Influence of liposomal drug entrapment on percutaneous absorption

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Summary

Results of permeation experiments involving finite dose diffusion cells with hairless mouse skin as the membrane indicate that neither intact liposomes nor the phospholipid of which they are comprised diffuses across the skin. Lipophilic drugs like progesterone and hydrocortisone, which are intercalated within the bilayer structure of the phospholipid in multilamellar liposomes, seem to pass through the skin with comparable facility to free drug (comparable mass transfer coefficients). On the other hand, highly polar glucose entrapped in the aqueous compartments of the liposome is poorly available for transport. The results of in vitro release rate studies and theoretical calculations indicate that the very slight flux of liposomally incorporated glucose seen experimentally is attributable to a slow release rate of glucose out of the liposome followed by relatively rapid skin permeation of the free solute. On the other hand, for hydrophobic progesterone and hydrocortisone the experimental results and supportive theoretical analysis suggest direct transfer of drug from liposome to the skin. Considering this mechanism and owing to increased soluble payloads of lipophilic drugs through liposomal incorporation, more total drug may be delivered through skin via liposomes relative to simple aqueous solutions.

Introduction

The potential application of liposomes (concentric bilayer lipid vesicles) as drug carriers has been recently reviewed (Patel and Ryman, 1981; Poste, 1983). Most

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studies have been directed towards the use of liposomes as drug carriers in oral or parenteral administration. Use of liposomes for topical applications has received little attention until recently. Mezei and Gulasekharam (1980, 1982), Mezei and Singh (1983), and Schaeffer and Krohn (1982) reported the potential use of liposomes in topical applications for the skin and eyes. These reports indicate that liposomes facilitate the passage of drugs through skin and it has even been suggested by Mezei and Gulasekharam (1980) that the liposomes traverse the skin and act as drug carriers. This suggestion has been received critically as it is difficult to envision how particles as large as liposomes can diffusionally pass through the skin's dense horny layer.

Therefore, we have undertaken some range finding studies to determine the extent to which liposomes promote the permeation of chemicals through skin, the possible mechanisms involved in permeation and, further, to assess whether liposomes themselves pass through the skin barrier. A quantitative and mechanistic approach is taken.

Experimental

Materials

Dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), stearylamine (SA), dicetylphosphate (DCP) and cholesterol (CHOL) were purchased from Sigma Chemicals (St. Louis, MO). Cholesterol was purified by recrystallization 3 times from ethanol solution; all other lipids were used without further purification. Hydrocortisone was supplied by Upjohn (Kalamazoo, MI). Progesterone was purchased from Aldrich Chemicals (Milwaukee, WI). [^{14}C]Glucose (5 mCi/mmol), [^{3}H]hydrocortisone (95.2 Ci/mmol), [^{3}H]progesterone (55 Ci/mmol) and [^{14}C]DPPC (115 mCi/mmol) were purchased from New England Nuclear (Boston, MA). All other chemicals were of reagent grade. The lipids were dissolved in chloroform and stored under nitrogen at $-20°C$. Normal saline from Abbott Labs. (N. Chicago, IL) was used. Water was triply distilled in an all-glass still.

Preparation of liposomes

The lipid in chloroform solution was transferred to 10 ml round-bottom flasks and the solvent was evaporated with nitrogen. Hydrocortisone or progesterone (0.1% final concentration) with or without radio-tracer was also dissolved in chloroform and dried with the lipid in the same flask. The flask was rotated by hand to produce a thin layer of lipid at the bottom of the flask. The flask was left overnight under vacuum to remove the residual solvent. The lipid film was then suspended at the phase transition temperature of the saturated lecithin component in a sufficient amount of normal saline by vortexing for 10 min to yield a final concentration of about 65 μmol lipid per ml. For experiments involving glucose, normal saline containing radiolabeled glucose (2 μCi) was added. In some studies stearylamine or dicetylphosphate (10 mole%) was added to give a net positive or negative charge to

the liposomes. In yet another study liposomes were prepared with 30 mole% cholesterol. The liposomal suspensions were centrifuged 3 times for 20 min at 10,000 rpm. Each time the supernatant was removed to discard small particles and the liposomal pellet resuspended in fresh normal saline. The washed liposomal preparation was then used for subsequent experiments. No determination of final solute to lipid ratio was performed. Liposomes thus formed were found to be multilamellar as determined by negative stain electron microscopy.

The volume fraction of all liposome preparations, determined by centrifuging 1 ml of the liposomal suspension in a graduated centrifuge tube at 10,000 rpm for 60 min, was found to be approximately 0.075. The average size of blank liposomes was 0.8 μm in diameter (range of 0.4–1.0 μm) as determined by negative stain electron microscopy.

