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Simultaneous Vascular and Luminal Perfusion of Rat Small Intestine

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A method is described which allows simultaneous vascular and luminal perfusion of the jejunal segment of rat intestine. Perfluorochemical artificial blood (FC-43 Emulsion) was utilized as a vascular perfusion medium. The viability of this preparation can be maintained for 1 h as indicated by morphological findings, competitive inhibition between L-phenylalanine and L-methionine, and the transport of L-phenylalanine against a concentration gradient. This preparation was used for investigation of the transport behavior of several amino β-lactam antibiotics.

Keywords——intestinal absorption; vascular perfusion; luminal perfusion; L-phenylalanine; ampicillin; cephalexin; cephradine

There have been numerous attempts[1] to study the rat and the mouse intestine as isolated tissue sustained by vascular perfusion. Many of these methods utilized whole blood or erythrocytes obtained from test animals as an oxygen carrier, and Windmueller *et al.*[1a] developed a procedure in which norepinephrine and a glucocorticoid are used for the maintenance of viability. These methods, however, require a large quantity of fresh blood or erythrocytes, as well as time-consuming procedures for preparation of the perfusate. Furthermore, it is desirable to exclude any additive such as a vasodilator or hormone, since the additives may interact with test drugs in the process of absorption. We have developed a simple procedure for perfusion of rat intestine by using perfluorochemical artificial blood as a perfusate, which does not require erythrocyte and additives. This report describes the technique and the characteristics of this preparation.

Experimental

Animals——Male Wistar rats (180—200 g) were fed commercial diet (Oriental Yeast Industries, MF) and water *ad libitum*. Food was withdrawn 18—21 h before the experiment but water was available *ad libitum*.

Materials——Ampicillin anhydrous (Takeda Chemical Industries, Osaka, Japan), cephalexin monohydrate (Shionogi & Co., Tokyo, Japan), and cephradine dihydrate (Sankyo Co., Tokyo, Japan) were kindly supplied by the manufactures. Perfluorochemical artificial blood (FC-43 Emulsion) was purchased from the Green Cross Co. (Osaka, Japan). L-Phenylalanine and L-methionine were from Wako Pure Chemicals (Tokyo, Japan). Heparin-Na was from Shimizu Seiyaku Co. (Shimizu, Japan). All other chemicals were of reagent grade, and were used without further purification.

Perfusion Media——FC-43 Emulsion (290—300 mOsm, pH 7.4) was used as a vascular perfusion medium. The composition of FC-43 Emulsion was as follows (%, w/v): FC-43 (perfluorotributylamine), 20.0; Pluronic F-68 (polyoxypropylene–polyoxyethylene copolymer), 2.56; NaCl, 0.6; KCl, 0.034; $MgCl_2$, 0.02; $CaCl_2$, 0.028; $NaHCO_3$, 0.21; glucose, 0.18; hydroxyethyl starch, 3.0.

The luminal perfusion medium was modified Ringer solution[2] (290 mOsm, pH 6.8).

Perfusion Apparatus——A shematic diagram of the combined intraluminal circulation and vascular perfusion method is shown in Fig. 1. Perfusates for the lumen and the vessel were warmed at 37 °C with a water jacket, and the perfusate for the vessel was oxygenated with a mixture of moist 95% O_2–5% CO_2 during the experimental period. The

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luminal perfusate (10 ml) was recycled to the reservoir by a glass pump (Tokyo Rika Kikai Co., Tokyo, Japan, model GMW) at a flow rate of 4 ml/min. One-tenth milliliter of the medium was pipetted from the reservoir at various times. The vascular perfusate was infused by single-pass perfusion by using a micro tubing pump (Tokyo Rika Kikai Co., Tokyo, Japan, model MP-3) at a rate of 0.8 ml/min, and the effluent was collected from the venous cannula as shown in Fig. 1 at fixed intervals of time.

Surgical Procedure——Rats were anesthetized with sodium pentobarbital (i.p., 3 mg/100 g body weight), and the surgery and absorption experiments were performed under this anesthesia. The small intestine was exposed *via* a midline abdominal incision, and about 30 cm length of proximal gut was gently removed from the surrounding mesentery. About 10 cm of jejunum (below 20 cm from the pylorus), with a pair of mesenteric vessels, was then selected. The content in the lumen was washed out with 10 ml of warm saline and the surface was blotted gently with the gauze. Inflow and outflow glass cannulas for the luminal perfusion were tied in the lumen. The surrounding mesenteric vessels unrelated to the perfusion were ligated.

