The one-compartment model appeared to describe adequately the data obtained after oral administration, but calculation of the absorption rate constant failed in computing with some sets of data from individual subjects after sublingual administration. It was likely, therefore, that after the absorption phase, a very rapid distribution phase preceded the linear elimination phase of the concentration–time relationships but that there were insufficient early sampling times to allow its detection. A simple one-compartment model, therefore, is probably not adequate to describe the pharmacokinetics of isosorbide dinitrate.

Moreover, isosorbide dinitrate is absorbed completely from the GI tract after oral doses (12). Although the reduced bioavailability of isosorbide dinitrate from the standard tablet after oral administration could have been formulation related, the decreased bioavailability after oral doses probably was due to rapid metabolism by the liver after absorption into the hepatoportal system, the drug being subjected to a considerable first-pass effect as occurs for certain other drugs (21–23). Rapid metabolism also explains the very large volumes of distribution and clearances of isosorbide dinitrate by applying a simple one-compartment model to the data. An adequate model of the pharmacokinetics of isosorbide dinitrate must include a pass effect and can be constructed only from a more intensive study of plasma concentrations of the drug and its metabolites after different routes of administration.

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Systems Approach to Vaginal Delivery of Drugs IV: Methodology for Determination of Membrane Surface pH

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Abstract □ A physical model including a diffusional layer in series with the membrane was developed for studying the possible differences between the pH at the membrane surface and that in the bulk solution. Both the membrane-secreted substances (acids and bases) and buffer constituents in the bulk solutions are assumed to contribute to the surface pH. Equations derived for this situation, together with experimental determinations of the acidic dissociation constant of the secreted material, the total secretion flux, the flux of total secreted acidic species, and the diffusional layer thickness, allow estimates to be made of the pH at the membrane surface. With the rabbit vagina, the membrane surface pH was close to that of the bulk solution in most cases. These results were supported by the fact that the absorption of 1-alkanoic acids in pH 2.2 phosphate buffers was relatively constant over the buffer concentration range of 0.003–0.1 *M* phosphate.

Keyphrases □ Drug delivery, vaginal—membrane surface pH determined, compared to bulk solution pH, rabbits □ pH—membrane surface compared to bulk solution, vaginal drug delivery model, rabbits □ Vaginal drug delivery—model, membrane surface pH determined, compared to bulk solution pH, rabbits

The objectives of the present investigations were to develop suitable methodology in an appropriate animal system to obtain firm baseline data on vaginal absorption, to delineate the general barrier properties of the vaginal mucosa, and to develop quantitative integrated models describing both the release of drug from vaginal devices and the subsequent drug absorption.

The first paper (1) in this series described a method for evaluating drug absorption in the vagina, using the rabbit doe as a prototype animal. A rib-cage-type cell, which provided a closed absorptive compartment in the vaginal tract, was designed and surgically implanted in the rabbit. Drug absorption was determined by perfusing the drug solution through this system and following the time changes in drug concentration in the system. The study showed that the method generally affords good precision

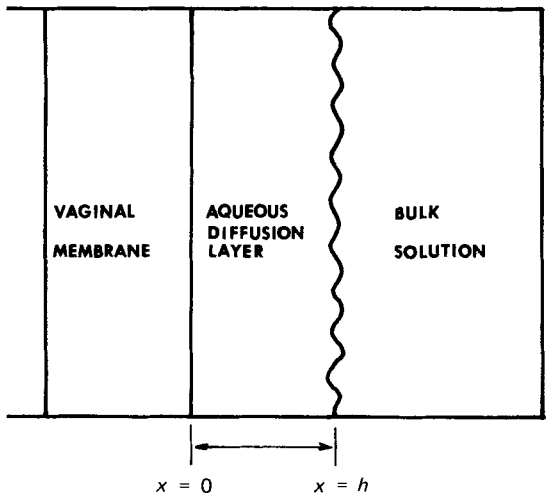

Figure 1—*Physical model for consideration of surface pH. Aqueous diffusion layer is in series with the membrane.*

and should provide a sound basis for meeting the objectives of this research.

The second paper (2) reported the results of the investigation of a physical model for the vaginal absorption barrier, utilizing experimental data on the absorption of the 1-alkanols from methanol to 1-octanol. The analysis of the data showed that experimental results were in reasonably good agreement with the model in which the absorption barrier consists of an aqueous diffusion layer in series with a membrane having two parallel pathways for solute transport—a lipoidal pathway and an aqueous pore pathway.

