Biochimica et Biophysica Acta 854 (1986) 191–197
Elsevier

BBA 72879

Determination of the membrane permeability coefficient and the reflection coefficient by the two-dimensional laminar flow model for intestinal perfusion experiments

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(Received April 29th, 1985)
(Revised manuscript received August 26th, 1985)

Key words: Intestinal absorption; Permeability coefficient; Reflection coefficient; Unstirred layer; Two-dimensional Laminar flow; (Rat intestine)

We performed single perfusion experiments in the small intestine of rats in order to prove that the two-dimensional laminar flow model is suitable to determine the membrane permeability coefficient and the reflection coefficient. We used progesterone as an aqueous-diffusion-limited drug, urea as a membrane transport-limited drug and the tritiated water as an intermediate substance. The membrane permeability coefficient for progesterone was calculated to be $3.6 \cdot 10^{-4}$ cm/s. This value did not change when the thickness of the aqueous diffusion layer was altered by increasing the perfusion rate 10-fold. It was directly demonstrated that the two-dimensional laminar flow model was suitable to analyze the data of intestinal perfusion experiments. Membrane permeability coefficients for urea and tritiated water were determined to be $3.4 \cdot 10^{-5}$ cm/s and $8.9 \cdot 10^{-5}$ cm/s, respectively. In the presence of water absorption with the hypotonic perfusion solution, the reflection coefficient for urea was 0.84. This value is thought to be theoretically reasonable, suggesting the usefullness of the two-dimensional laminar flow model to obtain the reflection coefficient in the intestinal membrane.

Introduction

The aqueous diffusion layer, that is, the unstirred water layer, is significant in permeability studies with biological membranes [1]. Some investigators have reported, in the small intestine, the effect of the aqueous diffusion layer on the membrane permeability coefficient for substances absorbed by a passive transport or on the Michaelis constant and the maximal transport velocity for substances absorbed by a carrier-mediated transport. The effect of the aqueous diffusion layer on the Michaelis constant and the maximal transport velocity were considered theoretically by the film model, to analyze the data generated using in vitro preparations [2–7]. Thomson et al. [8–10] have demonstrated experimentally the existence of the aqueous diffusion layer and its effects on kinetic constants using an in vitro technique.

Komiya et al. [11] adapted this film model to intestinal perfusion experiments. In this model, the thickness of the aqueous diffusion layer was assumed to be constant. This assumption is incorrect because the thickness of the aqueous diffusion layer gradually increases along the axial direction

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of the intestinal tract. On the other hand, Winne [12] and Elliott et al. [13] adapted the solution of the Graetz problem in heat transport to intestinal perfusion experiments. Amidon et al. [14] demonstrated that the flow of the perfusion was laminar by residence time distribution analysis. We developed a theoretical model called the two-dimensional laminar flow model by which the reflection coefficient can be determined from the data of perfusion experiments in the presence of water absorption or secretion [15,16]. Furthermore, this model also enables us to assess the effects of the aqueous diffusion layer, the passive diffusion and the water movement on the kinetic constant of carrier-mediated transport processes [17].

The aim of the present study was to show experimentally that the two-dimensional laminar flow model is suitable for the determination of the membrane permeability coefficient and the reflection coefficient in intestinal perfusion experiments.

Theory

Two-dimensional laminar flow model. For details of the two-dimensional laminar flow model, see Ref. 16, Miyamoto et al. Following is a brief description only of the theory behind the model. Assuming that the intestinal tract is a constant radial tube, the axial flow is laminar and the fluid flowing through the intestinal membrane is uniform throughout, a non-steady-state equation of continuity of a given substance in cylindrical coordinates is,

$$\frac{\partial c}{\partial t} + v\frac{\partial c}{\partial r} + u\frac{\partial c}{\partial x} = D\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c}{\partial r}\right) \tag{1}$$

where c, r, x, t, D, v and u are the concentration of the substance, the radial coordinate, the axial coordinate, the time, the diffusion constant, the velocity of the radial component and the velocity of the axial component, respectively. We used an approximate solution of incompressible laminar flow in a porous tube reported by Yuan and Finkelstein [18] for the radial component of velocity only, and the axial component of velocity was calculated by the volume balance. However, the definition of the radial component of velocity is reversed as compared to that reported by Yuan and Finkelstein. In our model, (+) means absorption and (−) means secretion. The phenomenological equation by Kedem and Katchalsky [19], which is suitable for the description of passive transport of a substance through the biological membrane, was applied to the boundary condition as follows, supposing that the concentration of the substance in the intestinal blood is zero.

