enzyme rate constant from the coupled diffusional transport-bioconversion kinetic constant.

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Physical Model Approach to Gastrointestinal Absorption of Prostaglandins III: *In Situ* Rat Intestinal Absorption of Dinoprostone

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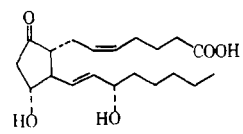
Received October 18, 1976, from the **College of Pharmacy, University of Michigan, Ann Arbor, MI 48109* and the [‡]*Upjohn Co., Kalamazoo, MI 49001.* Accepted for publication February 16, 1977. Present addresses: [¶]Merck, Sharp & Dohme, West Point, PA 19486 and the [§]Upjohn Co., Kalamazoo, MI 49001.

Abstract □ *In situ* absorption studies with dinoprostone in the rat jejunum were carried out to provide a quantitative mechanistic insight of the absorption process. The variables included buffer pH (3.5-9.5), buffer capacity, hydrodynamics in the lumen, and concentration. The disappearance kinetics from the lumen was first order. The rate decreased with increasing pH in a sigmoidal manner and reached a minimum at about pH 9. These results indicate the effects of the partitioning of nondissociated species in the lipoidal membrane and transport across aqueous pores. The rate was higher with the higher degree of agitation of the lumenal solution. Between two hydrodynamic situations, the differences in the rates were large at pH 4.5 where the transport was largely aqueous diffusion controlled and then tended to become smaller with increasing pH where the transport became effectively membrane controlled. The 15-oxo- and 13,14-dihydro-15-oxo metabolites of dinoprostone were found. The physical model was applied to quantify the permeability coefficients of the aqueous diffusion layer and the aqueous pores of the membrane and the effective membrane transport-bioconversion permeability coefficient at various pH values. The overall absorption process of dinoprostone was similar to that of the less lipophilic dinoprost reported earlier and also more rapid. Hence, baseline absorption studies were completed with two major reference prostaglandins from which estimations of intestinal absorption can be made for their analogues and derivatives.

Keyphrases □ Absorption, GI—dinoprostone, rat jejunum, effect of buffer pH, buffer capacity, lumenal hydrodynamics, and concentration □ Dinoprostone—GI absorption, effect of buffer pH, buffer capacity, lumenal hydrodynamics, and concentration □ Prostaglandins—dinoprostone, GI absorption, effect of buffer pH, buffer capacity, lumenal hydrodynamics, and concentration, rat jejunum □ Oxytocic agents—dinoprostone, GI absorption, effect of buffer pH, buffer capacity, lumenal hydrodynamics, and concentration, rat jejunum

In situ absorption studies in the rat jejunum have been carried out to provide a quantitative mechanistic insight into the intestinal absorption of dinoprostone (I)[1]. These studies were intended to serve as baselines for comparisons between dinoprostone and its analogues and derivatives and other natural prostaglandins such as dinoprost (II).



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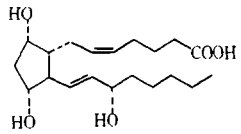

II

Magee *et al.* (1) were the first to study the *in situ* rat intestinal absorption of dinoprostone by the Doluisio *et al.* (2) technique. With the entire small intestines and 0.01 M phosphate buffer (pH 6.4), the disappearance half-life from the intestinal solution was 40 min. The 15-oxo metabolite was found in the lumen. No mechanistic interpretation on the absorption process was made.

Recently, *in situ* absorption studies with dinoprost in the rat jejunum, using a modified Doluisio technique, showed that the absorption rate was first order and was dependent on the pH and hydrodynamics in the intestinal lumen (3). No metabolism involving 15-hydroxyprostaglandin dehydrogenase occurred in the intestinal lumen and at the membrane surface; however, metabolism occurred within the biomembrane. The transport mechanism involved simultaneous passive diffusion and enzymatic bioconversion in the membrane. The quanti-

[1] Dinoprostone is prostaglandin E_2; dinoprost is prostaglandin $F_{2\alpha}$.

