CHAPTER - 4

EFFECT OF LIQUID CRYSTALLINE CREAM ON THE EPIDERMAL LIPIDS IN RAT PUP SKIN DURING PERCUTANEOUS ABSORPTION

In most of the skin disorders, topical drug therapy is preferred to systemic therapy, because there is no danger of systemic toxic side effects such as gastrointestinal absorption and hepatic first pass effect. Moreover, through topical application, only sufficient controlled amount of drug reaches the appropriate affected site at the right time and as often required. Apart from the skin diseases, many other body disorders could be treated through drugs delivered through the skin. Modern research has lead to the development of transdermal drug delivery systems (TDS), which are polymer based adhesive skin patches with a reservoir of drugs that needs continuous and prolonged administration such as scopolamine, clonidine, nitroglycerine, estradiol and testosterone.

Diffusion of the drug depends on the microenvironment of the skin. During the flux of drug and drug vehicles through the skin, they have to traverse through three barriers, viz :

1. Stratum corneum (SC) intercellular lipids, that are hydrophobic in nature and therefore only lipophilic drugs can be transferred through these domains.

2. Stratum corneum - stratum granulosum (SC-SG) interphase, where the drug has to face a living tissue which is aqueous in nature. Here penetration will depend on the molecular size of the permeant and the hydrophilic diffusional characteristics of the drug.

3. The drug further encounters the dermal vasculature where it has to pass through the endothelial lining of blood vessels and once inside, the drugs can be removed efficiently to other parts of the body (Hadgraft, 1987).

The rate of drug delivery can be influenced by using vehicles which act as penetration enhancers or by using an occlusive effect on the skin which involves application of a plastic wrap to hold the drug and its vehicle in close contact with the skin. TDS using occlusive effect, could give an allergic dermatological side effect (Boddé et al., 1987). Chemicals like propyleneglycol, urea, dimethylsulfoxide can enhance penetration of other substances in various topical formulations (Hannuksela, 1987).

Skin also shows a regional variation to drug penetration. The scrotal, facial , axilla and scalp skin are more permeable than the forearm. This could be attributed to the variation in the distribution of all major lipid species especially the neutral lipids present in the SC of the epidermis (Lampe et al., 1983). Nemanic and Elias (1980), Hadgraft (1987), and other group of workers have shown that the neutral lipid enriched intercellular spaces of SC might constitute a primary preferential pathway for the percutaneous transport of topically applied, lipid soluble substances. Hair follicular route is another preferential pathway in normal, unperturbed skin (Sharata and Burnette, in press). They have also shown that, in a chemically perturbed skin, the heavy metal tracers are even seen intracellularly. This increases the surface area for interaction and adsorption of the tracer with cellular proteins.

Binding of the permeant with proteins could extend lag time for the activity of drug. Stoughton (1965), Munro (1969), Scheuplein and Ross (1974), Vickers (1980) and Clanachan and Foreman (1984), have proposed that this mechanism can create 'reservoirs' in the horny layers of the skin for steroids, griseofulvin and hexachlorophene. Elias and coworkers

(1989), have postulated that the increased surface area resulting from the intercellular broad lipid sheets in the SC account for the skins reservoir function. Elias (1987), has suggested that the enzymes, like acid phosphatases, lipases and proteases secreted by the lamellar bodies may be active in the degradation of topically applied drugs or lead to formation of prodrugs. These prodrugs could then have an access, or they could penetrate, into deeper region of the skin through the lipophilic barrier and will be metabolized to active drug at their site of action.

The permeant and/or the penetration enhancer, during its course of diffusion may modify the structural lipids in the intercellular domains (Hadgraft, 1987). Golden et al. (1987), have suggested that the penetrant enhancers should have properties similar enough to SC lipids to allow significant partitioning into these domains, yet dissimilar enough to maximally disrupt lipid packing. This alteration in the intercellular spaces may probably be a result of increased lipid fluidity.

