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CONTROLLED DRUG RELEASE FROM A NOVEL LIPOSOMAL DELIVERY SYSTEM

by

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CONTROLLED DRUG RELEASE FROM A NOVEL LIPOSOMAL DELIVERY SYSTEM

Victoria M. Knepp

Controlled drug delivery from a liposomal-based reservoir device has been achieved. The system consisted of a thin agarose gel containing a disperse liposome formulation in which drug was sequestered. The gel was supported on an impermeable plastic backing and the release of a model drug (progesterone (PG)) was monitored, as a function of lipid formulation, (a) directly into an aqueous "sink', and (b) across excised hairless mouse skin into a similar aqueous receiver compartment. While the release rate of PG from an agarose matrix decreased rapidly over 24 hours, with over 90% delivered, the liposomal devices imposed zero-order release of PG for periods of up to 48 hours. The systems were also able to modulate the kinetics of PG delivery across the in-vitro skin model, and in-vivo through the skin of hairless guinea pigs.

To establish the physicochemical basis by which the system controls the release of drug into aqueous buffer solution, the observations of release kinetics were extended to four steroidal compounds spanning a range of physicochemical properties. Release rates obtained from the liposomal systems were compared with the kinetics of steroid transport across simple organic liquid-aqueous solution interfaces. It was concluded that the mechanism by which the liposomal device controls drug release involves slow transport of drug at the lipid bilayer-aqueous phase boundary, rather than slow diffusion through the lipid-agarose matrix.

R.H. Guy

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CHAPTER I - INTRODUCTION

1. Anatomy and Physiology of Human Skin

The following is a brief overview of the anatomy and physiology of the skin. For a more detailed description, the reader is directed to the publications of Jakubovic and Ackerman (1985), Odland (1983), and Montagna and Parakkal (1974).

The skin is a multilayered organ that can be divided into three tissue compartments: the epidermis, dermis, and subcutaneous tissue (Figure 1).

1 Subcutaneous Tissue - This sheet of fibrofatty material separates the skin and the underlying deep fascia. It provides a thermal barrier, a mechanical cushion and a reserve depot of calories.

2. Dermis - This layer consists of collagenous connective tissue, elastic fibers and ground substance within which are embedded nerves, blood and lymph vessels, and pilosebaceous, apocrine and eccrine sweat glands. The fully formed dermis may be sub-divided into two parts: (1) The adventitial dermis, a thin zone immediately beneath the epidermis which is characterized by collagen and elastic fibers, ground substance, and a highly developed microcirculation composed of arterioles, capillaries and venules. This region of the dermis is of particular interest to the pharmaceutical scientist, because the capillary bed functions as an effective sink for molecules diffusing through the epidermis. (2) The larger component of the dermis, which extends from the base of the adventitial dermis, and contains elastic fibers interspersed with thick collagen bundles oriented parallel to the skin surface.

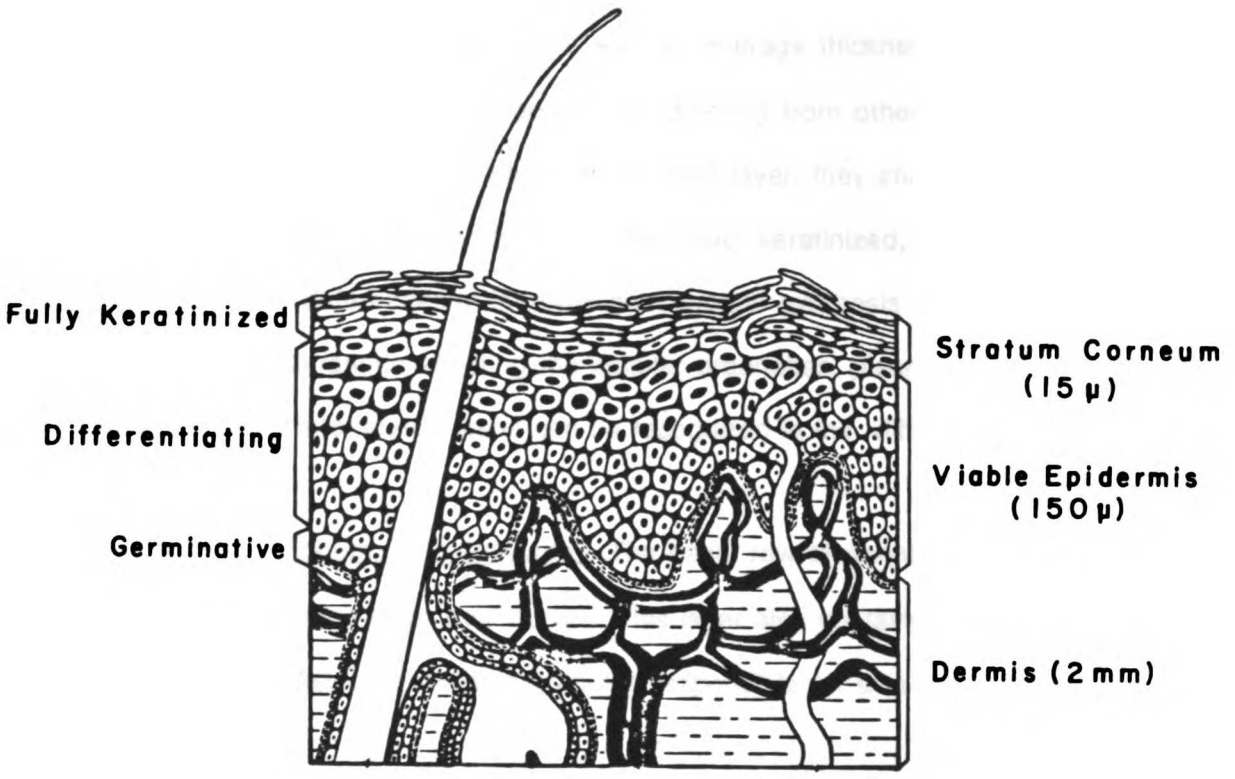


Figure 1. Schematic of human skin.

3. Epidermis - This is the thinnest layer, with an average thickness of approximately 100 um. It is stratified, squamous epithelium, differing from other such tissues in that, as the cells ascend from the basal proliferative layer, they change from metabolically active viable entities to dense, flattened, keratinized, corneocytes. This complex process of differentiation involves both protein synthesis (of keratin and keratohyalin) and alterations affecting the cellular organelles, plasma membrane and desmosomes. Four interrelated areas can be recognized in the differentiation process:

a. Stratum Germinativum - The basal row of germinal keratinocytes are columnar cells containing large oval nuclei. This layer also contains melanocytes, which produce and distribute melanin granules responsible for skin pigmentation and UV radiation protection.

b. Stratum Spinosum - These suprabasal keratinocytes are polygonal in shape and are called spinous because of the delicate spinelike processes (desmosomes) that appear to cross the intercellular spaces and form contacts between adjacent keratinocytes (Elias, 1981). By linking adjacent cells, these desmosomes maintain the integrity of the epidermis. Between desmosomes, interstitial fluid separates neighboring cells and permits nutrient and oxygen transfer. The cells in this layer actively synthesize tonofilaments, precursors of the keratinous protein within the cornified cells of the stratum corneum.

c. Stratum Granulosum - In this, the last viable layer, epidermal lamellar bodies (Odland bodies, keratinosomes, membrane coating granules) can be identified. Originally synthesized in the stratum spinosum, the lamellar bodies contain

polysaccharides (Hayward and Hackerman, 1973) hydrolytic enzymes (Weinstock and Wilgram, 1970) and lipids (Breathnach and Wylie, 1966; Elias et. al., 1977) . In the mid to upper stratum granulosum, these lamellar bodies migrate to the cell periphery and expel their contents into the intercellular space. Also formed in the stratum spinosum but more prominent in the stratum granulosum, are the keratohyalin granules. These are electron-dense bodies, lacking internal structure and composed of two types of proteins. These proteins, one rich in cysteine the other in histidine, form the fibrous, insoluble keratin molecules found in the stratum corneum.

d. Stratum Corneum - This final superficial layer of the epidermis consists of flattened, polygonal, fully differentiated keratinized dead cells. These cells are filled with keratin - a sulfur-poor fibrous protein. The thickened cell membrane and matrix consist of sulfur-rich amorphous proteins, with disulfide linkages producing stability (Matolsty, 1976). Within the intercellular spaces freeze fracture electron microscopy (Elias and Friend, 1975) reveals multiple membrane bilayers arranged in broad lamellae. It is assumed that these lamellae, rich in neutral lipids and ceramides, are derived solely from catabolized lamellar body contents (Elias and Friend, 1975; Elias et. al. 1977a; Elias et al, 1977b), and that the progressive accumulation of their extruded contents results in an increase in the intercellular space from less than 1% of the total tissue volume to 5 to 30% (Elias, 1981). Thin layer chromatography (Lampe et al, 1983 (a),(b)) reveals that a striking shift in composition from polar to neutral lipids occurs during differeniation from the stratum granulosum to the stratum corneum (Table 1).

4. Appendages - Found predominantly in the dermis and epidermis, these include the eccrine and apocrine sweat glands, the hair follicles, and sebaceous glands.

Fraction	Stratum Spinosum	Stratum Granulosum	Stratum Corneum
Phospholipids	44.5	25.3	4.9
Cholesterol Sulfate	2.4	5.5	1.5
Neutral Lipids	51.0	56.5	74.8
Free Sterols	11.2	11.5	14.0
Free Fatty Acids	7.0	9.2	19.3
Triglycerides	2.4	24.7	25.2
Sterol Esters	5.3	4.7	5.4
Squalene	4.9	4.6	4.8
n-Alkanes	3.9	3.8	6.1
Sphingolipids	7.3	11.7	18.1
Glucosylceramides	3.5	5.8	tr.
Ceramides	3.8	8.8	18.1

TABLE 1. Variations in lipid composition (weight %) during human epidermal differentiation (Adapted from Lampe et al, 1983 (a) and(b)).

a. Eccrine sweat glands - These are found over the entire body surface (except mucous membranes), their density varying considerably with skin site. The average fractional surface area occupied by the openings to these glands is greater than or equal to 10^{-5} (Scheuplein, 1967). A typical gland consists of a secretory coil in the lower dermis and subcutaneous tissue, with a duct leading through the dermis and epidermis where it exits as a small pore on the surface. The purpose of these glands is to assist in thermoregulation.

b. Apocrine sweat glands - These epidermal appendages are located in the axilla (armpit), perianal region, and the areola of the breasts. They are approximately 10 times larger than eccrine sweat glands, and secrete an oily fluid containing lipids, lipoproteins and saccharides. Considered vestigial secondary sex organs, these glands have no apparent function.

c. Hair - Hair follicles are invaginations of the superficial epidermis, which slant into the dermis, and occasionally, the subcutaneous fat. They develop over the entire skin surface except the palms, soles, lips, and parts of the sex organs, with an average fractional surface area of 0.1% (Scheuplein, 1967).

d. Sebaceous Glands - These are found primarily on the face, back, chest and scalp. They open into the hair follicle and secrete a lipoidal mixture of free fatty acids and alcohols, triglycerides, cholesterol and hydrocarbons.

5. Cell Turnover Time - Although there are difficulties in calculating epidermal turnover time, studies using labelled DNA (Halprin, 1972) have shown that it takes approximately fourteen days for a cell to transit from the basal layer to the stratum

corneum. Experiments employing radioactive glycine indicate that the normal residence time of a cell in the stratum corneum is thirteen to fourteen days (Rothberg et al, 1961). Therefore, in healthy skin, the total turnover time from stratum germinativum to stratum corneum is 28 days.

2. Transdermal Penetration

Location of Barrier - By the middle of the nineteenth century it was recognized that the layers of the skin were not equally permeable. Early experiments (Blank, 1953), in which the stratum corneum was progressively stripped with cellophane tape, showed that water permeation increased dramatically when this membrane was completely removed. The observation was originally interpreted to mean that the barrier was located at the base of the stratum corneum (the so-called stratum conjunctum); however, this hypothesis was later refuted by further, more detailed, experiments (Loveday, 1961 ; Flynn, 1981) which showed that the stratum corneum is of relatively uniform resistance.

Pathways of Permeability - Once a compound has been deposited on the skin, there are several potential routes of penetration through the stratum corneum (Figure 2). Although there has been considerable effort to determine the mechanism(s) by which compounds penetrate the skin, there has, until quite recently, been little agreement among investigators as to the relative importance of the possible pathways.

1. Appendageal - Drugs applied to the surface of the skin contact the orifices of the sweat glands and hair follicles directly. These ducts are lined with stratified squamous epithelia, through which the compound can presumably penetrate with ease. Investigators favoring this route of penetration cite the rapid diffusion of charged dyes

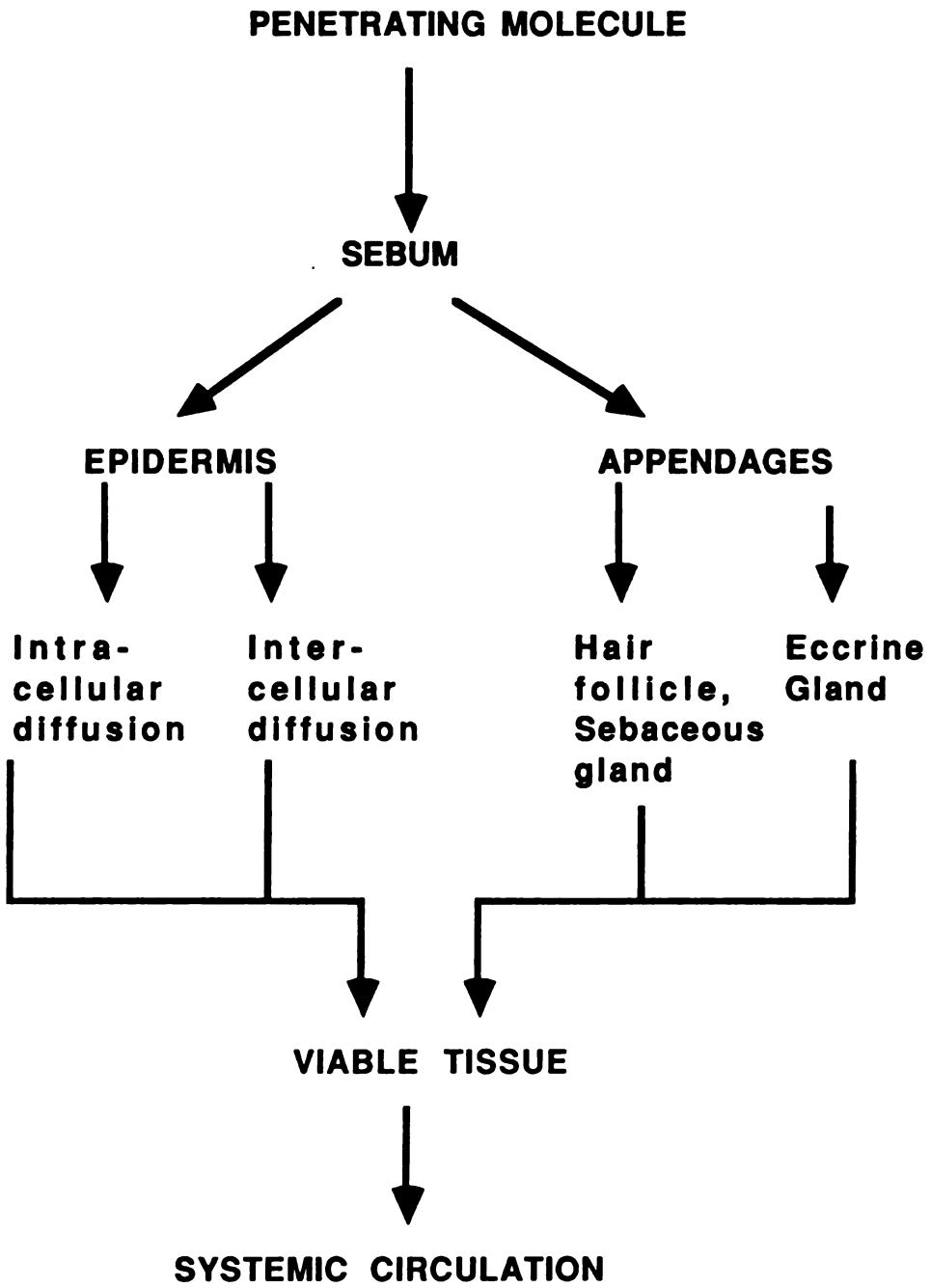


Figure 2 - Potential routes of penetration through full-thickness skin. (Modified from Katz and Poulsen, 1971)

through the sweat glands when an electric potential is applied to the skin (Abramson and Gorin, 1940), and the preferential staining of hair follicles by certain dyes (Rothman,1954). However, the relatively small surface area occupied by the appendages (between 0.1 and 1% of the total area of the skin), and the fact that a molecule must diffuse against an outward flow of fluid through these glands, argue against this pathway as a major mode of transport. Current opinion is that there is little evidence supporting appendageal transport as a major contributing pathway for the passive diffusion of compounds through the stratum corneum (although the route may serve as an early "shunt" mechanism for small molecules (Scheuplein et al, 1969)).

2. Transepidermal - It is generally agreed, therefore, that most molecules enter the skin by traversing the architecture of the stratum corneum (Katz and Poulsen, 1971; Barry 1981). The stratum corneum can be idealized as a "brick and mortar" wall (Flynn, 1983) (Figure 3), where the "bricks" represent the fully keratinized cells, and the "mortar" the interstitial lipid bilayers. It follows that transport can occur either: (a) via a direct path involving partitioning into and out of the cells and diffusion through both inter- and intra-cellular spaces (transcellular path), or, (b) with the molecule partitioning into and diffusing through the interstitial lipids only (intercellular).

a. Transcellular - Earlier proponents of this route viewed the skin as a functionally isotropic phase (a lipid-protein gel) into which small molecular weight compounds dissolved with strong chemical interactions, and through which diffusion occured slowly (Katz and Poulsen, 1971; Idson, 1975). A major reason for the acceptance of this view was that the volume of the intercellular lipids was then estimated to be only 0.01 - 0.1% of the total volume of the stratum corneum (Idson, 1975). However, more recent studies have demonstrated that the actual intercellular volume is

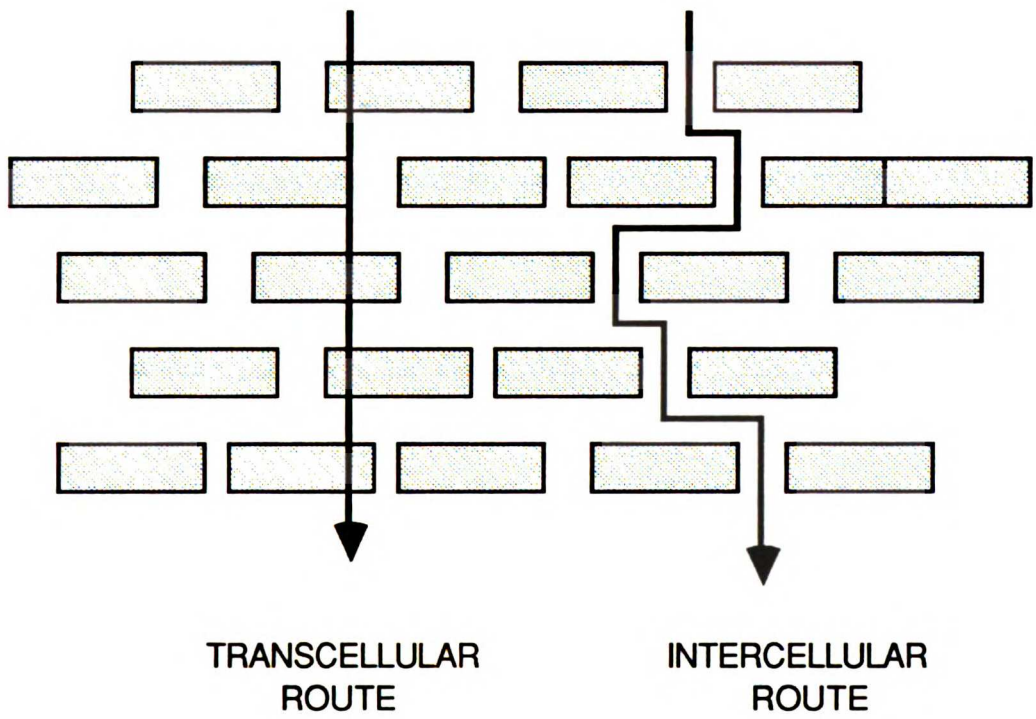


Figure 3 - "Brick and mortar" representation of the skin

5% to 30% of the total (Elias and Leventhal, 1979). Currently, the transcellular route is believed by some investigators to be a "polar pathway" through which hydrophilic compounds diffuse via water that is substantially bound in the hydrated protein mass of the keratinocytes (Flynn, 1985). The existance of this path is based upon the higher than expected permeation rates of polar substances, and the passage of ionic species through the skin. The low diffusivities of polar substances are attributed to the density of the keratinocytes and to the structure of the bound water.

b. Intercellular - Although initially disregarded as insignificant, many investigators now consider this to be the primary route of diffusion for lipophilic molecules. The importance of the interstitial lipids was demonstrated by Sweeney and Downey (1970), who showed that disruption or removal of the interstitial lipids by organic solvents reduced the resistance to solute transport. Elias et al (1981) found that the penetration of both salicylic acid and water was inversely correlated to the lipid content of the stratum corneum, and was not influenced by the number of cell layers or the thickness of the membrane. On a mechanistic level, investigators (Knutson et al, 1985; Golden et al, 1987) examined the role of intercellular lipid bilayer fluidity in regulating the transdermal flux of certain compounds using differential scanning calorimetry and Fourier transform infrared spectroscopy. Both calorimetric and spectroscopic techniques associated enhanced permeability of compounds with transitions involving the hydrocarbon chains of the stratum corneum lipids. It appears that as the hydrocarbon chains become more disordered or "fluid", the viscosity of this region is diminished, thus reducing the diffusional resistance of these domains to a lipophilic compound (Figure 4), and enhancing its permeability.

In conclusion, present theory suggests two potential pathways for solute transport across the stratum corneum. The so called "polar" pathway has a phenomenologic basis,

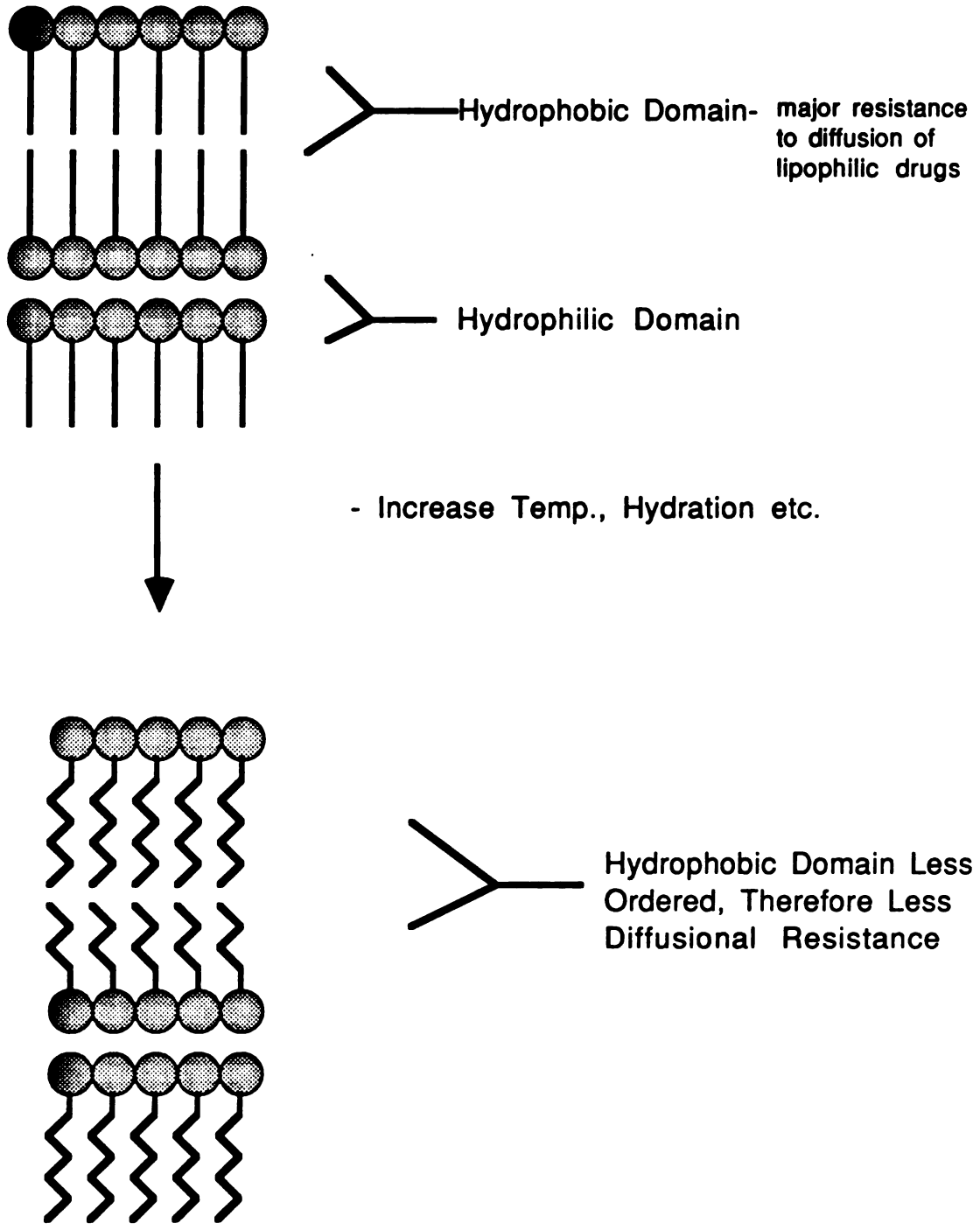


Figure 4 - Mechanism of temperature and hydration induced changes on stratum corneum permeability. (Adapted from Knutson et al, 1985)

and has been invented to explain the anomalous permeability of certain hydrophilic compounds. Proponents of this pathway also cite studies which indicate that alterations in keratin structure produced by either thermal burns (Flynn, 1985) or DMSO (Oertel, 1977) increase the permeability of the skin. However, it is unclear whether the conformational changes themselves are responsible for the decreased resistance of the stratum corneum, or whether it is due to perturbations in the lipid bilayers caused by unfolding of the protein. The interstitial pathway, on the other hand, has been characterized to a much greater extent. Oertel (1977) has shown that removal of the interstitial lipids by organic solvents does not effect the conformation of the stratum corneum proteins, and has concluded that the intercellular route operates as a separate pathway. Finally, mechanistic studies by Golden et al (1987) and Knutson et al (1985) have correlated increased fluidity of the acyl chains of the lipid bilayer with increased permeability, and have thereby established a persuasive cause - effect relationship.

