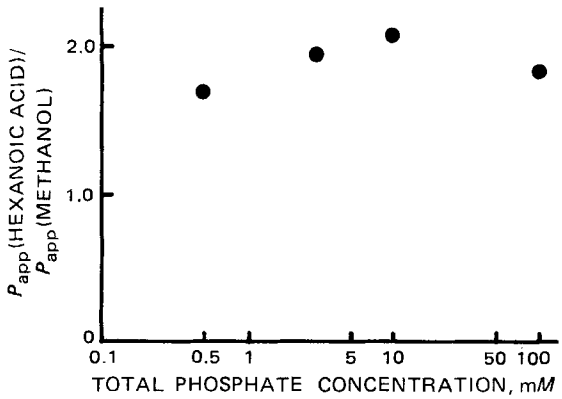

Figure 4—*Plot of the normalized permeability coefficient, P_{app} (hexanoic acid)/P_{app} (methanol), as a function of buffer concentration. The P_{app} is the apparent permeability coefficient.*

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

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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.

Table I—Compositions of 1 Liter of 0.1 *M* Buffer[a] at Designated pH

Component	pH 3	pH 6	pH 8
$NaH_2PO_4 \cdot H_2O$	13.8 g	13.8 g	0.69 g
Na_2HPO_4	—	—	13.5 g
1 *N* NaOH	—	16 ml	—
1 *N* HCl	12 ml	—	—

[a] Adjusted to isotonic by adding sodium chloride.

Perfusion System and Absorption Study Procedure—The perfusion system, as described previously (1, 2), consisted of an outside solution reservoir and a rib-cage cell surgically implanted in the vaginal tract. The entire perfusion path was made of glass tubing and stainless steel, except the short portion [about a 2.54-cm (1-in.) polyethylene tube] between the rib-cage cell and the fenestra rotunda. The rib-cage cell provided an interior volume of 8.1 cm^3 and a geometrical surface area of 22.5 cm^2. It was inserted through the wall of the upper part of the vaginal tract after an appropriate incision.

The procedure for an absorption experiment was essentially the same as described previously (2). Experiments were started 2 days after surgical implantation of the cell. For each rabbit, experiments were conducted on each day successively for 5 or 6 days. The rabbit was anesthetized with pentobarbital sodium, 25 mg/kg, and the perfusion system was connected through the fenestra rotunda. The vagina was first washed with normal saline solution for about 5 min at a flow rate of 35 ml/min and then for an additional 2 min with the buffer solution to be used in the experiment.

In all experiments (except that with polyethylene glycol 4000), ^3H-labeled methanol was run as a reference together with the acid of interest. After the vagina was washed with buffer, the drug solution was introduced by turning the three-way switch. The drug solution became well mixed with the buffer in the whole system after about 3 min (1). Then a zero-time sample was taken, and the experiment was continued for 1 hr during which samples were taken periodically and analyzed for both activities with a liquid scintillation counter[3] employing a dioxane cocktail[4].

RESULTS AND DISCUSSION

Water Flux in Rabbit Vagina—^3H-Labeled polyethylene glycol 4000 was used as a marker to determine the water flux with the three buffer systems (Table I) in the rabbit vagina. Three rabbits were used for each buffer. The water flux was not significant within the 1-hr experiment (Fig. 1 and Table II).

Permeability Coefficient of Methanol ($P_{app}^{methanol}$) with Three Buffers—Nine rabbits were equally divided into three groups for studying the permeability coefficient of methanol in the three buffers (pH 3, 6, and 8). Five experiments were carried out on 5 successive days for every rabbit (Table III). No significant differences in the permeability coefficient of methanol were found among the three buffers.

