Composite Membrane Estradiol Implant

RENÉ BLOCH ˣ, PERRY F. KRAICER*, HANA BINDER, and ESTHER LÖBEL

Abstract □ The diffusion and permeability of large organic molecules through polymeric films were investigated, specifically the passage of estrogen through films of polyethylene, silicone, or a composite of these two films. Such systems are of interest because of the possibility of constructing a biocompatible implant that releases a hormone at a controlled and desired rate. Diffusion and permeability coefficients through implants of a desirable size—successfully tested in rats—were measured by the "time lag" method of diffusion, and solubility coefficients were determined by the method of sorption kinetics. Independently derived values were in good agreement. An interesting observation was made with implications for the controlled release from membrane-based drug delivery systems. Permeability coefficients of estrogen are orders of magnitude higher when estrogen is present as a solid rather than in a dilute solution, while diffusion coefficients are equal. This finding is explained by the considerably higher partial vapor pressure of estrogen in the former case. In fact, control of the partial vapor pressure of the encapsulated drug is a powerful method for controlling its release rate.

Keyphrases □ Estradiol—polyethylene–silicone composite implant, diffusion, permeability, and solubility coefficients, release patterns □ Polymeric films—polyethylene–silicone composite, use as estradiol timed-release implant □ Estrogens—release patterns of estradiol from polyethylene–silicone composite implant □ Implants—estradiol polyethylene–silicone composite, use as a timed-release device

The development of an implant that would permit timed release of steroids has been the subject of numerous publications (1–7). In general, it has been found that the best encapsulating material for this application is a silicone elastomer. The physicochemical properties governing the rate of steroid release through a silicone membrane are most favorable (6–8). Other characteristics of the silicone that recommend its use for implants are its compatibility with tissues, mechanical strength, and flexibility.

For certain applications, however, the rate of release from a silicone elastomer implant is too great. For example, for estrogens it would be advantageous to restrict the rate of release to micrograms per day while retaining the outer coating of silicone elastomer with its excellent biocompatibility.

In this study, it was shown that a composite membrane of polyethylene coated with silicone elastomer is suitable for the fabrication of such implants. Estradiol (free alcohol) was chosen since it is the natural estrogen and therefore would be rapidly catabolized.

The diffusion behavior of estradiol in polyethylene and silicone membranes was evaluated by using both dry powdered estradiol and ethanolic solutions. On the basis of the diffusion behavior, implants suitable for rats were constructed and tested.

THEORETICAL

Transport across membranes as used in this study is governed by Fick's first law. From this law the release rate of a device containing a permeant at a defined concentration, C, can be computed by inserting the appropriate boundary conditions:

$$\frac{dM_t}{dt} = \frac{ADK\,\Delta C}{l} \qquad \text{(Eq. 1)}$$

where M_t is the mass of drug released, dM_t/dt is the steady-state release rate, A is the membrane surface, the product DK is the membrane permeability as D is the diffusion coefficient, and K is the partition coefficient.

The mathematical expression for the time lag, L (11), which can be derived from differentiation of the steady-state expression, is:

$$L = \frac{l^2}{6D} \qquad \text{(Eq. 2)}$$

By plotting M_t against time, the intercept L of the steady-state portion of the plot on the time axis gives the diffusion coefficient D. From the slope of the linear part dM_t/dt, the permeability coefficient DK can be computed.

Independently, the diffusion and solubility coefficients were determined by adsorption and desorption of steroid vapors into the membrane.

The following equation for sorption kinetics (11) limited to very short times was used:

$$\frac{M_t}{M_\infty} = \frac{4}{l}\frac{\sqrt{Dt}}{\sqrt{\pi}} \qquad \text{(Eq. 3)}$$

where M_t is the amount of drug adsorbed at time t, and M_∞ is the maximum amount of drug adsorbed into the membrane. From this equation, by plotting M_t/M_∞ *versus* t or $\sqrt{t}$, the diffusion coefficient can be computed from the initial slope.

The solubility, S, of the steroid in the membrane can be calculated from the maximum amount adsorbed, M_∞, per unit membrane (grams of steroid per gram of polymer).

In cases where a polyethylene membrane was coated with a silicone membrane, it was assumed that the diffusion through the silicone membrane could be neglected because the permeability through the polyethylene was a few orders of magnitude smaller than through the silicone and was, therefore, the rate-determining step.

