

Rate of Gastric Emptying of Phenol Red in a Rabbit<sup>1a,b)</sup>SHIGERU GOTO,<sup>2a)</sup> TETSUYA YAMAGATA,<sup>2b)</sup> OSAMI TSUZUKI,  
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The purpose of this study was to determine the gastric emptying pattern of Phenol Red in solution form and to consider the apparent effect of few drugs on the rate of emptying through the pylorus of orally administered Phenol Red using rabbits. It was found that the gastric emptying of Phenol Red was regarded as exponential in form and the gastric response of rabbits was almost unchanged for two months. Aminopyrine delayed considerably the gastric emptying of Phenol Red from kinetical point of view. Caffeine which has increasing effect on human gastric secretion did not affect the rate of emptying of Phenol Red.

Plasma concentration of aminopyrine at the initial stage after its oral administration in solution was found to increase in rabbits treated simultaneously with barbital compared to control rabbit administered aminopyrine alone.<sup>3)</sup> It was also found in a previous work<sup>1b)</sup> that an increased rate of gastric emptying of aminopyrine induced by barbital could be considered as a likely reason for such a positive effect of barbital. The effect of orally administered bile salts on the pattern of gastric emptying of drugs was examined by Gibaldi, *et al.*<sup>4,5)</sup> and Hayton has found that SKF-525A decreases the gastric emptying rate polyethylene glycol 4000 and sulfacetamide in rat.<sup>6)</sup> These studies seem to indicate the necessity for considering the influence of simultaneously administered drugs on the gastric emptying since if the intestinal absorption is very fast and a gastric absorption is negligible, passage of a drug through the pylorus may become rate limiting in the absorption process as shown in Chart 1. Although much work has been done on the effect of various drugs on the gastric emptying in rats, mice, and humans, there has been a few reports on the systematic investigation on that in rabbit.

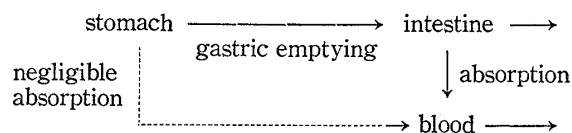


Chart 1. Drug Transfer in Gastrointestinal Tract

The present study was designed to investigate quantitatively the gastric emptying of Phenol Red alone and the effect of few drugs on the gastric emptying of Phenol Red from kinetical point of view, using rabbit.

## Experimental

**Procedure**—Male rabbits weighing between 2.5 and 3.3 kg were fasted for 24 hr but given water freely. A vinyl tube (0.5 × 30.0 cm) was inserted into rabbit stomach and 50–100 ml of warm water (37°) was instilled. The fluid was rapidly withdrawn by suction with a syringe. This procedure (gastric washout)

- 1) a) This paper forms Part V of a series entitled "Effect of Combination of Pharmaceuticals on Gastrointestinal Absorption"; b) Part IV: S. Goto, O. Tsuzuki, and S. Iguchi, *J. Pharm. Sci.*, **61**, 945 (1972).
- 2) Location: a) *Tsushima, Okayama*; b) *Tashiro, Tosu, Saga*; c) *Katakasu, Higashi-ku, Fukuoka*.
- 3) S. Goto, O. Tsuzuki, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **19**, 944 (1971).
- 4) S. Feldman and M. Gibaldi, *Gastroenterology*, **54**, 918 (1968).
- 5) S. Feldman, R.J. Wynn, and M. Gibaldi, *J. Pharm. Sci.*, **57**, 1493 (1968).
- 6) W.L. Hayton and G. Levy, *Life Sciences, Pt. 1 Physiol. Pharmacol.*, **10**, 691 (1971).

was repeated five times and then the stomach content was removed as completely as possible. After washing, 100 ml of isotonic solution containing one of the drugs and Phenol Red was instilled into the stomach through the vinyl tube during approximately 1 min. Mixing of the sample solution in the rabbit stomach was completed by moving the syringe vertically, 1 ml of the sample solution was collected from stomach through the vinyl tube and the starting time of experiment was recorded. The sample solution was analyzed, and the initial concentration of Phenol Red ( $c_0$ ) and the initial volume of gastric content ( $v_0$ ) were calculated. Then the vinyl tube was removed. After a specified time, the vinyl tube was passed into the stomach again and the gastric content was withdrawn by gentle aspiration with a syringe (concentration of Phenol Red and volume of gastric content expressed as  $c_1$  and  $v_1$ , respectively). To remove any residual gastric content, 100 ml of warm water was injected into the stomach through the vinyl tube, this washing was recovered, and the volume of residual gastric content was calculated from its Phenol Red concentration ( $c_2$ ). The residual volume ( $v_2$ ) was calculated from Eq. (1).

$$v_2 = 100c_2/(c_1 - c_2) \quad (1)$$

The rabbits were used repeatedly at intervals of 7 days. The administered amounts of drugs are indicated in Fig. 3 and Table III.

