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Transdermal Dual-Controlled Delivery of Testosterone and Estradiol: (I) Impact of System Design

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Abstract

A multilaminate-type transdermal drug delivery (m-TDD) system was designed with the objective of delivering testosterone and estradiol simultaneously but at different daily dosage rates. To achieve such a dual-controlled transdermal delivery, skin permeation enhancer and permselective membrane were incorporated into the system. The results demonstrated that skin permeation enhancer, which is incorporated into the testosterone reservoir

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layer, and permselective membrane, which is added onto the drug-releasing surface of estradiol reservoir layer, can both alter the overall rate of skin permeation for both drugs. The skin permeation enhancer was observed to enhance the skin permeation rate of testosterone, while the permselective membrane was shown to reduce the skin permeation rate of estradiol. The addition of permselective membrane was observed to modify the release kinetics of estradiol from the matrix diffusion-controlled drug delivery to membrane permeation-controlled drug delivery. Other factors which may affect the deliver rate of drug, e.g., the thickness of permselective membrane and loading dose of drugs, were also studied. Furthermore, evaluation of physical stability of this m-TDD system demonstrated that the inter-layer migration of drugs is at minimum.

Introduction.

One of the major advances in pharmaceutical research and development in recent years has been the successful development and commercialization of several transdermal rate-controlled therapeutic systems, which utilize the controlled drug release technologies to control the delivery of therapeutic agent(s) to the systemic circulation through the intact skin, the so-called "transdermal controlled drug administration" (1). These controlled drug release technologies can be classified into four main categories: (a) membrane-moderated, (b) adhesive dispersion-type, (c) matrix diffusion-controlled, and (d) microreservoir dissolution-controlled transdermal drug delivery system (2). The drug candidates which have been evaluated for their potential use in transdermal systemic medication cover a wide range: from anti-

hypertensive, antianginal, antihistamine, anti-inflammatory and analgesic agents to antiarthritic, steroidal and contraceptive drugs (2).

Different drugs have difference not only in their physicochemical properties, but also in their pharmacological activities. Furthermore, the therapeutic dosage range of a drug may be varied from one clinical application to another . These variations become even more complicated when two drugs or more are delivered simultaneously, which may require different kind of controlled drug release technology or even use a more complex system design in order to deliver multiple drugs from one drug delivery system. In this series of studies, two naturally-occurring sex hormones, testosterone and estradiol, were chosen as the model drugs to investigate the feasibility of delivering, simultaneously, two drugs with rather different daily dosage rate requirements. For achieving male contraception, as one example of the biomedical applications, testosterone needs to be delivered intramuscularly at daily dose as high as 25 mg/day (as propionate) (3), while estrogen (as ethinylestradiol) needs to be administered orally only at a relatively low daily dose of about 30 mcg/day (4). Synergistic action of testosterone and estradiol in inhibiting spermatogenesis was studied in rats by delivery them from a subdermal sustained-release device (5) and results indicated that testosterone (e.g.,30 mcg/day) and estradiol (e.g. 0.01 mcg/day) markedly inhibite spermatogenesis when they are administrated simultaneously, but fail significantly to attain azoospermia when they are given alone. However, the contraception efficacy of this combination in humans needs to be further investigated clinically with optimized daily dosage rate.

In the first part of this investigation, various factors which may affect the dual-controlled delivery of testosterone and estradiol from transdermal drug delivery system were studied; and the results are discussed in this report.

Experimental.

Materials. The following chemicals were used as obtained: testosterone, estradiol, n-decanol (obtained from Sigma Chemical Company, St. Louis, Mo.), polyacrylate adhesive (obtained from National Starch and Chemical corporation, Bridgewater, N.J.), polyisobutylene (obtained from BASF Corporation, Chemicals division, Holland, Michigan).

Male hairless mice (HRS/J strain, 5-7 weeks old) were obtained biweekly (from Jackson Laboratories, Bar Harbor, Maine).

HPLC assay. The high performance liquid chromatographic system used is equipped with a Programmable System Controller (Model 721, Waters Associates, Milford, Mass.), two reciprocating piston pumps (Model 510, Waters Associates), a Wisp auto-injector system (Model 712, Waters Associates), a Programmable Variable Wavelength UV/VIS detector (Model 783, Kratos Analytical Instruments, Ramsey, N.J.) operating at a wavelength of 225 nm, a reversed-phase u-Bondapak C^{18} column (15 cm x 3.0 mm I.D., Waters Associates), and a Data Module recorder (Model 730, Waters Associates). A solvent system prepared from acetonitrile and water at a ratio of 3:1 was used as mobile phase at a flow rate of 0.8 ml/min with column temperature maintained at ambient.

Under these conditions, estradiol and testosterone can be chromatographically separated and shown in well-defined peaks with

retention time of 5.0 min. and 5.8 min., respectively. The detection limit of this HPLC method is 2.5 ng for estradiol and 1.0 ng for testosterone, with high degree injection-to-injection reproducibility in the peak height response (intraday variation < 3.0% and interday variation < 5.0%).