Heparin (50 heparin units) was injected into the jugular vein, and loose ties were placed around the mesenteric artery and vein. The proximal mesenteric artery was ligated and a small incision was made with a needle. The inflow polyethylene cannula (cut at an angle at the end; outside diameter, 1 mm) was then inserted immediately into the mesenteric artery while passing the vascular perfusate, and secured with the arterial tie. Finally, the outflow cannula (cut at an angle at one end; outside diameter, 1.3 mm) was inserted into the mesenteric vein and secured.

The absorption experiment was started after the mesenteric blood had been displayed completely with the vascular perfusate (FC-43). The intestine was covered with saline-soaked gauze and kept at 37 °C by means of a heat lamp.

Electron Microscopy——samples for scanning electron microscopy were taken before and after a perfusion period of 1 h. Specimens were fixed with glutaraldehyde and dried by using conventional techniques. They were examined with a Hitachi S-450 scanning electron microscope.

Analytical Methods——L-Phenylalanine and β-lactam antibiotic were determined by high-performance liquid chromatographic (HPLC) methods. To remove interfering substances in the vascular perfusate, the vascular samples were centrifuged at $25000 \times g$ for 20 min, and the supernatant (1 ml) was shaken with 1 ml of chloroform for 10 min. The mixture was centrifuged at $900 \times g$ for 5 min, and an appropriate volume of the aqueous layer was injected into the HPLC apparatus.

A liquid chromatograph (Hitachi 635 A) equipped with a multi-wavelength detector (Hitachi 638-41) was used. For the determination of aminocephalosporins, Hitachi gel #3053 (ODS, 25 cm × 4 mm i.d.) was used as the stationary phase, with a mobile phase of 0.05 M KH_2PO_4 solution–methanol (85 : 15, v/v). For the determination of L-phenylalanine, Hitachi gel #3056 (ODS, 25 cm × 4 mm i.d.) and a mixed solution of the above components (95 : 5, v/v) were used. The wavelength was set at 260 nm and 256 nm for aminocephalosporins and L-phenylalanine, respectively. In the case of ampicillin or aminocephalosporins at concentrations below about 1 μg/ml in the medium, an HPLC technique[3] using a fluorometric detector was employed.

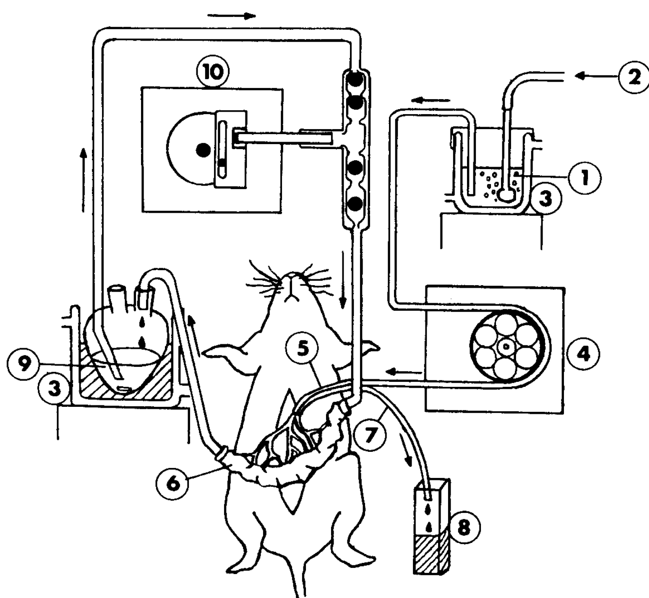

Fig. 1. Schematic Diagram of the Combined Intraluminal and Vascular Perfusion Method in the Rat Jejunal Segment

1, vascular perfusion medium (37 °C); 2, gas (95% O_2–5% CO_2); 3, water jacket; 4, pump for vascular perfusion; 5, inflow cannula; 6, upper jejunal segment (10 cm); 7, outflow cannula; 8, collecting vessel for venous outflow; 9, intraluminal perfusion medium (37 °C); 10, pump for intraluminal perfusion.

