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# Controlled Drug Release from Polymeric Delivery Devices III: *In Vitro–In Vivo* Correlation for Intravaginal Release of Ethynodiol Diacetate from Silicone Devices in Rabbits

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**Abstract** □ Forty female rabbits were implanted with silicone vaginal devices containing ethynodiol diacetate for up to 8 weeks. As predicted from *in vitro* studies, a $Q - t^{1/2}$ (matrix-controlled) release profile was observed *in vivo*. The *in vivo* drug release profile was compared with *in vitro* data measured at three hydrodynamic conditions, and the diffusional resistance across the vaginal wall was estimated. Drug released from silicone devices yielded a prolonged plasma level when compared with data following intravaginal or intravenous administration of a solution dose. The rate constant for elimination was unchanged. The plasma concentration of the drug was related to the intravaginal drug release profile both theoretically and experimentally and was above the concentration required to inhibit fertilization.

**Keyphrases** □ Drug delivery systems—controlled release, polymeric devices, ethynodiol diacetate in silicone matrix, *in vitro–in vivo* correlation of intravaginal release, rabbits □ Ethynodiol diacetate—intravaginal release from silicone matrix, *in vitro–in vivo* correlation, rabbits □ Silicone matrix—ethynodiol diacetate delivery system, *in vitro–in vivo* correlation for intravaginal release, rabbits □ Vaginal devices—controlled release of ethynodiol diacetate from silicone matrix, *in vitro–in vivo* correlation, rabbits

An *in vitro* drug release system, which allows a direct and rapid characterization of a drug release profile and mechanism, was recently reported from this laboratory (1). Two types of drug release mechanisms, matrix controlled and partition controlled, were observed when the drug release profiles of ethynodiol diacetate from silicone devices were followed daily in this system (2).

To develop a device with a desirable, long-acting, drug release profile, it is necessary to examine intravaginal drug release mechanisms in animals to establish an *in vitro–in vivo* correlation. This paper reports results of drug release studies with silicone devices containing ethynodiol diacetate placed in the vaginal tracts of 40 rabbits for up to 8 weeks.

## EXPERIMENTAL

***In Vitro* Release Studies**—The apparatus for *in vitro* drug release studies and the assay of drug samples were essentially the same as those reported previously (1). The ring-shaped silicone device, containing 112.6 mg/cm$^3$ of ethynodiol diacetate[1], was mounted in the arms of a Plexiglas holder and then rotated at 81 or 30 rpm or held stationary (to simulate more closely the status of implants in the vaginal lumen) in 150 ml of a 75% polyethylene glycol 400 solution as the elution medium at 37°. The solution was mixed well prior to sampling, and the drug concentration in the medium was assayed daily (1, 2). The reproducibility of the *in vitro* $Q/t^{1/2}$ profiles measured at various dates within 1 year was excellent (Table I).

***In Vivo* Release Studies**—The same silicone devices as those used in the *in vitro* drug release studies were cut into sections of 1 cm in length. One segment was inserted into the anterior vagina of each of 40 young adult New Zealand white female rabbits *via* a midventral laparoelytrotomy. It was anchored with a single polyethylene suture knotted on one side of the implant (through the implant perpendicular to the long axis) and then drawn through the vaginal wall and knotted to a 1-cm section of medical-grade tubing[2].

[1] SC-11800.
[2] Silastic, Dow Corning Corp., Midland, Mich.

Table I—Reproducibility of *In Vitro* Release Profiles ($Q/t^{1/2}$) of Ethynodiol Diacetate from Silicone Vaginal Devices[a]

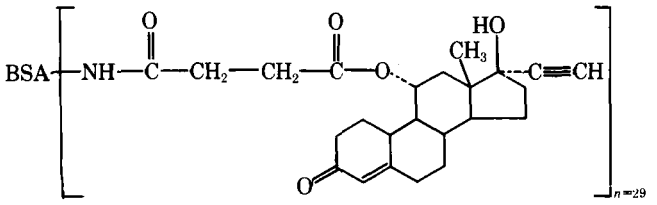
| Experiments | Age of Devices, days | $Q/t^{1/2}$, mg/cm²/day$^{1/2}$ |
|---|---|---|
| 121 | Initial | 3.40 |
| 122 | 312 | 3.135 |
| 123 | 312 | 2.985 |
| 124 | 312 | 2.966 |
| 127 | 331 | 3.34 |
| Mean (±*SD*) = 3.1652 (±0.185) | | |

[a] Intravaginal devices were rotated at constant 81 rpm in 150 ml of 75% polyethylene glycol 400 at 37°.

At various intervals, three to six rabbits were sacrificed. The implants were removed, cut into thin slices, and extracted by constant shaking for 2 days in 25 ml of spectral grade methanol. Drug content in the methanolic solution was assayed spectrophotometrically as described earlier (1, 2), and residual drug content was calculated. The results on the residual drug content and the amount of drug released per unit area are shown in Table II.

