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Effect of Carbachol on Intestinal Absorption of Aminopyrine, Caffeine and Salicylic Acid during *in Situ* Recirculating Perfusion in Rats

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The effect of carbachol on the absorptions of caffeine, aminopyrine and salicylic acid in the rat small intestine was studied, using an *in situ* recirculating perfusion method.

Carbachol, either added to the perfusate or injected intravenously, remarkably reduced drug absorption. Upon the addition of carbachol, the perfusate volume of intestine decreased markedly and there was a positive correlation between the perfusate volume and the drug absorption rate. No physicochemical interaction, as determined by measurement of the partition coefficient between isoamyl acetate or benzene and phosphate buffer (pH 7.0), was noted between the drugs and carbachol in solution.

Our results suggest that the carbachol-induced decrease in drug absorption is due to a reduction in the perfusate volume in the intestine upon recirculating perfusion.

Keywords—smooth muscle contraction; perfusate volume in intestine; intestinal absorption; recirculating perfusion method; carbachol; aminopyrine; caffeine; salicylic acid; rat

Intestinal drug absorption is affected by factors related to the physiological condition of the experimental animals, *e.g.*, the gastric emptying rate and water flux due to osmotic pressure. While many studies on the effect of drugs on gastrointestinal (GI) motility have been reported, to our knowledge, there are few reports on the effect of intestinal muscle contraction on intestinal drug absorption.¹⁻³⁾

Drug absorption essentially parallels the surface area of GI segments contacted by the drug. However, measurement of the area available for absorption and of the time required for transit from the intestinal segments to the body is very difficult and no appropriate method has been devised to date. Although the perfusate volume in the intestine is not necessarily proportional to the surface area of the intestinal segment, the intestinal volume may represent an important parameter in studies of drug absorption from the small intestine by the recirculation method.

We examined the relationship between drug absorption and perfusate volume in the rat intestine, using an *in situ* recirculating perfusion technique. We obtained new results on the effect of carbachol on drug absorption, with aminopyrine, caffeine and salicylic acid as test drugs.

Experimental

Materials—Caffeine (CA, mp 237—238°C), aminopyrine (AM, mp 107—109°C), salicylic acid (SA, mp 210—212°C), and carbachol (mp 200—202°C) were used. Other reagents were of analytical reagent grade. Organic solvents were distilled prior to use.

Animals—Male Wistar rats were fasted overnight with water *ad libitum* and anesthetized by intraperitoneal injection of 0.5 ml of 1.3% pentobarbital sodium per 100 g body weight. In the recirculating perfusion experiments, rats weighing 70—240 g were used.

***In Situ* Recirculating Perfusion Method**—A modification of the method of Schanker *et al.*⁴⁾ was used. Anesthetized rats were laparotomized by a midline incision, the bile duct was ligated and the exposed small intestine was cannulated with polyvinyl tubing at the proximal end of the duodenum and the distal end of the ileum. The intestine was washed with approximately 30 ml of 37°C saline and then the intestinal tract was perfused with 80 ml of a 2 mM drug solution, using a perfusion apparatus (Tokyo Riken perfusion pump, model D.M.) which allowed the drug solution to flow from the duodenum to the ileum at 37°C at a

flow rate of 2.5 ml/min. After 5 min perfusion, 1 ml aliquots of the perfusate were removed at 5 min intervals for 30 min, or once after 30 min, and analyzed. Phenol red,^{4,5)} the intestinal absorption of which is negligible, was used as a volume indicator. The drug solution in the flask was mixed with the continuous introduction of air throughout the experiment.

Determination of Perfusate Volume in the Intestine—A laparotomized rat prepared for recirculating perfusion was placed on a plate mounted on a graduated (0.5 g) scale as shown in Fig. 1. After the start of recirculation, the total weight (W_1) was recorded, and simultaneously, 0.5 ml of perfusate was pipetted to determine the water flux. The total amount of fluid absorbed from the intestinal tract or excreted into the intestine (F_t) was calculated by means of the equation;

$$F_t = V_0 - V_0 \frac{C_0}{C_t} \quad (1)$$

where V_0 is the perfusate volume at time 0 and C_0 and C_t are the phenol red concentrations at time 0 and at time t , respectively. If fluid from the body is excreted into the intestine, the value of F_t is negative. After the final perfusate sampling, the remaining perfusate in the intestinal tract was removed as completely as possible through passing air and simultaneously rubbing the abdominal region. Thereafter, the weight shown on the scale (W_2)

