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# Liposomes as a topical drug delivery system

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## Contents



## Summary

In the past decade, liposomal formulations have been extensively employed to enhance the efficiency of drug delivery via several routes of administration. In a number of instances, liposomal drug formulations have been shown to be markedly superior to conventional dosage forms, especially for intravenous and topical

Abbreviations: CSA, [$^3$H]ciclosporin; DPPC, dipalmitoylphosphatidylcholine; EGF, epidermal growth factor; FA, flufenamic acid; GC, glycosylceramide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; HSV, *herpes simplex* virus; HSV-1, *herpes simplex* virus type 1; HSV-2, *herpes simplex* virus type 2; MLV, multilamellar vesicle; PA, palmitic acid; PC, phosphatidylcholine; SOD, superoxide dismutase; TGF-β, transforming growth factor-β.

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modes of administration of drugs. The major advantages of topical liposomal drug formulations accrue from their demonstrated ability: (i) to reduce serious side effects and incompatibilities that may arise from undesirably high systemic absorption of drug; (ii) to enhance significantly the accumulation of drug at the site of administration as a result of the high substantivity of liposomes with biological membranes; and (iii) to readily incorporate a wide variety of hydrophilic and hydrophobic drugs. Liposomes are also non-toxic, biodegradable and are readily prepared on a large scale. This paper presents a review of topically applied liposomal formulations with emphasis on the evaluation of liposomal systems in a wide variety of animal models and human skin using both in-vivo and in-vitro techniques. The mechanism by which liposomes facilitate deposition of drugs into the skin and potential applications of topically applied liposomes are discussed.

## I. Introduction

Liposomes are microscopic vesicles composed of one or more lipid bilayers arranged in concentric fashion enclosing an equal number of aqueous compartments [1]. Various amphipathic molecules have been used to form the liposomes, and the method of preparation can be tailored to control their size and morphology. Drug molecules can either be encapsulated in the aqueous space or intercalated into the lipid bilayer; the exact location of a drug in the liposome will depend upon its physicochemical characteristics and the composition of the lipids [2,3].

Excellent reviews on the physicochemical characterization and preparation of liposomes using a wide variety of techniques are available [4–6]. Liposomes have shown great potential as a drug delivery system. An assortment of molecules, including peptides and proteins, has been incorporated in liposomes, which can then be administered by different routes [7–9]. Due to their high degree of biocompatibility, liposomes were initially conceived of as delivery systems for intravenous delivery. It has since become apparent that liposomes can also be useful for delivery of drugs by other routes of administration.

## II. Studies on the use of liposomes for topical delivery of drugs

A particularly promising area concerns topical administration using liposomal drug formulations. The first report indicating that topical application of liposomally encapsulated drugs altered drug deposition was presented at the FIP 1979 congress [10]. This was the first of a series of papers indicating that liposomal entrapment of a drug increased its deposition in the skin and reduced its absorption into the central blood supply. Mezei and Gulasekharam [11] reported that topical application of liposomal triamcinolone acetonide for 5 days resulted in a drug concentration in the epidermis and dermis four times higher than that obtained using a control ointment, while urinary excretion of the drug was diminished. Therefore, their results indicated that the use of liposomes might be useful for increased local activity while diminishing the percutaneous absorption of the drug.

Vermorken et al. [12] tested the effect of topical application of dihydrotestosterone encapsulated in liposomes and as an acetone solution on the hamster flank organ. These authors reported that the systemic absorption of dihydrotestosterone from the liposomal system was negligible whereas significant absorption was observed from the acetone solution. Since they were only concerned with systemic absorption, they reported no advantages of the liposome system over the acetone solution in achieving the desired biological effect.

Mezei et al. [13] compared the deposition of topically applied gels of free and liposomally entrapped triamcinolone in rabbit skin. They found that application of the liposomal gel resulted in a concentration of triamcinolone acetonide approximately five times higher in the epidermis and three times higher in the dermis, than application of the free drug gel. The results of this study and those reported earlier by the same researchers [10,11] suggested to them the inherent potential of liposomes as a selective drug delivery system for cutaneous application.