**Radioimmunoassay**—Tokuda *et al.* (3) reported that the stepwise hydrolysis of the acetate moieties of ethynodiol diacetate gives its 3β,17β-diol metabolite. This metabolite is rapidly transformed to the 3-keto derivative, norethindrone (Table III), the major metabolite of ethynodiol diacetate.

*Preparation of 11α-Hydroxynorethindrone–Bovine Serum Albumin Conjugate*—The hemisuccinate of 11α-hydroxynorethindrone was prepared first and then conjugated to bovine serum albumin (BSA). Analysis of the conjugate by UV spectroscopy and nitrogen analysis indicated that each conjugate molecule was composed of 29 steroid molecules to one bovine serum albumin molecule with the following chemical structure:




*Preparation of Antiserums*—New Zealand rabbits were immunized with norethindrone–bovine serum albumin conjugate by the technique described by Vaitukaitus *et al.* (4). The resultant antiserums were characterized, divided into several small aliquots, and stored at −80°.

*Immunoassay Procedure*—The assay was carried out in glass tubes (12 × 75 mm), and 0.1% gelatin–phosphate buffer (0.05 *M* phosphate and 0.15 *M* NaCl at pH 7.5) was utilized as the diluent for all dilution preparations of antiserums, standards, and samples. To each tube, 10,000 dpm of 1,2-$^3$H-norethindrone (20 Ci/mmole), 200 μl of diluted antiserum (capable of binding 40% of the labeled antigen added), and 20 μl of sample serum (to calibrate the standard curve, 20 μl of control rabbit serum and 10–1000 pg of crystalline norethindrone were used instead) were added and mixed (vortex).

Duplicate sets of the binding mixture were prepared and incubated at 4° for 12 hr. Antibody-bound antigen was then separated from the unbound antigen by the addition of 0.5 ml/tube of dextran-coated charcoal (prepared from 50 mg of dextran[3] and 500 mg of charcoal in 50 ml of phosphate buffer). The mixtures were immediately mixed and then centrifuged at 2000×*g* and 4° for 10 min. The resultant supernate was mixed with 15 ml of scintillator[4] in glass vials of low background. The radioactivity of each sample was then determined[5].

Conversion of counts per minute to disintegrations per minute was accomplished by the external standard channel ratio method. Disintegrations per minute data were evaluated and converted to the corresponding drug concentration by a computer program utilizing a logit–log transformation of standards.

[3] Pharmacia T-70.
[4] PCS, Amersham/Searle Corp., Arlington Heights, Ill.
[5] Nuclear Chicago Mark II scintillation counter.

Table II—Intravaginal Release of Ethynodiol Diacetate from Silicone Devices Implanted in Rabbits

| Days[a] | Number of Rabbits | Amount of Drug Remaining in Devices, mg (±*SD*) | Cumulative Amount of Drug[b] Released per Square Centimeter, mg/cm² (±*SD*) |
|---|---|---|---|
| Initial | — | 30.428 (0.83) | 0.0 |
| 7 | 3 | 25.948 (2.40) | 2.239 (0.97) |
| 14 | 3 | 17.660 (0.78) | 5.210 (0.32) |
| 25 | 5 | 13.964 (3.80) | 6.720 (1.60) |
| 42 | 6 | 4.997 (6.70) | 10.380 (2.70) |
| 49 | 4 | 0.083 (0.09) | 12.386 (0.04) |
| 52 | 4 | 1.238 (0.38) | 11.914 (0.15) |

[a] Five rabbits were implanted with devices (containing no ethynodiol diacetate) and sacrificed at various dates as the references. [b] Total surface area of the device used is 2.45 cm² each.

A measure of the accuracy of the immunoassay technique is illustrated in Fig. 1. Crystalline norethindrone was added to control rabbit serum and assayed as an unknown. A linear relationship was observed between added and recovered norethindrone, with a tendency for overestimation at high concentrations.

The specificity of the anti-11-hydroxynorethindrone antiserum was demonstrated by examining its cross-reactivity with ethynodiol diacetate and its seven major metabolites. As shown in Table III, this antiserum specifically reacted only with norethindrone.

The cross-reactivity of 11-hydroxynorethindrone antiserum to progesterone, 20α-hydroxyprogesterone, estradiol, corticosterone, hydrocortisone, and testosterone was also examined. These common steroids showed no cross-reactivity except for testosterone, which has a cross-reactivity of only 0.15%.