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Results and Discussion

Viability of the Vascularly Perfused Segment

The viability of the preparation was examined in terms of several criteria, namely the preservation of an intact morphology, the ability to actively transport L-phenylalanine, and low leakage from the vascularly perfused segment.

After 1 h of perfusion the morphology of the microvilli did not show any abnormality compared to the non-perfused control, as shown in Fig. 2.

Active transport, *i.e.* carrier-mediated transport against a concentration gradient, of sugars and amino acids is indicative of intact functioning of *in vitro* preparations. Therefore, the transport behavior of L-phenylalanine was examined. Two types of experiments were carried out. In the first, the inhibitory effects of L-methionine on the absorption of L-phenylalanine were examined by using the vascularly perfused preparation and an intact intestinal loop. The loop was prepared according to the method of Levine and Pelikan.[4]

As shown in Fig. 3, the extents of transport of L-phenylalanine in the absence of L-methionine were almost the same in both cases. Although there was some difference in the degree of inhibition, L-phenylalanine transport was reduced markedly by L-methionine in both cases. Furthermore, L-phenylalanine appearance in the vascular bed was also reduced significantly by L-methionine (Fig. 4).

In order to demonstrate that L-phenylalanine was transported "uphill", a further experiment was conducted in which 1 mM L-phenylalanine was present in both the vascular and luminal perfusion media. Single-pass luminal and vascular perfusion was carried out. As shown in Fig. 5, after the luminal perfusion was started the concentration of L-phenylalanine in the vascular effluent became higher than the initial value, and thereafter the uphill transport was maintained. A high concentration ratio (about 1.2) was maintained until 60 min (data not shown).

From these results, it was confirmed that the biological functions (such as carrier-mediated transport) were well maintained in the vascularly perfused intestine.

The leakage of the test compound from the vascularly perfused segment was then examined. After 1 ml of drug solution (cephalexin, 150 μM) had been injected into the intestinal loop, vascular perfusion was carried out for 1 h, and the amounts of drug in the loop, in the tissue, and in the venous effluent were measured by the HPLC method[3] using a fluorometric detector. The amounts of drug in the loop and the tissue were also compared

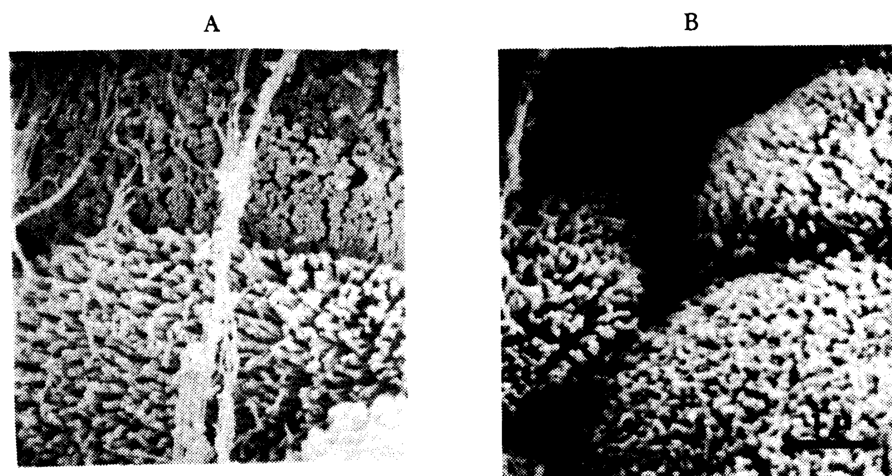

Fig. 2. Scanning Electron Micrograph of the Microvilli before (A) and after (B) Luminal and Vascular Perfusion of a Jejunal Segment for 1 h (12000 ×)

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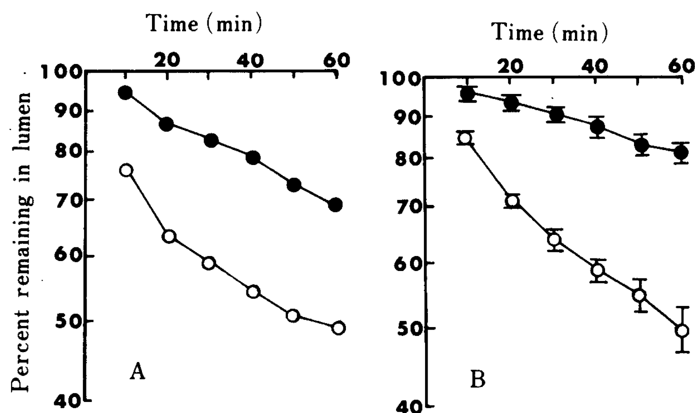