Determination of drug concentration in the sample solutions was carried out by first measuring the peak height of drugs and then computing the concentration (in mcg/ml) from the calibration curves constructed from a series of standard solutions ($r > 0.999$).

Device Fabrication. The drug reservoirs were made by first weighing out the drug and enhancer in a disposable glass bottle, mixing them gentlely with adequate amount of adhesive solution, using a laboratory rotator, to form a drug adhesive solution or dispersion, then coating the drug-adhesive solution (or dispersion) on a drug-impermeable substrate and drying the resultant film overnight at ambient. Permselective membrane was obtained by casting the drug-free polyisobutylene solution on a release liner and then drying at ambient. Very uniform membranes with controlled thickness were obtained for both the drug reservoir layer and for the permselective membrane. The final drug delivery system was fabricated by laminating these membranes together to form multilayered laminate (Fig. 1) and cut it into 5-cm^2 multilaminate transdermal drug delivery (m-TDD) devices.

Physical Examinations. Microscopical examination was conducted with Laborlux 11 Pol microscopy (Kramer Scientific Corporation, Yonker, N.Y.). DSC study was conducted with a Delta series 7 differential scanning calorimeter system (Perkin-Elmer Corporation, Normalk, Connecticut).

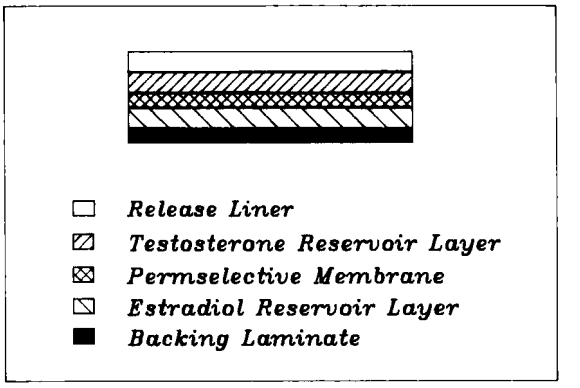

Figure 1: Schematic Illustration of Multilaminate-type Transdermal Drug Delivery (m-TDD) System for Simultaneous, Controlled Skin Permeation of Testosterone and Estradiol.

In-Vitro Release. One 5-cm^2 device was mounted onto each half-cell of one Valia-Chien permeation system with the drug-releasing surface facing the cell. An aliquot (3.5 ml) of the aqueous solution of 40% PEG 400 in normal saline (V/V) was filled into each half-cell as the drug elution medium. Samples (1 ml each) were withdrawn at predetermined time intervals and each half-cell was refilled with same volume of fresh (drug-free) elution medium to maintain the same solution volume. Concentrations of both testosterone and estradiol in each sample were analyzed by the HPLC method described above.

In-Vitro Skin Permeation. The hairless mouse was sacrificed just prior to the experiment by cervical dislocation. Full thickness skin specimen was excised and its dermal surface was carefully cleaned. One piece of the skin specimen was then laid evenly on each of the half-cells with its dermis facing the

solution compartment. One unit of m-TDD device was applied onto the skin with its drug-releasing surface in intimate contact with the stratum corneum. The two half-cells were then clamped together. An aliquot (3.5 ml) of the aqueous solution of 40% PEG 400 in normal saline (V/V) was filled into each half-cell as the drug elution medium. Samples (100 ul each) were withdrawn at predetermined times and assayed for both testosterone and estradiol concentration by the HPLC method.

Calculation. The skin permeation rate was calculated from the slope of the linear region of the permeation profile. Lag time was calculated from the intercept on the time axis by extrapolation from the steady-state skin permeation profile. The release flux was calculated from the slope of the linear region of the Q vs. $t^{1/2}$ release profile.

Results and Discussion.

As expected from the system design (Fig. 1) both testosterone and estradiol are delivered from the m-TDD device at zero-order kinetics, but permeate through the skin at different rates, i.e. testosterone shows a much higher rate of skin permeation than that of estradiol (Fig. 2). Due to the complexity of the system design, any variation in the system design may affect the delivery rate of testosterone and/or estradiol. Some rate-limiting factors in system design were extensively studied and results obtained are discussed as follows:

Modification in Controlled Release Kinetics of Estradiol. According to the primary objective of this series of investigations,