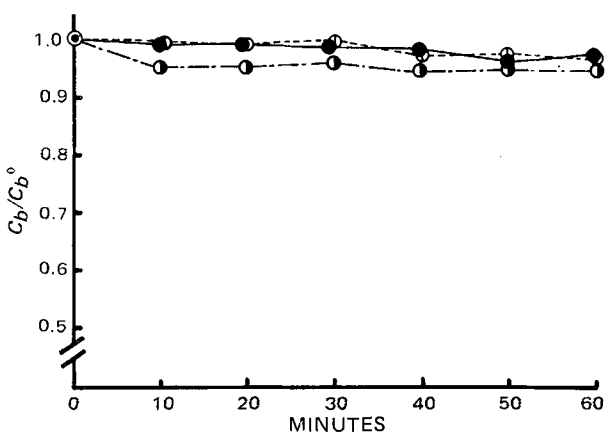

Figure 1—*Plots of polyethylene glycol 4000 concentration as a function of time. The C_b and C_b^0 represent the polyethylene glycol 4000 concentration at times t and 0, respectively. Key: ●, pH 3; ◑, pH 6; and ⊙, pH 8.*

[3] Model LS 200, Beckman Instruments, Fullerton, Calif.

[4] Prepared by dissolving 6 g of 2,5-diphenyloxazole and 100 g of naphthalene and diluting to 1000 ml with dioxane.

Table II—Percentage of Recovered Polyethylene Glycol after 1 hr of Circulation in the Rabbit Vagina

	pH 3	pH 6	pH 8
Rabbit 1	89	97	97
Rabbit 2	90	88	96
Rabbit 3	88	93	95
Mean	89	93	96

Table III—($P_{app}^{methanol} \pm SD$) × 10^4 cm/sec in Three Different Buffer Systems

	pH 3	pH 6	pH 8
	1.11 ± 0.32	1.07 ± 0.14	1.50 ± 0.21
	2.42 ± 0.28	2.26 ± 0.71	1.51 ± 0.19
	2.47 ± 0.30	1.31 ± 0.22	1.59 ± 0.43
Mean ± *SD*	2.00 ± 0.77	1.55 ± 0.63	1.53 ± 0.05

Absorption of 1-Alkanoic Acids—The absorption of the acids (from acetic to decanoic) was studied at pH 3, 6, and 8 in the same buffers. Three rabbits were used for each pH, and all acids were studied in each rabbit at the assigned pH. The order of the experiments was different for each rabbit in each pH group, but the same order combination was used for each pH group.

The data were plotted as log (C_b/C_b^0) *versus* time, where C_b^0 is the drug concentration at time zero. Without exception, the plots were linear for all acids (Fig. 2). This first-order pattern could be expressed by:

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

and:

$$K_u = P_{app}\left(\frac{A}{V}\right) \qquad \text{(Eq. 2)}$$

where C_b is the concentration of the drug in the bulk, K_u is the first-order rate constant, A is the effective surface area of the vaginal tract, V is the volume of the perfusion solution, and P_{app} is the apparent permeability coefficient.

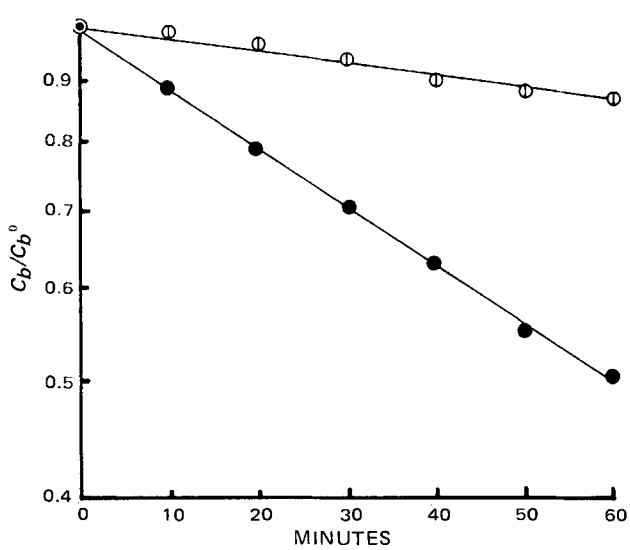

Figure 2—*Semilogarithmic plot of the acid concentration in solution as a function of time. Key: ⊙, acetic acid, pH 8; and ●, hexanoic acid, pH 3.*

Table IV—($P_{app} \pm SD$) × 10^4 cm/sec at Three Different pH Values[a]

Acid	pH 3	pH 6	pH 8
Acetic	2.39 ± 1.40	1.07 ± 0.20	0.49 ± 0.15
Butyric	3.31 ± 1.91	2.89 ± 0.95	0.48 ± 0.04
Hexanoic	3.68 ± 1.18	3.57 ± 0.59	1.15 ± 0.17
Octanoic	3.92 ± 1.48	3.57 ± 0.93	1.87 ± 0.18
Decanoic	—	—	1.89 ± 0.32

[a] Each value is a mean of three experiments involving three different rabbits.

