CHAPTER - 2

PARAKERATOSIS OF RAT TAIL SKIN AS A MODEL FOR PSORIASIS : A COMPARATIVE HISTOCHEMICAL STUDY

Lack of animal model systems to study the skin barrier function/dysfunctions for in vitro and in vivo studies of pathological conditions, and for development in the field of topical medication (i.e. effective transepidermal drug delivery) has been a hindrance to the progress in this area. Much of the studies make use of Cadaver skin (Blank, 1952) or Stratum Corneum sheets. With a new understanding about difference in barrier properties of various animal groups, it is now important that specific animal models should be evaluated and developed for specific purpose e.g. the avian skin as a dry skin model (Lachyanker, 1987), diazacholesterol induced ichthyosis in hairless mouse (Elias et al., 1983), domestic pig skin as an animal model of human dry skin (Bisett and McBride, 1983), squamate epidermis in percutaneous absorption studies (Roberts, 1986) and rat tail skin as a model for psoriasis (Bladon et al., 1986; Wrench and Britten, 1975; Spearman and Hardy, 1977). Though a number of models, like induction of hyperplasia by adhesive tape stripping (Hunter and Skerrow, 1981) and normal human buccal epithelium (Harrison and Skerrow, 1982) have been proposed to study psoriasis, no pathological model is yet known. Thus, the 'normal' parakeratotic condition in the rat tail skin remains the only present alternative (Schweizer and Marks, 1977; Bladon et al., 1986) to study psoriasis.

Parakeratosis, a form of abnormal epidermal keratinizing pattern is characterized by an absence of the granular layer in the epidermis. The state of normal differentiation is altered. In pathological skin conditions like psoriasis one of the reasons for parakeratosis could be a substantial increase in the mitosis and decrease in the transit time of the differentiating keratinocyte during cornification (Weinstein et al., 1983).

The normal adult rat tail skin has alternate rings of ortho and parakeratotic stratum corneum. The orthokeratotic interscale region around the hair follicle shows a presence of granular layer and the stratum corneum (SC) is histologically similar to that of normal human epidermis. The parakeratotic, scaly region on the other hand shows an absence or a thin granular layer and also nucleated cells in the SC. These regions are histologically similar to parakeratosis seen in the psoriatic plaques (Bladon et al., 1986).

Jarett (1959) has reported that the parakeratosis sets in by 7th day after birth. Histological changes during the development of this scaly pattern is also investigated/observed. Alternate rings of ortho and parakeratotic regions become apparent by 9th day after birth. Many extrinsic and intrinsic factors like dermal cells, certain hormones and vitamin A etc. help in epidermal modulation (Spearman, 1981).

Hennings (1980) has shown that calcium could regulate the proliferation and differentiation of epidermal cells in culture. A significant accumulation of intercellular Ca^{++} in midgranular layer, coupled with Ca^{++} influx in upper granular layer suggests that changes in intracellular Ca^{++} concentration may may regulate epidermal differentiation. Ca^{++} is also seen to be associated with LB disc membranes and lamellar contents, which further indicates that these cations may contribute to the lamellar body secretion and to the formation of intercorneocyte membrane bilayers (Menon et al., 1985). The parakeratotic, suprabasal layers of the psoriatic skin shows an increase in the concentration of Ca^{++} which indicates a loss of the normal concentration gradient of Ca^{++} that programs terminal differentiation (Menon and Elias,

1990). Calmodulin, (a Ca^{++} binding protein) levels are also high in psoriatic epidermis (Tucker et al., 1984 and Fairley et al., 1985).

Chlorotetracyclin (CTC) a Ca^{++} chelating fluorescent probe, has been used to localize Ca^{++} ions in neural crest cells (Moran, 1983) and in the membranes (Wolniak et al., 1980) using fluorescent microscopy. This technique was therefore used to compare the Ca^{++} distribution in psoriatic skin and parakeratotic region of the rat tail skin.

Lipids are also known to play a very important role in the differentiation and desquamation of the keratinocytes (discussed in detail in Introduction). Any anomaly in the processing of these lipids during keratinization could lead to abnormal differentiation, cohesion and dyshesion of corneocytes (Chapter 1). Nile red, a fluorescent neutral lipid probe, was thus used to obtain the lipid profile in parakeratotic epidermis of psoriatic and rat tail skin and the orthokeratotic epidermis of the tail skin.

There are changes in the pattern of keratins in living layers of epidermis and stratum corneum (Dale and Stern, 1975; Dale et al., 1976; Baden and Lee, 1978; Skerrow and Hunter, 1978; Fuchs and Green, 1980; Skerrow and Skerrow, 1983). Newly synthesized mRNAs encoding both the type I and type II class of keratins seem to be produced in a differentiating cell (Fuchs and Hanukoglu, 1966). Once a cell passes through the spinous layer of the epidermis and enters the stratum granulosum (SG), its biosynthetic activity ceases and hydrolytic processes become highly active (Lavker and Matoltsy, 1970). During differentiation, with the onset of keratin synthesis, there is a loss in the cell's ability to replicate DNA

(Kirscher and Furlong, 1967) and there is an active removal of DNA from the keratinizing cells during maturation. mRNA also codes for the proteins like histidine rich protein (HRP), cysteine rich protein (CRP), proline rich protein (PRP) and arginine rich protein (ARP) of keratohyalin granules, which are dispersed in the interfilamentous spaces (Fukuyama and Epstein, 1986). Their precise function is not yet clear. Thus nuclear reorganization may contribute to selective transcription in different stages of cell maturation. An attempt was thus made to compare the DNA and RNA profile in the psoriatic skin and parakeratotic areas of the tail skin, using acridine orange (Culling, 1974).

