Mechanisms of Intestinal Absorption of the Antibiotic, Fosfomycin, in Brush-Border Membrane Vesicles in Rabbits and Humans

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In order to clarify the mechanism of intestinal absorption of an antibiotic, fosfomycin (FOM), the uptakes of FOM by rabbit and human small intestinal brush-border membrane vesicles (BBMV) were studied. The initial uptake of FOM by BBMV at 15 s was saturable at a higher concentration of FOM. The kinetic parameters at 37 °C of the saturable uptake expressed by the Michaelis–Menten equation were $K_t = 5.17$ mM and $J_{\max} = 3.88$ nmol/15 s/mg protein for rabbits, and $K_t = 4.03$ mM and $J_{\max} = 1.90$ nmol/15 s/mg protein for humans. The most efficient uptake was observed in the presence of both inward-directed Na^+ - and H^+ -gradients in both mammals. The uptake of FOM was inhibited by inorganic phosphate, FOM glycol which is a degradation product of FOM in the gastric juice and specific inhibitors of phosphate transport such as arsenate and phosphonoacetic acid. These findings confirmed that FOM absorption from rabbit and human small intestines is associated with the phosphate transport system. These transport phenomena of FOM are in close agreement with those obtained previously in rat BBMV studies. Judging from the results obtained for three mammalian species, rat, rabbit and human, it was concluded that carrier-mediated transport *via* the phosphate transport system is a very important pathway of intestinal absorption of FOM.

Keywords — fosfomycin; antibiotic; intestinal brush-border membrane vesicle; intestinal uptake; carrier-mediated transport; sodium-cotransport; species difference; rabbit; human

Introduction

Fosfomycin ((-)(1R, 2S)-1, 2-epoxypropylphosphonic acid, FOM) is a highly hydrophilic, small-molecule antibiotic (M_r , 138.1) which includes a phosphoric acid group in its structure. FOM is frequently used clinically by the oral and intravenous administration routes to treat various infectious diseases because its bacteriocidal mechanism is very unique.¹⁾ Although there have been some reports regarding FOM absorption²⁻⁵⁾ after oral dosing, the mechanism of the intestinal absorption of FOM remains obscure. To clarify the mechanism of small intestinal absorption of FOM, we have already investigated intestinal transport of FOM by using brush-border membrane vesicles (BBMV),⁶⁾ with *in situ* intestinal single-pass perfusion⁷⁾ and voltage-clamp⁸⁾ studies. To date, the following results have been obtained: 1) in the rat small in-

testinal BBMV, the transport of FOM is saturable and associated with a carrier-mediated system; 2) its driving force is inward-directed Na^+ - and H^+ -gradients; and 3) the carrier-mediated transport of FOM is related to the phosphate transport system. In the rat *in situ* perfusion study,⁷⁾ the absorption of FOM was found to be saturable and inhibited by inorganic phosphate, as in the BBMV study.⁶⁾ Furthermore, in the voltage-clamp study⁸⁾ using the intestinal segments from rats and rabbits, it was found that the absorption proceeds *via* a transcellular route rather than a paracellular route under physiological conditions. The transcellular transport of FOM was significantly decreased by inorganic phosphate in both rats and rabbits, suggesting that carrier-mediated transport proceeds in rabbits as well as in rats. However, the intestinal absorption mechanism of FOM in humans is not clear. It is very important for clini-

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cal therapeutics to clarify the absorption mechanism in the human intestine. In this study, we prepared BBMVs from human and rabbit small intestines, and compared the intestinal uptake mechanism of FOM among the three species of rat, rabbit and human.