$$W_3 = W_2 - F_{\text{final}} \quad (2)$$

was recorded. F_{final} is the absorbed or excreted fluid at the end of the experiment and W_3 is really equal to the weight excluding the perfusate in the intestinal tract at time 0. The perfusate volume (V , in ml; the density of the perfusate used in this experiment was 1.01 g/cm³) in the intestinal tract at a given time, V_t , was calculated by means of the equation;

$$V_t = W_1 - (W_3 + F_t) \quad (3)$$

The weight of the excreted urine and feces during this experiment is included in W_1 . These materials were not removed from the balance plate holding the animal.

Determination of the Partition Coefficient—To 10 ml of each drug solution, 10 ml of organic solvent (benzene or isoamyl acetate) was added and the mixture was vigorously shaken in an automatic shaker for 30 min at 37°C to achieve equilibration. The drug content in the aqueous layer was determined and the partition coefficient was calculated from this value.

Analytical Methods—a) Phenol Red: To 0.5 ml of the sampled perfusate, 5 ml of 1 N NaOH was added. This resulted in an immediate color change to reddish-purple. The developed color was determined spectrophotometrically at a wavelength of 550 nm.

b) AM: The colorimetric method of Ono *et al.*⁶⁾ was used. To 0.5 ml of the sampled perfusate were added 2 ml of ammonium chloride buffer (pH 8.0), 1 ml of 2% phenol and 2 ml of 1% $K_3Fe(CN)_6$. The mixture was allowed to stand for 30 min at room temperature and then shaken vigorously for 1 min in the presence of 10 ml of $CHCl_3$. The colored product of AM was extracted and the aqueous layer was removed. The $CHCl_3$ solution was filtered through dry filter paper, and the developed color was determined at 460 nm.

c) CA: To 0.5 ml of the sampled perfusate, 1 ml of thiopental solution was added as an internal standard. The mixture was filtered through a Millipore filter (0.5 μ). The filtrate (10 μ l) was subjected to high-speed liquid chromatography (HLC) on a Hitachi 633-A liquid chromatograph (column size 2.0 mm i.d. \times 500 mm) with Hitachi gel 3010 and an ultraviolet absorption photometer set at a wavelength of 280 nm. The mobile phase consisted of CH_3OH and H_2O (7.8: 2.2), and the flow rate was 1.5 ml/min.

d) SA: Determination was as described for CA, except that AM was used as the internal standard and photometric determination was at 250 nm.

Carbachol: Determination was done according to the Magnus method.⁷⁾ The isolated ileum of a male ddN strain mouse weighing approximately 25 g was suspended in 30 ml of aerated Tyrode solution at a temperature of 30°C. Muscle movements were recorded isotonicly on a kymograph and the ratio of ileal contractions was used to determine the carbachol concentration.

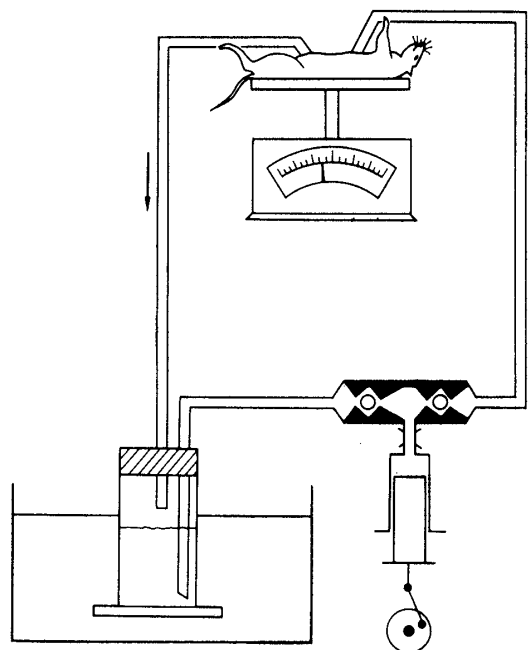


Fig. 1. Experimental Arrangement for Determining Rates of Absorption of Drugs from Rat Intestine by *in Situ* Recirculating Perfusion

Results and Discussion

Effect of Carbachol on Intestinal Drug Absorption

Male Wistar rats weighing from 210 to 230 g were perfused *in situ* with phosphate isotonic buffer (pH 7.0), according to the recirculating perfusion method. The effect of carbachol, present in the perfusate, or administered intravenously (*i.v.*) via the carotid artery, on drug absorption is shown in Fig. 2. Absorption of AM, CA and SA was reduced most markedly in rats that had received carbachol *i.v.* Intestinal drug absorption is affected by the water flux in the intestinal tract; water absorption increases, while water excretion decreases.⁸⁻¹⁰ In the present study, the use of phenol red as a volume indicator revealed that the volume of the recirculating perfusate was slightly decreased in a linear fashion during 30-min perfusion. However, the water flux did not differ measurably, irrespective of the absence or presence of carbachol in the perfusate, or of the *i.v.* administration of carbachol (Fig. 2).