Differential scanning calorimetry

Thermograms were obtained using a Perkin Elmer differential scanning calorimeter (Model DSC-2C) with thermal analysis data station for data analysis. Liposomes were prepared as previously described. Samples were placed in sample pans for volatile matter and were properly sealed. Each sample was usually 16 μl and contained approximately 1 μmole lipid. An equal amount of buffer was placed in the reference pan. All the scans were obtained at a heating rate of 5°C/min and a range setting of 1 mcal/s. Indium standard and water were used to calibrate the calorimeter.

In vitro release rates of entrapped solute

About 5 ml of liposomal suspension containing entrapped solutes were prepared in a manner described earlier. Exactly 200 μl samples were obtained with time and centrifuged for 60 min at 10,000 rpm. The supernatant liquid was assayed by liquid scintillation counting (Beckman LS 9000).

Skin permeation studies

Sixty-day-old, male hairless mice (SKH hr-1 strain) supplied by Skin and Cancer Hospital (Philadelphia, PA) were used. Appropriate size sections of full-thickness skin were excised from the abdominal surface and mounted in Franz diffusion cells (Crown Glass, Somerville, NJ). The diffusional cross-sectional area of the skin was 0.785 cm^2. A 200 μl liposomal suspension was placed on the skin in the donor compartment and the receiver compartment was filled with 5.2 ml normal saline and was well stirred. Water at 37°C was circulated throughout the water jacket of the diffusion cell. Microliter samples were withdrawn from the receiver compartment every hour for a 60-h period and counted in the liquid scintillation spectrometer.

From the plots of the concentration of permeant appearing in the receiving chamber with time, the permeability coefficients were calculated. The flux of mass from the donor compartment is equal to the appearance rate of mass in the receiver.

$$J = AP_eC_D = V \cdot \frac{dC}{dt} \tag{1}$$

where J is flux, mass/h; A is area of skin, cm^2; P_e is effective permeability coefficient, cm/h; C_D and C are the drug concentrations in the donor and receiver chambers, respectively, mass/cm^3; V is volume of receiving chamber, cm^3; and t is time, h. Experimentally, C_D does not change appreciably during the experimental period; therefore, sink conditions in the receiver side essentially prevail. Consequently, the effective permeability coefficient is determined by

$$P_e = \frac{V}{AC_D}\left(\frac{dC}{dt}\right)$$ (2)

Results

Thermograms for the DPPC blank liposomes and liposomes containing glucose hydrocortisone and progesterone are shown in Fig. 1. The entrapment of glucose did not effect the baseline thermogram of DPPC liposomes. Hydrocortisone and progesterone, when incorporated in the liposomes, lowered the main transition temperature by $1-2°C$ and also eliminated the pretransition peak at $36°C$.

The in vitro release kinetics of glucose, hydrocortisone and progesterone from DPPC liposomes are shown in Fig. 2. It is seen that there was an initial rapid release
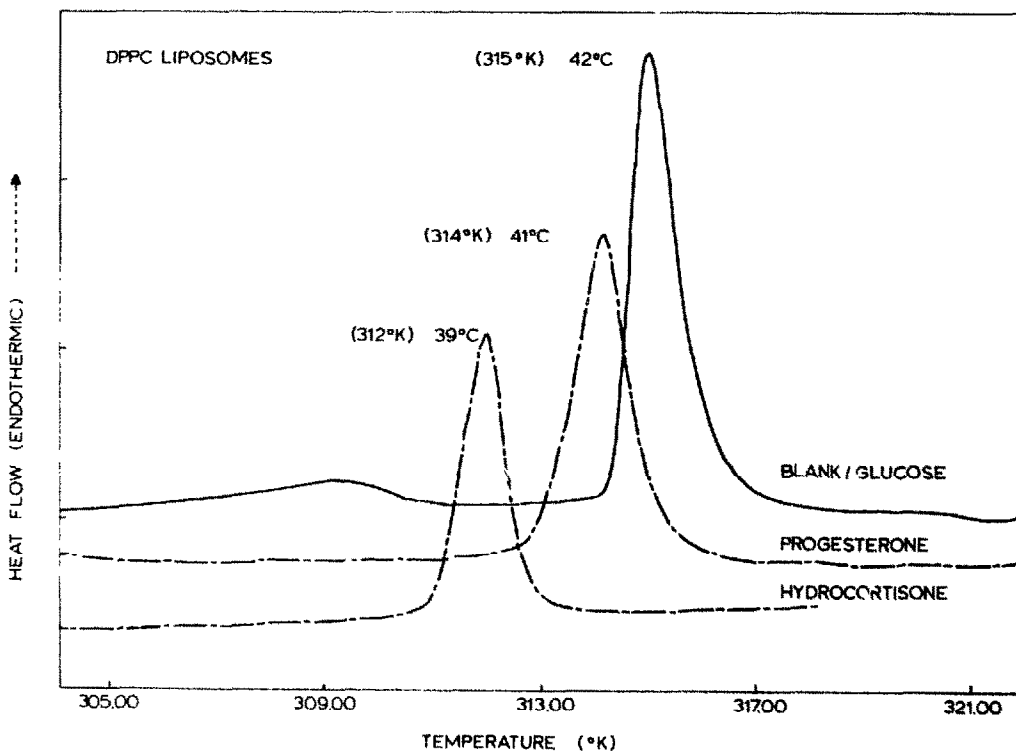

