Physical Model Approach to Gastrointestinal Absorption of Prostaglandins II: *In Situ* Rat Intestinal Absorption of Dinoprost

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Abstract □ *In situ* absorption studies with dinoprost in the rat jejunum were carried out using a modified Doluisio technique. The absorption rate was first order. There was a sigmoidal decrease in the rate with increasing buffer pH (from 3.5 to 9.5), which strongly indicated the partitioning of weak acid species into the lipoidal membrane. An asymptotic minimum rate was attained from buffer pH 7.5 to 9.5, operationally indicative of transport of anions across aqueous pores. The importance of the aqueous diffusion layer on the mucosal side of the membrane was evident; rates at pH 3.5 and 4.5 were faster at high agitation hydrodynamics in the lumen solution. Preliminary studies showed that there was no metabolism in the lumenal solution and that metabolism occurred within the membrane. The transport mechanism involved simultaneous passive diffusion and bioconversion in the membrane because (*a*) a 1.5 × 10^4-fold range in dinoprost concentration (0.014–210 μM) showed no saturable carrier-mediated tendency on the rate, (*b*) iodoacetic acid and indomethacin did not inhibit the absorption rate, and (*c*) the shape of the absorption-pH profiles was suggestive of passive diffusion. The prostaglandin did not have apparent adverse membrane and vascular effects under the conditions employed. The quantification and factorization of the physically meaningful transport parameters were accomplished using the physical model previously described. The permeability coefficients of the aqueous diffusion layer for the oscillation and static hydrodynamic situations were 0.8 × 10^{-4} and 1.7 × 10^{-4} cm/s, respectively. The effective transport-bioconversion permeability coefficients of the membrane were 2.57 × 10^{-4} cm/s at buffer pH 3.5 and 1.46 × 10^{-4} cm/s at pH 4.5; the effective permeability coefficient of the aqueous pores was 1.5 × 10^{-5} cm/s. Depending upon the buffer pH, 3.5 or 4.5, and the two hydrodynamic situations, the total transport rate was 45–76% aqueous diffusion controlled.

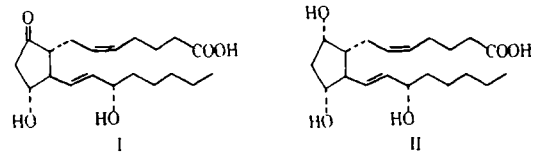
Keyphrases □ Dinoprost—GI absorption, effect of concentration, pH, buffer capacity, and hydrodynamics, rats □ Absorption, GI—dinoprost, effect on concentration, pH, buffer capacity, and hydrodynamics, rats □ Prostaglandins—dinoprost, GI absorption, effect of concentration, pH, buffer capacity, and hydrodynamics, rats

The purpose of this paper is to provide a quantitative insight into some aspects of the absorption process of dinoprost[1] in rat intestines and to identify and assess potential physiological reactions of the membrane and mesenteric circulatory system that may compromise the attainment of an unambiguous physicochemical interpretation of the absorption mechanism. This goal is only part of the larger objectives of a rational biopharmaceutical development of oral delivery systems of the prostaglandins and the molecular design of prostaglandin derivatives and analogues.

To provide the conceptual basis for the biopharmaceutical design, special emphasis is placed on the *in situ* experiments with dinoprost, on the transport processes, and on quantification of the transport and enzymatic kinetic parameters.

Absorption of dinoprost (II), dinoprostone (I), and the methyl ester of the 15-methyl derivative of dinoprost is accompanied by extensive metabolism in the small intestines of the rat (1). Similar results were found in absorption experiments using intact intestinal loops of dogs (2). Using 1-[^{14}C]prostaglandin E_1, Parkinson and Schneider (3) found that only minute amounts of the intact weak acid survived the transport from the mucosal to the serosal fluid of rat jejunum segments *in vitro*. They attributed the loss of the ^{14}C-label to an oxidative process leading to the formation of carbon dioxide. Prostaglandin E_1 is metabolized by homogenates of both rat and dog stomach and jejunum (4). Intestinal absorption of prostaglandin E_1 and dinoprost *via* the lymphatic system appear to be insignificant (2, 5).

The absorption, distribution, excretion, and metabolism of dinoprostone were studied in female rats following oral administration (6). Recently, Bito (7) reviewed the literature and made postulations on the membrane carrier-mediated transport mechanism of the prostaglandins with respect to specific tissues and tissue functionality. Although the transport of dinoprost across the rabbit vagina appears to be a saturable carrier-mediated process, definitive mechanistic studies on intestinal transport in animals are lacking.



The literature on the chemistry, metabolism, and biology of prostaglandins is extensive and was reviewed previously (8). Prostaglandins are substrates to NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase, Δ^{13}-reductase, β-oxidase, and ω-oxidase (9–11). The bioconversion to the 15-oxo and then the 13,14-dihydro-15-oxo forms is sequential. The pK_a of dinoprost is 4.9, and the solubility of the free acid in water is 1.5 mg/mL. The critical micelle concentration (CMC) increases with pH and is ~4 × 10^{-3} M at pH 3 and ~2 × 10^{-2} M at pH 6.0 (12).

Aqueous solutions of a 5-mg/mL dinoprost equivalent as the tromethamine salt are stable for at least 3 months at room temperature and pH 7–9. No decomposition of 15-mg/mL aqueous solutions at about pH 6.7 was found over 7 d and a 4–47°C temperature range (13). Epimerization to the 15-epi derivative of dinoprost occurs at very low pH, but there are no kinetic data. However, studies in the epimerization kinetics of 15(*R*)- and 15(*S*)-15-methyl derivatives of dinoprost methyl ester serve as a useful guideline (14). When each analogue was incubated in an acidic medium, each epimerized at C-15 to give approximately a 1:1 mixture of the 15(*R*)- and 15(*S*)-forms. About 50% epimerized in 1 h at pH 1.36, and 5% epimerized in 3 h at pH 3.12.

