INTRODUCTION

The vertebrate skin is the largest organ system that provides a first line defense to protect the body against environmental aggressions. During the course of evolution, there has been a profound alteration among the vertebrate skin. The skin acts as an effective barrier to water loss, as an important thermoregulatory organ, depicts sexual dimorphism and has a multitude of other less obvious, yet important functions.

The functions and dysfunctions of the skin, so important in skin care (cosmetic industry), treatment and control of pathogenic skin microflora (dermatology), formulation of topical applications and transcutaneous drug delivery (pharmaceutical industry) etc: can be appreciated, once the structural and functional organization of skin is elucidated.

Embryologically, morphologically and physiologically the skin is a compound organ having two distinct components; the dermis and the epidermis.

Epidermis is in direct contact with the environment and plays an important role to maintain the internal equilibrium of the body in all habitats. In terrestrial animals, the major biological role of the epidermis is to form an effective barrier against transcutaneous water loss thus preventing dessication and making survival possible.

The shape, size and function of the epidermis and its appendages are significantly different in various vertebrate classes, but still they possess common properties. With advancing age, both the epidermis and dermis and its appendages undergo marked changes (Lavker et al., 1987). Epidermis consists of epithelial cells that are derived from ectoderm and is

attached to the dermis which is a mesodermal derivative. Mcloughlin (1963), showed that mesenchyme is required both as an inert backing for normal epidermal growth and for organization of epidermal development. Thus, epidermal-dermal interactions are essential for the maintenance of a healthy integument.

Due to daily wear and tear of the body cells, the outermost cells of skin layers get cast off. To balance this loss, and maintain a healthy skin, a continuous normal process of formation of these cells has to take place (Elias, 1983). This process is affected by various factors, like temperature and humidity of the environment (Mathias, 1981), secretion of enzymes and toxins by cutaneous microflora and through hereditary factors leading to chromosome linked disorders, like ichthyosis vulgaris, X-linked ichthyosis, lamellar ichthyosis, epidermolytic hyperkeratosis and congenital ichthyosiform erythroderma (MacGuire, 1982; Williams and Elias, 1981; Epstein et al., 1981).

The epidermis consists of epithelial cells that differ from one another in shape, composition, function and potentiality. It is an avascular tissue, receiving nourishment from the dermis by diffusion of substances through the basal lamina (Matoltsy, 1986).

The epidermis can be basically divided into two:the inner malpighian layer and the outer cornified layer. The malpighian layer maintains the tissue by proliferation and differentiation of its cellular components. The inner surface of the epidermis may be smooth or rough, forming branching ridges and mounds i.e. rete pegs (Montagna and Parakkal, 1974). Thus, the

thickness and the architecture of the epidermis varies from site to site in relation to its surface and undersurface structures. In general, the epidermis is thicker over the glabrous skin than over the hairy skin.

The successive layers of epidermis are formed by stratum basale (B), stratum spinosum (SS), stratum granulosum (SG) and stratum corneum (SC), which are stacked over the dermis. The basal layer may consist of single or multiple rows of cuboidal cells with a large nucleus and little cytoplasm. The spinous cels form multiple rows, are polyhedral and have more amount of cytoplasm. The most characteristic component of this layer is the presence of lamellar bodies (LB) or membrane-coating granules (MCG) (Matoltsy and Parakkal, 1965). These cells appear to be connected by intercellular bridges called 'prickles'. Granulosum cells also form numerous layers and are larger than spinous cells. They tend to flatten in the upper portion of the epidermis. The typical characteristic feature of granular cells is the presence of keratohyalin granules (KHG). Stratum corneum is composed of stacks of interlocking, tightly packed, vertical columns of polyhedral flattened cells called corneocytes, filled with amorphous material and with thickened envelopes (Mackenzie, 1969; Christophers, 1971; Menton et al., 1971).

The basal layer is made of heterogenous cells consisting of actively dividing 'stem cells' or proliferative cells' which are capable of only a few transit divisions, nondividing, 'postmitotic maturing cells' and 'non-cycling' cells in Go phase (Wright, 1983; Weinstein et al., 1984). The mitotic cells are also found in suprabasal layers of epidermis (Penneys et al., 1984). Mitosis occurs scattered throughout the epidermis, and the

daughter cells enter the differentiation phase. During this phase the cells migrate towards the SC (terminal stage) and are finally shed off. In the course, the keratinizing cell phases through a synthetic and a transformation phase (Matoltsy, 1976).