In the next phase, which is to explore the vaginal absorption behavior of ionizing solutes, it became apparent that an understanding of the membrane surface pH was necessary before a rational study of any physical model for the absorption of weakly ionized drugs (*e.g.*, weak acids and weak bases) could be undertaken. Therefore, the purposes of this study were to develop a method for evaluating the surface pH and to employ it to assess the possible importance of surface pH in analyzing the 1-alkanoic acid vaginal absorption data with the physical models.

THEORY

A schematic diagram of the physical model is shown in Fig. 1. There are the simultaneous fluxes of secretions from the membrane surface across the aqueous diffusion layer and fluxes of the buffer species in the aqueous diffusion layer.

It is assumed that the buffer species (*e.g.*, phosphate) are essentially conserved within the vagina, *i.e.*, there is little or no absorption of buffer species.

It is assumed that the membrane secretes two species, H_2X and HX^-, of a substance by some undefined mechanism. As shown later, the measured acid dissociation constant(s) of H_2X is very close to that for carbonic acid; therefore, H_2X and HX^- might be H_2CO_3 and HCO_3^-, respectively. However, it is unnecessary to specify the exact chemical nature of the secreted material in surface pH (pH_s) analysis.

For the present discussion, assume that phosphate is the buffer in the bulk solution and consider the bulk pH range of 2–8. Then the following six equations (steady state) may be written for the diffusion and chemical equilibrium reaction occurring in th aqueous diffusion layer:

$$K_1 = \frac{(H_2P)(H)}{(H_3P)} \quad \text{(Eq. 1)}$$

$$K_2 = \frac{(HP)(H)}{(H_2P)} \quad \text{(Eq. 2)}$$

$$K_X = \frac{(HX)(H)}{(H_2X)} \quad \text{(Eq. 3)}$$

$$D_{H_3P}\frac{d(H_3P)}{dx} + D_{H_2P}\frac{d(H_2P)}{dx} + D_{HP}\frac{d(HP)}{dx} + D_P\frac{d(P)}{dx} = 0 \quad \text{(Eq. 4)}$$

$$J_{TX} = D_{H_2X}\frac{d(H_2X)}{dx} + D_{HX}\frac{d(HX)}{dx} \quad \text{(Eq. 5)}$$

$$J_{TA} = D\frac{d(H_3P)}{dx} + D_{H_2P}\frac{d(H_2P)}{dx} + D_{HP}\frac{d(HP)}{dx} + D_H\frac{d(H)}{dx} + D_{H_2X}\frac{d(H_2X)}{dx} \quad \text{(Eq. 6)}$$

where (H_3P), (H_2P), (HP), and (P) = concentrations of phosphate buffer species, with the valence of the ions omitted for convenience; (H_2X) and (HX) = concentrations of secretion species of a diprotic acid, with the valence of the ions omitted for convenience; (H) = hydrogen-ion concentration; D = aqueous diffusion coefficient; K = dissociation constant; J_{TX} = total flux of the secretion buffer species per unit surface area; and J_{TA} = total flux of acidic species including drug solution buffer species and hydrogen ions per unit surface area.

The integration of Eqs. 4–6 yields:

$$(H_3P)_s + (H_2P)_s + (HP)_s + (P)_s = (H_3P)_b + (H_2P)_b + (HP)_b + (P)_b \quad \text{(Eq. 7)}$$

$$J_{TX} = \frac{D}{h}[(H_2X)_s - (H_2X)_b + (HX)_s - (HX)_b] \quad \text{(Eq. 8)}$$

$$J_{TA} = \frac{D}{h}[3(H_3P)_s - 3(H_3P)_b + 2(H_2P)_s - 2(H_2P)_b + (HP)_s - (HP)_b + (H_2X)_s - (H_2X)_b + (H)_s - R(H)_b] \quad \text{(Eq. 9)}$$

where h = effective thickness of the aqueous diffusion layer, and s and b = subscripts denoting the membrane surface and the bulk solution boundaries, respectively.

The aqueous diffusion coefficients of all buffer species have been taken to be equal to D, except for the hydrogen ions for which $R = D_H/D$. At low pH, <8, $(P)_b$ and $(P)_s$ are negligible. Then Eq. 7 is reduced to:

$$(H_3P)_s + (H_2P)_s + (HP)_s = (H_3P)_b + (H_2P)_b + (HP)_b \quad \text{(Eq. 10)}$$

Combining Eqs. 9 and 10 yields:

$$J_{TA} = \frac{D}{h}[2(H_3P)_s - 2(H_3P)_b + (H_2P)_s - (H_2P)_b + (H_2X)_s - (H_2X)_b + R(H)_s - R(H)_b] \quad \text{(Eq. 11)}$$