EXPERIMENTAL SECTION

Materials and Apparatus—Dinoprost tromethamine and the 15-oxo and 13,14-dihydro-15-oxo derivatives were used without further purification[2]. [9-^{3}H]Dinoprost(prostaglandin $F_{2\alpha}$)(9.2 Ci/mmol) was obtained from two sources[2,3]. Other radiolabeled chemicals[3] included ^{14}C-labeled 1-butanol (10 mCi/mmol), 1-octanol (10 mCi/mmol), and benzoic acid (5 mCi/mmol). Indomethacin and iodoacetic acid also were used. The isotonic buffers are

[1] Dinoprost is prostaglandin $F_{2\alpha}$; dinoprostone is prostaglandin E_1.

[2] Supplied by The Upjohn Co., Kalamazoo, Mich.

[3] Aquasol, New England Nuclear, Boston, Mass.

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Table I—Standard Isotonic Buffer Solutions

Component[a]	pH 3.5	pH 4.5	pH 6.0[b]	pH 7.5[b]	pH 9.5[b]
Citric acid monohydrate, g	14.75	11.03	—	—	—
Dibasic potassium phosphate, anhydrous, g	10.38	16.53	—	—	—
Potassium sulfate, g	7.0	2.63	11.93	11.93	9.13
Monobasic potassium phosphate, anhydrous, g	—	—	9.07	9.07	—
Boric acid, g	—	—	—	—	10.33
Distilled water[c]	*q.s.*	*q.s.*	*q.s.*	*q.s.*	*q.s.*

[a] Reagent-grade chemicals were used without further purification. All solutions were adjusted to 300–310 mOsm/kg with potassium sulfate. For buffer solutions less than the buffer capacities of the standard preparations, the concentrations of buffer reagents were reduced proportionally and adjusted to isoosmotic conditions with potassium sulfate. [b] The pH was adjusted with 1 M NaOH. [c] The final volume was 1 L.

listed in Table I. A commercial scintillation cocktail[3] was used. A liquid scintillation counter[4], research pH meter[4], osmometer[5], two-directional flow pump[6], and a centrifuge[7] were used.

***In Situ* Absorption Procedures**—Male Sprague–Dawley rats, 220–275 g, fasted for 12–16 h, were anesthetized with 20% urethane solution, 1–2 mg/g ip. An appropriate 22-cm section of the jejunum was selected, washed profusely with normal saline, cannulated, connected to two 5.0-mL syringe barrels and the two-directional pump. The last wash consisted of the buffer intended for the ensuing experiment.

Exactly 2 mL of the radiolabeled solute in isotonic buffer was introduced into the lumen of the jejunum. With the aid of the flow pump, 0.5 mL of solution was pushed up the barrel of one syringe at the rate of 0.075 mL/s, up the other syringe, and finally up the first syringe, whereupon a 20-μL sample was withdrawn with an automatic pipet for assay. This first sample was regarded as the time zero sample, which was 30–35 s after the initial introduction of the 2-mL drug solution into the intestinal lumen.

The initial oscillation of the lumenal solution was done to obtain a uniform mixing of the 2-mL drug solution with the residual solution in the gut lumen from the final wash. The apparent surface area to volume ratio was 11.2[8]. All absorption experiments were carried out at 37°C.

In experiments involving static hydrodynamic conditions, the drug remained in the lumen until, at regular 150-s sampling intervals, 0.5 mL of the lumenal solution was pushed up into the barrel of the syringe with the pump at 0.075 mL/s, and a 20-μL sample was withdrawn. Then the solution was pumped back into the lumen and remained there until the next regular sampling interval. This sampling procedure took no more than 10 s, and at least 75% of the solution volume always remained in the lumen. The regularity of the sampling time interval under these "static" experimental conditions was necessary physicochemically to maintain some constancy in the hydrodynamics of the solution in the gut lumen. This constancy is important when the transport rate is influenced significantly by the permeability of the unstirred aqueous layer.

Chromatography—For TLC, precoated silica gel F-250 plates[9] were developed in ethyl acetate–acetic acid–isooctane–water (90:20:50:100, by volume) (15). Dinoprost and its metabolites were readily observed by spraying with 15% ammonium sulfate. Zones of radioactivity were quantified in the scintillation cocktail with 0.5-cm scrapings. With this TLC system, no impurities in the original [^{3}H]dinoprost solutions were detected.

During the *in situ* intestinal absorption studies, samples from the lumenal solution, blood, and small intestine were taken. Lumenal solution or blood, 0.1 mL, was extracted two to three times with 2 mL of ethyl acetate, concentrated by evaporation with a nitrogen stream, and then spotted. A 1-cm intestinal segment, previously washed with normal saline, was excised, homogenized in the cold, extracted three times with 2 mL of ethyl acetate, concentrated with a nitrogen stream, and spotted.

Qualitative and quantitative analyses of samples from the lumenal solution and the blood (after extraction with ethyl acetate) were performed by HPLC[10]

[4] LS-200 spectrometer; Beckman.
[5] Advanced Instruments, Newton Highlands, Mass.
[6] Model RRP-G-150, Fluid Metering, Oyster Bay, N.Y.
[7] Model SS-3 automatic, Ivan Sorvall, Norwalk, Conn.
[8] The apparent surface area to volume ratio for a 22-cm intestinal segment and 2.2 mL of solution (2.0 mL of isotonic buffer solution of radiolabeled solute and 0.2 mL of residual wash solution in the lumen) is 11.2 This value is calculated by considering the intestinal lumen as a right cylinder:

$$\frac{A}{V} = 2\sqrt{\frac{\pi h}{V}}$$

where A/V is the apparent surface area to volume ratio and h is the length of the intestinal segment.
[9] Analtech, Newark, Del.
[10] Du Pont model A-20 liquid chromatograph.