Materials and Methods

Materials — [^3H]-Labeled FOM ([^3H]-FOM, 2.4 GBq/mmol) was purchased from Amersham International, Ltd. (U.K.), and unlabeled fosfomycin and FOM glycol (FOM-Gly) were supplied by Meiji Seika Kaisha, Ltd. (Yokohama, Japan). [^{14}C]-Labeled D-glucose ([^{14}C]-D-glucose, 0.29 GBq/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). Phosphonoacetic acid (PAA), bovine serum albumin (BSA, fraction V), ouabain, phosphoenolpyruvate monopotassium salt, EGTA (ethyleneglycol-bis(β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid), lactic dehydrogenase (type XI), pyruvate kinase (type III), adenosine 5'-triphosphatase (ATPase) (grade IV) and cytochrome c (type III) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Carbonylcyanide-4-trifluoromethoxyphenylhydrazine (FCCP) was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI, U.S.A.). Nicotinamide adenine dinucleotide, reduced form (NADH) and adenosine 5'-triphosphate (5'-ATP) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Seikagaku Kogyo Co., Ltd. (Tokyo, Japan), respectively. All other chemicals were of reagent grade and used without further purification.

Preparation of Rabbit and Human Intestinal BBMVs — New Zealand white male rabbits weighing about 2.5 kg (Saitama Jikken Dobutsu Ltd., Saitama, Japan) were used. The small intestines were removed in a length of about 40 cm from the pylorus. Human intestines were obtained at the time of pancreaticoduodenectomy and used from the lower duodenum to the jejunum, about 20–30 cm in length. The intestines which were obtained from rabbits or humans were immediately flushed with ice-cold saline and divided into 10–15 cm lengths. The segments were opened longitudinally beyond the mesen-

terium. The rabbit intestinal mucosa was scraped with a slide glass while a metal spatula was used for the human intestines. Then the mucosa was frozen in liquid N_2 and stored at -80°C until used. All experiments were performed within 2 months after tissue harvest.

BBMVs were prepared with a modification of Kessler's divalent cation precipitation technique,⁹ which was described in detail previously.¹⁰ The final membrane vesicles were suspended in 20 mM Tris-HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.5) or 20 mM Tris-MES (2-[*N*-morpholine]ethanesulfonic acid) buffer (pH 6.0) containing 100 mM KCl and 100 mM mannitol.

Uptake Experiment — The uptakes of [^{14}C]-D-glucose and [^3H]-FOM by BBMVs were measured by a rapid filtration technique as described previously by Tsuji *et al.*¹⁰ Briefly, the uptake was initiated by the addition of 20 μl of BBMVs to 80 μl of incubation solution (the composition is noted in each figure legend). Experiments were conducted at 37°C . At designated times, the uptake reaction was terminated by adding 1 ml of ice-cold stop solution (200 mM mannitol, 100 mM NaCl and 20 mM Tris-HEPES at pH 7.5). The reaction mixture was immediately applied to a Millipore membrane filter (HAWP, 0.45 μm) under a vacuum. The filter was rapidly rinsed twice with 4 ml of ice-cold stop solution and immersed in a scintillation cocktail for the determination of radioactivity. The amount of [^3H]-FOM and [^{14}C]-D-glucose taken up by the BBMVs was expressed as pmol (or nmol)/mg protein. All experiments were repeated three to five times, and the data are presented as the mean $\pm$ S.E.

Assay Methods — The purity of each brush-border membrane prepared was determined from the enzyme activities which are known to localize in certain organelle of the cells. Leucine aminopeptidase and alkaline phosphatase were measured using enzyme assay kits which are commercially available by Wako Pure Chemical Industries, Ltd. Na^+/K^+ ATPase, cytochrome c oxidase and NADH cytochrome c reductase were determined by the methods of Scharschmidt *et al.*,¹¹ Cooperstein and Lazarow,¹² and Hryciay and Prough,¹³ respectively. The protein

concentration was measured by the method of Bradford,¹⁴⁾ using a Bio-Rad protein assay kit with BSA as the standard.

The radioactivities of [³H]FOM and [¹⁴C]D-glucose were measured with a liquid scintillation counter (Aloka LSC-903, Tokyo, Japan). Cleasol-I (Nakarai Tesque, Inc, Kyoto, Japan) was used as a scintillation cocktail, and quenching was corrected by the external standard method. The osmolarity of each medium was measured with an osmometer (Voger Osmometer OM801, Germany).