Equations 1–3, 8, 10, and 11 represent six independent equations. All bulk concentrations are known. The effective diffusion layer thickness, h, is 0.035 cm, as previously determined (2) from the alcohol absorption studies. The diffusion coefficients are taken to be 1×10^{-5} cm^2/sec, and R is in the order of 8 under swamping electrolyte conditions (3). The parameters K_X, J_{TX}, and J_{TA} are experimentally determined. Thus, there are six unknowns: $(H_3P)_s$, $(H_2P)_s$, $(P)_s$, $(H_2X)_s$, $(HX)_s$, and $(H)_s$. The J_{TX} and J_{TA} are determined for the experimental situations in which the phosphate buffer is perfused in the rabbit vagina. Subsequently, the surface concentrations of all buffer species and the hydrogen-ion concentration can be calculated. All of these results are examined in the light of the experimental aliphatic carboxylic acid absorption rates and bulk pH values.

EXPERIMENTAL

Materials and Apparatus—^{14}C-Labeled hexanoic acid[1] and tritium-labeled methanol[2] were used without further purification. The radioactivity of the sample was determined by a liquid scintillation counter[3], using a cocktail prepared as described previously (2). The pH was measured by a pH meter[4].

Absorption Procedure and Perfusion System—The absorption procedure and perfusion system were the same as described previously (2). Mature female New Zealand White rabbits, 3.5–5.5 kg, were used. The perfusion system consisted of a reservoir and a rib-cage cell surgically

[1] International Chemical & Nuclear Corp., Irvine, Calif.
[2] New England Nuclear Corp., Boston, Mass.
[3] Model LS 200, Beckman Instruments, Fullerton, Calif.
[4] Research pH meter, Beckman Instruments, Fullerton, Calif.

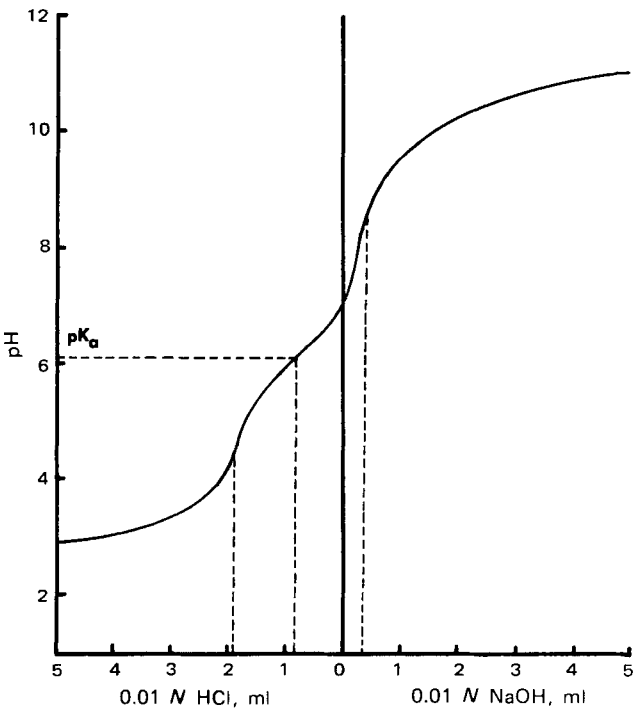

Figure 2—*Titration curve of the saline solution that was circulated in the rabbit vagina for 1 hr.*

implanted in the vagina. The entire path was made of glass and stainless steel, except a short connection [about a 2.54-cm (1-in.) polyethylene tube] between the rib-cage cell and the circuit.

Experiments were scheduled beginning 2 days after the surgical implantation of the rib-cage cell. Two or three experiments were carried out on each day, but the total time interval of an experiment did not exceed 2 hr. The rabbit was anesthetized with pentobarbital sodium (25 mg/kg), and the perfusion system was connected through the fenestra rotunda. The vagina was washed with normal saline solution for about 5 min at 35 ml/min and then with the buffer solution to be used in the experiment. Finally, the drug solution in the reservoir was introduced to the circuit, and the sample was taken periodically at 10-min intervals. The pH was measured before each sample was taken.

Determination of Acid Dissociation Constant, K_X, for Secreted Material—Thirty-five milliliters of 0.9% sodium chloride solution was circulated in the rabbit vagina for 1 hr. Two 10-ml samples were taken. One was titrated with 0.01 *N* NaOH and the other with 0.01 *N* HCl.