Fig. 1. Thermograms of multilamellar DPPC liposomes in the absence or presence of entrapped glucose, hydrocortisone and progesterone.
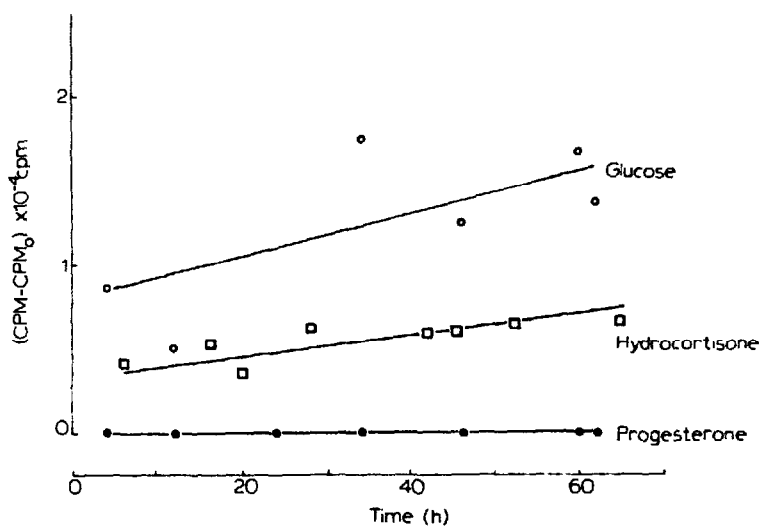
Fig. 2. In vitro release kinetic plots of glucose, hydrocortisone and progesterone from DPPC liposomes.
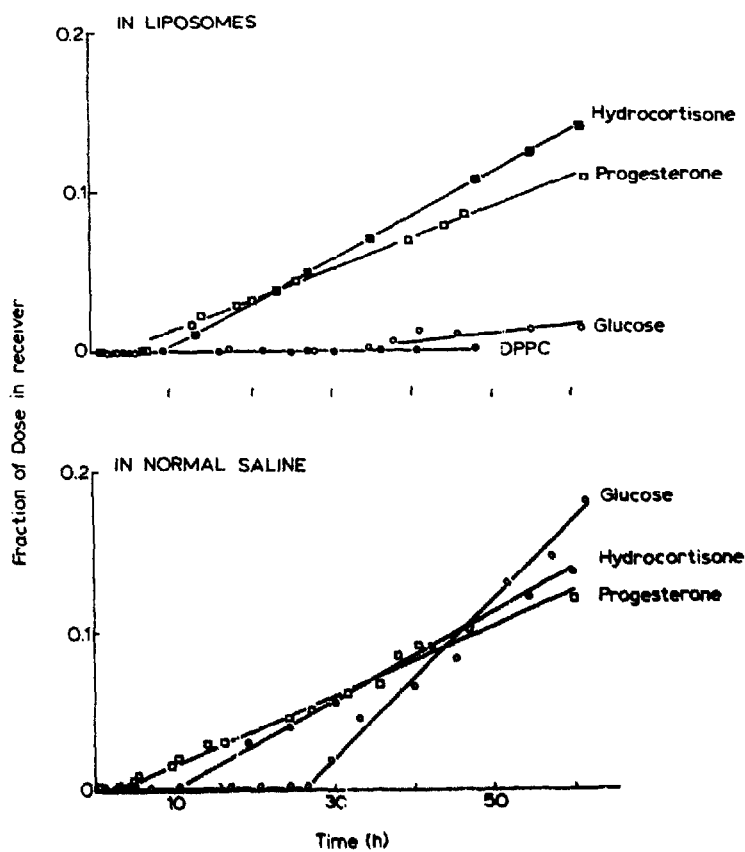

Fig. 3. Fraction of permeants appearing in the receiver compartment with time from solutes in normal saline solutions and liposomal suspensions.