$$N = \{P_m + 0.5(1-\sigma)v_0\}C \tag{2}$$

where N, P_m, σ and v_0 are the molar flux, the membrane permeability coefficient (cm/s), the reflection coefficient and the radial velocity at the intestinal membrane (cm/s), respectively. Dividing the calculation field to small finite difference grids and calculating this time-dependent equation using a time-stepping procedure, we regarded the asymptotic behavior at sufficiently long time as the steady-state solution.

To analyze the data of perfusion experiments, the maximal velocity at the inlet of the small intestine, u_0 (cm/s), the radial velocity at the intestinal membrane, v_0 (cm/s) and the radius of the small intestine, R (cm) were calculated as follows,

$$u_0 = Q \div \pi R^2 \div 60 \cdot 2 \tag{3}$$

$$v_0 = W \div (2\pi R \times L) \div 60 \tag{4}$$

$$R = \text{outer circumference} \times 0.83 \div 2\pi \tag{5}$$

where Q, W and L are the perfusion rate (ml/min), the water absorption rate (ml/min per L cm intestine) and the length in cm of the small intestine used in perfusion experiments, respectively. Eqn. 5 means that the inner circumference is determined by multiplying the outer circumference by 0.83 [20].

Determination of the membrane permeability coefficient and the reflection coefficient by the graphic method. Theoretical curves of absorption rate vs. the membrane permeability coefficient or the reflection coefficient were drawn using the values obtained by changing the membrane permeability coefficient or the reflection coefficient of the boundary condition (Eqn. 2) in the two-dimensional laminar flow model (Eqn. 1). We used the following values in these calculations; the water kinetic viscosity, $6.95 \cdot 10^{-3}$ cm^2/s; the diffusion constants for progesterone, urea and tritiated

water, $8.00 \cdot 10^{-6}$ cm^2/s [11], $1.43 \cdot 10^{-5}$ cm^2/s and $2.89 \cdot 10^{-5}$ cm^2/s [21], respectively. The experimental absorption rates were calculated from the perfusion data as follows,

$$\text{Absorption rate (nmol/min per } L \text{ cm)} = C_{in} \cdot Q - C_{out}(Q - W) \quad (6)$$

where C_{in} is the inflow concentration (nmol/ml) and C_{out} is the outflow concentration (nmol/ml). We determined the membrane permeability coefficient and the reflection coefficient from the theoretical curves using the experimentally calculated absorption rates.

Film model. To compare the resistance of the membrane with that of the aqueous diffusion layer and to calculate the effective thickness of the aqueous diffusion layer, δ (μm), we used the film model [11], as follows,

$$C_{out} = C_{in} \exp\left(-\frac{2\pi RL}{Q/60} P_{app}\right) \quad (7)$$

$$\frac{1}{P_{app}} = \frac{1}{P_m} + \frac{1}{P_{aq}} \quad (8)$$

$$\text{Resistance of aqueous diffusion layer (\%)} = \frac{1/P_{aq}}{1/P_{app}} \times 100 \quad (9)$$

$$\text{Resistance of membrane (\%)} = \frac{1/P_m}{1/P_{app}} \times 100 \quad (10)$$

$$\delta(\mu\text{m}) = \frac{D}{P_{aq}} \times 10^4 \quad (11)$$

where P_{app} and P_{aq} are the apparent membrane permeability coefficient (cm/s), and the permeability coefficient of the aqueous diffusion layer (cm/s), respectively. We determined the apparent membrane permeability coefficient, using Eqn. 7 for each of the experimental data. Using this value of the apparent membrnae permeability coefficient obtained by the two-dimensional laminar flow model, we determined the permeability coefficient of the aqueous diffusion layer using Eqn. 8. Resistances of the aqueous diffusion layer and the membrane were calculated using Eqns. 9 and 10, and the effective thickness of the aqueous diffusion layer was calculated using Eqn. 11.

