= (slope × concentration) + intercept]. Calculations were performed on a programmable calculator[7].

The data in Table II demonstrate the utility of HPLC in the analysis of I and II in biological fluids at plasma and urine concentration levels.

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In Vitro and *In Vivo* Considerations of a Novel Matrix-Controlled Bovine Progesterone-Releasing Intravaginal Device

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Abstract □ An *in vitro* system measuring progesterone release from silicone matrixes into serum is described. Drug release followed a matrix-controlled diffusion model in which the cumulative quantity released was linear with the square root of time. A bovine intravaginal device, consisting of a steel coil coated on both sides with a progesterone silicone matrix, was used as an *in vivo* drug delivery system. *In vivo* drug release also was matrix controlled, with rates comparable to those obtained *in vitro*. However, lag times of 1.7 and 4.2 days before reaching the steady-state rate were obtained for the outer and inner surfaces, respectively. The combination of effects resulted in a pseudo-zero-order drug-releasing device in which cumulative drug released was linear with time for at least 16 days.

Keyphrases □ Progesterone—release from silicone matrixes, *in vitro* system compared to bovine intravaginal device *in vivo* □ Silicone matrixes—release of progesterone, *in vitro* system compared to bovine intravaginal device *in vivo* □ Drug delivery—progesterone from silicone matrixes, *in vitro* system compared to bovine intravaginal device *in vivo* □ Vaginal devices, bovine—progesterone in silicone matrix, compared to *in vitro* system □ Hormones—progesterone, release from silicone matrixes, *in vitro* system compared to bovine intravaginal device *in vivo*

Intravaginal devices coated with a progesterone silicone matrix were used successfully (1–3) to control the bovine estrus cycle and to synchronize estrus by regulating pituitary release of gonadotropic hormones. Estrus occurs at a predictable time upon removal of the device developed in these laboratories 9–21 days after intravaginal implantation. The drug release rate profile of this device is defined in this report through correlation of *in vitro* and *in vivo* studies.

EXPERIMENTAL

Matrix Preparation—Progesterone silicone matrixes at various drug concentrations were prepared by first mixing the required amount of micronized drug into silicone fluid 360[1] and then adding Silastic elastomer 382[1]. The mixture was blended for 30 min to ensure random distribution of drug before adding the polymerizing catalyst, stannous octanoate[1].

***In Vitro* Study**—Matrixes used in the *in vitro* studies were prepared by streaking the silicone drug mixture containing catalyst onto fiber glass cloth and allowing it to cure. A template was used to obtain a uniform thickness of 1.5 mm. The cured matrixes were cut accurately into 2 × 2-cm pieces, mounted onto stainless steel plates with medical adhesive[1], and allowed to dry for 48 hr.

Two types of *in vitro* experiments were performed. The first followed the release of progesterone as a function of time from a silicone matrix containing 6.75% drug. The second concerned the release rate as a function of drug concentration using 5, 7.5, and 10% progesterone in silicone matrixes. The 6.75% matrix was prepared from a different lot of silicone liquid and elastomer, restricting direct comparison of the two *in vitro* experiments.

In both experiments, a specified number of matrixes mounted on stainless steel were individually placed in 25 × 150-mm glass tubes. To each tube, 15 ml of sheep serum[2] and 2 drops of toluene were added to retard microbial growth. Each tube was capped with a rubber stopper, placed on a shaker, and incubated at 37°. Serum changes at the end of Days 1, 2, 3, 4, 7, 11, 15, and 18 for one set of matrixes were matched with serum changes at the end of Days 0.1, 0.2, 0.3, 0.7, 1, 1.3, 2, 2.3, 3, 4, 5, 8, 12, 15, 18, and 20 for a duplicate set of matrixes.

The progesterone released in selected mounted matrixes was determined at various scheduled times by analyzing the amount of drug re-

[1] Dow Corning Corp., Midland, MI 48640.

[2] International Scientific Industries, Cary, IL 60013.

Table I—Comparative *In Vitro* Progesterone Release Rates from 5, 7.5, and 10% Silicone Matrixes Incubated 21 Days at 37° in Sheep Serum

Parameter	Progesterone Matrix Concentration		
	5%	7.5%	10%
Rate ($k = Q/t^{1/2}$ mg/cm day$^{1/2}$)	1.924	2.164	2.436
Confidence limit ($p < 0.05$), ±%	10.7	6.2	8.3
Extrapolated lag time, hr	5.0	4.5	5.0
Correlation coefficient (r)	0.969	0.987	0.980

maining and calculating the difference from the initial content (determined by percent progesterone of total matrix weight) of the particular mounted matrix analyzed. Since there were no significant differences in results between the two serum changing schedules and at no point did the released progesterone exceed 10% of its maximum solubility in serum, near perfect sink conditions existed throughout the experiment.