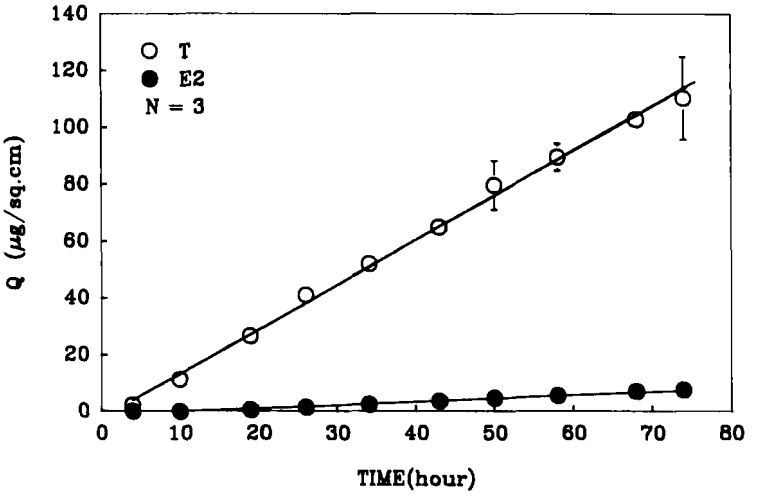

Figure 2: Zero-order Permeation Profiles of Testosterone (T) and Estradiol (E_2) across the Hairless Mouse Skin following Controlled Delivery from the m-TDD Device.

estradiol is expected to be delivered and controlled at a low rate of delivery by using a permselective membrane made of polyisobutylene. To achieve this goal, a polyisobutylene membrane with controlled permeability to estradiol and thickness was laminated onto the drug-releasing surface of estradiol reservoir layer to form bilayer device. The release profiles of estradiol from the monolayer device (containing estradiol reservoir layer alone) and from the bilayer device (containing the composite of permselective membrane/estradiol reservoir layer) are compared in Figure 3. The observation of a linear relationship between the cumulative amount (Q) of estradiol released from the monolayer device and the square root of time indicates that the mechanism of estradiol release from the monolayer device follows the matrix diffusion-controlled drug release process as described by the

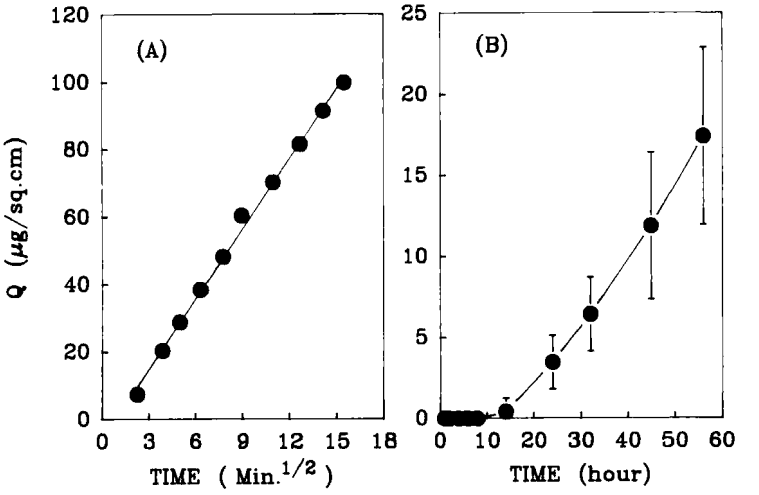

Figure 3: Effect of Permselective Membrane on the Cumulative Release Profiles of Estradiol.
Keys: (A) Q vs $t^{1/2}$ release profile (without permselective membrane; n=3); (B) Q vs t release profile (with permselective membrane; n=3).

classical Higuchi relation (6, 7). On the other hand, the release profile of estradiol, at steady state, from the bilayer device was observed to follow a linear Q vs. t relationship after a lag time. The observed linearity means that the release of estradiol from the bilayer device follows a zero-order release kinetics. In other words, the release of estradiol from the bilayer device is kinetically controlled by the permselective membrane which plays a rate-limiting role in controlling the rate of estradiol release, so the release profile has been modified to deliver at membrane permeation-controlled drug delivery (Q vs. t pattern) from matrix diffusion-controlled drug delivery (Q vs. $t^{1/2}$ pattern).

Effect of Permselective Membrane on Estradiol Skin Permeation. It is commonly known that the greater the resistance a drug molecule encounters in its course of diffusion (or skin permeation), the lower its rate of diffusion (or permeation) will be. Additionally, the total resistance which a drug molecule has to overcome during its course of skin permeation is known to be the reciprocal of the overall permeability of the skin to the drug. This total resistance can be expressed mathematically as the sum of the individual resistance (8, 9) as follows:

$$R_s = R_{sc} + R_e + R_{pd} = \frac{1}{P_{sc}} + \frac{1}{P_e} + \frac{1}{P_{pd}} \qquad (1)$$

where the subscripts s, sc, e, pd represent the skin, stratum corneum, viable epidermic, and the papillary layer of the dermis. Previous studies (10) have demonstrated that the diffusional resistances from the viable epidermis and papillary layer of dermis are negligibly small as compared to that from the stratum corneum. Therefore, Equation (1) can be simplified as :

$$R_s = R_{sc} = \frac{1}{P_{sc}} = \frac{h_{sc}}{D_{sc}\,K_{sc}} \qquad (2)$$

where h, D and K stand for the thickness of stratum corneum, the diffusion coefficient of drug in the stratum corneum, and the partition coefficient of drug between stratum corneum and vehicle (TDD system), respectively; and the subscripts s and sc have the same meaning as in Equation (1).

