extracted and then reacted with sodium hydroxide to give sufficient fluorescence for detection in serum or tissue samples.

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Vaginal Drug Absorption in Rhesus Monkeys I: Development of Methodology

E. OWADA, C. R. BEHL, S. S. HWANG, L. SUHARDJA, G. L. FLYNN, N. F. H. HO, and W. I. HIGUCHI ˣ

Abstract □ Earlier reports from these laboratories described a procedure for determining vaginal drug absorption in the rabbit based upon a perfusion system, and data on the vaginal absorption of the straight-chain aliphatic alcohols and carboxylic acids were given. These studies have been extended to the rhesus monkey. Rib-cage-type cells were designed for intravaginal insertion through the vulval orifice and to fit the specific dimensions of the monkey vagina. The general design of the cell was similar to that used in the rabbit vaginal absorption studies. The perfusion system was checked by using ^{3}H-polyethylene glycol 4000, and no significant leaks from the cell were found. The absorption of the alcohols followed first-order kinetics. The computed apparent permeability coefficients for the alcohols were of comparable magnitude to those previously reported for the rabbit vaginal membrane.

Keyphrases □ Absorption, vaginal—various straight-chain aliphatic alcohols, perfusion system designed, rhesus monkeys □ Vaginal absorption—various straight-chain aliphatic alcohols, perfusion system designed, rhesus monkeys □ Perfusion systems—vaginal drug absorption, various straight-chain aliphatic alcohols, rhesus monkeys □ Alcohols, straight-chain aliphatic, various—vaginal absorption, perfusion system, rhesus monkeys

Research efforts in these laboratories have been based upon the premise that the pivotal event in regional or systemic therapy *via* the vaginal absorption route may be drug transport across the vaginal membrane. A method was described (1) for evaluating drug absorption in the vagina, using the rabbit doe as a prototype animal. A rib-cage-type cell, which provided a closed absorption compartment in the vaginal tract, was designed and surgically implanted. Drug absorption was determined by perfusing the drug solution through this system.

In subsequent reports, the absorption of straight-chain aliphatic alcohols (2) and aliphatic carboxylic acids (3) was described. The data agreed with a physical model involving a diffusion layer in series with a membrane consisting of two parallel pathways: a lipoidal pathway and an aqueous "pore" pathway. The surface pH of the rabbit vaginal membrane under the conditions prevailing during the experiments could be well approximated by the pH of the bulk lumenal solution (4).

The rabbit doe was chosen for these studies because it does not exhibit an estrous cycle and the vaginal tissues exhibit a constancy in their histological, biochemical, and physiological properties not seen with other rodents and most other mammals (5). The lack of a sexual cycle in the rabbit was expected to provide minimum variability in the membrane permeability behavior (1–4).

In the human female, however, the secretion of estrogenic hormones in the ovarian cycle induces cyclic changes in the histology, biochemistry, and physiology of the vaginal tissues; therefore, the vaginal membrane might exhibit a corresponding cyclic barrier behavior. Thus, the rationale for the present investigation was to extend the studies to a sexually cyclical animal, closely approximating the human female with regard to ovarian-induced membrane changes. The Macaques rhesus species of the higher primates was selected. The rhesus has an ovarian cycle of approximately 28 days, as does the human female. It is widely believed by researchers in the fertility area that the rhesus and human reproductive systems are functionally similar in that they have comparable anatomy and phys-

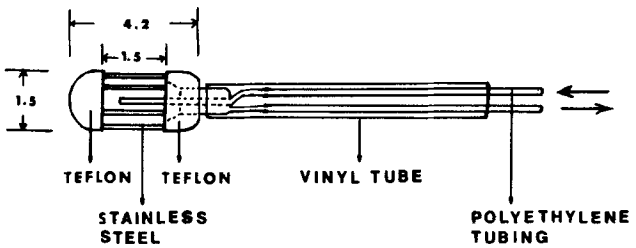

Figure 1—*Drawing of a typical rib-cage cell, which is inserted into the vagina of the monkey through the vulval orifice (all dimensions are given in centimeters for the medium size cell).*

iology (6). Therefore, the female rhesus is believed to provide an excellent model for the human female.

The purpose of the present report is to describe a method for the determination of the vaginal membrane permeability using the female rhesus monkey and straight-chain aliphatic alcohols as model compounds.