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Table I—*In Situ* Absorption of [^{3}H]Dinoprostone in the Rat Jejunum [a]

Hydrodynamic Condition	Buffer pH	Buffer Capacity	Number of Experiments	$k_u \times 10^4$ s^{-1} ±SD
Oscillation, 0.075 mL/s	3.5	Std	5	14.59 (1.50)
		1.5 Std	3	16.60 (0.40)
	4.5	Std	2	9.81 (0.29)
		1.5 Std	9	11.94 (0.58)
	6.0	Std	13	7.63 (0.45)
	6.5	Std	4	6.22 (0.68)
	7.5	Std	2	4.60 (0.21)
		1.5 Std	4	2.98 (0.35)
	8.5	Std	2	2.05 (0.64)
	9.5	Std	4	2.00 (0.77)
Static, 150-s intervals	3.5	Std	3	8.60 (0.77)
		1.5 Std	3	8.78 (0.38)
	4.5	Std	2	6.67 (0.51)
		1.5 Std	6	7.81 (0.72)
	5.0	Std	6	5.44 (0.38)
	6.5	Std	2	4.59 (0.30)
	7.5	Std	2	3.47 (0.06)
		1.5 Std	2	2.70 (0.28)
	8.5	Std	2	2.2 (0.25)
	9.5	Std	2	1.93 (0.25)

[a] Initial concentration of [^{3}H]dinoprostone was 5×10^{-4} mg/mL or 1.7×10^{-6} mg/mL. The k_u is the apparent first-order transport rate constant. Std means standard buffer capacity (see Ref. 3 for buffer formula).

fication and factorization of the permeability coefficients of the aqueous boundary layer in front of the membrane and the passive diffusional-bioconversion and aqueous pore pathways of the membrane were accomplished using the physical model derived previously (4).

EXPERIMENTAL SECTION

In situ absorption studies with dinoprostone in the rat jejunum were carried out by a modified Doluisio technique (4). The experimental variables included a wide range of buffer pH (3.5–9.5), buffer capacity, dinoprostone concentration, and hydrodynamic situations. Dinoprostone[2], [^{3}H]dinoprostone[3], 1-[^{14}C]butanol[3], and *n*-[^{14}C]butyric acid[3] were used without further purification. All buffer and rinsing solutions were isoosmotic.

Male Sprague-Dawley rats, 220–275 g, were used. A 22-cm segment of jejunum was exposed and cannulated. All experiments were conducted at 37°C.

To assess membrane damage, villous immobility, or vasoconstriction caused by concentrations of prostaglandins that might be used in subsequent experiments, the jejunal absorption of 1-[^{14}C]butanol, 1-[^{14}C]octanol, and *p*-[^{14}C]aminobenzoic acid was followed in the presence and absence of experimental concentrations of dinoprostone at constant hydrodynamic conditions. The effect on intestinal secretions was observed by following the absorption of *n*-[^{14}C]butyric acid in the absence and presence of dinoprostone at a constant hydrodynamic condition.

In the *in situ* absorption studies, exactly 2 mL of radiolabeled solute in isotonic buffer was introduced into the lumen of the jejunum by means of the pump. To control the hydrodynamics, the experiments were conducted by the so-called static and oscillation methods. In the oscillation method, the lumenal solution was continuously pumped back and forth at 0.075 mL/s. All sampling points were at 150-s intervals.

At all times, at least 80% of the drug solution remained in the lumenal segment. The initial tracer concentration of [^{3}H]dinoprostone was 0.1 μCi/mL of drug-buffer solution, equivalent to 5×10^{-4} mg/mL. Twenty microliters of lumenal solution was taken at intervals with the aid of an automatic pipet and transferred into 10 mL of a liquid scintillation[4] cocktail[5].

TLC was performed on silica gel plates and developed with the A-IX system (5). Quantitative determinations of radioactivity on the silica gel plates were done from 0.5-cm section scrapings in the scintillation cocktail[5].

THEORETICAL SECTION

Recently, a physical model was derived for the stimultaneous passive transport and bioconversion of dinoprost across the intestinal membrane. It was consistent with the *in situ* absorption studies in the rat jejunum. Because of the similarities of the intestinal absorption of dinoprostone to dinoprost, the identical model is applied here (3, 4).

The disappearance rate in the intestinal lumen follows an apparent first-order transport process:

$$\frac{dC_b}{dt} = -k_u C_b \qquad \text{(Eq. 1)}$$

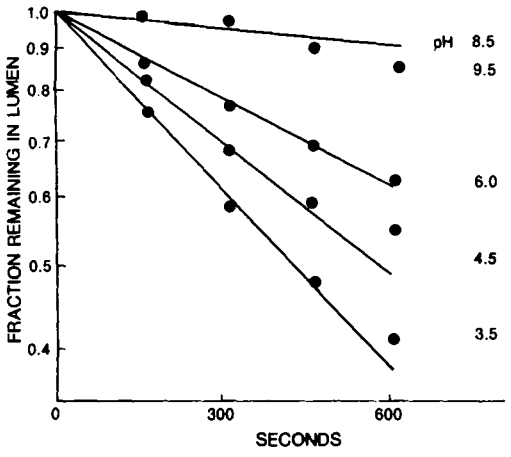

Figure 1—*In situ absorption of dinoprostone at various buffer pH values and high buffer capacity under oscillating (0.075 mL/s) hydrodynamics in the rat jejunum.*