Ganesan et al. [14], using liposomal formulations in in-vitro diffusion experiments with hairless mouse skin, found that neither intact liposomes nor the phospholipid of which they are comprised diffuse across the skin. They also reported that lipophilic drugs such as progesterone and hydrocortisone, which are expected to be intercalated within the bilayer structure of phospholipid multilamellar liposomes, passed through the skin with comparable facility to the free drug. The effect of application of liposomal progesterone in reducing the rate of hair growth in idiopathic hirsutism was reported by Rowe et al. [15].

Patel [16] suggested that liposomes can be used for the sustained release of drugs into the epidermis when applied topically. For example, when free and liposomally entrapped [$^3$H]methotrexate was applied topically onto the skin of nude mice, percutaneous absorption of drug (as determined by the amount of radiolabel in the blood) was greatly reduced by liposomal encapsulation. Furthermore, the retention of [$^3$H]methotrexate in the skin was 2–3-fold higher from the liposomal form than the free form, again suggesting a localization of the liposomes in the epidermis, where a sustained release of methotrexate takes place.

Methods of production of liposomes and their potential in topical application, especially in the field of cosmetics and dermatology, have been reviewed by Siciliano [17]. Westerhof [18] reviewed the possible use of liposomes as a dynamic dosage form in dermatology and suggested that liposomal preparations could provide a reservoir for the drug and permit its sustained and regular release into the skin. The author suggested that the prolonged release from liposomal delivery systems might be useful in the treatment of a variety of skin lesions.

Komatsu et al. [19] examined the in-vivo percutaneous penetration of butylparaben and dipalmitoylphosphatidylcholine (DPPC) from liposomal suspensions in guinea pigs by autoradiography. They reported that butylparaben penetrated through the skin whereas DPPC was scarcely detected in the body, suggesting that the liposomes themselves remained on the skin surface. They also examined the in-vitro percutaneous penetration of butylparaben and DPPC from the liposomal formulations using flow-through diffusion cells in order to quantitate the effect of liposomes on drug penetration [20]. They found that the amount of butylparaben that

penetrated the skin of guinea pigs from the liposomal suspension decreased as the lipid content increased, suggesting that butylparaben concentration in the outer aqueous phase of the liposomes determined the extent of percutaneous penetration.

Nishihata et al. [21] reported that the in-vitro penetration of diclofenac, formulated as a gel, through rat dorsal skin was poor. This lack of transport was attributed to the poor permeability of the drug through the *stratum corneum*. However, co-administration of hydrogenated soya phospholipid liposomes increased the concentration of drug in the subcutaneous tissue as well as its permeation through the skin. Transdermal delivery of bunazosin HCl and the enhancing effect of egg yolk lecithin were examined by Kato et al. [22] using in-vitro hairless mouse skin preparations as well as in-vivo systemic absorption studies with rabbits. They found an enhancement effect of lecithin on the penetration of bunazosin HCl into the skin in both in-vitro and in-vivo studies.

The applicability of liposomal hydrocortisone as a selective drug delivery system for cutaneous administration of glucocorticoids has been studied by Wohlrab and Lasch [23]. Topical application of a liposomal preparation of hydrocortisone resulted in higher concentrations of drug in the individual layers of human skin as compared to a similar application of hydrocortisone formulated as an ointment.

Jacobes et al. [24] examined the effect of pre-treating skin with phosphatidylcholine (PC), applied as a liposomal suspension, on the bioavailability of four formulations of corticosteroids with different inherent potencies. At the end of the second week of corticosteroid application, the blanching response to all four formulations on the PC liposomal-treated arms was significantly higher than on the control-treated arms. They suggested that the applied phospholipids either supplement the lipid content of the skin or provide a thin film in intimate epidermal contact. It was argued that such a film may promote hydration of the *stratum corneum* and also provide an environment into which corticosteroids initially partition before a subsequent, more controlled release to the underlying tissue.

Gregory et al. [25] investigated the effect of locally applied, biosynthetic human epidermal growth factor (EGF) and transforming growth factor-β (TGF-β) on tensile strength of experimental incisions. A single dose of EGF in saline failed to increase tensile strength over controls. A single dose of EGF incorporated into multilamellar liposomes produced a 200% increase in wound tensile strength over controls between 7 and 14 days, suggesting that the liposomal formulation provided prolonged local delivery of EGF to the wound and increased early tensile strength.

