CHAPTER - 1

PSORIASIS : A DEVELOPMENTAL PROBLEM
ABNORMAL LAMELLAR BODY AND CORNEOCYTE COHESION IN THE HYPERPROLIFERATIVE SKIN

Psoriasis is an inflammatory, hyperproliferative, human skin disease characterized both by a significant increase in the rate of cell division and a substantial (eight fold) decrease in the transit time of the differentiating keratinocyte through the viable compartment of the epidermis (Weinstein et al., 1983). This disease begins as an erruption of small macules and papules. The lesions are rich red or pink and covered with fine, dry silvery-white scales. As the lesions increase in size, they become more scaly and may coalesce with others forming irregular plaques with polycyclic borders. Lesions are well defined with sharply delineated edges, which are raised from the adjoining skin and are easily palpable. The eruption is usually symmetrical (Atlas of Chronic Dermatoses, 1985). Moreover, the differentiation of keratinocytes here is characterized by parakeratosis rather than orthokeratosis (Brody, 1962).

Psoriasis is a common chronic disease with complex heredity and an as yet unknown aetiology. One or more primary and genetically determined defects (autosomal inherited dermatoses) may be present. The lesions may possibly be elicited and/or exacerbated by trauma, infection, endocrine, metabolic and climatic factors. Emotional stress, excessive intake of alcohol and some drugs (antimalarials) are known to aggravate psoriasis (Atlas of Chronic Dermatoses, 1985).

The basis for this skin disorder is usually attributed to defects in :

1. The synthesis of keratin polypeptides at various layers of epidermis.
2. The rate of cell transit through the epidermis.

3. Synthesis of nonfibrous protein (keratohyalin or filaggrin) which accumulates in stratum granulosum (SG).

4. Biochemical / metabolic pathways.

5. Degradation of all intracellular organelles.

6. Normal cohesion and dyshesion of corneocytes that would lead to altered desquamation.

Biochemical studies related to transmembranous signal transducing systems have revealed a number of defects in psoriatic skin. These include, reduced cAMP cascade activity (Voorhees and Duell, 1971), high lesional cGMP levels (Voorhees et al., 1973), increased calmodulin levels (Kerkhof and Pej, 1983 and Tucker et al., 1985), increased Ca^{2+} levels in suprabasal cell layers and a drastic alteration in the calcium gradient within the lesional but not the uninvolved epidermis (Menon and Elias, 1990), increased phospholipase C activity (Bartel et al., 1987), increased tyrosine kinase activity (Gentleman et al., 1984) and increased epidermal growth factor binding in the upper layer of involved epidermis (Nanney et al., 1986). The role of phospholipases, kinases and eicosanoids as molecular mediators in psoriasis is also been studied (Kragballe et al., 1987).

In the process of keratinization the cells move up in the SC, during which the cohesive forces are reduced due to desmosome degradation and lipid modifications with the ultimate dyshesion and sloughing of individual cells (Bowser and White, 1985). The importance of corneosomes and desmosomes in normal desquamation has been recently described (Chapman and Walsh, 1990). Abnormally high number of desmosomes in psoriatic epidermis could

lead to the excessive cohesion of corneocytes and thus the scaling of skin (Skerrow and Skerrow, 1990; Skerrow et al., 1988).

The crucial role of intercellular lamellar lipids of the corneocytes in maintaining the epidermal barrier to water loss has been well accepted by now. Removal of these lipids by surfactants and organic solvents induce dry skin formation, characterized by decrease in water content (Blank and Shappirio, 1955). The role of lamellar body (LB) secretion in maintaining the epidermal barrier has been extensively studied by Landmann (1986, 1988) (discussed in detail in the chapter of Introduction). The extruded contents of the LBs fill the inercellular domains of lower SC as disk like structures. The transformation of these disks into broad sheets towards the outer SC is modulated by an array of hydrolytic enzymes including lipases. The concommitant action of lipases on the lipid bilayers and proteases on desmosomal components may be the basis for normal desquamation and dyshesion of the corneocytes. The barrier properties of the SC depends on the state of cohesion between the cells and upon the organization of intercellular lipid species. Thus, any abberation in the secretion or maturation of the LBs could lead to abnormal cohesion and barrier properties of SC.

A defect in the LBs has been observed by different group of workers in EFAD (essential fatty acid deficiency) conditions (Wertz et al., 1983; Hou et al., 1989). A direct role for linoleic acid, an essential fatty acid in epidermal barrier function has been proposed by Elias (1980). The importance of free fatty acids and fatty acids of triacylglycerols (TAG) in the differentiating corneocytes and a defect in the epidermis, present a

new evidence that the abnormality of lipid metabolism can influence the process of desquamation in SC (Nicollier et al., 1986). There is an increased cholesterol sulfate content in the SC of X-linked ichthyosis (Williams and Elias, 1981). Brody (1962) has reported the presence of vacuoles, with excess of neutral lipids, particularly TAG in psoriasis.