## RESULTS AND DISCUSSION

***In Vivo* Drug Release Studies**—The intravaginal drug release data (Table II) indicate that the longer the period of implantation, the lower the drug content in the device and the greater the quantity of drug released. If the release of ethynodiol diacetate from the silicone vaginal devices implanted in the vaginal tract of rabbits is assumed to be predominately matrix controlled (1) and not partition controlled (2), then the cumulative amount of drug released ($Q$) in a rabbit's vagina should be defined by:

$$Q = \sqrt{D_m(2A - C_s)C_p t} \qquad \text{(Eq. 1)}$$

where $Q$ is the cumulative amount of drug released from a unit surface area of device (grams per square centimeter); $A$ is the initial amount of solid drug impregnated in a unit volume of device

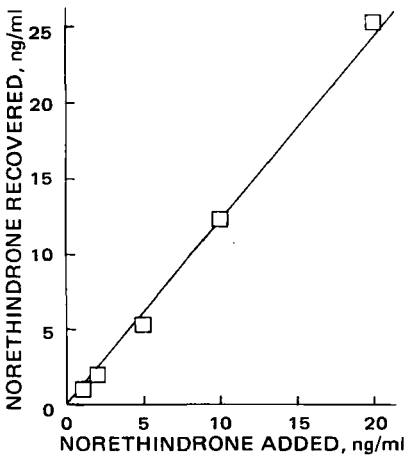
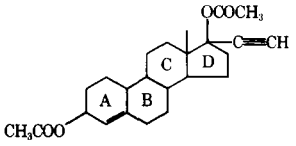
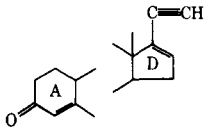
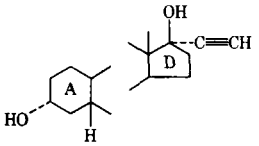
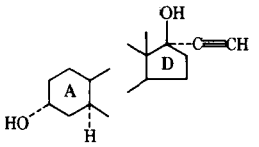
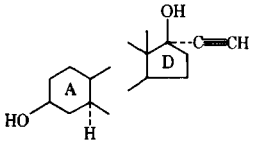
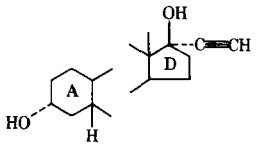
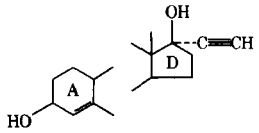
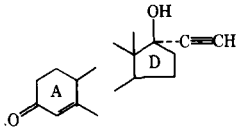



**Figure 1**—*Linear relationship between norethindrone added to control serum* versus *norethindrone determined by radioimmunoassay. Each data point is a mean of eight determinations.*

**Table III—Cross-Reactivity of Anti-11-hydroxynorethindrone Antiserum[a] to Ethynodiol Diacetate and Its Metabolites[b]**

| Ethynodiol Diacetate Metabolite | Molecular Structure | Reactivity[c], % |
|---|---|---|
| Ethynodiol diacetate |  | N.R.[d] |
| Metabolites: | | |
| I |  | 0.58 |
| II |  | 1.22 |
| III |  | 1.50 |
| IV |  | 1.63 |
| V |  | 2.50 |
| VI |  | 6.00 |
| VII[e] |  | 100.00 |

[a] 2-4-12-73-ws; a dilution of 1:10,000 was used. [b] Ref. 9. [c] Based on 50% inhibition of binding assay. [d] No reactivity could be detected. [e] Norethindrone.

(grams per cubic centimeter); $D_m$ is the effective diffusivity of the drug species in the matrix; $C_s$ and $C_p$ are the solubilities of the drug in the elution medium and in the silicone polymer phase, respectively; and $t$ is time.

According to Eq. 1, the cumulative amount ($Q$) of ethynodiol diacetate released from a unit surface area of device should be linearly proportional to the square root of the implantation time ($t^{1/2}$) as shown in Fig. 2. By using Eq. 2:

$$Q/t^{1/2} = \sqrt{D_m(2A - C_s)C_p} \qquad \text{(Eq. 2)}$$

the intravaginal $Q/t^{1/2}$ profile was estimated from the slope of the $Q$ *versus* $t^{1/2}$ plot to be 2.10 mg/cm$^2$/day$^{1/2}$.

The drug molecules released from the silicone vaginal device are presumably dissolved in the vaginal fluid surrounding the implanted device. They are then transported across the vaginal membrane

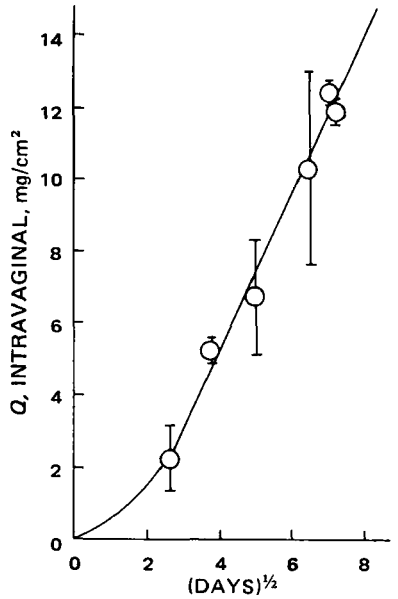



**Figure 2**—*Linear relationship between the cumulative amount of drug released* (Q) *in the rabbit's vagina and the square root of time* ($t^{1/2}$) *following Eq. 1. The data in Table II (fourth column) were plotted. The* $Q/t^{1/2}$ *value for this intravaginal device was calculated from the slope, following Eq. 2, as 2.10 mg/cm*$^2$*/day*$^{1/2}$.