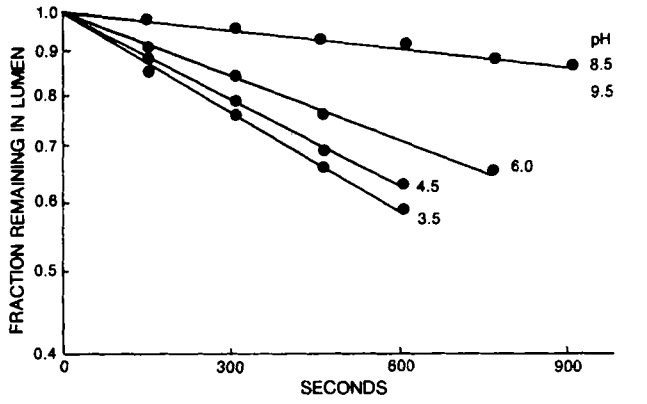

Figure 2—*In situ absorption of dinoprostone at various buffer pH values and high buffer capacity under static conditions (150-s intervals) in the rat jejunum.*

[2] The Upjohn Co.
[3] New England Nuclear Co.
[4] Beckman model LS 200 spectrometer.
[5] Aquasol.

Table II—Assessment of the Presence of Metabolism and Impurity Compounds on the Determination of the Absorption Rate Constant of Dinoprostone in the Rat Jejunum

Buffer pH	Hydrodynamic Condition	$k_u \times 10^4$ s^{-1} Uncorrected	Corrected
3.5	Static	9.1	8.4
	Oscillation	17.0	16.9
4.5	Static	8.9	8.5
	Oscillation	12.0	11.7
6.0	Static	6.2	5.9
	Oscillation	7.4	7.3

and:

$$k_u = \frac{A/V}{\frac{1}{P_{aq}} + \frac{1}{\frac{KX_s\sqrt{kD}}{\tanh(L\sqrt{k/D})} + P_p}} \quad \text{(Eq. 2)}$$

where C_b is the total dinoprostone concentration in the bulk lumenal solution; t is the time in s; k_u is the apparent first-order absorption rate constant in s^{-1}; P_{aq} is the permeability coefficient of aqueous diffusion layer; P_p is the permeability coefficient of the aqueous pores of the membrane; L is the membrane thickness; k is the apparent first-order enzyme rate constant in s^{-1}; D is the diffusion coefficient of dinoprostone in the membrance (cm^2/s); A is the apparent lumenal surface area; X_s is the fraction of nondissociated dinoprostone species at the membrane surface; K is the lipoidal membrane-aqueous partition coefficient of the nondissociated prostaglandin acid; V is the volume of lumenal solution. The integration of Eq. 1 between the limits of the initial total concentration $C_b(0)$ and $C_b(t)$ leads to the semilogarithmic expression:

$$\ln C_b = \ln C_b(0) - k_u t \quad \text{(Eq. 3)}$$

By using Eq. 2, one can readily determine the apparent absorption rate constants under varying pH and hydrodynamic conditions in the lumen. In turn, the permeability coefficients of the aqueous diffusion layer and the membrane itself can be factored out.

The permeability coefficients of the aqueous diffusion layer are found as follows. Under two hydrodynamic conditions at constant buffer pH, two rates of absorption are expected. Accordingly, this situation is described by the following set of linear algebraic equations:

$$\frac{1}{P_{aq}} + \frac{1}{P_{e,m}} = \frac{A/V}{k_u} \quad \text{(Eq. 4)}$$

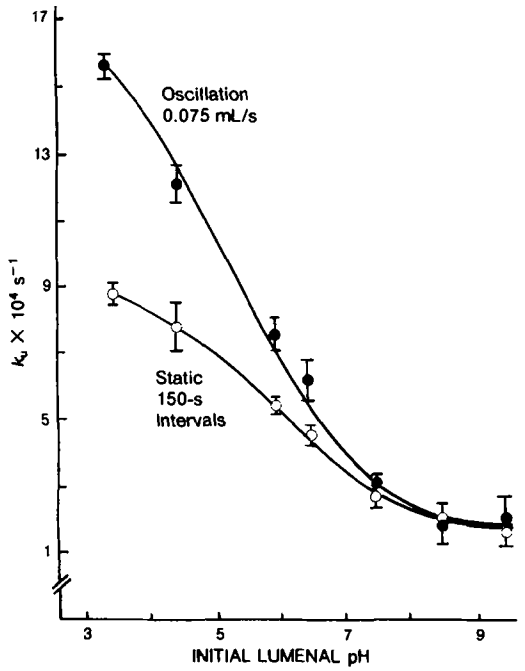

Figure 3—*Apparent absorption rate constants versus high buffer capacity pH under two hydrodynamic conditions for dinoprostone in the rat jejunum.*

