Effect of Pre-administered Fats and Fatty Acids on the Intestinal Absorption of Tryptophan in the Rat[1]

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The effects of pre-administered fats and fatty acids on the absorption of tryptophan from the rat small intestine were investigated using *in situ* recirculation and *in vitro* everted sac techniques. Pre-administration of octanoic acid significantly inhibited the absorption of L-tryptophan, and this effect was not rapidly reversible. Trioctanoin, oleic acid, methyl octanoate, and octyl alcohol modestly inhibited the absorption of L-tryptophan, while triolein and olive oil did not significantly affect. The maximal transport capacity (V_{max}) seemed to be affected rather than Michaelis constant (K_m) by the administration of octanoic acid. Pre-administration of octanoic acid also inhibited the transport of L-tryptophan *in vitro*. In contrast, the absorption of D-tryptophan was partially inhibited by octanoic acid. With the pre-administration of octanoic acid there was an increase in the amount of protein released into the perfusate when compared to the control or the administration of other lipids. It is possible that the effect of pre-administered octanoic acid on the intestinal absorption of tryptophan may be due to the loss of structural and/or functional integrity of the intestinal membrane.

During the course of an investigation concerning the effects of fatty acids on the drug absorption from rat small intestine,[3] it became also desirable to explore the effects of dietary lipids to actively absorbed compounds.

Evidence has been obtained that several alcohols inhibited the active transport of amino acids in the small intestine.[4,5] Holtzapple, *et al.*[6] have reported that amino acid accumulation in jejunal segments prepared from rats given octanoic acid intragastrically was enhanced, when compared to the uptake in control segments. Ahmed, Walker,[7] Johns, and Bergen[8] have shown that essential fatty acid deficiency exhibited a marked effect on the ability of rat intestine to accumulate amino acids. Reiser and Christiansen[9] have determined the ability of lipid extracted from the intestinal mucosa to bind valine.

The purpose of the present investigation was to study the effect of pre-administered fats and fatty acids on the absorption of amino acids from the perfused rat small intestine. L-Tryptophan and D-tryptophan were chosen as model compounds for this purpose.

Experimental

Materials——L-Tryptophan and D-tryptophan were obtained from Wako Pure Chemical Industries, Ltd. Octanoic acid, oleic acid, trioctanoin, triolein (Tokyo Kasei Kogyo Co., Ltd.), octyl alcohol, methyl octanoate, and olive oil (Nakarai Chemicals, Ltd.) were used as supplied. All other chemicals were of analytical grade and were obtained commercially.

1) Presented to the 94th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April 1974.
2) Location: *Kasumi 1–2–3, Hiroshima.*
3) K. Inui, M. Horiguchi, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), 22, 1781 (1974); K. Inui, M. Shintomi, R. Hori, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), 24, 2504 (1976).
4) T. Chang, J. Lewis, and A.J. Glazko, *Biochim. Biophys. Acta,* 135, 1000 (1967).
5) Y. Israel, J.E. Valenzuela, I. Salazar, and G. Ugarte, *J. Nutr.,* 98, 222 (1969).
6) P. Holtzapple, W. Berman, and S. Segal, *Gastroenterol.,* 62, 849 (1972).
7) S. Ahmed and B.L. Walker, *Biochim. Biophys. Acta,* 255, 815 (1972).
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Preparation of Tryptophan Solutions——The composition of isotonic buffer solution (pH 6.5) used as the perfusion medium for the absorption experiments was NaH_2PO_4–Na_2HPO_4 and the incubation medium for *in vitro* experiments was a modified Krebs-phosphate buffer (pH 6.5) without calcium or magnesium,[10] saturated with oxygen–carbon dioxide (95: 5 v/v). The specified amounts of tryptophan were dissolved in the above mediums.

Animals——Male Wistar albino rats, weighing 150—180 g and fasted for 16—20 hr, were used in all experiments. In case of pre-administration of fats and fatty acids, the animals were given intragastrically 1 ml of various lipids, and the absorption studies were carried out at 1, 3, 6, and 9 hours later.