In search of improved drug vehicles, liposomes (Mezei, 1987), and liquid crystalline creams (Boddé et al., 1987) as potential drug carriers have been investigated. Liquid crystalline creams have the ability to take up water from the skin. Skin irritation from occlusion can thus be avoided. Non ionic surfactants are known to show no, or minimal skin irritation.

Brij 99 [polyoxyethylene (10)-oleyl ether] is a non ionic surfactant. Liquid crystalline cream has been prepared using Brij 99 and water. Depending on the surfactant / water ratio, these mixtures can adopt a lamellar (low water content: 15-40%) or a hexagonal (higher water content:

30-60%) gel structure (Junginger et al., 1987). The same group of workers have suggested that the creams having a low initial water content, take up water from the skin after application and have a low drug release rate, while the creams having high water content shows highest drug release rate. Brij 99 is known to increase the permeability of SC, probably by fluidization of its lipid components (Junginger et al., 1987).

Thus, it was deemed worthwhile to check the route of permeation of liquid crystalline creams, which are used to enhance permeation after epicutaneous application. An attempt is also made to study the effect of such creams on the secretion of lamellar bodies, the extruded contents of which forms the basis of barrier, and also on the intercellular lipids of the stratum corneum, ultrastructurally. Neonatal rat pup skin was used to avoid the interference of hair follicular route of permeation.

MATERIALS AND METHODS

Preparation of the cream : Liquid crystalline cream was prepared by using Brij 99 and double distilled water. The water content was 30%. This mixture formed a lamellated gel structure, which was then used for topical application.

Treatment : Rat pups, one day old, were chosen for the topical treatment with 70% Brij 99. 20 mg of cream was applied twice daily for 3 days on the dorsal skin of the rat pups. The pups were kept separately from the mother for atleast one hour to allow sufficient time for permeation of the cream. Loss of the cream by licking of the pups by mother is also thus avoided.

Fluorescence studies : Brij 99 was mixed with FITC (Fluorescein isothiocyanide) (Sigma Chemicals), to check whether Brij 99 permeates the skin, when applied topically. Frozen sections were then taken (12 μ), and observed under the fluorescence microscope.

Ultrastructural studies : By 4th day the rat pups were sacrified (as hair growth starts by the 4th day after birth) and a piece of dorsal skin was removed and minced with a surgical blade into small pieces ($<$ 5 mm), fixed in glutaraldehyde - paraformaldehyde fixative and processed routinely as described in Chapter 1.

RESULTS

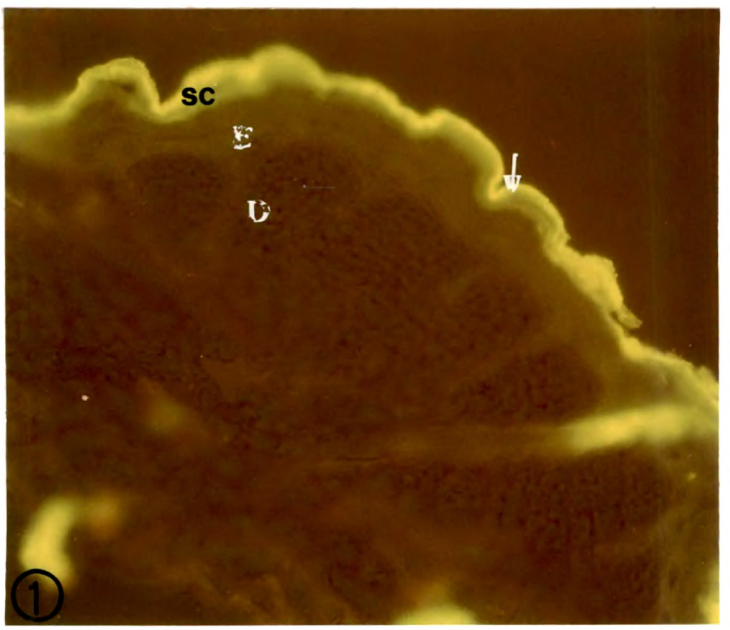
The neonatal skin did not show any morphological change in the texture, after Brij 99 treatment.