TABLE 1

PERMEABILITY COEFFICIENTS OF MODEL SOLUTES IN NORMAL SALINE AND DPPC LIPOSOMES

System	Permeability coefficients $\times 10^4$ (cm/h) (S.D.)		
	Glucose	Hydrocortisone	Progesterone
Dilute solution in normal saline	14.0 (4.0)	5.4 (2.1)	6.3 (1.2)
Externally added solute to blank DPPC liposomes		3.7 (0.99)	
DPPC liposome-entrapped solute	0.006 (0.001)	3.0 (2.8)	4.6 (0.34)

Standard deviation represents 3–4 replicate experiments.

of glucose and hydrocortisone from the liposomes followed by an apparent zero-order release. The slope of the plot for glucose is 147 cpm/h ($r = 0.78$) whereas the release rate for hydrocortisone was 42 cpm/h ($r = 0.78$). The scatter in the data is primarily attributed to the experimental sensitivity in following the small changes of the radiolabeled solute in the bulk liquid with time; less than 5% of the drug was released in 60 h. There was no measurable amount of progesterone outside the liposomes over the entire course of the experiment.

Fig. 3 shows the appearance of permeant with time for progesterone, hydrocortisone and glucose applied on the skin as a dilute solution in normal saline or as DPPC liposomally entrapped system. It is significant that, for blank DPPC liposomes containing radioactive lipid, no lipid could be detected in the receiver chamber up to 48 h. As can be seen in Table 1, the effective permeability coefficients of the liposomally entrapped steroids are essentially identical to the permeability coefficient of the free steroid in aqueous solution. However, liposomal entrapment of glucose results in a permeability coefficient which is several orders in magnitude smaller than that for the free solute case.

TABLE 2

PERMEABILITY COEFFICIENTS OF LIPOSOME-ENTRAPPED PROGESTERONE IN VARYING LIPID COMPOSITION

Liposomal lipid composition	Permeability coefficients $\times 10^4$ (cm/h) (S.D.)
DMPC	1.75 (0.20)
DPPC	4.60 (0.34)
DSPC	6.80
DSPC : CHOL (2 : 1) *	6.70 (0.60)
DPPC : DCP (9 : 1) *	9.00 (0.37)
DPPC : SA (9 : 1) *	5.20 (0.42)

* The ratio is on a molar basis.

To explore the influence of liposomal charge and lipid composition on percutaneous transport, progesterone was entrapped in DMPC, DSPC and DPPC liposomes in combination with dicetylphosphate or stearylamine. The results of these experiments are found in Table 2. It is notable that the permeability coefficients are seen to be relatively indifferent to the composition of the liposomes.

Discussion

Physicochemical properties of the liposomes

Interaction of encapsulated drugs with the lipid component of the liposomes may alter the physicochemical properties of the liposomes which, in turn, would influence the drug transfer from the liposomes and the bioavailability of the drug. Differential scanning calorimetry is useful in characterizing physical properties of bilayers by following the lipid phase transitions (Ladbrooke and Chapman, 1969; Cater e. al., 1974). The transition temperature profile not only provides information as to possible 'fluidization' or 'solidification' of the bilayer, but also allows for the determination of critical thermodynamic properties (temperature range and enthalpy of transition). It also indicates whether the whole bilayer or part of it undergoes a change. The very fact that the phase transition profile of a lipid bilayer is modified by an additive is direct proof of perturbation of the bilayer organization (Jain and Wu, 1977).

Glucose, which presumably concentrates exclusively in the aqueous compartments of the liposomes (Bangham et al., 1965; Demel et al., 1968; Papahadjopoulos e al., 1971), is without effect on the thermograms, indicating that it does not perturb the fundamental crystallinity of the bilayer. In contrast, progesterone and hydrocortisone at a total concentration of 0.1% induce readily observable changes in the thermograms indicating that they are associated with the bilayer. The 37°C temperature of the skin permeation experiments lies below the main transition temperature where the DPPC hydrocarbon chains 'melt' even in the presence of progesterone and

TABLE 3

SUMMARY OF PERMEABILITY COEFFICIENTS AND MECHANISMS

Permeability coefficient (cm/hr)	Glucose	Hydrocortisone	Progesterone
P_c	6×10^{-7}	4×10^{-4}	4.6×10^{-4}
P_s	14×10^{-4}	5.4×10^{-4}	6.3×10^{-4}
P_{hl}	6.2×10^{-9}	3.7×10^{-9}	0
P^*	~ 0	3.95×10^{-4}	4.6×10^{-4}
Mechanism	Free solute (100% liposome released-controlled)	~ 99% liposome/skin solute transfer-controlled; ~1% free solute-controlled	100% liposome/skin solute transfer-controlled

hydrocortisone. Those liposomes containing cholesterol would also be expected to be of the solid type (Ladbrooke and Chapman, 1969). Thus, all transport experiments were carried out with 'gel phase' or 'solid' liposomes. It seems that liposome crystallinity per se may be of little consequence here as DMPC liposomes containing progesterone have transition temperatures well below 37°C and progesterone permeates skin from these liposomes at somewhat similar rates found with the solid liposomes (Table 3).