Procedure of *in Situ* Absorption Experiments——The procedure of *in situ* absorption experiment from the rat small intestine was the same as reported in the previous papers.[3] The animals were anesthetized with sodium pentobarbital. The abdomen was opened by a midline incision, and the small intestine, from the proximal end of the duodenum to the distal end of the ileum, was cannulated proximally and distally. The bile duct was ligated in all experiments. The small intestinal contents were washed out with physiological saline (about 60 ml) until the effluent became clear. Forty milliliters of tryptophan solution was recirculated through the intestine at a rate of 5 ml/min. After 30 min, the perfusate was withdrawn as completely as possible, and washed with physiological saline. The washings were combined to the perfusate and made up to 100 ml with physiological saline. Blood samples were collected immediately from the carotid artery at the end of absorption experiments. The amount absorbed was calculated by the difference in amount of tryptophan between the initial and the final solutions. In some experiments, 40 ml of buffer alone was recirculated similarly in order to determine the amount of protein released into the perfusate for 30 min.

Procedure of *in Vitro* Everted Sac Method——The jejunum was removed, everted, and tied into sacs about 7 cm long according to the method of Wilson and Wiseman.[11] Each sac was filled with 1 ml of Krebs-phosphate buffer containing L-tryptophan. The sacs were placed in a 25 ml Erlenmeyer flask containing 5 ml of the same solution as inside the sac and incubated at 37° for 60 min in a water bath with shaker. After the incubation the sacs were removed from the flasks and excess incubation medium removed by touching them on the sides of a glass beaker. The sacs were then weighed, the inside fluid drained and collected, and the empty sacs reweighed. The ratio after incubation of the concentration of L-tryptophan between serosal and mucosal fluid was determined.

In some experiments, initial uptake of L-tryptophan into the everted intestine was estimated. The whole small intestine was everted, tied both ends, and incubated in 10 ml of buffer solution containing L-tryptophan at 37°. No solution was placed in the serosal side. Aliquots (0.5 ml) of mucosal solution were taken periodically at 1, 2, 3, and 5 min after the incubation was started. The uptake of tryptophan into the intestine was calculated by the disappearance of tryptophan from the initial incubation solution.

Analytical Methods——Tryptophan was determined by procedure A of the method of Spies and Chambers.[12] In the case of blood samples, 1 ml of plasma was deproteinized by the addition of 1 ml of 30% trichloroacetic acid and centrifuged. One milliliter of the supernate was used for the estimation. Protein determination of the perfusate was made by the method of Lowry, *et al.*[13] Bovine serum albumin (Fraction V, Seikagaku Kogyo Co., Ltd.) was used as a standard.

Results and Discussion

The active intestinal transport of tryptophan has been studied in detail by many investigators.[14-18] The intestinal absorption of L-tryptophan is described by a saturable process that conforms to Michaelis–Menten kinetics. D-Isomer is also absorbed from the intestine with minor contribution of the same specialized transport system. In the present investigation, the absorption rate of L-tryptophan was considerably high at lower concentration. Therefore, 30 min was mainly selected for the period of *in situ* absorption study.

Effect of Pre-administration of Various Lipids on the Intestinal Absorption of L-Tryptophan

The apparent effects of pre-administered lipids on the absorption of 5 mM L-tryptophan from the rat small intestine at pH 6.5 is summarized in Table I. Absorption experiments

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14) R.P. Spencer and A.H. Samiy, *Am. J. Physiol.*, **199**, 1033 (1960).
15) K. Kakemi, H. Sezaki, M. Nakano, and E. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **18**, 2176 (1970).
16) S.G. Schultz, L. Yu-Tu, and C.K. Strecker, *Biochim. Biophys. Acta*, **288**, 367 (1972).
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TABLE I.   Effect of Pre-administration of Various Lipids on the
Absorption of L-Tryptophan from Rat Small Intestine