Fluorescent studies showed that Brij 99 does permeate the skin via the intercellular spaces of the corneocytes, as fluorescence was observed in these domains. When applied on pups with slight hair growth, it was seen to permeate along the hair follicles also. The dermis also showed fluorescence, which implies that Brij 99 even crosses the epidermal - dermal barrier (Fig. 1,2).

Ultrastructural studies confirmed the earlier findings regarding the route of permeation of drugs and penetration enhancers / vehicles. Brij 99 also seems to permeate through the SC, by traversing through the intercellular spaces and not transcellularly (Fig. 3). Our studies revealed that Brij 99 causes extensive domain separation within the SC bilayers (Fig. 4).

Fig. 1 : Fluorescence micrograph of rat pup epidermis after topical application of water + FITC (control). Bright yellow fluorescence is seen only in the corneal layers (arrow). X 50.

Fig. 2 : Fluorescence micrograph of rat pup epidermis after topical application of 70% Brij 99 + FITC (experimental). Fluorescence seen even in lower epidermis and dermis, indicating permeation of Brij 99. X 50.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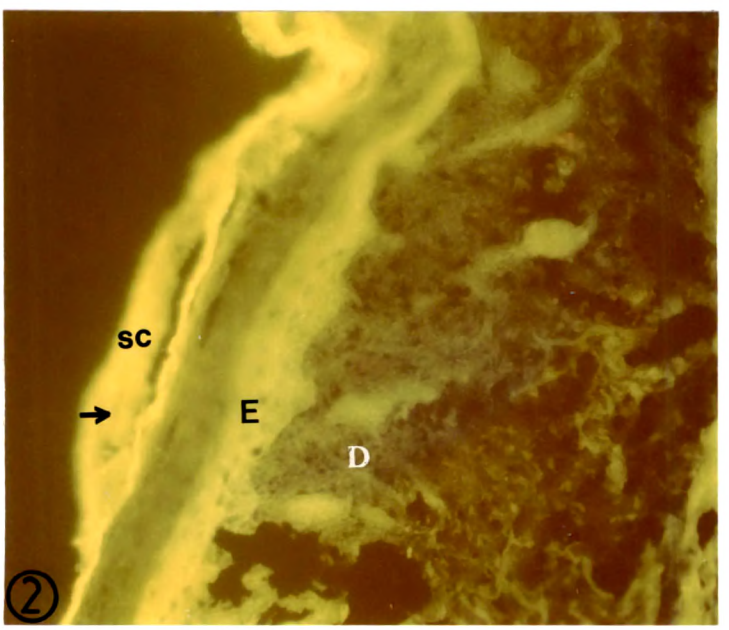
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Fig. 3-8 : Electron micrographs of Brij 99 treated rat pup epidermis.

Fig. 3 : Upper corneocyte layer showing permeation of Brij 99, through the intercellular domains (arrow). X 63,000.