Data Analysis — The kinetic parameters of FOM uptake by BBMV were estimated by the previously described method.⁶⁾ That is, J , the amount of initial uptake of FOM by BBMV, was expressed by Eq.1.

$$J = J_{\max} \times C/(K_t + C) + k_d \times C \quad (1)$$

where C represents the concentration of FOM in the incubation medium, $J_{\max}$ and K_t are the maximum uptake rate and the apparent Michaelis constant for a carrier-mediated process, respectively, and k_d is the first-order rate constant for an apparently nonsaturable term.

Statistical Analysis — The level of statistical significance was assessed using Student's t -test. A significant difference was defined as a p value of less than 0.05.

Results

Purity of Membrane Vesicles and D-Glucose Uptake

The purity of membrane vesicles was assessed by the ratio of the activity of enzymes in the crude homogenate and the BBMV fraction. In the rabbit, the activities of leucine aminopeptidase and alkaline phosphatase, which are localized in the brush-border membrane, were enriched 28.2- and 24.7-fold, respectively, in the BBMV compared with the crude homogenate. The enrichments of activities of $\text{Na}^+ - \text{K}^+$ ATPase, cytochrome c oxidase and NADH cytochrome c reductase, which are localized in basolateral membrane, mitochondrial membrane and microsome fraction, respectively, in BBMV fraction relative to the crude homogenate were

0.37-, 0.13- and 0.04-fold, respectively. The specific enzyme activities in the human BBMV were similar to those in the rabbit. Namely, enrichment factors of leucine aminopeptidase and alkaline phosphatase in the BBMV fraction over the crude homogenate were enriched 20.0- and 23.6-fold, respectively, and those of the other enzymes, $\text{Na}^+ - \text{K}^+$ ATPase, cytochrome c oxidase and NADH cytochrome c reductase, were 0.24-, 0.09- and 0.02-fold, respectively. These results indicate that contamination of the basolateral membrane, mitochondrial membrane and endoplasmic reticulum to the final BBMV fraction was minimal during the preparation of the vesicles from both rabbit and human intestinal homogenates. Furthermore, D-glucose uptake, which was used as a criterion for the transport activity of BBMV prepared, showed a Na^+ -gradient dependent "overshoot" phenomenon in both the rabbit and human BBMV. The D-glucose uptake values at the "overshoot" peak at 30 s were approximately 8 and 2.5 times higher than the equilibrium uptakes measured at 60 min in the rabbit and human BBMV, respectively.

Fig. 1. Effect of Osmolarity of the Extravesicular Medium on the Uptake of FOM by Human Intestinal BBMV. BBMV were preloaded with 20 mM Tris-HEPES buffer (pH 7.5) containing 100 mM KCl and 100 mM mannitol. The uptake of [³H]FOM at a concentration of 0.14 mM was measured at 37 °C for 180 min by incubating BBMV (approximately 100 μ g protein) in Tris-MES buffer (pH 6.0) containing 100 mM NaCl and variable concentrations of mannitol (100–450 mM). The solid line was generated by the linear regression analysis.

Each point represents the mean $\pm$ S.E. of 3–5 experiments.

Fig. 2. Concentration Dependence of the Uptake of FOM by (a) Rabbit Intestinal BBMV and (b) Human Intestinal BBMV

The conditions for preloading of BBMV were the same as described in the legend for Fig. 1. The uptake of the various concentrations of FOM (0.14–20 mM) was measured at 37 °C for 15 s by incubating BBMV in 20 mM Tris–MES buffer (pH 6.0) containing 100 mM NaCl and an appropriate concentration of mannitol to adjust the osmolarity. Each point represents the mean $\pm$ S.E. of 3–5 experiments. When the S.E. was small, it was included in the symbol. The solid and broken lines indicate the total and carrier-mediated uptakes, respectively, and were generated by using the PCNONLIN program-fitted parameters listed in the text.