Methods and Materials

Animals and preparations. Wistar male rats, 329 $\pm$ 12 g ($n = 24$), were anesthetized with urethane (4.5 ml/kg intraperitoneally, 25% solution). After a midline abdominal incision was made, a jejunal loop was taken out. Both ends of the loop, which was 10 cm long, measured by a thread soaked in saline and 29 $\pm$ 8 cm ($n = 24$) distant from the duodeno-jejunal flexure, were cannulated. After injection of heparin (1000 U/ml, 0.22 ml) and phentolamine (2.5 mg/ml, 0.2 ml) into the right jugular vein, the jejunal vein of the intestinal loop was cannulated with polyethylene tubing (inner diameter = 0.1 cm). The amount of lost blood was substituted by concomitant blood infusion into the right jugular vein to help bring the concentration of any substance in the blood to zero. The blood for these infusions was drawn from an other three or four rats immediately before the experiment. The blood pressure was monitored at the left carotid artery. The jejunal loop was put on the flat plate, covered with a gauze soaked in saline and was kept at 37°C with a heat lamp. The opening of the outflow tubing was settled as high as the jejunal loop to prevent hydrostatic pressure on the loop. The outer circumference was measured by a thread leading around the loop through a small hole in the mesentery, attached close to the surface when the loop was filled with the perfusion buffer. For the perfusion study of progesterone, we used siliconized glass apparatuses to prevent adsorption. The intestinal loop was perfused with radiolabelled progesterone, urea or water and the inflow (C_{in}) and outflow concentration (C_{out}) were measured at steady state.

Materials. [^{14}C]Progesterone, [^{14}C]urea, [^{14}C]inulin or [^{3}H]inulin and tritiated water were purchased from New England Nuclear (Boston, MA). All other reagents were of analytical grade. The labelled and unlabelled substances were dissolved in Krebs-Henseleit bicarbonate medium containing 4.7 mM KCl, 2.54 mM $CaCl_2$, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 24.9 mM $NaHCO_3$ and a suitable amount of NaCl for the osmolarity. Inulin was used as a volume indicator in the lumen. The radioactivity in the perfusion solution was counted by liquid scintillation procedure.

Results and Discussion

Membrane permeability coefficient

Fig. 1 represents a typical plot of the change in the outflow-inflow concentration ratio (C_{out}/C_{in}) with time in the case of the perfusion experiments of progesterone under the conditions; $Q = 0.151$ ml/min, $C_{in} = 31.8$ nmol/ml. The absorption rate seemed to be in steady state after 45 min. In all experiments, we measured the outflow concentration (C_{out}) at steady state and calculated the experimental absorption rate using Eqn. 6. Table I shows the data of the progesterone perfusion experiments. The perfusion rate was increased 10-fold to examine whether the membrane permeability coefficient of progesterone is altered by changes in the effective thickness of the aqueous diffusion layer. When the perfusion rate was 0.151 ml/min, C_{out}/C_{in} was 0.641 ± 0.008, the absorption rate was 1.72 ± 0.03 nmol/min per 10 cm and the outer circumference was 1.65 cm. When the perfusion rate was 1.47 ml/min, C_{out}/C_{in} was 0.919 ± 0.026, the absorption rate was 3.31 ± 0.45 nmol/min per 10 cm and the outer circumference was 1.61 cm. We could not observe the distention of the loop even when the perfusion rate was 1.47 ml/min.

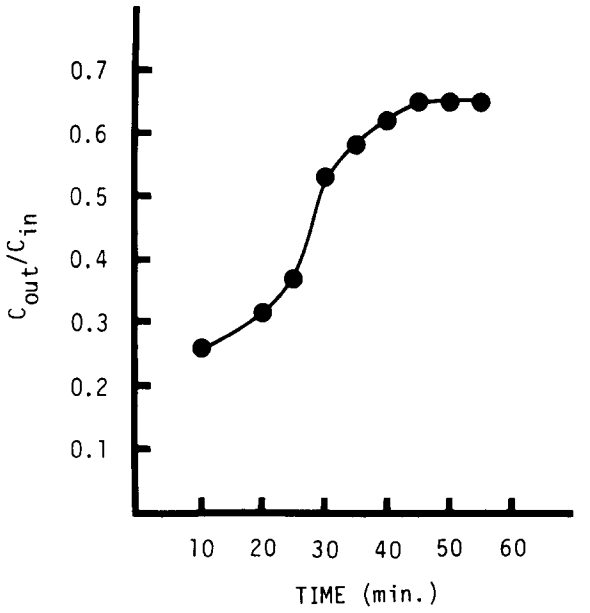

Fig. 1. Typical outflow to inflow concentration ratio (C_{out}/C_{in}) profiles, with time, in single perfusion experiments. The inflow concentration of progesterone is 31.8 nmol/ml. The perfusion rate is 0.151 ml/min.