In the skin permeation of estradiol delivered from a m-TDD device with system design as shown in Figure 1, it also has to overcome the diffusional resistances from the permselective

membrane as well as the upper testosterone reservoir layer before it reaches the surface of stratum corneum. So, the total diffusional resistance becomes the sum of the diffusional resistances from the stratum corneum, permselective membrane and testosterone reservoir layer. As observed earlier in the release studies of estradiol (Fig. 3), the diffusional resistance of the testosterone reservoir-containing adhesive matrix to estradiol diffusion is also expected to be much smaller than that of the permselective membrane. Therefore, the total diffusional resistance which estradiol has to overcome in its skin permeation from the m-TDD device (Fig. 1) can be expressed as:

$$R_T = R_B + R_s = R_B + R_{sc} \qquad (3a)$$

or the apparent overall permeability as :

$$P_T = \frac{1}{R_T} = \frac{1}{\frac{1}{P_B} + \frac{1}{P_{sc}}} = \frac{1}{\frac{h_B}{D_B K_B} + \frac{h_{sc}}{D_{sc} K_{sc}}} \qquad (3b)$$

where subscript T stands for apparent overall permeability, B for permselective membrane.

Typical skin permeation profiles of estradiol following delivery from a monolayer device (contains only estradiol reservoir layer) and bilayer device (contains composite of permselective membrane and estradiol reservoir layer) are compared in Fig. 4. The results show that the addition of the permselective membrane does not modify the zero-order skin permeation profile of estradiol, but it has significantly reduced the skin permeation rate of estradiol (0.62 ± 0.12 mcg/cm^2/hr vs. 0.15 ± 0.02 mcg/cm^2/hr). The lag time has also been prolonged from about 5.56 ($\pm$ 0.62) hours to 20.40 ($\pm$ 5.53) hours. The observations indicate

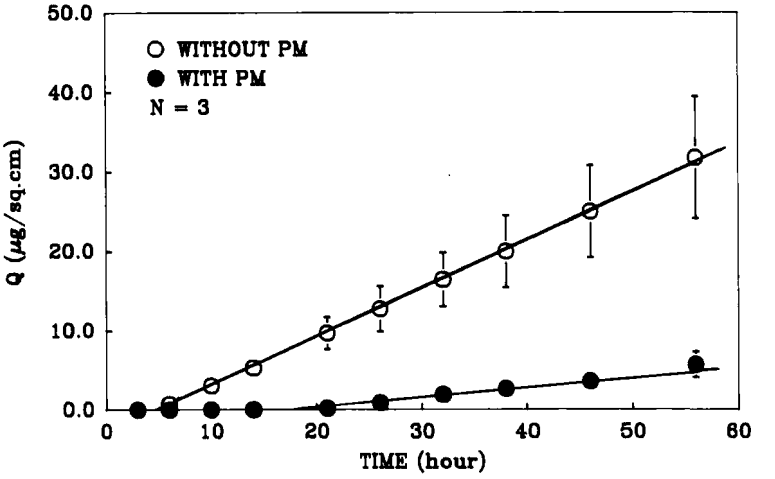

Figure 4: Effect of Permselective membrane (PM) on the Cumulative Permeation Profiles of Estradiol through Hairless Mouse Skin.

that the permeability of the permselective membrane contributes greatly to the apparent overall rate of estradiol permeation that it also brings the permeation of estradiol under the control of the permeability of the permselective membrane, in addition to the intrinsic permeability of skin to estradiol.

If it is true that the permeation of estradiol is mainly under the control of this permselective membrane, Equation (3b) then can be further simplified to:

$$P_T = \frac{D_B K_B}{h_B} \qquad (4)$$

If the sink condition is well maintained throughout the course of skin permeation studies, i.e., $C_b = 0$, and there is enough amount of drug in the system to maintain the concentration in the system

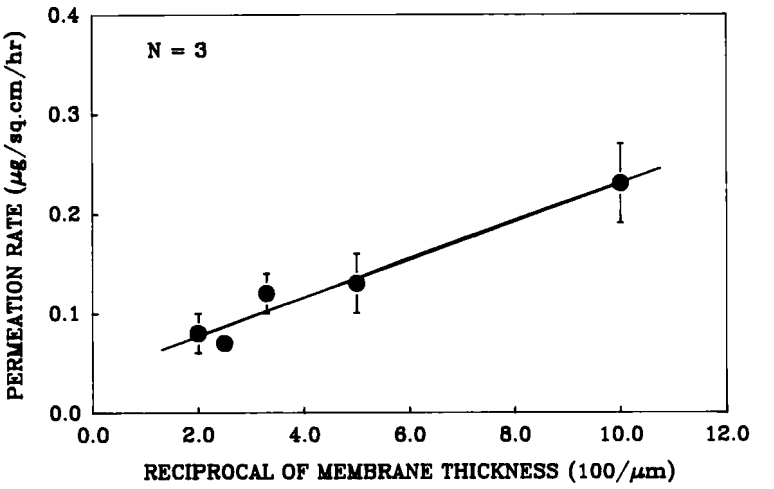

Figure 5: Effect of Thickness of Permselective Membrane on Skin Permeation Rate of Estradiol.