Table III—*In Situ* Absorption of 1-[^{14}C]Butanol and 1-[^{14}C]Octanol in the Presence and Absence of Dinoprostone at pH 4.5 and 6.0 under Oscillation (0.075 mL/s) Hydrodynamics; Assessment of Effects on the Aqueous Diffusion Layer, Membrane, and Vascular System

Buffer pH	Rat	Order of Experiment[a]	Dinoprostone, μg/mL	$k_u \times 10^3$ s^{-1}
		1-[^{14}C]Butanol		
4.5	A	1	0	1.9
		2	1.76	1.4
	B	1	1.76	1.8
		2	0	1.6
6.0	C	1	0	1.4
		2	1.76	1.4
	D	1	1.76	1.2
		2	0	1.2
		1-[^{14}C]Octanol		
4.5	A	1	0	2.6
		2	1.76	2.5
	B	1	1.76	2.5
		2	0	2.7
6.0	C	1	0	2.8
		2	1.76	2.7
	D	1	1.76	3.0
		2	0	2.7

[a] In the same jejunal segment.

$$\frac{1}{P_{aq}'} + \frac{1}{P_{e,m}} = \frac{A/V}{k_u'} \quad \text{(Eq. 5)}$$

where the effective permeability coefficient of the membrane, $P_{e,m}$, is:

$$P_{e,m} = \frac{KX_s\sqrt{kD}}{\tanh(L\sqrt{k/D})} + P_p \quad \text{(Eq. 6)}$$

Here it is noted that the first-order rate constant, k_u, and the permeability coefficient of the aqueous diffusion layer, P_{aq}, are different from k_u' and P_{aq}' due to the difference in hydrodynamic conditions but that the effective permeability coefficient of the membrane is not affected by the hydrodynamics in the lumenal solution. Subsequently, if one knows the value of the ratio between P_{aq} and P_{aq}', *i.e.*:

$$\frac{P_{aq}'}{P_{aq}} = r^* \; (P_{aq}' > P_{aq};\; k_u' > k_u) \quad \text{(Eq. 7)}$$

where $r^* \geq 1.0$, it follows that:

$$\frac{1}{P_{aq}} + \frac{1}{P_{e,m}} = \frac{A/V}{k_u} \quad \text{(Eq. 8)}$$

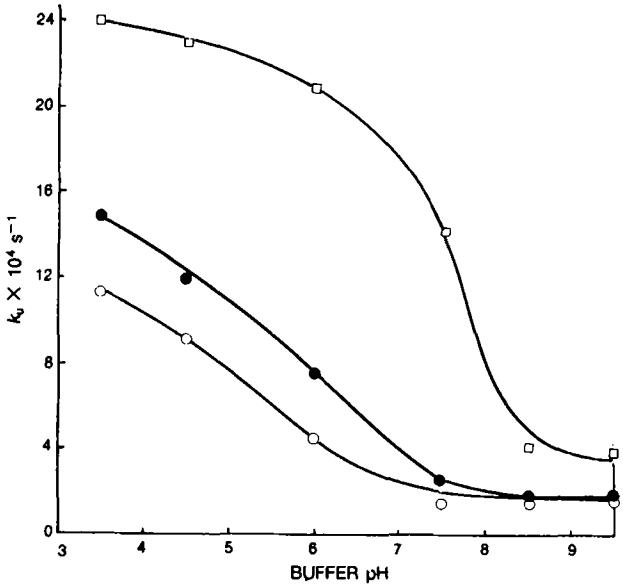

Figure 4—*Comparison of the absorption rate constant-buffer pH profiles of dinoprostone (●), dinoprost (○), and n-butyric acid (□) in the rat jejunum. The pK_a of these acids is 4.9.*

Table IV—*In Situ* Absorption of *n*-[^{14}C]Butyric Acid in the Rat Jejunum in the Presence and Absence of Dinoprostone at Buffer pH 4.5 and 9.5 under Oscillation (0.075 mL/s) Hydrodynamics [a]

pH	Dinoprostone, μg/mL	$k_u \times 10^3$ s^{-1}
4.5	0	3.13
		2.89
	0.5	2.96
		2.63
9.5	0	0.52
		0.52
	0.5	0.5
		0.51

[a] $n = 2$.

$$\frac{1}{r^* P_{aq}} + \frac{1}{P_{e,m}} = \frac{A/V}{k_u'} \qquad \text{(Eq. 9)}$$

Hence, one has a system of two equations in two unknowns, which can be solved in the usual manner. The r^* was determined previously for a number of hydrodynamic situations in the rat intestinal tract using the 1-alkanol series (6).