Determination of H_2X and HX^- Secretion Rates, J_{TX}—Thirty-five milliliters of phosphate buffer (0.001, 0.003, or 0.01 *M*) or 0.9% sodium chloride was circulated as usual in the system for 23, 43, and 63 min. The

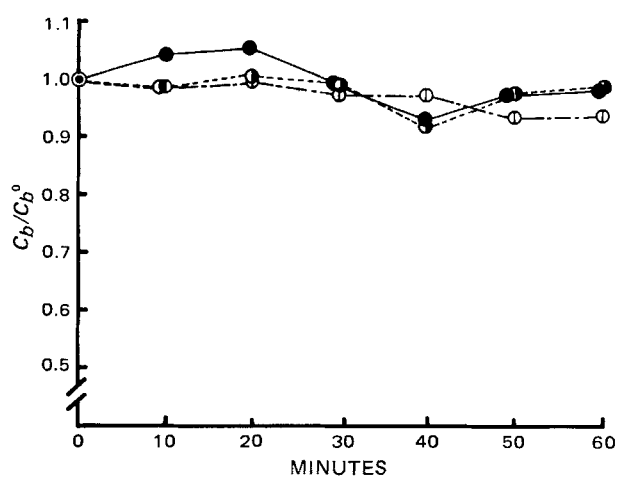

Figure 3—*Plots of phosphate concentration as a function of time. The* C_b *and* C_b^0 *represent the phosphate concentration at time* t *and 0, respectively. Key:* ●, *100 mM;* ◑, *10 mM; and* ⊙, *3 mM.*

Table I—Results of Typical Secretion Rate Determinations at Different Time Intervals

Solution Used	Secretion Rate, μEq/min/cm²		
	23 min	43 min	63 min
0.9% NaCl	0.13	0.11	0.10
3 m*M* Phosphate isotonic buffer (initial pH 3.0)	0.07	0.12	0.08

solutions were then titrated to determine the total acid and base content. The total phosphate concentration was determined by a colorimetric method (4). The difference between the total acid/base of the solution and the total acid/base calculated for the phosphate buffer was attributed to secretions.

Determination of Total Acid Flux, J_{TA}—Let *B* and *C* be the initial amounts of the acidic and basic species of the buffer (at pH close to 3, these will be H_3PO_4 and $H_2PO_4^-$, respectively). Let *Y* and *Z* be the amounts of H_2X and HX^-, respectively, secreted in the time interval, *t*. Then the reaction shown in Scheme I takes place at time *t*:

$$\underset{(B-\delta)}{H_3PO_4} + \underset{(Z-\delta)}{HX^-} \rightleftharpoons \underset{(C+\delta)}{H_2PO_4^-} + \underset{(Y+\delta)}{H_2X}$$

Scheme I

where δ is the amount of reaction taking place. Since the pH of the solution is known at time *t*:

$$K_X = \frac{(H)(Z-\delta)}{(Y+\delta)} \qquad \text{(Eq. 12)}$$

$$K_1 = \frac{(H)(C+\delta)}{(B-\delta)} \qquad \text{(Eq. 13)}$$

where (H) is the hydrogen-ion concentration, K_X is the acid dissociation constant for the secreted material, and K_1 is the first dissociation constant for phosphoric acid. The value of δ is determined by Eq. 13 because *B* and *C* are both known. Thus, *Y* (and *Z*) may be determined from Eq. 12 and the fact that $Y + Z$ is known from the J_{TX} determination discussed previously. The total acid flux, J_{TA}, can then be calculated from *Y*, *t*, and *A*, the surface area of the vaginal membrane, by:

$$J_{TA} = \frac{Y}{tA} \qquad \text{(Eq. 14)}$$

RESULTS AND DISCUSSION

A typical titration curve of the vaginal secretion obtained by perfusing normal saline solution in the vagina for 60 min is shown in Fig. 2. From five experiments, the effective pKa was 6.3 ± 0.1. These data also were used to estimate a second pKa of around 10. These pKa values are close to those for carbonic acid.

The absorption of the phosphate species over the concentration range of 0.003–0.10 *M* at pH 3 was insignificant during 1 hr (Fig. 3). Therefore, it was concluded that the total phosphate is essentially conserved within the usual experimental period.

The secretion rates were relatively constant during the experiments (Table I). The titratable secreted acid was essentially zero, and this value was determined by Eqs. 12 and 13.

The surface pH for the phosphate buffer at bulk pH 2 and 3 could then be calculated (Table II). The surface pH was not greatly different from the bulk pH in these instances over the buffer concentration range of 0.001–0.1 *M*. These results were supported by 1-hexanoic acid absorption studies where the absorption rate at pH 2.1 was independent of the buffer concentration (Fig. 4).