greater than the bulk concentration in the receptor solution, i.e., $C_p >> C_b$, the apparent permeation rate of estradiol can be expressed as:

$$(dQ/dt) = P_T (C_p - C_b) = \frac{C_p D_B K_B}{h_B} \qquad (5)$$

where C_p and C_b represent concentration of drug in the permselective membrane and in the bulk solution, respectively. Therefore, the apparent permeation rate of estradiol in Equation (5) should be an inverse function of the thickness of permselective membrane (h_B), or the change in skin permeation rate (dQ/dt) should be proportional to the reciprocal of the membrane thickness. In order to find out this permeation rate vs. membrane thickness relationship, the effect of variation in membrane thickness on estradiol permeation rate was studied. The results in Figure 5 demonstrate that as expected from Equation (5), a linear

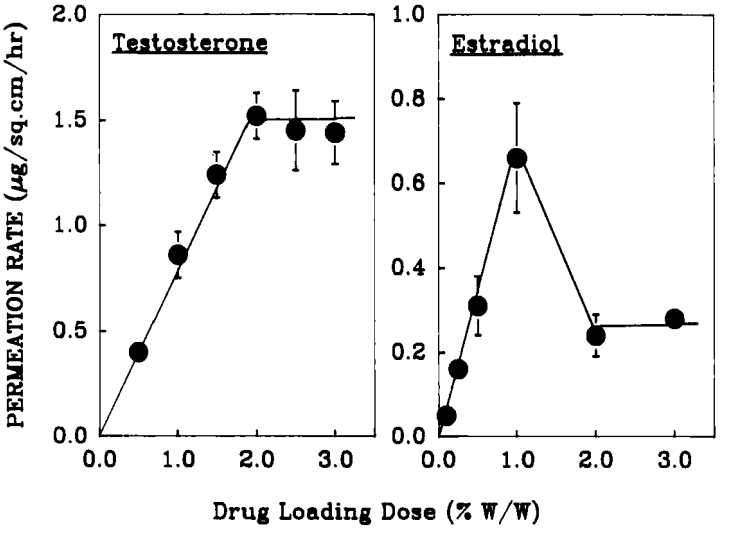

Figure 6: Dependency of Permeation Rate on Loading Dose of Testosterone and Estradiol.

relationship exits between the permeation rate and the reciprocal of the membrane thickness. This further suggests that the skin permeation of estradiol is highly controlled by the permeability of permselective membrane.

Dependency of Permeation Rate On Drug Loading Dose. The effect of drug loading dose on the permeation rates of testosterone and estradiol is shown in Figure 6. Results indicate that linear relationship exists between the skin permeation rate and the loading doses of testosterone and estradiol. In case of testosterone, the good linearity is observed in the dose range of up to 2% (W/W), beyond which the intrinsic permeability of the stratum corneum becomes rate-limiting and the skin permeation rate of testosterone reaches the plateau level at loading doses greater than 2% (W/W). On the other hand, different behavior is observed

for the skin permeation rate profile of estradiol, which increases linearly with loading dose for up to 1% (W/W) and then decreases as loading dose increases to 2-3% (W/W). This unusual phenomenon is apparently not the result of limitation by intrinsic skin permeability. To gain better understanding to this phenomenon, microscopic examination and DSC study were initiated to investigate the possibility of recrystallization and formation of crystals with different physicochemical characteristics.

Microscopic examination indicated that recrystallization of estradiol occurred at a loading dose of 2% (W/W) or higher when it was incorporated in the solution of polyacrylate adhesive polymer as well as in the course of solvent evaporation. Recrystallization was observed to occur in the adhesive solution with formation of tabular-shaped crystals from the original powder form (Fig. 7). As solvent evaporated, tiny needle-shaped crystals were observed to form and accumulate on the surface of the tabular crystals. This observation suggests that the crystals formed by recrystallization may act as the core-crystals to induce more drug to recrystallize on their surface as the solvent system evaporates. As the solvent was totally removed and clusters of crystals were formed to exist in the dried adhesive polymer matrix. Thereby, the concentration of drug in the solution state (C_p) could become much lower than the saturation solubility of the drug in the adhesive.