The permeability coefficient of the aqueous pores of the membrane is determined at buffer pH $\gg$ pK_a. In this situation, X_s approaches zero, causing Eq. 2 to become:

$$k_u = \frac{A}{V} \frac{1}{\frac{1}{P_{aq}} + \frac{1}{P_p}} \qquad \text{(Eq. 10)}$$

RESULTS AND DISCUSSION

General Absorption Experiments—Dinoprostone absorption at various pH values at high buffer capacity and at two hydrodynamic conditions is shown in Figs. 1 and 2. These plots reflect the change in the total radioactivity in the lumenal solution with time. The decrease in rate concomitant with the increase in pH strongly indicates the importance of the partitioning of nondissociated species of dinoprostone (pK_a ~4.9) into the lipoidal diffusion-bioconversion pathway of the membrane as part of the total transport process.

By comparing Figs. 1 and 2, one notices the marked influence of the aqueous diffusion layer on transport kinetics. The hydrodynamics of the oscillation procedure produced a larger effect on the slope than did the hydrodynamics of the static procedure with 150-s sampling intervals; the higher degree of agitation produced a thinner aqueous diffusion layer on the mucosal side of the membrane.

Table I summarizes the absorption experiments using tracer dinoprostone concentrations at various hydrodynamic conditions, buffer pH, and buffer capacities. The buffer capacity effect on the absorption rate constant is evident. For example, at the nominal buffer pH of 4.5, the rate constant was higher at a high buffer capacity than at a lower capacity. This result is attributed to the relative differences in the surface pH and bulk lumenal pH, which depends on the net fluxes of intestinal secretions and bulk buffer species across the aqueous diffusion layer (6). The bulk pH initially at pH 4.5 increased slower with time the higher buffer capacity than with the lower capacity solution. In contrast, in the pH region beyond 6.0–6.5, the bulk pH (*e.g.*, 7.5) tended to decrease slower with time with the high buffer capacity than the lower buffer capacity solution and converged to approximately pH 6.3. These results emphasize the need to have sufficiently high buffer capacity solutions for baseline control of the surface pH relative to the initial bulk pH.

Figure 3 is a plot of k_u *versus* buffer pH at high buffer capacities taken from the results in Table I. The relative differences in the k_u values for the oscillation and static hydrodynamic cases become smaller as the buffer pH increased. At pH 9.5, the rate constants were about the same. These results were expected

Table V—*In Situ* Absorption of *p*-[^{14}C]Aminobenzoic Acid in the Presence and Absence of Dinoprostone at pH 8.5 under Oscillation (0.075 mL/s) Hydrodynamics: Assessment of Effects on the Aqueous Pore Pathway of the Membrane

Order of Experiment [a]	Rat A Dinoprostone, μg/mL	Rat A $k_u \times 10^4$ s^{-1}	Rat B Dinoprostone, μg/mL	Rat B $k_u \times 10^4$ s^{-1}
1	0	3.5	1.76	4.2
2	1.76	3.6	0	3.8

[a] In the same jejunal segment.

Table VI—Effect of Dinoprostone Concentration on the Absorption at pH 6.0 and Oscillation Hydrodynamics

Rat	Order of Experiment [a]	Dinoprostone, mg/mL	$k_u \times 10^4$ s^{-1}
A	1	8.0×10^{-7}	7.3
	2	8.0×10^{-7}	7.9
B	1	1.6×10^{-5}	8.0
	2	1.6×10^{-5}	6.8
C	1	5.0×10^{-4}	7.2
	2	5.0×10^{-4}	7.9
D	1	1.0×10^{-3}	7.1
	2	1.0×10^{-3}	5.1

[a] In the same jejunal segment.

Table VII—Estimation of the Permeability Coefficient of the Aqueous Diffusion Layer (P_{aq}) [a]

Rat	Buffer pH	Hydrodynamic Condition	Average $k_u \times 10^4$ s^{-1}	$P_{aq} \times 10^4$ cm/s Static	$P_{aq} \times 10^4$ cm/s Oscillation
A	6.0	Static	5.44	0.89	1.88
		Oscillation	7.63		
B	6.5	Static	4.59	0.83	1.74
		Oscillation	6.22		

[a] Average $P_{aq(static)} = 0.86 \times 10^{-4}$ cm/s. Average $P_{aq(oscillation)} = 1.82 \times 10^{-4}$ cm/s. The P_{aq} value was calculated with the aid of Eqs. 8 and 9 with $A/V = 11.2$ and $r^* = 2.12$.

since the total transport rate is more aqueous diffusion controlled at low pH and more effectively membrane controlled with increasing pH. The shapes of the profiles are similar in character to those reported earlier for dinoprost (4). These prostaglandins are known to undergo metabolism in the jejunal membrane to, at least, the 15-oxo and 13,14-dihydro-15-oxo metabolites.