EXPERIMENTAL

Animals—Mature female Macaques rhesus monkeys (4–9 years old and 4–6 kg) known to have menstrual cycles were employed. Their vaginal tracts ranged from 1.3 to 1.7 cm in diameter and were approximately 4 cm in length. The animals were maintained under regular animal health care in an air conditioned building (25°) with free access to water and a normal diet.

The condition of the monkeys was monitored by visual examination throughout the studies. The overall procedure did not appear to affect the appetite or temperament of the animals, nor was there any significant change in body weight. Vaginal examinations revealed no vaginal infections due to the inserted rib-cage cell in any monkey.

Materials—All materials were used without further purification. A liquid scintillator was prepared by dissolving 18 g of 2,5-diphenyloxazole[1] and 300 g of naphthalene[2] in 3 liters of dioxane[2]. The buffer solution of

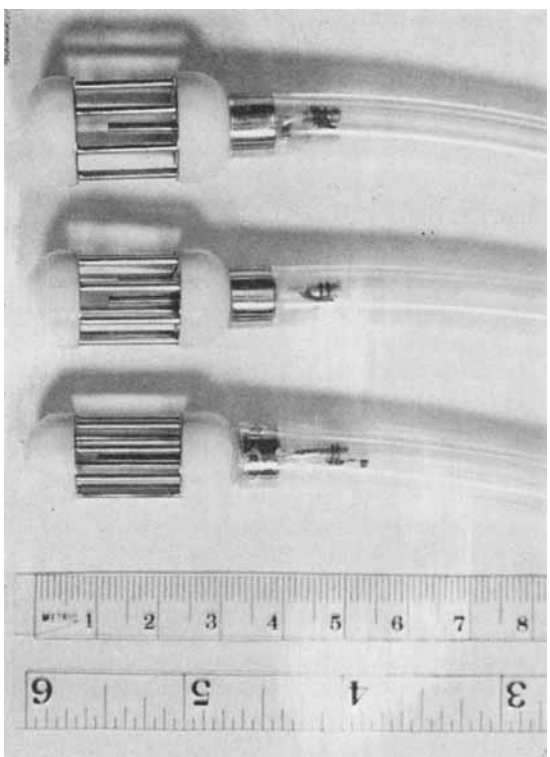

Figure 2—*Photograph of three rib-cage cells.*

[1] Isolab Inc., Akron, Ohio.
[2] Matheson, Coleman and Bell, Norwood, Ohio.

Table I—Dimensions of the Three Rib-Cage Cells Designed for Intravaginal Insertion

Cell	Length of Stainless Steel Rods, cm	Diameter of Ends, cm	Volume within Cage, ml	Surface Area, cm^2
Small	1.53	1.30	1.83	6.10
Medium	1.53	1.50	2.43	7.03
Large	1.53	1.70	3.13	7.98

pH 6.0 was prepared by dissolving 13.8 g of monobasic sodium phosphate[2] in 16.0 ml of 1.0 *N* sodium hydroxide[3] and adding distilled water to give a final volume of 1 liter. The buffer solution was then made isotonic by adding sodium chloride[3].

Design of Rib-Cage Cell—The rib-cage cell was designed to fit the vaginal dimensions of the monkey in such a way as to provide a constant volume and surface area in the absorption compartment (Fig. 1). The general design was similar to that used in the rabbit vaginal absorption studies (1). The cell was constructed with two smooth endings[4] connected by eight stainless steel rods. The stainless steel inlet and outlet for the perfusate were installed through one ending from which polyethylene tubes were led through vinyl tubing, for protection and convenient handling, to the rest of the perfusion system.

The dimensions of the cells were adjusted to fit each specific monkey, and the cell was constructed so it could be inserted through the vulval orifice without difficulty. The dimensions of three such cells (Fig. 2) are presented in Table I.

Perfusion System and General Procedure—The perfusion system consisted of two sections (Fig. 3). The first included the rib-cage cell, which was intravaginally inserted. The second section, external to the animal, consisted of a rotating and reciprocating pump[5] and a constant-temperature solution reservoir. The entire perfusion path in the system was glass tubing and stainless steel, except for the connections, which were polyethylene tubing[6]. A washing device and drain flasks were connected through two three-way stopcocks to the reservoir. During the experiments, the two sections were securely attached to provide a closed system for perfusion.

The monkeys were anesthetized by administering approximately 20 mg/kg im of ketamine hydrochloride[7]. The level of anesthetization was maintained by administering additional doses of ketamine every 0.5 hr.

