Development of an Estriol-Releasing Intrauterine Device

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Abstract □ Estriol-releasing intrauterine devices were developed for experimental use in animals and humans. The devices consist of a reservoir containing the steroid surrounded by a rate-limiting polyurethane membrane. After an initial transient, the drug is released from the device at a constant rate for 1 year or more; devices with a much longer release period can be fabricated readily. A constant release rate is achieved by maintaining solid drug in the reservoir and good physical contact between the drug and the inside wall of the device. The methods used to fabricate the devices are described along with release rate and stability data.

Keyphrases □ Intrauterine devices—estriol releasing, prepared, release rate and stability evaluated □ Estriol—delivered by intrauterine devices, release rate and stability evaluated □ Dosage forms—intrauterine devices, estriol releasing, prepared, release rate and stability evaluated □ Estrogens—estriol, delivered by intrauterine devices, release rate and stability evaluated

Scommegna *et al.* (1) first described the preparation and use of a drug-releasing intrauterine device (IUD) for fertility control. A low dose of a steroid, applied locally to the entrometrial tissue of the uterus, provided a contraceptive effect by a mechanism other than simple irritation with its concomitant side effects. The delivery of the naturally occurring steroid progesterone at a constant dosage rate of 70 μg/day (or 26 mg/year) from such an IUD can maintain contraception (2–7). This dosage rate is well below that required to maintain contraception when the same drug is given systemically.

This paper describes the development and *in vitro* behavior of two similar estriol-releasing IUD's. One device is intended for use in experimental studies in animals; the other is intended for use in humans, following approval by the Food and Drug Administration. Prior animal studies (8) suggested that estriol is effective at concentrations lower than those required with progesterone, suggesting that such a device could be effective *in vivo* for several years.

The design of the human device closely follows that developed earlier (5), which, in turn, was based on the "T" shape first proposed by Tatum (9). The design of the device and its approximate dimensions are presented in Fig. 1. The devices for use in animals consisted essentially of only the stem of the T shown in Fig. 1. That is, they were rod-shaped devices, with a nominal outside diameter of 2–3 mm and lengths of 1–2 cm.

Devices with a range of release rates from 0.5 to 12.5 μg/day were required for use in animals to study the effect of release rate on contraceptive efficacy, tissue development, and other physiological parameters. Furthermore, the release rates had to be essentially invariant over the intended period of use, *i.e.*, from several months to 1 year or more.

THEORETICAL

Constant or zero-order release of a drug from a device can be achieved by maintaining a constant thermodynamic driving force across a fixed, rate-limiting membrane. This concept follows from Fick's first or steady-state law. Thus, the flux, J, across a membrane in one dimension is given by:

$$J = D\,\Delta C_m/l \qquad \text{(Eq. 1)}$$

where ΔC_m is the difference in drug concentration within the membrane of thickness l, and D is the diffusion coefficient of the drug in the membrane. The drug concentration within the membrane is simply related to that in the external phases in contact with the membrane through a partition coefficient, K. Thus, Eq. 1 can be restated in more useful terms as:

$$J = DK\,\Delta C/l \qquad \text{(Eq. 2)}$$

where ΔC is the concentration difference in the two solutions external to the membrane. Therefore, the flux will be constant as long as ΔC is maintained constant.

The developed devices were conceptually of this type. Solid drug was contained within the reservoir created by the tube-shaped stem of the T shown in Fig. 1. The steady-state release rate from such a cylindrical reservoir device is given by (10):

$$Q = \frac{2\pi hDK\,\Delta C}{\ln(r_o/r_i)} \qquad \text{(Eq. 3)}$$

where Q is expressed in mass per unit time (*e.g.*, micrograms per day), h is the filled length of the cylinder, and r_o and r_i are the outer and inner radii, respectively, of the cylinder. With solid drug present in the reservoir, the internal concentration is fixed; when the IUD is in use *in vivo*, the external concentration also is fixed at essentially zero. Thus, the release rate remains constant and is determined only by device dimensions and the permeability of the rate-limiting membrane.