The synthetic phase can be identified by the formation of specific products such as MCG, KHG, synthesis of specific proteins and lipids, appearance of protein filaments and formation of a marginal band. The protein filaments assemble to form protofibrils and thus contribute in the formation of the cytoskeleton. The association of these filaments into bundles (tonofibrils) and their attachment to desmosomal plaques enables the malpighian layer to respond to mechanical stress. These filament bundles also provide a framework for deposition of the material forming the KHGs (Matoltsy, 1986).

Keratins form the majority of the proteins synthesized during synthetic phase. Individual keratins are a highly conserved family of polypeptides with great internal similarity. Individual polypeptides form a triple helix which in turn form larger aggregates in the presence of stratum corneum basic protein (Steinert et al., 1976). There are several major and minor types of keratin polypeptides, which are produced during the course of keratinization in human epidermis. Each keratin appears to be translated from its unique mRNA (Fuchs and Green, 1979). Physical durability and chemical stability of keratins depends on two main factors, the linkages between the polypeptide chains and the degree of polymerization of chains. Normal epidermal keratin has fewer disulphide linkages, but its stability

and pliability is probably due to a high degree of polymerization (Jarrett et al., 1959).

At the end of the synthetic stage the cells get transformed into the corneal cells. During this process the hydrolytic enzymes are secreted, that selectively attack and lyze all organelles including the nucleus, mitochondria, rough endoplasmic reticulum, ribosomes and golgi apparatus (Lavker and Matoltsy, 1970). T-cells engulf the degraded mass of cell organelles. Keratohyalin granules coalesce to form large dense masses and filament bundles condense at the cell periphery. Modification of these protein takes place (Bowden et al., 1984) and finally the keratohyalin masses disperse, mix with remnants of cell organells and penetrate the interfibrillar spaces of filament bundles.

During the terminal stage the filaments and other remaining materials gradually consolidate into a fibrous - amorphous matrix. The desmosomes are degraded and finally the outermost epidermal cells are given off.

Cell to cell adhesion in a viable epidermis is usually attributed to desmosomes. Their characteristic extracellular, trilaminar structure is modified into an electron-dense plug in the SC (Skerrow et al., 1989). These complex structures comprise 5% of the cell surface of basal cells, 25% of spinous cells and 35% of granular and SC cells (Arnn and Stachelin, 1979). Chapman and Walsh (1990), have recently developed a pig skin model to determine the role of corneosomes (modified desmosomes in SC), in desquamation.

Glycolipids (Huang, 1978), protein and calcium (Bisett et al., 1987) and extracellular MCG derived lipid lamellae (Cox and Squier, 1986; Smith et al., 1982) also are known to contribute in cell adhesion and cohesion of corneocytes. Corneosomes are lost at stratum disjunctum. Corneosome breakdown commences with an electron lucent band forming between the plug and lipid envelope, which is then degraded (Chapman and Walsh, 1990), ultimately leading to desquamation. Abnormal corneosome retention is shown to be one of the reasons for defective keratinocyte adhesion in psoriatic epidermis (Skerrow and Skerrow, 1990; Skerrow et al., 1988).

Study of the 'Permeability Barrier of Skin' is in limelight for the last two decades. The permeability barrier does not entirely inhibit the flow of water through the skin. A small amount is continuously lost to the environment. This water loss is called 'insensible perspiration' and amounts to 0.2 - 0.4 mg cm^2/hr. at 30°C, which is referred to as trans-epidermal water loss (TEWL).

For a long time, it was known that the barrier to TEWL resides in SC, the outermost, terminally differentiated, keratinized layer of epidermis. Nevertheless, until about a decade ago, it was believed that the SC is a homogenous sheet passively occluding and curtailing TEWL much like a plastic wrap and that the route of entry of whatever substance that penetrated SC was transcellular (Middleton, 1968; Scheuplein and Blank, 1971). Accordingly, hydrophilic substances traversed the SC via the keratin matrix while the lipophilic substances passed via the lipid surrounding the keratin filaments, within individual corneocytes (Michael et al., 1975). These concepts were based on the appearance of SC as loose

squames with a "basket-weave" pattern as shown by conventional techniques of both light and electron microscopy.