A disturbance in the maturation of LBs is also seen in 'dry', noneczematous skin of patients with atopic dermatitis (Werner et al., 1987). This study was thus conducted to ultrastructurally investigate the alterations in the intercellular SC lamellae and the lamellar body assembly and structure, due to increased cell turnover in psoriatic lesions, which is responsible for abnormal SC lipid composition. Histochemical and electron microscopic techniques were used to compare the lipid profile in the involved vs. uninvolved epidermis of the psoriatic epidermis, in the psoriatic patients.

MATERIALS AND METHODS

Tissues : Fresh biopsy samples of psoriatic lesions from patients undergoing no previous treatment, uninvolved skin from patients, as well as normal skin from volumteers were obtained with known consent (Courtesy of Dermatology Department, Medical College, Baroda). Tissue samples were immediately frozen in a cryostat (-28°C) for light microscopic histochemical studies, while appropriate fixation technique was carried out for samples used for electron microscopic studies.

Light Microscopy : Frozen sections 10-15 μ were cut and rinsed in 50% alcohol. Sections were stained with Fat red 7B (saturated solution in 70% ethanol), for 10-15 minutes. Sections were washed in water and mounted

in glycerine jelly, and then observed under light microscope (Pearse, 1968). Neutral lipids stain pinkish-red.

1 μ sections were cut on Sorvall microtome and stained with toluidine blue and observed under light microscope.

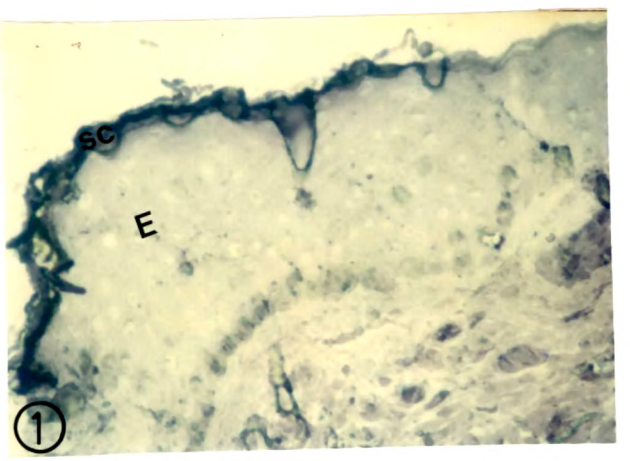
Electron Microscopy : Tissue samples were immersed in cold glutaraldehyde-paraformaldehyde fixative. Most of the dermis was removed with a surgical blade, and the epidermis was minced finely (< 0.5 mm), while immersed in the fixative and kept at room temperature for one hour. Samples were then washed repeatedly with 0.1 M cacodylate buffer (pH 7.2) and post fixed in ruthenium tetroxide (0.5% containing 1% potassium ferrocyanide) at pH 7.1 for 1 hour at 4°C (Pelttari, 1979). Tissues were routinely dehydrated with ethanol series and embedded in Spurr's low viscosity resin (Spurr, 1969). Ultrathin sections were viewed under transmission electron microscope (TEM) (Jeol SX, operated at 80 KV) after double staining with uranyl acetate and lead citrate.

RESULTS

The histochemical distribution pattern of neutral lipids in the uninvolved psoriatic epidermis resembled the normal human epidermis, showing predominance of neutral lipid in the SC interstices, and phospholipids in the viable epidermis (Fig. 3). However, in the involved lesional psoriatic epidermis, the scale forming corneocytes showed intracellular retention of neutral lipid droplets, while the intercellular spaces stained much less intensely (Fig. 4). Comparable results are observed ultrastructurally, wherein the corneocytes show retention of nuclear material, neutral lipid

Fig. 1 : Histological section of uninvolved psoriatic skin, showing a normal structure of epidermis. X 315.