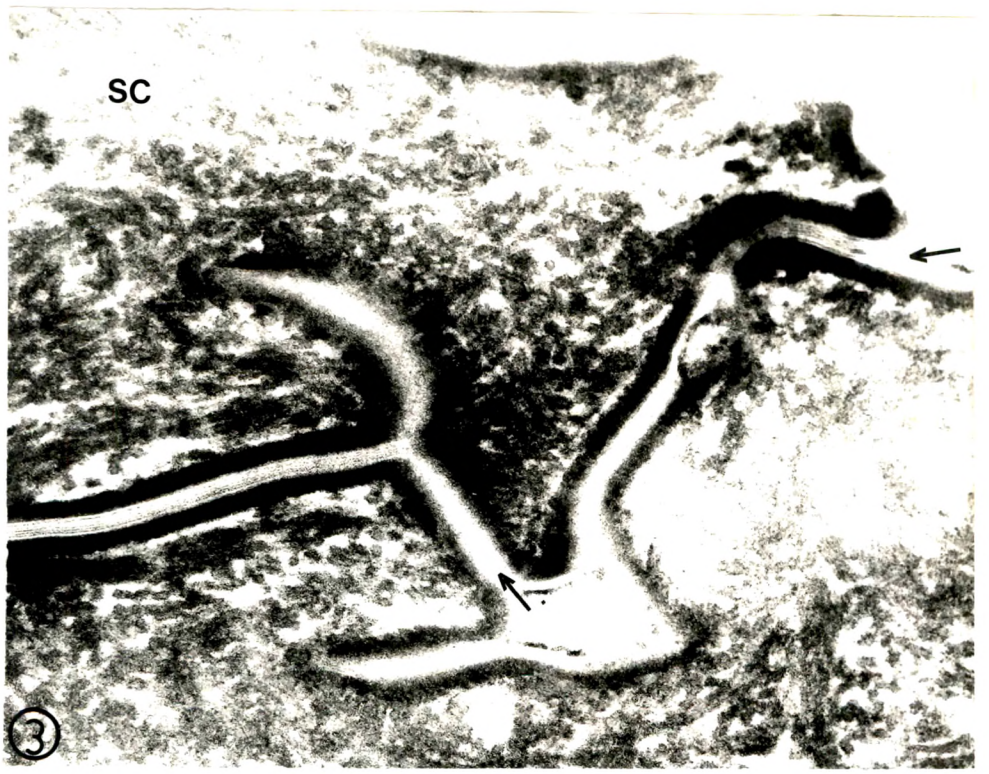
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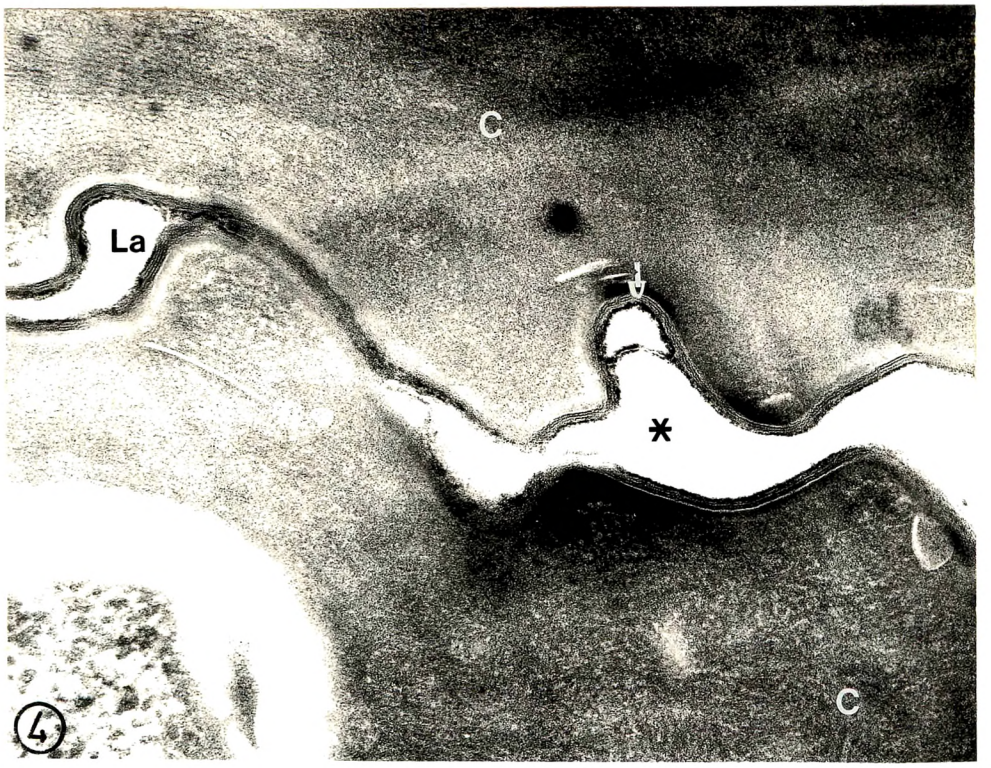
Fig. 4 : Excessive domain separation (*) in the stratum corneum bilayers seen (arrow). Dialation of 'lacunae' is visible. X 63,000.