3. Transdermal Drug Delivery

The concept of a small topically administered bandage containing, and capable of, delivering sufficient medicine for a day or longer is both elegant and attractive. An alternative to oral administration is, in many cases and for large numbers of individuals, an important option that is infrequently available. The control of drug input, which is potentially available with transdermal delivery (Figure 5), is also alluring. The saw-tooth profile of drug concentration in the biophase vs. time, characteristic of conventional dosing regimens, can be damped by administration via the skin. A clear advantage for drugs of narrow therapeutic index is thereby implicated (Shaw and Theeuwes, 1985).

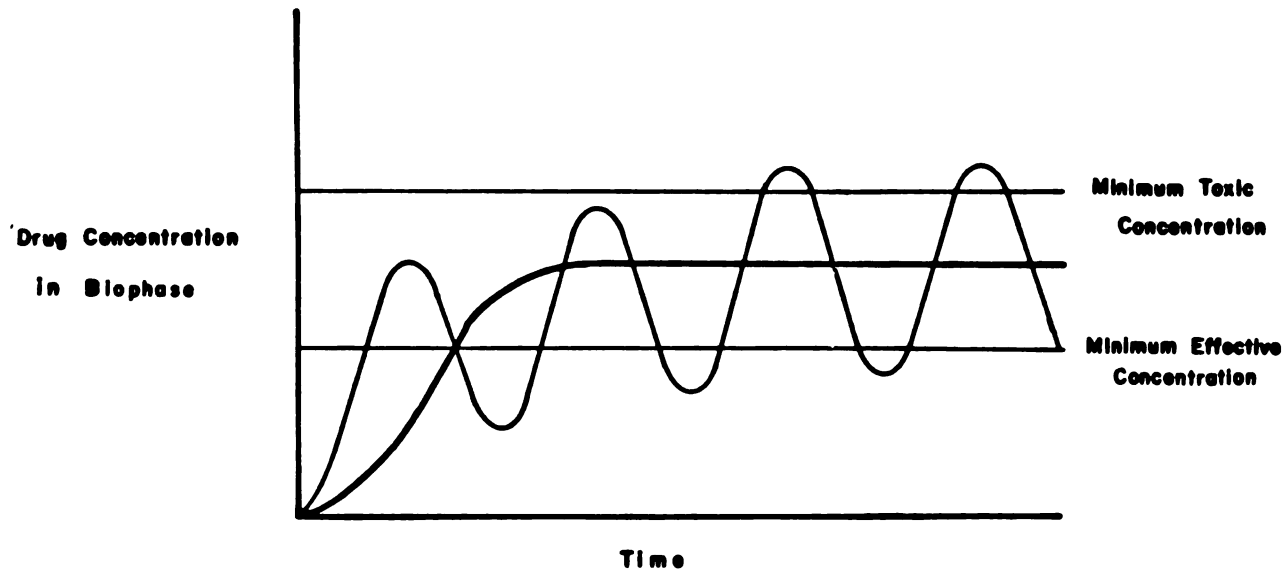


Figure 5. Schematic representation of drug levels in the biophase as a function of time following conventional (e.g. oral) multiple dosing (oscillating line) and sustained (e.g. transdermal) drug delivery (horizontal line).

Enthusiasm for topical drug input to elicit systemic effect must be tempered, however, by recognition of the fact that skin represents one of the most formidable barriers of biology. It is also a tissue which registers insult in a manner designed to discourage repeat events. Hence, transdermal delivery is presently limited to potent drugs which elicit no, or minimal, local irritating effects (Guy and Hadgraft, 1985). A few simple calculations serve as an initial guide to the feasibility of transdermal delivery for some representative "candidate" compounds. Assume, for example, that a trandermal device exists which is capable of providing zero-order delivery to the skin surface at a rate (k_o; $\mu g/cm^2/hr$) slightly less than the maximum flux (J_m; $\mu g/cm^2/hr$) of a model drug across the stratum corneum. Taking $J_m = 35\ \mu g/cm^2/hr$, then a value of $k_o = 25\ \mu g/cm^2/hr$ is appropriate (Good, 1983). If the target plasma concentration of the drug is C^{ss} $\mu g/ml$, it follows that Equation 1 must hold at steady state:

$$A \cdot k_o = Cl \cdot C^{ss} \qquad (1)$$

where A (cm^2) is the area of the patch and Cl (ml/hr) is the drug clearance. Setting k_o less than J_m is desirable to retain drug input control within the delivery system and to avoid variability associated with differential skin permeabilities within a patient population (Good, 1983). Given this constraint, Equation 1 contains only A as a manipulatable parameter. In other words, if inherent skin permeability cannot be increased in some way, the the input function can be maneuvered only within the confines of $k_o < J_m$ and that A be "reasonable".

The limitations can be emphasized with reference to Table 2 in which an initial pharmacokinetic feasibility assessment of transdermal delivery is performed for an arbitrary selection of compounds. Clearances and target therapeutic plasma levels were

Drug	Cl (L/hr)[a]	C^{ss} (μg/ml)	$A^{min}(cm^2)$[b]
Acetaminophen	23.1	15	13,846
Aspirin	29.1	150	174,510.0
Cimetidine	48.5	1.0	4.0
Clonidine	12.0	0.001	0.48
Estradiol	66.9	0.0001	0.54
Scopolamine	43.3	0.0002	0.35
Nitroglycerin	4,213.4	0.0001	17.0

[a] Calculated for a 70 kg adult

[b] The total body surface area of a 70 kg, 1.8m tall adult is approximately 19,000 cm^2. The values in this column are calculated using Equation 1 with k_o = 25 μg/cm^2/hr.

Table 2. Feasibility screen for representative transdermal delivery candidates.

obtained from the literature (Benet and Sheiner, 1980), k_o was fixed at 25 $\mu g/cm^2/hr$, and the value of A required by solution of Equation 1 was calculated for each drug. The results clearly indicate the nonfeasible candidates (aspirin, acetominophen, cimetidine, indomethacin) for which A is unacceptably large. In the case of aspirin, for example, the calculated A is approximately nine times that of a normal adult's skin surface area! For economic and practical reasons, a delivery system area of 50 cm^2 is a reasonable upper limit. The remaining drugs pass the initial screen, although it should be emphasized that the theophylline calculation is for pediatric purposes only (Evans et al, 1985). For clonidine, digoxin, estradiol, and scopolamine, A is very small and a reduction in k_o is indicated so that a patch of manageable dimensions can be fabricated. This is exactly the strategy that has been followed in the development of the marketed clonidine, estradiol, and scopolamine systems.

However, this simplistic approach leaves a number of questions unanswered. Is a percutaneous flux of 25 $\mu g/cm^2/hr$ possible for all compounds? Probably not, and some degree of input control may therefore need to be sacrificed. How long will be required for the attainment of C_t following application of the patch? The half-life of the drug can be of some use here, but if absorption through the skin is slow (the usual case), an unacceptably long approach to the target level may be apparent. Which of the "feasible candidates will elicit a local irritating effect on the skin? There are no simple nor reliable methods to predict skin irritation from a compound's structure and properties unless information pertinent to a closely related homolog or analog is known (Benezra et al, 1985). Clearly then, transdermal drug delivery presents nontrivial challenges (independent of formulation itself) while offering certain unique opportunities. However when these are placed in perspective, strategies can be developed to evaluate the likelihood of successful drug moieties.

3.1 Advantages and Disadvantages of Transdermal Drug Delivery

Drug delivery via the skin to elicit systemic effect offers several advantages over more conventional methods of administration, including the following (Guy and Hadgraft, 1985):

1. Steady-state drug concentrations within the therapeutic window can be maintained. The "peaks and valleys" associated with conventional multiple-dosing regimens are avoided (Figure 5). This precise control over plasma drug concentration enables the selectivity of drug action to be enhanced and decreases unwanted side - effects.

2. Gastrointestinal tract variables such as erratic or incomplete absorption are circumvented. Hepatic first-pass metabolism, which can severely limit the systemic availability of a drug, is eliminated.

3. An alternative route to oral administration is provided for those situations in which patient variables (e.g. geriatric or pediatric cases, nausea and vomiting symptoms) preclude conventional dosing.

4. A substitute parenteral form of therapy is possible without the inconvenience and anxiety associated with IV infusions, boluses, or IM injections.

5. Transdermal therapeutic systems are able to extend significantly the duration of action of many drugs, thereby reducing the frequency of drug dosing necessary with conventional dosage forms. This reduction may lead to enhanced patient compliance and, consequently, more effective therapy.

6. When medical needs demand, therapy can be terminated quickly and simply by removing the system.

These important attributes of transdermal drug delivery are counterbalanced by a number of major drawbacks, any one or more of which may be sufficient to preclude its use:

1. The skin is an excellent barrier to chemical penetration into the body. The stratum corneum is a tough, resilient, hydrophobic membrane through which drug diffusion is slow. In order to gain systemic access, a transporting molecule must breach this layer and then partition into the much more aqueous viable epidermis. Balanced physicochemical properties (i.e. reasonable solubility in oil and in water, moderate or low molecular weight, conservative lipid-water partitioning characteristics) are prerequisite for a successful penetrant, therefore.

2. As percutaneous absorption is slow, the drug must be pharmacologically potent because the concentration of active species in the biophase will be low. Currently, very few drugs whose effective plasma concentrations exceed 1 to 10 ng/ml are seriously considered for delivery transdermally.

3. In addition to the above pharmacokinetic limitation, there may exist a potentially more restrictive pharmacodynamic disadvantage. The excellent barrier nature of the skin means that continuous transdermal delivery will produce rather steady drug concentrations in the biophase. Such a situation is not necessarily optimal from a pharmacological point of view and may exacerbate the potential for tolerance development, as has been observed with the continuous transdermal delivery of

nitroglycerin (for a more in-depth discussion of nitrate tolerance, the reader is directed to a review by Knepp et al, 1987 and references therein).

4. Local, unwanted biological effects may also occur. The drug and/or contact system of the transdermal device must not elicit irritant or allergic reactions within the skin at the site of application. Cutaneous binding and metabolism remain, at this time, unknowns, the significance of which await clearer demarcation and quantitation.

5.. Transdermal delivery systems are relatively expensive compared to conventional dosage forms. They may contain large amounts of drug, of which only a small percentage may be used during the application period.

3.2 Types of Transdermal Therapeutic Systems

The objective of a transdermal therapeutic system is to deliver drug into the body at a controlled, efficacious rate such that inter- and intrapatient variations in skin permeability are overcome. Thus, the rate-limiting step in transdermal drug absorption is ideally provided by the delivery system and not the skin. At this time, a number of transdermal delivery systems have been described. They may be classified broadly into three general categories:

1. Membrane Moderated - A reservoir containing the drug is enclosed on all sides, bar that through which drug is released, by an impermeable laminate (Figure 6). The releasing face of the reservoir is covered by a rate-controlling polymeric membrane. Different release rates are achieved by variation of the polymer composition and the thickness of the membrane. Devices of this type are manufactured for scopolamine, nitroglycerin, clonidine, and estradiol.

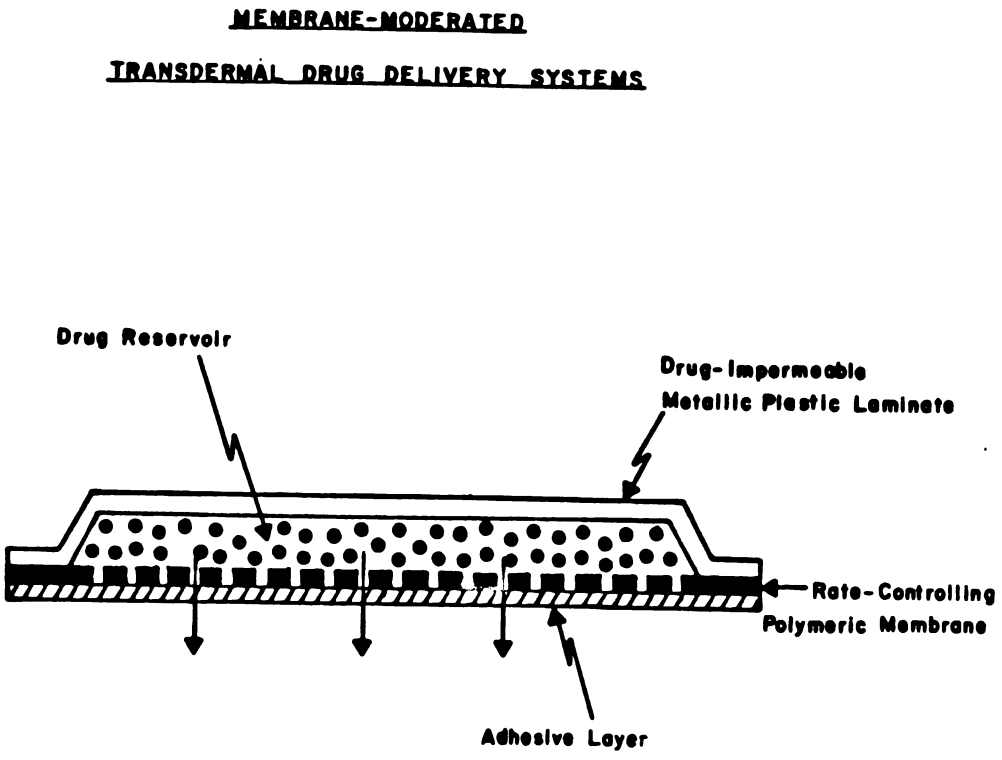


Figure 6. Diagram of a membrane-moderated transdermal drug delivery system.

2. Matrix Diffusion Controlled - The reservoir is manufactured by homogenously dispersing the drug in a polymer matrix which is then molded into a disc with a defined surface area and thickness (Figure 7). Drug release from the device into the body is controlled by diffusion through the matrix reservoir material. Currently there are three nitroglycerin transdermal systems of this type on the market.

3. Microsealed - The microsealed category is represented by the Nitro-Disc (Searle) device (Figure 8). In this system, the reservoir is formed by dispersing nitroglycerin adsorbed to lactose in a hydrophilic solvent system of 10 to 30% (v/v) polyethylene glycol in distilled water, which is subsequently distributed in a silicone elastomer by mechanical force to form thousands of microscopic drug compartments. Drug release is controlled by diffusion through the polymeric matrix.

3.3 Drug Selection

The choice of a compound for transdermal delivery depends upon a number of factors which may be grouped conveniently into two categories: biological and physicochemical (Guy and Hadgraft, 1987).

Biological Criteria

1. The drug must be potent, requiring a parenteral daily dose of milligrams or less. In most cases, this limitation translates into an effective plasma concentration in the μg/ml range.

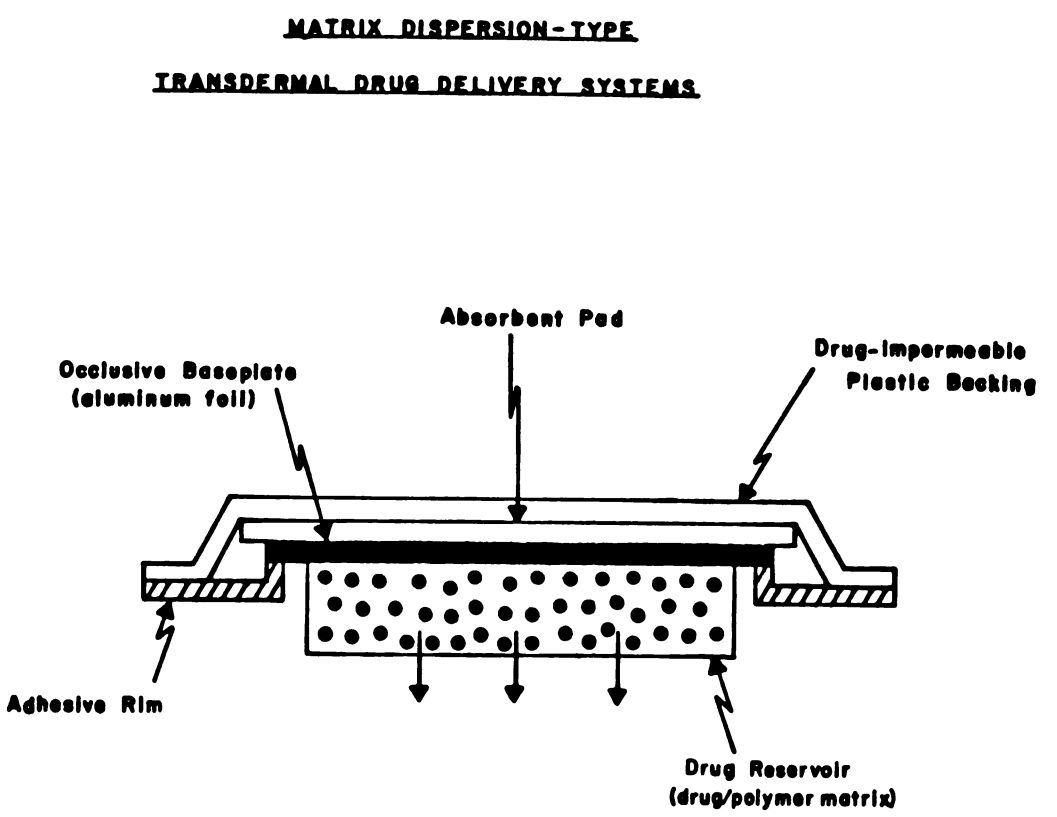


Figure 7. Diagram of a matrix dispersion-type transdermal drug delivery system.

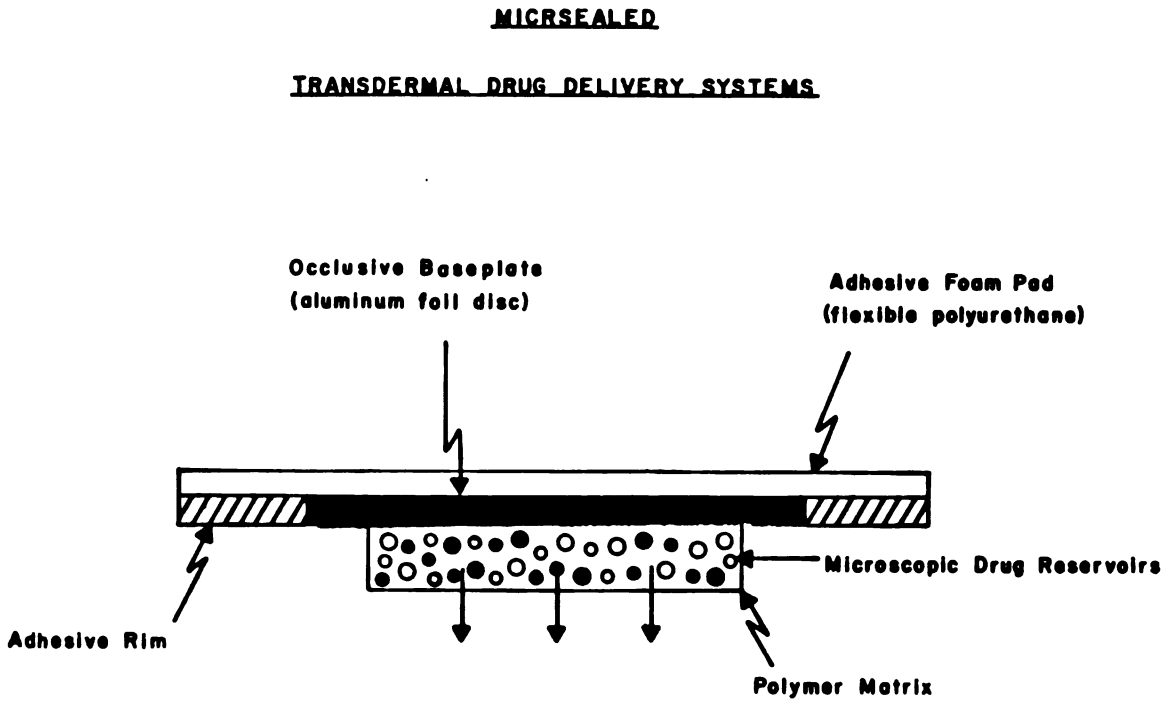


Figure 8. Diagram of the microsealed transdermal drug delivery system.

2. Drugs subject to an extensive hepatic first-pass effect on oral dosing may benefit by transdermal administration. Dose amount and dosing frequency may be significantly reduced in this way.

3. As for all forms of sustained or prolonged delivery, drugs with short (rather than long) biological half-lives are most appropriate.

4. The drug should not elicit a major cutaneous irritant or allergic response. The definition of "major" in this context is difficult to specify. The clonidine system, for example, has been launched successfully despite a relatively high rate of irritancy provocation (Grattan and Kennedy, 1985).

5 Because transdermal delivery typically provides constant drug input, it is important that the pharmacological effect of the agent be suited to this absorption pattern. The possible induction of tolerance must be carefully monitored, therefore.

6. Sensitivity of the drug to cutaneous metabolism (Noonan and Wester, 1985) within the viable epidermis or to degradation by surface microflora (Denyer et al, 1984) is clearly undesirable. Current understanding of these areas is sketchy, and methods for their evaluation are poorly developed.

Physicochemical Criteria

Physicochemical Criteria

The sequential events that a drug must undergo in order to become systemically available following application in a transdermal device are (Guy and Hadgraft, 1985):

1. Transport within the delivery system to the device-skin surface interface.
2. Partitioning from the delivery system into the stratum corneum.
3. Diffusion through the stratum corneum.
4. Partitioning from the stratum corneum into the viable epidermis
5. Diffusion through the viable tissue.
6. Uptake by the cutaneous microcirculation and subsequent systemic distribution.

It follows that diffusion and partitioning are the key physical processes pertinent to transdermal delivery.

Diffusion: Drug transport is determined primarily by the molecular size and the level of interaction with the medium through which diffusion is taking place (viz. delivery system, stratum corneum, viable epidermis). Most currently used drugs have molecular weights (M) less than 1000 daltons and the effect of size on diffusion coefficient may be adequately described by a power dependency (e.g., the Stokes-Einstein equation: D is proportional to $M^{-1/3}$) or sometimes, by an exponential function (Berner, 1984). Generally these relationships predict that D is much less sensitive to M than, for example, to the viscosity of the medium through which the drug is diffusing.

Partitioning: The partitioning criteria for a transdermal candidate are demanding. The molecule must favor the stratum corneum over the device, and the relative affinity of the drug for stratum corneum and viable tissue must be reasonably balanced. Extreme partitioning characteristics are not conducive to successful drug delivery via the skin. Correlations between skin absorption and various oil-water partition coefficients have been reported. Percutaneous penetration through human skin has been related to both heptane-aqueous buffer (Bartek et al, 1972) and octanol-water partition coefficients (Roberts et al, 1977). Linear free energy relationships have been established between steroid absorption across human skin and benzene solubility (Anjo et al, 1980) and log K (octanol/water) (Lien and Tong, 1973). The in-vitro transdermal penetration of a series of n-alkanols has been compared to the corresponding values of K (ether/water) (Blank et al, 1967, Flynn et al, 1981). A linear correlation was found up to octanol, but the behavior of subsequent homologs indicated that a change of rate-limiting step was occurring when the penetrant hydrophobicity reached a certain level (Figure 9). Mechanistically, a plausible explanation for this observation is that stratum corneum to viable epidermis transfer becomes a slower process than stratum corneum permeation for very lipid-soluble drugs. Therefore, an oil-water partition coefficient is a useful qualitative indicator of penetration, the reliability of which is least at the extremes of solute partitioning behavior.

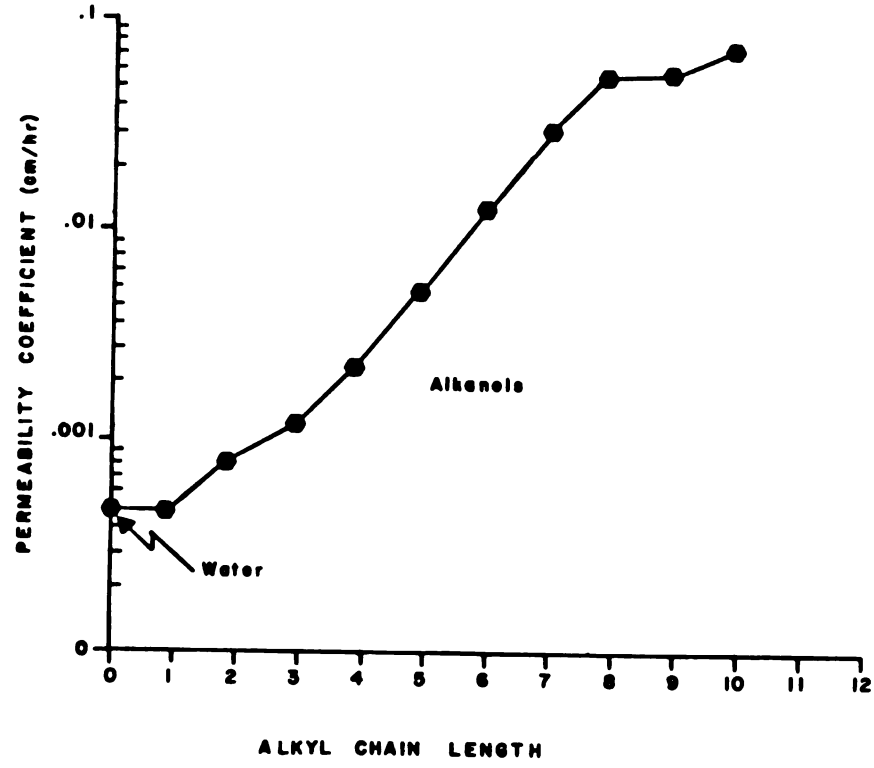


Figure 9. Skin permeability coefficients plotted vs. alkyl chain length for a series of n-alkanols.

3.4. Penetration Enhancement

To extend the therapeutic potential of transdermal drug delivery, it would be advantageous to have a reversible means of reducing the barrier to percutaneous penetration (Barry, 1983). This may be achieved to a certain degree using penetration enhancers or accelerants. However, it is often difficult to determine whether these additives affect the relelase of drug from the formulation or have a genuine effect on the stratum corneum. For example, propylene glycol has been quoted as a penetration enhancer, and has also been shown to alter the in-vitro release rate of steroids by its effect on the thermodynamic activity of the drug (Katz and Poulsen, 1971). These two effects are difficult to separate and may be complimentary. In certain circumstances, on the other hand, a penetration enhancer may decrease the barrier function of the skin, but at the same time reduce the thermodynamic activity of the drug. If this occurs, the formulation may have no apparent benefit. A useful working definition, therefore, may be that a penetration enhancer is an agent which, under conditions of constant drug thermodynamic activity, increases the permeability of the skin without severe irritation or damage to its structure.