Skin permeation of liposomal systems

The failure of radiolabeled phospholipid in blank and hydrocortisone-entrapped liposomes to reach the receiver compartment shows unequivocally that these liposomes do not pass intact through the hairless mouse skin. It suggests that DPPC molecules are so locked into the liposomal structure that no appreciable free phospholipid exists to partition into the skin and permeate by this mechanism. The alternative explanation is that free DPPC is incapable of permeation which, although not tested, appears improbable because of its small molecular size and other physicochemical properties unfavorable for permeation.

Although liposomes clearly do not pass through the skin, they do induce remarkably different permeation behaviors of solutes entrapped within them. It appears that lipophilic drugs, like progesterone and hydrocortisone, pass through the skin with comparable facility to free drug (comparable mass transfer coefficients). Since the soluble payloads of lipophilic drugs can be increased through liposomal incorporation, more total drug may be delivered through skin via liposomes relative to simple aqueous solutions. Addition of 'empty' liposomes to free hydrocortisone in normal saline had no apparent influence on the permeation of the drug indicating that entrapment of the drug by liposomes is an important factor.

The preponderance of evidence does not support fusion of the liposomes to the skin surface as a mechanism of solute transport. Firstly, neither phospholipid molecules nor entrapped glucose molecules (other than the free glucose) appear on the receiver side. Secondly, the permeation of progesterone is not very dependent on liposome fluidity or charge as would be expected for fusion mechanism. If fusion was a dominant mechanism, the effective permeability of entrapped glucose should approach the values of the free solute, where in fact the permeability coefficient for entrapped glucose is about 3 orders of magnitude lower than that of free glucose. In our studies, the charge on the liposomes appears to have little influence on the overall permeation process with skin, although it has been reported to influence corneal penetration (Schaeffer and Krohn, 1982).

Theoretical analysis

The results of the foregoing permeation studies with DPPC liposomes containing model solutes (glucose, hydrocortisone and progesterone) suggests 3 probable mechanisms (see Appendix and Fig. 4): (1) release of liposome-entrapped solute and percutaneous absorption of the free solute; (2) release of liposome-entrapped solute
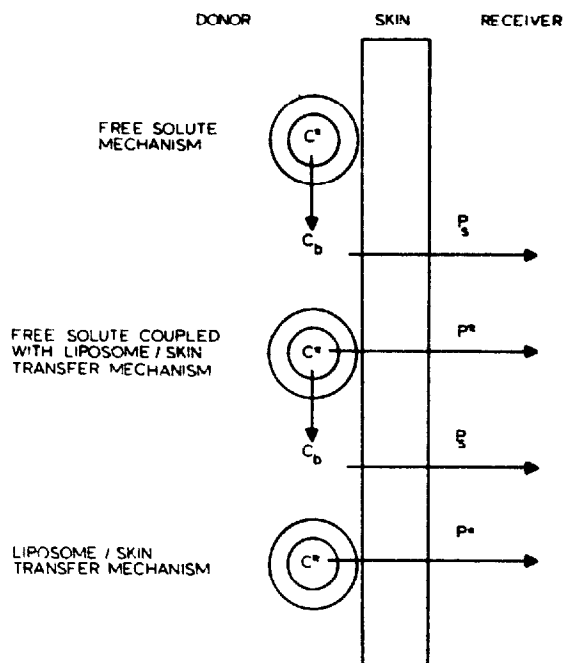
Fig. 4. Schematic description of various mechanisms in the skin permeation of liposome-entr.pped solutes. Liposomes are not absorbed intact nor fused with stratum corneum. See text for definitions of the permeability coefficients.

coupled with skin permeation of free solute and also direct liposome/skin solute transfer; and (3) skin permeation involving liposome/skin solute transfer. The first mechanism would be expected to apply to glucose, a hydrophilic solute entrappcd in the aqueous phase of the liposomes; while the proposed third mechanism migl t be appropriate for progesterone, a hydrophobic solute located principally in the lipid bilayers.