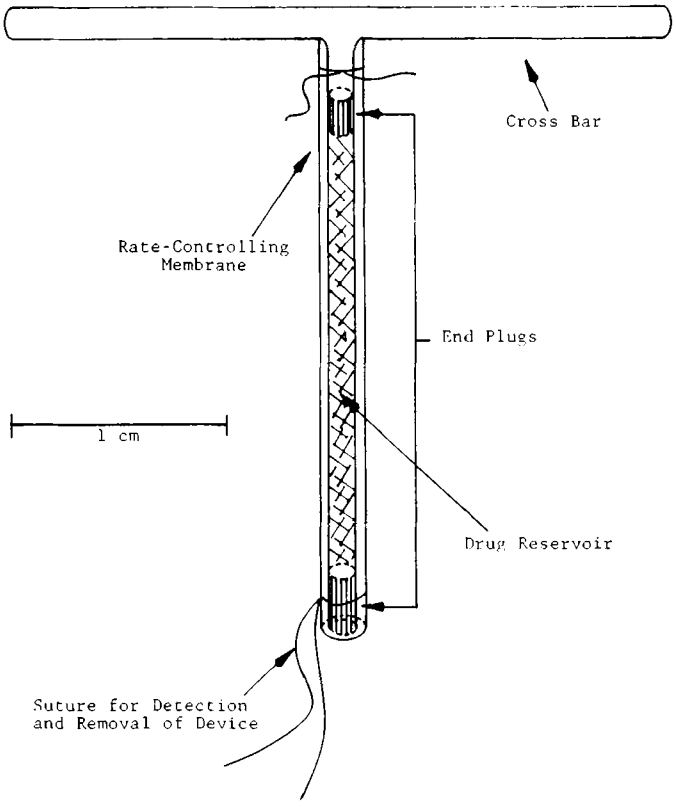

Figure 1—*Schematic of the drug-delivering IUD.*

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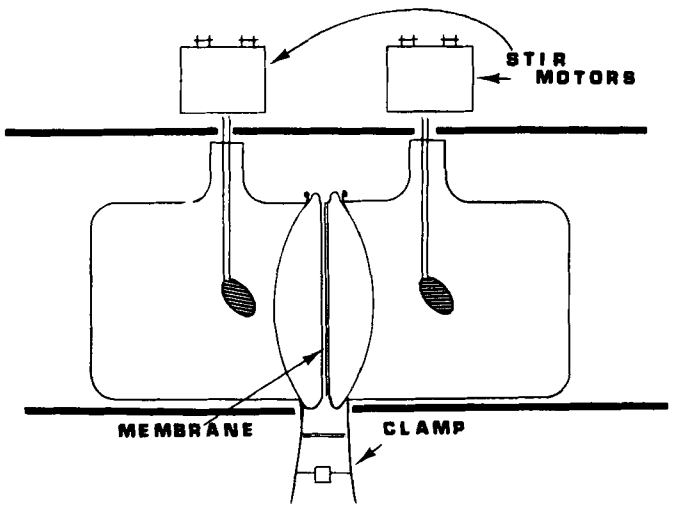

Figure 2—*Permeability cell.*

EXPERIMENTAL

Constraints—Several constraints on the device affected material selection and the final design:

1. The reservoir must hold sufficient drug to achieve constant release for the intended period of use. For devices releasing 12.5 μg of estriol/day for more than 1 year, the reservoir must be sufficiently large to hold at least 5 mg of estriol; *i.e.*, the reservoir volume must be at least 5×10^{-3} cm^3. Because of the necessity to include excipients, the minimum acceptable reservoir volume is about 10×10^{-3} cm^3.
2. The device must be flexible, so as to be nonirritating, but sufficiently strong to withstand uterine contractions without expulsion or failure of the device walls. Furthermore, the walls must be sufficiently thick that they can be fabricated to a reproducible thickness. In practice, these constraints limited the usable range of wall thicknesses to 0.15–0.50 mm.
3. The portion of the reservoir to be filled with drug must be less than the total length of the device, to allow for plugging the ends, but sufficient to allow the reservoir to be filled reproducibly. The desired range of filled reservoir lengths is 5–15 mm.

With reference now to Eq. 3, these constraints and the required range of drug release rates define the quantities Q, h, r_o, and r_i. This equation then defines the requisite permeability of the membrane to the drug. In the present work, a "normalized flux" is used to express this permeability. The normalized flux is the flux, expressed in micrograms per square centimeter per day, through a flat sheet membrane 1 mm thick when one side of the membrane is exposed to a saturated solution of the permeant and the other side is maintained free of permeant. The normalized flux is thus $DK\ \Delta C$ (or Jl in Eq. 2), and it has dimensions micrograms-millimeters per square centimeter-day. The permissible range of normalized fluxes that will satisfy all device constraints is 1–6 μg-mm/cm^2-day, and the desired value is approximately 4 μg-mm/cm^2-day. With a single rate-limiting membrane material, all the required release rates can be achieved, by appropriate changes in reservoir length and wall thickness, if the permeability of the membrane to the drug is of this magnitude.

Permeabilities—Membrane permeability measurements were carried out in conventional, two-compartment, stirred glass cells at 37° (Fig. 2).

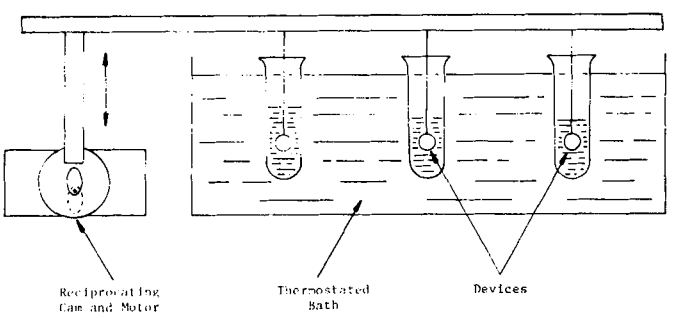

Figure 3—*Apparatus used to measure release rates from devices.*

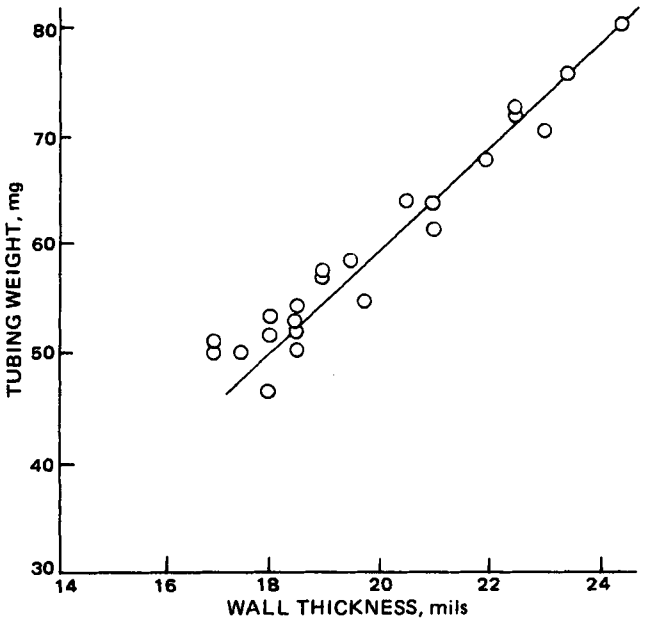

Figure 4—*Weight* versus *wall thickness for 20-mm long 45-mil bore Polymer 12 tubing.*

One compartment was filled with a saturated solution of estriol while the other compartment was maintained at a concentration (<1 ppm) well below saturation (~15 ppm for estriol). Stirring sufficient to minimize boundary layer effects was maintained in both cell compartments. The low concentration compartment was sampled periodically by pipetting a small, known aliquot and replacing it with fresh solution.