into a biological sink maintained by hemoperfusion. In such a case, the rate of drug permeation ($dQ'/dt$) across the vaginal membrane (with an aqueous diffusion layer on each side of the membrane) should be directly proportional to the drug concentration ($C_v$) in the vaginal fluid (as the driving force for drug permeation) and inversely proportional to the total diffusional resistance ($\Sigma R$) encountered by the drug molecules along the way across the vaginal wall:

$$\frac{dQ'}{dt} = \frac{C_v}{\Sigma R} \qquad \text{(Eq. 3)}$$

The total diffusional resistance (5) is the sum of the diffusional resistances as follows:

$$\Sigma R = R_l + R_v + R_c \qquad \text{(Eq. 4}a\text{)}$$

where $R_l$ and $R_c$ are the diffusional resistances across the aqueous diffusion layer on the luminal side and on the circulation side of the vaginal membrane, respectively; and $R_v$ is the diffusional resistance across the vaginal membrane itself. Equation 4*a* is equivalent to:

$$\Sigma R = \frac{\delta_l}{D_l K_{l/l}} + \frac{\delta_v}{D_v K_{v/l}} + \frac{\delta_c}{D_c K_{c/v}} \qquad \text{(Eq. 4}b\text{)}$$

where $\delta$, $D$, and $K$ represent the thickness, diffusivity, and partition coefficient across two contacting phases, respectively; and the subscripts $v$, $l$, and $c$ stand for vaginal membrane and the aqueous diffusion layers on the luminal and the circulatory hemoperfusion sides of the vaginal barrier, respectively.

The instantaneous rate of drug release at time $t$ may be derived by differentiating Eq. 1 with respect to time:

$$\frac{dQ}{dt} = \frac{1}{2}\sqrt{D_m(2A - C_s)C_p/t} \qquad \text{(Eq. 5)}$$

At steady state, the rate of drug release ($dQ/dt$) should be equal to the rate of drug permeation ($dQ'/dt$). Equating Eq. 3 with Eq. 5 yields Eq. 6:

$$\Sigma R[D_m(2A - C_s)C_p]^{1/2} = 2C_v(t)^{1/2} \qquad \text{(Eq. 6)}$$

Equation 6 indicates that the drug concentration ($C_v$) in the vaginal fluid decreases with the square root of time ($t^{1/2}$) as the drug molecules diffuse actively to a sink; that is, under a matrix-controlled process, the magnitude of $2C_v(t)^{1/2}$ is a constant since both $\Sigma R$ and $[D_m(2A - C_s)C_p]^{1/2}$ are constants under a controlled con-

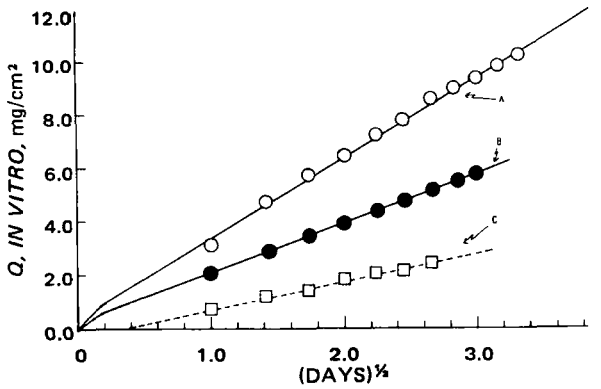



**Figure 3**—*Linear relationship between the cumulative amount of drug release (Q) and the square root of time ($t^{1/2}$) obtained from the* in vitro *drug release studies conducted at the rotation speed of 81 (A), 30 (B), and stationary state (0) (C) rpm. Each data point represents an average of three determinations. The slope ($Q/t^{1/2}$) was estimated as 3.1652 (A), 1.862 (B), and 1.065 (C) mg/cm²/day$^{1/2}$, respectively.*

dition. Equation 6 also states that the value of $C_v$ is directly proportional to the magnitude of the total diffusional resistance ($\Sigma R$) when the same silicone vaginal device is implanted in a different animal with a different value of $\Sigma R$. Therefore, the greater the magnitude of $\Sigma R$, the higher the value of $C_v$ since the intravaginal permeation of the drug is inhibited. The validity of Eq. 6 will be further analyzed later.