Glucose

The skin permeability coefficient, P_s, for glucose in aqueous solution was previously found to be 1.4×10^{-3} cm/h, while the effective permeability coefficien, P_e, for the liposome-entrapped glucose and skin was 6×10^{-7} cm/h. With the u e of Eqn. A-8 in the Appendix, it is seen that

$$\frac{aA}{3\epsilon V_D P_{bl}} = \frac{1}{P_e} - \frac{1}{P_s} \approx \frac{1}{6 \times 10^{-7}} \tag{3}$$

In other words, release of glucose from the liposomes is the rate-determiniug st:p in the overall skin permeation kinetics of glucose. Subsequently, the mass tra isfer coefficient, P_{bl}, for glucose across the bilayers of the multilamellar liposomes c: n be readily calculated. With $A = 0.785$ cm², $\epsilon = 0.075$, $V_D = 0.2$ ml and average a $= 0.4 \times 10^{-4}$ cm, P_{bl} is 4.2×10^{-10} cm/h.

In vitro release studies indicate that the kinetics are very slow (Fig. 2). From the slope, P_{bl} is estimated by Eqn. A-22 to be 6.2×10^{-9} cm/h and, when compared to 4.2×10^{-10} cm/h obtained from the skin permeation studies, the in vitro P_{bl} is an order of magnitude larger. The disparity in P_{bl} by a factor of 15 takes on less significance in light of the 2000-fold reduction in the effective skin permeability coefficient for the entrapped glucose as compared to free glucose. Thus, it is concluded that the experimental and theoretical studies are supportive of the free solute mechanism for the skin permeation of liposomally entrapped glucose wherein the release rate from the multilamellar liposome is the rate-determining step. It should be noted that even a 50-fold smaller value for P_s found in less hydrated skin in our laboratories has negligible effect on overall kinetics and mechanistic interpretation of skin transport of this polar substance in liposomes.

Progesterone

The skin permeability of progesterone from the liposome system ($P_e = 4.6 \times 10^{-4}$ cm/h) is nearly the same as that for the dilute aqueous solution ($P_s = 6.3 \times 10^{-4}$). In determining the mechanism the free solute absorption model used to analyze the glucose permeation studies is initially assumed. Upon employing Eqn. A-8, P_{bl} is estimated to be 1.2×10^{-6} cm/h, which predicts that the permeability of the lipid bilayers should be larger than that of glucose. Contrary to this prediction, the in vitro release kinetics of progesterone intercalated in the bilayers was immeasurably slow (Fig. 2). Consequently, this suggests a direct liposome/skin transfer mechanism in the skin permeation of liposomally entrapped progesterone.

In performing the calculations for P_{bl}, the average radius of 0.4 μm used for the glucose-entrapped liposomes was also used here for progesterone. Insofar as the effect of additives on liposomal size is concerned, small amounts of uncharged substances ($< 15\%$), which enter and accumulate within the bilayer structure, do not affect the morphological features of multilayer vesicles including particle size (Bangham et al., 1965; Sessa and Weissmann, 1968). Furthermore, polar additives which concentrate in the aqueous compartments also are without effect on liposomal morphology except when the liposomes are under the influence of very large osmotic stress (Weissmann et al., 1965). The 1% glucose (hypotonic) in the present studies falls well out of the osmotic differential necessary to alter liposomal size.

Hydrocortisone

The skin permeability of liposomally entrapped hydrocortisone is 4×10^{-4} cm/h, which is essentially identical to the progesterone case ($P_e = 4.6 \times 10^{-4}$ cm/h), and the permeability of hydrocortisone from aqueous solution is 5.4×10^{-4} cm/h. Furthermore, in vitro studies indicate slow leakage rates from the multilamellar liposomes. Here, P_{bl} is about 3.7×10^{-9} cm/h, which is approximately three-fold slower than that found for glucose and, on the other extreme, more pronounced as compared to progesterone. Taken together, these findings suggest that both the free solute and liposome/skin transfer mechanisms are operating. With the aid of Eqn. A-13 and the aforementioned permeability coefficient values, P* is found to be 3.95×10^{-4} cm/h. Thus, about 99% of the hydrocortisone permeates the skin by the

liposome/skin solute transfer pathway and the small remainder by the free solute mechanism within which it is liposome release rate-controlled. The fact that an illary experiments utilizing a dilute volume fraction of liposomes containing hydr cortisone ($\varepsilon = 0.038$) gave the same P_e values as the higher liposome concentration case ($\varepsilon = 0.075$) provides additional support to the liposome/skin transfer mechanism as the principal transport pathway. If the free solute mechanism was highly inv olved, one would expect P_e to be a function of the ratio of free solute to total solute concentration.