***In Vivo* Study**—Intravaginal devices were prepared by injection molding the silicone drug mixture containing catalyst around a 3.5 × 28.5 × 0.1-cm stainless steel strip set in a die such that both sides of the strip had 1.5 mm of matrix attached. The cured progesterone silicone device was then formed into a coil with a diameter of ~4 cm and a length of ~12 cm before intravaginal implantation into cows as described by Roche (1).

Devices were removed at the end of Days 2, 6, 9, 12, and 16. The silicone matrix of the inside and outside surfaces was peeled from the steel and analyzed for progesterone content. As was done in the *in vitro* studies, the progesterone released was determined by difference from the initial progesterone content of the device.

Analytical—Progesterone was extracted from the silicone matrixes by soaking in ethyl acetate, and the extracted drug was analyzed by flame-ionization GLC using bis(2-ethylhexyl) sebacate[3] as an internal standard. The column was a 0.31-cm × 1.22-m (0.125-in. × 4-ft) glass U-tube[4] containing 3% Dexsil on Chromosorb W Hp[3]. The flow rates (milliliters per minute) were: air, 300; hydrogen, 25; and nitrogen, 40. The temperatures were: injection, 250°; column, 240°; and detector, 250°.

The method described is accurate with 100% recovery on spiked samples and a coefficient of variation less than ±3%.

RESULTS AND DISCUSSION

The release rate of drugs suspended in a stationary matrix was described theoretically (4) to be a linear function of the square root of time. With the assumptions that (*a*) a quasi-steady state exists, (*b*) the drug particles are small compared to the average distance of diffusion, and (*c*) perfect sink conditions exist in the external media, the basic equation of interest for planar systems is:

$$Q = [D(2A - C_s)C_s t]^{1/2} \quad \text{(Eq. 1)}$$

where Q is the amount of drug released after time t per unit of exposed area, A is the initial drug concentration, C_s is the solubility of the drug in the matrix, and D is the effective diffusivity through the matrix.

For a given matrix system, D, A, and C_s remain constant; therefore, Eq. 1 can be expressed as:

$$Q = kt^{1/2} \quad \text{(Eq. 2)}$$

where the release rate constant:

$$k = [D(2A - C_s)C_s]^{1/2} \quad \text{(Eq. 3)}$$

Results of the first *in vitro* experiment (Fig. 1), using the 6.75% progesterone silicone matrix, dramatically demonstrate the relationship in Eq. 2, with a matrix-controlled rate constant, k, of 2.425 mg/cm^2 day$^{1/2}$ ± 4% ($p < 0.05$ and $r = 0.995$). Extrapolation to the x axis indicates a lag time of 6 hr before reaching steady state. This lag time is generally seen in systems of this type, and it has been attributed to drug release being controlled by a matrix-boundary diffusion layer model in the early stages (5).

The Higuchi equation describing matrix-controlled drug release (Eq. 1) also dictates that the rate constant should be proportional to the square root of drug concentration as long as $2A$ is much greater than C_s. This

[3] Applied Science Laboratories, State College, PA 16801.
[4] Varian 2100.

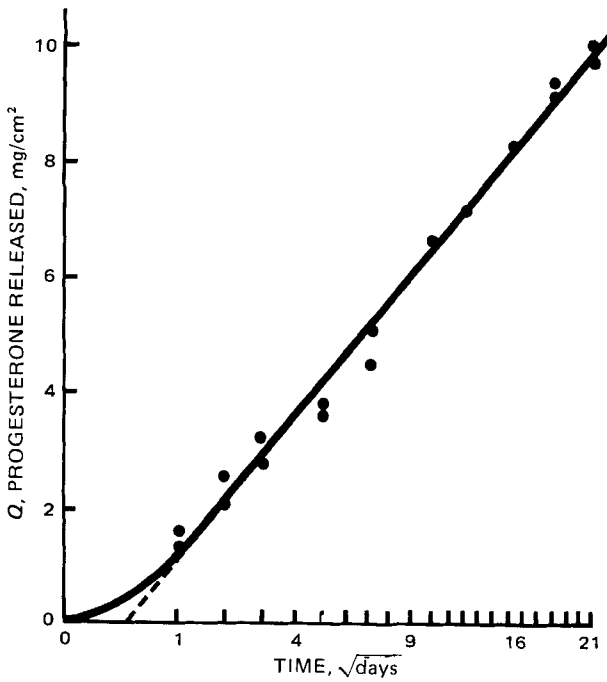

Figure 1—In vitro *progesterone release as a function of the square root of time from a silicone matrix containing 6.75% drug incubated at 37° in sheep serum. Matrix-controlled release rate* ($k = Q/t^{1/2}$ *mg/cm*^{2} *day*$^{1/2}$) *is 2.425 ± 4% (*$p < 0.05$ *and* $r = 0.995$*). Based on extrapolated intercepts, there is a 0.25-day lag time before reaching steady state.*

