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Transdermal Dual-Controlled Delivery of Testosterone and Estradiol: (II) Enhanced Skin Permeability and Membrane-Moderated Delivery

Jian-wei Yu[+], Te-yen Chien and Yie W. Chien[*]

Controlled Drug-Delivery Research Center
Rutgers - The State University of New Jersey
College of Pharmacy, Busch Campus
Piscataway, New Jersey 08855-0789

Abstract

In the second report of this series of investigations, series of alkanols, alkanoic acids and propyl alkanoates were evaluated for their potential as skin permeation enhancers for testosterone and estradiol delivered from a multilaminate-type transdermal drug delivery (m-TDD) system. Results indicated that permeation rates vary with the length of alkyl chain and the maximum enhancement

[+] Recipient of WHO Fellowship. Current address: National Institutes of Pharmaceutical Research and Development; P.O. Box 948, Beijing, People's Republic of China.

[*] To whom all the correspondences should be addressed.

effect is attained with alkanol having n=6-8 (n is the number of methylene groups), alkanoic acid having n=8-10, and propyl alkanoate having n=10-12. The enhancing effect of series of sorbitan esters and polysorbates on the skin permeation of testosterone was also studied; and the results indicated that sorbitan esters are effective skin permeation enhancer, while polysorbates are not. The effect of enhancer loading on the skin permeation rates of testosterone and estradiol, the barrier property of permselective membrane, the dosage rate ratio of testosterone/estradiol was extensively studied; and the effect of variation in the location of enhancer in the m-TDD system on the skin permeation of estradiol and testosterone was also investigated. The permselective membrane has been shown to be effective in controlling the delivery and skin permeation of estradiol. By simultaneous application of an appropriate skin permeation enhancer in testosterone reservoir layer and a permselective membrane with controlled thickness sandwiched between the estradiol- and testosterone-reservoir layer, the skin permeation rates of testosterone and estradiol from the m-TDD system can be modulated and the dosage rate ratio of testosterone/estradiol can be varied to suite for a particular therapeutic application.

Introduction.

Skin is one of the most extensive and readily accessible organs of human body (1). Recently, its potential of serving as the port of systemic administration for a number of systemically-active drugs has received a growing recognition (2-4). However, the skin is an organ which serves as a protective barrier to protect the

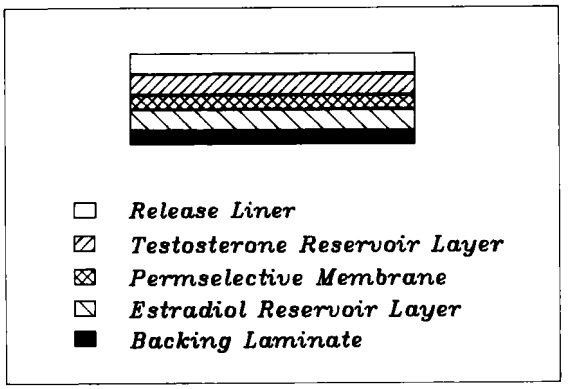

Figure 1: Schematic Illustration of the Multilaminate-type Transdermal Drug Delivery (m-TDD) System Developed, in Which Various Structural Components Are Shown.

body from physical, chemical or microbial attacks from its surrounding environment. So, a drug molecule will encounter several diffusional resistances in the course of skin permeation (5, 6).

To be therapeutically beneficial for systemic medication, the barrier properties of the skin must be modified such that the drug can be administered at a rate sufficiently high to achieve a therapeutically-effective level in the body. There are several approaches which can be utilized to alter the barrier properties of the skin and so the transdermal permeation rate of drugs (6, 7). One of the useful approaches is the co-administration of skin permeation enhancer (6, 7) which has been increasingly used to improve the transdermal delivery rate of drugs from transdermal drug delivery devices (6-14).

In the first article of this series of investigations, we reported the successful development of a multilaminate-type transdermal drug delivery (m-TDD) system (Fig. 1) for the dual-

controlled delivery of testosterone and estradiol (15). In this investigation, we evaluated the potential of series of alkanols, alkanoic acids, propyl alkanoates, sorbitan esters and polysorbates as skin permeation enhancers to promote the skin permeation rate of testosterone delivered simultaneously with estradiol from a m-TDD system. In addition to modification of skin permeability with the use of skin permeation enhancer, the effect of a permselective membrane, which was sandwiched in-between testosterone- and estradiol-reservoir compartments in the m-TDD system, on the skin permeation rate of estradiol was also studied. By this system design, the skin permeation rates of testosterone and estradiol can be varied simultaneously and optimized. This approach permits one to deliver testosterone and estradiol from the m-TDD system at an optimized dosage rate ratio for a specific clinical application. The results are discussed in this report.

Experimental.

Materials - Testosterone, estradiol, alkanols, alkanoic acids, propyl alkanoates, sorbitan esters (from Sigma Chemical Company, St. Louis, Mo.), polysorbates (from ICI Americas Inc. Wilmington, DE), polyacrylate adhesive (from National Starch and Chemical corporation, Bridgewater, N.J.) polyisobutylene (from BASF Corporation, Chemicals division, Holland, Michigan) were used as obtained.