This concept has recently been replaced by the two compartment model of SC, the evidence for which came by investigations using a number of modern techniques (Reviewed by Elias, 1983), such as (1) Frozen sections swollen with alkali examined under phase contrast microscope showing regular alignment of corneocytes (Mackenzie, 1975). (2) Fluorescence microscopy. (3) Lipid histochemical and biochemical analysis (Elias, et al., 1977; Grayson et al., 1982). (4) Freeze fracture (Elias et al., 1975; 1977) and (5) Ultrastructural enzyme cytochemical studies (Elias et al., 1988).

Together, these studies documented that lipids are sequestered in the interstices of SC giving rise to 'brick and mortar' organization of SC. In this analogue, the protein enriched, lipid depleted corneocytes form the 'bricks' and the hydrophobic lipids sequestered in the intercellular spaces forms the 'mortar'.

The intercellular lipid layers in between the corneocytes originate from the lamellar bodies (LBs), also known as membrane-coating granules (MCG), keratinosomes or Odland bodies (Matoltsy, 1965). These are specific products of keratinizing epithelia and represent the most useful marker of synthetic stage.

Lamellar bodies are small ovoid (0.2 - 0.3 m), membrane bound organelles, elaborated by the viable epidermis from the spinous layer upwards (Wolff-Schreiner, 1977). Lamellar bodies possess an organized structure characterized by stacks of small disks of about 20A° diameter and separated by dense lines (Matoltsy, 1965; Farbman, 1964; Martinez and Peters, 1971; Elias and Friend, 1975; Lavker, 1976; Wolff and Schreiner, 1976). In all LBs, successive major dense lines are connected at their ends in such a way that they fold back on themselves and enclose a minor dense line. These disks are bound individually by half a major dense line (Landmann, 1980). This is called a 'Landmann Unit'. Lamellar bodies are thought to arise from the golgi apparatus (Matoltsy and Parakkal, 1965), or from endoplasmic reticulum (Hayward, 1979). In many intercellular sites, the basic unit of the LB expands to a double or triple unit, by the addition of additional lamellae. In contrast, it can also become attenuated particularly at the lateral margins and at three cell junctures, by the deletion of one or more lamellae (Hou et al., 1989). Such a site-specific loss of membranes may result in a relatively lipid depleted pathway, for the passage of hydrophilic or amphipathic molecules, i.e. relatively 'leaky zones' (Elias, 1991).

The LB contents in the normal course are secreted into the intercellular domains by fusion with plasma membrane, at the SC-SG interface, during terminal differentiation of epidermal cells (Frithiof, 1970; Hashimoto, 1971; Ricardo-Martinez and Peters, 1971). After being discharged into the intercellular space, the lamellae coalesce and form broad sheets in the lower SC which are parallel to the surface of the horny cells (Elias and Friend, 1975; Lavker, 1976; Landmann, 1980). Based upon their apparent

lipid (Elias et al., 1979) and sugar (Elias et al., 1979; Hayward and Hackemann, 1973) content, their ability to exclude injected water soluble tracers (Elias and Friend, 1975; Elias et al., 1977), their variations in structure (Elias and Brown, 1978) and apparent lipid content (Wertz et al., 1983) in essential fatty acid deficiency (EFAD), a role for LB in the permeability barrier has been proposed (Odland and Holbrook, 1981; Wertz and Downing, 1982; Elias, 1983). On the other hand, the cytochemical (Odland and Holbrook, 1981; Gonzales et al., 1976) and biochemical (Freinkel and Traczyk, 1981) demonstration of hydrolytic enzyme activity, suggests that LBs may be lysosomes that could regulate desquamation (Wolff-Schreiner, 1977).

