Systems Approach to Vaginal Delivery of Drugs I: Development of *In Situ* Vaginal Drug Absorption Procedure

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Abstract □ In the framework of the development of drug delivery systems for locally administered contraceptive drugs, a reliable method that can afford quantitative evaluation of drug absorption behavior was explored using the rabbit doe. A system was constructed based upon perfusing the drug solution in the vaginal tract. For this purpose, a "rib-cage"-type cell was constructed and surgically implanted in the rabbit prior to an experiment. The primary purpose of the present paper is to evaluate the method, including the surgical operation and the perfusion system. The absorption experiments were carried out using *n*-butanol-1-^{14}C as the model solute to survey the reproducibility of the absorption behavior. Experiments were conducted with a number of rabbits on several successive days to determine the day-to-day and animal-to-animal variations. The drug disappearance in the reservoir followed first-order kinetics from which the apparent permeability coefficient was calculated. The results indicated that a set of experiments may be carried out on a single animal and that the method generally affords rather high precision.

Keyphrases □ Vaginal drug delivery system—development of *in situ* vaginal drug absorption procedure, equipment and equations, rabbits □ Drug delivery systems—contraceptive drugs, development of *in situ* vaginal drug absorption procedure, equipment and equations, rabbits □ Contraceptive drugs—procedure to evaluate drug absorption from vagina, equipment and equations, rabbits □ Absorption, drug—*in situ* vaginal procedure, equipment and equations, rabbits

The development of reliable contraceptive systems intended for local vaginal administration will require in large measure the understanding of the complex processes of drug absorption and interactions between the drug delivery device (formulation) and the vaginal tract. For the drug delivery process, the following may be considered as critical: (*a*) the release of drug, (*b*) the permeability of the vaginal membrane, (*c*) drug transport to the site of action, and (*d*) drug reaction with the "bioreceptor."

From current studies of drug transport across the buccal membrane (1, 2), the intestinal mucosa (3, 4), and skin (5), which appears to be morphologically similar to the vaginal membrane, it may be inferred that either the release or the absorption steps can be, and likely are, the determinants of the relative biological activities within a given class of compounds. For certain drug delivery systems (*e.g.*, the silicone matrixes), the mechanisms for drug release are now reasonably well understood (6, 7). In contrast, little, except for some grossly qualitative information (8–10), is available on the rates and mechanisms of mass transport in the vagina. Without a more rigorous definition of this step in the total drug release and absorption picture, the rational drug delivery in the vaginal tract is not possible. In other words, an understanding of the mechanism of drug transport and interactions subsequent to the release from the vaginal device should make possible the *a priori* development of maximally effective contraceptive drug delivery systems.

The purposes of the present report are to describe and assess a method for the determination of vaginal permeabilities, using the rabbit doe as the prototype animal and *n*-butanol as a test compound.

EXPERIMENTAL

Animals—Mature female New Zealand White rabbits (7–9 months old, 4.5–5.0 kg), which were parous, were used. With this weight range, the vaginal tract was about 2 cm in diameter and 8 cm in length, with relatively uniform dimensions. The rabbits were maintained in a temperature-controlled room (25°) with free access to water and normal pelleted food diet.

Perfusion System—The perfusion system consists of two sections (Fig. 1). The first includes the "rib-cage" cell, surgically implanted in the vaginal tract, and the fenestra rotunda (Figs. 2 and 3). The second section is maintained on the laboratory bench and involves a rotating and reciprocating pump[1] and a constant-temperature solution reservoir. The entire perfusion path in the system is of glass tubing and stainless steel material, except for the portions between the cage and the fenestra rotunda, which was polyethylene tubing[2]. The washing device and drain bottles are connected through two three-way stopcocks to the reservoir. During the experiments the two sections are securely attached to provide a closed system for perfusion.

Rib-Cage Cell—The rib-cage cell was designed to fit the rabbit's vaginal dimensions in a way that provides a constant volume and surface area in the absorption compartment (Fig. 3). The cell was constructed with two smoothened Teflon endings connected by eight stainless steel rods. The geometrical volume and surface area enclosed by this cell and surrounding vaginal wall are 8.1 cm^3 and 22.5 cm^2, respectively. The stainless steel inlet and outlet for the perfused solution were installed through one Teflon ending, from which polyethylene tubings were led to the fenestra rotunda. Because of anatomical obstruction, the rib-cage cell could not be inserted intravaginally. Thus, the cell had to be implanted surgically into the vaginal tract.

Fenestra Rotunda—The fenestra rotunda, made of a medical grade silicone rubber sheet[3], was intended to provide a connection shut-off route between the two sections of the perfusion system (Fig. 3). It also assists in positioning the rib-cage cell in the vaginal tract. The solution is perfused through the rib-cage cell through the stainless steel inlet and outlet of the fenestra rotunda, which is permanently fixed on the abdomen of the animal.