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

HPLC assay - A high performance liquid chromatographic system (Waters Associates, Milford, Mass.) was used, which is equipped

with a Programmable System Controller (Model 721), two reciprocating piston pumps (Model 510), a Wisp auto-injector system (Model 712), reversed-phase u-Bondapak C_{18} column (15 cm x 3.0 mm I.D.), and a Data Module recorder (Model 730) and a Programmable Variable Wavelength UV/VIS detector (Model 783, Kratos Analytical Instruments, Ramsey, N.J.) operating at a wavelength of 225 nm. A mixture of acetonitrile and water (3:1) was used as the mobile phase.

At flow rate of 0.8 ml/min with column temperature at ambient, testosterone and estradiol were separated and detected 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 of injection-to-injection reproducibility in the peak height response (intra-day variation < 3.0% and inter-day variation < 5.0%).

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

Device Fabrication - The drug reservoirs were prepared by first weighing out testosterone (or estradiol) (with or without skin permeation enhancer) in a disposable glass bottle, mixing them well, adding an appropriate amount of polyacrylate adhesive (50%) solution, rotating gently, using a laboratory rotator, to form a drug/adhesive solution (or dispersion); then coating the drug/adhesive combination on a drug-impermeable backing laminate or release liner and drying overnight at ambient to form drug reservoir compartment. Permselective membrane was fabricated by

first preparing the polyisobutylene (10%) solution and then casting it on a release liner and then drying at ambient. Very uniform film were obtained for both the drug reservoir compartment and for permselective membrane. The m-TDD system was obtained by laminating these membranes together and cut it, using a steel die cutter, into 5 cm^2 patches.

In-Vitro Skin Permeation studies - The hairless mouse was sacrificed just prior to the experiment by cervical dislocation. Skin samples in full thickness was removed and its dermal surface was carefully cleaned. Two pieces of the skin were then laid evenly on a pair of the half-cells with their dermis facing the solution compartment and their stratum corneum facing each other. One unit of m-TDD patch was applied onto each skin with its drug-releasing surface in intimate contact with the stratum corneum surface. 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) were withdrawn at predetermined times and assayed for both testosterone and estradiol by the HPLC method outlined above.

Calculation - The skin permeation rate was calculated from the slope of the linear region of the permeation profile. The extent of enhancement can be expressed as enhancement factor which was calculated from the following relationship:

$$\text{Enhancement factor} = \frac{(\text{Normalized skin permeation rate})_{\text{enhancer}}}{(\text{Normalized skin permeation rate})_{\text{control}}}$$

The dosage rate ratio of testosterone over estradiol was calculated from the following relationship:

$$\text{Dosage Rate Ratio } (T/E_2) = \frac{(\text{Normalized skin permeation rate})_T}{(\text{Normalized skin permeation rate})_{E2}}$$

Results and Discussion.

The Alkyl Chain Length Dependency of Enhancement Effect.

Earlier studies conducted in this laboratory and others have demonstrated that alkanols, alkanoic acids and their esters are capable of improving the transdermal delivery of several classes of drugs (7), like progesterone (8), indomethacin (9), nitroglycerin (10), naloxone (11), hydromorphone (12), verapamil (13) and progestin-estradiol combination (14). In this investigation, series of alkanols, alkanoic acids, and propyl alkanoates were also evaluated to study whether or not they are also capable of enhancing the permeation rate of testosterone across the skin of male hairless mouse. In Figure 2, the effect of alkanols, alkanoic acids and propyl alkanoates on the skin permeation profiles of testosterone is compared. Results indicate that the skin permeation of testosterone has been enhanced to various extent by these chemicals, while the zero-order skin permeation profile is still maintained. The extent of enhancement appears to be dependent upon the number of methylene (CH_2) group in the alkyl chain and the terminal functional group of these chemicals (Fig. 3).

The comparisons outlined in Figure 3 suggest that in the alkanol series (Fig. 3A), the increase in the alkyl chain length leads to a gradual increase in the enhancement of testosterone permeation rate; the extent of enhancement reaches the peak level at n=8 (decanol), and further increase in alkyl chain length, the

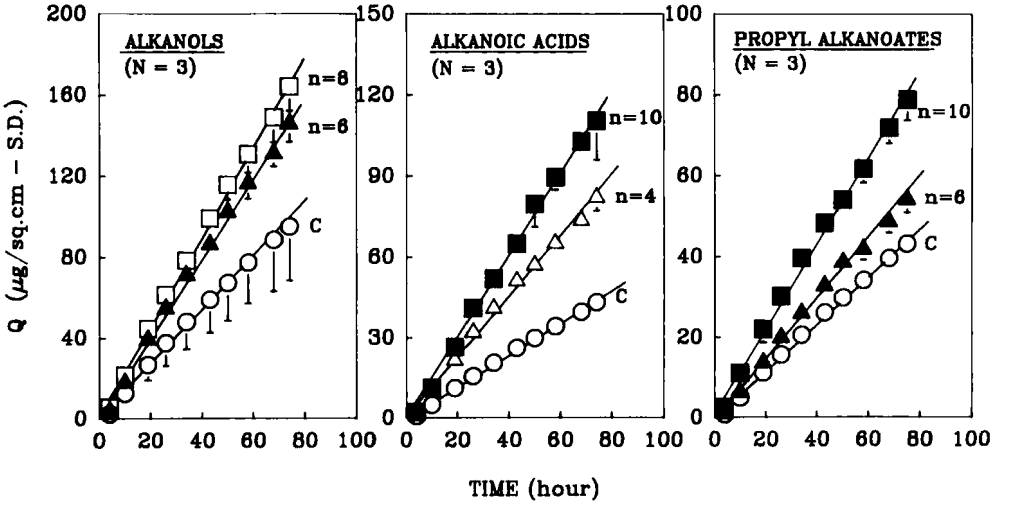

Figure 2: Skin Permeation Profiles of Testosterone and Enhancing Effect of Alkanols, Alkanoic acids and Propyl Alkanoates As A Function of Alkyl Chain Length.

keys: n = number of methylene (CH_2) group in the alkyl chain; C: as the control (without enhancer).