***In Vitro* Drug Release Studies**—A series of parallel *in vitro* drug release studies using similar devices was conducted at low rotation speed (81 or 30 rpm) and at a stationary state to simulate more closely the status of implants in the vaginal lumen. The *in vitro* release of ethynodiol diacetate followed a linear $Q - t^{1/2}$ relationship in all three cases (Fig. 3). The magnitude of the $Q/t^{1/2}$ values calculated for the rotating devices (Table IV, fourth column) are 2.97 (81 rpm) or 1.75 (30 rpm) times higher than that for a stationary one. Since $D_m$, $A$, $C_s$, and $C_p$ were the same in all three *in vitro* studies, the 1.75–2.97-fold difference in the magnitudes of the $Q/t^{1/2}$ value between the rotating and the stationary devices indicates the existence of a rate-limiting parameter, *i.e.*, a hydrodynamic diffusion layer on the immediate surface of the devices.

When a device is immersed stationary in a solution, a diffusion layer is established on the immediate surface of the device. The effective thickness of this diffusion layer (6) varies with the square root of time as follows:

$$(\delta_l)s = \sqrt{D_l \pi t} \qquad \text{(Eq. 7)}$$

If this device is rotated at a constant speed, convection results. The convective transport of the drug to and from the device surface is much faster than when natural diffusion is operating on a stationary device, because the concentration gradient of drug extends over a thinner diffusion layer (7). The effective thickness of the diffusion layer on the rotating device becomes time independent and may now be defined by the Levich equation:

$$(\delta_l)r = 1.62 D_l^{1/3} v^{1/6} w^{-1/2} \qquad \text{(Eq. 8)}$$

where $v$ and $w$ represent the kinematic viscosity of the elution medium and the angular rotation speed of the silicone device, respectively; and the subscripts $s$ and $r$ stand for stationary and rotation states, respectively. This equation was developed originally for the relationship of the thickness of the convective diffusion layer at a rotating disk and also was successfully applied to the case of a polymeric membrane-covered rotating disk (8). Using Eq. 8, the magnitude of $(\delta_l)r$ on a rotation device can be calculated if $D_l$, $v$, and $w$ are known or predetermined. The $(\delta_l)r$ values were calculated and are given in Table IV.

In the *in vitro* elution studies, the elution medium was maintained at sink conditions throughout the experiment. In this case, only the thickness of the hydrodynamic diffusion layer ($\delta_l$) exists between the surface of the device and the bulk of the elution medium. Both the $R_v$ and $R_c$ terms in Eq. 4 may be dropped, and Eq. 4 may be reduced to:

$$\Sigma R = R_l = \frac{\delta_l}{D_l} \qquad \text{(Eq. 9)}$$

where $k_{l/l} = 1$ and Eq. 6 becomes:

$$\delta_l[D_m(2A - C_s)C_p]^{1/2} = 2D_l C_v(t)^{1/2} \qquad \text{(Eq. 10)}$$

Equation 10 indicates that at a given value of $2D_lC_v(t)^{1/2}$, the magnitude of $[D_m(2A - C_s)C_p]^{1/2}$ should be inversely proportional to the effective thickness, $\delta_l$, of the diffusion path on the immediate surface of the silicone device. In other words, the larger the value of $\delta_l$ on a device, the less is the amount of drug released at a given time (the lower the magnitude of $Q/t^{1/2}$). This relationship is shown by the data of Table IV. For instance, the magnitude of $Q/t^{1/2}$ for the device rotating at 81 rpm (3.165 mg/cm²/day$^{1/2}$) is 1.7 times higher than that for the device rotating at 30 rpm (1.862 mg/cm²/day$^{1/2}$).

This difference in the magnitudes of $Q/t^{1/2}$ may be explained by comparing the value of $\delta_l$ for the device rotating at 81 rpm (154.7 μm) to that for the device rotating at 30 rpm (254.0 μm). The $\delta_l$ value for the higher rotating device (81 rpm) is 1.64-fold lower than that for the lower rotating one (30 rpm). When all other experimental conditions are the same, the thickness of the diffusion layer ($\delta_l$) becomes the rate-determining parameter in the process of drug release (Eq. 10). The increase in the thickness of the diffusion layer, due to the reduction in the angular rotation speed of the device, tends to decrease proportionally the magnitude of $Q/t^{1/2}$.

***In Vitro–In Vivo* Correlation**—Analysis of the intravaginal drug release data (Fig. 2) yields an *in vivo* $Q/t^{1/2}$ value of 2.10 mg/cm²/day$^{1/2}$. This value is between those observed for the two *in vitro* rotation experiments (Table IV). Since the smooth muscle of the vaginal lumen shows a periodic constriction and dilatation, it may be assumed that the implanted silicone device was moved

Table IV—Comparison of *In Vivo* and *In Vitro* Data on $Q/t^{1/2}$

| Conditions | $n$, rps | $\delta_l$[a], cm × 10⁴ | $Q/t^{1/2}$ Observed, mg/cm²/day$^{1/2}$ |
|---|---|---|---|
| *In Vitro* | | | |
| 81 rpm | 1.328 | 154.7 | 3.165 |
| 30 rpm | 0.500 | 254.0 | 1.862 |
| Stationary | 0.0 | — | 1.065 |
| *In Vivo*, intravaginal | | | 2.10 |

[a] Calculated from $\delta_l = 1.62 D^{1/3} v^{1/6} W^{-1/2} = 4.5027 \times 10^{-4} \times W^{-1/2}$, where $W = 2n\pi$.