Further investigation by DSC study indicates that the peak temperature for the melting of estradiol has increased for only 0.04 $^{\circ}C$, while the heat fusion increases about 2 J/gm . Therefore, the decrease in the skin permeation rate of estradiol at high loading doses may be resulted from the reduction in drug concentration (C_p) in the polyacrylate adhesive polymer, due to

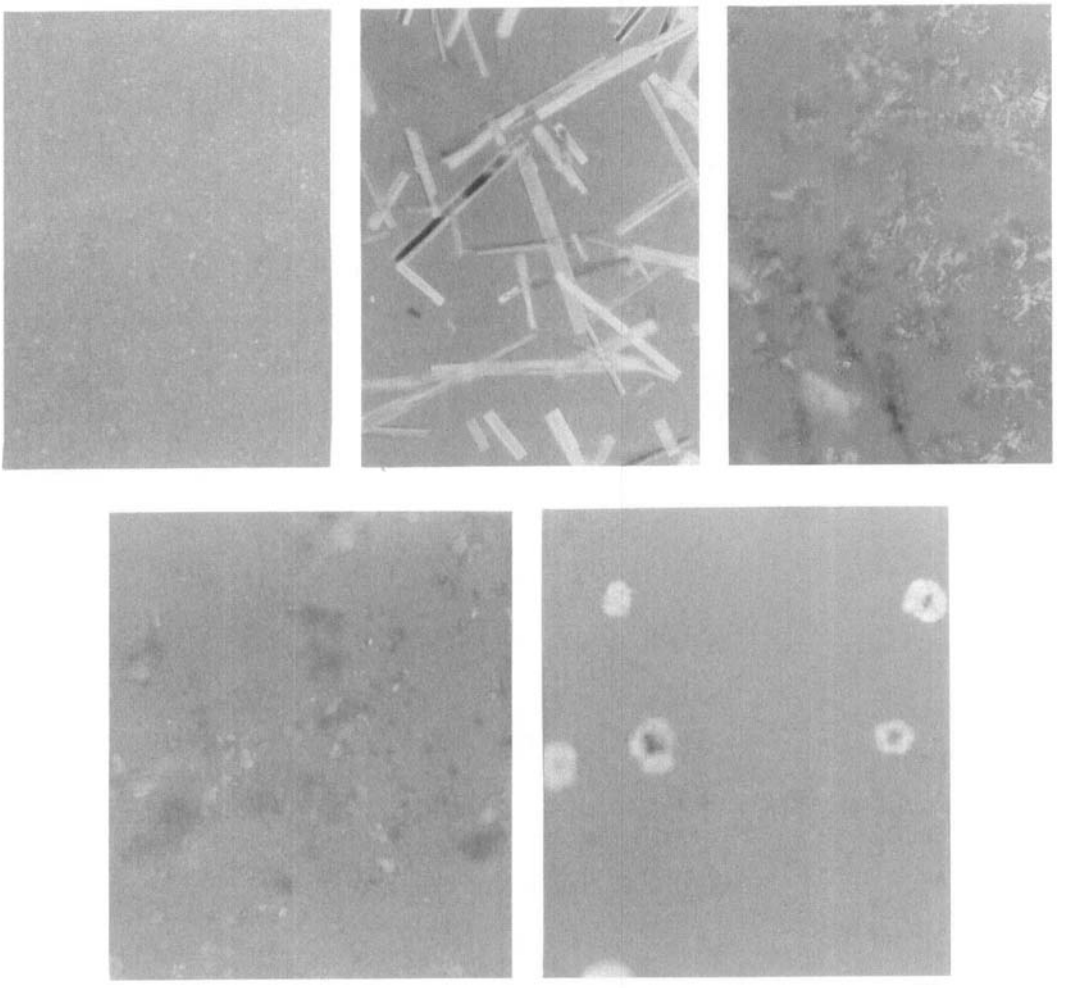

Figure 7: Photomicrographs of Crystals of Estradiol (A, B, C) and Testosterone (D, E) (100x).

Keys: (A) Original powder form of estradiol; (B) Crystals of estradiol formed from recrystallization in the polyacrylate adhesive solution before being dried. (C) Estradiol crystals observed in the dried polyacrylate adhesive matrix. (D) Original powder form of testosterone. (E) Testosterone crystals observed in the dried polyacrylate adhesive matrix.

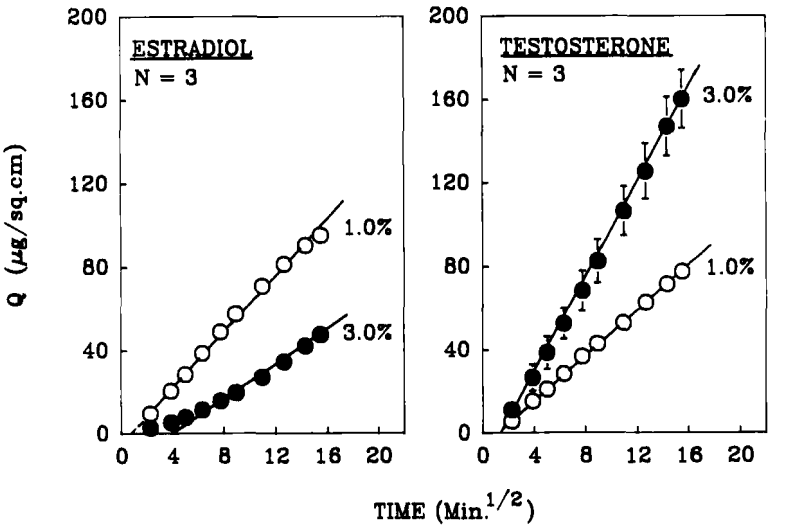

Figure 8: In Vitro release profiles of estradiol and testosterone from TDD system and the effect of drug loading dose.

recrystallization, and the increase in the heat fusion required for dissolution of new estradiol crystals in the adhesive matrix.