should be the case in the progesterone-silicone matrix system for which Roseman (6) reported a C_s value of 0.513 mg/ml. The relationship may be expressed as:

$$\frac{k_1}{k_2} = \frac{\sqrt{A_1}}{\sqrt{A_2}} \quad \text{(Eq. 4)}$$

Chien *et al.* (7) reported this relationship in a study of ethynodiol diacetate in silicone matrixes. Results of the second *in vitro* experiment, comparing progesterone release from matrixes containing 5, 7.5, and 10% drug, are shown in Table I. The rate constants show increasing values with drug concentration as predicted, although the magnitude of increase is somewhat less than that expected from Eq. 4. When using the 7.5% rate

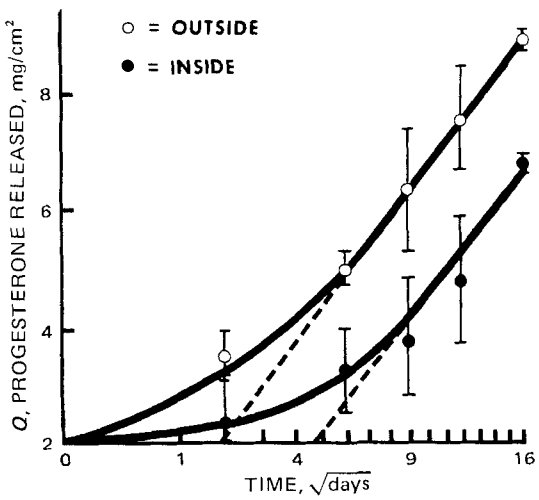

Figure 2—In vivo *cumulative progesterone release* ±SD *as a linear function of the square root of time from separate outside and inside surfaces of a silicone matrix intravaginal device containing 7.8% drug implanted in cows. The following matrix-controlled release rates (in milligrams per square centimeter day*$^{1/2}$*) were calculated from the 6-, 9-, 12-, and 16-day means shown in Table II: outside rate,* $k_o = 2.597 \pm 23\%$ ($p < 0.05$ *and* $r = 0.998$*); and inside rate,* $k_i = 2.359 \pm 87\%$ ($p < 0.05$ *and* $r = 0.959$*). Based on extrapolated intercepts, there are 1.7- and 4.2-day lag times before reaching steady state for the outside and inside surfaces, respectively.*

Table II—*In Vivo* Progesterone Release from the Outside and Inside Surfaces of 7.8% Silicone Matrix Intravaginal Coils Implanted in Cows

Day	Device Section	Cumulative Progesterone Released per Unit of Exposed Area, mg/cm²						Mean	±SD
2	Outside	2.02	1.83	1.18	1.08	—	—	1.53	0.47
	Inside	1.23	0.36	0.56	−0.83[a]	—	—	0.33	0.86
	Total	1.62	1.10	1.37	0.13	—	—	1.06	0.75
6	Outside	3.42	3.06	2.66	3.01	—	—	3.04	0.31
	Inside	0.94	1.98	0.16	1.58	—	—	1.17	0.80
	Total	2.18	2.52	1.41	2.29	—	—	2.10	0.48
9	Outside	3.93	5.39	3.25	—	—	—	4.19	1.09
	Inside	1.16	2.79	1.03	—	—	—	1.66	0.98
	Total	2.55	4.09	2.14	—	—	—	2.93	1.03
12	Outside	5.46	5.10	5.67	7.25	5.71	4.33	5.59	0.96
	Inside	2.56	2.95	4.07	0.75	3.06	3.20	2.77	1.10
	Total	4.01	4.03	4.87	4.00	4.39	3.77	4.18	0.39
16	Outside	6.86	7.15	7.03	—	—	—	7.01	0.15
	Inside	4.68	5.08	4.74	—	—	—	4.83	0.21
	Total	5.77	6.12	5.89	—	—	—	5.92	0.18

[a] Negative value results from positive variation in actual progesterone content of individual implant from assumed initial progesterone content. Thus, percent drug remaining after a 2-day implantation was greater than the theoretical 7.8%. Since averages are used in determining drug release, the negative value must be included so as not to bias the final result.

as reference, the 5 and 10% rates are 108 and 97.9% of expected theory.