The monkey was positioned on an operating table with the dorsal side up. The rib-cage cell was inserted into the monkey's vagina with care and connected by tubing to the rest of the perfusion system (Fig. 4). A sodium chloride irrigation solution was passed through the vagina for approximately 15 min at a flow rate of 20 ml/min to remove any exfoliated cells and other debris and secretions. Then the saline was replaced by the

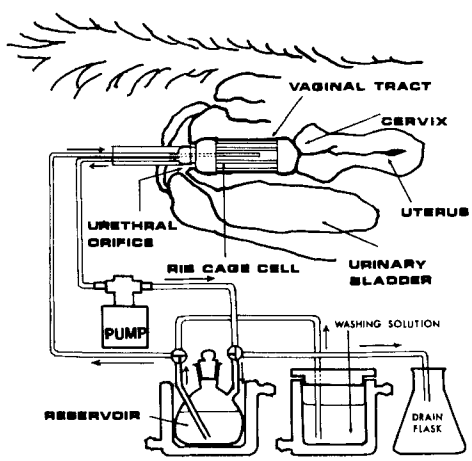

Figure 3—*Diagram of the perfusion system.*

[3] Mallinckrodt Chemical Works, St. Louis, Mo.
[4] Teflon (du Pont).
[5] FMI lab pump, model RRP 2G 150, Fluid Metering Inc., Oyster Bay, N.Y.
[6] Intramedic PE 205, Clay Adams, Division of Becton, Dickinson & Co., Parsippany, NJ 07054.
[7] Vetalar, Parke-Davis and Co., Detroit, Mich.

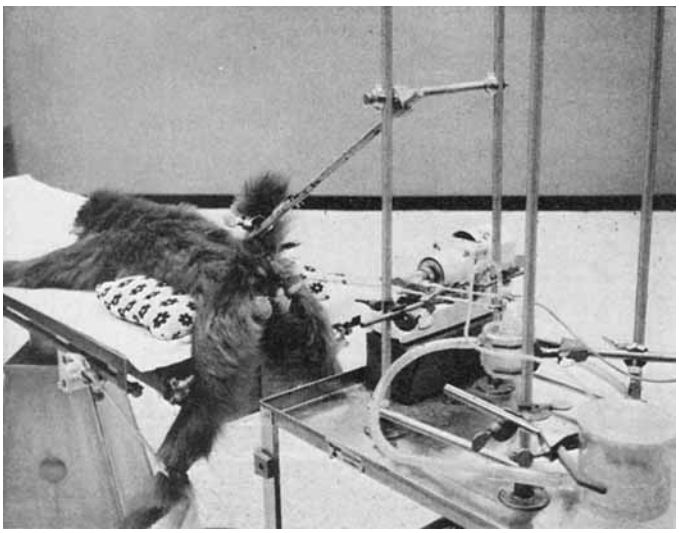

Figure 4—*Entire setup under typical experimental conditions.*

buffer solution used in the experiments, warmed to 37°, and the washing was continued for an additional 5 min. The washings were not circulated but were collected and discarded.

Independently, 5 ml of a perfusate concentrate solution was placed in the reservoir at the perfusion system, which could be isolated from the rib-cage cell and lines leading to it by a set of stopcocks. Upon completion of the rinsing procedure, the contents of the reservoir were introduced into the perfusion circuit, which contained about 10 ml of additional buffer in the cell and lines, *via* the stopcocks. This manipulation also formed a closed perfusion loop consisting of a total volume of approximately 15 ml in the reservoir, tubing, and rib-cage cell. This procedure was employed in studies checking the tightness of fit of the cell using radiolabeled polyethylene glycol 4000 (I) and in the permeation studies using the alcohols.

In all experiments, the flow rate through the pump was 20 ml/min. In order that the solution in the system would be mixed well, a 10-min period was allowed before the first sample was withdrawn. Further samples were withdrawn at 10-min intervals up to 80 min. At the end of a perfusion run, the solution was washed out with isotonic saline introduced into the system *via* the three-way stopcocks (polyethylene glycol 4000) or quantitatively collected (alcohols). In the polyethylene glycol 4000 experiments, the wash was collected and a mass balance on the radiolabeled compound was obtained after bringing the wash volume up to 250 ml. The residual I concentration was determined radioisotopically by using a liquid scintillation counter[8].

In the experiment with I, its perfusate concentration was 2.5×10^{-4} *M*. Initial concentrations were 5×10^{-6}, 4.8×10^{-5}, and 4.1×10^{-5} *M* for ^{3}H-methanol, ^{14}C-1-butanol, and ^{14}C-1-octanol, respectively. ^{3}H-Methanol was used as a reference permeant, and its absorption was followed simultaneously with that of the ^{14}C-labeled alcohols using a dual-label analytical procedure (2).