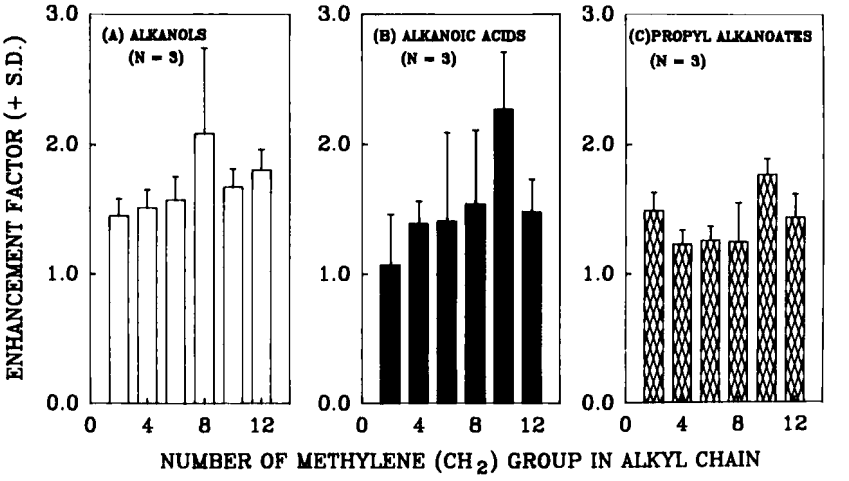

Figure 3: Variation in Enhancement Factor as A Function of Alkyl Chain Length.

efficiency of enhancement is slightly reduced. This observation is quite similar to the results reported previously for progesterone delivered transdermally through hairless mouse skin (8), and for the permeation of naloxone (11) and progestin-estradiol combinations (14) through human cadaver skin. The enhancement in the skin permeation rate of testosterone also shows dependency on the alkyl chain length of alkanoic acid (Fig. 3B); and, the maximum enhancement effect has been achieved at n=10 (lauric acid). Similar results were also reported for the enhanced permeation of progesterone (8), nitroglycerin (10) and verapamil (13) through hairless mouse skin and of naloxone (11) and progestin/estradiol combination (14) through human cadaver skin by alkanoic acids.

In the propyl alkanoate series (Fig. 3C), the maximum enhancement has been achieved with propyl laurate (n=10). However, propyl laurate is not so effective as lauric acid (n-LA). The results appear to indicate that with the free carboxylic acid (-COOH) group as the terminal group is more effective in enhancing the skin permeation of testosterone than that with the propyl ($-C_3H_7$) group as the terminal group. Similar results were also observed for progesterone (7) and nitroglycerin (10), in which maximum enhancement was achieved by decanoic acid (n=8) and the esterification of decanoic acid to propyl decanoate has reduced substantially the extent of skin permeation enhancement of progesterone and nitroglycerin. All these observations seem to suggest that the chemical structure of a skin permeation enhancing agent as well as its alkyl chain length are critical factors which determine the effectiveness of skin permeation enhancement.

In order to gain a better understanding of any possible structure-activity relationship and other possible factors which may also influence the enhancement efficiency of a skin permeation

Table I. Factors influencing enhancement effect of enhancer

Drug	Vehicle	Skin	n of Maximum Enhancement effect in A	B	C
Testosterone	PAM	HMS	8	10	10
Progesterone(8,14)	SPM	HMS	8	6	8-12
Indomethacin(9)	SPM	HMS	6	8	12
Nitroglycerine(10)	SPM	HMS	-	8	8
Naloxone(11)	PGS	HCS	8-10	10	12

A: $CH_3(CH_2)_nCH_2OH$
B: $CH_3(CH_2)_nCOOH$
C: $CH_3(CH_2)_nCOOC_3H_7$
PGS: Propylene Glycol solution
PAM: polyacrylate adhesive matrix
SPM: silicon polymer matrix
HMS: hairless mouse skin
HCS: human cadaver skin

enhancer, the results of this series of investigations and some literature reports are summarized in Table 1. The data suggest that the maximum enhancement effect is often achieved by alkanol, alkanoic acid and propyl alkanoate with alkyl chain length consisting of 6-12 methylene groups.These data seem to imply that alkanol with n=6-8, alkanoic acid with n=8-10, and propyl alkanoate with n=10-12 are most effective in modifying skin permeability. In addition, some kind of relationship may also exist between the extent of enhancement and the molecular structure of the permeant itself. Other factors such as vehicles, skin species, etc. may also affect the enhancement efficiency of skin permeation enhancer.