In transdermal delivery, a penetration enhancer will, ideally, improve the rate of absorption of drugs into the systemic circulation. This possibility would considerably increase the number of drugs which could be delivered by this route. However, in order to utilize penetration enhancers, there are several criteria which should be fulfilled. These have been outlined by Katz and Poulsen (1971), and Barry (1983).

1. A penetration enhancer (PE) should elicit no pharmacological response.
2. A PE should act immediately with a predictable duration and its action should be reversible.

3. A PE should be chemically and physically stable and be compatible with all the components of the formulation.
4. A PE should be colorless, odorless, and tasteless.
5. A PE should be nontoxic, nonallergenic, and nonirritant.

There are, of course, very few chemicals which have all the above attributes, an a certain degree of compromise may be necessary in order to pursue a potential candidate PE. Certain molecules have shown penetration enhancing effects:

Water - In nearly all instances, hydrated skin is more permeable than dry skin. It is well recognized in the dermatological field that occlusion promotes the topical pharmacological effect of a number of drugs (e.g. steroids), although the effect is not necessarily ubiquitous. The mechanism by which water exerts its effects has not yet been established, but studies by Knutson et al (1985) show that increased hydration resulted in increased fluidity of the stratum corneum lipid bilayers.

Ethanol - Ethanol is a putative penetration enhancer, and is actually an integral component of the Estraderm transdermal delivery system for estradiol. It appears that lipid extraction is a key component of ethanol's enhancing effect (Kai et al, 1988).

Fatty acids and alcohols - These are particularly useful enhancers when they are applied with a cosolvent such as propylene glycol (Cooper, 1984). The optimum chain length for enchacement is approximately C-12 (Aungst et al, 1986); and cis-unsaturated analogs are more effective penetration enhancers than trans-unsaturated or saturated fatty acids (Golden et al, 1987; Cooper, 1984). Oleic acid for example, a C-18 fatty acid with a cis double bond at C-9, is a well recognized enhancer. Its mechanism of action, as studied by differential scanning calorimetry and fourier transform infrared

spectroscopy (Golden et al, 1987) appears to be related to the ability of this "kinked" molecule ot disrupt the stratum corneum intercellular lipid domains.

Finally, mention should be made of two alternative methods to acheive penetration enhancement: the use of prodrugs and iontophoresis. Neither of these approaches can claim any existing applications in transdermal drug delivery for systemic effect. The philosophy of the prodrug concept is generally straightforward in its use with respect to the skin: to prepare a labile, more lipophilic precursor of the drug which has improved skin permeability but rapidly hydrolyzed once it has breached the stratum corneum. The area of topical prodrugs has been reviewed in some depth (Barry et al, 1984). Iontophoresis has been recognized as a means of driving charged molecules across lipoidal membranes for a considerable time. There is evidence to indicate that ionic species can be driven across the skin by this technique (Tyle, 1986), however the problems associated with both formulation of suitable delivery systems and long-term passage of current at a specific skin site remain unresolved. It follows, therefore, that a significant amount of work remains to be performed before the potential use of these alternative approaches is clearly defined.

4. Liposomes

Liposomes are lyotropic smectic mesophases of membrane-like lipids, capable of forming enclosed structures that are relatively impermeable to entrapped ions (Bangham et al, 1974). They are classified by the number of bilayers which enclose the sequestered volume: unilamellar liposomes have a single bilayer, multilamellar liposomes have several. Unilamellar vesicles can be further subdivided on the basis of size; those with a diameter less than 1000 Å are considered small unilamellar vesicles (SUV) whereas those with greater diameters are designated large unilamellar vesicles

(LUV). Although usually conceptualized as an onion-like arrangement of concentric spheres, multilamellar vesicles (MLV) actually exist in a number of morphological configurations; for example as an LUV or MLV enclosing a number of SUV.

For a more comprehensive review of liposomes, the reader is directed to a number of books and review articles describing the general properties of liposomes and their use as model membranes (Bangham, 1972; Bangham et al, 1974), their preparation and characterization (Szoka and Papahadjopoulos, 1980,1981) and their use as therapeutic drug carriers (Ostro, 1987; Gregoriadis, 1973 a&b).

5. Background and Objectives of This Research

The potential utility of liposomes in topical formulations had been investigated prior to the initiation of this work. In an initial set of studies using rabbits in vivo, Mezei and Gulasekharam (1980,1982) considered the applicability of liposomes as drug carriers for the topical route of administration. From their experiments, they concluded that liposomal encapsulation could favorably alter drug disposition, selectively decreasing drug levels at the site of adverse effect while concentrating drug at the site of action. The suggestion, implicit in this early work, that intact liposomes could cross the stratum corneum, was questioned by the subsequent investigations of Ganesan et al (1984) and Ho et al (1985). Performing straightforward in vitro permeation experiments using excised hairless mouse skin, it was clearly demonstrated that neither intact liposomes, nor the phospholipid of which they were comprised, diffused through the barrier. Physical model analysis of their results, based upon delivery of progesterone and hydrocortisone, suggested a direct transfer of solute from liposome to skin and prompted the conclusion that more drug may be delivered percutaneously from liposomes than from simple aqueous solutions. Finally, Wester et al (1984) briefly described a

prototypal liposome transdermal system, from which estradiol delivery to rhesus monkeys in vivo was monitored and compared to intravenous and topical acetone solution controls. Absorption was quantified by measurement of ^{14}C-drug plus metabolites in the urine. The results suggested that constant drug input was possible for a three day period following application of the device.

It is the objective of the research described in this thesis to develop further the latter prototypal system, to establish the physicochemical basis by which it controls drug release into buffer solution, and to determine the mechanism by which it controls drug delivery to the skin . While it seems that liposomes cannot carry drugs across the skin, the unique amphipathic nature of their structure offers a reservoir system sympathetic to high concentrations of drugs spanning a wide range of physicochemical properties. Co-incorporation of both the active species and, for example, a penetration enhancer of quite different chemistry is also possible because of the bi-functional environment of an aqueous lipid vesicle suspension. In addition, the bilayer structure may provide a means to modulate drug release through the judicious selection of lipid formulation. An opportunity exists,therefore, to explore and to take advantage of a number of potentially useful determinants of controlled drug delivery.

Chapter II - Release Kinetics into Aqueous Buffer

1. Objective

To elucidate the mechanism by which the system controls the release of a model lipophilic drug (progesterone (PG)) into aqueous buffer.

Release profiles from the following systems were obtained:

1. Free PG
2. PG associated with egg phosphatidylcholine (EPC) liposomes
3. PG associated with EPC-cholesterol (2:1) liposomes
4. PG associated with Intralipid emulsion
5. PG associated with dimyristoyl phosphatidylcholine (DMPC) liposomes
6. PG associated with dioleoyl phosphatidylcholine (DOPC) liposomes
7. PG associated with dipalmitoyl phosphatidylcholine (DPPC) liposomes

2. Materials and Methods

Progesterone (PG) and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO); to enable the analysis of drug in the release experiments, ^{14}C-labeled PG (R.P.I. Corp., Mount Pleasanton, IL; 1.85MBq/umole) was incorporated into each formulation during the preparation procedure. Lipids were purchased from either Sigma Chemical Co. or Avanti Polar Lipids (Birmingham, AL), and were checked for purity by thin layer chromatography on silica gel plates with a mobile phase of chloroform:methanol:water (16:5:1). Intralipid 10% parenteral emulsion was acquired from Cutter Laboratories (Berkeley, CA). SeaPlaque® agarose and Gelbond® backing material were obtained from FMC Corp. Marine Colloids Division (Rockland, ME).

2.1. Preparation of multilamellar vesicle suspensions

Lipid, progesterone, and when appropriate, cholesterol were deposited from chloroform solution onto the sides of a round bottom flask by rotary evaporation. The materials were then resuspended by addition of sodium chloride (0.1M)-EDTA (0.1mM) buffer (pH 6.5) with constant nitrogen flushing and intermittent vortexing for 1 hour, at a temperature at least 10°C above the main transition temperature of the lipids. The final concentration of lipid in the suspension was 80 umole/ml; the final concentration of PG was 8 umole/ml.

2.2. Preparation of large unilamellar vesicle suspensions

Large unilamellar vesicles were prepared by reverse phase evaporation using the method of Szoka and Papahadjopolous (1978). Briefly, PG and lipid were dissolved in chloroform in a 50 ml round bottom flask, and the solvent was removed by rotary evaporation under reduced pressure. The system was then purged with nitrogen, and the lipid-PG mixture redissolved in diethyl ether (3:1 ratio of organic solvent phase to final aqueous phase). The aqueous phase was added, and the system (which was kept continuously under nitrogen) was sonicated for approximately 5 minutes until a single phase opalescent suspension was obtained The mixture was again placed on the rotary evaporator, and the organic solvent removed under reduced pressure. The resulting unilamellar vesicles were allowed to anneal for 1 hour at a temperature no less than10°C above the main transition temperature of the constituent phospholipid.

2.3. Preparation of devices

a. Liposome devices - A 4% agarose-aqueous buffer solution was heated to 67°C (the melting point of agarose). Then equal volumes (0.5 ml) of melted agarose and

multilamellar liposome suspension were combined and mixed thoroughly. The mixture was poured into a 2 cm diameter Teflon O-ring mold set up on the Gelbond® backing material. The patch was allowed to cool and solidify, and the O-ring was removed (Figure 10). The devices produced had areas of 3.14 cm^2 and were approximately 0.3 cm in width.

b. Emulsion devices - Emulsion patches were prepared by equilibrating 0.1ml of Intralipid emulsion (containing 40 umole lipid in the form of soybean oil emulsified with EPC) with the appropriate amount (4 umole) of PG for 24 hours. The resultant mixture was then combined with 0.9 ml of melted agarose solution and devices prepared as above.

c. "Free" PG devices - Melted agarose (0.5 ml) was combined with an equal amount of aqueous buffer containing 4 umole of PG. Devices were prepared as described above.

All systems were stored at 4°C for $\leq$ 24 hours prior to testing. No dependence of release characteristics on storage time was observed.

2.4. PG Release kinetics

a. Flow Through System - The release of PG from the seven delivery systems was monitored using a fully automated, continuously perfused, in vitro diffusion cell system (Figure11). The devices were clamped between the upper and lower halves of the glass permeation cells (Figure 12), such that the releasing surface of the system faced into the lower receptor chamber. This latter compartment was continously perfused via a cassette pump at 10 ml/hr with sodium chloride (0.1 M) - EDTA (0.1mM) buffer solution. Samples of perfusate were collected hourly on a

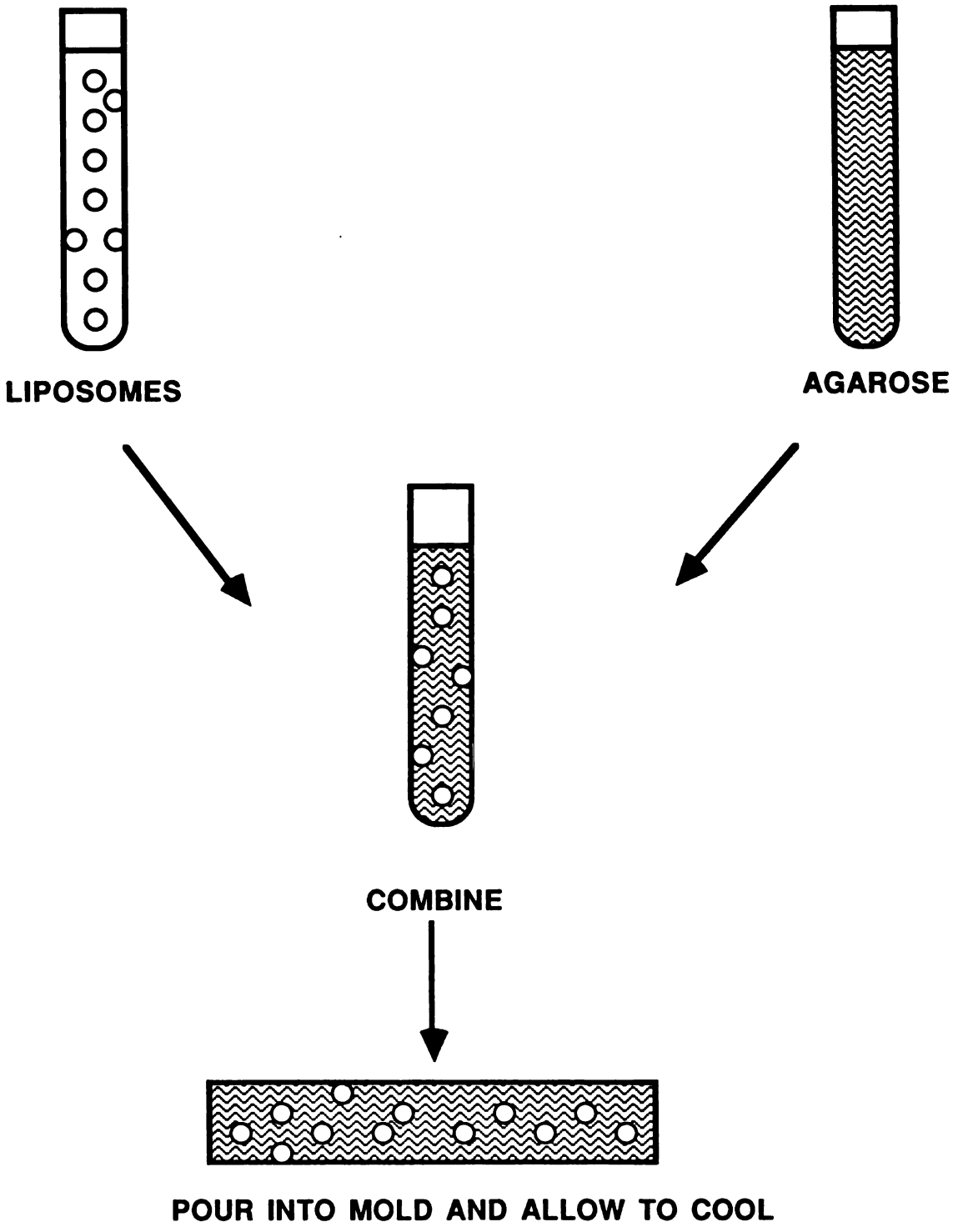


Figure 10. Schematic of method of making liposomal device.

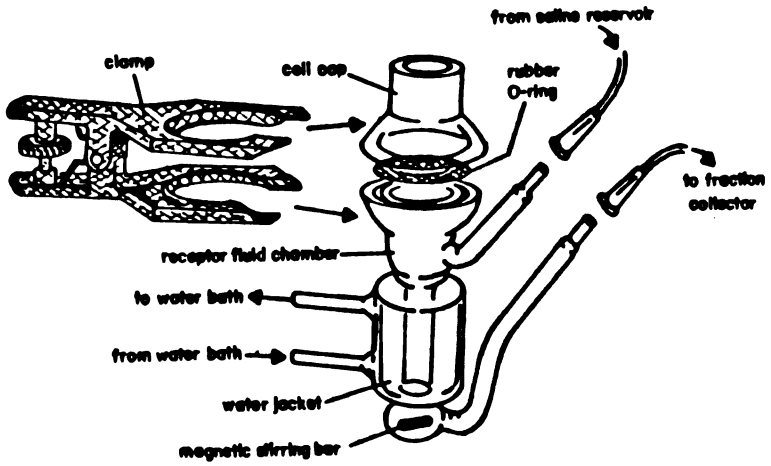


Figure 11. In vitro diffusion cell.

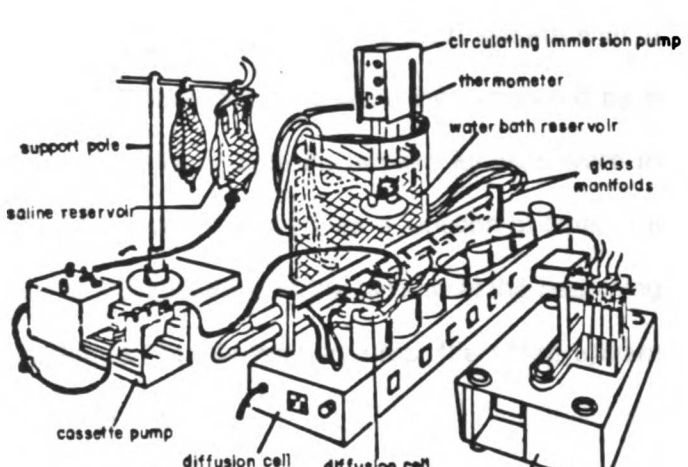


Figure 12. In vitro diffusion cell apparatus.

fraction collector for up to 48 hours. The receptor phase of the diffusion cell was stirred with a teflon-coated magnetic bar throughout the experiment. A thermostatting jacket, through which water at 35°C was passed, provided isothermal conditions for the release process. Analysis of the samples involved mixing with an appropriate amount of scintillation cocktail and measuring ^{14}C-radioactivity in a liquid scintillation counter. At the end of a run, PG which had not been released was extracted from the device with ethanol by incubating each system with 10 ml of solvent for I to 24 hours. The ethanol fractions were then analyzed by liquid scintillation counting. For each delivery system, release kinetics were measured in quadruplicate. PG release has been expressed, as a function of time, in terms of the percent of the total amount of PG contained in the system at the beginning of the experiment. For runs performed at low temperatures, the entire apparatus was set up in a cold room maintained at 4°C.

2.4.b. Static System - To minimize an apparent lag time caused by slow equilibration of penetrant in the flow through system, the release of PG from the "free" and DMPC formulations was also determined in a static cell system. As described above, the devices were clamped between the upper and lower halves of single-port Franz diffusion cells, the receptor chamber of which was filled with buffer solution. Following commencement of transport, the entire volume (10 ml) of the receptor chamber was removed and replenished with fresh buffer solution at 15 minute intervals. Analysis of PG was performed by liquid scintillation counting as above. Steady-state PG release in the static system was indistinguishable from that measured in the flow-through configuration, for both of the systems examined.

2.5 Differential scanning calorimetry (DSC)

DSC measurements were made with a Perkin Elmer DSC-2 calorimeter operating at a sensitivity of 2 mcal/sec and a scanning rate of 5°C/min. Samples were concentrated by centrifugation (15,000 rpm for 5 minutes) in an Eppendorf centrifuge, the supernatant was discarded, and 14 ul of the pellet were sealed in an aluminum sample pan. An equal amount of buffer was placed in the reference pan. Three heating and cooling scans were run for each sample.

3. Results

DSC thermograms for the pure DMPC liposomes and liposomes containing 10 mole% PG are shown in Figure 13. The transition temperature (Tc) of pure DMPC vesicles, as measured by extrapolation of the rising endothermic curve to the baseline, was 23.5 °C. This value agrees well with those reported in the literature (Silvius, 1982). Steroid incorporation broadened the endothermic peak, and lowered the onset of Tc by approximately 2°C. The same quantitative effect has been reported for 10 mole% PG - DPPC suspensions (O'Leary et al, 1984), where the onset of Tc was lowered from 41°C to 39°C. The low transition temperatures of EPC and DOPC liposomes (-5°C and -18°C respectively), precluded the necessity of obtaining DSC measurements, because experiments were not performed below the Tc of these liposomes.

Progesterone release curves into buffer from the seven formulations are shown in Figures 14 through 20. Each figure includes the mean (± s.d.) release rate of drug as a function of time, and a plot of the average cumulative amount of PG liberated from the delivery system over the course of the experiment. The cumulative amounts released from the different devices in 24 hours are in Table 3. Three

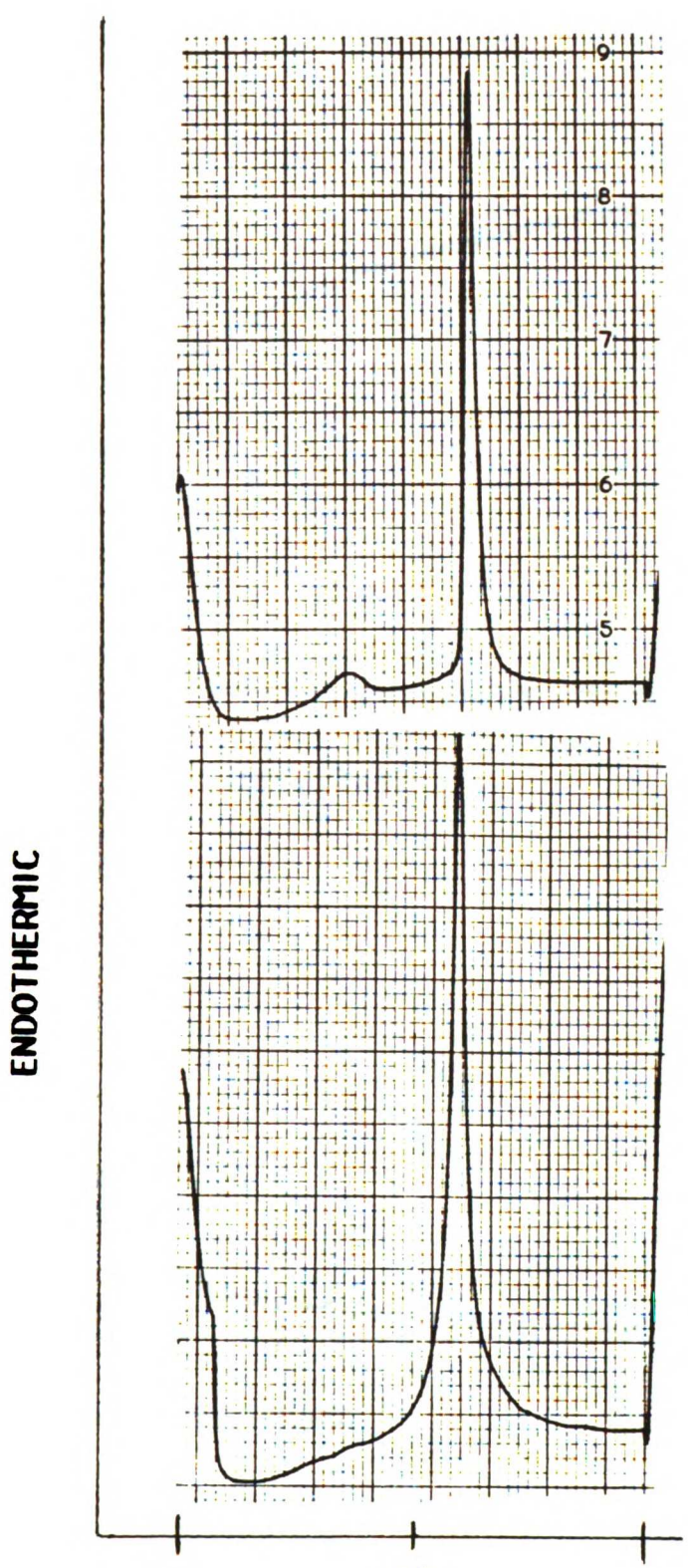


Figure 13. DSC thermogram of (a) pure DPMC vesicle suspension and (b) 10% PG - DMPC suspension.

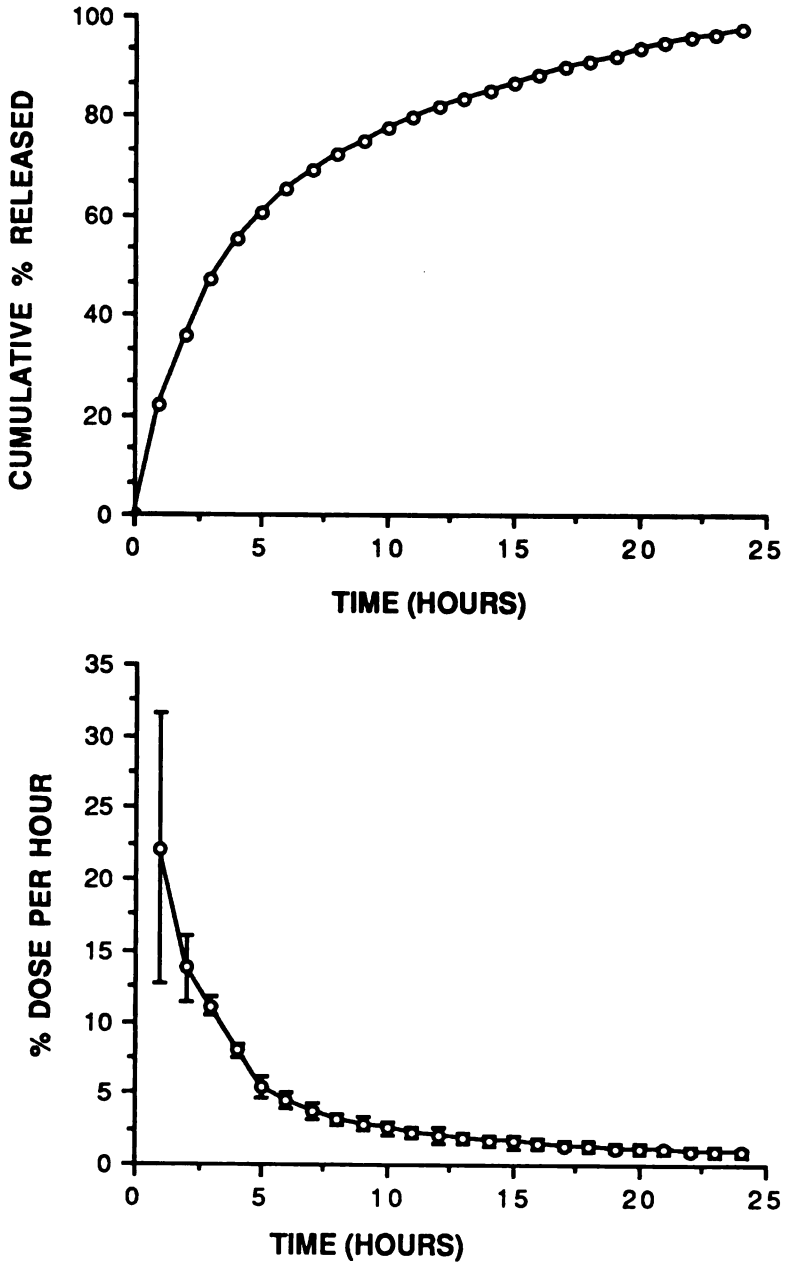


Figure 14. PG release kinetics from device (1) (PG alone).