**Determination of Phenol Red**—To 1 ml of the sample solution, 4 ml of 1% NaOH was added and the color of the solution changed immediately to reddish purple. The absorbance was measured at 550 nm using a spectrophotometer.

**Calculation of Values**—The equations proposed by Hunt and Spurrell<sup>7,8)</sup> and by Gloor and Heinkel<sup>9)</sup> were used as summarized by Mizuno.<sup>10)</sup>

The residual amount of Phenol Red (in mg) at the end of the experiment:

$$\text{Phenol Red} = c_1(v_1 + v_2) \quad (2)$$

The volume of gastric content leaving the stomach during the experimental period ( $v_e$ , in ml)<sup>11)</sup>:

$$v_e = (c_0v_0 - \text{Phenol Red}) / (c_0 + c_1) / 2 \quad (3)$$

The volume of gastric juice secreted during the experimental period ( $v_s$ , in ml):

$$v_s = v_1 + v_2 + v_e - v_0 \quad (4)$$

## Result and Discussion

### Kinetics of Gastric Emptying of Phenol Red

In the administration of Phenol Red alone, recovery of the residual amount of Phenol Red and the volume of gastric content containing gastric juice secreted during any time

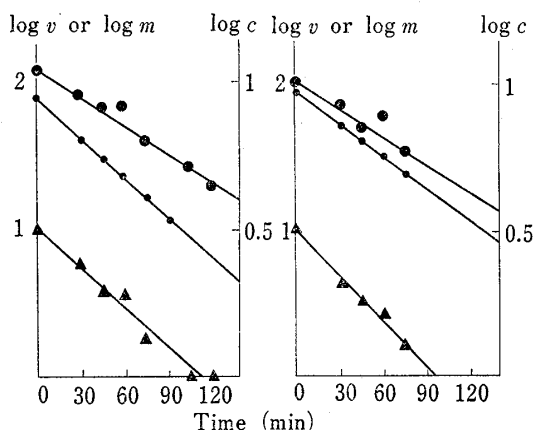


Fig. 1. Gastric Emptying Pattern of Phenol Red (10 mg) in Rabbit

- : volume of gastric content ( $v$ )
- ▲—: amount of Phenol Red ( $m$ )
- : concentration of Phenol Red ( $c$ )

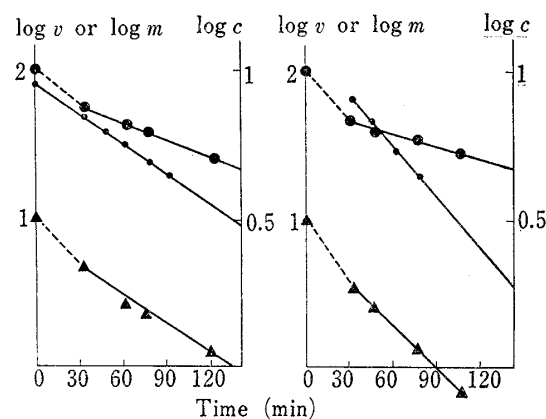


Fig. 2. Gastric Emptying Pattern of Phenol Red (10 mg) in Rabbit. Refer to Fig. 1 for the Symbols noted in This Graph

- 7) J.N. Hunt and W.R. Spurrell, *J. Physiol.*, **113**, 157 (1951).
- 8) J.N. Hunt, *J. Physiol.*, **113**, 169 (1951).
- 9) M. Gloor and K. Heinkel, *Med. Welt*, **7**, 1 (1967).
- 10) S. Mizuno, *Nippon Shokakibyō Gakkaishi*, **66**, 847 (1969).
- 11) This arithmetical method is an approximation.

TABLE I. Apparent First-order Decreasing Rate Constant for Gastric Emptying in the Rabbit