Procedure for Implantation of Rib-Cage Cell—The procedure for the implantation of the cell involved a surgical operation near the cervical end of the vaginal tract (Fig. 2). For the operation the rabbit was anesthetized with pentobarbital, and the abdomen was clipped and sterilized with iodine tincture. A central line incision of 6–7 cm in length was made in the lower portion of the abdo-

[1] FMI lab pump, model RRP 2G 150, Fluid Metering Inc., Oyster Bay, N.Y.
[2] INTRAMEDIC PE 205, Clay Adams, Division of Becton, Dickinson & Co., Parsippany, NJ 07054
[3] Silastic, Dow Corning Corp., Midland, Mich.

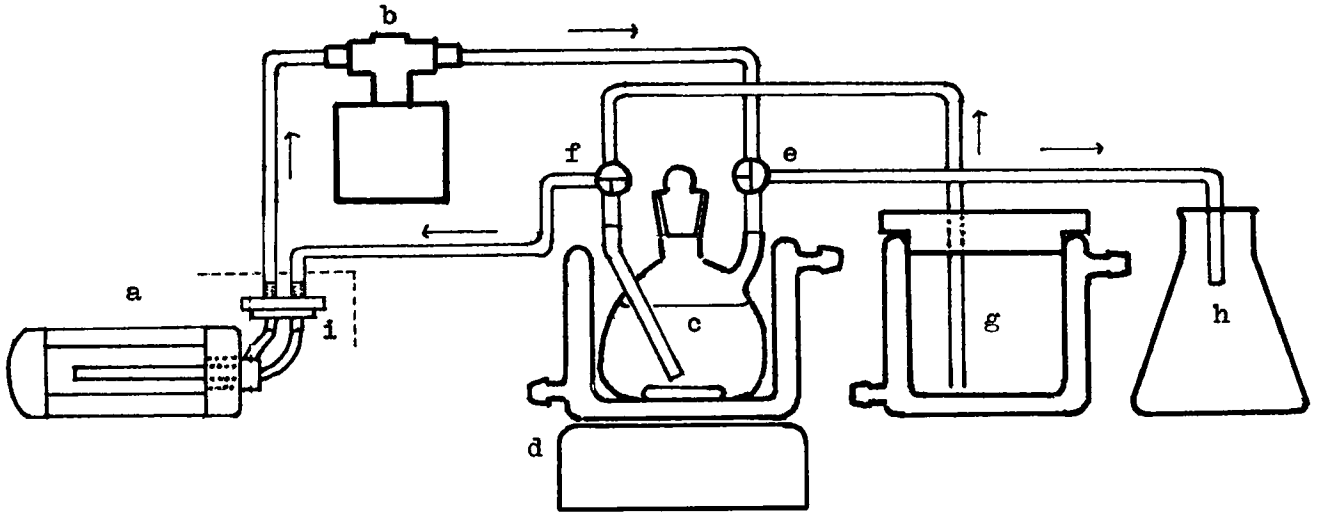


Figure 1—*Diagram of perfusion system. Key: a, rib-cage cell; b, perfusion pump; c, reservoir of solution; d, magnetic stirrer; e, three-way stopcock to returning and drain; f, three-way stopcock to circulating and washing; g, reservoir of washing solution; h, drain beaker; and i, fenestra rotunda.*

men, exposing the vaginal tract. Then the wall of the upper part of the vagina (about 0.5–~1 cm from cervixes) was opened to an appropriate length to insert the rib-cage cell, which was soaked and sterilized in advance in a surgical anti-infection solution. The incised surface of the vaginal wall was then closed by stitching with surgical gut suture (0-0). The polyethylene tubes passing through the vaginal wall were properly adjusted in length and led to the fenestra which, in turn, was positioned and stitched onto the abdominal surface. A silk string was led from the rib-cage cell along the polyethylene tubes and tied to the fenestra rotunda to prevent the cell from slipping when the rabbit assumes the normal position after the operation. The string also serves to minimize the tension on the polyethylene tubings. After the operation, an oxytetracycline hydrochloride solution was injected (10 mg/kg im) to prevent systemic infections. The whole perfusion system was then irrigated by a normal saline solution to remove blood left from the operation. This also served to confirm that the perfusing solution was able to circulate freely throughout the system.

Theoretical Analysis of Circulation Dynamics for Perfusion System—The perfusion system basically consists of a rib-cage cell, a reservoir of solution, and a pump (Scheme I). Compartment I in Scheme I is the reservoir of external solution where drug samples are taken, and Compartment II is the solution of the rib-cage cell in the vagina.