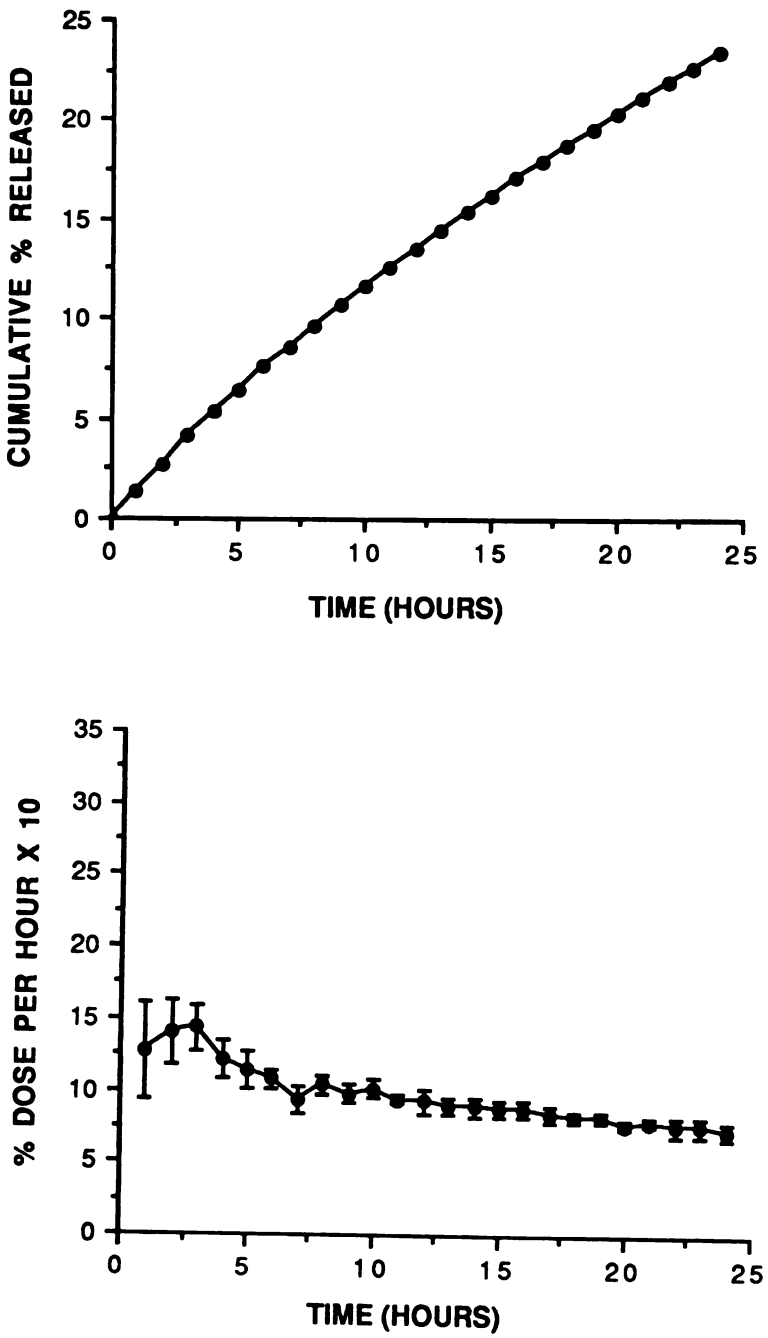


Figure 15. PG release kinetics from device (2) (PG-EPC liposomes).

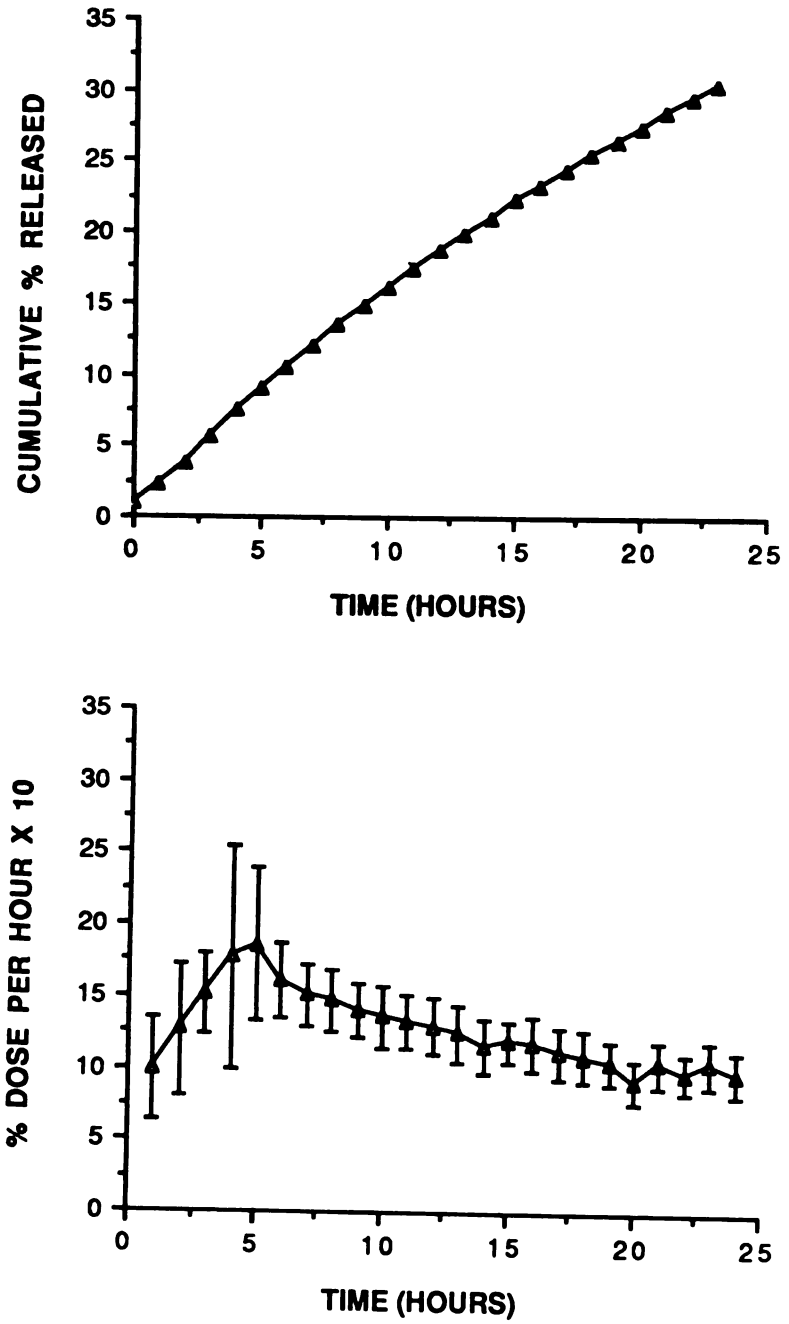


Figure 16. PG release kinetics from device (3) (PG-EPC-cholesterol liposomes).

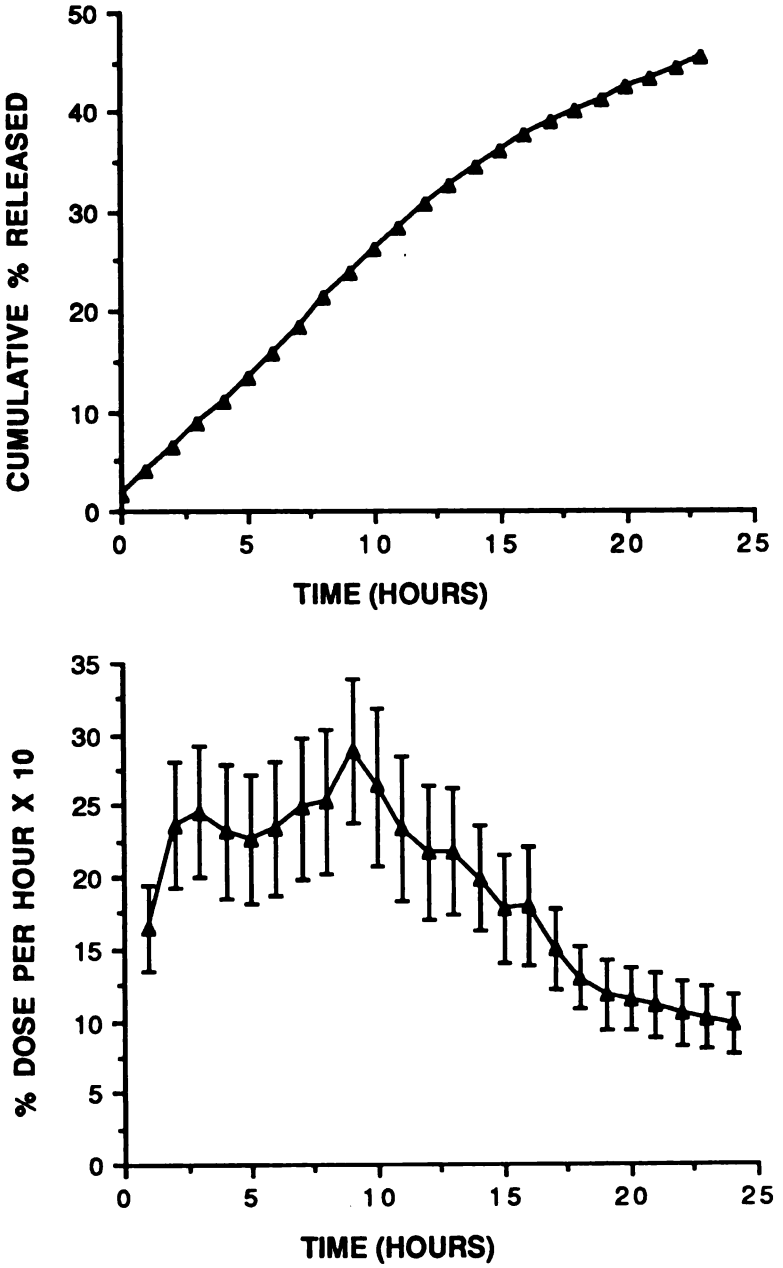


Figure 17. PG release kinetics from device (4) (PG-Intralipid emulsion).

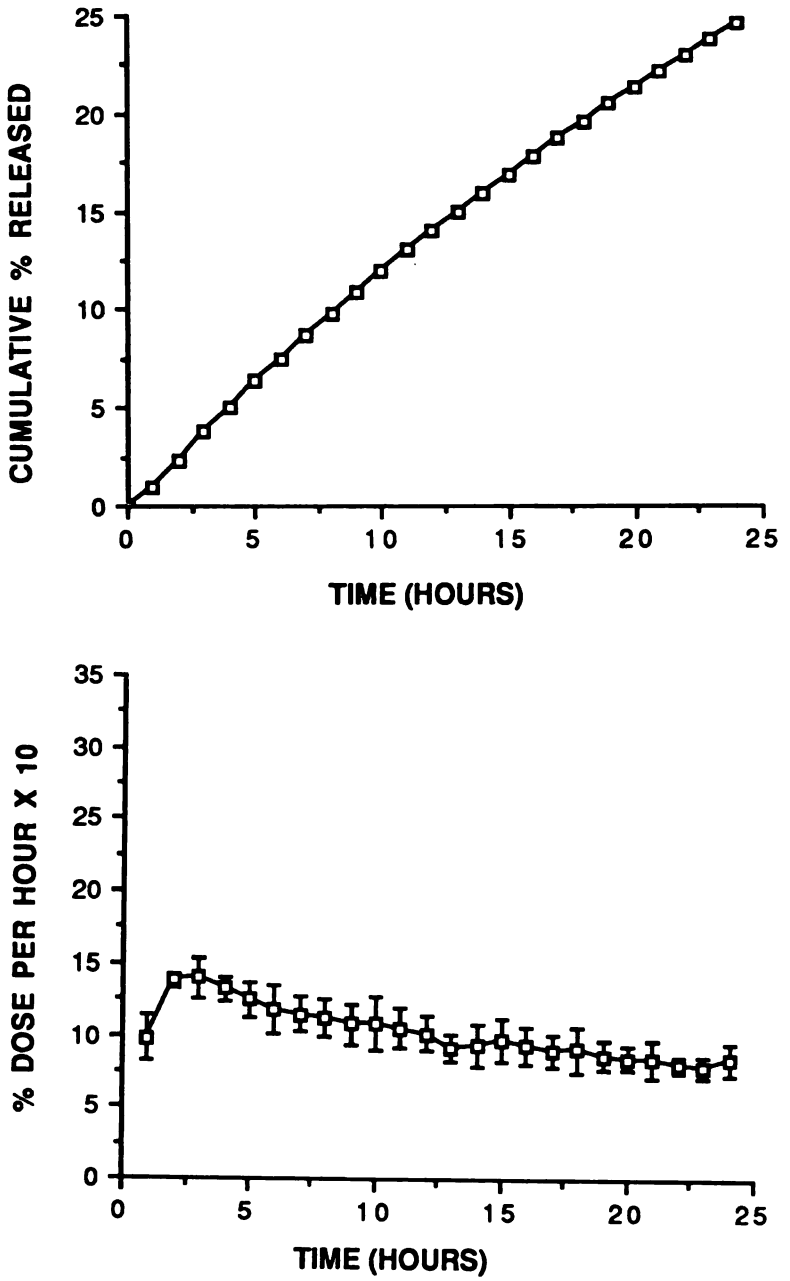


Figure 18. PG release kinetics from device (5) (PG-DMPC liposomes).

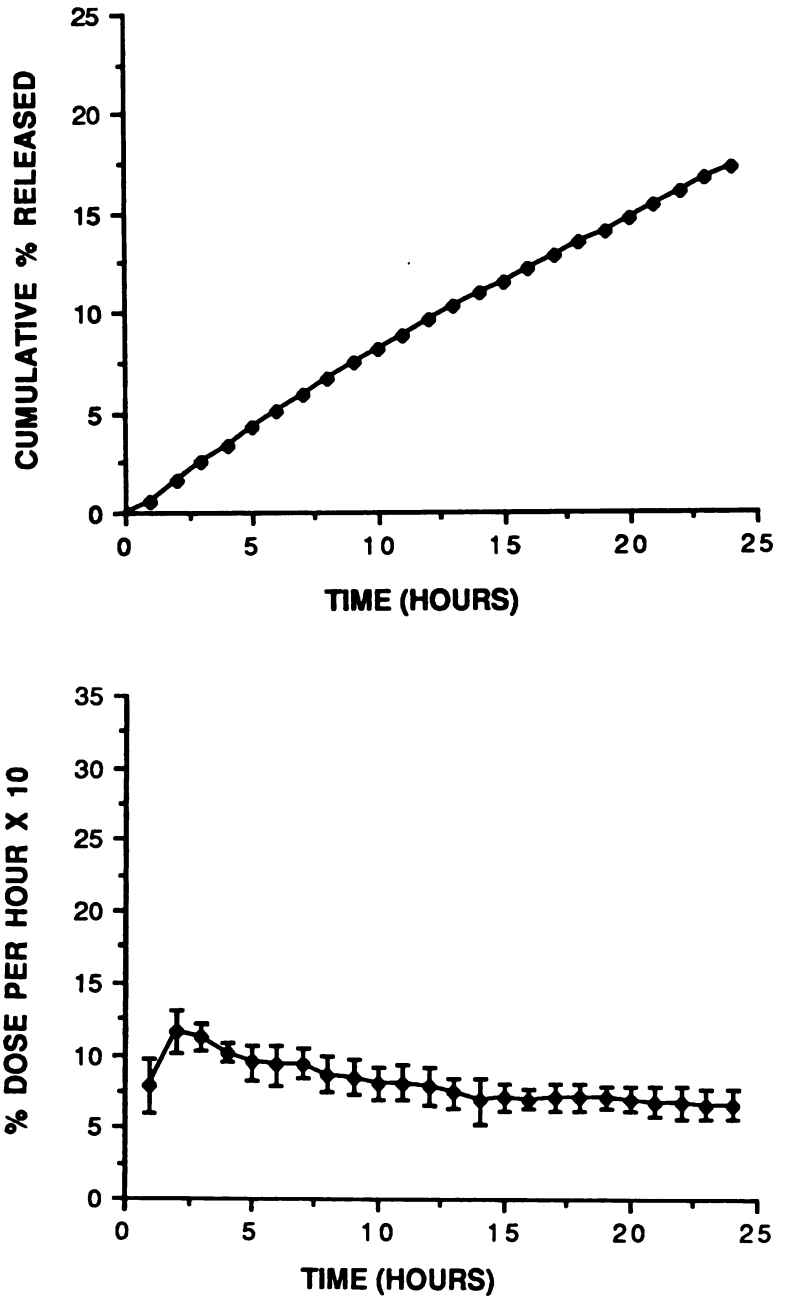


Figure 19. PG release kinetics from device (6) (PG-DOPC liposomes).

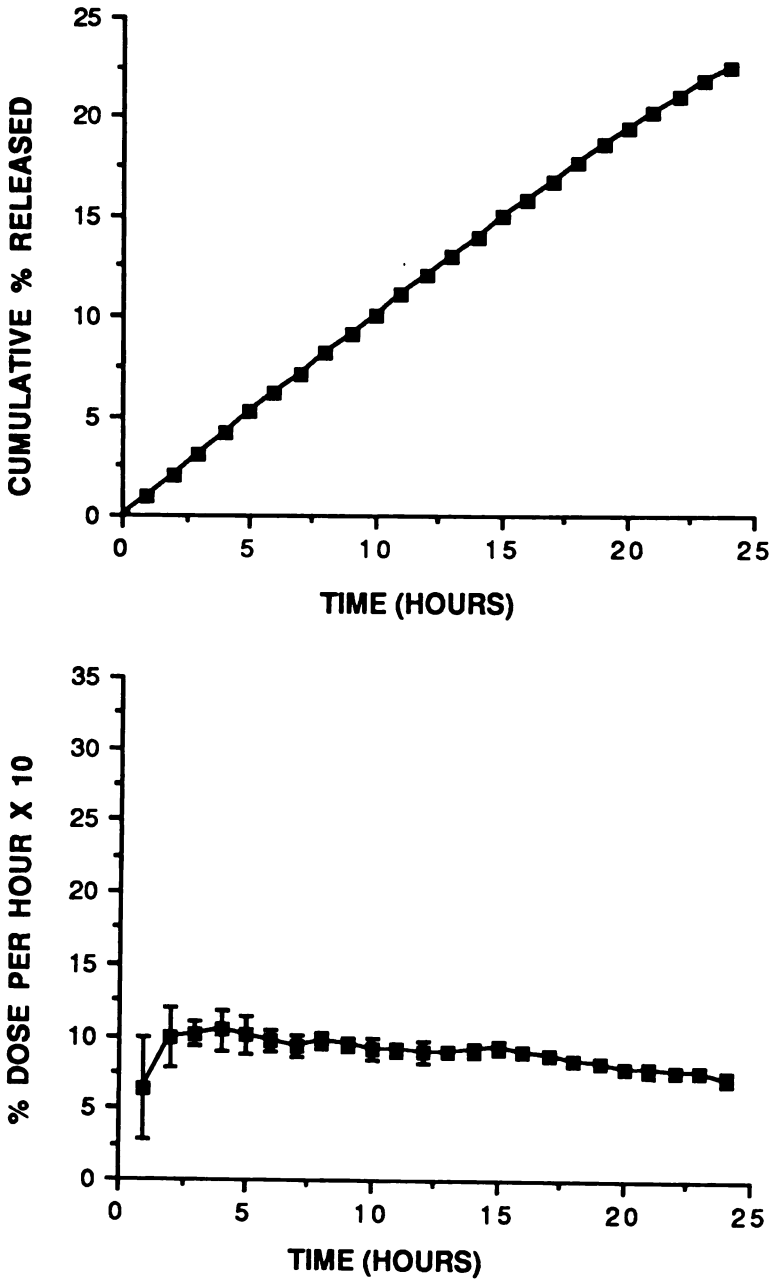


Figure 20. PG release kinetics from device (7) (PG-DPPC liposomes).

Formulation	Tc (°C)[a]	CCL/#DB[b]	% Dose Released[c]
PG Alone	- - -	- - -	90.4 ± 5.5
PG/EPC Liposomes	- 5	mixed	24.8 ± 3.8
PG/CHOL/EPC Liposomes	- - -	- - -	26.3 ± 1.5
PG/Intralipid® Emulsion	- - -	- - -	48.4 ± 1.9
PG/DMPC Liposomes	2 3	1 4 / 0	24.6 ± 2.4
PG/DPPC Liposomes	4 1	1 6 / 0	21.2 ± 1.1
PG/DOPC Liposomes	- 2 2	1 8 / 1	15.9 ± 5.1

[a] Transition Temperature of phospholipid. [b] Carbon Chain Length/Number of Double Bonds.
[c] Mean ± standard deviation of 4 to 6 experiments.

Table 3. Cumulative amounts of PG, expressed as mean % (± s.d.; n = 4-6) of initially encapsulated dose, released in 24 hours from 7 delivery systems into buffer.

important points are immediately apparent: (1) all of the lipid formulations released significantly ($p < 0.01$) less PG than the "free" system in 24 hours; (2) the liposomal systems liberated PG at essentially identical rates; however, the emulsion patch released significantly more PG during 1 day; and (3) the lipid-containing formulations all released PG with zero-order kinetics for at least part of the experiment. The liposome systems released drug at approximately 1%/hr for 24 hours. The emulsion patch released PG at about 2.4%/hr for 0-10 hours; the release rate then declined gradually over the next several hours. To determine whether liposomes themselves were able to diffuse through the agarose matrix and reach the receptor phase, DPPC patches containing ^{14}C-labelled lipid were prepared and were allowed to remain in contact with continuously perfused buffer for 48 hours. No radioactivity was detected in the buffer solution, confirming that the liposomes are spatially immobilized within the agarose matrix.

4. Discussion

4.1. Differential scanning calorimetry (DSC)

DSC provides information on the physical properties of liposomal bilayers through measurement of the temperature and width of lipid phase transitions (Ladbrooke and Chapman, 1969). The DSC profile indicates the fluidity of the bilayer as a function of temperature and allows perturbations of bilayer organization (e.g. by added compounds) to be evaluated (Jain and Wu, 1977). The objectives of the DSC experiments were: (1) to determine the extent to which PG interacts with liposomal bilayers, and (2) to quantify the effect of PG incorporation on liposome transition temperature.

The low water solubility (10-15 ug/ml), and high octanol-water partition coefficient (ca. 10^4 (Leo et al, 1971)) of PG suggest that essentially all of the

steroid, colyophilized with lipid in a vesicle dispersion, remains in the lipid phase. Figure 12 shows that the incorporation of 10 mole% PG into DMPC liposomes broadens the endothermic lipid melting peak, and lowers the transition temperature by approximately 2°C. Similar results for DPPC liposomes have been reported (O'Leary et al, 1984). Melting thermograms exhibiting broadened transitions and lowered transition temperatures are characteristic of bilayers modified by the incorporation of solutes into the acyl chain region of the bilayer (Jain and Wu, 1977). The DSC observations results are further supported by Raman spectroscopic measurements, which also reveal that progesterone is localized within the acyl chain region of the bilayer (O'Leary, 1984). It can be concluded, therefore, that: (1) PG interacts with, and is intercalated in, the liposomal bilayer, and (2) this interaction lowers the phase transition temperature of DMPC and DPPC liposomes by approximately 2°C. Hence, experiments performed at 35°C for the DPPC formulation and at 4°C with the DMPC formulation examined the efflux of PG from "solid" liposomes.

4.2. Release Data

The PG release data into buffer (Figures 14-20) show that the lipid formulations are able to modulate the delivery kinetics of drug. Table 3 indicates that the preparative techniques employed produce devices of reproducible release characteristics.

1. Emulsion Device

Examination of Table 3 and Figure 17 reveals that the cumulative amount of PG released in 24 hours from the Intralipid device falls between that of the liposomal and the "free" devices. Detailed interpretation of these results is difficult however, because the emulsion is not well characterized. Since egg lecithin is used as an

emulsifing agent in Intralipid, there is a strong possibility that the formulation includes multilamellar liposomes. To what extent these are present, though, cannot be elucidated, and comments on the nature of the release profile cannot progress beyond the speculative.

2. Liposomal Devices

Statistical analysis of the data presented in Table 3 revealed no significant difference in the cumulative amount of PG released in 24 hours between the liposomal systems.

a. Effect of Cholesterol

Adding cholesterol normally "tightens" a fluid phospholipid bilayer rendering the membrane less permeable to hydrophilic solutes (de Gier et al, 1968; Papahadjopoulos et al, 1971). This appears to be due to a condensing effect on the lipid fatty acid acyl chains at temperatures above that of the gel-liquid crystalline phase transition (Oldfield et al, 1978; Brown and Selig, 1978). Overall, the result is to decrease the effective diffusion coefficient of a solute within the bilayer. The results in Figures15 and 16 and Table 1 demonstrate, however, that the incorporation of 33 mole% cholesterol into the liposome formulation does not effect the efflux of PG. This may be explained by (a) the inability of cholesterol to exert a condensing effect on a bilayer containing10 mole% PG, or (b) the fact that PG release is independent of the microviscosity of the liposomal bilayer (see below).

b. Effect of Acyl Chain Order

In these experiments, we have studied PG release from a range of phosphatidylcholine-based liposomes. The head groups are consistent, but the acyl chain regions of the different phospholipids used vary considerably. As a result, the phase transition temperatures (Tc's) of the liposomes prepared span a range that

includes the experimental temperature (see above). Despite the significant differences in the physicochemical properties of the lipids, an analysis of variance reveals that the release of PG from multilamellar vesicles is independent of the lipid used (Table 3). Further experiments were performed in which PG release data was obtained from DMPC vesicles at temperatures above and below the Tc (see Figure 21). No significant differences between the release kinetics at 4°C and 35°C were found. Two explanations are consistent with these results: (1) below the phase transition temperature a lateral phase separation occurs in which PG-phospholipid clusters, whose fluidity is invariant over a wide temperature range, coexist with regions of pure, solid phospholipid. Such behavior has been observed in both androstane-lecithin and cholesterol-lecithin mixtures below Tc (Sackmann and Trauble, 1972 a & b; Trauble and Sackmann, 1972; Darke et al, 1972; Owicki and McConnell, 1980) or, alternatively, (2) PG efflux is primarily influenced by its association with a lipophilic medium and is not affected by the physical state (gel versus liquid crystalline) of the lipid acyl chains in which it resides. Further experiments (i.e. electron spin resonance measurements) are needed to distinguish between these two possibilities.

c. Release from multilamellar vesicles (MLVs) vs large unilamellar vesicles (LUVs)

Examination of Figure 22 reveals that PG efflux from a device containing EPC LUV's is identical to that from an EPC MLV device. These results suggest that drug diffusion between lamellae (within a multilamellar vesicle) is fast, relative to drug release at the outermost bilayer.