In following dinoprostone absorption, there was always some concentration of extraneous tritium-labeled compounds in the intestinal lumen as well as in the original stock solution[6] as determined by quantitative TLC. These concentrations were always about 10% of the total activity at all sampling intervals during the absorption experiments. Furthermore, the back-diffusion of the 15-oxo derivative of dinoprostone from the membrane into the lumen was detected with time. *In vitro* studies in rat intestinal secretions at pH 6.0 indicated that no metabolism was occurring. The rate constants taken from first-order absorption–time plots corrected for nondinoprostone substances were no different from constants from uncorrected plots (Table II).

Assessment of Aqueous Diffusion Layer, Membrane, and Vascular Effects of Dinoprostone on Absorption—The absorption of various selected radiolabeled solutes (1-butanol, 1-octanol, *n*-butyric acid, and *p*-aminobenzoic acid) was carried out under various pH conditions in the presence and absence of dinoprostone to evaluate possible effects of the postaglandin on the aqueous diffusion layer, membrane permeability, and vascularity of the intestines affecting the blood sink. 1-Butanol and *n*-butyric acid were chosen because their absorption rates are known to be membrane controlled; the absorption rate of 1-octanol is controlled by diffusion across the aqueous boundary layer (6). Successive experiments were often performed in the same jejunal segment of each rat since within-rat variations are usually smaller than between-rat variations.

When using 1-[^{14}C]butanol to focus principally on the membrane effects and 1-[^{14}C]octanol to focus on the aqueous diffusion layer and vascular effects,

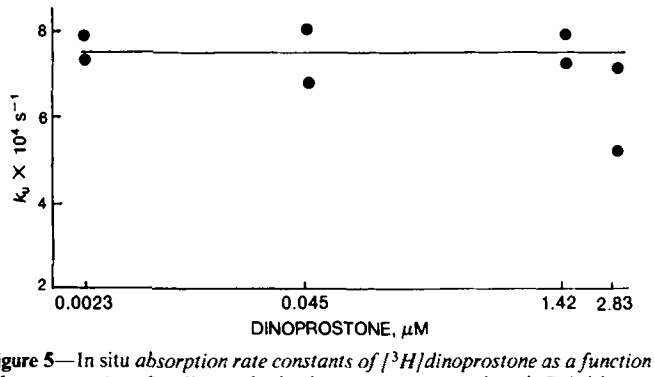

Figure 5—In situ *absorption rate constants of [^{3}H]dinoprostone as a function of concentration. Oscillation hydrodynamics were employed. Solid line is the average rate constant.*

[6] One impurity in the original stock solution had the same R_f as PGA.

Table VIII—Comparison of Diffusional Rates across the Aqueous Layer Barrier in Front of the Mucosal Membrane to the Total Absorption Rates

Hydrodynamic Condition	$P_{aq} \times 10^4$ cm/s	Buffer pH	$k_u \times 10^4$ s^{-1} Experimental	$k_u \times 10^4$ s^{-1} Maximum[a]	Fraction of Absorption Rate Controlled by Aqueous Diffusion Layer
Oscillation, 0.075 mL/s	1.82	3.5	16.6	20.38	0.81
		4.5	11.94	20.38	0.59
		6.0	7.63	20.38	0.37
		7.5	2.98	20.38	0.15
		8.5	2.05	20.38	0.10
		9.5	2.0	20.38	0.10
Static, 150-s interval	0.86	3.5	8.78	9.63	0.91
		4.5	7.81	9.63	0.81
		6.0	5.44	9.63	0.56
		7.5	2.70	9.63	0.28
		8.5	2.2	9.63	0.23
		9.5	1.93	9.63	0.20

[a] $k_{u,max} = \frac{AP_{aq}}{V} = 11.2\,P_{aq}$.