The overall process of liposome/skin solute transfer and permeation of both steroids are seen to occur with about equal facility. This mechanism is support ed by a study involving the permeation of liposomally entrapped, radiolabeled chole sterol and β-sitosterol across a silicone rubber membrane in a well-stirred, two-ch mber diffusion cell (Kreuter et al., 1981). Their results suggested a concerted two-step process involving a 'collision complex' followed by a transfer of solute thro igh a thin hydrophilic layer into the membrane surface to explain the overall phenc mena and differences in permeability coefficients of cholesterol and the more hydrop iobic β-sitosterol. In our diffusion cell system the liposomes of low volume fr ction ($\varepsilon = 0.075$) are in a low volume, unstirred liquid and presumably, all have settl d on the skin membrane. The existence of a thin hydrophilic layer between the lipc some and skin cannot be entirely excluded.

General remarks

This theoretical analysis is summarized in Table 3. It sheds light on unders anding why liposomes are able to enhance the skin permeation rates of a steroid be yond that for the steroid in aqueous solution. In general, the permeation rate per unit area is equal to the product of the effective permeability coefficient and the applied concentration, i.e.

$$J_s = P_e C \tag{4}$$

While P_e for the liposomally entrapped steroid may be somewhat smaller than P_e for the free steroid in aqueous solution, one gains by the many-fold higher solubility in liposomes (Arrowsmith et al., 1983). The aqueous solubilities for the two steroids are only 12 $\mu g/ml$ for progesterone and 285 $\mu g/ml$ for hydrocortisone. The interfacial transfer occurs for liposomally entrapped steroids by 'collision complex transfer' between the steroid intercalated in the liposomal bilayer and the surface phases of the stratum corneum.

In contrast to hydrophobic solutes entrapped in liposomes (aqueous and lipid regions), it is expected that hydrophilic solutes, which are entrapped principal y in the aqueous spaces of liposomes, would have significantly lower permeation ates than its aqueous solution counterpart. Here, the skin uptake rates will depend la gely upon the free solute concentration which is governed by the very slow leakage ates from liposomes. The interfacial transfer of the free solute involves str tum corneum/water partitioning.

From a mechanistic and thermodynamic point of view, our theoretical analyses are consistent with the general observations of Mezei and Singh (1983) and Schaeffer and Krohn (1982) that corneal permeability of hydrophobic solutes is enhanced when they are liposomally entrapped and that the permeation of hydrophilic solutes is not enhanced by liposomal delivery.

Appendix: physical models

A variety of physical models is shown in Fig. 4 and will be developed. The models assume that the liposomes are not absorbed intact nor fused with the membrane. The liposomes are either unilamellar or multilamellar and monodispersed in size. Sink conditions are also assumed.

Free solute mechanism

This model involves the leakage of liposome-entrapped solute into the bulk aqueous medium and skin permeation of the free solute.

The release rate of solute from the liposomes is described by:

$$J_{lip} = 4\pi a^2 n P_{bl}(C^* - C_b) \tag{A-1}$$

where J_{lip} is the release rate, mass/h; C^* and C_b are the solute concentrations in the liposomes and bulk aqueous medium, respectively, mass/cm^3; P_{bl} is the permeability coefficient of the solute for the lipid bilayer(s), cm/h; a is the radius of the liposome, cm; and n is the total number of liposomes.

The flux of free solute across the skin into the sink of the receiver compartment is:

$$J_s A P_s C_b \tag{A-2}$$

Here, J_s is the skin permeation rate, mass/h; P_s is the permeability coefficient of the skin, cm/h; and A is the diffusional area of the skin, cm^2.

Because of the steady-state continuity of mass flow, $J_{lip} = J_s$. Hence,

$$C_b = \frac{4\pi a^2 n P_{bl} C^*}{A P_s + 4\pi a^2 n P_{bl}} \tag{A-3}$$

whereupon the substitution of C_b into Eqn. A-2 yields:

$$J_s = \frac{1}{\dfrac{1}{4\pi a^2 n P_{bl}} + \dfrac{1}{A P_s}} \cdot C^* \tag{A-4}$$

The number of relatively monodispersed size liposomes is approximated by

$$n = \frac{\varepsilon V_D}{V_{lip}} = \frac{3\varepsilon V_D}{4\pi a^3}$$ (A-5)

where V_D is the volume of liposome suspension on the donor side of the skin; ε is the volume fraction of liposomes; and V_{lip} is the volume of a liposome of rad us a. Furthermore, the skin permeation rate is equal to the appearance rate:

$$J_s = V\frac{dC}{dt}$$ (A-6)

wherein C is the concentration of permeant on the receiver side, mass/cm³; V is the volume of liquid, cm³; and t is the time.