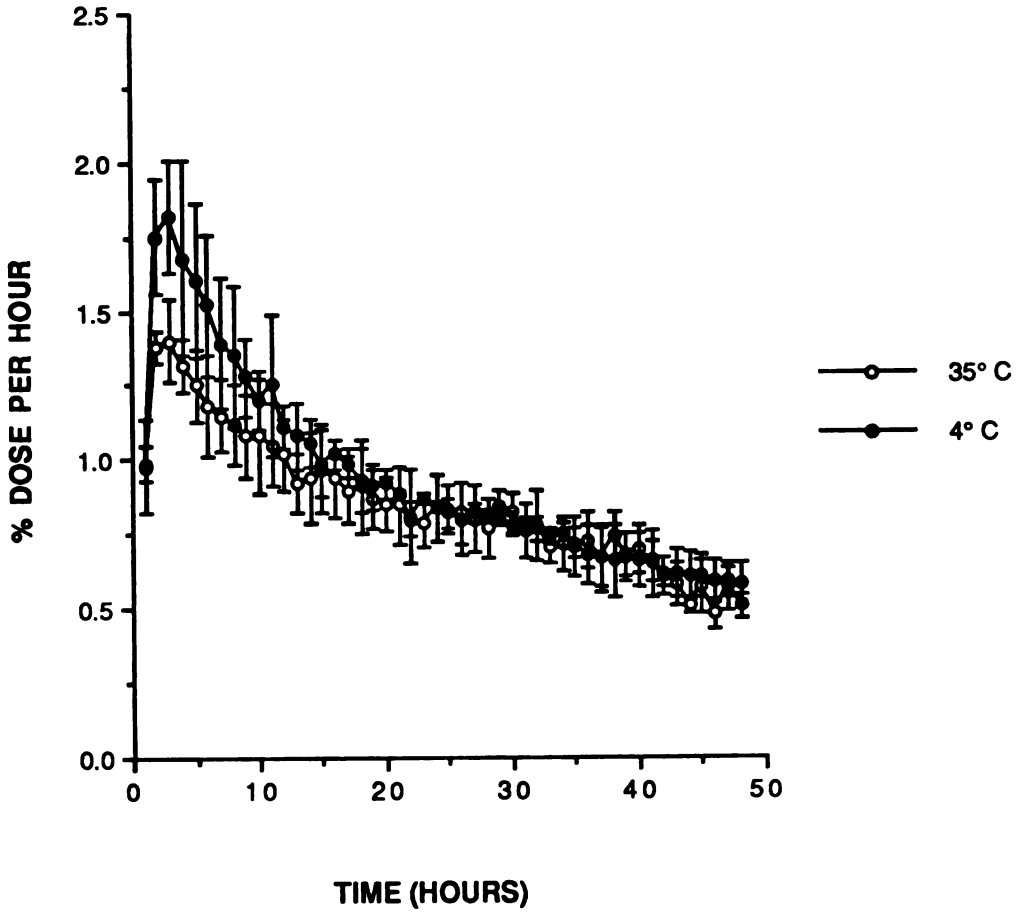


Figure 21. PG release kinetics from PG-DMPC device (5), at 4°C and 35°C (mean ± s.d.; n = 4).

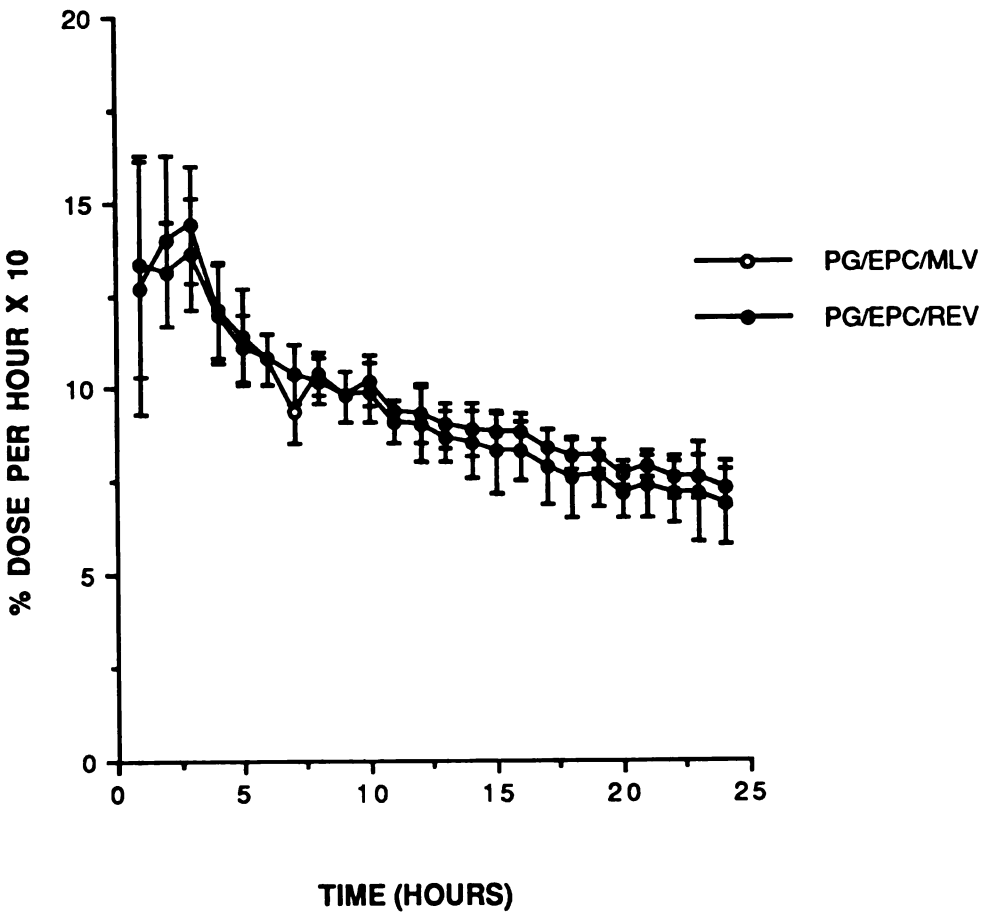


Figure 22. PG release kinetics from PG-EPC MLV device and PG-EPC REV device (mean ± s.d.; n = 4).

4.3. Mechanism of release

Two mechanisms of PG release control have been examined theoretically: (1) slow diffusion of PG through the lipid-agarose matrix, and (2) slow interfacial transfer of drug from the hydrocarbon region of the bilayer to the surrounding aqueous phase. Mathematically, these mechanisms can be modelled as follows:

a. Diffusion control

If it is first assumed (Fig 23a) that a lipid-agarose system provides a uniform matrix, through which PG diffuses slowly with an effective diffusion coefficient D_{eff} and that this slow transport process controls drug release, then the form of the release profile can be deduced. This is accomplished by solving Fick's Second Law of Diffusion

$$\frac{\partial C}{\partial t} = D_{eff}\left(\frac{\partial^2 C}{\partial x^2}\right) \qquad (1)$$

with the initial condition that PG is uniformly dispersed within the device at the beginning of the experiment:

$$C = C_o \text{ at } t = 0,\ 0 < x < L$$

and the two boundary conditions:

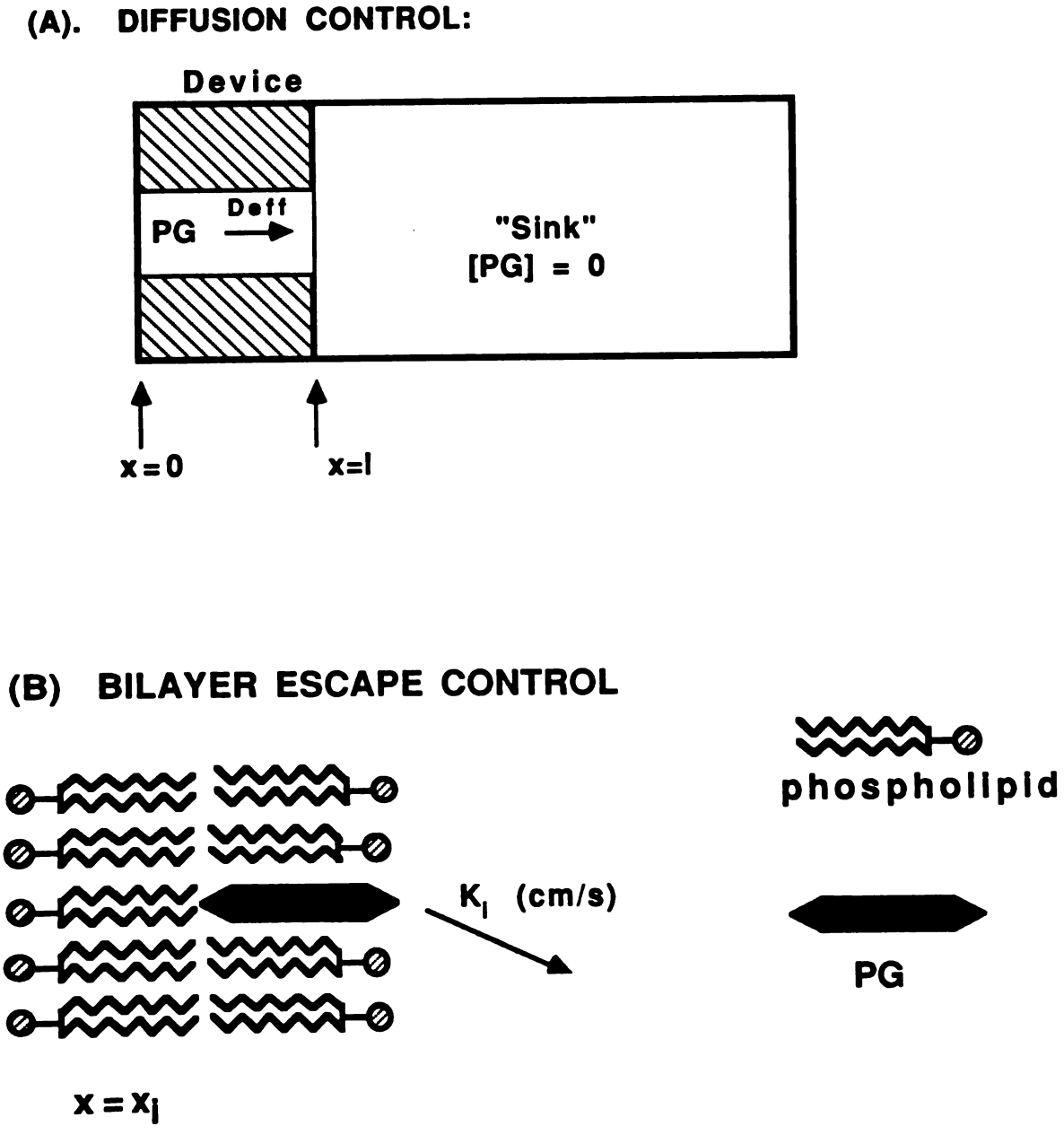


Figure 23. Putative mechanisms of PG release from liposomal delivery systems: (a) diffusion controlled and, (b) bilayer "escape" controlled.

1. The receptor chamber is a perfect "sink" for PG, that is:

$$C = 0 \text{ at } x = L, \quad t \geq 0$$

2. There is no loss nor replenishment of PG at the unexposed surface of the device:

$$\frac{\partial C}{\partial x} = 0 \text{ at } x = 0, \quad t > 0$$

Limiting our interest to the early phase of the release behavior (the first 24 hours), during which time less than 30% of the drug has been liberated, it can be easily shown (Crank, 1975) that the solution to equation 2, with boundary conditions specified by equations 2-4, is:

$$\%D = (100)\frac{2}{L}\left(\frac{D_{eff}\,t}{\pi}\right)^{\frac{1}{2}} \qquad (2)$$

i.e., the cumulative % dose (%D) released is proportional to the square root of time. In Figures 24 and 25 the average (± s.d.; n=4) cumulative % dose released from the free PG and the PG-DMPC systems are plotted against the square root of time. The plot for the free system is linear, indicating that diffusion of PG through the agarose matrix is rate limiting. However, for the PG-DMPC device, the graph is significantly non-linear, questioning, therefore, the validity of this release mechanism.

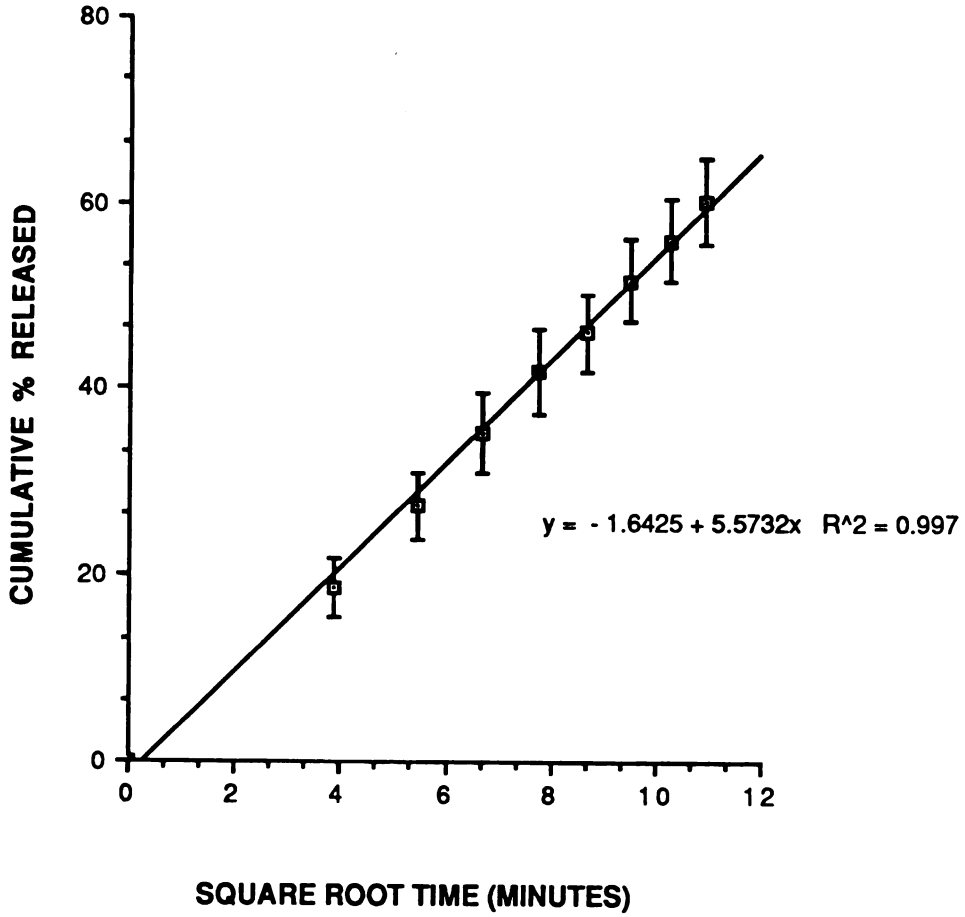


Figure 24. Linearity of PG release from "free" PG systems plotted vs. the square root of time (mean ± s.d.; n = 4).

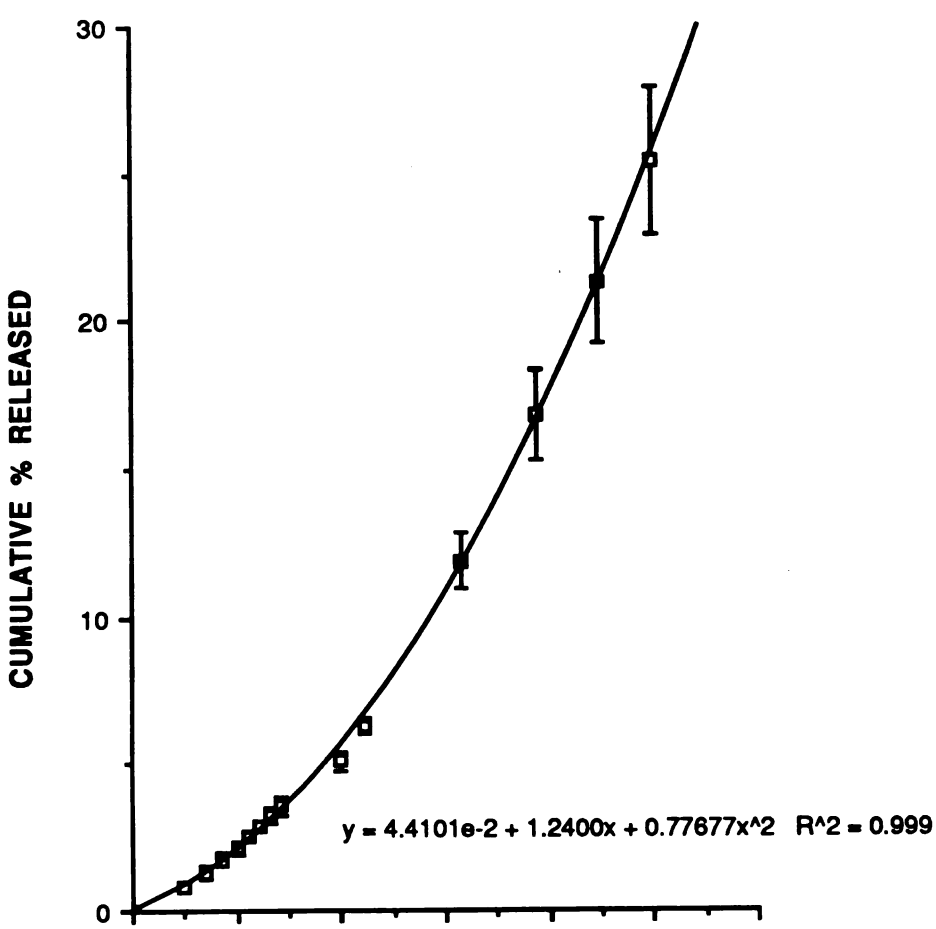


Figure 25. Non-linearity of PG release from PG-DMPC systems plotted vs. the square root of time (mean ± s.d.; n = 4).

b. Interfacial control

An alternative interpretation may be proposed which recognizes the biphasic nature of the liposome-agarose matrix and which postulates a different rate-limiting process. As discussed above, in the PG-liposome formulations, essentially all of the drug is initially associated with the liposomal bilayer. The alternative model for release (Figure 23b) proposes that the interfacial "escape" of PG from the bilayer is a slow process (determined by a heterogenous transport coefficient (k_{AL}, cm/sec)), relative to the subsequent diffusion of the drug through the aqueous gel network into the receptor phase. In this case, the total flux (J) of PG into the receptor phase is equal to the combined rate of escape of drug from the liposome ensemble, i.e.,

$$J = \sum_{j=1}^{n} J_j^L \qquad (3)$$

where J_j^L is the flux of PG out of the jth liposome. If interfacial transport is indeed the rate limiting step, then J can be re-expressed as follows:

$$J = k_{AL} \sum_{j=1}^{n} A_j C_j^l \qquad (4)$$

where C_j^L is the PG concentration in, and A_j is the surface area of, the jth liposome. Assuming that the vesicles are of comparable size (A_j = constant = A, and the total liposome surface area is $nA = A_T$), then it should also be true that C_j^L is constant (C^L) from liposome to liposome (since PG is evenly distributed at t = 0). Hence

$$J = (A_T)\ (k_{AL})\ (C^L) \qquad (5)$$

that is, the rate of PG release is predicted to be constant with time for the early portion of drug efflux when the level of liposomal PG is not significantly depleted. Therefore, the cumulative % released when plotted against time, should yield a straight line. Figure 26 provides experimental support for the model described by Equation 8. It may be concluded, therefore, that our data are consistent with the hypothesis that PG release from liposomal bilayers is determined kinetically by a process corresponding to very slow partitioning of the drug into the surrounding aqueous medium. It should be stated that this second interpretation assumes that PG is exclusively associated with the liposomal bilayer at t = 0 and that PG depletion from the vesicles over the time period considered is small. This approximation is justified because of the very high lipid-water partition coefficient of the drug. For less lipophilic molecules, the "interfacial" interpretation will require that the transport equations for drug in both the lipid and aqueous environments of the patch be solved simultaneously (Bodde and Joosten, 1985).

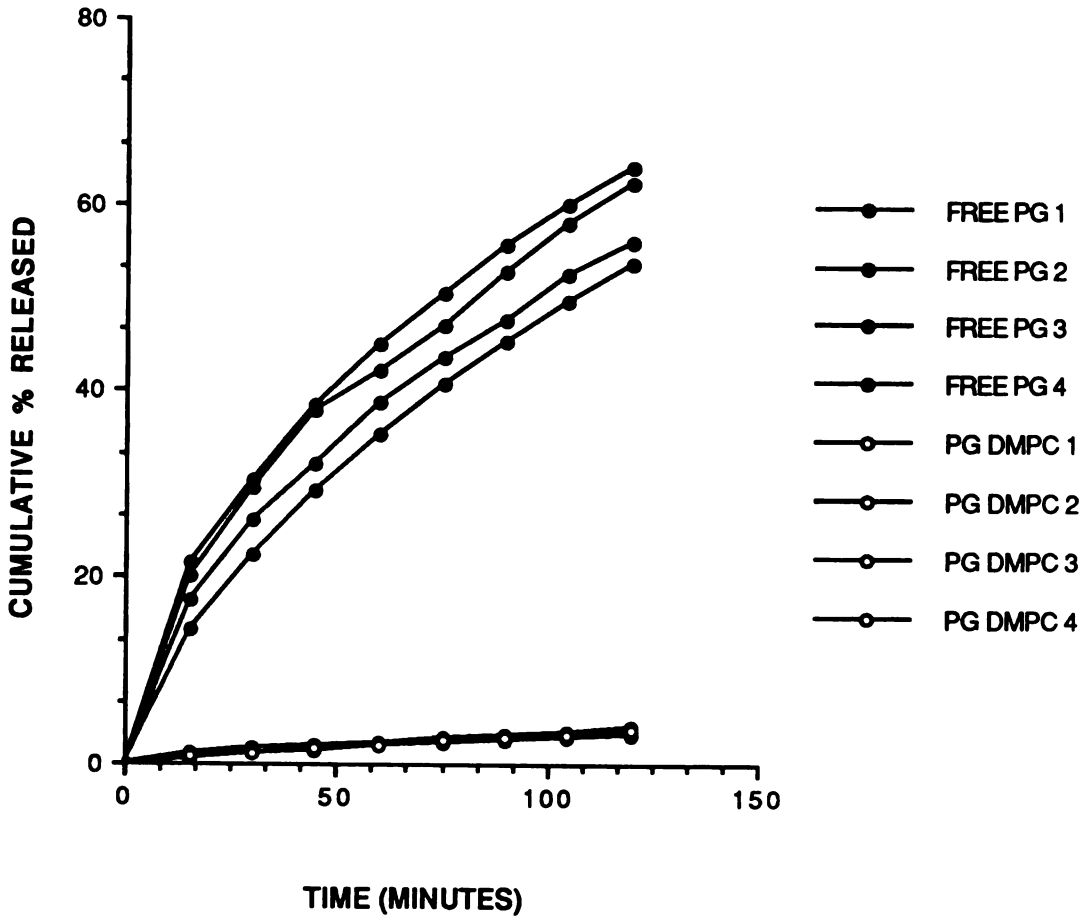


Figure 26. Representative plots of the cumulative amount of PG released from the "free" (n=4) and PG-DMPC (n=4) systems plotted vs. time.

Chapter III - Transdermal Delivery Characteristics of the Device

1. Objectives

To determine the mechanism by which the system controls the transdermal flux of PG: (a) in-vitro across hairless mouse skin, and (b) in-vivo in hairless guinea pigs.

The in-vitro transdermal delivery characteristics of the following systems have been studied:

1. Free PG
2. PG associated with EPC liposomes
3. PG associated with DMPC liposomes
4. PG associated with DPPC liposomes
5. PG associated with DOPC liposomes
6. PG and oleic acid (OA) associated with DPPC liposomes
7. PG and stearic acid (SA) associated with DPPC liposomes

In-vivo, transdermal delivery was studied from the following devices:

1. Free PG
2. PG-EPC Liposomes
3. PG-DPPC Liposomes

2. Materials and Methods

Progesterone (PG), oleic acid (OA) and stearic acid (SA) were purchased from Sigma Chemical Co. (St. Louis, MO); to enable the analysis of drug in the release experiments,

^{14}C-labeled PG (R.P.I. Corp., Mount Pleasanton, IL; 1.85MBq/umole) was incorporated into each formulation during the preparation procedure. Lipids were purchased from Avanti Polar Lipids (Birmingham, AL), and were checked for purity by thin layer chromatography on silica gel plates with a mobile phase of chloroform:methanol:water (16:5:1). SeaPlaque agarose and Gelbond backing material were obtained from FMC Corp. Marine Colloids Division (Rockland, ME). Hairless guinea pigs (Hartley) were obtained from Charles River (Wilmington, MA). Hairless mice (SKH:HR-1; aged 5-15 weeks) were obtained from the Skin Cancer Hospital, Philadelphia, PA . Hilltop chambers were purchased from Hilltop Inc (Cincinnati, OH) and Tegaderm™ adhesive dressing was aquired from 3M Company (St. Paul, MN).

2.1. Preparation of MLVs

Lipid, progesterone, and when appropriate, oleic or stearic acid were deposited from chloroform solution onto the walls of a round bottom flask by rotary evaporation. The materials were then resuspended by addition of sodium chloride (0.1M) - EDTA (0.1mM) buffer with constant nitrogen flushing and intermittent vortexing for 1 hour at a temperature at least 10°C above the main transition temperature of the lipids. The final concentration of lipid in the suspension was 80 umole per ml; the final concentration of PG was 8 umole per ml.

2.2. In-vitro transdermal studies

The devices prepared for in-vitro transdermal flux studies were slightly smaller than those used for the release into buffer experiments. This reduction in area allowed the systems to be placed on the skin surface with ease at the start of the experiment. The systems were made by adding 0.3 ml of agarose to 0.3 ml of PG formulation, pouring into an appropriate mold and allowing the mixture to cool. The resulting devices had a releasing surface area of 2.0 cm^2, and were 0.3 cm thick.

Transdermal transport of PG was followed using the same in vitro flow-through diffusion cell apparatus described previously (Chapter 2); however, full thickness hairless mouse skin was interposed between the delivery system and the receptor chamber.