For most membrane transport studies of weak electrolytes conducted

Table II—Calculated Surface pH as a Function of Bulk pH and Total Phosphate Concentration

Bulk pH	Surface pH at Various Concentrations of Total Phosphate		
	100 m*M*	10 m*M*	1 m*M*
2.0	2.0	2.0	2.0
3.0	3.1	3.2	3.3

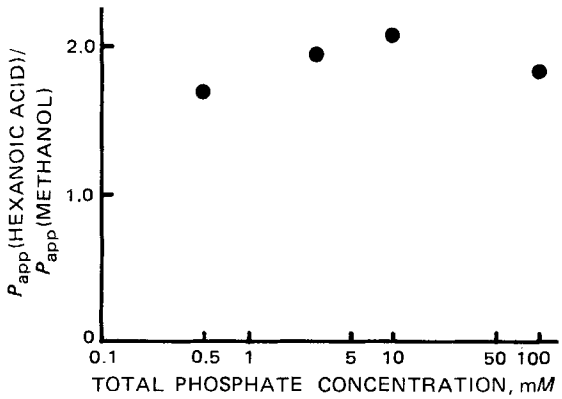

Figure 4—*Plot of the normalized permeability coefficient, P_{app} (hexanoic acid)/P_{app} (methanol), as a function of buffer concentration. The P_{app} is the apparent permeability coefficient.*

in these laboratories, the pH at the membrane surface was assumed to be the same as the pH in the bulk solution. This assumption has now been shown to be a reasonable approximation when the lumenal solution possesses a minimal modest buffer capacity in the case of the rabbit vagina because the rate of secretion (acids or bases) from the membrane is relatively small. In contrast, Desai (5) showed that the surface pH is significantly different from the bulk lumenal pH in *in situ* intestinal absorption experiments with *n*-butyric acid in the rat jejunum. In these studies with the rat intestine, wide ranges of initial buffer pH's (4.5–9.5), buffer capacities, and hydrodynamic conditions were employed in which the flux of buffer secretions across the aqueous diffusion layer was significant and the prevailing surface pH influenced the absorption of *n*-butyric acid.

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Systems Approach to Vaginal Delivery of Drugs V: *In Situ* Vaginal Absorption of 1-Alkanoic Acids

S. HWANG, E. OWADA, L. SUHARDJA, N. F. H. HO, G. L. FLYNN, and W. I. HIGUCHI [x]

Abstract □ The vaginal absorption of a homologous series of ionizable compounds, the 1-alkanoic acids, was studied using a perfusion method with a rib-cage cell surgically implanted in the rabbit vagina. The absorption rates of these compounds followed first-order kinetics. The physical model previously used for the 1-alkanols, but accounting for the pKa and pH effects in the present case, was employed in the analysis of the carboxylic acid data. The aqueous diffusion layer thickness was 0.031 cm. The permeability coefficient for the lipoidal pathway increased 3.5-fold per methylene group. Both values agree reasonably well with those obtained in the alcohol study.

Keyphrases □ Drug delivery, vaginal—*in situ* absorption of 1-alkanoic acids, rabbits □ Absorption, vaginal—1-alkanoic acids, rabbits □ Acids, 1-alkanoic—vaginal absorption, rabbits

The first paper (1) of this series described a methodology for studying the absorption of solutes in the rabbit vagina. A rib-cage cell is implanted in the rabbit vagina, and an appropriate perfusion system is used. The absorption of a homologous series of 1-alkanols was studied (2) employing this technique. The data were analyzed by a physical model that involves a diffusion layer in series with a membrane consisting of two parallel pathways—a lipoidal and an aqueous pore pathway. Most recently (3), it was found that the surface pH of the rabbit vaginal membrane under experimental conditions was well approximated by the pH of the lumenal solution.

The general perfusion technique has now been used to study the permeability behavior of a homologous series of 1-alkanoic acids in the rabbit vaginal membrane. These compounds were expected to exhibit a lumen solution pH dependence for absorption and a chain length dependence. The same physical model as was used (2) with the 1-alkanols appeared to describe adequately the primary features of the absorption behavior of the 1-alkanoic acids in the rabbit vagina.

EXPERIMENTAL

Animals—Mature female New Zealand White rabbits, 3.5–5.0 kg, were maintained in a temperature-controlled animal room (25°) with free access to water and a regular diet.

Materials—^{14}C-Labeled acetic[1], butyric[1], octanoic[1], hexanoic[2], and decanoic[2] acids, ^{3}H-labeled polyethylene glycol[1] (mol. wt. ~4000), and ^{3}H-labeled methanol were used without further purification. Buffers of 0.1 *M* phosphate at pH 3, 6, and 8 were prepared (Table I).

[1] New England Nuclear Corp., Boston, Mass.
[2] International Chemical and Nuclear Corp., Irvine, Calif.