The permeability coefficients of acids at various pH values calculated by Eq. 2 are summarized in Table IV. The ratio of $P_{app}/P_{app}^{methanol}$ is summarized in Table V. (The data for decanoic acid at pH 3 and 6 are not presented, because solute losses to the perfusion system were large for this compound under these conditions.) The relative standard deviations for the ratio were generally less than for the permeability coefficient itself, showing the value of having the reference solute, methanol, run simultaneously with the acid.

Figure 3 is a plot of the mean ratio as a function of the carbon number for the acids. For pH 3 and 6, the ratio reached a plateau between butyric and hexanoic acids. For pH 8, the ratio increased from acetic to decanoic acid.

Proposed Physical Model and Analysis of Acid Data—As previously reported (2), the rabbit vaginal absorption barrier may be considered to consist of two barriers in series, an effective aqueous barrier and a principal membrane barrier, with two parallel pathways: a lipoidal pathway and an aqueous pore pathway. The model is schematically illustrated in Fig. 4. Mathematically, the apparent permeability, $P_{app}(n,\text{pH})$, can be expressed as a function of the permeability coefficient for the aqueous barrier, $P_{aq}(n)$, and of the permeability coefficient for the membrane $P_m(n,\text{pH})$, where n is the number of carbon atoms in the compound of interest:

$$P_{app}(n,\text{pH}) = \frac{1}{\frac{1}{P_{aq}(n)} + \frac{1}{P_m(n,\text{pH})}} \qquad \text{(Eq. 3)}$$

The value of $P_{aq}(n)$ is assumed to be equal to $D_{aq}(n)/h$, where $D_{aq}(n)$ is the diffusion coefficient of the compound and h is the effective diffusion layer thickness. The Stokes–Einstein relationship was used to account for the difference in D_{aq} for the different compounds in the homologous series.

By assuming that the ionic form of the acid cannot be transported through the lipid pathway and that the undissociated acid molecule can go through both the pore and the lipid pathways, $P_m(n,\text{pH})$ can be expressed as a function of the fraction of undissociated acid, X, the permeability coefficient of the lipid pathway, P_l, and the permeability coefficient of the pore pathway, P_p:

$$P_m(n) = XP_l(n) + P_p(n) \qquad \text{(Eq. 4)}$$

$$X = \frac{(H)_s}{K_a(n) + (H)_s} \qquad \text{(Eq. 5)}$$

where $(H)_s$ is the membrane surface ($x = 0$) hydrogen-ion concentration, and $K_a(n)$ is the dissociation constant of the acid. The surface hydrogen-ion concentration in these experiments should be well approximated by the bulk hydrogen concentration, (H) (3).

Following the concept of incremental partition coefficient (4, 5) yields:

$$P_l(n) = P_l^0 \times 10^{\pi n} \qquad \text{(Eq. 6)}$$

where P_l^0 is the permeability coefficient of the lipoidal pathway for the hypothetical acid with zero carbon atoms.

The term $P_p(n)$ is a function of the diameter of the pore and the molecular size and shape of the permeant and its diffusion coefficient. However, it is assumed to be a constant in the present calculations since this approximation does not significantly affect our considerations. Thus, Eq. 3 may be rewritten as:

$$P_{app}(n,\text{pH}) = \frac{1}{\left[\frac{MV(n)}{125}\right]^{1/3}\frac{h}{D(6)} + \frac{1}{\frac{(H)}{K_a + (H)}P_l^0\,10^{\pi n} + P_p}} \qquad \text{(Eq. 7)}$$

where $MV(n)$ is the molar volume of the acid with n carbon atoms, 125 is the molar volume of hexanoic acid[5], and $D(6)$ is the diffusion coefficient of hexanoic acid at 37°[6].

By using the proposed model, the following assessments were made.