2.3 In-vivo studies

A 4% agarose-buffer solution was heated to its melting temperature of 67°C. Then equal volumes (0.3 ml) of melted agarose and liposome suspension were combined, mixed thoroughly, poured into a 2.0 cm^2 Hilltop™ chamber lined with aluminum foil, and allowed to cool. The resulting patches were then adhered to the backs of hairless guinea pigs with Tegaderm™ dressing for 48 hours. Urine and feces were collected for 5 days, analyzed for ^{14}C-radioactivity, and the cumulative amount of drug excreted was determined.

3. Results

3.1 In-vitro

The rates of appearance of PG in the receptor chamber when delivered across hairless mouse skin from formulations 1-5 are shown in Figures 27 and 28. The association of PG with EPC and DOPC liposomes halves the transdermal delivery of the drug when compared to the "free" formulation. Incorporation of PG into the DMPC and DPPC devices lowers skin transport relative to the "free" formulation by an order of magnitude.

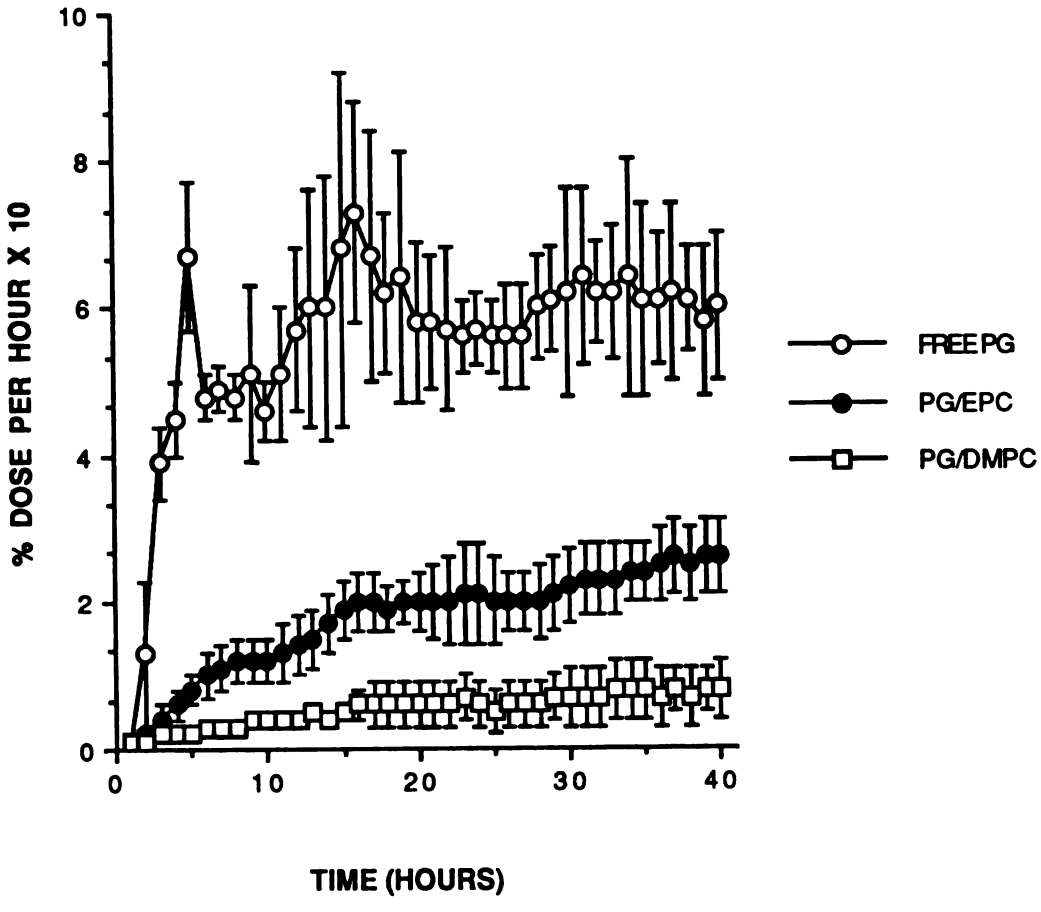


Figure 27. Transdermal delivery rate (mean ± s.d.) of PG across hairless mouse skin in-vitro from devices (1) (PG alone), (2) (PG-EPC liposomes) and (3) (PG-DMPC liposomes).

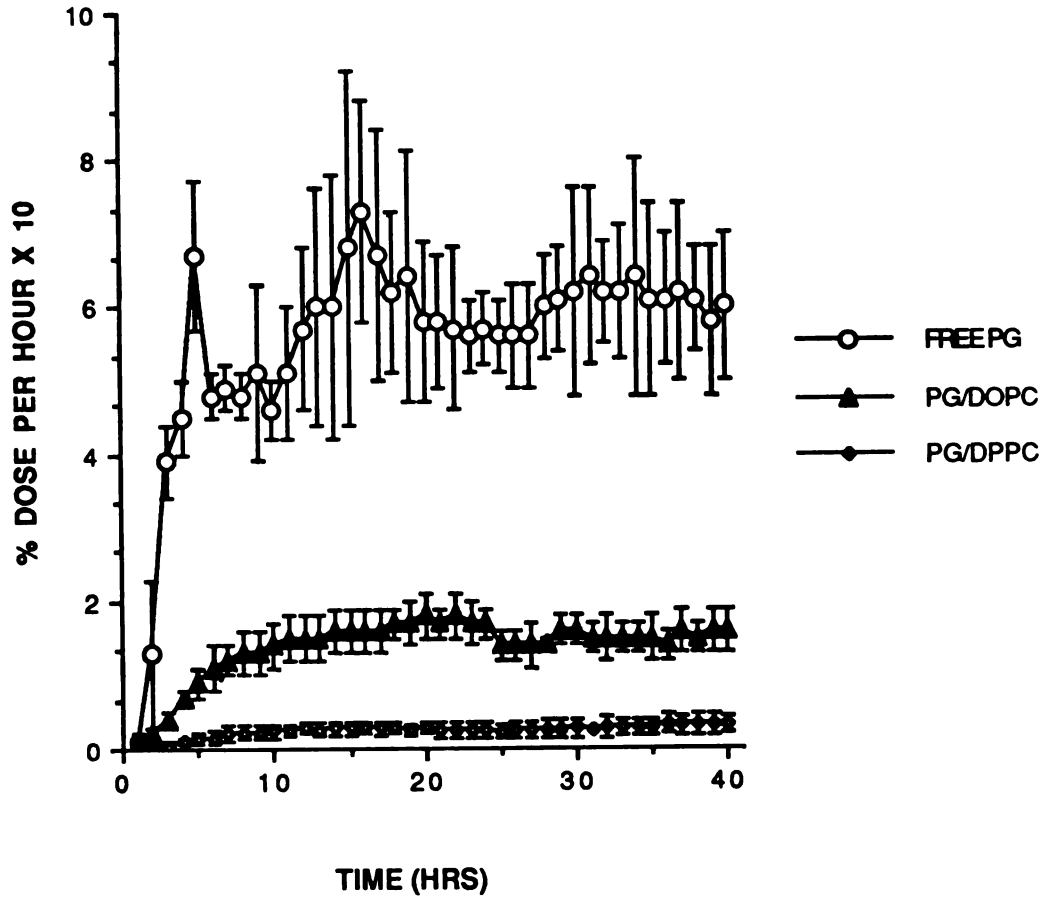


Figure 28. Transdermal delivery rate (mean ± s.d.) of PG across hairless mouse skin in-vitro from devices (1) (PG alone), (4) (PG-DPPC liposomes), and (5) (PG-DOPC liposomes).

3.2. In-vivo

Table 4 summarizes the cumulative amounts of PG delivered in vivo over a 24 hour time period. The "free" PG system delivered 92.7 ± 0.2% of its payload , the EPC and DPPC systems delivered 32.9 ± 4.2% and 44.0 ± 4.8% respectively.

4. Discussion

4.1. In vitro transdermal delivery

The delivery of PG across hairless mouse skin in vitro demonstrates how the liposomal patches may be used to control transdermal absorption. Comparison of Figure 27 and Figure 14 (previous chapter) demonstrates that the appearance of drug in the receptor chamber, following administration to the skin in the "free" PG patch (device 1) is skin-controlled. In this case, the skin acts as a rate-limiting membrane and the delivery system constitutes a reservoir of PG. After a relatively short lag period, transport follows Fick's first Law of Diffusion and a steady-state flux of 0.45% per hour is observed. Presentation of PG associated with either EPC or DOPC liposomes (devices (2) and (5); Figures 27 and 28), slows the transdermal throughput to approximately one half of the value obtained with device (1). The DMPC and DPPC systems ((3) and (4)) show an unexpected pattern of behavior (Figures 27 and 28). Despite the fact that PG release into aqueous buffer from DMPC and DPPC systems is identical to that from EPC and DOPC systems, the DMPC and DPPC liposomes slow transdermal throughput of drug by almost an order of magnitude relative to the EPC and DOPC vesicles.

Because the liposomes in the agarose delivery systems are immobilized within the gel network, it is impossible for PG to be transported across the skin in association with the vesicle. This was confirmed by fabricating DPPC liposomes using ^{14}C-labelled lipid. In agreement with earlier work (Ganesan et al, 1984; Ho et al, 1985), no radioactivity was detected in the receptor phase or in the skin during 48 hours post-application of the

Formulation	Cum % Absorbed in-vivo	Cum % released in-vitro
Free PG	92.7 ± 0.2	≈ 100
PG-EPC	32.9 ± 4.2	41.1 ± 2.8
PG-DPPC	44.0 ± 4.8	43.1 ± 5.6

Table 4. Comparison of the cumulative % of the applied dose of PG absorbed in-vivo rrom the "free", EPC and DPPC devices in 48 hours (mean ± s.d.; n=4), and the cumulative % of the dose released into buffer in-vitro during the same time period.

^{14}C-labelled patches to hairless mouse skin. Therefore, alterations in PG delivery are not caused by differences in liposome transport across the skin. Rather, we hypothesize that impurities (namely, cis-unsaturated fatty acids, see below) which are present in the EPC and DOPC systems, are co-delivered with PG to the skin and alter the cutaneous barrier.

Examination of the literature reveals a number of studies which indicate that cis-unsaturated fatty acids, including, specifically, oleic acid, act as skin penetration enhancers (Cooper, 1982; Cooper et al,1984; Golden et al, 1986), whereas the fully saturated counterparts do not cause appreciable barrier compromization. Typically, free fatty acids are found, albeit in very small amounts, in commercially supplied phospholipid preparations. It may be suggested, therefore, that the EPC and DOPC suspensions (whose cis-unsaturated fatty acid impurities would correspond in composition to the acyl chains of the component phospholipids) serendipitously co-deliver molecules which enhance the penetration of PG through the skin.

To establish this causative link, PG-DPPC delivery systems, which contained either 1 mole% oleic (cis 9-octadecenoic) acid or 1 mole% stearic (octadecenoic) acid, were prepared. In preliminary experiments, we first confirmed that PG release rates into buffer from the DPPC-OA and DPPC-SA systems were essentially identical to that from the simple DPPC device (Table 5). Transdermal PG delivery from the fatty acid-containing liposomes was then assessed. The results in Figure 29 show that the DPPC-OA system delivered PG at a rate comparable to the egg-PC and DOPC formulations, whereas the DPPC-SA device provided PG throughput similar to that achieved from the basic DPPC liposome reservoir. The role of cis-unsaturated fatty acids in penetration enhancement is strongly implicated by this data and provides persuasive circumstantial evidence to explain the results for PG delivery from the egg-PC and DOPC systems.

Formulation	Tc (°C)[a]	CCL/#DB[b]	% Dose Released[c]
PG - DPPC Liposomes	41	16/0	21.2 ± 1.1
PG-OA-DPPC liposomes	41	16/0	23.1 ± 2.5
PG-SA-DPPC liposomes	41	16/0	22.0 ± 2.2

[a] Transition Temperature of phospholipid [b] Carbon Chain Length/Number of Double Bonds
[c] Mean ± standard deviation of 4 to 6 experiments

Table 5. Cumulative amounts of PG, expressed as mean cumulative % (± s.d.) of initially encapsulated dose, released in 24 hours from 3 DPPC formulations into buffer at 35°C.

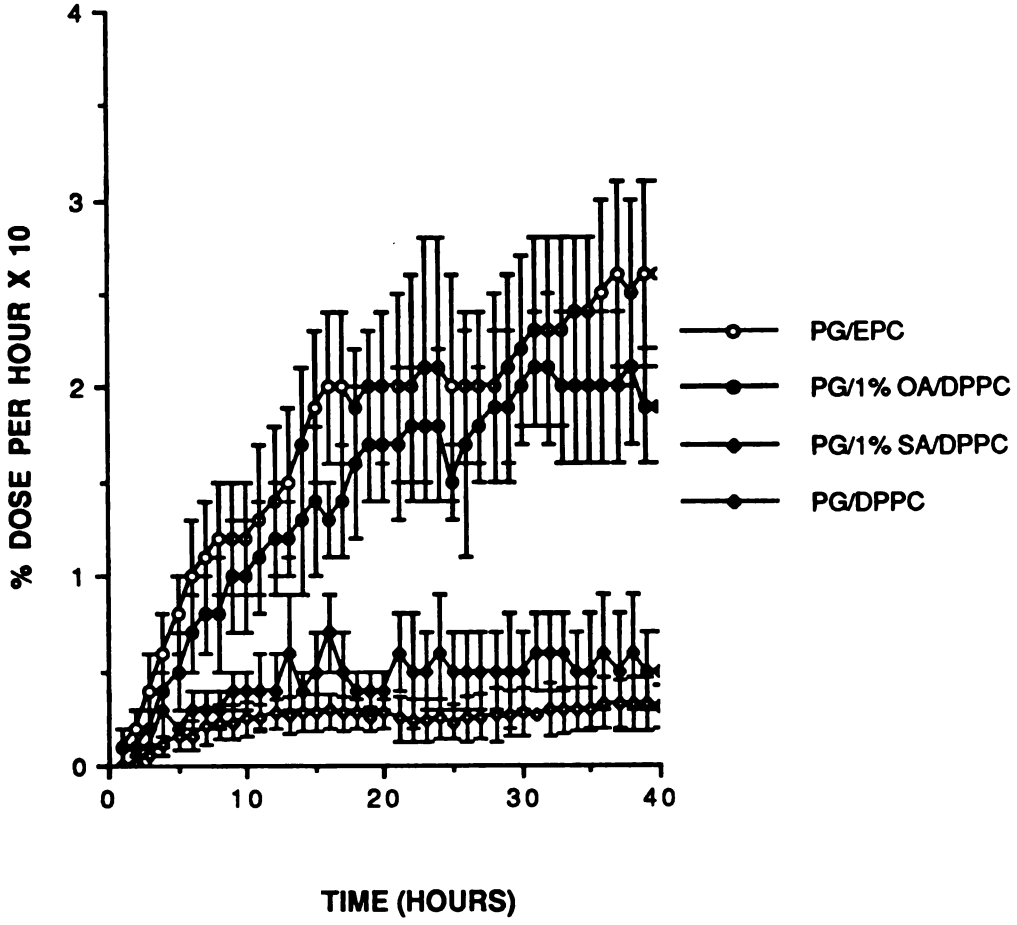


Figure 29. Transdermal delivery rate (mean ± s.d.) of PG across hairless mouse skin in-vitro from devices (2) (PG-EPC liposomes), (5) (PG-DPPC liposomes), (6) (PG-OA-DPPC liposomes), and (7) (PG-SA-DPPC liposomes).

We then examined the dose-response relationship with respect to the penetration-enhancing properties of the DPPC liposome formulation containing oleic acid. Figure 30 shows the transdermal flux of PG observed when delivered from DPPC-OA liposomal formulations containing different OA concentrations (0.1, 1, and 10 mole% of phospholipid concentration). Essentially equivalent (enhanced) PG delivery rates were observed despite the level of cis-unsaturated fatty acid in the formulation varying over two orders of magnitude. However, this saturability of enhancement has been previously observed (Chien et al, 1987). Furthermore, Mak et al (1988 (a), (b)) have demonstrated comparable results in vivo in humans using attenuated total reflectance Fourier transform infrared spectroscopy. These observations, and earlier research (Golden et al 1987), have shown that cis-unsaturated fatty acids enhance percutaneous absorption via a mechanism involving fluidization of the stratum corneum intercellular lipid domains. It is reasonable to expect that such an effect is saturable, i.e. that the freedom of motion of the lipid acyl chains in the intercellular lamellae can only be increased up to a limiting value. Furthermore, one also anticipates that the OA dose response curve will be dependent upon the skin source (hairless mouse versus human) and the delivery vehicle (liposomal, propylene glycol, ethanol, etc.) employed. For example, Cooper (1984) determined that approximately 14 mole% oleic acid in a propylene glycol vehicle increases the flux of salicylic acid through human epidermis 10 fold. Although this concentration is approximately 5 fold higher than the highest concentration of oleic acid used here, human epidermis is, on average, 43 times thicker than full-thickness hairless mouse skin (Wester and Maibach, 1985).

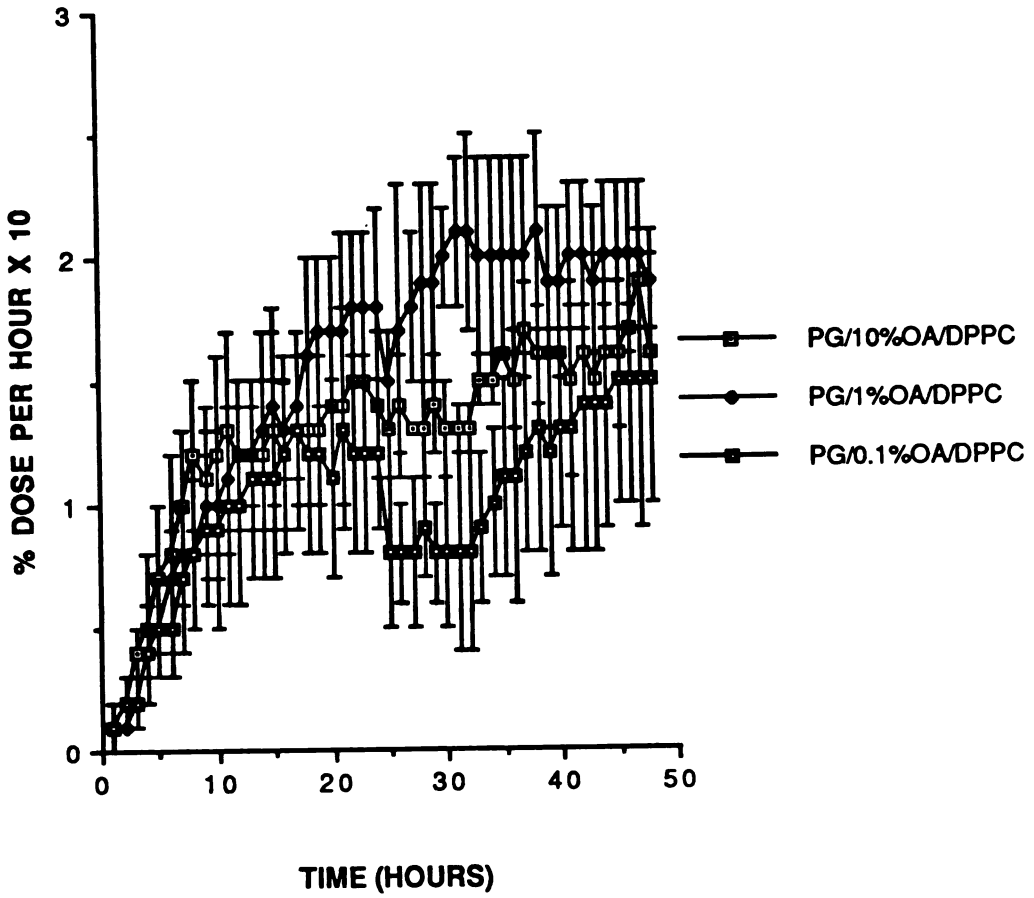


Figure 30. Transdermal delivery rate (mean ± s.d.) of PG across hairless mouse skin in-vitro from PG-DPPC devices containing 0.1, 1.0, and 10.0 mole% (of phospholipid concentration) OA.

Finally, in comparing the results of this work with previous studies involving topical application of liposomal formulations, a noteworthy observation should be made. The previously published (Ganesan et al, 1984; Ho et al, 1985) rate of PG transport across hairless mouse skin from normal saline - approximately 0.47% per hour - agrees closely with that observed in this work when PG is delivered "free" from device (1) (Figure 27). On the other hand, the earlier research indicated that transdermal delivery of PG from an aqueous suspension of DPPC liposomes was about 0.16% per hour for the period 10 to 50 hours post-application (Ganesan et al, 1984; Ho et al, 1985). This figure is much higher than that found for device (4) (0.03% per hour), the corresponding liposomal system in this study. Because of this discrepancy, we prepared a DPPC liposome suspension (identical to those used in the agarose devices), and applied 0.5 ml to triplicate samples of hairless mouse skin. The cells were occluded using clear plastic wrap, which was pressed down onto the skin surface such that a negligible air space remained between the cover and the skin. PG flux was monitored as before and the results are shown in Figure 31. The data are very reproducible and are similar to the results for device (4) as shown in Figure 31. The earlier work (Ganesan et al, 1984; Ho et al, 1985) postulated that PG delivery across skin from DPPC liposomes was mediated via a direct transfer of steriod between the lipid bilayer and the lipid phase of the stratum corneum. In device (4), such a collision complex transfer ((Ganesan et al, 1984; Ho et al, 1985; Kreuter et al, 1981) is impossible because of the spatial fixation of the liposomes in the agarose gel. With our DPPC suspension, for which this mechanism is theoretically possible, we find comparable PG delivery kinetics to those from device (4), implying a common rate-limiting process, namely slow PG transit out of the liposomes into the surrounding aqueous space. Exactly why our suspension data differ from the earlier results (Ganesan et al, 1984; Ho et al, 1985) is not known. One possibility is contamination of DPPC by lysophosphatidylcholine (lyso-PC). Preliminary experiments undertaken during this work revealed faster and much more

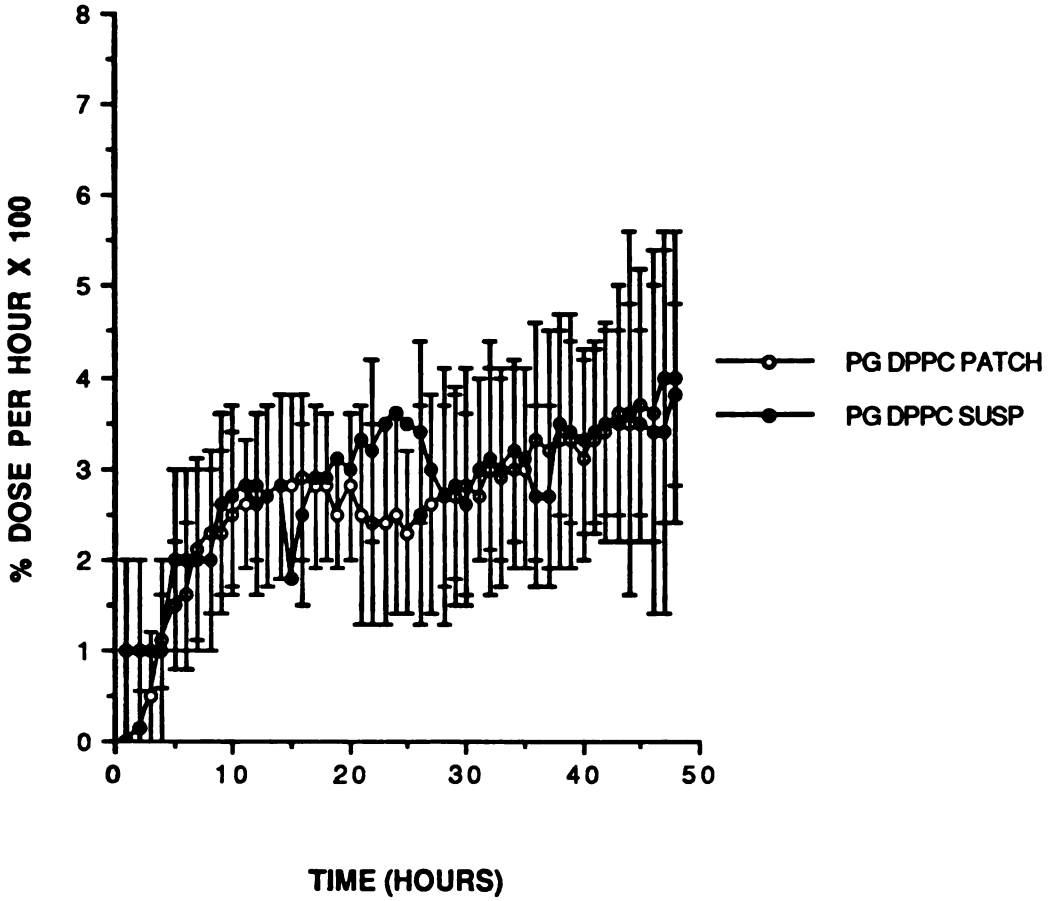


Figure 31. Transdermal delivery rate (mean ± s.d.; n = 4) of PG across hairless mouse skin in vitro from (a) PG-DMPC device and, (b) an aqueous suspension of PG-containing DPPC liposomes under occlusion.

variable PG delivery from a supposedly pure DPPC liposome suspension. Thin layer chromatography, however, revealed significant lyso-PC impurity. Repeating the experiment with a new and purified batch of DPPC produced the data in Figure 31. Therefore we conclude that PG delivery from liposomes across hairless mouse skin is independent of liposome presentation (agarose gel versus aqueous suspension) and does not invlolve direct transfer of PG on collision between liposome and skin.

4.2 In vivo transdermal delivery

Similar to the in-vitro situation, relative to the "free" PG system, the EPC and DPPC devices were able to reduce significantly the amount of PG delivered transdermally in a 48 hour period. However, in contrast to the in-vitro result, no significant difference in the amount of transdermally delivered PG was observed between the two lipid formulations. Two reasons could account for this observation: (1) significant irritation (as evidenced by erythema and blisters) was visible upon removal of both the EPC and DPPC systems (this was not observed with the "free" devices, therefore it appears that constituents in the liposomal devices are responsible). It is well known that irritated skin offers significantly less resistance to the passage of substances than intact skin, and it would not be unreasonable to hypothesize that this effect is overshadowing any penetration enhancement caused by free fatty acids. (2) Comparison of the cumulative amount of PG released into buffer in 48 hours (without skin) from the EPC, DPPC, and "free" systems with that which penetrated through hairless guinea pig skin in-vivo in a comparable time, revealed no significant difference (Table 6). Therefore, even intact hairless guinea pig skin may not provide much resistance to the passage of PG, however in-vitro studies would have to be performed to support this hypothesis. In any case, these studies highlight some of the problems encountered in comparing in-vitro and in-vivo results, particularly when the comparison is between two different animal models.