Fig. 2 : Histological section of psoriatic involved skin, showing rete pegs, increased thickness of corneal and viable layer, and an absence of granular layer. X 200.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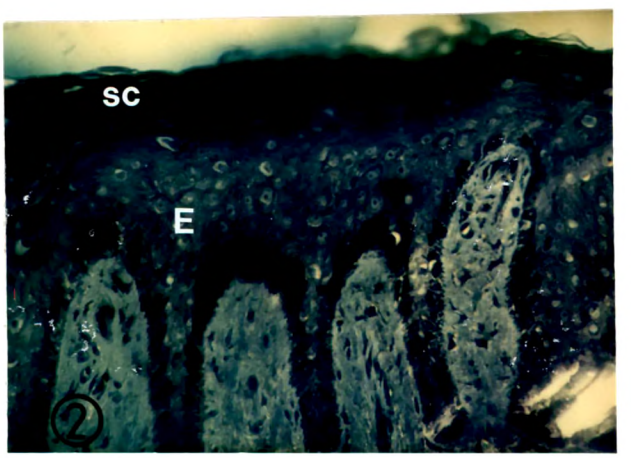
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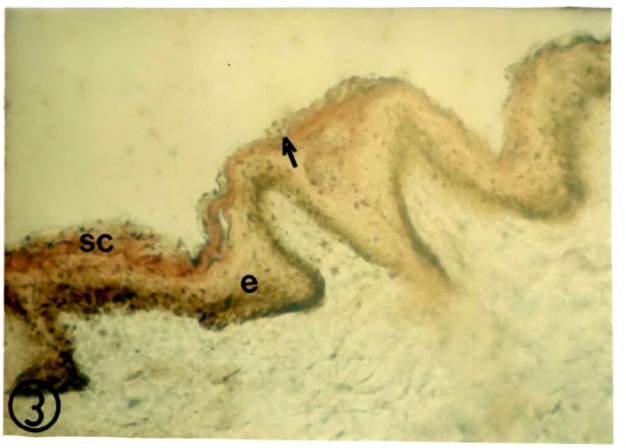
Fig. 3 : Histological localization of neutral lipids in uninvolved psoriatic skin, showing neutral lipid (arrow) staining in the stratum corneum, with negligible staining in the lower epidermis. X 250.

Fig. 4 : Histochemical localization of neutral lipids in involved psoriatic scale showing intracellular localization of neutral lipid droplets. Lipid staining is negligible in intercellular spaces. X 80.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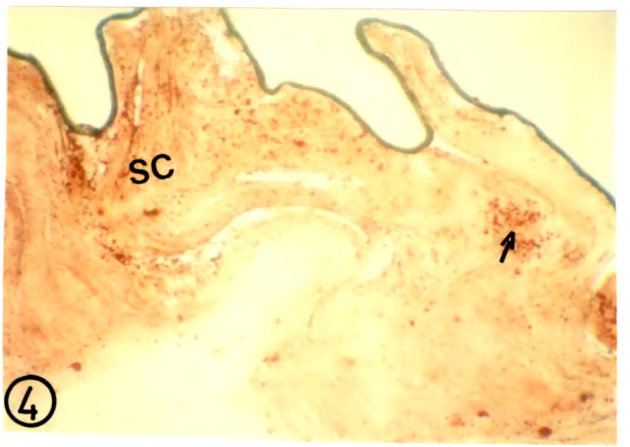
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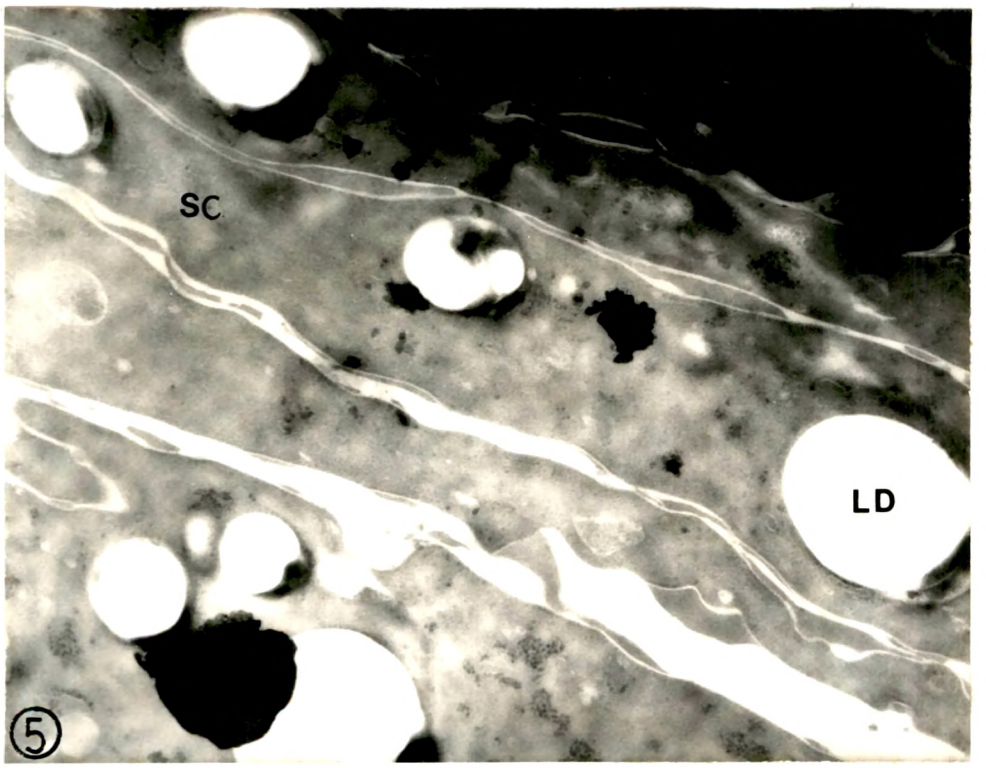
Fig. 5-11 : Electron micrographs of involved and uninvolved psoriatic epidermis.