(data not shown). Therefore, the purification and function for “uphill transport” in BBMV were proper in this study.

Effect of Medium Osmolarity on FOM Uptake

In order to know the extent of adsorption of FOM to the BBMV, the effect of medium osmolarity was investigated.

The result is shown in Fig. 1. FOM uptake by the human BBMV decreased with the increase in osmolarity of the incubation medium at equilibrium (180 min). The extrapolated uptake of FOM at infinite osmolarity was approximately zero. This result indicates that the binding of FOM to BBMV is negligible and that FOM is taken up into intravesicular space with negligible binding to the membrane surface.

Concentration Dependence of FOM Uptake

The concentration dependence of the initial uptake rate of FOM was investigated to clarify the affinity for the carrier and the capacity of the saturable component involved in FOM transport. The uptake rate of FOM by BBMV was obtained from the uptake at 15 s and at FOM concentration from 0.14 to 20 mM in the presence of both inward-directed Na^+ - and H^+ -gradients. As shown in Figs. 2a) and 2b),

the uptake of FOM involved saturable and non-saturable processes. The kinetic parameters calculated from Eq. 1 by a nonlinear regression program of PCNONLIN (SCI Software, Lexington, U.S.A.) were $K_t = 5.17 \pm 0.71$ mM, $J_{\max} = 3.88 \pm 0.41$ nmol/mg protein/15 s, $k_d = 0.432 \pm 0.013$ nmol/mg protein/15 s/mM for the rabbit and $K_t = 4.03 \pm 0.49$ mM, $J_{\max} = 1.90 \pm 0.16$ nmol/mg protein/15 s, $k_d = 0.375 \pm 0.006$ nmol/mg protein/15 s/mM for the human. In this study, we reevaluated the previous transport data obtained for the rat BBMV, because the nonlinear regression analysis method used for rat BBMV were different from those for humans and rabbits. Although in the previous paper the mean value of uptake rate ($n = 8$) at each concentration were used, in the present study, the number of data for calculating the kinetic parameters was changed to all transported data ($n = 36$ –50), because the concentration points measured for FOM uptake were fewer for humans than those for rats and rabbits, and the nonlinear regression analysis program was changed to PCNONLIN for a personal computer from NONLIN. The kinetic parameters were recalculated and the following parameters for 15

Fig. 3. Time Course of FOM Uptake by (a) Rabbit Intestinal BBMVs and (b) Human Intestinal BBMVs

BBMVs were preloaded with 20 mM Tris-HEPES buffer (pH 7.5; ●, ▲) or 20 mM Tris-MES buffer (pH 6.0; ○, △) containing 100 mM KCl and 100 mM mannitol. The uptake of [3 H]FOM at a concentration of 0.14 mM was measured at 37 °C by incubating BBMVs in 20 mM Tris-MES buffer (pH 6.0) in the presence of 100 mM NaCl (●, ○) or 100 mM choline chloride (▲, △), and 100 mM mannitol. Each point represents the mean $\pm$ S.E. of 3–5 experiments. When the S.E. was small, it was included in the symbol.

s were obtained: $K_t = 6.67 \pm 1.70$ mM, $J_{\max} = 1.72 \pm 0.37$ nmol/mg protein/15 s, $k_d = 0.148 \pm 0.011$ nmol/mg protein/15 s/mM. We obtained different parameters from those previously reported for rats, because it may be due to the different number of each concentration data point, the deviation of the FOM uptake at higher concentrations, and on changing the computer program.