EXPERIMENTAL

Diffusion and Sorption Experiments—Composite membranes of polyethylene[1] coated with a silicone rubber[2] were prepared by dissolving the silicone elastomer latex plus polymerization catalyst in chloroform at a concentration of 25% and by spreading a thin film on a polyethylene sheet with a membrane casting knife. A uniform layer was obtained with a dry thickness of 60–80 μm. Heat-sealed polyethylene bags were dipped in the same silicone coating solution and then floated overnight on a saturated aqueous sodium chloride solution, when polymerization of the silicone was complete.

The diffusion of estradiol[3] through silicone[4] rubber membranes, polyethylene films, and composite polyethylene membranes coated with silicone was measured by using a thermostated diffusion cell of Perspex in which the membrane separated two compartments. One compartment was filled with a dilute solution of estradiol in ethanol, and the other was filled with ethanol. Solutions were mag-

[1] Plastics U.S. Ltd., Holon, Israel.
[2] Silastic 382 medical grade elastomer, Dow Corning Corp., Midland, Mich.
[3] Ikapharm, Israel.
[4] Midland Silicones Ltd., Reading, U.K.

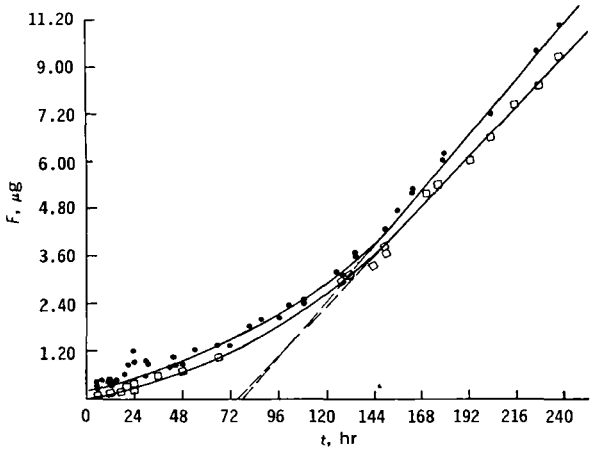

Figure 1—*Total amount, F, of estrogen diffusing through a polyethylene bag as a function of time. Key: ●,□, duplicate experiments with two polyethylene bags.*

netically stirred. The amount of estrogen appearing after diffusion into the second compartment as a function of time was analyzed. Diffusion experiments were made at 25°.

In a different type of experiment, 25 mg of estradiol was sealed by heat into a polyethylene bag with a surface area of 2 cm^2. The estrogen diffusing from the inside of the bag into a bath of alcohol as a function of time was analyzed. Some of these bags were coated with silicone.

Adsorption and desorption of estrogen on membranes were measured with an automatic recording vacuum ultramicrobalance[5] (with an accuracy of $\pm 1 \times 10^{-9}$ g) connected to a vacuum system containing solid estrogen. The membrane adsorbing or desorbing estrogen as a function of time was weighed. Estradiol was assayed by UV spectrophotometry[6] or by GC[7] of the diacetyl derivative (9, 10).

Animal Experiments—The ability of double-membrane encapsulated implants to provide physiological levels of estrogen was tested in ovariectomized adult albino females rats[8] (4–5 months of age). They were ovariectomized through a pair of lumbar incisions using ether anesthesia. Their vaginal smears showed persistence of leukocytes for 21 days.

A subcutaneous implant containing estradiol was made; after ether anesthesia, a small incision was made in the skin at the nape of the neck. The implant was placed under the scalp, between the ears.

RESULTS AND DISCUSSION

Diffusion and Sorption Kinetics—A typical time lag diffusion experiment showing the flux of estradiol through two polyethylene membranes as a function of time is illustrated in Fig. 1. The total

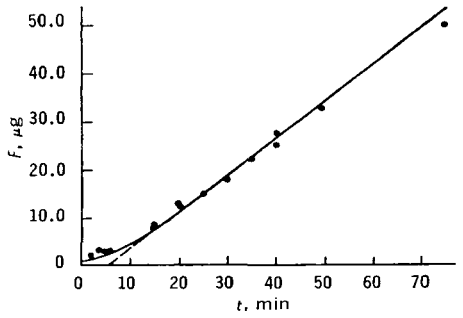

Figure 2—*Total amount, F, of estrogen diffusing through a silicone membrane as a function of time.*

[5] Cahn electrobalance.
[6] Beckman UV spectrophotometer.
[7] Packard model 803 gas chromatograph.
[8] University of Tel-Aviv breeding colony.