The purposes of the following mathematical description and analysis of the perfusion system are to determine: (*a*) the lag time after which the circulation dynamics of the system are at steady-state, (*b*) the steady-state relationship of the drug solution in the

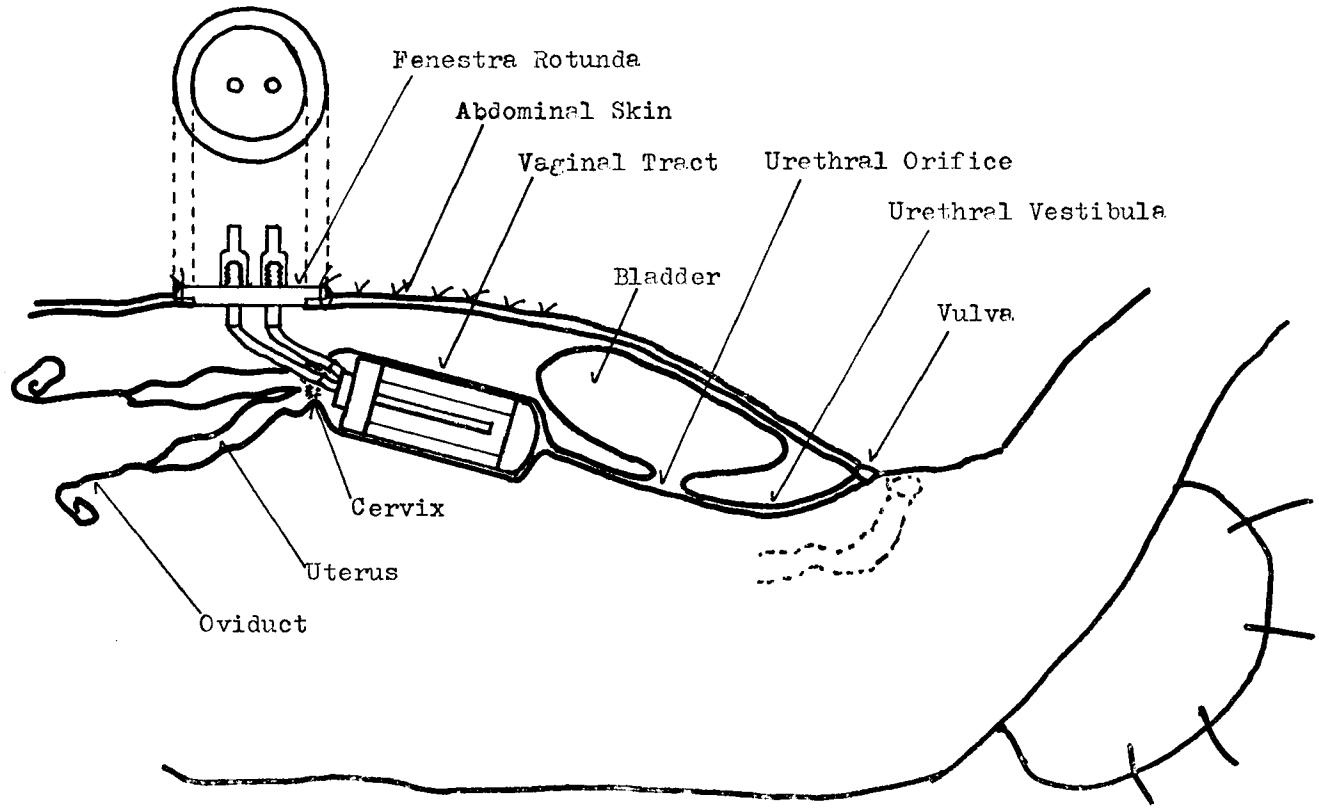


Figure 2—*Position of implanted rib-cage cell in vaginal tract.*

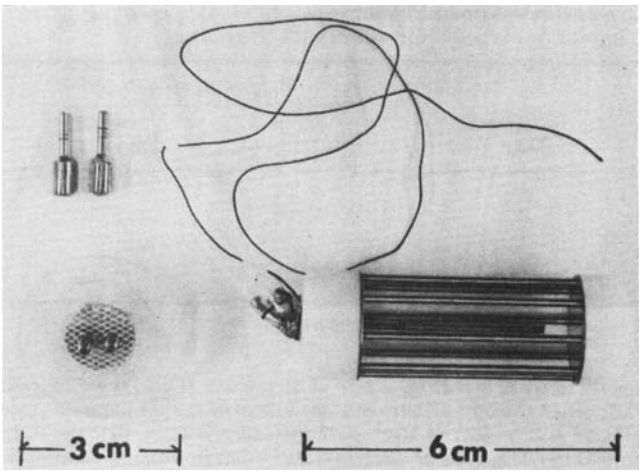


Figure 3—*Surgically implanted parts. Left: fenestra rotunda with the stainless steel inlet and outlet. Right: rib-cage cell with polyethylene tubings and silk string attachments.*