Effect of Sorbitan Esters and Polysorbates on the Skin Permeation.

Sorbitan esters (Spans) and polysorbates (Tweens) are two series of nonionic surface-active agents with some common chemical structure but different hydrophilicity. The incorporation of polyoxyethylene chains into structure of the hydrophobic property-predominated sorbitan esters yields the hydrophilic property-predominated polysorbates. Therefore, the major difference between

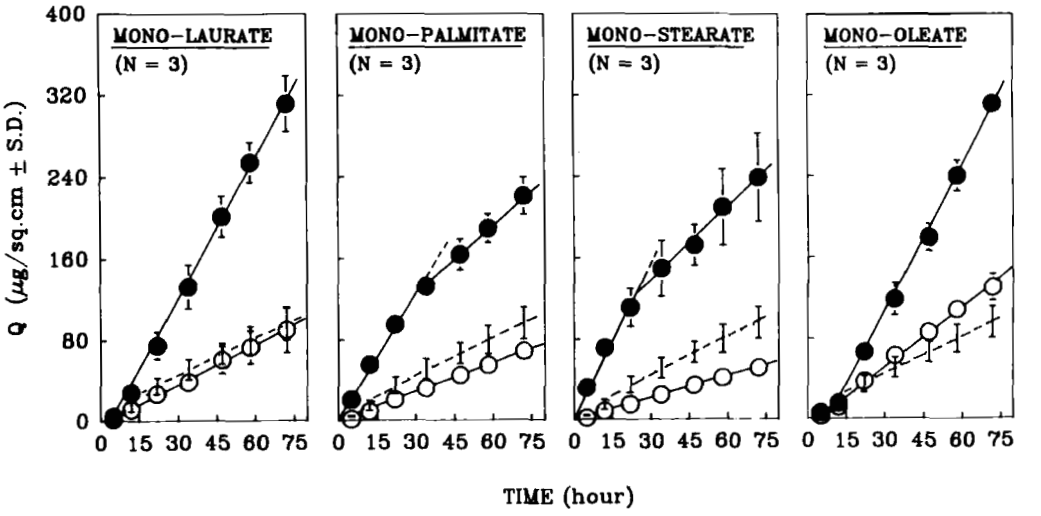

Figure 4: Skin Permeation Profiles of Testosterone and Enhancing Effect of Various Sorbitan Derivatives.
keys: (----) Control (without additive); (●) Sorbitan Esters; (○) Polysorbates.

them lies in the value of hydrophile-lipophile balance and this difference may probably show different effect on the skin permeation of drugs, including testosterone in this investigation.

Effect of sorbitan esters and polysorbates on the skin permeation of testosterone is compared in Figure 4. Results demonstrate that all the sorbitan esters evaluated increase significantly the skin permeation of testosterone, while polysorbates mostly decrease the skin permeation of testosterone. This indicates that the hydrophobic sorbitan esters may facilitate the partitioning of testosterone from its polyacrylate adhesive reservoir onto the surface of stratum corneum while polysorbates may reduce this partitioning process as a result of their hydrophilic property.

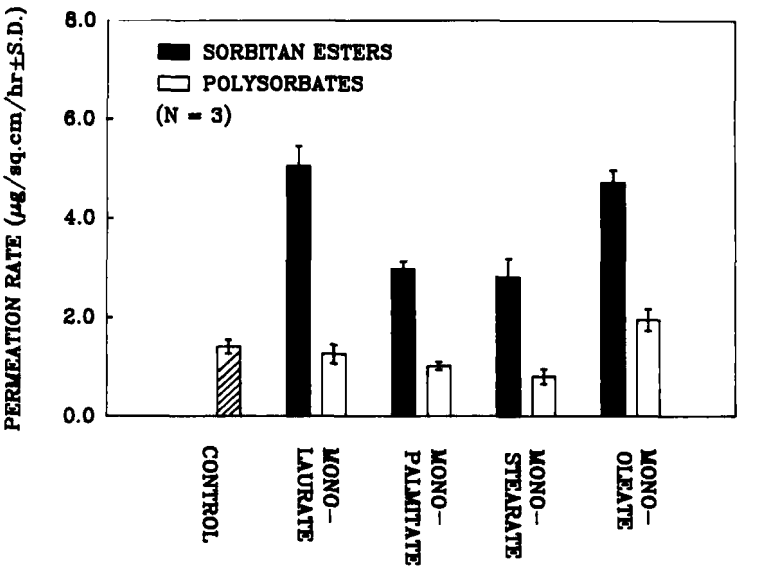

Figure 5: Effect of Sorbitan Esters and Polysorbates on the Skin Permeation Rate of Testosterone.
keys: (▨) Control; (■) Sorbitan Esters; (□) Polysorbates.

Although the difference between sorbitan esters and polysorbates seems to be predominated by their HLB value, their effect on the skin permeation of testosterone is not really dependent upon the HLB value. In this study, the four mono-esters of both series were chosen in order to investigate the possible effect of the ester chain. The results compared in Figure 5 indicate that mono-laurate and mono-oleate of sorbitan are much effective in enhancing the skin permeation rate of testosterone than the other two esters. On the other hand, in the series of polysorbates, except the mono-oleate which has slightly enhanced the skin permeation of testosterone, all other esters have actually reduced the skin permeation of testosterone.