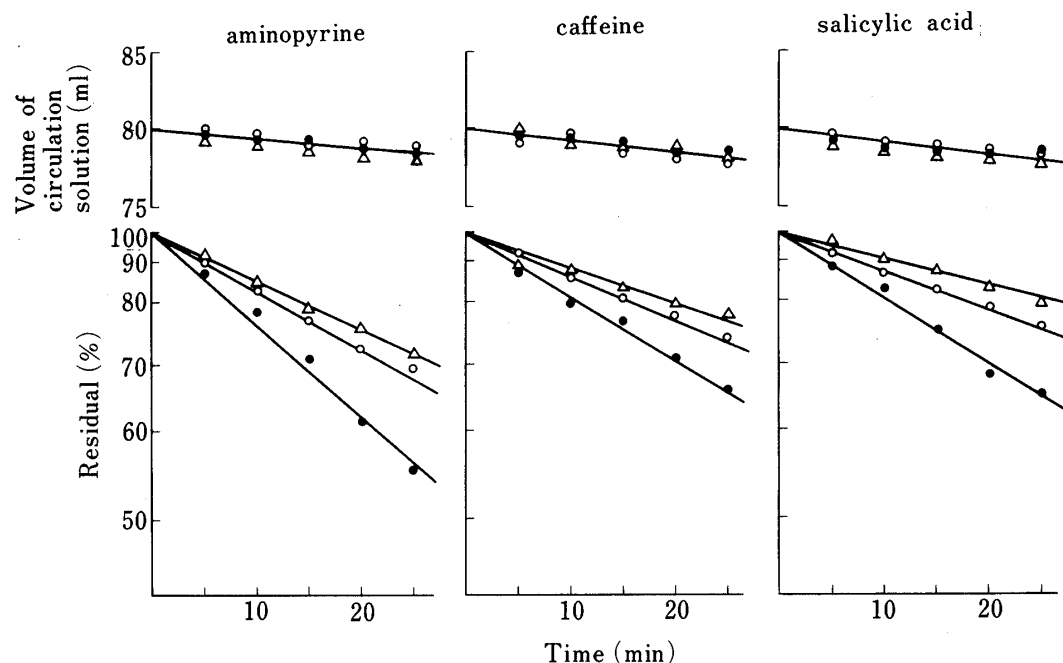


Fig. 2. Semilogarithmic Plots of Residual Aminopyrine, Caffeine and Salicylic Acid in the Rat Small Intestine

Key ●; 2 mM drugs alone,
○; 2 mM drugs+1 mM carbachol in perfusate,
△; 2 mM drugs+0.1 mg/kg carbachol administered *i.v.*

Intravenous administration of carbachol was carried out five minutes before recirculation. The indicated values represent the means of five rats. The upper graph represents the volume of circulating solution at the same time.

Volume in the Intestine during Recirculating Perfusion

To study the effect of smooth muscle contraction on drug absorption, the perfusate volume in the intestinal tract was measured. Control rats were perfused with a solution consisting of phenol red and isotonic phosphate buffer. In these rats, the perfusate volume in intestine increased slightly in a linear fashion during 50-min perfusion (Fig. 3). Fourteen rats were divided into 2 groups of 7 rats each and after phenol red-phosphate buffer perfusion for 25 min to accustom the intestine to the experimental conditions, 0.1 mM or 1 mM carbachol was added to the recirculating perfusate. In the 1 mM carbachol group, the effect of carbachol became manifest within 5 min and a distinct decrease in the perfusate volume in intestine was noted. This decreased volume level was essentially unchanged for the remainder of the 50-min observa-