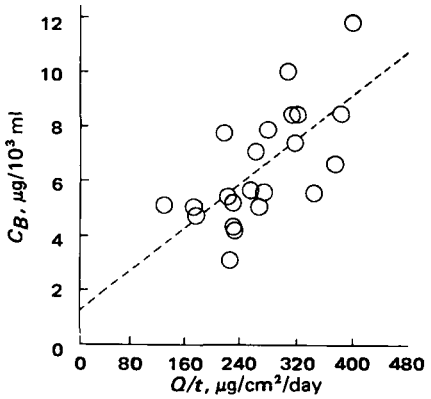
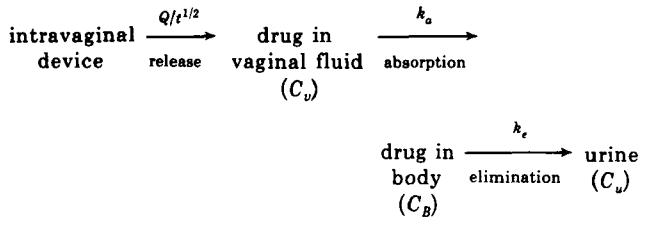



**Figure 4**—*Correlation between the plasma concentration ($C_B$) of norethindrone, the major metabolite of ethynodiol diacetate, in each rabbit and the flux (Q/t) of ethynodiol diacetate released from a silicone vaginal device at a given time. The slope = 1.96 (±0.49) × 10⁻⁵ day/cm, and the intercept = 1.199 × 10⁻³ μg/ml; F = 16.583 [F1, 20 (α = 0.01) = 8.10].*




*Scheme I*

slightly in a periodic fashion. Therefore, a diffusion layer ($\delta_l$) (on the lumen side of the vaginal wall) with an effective thickness smaller than 254 μm (slow rotation state) and larger than 154.7 μm (high rotation state) may be expected. Literature data do not allow the calculation of the total diffusional resistance term ($\Sigma R$) of Eq. 4. The absence of a known $\Sigma R$ makes the prediction of *in vivo* release from *in vitro* data impossible. However, the data may be utilized to establish a proportionality between *in vivo* and *in vitro* $Q/t^{1/2}$ profiles. This proportionality should be equivalent to the ratio of *in vitro* diffusional resistance ($R_l$) over *in vivo* total diffusional resistance ($\Sigma R$) as follows:

$$\frac{R_l}{\Sigma R} = \frac{(Q/t^{1/2})\ in\ vivo}{(Q/t^{1/2})\ in\ vitro,\ 30\ rpm} = \frac{2.10}{1.862} = 1.13 \quad \text{(Eq. 11)}$$

Such a proportionality may be useful for predicting the magnitude of $(Q/t^{1/2})$ *in vivo* from the *in vitro* drug release profiles measured under controlled conditions (when $R_l$ is constant).

**Pharmacokinetics of Intravaginal Absorption**—If the absorption, distribution, and elimination of ethynodiol diacetate after its release from an intravaginal device follow Scheme I, then the instantaneous rate of change in the body concentrations of drug may be expressed as:

$$\frac{d(C_B)}{dt} = k_a C_v - k_e C_B \quad \text{(Eq. 12)}$$

where $k_a$ and $k_e$ are the respective rate constants for absorption and elimination, and $C_v$, $C_B$, and $C_u$ are the drug concentrations in the vagina, body (including blood, tissues and related compartments), and urine, respectively. Equation 12 may be transformed to Eq. 13 by substituting Eq. 2 into Eq. 6 and then for the $C_v$ term in Eq. 12:

$$\frac{d(C_B)}{dt} = \left(\frac{k_a \Sigma R}{2}\right)\left(\frac{Q}{t}\right) - k_e C_B \quad \text{(Eq. 13)}$$

At steady state, the change in the body concentration of the drug will be very small, that is, $dC_B/dt \simeq 0$, and the body concentration of norethindrone ($C_B$), the major metabolite of ethynodiol diacetate (9), may be related to the amount of ethynodiol diacetate ($Q$) released at time ($t$) as follows:

$$C_B = \left(\frac{k_a \Sigma R}{2k_e}\right)\left(\frac{Q}{t}\right) \quad \text{(Eq. 14)}$$

Equation 14 states that the plasma concentration of norethindrone in each rabbit should be directly proportional to the flux of ethynodiol diacetate released from the vaginal devices ($Q/t$) at a given time. The result is illustrated in Fig. 4. When the correlation of $C_B$ with $Q/t$ was submitted to an $F$ test, the analysis resulted in an $F$ value of 16.583, indicating that the correlation was statistically significant at the 99% confidence level ($F1, 20\ [\alpha = 0.01] = 8.10$).