external sampling compartment to that in the vaginal compartment at any time, and, consequently, (c) the steady-state vaginal absorption rate constant. Equations 1 and 2 give the rates of change in the concentrations of Compartments I and II, respectively:

$$\frac{dC_I}{dt} = -\frac{R_f}{V_I}(C_I - C_{II}) \qquad \text{(Eq. 1)}$$

$$\frac{dC_{II}}{dt} = \frac{R_f}{V_{II}}(C_I - C_{II}) - \frac{P_f}{V_{II}}C_{II} \qquad \text{(Eq. 2)}$$

where C_I and C_{II} are the concentrations of Compartments I and II, respectively; R_f (cubic centimeters per second) is the circulating rate of the solution; V_I and V_{II} are the volumes of Compartments I and II, respectively; and P_f (cubic centimeters per second) is the absorption rate coefficient which corresponds eventually to the apparent permeability coefficient of the vaginal mucosal membrane. The total concentration change of drug in the perfusing solution can be given by:

$$\frac{dC_t}{dt} = \frac{V_I}{V_t}\frac{dC_I}{dt} + \frac{V_{II}}{V_t}\frac{dC_{II}}{dt} \qquad \text{(Eq. 3)}$$

where C_t is the total concentration in the perfusing solution, and V_t is the total volume of the perfusing solution ($V_I + V_{II}$).

For a two-compartment linear system, the equations of the system are two simultaneous first-order linear differential equations in two variables. The solution for these equations is given by:

$$C_I = \frac{K_1}{V_I}e^{-m_1 t} + \frac{K_2}{V_I}e^{-m_2 t} \qquad \text{(Eq. 4)}$$

$$C_{II} = K_1\left(\frac{1}{V_I} - \frac{m_1}{R_f}\right)e^{-m_1 t} + K_2\left(\frac{1}{V_I} - \frac{m_2}{R_f}\right)e^{-m_2 t} \qquad \text{(Eq. 5)}$$

where:

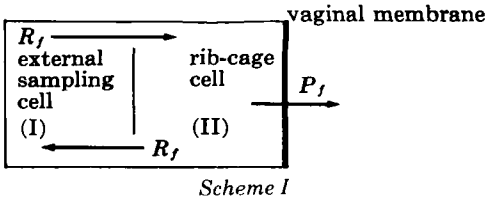
$$m_1 = \frac{R_f V_t + P_f V_I + \sqrt{(R_f V_t + P_f V_I)^2 - 4R_f P_f V_{II} V_I}}{2V_I V_{II}} \qquad \text{(Eq. 6)}$$

$$m_2 = \frac{R_f V_t + P_f V_I - \sqrt{(R_f V_t + P_f V_I)^2 - 4R_f P_f V_{II} V_I}}{2V_I V_{II}} \qquad \text{(Eq. 7)}$$

and K_1 and K_2 are determined by the initial conditions. From the initial conditions, $C_I = C_0$ and $C_{II} = 0$ at $t = 0$. Therefore:

$$K_1 = \frac{(V_I m_2 - R_f)C_0}{m_2 - m_1} \qquad \text{(Eq. 8)}$$

$$K_2 = \frac{(R_f - V_I m_1)C_0}{m_2 - m_1} \qquad \text{(Eq. 9)}$$



Scheme I

where C_0 is the initial concentration.

After substituting Eqs. 8 and 9 into Eqs. 4 and 5 and then taking their ratios:

$$\frac{p_2 C_{II} - q_2 C_I}{q_1 C_I - p_1 C_{II}} = \frac{e^{-m_1 t}}{e^{-m_2 t}} \qquad \text{(Eq. 10)}$$

where:

$$p_1 = R_f(V_I m_2 - R_f) \qquad \text{(Eq. 10a)}$$

$$p_2 = R_f(R_f - V_I m_1) \qquad \text{(Eq. 10b)}$$

$$q_1 = (V_I m_2 - R_f)(R_f - V_I m_1) \qquad \text{(Eq. 10c)}$$

$$q_2 = (R_f - V_I m_2)(R_f - V_I m_1) \qquad \text{(Eq. 10d)}$$

Accordingly, the relationship between C_I and C_{II} can be expressed as:

$$C_{II} = \frac{q_2 e^{-m_2 t} + q_1 e^{-m_1 t}}{p_2 e^{-m_2 t} + p_1 e^{-m_1 t}} C_I \qquad \text{(Eq. 11)}$$