Test films for permeability measurements were prepared by conventional methods, *i.e.*, melt pressing or solution casting. Some films were used as supplied by the manufacturer. Others were annealed in air to alter their permeability. Silicone rubber films were prepared by melt pressing the unvulcanized polymer in the presence of a peroxide catalyst at 100° to effect cross-linking.

Release rate measurements on assembled devices were made by periodically suspending the devices from a reciprocating arm in 25-ml vials of distilled water at 37° for about 1 day (Fig. 3). This elution solution then was analyzed. Between release rate measurements, the devices were stored in a flowing water bath at 37°.

The permeability and release rate data were obtained using radiolabeled estriol and standard scintillation counting techniques.

A number of rubbery polymers were initially screened for their permeability to estriol, including three grades of silicone rubber, chlorinated polyethylene, three grades of ethylene–vinyl acetate copolymers of varying vinyl acetate content, and several ester-based and ether-based polyurethanes.

Device Fabrication—Two types of devices were prepared: animal devices, which were simple tubes containing the drug and plugged at both ends, and human devices, which were similar filled tubes sutured to an inert cross bar (Fig. 1).

The tubing was made by dip coating stainless steel rods with a 10% (w/v) solution of polyurethane Polymer 12[1] in tetrahydrofuran. Typically, a rod was immersed in the viscous polymer solution and then slowly and smoothly withdrawn vertically from the solution with a motor drive. The tetrahydrofuran was allowed to evaporate, and the dipping was repeated a sufficient number of times to build up a wall of sufficient thickness. Once completed, the tubing was removed from the rod by swelling it by brief immersion in ethanol or methanol. About 30 cm of the rod was coated with polymer, and there was a slight variation in thickness along the length of the tubing caused by gravity flow of the polymer solution during the removal and drying steps. Therefore, the tubing was cut into short lengths, somewhat longer than required for the devices. Thickness variation along these segments was not significant.

All the required devices could be fabricated from tubing made with stainless steel rods of three different diameters: 29, 45, and 63 mils (0.73, 1.13, and 1.58 mm, respectively); compensation for wall thickness variations could be made through variations in the filled length. Initially, the

[1] Estane 5714.

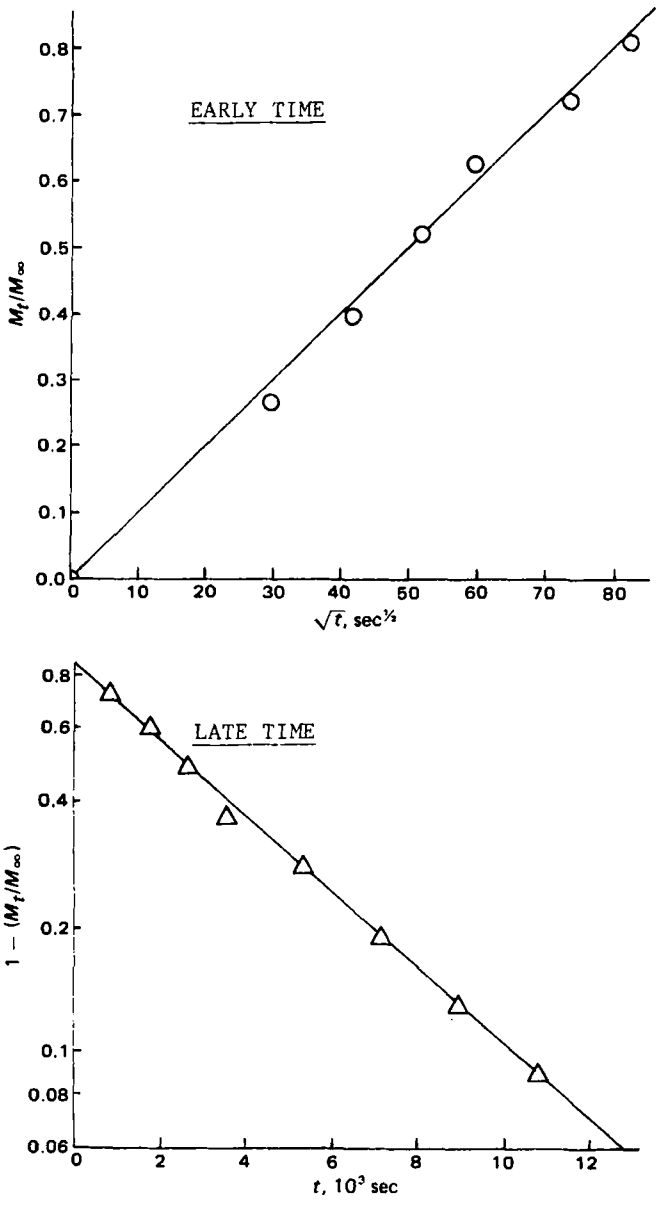

Figure 5—*Estriol desorption from Polymer 12 membranes.*

wall thickness was determined by measuring the outside diameters individually with a microscope. Later, however, a simpler and more reliable method was developed in which tubing of 20 mm length was weighed and a correlation between weight and wall thickness was established (Fig. 4). Tubing of a given inside diameter was then simply weighed, and its wall thickness was read off the curve. The release rate was then fixed by appropriate adjustment in the length of the tubing to be filled.

Devices were prepared by: (*a*) taking a piece of tubing of the correct length and selected wall thickness, (*b*) creating a plug of nominally 2-mm length in one end with a room temperature-curing silicone resin[2], (*c*) filling the tubing with powdered estriol or a suspension of estriol in a suitable drug carrier, including 6–10% (w/w) of barium sulfate added to render the devices radioopaque, and (*d*) sealing the other end with another 2-mm plug of the silicone resin. A short length of 4–0 nylon monofilament, to be used to remove the devices from the animals, was then sutured through one plugged end.

Several liquid carriers for the estriol in the reservoir of the device were evaluated, including water, cocoa butter, and a triglyceride[3]. The evaluation was based on release rate measurements made with devices containing a fixed amount of carrier but decreasing amounts of drug to duplicate the depletion that would occur in use.