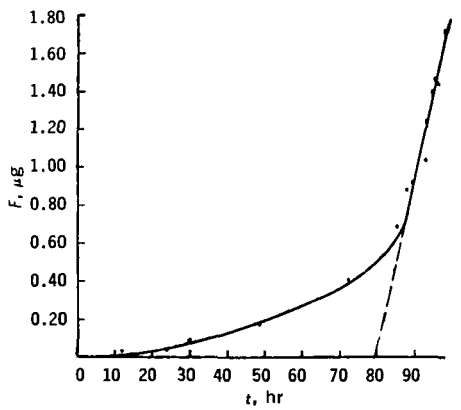

Figure 3—*Total amount, F, of estrogen diffusing through a polyethylene membrane coated with silicone as a function of time.*

areas of the polyethylene bags were 3.7 and 3.2 cm^2, respectively. The membrane thickness was 96 μm. From this plot, the diffusion coefficient, in addition to the solubility of estradiol in polyethylene (being equivalent to its partition coefficient from a saturated solution), was computed.

Figure 2 shows a similar experiment for a silicone membrane having an area of 19.6 cm^2 and a thickness of 70 μm. The same plot for a composite polyethylene–silicone membrane, the polyethylene being exposed to the estrogen solution, is shown in Fig. 3. A typical sorption kinetic experiment showing the fractional amount of estrogen adsorbed and desorbed in a silicone membrane as a function of time is illustrated in Fig. 4. Figure 5 shows the same data for a polyethylene film; the fractional release is plotted as a function of the square root of time and straight lines are observed. From these plots, diffusion coefficients and solubilities for estrogen in the various membranes used also were computed. In the case of sorption experiments, the estrogen was always in a solid form, but its relatively high vapor pressure (10^{-5} mm Hg) enabled it to be evaporated into the vacuum system connected to the microbalance, which was used to weigh the membranes during the experiment.

A summary of fluxes, diffusion coefficients, and solubilities com-

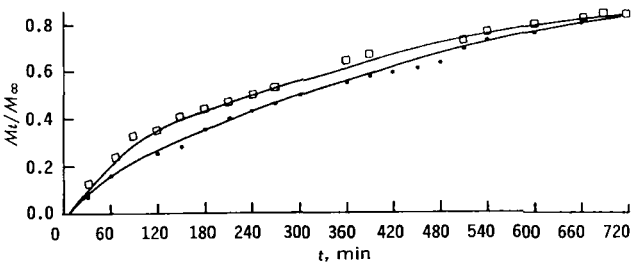

Figure 4—*Adsorption and desorption of estrogen on a silicone membrane as a function of time. Key: □, adsorption; and ●, desorption.*

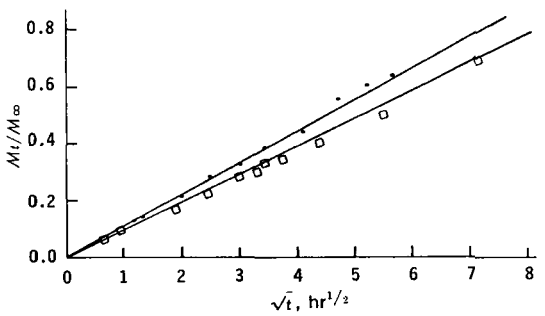

Figure 5—*Adsorption and desorption of estrogen on a polyethylene membrane as a function of time. Key: □, adsorption; and ●, desorption.*

Table I—Solubilities, Diffusion Coefficients, and Fluxes for Estrogen[a] through Silicone Membranes at Thickness l

Method[b]	$S \times 10^3$	$D \times 10^7$ cm²/min	$J \times 10^2$ μg/min cm²	C, %	l, μm	Temperature	System
s.k.	2.27	1.76			70	33°	Membrane–vapor
s.k.	2.55	1.45			70	30°	Membrane–vapor
s.k.	1.07	1.65			70	26°	Membrane–vapor
s.k.	0.99	1.05			70	25°	Membrane–vapor
t.l.		6.9	6	0.166	50	25°	Solution–membrane solvent
t.l.		4.7	5	0.128	70	25°	Solution–membrane solvent
t.l.		3.6	3.5	0.102	70	25°	Solution–membrane solvent

[a] The feed system is either an estrogen solution in alcohol or solid estrogen in powder form. [b] s.k. = sorption kinetics; t.l. = time lag method.