RESULTS AND DISCUSSION

Tightness of Rib-Cage Cell—The experiments with ^{3}H-I were aimed at assessing whether the cell was leakproof as used and whether there was

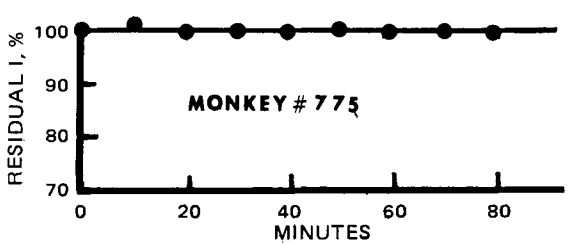

Figure 5—*Typical plot of change in concentration of I as a function of perfusion time.*

[8] Model LS 200, Beckman Instruments, Inc., Scientific and Process Instruments Division, Fullerton, Calif.

Table II—Percent Recovery Values for Polyethylene Glycol 4000 Obtained with the Three Rib-Cage Cells

Monkey	Cell Used	I Recovered, %
775	Small	90.2
774	Medium	90.3
788	Large	91.1

a significant or measurable amount of water gained or lost during the experiment. Compound I, being a large and very polar molecule, is essentially unabsorbed. Its constancy in concentration over the lifetime of a typical run coupled with total recovery is adequate evidence of the absence of *in situ* leakage. Gradual increases in the concentration of I coupled with its total recovery would suggest water absorption. Constancy of concentration without total recovery would suggest leakage.

The percent residual I values were plotted as a function of the perfusion time. A typical plot is shown in Fig. 5. The data generally indicated no significant water flux across the vaginal membrane. The computed values of I recovery (Table II) indicated no significant leakage from the cell.

Absorption of Alcohols—The primary purpose of these experiments was to assess the utility of the perfusion procedure and system for measuring vaginal permeability. Consequently, in these experiments, the periodicity of the vaginal cycle, which is presumed to affect vaginal permeability, was neglected. Rather, each run constituted an absolute test of the procedure; first-order absorption patterns previously seen in the rabbit work were expected and sought.

The logarithm of the percent residual alcohol concentrations was plotted as a function of the perfusion time (Figs. 6 and 7). The linear relationship obtained on a semilogarithmic scale indicates that the drug disappearance followed a first-order pattern. The rate of disappearance can thus be expressed by:

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

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

where C_b is the concentration of the drug in the bulk solution, K_u is the first-order rate constant, A is the effective surface area of the vaginal membrane exposed to the drug solution and is dependent on the geo-

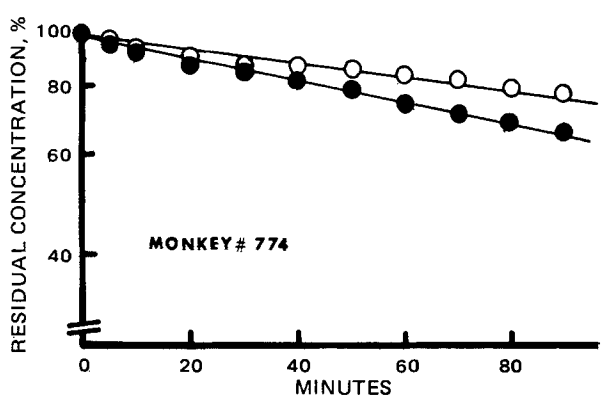

Figure 6—*Typical plots of the change in the concentrations of methanol and 1-butanol in the sampling reservoir as a function of perfusion time.*

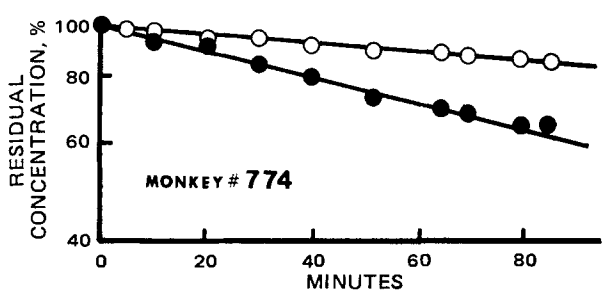

Figure 7—*Typical plots of the change in the concentrations of methanol and 1-octanol in the sampling reservoir as a function of perfusion time.*

Table III—Apparent Permeability Coefficient, P_{app}, Determined for the Vaginal Absorption of the Alcohols

Monkey	$P_{app} \times 10^4$, cm/sec		
	Methanol	1-Butanol	1-Octanol
774	0.80	1.44	2.18
775	1.16	1.77	3.52
778	0.75	0.96	2.85

metrical surface area of the cell used, V is the total volume of the drug solution, and P_{app} is the apparent permeability coefficient. The ratio of the surface area to the volume depended upon the cell employed.