Grayson et al. (1985) have partially characterized the lamellar body-enriched fractions from neonatal mice. The enzymic fraction is enriched in certain hydrolytic enzymes like acid phosphatase, carboxypeptidase, cathepsin B, acid lipase, sphingomyelinase and phospholipase A. This fraction was surprisingly devoid of all sulphatases, β-glucuronidases and the non-lysosomal protease plasminogen activator, glycosidases are however, present (Wertz et al., 1989; Nemanic et al., 1983).

Several features of this model are still speculative, as functions of some enzymes like proteases and acid phosphatases has not yet been investigated. Chapman et al. (1989) has shown that an acidic environment results both from the deposition of LB contents, and from the insertion of proton pumps in the plasma membrane in association with LB exocytosis. This could lead to protease activation, which can further cause conversion of proenzymes to active forms of LB derived hydrolases, leading to the

composition and structural changes known to occur in the intercellular spaces of the lower SC (Elias et al., 1988).

Lamellar bodies are rich in phospholipids, free sterols and glycosphingolipids, but not in other neutral lipids or ceramides (Grayson et al., 1985; Frienkel et al., 1985; Wertz et al., 1985; Williams et al., 1987).

In human SC lipids, the free or esterified fatty acids have chain length varying from 14-24 carbon atoms (Prottey, 1976). Amongst them C_{16} and C_{18} chains predominate. In EFAD conditions, emulsions rich in linoleic acid restored a distribution of fatty acids, identical to that in normal skin (Elias et al., 1980). Here, there is an omission of certain polyunsaturated fatty acids (n-6 series) from the diet of weaning rodents, that leads to abnormalities in keratinization, characterized by epithelial hyperplasia and scaling dermatoses (Brod, 1991). Oleic acid is formed in place of linoleic acid. This has given rise to the suggestion that linoleic acid may play a direct role in barrier function by incorporating two unique sphingolipids viz. acylglucosylceramides and acylceramides.

Epidermal ceramides contain sphingosine or phytosphingosine bases in amide linkage with non-hydroxy, α-hydroxy or ω-hydroxyacids. In the ceramides that contain ω-hydroxyacids, the ω-hydroxyl groups are esterified with fatty acids, predominantly linoleic, to form unusual O-acylceramides (Downing et al., 1987). These O-acylceramides are thought to serve a specialized function of major importance in maintaining the multi-bilayer organization of the barrier lipids (Abraham et al., 1985). This molecule prefers an extended configuration in which the ω-hydroxacyl chain extends all the way through a typical bilayer and linoleoyl

chain is free to insert into a second closely apposed bilayer. This sort of interaction can account for the observed stacking of the extracellular bilayers of SC (Downing et al., 1989).

Glycolipids are also known to mediate cell adhesion of animal cells (Huang, 1978).

Cholesterol sulfate also comprises of 2-3% of the total epidermal lipids (Lampe et al., 1983; Cox and Squier, 1986; Epstein et al., 1984; Long et al., 1985), and it plays an important role in corneocyte cohesion - desquamation phenomenon. This assertion is based on the abnormal degradation of cholesterol sulfate to cholesterol, resulting from a genetic lack of steroid sulfatase in recessive X-linked ichthyosis (RXLI) (Shapiro et al., 1978; Epstein et al., 1981; Williams and Elias, 1981). The failure of desulfation results in accumulation of cholesterol sulfate (10 times more) and lack of cholesterol leads to hyperkeratosis in these patients due to corneocyte cohesion.

Finally, the invariable occurence of significant quantities (2-5%) of n-alkanes in the SC is noteworthy in light of the importance of these substances for barrier function in plants and insect integument (Hadley, 1980). Large quantities of n-alkanes are found in untreated ichthyotic scale, suggesting an endogenous origin of this compound (Williams and Elias, 1982). Whether n-alkanes are incidental coproducts of epidermal lipid metabolism, or subserve a specific function in skin still remains to be seen.

