ml of $CHCl_3$ for 30 sec. The colored product of aminopyrine (and/or extremely small quantity of 4-aminoantipyrine in plasma) was extracted and then the aqueous layer was removed. After drying over Na_2SO_4, the concentration of $CHCl_3$ layer was determined at 460 nm.

Results and Discussion

Fig. 1 represents the plasma concentration curve after the oral administration of 20 mg/kg or 50 mg/kg of aminopyrine. The maximum plasma concentration of aminopyrine was recognized at 30 min after the administration of lower dose. However, the peak level of higher dose occurred at 90 min. Therefore, both the magnitude of the peak level and the time at which the peak level occurs seem to be dose dependent.

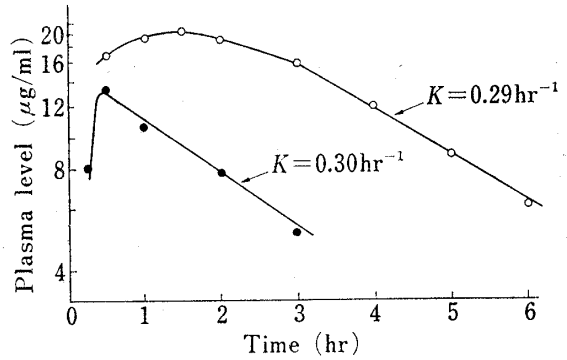

Fig. 1. Mean Plasma Levels of Aminopyrine after Oral Administration in Rats

—●—: aminopyrine 20 mg/kg
—○—: aminopyrine 50 mg/kg
Each point represents the mean of 8 rats.

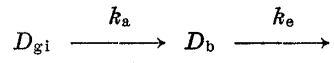

Chart 1

D_{gi} and D_b: the amounts of drug in the gastrointestine and in the body
k_a and k_e: absorption and elimination rate constants

When a drug is orally administered into the gastrointestine, the simplest model can be generally used to describe the absorption and elimination of drug as shown in Chart 1. In this model, the differential equation for the amount of drug in the body will be expressed by the following equation;

$$\frac{dD_b}{dt} = k_a D_{gi} - k_e D_b \qquad (1)$$

When equation (1) is equal to zero, the rates of entry and elimination are equal. Thereby a time $t(B_{max})$ at which the maximum plasma level occurs can be easily calculated as follows;

$$t(B_{max}) = \frac{\ln (k_a/k_e)}{k_a - k_e} \qquad (2)$$

6) S. Feldman, R.J. Wynn, and M. Gibaldi, *J. Pharm. Sci.*, **57**, 1493 (1968).
7) S. Ono, R. Onishi, M. Tange, K. Kawamura, and T. Imai, *Yakugaku Zasshi*, **85**, 245 (1965).

From the equation, the time $t(B_{max})$ should be essentially independent upon the drug dose. This is apparently inconsistent with our experiment. In order to solve this problem, it is considered that the delay of $t(B_{max})$ must have occurred by the decrease of absorption rate of aminopyrine or the increase of elimination rate of aminopyrine. In two cases shown in Fig. 1, it is observed that the elimination of aminopyrine proceeds by the first order reaction, since two straight lines after 30 and 180 min were pararel and both of the rate constants agree closely. Moreover, the area around the peak level of higher dose was flatter than that of lower dose. It seems to be considered that the elimination of aminopyrine depends on the kinetics of Michaelis–Menten type or the capacity-limited process which seems to be a zero order process at higher drug levels, but first order at lower drug levels.

In order to investigate the kinetics of Michaelis–Menten type, 50 mg/kg of aminopyrine was given by the intravenous administration in rat. The plasma concentration of aminopyrine decreased rapidly and the process of elimination obeyed first order kinetics shown in Fig. 2. The elimination rate constant, 0.36 hr,$^{-1}$ was slightly greater than that of oral administration, and the plasma concentration of aminopyrine after intravenous administration was significantly higher than that after oral administration (Fig. 1). From the result, it is clarified that the elimination of aminopyrine does not depend on Michaelis–Menten process in this case.

The gastrointestinal absorption of aminopyrine has been studied by Shanker, *et al*,[8,9] who observed that aminopyrine was absorbed rapidly from the small intestine but little from

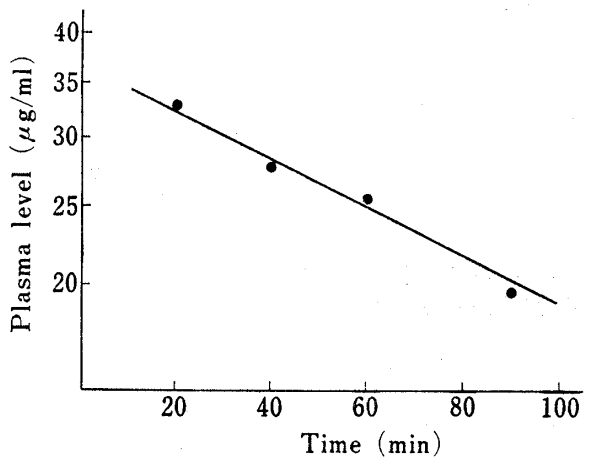

Fig. 2. Mean Plasma Level of Aminopyrine after Intravenous Administration (50 mg/kg) in Rat

The value represents the mean of 6 experiments.