Lipid	% absorbed in 30 minutes
Control	53.1 ± 4.9
Octanoic acid	$28.6 \pm 3.3^{a)}$
Oleic acid	$44.7 \pm 5.1^{b)}$
Trioctanoin	$41.4 \pm 3.2^{a)}$
Triolein	$48.0 \pm 4.4^{c)}$
Olive oil	$51.5 \pm 4.4^{c)}$
Octyl alcohol	$42.4 \pm 2.1^{d)}$
Methyl octanoate	$45.0 \pm 2.9^{d)}$

concentration of L-tryptophan = 5 mM
Various lipids (1 ml) were administered intragastrically 3 hr before absorption experiments.
Each value is the mean ± S.D. in at least 4 animals.
significantly different from control:
a) $p < 0.001$ b) $p < 0.02$ c) not significant $p > 0.05$ d) $p < 0.01$

were carried out at 3 hours after intragastric administration of lipids (1 ml). Pre-administration of octanoic acid significantly inhibited the absorption of L-tryptophan compared to the control experiment. In contrast, pre-administration of trioctanoin and oleic acid modestly inhibited the absorption of L-tryptophan, while long chain triglycerides such as triolein and olive oil did not significantly affect the absorption of L-tryptophan. Octyl alcohol and methyl octanoate had a little inhibitory effect. Then, the effects of octanoic acid and trioctanoin on the intestinal absorption of tryptophan were further investigated.

The time course of the effect of pre-administered octanoic acid and trioctanoin on the intestinal absorption of 5 mM L-tryptophan is shown in Fig. 1. As is evident from the figure,
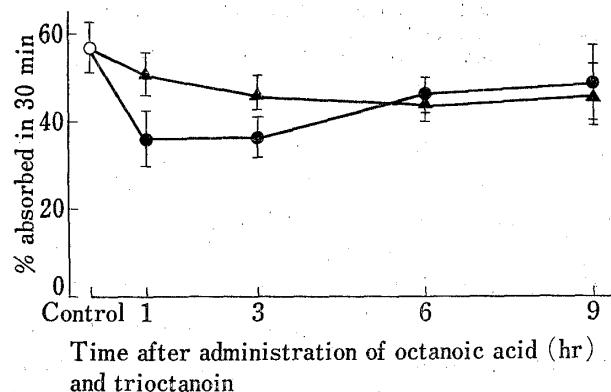

Fig. 1.   Time Course of the Effect of Pre-administered Octanoic Acid and Trioctanoin on the Intestinal Absorption of L-Tryptophan

concentration of L-tryptophan = 5 mM
—○—: control
—●—: pre-administration of octanoic acid
—▲—: pre-administration of trioctanoin
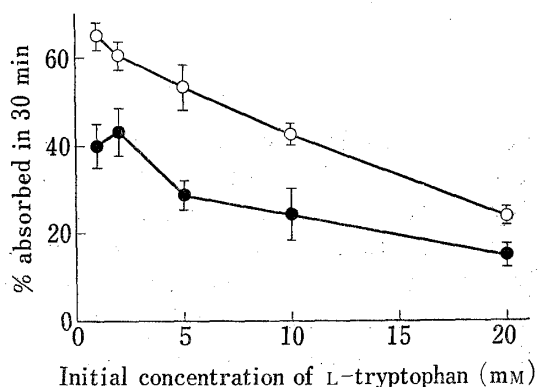Each point represents the mean ± S.D. in at least 4 animals.

Fig. 2.   Effect of Pre-administered Octanoic Acid on the Intestinal Absorption of L-Tryptophan at Various Initial Concentrations

—○—: control
—●—: pre-administration of octanoic acid
Octanoic acid (1 ml) was administered intragastrically 3 hr before absorption experiments.
Each point represents the mean ± S.D. in at least 3 animals.

octanoic acid inhibited more rapidly the absorption of L-tryptophan than trioctanoin did. Absorption-inhibiting effect by octanoic acid was decreased gradually and the curve approached to the control value at 9 hours later. Abundant intestinal contents, which contained the administered lipids, were observed in the lumen 3 hours after intragastric administration, but little of them were observed in the lumen 9 hours after intragastric administration.