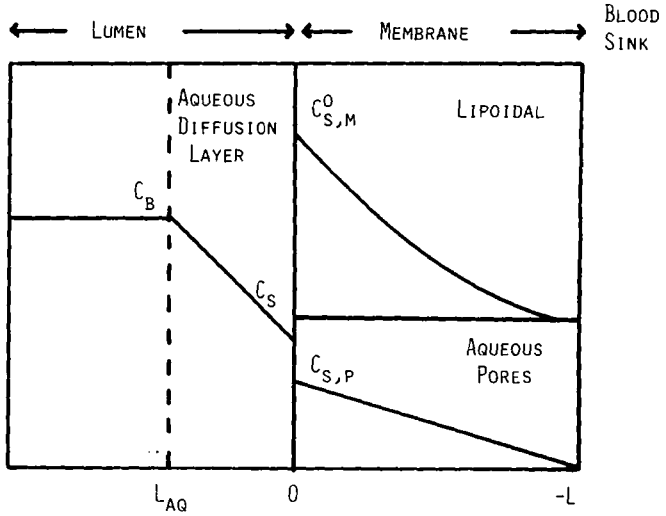

Figure 1—*Physical model for the simultaneous intestinal membrane transport of dinoprost. An aqueous diffusion layer is in series with the membrane consisting of two parallel pathways: the diffusional transport-bioconversion pathway and the aqueous pore pathway. The model depicts the boundary concentrations and distances.*

after conversion of the prostaglandins to the *p*-nitrophenacyl esters as described previously (16).

THEORETICAL SECTION

As will be seen, the following physical model (17) is consistent with the dinoprost absorption in the rat jejunum (Fig. 1). It consists of an aqueous diffusion layer (often referred to as the unstirred aqueous layer) in the lumen in series with the intestinal membrane followed by the blood sink. Operationally, the membrane is comprised of two parallel pathways: the simultaneous diffusional transport-bioconversion pathway in the lipoidal fraction of the membrane and the aqueous pore pathway. It is assumed that the nondissociated and anionic species have equal facility across the aqueous pores. This approach leads to the semilogarithmic expression:

$$\ln C_b = \ln C_b(0) - k_u t \qquad \text{(Eq. 1)}$$

where C_b are the total drug concentration remaining in the lumenal solution at time t, and $C_b(0)$ is the initial concentration. The apparent first-order absorption rate constant, k_u, is given by:

$$k_u = \frac{A}{V} \cdot \frac{1}{\frac{1}{P_{aq}} + \frac{1}{P_{e,m}}} \qquad \text{(Eq. 2)}$$

where A/V is the apparent surface area to solution volume ratio, and P_{aq} and $P_{e,m}$ are the effective permeability coefficients of the aqueous diffusion layer and the membrane, respectively. Furthermore:

$$P_{e,m} = \frac{\alpha K X_s \sqrt{kD}}{\tanh\left(L\sqrt{k/D}\right),} + P_p \qquad \text{(Eq. 3)}$$

where K is the membrane lipoidal biophase–aqueous partition coefficient, X_s is the fraction of nondissociated species at the membrane surface, L is the thickness of the membrane, D is the diffusion coefficient in the membrane, k is the apparent first-order bioconversion rate constant, P_p is the permeability coefficient of the aqueous pores, and α is the volume fraction of the diffusional transport-bioconversion pathway of the lipoidal membrane.

The concentration profile in the diffusional transport-bioconversion pathway is described by:

$$C_m = \frac{C_{m,s} \sinh\left[\sqrt{k/D}(L + x)\right]}{\sinh\left(L\sqrt{k/D}\right)} \qquad (0 \geq x \leq -L) \qquad \text{(Eq. 4)}$$

where $C_{m,s}$ is the concentration of the drug at the surface on the membrane side. Thus, this model assumes that only intracellular metabolism is significant and that cavital and membrane digestion is insignificant (17, 18).

RESULTS AND DISCUSSION

General Absorption Studies—The absorption of [^{3}H]dinoprost at various isotonic buffer values pH and high buffer capacity under the continuous oscillation procedure is shown in Fig. 2. There was a logarithmic change in the concentration remaining in the lumen of the jejunum with time. The slopes decreased with increasing pH. At pH 7.5 and 9.5, the finite rate constants of

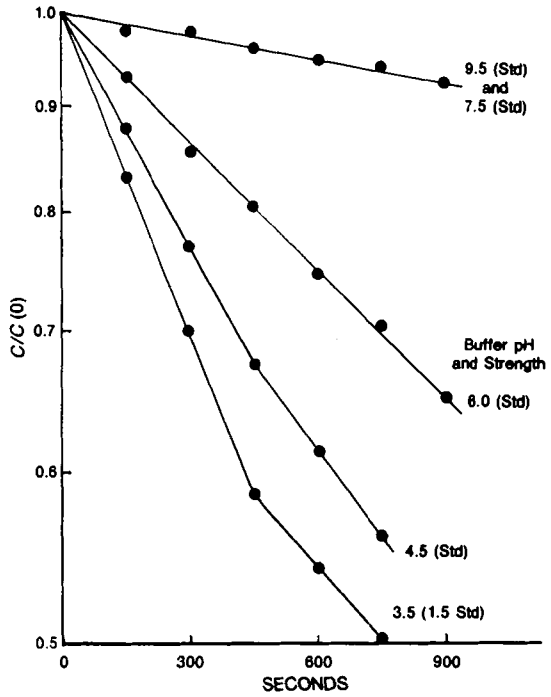

Figure 2—*Semilogarithmic change in the fraction of [^3H]dinoprost remaining in the lumen with time.*

the anionic species were about the same. The absorption at pH 3.5 and 4.5 tended to depart from linearity at later times, most likely due to the flow of intestinal secretions changing the surface and bulk pH with time.

The marked influence of the aqueous diffusion layer on the transport kinetics is observed in the first-order plots in Fig. 3. All other factors being constant, the hydrodynamics produced by the oscillation of the lumenal solution had a larger effect on the slope than the hydrodynamics produced by the static method with 150-s sampling intervals. The higher the degree of agitation, the thinner the aqueous diffusion layer on the mucosal side of the intestinal membrane (19).