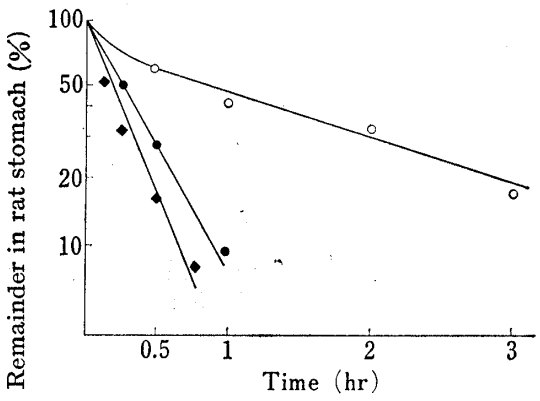

Fig. 3. Effect of Aminopyrine on Gastric Emptying Rate of Phenol Red in Rats

—◆—: control
—●—: administration of aminopyrine (20 mg/kg)
—○—: administration of aminopyrine (50 mg/kg)
Each point represents the mean of 8 rats.

TABLE I. Absoroption Rates of Aminopyrine from Rat Stomach and Small Intestine, *in Situ*

	pH in solution	Absorption rate (%)[a]			
Stomach[b]	1.2	0.0	0.0	0.0	0.0
	7.2	7.6	6.7	7.6	8.9
Small intestine[b]	7.2	53.8	40.0	38.2	41.5

a) in one hour *b*) initial concentration 1×10^{-3}M

8) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **126**, 283 (1959).
9) L.S. Shanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).

the stomach in rat. By means of intestinal recirculating perfusion method and ligated stomach method, the absorption of aminopyrine in rat was investigated to reaffirm. As shown in Table I, the absorption rate of the drug in the small intestine was much greater than that in the stomach. Consequently, it is probable that the absorption rate of aminopyrine may be related with the rate at which the drug leaves the stomach and enters into the duodenum, *i.e.* the gastric emptying rate. In consideration of the delay of maximum plasma concentration of aminopyrine due to higher dose, the gastric emptying rate is the most important factor. In a previous paper,[1b] it has been shown that the rate of gastric emptying of Phenol Red in rabbit decreased remarkably by aminopyrine. Fig. 3 showed the recovery of Phenol Red from the rat stomach as a function of time after simulataneous gastric intubation with aminopyrine. A distinct delay was also observed in the gastric emptying of Phenol Red in the presence of 50 mg/kg of aminopyrine. However, no variation was found measurably in the case of 20 mg/kg of aminopyrine as compared with the control rat (Phenol Red alone). For example, in the case of lower dose, 70% of Phenol Red was emptyed within 0.5 hr, whereas in the case of higher dose, about 2 hr were necessary to empty the same amount of dye.

Therefore, it is concluded that in the case of 50 mg/kg of aminopyrine the delay of the time at which the peak level occurs is caused by the pharmacologic effect of aminopyrine which is the inhibition of gastric emptying to delay the transfer of aminopyrine itself, and that a gastric emptying may be the rate-limited step in the absorption.

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Synthesis of Tryptic Peptides of Hen Egg Lysozyme (Positions 1—5 and 69—73) and Related Peptides[1]

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(Received March 25, 1974)

Joullié, *et al*[3] have claimed that three tryptic peptides of hen egg lysozyme, H–Lys–Val–Phe–Gly–Arg–OH (positions 1—5), H–Thr–Pro–Gly–Ser–Arg–OH (positions 69—73) and H–Phe–Glu–Ser–Asn–Phe–Asn–Gln–Ala–Thr–Thr–Asn–Arg–OH (psitions 34—45),[4] inhibited the action of histamine on isolated guinea pig ileum at a concentration of 1×10^{-7} g/ml.

In the present communication, we describe the synthesis of H–Lys–Val–Phe–Gly–Arg–OH, H–Lys–Val–Tyr–Gly–Arg–OH (amino terminal pentapeptide of turkey egg lysozyme),[5] its

1) Symbols for amino acid derivatives and peptides used in this text are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature; *Biochem. J.*, **126**, 773 (1972). Other abbreviations: DCC=dicyclohexylcarbodiimide, DMF=dimethylformamide, CMC=carboxymethyl cellulose.

2) Location: *Komatsushima, Sendai, 983, Japan*; *a*) Present address: *Yoshitomi Seiyaku Co., Nihonbashi, Chuo-ku, Tokyo.*

3) M. Joullié, M. Laurre, G. Maillard and P. Muller, Fr. Patent 1549157 (1968) [*C.A.* **72**, 3775*d* (1970)].

4) For the sequence according to J. Jolles, J. Jauregui-Adell, I. Bernier and P. Jolles, *Biochim. Biophys. Acta*, **78**, 668 (1963) read H–Phe–Glu–Ser–Asn–Phe–Asn–Thr–Gln–Ala–Thr–Asn–Arg–OH according to R. Canfield, *J. Biol. Chem.*, **238**, 2698 (1963) and R. Canfield and A.K. Liu, *ibid.*, **240**, 1998 (1965).

5) J.N. LaRue and J.C. Speck, Jr., *J. Biol. Chem.*, **245**, 1985 (1970).