Fig. 5 : Continuous channel (arrow) in the stratum corneum interstices seen for the passage of the vehicle. X 30,000.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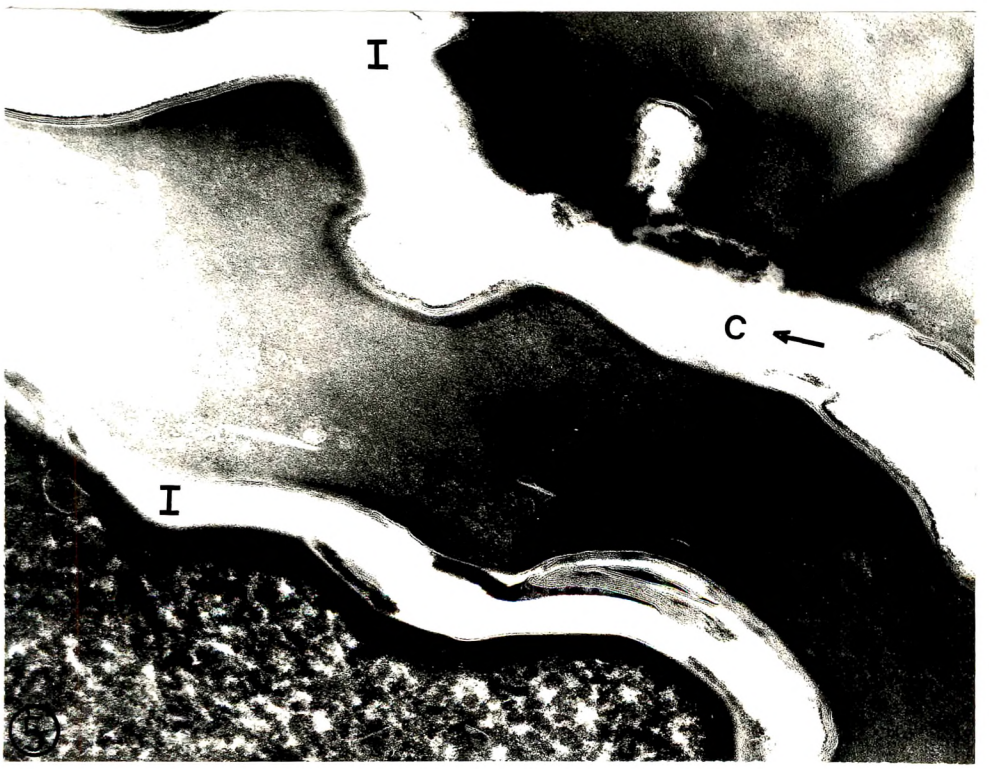
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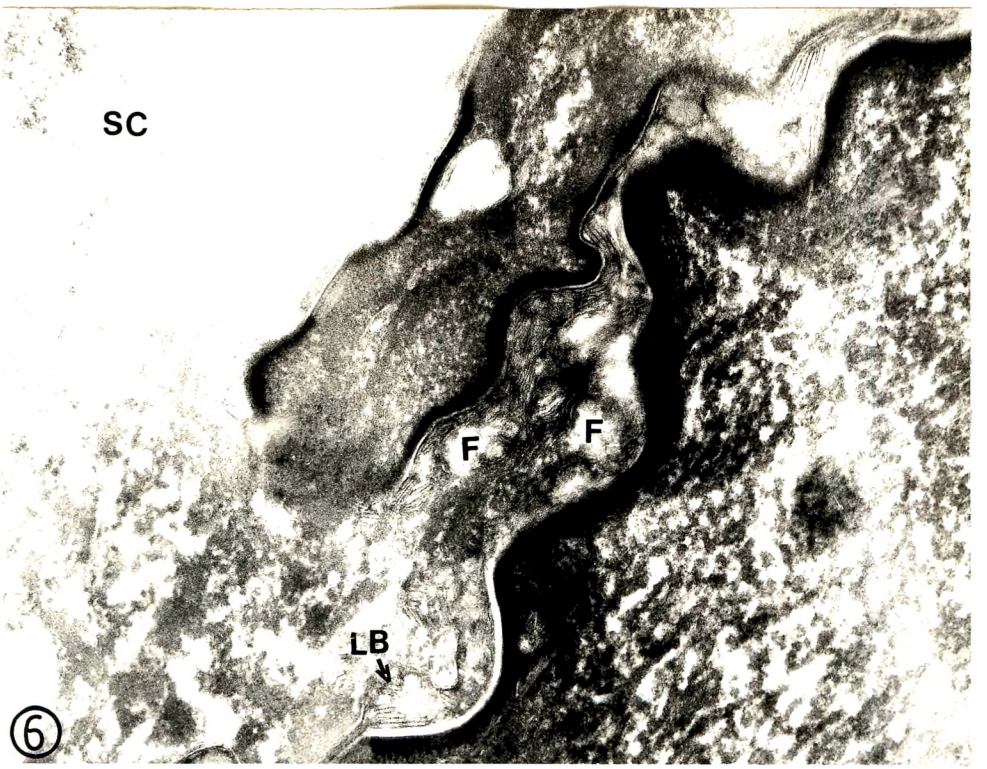
Fig. 6 : Large focal areas of non-lamellar domains in the lamellar bodies evident within the lower stratum corneum. X 57,500.