Table II—Solubilities, Diffusion Coefficients, and Fluxes for Estrogen[a] through Polyethylene Membranes of Thickness l

Method[a]	$S \times 10^4$	$D \times 10^9$ cm²/min	$J \times 10^5$ μg/min cm²	C, %	l, μm	Temperature	System
s.k.	0.94	1.28			96	25°	Membrane–vapor
s.k.	1.75	2.7			96	25°	Membrane–vapor
s.k.	1.34	3.7			96	25°	Membrane–vapor
s.k.	1.67	2.3			96	25°	Membrane–vapor
t.l.		3.4	0.77	0.102	96	25°	Solution–membrane solvent
t.l.		6.7	1.97	0.105	38	25°	Solution–membrane solvent
t.l.		4.4	1.4	0.105	53	25°	Solution–membrane solvent
t.l.		3.3	26		96	25°	Solid–membrane solvent
t.l.		3.4	28		96	25°	Solid–membrane solvent

[a] The feed system is either an estrogen solution in alcohol or solid estrogen in powder form. [b] s.k. = sorption kinetics; t.l. = time lag method.

puted from both time lag and sorption kinetic experiments is given in Tables I and II. It can be seen that the diffusion coefficients obtained from the time lag method and from sorption kinetics were different. From sorption kinetics, they were 2.5 times smaller than those found by the time lag method for silicone membranes and 1.3 times smaller for polyethylene membranes. The fact that different results for the diffusion coefficient have been obtained by different measurement methods is not extraordinary but in line with other findings (12, 13). A plausible explanation (12) is that nonstationary states of flow may not yield meaningful values of the interdiffusion coefficients for internally viscous polymeric media, since relaxation processes may take place in the polymer matrix which, in turn, influence the diffusion coefficient. In a steady-state measurement, all relaxation processes must have died away before a steady state can be established. The diffusion coefficient for estrogen through a silicone membrane depends on its concentration in solution.

Table III summarizes a comparison of fluxes of estrogen in a 0.1% alcohol solution through a polyethylene membrane and through a silicone-coated polyethylene membrane. It is apparent that coating with silicone does not affect the flux of the drug through polyethylene. Furthermore, solid estradiol yields a flux that is 35 times higher than that of estrogen in a solution of 0.1%.

Table III—Comparison of Fluxes of Estrogen through a Polyethylene Membrane and through a Polyethylene Membrane Coated with Silicone

J, g cm^{-2} min^{-1}	Diffusion System
7.7×10^{-6}	Solution–polyethylene–solvent
7.6×10^{-6}	Solution–polyethylene:silicone–solvent
2.6×10^{-4}	Solid–polyethylene–solvent
2.3×10^{-4}	Solid–polyethylene:silicone–solvent

Table IV—Diffusion Coefficients Obtained from Time Lag Experiments as a Function of Estrogen Concentration for Silicone Membranes with a Defined Thickness

l, μm	$D \times 10^7$, cm²/min	C, %
70	3.6	0.102
70	4.7	0.128
50	7	0.166

(A saturated solution of estradiol in alcohol is 0.23%.) This surprising result is explained by the higher vapor pressure of pure solid estradiol than of the 0.1% estrogen dissolved in alcohol and by the change of phase from a gas to a liquid system.

The fluxes of estradiol through the membrane as a function of its concentration in a solution and membrane thickness were also determined and found to be in agreement with Fick's law (Table IV). The distribution coefficient of estrogen between alcohol and silicone as computed from the time lag experiment was found to be 0.47; for polyethylene, it was 0.011.

Animal Experiments—In a preliminary study, 12 rats were used. Two days after insertion of silicone-coated polyethylene bags containing estradiol, their vaginal smears became estrous—cornified cells plus some nucleated epithelial cells. The smears remained persistently estrous for 6 months. At autopsy, the uteri were found to be large. The only sign of pathology was gross enlargement of the pituitary in eight of the 12 rats. The persistence of the estrous response indicated that the implants were capable of sustained release of effective quantities of estradiol.

In a second test, eight rats received estradiol implants; six others served as ovariectomized controls. From the 2nd day after implantation, all eight rats bearing implants had persistently cornified vaginal smears. The rats were killed 10 days after insertion of the implants. Weights of the uteri, pituitaries, and adrenals were recorded. The animals were also weighed on the day of ovariectomy, the day of implantation, and the day of autopsy.