All devices were sterilized with pure ethylene oxide for 15 hr by standard procedures.

Clinical devices and the individual components were subjected to a series of stability tests. The purity and stability of the estriol prior to and during these tests were determined by standard GLC and TLC methods. The tests were run for 6 months at storage temperatures of 0, 25, 37, and 50°. The stability of the components was checked individually, in combination with one another, and in the presence of water.

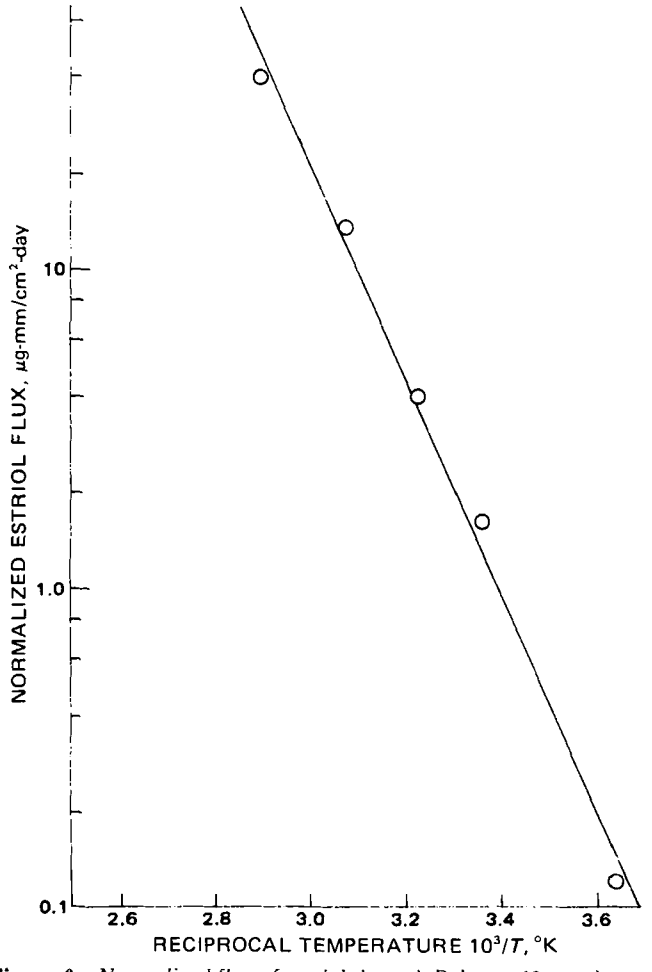

Figure 6—*Normalized flux of estriol through Polymer 12 membranes* versus *reciprocal temperature.*

RESULTS AND DISCUSSION

Permeabilities—Membrane permeabilities, expressed in terms of the normalized flux, are presented in Table I. The tabulated results are generally averages of two runs on separate films; reproducibility was generally quite good. Of the membranes tested, only the polyurethanes had the requisite permeability (Table I). The ester-based polyurethanes were discounted because of their instability to hydrolysis during use *in*

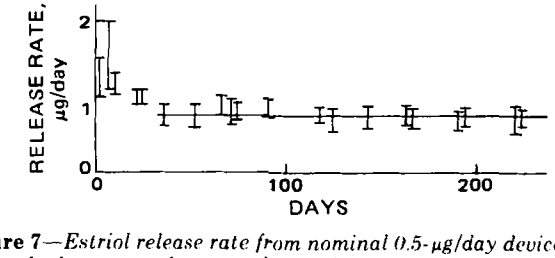

Figure 7—*Estriol release rate from nominal 0.5-μg/day devices. The range of release rates from two devices is shown.*

[2] Dow Corning Silastic A.
[3] Dynamit Nobel Witespol H-5.

Table I—Permeability of Polymers to Estriol

Polymer Generic Name	Normalized Flux, μg-mm/cm²-day
Silicone rubber[a]	<0.1
Silicone rubber[b]	<0.1
Silicone rubber[c]	<0.1
Chlorinated polyethylene[d]	<0.1
Chlorinated polyethylene[e]	<0.1
Ethylene vinyl acetate[f] (9% vinyl acetate)	<0.1
Ethylene vinyl acetate[g] (18% vinyl acetate)	~0.1
Ethylene vinyl acetate[h] (40% vinyl acetate)	0.8
Polyurethane (ester)[i]	<0.1
Polyurethane (ester)[j]	0.4
Polyurethane (ester)[k]	2.7
Polyurethane (ester)[l]	0.3
Polyurethane (ether)[m]	1.9
Polyurethane (ether)[n]	0.3
Polyurethane (ether)[o]	3.3

[a] Dow Corning, 46514. [b] Dow Corning, 46515. [c] Dow Corning, 46516. [d] Dow Chemical, 3623. [e] Dow Chemical, 4213. [f] USI, UE638. [g] USI, UE630. [h] DuPont, Elvax 40. [i] Goodrich, Tuftane 800. [j] Uniroyal, Roylar E9. [k] Goodrich, Estane 5702. [l] Goodrich, Estane 5710. [m] Uniroyal, Roylar 84N. [n] Upjohn, Pellethane 80A. [o] Goodrich, Estane 5714.

vivo (11). Therefore, a more detailed study of the permeability of ether-based polyurethanes was carried out (Table II).

Several materials exhibited the required permeability. However, the permeability of Polymer 10 and 11 films was history dependent. When the manufacturer-supplied film was heated in air for several hours at 120°, the permeability to estriol increased severalfold. Polymer 12 appeared to offer the best combination of properties, and all the devices were prepared from this material.

To characterize further the Polymer 12 membranes, the solubility and diffusivity of estriol were measured by the sorption–desorption method (12). A film of the polymer was immersed in a saturated aqueous solution of estriol until equilibrium was obtained and the film was saturated with drug. The film was removed from the solution, rapidly rinsed to remove any solution adhering to the surface, and placed in a large volume of pure water. The rate of desorption of drug from the film was measured by periodically removing and analyzing samples of the water.