Fig. 5 : Corneocytes from psoriatic scale showing retention of neutral lipid droplets. X 63,000.

Fig. 6 : Corneocyte from psoriatic plaque, showing intracellular retention of lamellar bodies with a typical lamellated pattern (arrow). X 80,000.



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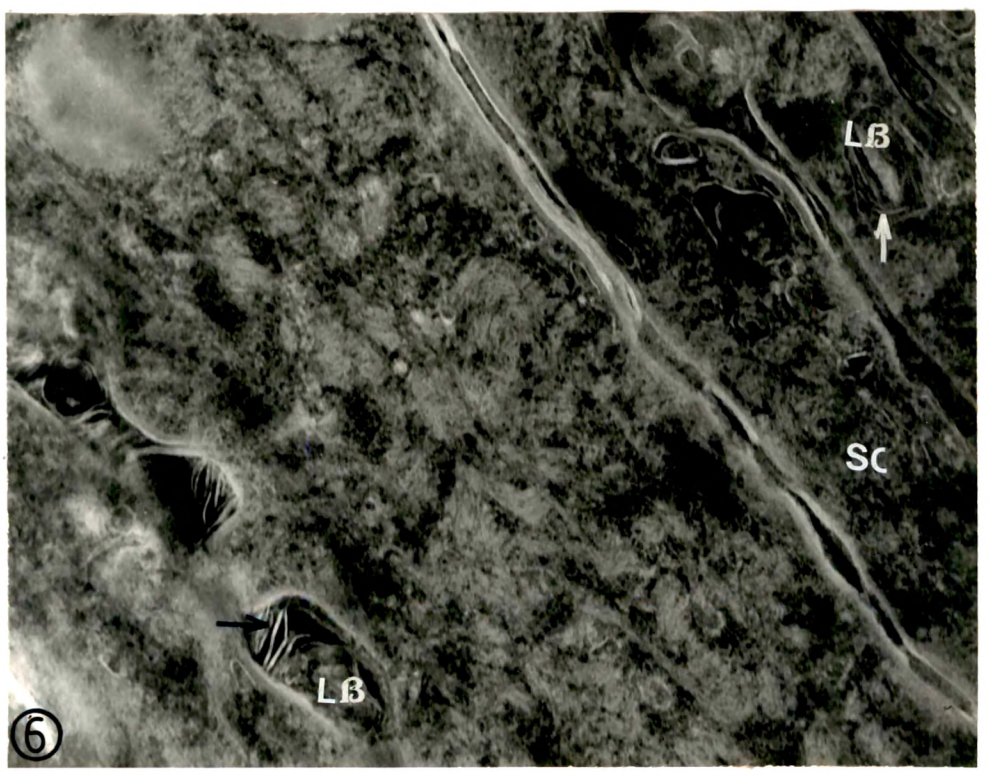

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droplets (Brody, 1962) and also lamellar bodies having typical lamellated structure (Fig. 5,6). Abnormal retention of desmosomes is also seen in the psoriatic scale as observed earlier (Skerrow et al., 1988).

In this study, ultrastructural observations using ruthenium tetroxide (RuO_4), for the first time revealed, an anomaly in the structure of all the LBs in the upper transitional layers. These abnormal LBs are heterogenous in their morphology, some show clefts between the lamellae (Fig. 7), with empty appearing spaces, while others display whorl-like structures within an electron lucent core. Uninvolved epidermis showed a normal lamellar structure of the LBs in the granular layer (Fig. 9).

The normal human epidermis and the uninvolved epidermis, showed normal structure and pattern of exocytosis of the LBs at the SC-SG (stratum corneum stratum granulosum) interface (Fig. 8). These LB derived disks form broad bilayered sheets in the interstices of the lower 2-3 layers of SC (Fig. 10), as reported earlier (Landmann, 1986).

The psoriatic epidermis on the other hand observed, a strikingly abnormal pattern of arrangement of lipid bilayers in the SC intercellular spaces, in contrast to normal and uninvolved human epidermis. The secreted LB contents form bilayers within the SC extracellular domains - but are abnormal in containing large lacunae in between (Fig. 11). The bilayers do not show a compact arrangement, as seen in normal skin. These bilayers are rather seen to be disarranged in whorls and broken up in many loci. In the extracellular spaces, the incomplete processing of LB derived disks, leads to abnormal bilayers structures that do not completely fill the intercellular domains, by formation of continuous broad bilayered sheets.