To inhibit fertilization effectively, a rabbit required a subcuta-

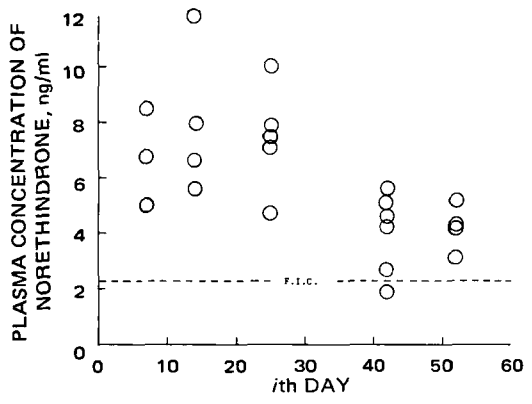



**Figure 5**—*Plasma concentration of norethindrone from each rabbit measured by radioimmunoassay at the time of sacrifice and removal of the intravaginal device. The fertilization inhibitory concentration (FIC) of 2.27 ng/ml, resulting from the daily subcutaneous injection of 30–50 μg of ethynodiol diacetate per rabbit for 6 days prior to mating and 1 day after reduced fertilization, is also shown. Most data resulting from the intravaginal absorption of ethynodiol diacetate in silicone devices are higher than the FIC required.*

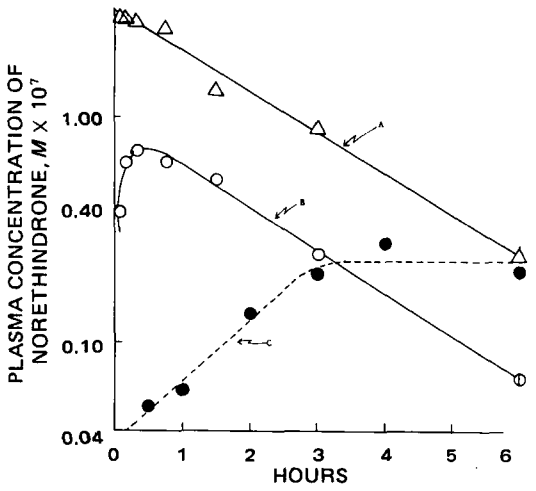



**Figure 6**—*Time course of plasma norethindrone concentration after the administration of a single solution dose (1 mg/0.2 ml) of norethindrone via intravenous injection (A) and ethynodiol diacetate via intravaginal absorption (B), and intravaginal absorption of ethynodiol diacetate from a silicone vaginal device (C) (A = 0.1126 g/cm³). The rate constants for absorption ($k_a$) are: 10.6 (B) and 0.244 (C) hr⁻¹. The rate constants for elimination ($k_e$) are 0.40 (A) and 0.42 (B) hr⁻¹.*

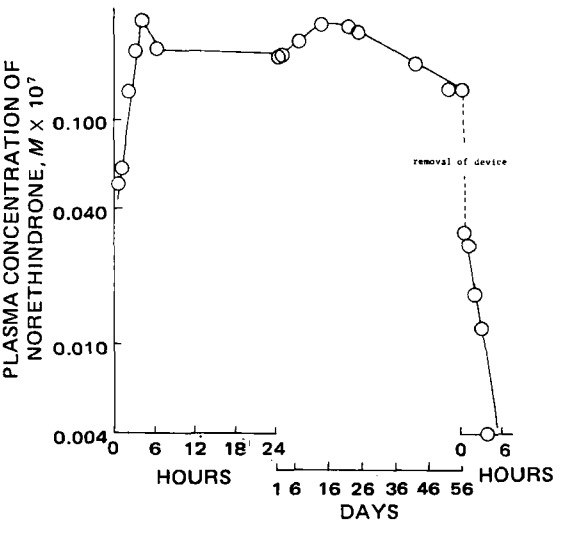



**Figure 7**—*Overall plasma norethindrone concentration profile after the intravaginal implantation of silicone devices containing ethynodiol diacetate in rabbits for up to 56 days. The $k_a$ and $k_e$ values are 0.244 and 0.511 hr⁻¹, respectively. The $k_e$ value after the removal of the devices (elimination of the residual drug content in the body) is 0.436 hr⁻¹.*