Table IX—Factorization of Effective Permeability Coefficients of the Jejunal Membrane at Various Buffer pH Values into the Permeability Coefficients of the Aqueous Pores and Principal Membrane Pathway

Hydrodynamic Condition	Buffer pH	Average $k_u \times 10^4$ s^{-1}	Permeability Coefficients $\times 10^4$ cm/s P_{aq}	$P_{c,m}$[a]	P_p	P_m[a]
Oscillation 0.075 mL/s	3.5	16.6	1.82	7.98	0.2	7.78
	4.5	11.94	1.82	2.57	0.2	2.37
	6.0	7.63	1.82	1.09	0.2	0.89
	7.5	2.98	1.82	0.31	0.2	0.11
	8.5	2.05	1.82	0.2	0.2	0
	9.5	2.0	1.82	0.2	0.2	0
Static 150-s interval	3.5	8.78	0.86	—	0.2	—
	4.5	7.81	0.86	—	0.2	—
	6.0	5.44	0.86	1.12	0.2	0.92
	7.5	2.70	0.86	0.33	0.2	0.13
	8.5	2.2	0.86	0.2	0.2	0
	9.5	1.93	0.86	0.2	0.2	0

[a] $P_{c,m}$ is defined by Eq. 6, and $P_m = \frac{KX_s\sqrt{kD}}{\tanh(L\sqrt{k/D})}$.

the absorption of these solutes was not affected by 1.76 μg/mL of dinoprostone at pH 4.5 and 6.0 (Table III). These pH conditions were chosen since the absorption is pH dependent (Table I) while the alkanol absorption is pH independent. Furthermore, when using *n*-butyric acid at pH 4.5 to assess the lipoidal and aqueous pore pathways of the membrane and *n*-butyric acid and the larger molecular size *p*-aminobenzoic acid at high pH to assess possible changes in the aqueous pores, a 1.76-μg/mL concentration did not have any apparent effect (Tables IV and V). As will be seen later, absorption was not influenced by concentrations up to 1×10^{-3} mg/mL (equivalent to 2.83 μM). From all of these results together, it is concluded that dinoprostone does not have significant adverse effects on the membrane system so that an unambiguous quantification and interpretation of the membrane permeability coefficients can be made.

Mechanism of Dinoprostone Absorption in Intestines—Since dinoprost is transported across the lipoidal membrane by a simultaneous passive diffusion-bioconversion kinetic process (3, 4), this is good reason to expect that dinoprostone follows the identical mechanism. For comparison, the absorption rate constant *versus* buffer pH plots are shown in Fig. 4 for both prostaglandins. Although *n*-butyric is only passively absorbed (6), it is included here to point out the similarities of the general pH-partition phenomena with *n*-butyric acid to the prostaglandins.

As shown in Fig. 4, the absorption rate constants of dinoprost from pH 3.5 to 7.5 were comparatively less than those of the more lipophilic dinoprostone at comparable pH. They tended to converge at about pH 8.5, as might be expected for the transport of the anions across the aqueous pore pathway. In contrast, the absorption-pH profile of *n*-butyric acid was higher than that for the prostaglandins and was also shifted more to the right. The asymptotic minimum absorption rate of *n*-butyric acid across the aqueous pore at pH 8.5 and higher was faster than the prostaglandins by a factor of two. In summary, the comparative profiles of these three weak acids represent the net interplay of the pH and permeabilities of the aqueous diffusion layer barrier in front of the membrane and the membrane itself.

The possibility of a facilitated transport process is virtually eliminated by the absorption data found in Table VI and Fig. 5. Contrary to the theory of the facilitated mechanism, a relatively constant absorption rate at pH 6.0 was found over a 1.25×10^3-fold range of dinoprostone concentrations (from 8×10^{-4} to 1.0 μg/mL).

Permeability Coefficients—The permeability coefficients of the aqueous diffusion layer, estimated from the pH 6.0 and 6.5 absorption data, were 0.86×10^{-4} cm/s for the static hydrodynamics and 1.82×10^{-4} cm/s for the oscillation case (Table VII). The data at pH 3.5 and 4.5 were not employed, since the absorption rates are highly aqueous diffusion controlled where Eqs. 8 and 9 become insensitive. The coefficients compare well with the 0.8×10^{-4} and 1.7×10^{-4} cm/s found in the dinoprost studies (3). Using the P_{aq} values, one gets estimates on how much the total absorption rate is aqueous diffusion controlled or membrane controlled (Table VIII). At low pH, the absorption is governed by diffusion in front of the membrane, while at higher pH it is controlled more by the membrane.

With the average $k_u = 2.0 \times 10^{-4}$ s^{-1} at high pH and Eq. 10, the permeability coefficient of the aqueous pores for the dinoprostone anions is 2.0×10^{-5} cm/s. In perspective, P_p is 1.5×10^{-5} cm/s for dinoprost. Since the permeability coefficients of the aqueous diffusion layer and the aqueous pores of the membrane are known, the factorization of the effective permeability coefficient for the simultaneous membrane transport and bioconversion of dinoprostone, *i.e.*, $KX_s\sqrt{kD}/\tanh(L\sqrt{k/D})$, is readily attained. Table IX summarizes the quantitative estimates of all of the permeability coefficients.