Fig. 7 : The stratum corneum - stratum granulosum interface, showing lamellar material, interspersed with non-lamellar lipid droplet. X 57,500.



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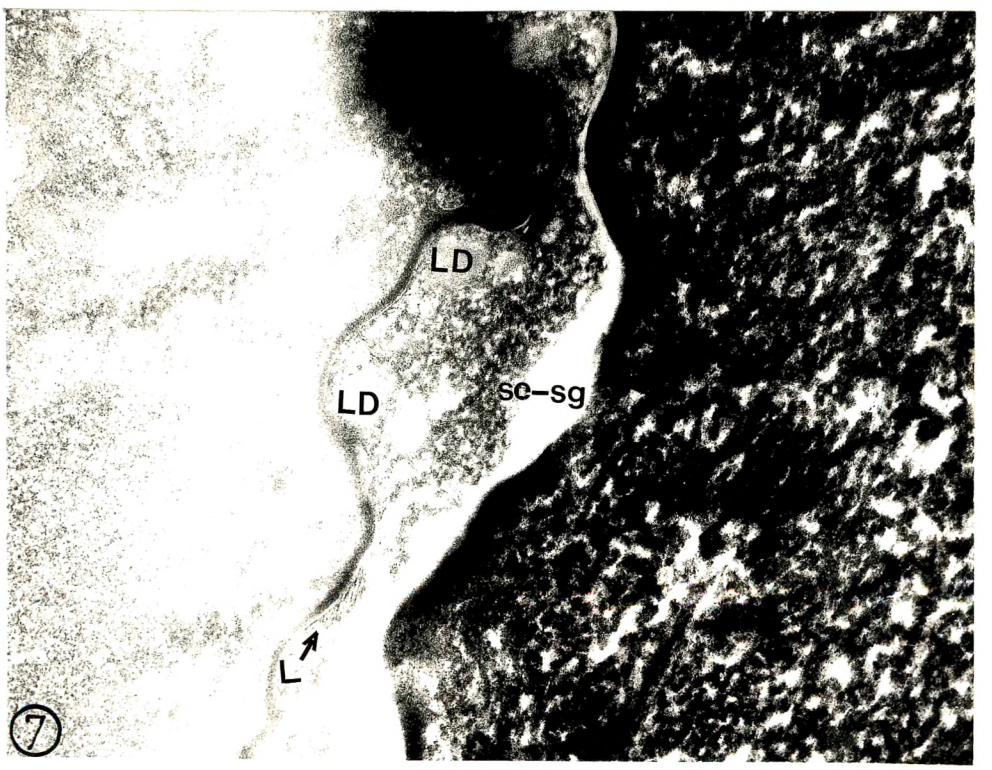

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Fig. 8 : Another area of the stratum corneum - stratum granulosum interface, where the secreted discs in the stratum corneum domains do not show tight packing (arrow) of lipid lamellae. X 67,500.