To summarize, all the above mentioned lamellar contents are discharged into the intercellular space at SC-SG interface and is acted upon by an array of hydrolytic enzymes, where the more polar lipid species are converted to nonpolar species. During this transition, following products are formed (Elias, 1989).

$$\text{Glycolipids} \xrightarrow{\text{glycosidases}} \text{Ceramides}$$

$$\text{Cholesterol sulfate} \xrightarrow{\text{steroid sulfatase}} \text{Cholesterol}$$

$$\text{Phospholipids} \xrightarrow{\text{phospholipases}} \text{Diglycerides + FFA}$$

$$\text{Triglycerides} \xrightarrow{\text{acid lipases}} \text{FFA}$$

These lipids then contribute to the formation of broad membrane bilayers by membrane-fusion process (Landmann, 1986).

Apart from lipids, calcium is known to have a regulatory activity in the proliferation and differentiation of epidermis. Calcium acts as an intracellular second messenger and conducts multifarious functions (Carafoli and Penniston, 1985). Hennings (1980), has shown that the proliferation of epidermal cells in culture can be accelerated by reducing the level of this cation in the medium. Low concentration also prevents stratification of the cells by accelerating detachment of the corneocytes. Increased calcium levels induce terminal differentiation of mammalian keratinocytes (Hennings et al., 1980). Calcium influx triggers several terminal events related to cornification (Elias et al., 1988; Hennings et al., 1981). Translocation of this cation from intercellular and organelle reservoirs to cytosol (Menon et al., 1985) may regulate epidermal differentiation. Thus sequestration of

Ca^{+2} to specific cellular compartments is tightly regulated.

Calmodulin is the major binding protein of calcium and modulates many of the effects of calcium. Calcium-calmodulin complexes are involved in the release of arachidonic acid. This arachidonic acid is then metabolized to inflammatory mediators, the eicosanoids. Phospholipase A_2 is activated by calcium and this activity is mediated through calmodulin (Fairley, 1988). Calmodulin (Tucker et al., 1984; Fairley et al., 1985), calcium (Menon and Elias, 1990) and phospholipase A_2 have been shown to be elevated in psoriatic skin. This evidence taken together suggests that the interaction of calcium, its binding proteins and arachidonic acid metabolites may be of great importance in hyperproliferative states.

During keratinization, all the above mentioned processes have to be intricately regulated. Any anomaly during this normal course of differentiation will lead to abnormal functioning of the epidermis. Most of the skin disorders, and adaptation of the animal to TEWL is reflected in epidermal morphology and functions (Matoltsy, 1986). Thus epidermis was chosen as the subject of present investigation.

In this project, an attempt is made to study the role of epidermal lipids derived from LBs in adaptation of the animal to various climatic conditions. The effect of glycerol, a skin conditioner and Brij 99 (used for making liquid crystalline creams), on the LB secretion and the mortar lipids was also carried out. The in vivo route of permeation of Brij 99 as a vehicle was observed using ultrastructural studies. In vitro permeation of water in epidermis using tritiated water was carried out by constructing permeability cells. Abnormality in epidermal proliferation and scaling caused

due to an anomaly in cohesion of corneocytes was studied in psoriatic skin. Ultrastructural observations on LB secretion, intercellular lipids, desmosomes and organelle degradation during abnormal process of differentiation in psoriatic epidermis was also done. The details of these investigations are individually discussed in respective chapters.

Epidermal lipids help the animal to adapt itself to its natural habitat. Peculiar epidermal adaptation is seen in marine mammals (cetaceans). They need a functional barrier, as the sea water is hypertonic and they also have to retain all water derived from metabolic sources. Cetacean epidermis is a 'lipokeratinocyte'. It generates both intercellular and intracellular lipids (Elias et al., 1987; Menon et al., 1986). Intercellular lipids provide not only a barrier against water loss and salt ingress, but they also probably contribute to streamlining, since sphingolipids remain glycosylated at all levels of marine mammal SC providing a slippery outer surface (Menon et al., 1986). Intracellular lipid droplets help in generation of calories (as external water temperature is very low) and metabolic water.

Thicker skin, sparse hair, density of sweat and sebaceous glands are shown to contribute towards water loss in the skin of buffalo. The epidermis of the buffalo is papillomatous and supplied with blood capillaries (Badreldin and Ghany, 1954). In camel the cuboidal cells of sweat glands have microvilli that could help minimize water loss, through sweating (Dougbag, 1987). This instigated the exploitation of buffalo skin for the current study, as these animals show typical adaptation to wallowing habit (Chapter 6).