CONCLUSIONS

Baseline intestinal absorption studies of dinoprostone in the rat were established, and the results were mechanistically consistent with dinoprost. These two important prostaglandins provide the reference compounds from which the *in situ* absorption and metabolism in the intestinal membrane of their respective derivatives and analogues can be studied on a predictive level *via* the physical model approach. Such studies are in progress.

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Influence of Mode of Intravenous Administration and Blood Sample Collection on Rat Pharmacokinetic Data

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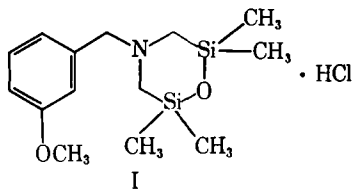
Abstract □ The influence of the mode of intravenous dosing and blood sample collection on the pharmacokinetics of 4-[(3-methoxyphenyl)-methyl]-2,2,6,6-tetramethyl-1-oxa-4-aza-2,6-disilacyclohexane hydrochloride (I) was studied in the rat. Blood samples obtained from the tail and by exsanguination following injection of the ^{14}C-labeled drug into the caudal vein, the jugular vein, and the heart were analyzed for total radioactivity, and the concentration profiles from the different treatments were compared. Dosing and sampling from the tail vein resulted in significantly different blood levels (and related pharmacokinetic parameters) when compared to other methods, probably attributable to a local depot effect. Intracardiac administration tended to cause higher drug levels in the heart than intravenous doses, although no significant differences were found between the respective blood concentrations. The results showed that caudal vein injection is a simple and adequate method of intravenous administration in rats designated for exsanguinated blood and tissue collection. For serial blood sampling in individual animals, the dose may be given *via* the jugular vein and the blood collected from the cut tail. These methods require little or no surgical preparations and are particularly suitable for prolonged sampling in studies where a relatively large number of animals are involved.

Keyphrases □ Pharmacokinetics—influence of mode of intravenous administration and blood sample collection, rat □ Dosing—pharmacokinetics, mode of intravenous administration, blood sample collection

During the developmental process of a potential therapeutic agent, pharmacokinetic studies are often conducted at the same time as toxicity trials. While the oral route of administration is invariably used in these studies in order to be consistent with the toxicity tests, additional experiments employing intravenous doses are usually performed so that important pharmacokinetic parameters such as the volume of distribution and absolute oral bioavailability or absorption can be determined. In the rat, the caudal vein is often used as the site of injection. In contrast, injection *via* the jugular or femoral vein requires surgical exposure of the vein (1) and can be cumbersome in studies involving a large number of animals. Drugs are also administered by simple cardiac puncture, but the results may not be truly representative of an intravenous dose. Potential complications such as embolization and excessive local drug toxicity are further limitations of the intracardiac route. Intraperitoneal injection, commonly used for the administration of compounds to small animals, is different from intravenous dosing in that drug absorption from the peritoneal cavity is slower (2) and entails passage into the portal circulation, resulting in incomplete systemic bioavailability (3).

In recent years, numerous sensitive analytical methods that require only microsamples of blood have been developed, and pharmacokinetic data from individual rats or similar small animals can be obtained by serial sample collection. Several methods using a chronic indwelling catheter to facilitate repeated blood collection have been described (4–6), but the animal preparation procedures are elaborate and tedious and are incompatible with prolonged sampling periods in studies involving a large number of animals. Other methods of vascular access and their potential complications have been described in a recent review (7). A simple alternative to cannulation is the nonsurgical method of bleeding the rat from the cut end of its tail. However, due to the relatively low regional blood flow (8, 9), which could result in delayed mixing of the administered drug, the validity of tail concentration data remains unclear.

Using a new skeletal muscle relaxant, 4-[(3-methoxyphenyl)-methyl]-2,2,6,6-tetramethyl-1-oxa-4-aza-2,6-disilacyclohexane hydrochloride[1] (I), as a test compound, the present study was undertaken to examine the effect of the mode of intravenous dosing and blood sample collection on pharmacokinetic data in the rat. Blood was obtained from the tail and by exsanguination following injection of the ^{14}C-labeled drug into the caudal vein, the jugular vein, and the heart. Radioactivity concentration in the heart tissue after intracardiac administration was also measured for comparison with existing intravenous data.



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EXPERIMENTAL SECTION

Dose Administration and Sample Collection—Male Sprague–Dawley rats, average weight 250 g, were used. They were housed individually in standard cages and had free access to food and water at all times. The rats were divided randomly into three groups of 24 each for drug administration *via* the caudal vein, the jugular vein, and the heart.

Radioactive I was labeled with carbon-14 at the methylene group of the

[1] Compound 58-112 hydrochloride; Sandoz, Inc., East Hanover, N.J.

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