The potential of a liposomal local anesthetic formulation to provide topical anesthesia of the intact skin was investigated by Gesztes and Mezei [26]. They studied the topical anesthetic effects of liposomal and free (Pontocaine cream) tetracaine preparations by the pinprick technique in adult volunteers. For the liposomal preparation, tetracaine base, at 0.5%, was encapsulated into multilamellar phospholipid vesicles. They found that while the liposomal formulation provided long-lasting anesthesia of the skin even at low drug concentrations, the Pontocaine cream was ineffective.

Kimura et al. [27] studied the use of lipid disperse systems, containing soybean

phosphatidylcholine (PC) and glycosylceramide (GC) as the lipid components, to enhance the percutaneous penetration of flufenamic acid (FA) through rat abdominal skin using both in-vitro penetration and in-vivo absorption studies. They found that the in-vitro penetration of FA from a buffer suspension containing no lipid was poor but the uptake was markedly enhanced when FA was formulated as a PC dispersion. An enhanced absorption of FA from lipid disperse systems was also obtained in their in-vivo studies.

The effect of a single exposure to ultraviolet radiation on skin superoxide dismutase (SOD) activity was examined in mice by Miyachi et al. [28]. A significant decrease in SOD activity was observed 24 and 48 h after ultraviolet irradiation. The SOD levels returned to the normal level by the 72 h time period. Decreased SOD activity after ultraviolet exposure was lessened by pretreatment of skin with liposomal SOD. The authors suggested that this protective effect of the encapsulated SOD may have potential clinical application for photodermatologic reactions.

Rodney et al. [29] investigated the therapeutic and immunologic effects of the topical application of a liposome preparation containing both a macrophage activator, muramyltripeptidylethanolamine, and a recombinant antigen, glycoprotein D of HSV-1. This preparation was tested in vitro for its ability to stimulate peripheral blood lymphocytes and in vivo for the control of recurrent *herpes genitalis* in guinea pigs. They found that the liposomal antigen adjuvant preparation was capable of enhancing antigen specific lymphocyte stimulation, which may be related to the observed 75% suppression of the frequency and severity of reactivation of recurrent HSV-2 *genitalis* compared with that of placebo controls.

## III. Liposomes in cosmetics

Recently, a great deal of interest in the use of liposomes in skin gels or skin creams has been generated in the field of cosmetics. Vegetable phospholipids are widely used for topical applications in cosmetics and dermatology, since they have a high content of esterified essential fatty acids, especially linoleic acid which is believed to increase the barrier function of the skin and decrease water loss within a short period of time after application [30, 31]. Soya phospholipids or other vegetable phospholipids, due to their surface activity and their ability to form liposomes, are also an ideal source for possible transport of linoleic acid into the skin. Lautenschlager et al. [32,33] discussed the potential use of liposomes derived from soya bean phospholipids in cosmetics. They predicted that liposome technology offers great opportunities for several new cosmetic products and that cosmetic developers would now have to deal very intensively with questions of raw material selection, characterization of raw and finished formulations, and clinical safety of these unique formulations. They suggested that soya phospholipids in the form of liposomes satisfy many of these requirements.

## IV. Mechanism of liposomal action

Although the use of liposomal drug formulations for topical application has been steadily increasing, few studies have been undertaken in order to explain the mech-

anism of liposomal action on drug transfer into the skin and ultimately, its improved therapeutic effect. Most in-vitro transport studies, which typically concern themselves with permeation of drug *through* the skin, do not focus on the extent of drug accumulation in the various skin strata. In order to evaluate formulation effects on the treatment of dermatological diseases by topical application, a knowledge of such tissue levels is crucial, since it is expected that for a formulation to be most effective it should facilitate increased drug levels in the epidermis.

The *stratum corneum* of humans, mice and pigs have shown to be devoid of phospholipids. The lipid composition is rather non-polar in nature and consists primarily of ceramides (≈40%), cholesterol (≈25%), fatty acids (≈25%) and cholesteryl sulfate (≈10%). Such lipid compositions has been referred to as 'skin lipids' and they are arranged in bilayer sheet structures that fill the intercellular space in the *stratum corneum*. The pathway for the transport of water and other drugs is believed to reside mainly in these bilayer structures. Removal of these bilayer sheets either by solvent treatment [34] or by successive tape stripping [35] increases the permeability of water, suggesting a decreased barrier function.