Time Course and Influence of Sodium- and Proton-Gradients on FOM Uptake

In order to explore the driving force involved in FOM uptake, the time course of FOM uptake by the rabbit and human intestinal BBMVs was investigated in the presence and absence of Na^+ - and/or H^+ -gradients. As shown in Figs. 3a) and 3b), FOM was taken up by the rabbit and human BBMVs most efficiently in the presence of both inward-directed Na^+ - and H^+ -gradients. The rates of uptakes by the BBMVs were linear until initial 30 s. The order of the amount of uptake by the rabbit BBMVs was: presence of both Na^+ - and H^+ -gradients $>$ presence of Na^+ -gradient and absence of H^+ -gradients $>$ absence of Na^+ -gradient and presence of H^+ -gradients $>$ absence of both Na^+ - and H^+ -gradients. On the other hand, the result for the human BBMVs was different

from that for the rabbit BBMVs. That is, the order of the amount of uptake was: presence of both Na^+ - and H^+ -gradients $>$ absence of Na^+ -gradient and presence of H^+ -gradient $>$ presence of Na^+ -gradient and absence of H^+ -gradient = absence of both Na^+ - and H^+ -gradients.

The effect of a protonophore, FCCP on FOM uptake was examined. As shown in Table I, by the addition of FCCP the uptake decreased significantly in the presence of an inward-directed H^+ -gradient, whereas no effect of FCCP was observed in the absence of an H^+ -gradient in both mammals. The effect of the extravesicular and intravesicular pHs is shown in Table I. That is, the uptake of FOM was more efficient at low pH (pH = 6.0) than high pH (pH = 7.5) in the absence of pH gradient across the membrane.

Influence of Phosphate Transport Inhibitors and FOM-Gly

In our previous report,⁶⁾ it was shown that the FOM transport is partially related to a phosphate transport in the rat small intestine. Then FOM uptake was studied in the presence and absence of several compounds involved in the phosphate transport system and D-glucose⁹⁾ which is known to transport through an Na^+ -dependent system that is different from the phosphate trans-

TABLE I. Effect of FCCP on FOM Uptake by the Rabbit and Human Intestinal BBMV

	pH _{in} /pH _{out}	Uptake of FOM (pmol/mg protein/15 s) ^{a)}			
		Control	(n)	+ FCCP	(n)
Rabbit	6.0/6.0	64.3 ± 1.8	(4)	62.1 ± 2.3	(5)
	7.5/7.5	52.6 ± 0.8	(4)	50.5 ± 1.5	(5)
	7.5/6.0	93.6 ± 3.1	(4)	79.4 ± 4.1 ^{c)}	(5)
Human ^{b)}	6.0/6.0	43.7 ± 2.3	(5)	38.1 ± 2.4	(5)
	7.5/7.5	22.7 ± 0.5	(4)	21.0 ± 0.6	(5)
	7.5/6.0	72.1 ± 1.8	(4)	61.2 ± 3.3 ^{c)}	(4)

BBMV were preloaded with 20 mM Tris-HEPES (pH 7.5) or 20 mM Tris-MES buffer (pH 6.0) containing 100 mM KCl and 100 mM mannitol. The uptake of [³H]FOM at a concentration of 0.14 mM was evaluated at 37 °C for 15 s for rabbit and human BBMVs by incubating BBMVs in 20 mM Tris-MES buffer (pH 6.0) or 20 mM Tris-HEPES buffer (pH 7.5) containing 100 mM NaCl and 100 mM mannitol, with simultaneous addition of FCCP dissolved in ethanol (+ FCCP). In the absence of FCCP (control), only ethanol was added. The final concentrations of FCCP and ethanol were 50 μM and 0.5%, respectively.

a) Each value represents the mean ± S.E.; n is the number of experiments. b) Uptake values for 15 s were calculated from the amount of uptake for 30 s. c) *p* < 0.05 vs. the control study.

port system. Table II shows the results obtained for the rabbit and human BBMVs. In the presence of 10 mM phosphate, FOM uptake was reduced to about 55% and 61% of the control in the rabbit and human BBMVs systems, respectively. The inhibitory effect was dependent on the concentration of inorganic phosphate for both mammals. FOM-Gly, which is a degradation product of FOM in the stomach juice, also inhibited the FOM uptake. Arsenate and PAA, which are known as the phosphate transport inhibitors, also significantly inhibited the FOM uptake. The

inhibitory effects were not as remarkable in the human as in the rabbit. On the other hand, D-glucose hardly inhibited FOM transport in both mammals.