The diffusion coefficient was calculated by applying Fick's second law. There are two approximate solutions to this law for the present boundary conditions, an early time solution given by:

$$\frac{M_t}{M_\infty} = 4(Dt/\pi l^2)^{1/2} \qquad \text{for } 0 \le M_t/M_\infty \le 0.6 \qquad \text{(Eq. 4)}$$

and a late-time solution given by:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\exp(-\pi^2 Dt/l^2) \qquad \text{for } 0.4 \le M_t/M_\infty \le 1.0 \quad \text{(Eq. 5)}$$

where M_t is the amount of drug desorbed after time t, M_∞ is the total amount desorbed, and l is the thickness of the film.

Typical plots of experimental data, presented in the manner suggested by Eqs. 4 and 5, are shown in Fig. 5. From such plots, the diffusion coefficient of estriol in Polymer 12 was calculated to be 2×10^{-9} cm²/sec at 30°. The solubility of the drug in the polymer, obtained from the same data, is 0.2% (w/w).

An Arrhenius plot of the normalized flux *versus* temperature is presented in Fig. 6. The slope of the line yields an activation energy of 7 kcal/mole. This value is relatively low, reflecting the rubbery nature of the polymer. Thus, a 1° change in body temperature at 37° would produce a 6% change in release rate, an amount too small to be of concern.

Table II—Estriol Permeability of Several Ether-Based Polyurethanes

Polymer[a]	Method of Preparation	Normalized Flux, μg-mm/cm²-day
1	Melt pressed	16
2	Melt pressed	5.8
3	Solution cast[b]	5.4
4	Solution cast	3.7
5	Solution cast	7.7
6	Solution cast	2.9
7	Melt pressed	6.3
8	Melt pressed	5.5
9	Melt pressed	1.3
10	Supplied as film	0.3–0.6
10	Film heat treated at 120° for 12 hr	2.4
10	Solution cast	2.2
11	Melt pressed	1.4
12	Melt pressed	3.3
12	Solution cast	3.9

[a] Suppliers and trade names are: Polymer 1, K. J. Quinn, PE-49; Polymer 2, K. J. Quinn, PE-55; Polymer 3, K. J. Quinn, PE-55; Polymer 4, K. J. Quinn, PE-18; Polymer 5, K. J. Quinn, PE-19; Polymer 6, Hooker Chemical, Rucothane P-602-3; Polymer 7, Uniroyal, Roylar A-895; Polymer 8, Goodrich, Tuftane TF-410; Polymer 9, American Cyanamid, Cyanaprene 9341; Polymer 10, Upjohn, Pellethane 80A; Polymer 11, Upjohn, Pellethane 55D; and Polymer 12, Goodrich, Estane 5714. [b] All solution-cast films in this table were cast from tetrahydrofuran solutions.

Release Rates—*Animal Devices*—Release rates from animal devices filled with dry estriol are presented in Figs. 7–9. The various release rates were achieved by varying the wall thickness of the device as well as the length of the reservoir. Long-term constant release rates were obtained with all of the devices, usually in reasonable agreement with the target release rates.

Not readily apparent in the figures is the fact that there was generally a complex "burst effect." This effect is more clearly evident in the early portion of the release curves; one is shown in more detail in Fig. 10. There was an obvious peak in the release rate at about 3 days. After approximately 10 days, the rate reached a steady-state value.

For devices freshly prepared, one would expect a "time lag" effect where the release rate is initially less than the steady-state value. For devices stored under conditions where the estriol is not eluted (*e.g.*, stored in air), one would expect a burst effect where the release rate is initially higher than the steady-state rate (10). Because of the way the devices were stored, the observed release rates did not follow either simple pattern.

The device depicted in Fig. 10 had been stored in air at room temperature for 2–4 weeks before the release rate measurements were begun. During this time, the polyurethane walls became saturated with drug at room temperature. When the device was then taken to 37° for release rate measurements, a new equilibrium was approached. It was this discontinuity in estriol concentration in the wall of the device that resulted in the peaked release rate curve shown in Fig. 10. This behavior is typical of that obtained throughout this study, although the magnitude and duration of the burst effect depended on the device geometry. A similar effect was observed in *in vitro* measurements of release rates of testosterone from silicone rubber implant devices (13).

The burst effect was examined in some detail, because of its effect on the early release rate profiles from devices. The time for the flux through a cylinder to reach its steady-state value is equal to about four "time lags"

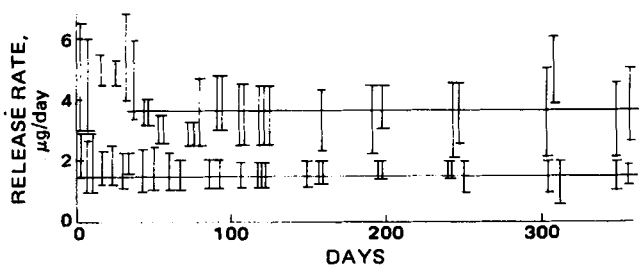

Figure 8—*Estriol release rate from nominal 1.25- and 3.75-μg/day devices. The range of release rates from two devices is shown.*

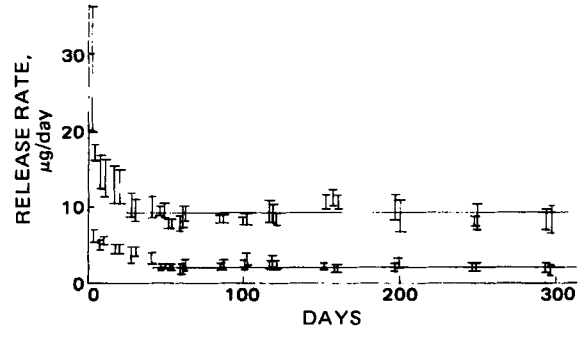

Figure 9—*Estriol release rate from nominal 3- and 9-μg/day devices. The range of release rates from three devices is shown.*