Table II is a summary of the absorption experiments at tracer concentrations in the jejunum; the results are also illustrated in Fig. 4. The k_u-pH

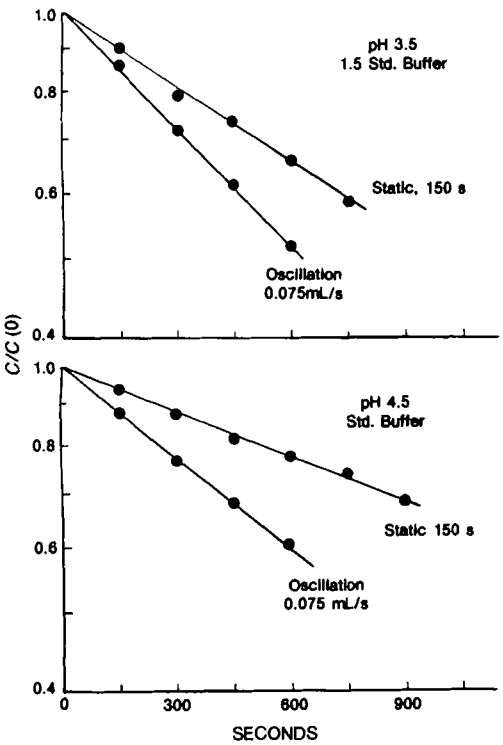

Figure 3—*Comparison of the* in situ *absorption of dinoprost at buffer pH 3.5 and 4.5 under two hydrodynamic conditions.*

Table II—*In Situ* Absorption of Dinoprost (5×10^{-3} μg/mL) in Rat Jejunum at 30°C: Apparent Rate Constants [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	Standard	3	11.49 (0.05)
		1.5 Standard	1	11.1
	4.5	Standard	4	9.24 (0.70)
	6.0	Standard	5	4.64 (0.14)
	7.5	Standard	3	3.86 (0.18)
		1.5 Standard	3	1.56 (0.15)
	8.5	1.5 Standard	1	1.8
	9.5	Standard	2	1.51 (0.08)
Static, 150-s intervals	3.5	1.5 Standard	2	6.37 (0.35)
	4.5	Standard	2	5.50 (0.22)
	6.0	Standard	1	3.46
	7.5	1.5 Standard	2	1.48 (0.4)

[a] This table is a summary of all experiments with tracer concentrations of [^{3}H]dinoprost.

profiles were similar to those expected for weak acids such as the 1-alkanoic acids (20). At low pH, the absorption under the higher degree of agitation of the solution was greater and less aqueous diffusion controlled than the absorption under less agitation. The differences in the absorption rate constants between the two hydrodynamic conditions became smaller with increasing pH. This result is attributed to the increasing importance of the membrane as the rate-controlling transport barrier. From about pH 7.5 and higher, the k_u-pH profiles for the two hydrodynamic situations converged to an asymptotic minimum. In an operational way, this result is indicative of the rate-limiting transport of the anions across the aqueous pores of the jejunal membrane.

Physiological Effects of Dinoprost on Membrane System—The objectives of the following studies were to identify and to assess potential physiological membrane reactions to dinoprost that may compromise an unambiguous biophysical interpretation and quantification of the absorption mechanism. These objectives were accomplished by following dinoprost absorption at varying concentrations repeatedly within the same intestinal segment of a rat and also between rats and by absorption experiments with selective permeating species in the absence and presence of dinoprost.

Tables III and IV show the results of successive absorption experiments in the same jejunal segment of a rat with various buffer pH combinations and with prostaglandin concentration combinations (up to 10 μg/mL) at pH 6. Taken together, the results indicated that pH and dinoprost did not have adverse effects on the membrane under the experimental conditions. The within-rat variation was small. A 1.5×10^4-fold increase in the concentration, corresponding to 0.014 and 210 mM, respectvely, did not influence the absorption rate constant.

The absorption rates of 1-[^{14}C]butanol in the jejunum in the absence and

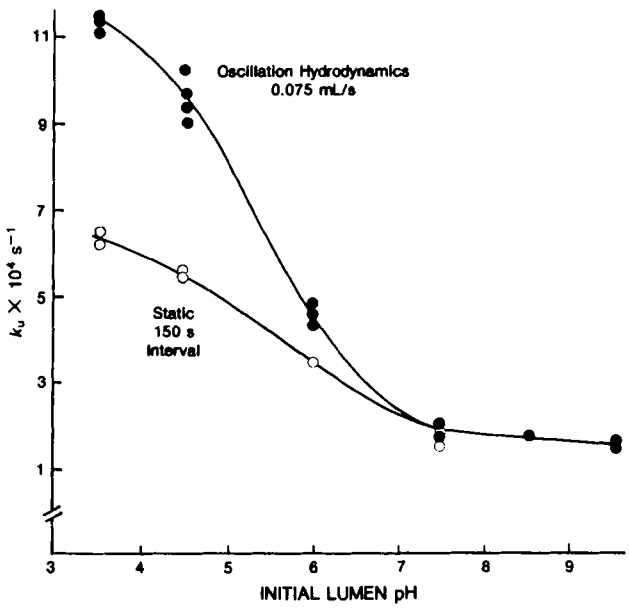

Figure 4—*Apparent first-order rate constants* versus *high buffer capacity pH under two hydrodynamic conditions in the rat jejunum.*

Table III—Successive Absorption Experiments of [^{3}H]Dinoprost in Same Jejunum Segment at Various Buffer-pH Combinations [a]

Order of Experiment	Rat A		Rat B		Rat C	
	Buffer pH	$k_u \times 10^4$ s^{-1}	Buffer pH	$k_u \times 10^4$ s^{-1}	Buffer pH	$k_u \times 10^4$ s^{-1}
1	3.5	11.51	4.5	10.42	7.5	4.08
2	9.5	1.43	7.5	3.65	4.5	9.1
3	3.5	11.43	4.5	8.8	7.5	3.86

[a] All buffers were of standard buffer capacities. The concentration of [^{3}H]dinoprost was 5×10^{-3} μg/mL.