Discussion

It has been regarded that for some drugs, such as β-lactam antibiotics^{10,15,16)} and new quinolone antibacterial agents,¹⁷⁾ intestinal absorption is performed by specific carrier-mediated mechanisms across the brush-border membrane. We

TABLE II. Effect of Various Compounds on the Uptake of FOM by the Rabbit and Human Intestinal BBMVs

Inhibitor	(mM)	Rabbit % of control ^{a)}	<i>p</i> ^{b)}	Human % of control ^{a)}	<i>p</i> ^{b)}
Control		100		100	
+ Pi	(1)	74.6 ± 5.6	<i>d)</i>	71.2 ± 5.7	<i>c)</i>
+ Pi	(10)	55.2 ± 1.6	<i>d)</i>	60.6 ± 4.9	<i>d)</i>
+ FOM-Gly	(10)	71.9 ± 2.8	<i>d)</i>	53.3 ± 5.4	<i>d)</i>
+ Ars	(10)	86.2 ± 1.0	<i>c)</i>	74.5 ± 10.6	<i>c)</i>
+ PAA	(20)	81.5 ± 1.4	<i>c)</i>	53.5 ± 9.1	<i>d)</i>
+ D-glucose	(10)	94.8 ± 2.4	<i>e)</i>	92.0 ± 10.8	<i>e)</i>

The conditions for preloading of BBMVs were the same as described in the legend for Fig. 1. The uptake of [³H]FOM at a concentration of 0.14 mM was measured at 37 °C for 15 s for rabbit BBMVs and 30 s for human BBMVs by incubating BBMVs in 20 mM Tris-MES buffer (pH 6.0) containing 100 mM NaCl, an inhibitor and an appropriate concentration of mannitol to adjust the osmolarity to the control level. The concentration of each inhibitor is indicated in parentheses. Pi, phosphate; FOM-Gly, fosfomycin glycol; Ars, arsenate; PAA, phosphonoacetic acid; a) Each value represents the mean ± S.E. of 3–5 experiments; b) *p*, significance; c) *p* < 0.05 vs. the control study; d) *p* < 0.01 vs. the control study; e) not significant.

have already shown using rat small intestinal BBMV that the intestinal absorption of FOM is carried out by a carrier-mediated mechanism.⁶⁾ It is very important to know whether or not the mechanism is common to other mammals, including human. Few comparative studies of species differences in the intestinal absorption mechanism of drugs have been performed, and the studies of the intestinal absorption mechanism of drugs in humans are especially negligible. The transport mechanisms of only endogenous or nutrient compounds such as amino acids,^{18,19)} vitamins,^{20,21)} D-glucose²²⁾ and inorganic compounds^{23,24)} have been investigated by using human intestinal BBMV. Therefore, we tried to clarify the absorption mechanism of FOM using human BBMV in comparison with the results obtained for rabbits under the same experimental conditions and for rats in our previous study.⁶⁾

In the previous study, we elucidated the intestinal absorption mechanism of FOM with the use of BBMV from rats⁶⁾ to be as follows: 1) FOM is transported by a carrier-mediated mechanism across the brush-border membrane, 2) the driving force for FOM transport is Na^+ - and H^+ -gradients across the membrane and 3) the transport mechanism is associated with the phosphate transport system.