Fig. 3. Effects of L-Methionine on L-Phenylalanine Disappearance from Intact (A) and Vascularly Perfused (B) Rat Jejunal Segment

L-Phenylalanine (1 mM) was recycled through the jejunal segment in the absence (○) or in the presence (●) of L-methionine (20 mM). The results obtained from the intact segments are expressed as means of two experiments. The results obtained from the vascularly perfused segments are expressed as means ± S.E.M. of four experiments.

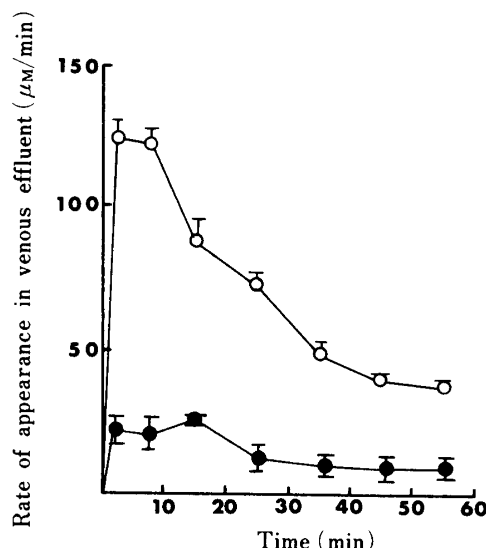

Fig. 4. Effects of L-Methionine on L-Phenylalanine Appearance in the Venous Effluent

L-Phenylalanine (1 mM) was recycled through the jejunal segment in the absence (○) or in the presence (●) of L-methionine (20 mM). The results are expressed as means ± S.E.M. of four experiments.

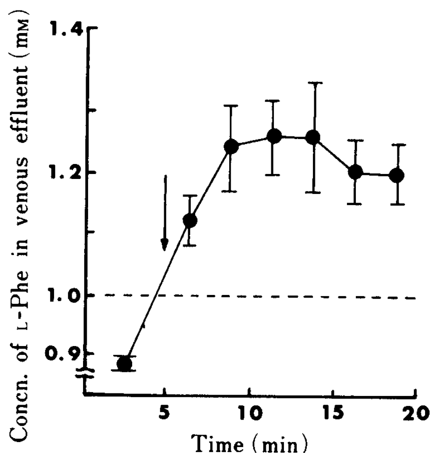

Fig. 5. Transport of L-Phenylalanine against a Concentration Gradient

Luminal and vascular perfusion with medium containing 1 mM L-phenylalanine was carried out. The luminal perfusion was started at the time shown by the arrow (5 min) while perfusing the vascular medium. The results are expressed as means ± S.E.M. of four experiments.

with those obtained from the intact loop. As shown in Table I, the recovery of the drug from the vascularly perfused segment was almost complete, and the values were in good agreement with those obtained from the intact intestinal loop. From these results, it is considered that the vascularly perfused small intestine remains viable for at least 1 h.

Absorption Properties of Cephalexin, Cephradine, and Ampicillin

The preparation was used to investigate absorption differences between aminocephalosporins (cephalexin and cephradine) and ampicillin. Figure 6 shows the disappearance profiles (log scale) from the intestinal lumen and the rate of appearance in the venous effluent of these drugs. Amino-cephalosporins disappeared rapidly from the lumen and appeared more markedly in the venous effluent compared to ampicillin. The superior absorption of these aminocephalosporins is well known,[5] and the results obtained in the present study agreed well with those observations. This technique directly demonstrated that there were significant

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Table I. Recovery of Cephalexin from Vascularly Perfused and Non-vascular Perfused Rat Intestine

	Perfused intestine	Non-vascular perfused intestine
Percent of drug remaining in intestinal lumen	23.6±1.5	22.7±0.7
Percent of drug accumulated in intestinal tissue	52.0±1.2	51.5±2.1
Percent of drug appearing in vascular bed	23.2±1.2	—
Total percent of drug recovered	98.8±1.0	—

The results are expressed as means±S.E.M. of four or five experiments.