Fig. 7 : Psoriatic epidermis with structural abnormality of the lipid lamellae in virtually all the lamellar bodies, in the upper transitional layer. Note the lamellar bodies having empty spaces, while some display whorl-like structures with an electron lucent core. X 75,600.

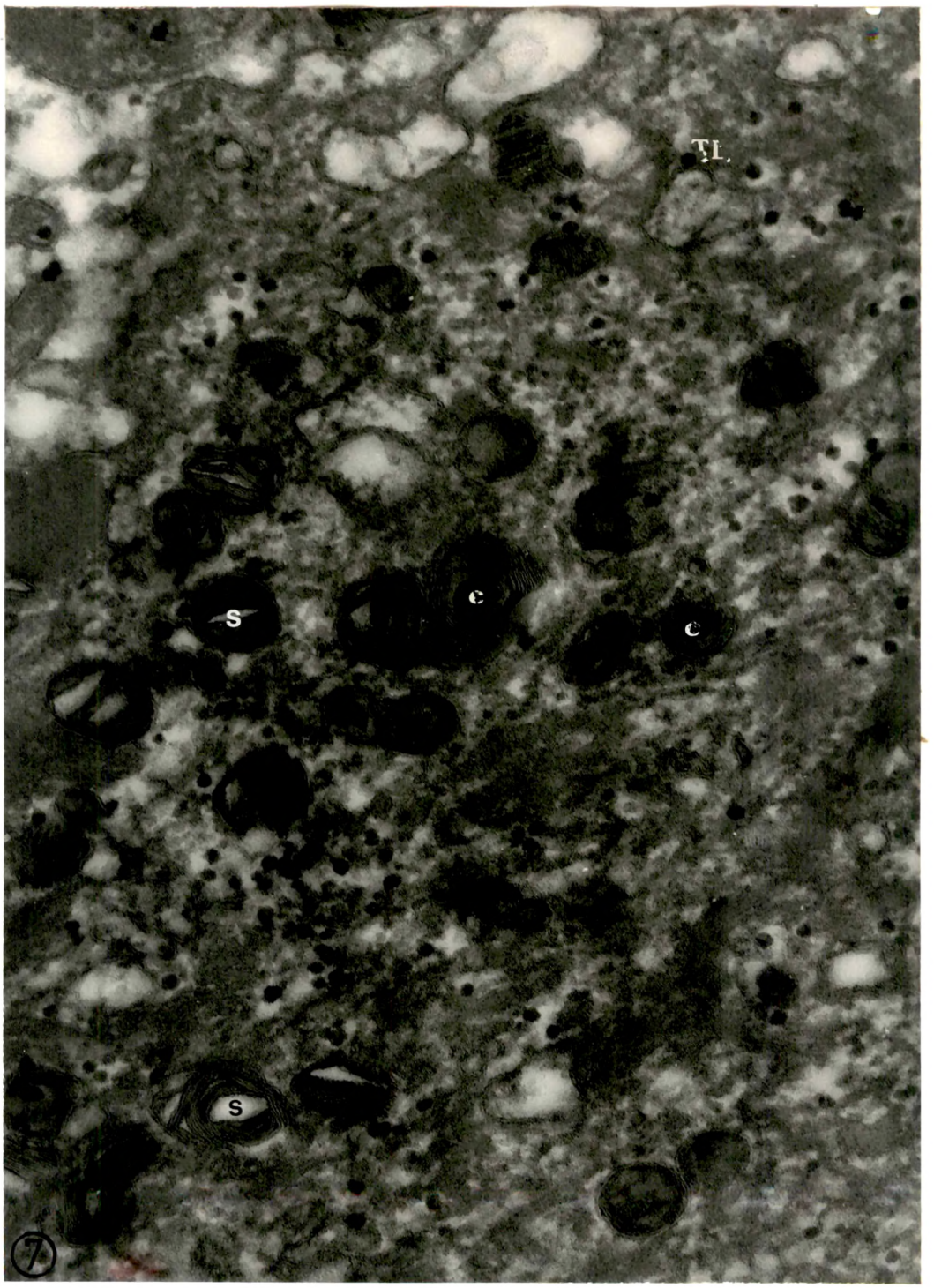
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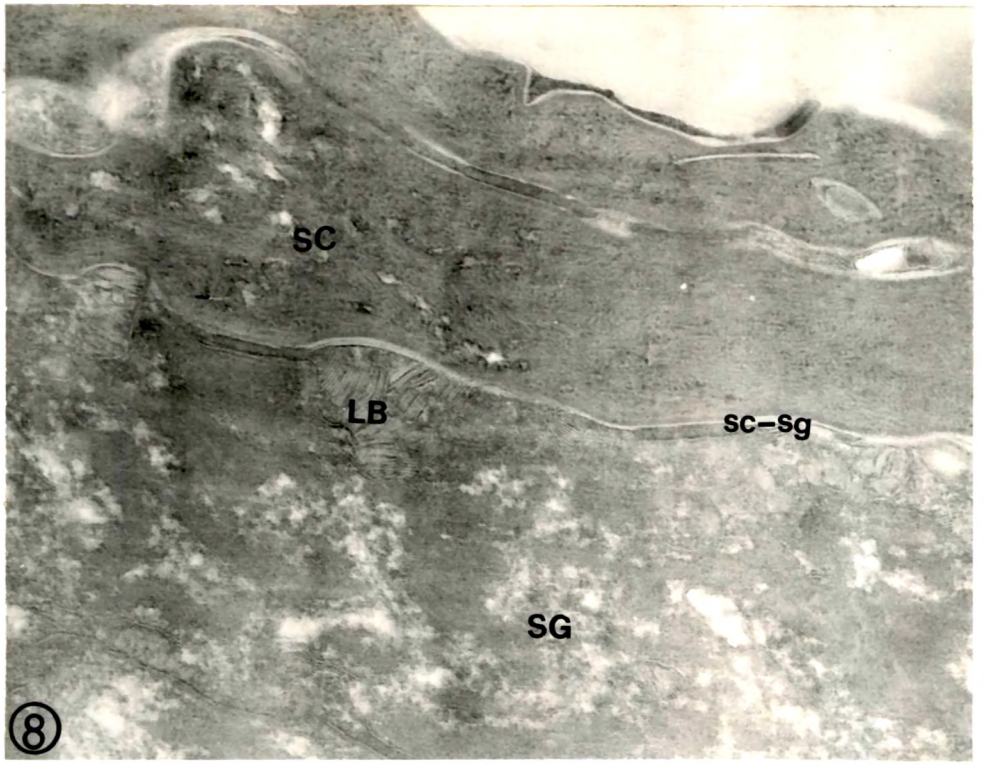
Fig. 8 : Normal human epidermis, showing a normal pattern of structure and extrusion or lamellar body contents into the stratum corneum - stratum granulosum interface. X 1,26,000.