It is presumed that a certain existence of octanoic acid on the mucosal surface is necessary for this inhibitory effect of octanoic acid. Therefore, the following experiments were carried out at 3 hours after intragastric administration of octanoic acid.

Effect of Pre-administered Octanoic Acid on the Intestinal Absorption of L-Tryptophan

Effect of pre-administered octanoic acid on the intestinal absorption of L-tryptophan is given as a function of tryptophan concentration in the perfusion solution in Fig. 2 and 3.
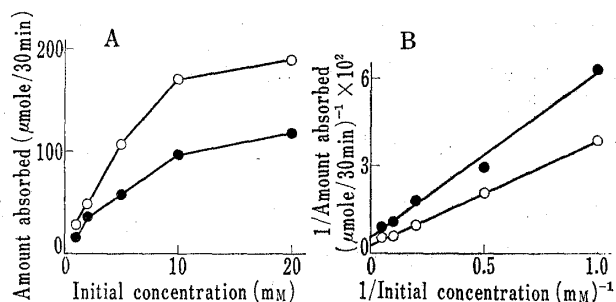

Fig. 3. Effect of Pre-administered Octanoic Acid on the Intestinal Absorption of L-Tryptophan A: Relation between the Initial Concentration and the Absorption Rate of L-Tryptophan B: Line-weaver–Burk Plots

Data were calculated from the values shown in Fig. 2.
—○—: control
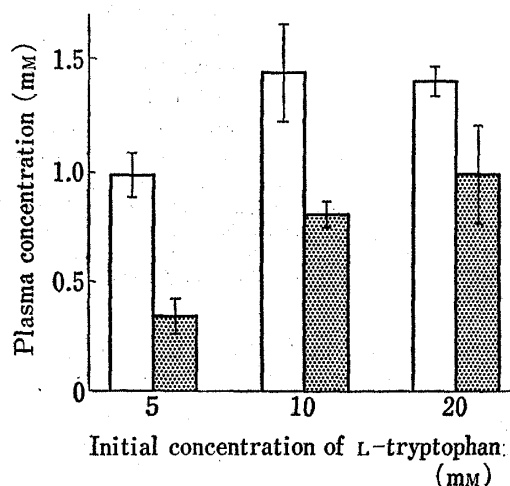—●—: pre-administration of octanoic acid

Fig. 4. Plasma Concentration of L-Tryptophan after Intestinal Absorption Experiments at Various Initial Concentrations

▢: control
▨: pre-administration of octanoic acid
Plasma concentration was measured following the recirculation of L-tryptophan solution through the intestine for 30 min.
Each column represents the mean ±S.D. in at least 3 animals.

In control rats, it is evident from the figures that the rate of absorption of L-tryptophan is not proportional to the initial concentration of the amino acid and that the absorption of L-tryptophan can be adequately described by a saturable process conforming to Michaelis–Menten kinetics. In the case of pre-administration of octanoic acid, the absorption of L-tryptophan also displayed a saturation kinetics and was inhibited to varying extents within the concentration range employed in this study. As can be seen in Fig. 3, administration of octanoic acid had little effect on the affinity (K_m) of the transport mechanism for L-tryptophan. In contrast, the maximal transport capacity (V_{max}) was rather decreased by administration of octanoic acid.

Possible structural or functional alterations, which could be caused by the pre-administration of octanoic acid and which may affect the apparent permeability of the intestinal mucosa to L-tryptophan, are thickness of unstirred layers on the luminal side of the epithelial cell,[19,20] mucosal blood flow,[21] and impairment of carrier-mediated transport systems in the intestinal mucosa. Winne,[19] Wilson and Dietschy[20] have reported that the apparent Michaelis constant (K_m) of a carrier-mediated transport system in a membrane is determined too high when an unstirred layer is increased. In this study, however, V_{max} seemed to be affected rather than K_m by the administration of octanoic acid. Thereby, the absorption-inhibiting effect of octanoic acid cannot be attributed to the increase of unstirred layers.