The control animals weighed 178 ± 4.6 g on the day of ovariectomy. By the day of autopsy, they had gained 49.7 ± 2.3 g, an average of 1.4 g/day. Estrogen-treated rats weighed 187 ± 4.6 g initially; by 25 days after ovariectomy, they had gained 34.2 ± 2.7 g, an average of 1.4 g/day. During the 10 days following insertion of the implants, they lost 18.2 ± 2.7 g. This weight loss is believed to reflect the increased activity and decreased appetite associated with estrus. Ovariectomized rats are known to fatten due to relative inactivity.

Table V—Effect of an Estradiol Implant on Organ Weights (in mg/100 g Body Weight) of Ovariectomized Rats

Organ	With Implant	Control
Uterus (drained)	235 ± 15.4	47 ± 3.4
Pituitary	9.3 ± 0.36	6.1 ± 0.44
Adrenals	26 ± 4.4	21 ± 0.87

The estradiol released from the implant maintained the weights of the uteri, increased pituitary weights significantly, and had no effect on the adrenal weights (Table V). Five of the eight uteri from the estrogen-treated rats were distended with fluid at autopsy—further evidence of unopposed estrogen action. All of these findings confirm that the implants were able to maintain a physiologically significant level of estradiol in the treated rats.

No inflammations were observed after 8 months of subcutaneous residence.

CONCLUSIONS

The diffusion, flux, and solubility coefficients of estrogen are 100, 1000, and 10 times, respectively, higher through silicone than through polyethylene. The values of diffusion coefficients of estrogen through the membranes derived independently by the time lag method and by sorption kinetics were in good agreement. Coating of silicone on polyethylene had no influence whatever on the flux of estrogen through it.

An interesting phenomenon observed during this study was that permeability coefficients through the membranes were orders of magnitude higher when estrogen was present as a solid rather than in solution, while the diffusion coefficients found by the time lag method were equal in both cases. This finding can be explained by the fact that a larger concentration gradient within the membrane is established in the former case partly because the absolute concentration may be higher in the vapor phase above the solid and partly because of larger distribution coefficient in favor of the membrane. The existence of this phenomenon indicates that control of the partial vapor pressure of the encapsulated drug is a powerful method for controlling its release rate.

An implant of desirable size regulating the flux of estrogen through a polyethylene membrane, coated with silicone, was prepared based on these findings. This implant can be successfully used in rats.

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Hypothermic Response following Administration of 2-Amino-4-pentenoic Acid (Allylglycine)

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Abstract □ Intraperitoneal administration of allylglycine resulted in a hypothermic response in rats. At a dosage of 90 mg/kg, a significant decrease in temperature was noted at 4 and 8 hr after administration, but no significant difference was noted at 12 hr. The same dose administered intraperitoneally to decapitated rats did not result in a hypothermic response, and intraventricular administration resulted in a rapid onset of hypothermia after 1 hr. These findings are indicative of a central site of action for the allylglycine-induced hypothermia. Quantitative assay of hypothalamic monoamines (norepinephrine, serotonin, and dopamine) did not show any significant changes at 4, 8, and 12 hr postadministration when compared to controls. A significant decrease in hypothalamic γ-aminobutyric acid was noted at each of these time points when compared to controls. These data suggest an important role for γ-aminobutyric acid in mammalian thermoregulatory control.

Keyphrases □ Allylglycine (2-amino-4-pentenoic acid)—effect of intraperitoneal and intraventricular administration on hypothermic response in rats □ Hypothermia—effect of intraperitoneal and intraventricular administration of allylglycine, rats □ Thermoregulation—effect of allylglycine intraperitoneal and intraventricular administration on hypothermic response, rats

Feldberg and Myers (1) first proposed that body temperature was regulated by a fine balance in the release of catecholamines and serotonin in the anterior hypothalamus. The hypothesis was based on the responses to intraventricular injection of these agents in the cat. Subsequent investigations showed that there is a distinct species variation in the temperature response, both in magnitude and direction of change, when these agents are administered by the intraventricular route. Feldberg and Lotti (2) showed that intraventricular administration of small doses of norepinephrine in the rat resulted in an increased body temperature, while larger doses had an opposite action. Intraventricular or intracisternal administra-