Fig. 9 : Uninvolved psoriatic epidermis displaying a normal structural pattern of lipid lamellae in the lamellar bodies, in the transitional layers. X 50,000.



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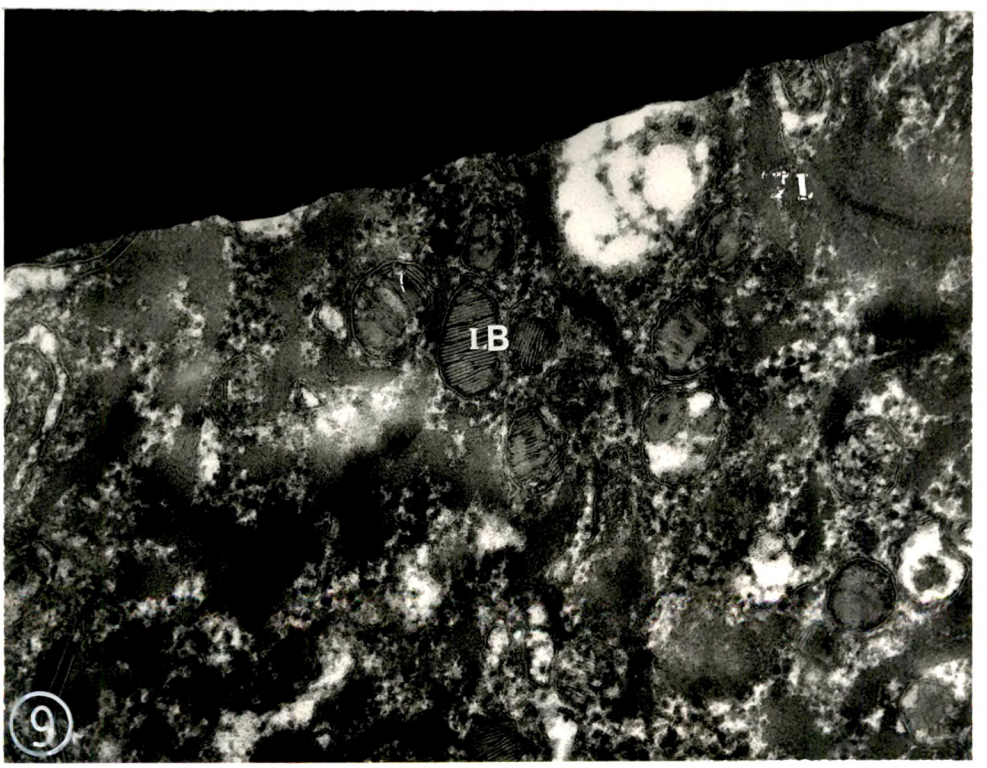