Substituting Eq. 11 into Eq. 3:

$$\frac{dC_I}{dt} = -\frac{P_f(q_2 e^{-m_2 t} + q_1 e^{-m_1 t})}{V_I(p_2 e^{-m_2 t} + p_1 e^{-m_1 t}) + V_{II}(q_2 e^{-m_2 t} + q_1 e^{-m_1 t})} C_I \qquad \text{(Eq. 12)}$$

After a lag time, the circulation dynamics of the perfusion system will be at steady state so the ratio C_{II}/C_I in Eq. 11 converges to q_2/p_2. Also, at steady state Eq. 12 reduces to:

$$\frac{dC_I}{dt} = -kC_I \qquad \text{(Eq. 13)}$$

$$k = \frac{P_f q_2}{V_I p_2 + V_{II} q_2} \qquad \text{(Eq. 14)}$$

where k is the first-order rate constant.

Under the condition where the perfusion rate, R_f, is much greater than the membrane absorption rate, P_f, it may be assumed that the rate change of Compartment I equals that of Compartment II; that is:

$$-\frac{R_f}{V_I}(C_I - C_{II}) = \frac{R_f}{V_{II}}(C_I - C_{II}) - \frac{P_f}{V_I}C_{II} \qquad \text{(Eq. 15)}$$

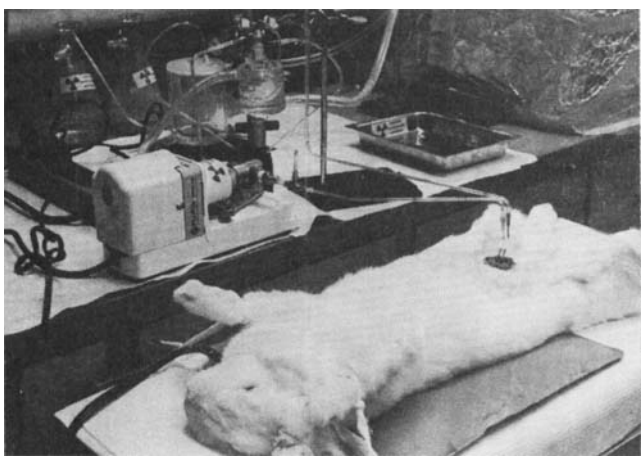


Figure 4—*Layout of perfusion system during an absorption experiment.*

Table I—Influence of Perfusion Rate to Concentration Difference between Compartments I and II and to the First-Order Rate Constant[a]

R_f, ml/min	P_f/R_f	Lag Time, sec	Upper Limit of C_I/C_{II}	C_I/C_{II}	k, sec^{-1}
35	0.38	75	1.38	1.31	5.34×10^{-3}
	0.038	105	1.038	1.03	6.47×10^{-4}
25	0.54	105	1.54	1.45	4.94×10^{-3}
	0.054	165	1.056	1.04	6.41×10^{-4}
15	0.90	150	1.90	1.77	4.17×10^{-3}
	0.090	180	1.090	1.07	6.28×10^{-4}

[a] For these calculations, these dimensional constants were used: $k_{max} = (P_f/V_I) = (AD/V_I h) = 6.62 \times 10^{-3}$ sec^{-1}, A = surface area (22.5 cm^2), D = diffusion coefficient in aqueous phase (1×10^{-5} cm^2/sec), h = thickness of aqueous diffusion layer (10^{-3} cm), V_I = volume of Compartment I (26 cm^3), and V_{II} = volume of Compartment II (8 cm^3).

or:

$$C_{II} = \frac{1}{1 + \frac{V_I P_f}{V_I R_f}} C_I \qquad \text{(Eq. 16)}$$

When substituting Eq. 13 into Eq. 1:

$$\frac{dC_I}{dt} = - \frac{1}{\frac{V_I}{P_f} + \frac{V_{II}}{R_f}} C_I \qquad \text{(Eq. 17)}$$

According to the assumption, the second term of the denominator of the first-order rate constant would be negligible so that the rate of change in drug concentration in the external sampling compartment would be principally due to absorption by the vaginal membrane.

From Eq. 2, the rate change of the concentration of Compartment II should be negative for all time and therefore $1 \leq (C_I/C_{II}) < 1 + (P_f/R_f)$. The upper limit of the concentration ratio between Compartments I and II could be estimated from the introduction of reasonable values for the exposed area, the diffusion coefficient in the aqueous phase, and the thickness of the aqueous diffusion layer (unstirred aqueous layer) into P_f according to the physical model study of membrane transport (11).

Table I shows the influence of the perfusion rate to the first-order rate constant and to the concentration difference between the external sampling compartment and the rib-cage cell compartment. The ratio between C_I and C_{II} becomes constant after a certain lag time and its magnitude is dependent upon the perfusion rate constant, R_f, relative to the absorption rate constant as described by P_f.