TABLE I

INTESTINAL ABSORPTION AND MEMBRANE PERMEABILITY COEFFICIENT OF PROGESTERONE

The inflow concentration (C_{in}) was 31.8 nmol/ml and the length of the small intestine used in the perfusion experiments (L) was 10 cm at both perfusion rates. The membrane permeability coefficient of progesterone was determined according to the method shown in Fig. 2 using all the perfusion experiment data listed here. The diffusion constant for progesterone used for calculation was $8.00 \cdot 10^{-6}$ cm^2/s. Values are means ± S.D. ($n = 5$).

	Perfusion rate	
	0.151 ml/min	1.47 ml/min
C_{out}/C_{in}	0.641 ± 0.008	0.919 ± 0.026
Absorption rate (nmol/min per 10 cm)	1.72 ± 0.03	3.31 ± 0.45
Intestinal radius (cm)	0.218 ± 0.015	0.213 ± 0.009
Membrane permeability coefficient ($\times 10^{-4}$ cm/s)	3.60 ± 0.35	3.57 ± 1.38

Fig. 2 represents the theoretical curves of the absorption rate vs. membrane permeability coefficient calculated by the two-dimensional laminar flow model at two different perfusion rates; 0.151 ml/min and 1.47 ml/min. We determined the membrane permeability coefficient for progesterone from Fig. 2 using the experimentally determined absorption rates at both perfusion rates. In spite of the perfusion rate change, the membrane permeability coefficient for progesterone remained $3.6 \cdot 10^{-4}$ cm/s (Table I).

Table II shows that the data of the perfusion experiments with urea and tritiated water and the membrane permeability coefficients obtained by the graphic method based on the two-dimensional laminar flow model. The membrane permeability coefficients for urea and tritiated water were determined as $3.4 \cdot 10^{-5}$ cm/s and $8.9 \cdot 10^{-5}$ cm/s, respectively.

The membrane permeability coefficient obtained from the data of the intestinal perfusion experiments depends on the analyzing model which deals with the aqueous diffusion layer. So, it is difficult for us to judge which model is most

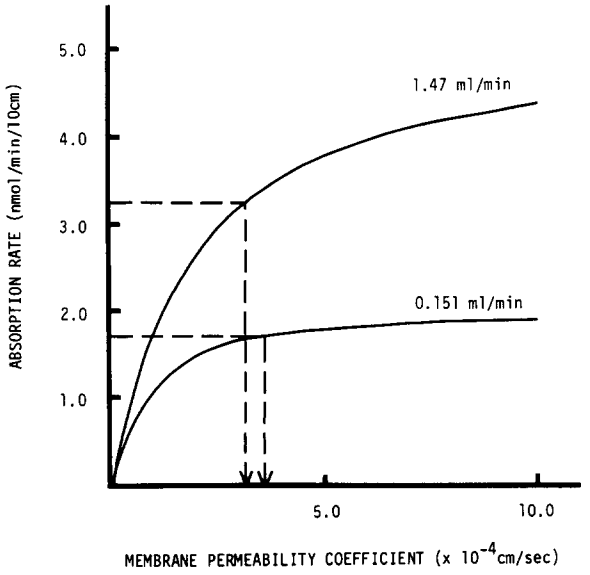

Fig. 2. Theoretical curves calculated from the data of the progesterone experiments listed in Table I, as a function of the perfusion rate. Dotted lines represent typical examples of the graphic method at each perfusion rate to determine the membrane permeability coefficient.

suitable for the perfusion experiment of the small intestine. In this study, we changed the perfusion rate to alter the thickness of the aqueous diffusion layer and obtained the same membrane permeability coefficient of progesterone at the two different perfusion rates, although progesterone was thought to be the aqueous-diffusion-limited drug. Therefore, the data suggest that the two-dimensional laminar flow model is useful for analyzing the data of the intestinal perfusion experiment to determine the membrane permeability coefficient without the interference of the aqueous diffusion layer.