The ability of the *stratum corneum* to act as a reservoir for drug transport through the skin was amply demonstrated by Rougier et al. [36], who reported that the absorption of a variety of drugs through the skin was proportional to the amount of drug recovered in the *stratum corneum* following 30 min topical application. It is therefore important to examine the extent of drug accumulation in the various strata of the skin in addition to estimating percutaneous absorption profiles. A common procedure for the determination of drug levels in the skin strata involves stripping of treated skin with adhesive tape. With the use of appropriately radiolabeled drugs and liposomal lipids, it has been possible to obtain both drug and liposomal lipid distributions in the various strata of the skin.

The experimental procedure adopted in our in-vitro studies [37,38] with full thickness hairless mouse and guinea pig skin was as follows. Briefly, the skin was mounted on a Franz diffusion cell with a nominal surface area of 2 $cm^2$ and a receiver compartment with a 7 ml capacity (Crown Glass, Somerville, NJ ). The epidermal side of the skin was exposed to ambient conditions while the dermal side was bathed by a 0.05 M isotonic HEPES buffer (pH=7.4). The receiver solution was stirred continuously using a small Teflon-covered magnet. Care was exercised to remove any air bubbles between the underside of the skin and solution in the receiver compartment. The temperature of the receiver was maintained at 37°C. Following mounting of the section of skin, 200 μl of the test formulation was applied to the epidermal surface. A smaller amount of formulation was found to be insufficient to ensure uniform spreading across the entire exposed surface of the skin in the cell. A minimum of three cells was used for each formulation and duplicate experiments were carried out using sections of skin from different skin specimens for each formulation. All experiments were carried out with non-occluded donor compartments. At predetermined time periods the experiments were stopped and the diffusion set-up was dismantled for assay of radiolabeled drug and lipids. Upon dismantling the donor compartment of the cell was rinsed carefully 5 times with 0.5 ml HEPES buffer (pH 7.4). The skin was then removed

and it too was rinsed twice with 3 ml of the same buffer. The washing procedure was found to be sufficient to remove more than 99% of the formulation when determined at time zero. All washings were collected and assayed for radiolabel. Following the rinsing procedure, the skin patch was mounted on a board and a piece of adhesive tape (Scotch Magic Tape, 810, 3M Commercial Office Supply Division, St. Paul, MN), 1.9 cm wide and about 6 cm long, was used to strip the skin. The tape was of sufficient size to cover the area of skin that was in contact with the formulation. Nine strippings were carried out for each specimen and each strip was analyzed separately for radiolabelled drug and lipid. The amount adhering to the *stratum corneum* surface is determined by analysis of the first two strippings and the amount in the deeper *stratum corneum* is determined by analysis of the remaining strips. The amount of drug and lipid penetrating the deeper skin strata is determined by analysis of the remainder of the stripped full thickness skin. A mass balance of more than 95% was achieved after the donor compartment, and the skin rinses were taken into account. Assay of the donor and skin rinses and receiver solutions were carried out after addition of about 15 ml of Ecolite scintillation cocktail to each system. The tape strippings and the skin remaining after stripping were assayed as follows. Each sample was placed in a combustion cone and burnt in a tissue oxidizer (Model 306 Packard oxidizer, Packard Instrument, Downers Grove IL). The separated radionuclides were then assayed using a scintillation counter. A variety of liposomal formulations were examined in both an in-vivo and an in-vitro study. These included phospholipid-based liposomes and liposomes prepared from mixtures similar in composition to the *stratum corneum* lipids, termed 'skin lipids'.

The formulation of stable liposomes from mixtures of 'skin lipid' has been documented by us and others [37–39]. Weiner et al. [37] were the first to report that 'skin lipid' liposomes are effective drug delivery systems. They demonstrated that topical application of α-interferon entrapped in 'skin lipid' liposomes showed a greater reduction of lesion scores than interferon entrapped in liposomes prepared from phospholipid mixtures, when tested in a HSV-1 guinea pig model. Weiner et al. [37] also reported that the method of preparation affected the efficacy of interferon action.