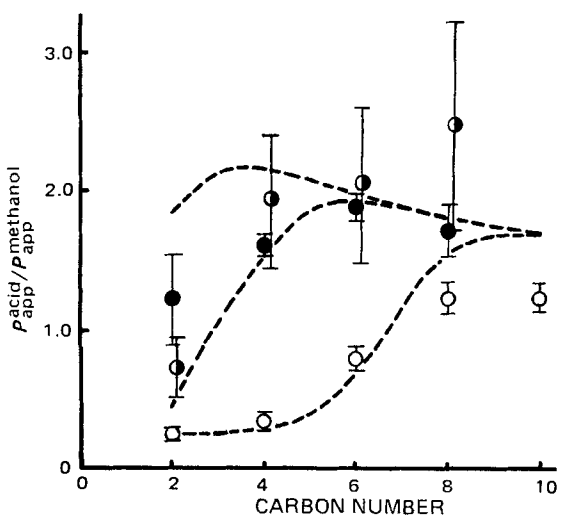

Figure 3—*Effect of carbon number on the normalized permeability coefficient,* $P_{app}^{acid}/P_{app}^{methanol}$, *at pH 3 (●), 6 (◑), and 8 (○).*

Table V—$P_{app}^{acid}/P^{methanol} \pm SD$ at Three Different pH Values[a]

Acid	pH 3	pH 6	pH 8
Acetic	1.22 ± 0.33	0.73 ± 0.23	0.25 ± 0.04
Butyric	1.62 ± 0.08	1.94 ± 0.48	0.34 ± 0.06
Hexanoic	1.89 ± 0.10	2.06 ± 0.56	0.81 ± 0.09
Octanoic	1.74 ± 0.18	2.49 ± 0.75	1.24 ± 0.12
Decanoic	—	—	1.26 0.10

[a] Each value is the mean of three experiments involving different rabbits.

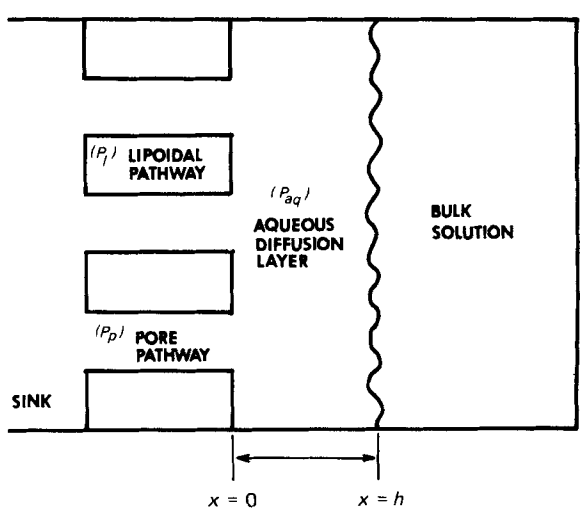

Figure 4—*Schematic model of the vaginal membrane as a transport barrier. Aqueous diffusion layer is in series with the membrane consisting of parallel lipoidal and aqueous pore pathways.*

[5] Hexanoic acid was arbitrarily chosen as a reference to allow for the Stokes–Einstein molar volume dependence for the diffusivity.

[6] It is calculated from the 25° value (6) by the Stokes–Einstein equation.

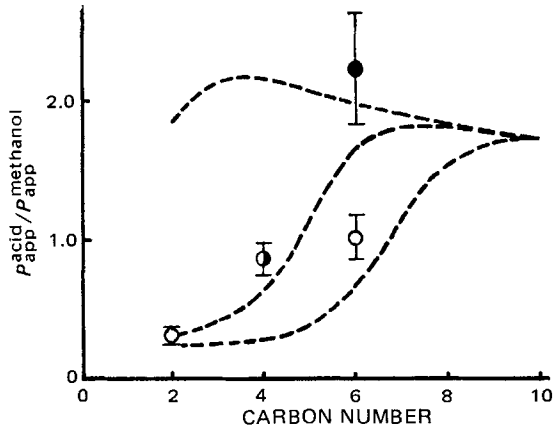

Figure 5—*Further test of the model. Theoretical curves are based on the results in Fig. 3. Key:* ●, *pH 3;* ◑, *pH 7; and* ○, *pH 8.*

At pH 3 and 6, the absorption rates for all acids (except acetic) were mainly controlled by the aqueous diffusion layer. At pH 8, the absorption rates of the lower acids were mainly controlled by the membrane. More specifically, both acetic and butyric acids were controlled by the pore pathway.