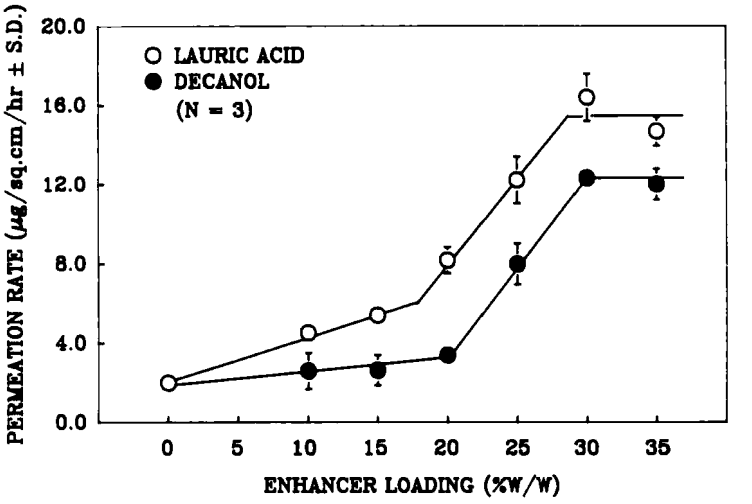

Figure 6: Effect of Enhancer Loading on Skin Permeation Rate of Testosterone.

Effect of Concentration of Skin Permeation Enhancer.

In the first phase of evaluation, lauric acid and decanol were identified as the skin permeation enhancers for improving the skin permeation of testosterone (Fig.3). To optimize the enhancing effect of these enhancers, studies were initiated to investigate the skin permeation profile of testosterone from m-TDD system containing various concentrations of an enhancer in the testosterone reservoir compartment. Results in Figure 6 indicate that the skin permeation rate of testosterone is dramatically increased as the concentration of enhancers in the m-TDD system increases from 15-20% (W/W) to 30% (W/W). The dependence of skin permeation rate on enhancer concentration is very similar between lauric acid and decanol, except that lauric acid is more effective than decanol in promoting the skin permeation of testosterone. The maximum rate of skin permeation for testosterone was achieved at a enhancer concentration of 30% (W/W) for both types of enhancers.

Membrane-Controlled Transdermal Delivery of Estradiol.

One of the objectives for this investigation is to achieve a controlled delivery of estradiol at a lower skin permeation rate by using a permselective membrane to modulate its permeation, while testosterone is delivered at a higher skin permeation rate by the enhancing effect of skin permeation enhancer incorporated in the testosterone reservoir compartment; So a high daily dosage rate ratio of testosterone over estradiol can be achieved. In the earlier studies (15), we observed that the skin permeation rate of estradiol is decreased linearly with the increased thickness of permselective membrane. In this study, a permselective membrane with thickness of 20nm was used. The effect of skin permeation enhancer on the barrier properties of the permselective membrane were evaluated by studying the effect of different enhancers incorporated in the testosterone reservoir layer, at varying concentrations, on the skin permeation rate of estradiol..

As reported earlier in Figure 6, the concentration of skin permeation enhancer plays a very important role in modifying the skin permeability for testosterone, which was increased as much as eight folds by lauric acid and six folds by decanol at the enhancer concentration of 30% (W/W). This indicates that the skin permeation rate of testosterone could be greatly promoted by the use of enhancer. The results in Figure 7 show that the skin permeation rate of estradiol is increased only slightly by lauric acid and decanol, but the increase is a linear function of enhancer concentration. The observation of linear increase in estradiol permeation rate with the increase in enhancer concentration seems to suggest that the enhancer used could also alter the permeability of the polyisobutylene membrane, to some extent, to the permeation of estradiol; and lauric acid appears to affect the estradiol permeability of the polyisobutylene membrane more than decanol.

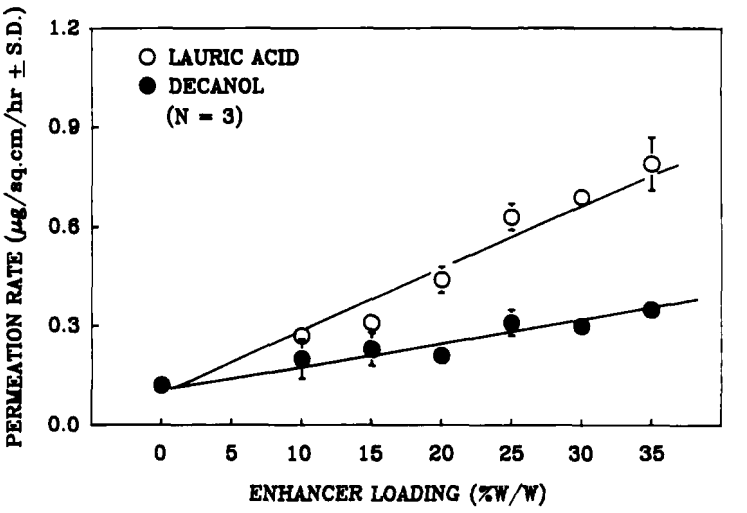

Figure 7: Effect of Enhancer Loading on Skin Permeation Rate of Estradiol.

Controlled Dosage Rate Ratio of Testosterone/Estradiol.