In an effort to understand effects of liposomal composition and method of preparation on the deposition of interferon into the *stratum corneum* and deeper strata of the skin the topical delivery of several interferon formulations was evaluated by Egbaria et al. [38] using in-vitro diffusion experiments. It was found that application of liposomes prepared from 'skin lipids' resulted in almost twice the amount of interferon deposited in the deeper skin layers than application of liposomes prepared from phospholipids. They also showed that topical application of 'skin lipid' liposomes prepared by the dehydration/rehydration method showed twice the effectiveness of those prepared by the reverse-phase evaporation method with respect to their ability to deposit interferon into the skin strata where the basal cell layers reside. The in-vitro results were consistent with the in-vivo effects.

The recent work of Egbaria et al. [38] and Weiner et al. [37] has revealed several aspects related to the mechanism by which liposomal entrapment enhances local

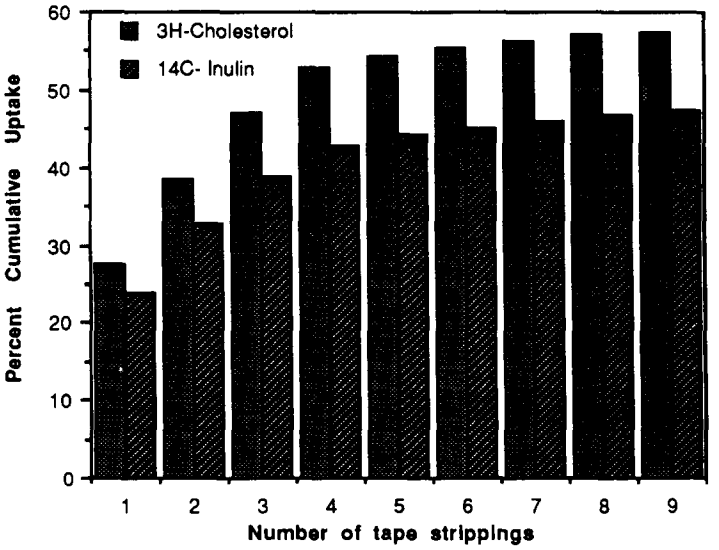


Fig. 1. 24-h in-vitro uptake of [$^3$H]cholesterol and [$^{14}$C]inulin from egg phosphatidylcholine/cholesterol/phosphatidylserine (molar ratio, 1.0:0.5:0.1) MLV in various strippings of hairless guinea pig *stratum corneum*.

activity of topically applied drugs.

(i) When [$^{14}$C]inulin is incorporated into liposomes containing [$^3$H]cholesterol as a lipid marker, the ratio of $^{14}$C- and $^3$H-label hardly changes in various layers of the *stratum corneum* (Fig. 1). It therefore appears that liposomes are able to carry their entrapped solutes into the skin, even to a depth beneath the *stratum corneum* (Fig. 2). Inulin is a water-soluble marker, and when it is applied as an aqueous solution it is essentially incapable of permeating the skin (Fig. 3). The maintenance of the inulin/lipid ratio, together with the observation that there is no breakthrough of inulin into the receiver compartment, even after 24 h, further suggests that a significant amount of water associated with the liposomal bilayers is carried into the different layers of the *stratum corneum* and even into the deeper skin strata (Figs. 1–3).

(ii) The ratio of radiolabeled lipids of the liposomal preparation was essentially maintained throughout the skin strata (Figs. 4 and 5). This strongly suggests a molecular mixing of the liposomal bilayers with those of the *stratum corneum* 'bilayers' (Figs. 6 and 7). The observation that significantly larger amounts of drug and lipid are found in the deeper strata (residual skin) for 'skin lipid' systems suggests that mixing of the liposomal bilayers with the *stratum corneum* 'bilayers' was more extensive with 'skin lipid' liposomes than with phospholipid liposomes. It is argued that the greater ease of mixing of 'skin lipid' liposomal bilayers is not surprising, since its composition is similar to that of the *stratum corneum* 'bilayers'. This greater facility of mixing also explains the greater efficiency of penetration of ciclosporin [40] and interferon [38] into deeper strata of the *stratum corneum* from 'skin lipid' liposomes as compared to phospholipid-based liposomes.