Table IV—Successive Absorption Experiments at pH 6.0 in Same Jejunum Segment at Various Dinoprost Concentration Combinations [a]

Order of Experiment	Rat A		Rat B		Rat C		Rat D	
	Concentration, μg/mL	$k_u \times 10^4$ s^{-1}	Conc., μg/mL	$k_u \times 10^4$ s^{-1}	Conc., μg/mL	$k_u \times 10^4$ s^{-1}	Conc., μg/mL	$k_u \times 10^4$ s^{-1}
1	5×10^{-3}	4.28	1	4.79	10	5.1	100	4.37
2	1	4.57	5×10^{-3}	4.64	10	5.0	100	4.41
3	5×10^{-3}	4.44	1	5.96	—	—	100	4.86

[a] Concentration of 1, 10, and 100 μg/mL refers to dinoprost tromethamine salt. Tracer [^{3}H]dinoprost concentrations of 5×10^{-3} μg/mL were added.

Table V—Absorption of 1-[^{14}C]Butanol in the Jejunum at pH 6.0 in the Absence and Presence of Dinoprost [a]

Concentration of Nonlabeled Dinoprost Tromethamine Salt[b], μg/mL	N	$k_u \times 10^3$ s^{-1} for 1-Butanol
0	4	1.59 (0.16)
10	2	1.51 (0.2)
100	3	1.61 (0.13)

[a] Oscillator (0.075 mL/s) hydrodynamics were used. The 10 and 100 μg/mL are equivalent to 21 and 210 μM, respectively.

presence of dinoprost were not statistically different (Table V), providing further support that there were no significant adverse membrane and vascular effects to transport. There was also no effect on the transport of 1-[^{14}C]octanol at pH 4.5 (Fig. 5). The fact that the absorption of [^{14}C]benzoate anions at pH 8.5 was not influenced by the presence of dinoprost indicated that the prostaglandin did not significantly affect the aqueous pore pathway of the jejunal membrane (Table VI). It is concluded that dinoprost does not have apparent adverse membrane and vascular effects under the experimental conditions employed.

Mechanism of Membrane Transport—It was postulated that the transport of dinoprost in the rat occurs by passive transport across the aqueous diffusion layer and then by the simultaneous passive membrane transport and bioconversion mechanism. The k_u-pH plots in Fig. 4, which are of the kind predicted by the pH-partition-aqueous diffusion layer hypothesis for the passive absorption of weak acids, serve as part of the basis for the postulation of the passive mechanism of the total transport process across the jejunal membrane *per se*. Comparison of the absorption-pH profiles of dinoprost and *n*-butyric acid (Fig. 6) illustrates this point.

The following results provide additional evidence that the absorption does not involve a membrane-saturable process. The rate constant of dinoprost at pH 6 and 7.5 was not concentration dependent with concentrations from 1.4 $\times 10^{-2}$ to 200 μM (a 1.5×10^4-fold range) (Table VII and Fig. 7). Furthermore, the absorption was not affected by iodoacetic acid and indomethacin, substances that inhibit the carrier-mediated transport of natural prosta-

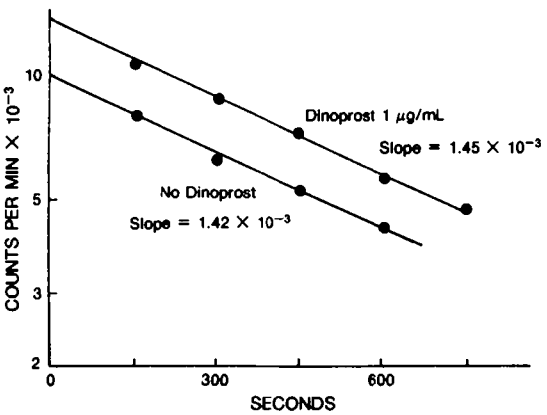

Figure 5—*Semilogarithmic plots of the concentration of 1-[^{14}C]octanol remaining in the lumen of the jejunum with time in the presence and absence of dinoprost under static (150-sec sampling interval) hydrodynamics.*

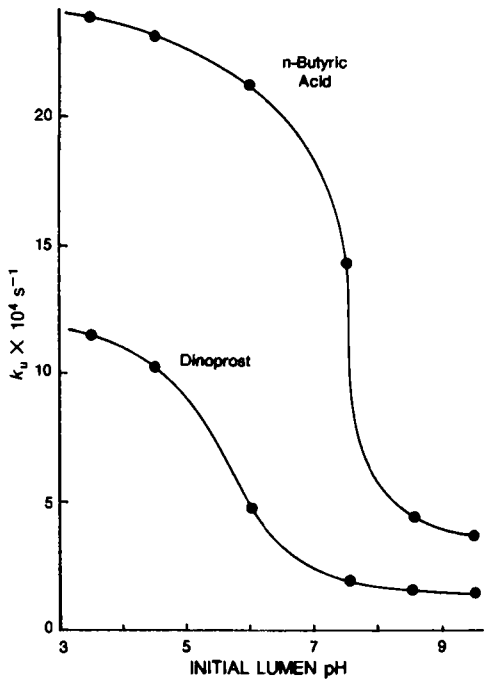

Figure 6—*Comparison of the absorption-pH profiles of dinoprost and* n-*butyric acid under oscillation (0.075 mL/s) hydrodynamics in the rat jejunum. Dinoprost undergoes simultaneous diffusional transport-bioconversion kinetics in the membrane while* n-*butyric acid is passively absorbed.*

Table VI—*In Situ* Absorption of [^{14}C]Benzoate at pH 8.5 in the Absence and Presence of Dinoprost in the Jejunum [a]

Order of Experiment[b]	Rat A		Rat B		Rat C	
	Dinoprost, μg/mL	$k_u \times 10^4$ s^{-1}	Dinoprost, μg/mL	$k_u \times 10^4$ s^{-1}	Dinoprost, μg/mL	$k_u \times 10^4$ s^{-1}
1	0	6.36	0	5.32	1	5.85
2	1	6.80	1	4.74	0	4.22
3	0	6.45	0	5.29	1	5.22

[a] Oscillation (0.075 mL/s) hydrodynamics were employed. The pH 8.5, 1.5 standard buffer capacity, was used. Dinoprost was used as the tromthamine salt. [b] In the same 22-cm jejunal segment.