Since the efficient alteration of skin permeability to testosterone by the use of skin permeation enhancer (Fig. 6) and the effective control of estradiol delivery rate by the use of a permselective membrane (Fig. 7), it has become possible that testosterone and estradiol could be delivered at different rates by varying the type and concentration of enhancer incorporated in the testosterone reservoir layer and the composition and thickness of permselective membrane added (Fig 1). This results in an increase in the skin permeation rate of testosterone and a reduction in the skin permeation rate of estradiol, which allows one to optimize the dosage rate ratio of testosterone over estradiol.

The data summarized in Figure 8 demonstrate that the dosage rate ratio of testosterone over estradiol could be varied

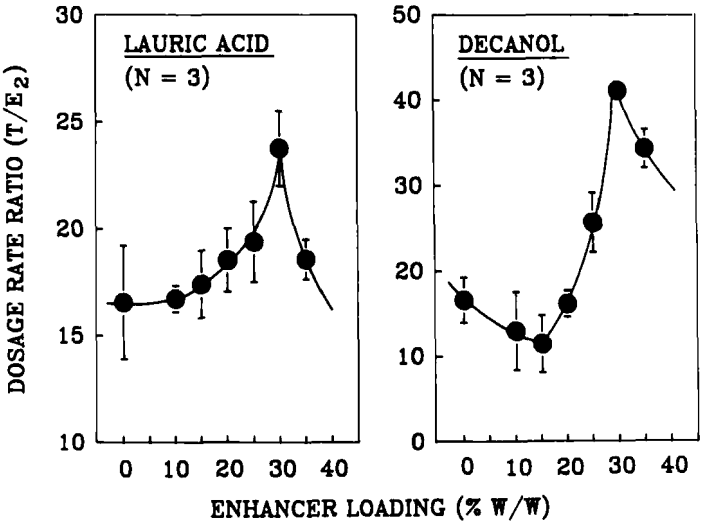

Figure 8: Effect of Enhancer Loading on Dosage Rate Ratio of Testosterone over Estradiol (T/E_2).

significantly by varying the type and the concentration of enhancer incorporated into testosterone reservoir layer. The results in Figure 8 suggest that even though lauric acid and decanol both promote the skin permeation rate of testosterone in a relatively parallel manner (Fig. 6), decanol alters the dosage rate ratio in a different pattern and with greater effect than lauric acid as enhancer concentration varies. This difference could be attributed to the difference between lauric acid and decanol in influencing the permeation of estradiol through the permselective membrane (Fig. 7). Therefore, the dosage rate ratio of testosterone over estradiol was changed at a greater extent by decanol than by lauric acid as increasing the concentration of enhancer (Fig. 8).

Synergistic Effect of Enhancers.

It has been reported that the skin permeability could be further improved by incorporating a combination of two or more

Table II. Effect of combination of lauric acid and decanol on skin permeation rate of testosterone and estradiol

Lauric Acid (% W/W)	Decanol (% W/W)	Permeation rate($ug/cm^2/hr \pm$ S.D.) Testosterone	Estradiol
0	0	1.97 (±0.29)	0.09 (±0.01)
10	0	4.92 (±0.33)	0.14 (±0.03)
10	5	7.89 (±1.82)	0.41 (±0.05)
10	10	8.41 (±0.91)	0.58 (±0.07)
10	15	7.24 (±0.58)	0.75 (±0.04)
10	20	12.61 (±0.99)	0.74 (±0.09)
0	10	2.81 (±0.72)	0.09 (±0.01)
5	10	5.90 (±0.90)	0.30 (±0.04)
10	10	8.41 (±0.91)	0.58 (±0.07)
15	10	8.30 (±0.64)	0.65 (±0.11)
20	10	13.00 (±1.77)	0.68 (±0.08)

different types of enhancers (12). In the present investigation, this feasibility was also explored and the effect of two series of combinations of lauric acid and decanol on the skin permeation of testosterone and estradiol was studied. Data are summarized in Table 2. In the first series of experiments, lauric acid was kept at a constant concentration of 10% (W/W) and the concentration of decanol was varied from 5% to 20% (W/W); In the second series of experiments, decanol was kept at 10% (W/W) and the concentration of lauric acid was varied from 5% to 20% (W/W). Results suggest that the combinations do not produce any synergistic effect on the enhancement of skin permeability; Furthermore, no significant difference between these two series of combinations is observed in their effects on the skin permeation rate of testosterone and on the barrier property of permselective membrane to estradiol permeation when the total concentration of the combination is greater than 20% (W/W).

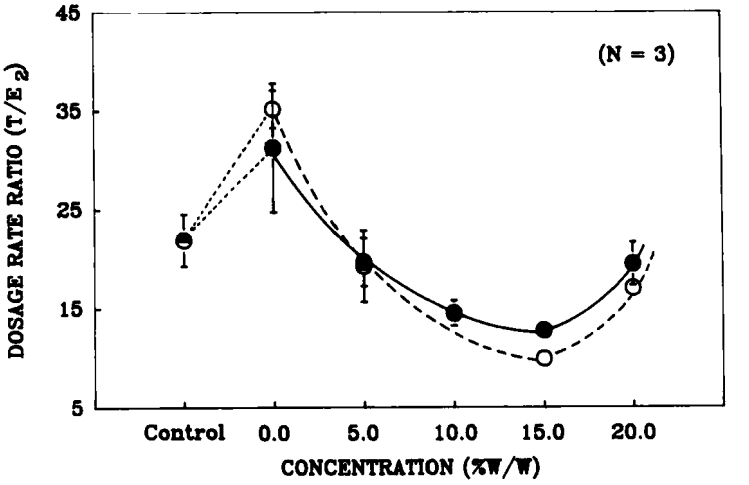

Figure 9: Effect of Combination of Enhancers on Dosage Rate Ratio of Testosterone over Estradiol.
keys: (◯) 10% lauric acid + % decanol; (●) 10% decanol + % lauric acid; (◓) Control (no enhancer).