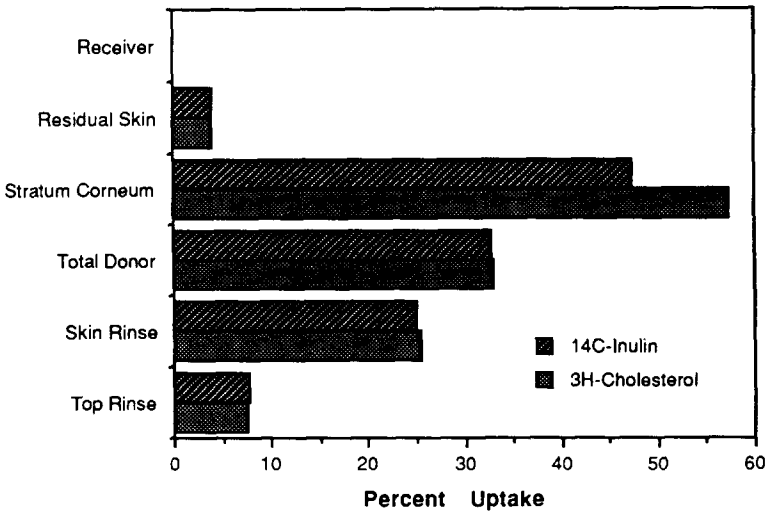


Fig. 2. 24-h in-vitro distribution of [$^{3}$H]cholesterol and [$^{14}$C]inulin from egg phosphatidylcholine/cholesterol/phosphatidylserine (molar ratio, 1.0:0.5:0.1) MLV with hairless guinea pig skin in various compartments.

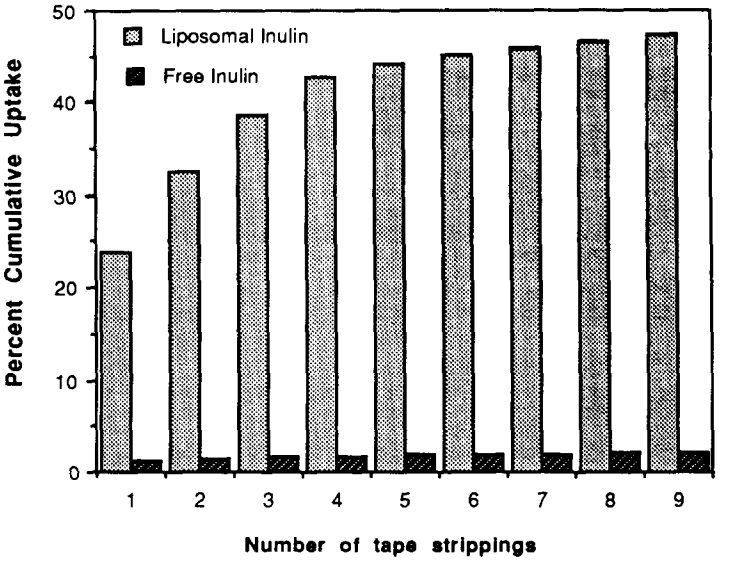


Fig. 3. Comparison of the 24-h in-vitro uptake of [$^{14}$C]inulin from aqueous solution and from egg phosphatidylcholine/cholesterol/phosphatidylserine (molar ratio, 1.0:0.5:0.1) MLV in various strippings of hairless guinea pig *stratum corneum*.

(iii) The uptake and distribution of ciclosporin from emulsions having an emulsifier composition similar to the lipid composition of phospholipid liposomal formulations is markedly reduced (Fig. 8). This finding suggests that for efficient drug