The best fit theoretical curves of $P_{app}/P_{app}^{methanol}$ *versus* n were deduced using Eq. 6 and by varying the parameters P_p, P_1^0, π, and h. A $P_{app}^{methanol}$ value of $(1.69 \pm 0.55) \times 10^{-4}$ cm/sec was used. This value, as mentioned earlier, was based on experiments with nine rabbits. The best fit set of curves is shown in Fig. 3, and these curves were based on the best parameter values $P_p = 4.6 \times 10^{-5}$ cm/sec, $P_1^0 = 6.8 \times 10^{-5}$ cm/sec, $\pi = 0.55$, and $h = 0.031$ cm.

The principal features of the proposed model are consistent with the experimental data (Fig. 3). The rather large standard deviations with the animal experiments preclude making a strong statement about the general quantitative validity of the proposed model. However, the π value of 0.55 ± 0.10 and the h value of 0.031 cm are believed to be physically significant.

Further Test of Model—Following the initial studies (Fig. 3), it was decided to pursue another study of limited scope to test the model. According to the theoretical predictions (Fig. 3), butyric acid at pH 7 was expected to be in a sensitive region of the $(P_{app}/P_{app}^{methanol})$ *versus* carbon number curve. Thus, an experiment involving butyric acid at pH 7 was expected to be a good further test of the model. Accordingly, a set of experiments involving three rabbits was designed and performed. Results of the new experiments were in good agreement with the predictions of the model based on the previous experiments (Fig. 5); these results were uniformly slightly higher but essentially within the experimental variations. Table VI shows that if only the results of the new experiments are used in the calculations with the model, a π value of 0.55 ± 0.06 is obtained, in excellent agreement with π values found with the first set of experiments (Fig. 3).

Table VI—Apparent Permeability Coefficient for the Acids and the Values of π for Three Rabbits

Acid	$P_{app} \times 10^4$ cm/sec		
Hexanoic acid, pH 3	4.69	6.24	6.71
Acetic acid, pH 8	0.46	0.50	0.54
Hexanoic acid, pH 8	1.13	1.64	1.97
Butyric acid, pH 7	1.05	1.32	1.32
Calculated π	0.50	0.54	0.62

Table VII—Physical Model Constants for the Aliphatic Acids and the Alcohols

Constant	Acid	Alcohol
P_p	4.6×10^{-5} cm/sec	1.9×10^{-4} cm/sec
P_1^0	6.8×10^{-5} cm/sec	1.5×10^{-6} cm/sec
h	0.031 cm	0.035 cm
π	0.55 ± 0.10	0.40 ± 0.15

Comparison of Results of Aliphatic Alcohols and Carboxylic Acids—These results with the acids compared favorably with those obtained with the alcohols. A summary of all physical constants for both series is presented in Table VII. The diffusion layer thickness, h, and the methylene group incremental constant, π, were in good agreement between these two homologous series. However, the permeability coefficient of the aqueous pore, P_p, for acids was significantly smaller than that for alcohols. This finding suggests two possibilities:

1. The "aqueous" pore phase may not be truly aqueous but somewhat semipolar in character and itself have a modest π value associated with it, or the aqueous pore may be negatively charged so that anions have more difficulty in being transported *via* this pathway.
2. As discussed previously (2), there may be an effective third parallel pathway in the membrane which may be characterized as being "lipoidal" but have a π value significantly less than that for the primary lipoidal pathway. This pathway may allow polar neutral species (*e.g.*, methanol) to go through with relative ease compared to ions like acetate or butyrate.

The methylene group incremental constant, π, had values close to that for the partition coefficient obtained in the 1-octanol–water system, $\pi = 0.5$, as reported by Hansch and Anderson (7). However, the difference in P_1^0 values between acid and alcohol was much larger than that expected by extrapolation of the octanol–water data for the alcohols and the fatty acids (8). Therefore, as might be expected, the similarity between the lipoidal pathway in rabbit vagina and octanol is not total.

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