Testosterone recrystallization was also observed to occur when the loading dose was increased, but occurs only as loading dose was higher than 3% (W/W). Microscopic examination also showed that the clusters of crystals formed by recrystallization was characteristically similar to the original powder form (Fig.7, D&E). Obviously, the observed levelling off in the skin permeation rate of testosterone at loading dose greater than 2% (W/W) is not the result of recrystallization.

Release kinetics of estradiol and testosterone from their respective drug reservoir layers was also studied to evaluate the effect of drug loading dose on the release flux. Results in Figure 8 indicate that as the loading dose increases from 1% to 3%, the release flux of testosterone increases from 5.40 (± 0.25) to 11.38

($\pm$ 0.67) mcg/cm^2/min$^{1/2}$, whereas the release flux of estradiol decreases from 6.60 ($\pm$ 0.33) to 3.46 ($\pm$ 0.19) mcg/cm^2/min$^{1/2}$. The results obtained apparently substantiate the conclusion that the observed reduction in the skin permeation rate of estradiol at higher loading dose ($\geq$ 2%) (Fig. 6) is due to the formation of new estradiol crystals, by recrystallization process, which require higher heat of fusion to dissolve in the adhesive matrix and the decrease in drug concentration (C_p) in the adhesive polymer.

Effect of Permeability Modification On Skin Permeation Rate. Equation (5) indicates that drug permeation rate (dQ/dt) should be dependent on the overall permeability coefficient (P_T) as the drug concentration (C_p) in the drug delivery device is maintained at constant level. So, any variation in the overall permeability coefficient will lead to a proportional change in the permeation rate. If $(dQ/dt)_i$ and P_i represent, respectively, the intrinsic skin permeation rate and permeability coefficient for a drug, while $(dQ/dt)_m$ and P_m represent, respectively, the modified skin permeation rate and permeability coefficient; Then, the following relationship should exist:

$$\frac{(dQ/dt)_m}{(dQ/dt)_i} = \frac{P_m}{P_i} \qquad (6)$$

In this series of studies, the effects of modification in skin permeability, by the use of skin permeation enhancer, on the skin permeation rate of testosterone and, by the use of permselective membrane, on the skin permeation rate of estradiol were studied. The linear relationships obtained (Fig. 9) demonstrate that the modified skin permeation rates of both

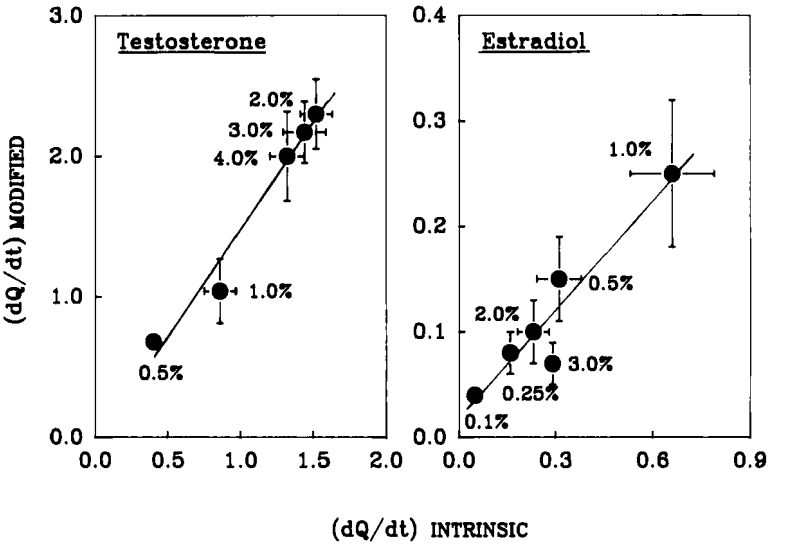

Figure 9: Correlation between Modified and Intrinsic Permeation Rate at Various Loading Doses; n-Decanol, a skin permeation enhancer, was incorporated into testosterone reservoir layer and permselective membrane was added onto drug-releasing surface of estradiol reservoir layer to modify skin permeation rate of testosterone and estradiol, respectively (n=3).

testosterone and estradiol determined at various drug loading doses are well correlated with their corresponding intrinsic skin permeation rates. The results suggest that the overall skin permeabilities for testosterone and estradiol permeation have been modified, respectively, by skin permeation enhancer and by permselective membrane at the same extent for all the dose levels studied as expected from Equation (6).