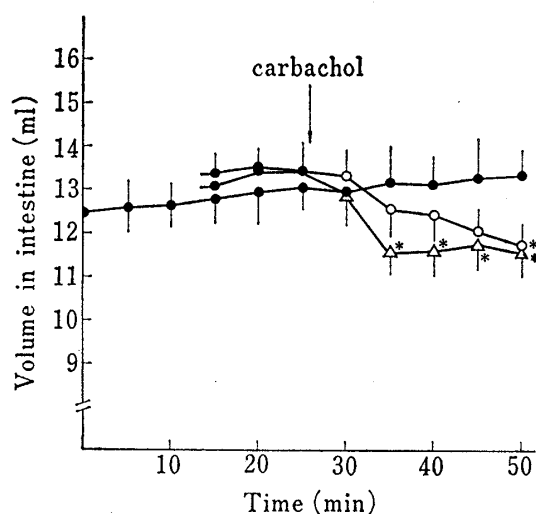


Fig. 3. Time Course of the Recirculating Perfusion Volume in Rat Small Intestine

Key ●; phenol red-phosphate buffer perfusate (control),
○; perfusate+0.1 mM carbachol,
△; perfusate+1 mM carbachol.

Each point represents the mean \pm S.E. for seven rats. An arrow shows the point of addition of carbachol in perfusate.

* Significantly different ($p < 0.05$) from control and pre-carbachol levels.

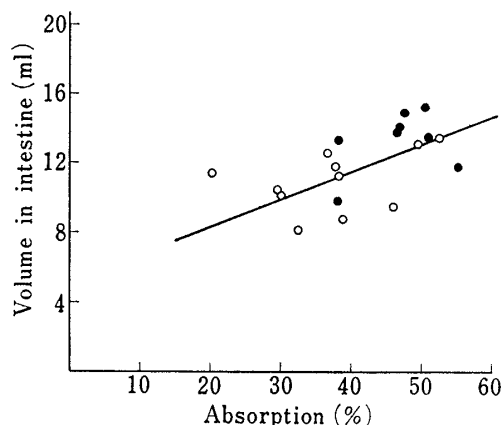


Fig. 4. Relationship between Perfusate Volume in the Intestine and Aminopyrine Absorption

Key ●; aminopyrine, 2 mM,
○; aminopyrine, 2 mM+carbachol, 1 mM.

The volume in the intestine is the mean value determined at intervals of 5 min for 30 min. The drug absorption (%) was determined at 30 minutes after the beginning of circulation.

$$y = 0.183x + 4.137 \quad (r = 0.648, n = 19)$$

tion period; it was significantly different ($p < 0.05$) from the pre-carbachol level and the control level. In the 0.1 mM carbachol group, the perfusate volume in the intestine decreased gradually; at 25 min after the addition of carbachol, the volume in the intestine was significantly different ($p < 0.05$) from the pre-carbachol level and the control level (Fig. 3).

Relationship between Perfusate Volume in the Intestine and Drug Absorption

As shown in Figs. 4–6, drug absorption in the 30 min after the start of recirculating perfusion in the absence or presence of 1 mM carbachol appeared to be related to the perfusate volume

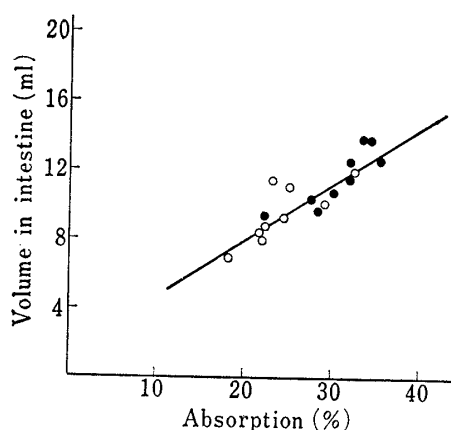


Fig. 5. Relationship between Perfusate Volume in the Intestine and Caffeine Absorption

Key ●; caffeine, 2 mM,
○; caffeine, 2 mM+carbachol, 1 mM.

For determination of the volume in the intestine and the drug absorption (%), see Fig. 4.

$$y = 0.324x + 1.647 \quad (r = 0.849, n = 18)$$

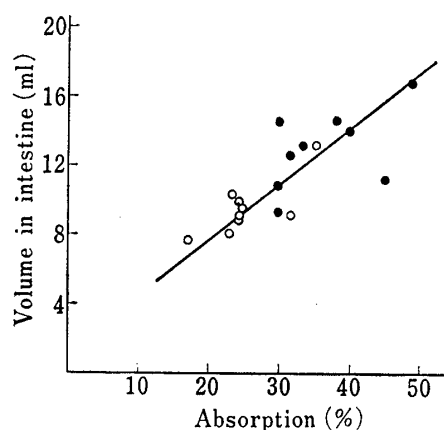


Fig. 6. Relationship between Perfusate Volume in the Intestine and Salicylic Acid Absorption

Key ●; salicylic acid, 2 mM,
○; salicylic acid, 2 mM+carbachol, 1 mM.