Abbreviations :

Lipid lamellae - L; Lipid droplet - LD: Stratum corneum - SC; Corneocyte - C; Stratum corneum interstices - I; Lacunae - La; Channels - C; Viable epidermis - E; Dermis - D; Stratum corneum - Stratum granulosum interface - sc-sg; Focal areas - F.


SC
L
SG
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Infact, an almost continuous channel of considerable dimension is formed by formation of 'lacunae' in between the bilayers (Fig. 5). These lacunae are formed by dialation of the space present within the bilayers (Fig. 4).

Brij also affects the secreted lamellar body contents in the intercellular spaces. The secreted disks in the lower most SC domains, shows alterations where the normally tight packing of disks is absent and large focal areas of nonlamellar domains are seen (Fig. 6,8). The bilayers can be seen interspersed with nonlamellated lipid material (Fig. 7,8). Bilayers are even seen to be retained in the outermost layer of the SC (Fig. 3).

DISCUSSION

Imokawa and Hattori (1985) have shown that lipid solvent treatment, leads to a loss of structural intercellular lipids of the SC leading to an impaired water holding property (osmotic property) of the SC. Surfactants and detergents (Imokawa and Takeuchi, 1976; Kawai and Okamoto, 1982; Imokawa, 1980) are known to perturb the epidermal barrier and permit ready percutaneous movement of normally excluded substances. Some of these disrupting agents are of practical therapeutic interest to Dermatology, eg. DMSO (Dimethylsulfoxide) and sodium lauryl sulfate can enhance delivery of medications through the SC.

Middleton (1969), had suggested that the electrolyte penetration pathway was not through the SC, but that they pass between the cells. Furthermore, hydration and temperature induced changes in SC permeability causes changes in lipid fluidity, suggesting a correlation between transdermal flux and stratum corneum lipid disorder (Knutson et al., 1985). This suggests

that transdermal drug flux may be finally related to SC lipid structure (Golden et al., 1987).

Most of the vehicles are known to cause skin irritation thereby leading to dry skin conditions and further causing inflammation. Therefore, trans-epidermal drug delivery continues to be a challenging prospect. A number of new approaches including use of liquid crystalline creams and liposomes having minimal side effects are therefore being tried to deliver drugs through the skin.

Brij 99, a vehicle used in the formulation of liquid crystalline cream, has to pass through the lipid barrier present in the SC. Brij, probably increases SC permeability by fluidization of its lipid components (Junginger et al., 1987). From our observations, we therefore propose that Brij 99 modifies the organization of the SC intercellular lipids. The lacunae in between the lipid bilayers indicate a direct route for the penetrant to have an access to the lower layers of the epidermis. This can also serve to form an excellent reservoir for the drug (miscible in these creams) in the SC. A large surface area is therefore available for the drug to bind to the SC domains, and a slow release of the drug as proposed by Junginger et al. (1987) could thus give enough time for the drug to be acted upon by the enzymes that are secreted in the SC-SG domains. This could also facilitate the formation of prodrugs.

This surfactant is seen to act on the lamellar lipids, which ultimately loose their lamellar structure probably by formation of small micelles and aggregating into the intercellular domains to form focal areas of nonlamellar

lipid material. This could facilitate the permeation of Brij 99 into the epidermis. The retention of bilayers in the outermost SC (also seen in psoriatic epidermis - Chapter 1), could be due to change in lipid fluidity which can result in the formation of an altered diffusional resistance of epidermis to water flux.

This study thus gives a clue as to how penetrants and penetration enhancers / vehicles may cause modification in the structural organization of SC intercellular lipids. Though hydrogels, do not give any unwanted side effects such as skin irritation, to have a proper insight into the effectiveness of the liquid crystalline creams as potential vehicles for drug delivery, biochemical studies on the effect of these surfactants on lamellar lipids, are still needed to be done.