On the other hand, avian plumage helps in maintaining the body temperature of the animal by providing a layer of insulation, with a trapped layer of humid air below. It also provides protection to the bird. The presence of plumage seems to alleviate the need for 'bricks', since avian corneocytes are much more attenuated than their mammalian counterparts. This gives rise to a scaly appearance of the skin and thus instead, a straw-mortar model is suggested for birds (Menon et al., 1986).

Aves in general have higher TEWL than mammals. This is correlated to the fact that avians also form LBs like the mammalian epidermis, but the contents of these organelles are converted to neutral lipid (NL) droplets prior to secretion in the SC intercellular spaces (Menon et al., 1986). Secretion occurs instead under conditions of xeric stress as observed in zebra finches (xeric species) (Elias et al., 1987), where these lipids fortify the barrier. These intercellular lipids may not be required for barrier function, but may participate in the SC's antimicrobial activity and possibly in imparting emolliency to the plumage and SC (Menon, 1984).

Avian skin, especially the pigeon skin, due to its scaly nature can be taken as a model for dry and scaly skin conditions (Lachyanker, 1987). Thus, in the present study pigeon apterial skin was chosen to study the effect of 'glycerol', a skin conditioner with regards to the LB secretion and its consequent effect on permeability barrier (Chapter 3).

Water is known to be a good plasticizer for SC (Blank, 1952). Application of skin moisturizers increase the amount of water content in the SC (Wu et al., 1983). Glycerol is commonly used as a skin conditioner. Topical

application increases skin levels of glycerol. At 80% or more concentrations glycerol is as effective as petrolatum (Bisett and McBride, 1984).

Friberg and Osborne (1985) proposed that maintaining the lipids in liquid crystalline state is required for optimal barrier function in preventing water loss. In a dry atmosphere glycerol acts as a skin moisturizer by inhibiting the transition of SC lipids from liquid to solid crystals, rather than by acting as a humectant (Froebe et al., 1990).

Decades ago, the water binding capacity of the SC was put forward by Middleton (1968). Imokawa and Hattori (1985), later proposed the role of structural lipids, in water holding capacity of the SC. The lamellar structure serves as a water modulator in the SC (Imokawa et al., 1986, 1989, 1991). This evidence is based on the fact that ceramides, the major components of SC lipids are main determinants for water holding capacity and permeability barrier. Ceramides with relatively short, unbranched and saturated alkyl chain length are mainly associated with the water-holding function. On the other hand, acylceramides with linoleic acid, or ceramides with long alkyl chain length serve as a permeability barrier (Imokawa et al., 1989).

With experiments on water permeation of reaggregated SC with model lipids, Friberg et al. (1990) have supported earlier hypothesis, that the lipid barrier to water penetration of the SC is determined by the structural organization of the lipids, and not by the exact chemical structure of the individual species. Thus, in vitro permeation of water through the epidermal sheets is conducted in the present study by constructing permea-

bility cells (Chapter 5), to check if this device could be used to study in vitro water permeation in the epidermis.

Skin from various body sites has different permeability properties (Scott et al., 1991) and could be related to interspecies differences in skin structure. Scientists argue that, the excellent barrier properties of the skin are 'frustrating' and need to be overcome to develop transdermal penetration of drugs, as a route of entry. Accordingly, therapeutic variable can be divided into three categories, which are interdependent: viz. pharmaceutical factors, biological behaviour of the drug in the given vehicle and treatment modalities. Without considering the different interpretations about mechanisms acting on skin permeability, it is well established that the main barrier is constituted by the SC, which also acts as a 'reservoir' for compounds topically applied (Rougier, 1987).

It is possible that some molecules will be bound to sites in the skin (Foreman and Clanachan, 1984). The NL sequestered in the SC interstices, forms the basic pathway for percutaneous transport of topically applied lipid soluble substances (Nemanic and Elias, 1980; Loth, 1991) by spreading and by capillary action. The water molecules can hydrate the polar head groups of the lipids forming thinner or thicker films between the lipid bilayer. Polar penetration enhancers also interact with the polar head groups of the lipids influencing their hydration and the relative order of molecular packing (Loth, 1991). Lipid penetration enhancers intercalate into the non-polar region of the bilayers by disturbing their molecular packing.