The results of some typical P_{app} determinations are summarized in Table III. As can be seen, the values increased from methanol through octanol for all monkeys used. This finding is in qualitative accord with the results obtained in the rabbit vaginal absorption studies. Furthermore, the P_{app} values for the monkey were approximately of the same order of magnitude as those obtained with the rabbit system (2). However, since these experiments were carried out with little regard for the menstrual cycle effects, the results in Table III mainly represent a demonstration of the feasibility of the experiments. A rather systematic study of the menstrual cycle effects, that is, the influence of the phase of the cycle and the influence of the alkyl chain length as a function of the menstrual cycle, will be reported[9] (7).

In separate experiments, as was found in the rabbit studies, there were no significant losses of the alcohols in the perfusion system through, for example, adsorption when the rib-cage cell was bypassed.

[9] Presented at the APhA Academy of Pharmaceutical Sciences, New Orleans meeting, Apr. 1976 (abstract 54).

In conclusion, this study has shown that a relatively simple procedure involving perfusion and a special cell inserted into the vagina may be used to carry out vaginal membrane permeability experiments in the rhesus monkey. The procedure should be well suited for determining vaginal membrane permeability coefficients over a wide range of conditions, especially as a function of the menstrual cycle employing, wherever possible, the particular monkey as its own control.

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Aqueous Chamber Drug Distribution Volume Measurement in Rabbits

JOSEPH M. CONRAD and JOSEPH R. ROBINSON [x]

Abstract □ A method was developed for aqueous chamber drug distribution volume measurement in the albino rabbit, and the apparent volume of distribution was determined for inulin, pilocarpine alkaloid, and 1-hexanoic acid. The method consists of injecting a suitable concentration of drug, in an appropriate volume of fluid, into the anterior chamber of the eye and monitoring the decline in drug concentration as a function of time by periodic sampling of the aqueous humor. Graphical analysis of the resulting data yields both the apparent volume of distribution and the turnover rate constant of the aqueous humor. The technique does traumatize the eye, causing formation of plasmoid aqueous, which does not interfere with the apparent drug distribution volume measurement or the determination of aqueous humor turnover. Inulin was used to determine the physiological aqueous volume, 287 μl, in good agreement with literature values. The turnover rate constant was 0.016 min^{-1}, also in good agreement with literature values. The apparent volume of distribution for pilocarpine alkaloid was 575 μl in albino eyes and 760 μl in pigmented irides; for 1-hexanoic acid in albino eyes, it was 760 μl. For pilocarpine alkaloid, literature citations on the fraction of dose absorbed have been based on an assumed apparent volume of distribution of 250–300 μl. Therefore, a factor of two error has been introduced when using albino eyes and a factor of almost three has been introduced when using pigmented eyes. The implication of the apparent volume of distribution for pilocarpine in its ocular disposition is discussed, as is the unexpected observation that pilocarpine alkaloid apparently inhibits formation of plasmoid aqueous and follows one-compartment kinetics during these studies. It is shown that the one-compartment kinetics for pilocarpine are due to its biological activity in the aqueous chamber.

Keyphrases □ Drug distribution—aqueous chamber drug volume, inulin, pilocarpine, and 1-hexanoic acid, rabbit eyes □ Inulin—aqueous chamber volume of distribution, rabbit eyes □ Pilocarpine—aqueous chamber volume of distribution, rabbit eyes □ 1-Hexanoic acid—aqueous chamber volume of distribution, rabbit eyes □ Ocular volumes of distribution—aqueous chamber, pilocarpine, inulin, and 1-hexanoic acid, rabbit eyes

To make meaningful quantitative statements about the bioavailability of an ophthalmic preparation, it is necessary to know the apparent volume of distribution for that drug in the eye. At present, the ophthalmic literature contains statements on the bioavailability of topically applied ocular drugs that are based, at best, only on an approximation to the actual aqueous humor volume, ignoring all other factors, and, at worst, on poorly controlled clinical studies