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20) F.A. Wilson and J.M. Dietschy, *Biochim. Biophys. Acta*, **363**, 112 (1974).
21) D. Winne, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **277** 113 (1973).

To verify that the disappearance of L-tryptophan from the intestinal perfusion solution reflects its absorption, the concentration of tryptophan in the plasma was determined at the end of absorption experiments. Figure 4 depicts plasma tryptophan concentration obtained following the recirculation of L-tryptophan solution through the intestine for 30 min. The concentration of tryptophan in the plasma was significantly decreased by the administration of octanoic acid. This finding shows that the decline in tryptophan concentration in the intestinal perfusion solution reflects absorption of the amino acid and that octanoic acid inhibit this process.

To estimate the relative absorption rate more precisely, the time course of 5 mM L-tryptophan absorption affected by pre-administration of octanoic acid was studied during 60 min. It appeared that the inhibitory effect of octanoic acid continued within this period. In another experiment, the absorption of 5 mM L-tryptophan was studied 1 hour after washing in the intestinal lumen of octanoic acid administered rats. The absorption of L-tryptophan was similarly inhibited by this treatment. It is therefore conceivable that the absorption-inhibiting effect of octanoic acid is not rapidly reversible.

In order to further investigate the inhibitory effect of octanoic acid in detail, *in vitro* experiments were performed. The initial uptake of 0.25 mM L-tryptophan into the everted intestine is shown in Fig. 5. As in the *in situ* absorption studies, the initial uptake of L-tryptophan into the intestine was inhibited by the administration of octanoic acid.
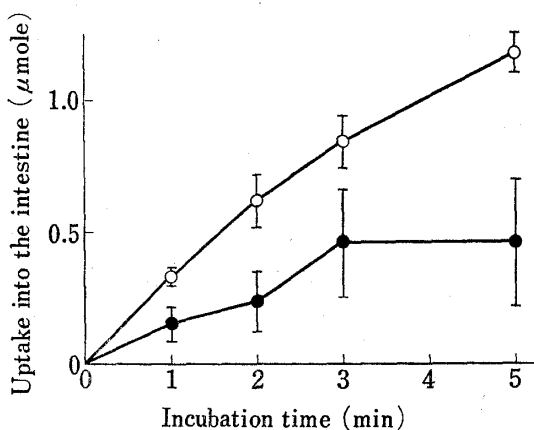

Fig. 5. Effect of Pre-administered Octanoic Acid on the Initial Uptake of L-Tryptophan into the Everted Intestine

concentration of L-tryptophan=0.25 mM

—○—: control
—●—: pre-administration of octanoic acid

Octanoic acid (1 ml) was administered intragastrically 3 hr before sacrifice.
Each point represents the mean ±S.D. in at least 4 experiments.
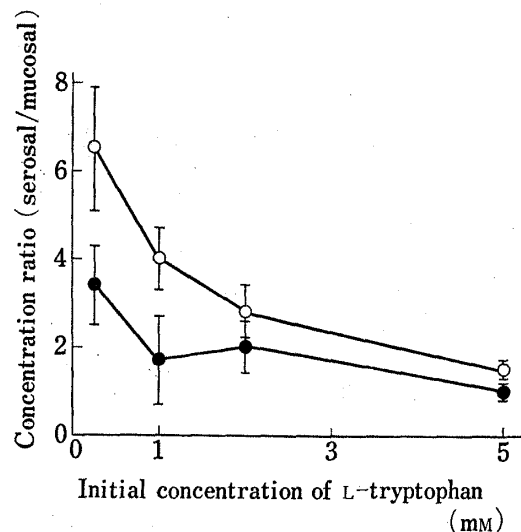

Initial concentration of L-tryptophan (mM)

Fig. 6. Relation between the Initial L-Tryptophan Concentration and the Concentration Ratio of Serosal to Mucosal Fluid

—○—: control
—●—: pre-administration of octanoic acid
Octanoic acid(1 ml) was administered intragastrically 3 hr before sacrifice.
Each point represents the mean ±S.D. in at least 4 experiments.