We calculated the resistances of the aqueous diffusion layer and the membrane, and the effective thickness of the aqueous diffusion layer by the film model using Eqns. 9, 10 and 11 (Table III). With regard to progesterone, the resistance of the aqueous diffusion layer was 77% and that of the membrane was 23%, when the perfusion rate was 0.151 ml/min. When the perfusion rate was 1.47 ml/min, the resistances of the aqueous diffusion layer and the membrane were 57% and 43%, respectively. It is reasonable that the resistance of the aqueous diffusion layer decreases as the perfusion rate becomes higher. The effective thickness of the aqueous diffusion layer was calculated to be 755 μm when the perfusion rate was 0.151 ml/min and 292 μm when the perfusion rate was 1.47 ml/min. At the same perfusion rate (0.122 ml/min), the effective thicknesses of the aqueous diffusion layer of urea and tritiated water were 151 μm and 923 μm, respectively. Hence, the effective thickness of the aqueous diffusion layer may depend on both the membrane permeability coefficient and the diffusion constant as well as the perfusion rate.

TABLE II

INTESTINAL ABSORPTION AND THE MEMBRANE PERMEABILITY COEFFICIENTS OF UREA AND TRITIATED WATER

The perfusion rate was 0.122 ml/min in both experiments. The length of the small intstine used in both perfusion experiments (L) was 10 cm. The membrane permeability coefficients were determined graphically as shown in Fig. 2. The diffusion coefficients, for urea and tritiated water, used for calculation were $1.43 \cdot 10^{-5}$ cm^2/s and $2.89 \cdot 10^{-5}$ cm^2/s, respectively. Values are means $\pm$ S.D. ($n = 5$).

	Urea	Tritiated water
C_{in}	10 (nmol/ml)	100 (nCi/ml)
C_{out}/C_{in}	0.804 ± 0.035	0.634 ± 0.017
Absorption rate	0.206 ± 0.051 (nmol/min per 10 cm)	4.27 ± 0.45 (nCi/min per 10 cm)
Membrane permeability coefficient ($\times 10^{-5}$ cm/s)	3.43 ± 1.17	8.87 ± 0.67

TABLE III

RESISTANT RATIO BETWEEN THE AQUEOUS DIFFUSION LAYER AND THE INTESTINAL MEMBRANE, AND THE EFFECTIVE THICKNESS OF THE AQUEOUS DIFFUSION LAYER

The resistance of the aqueous diffusion layer and the membrane, and the effective thickness of the aqueous diffusion layer were calculated using Eqns. 9–11 for the film model.

	Perfusion rate (ml/min)			
	Progesterone		Urea	Tritiated water
	0.151	1.47	0.122	0.122
Resistance of aqueous diffusion layer (%)	77	57	3	22
Resistance of membrane (%)	23	43	97	78
Effective thickness of aqueous diffusion layer (μm)	755	292	151	923

Reflection coefficient

To determine the reflection coefficient in the intestinal membrane, it is necessary to perform the perfusion experiment in the presence of intestinal water absorption or secretion. We investigated the degree of change in water absorption and secretion as a function of the change in osmolality of the perfusion solution (Fig. 3). The ordinate is represented by the radial velocity at the intestinal membrane calculated using Eqn. 4 under the conditions; $L = 10$ cm and $R = 0.213$ cm. No matter how much the osmolality of the perfusion solution was changed, the radial velocity at the intestinal membrane did not change more than $\pm 2.0 \cdot 10^{-5}$ cm/s. As we pointed out in a previous study [16], it is necessary that the water movement occurs in the same order of the membrane permeability coefficient to determine the reflection coefficient. Since the membrane permeability coefficient for urea is $3.43 \cdot 10^{-5}$ cm/s, the reflection coefficient for urea can be conveniently calculated.

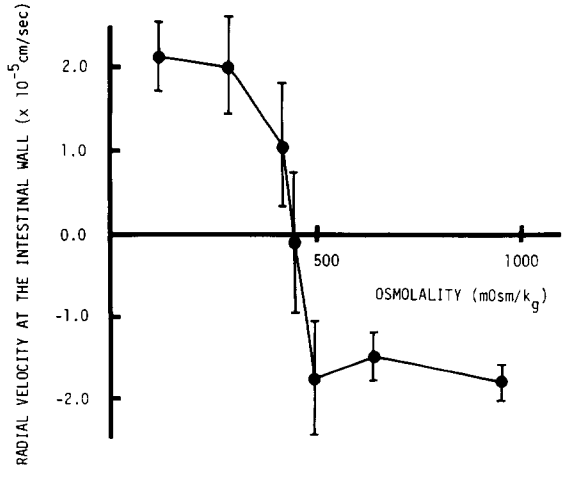

Fig. 3. Change in the water absorption rate in the rat intestine as a function of the osmolality. The osmolality in the perfusion solution was changed by alteration of the NaCl concentration. The ordinate is the radial velocity at the intestinal membrane calculated by Eqn. 4 under the conditions; $L = 10$ cm, $R = 0.213$ cm. Points and bars represent the mean $\pm$ S.D. ($n = 3$).