Table VII—*In Situ* Absorption of [^{3}H]Dinoprost at Various Concentrations and Buffer pH [a]

Buffer pH	Buffer Capacity	Number of Experiments	Dinoprost Concentration, μg/mL	$k_u \times 10^4$ s^{-1} (±*SD*)
6.0	Standard	3	5×10^{-3}	4.45 (0.18)
		2	1	5.11 (0.75)
		2	10	5.05 (0.07)
		3	100	4.61 (0.26)
7.5	1.5 Standard	3	5×10^{-3}	1.56 (0.15)
		3	1	1.81 (0.17)

[a] Oscillation (0.075 mL/s^{-1}) hydrodynamics were employed. The dinoprost concentrations were 1-100 μg/mL of dinoprost tromethamine with 5×10^{-3} μg/mL of [^{3}H]dinoprost added.

Table VIII—Absorption of [^{3}H]Dinoprost in the Absence and Presence of Indomethacin at Buffer pH 7.5 [a]

Order of Experiment	Rat A Indomethacin, M	Rat A $k_u \times 10^4$ s^{-1}	Rat B Indomethacin, M	Rat B $k_u \times 10^4$ s^{-1}
1	0	1.99	10^{-5}	1.71
2	10^{-5}	1.83	0	1.74
3	0	1.71	10^{-5}	1.61

[a] Oscillation (0.075 mL/s) hydrodynamics were employed. The buffer capacity was 1.5 standard. The dinoprost concentration was 1 μg/mL of dinoprost tromethamine with 5×10^{-3} μg/mL of [^{3}H]dinoprost added. The experiments were performed in the same 22-cm jejunal segment.

Table IX—*In Situ* Absorption of [^{3}H]Dinoprost in the Absence and Presence of Iodoacetic Acid at Buffer pH 6.0 [a]

Order of Experiment	Rat A Iodoacetic Acid, mM	Rat A $k_u \times 10^4$ s^{-1}	Rat B Iodoacetic Acid, mM	Rat B $k_u \times 10^4$ s^{-1}	Rat C Iodoacetic Acid, mM	Rat C $k_u \times 10^4$ s^{-1}
1	0	4.50	2	4.83	10	4.64
2	2	4.77	0	4.81	--	—
3	0	4.80	2	4.73	—	—

[a] Oscillation (0.075 mL/s^{-1}) hydrodynamics were employed. The buffer capacity was 1.5 standard. The [^{3}H]dinoprost concentrations were 5×10^{-3} μg/mL for rats A and B and 0.1 μg/mL for rat C.

glandins (21) (Tables VIII and IX). Indomethacin also is a prostaglandin synthetase inhibitor.

Preliminary Metabolism Studies—These studies were carried out to characterize generally dinoprost metabolism in the first 20 min of *in situ* rat intestinal absorption experiments with pH 6.0 buffer. In the first 5 min, <1.0% of the 15-oxo and 13,14-dihydro-15-oxo forms appeared in the lumenal solution (Fig. 8); in 20 min, ~10% of these major metabolites was present. No metabolism occurred *in vitro* in rat blood and the lumenal secretion solution (Fig. 9). However, dinoprost and its two major metabolites were found in the intestinal segments and the blood in the *in situ* absorption studies. If β-oxidation products were present, they could not be detected by the chromatographic techniques employed.

The results of the preliminary *in vitro* and *in situ* studies together indicate

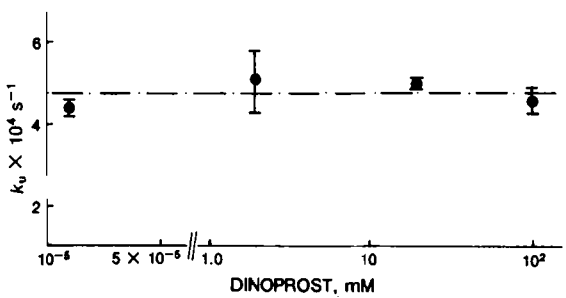

Figure 7—*Effect of dinoprost concentration on the intestinal absorption rate constant at pH 6.0 and oscillation hydrodynamics. The bars represent the standard deviations; the dash line is the mean rate constant for all concentrations, which is equal to 4.8 × 10^{-4} s.*

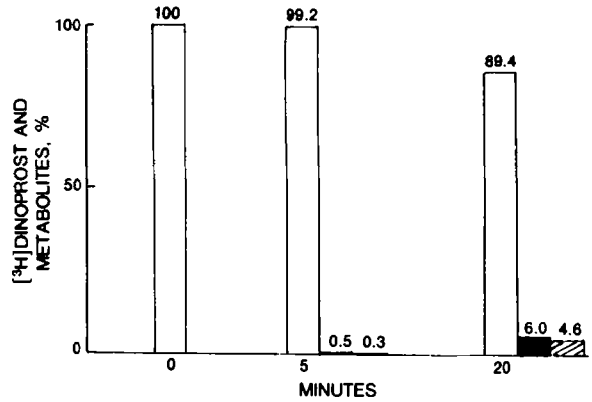

Figure 8—*Percent of radiolabeled dinoprost and metabolites found in the lumen of the rat jejunum* in situ *with time. Key:* □, *dinoprost;* ■, *15-oxo metabolite; and* ▨, *13,14-dihydro-15-oxo metabolite.*

that there is no dinoprost metabolism in the lumen; metabolism by 15-hydroxyprostaglandin dehydrogenase and Δ^{13}-reductase occurs in the membrane and there is a back-diffusion of metabolites into the lumen. The possibility of membrane contact metabolism seems unlikely, since the enzyme systems usually associated with the membrane surface include the carbohydrases, peptidases, lipases, hydrolases, and isoenzymes of alkaline phosphatase (18). Studies are currently directed toward determining, through the use of inhibitors, whether enzymes relevant to prostaglandin metabolism are present at the membrane surface.