**Table V—Comparison of Calculated and Observed Plasma Concentrations of Norethindrone**

| Days | Plasma Concentration of Norethindrone, ng/ml: Calculated[a] | Observed[b]: Range | Observed[b]: Mean (±SD) | Observed/Calculated |
|---|---|---|---|---|
| 22 | 8.77 | 6.15–9.41 | 7.78 (1.63) | 0.89 |
| 56 | 5.49 | 2.32–7.42 | 4.12 (1.80) | 0.75 |

[a] Calculated from $C_B = (k_a \Sigma R)/2k_e \times (1.13) \times (Q/t)$ *in vitro* = 22.11 $(Q/t)$ *in vitro* (ng/ml). [b] Data of four to six rabbits for each scheduled interval.

neous dose of 30–50 μg/day of ethynodiol diacetate for 6 days prior to and 1 day after mating. These doses normally resulted in a maximum plasma concentration of 2–3 ng/ml of norethindrone in a 4-kg rabbit. The latter concentration is referred to as the fertilization inhibitory concentration (FIC). As shown in Fig. 5, the release of ethynodiol diacetate from implanted silicone devices resulted in a plasma concentration of norethindrone well above the required FIC. This concentration was maintained for up to 8 weeks.

The plasma norethindrone profile after the intravaginal absorption of ethynodiol diacetate from a solution dose of 1 mg/0.2 ml is compared to the profile after an intravenous injection of an equivalent dose of norethindrone in Fig. 6. As illustrated, the intravaginal dose of ethynodiol diacetate (curve B) was rapidly absorbed ($k_a = 10.6\ hr^{-1}$) and showed a peak plasma level of norethindrone within 20 min after administration. The observed elimination rate constant ($k_e = 0.42\ hr^{-1}$) agreed with the value of $0.40\ hr^{-1}$ found for the intravenous dose of norethindrone (curve A) in the same animal. From the ratio of the area under the curve ($AUC$) for intravaginal absorption over that for the intravenous dose, the availability of norethindrone from an intravaginal dose of ethynodiol diacetate was calculated to be 34.76%.

The plasma level of norethindrone following intravaginal absorption from a vaginal device containing ethynodiol diacetate is also shown in Fig. 6 (curve C). The rate of appearance of drug in plasma was sustained (C in Fig. 6), and the plasma level of norethindrone was significantly prolonged (Fig. 7). After removal of the device, the residual drug content in the body (Fig. 7) was eliminated with a rate constant ($0.436\ hr^{-1}$) comparable to those seen after the intravenous and intravaginal administrations.

Figure 7 illustrates the overall plasma norethindrone concentration profile after the implantation and removal of the silicone vaginal devices. A prolonged drug level was maintained for up to 56 days. After the removal of the device, the residual plasma drug content was eliminated as fast as after a solution dosage.

The rate constants for absorption ($k_a = 10.6\ hr^{-1}$) and for elimination ($k_e = 0.42\ hr^{-1}$), determined earlier from the intravaginal absorption study of a solution dose of ethynodiol diacetate, may be applied into the slope ($k_a \Sigma R/2k_e$) of the $C_B$ *versus* $Q/t$ profile to estimate the total diffusional resistance ($\Sigma R$) across the vaginal membrane of the rabbit:

$$\Sigma R = \frac{\delta_l}{D_l k_{l/l}} + \frac{\delta_c}{D_c k_{v/l}} + \frac{\delta_c}{D_c k_{c/v}} \quad \text{(Eq. 15a)}$$

$$= \frac{2ke \times 1.96 \times 10^{-5}}{k_a} \quad \text{(Eq. 15b)}$$

$$= 1.55 \times 10^{-6}\ \text{day/cm} \quad \text{(Eq. 15c)}$$

The resultant $\Sigma R$ value will be useful as a working factor for predicting plasma norethindrone levels ($C_B$) from the *in vivo* release profile ($Q/t$) of ethynodiol diacetate (using Eq. 14). The *in vivo* $Q/t^{1/2}$ value may be estimated from *in vitro* drug release data after a correction factor of 1.13 is made (Eq. 11). This information should facilitate the future development of a suitable long-acting delivery system, directly from short-term *in vitro* drug release experiments, for the vaginal administration of ethynodiol diacetate. For instance, based on a 1-week *in vitro* experiment, it was predicted that a plasma concentration of norethindrone at 8.77 and 5.49 ng/ml will be found after 22- and 56 days of implantation, respectively (Table V). Intravaginal implantation of such devices in 10 rabbits for 22 and 56 days resulted in a plasma concentration of 7.78 (±1.63) and 4.12 (±1.80) ng/ml, respectively (Table V).

In conclusion, in developing a long-acting delivery system for a given drug, a study should be performed to evaluate the *in vitro–in vivo* correlation of both the mechanisms and the rates of drug release. This study should also measure the pharmacokinetic parameters for the absorption, distribution, and elimination of the drug *via* a given route of administration. The information so gained can then be applied to additional studies to develop a device that delivers the drug at a programmed rate, thereby maintaining constant plasma drug levels for an optimum duration of treatment.

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