Under the maximum absorption rate situation where the maximum rate constant $k_{max} = 6.62 \times 10^{-3}$ sec^{-1}, the ratio $C_I/C_{II} = 1.31$ when $R_f = 35$ ml/min and $C_I/C_{II} = 1.45$ when $R_f = 25$ ml/min. This maximum absorption rate would correspond to the situation in which the rate is governed by the unstirred aqueous layer in front of the vaginal membrane and not by the vaginal membrane itself. A conservative value of 10 μm thickness for the unstirred layer is employed in the calculations. On the other hand, when the absorption rate constant is indicative of a more membrane-controlled case, *i.e.*, $k = 6.62 \times 10^{-4}$ sec^{-1}, the ratio C_I/C_{II} is more nearly unity at a perfusion rate of 35 ml/min. Thus, the *apparent* first-order absorption rate constant (uncorrected for perfusion rate effects) will be 6.47×10^{-4} sec^{-1} instead of 6.62×10^{-4} sec^{-1}. In conclusion, this type of analysis aids in determining the appropriate perfusion rates for the different situations in the experiment to follow.

Table II—Apparent Permeability Coefficients[a], P_{app}, of Vaginal Absorption for *n*-Butanol (Rabbit 1)

Day	P_{app} (cm/sec) $\times 10^4$ First Experiment	Second Experiment
1	1.91	1.65
2	1.95	2.21
3	2.09	1.98
4	2.18	2.69
5	2.18	2.50

[a] Arithmetic mean = 2.13 ± 0.28 *SD*.

Table III—Apparent Permeability Coefficients[a], P_{app}, of Vaginal Absorption for *n*-Butanol (Rabbit 2)

Day	P_{app} (cm/sec) $\times 10^4$ First Experiment	Second Experiment
1	2.07	1.67
2	2.07	2.28
3	—	—
4	—	—
5	2.04	1.55

[a] Arithmetic mean = 1.94 ± 0.26 *SD*.

Procedure for Absorption Studies—The absorption experiments were carried out on 5 successive days starting 3 days after surgery. On each day, two successive experiments were conducted with each rabbit. This procedure allows more experiments to be conducted within a short period without exhausting the animal.

The rabbit was anesthetized with sodium pentobarbital, 25–50 mg/kg, and connected to the perfusion system through the stainless steel inlet and outlet of the fenestra rotunda (Fig. 4). A heating pad was kept under the rabbit to maintain body temperature during the experiments. The vagina was then washed by perfusion with a normal saline solution for 10 min at a flow rate of 35 ml/min. Then the saline was replaced by the buffer solution to be used in the experiment and the washing was continued for an additional 5 min. The buffer was 0.115 *M* phosphate buffer (pH 6.0) made isotonic with sodium chloride.

With the system filled with buffer solution, the stopcocks were switched to introduce the reservoir and its contents (20 ml of buffer) into the system and the perfusion was started. All perfusion experiments were carried out under the flow rate of 35 ml/min at 37°. Two minutes later, an aliquot of a saline solution of *n*-butanol-1-^{14}C was rapidly injected into the reservoir and the first sample (zero time) was then taken after 2 min more. The losses in the butanol concentration were followed by taking 0.05-ml samples from the reservoir every 10 min for about 1 hr. After the first experiment on each day, the system was emptied of the drug solution and washed with saline for 15 min and then with the buffer solution for an additional 5 min. This washing procedure was sufficient to remove all drug from the system. A sample taken from the reservoir before starting the second absorption experiment showed that no drug was present. The butanol activity in the samples was determined by means of a liquid scintillation counter[4] using dioxane solution[5].

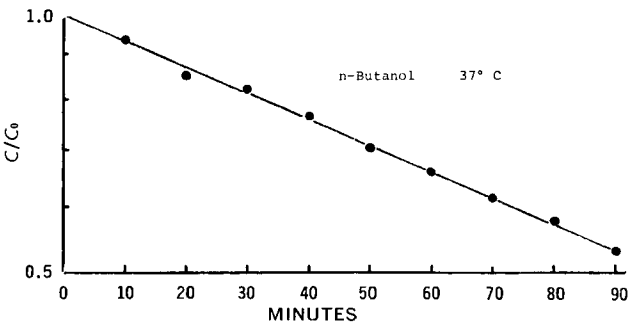


Figure 5—*Typical plot of the concentration change in the sampling reservoir as a function of time showing the first-order kinetics.*

[4] Beckman LS 200.

[5] The solution consisted of: 2,5-diphenyloxazole, 8 g; naphthalene, 100 g; and dioxane, *q.s.*, 1000 ml.