Rabbit No.	Rate constant ( $\times 10^2 \text{ min}^{-1}$ )			
	$k_m$	$k_v$	$k_c$	
			Calcd.	Found
1	2.2	1.5	0.7	—
2	2.3	0.8	1.5	—
3	2.5	1.7	0.8	0.6
4	2.1	1.4	0.7	1.0
5	1.8	0.9	0.9	0.8
6	2.3	0.8	1.5	1.4
7	2.3	1.5	0.8	0.9
8	1.8	1.3	0.5	—
9	3.0	1.8	1.2	—
10	2.5	1.5	1.0	—
11	2.7	1.3	1.4	—
12	2.4	1.8	0.6	—

between instillation and withdrawal as a function of time after intubation are summarized in Fig. 1 and 2. These straight lines which are semilogarithmic plots of gastric retention versus time indicate that the gastric emptying of Phenol Red and the volume of gastric content in the rabbit proceed by apparent first-order kinetics.<sup>12)</sup> This result is consistent with the results of Hunt and MacDonald<sup>13)</sup> using the serial test meal method in a human. The apparent first-order rate constants calculated from the slope of the lines are summarized in Table I. Twelve rabbits were used in the series of this experiment and the average rates were found to be  $0.023 \text{ min}^{-1}$  for the residual amount of Phenol Red and  $0.014 \text{ min}^{-1}$  for the volume of gastric content. Fig. 2 also shows that the rapid decline of the gastric emptying at initial stage is followed by a slower exponential decline, *i.e.*, the extended line does not go through the point of administered value corresponding to zero time. Hunt has already reported that a similar phenomenon was observed in his liquid meal test using human stomach.<sup>7)</sup> It was assumed therefore that the movement of human stomach has already started before administration of serial test meal, and such a stomach must be called "quick starter stomach" as suggested by Hunt. This explanation may also be applied in the case of the rabbit. On the other hand, "slow starter stomach", which has been observed in his experiment, could not be found in our case. Since each plot in Fig. 1 and 2 consists of the value obtained on different days, 30 or 40 days were required for the completion of each line. Namely, by synthesizing the data obtained from each withdrawal on the same rabbit into one record a complete picture of gastric activity of the experimental rabbits may be formed. However, attention should be paid on changes in the conditions of the body, environment, and familiarity to administered drugs during the experimental period since these conditions may have a delicate effect on the measured values. In order to find such an effect, concentration of Phenol Red in gastric content was determined by analyzing the sample solution obtained from the rabbit stomach successively on a defined day. These results are plotted on the semilogarithmic graph and shown in Fig. 1 and 2. The concentration of Phenol Red declines undoubtedly in an exponential fashion with time, *i.e.*,  $c=c_0e^{-kt}$ .

12) According to Hopkins's report (A. Hopkins, *J. Physiol.*, **182**, 144 (1966)), the square-root pattern is more satisfactory description of gastric emptying than the hitherto accepted exponential pattern.

13) J.N. Hunt and I. Mac Donald, *J. Physiol.*, **126**, 459 (1954).

On the other hand, the decreasing rate for concentration of Phenol Red will be expressed by the following equation:

$$\begin{aligned} -\frac{dc}{dt} &= -\frac{d}{dt} \frac{m}{v} = -\left(v \frac{dm}{dt} - m \frac{dv}{dt}\right) / v^2 \\ &= -\frac{1}{v} \frac{dm}{dt} + \frac{c}{v} \frac{dv}{dt} \end{aligned} \quad (5)$$

where  $c$  is the concentration of Phenol Red at time  $t$ ,  $m$  is the residual amount of Phenol Red at time  $t$ , and  $v$  is the volume of gastric content at time  $t$ . Because the straight lines in Fig. 1 and 2 represent  $-dm/dt = k_m m$  and  $-dv/dt = k_v v$ , respectively, and the product of  $c$  and  $v$  is equal to the residual amount of Phenol Red, Eq. (5) can be arranged to the following equation:

$$-\frac{dc}{dt} = (k_m - k_v)c \quad (6)$$

Therefore, the plots for Phenol Red concentration should be a first-order relationship and the slope of the lines should represent a difference between the above two apparent rate constants,  $k_m$  and  $k_v$ . Some of the data in Table I also demonstrate the difference between the calculated and experimental values. These values agree closely, and it seems that the result of previous experiments requiring 1–2 months has a considerable reliability.

The tests were performed at weekly intervals with the same rabbit. For the first one or two months, the gastric emptying behavior was almost always reproducible, but there was some variation after three months. This fact will be obvious from Table II. A familiarity of the body to administered drugs, and physiological and structural changes induced by repeated administration of a drug may be considered as factors for some variation after three months. Hunt<sup>9)</sup> reported that the gastric response was almost unchanged for 3 years in two healthy human volunteers and that, in another three subjects, the alteration in the stress of life induced one consistent level of activity to a much lower consistent level.