The intestinal transport mechanism of FOM in rabbits identified in the present study is similar to that in rats. Namely, the kinetics of the initial uptake of FOM are consistent with two transport mechanisms operating in parallel: one is saturable at higher concentrations of FOM, with a half-saturation concentration of 5.2 mM, and the other is apparently nonsaturable. Initial uptake was pH- and Na^+ -dependent. FCCP, a protonophore, reduced the uptake in the presence of an inward-directed H^+ -gradient. In addition, in the presence of both the Na^+ - and H^+ -gradients, "overshoot" uptake was observed. These results suggest that the uptake of FOM by rabbit BBMV includes a carrier-mediated process energized by inward-directed Na^+ - and H^+ -gradients. Furthermore, the initial uptake of FOM was significantly reduced in the presence of inorganic phosphate transport inhibitors such as phosphate, arsenate, PAA and

FOM-Gly. All of these results indicate that FOM is taken up by the rabbit intestinal BBMV by a specific carrier-mediated mechanism which is common to a phosphate transporter. It is interesting that FOM-Gly, a degradation product of FOM usually formed by gastric acid, inhibited FOM uptake, suggesting that FOM-Gly has a binding affinity to the same carrier as FOM. A similar effect of FOM-Gly was observed in the study of *in situ* rat intestinal single-pass perfusion.⁷⁾ However, up to the present it is not clear whether FOM-Gly is transported by the carrier or not. In human BBMV, the "overshoot" uptake was not observed on FOM uptake. The lack of "overshoot" uptake was similar to the uptake of phosphate in human BBMV,²⁴⁾ although it is not clear why "overshoot" phenomenon was not observed. Possible reasons were that the human BBMV may be leaky owing to 2 times freezing of the brush-border membrane until uptake experiments and/or nonsaturable transport of FOM apparently covered the "overshoot" uptake because the saturable transport across the brush-border membrane may be slow. However, a very similar result was obtained also for human intestinal transport, showing saturation kinetics with a half-saturation concentration of 4.0 mM, pH- and Na^+ -dependences and specific inhibition by phosphate transport-related inhibitors.

The amounts of "overshoot" and equilibrium uptakes in rats⁶⁾ were different from those in humans or rabbits, which may be caused by the smaller vesicle size of rats than humans and rabbits owing to the different condition for the preparation of the BBMV, and by the slight change of membrane permeabilities of FOM itself and the driving forces, sodium and proton, of rats from those of the human or rabbit BBMV. But the values of kinetic parameters obtained in the present study, including the half-saturation concentration, maximum uptake rate and apparently nonsaturable first-order rate constant for FOM transport, were surprisingly similar among the three mammalian species. In addition, inhibitory studies (Table II and our previous report⁶⁾) suggest that the systems for FOM transport in all three mammals have the same substrate specificity. Therefore, the carri-

er protein of FOM in these mammals is suggested to have a very similar substrate binding site. Although the reason is not clear, in rabbits and humans the uptake of FOM increased at acidic pHs (pH 6.0 compared with pH 7.5) in the absence of a pH-gradient across the brush-border membranes was observed (Table I), whereas the pH itself had no effect on FOM uptake in rats.⁶⁾ Furthermore, as shown in Figs. 3a) and 3b) the sodium effect was not as clear in the human BBMV compared to that observed for the rabbit BBMV. Sodium effect in rat BBMV is similar to that in human BBMV.

These quantitative discrepancies for Na⁺- and pH- or H⁺-dependences of FOM uptake among the three mammalian species may suggest that the transporter involved in each mammal has a different preference for driving forces between sodium and proton.

In humans, the relative contribution of the carrier-mediated system is about 40 to 50% of the total uptake at mM-order concentrations, which was expected of the initial concentration taking the clinical single dose of FOM (500—1000 mg/man, *i.e.*, 3.6—7.2 mmol/man) with a glass of water. Although this value does not necessarily reflect the actual absorption because BBMV is the simplest model of intestinal absorption, the result strongly suggests that absorption *via* a specific transport carrier is never negligible.

In conclusion, the transport of FOM across the small intestinal brush-border membrane in rabbits and humans as well as in rats occurs *via* a carrier-mediated system in parallel with a non-saturable first-order process. The carrier-mediated process is dependent on Na⁺- and H⁺-gradients, is favored by an acidic pH and is associated with a phosphate transport system. Although the actual contribution of a carrier-mediated process in the whole small intestinal absorption of FOM is not clear, the findings of this study suggest that its contribution is significant.

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