Table IV—Apparent Permeability Coefficients[a], P_{app}, of Vaginal Absorption for *n*-Butanol (Rabbit 3)

Day	P_{app} (cm/sec) × 10^4 First Experiment	P_{app} (cm/sec) × 10^4 Second Experiment
1	2.11	2.16
2	1.71	2.10
3	2.17	1.80
4	2.38	2.30
5	2.37	2.25

[a] Arithmetic mean = 2.13 ± 0.21 *SD*.

RESULTS AND DISCUSSION

General Observations—Some general observations were made on the state of the animals after surgery and during the experimental period. The rabbits behaved normally in their actions and discharged urine and stool as usual. Losses in body weight of up to 10% were generally observed for the first 2–5 days after surgery. However, after this period, most animals showed an improvement in appetite and some began to gain weight. The abdominal incision wound showed considerable healing during this period. After completion of the perfusion experiments, the animals were sacrificed and the site of the surgery was examined. Conglutination was found in the area, indicating normal recovery after surgery and the regaining of normal blood circulation in the area. The vagina itself appeared normal except for what appeared to be a slight thickening of the wall.

In the initial design, the supporting stainless steel ribs of the rib-cage cell were covered with medical grade polyethylene tubing[2]. However, in these instances, inflammation (congestion and bleeding) of the mucosal surface of the vaginal tract was observed. In all subsequent perfusion experiments, the polyethylene sleeve was not used and no abnormalities were observed.

One important assumption in the design of the rib-cage cell was that the cell would form a leaktight chamber with the vaginal walls. This appeared to always hold true, since the volume of the perfusion solution remained constant with time. The success achieved may be attributed to the combination of having the cell dimensions matched to those of the vagina and of having negative pressure in the absorption chamber during the perfusion runs.

Although isotonic solutions were used in the absorption experiments, the possible net transfer of water with time was also determined with ^{14}C-polyethylene glycol 4000. Using isotonic buffered solution at pH 6.0, no loss of water was observed in 1 hr. Accordingly, the influence of the water flow on drug movement was assumed to be negligible.

In separate experiments involving no vaginal membrane, it was also established that there was no detectable absorption of *n*-butanol to the materials of the perfusion setup.

Absorption Experiments with *n*-Butanol—Figure 5 represents the results of a typical experiment conducted for 90 min. The linear relationship obtained on a semilogarithmic scale indicates that the drug disappearance follows a first-order pattern. Thus, the rate of disappearance could be expressed by:

$$\frac{dC_b}{dt} = -K_u C_b \qquad \text{(Eq. 18)}$$

$$K_u = \frac{A}{V} P_{app} \qquad \text{(Eq. 19)}$$

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 vaginal tract, V is the volume of the perfusion solution, and P_{app} is the ap-

Table V—Buffer Systems[a] Employed in Assessing the Effect of pH and Buffer Species on Membrane Permeability

pH 3.0

Citrate Phosphate Buffer:		Biphthalate Buffer:	
Citric acid monohydrate	16.81 g	Potassium biphthalate	10.21 g
Na_2HPO_4	7.12 g	1.0 *N* HCl	21.8 ml
10 *N* NaOH to adjust			

pH 6.0

Citrate Buffer:		Phosphate Buffer:	
Citric acid monohydrate	19.75 g	NaH_2PO_4	11.4 g
10 *N* NaOH to adjust		10 *N* NaOH to adjust	

pH 8.0

Borate Buffer:		Phosphate Buffer:	
Boric acid	12.37 g	Na_2HPO_4	7.1 g
10 *N* NaOH to adjust		1.0 *N* HCl	0.4 ml
Tromethamine Buffer:			
Tromethamine	0.608 g		
1.0 *N* HCl	27.9 ml		

pH 9.8

Borate Buffer:	
Boric acid	12.37 g
10 *N* NaOH to adjust	

[a] All buffer solutions were made up to 1000 ml and adjusted to isoosmotic conditions (300 mosmoles/kg) with sodium chloride.

Table VI—Effect of pH and Buffer on the Apparent Permeability Coefficient of *n*-Butanol in Vaginal Tract[a]

pH	Apparent Permeability Coefficient P_{app} × 10^4 cm/sec		
3.0	Citrate–Phosphate Buffer	Biphthalate Buffer	
	2.24	1.67	
	2.34	2.04	
	2.76	1.48	
	2.08	1.82	
	Av. 2.35	Av. 1.75	
6.0	Citrate Buffer	Phosphate Buffer	
	2.30	1.73	
	1.85	1.73	
	1.48	1.56	
	Av. 1.87	1.62	
		1.82	
		1.65	
		Av. 1.68	
8.0	Borate Buffer	Phosphate Buffer	Tromethamine Buffer
	0.83	1.64	2.35
	0.89	1.74	2.17
	Av. 0.86	Av. 1.69	1.43
			1.65
			1.81
			Av. 1.88
9.8	Borate		
	0.69		
	0.75		
	0.79		
	0.89		
	Av. 0.78		

[a] Each experiment was carried out for 1 hr.