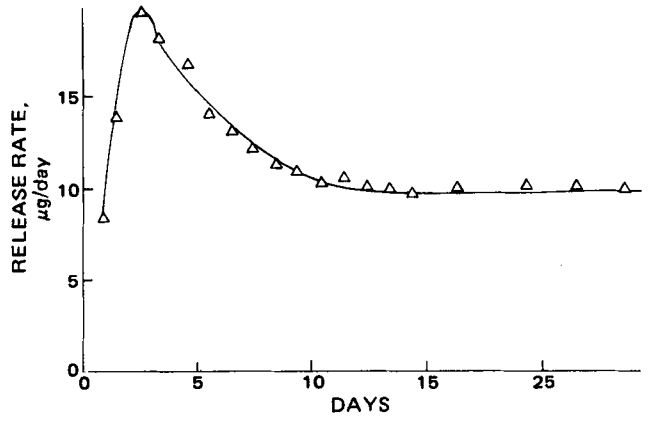

Figure 10—*Early time estriol release rate from a nominal 12.5-μg/day device.*

(14), *i.e.*:

$$\text{time to steady state} = \frac{r_i^2 - r_o^2 + (r_i^2 + r_o^2)\ln(r_o/r_i)}{D \ln(r_o/r_i)} \quad \text{(Eq. 6)}$$

where r_i and r_o are the inner and outer radii of the cylinder, respectively, and D is the diffusion coefficient. With this expression, the time to reach the steady-state release rate from the devices can be computed.

The results are shown in Fig. 11 as a plot of time to steady state *versus* r_o/r_i. Three plots are presented, one for each of the radii used. The time to steady state also is denoted on the figure. There was some variation in these times because of the range of wall thicknesses used to prepare each set of devices. The small-bore, thick-walled devices (*i.e.*, those with large r_o/r_i) with low steady-state release rates required a long time to reach steady state; *i.e.*, they exhibited a prolonged burst effect. The thin-walled devices with higher release rates had a shorter burst effect. Release rates measured after the burst effect agreed reasonably well with values predicted on the basis of permeability measurements.

Release rate data for the 12.5-μg/day animal devices are not presented here. The devices prepared for animal studies were, in fact, replicates of

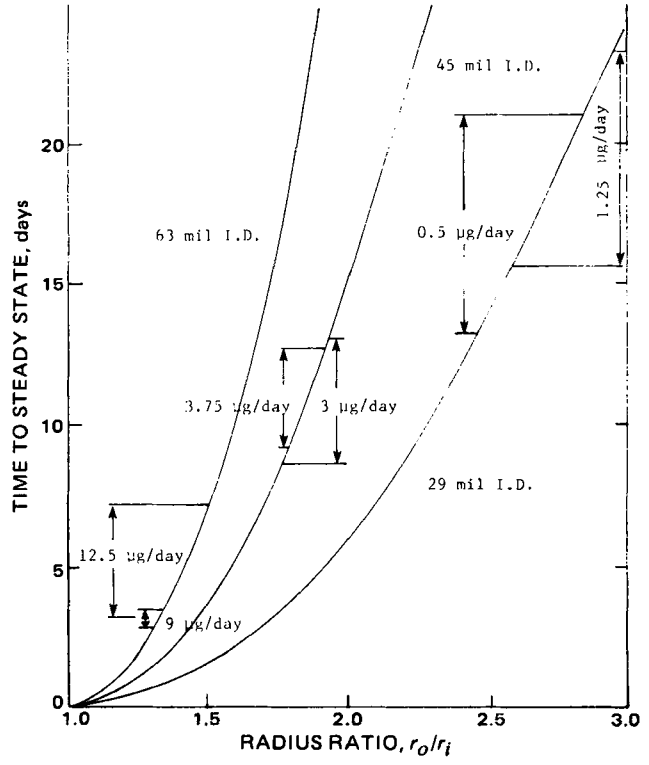

Figure 11—*Time to steady state for a cylinder* versus r_o/r_i. *The three curves refer to the three inner radii. The times to steady state for all of the actual devices are shown.*

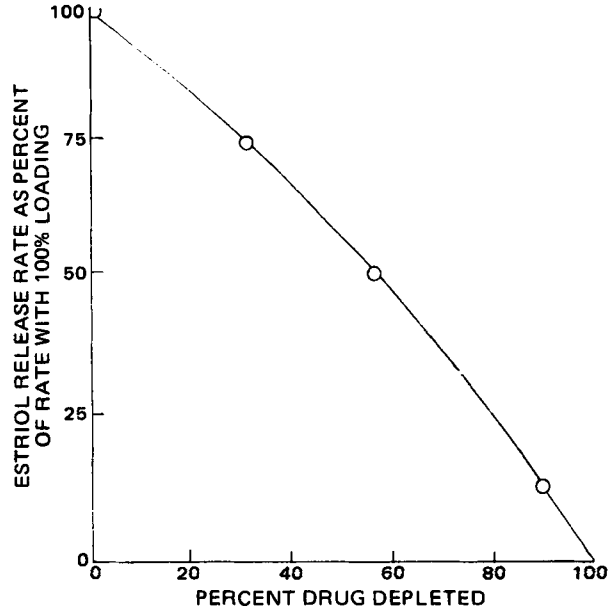

Figure 12—*Estriol release rate as a percentage of the release rate of a fully loaded device* versus *percent of drug depleted.*

the clinical devices. The release characteristics of these devices are discussed under *Clinical Devices.*

One can predict that the release rate from devices filled with only powdered drug will fall with time, because the contact between the drug and the inner wall of the device decreases as the device becomes depleted. This effect was simulated by preparing devices incompletely filled with drug. The measured steady-state release rates, expressed as a percentage of the release rate from a completely filled device, are plotted against the simulated fractional depletion of drug in Fig. 12. This phenomenon did not significantly affect the animal devices, because they contained a several years' supply of drug while the *in vivo* experiments were designed for a duration of only a few months. As discussed in more detail later, drug depletion and the resultant reduction in release rate would be important in devices intended for long-term use in humans.