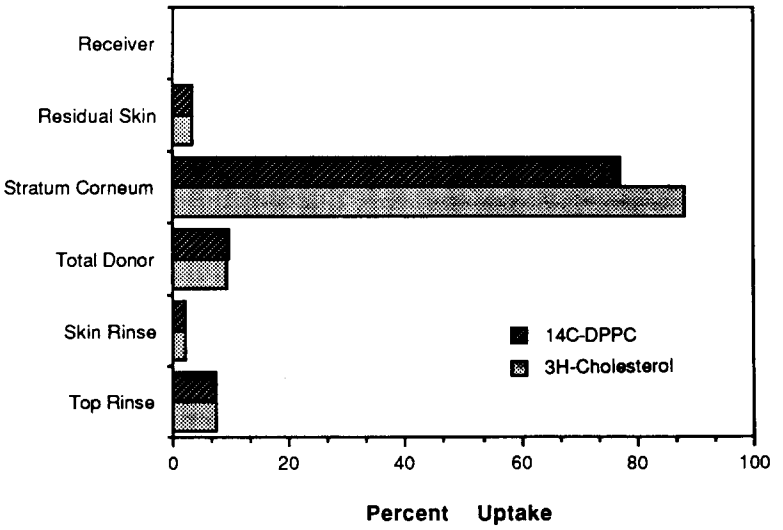


Fig. 4. 24-h in-vitro distribution of [$^3$H]cholesterol (L-3-phosphatidylcholine) and [$^{14}$C]DPPC (1,2-di[1-$^{14}$C]palmitoyl), lipid markers from egg phosphatidylcholine/cholesterol/phosphatidylserine (molar ratio, 1.0:0.5:0.1) MLV with hairless mouse skin in various compartments.

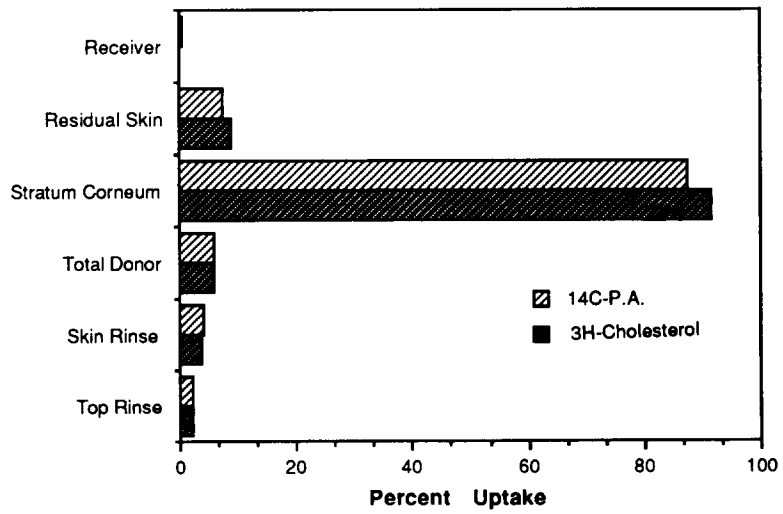


Fig. 5. 24-h in-vitro distribution of [$^3$H]cholesterol and [$^{14}$C]PA lipid markers from ceramide/palmitic acid/cholesterol/cholesterylsulfate (weight ratio, 4.0:2.5:2.5:1.0) MLV with hairless mouse skin in various compartments.

transfer to occur the lipid structure in the vehicle must be in a bilayer configuration. The molecular orientations of the lipids and the drug molecules would be quite different in an emulsion and a liposomal system. In the latter formulation ciclosporin is incorporated in the bilayer lipid structure, while in the former a fraction of the

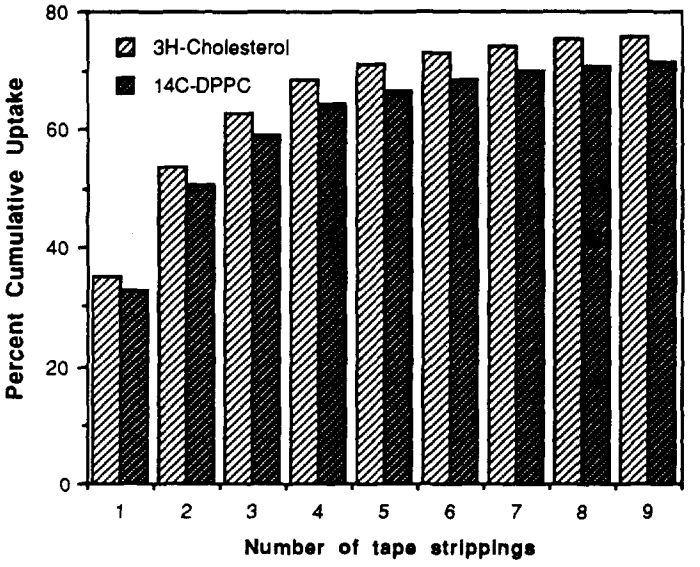


Fig. 6. 24-h in-vitro uptake of [$^3$H]cholesterol and [$^{14}$C]DPPC from egg phosphatidylcholine/cholesterol/phosphatidylserine (molar ratio, 1.0:0.5:0.1) MLV in various strippings of hairless mouse *stratum corneum*.