Chapter IV - Release Kinetics of Four Steroids: Comparison to Steroid Transport at Simple Organic Liquid - Aqueous Solution Interfaces

1. Objective

To establish the physicochemical basis by which the device controls the release of drug.

2. Introduction

It is the purpose of a drug delivery system to control the rate of release of the active agent into the body such that therapeutic efficacy is maximized while undesirable effects are minimized. In certain systems, e.g. membrane moderated transdermal drug delivery systems, rate control is achieved through a polymeric membrane, which is interposed between the drug reservoir and the body; alternatively, in implantable systems, for example, the drug is homogenously dispersed in a polymer matrix and slow diffusion from the polymer controls the rate of drug delivery. Obviously, in both of the latter examples, different drug release rates can be attained by changing the polymer composition , and/or the geometry (thickness, shape), of the device. Other systems (e.g. liposome suspensions, emulsions) are biphasic in nature, and the incorporated drug is distributed in, and partitioned between, hydrophobic and hydrophilic phases. The rate limiting step in drug release may be diffusion through either the lipid or aqueous phase, or transfer of drug between phases. To alter drug delivery rates from these systems requires first, therefore, that the step(s) controlling drug release be identified and the underlying mechanism of drug transport be clearly understood.

Recently, a prototypal transdermal drug delivery system, in which drug-loaded liposomes are dispersed as discrete entities within an aqueous gel matrix, has been

assessed (Knepp et al, 1988). Preliminary studies indicated that the release of progesterone from this system into an aqueous buffer solution was controlled by slow interfacial transport of the drug at the liposomal bilayer - aqueous gel interface. The kinetics of release were not consistent with drug release control via slow diffusion through the lipid - gel matrix. This paper to investigates further the release characteristics of this system, and extends the observations to four steroidal compounds spanning a range of physicochemical properties. In addition, release rates from the liposomal drug delivery systems are compared with the kinetics of steroid transport across simple organic liquid - aqueous solution interfaces. The latter measurements have employed a recently reported capillary tube method (Guy et al,1984; Hinz and Guy, 1987). In these experiments, a capillary containing radiolabelled solute dissolved in one phase is immersed in a large, well-stirred volume of an immiscible receptor phase. The rate constant for interfacial transfer at the mouth of the capillary can then be derived from the amount of solute released during the initial period of observation. It was hypothesized that a comparison of solute release from the two biphasic systems (liposomal versus simple liquid-liquid) would provide insight into the factors which govern the release of, primarily, lipophilic solutes intercalated in liposomal bilayers.

3. Materials and Methods

Steroids (progesterone (PG), testosterone (TST), estradiol (EST), and hydrocortisone (HCT)) and the organic solvents tetradecane (TDC) and isopropyl myristate (IPM) were purchased from Sigma Chemical Co. (St. Louis, MO). Radiolabelled steroids were purchased from New England Nuclear (Boston, MA); the purity of these materials was checked by thin-layer chromatography prior to use. Dimyristoylphosphatidylcholine was obtained from Avanti Polar Lipids

(Birmingham, AL). SeaPlaque® agarose and Gelbond® backing material were purchased from FMC Corp. (Rockland, ME).

3.1. Preparation of liposomal and "free" steroid delivery devices

Multilamellar liposomal suspensions containing 80 μmole/ml lipid and 0.8 μmole/ml steroid were prepared by standard procedures (Szoka and Papahadjopoulos, 1980). Briefly, lipid and the appropriate steroid were deposited from chloroform solution onto the sides of a round bottom flask by rotary evaporation. The materials were then resuspended by addition of sodium chloride (0.1M) - EDTA (0.1mM) buffer (pH 6.5) with constant nitrogen flushing and intermittent vortexing for 1 hour, at a temperature at least 10°C above the main gel-liquid crystalline phase transition temperature of DMPC (23°C). The liposomes formed in this way were then extruded through a 0.8 μm filter (Nucleopore, Pleasanton, CA) to obtain a reasonably homogenous size population. The final concentration of lipid in the suspension was 80 μmole per ml; the final concentration of steroid was 0.8 μmole per ml.

Drug delivery devices were prepared by thorougly mixing equal volumes (0.5 ml) of the liposome suspension and melted agarose (warmed to 67°C). The two components were then poured into a mold set up on the Gelbond backing material, and allowed to cool. The devices produced were 3.14 cm^2 in area and were 0.3 cm thick. Systems were stored for up to 24 hours at 4° C prior to use. It was found that storage in this way did not influence the drug release characteristics. Control, or "free", steroid delivery systems were prepared in an identical fashion after mixing 0.5 ml of an aqueous steroid solution (0.8 μmole/ml) with 0.5 ml of warmed agarose.

Steroid release from the delivery systems was monitored using a fully automated, continuously perfused, in vitro diffusion cell system (Knepp et al, 1988). The devices were clamped between the upper and lower halves of vertical glass diffusion cells (Figure 11), such that the releasing surface of the system faced into the (lower) receptor chamber. This receptor phase was stirred throughout the experiment with a Teflon-coated magnetic bar, and was continuously perfused with 0.1 M sodium chloride buffer (pH 6.5) at 10 ml/hour. A thermostatting jacket, through which water at 35° C was passed, provided isothermal conditions for the release process. Analysis of samples involved mixing with an appropriate amount of scintillation cocktail and measurement of ^{14}C-radioactivity in a liquid scintillation counter (Searle Mark III LSC model 6880). For each system, 6 replicates were run.

3.2. Capillary Tube Experiments

A small glass capillary (length 1.1-1.2 cm, internal diameter 0.84 mm) was filled with a solution of radiolabelled steroid in either isopropyl myristate (IPM) or tetradecane (TDC), such that the liquid surface was flush with the open end of the capillary. The capillary was then inserted into a wire support and lowered into 2 ml of an aqueous receptor phase contained within a scintillation vial, which had been prepositioned in a thermostatted water bath at 35°C. The receptor phase was stirred at 800 rpm by a magnetic flea. To allow the capillary to attain thermal equilibrium it was not submerged immediately, but was allowed sufficient time to reach 35° C by lowering it to approximately 90% of its length in the receptor phase.

At zero time, the capillary was fully immersed into the receptor phase to a depth of 0.5 cm and transport commenced. The duration of the experiments was between 150 and 600 seconds, at the end of which the capillary was removed. The receptor phase

was mixed with 10 ml of scintillation cocktail and the amount of transported solute was measured by liquid scintillation counting.

3.3. Diffusion Coefficient Measurements

Interpretation of the results from the liposomal and capillary experiments required knowledge of steroid diffusion coefficients in (a) the aqueous gel matrix (D_A), and (b) the capillary-entrapped organic liquid (D_o), respectively.

The cumulative amount of steroid released from the control ("free") systems increased linearly with the square root of time. The value of D_A was obtained in the normal way from the slope of this dependence (Knepp et al, 1988).

The organic phase diffusion coefficients were obtained from capillary experiments in which steroid transport from the organic liquid in the capillary was followed into a large, stirred volume of the same organic phase (i.e. the classic self-diffusion configuration) (Robinson and Stokes, 1951). Again, it was possible to calculate the appropriate D_o from the gradient of a graph of cumulative amount released versus the square root of time.

3.4. Partition Coefficient measurements

a. DMPC/Water - Multilamellar DMPC vesicles and an aqueous solution of radiolabelled steroid were equilibrated for 48 hours on a shaker bath at 35° C. Aliquots of the suspension were then removed and centrifuged for 30 minutes at 40,000 rpm. The amount in each phase was then determined on a mole fraction basis using a correction factor for the water trapped within the liposome pellet (Katz and Diamond, 1974 (a), (b)).

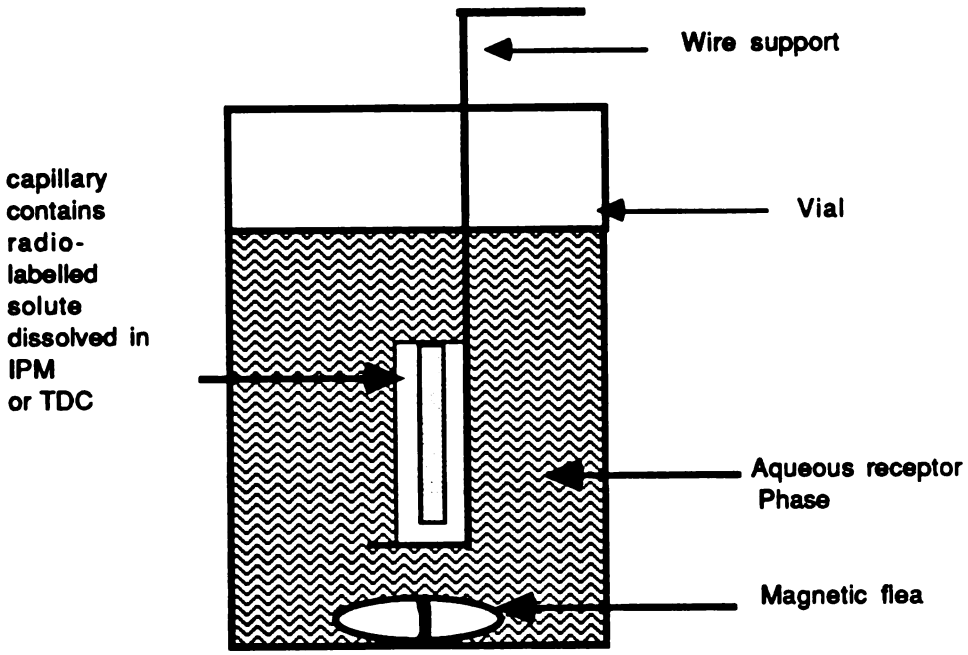
b. Organic liquid/Water - Aqueous solutions of radiolabelled steroid were added to equal volumes of either IPM or TDC, and shaken at 35° C for 48 hours. The samples were then centrifuged and each phase analysed for solute using liquid scintillation counting.

4. Theory

4.1. Capillary Tube

A schematic representation of the initial capillary tube experimental configuration is shown in Figure 32. Solute loss from the capillary as a function of time is described by the solution of Fick's second law of diffusion (with respect to the organic phase) using the boundary conditions indicated in the figure. Under the circumstances of short periods of transport ($t << 10^5$ seconds (Guy et al, 1984)) during which time only modest depletion of solute in the capillary has occurred, the cumulative amount (M) reaching the receptor phase at time t is given by (Hinz and Guy, 1987):

$$\frac{M_t}{\left(\frac{M^{\infty}}{L}\right)} = 2\left(\frac{D_o t}{\Pi}\right)^{1/2} + \left(\frac{D_o}{k_{AL}}\right)\left[\exp\left(\frac{k_{AL}^2 \cdot t}{D_o}\right)\operatorname{erfc}\left(\frac{k_{AL} \cdot \sqrt{t}}{\sqrt{D_o}}\right) - 1\right] \qquad (1)$$



FICK'S SECOND LAW OF DIFFUSION

$$\frac{\partial C_o}{\partial t} = D_o \left(\frac{\partial^2 C_o}{\partial x^2} \right)$$

BOUNDARY CONDITIONS

1. For $0 < x < l$, at $t = 0$, $\quad C_o = C^{\infty}$

2. At $x = 0$, $\quad \left(\frac{\partial C}{\partial x} \right)_o = 0$

3. At $x = l$, $\quad D_o \left(\frac{\partial C}{\partial x} \right)_l = - k_i C_{x=l}$

Figure 32. Diagram of the experimental configuration for the capillary technique.

where M^{∞} (= ALC^{∞}) is the total amount of solute in the capillary at t = 0. In the absence of a significant transport barrier at the interface at x = L, equation (1) reduces to the classic $t^{1/2}$ function:

$$\frac{M_t}{\left(\frac{M^{\infty}}{L}\right)} = A_{(\tau)}^{\infty} = 2\left(\frac{D_o t}{\Pi}\right)^{1/2} \quad (2)$$

where $A_{(t)}^{\infty}$ is the theoretical maximal release values of solute, which would be seen if no interfacial barrier existed.

4.2. Liposomal Delivery System

The liposomal drug delivery system is illustrated in Figure 33(a). The topological reduction of this representation to a two-phase system comprising a lipid internal compartment (the liposomes) and an aqueous external compartment (the agarose gel) is shown in Figure 33(b).

The configuration described by Figure 33 is a specific example of a generalized two-phase system addressed recently by Bodde and Joosten (1985). Only the external phase contacts the perfect "sink" receptor compartment at x = L. Solute is homogenously distributed within the internal phase, but this liposome-associated material cannot reach the receptor compartment without first partitioning into the external (aqueous gel) phase, and then, subsequently, diffusing (with diffusion coefficient = D_A) to the "sink" at x=L. The appearance of solute in the receptor compartment as a function of time is therefore obtained by solving the two fundamental transport equations:

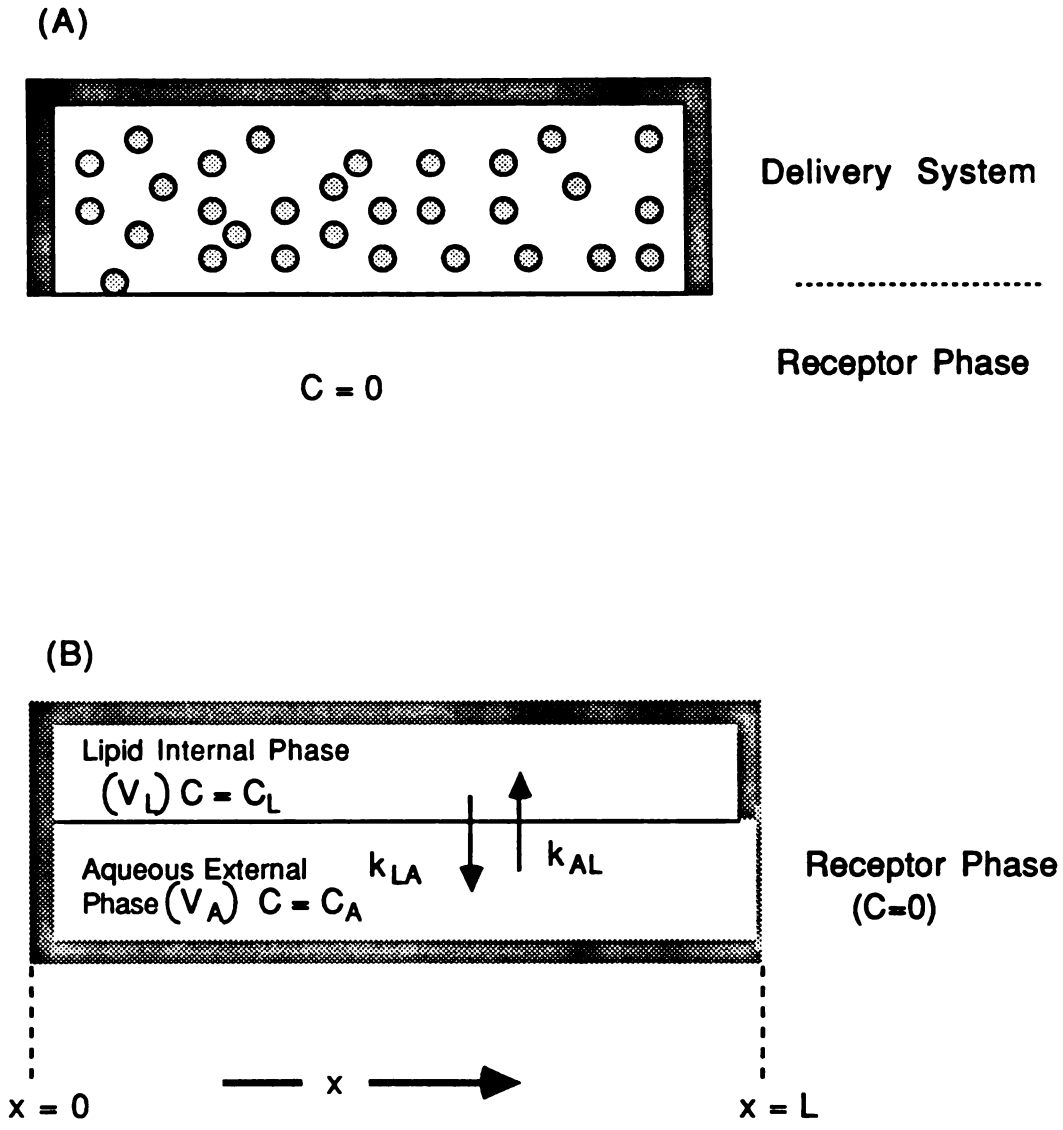


Figure 33. (a) Liposomal drug delivery system and, (b) topological reduction to a two-phase configuration of lipid internal (volume V_L) and aqueous external (volume V_A) compartments between which the solute transfers with interfacial permeabilities of k_{LA} and k_{AL} respectively.

$$V_L\left(\frac{\partial C_L}{\partial t}\right) = A\, k_{AL}\, C_A - A\, k_{LA}\, C_L \qquad (3)$$

$$V_A\left(\frac{\partial C_A}{\partial t}\right) = D_A\, V_A\left(\frac{\partial^2 C_A}{\partial x^2}\right) - A\, k_{AL}\, C_A - A\, k_{LA}\, C_L \qquad (4)$$

(where A is the interfacial area), with the appropriate boundary conditions:

(a) $C_A = 0$ at $x = L$, $t \geq 0$;

The receptor phase is a perfect "sink"

(b) $(\partial C_A/\partial x) = 0$ at $x = 0$, $t > 0$;

There is no supply nor depletion of solute at $x = 0$

(c) $C_L = C_L^0$ and $C_A = C_A^0$ at $t = 0$

The initial concentrations are related by the solute liposome-aqueous partition coefficient of the solute ($K = C_L^0/C_A^0 = k_{AL}/k_{LA}$).

The mathematics are most easily addressed using Laplace transforms and by recognizing that the desired outcome is an expression for the flux of solute out of the delivery system as a function of time, which can be determined experimentally from the slope of the regression line through the M_t/M^∞ vs time plot. Normalized for the area of contact between the external phase and the receptor compartment, this efflux (Q'(t)) is described by equations (5) and (6)

$$Q'(t) = - D_A \int_0^t \left(\frac{\partial C_A}{\partial X} \right)_{x = L} dt \qquad (5)$$

$$Q'(t) = - D_A \mathcal{L}^{-1} \left\{ \frac{1}{s} \left(\frac{\partial C_A}{\partial x} \right)_{x = L} dt \right\} \qquad (6)$$

By simple algebraic manipulation of equations (3) and (4) in Laplace space, it is easily shown that (Bodde and Joosten, 1985):

$$\left(\frac{\partial \bar{C}_A}{\partial x} \right)_{x = L} = \frac{\bar{C}_A^{\circ} \sqrt{\phi}}{s} \left(-\tanh L \sqrt{\phi} \right) \qquad (7)$$

where

$$\phi = \frac{1}{D_A} s + \frac{A k_{AL}}{V_A} \left[1 - \frac{1}{1 + \frac{s V_L}{A k_{LA}}} \right] \qquad (8)$$

For the situation in which solute diffusion in the external (agarose) phase is slower than aqueous - lipid partitioning but faster than lipid - aqueous partitioning ($Ak_{AL}/V_A > D_A/L^2 > Ak_{LA}/V_L$), a simple approximation to the full solution (Equation (6)) predicts a significant period of steady - state (zero -order) solute release from the delivery system (Bodde and Joosten, 1985). Explicitly, one obtains that, for $V_A/Ak_{AL} < t < V_L/Ak_{LA}$,

$$Q'(t) = C_L^{\circ}\left(\frac{A\, D_A\, k_{LA}}{V_A\, K}\right)^{1/2} \qquad (9)$$

5. Results

The data from the capillary tube experiments are summarized in Tables 6 and 7. These tables include the theoretical maximal release values of solute (A^{∞}), which would be seen if no interfacial barrier existed. The results show clearly that a significant interfacial barrier exists for all of the systems examined except EST/TDC and HCT/IPM.

Steroid release curves into buffer from the control (i.e. "free") devices are shown in Figure 36. Drug efflux was rapid, with 75 - 100% of the payload released in 24 hours. All of the DMPC formulations released significantly ($p < 0.001$) less steroid than the "free" devices during this time period (Figure 37); for PG and EST the amount released was reduced by a factor of 4. Table 9 summarizes the numerical data for all of the liposome devices, and includes the heterogenous rate constant (k_{LA}) for PG and EST transport across the lipid-aqueous interface calculated using equation (a). For TST and HCT interfacial kinetic transport constants could not be deduced because a significant period of zero-order release was not observed in the data collected. It appears, therefore, that the release of these two compounds from these devices is determined more by their rate of diffusion through the liposome-agarose matrix.

STEROID	$(10^2)\ M_t/M^{\infty}$ [b]	$(10^2)\ A^{\infty}$	$(10^6)\ D_o$ [c] (cm^2/s)	$(10^5)k_{LA}$ [d] (cm/s)
PG	0.4 ± 0.1	3.12	5.1	3.0
TST	1.7 ± 0.2	3.12	5.1	23.0
EST	3.0 ± 0.5	3.15	5.2	---

[a] Experiment duration = 150 seconds. [b] Mean ± standard deviation of 4 experiments.
[c] Evaluated as described in the experimental section. [d] Calculated from equation 1.

TABLE 6 - Capillary Tube Results for Tetradecane System[a]

STEROID	(10^2) M_t/M^{∞} [b]	(10^2) A^{∞} [c]	(10^6) D_o [d] (cm^2/s)	$(10^5)k_{LA}$ [e] (cm/s)
PG	0.3 ± 0.2	2.3	2.7	2.3
TST	1.1 ± 0.3	2.5	3.1	12.0
EST	0.4 ± 0.1	2.0	2.2	3.3
HCT	2.7 ± 0.5	1.9	1.9	- - -

[a] Experiment duration = 150 seconds. [b] Mean ± standard deviation of 4 experiments.
[c] Evaluated as described in the experimental section. [d] Calculated from Equation 1.

TABLE 7 - Capillary Tube Results for IPM System[a]

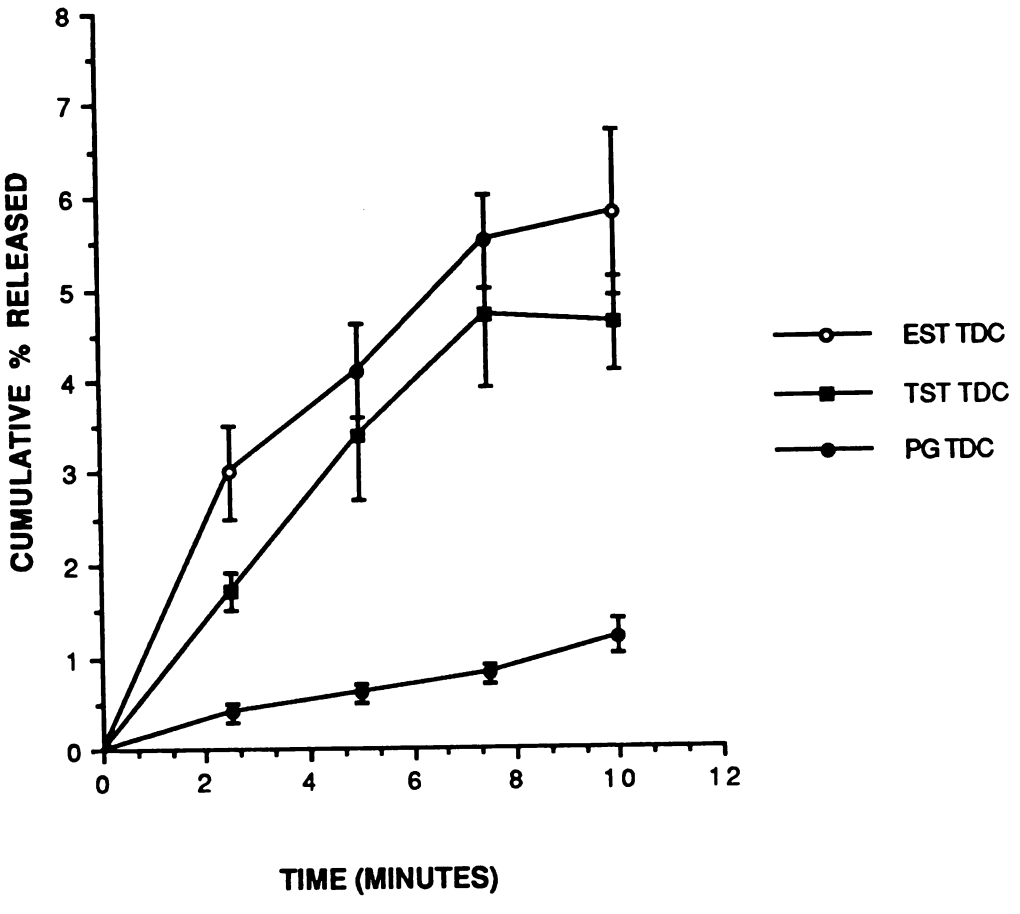


Figure 34. Release kinetics of steroids across a TDC-aqueous phase interface at 35°C. Each curve represents the mean ± s.d. of4 experiments.