For determination of the volume in the intestine and the drug absorption (%), see Fig. 4.

$$y = 0.350x + 1.108 \quad (r = 0.837, n = 18)$$

in the intestine. Irrespective of the drug tested, the scattered points obtained with and without carbachol were well separated from each other. In the presence of carbachol, the perfusate volume in the intestine was markedly decreased and drug absorption was reduced. Furthermore, there appeared to be a proportional relationship between the mean perfusate volume in the intestine and the drug absorption (%), and the data were analyzed by the least-squares method. The regression coefficients for CA and SA were similar (0.849 and 0.837, respectively) and greater than that for AM (0.648), but the difference was not statistically significant.

Table I shows the mean values \pm S.E. for perfusate volume in the intestine and drug absorption in the absence and presence of 1 mM carbachol. In the presence of carbachol, the perfusate volume in the intestine and drug absorption were decreased significantly ($p < 0.05$) as compared to the values obtained when the perfusate did not contain carbachol, but no marked change in the ratio of water flux was noted. There was no difference in rat body weight between the groups in the absence and the presence of carbachol.

TABLE I. Effect of Carbachol (1 mM) on the Drug Absorption and the Perfusate Volume in Rat Small Intestine

Drug (2 mM)	N	Body weight (g)	Intestinal volume ^{a)} (ml)	Absorption ^{a)} (%)	Ratio of water flux ^{b)}
AM {alone	8	226.6 \pm 12.2	13.1 \pm 1.5	47.1 \pm 6.2	0.975 \pm 0.023
AM {with carbachol	11	221.4 \pm 12.4	11.1 \pm 1.7*	38.6 \pm 9.1*	0.994 \pm 0.020
CA {alone	9	215.3 \pm 3.4	11.5 \pm 1.6	30.5 \pm 3.6	0.986 \pm 0.015
CA {with carbachol	9	220.6 \pm 4.2	9.6 \pm 1.5*	24.4 \pm 3.9*	0.990 \pm 0.007
SA {alone	9	229.9 \pm 6.6	13.1 \pm 2.1	35.0 \pm 6.9	0.976 \pm 0.006
SA {with carbachol	9	222.9 \pm 7.1	9.6 \pm 1.5*	24.5 \pm 5.0*	0.986 \pm 0.009

a) For determination of the volume in the intestine and the drug absorption (%), see Fig. 4.

b) Concentration of phenol red at $t=0$ /concentration of phenol red at 30 min.

* Significantly different from the "alone" of each group ($p < 0.05$).

Each value represents the mean \pm S.E.

N, number of rats; AM, aminopyrine; CA, caffeine; SA, salicylic acid.

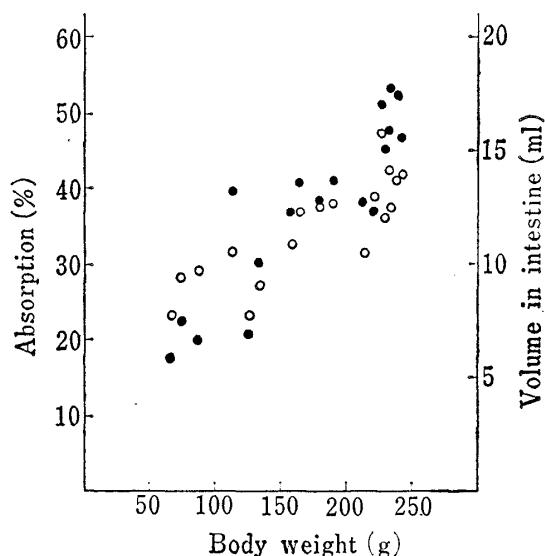


Fig. 7. Relationship between Body Weight, Perfusate Volume in the Intestine (○) and Aminopyrine Absorption (●)

For determination of the volume in the intestine and the drug absorption(%), see Fig. 4.