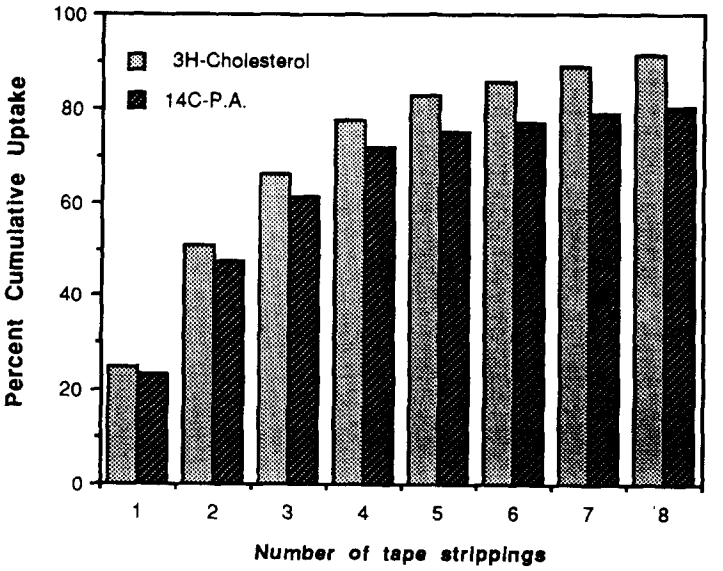


Fig. 7. 24-h in-vitro uptake of [$^3$H]cholesterol and [$^{14}$C]PA from ceramide/palmitic acid/cholesterol/cholesterylsulfate (weight ratio, 4.0:2.5:2.5:1.0) MLV in various strippings of hairless mouse *stratum corneum*.

drug would be dissolved in the oil phase and the remainder would associate with

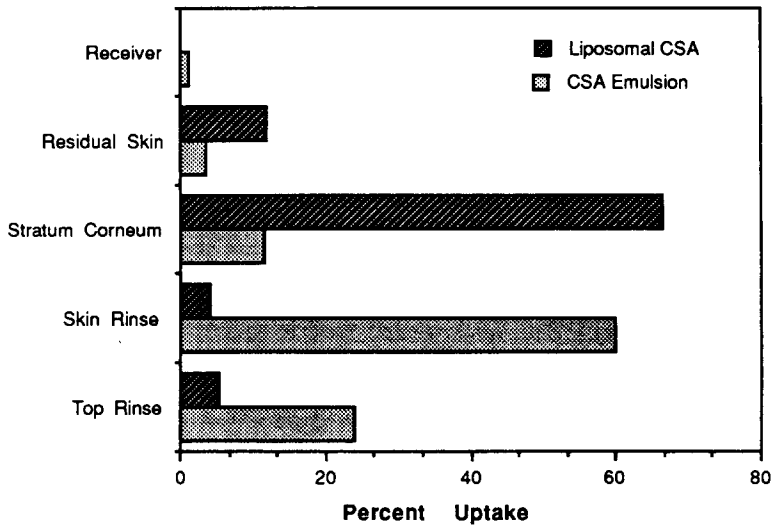


Fig. 8. Comparison of the 24-h in-vitro uptake of [$^3$H]ciclosporin (CSA) from egg phosphatidylcholine/cholesterol/phosphatidylserine (molar ratio, 1.0:0.5:0.1) MLV and from an emulsion in various strippings of hairless mouse *stratum corneum*.

the lipid emulsifier monolayer at the droplet interface.

## V. Conclusions

The combined results suggest that topically applied liposomal formulations, particularly those prepared from lipid mixtures of composition similar to the *stratum corneum*, would be an effective delivery system for the treatment of skin diseases. Since these liposomal formulations provide sustained, enhanced levels in deeper strata of the skin, they have the capacity to meter a sufficient quantity of drug into deeper tissue to treat the skin symptomology. Such metering should also reduce the incidence of undesirable side effects arising from systemic administration, or enhanced systemic absorption of drug after topical administration with vehicles (e.g., alcoholic solution) that disrupt the *stratum corneum* bilayer structure.

Detailed electron-microscopic investigations of the alterations of the *stratum corneum* bilayer structures coupled with autoradiographic studies are necessary to further elucidate the mechanism of liposomal action.

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