Therefore, Eqn. A-4 becomes

$$V\frac{dC}{dt} = \frac{1}{\dfrac{a}{3\varepsilon V_D P_{bl}} + \dfrac{1}{AP_s}} \cdot C^*$$ (A-7)

Upon rearrangement into a computational form to determine the effective permeability coefficient of the system from the linear plot of the amount of permeant appearing with time, one gets

$$\frac{V}{AC^*}\left(\frac{\Delta C}{\Delta t}\right) = P_e = \frac{1}{\dfrac{aA}{3\varepsilon V_D P_{bl}} + \dfrac{1}{P_s}}$$ (A-8)

It is assumed in Eqn. A-8 that C^* remains fairly constant throughout the experimental period. As can be seen, there are two limiting situations. When

$$\frac{aA}{3\varepsilon V_D P_{bl}} \gg \frac{1}{P_s}$$ (A-9)

by more than a factor of 20, release of solute from the liposomes is the rate-limiting step in skin permeation. The other extreme is the skin permeability-controlled case. The P_{bl} and P_s are experimentally accessible from skin permeation studies.

Concurrent free solute and direct liposome / skin mechanism

The rate of percutaneous absorption of drug is expressed by the sum of the permeation rates attributed to the drug solute in the bulk aqueous medium and also the direct transfer of solute between the liposome and skin.

$$J_s' = AP_s C_b + AP^*C^*$$ (A-10)

where P^* is the effective permeability coefficient for the liposome/skin transfer of solute and permeation processes, and the other terms have been previously described.

Since the permeation rate of the solute in the bulk fluid is dependent upon the release of solute from the liposomes, it follows that:

$$J_{lip} = J_s \qquad (A-11)$$

where J_{lip} is expressed by Eqn. A-1 and J_s by Eqn. A-2. Consequently, the substitution of Eqn. A-3 into A-10 yields:

$$J_s' = \left[P^* + \cfrac{1}{\cfrac{A}{4\pi a^2 n P_{bl}} + \cfrac{1}{P_s}} \right] AC^* \qquad (A-12)$$

or

$$\frac{V}{AC^*}\left(\frac{\Delta C}{\Delta t} \right) = P_e = P^* + \cfrac{1}{\cfrac{aA}{3\epsilon V_D P_{bl}} + \cfrac{1}{P_s}} \qquad (A-13)$$

An analysis of Eqn. A-13 shows that, in the event P^* is zero, the expression reduces to Eqn. A-8 for the free solute mechanism.

Direct liposome/skin mechanism

When percutaneous absorption of a hydrophobic compound from a liposome system is appreciable and the release kinetics into the bulk aqueous medium is insignificant, i.e. P_{bl} equals zero, then the direct liposome/skin transfer mechanism is a likely possibility. For this case, Eqn. A-13 becomes

$$\frac{V}{AC^*}\left(\frac{\Delta C}{\Delta t} \right) = P^* \qquad (A-14)$$

In vitro release kinetics

The rate of appearance of solute in the bulk aqueous phase from all liposomes under quasi-sink conditions is:

$$V_b \cdot \frac{dC_b}{dt} = 4\pi a^2 n P_{bl} C^* \qquad (A-15)$$

where the terms have been previously defined (Chowhan et al., 1972, 1973).

The total volume of the liposome suspension, V, is:

$$V = V_b + n V_{lip} \qquad (A-16)$$

and, in terms of volume fraction, the total volume of liposomes is:

$$nV_{lip} = \varepsilon V \qquad (A\text{-}17)$$

and the volume of the bulk liquid, V_b, is

$$V_b(1 - \varepsilon)V \qquad (A\text{-}18)$$

where ε is the volume fraction of liposomes. It follows from Eqn. A-17 that the total number of liposomes is:

$$n = \frac{3\varepsilon V}{4\pi a^3} \qquad (A\text{-}19)$$

When the solute concentration of the liposomes, C^*, has not changed appreciably with time, it may be determined by:

$$C^* = \frac{T}{nV_{lip}} \qquad (A\text{-}20)$$

where T is the initial amount of liposome-entrapped solute.

The substitution of Eqns. A-16–20 into 15 gives the following zero-order expressions:

$$\frac{dC_b}{dt} = \frac{3P_{bl}T}{a(1 - \varepsilon)V} \qquad (A\text{-}21)$$

and

$$C_b = C_b(0) + \left(\frac{3P_{bl}T}{a(1 - \varepsilon)V}\right)t \qquad (A\text{-}22)$$

from which the permeability coefficient of the bilayer(s), P_{bl}, can be determined.

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