Table IV shows the data of the perfusion experiments of urea in the presence of water absorption under the conditions of the hypotonic perfusion solution. Since the perfusate was condensed

TABLE IV

INTESTINAL ABSORPTION OF UREA IN THE PRESENCE OF WATER ABSORPTION

The osmolality in the perfusion solution was adjusted to 117 mosmol/kg. The perfusion rate was 0.122 ml/min in all experiments.

	Expt. No.			
	1	2	3	4
C_{out}/C_{in}	0.912	0.940	0.925	0.978
Absorption rate (nmol/min per 10 cm)	0.268	0.270	0.236	0.312
Water absorption rate ($\times 10^{-2}$ ml/min per 10 cm)	1.76	2.09	1.56	2.92

by water absorption, C_{out}/C_{in} increased to 0.94 as compared to 0.80 (Table III) in the absence of the water absorption, although the absorption rate also increased. Fig. 4 depicts the theoretical curve calculated using the data of Expt. 2 in Table IV by the two-dimensional laminar flow model. The reflection coefficient for urea was determined from the graph using the experimentally determined absorption rate of urea and the mean value of 0.84 ± 0.24 (S.D., $n = 4$) was obtained.

With regard to the reflection coefficient, the most reasonable model might be the one by which the reflection coefficient of tritiated water was calculated to be zero. However, the water movement in the small intestine was not more than $\pm 2.0 \cdot 10^{-5}$ cm/s as the radial velocity at the intestinal membrane, whereas the membrane permeability coefficient for water was $8.87 \cdot 10^{-5}$ cm/s. Therefore, the reflection coefficient for water could not be determined. The reflection coefficient for urea determined by Winne [22] was 0.14, which was smaller than that value determined by the two-dimensional laminar flow model in this study. However, our value of 0.84 agreed with the value of 0.81 determined by Lindemann and Solomon [23]. Reflection coefficients of many drugs reported by Winne seem to be underestimated, because not all the changes in absorption rate were due to the solvent drag. In our proposed model, even if the reflection coefficient equals 1, the absorption rate increases (or decreases) as water is absorbed (or secreted), for the concentration at the aqueous-intestinal membrane interface increases (or decreases) when water is absorbed (or secreted) [16]. Since the two-dimensional laminar flow model can calculate this change in the concentration at the aqueous-intestinal membrane interface, the reflection coefficient obtained with this model might approximate the true value.

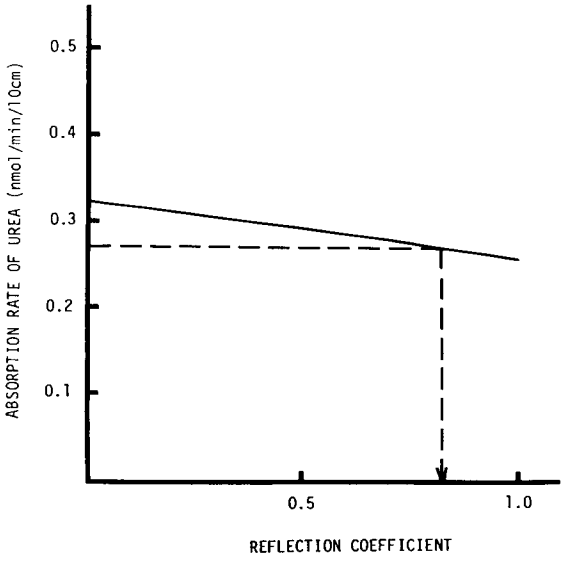

Fig. 4. Absorption rate of urea vs. reflection coefficient. The representative theoretical curve was calculated using the data of Expt. 2 in Table IV for the two-dimensional laminar flow model. The dotted line represents the graphic method to determine the reflection coefficient.

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