Clinical Devices—The problem of drug depletion would become acute for devices in use *in vivo* for many months. It can be solved, however, by incorporating a suitable liquid carrier within the reservoir. The carrier is maintained saturated with drug as long as any solid drug is present in

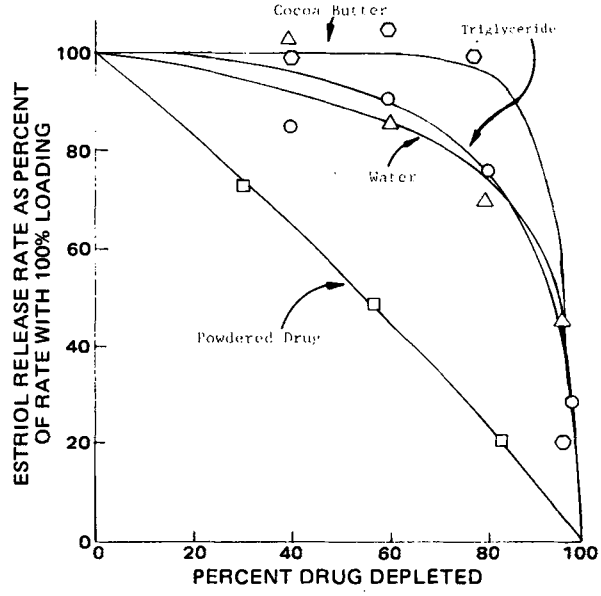

Figure 13—*Estriol release rate as a percentage of the rate of a fully loaded device* versus *drug depletion for devices incorporating different carrier agents.*

Table III—Summary of Device Release Rates

Type	Target Release Rate, μg/day	Actual Steady-State Release Rate, μg/day
Animal	0.5	0.7
	1.25	1.5
	3.0	3
	3.75	3.7
	9	9
	12.5	11
Human	12.5	11

the reservoir. The carrier thus provides intimate contact between the inside wall of the device and the reservoir. The concept of using a saturated solution to achieve a constant driving force within a reservoir dates back to Fick's original 1855 paper. The first application of this phenomenon to achieve controlled release, however, was in 1967 (15). It was further described in a U.S. patent in 1969 (16).

Figure 13 presents the results of carrier tests. Estriol release rate as a percentage of the rate of a fully loaded device is plotted *versus* the drug loading expressed as the percentage of drug depletion. All of the carriers provided a more nearly constant release over the device lifetime than did the dry-filled device. The best results were obtained with cocoa butter, which has a solubility for estriol of 70 μg/ml. Therefore, all of the clinical devices were prepared with cocoa butter in the reservoir.

Release rate data from cocoa butter-filled clinical devices are presented in Fig. 14. There was again a burst effect, following which zero-order release was observed for as long as the test was run. In these experimental human devices, sufficient drug was incorporated to sustain a constant release rate for more than 1 year. By simply incorporating more drug, a device lifetime of at least 5 years could be readily achieved.

Stability of Clinical Devices—GLC and TLC analyses of the estriol and cocoa butter present in the clinical devices showed that both agents were unaffected by the sterilization procedure. No degradation of the components was observed in the stability tests. The release rates of devices stored for 3 and 6 months are compared with the release rates obtained with devices placed on test within 2 weeks of preparation in Fig. 15. As can be seen by comparing these plots, there was no significant change in release rate on storage.

SUMMARY AND CONCLUSIONS

Estriol-releasing IUD's for use in animal studies and T-shaped devices that could be used in clinical trials were developed and subjected to stability tests. The release rates from these devices are summarized in Table III. The devices appear to be stable on long-term storage with respect to both release rate and the chemical nature of the drug and other components.

These devices consist of a simple reservoir containing the drug, surrounded by a rate-limiting polyurethane membrane. For long-term use, it is necessary to include a liquid carrier agent in the reservoir to assure intimate contact with the interior device walls. Devices containing sufficient estriol to provide a constant release rate of 12 μg/day for several years could be made by simple alterations in the geometry of the device.

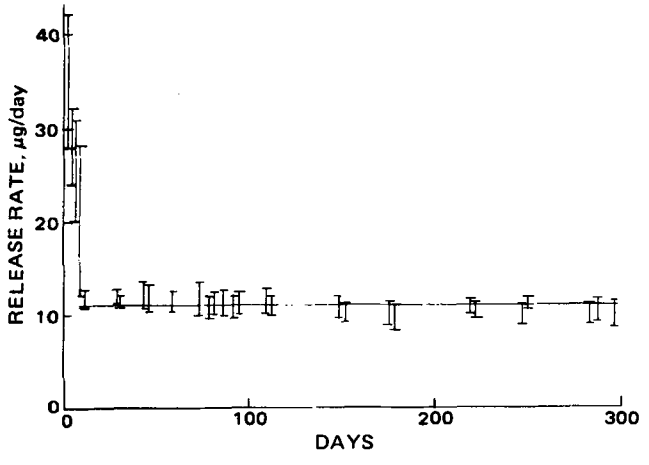

Figure 14—*Estriol release rate from clinical devices* versus *time. The range of release rates from four devices is shown.*