Permeability Coefficients—The theoretical physical model described is operationally consistent with the experimental evidence. Consequently, the permeability coefficients of the aqueous diffusion layer, the diffusional transport-bioconversion, and aqueous pore pathways of the jejunal membrane are readily found.

Between two absorption rate constants obtained at the same buffer pH but under different hydrodynamics in the lumen, the following relationships hold:

$$\frac{1}{P_{aq}} + \frac{1}{P_{e,m}} = \frac{A}{Vk_u} \qquad \text{(Eq. 5)}$$

$$\frac{1}{P_{aq}'} + \frac{1}{P_{e,m}} = \frac{A}{vk_u} \qquad \text{(Eq. 6)}$$

$$r^* = P_{aq}'/P_{aq} \qquad \text{(Eq. 7)}$$

The rate constant, k_u, and the permeability coefficient of the aqueous diffusion layer, P_{aq}, are different from k_u' and P_{aq}' because of the differences in the hydrodynamic conditions. In contrast, the effective permeability coefficient, $P_{e,m}$, is independent of the hydrodynamics. The ratio r^* already was determined for a number of hydrodynamic situations in the rat intestinal tract using the 1-alkanol series (19). Between the continuous oscillation (0.075-mL/s) and the static (150-s) sampling interval cases, r^* is 2.12.

As shown in Table X, the permeability coefficients of P_{aq} for the static and oscillation hydrodynamic situations are 0.8×10^{-4} and 1.7×10^{-4} cm/s,

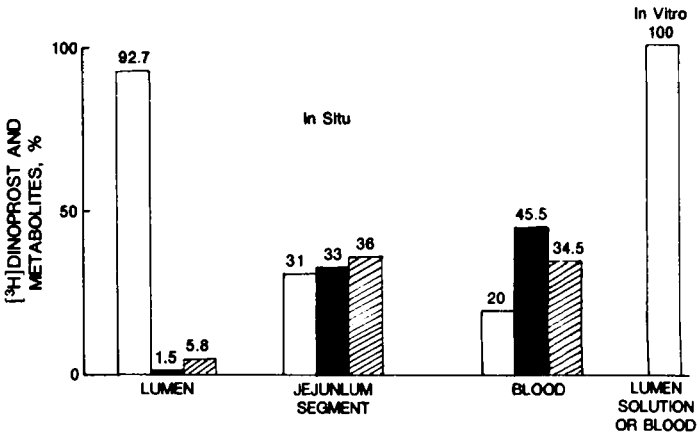

Figure 9—*Percent of radiolabeled dinoprost and metabolites found in the lumenal solution, jejunal gut segment, and blood after 20 min from* in situ *absorption experiments and in the lumenal solution and blood after 20 min exposure from* in vitro *experiments.*

Table X—Permeability Coefficients of the Aqueous Diffusion Layer [a]

Buffer pH	$k_u \times 10^4$ s^{-1} Static, 150 s	$k_u \times 10^4$ s^{-1} Oscillation, 0.075 mL/s	Permeability Coefficient $\times 10^4$ cm/s $P_{aq(static)}$	Permeability Coefficient $\times 10^4$ cm/s $P_{aq(oscillation)}$
3.5	6.61	11.09	0.8	1.64
4.5	5.66	8.65	0.8	1.64

[a] The P_{aq} for the static and oscillation situations and $P_{c,m}$ were calculated from the experimental rate constants with Eqs. 5 and 6. The r^* is 2.12, and A/V is 11.2.

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

Buffer pH	Buffer Capacity	Permeability Coefficient $\times 10^4$ cm/s $P_{c,m}$[a]	P_{pore}	$\frac{KX\sqrt{kD}}{\tanh(L\sqrt{k/D})}$
3.5	1.5 Standard	2.59	0.15	2.44
4.5	Standard	1.44	0.15	1.29
6.0	Standard	0.53	0.15	0.38
7.5	1.5 Standard	0.15	0.15	0
9.5	Standard	0.15	0.15	0

[a] Defined by Eq. 3.

respectively. The $P_{c,m}$ values are 2.57×10^{-4} cm/s at buffer pH 3.5 and 1.46×10^{-4} cm/s at pH 4.5. Since the aqueous diffusion coefficient of dinoprost is equal to 5.1×10^{-6} cm^2/s, the effective diffusion layer thickness is 310 μm for the oscillation hydrodynamics and 662 μm for the static situation.

The permeability coefficient of the aqueous pores, P_p, is determined from the experimental data in the limit of the buffer pH $\gg$ pK_a; thus:

$$k_{u,min} = \frac{A/V}{\frac{1}{P_{aq}} + \frac{1}{P_p}} \qquad \text{(Eq. 8)}$$

Using Tables II and X where the average $k_{u,min}$ is 1.51×10^{-4} s^{-1} and P_{aq} (oscillation) is 1.7×10^{-4} cm/s, one finds that P_p is 1.5×10^{-5} cm/s. In perspective (6), the P_p of the salicylate and *p*-aminobenzoate ions in the jejunum is 2.8×10^{-5} cm/s, and that of taurocholate anions is negligible. It is reasonable to assume that the P_p of the nondissociated dinoprost species is at least equal to, if not slightly larger than, the P_p of the anion. Consequently, the effective permeability coefficient of the principal transport-bioconversion pathway of the membrane, which is pH dependent, can be found to complete the factorization of the effective permeability coefficient of the membrane (Table XI).