Furthermore, the same concentration solution of L-tryptophan was placed in the mucosal and serosal sides, and the concentration ratio of serosal to mucosal fluid after 60 min incubation was determined. The relation between the initial L-tryptophan concentration and the concentration ratio of serosal to mucosal fluid is given in Fig. 6. The administration of octanoic acid decreased the ratio of L-tryptophan compared to the control.

Thus, octanoic acid also inhibited significantly the transport of L-tryptophan *in vitro*. From these results, there is little possibility that the absorption-inhibiting effect of octanoic acid is due to the change of mucosal blood flow.[21]

Effect of Pre-administered Octanoic Acid on the Intestinal Absorption of D-Tryptophan

To examine whether octanoic acid inhibited specifically the absorption of L-tryptophan, the effect of pre-administered octanoic acid on the absorption of D-tryptophan was studied. The results are shown in Table II. The absorption of D-tryptophan was lower than that of

TABLE II. Effect of Pre-administered Octanoic Acid on the
Intestinal absorption of D-Tryptophan

	% absorbed in 30 minutes	
	5 mM	10 mM
Control	15.8 ± 3.0	11.6 ± 1.3
Octanoic acid	$9.4 \pm 0.9^{a)}$	$9.5 \pm 2.1^{b)}$

Octanoic acid (1 ml) was administered intragastrically 3 hr before absorption experiments.
Each value is the mean $\pm$ S.D. in at least 4 animals.
a) significantly different from control, $p < 0.05$
b) not significant, $p > 0.05$

L-isomer, but was dependent on the concentration.[15] Octanoic acid inhibited the absorption of 5 mM D-tryptophan, while it did not significantly inhibit the absorption of 10 mM D-tryptophan. It is known that D-isomer is absorbed from the intestine with minor contribution of the specialized transport system. Therefore, the absorption of 10 mM D-tryptophan appears to be largely attributable to simple diffusion and seems to be little affected by the administration of octanoic acid.

Protein Release from the Perfused Rat Small Intestine

The absorption-inhibiting effect of the pre-administered octanoic acid may have resulted from structural alterations of the intestinal epithelium exposed to octanoic acid. To explore this possibility, the effect of pre-administered lipids on the release of protein from the small intestine, recirculated with pH 6.5 phosphate buffer alone for 30 min, was investigated. Table III lists the milligrams of protein released from the perfused intestine. As can be seen from

TABLE III. Effect of Pre-administered Lipids on the Protein Release
from the Perfused Rat Small Intestine

Lipid	Protein released (mg)
Control	9.0 ± 2.5
Octanoic acid	$21.2 \pm 5.5^{a)}$
Trioctanoin	$8.7 \pm 1.6^{b)}$
Oleic acid	$7.7 \pm 1.5^{b)}$

Various lipids (1 ml) were administered intragastrically 3 hr before perfusion experiments,
with buffer alone.
Each value is the mean $\pm$ S.D. of five animals.
a) significantly different from control $p < 0.005$
b) not significant $p > 0.2$

the data, with the pre-administration of octanoic acid there was an increase in the total amount of protein released when compared to the control or the administration of other lipids. It appears that octanoic acid may alter the composition of the intestinal membrane by producing an efflux of protein from the perfused intestine, resulting in an inhibition of the active transport to tryptophan.

On the basis of the *in situ* and *in vitro* studies, it is possible that the effect of pre-administered octanoic acid on the intestinal absorption of L-tryptophan may be due to the loss of structural and/or functional integrity of the intestinal membrane.