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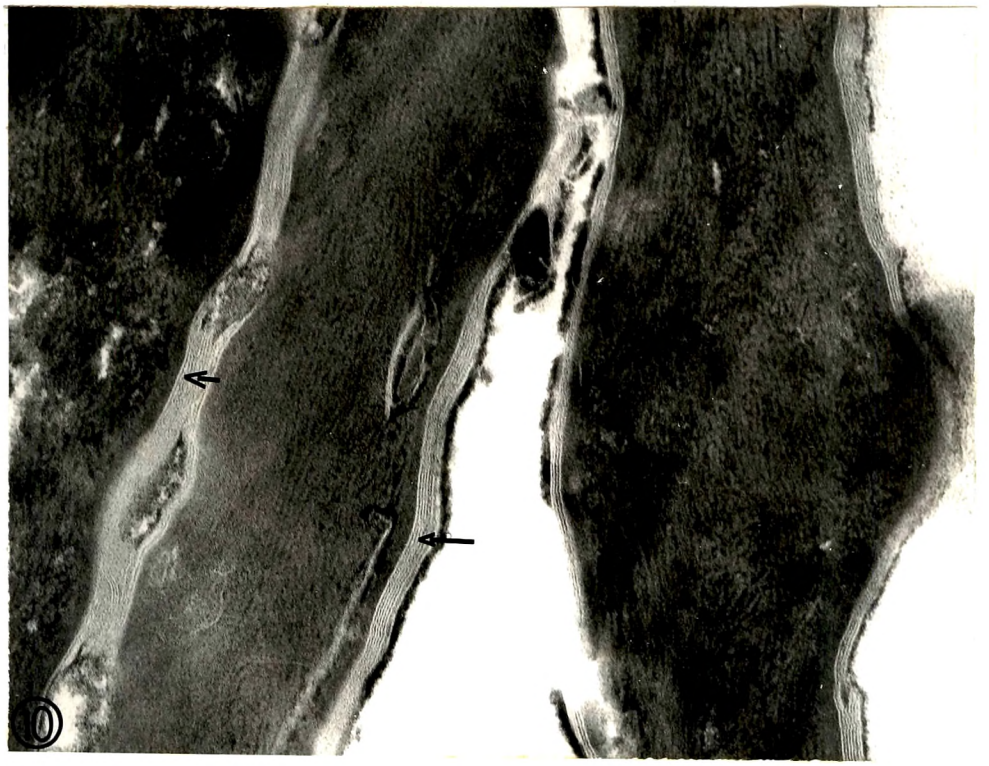
Fig. 10 : Uninvolved psoriatic corneocytes showing a normal number and arrangement of bilayered lipid sheets in the stratum corneum interstices (arrow). X 63,000.

Fig. 11 : A view of psoriatic (involved) corneocytes, showing abnormal number of lipid bilayers in the extracellular spaces (arrow). Note that these bilayers are disarranged in whorls and contain large lacunae in between (*). X 80,000.

Abbreviations :

Lamellar bodies - LB; Stratum corneum - SC; Corneocytes - C; Transitional layer - TL; Neutral lipid - NL; Empty space - S; Electron lucent core in lamellar body - **c**; Stratum corneum - stratum granulosum interface - sc-sg; Viable layers of epidermis - E; Granular layer - G.



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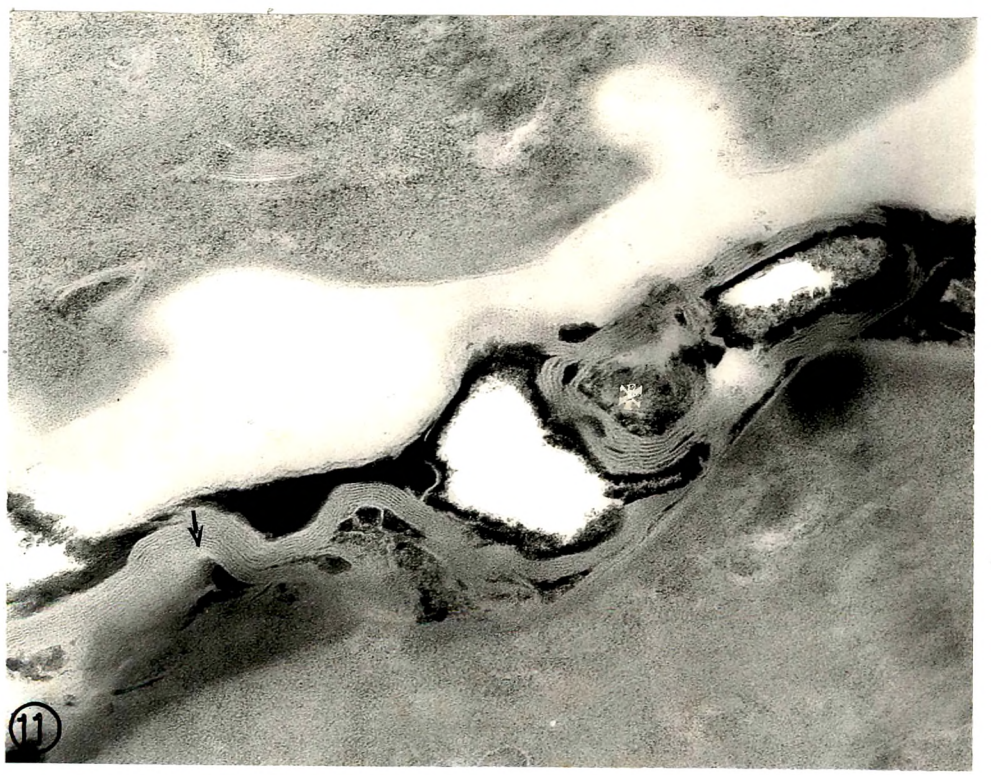