Effect of Permselective Membrane on Inter-layer Drug Diffusion. In addition to serving as a permeability-modifying membrane for

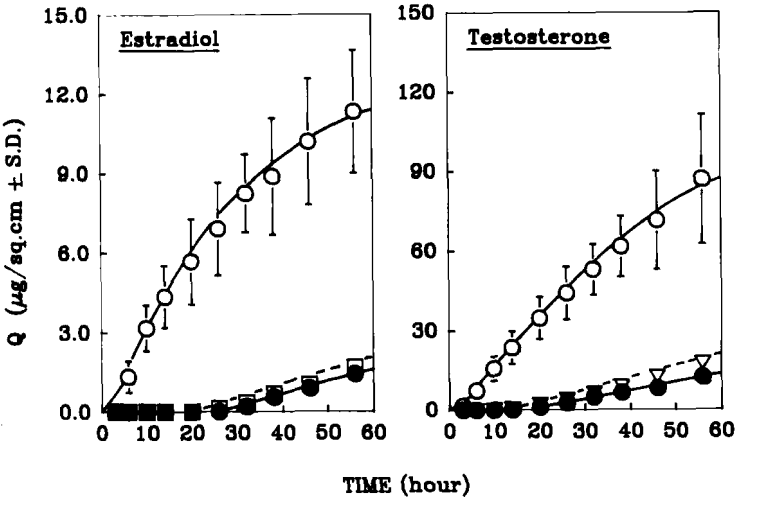

Figure 10: Effect of Permselective Membrane on Skin Permeation. Keys: (○) Estradiol (or testosterone) without permselective membrane in dual-direction diffusion; (●) Estradiol (or testosterone) with permselective membrane in dual-direction diffusion; (□) Estradiol with permselective membrane and testosterone reservoir layer on its diffusional path in one-direction diffusion; (▽) Testosterone with permselective membrane and estradiol reservoir layer on its diffusional path in one-direction diffusion.

estradiol release, the permselective membrane was also used to act as a barrier membrane to reduce the inter-layer migration of testosterone and estradiol. To evaluate the effectiveness of this barrier membrane to prevent or minimize the inter-layer drug migration and the physical stability of the drug delivery system, a dual-direction permeation study was performed. In this dual-direction permeation study, one unit of drug delivery system with

both release liner and backing laminate removed was sandwiched between a pair of half-cells with its testosterone-reservoir layer in intimate contact with the stratum corneum surface of the skin on the right-side half-cell and estradiol-reservoir layer with the stratum corneum surface of the skin on the left-side half-cell; and the two half-cells were then tightly clamped together. Results in Figure 10 demonstrate that incorporation of permselective membrane has significantly reduced skin permeation of both testosterone and estradiol. In consideration of the possibility that any change in concentration gradient on one direction may affect the permeation on the other direction, the results from one-direction skin permeation (with drug impermeable backing laminate on one side) were also plotted in Fig. 10 for comparison. The results in Figure 10 also imply that the permselective membrane has effectively played rate-limiting role in the overall skin permeation rate of estradiol as well as efficiently acted as a barrier membrane to minimize the inter-layer migration of testosterone into the estradiol reservoir layer.

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References

(1) 1982 Industrial Pharmaceutical R&D. Symposium on Transdermal Controlled Release Medication, Rutgers College of Pharmacy, Piscataway, New Jersey, January 14-15, 1982. Proceedings published in Drug Devel. Ind. Pharm., 9(4):497-744 (1983).

(2) Yie W. Chien; Developmental Concepts and Practice in Transdermal Therapeutic Systems. in "Transdermal Controlled Systemic Medication", Dekker, New York and Bassel (1987), Chap.2.

(3) P.R.K. Reddy and J.M. Rao; Reversible antifertility action of testosterone propionate in human males. Contraception, 5:295 (1972).

(4) H.E. Kulin and E.O. Reiter; Gonadotropin Suppression by Low Dose Estrogen in Men: Evidence for Differential Effects upon FSH and LH. J. Clin. Endocr. Met., 35:836 (1972).

(5) L. L. Ewing, C. Desjardins, D. C. Irby and B. Robaire; Synergistic Interaction of Testosterone and Estradiol Inhibits Spermatogenesis in Rats. Nature, 269:409 (1977).

(6) Yie W. Chien; Fundamentals of Controlled-Release Drug Administration. in "Novel Drug Delivery Systems", Dekker, New York and Bassel (1982), Chap.9.

(7) T. Higuchi; Mechanism of Sustained-Action Medication. J. Pharm. Sci., 52:1145 (1963).

(8) R. J. Scheuplein; On the Application of Rate Theory to Complex Multibarrier Flow Co-ordinates: Membrane, Permeability. J. Theoret. Biol., 18:72 (1968).

(9) J.Crank; Mathematics of Diffusion. Clerendon Press, Oxford, England (1967), Chap.IV.

(10) R. J. Scheuplein and I. H. Blank; Permeability of the Skin. Physiol. Rev., 51:702 (1971).