The extent to which the transport rate is influenced by the aqueous diffusion layer barrier in front of the mucosal membrane is presented in Table XII. As one would expect, the rate constant was more aqueous diffusion controlled at pH 3.5 than at pH 4.5, and the oscillation situation was less aqueous diffusion controlled as compared with the static situation. Although the degree of agitation of the lumenal solution at pH 3.5 produced by rapidly oscillating the solution was high, the rate was 60% diffusion controlled. At higher pH values, the rate was less aqueous diffusion controlled.

General Observations of Buffer Capacity on Absorption—The first-order absorption-time profiles at pH 3.5 and 4.5 tended to depart from linearity with time (Fig. 2). For cases where different buffer capacities at initial pH 3.5 and 7.5 were used, the rate constants varied with buffer capacity (Table II). Because of these observations and other intestinal absorption studies (22), all absorption studies were carried out at sufficiently high buffer capacities such that the initial rate constants at all buffer pH were independent of buffer strength.

To complete the baseline studies, additional experiments were carried out

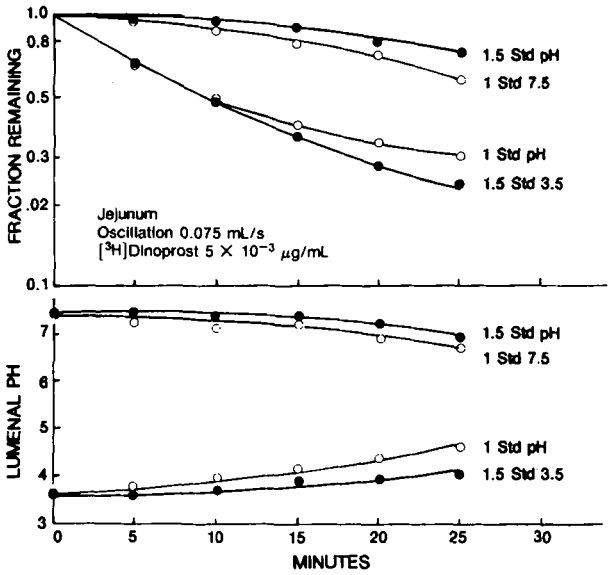

Figure 10—*Semilogarithmic change in the lumenal concentration of dinoprost and bulk pH change with time for pH 3.5 and 7.5 under oscillation hydrodynamics and two buffer strengths.*

wherein both the intestinal absorption and bulk lumenal pH changes with time were simultaneously followed at initial pH 3.5 and 7.5 and two buffer strengths. The first-order absorption curves at pH 3.5 were linear for a short time and then gradually decreased with time, while the bulk lumenal pH increased with time toward pH 6.0 (Fig. 10). These changes occurred more rapidly with the lower of the two buffer strengths. In both cases, the initial slopes in the absorption curves were about the same. In contrast, the increase in the absorption with time at pH 7.5 was accompanied wih a slow decrease in the bulk pH with time. These changes occurred faster at lower buffer capacities.

All of these observations are consistent with the mechanistic studies on the rat intestinal absorption of *n*-butyric acid (19) in which the factors influencing the dynamics of the surface pH, *i.e.*, the pH at the aqueous-membrane interface, were quantitatively assessed. Because the surface pH depends on the net effect of the fluxes of the intestinal buffer secretions and buffer species from the bulk solution, sufficiently high buffer capacity solutions in the lumen must be used to have a baseline control of the surface pH relative to the initial bulk pH.

CONCLUSIONS

The objectives of providing a broad insight into the absorption process and of identifying and assessing potential physiological reactions of the membrane to the prostaglandins that can compromise an unambiguous biophysical interpretation of the absorption mechanism *via* the physical mode approach have been met. *In situ* baseline absorption studies with [^{3}H]dinoprost in the rat jejunum were carried out using a modified Doluisio technique for better control of the hydrodynamics. The experimental variables included a wide range of buffer pH (3.5-9.5), buffer capacity, hydrodynamics, and prostaglandin concentrations. The physical model was applied to the results to obtain quantitative estimates of the permeability coefficients of the aqueous diffusion layer, the aqueous pore pathway, and the effective diffusional transport-bioconversion pathway of the membrane. Studies are in progress to distinguish further the occurrence of metabolism within the membrane from membrane contact metabolism and to obtain the factorization of the apparent *in situ*

Table XII—Influence of the Aqueous Diffusion Layer on the Transport Rate of Dinoprost in the Jejunum

Buffer pH	Hydrodynamic Condition[a]	$k_{u,exp} \times 10^4$ s^{-1}	$k_{u,max} \times 10^4$ s^{-1}	Percent of Rate Constant Aqueous Diffusion Controlled
3.5	Static, 150 s	6.61	8.96	74
	Oscillation	11.09	19.0	58
4.5	Static, 150 s	5.66	8.96	63
	Oscillation	8.65	19.0	46
6.0	Static 150 s	3.56	8.96	40
	Oscillation	4.67	19.0	25
7.5 and 9.5	Oscillation	1.51	19.0	8

[a] Static case: $k_{u,max} = AP_{aq}/V = 11.2 \times 0.8 \times 10^{-4} = 8.96 \times 10^{-4}$. Oscillation: $k_{u,max} = 11.2 \times 1.7 \times 10^{-4} = 19.0 \times 10^{-4}$.

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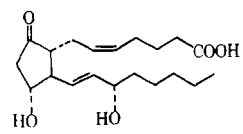
Received October 18, 1976, from the **College of Pharmacy, University of Michigan, Ann Arbor, MI 48109* and the [‡]*Upjohn Co., Kalamazoo, MI 49001.* Accepted for publication February 16, 1977. Present addresses: [¶]Merck, Sharp & Dohme, West Point, PA 19486 and the [§]Upjohn Co., Kalamazoo, MI 49001.

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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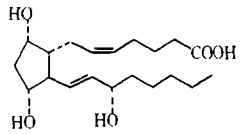

II

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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