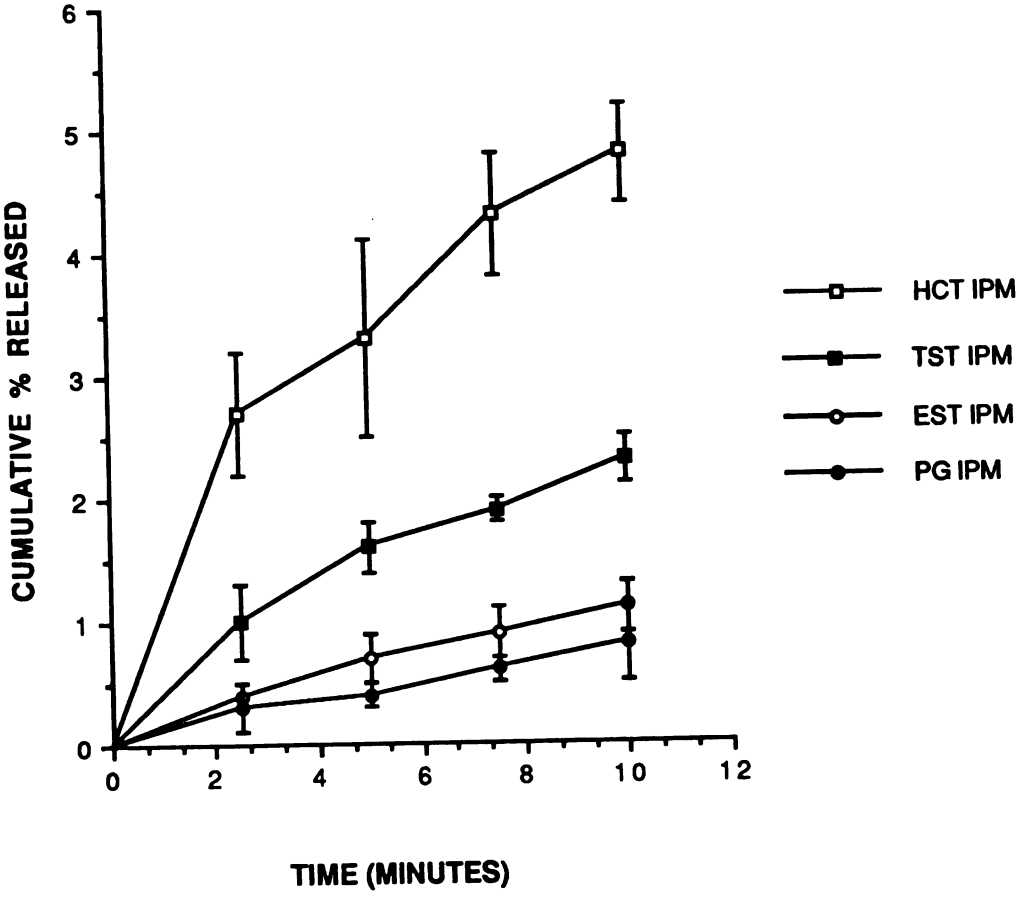


Figure 35. Release kinetics of steroids across an IPM-aqueous phase interface at 35°C. Each curve represents the mean ± s.d. of4 experiments.

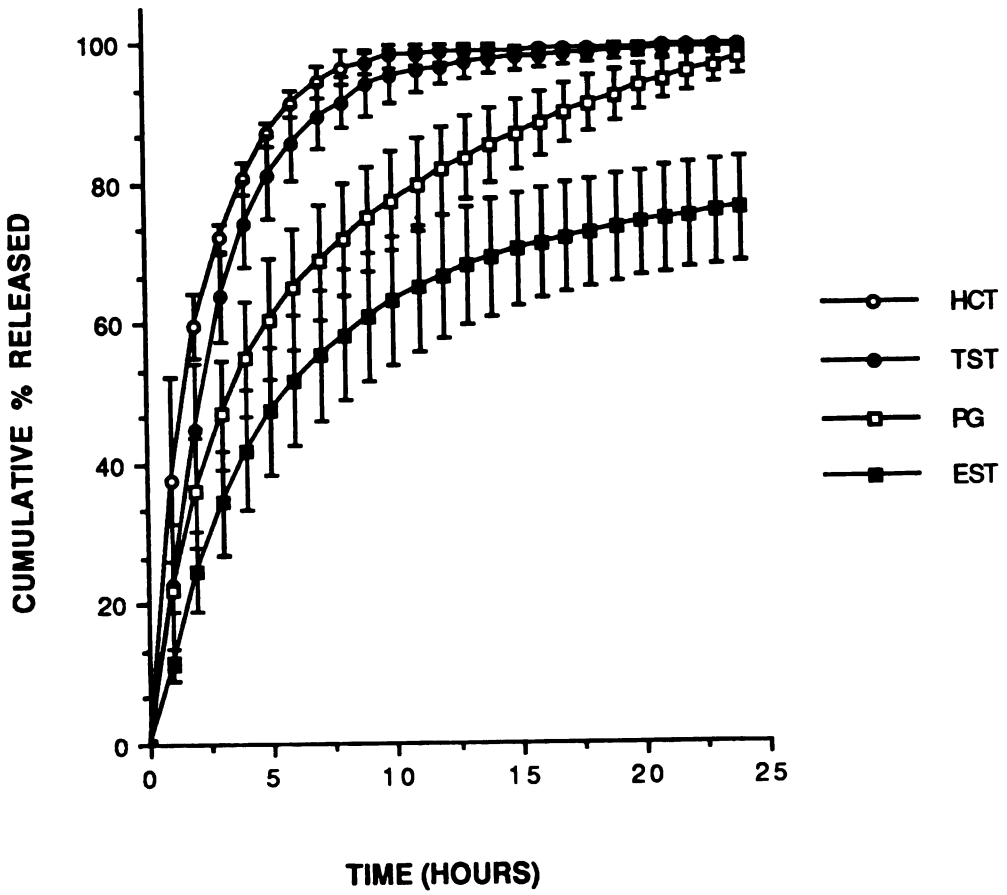


Figure 36. Release kinetics of steroids from "free", control devices. Curves are the mean ± s.d. of 6 experiments.

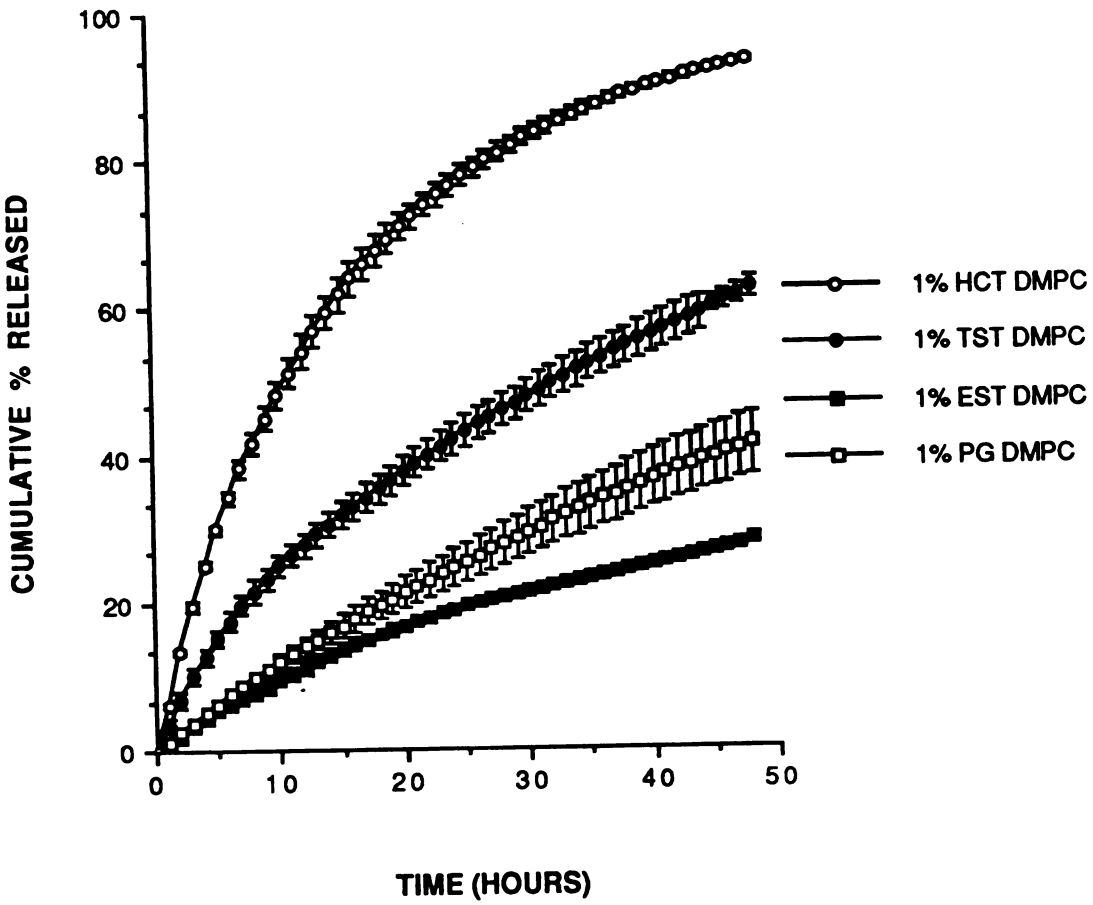


Figure 37. Release kinetics of steroids from DMPC liposome devices. Each curve represents the mean ± s.d. of 6 experiments.

STEROID	DMPC / AQUEOUS	IPM / AQUEOUS	TDC / AQUEOUS
PG	3.38	3.13	2.44
TST	2.66	2.07	0.86
EST	3.32	2.54	0.21
HCT	2.17	-0.08	-2.24

Table 8 - Log Partition coefficients of Steroids between various organic phases and water at 35 °C.

STEROID	$(10^2)\ M_t/M^{\infty}$ [b]	$10^6\ D_A$ [c] (cm^2/s)	$10^{11} k_{LA}$ [d] (cm/s)
PG	11.9 ± 1.0	5.2	6.8
EST	9.6 ± 1.0	5.2	4.9
TST	24.9 ± 1.5	5.5	- - -
HC	48.1 ± 1.8	4.1	- - -

[a] Experiment duration = 10 hours. [b] Mean ± standard deviation of 6 experiments.
[c] Evaluated as described in the experimental section. [d] Calculated from equation 9.

TABLE 9 - Results for DMPC System[a]

6. Discussion

We first examine the interfacial transfer kinetics observed in the capillary system (Figures 34 and 35). From TDC to water, the rank order of steroid efflux rates was HCT > EST > TST > PG; from IPM to water, the corresponding order was HCT > TST > EST ≈ PG. Tables I and II show that while TST and PG cross each of the two interfaces with approximately the same kinetics, the rate of EST transfer is highly influenced by the organic phase. Transport of EST at the TDC/water interface indicates no interfacial barrier, i.e. the appearance of EST in the aqueous phase is controlled only by its diffusion in the organic liquid in the capillary. On the other hand, there is a significant interfacial resistance to EST movement through the IPM/water interface. It may be suggested that this difference reflects a significantly altered capability of EST for hydrogen bonding in the two organic liquids. Whereas IPM can interact through H-bonding with EST, TDC cannot. This enhanced affinity of EST for IPM relative to TDC is reflected in the bulk partition coefficients shown in Table 8, and is manifested by the slower efflux kinetics in the capillary experiments (i.e. we may view the slower EST transfer at the IPM/water interface as a reflection of a reduced solute thermodynamic activity on the organic side of the phase boundary).

We now turn to the steroid release profiles from the liposomal and "free" (control) delivery systems (Figures 36 and 37). Efflux from the "free" devices allows calculations of the aqueous (gel phase) diffusion coefficients in Table 9. Figure 37 shows the effect of liposomal incorporation: in agreement with the theory described by Bodde and Joosten (1985), and outlined above, release of EST and PG shows a substantial period of zero-order kinetic behavior. Although the receptor phase appearance of TST and HCT is significantly slowed relative to the "free" controls, a truly constant release period is not observed. Therefore, as indicated in Table 9,

rate constants for steroid movement across the lipid - aqueous interface have been calculated using Equation (9) for EST and PG only.

The rank order of steroid release kinetics from the liposomal systems is HCT > TST > PG ≈ EST, which is a pattern similar to that found in the IPM/water capillary system. The trend is consistent with the bulk liposome/water partitioning data in Table 8 and emphasizes the apparently susbstantial interactions between the hydroxyl groups of EST and the DMPC head-group region.

The theoretical model, which has been used to analyze the liposome release data, predicts a steady-state (zero-order) steroid efflux rate for a period (T) defined by the inequality

$$\frac{V_A}{A\,k_{AL}} < T < \frac{V_L}{A\,k_{LA}} \qquad (10)$$

Using the appropriate values of V_A, V_L and A (discussed further below), and the interfacial transfer rate constants in Table IV, the values of T for PG and EST fall in the ranges of 0.33 - 32 hours and 0.5 - 44 hours, respectively. Inspection of Figure 37 shows that the experimental results are in reasonable agreement with these predictions.

The interfacial transfer rate constants from the capillary and liposomal systems reveal a striking difference in magnitude. For movement from the lipophilic to the aqueous phase, the model systems studied by the capillary procedure are characterized by kinetics on the order of 10^{-5} cm/sec, whereas the liposomal

results are approximately 10^{-11} cm/sec. The size of this disparity warrants further discussion and examination.

The precision of any measurement of an interfacial transfer coefficient depends upon the accuracy with which (a) the area of the interface is known, and (b) the contribution of other potential transport resistances (e.g. stagnant diffusion or membrane boundary layers) can be calculated. It is appropriate, therefore, to address these issues with respect to the liposomal systems studied in this work.

The surface area of the liposomes in the devices employed was determined by the method of Pidgeon and Hunt (1981). This calculation requires knowledge of the following parameters: the encapsulation ratio of the suspension (E_p, defined as the volume of aqueous phase encapsulated per volume of lipid), the partial molar volume of the lipid (TVL), and the average radius (r) of the liposomes contained within the system. The values of these parameters used in the area estimation were: E_p =1.5 (determined experimentally by sucrose encapsulation), TVL = 26.7 µl, and r = 0.4 µm. The surface area, evaluated from the equation (Pidgeon and Hunt, 1981)

$$TSA = \frac{3\,(E_p + 1)\,TVL}{r} \qquad (11)$$

was found to be 5.1 x 10^{11} μm^2. This figure can be compared to alternative assessments described by other workers. Schwartz and McConnell (1978), for example, estimated that 5-7% of the lipid in a spontaneously formed DMPC suspension is within the outer lamella. The total surface area of a dispersion is then calculable from the expression

$$A = (A_L)\ (N_{AV})\ (M_L)\ (F_o) \qquad (12)$$

where A_L is the area (70 $Å^2$) per DMPC molecule at 35°C (DeYoung and Dill, 1988), N_{AV} is Avagadro's number, M_L is the number of moles of lipid used to form the vesicle suspension, and F_o is the fraction of lipid contained in the external lamellae of the liposomes. Using M_L = 40 μmole, and assuming F_o = 0.05, Equation (12) predicts A = 8.4 x 10^{11} μm^2, a value in good agreement with the area determined above. In another estimation, Bangham et al (1967) estimated the surface area of an egg-PC multilamellar suspension. Correcting for the amount of lipid employed, a surface area of 6.2 x 10^{11} μm^2 is calculated for our suspension, again in good coincidence with the other calculations. It is apparent from this exercise, therefore, that no gross error in liposome interfacial area determination has been made and that this parameter cannot be implicated as the reason behind the disparate lipid-water transport coefficients observed in the capillary and liposome experiments.

With respect to the possibility that other transport processes are contributing significantly to the barrier to solute efflux from the liposomal devices, two "candidates" may be considered. Classically, one is always concerned about the existence of appreciable stagnant (or boundary) diffusion layers near a membrane surface. Previous work (Guy et al, 1984; Guy and Hinz, 1987) with the capillary system has established the negligible impact of a stagnant diffusion layer on solute efflux. The lipid-aqueous interfacial rate constants calculated for PG and EST transfer out of the liposomal system correspond to an interfacial resistance of about 10^{10} sec/cm. If, instead, this resistance was caused by an aqueous stagnant diffusion layer, then the thickness of this layer would be given by (10^{10} cm)(D_A) , where D_A is the solute diffusion coefficient in the aqueous gel phase. From Table IV, D_A ≈

10^{-6} cm^2/sec, and we would then calculate the hypothetical stagnant diffusion layer thickness to be $\approx 10^4$ cm or 100 meters! Clearly, this value is nonsensical and aqueous boundary layers are not, therfore, responsible for the lipid-water transport coefficients obtained. The second potential alternative resistance results from the fact that the vesicles employed are multilamellar. One may postulate, therefore, that the slow steroid release rates are due to the slow interbilayer transfer of drug within the liposome. In other words, once the outer lamella has been depleted of steroid, subsequent transport is controlled by the slow replenishment of the surface bilayer from the inner lipid layers. Three observations discredit this suggestion: (1) As mentioned above, the outer bilayer contains 5-7% of the total lipid. Assuming that the steroid is homogenously distributed throughout the liposome at the initiation of release, then the efflux profile should show a relatively rapid "burst" of 5-7% of the solute followed by a very slow subsequent release. This is not observed: steroid efflux proceeds with zero-order kinetics up to at least 30% release. (2) Parallel experiments using large unilamellar liposomes (LUVs) have been performed with PG as the solute (Knepp et al, 1988). A comparison of the release profiles of PG from LUVs and MLVs is shown in Figure 38; the curves are essentially identical. In this case, there can be no interbilayer transport event, and yet the efflux remains at the same slow and constant rate. (3) Other investigators (McLean and Phillips, 1981; Backer and Dawidowics, 1981; Bloj and Zilversmit, 1979) have reported that the transmembrane movement of sterols (e.g. cholesterol) is much faster than their desorption rate from the outermost bilayer.

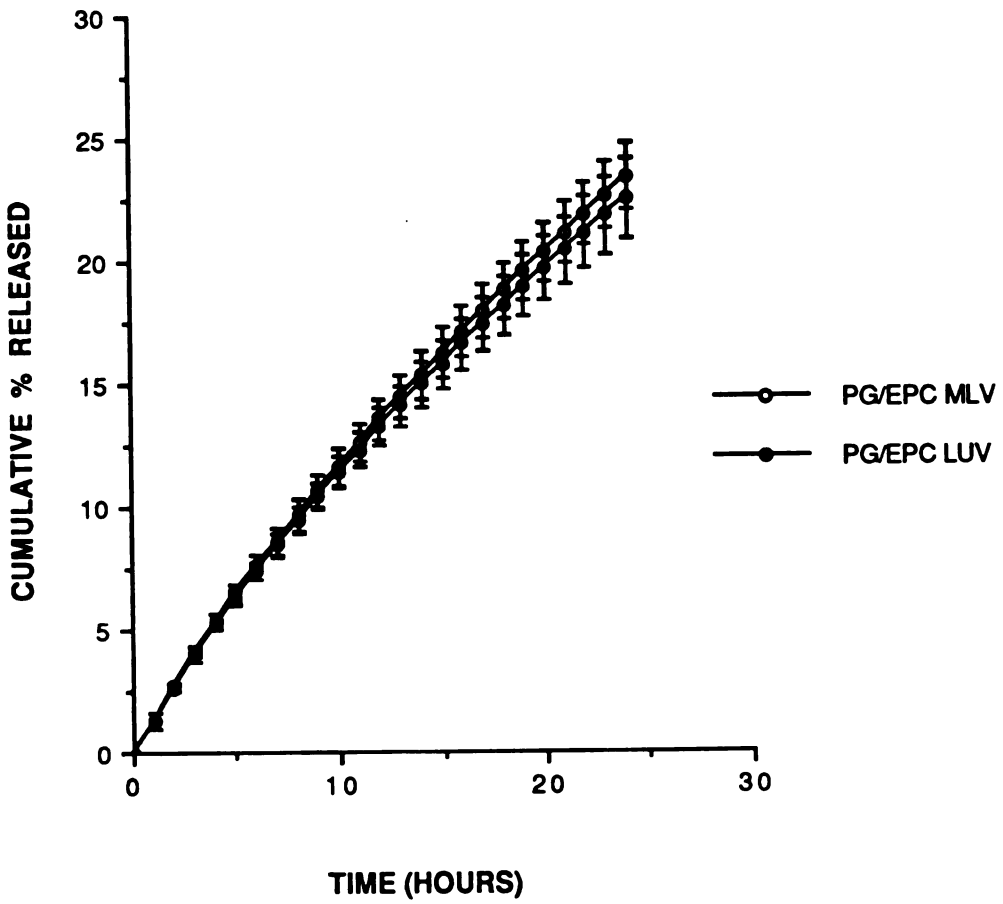


Figure 38. Release kinetics of PG from Egg PC large unilamellar (LUV) and multilamellar (MLV) devices. Curves are the mean ± s.d. of 6 experiments.

Credible alternative explanations for the slow release kinetics cannot be identified, therefore. On the contrary, other support for the values deduced can be proposed. For example, when the interfacial control mechanism was first suggested (Knepp et al, 1988), a simple model, based on Fick's first law of diffusion, was developed and predicted that the zero-order release rate (Q'(t)) of solute from the patch is given by

$$Q'(t) = A\, k_{LA}\, C_L \qquad (13)$$

This expression will be valid for the period during which relatively little depletion of steroid from the liposome reservoir has occurred (< 30%). For the PG system, $Q'(t) \approx 4 \times 10^{-3}$ μmole/hr; the total liposome surface area (A_L) was 5100 cm^2 (5.1×10^{11} μm^2); and the initial PG concentration in the lipid phase (C_L) was 13 μmole/cm^3. The latter value was determined from the total amount of PG incorporated into the system and the DMPC/aqueous phase partition coefficient (Table 8). Substituting these parameters into Equation (13), we calculate

$$k_{LA} = 1.7 \times 10^{-11} \text{ cm/sec}$$

a value in good agreement with that in Table 9. More significantly, the literature contains information which allows an independent determination of k_{LA} for progesterone. Ganesan et al, (1984) reported the efflux of PG from an aqueous suspension of dipalmitoyl-phosphatidylcholine (DPPC) liposomes. The quoted "permeability coefficient" for this process was 3.3×10^{-10} cm/sec. Given the rather indirect method by which the authors determined this parameter, its value is in respectable concurrence with our experimental result.

There is considerable evidence to support, therefore, the magnitude of different steroid interfacial transfer constants observed in the capillary tube and liposomal systems. What physical causes underly the relative values obtained? To transport across a lipid (or organic liquid) - aqueous interface, a molecule must (a) present itself on the lipid side of the boundary, (b) cross from this environment into a (presumably) ordered aqueous phase, and (c) move from this region away from the interface to a point at which the aqueous solution is indistinguishable from bulk water. In other words, interfacial transfer cannot be viewed realistically as a "one-step" process across a hypothetical, infinitely thin line separating two liquid phases.

To understand why interfacial transport in liposomal systems is markedly slower than that in the capillary experiment requires that we examine the nature of the organic and aqueous sides of the two interfaces studied. In the liquid-liquid system, a number of studies have inferred a hydrophobic effect-driven ordering of water molecules on the aqueous side of the interface (Fleming et al, 1983; Guy et al, 1982 (a) and (b); Guy and Honda, 1984). Perturbations of this structuring by added agents (e.g. urea, ethanol) has been observed (Guy et al, 1982 (a) and (b); Guy et al, 1984). The impact of the close proximity of the aqueous phase on the organic liquid, however, has not been commented upon nor rigorously assessed. To a first approximation, one assumes that the "interfacial" organic liquid behavior parallels that of the bulk phase. How do these observations compare to the phospholipid bilayer-water interface?

Consider first the acyl chain region. In the center of a bilayer, e.g. composed of DMPC, the viscosity has been determined to be about 1.5 poise, a value about 200 times greater than that of water and equivalent (approximately) to that of a light oil

(Edidin, 1981). However, from the center of the bilayer to the surface, ^{13}C - NMR has been used to demonstrate a 100 - 200 fold gradient of disorder (expressed as microviscosity) (Lee et al, 1976). The hydrocarbon chains near the headgroups are much more crystalline, therefore, than in the center of the bilayer. The liposomal interfacial transfer process initially involves, as a result, solute "extraction" from an organic environment which is much more structured than a simple liquid. This situation may be reasonably expected to result in a significant slowing of the phase transfer process.

At the headgroup region of a liposome, a very complex structure exists. The phosphatidylcholine headgroup binds water tightly: calorimetric experiments, for example, indicate that 10 moles of water per mole of synthetic lecithin are unfreezable at 0°C (Ladbrooke and Chapman, 1969); using deuterium NMR, it has been shown that 11 molecules of water are bound per polar headgroup (Finer and Darke, 1974). Furthermore, x-ray diffraction reveals that, at the headgroup region of DMPC bilayers, there is a hydrogen-bonded network which links phosphate groups and water molecules into infinite "ribbons" (Hauser et al, 1981); the result is a helical water structure extending parallel to the plane of the bilayer at the interface between polar headgroups. One deduces, therefore, that the aqueous side of the liposomal interface is extremely ordered and that the dipole-dipole forces responsible are considerably greater than the simple hydrophobic effect achieved in the simple liquid-liquid situation. The lipid-aqueous interface represents an exceptionally well-constructed edifice which a transporting solute must "puncture". Hence, it seems to us that the magnitude of the interfacial barriers observed in our liposomal systems are plausible and consistent with the independently documented biophysical properties of this region.

CHAPTER V - CONCLUSION

A liposomal drug delivery system has been developed which can control the release of a model drug, progesterone (PG), into aqueous buffer. Release of PG was independent of the physical state of the bilayer; changing the microviscosity of the hydrocarbon region by either temperature changes or cholesterol incorporation did not effect PG efflux. These observations support slow interfacial transfer of the drug from the liposomal bilayer into the surrounding aqueous media as the mechanism by which the system imposes constant release behavior. Simple mathematical modelling provided supporting evidence for this mechanism.

The liposome delivery systems have also demonstrated the ability to control the percutaneous absorption of PG. The transdermal input rate can be modulated by the lipid formulation employed; devices, which contained cis-unsaturated phospholipids, co-delivered fatty acid impurities that reduced the cutaneous barrier - PG input was thereby enhanced over that from devices containing saturated phospholipids. These results support a mechanism whereby the transdermal delivery of PG is controlled by the slow release of drug from the liposomal bilayer into the surrounding aqueous space (with subsequent uptake by the skin), as opposed to a collision-complex mediated mechanism proposed by previous investigators (Ganesan et al, 1984; Ho et al, 1985).

To examine further the physicochemical basis by which the system controls the release of drug, observations were extended to four steroidal compounds spanning a range of physicochemical properties. Release rates obtained from the liposomal systems were compared with the kinetics of steroid transport across simple organic liquid - aqueous solution interfaces. Qualitatively, the two systems were in

excellent agreement; quanititatively, however, the transport rate constants differed by almost 6 orders of magnitude. The slower rate constants obtained for the transport of steroids across the DMPC-aqueous interface may be attributed to: (1) solute "extraction" from a lipid environment which is much more ordered (i.e. viscous) than a simple organic liquid, and (2) an extremely complex and well-ordered aqueous structure at the DMPC-water interface.

Various areas of further study may be envisioned. Theoretically, the device has potential as a transdermal drug delivery system; however, stability testing and formulation refinement must be performed to assess whether the system would be practically useful. More importantly, further experiments are needed to probe the interfacial transfer kinetics of lipophilic solutes across phospholipid bilayer - aqueous solution interfaces. Systematic variation of the phospholipid headgroup region and investigations at temperatures above and below the lipid phase transition may (1) enable the thermodynamics of the interfacial transfer process to be evaluated, and (2) explain quantitatively the transport rate constants across simple liquid-liquid and complex bilayer-aqueous interfaces.

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