The lack of synergistic effect can be better visualized by looking into the effect of these two series of enhancer combinations on the dosage rate ratio of testosterone over estradiol (T/E_2). The results in Figure 9 suggest that all combinations produce a reducing effect on the dosage rate ratio of testosterone over estradiol; Both series of combinations yield very much the same degree of effect on the dosage rate ratio. Apparently, the dosage rate ratio of testosterone over estradiol has reached the maximum level with single enhancer and then decreased with the addition of a second enhancer. The dosage rate ratio first decreases as increasing the concentration of second enhancer in the combination for up to 15% (W/W), and then increases as the concentration goes beyond 15% (W/W). This phenomenon can be

well explained by the data outlined in Table 2, in that the skin permeation rate of estradiol increases substantially as increasing the concentration of second enhancer, reaches a plateau level at about 15% (W/W), while the skin permeation rate of testosterone first increases gradually as increasing the concentration of second enhancer for up to 15% (W/W) and is then suddenly promoted at greater extent as the concentration of second enhancer increases to 20% (W/W).

Barrier Property of Permselective Membrane to Testosterone Diffusion.

Question was raised previously (15) that the physical stability of this m-TDD system could be jeopardized by the incorporation of skin permeation enhancer. The results in Figure 7 have already demonstrated the possible effect of enhancer on the permeation of estradiol across the permselective membrane. To further investigate the barrier property of this permselective membrane to the inter-layer migration, a m-TDD system with a reversed configuration, in which testosterone- and estradiol-reservoir compartments are reversed in their locations (Fig. 1), was fabricated and its skin permeation studies were conducted. Results are compared in Figure 10 with those of normal configuration. The comparisons suggest that addition of permselective membrane does reduce the skin permeation rate of testosterone, especially at high enhancer concentration (e.g., 30%). The observation implies that testosterone also permeates through the permselective membrane and its rate of permeation increase as increasing the concentration of enhancer added. In the skin permeation of testosterone from a m-TDD system of normal configuration (Fig. 1), the diffusional resistance which

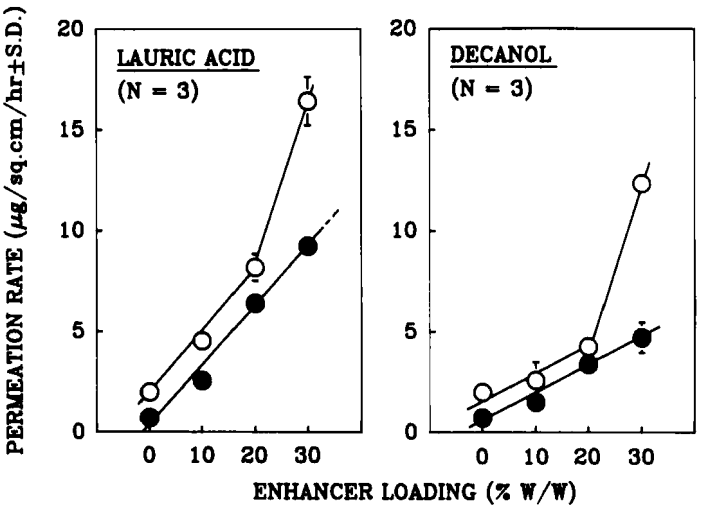

Figure 10: Effect of Enhancer on the Barrier Property of Permselective Membrane to Testosterone Permeation. keys: (○) Normal configuration; (●) Reversed configuration.

testosterone encounters during the course of skin permeation comes mainly from the barrier property of the skin, so the skin permeation rate is directly influenced by the addition of skin permeation enhancer. In the case of a m-TDD system with reversed configuration, however, testosterone molecules have to overcome one additional diffusional resistance, which is produced by the barrier property of the permselective membrane, before permeation through the skin. In this case, if the diffusional resistance of the skin is reduced by skin permeation enhancer used to the extent that it does not constitute the major diffusional resistance to drug permeation, the barrier property of the rate-controlling membrane will govern the skin permeation rate. Then, the skin permeation of testosterone will become under the control of the permselective membrane The difference in skin permeation rate data between normal