TABLE II. Change of Gastric Emptying Pattern During Experimental Period

Rabbit No.	Rate constant for gastric emptying ( $\times 10^2 \text{ min}^{-1}$ )		Gastric emptying pattern, 75 min after oral ad.		
	$k_m$	$k_v$	Residual phenol red (%)	Residual total volume (ml)	
5	Initial	1.8	0.9	22.0, 25.5	39, 41
	After 3 months	3.3	1.8	4.1	13
7	Initial	2.3	1.5	16.5	33
	After 3 months	3.5	2.8	7.0	16

### Effect of Aminopyrine Administered Simultaneously to Gastric Emptying of Phenol Red

Fig. 3 gives a quantitative picture of the progress of emptying from minute to minute, using aminopyrine as one of the simultaneously administered drugs. In the administration of aminopyrine, a smaller exponential decline in the amount of Phenol Red was found as compared with the control figure (Phenol Red alone), but the mechanism of this result is not obvious.

### Effect of Caffeine Administered Simultaneously to Gastric Emptying of Phenol Red

In relation to the important problem that the simultaneously administered drug could markedly affect gastric emptying, the effect of caffeine was examined using rabbit. Mizuno<sup>10)</sup> has reported that caffeine administered orally or intraperitoneally to a human significantly increased the gastric secretion and successively promoted the gastric emptying. It has been

explained that the human gastric secretion of hydrochloric acid and pepsin may be moderately stimulated and catalysed by caffeine which has a direct effect on the gastric mucosa in gastric antrum. The systemic effect through vagus of caffeine is produced rapidly with time after its invasion into the blood stream and gastric secretion is also increased, and this fact probably offers an alternate explanation for the increase of gastric emptying by caffeine.

Unexpectedly, any change in the secretory and motor activity of the rabbit stomach by caffeine was not observed as a result of the present experiment. The data in Table III demonstrate the secretion of gastric juice and residual amount of Phenol Red at 75 min after using various amounts of caffeine. The second test was performed after an interval of 20 min

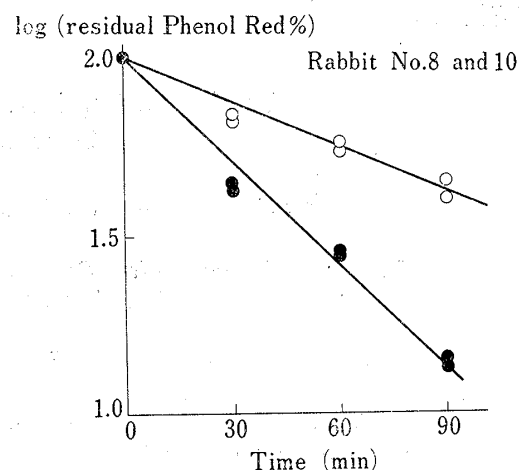


Fig. 3. Effect of Aminopyrine on Gastric Emptying Rate of Phenol Red (10 mg)

—●—: without aminopyrine  
—○—: administration of aminopyrine (900 mg)

TABLE III. Typical Data of Gastric Emptying Pattern at 75 Minutes (Rabbit No. 13)

	Control	Amount of caffeine administered (mg)					
		50		200	300		
		I <sup>a)</sup>	II <sup>a)</sup>		I <sup>a)</sup>	II <sup>a)</sup>	
Volume of solution administered (ml)	119	118	116	133	122	105	
Total residual volume (ml)	36	38	47	45	44	61	
Amount of Phenol Red administered (mg)	10.9	9.6	9.5	10.4	9.9	9.8	
Residual amount of Phenol Red (mg)	2.1	1.6	2.7	1.6	2.0	2.1	
-ditto- (%)	19.3	16.7	28.4	15.4	20.1	21.0	
Volume of gastric juice secreted (ml)	34	50	29	65	47	77	

a) The second experiment (II) was made 20 min after the completion of the first experiment (I).

after the completion of the first test using 50 or 300 mg of caffeine. No change was observed in any of the experiments. However Naito<sup>14)</sup> has found that the simultaneous oral administration of caffeine to a rabbit resulted in a significant increase in the blood level of aminopyrine. This phenomenon seems to emphasize the previously obtained hypothesis<sup>1b)</sup> that aminopyrine suppresses the transfer of Phenol Red to the duodenum, and simultaneously administered drugs (*e.g.*, barbital and caffeine), which have no effect on the gastric emptying of Phenol Red, can eliminate the effect of aminopyrine on the gastric emptying of Phenol Red and reduce the gastric emptying time of aminopyrine. A recent paper<sup>15)</sup> has reported the same experimental result in human subjects, that is, the gastric emptying of caffeine meals and water meals proceeded at the same rate up to 20 min at which time about 86% of the meal had left the stomach.

14) S. Naito, T. Awataguchi, and N. Ozaki, *Yakugaku Kenkyu*, **35**, 348 (1963).

15) T.E. Chvasta and A.R. Cooke, *Gastroenterology*, **61**, 838 (1971).