It is intriguing to speculate that an increase in drug concentration increases the diffusion pathway, thus decreasing the effective diffusion coefficient, D. Such a process is conceivable, because a "sponge effect" is created in the matrix as insoluble drug is removed by the matrix diffusion process. The higher the drug concentration, the more "holes" exist; consequently, there is an increased migration distance for soluble drug before passing into the external media. When using C_s at 0.513 (6), the effective diffusion coefficients are (Eq. 3) 0.0445, 0.0337, and 0.0285 cm²/day for the 5, 7.5, and 10% matrixes, respectively. The net result of such a decrease in the effective diffusion coefficients is a less than expected increase in rates with respect to increasing drug concentrations. There is no doubt, however, that the release of progesterone is matrix controlled.

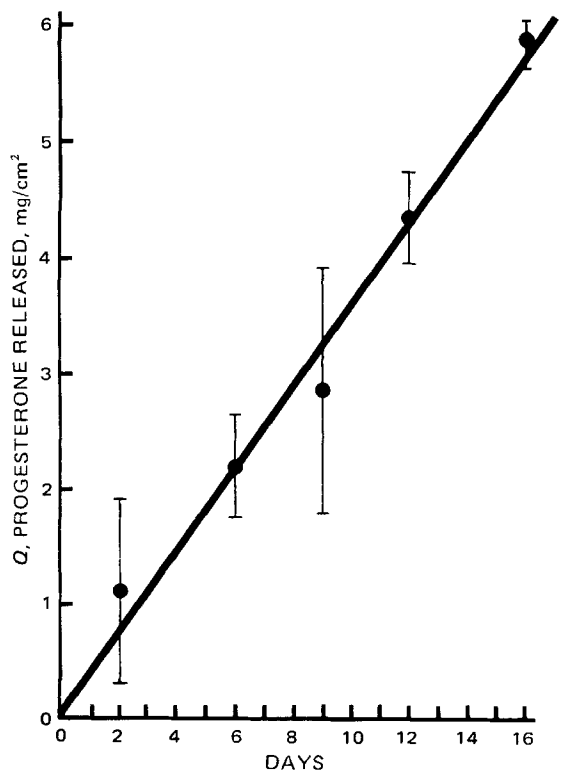

Figure 3—In vivo *cumulative progesterone release* ±SD *as a linear function of time from total outside and inside surfaces of a silicone matrix intravaginal device containing 7.8% drug implanted in cows. When using mean* Q *values shown in Table II including Day 0, the zero-order rate* (k_t = Q/t *mg/cm² day) is 0.357* ± *24%* ($p < 0.05$ *and* $r = 0.991$).

Results of the *in vivo* progesterone release from the outside and inside surfaces of 7.8% progesterone intravaginal devices implanted in cows are shown in Table II. The mean release values with respect to time (Q) are greater with less variation for the outer surface than for the inner surface of the coils at all time periods. The release rates as a function of the square root of time from the separate outside and inside surfaces are graphically shown in Fig. 2. When using the mean Q values from Days 6 through 16 in Table II, the following matrix-controlled release rates are calculated (in milligrams per square centimeter day$^{1/2}$): outside rate, $k_o = 2.597 \pm 23\%$ ($p < 0.05$ and $r = 0.998$); and inside rate, $k_i = 2.359 \pm 87\%$ ($p < 0.05$ and $r = 0.959$). These results are in excellent agreement with those obtained in the *in vitro* system and demonstrate progesterone release from the coils to be a matrix-controlled process *in vivo*.

Based on extrapolated intercepts, there are 1.7- and 4.2-day lag times before reaching steady state for the outside and inside surfaces, respectively. The shorter lag time for the outer surface accounts for its greater release values and less variation. The longer lag time for the inner surface likewise accounts for the smaller release values and greater variation. The difference between lag times may be attributed to physical phenomena such as wetting of the polymer surface, establishment of a steady-state diffusion gradient, and contact with the vaginal wall.

The most remarkable effect of the time lag difference between the two surfaces is the establishment of a pseudo-zero-order drug-releasing device. Indeed, as shown in Fig. 3, the amount of progesterone released per day is approximately uniform. When using the mean Q values including Day 0, the zero-order rate ($k_t = Q/t$ mg/cm² day) for 16 days is 0.357 ± 24% ($p < 0.05$ and $r = 0.991$). The net 16-day progesterone dose administered to the cow implanted with the described intravaginal device having a surface area of 224 cm² is 80 ± 19 mg/day ($p < 0.05$). This linear dose rate is achieved by superimposing *via* time lag the nonlinear matrix-controlled dose rate of the outer and inner surfaces of the implanted device.

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