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DISCUSSION

Understanding of the basic biology of LBs and how they influence desquamation is important because defective desquamation is a feature of many dermatologic conditions. The LB derived SC lipids principally consists of ceramides, cholesterol, cholesterol sulfate and free fatty acids, which are responsible for epidermal water barrier. Essential fatty acid deficiency (EFAD), leads to scaly skin conditions and also increases transepidermal water loss (TEWL). In EFAD diet, linoleic acid is replaced by oleic acid, which could be one of the reasons why the disk like structure disappears in the LBs of the treated animals (Wertz et al., 1983).

The relative volume of LBs was found to be significantly greater in the patients with atopic dermatitis (Werner et al., 1987). In this study, an abnormal differentiation and maturation of LBs is seen in the lesional epidermis. This and abnormal arrangement of the lamellae in the LB could be due an insufficient time period for the transition of the differentiating keratinocyte (Weinstein et al., 1983). This could lead us to conclude that probably a defect in the processing / metabolism of the lipids within the LB could trigger a chain of abberations in the terminal events associated with cornification.

The extruded lamellar contents form multiple intercellular bilayers in the lower SC, which provides efficient water barrier because of the crystalline array of the straight and predominantly saturated lipid chains (Downing et al., 1987). Also, the normal and ordered exfoliation of corneocytes, as they arrive at the SC surface presumably require hydrolysis of cholesterol sulfate to free cholesterol (Downing et al., 1987). Accumulation of cholesterol sulfate in the X-linked ichthyosis leads to scaliness (Williams and

Elias, 1981). Glycolipids are also known to mediate cell adhesion (Huang, 1978). Defect in the assembly of the extruded lamellar contents in EFAD is also attributed to glycolipids (Wertz and Downing, 1982).

These studies support our findings wherein the SC if the involved epidermis shows bilayers in the extracellular domains which remain incompletely processed and thus do not form the basic bilayer units leading to abnormal barrier function. These observations are supported by the current findings (Menon and Elias, 1990), where abnormal profile of calcium ions in the suprabasal layers, retention of this cation in the intercellular domains of the upper corneocytes is associated with failure of LB-derived disks to disperse normally, leading to abnormal adhesion of cells. The accumulated lipids of SC maintain an extensively defective bilayered configuration, compared to normal and uninvolved epidermis. This may be responsible for abnormal cohesion and scaliness of the psoriatic plaques.

Desmosomes in the viable epidermis provide the major mechanism of keratinocyte cohesion. Acid phosphatase, found in the LB and phospholipases probably mediate an attack on the desmosomes (Elias et al., 1988), in the stratum compactum, which may facilitate the subsequent release and degradation leading to sloughing of the keratinocytes (Bowser and White, 1985).

Secondly, an array of enzymes, like several types of lipases, including phospholipases and acid lipases are known to be present within the LBs (Frienkel and Traczyk, 1985; Grayson et al., 1985; Elias et al., 1988), which convert the lamellar polar lipids to more nonpolar species, in the SC

interstices (Elias et al., 1977; Elias, 1981). Histochemical observations have shown increased phospholipids in the SC of lesional epidermis and also neutral lipid droplets within the corneocytes, which further indicates an anomaly in the metabolism of the lamellar lipids. Furthermore, the psoriatic lesion is known to show alterations in the activity of phospholipase A_2 and phospholipase C (Forster et al., 1985; Bartel et al., 1987).

In conclusion, this study indicates that the psoriatic lesions shows a drastic alteration in the structure of lamellar bodies and lipid bilayers in the SC interstices, which could be due to abnormal metabolism of these lipids in the LBs, a probable alteration of the hydrolytic enzymes associated with LB, and also increased number of desmosomes. All these reasons together could possibly explain the barrier defect and abnormal cohesion of corneocytes in the psoriatic scale.