Table VII—Crossover Experiments Involving Various pH and Buffer Systems[a] (P_{app} × 10^4 cm/sec)

Day	First Experiment pH	First Experiment P_{app}	Second Experiment pH	Second Experiment P_{app}
1	3.0 (C)	2.24	9.8 (B)	0.79
2	9.8 (B)	0.89	6.0 (P)	1.82
3	6.0 (P)	1.65	3.0 (C)	2.34
4	3.0 (C)	2.76	9.8 (B)	0.75
5	9.8 (B)	0.69	6.0 (P)	1.73

[a] C = citrate buffer, P = phosphate buffer, and B = borate buffer.

parent permeability coefficient. The ratio of the surface area to the volume of solution is 0.66.

Absorption experiments were carried out with three rabbits (Tables II–IV). For Rabbits 1 and 3, the experiments were carried out and continued up to the 5th day with two successive runs each day. For Rabbit 2, there were some difficulties with the anesthesia on the 3rd and the 4th days so that experiments on these days were not performed. The data show overall good reproducibility of the method. Both the animal-to-animal and the day-to-day variations were statistically insignificant. Consequently, the method should be useful in carrying out quantitative experiments on a single rabbit as well as with a set of animals.

Effects of pH and Buffer Species on Permeability of Vaginal Membrane—For studies on the vaginal absorption of weak electrolytes, knowledge of the possible adverse effects of pH and buffer species on the integrity and permeability characteristics of the membrane is required. These effects were quantitatively assessed by absorption experiments utilizing *n*-butanol as the reference. The buffer systems employed are listed in Table V.

As can be seen in Table VI, the apparent permeability coefficient of *n*-butanol was the same at pH 3.0 with the phthalate buffer and at pH 6.0 and 8.0 with the phosphate buffers. In comparison, the average P_{app} values for the citrate buffer systems tended to be greater and those for the borate buffers were consistently lower. Although there were wider variations in the experimental results with the tromethamine buffer as compared to the variations with the other buffers at various pH, the average P_{app} was higher than the P_{app} for the phthalate and phosphate buffers. The pH of all buffer solutions changed no more than 0.1 unit in 1 hr.

Although it is not explicit in Table VI, the tabulations of the permeability coefficients are the overall results of crossover experiments involving pH and buffer systems. The crossover experiments gave reproducible and consistent results. As one example, an absorption experiment carried out at pH 6.0 with the phosphate buffer gave a higher P_{app} than that of an immediate followup experiment at pH 9.8 with the borate buffer on the same rabbit (Table VII). The result was the same when the order of the experiments was reversed.

These results show that the membrane is affected by both pH and buffer as adjudged by its permeability to *n*-butanol. There appears to be little effect on the membrane when phthalate and phosphate buffers are used. The citrate and borate buffers and, perhaps, the tromethamine buffer do affect membrane permeability. However, these effects upon the membrane appear to be reversible and not related to the integrity of the membrane.

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Timed Release from Polymeric Films Containing Drugs and Kinetics of Drug Release

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Abstract □ The preparation of cast films of ethylcellulose containing caffeine and salicylic acid is described. These films exhibit timed release of drugs. Release rates were found to agree with both the classical first-order equation (log drug retained against time) and diffusion-controlled release models, as exemplified by Higuchi's equations (drug release linearly related to square root of time). Mathematical analysis of the data shows that the release behavior actually conforms with the diffusion-controlled model. Literature results, reported as first order, for the release of cetylpyridinium chloride and benzalkonium chloride from polyamide films were analyzed similarly and shown to be diffusion controlled. Recommendations are made for presentation and routine treatment of drug release data to avoid ambiguity and provide useful biopharmaceutical information.

Keyphrases □ Ethylcellulose films containing caffeine or salicylic acid—timed release, first-order and diffusion-controlled models discussed □ Timed release of caffeine or salicylic acid from ethylcellulose films—first-order and diffusion-controlled models discussed □ Drug release from polymeric films—first-order and diffusion-controlled mechanisms discussed □ Polymer films containing drugs—kinetics of drug release, first-order and diffusion-controlled models discussed

The methods previously used in achieving timed-release formulations of drugs may be related to four types of processes: *(a)* coating the drug or preparation, *(b)* embedding the drug in fatty, plastic, or hydrophilic matrixes, *(c)* binding the drug to an ion-exchange resin, or *(d)* forming a complex or other chemical derivative of the drug (1). The possibility exists that incorporation of the drug into a polymer