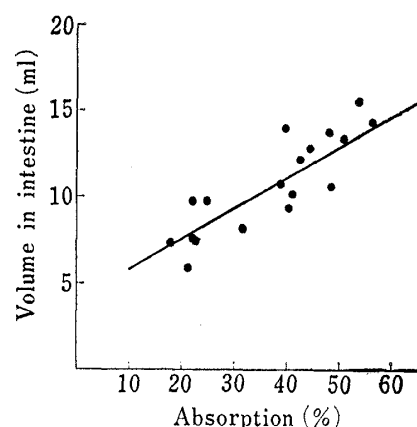


Fig. 8. Relationship between Perfusate Volume in the Intestine and Aminopyrine Absorption in Rats weighing 70 to 250 g

For determination of the volume in the intestine and the drug absorption(%), see Fig. 4.

$$y = 0.196x + 4.034 \quad (r = 0.849, n = 18)$$

We examined the relationships among the body weight and the mean perfusate volume in the intestine and AM absorption in rats (70—240 g) perfused for 30-min according to the recirculating perfusion method. As shown in Fig. 7, the greater the body weight, the higher were the values for perfusate volume in the intestine and AM absorption. The double correlation coefficient was $r(y_1, y_2, x) = 0.874$. The relationship between AM absorption and the mean perfusate volume in the intestine is illustrated in Fig. 8. The regression equation ($y = 0.196x + 4.034$) calculated by the least-squares method was similar to the equation ($y = 0.183x + 4.137$) obtained from Fig. 4. As in the examination of the relationship between perfusate volume in the intestine and AM absorption in the absence or presence of carbachol (Fig. 4), we found that AM absorption during 30-min recirculating perfusion was positively correlated with the volume of perfusate.

Lipid-aqueous Phase Partition Coefficient

Intestinal drug absorption is proportionate to the degree of hydrophobia, which is correlated with the partition coefficient between an organic solvent and water.¹¹⁾

Table II shows the partition coefficient for the different drug-containing perfusates in the absence or presence of 1 mM carbachol and the added organic solvents. The coefficients were not markedly affected by carbachol.

TABLE II. Apparent Partition Coefficients of Aminopyrine, Caffeine and Salicylic Acid between Organic Solvents and Phosphate Buffer with or without Carbachol at pH 7.0

Drug (2 mm)	Benzene		Isoamyl acetate	
	alone	with carbachol ^{a)}	alone	with carbachol ^{a)}
Aminopyrine	5.50	5.62	12.02	11.00
Caffeine	0.46	0.56	0.83	0.78
Salicylic acid	0.001	0.001	0.001	0.001

^{a)} Concentration, 1 mM.

In the present study, we examined the relationship between the perfusate volume in the intestine and drug absorption in the presence or absence of carbachol by the recirculating perfusion method.

We found that the carbachol-induced decrease in drug absorption was related to a decrease in the perfusate volume in the intestine (Fig. 2—6, Table I). Carbachol is stable in phosphate buffer (pH 7.0) at 37°C and is completely resistant to hydrolysis by intestinal choline esterase.¹²⁾ The poor absorption of quaternary ammonium compounds such as carbachol is thought to be due to their ionized character.¹³⁾ However, recirculating perfusion revealed that approximately 17% of 1 mM carbachol was absorbed *in situ* in the rat small intestine within 30 min. Quaternary ammonium compounds form stable complexes with various compounds in solution¹⁴⁾ and complex formation affects the intestinal drug absorption in various experimental animals.¹⁵⁾ If the observed effect of carbachol were due to complex formation in the perfusate, then *i.v.* administered carbachol should exert no effect on drug absorption from the small intestine. However, we noted that drug absorption was also decreased in rats injected *i.v.* with carbachol (Fig. 2).

Ochsenfact and Winne¹⁶⁾ have shown that mesenteric blood flow plays a role in the intestinal drug absorption of various animals, and Crouthamel *et al.*¹⁷⁾ reported that reductions in the mesenteric blood flow of dogs resulted in progressive impairment of sulfaethidol absorption. Carbachol extends the capillaries near the intestinal epithelium and increases the mesenteric blood flow;¹²⁾ suggesting that the observed decrease in drug absorption is not due to an effect of carbachol on mesenteric blood flow.

Intestinal drug absorption is affected by the physiological condition of the experimental animal. Furthermore, the co-administration of other drugs may affect the absorption of the test drug. Studies are under way to determine how smooth muscle contractions of the GI tract affect intestinal drug absorption.

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