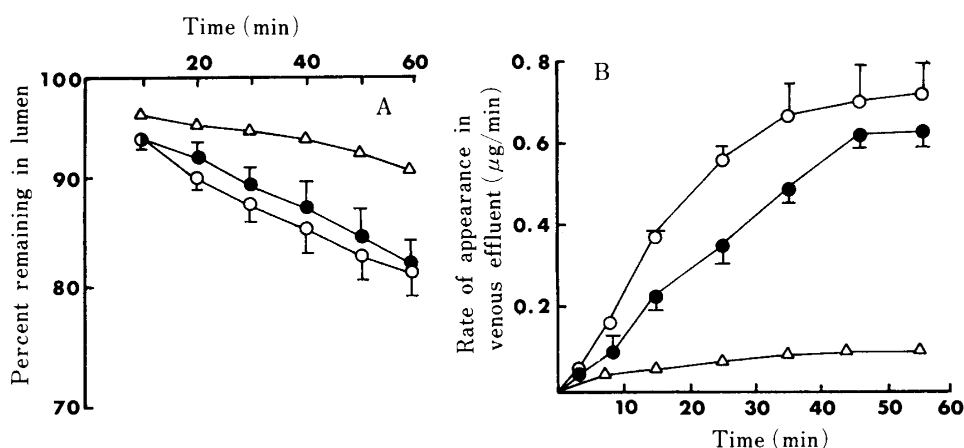

Fig. 6. Intestinal Absorption of Ampicillin (△), Cephalexin (●), and Cephradine (○) from Vascularly Perfused Rat Jejunal Segments

Each drug (150 μM) was recycled through the intestine. Disappearance from the lumen (A) and appearance in the venous effluent (B) are shown. The results are expressed as means of two or three (with S.E.M.) experiments.

differences in the venous appearance rate between aminocephalosporins and ampicillin.

The present method has several advantages. First, this method is very simple, since the vascular perfusate is an artificial blood and does not require time-consuming preparation, in contrast to perfusate containing whole blood or erythrocytes. Second, this method does not require the use of many rats. Third, the preparation of the segment is quick and technically easy. Therefore this vascularly perfused intestine should be useful as a preparation for directly investigating all the steps (disappearance, accumulation, and venous appearance) of drugs, as well as drug metabolism in the gut wall, blood-to-lumen movements, and the effect of blood flow on drug absorption.

References

1) *a)* H. G. Windmueller, A. E. Spaeth, and C. E. Ganote, *Am. J. Physiol.*, **218**, 197 (1970); *b)* J. M. J. Lamers and W. C. Hülsman, *Biochim. Biophys. Acta*, **275**, 491 (1972); *c)* P. J. Hanson and D. S. Parsons, *J. Physiol.* (London), **255**, 775 (1976); *d)* H. Ochsenfahrt, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **306**, 105 (1979); *e)* P. Wollenberg, V. Ullrich, and Rummel, *Biochem. Pharmacol.*, **32**, 2103 (1983).
2) S. G. Schultz, R. E. Fuisz, and P. F. Curran, *J. Gen. Physiol.*, **49**, 849 (1966).
3) K. Miyazaki, K. Ohtani, K. Sunada, and T. Arita, *J. Chromatogr.*, **276**, 478 (1983).

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4) R. R. Levine and E. W. Pelikan, *J. Pharmacol. Exp. Ther.*, **131**, 319 (1961).
5) K. Umeniwa, O. Ogino, K. Miyazaki, and T. Arita, *Chem. Pharm. Bull.*, **27**, 2177 (1979); A. Tsuji, E. Nakashima, I. Kagami, and T. Yamana, *J. Pharm. Sci.*, **70**, 768 (1981); K. Miyazaki, K. Ohtani, K. Umeniwa, and T. Arita, *J. Pharmacobio-Dyn.*, **5**, 555 (1982); T. Kimura, T. Yamamoto, M. Mizuno, Y. Suga, S. Kitade, and H. Sezaki, *ibid.*, **6**, 246 (1983).

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