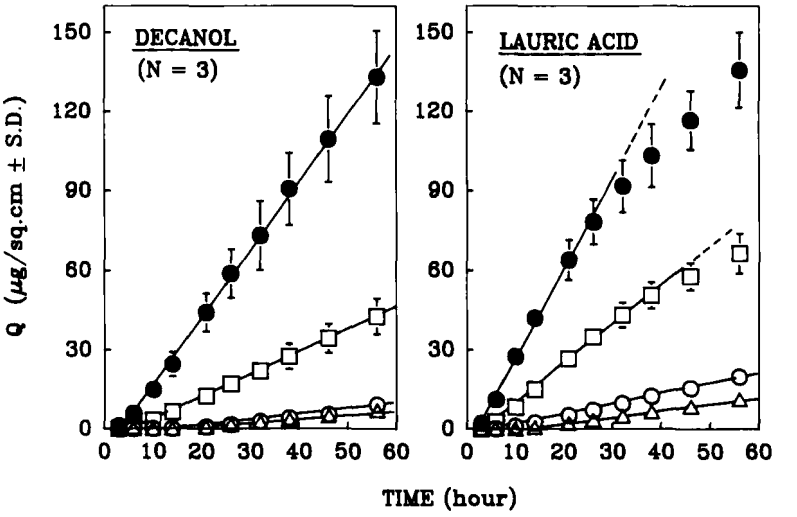

Figure 11: Effect of Enhancer Location in m-TDD system on Skin Permeation Profile of Estradiol.
keys: (◯) Enhancer in Estradiol reservoir Layer; (△) Enhancer in Testosterone reservoir Layer; (□) Enhancer in Both testosterone and estradiol reservoir Layer; (●) Monolithic Estradiol Patch Containing Enhancer as the Control.

and reversed configurations generated at 30% (W/W) enhancer concentration has demonstrated this. In other words, at high concentration of enhancer (e.g., 30% W/W) the permselective membrane becomes the rate-controlling membrane for testosterone in its skin permeation.

Effect of Enhancer Location on Drug Delivery.

The results discussed above indicate that incorporation of a skin permeation enhancer can modify not only the skin permeability to a drug penetrant, but also affect the barrier

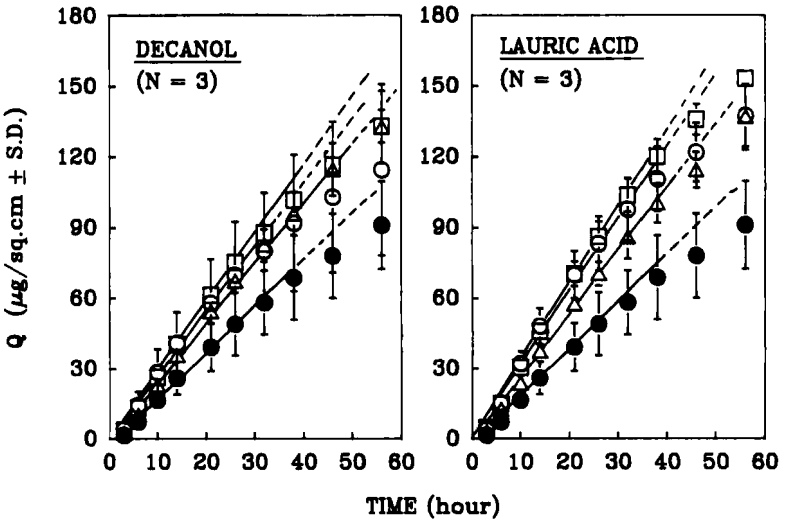

Figure 12: Effect of Enhancer Location in m-TDD System on Skin Permeation of Testosterone.
keys: (●) Control (without enhancer); (△) Enhancer in Estradiol Reservoir Layer; (○) Enhancer in Testosterone Reservoir Layer; (□) Enhancer in Both Estradiol and testosterone Reservoir Layers.

property of permselective membrane. To illustrate the effect of variation in the location of enhancer on the skin permeation of testosterone and estradiol, several m-TDD systems were fabricated with enhancers incorporated into either the estradiol reservoir layer, testosterone reservoir layer, or both estradiol and testosterone reservoir layers at a same concentration (e.g., 20% W/W).

The results shown in Figure 11 indicate that skin permeation profile of estradiol is dependent upon the combined barrier properties of the skin and the permselective membrane; the skin permeation profile of estradiol is remarkably reduced with the

addition of permselective membrane, and it is enhanced by the incorporation of enhancer into both the testosterone and estradiol reservoir layers. The data in Fig. 11 also show that the enhancement effect of enhancer on the skin permeation of estradiol depends on the location of enhancer. When the skin permeation enhancer was incorporated into either estradiol or testosterone reservoir layer, the enhancing effect is not as great as that achieved by having enhancer in both testosterone and estradiol reservoir layers. When the enhancer was incorporated into the testosterone reservoir layer, any enhancement in the skin permeation of estradiol was resulted mainly from the alteration of skin permeability. When the skin permeation enhancer was incorporated into the estradiol reservoir layer, however, the enhancing effect of skin permeation enhancer on the skin permeation of estradiol will depend primarily on its effect on the barrier property of permselective membrane. Incorporation of enhancer into both the estradiol- and testosterone-reservoir layers has effectively enhanced the skin permeation of estradiol, since the barrier properties of the skin and the permselective membrane were both altered.

The effect of the location of enhancer in the m-TDD system on the skin permeation of testosterone was also studied and the data are compared in Figure 12. Results indicate that no statistical difference was observed in the skin permeation profile of testosterone when enhancer was incorporated in either testosterone-or estradiol-reservoir layers or both; although all were substantially higher than that of m-TDD system containing no enhancer.

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