Liposomes (Mezei, 1987; Schäfer-Korting et al., 1989), steroids (Ebling and and Skinner, 1983), fatty acids as penetration enhancers (Golden et al., 1987), liquid crystalline creams (Junginger et al., 1987) and low density lipoproteins have been proposed as drug carriers (Vermeer, 1987).

The use of liquid crystalline creams, may be a good vehicle for transdermal drug penetration and prove to be 'skin friendly' in such a way that no skin irritation is normally caused due to unwanted occlusive effects. Liquid crystalline creams have been prepared, consisting of a mixture of Brij 96 [polyoxyethylene (10)-oleyl ether, a non-ionic surfactant] and water (Junginger et al., 1987). Chapter 4 deals with the investigation of the route of Brij 99, when applied topically. Effect of this vehicle on the structure of intercellular lipids and the LBs has also been analysed.

Psoriasis is a multifactorial disorder. The genetic defect, present at birth, is expressed alomst exclusively in the skin and has an expressed and a non-expressed phenotype. It requires additional factors to be manifested as lesional psoriasis. The expression of disease in a psoriatic subject at any given time is a composite of the basic defect, that gives rise to the excess epidermal proliferation seen in all skin of psoriatics and the subsequent mediator-regulated events that induce the lesions or the lesions induce (Krueger and Jorgensen, 1990).

As a consequence of the genetic defect, even in non-lesional psoriatic skin, a number of biochemical and histochemical abnormalities, such as capillary changes, increased levels of phospholipase A_2, plasminogen activator,

protein kinase C and increased mitotic indices of basal keratinocytes (Farber et al., 1987) are observed. Malfunctioning of a parakeratotic horny layer in dermatoses such as psoriasis and eczema, is demonstrated by increased TEWL and higher drug concentration.

Abnormal epidermal morphology (scaliness, dry skin) and functions (defective permeability barrier) are attributed to lipid abnormalities within the 'brick and mortar' organization of SC. In mammals with abnormal LBs, in deficiency conditions or hyperproliferative state, paucity of lipases in SC interstices correlate with visible scaliness. Thus, imposed functional demands modify the barrier through altered lipogenesis or lipid modulations for there is a common basis involving LB for structural and functional abnormalities in SC. These aspects have been studied in X-linked ichthyosis (Williams and Elias, 1981), atopic dermatitis (Werner et al., 1987) and in EFAD epidermis (Elias et al., 1980; Hou et al., 1989). Thus, this study was taken up to check if there is any anomaly in the secretion of the LB contents during differentiation of keratinocytes in the involved epidermis of the psoriatic patients (Chapter 1).

Development of animal models in the field of dermatology has always remained a challenge. Models are still in their infancy and not much is known, if the cadaver skin or non-human materials are adequate for screening, the fundamental physical chemistry and the effects of drug permeation enhancers, promoters or occlusive agents. Lack of animal models have been an hindrance in studying the pathological mechanisms underlying certain skin disorders, in percutaneous absorption studies and also in the field of dermatopharmacology. Though, a number of models

have been proposed and developed for various disorders, there still remains a big void in this area of research.

EFAD skin (Lowe and Stoughton, 1977), rat tail skin (Bladon et al., 1986; Wrench and Britten, 1975) and in vitro "skin equivalents" of the psoriatic tissue (Krueger and Jorgensen, 1990) are developed as experimental models for psoriasis. Though light microscopic histological evidences, show similarities in the parakeratotic areas of rat tail skin and psoriatic skin (Bladon et al., 1986), the histochemical/biochemical similarities, if any, are not yet explored. In present study therefore, a histochemical study of calcium, lipids and nucleic acid distribution in rat tail skin and the psoriatic skin is carried out (Chapter 2).

The study is aimed at expanding our current knowledge and gain new insights into the role of LBs in adaptation to climatic conditions, in skin disorders and also during vehicular permeation. Development of appropriate animal models for various skin dysfunctions, and more effective ways of barrier perturbation would go a long way in developing absorption promoters and more effective drug delivery.