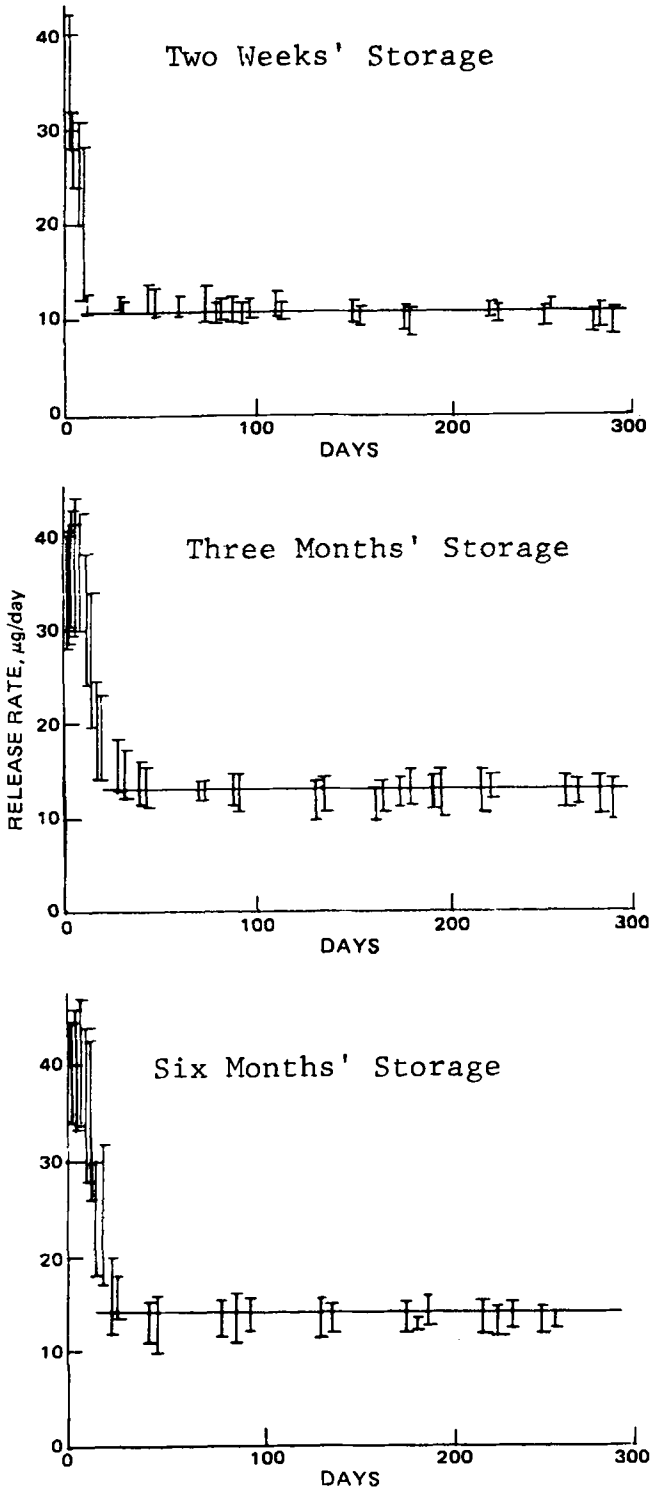

Figure 15—*Estriol release rate from clinical devices stored for 2 weeks, 3 months, and 6 months. The range of release rates from four, eight, and eight devices, respectively, is shown.*

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Pharmacokinetics of Morphine and Its Surrogates II: Methods of Separation of Stabilized Heroin and Its Metabolites from Hydrolyzing Biological Fluids and Applications to Protein Binding and Red Blood Cell Partition Studies

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Abstract □ The inhibition of the spontaneous hydrolysis of heroin in fresh dog plasma and blood ($t_{1/2}$ = 8 min) is effected by 10 mg of sodium fluoride/ml ($t_{1/2}$ = 40 min) and 35 μg of tetraethyl pyrophosphate/ml ($t_{1/2}$ = 415 min). Tetraethyl pyrophosphate is the inhibitor of choice and gives the same stability for heroin as in phosphate buffer. Aged plasma loses its enzymatic efficiency. Heroin in cerebrospinal fluid hydrolyzes at rates similar to those in buffer. Modified extraction procedures developed for enzyme-inhibited plasma at pH 4.5 have high extraction efficiencies (86–100%) and permit isolation of undegraded heroin from its metabolites. Separations of heroin and metabolites from enzyme-inhibited plasma were effected by described high-pressure liquid chromatographic systems and from TLC with elution of pertinent developed spots. Efficiencies of these TLC recoveries were 81 ± 1% for heroin and 82 ± 1% for morphine. Contrary to the literature, heroin has significant protein binding where 40% of that not bound to an ultrafiltration membrane is bound to dog plasma proteins. The apparent partition coefficient is 1.4 ± 0.2 between red blood cells and plasma water, and it is 0.8 ± 0.1 between red blood cells and dog plasma.

Keyphrases □ Heroin—hydrolysis in dog plasma and blood, effect of sodium fluoride and tetraethyl pyrophosphate, protein binding and partition coefficient evaluated □ Hydrolysis—heroin in dog plasma and blood, effect of sodium fluoride and tetraethyl pyrophosphate □ Enzyme inhibition—by sodium fluoride and tetraethyl pyrophosphate, dog plasma and blood, effect on heroin hydrolysis □ Protein binding—heroin in enzyme-inhibited dog plasma □ Partition coefficient—heroin between red blood cells and enzyme-inhibited dog plasma □ Narcotics—heroin, hydrolysis in dog plasma and blood, effect of enzyme inhibitors, protein binding and partition coefficient evaluated

3,6-Diacetylmorphine (heroin) is rapidly hydrolyzed in the body to 6-monoacetylmorphine and then to morphine (1–3). These latter compounds, along with morphine 3-glucuronide, are the major metabolites of heroin excreted in urine (4–8), although minor or negligible amounts of normorphine and its glucuronide as well as morphine 6-glucuronide and 6-acetylmorphine were reported in human urine after heroin administration (9–11).

BACKGROUND

Other than the heroin assay of Way *et al.* (2, 3) on homogenized mice after intravenous administration of heroin ($t_{1/2}$ for heroin = ~2.5 min), the inability to monitor heroin levels in plasma or biological fluid due to its fast hydrolysis and/or metabolism has prevented the study of heroin pharmacokinetics *per se*. Pharmacokinetic studies on heroin administration were thus limited to the estimation of half-lives of urinary excretions of the major heroin metabolites with apparent $t_{1/2}$ values of 1–3 hr (8, 10).

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