These three parameters viz. lipids, calcium and DNA/RNA profiles can give us a preliminary insight into the basic similarities, if any, in rat tail skin and psoriatic skin.

MATERIALS AND METHODS

Tissues were obtained from 4 day, 7 day and 27 day old rat tail skin. A punch biopsy sample was taken from patient having a typical psoriatic lesion. All the skin samples were immediately frozen at -28°C, in a cryostat microtome, embedded in OCT (Tissue tek II) and sectioned at 12 μ. Sections were stained with appropriate probes and observed under fluorescence microscope.

I. **Nile Red** : (Greenspan, 1985)

Stock solution : 1 mg Nile red powder was dissolved in 2 ml acetone and stored in dark at -10°C.

Working solution : 10 µl stock solution, mixed with one ml of 75% glycerol in water.

Staining : Frozen sections of psoriatic and rat tail skin, were stained with Nile red and observed in ultra-violet light, (excitation wavelength, 450-500 nm; emission wavelength, 528 nm) under Carl Zeiss fluorescence microscope.

Only hydrophobic lipids fluoresce with intense golden yellow fluorescence, while as phospholipids show a relative reddish-yellow fluorescence.

II. **Chlorotetracyclin (CTC)** : (Casewell, 1979)

Tissues were immersed in cold fixative containing 2% glutaraldehyde, 2% formaldehyde, 90 mM potassium oxalate and 1.4% sucrose at pH 7.4. Tissues were fixed overnight in this fixative, washed in double distilled water, and treated with 2% potassium pyroantimonate for 2 hours. Tissues were frozen and sections (12 µ) taken in a cryostat microtome. Sections were then exposed to 50 µg/ml, CTC in 2.5% glutaraldehyde. Control slides were treated with EGTA (100 µg/ml), before exposure to CTC.

Slides were washed with double distilled water and observed under Fluorescence microscope equipped with B224 (440 nm) excitation filter and G247 (510 nm) barrier filter. Ca^{++} sites emit bright yellow fluorescence.

III. **Acridine Orange** : (Culling, 1974)

Acridine orange is a metachromatic fluorochrome, which induces specific yellow emission of DNA and flame red emission of RNA at ultraviolet range.

Fresh frozen sections were exposed to 0.1% Acridine orange in 0.1 M sodium phosphate buffer (pH 6.0), differentiated with 10 mM $CaCl_2$ and then washed with 0.1 M sodium phosphate buffer. Sections were observed under fluorescence microscope with B224 (410 nm) excitation and G247 (510 nm) cut off filter.

For Histology :

1 μ sections of tissues embedded in Spurr's, epon-epoxy medium were taken (Chapter 1), stained with toluidine blue and observed under Carl Zeiss light microscope.

RESULTS

Histology :

In rat tail skin, parakeratosis sets in by 7th - 9th day of post natal development. Small depressions in the epidermis during this period is observed. These notches further develop forming the orthokeratotic (normal pattern of keratinization) areas, and the parakeratotic (abnormal pattern of keratinization) area. During this process, a gradual decrease of the granular layer in the parakeratotic (interscale region) areas was seen (Fig. 1,2).

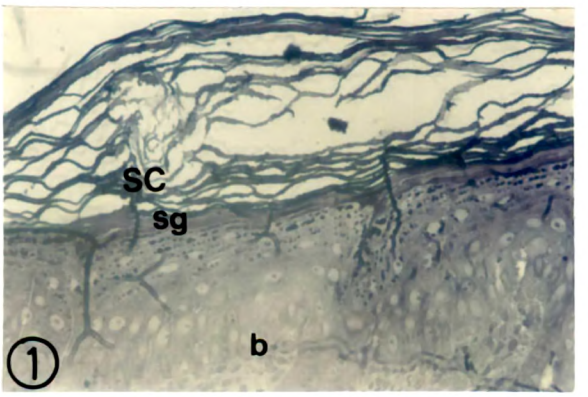
In adult tail skin, the granular layer is either one layer thick or absent, in the parakeratotic areas. Orthokeratotic areas showed a normal pattern of granular layers i.e. about 3-4 layers thick (Fig. 3). Psoriatic epidermis also shows an absence of granular layer, as seen earlier by Brody (1962) (Refer Fig. 2, Chapter 1).

Fig. 1-3 : Histological view of rat tail skin under light microscope.

Fig. 1 : 4 day tail epidermis showing presence of granular layer. X 200.

Fig. 2 : 7 day tail epidermis showing beginning of parakeratosis (arrow) and a reduction of granular layer. X 200.

Fig. 3 : 27 day old (adult) tail epidermis showing orthokeratotic and parakeratotic zone. Parakeratotic layer shows absence of granular layer, while or orthokeratotic zone shows presence of granular layer. X 200.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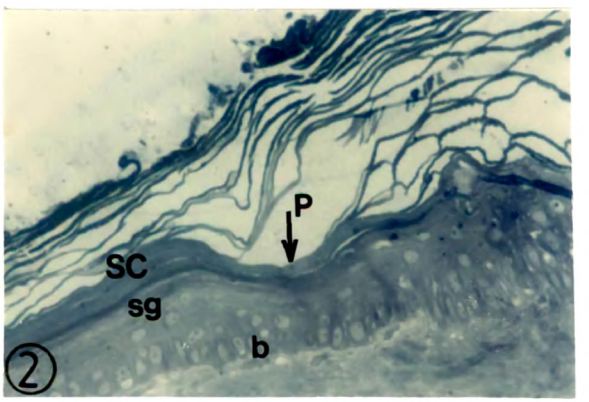
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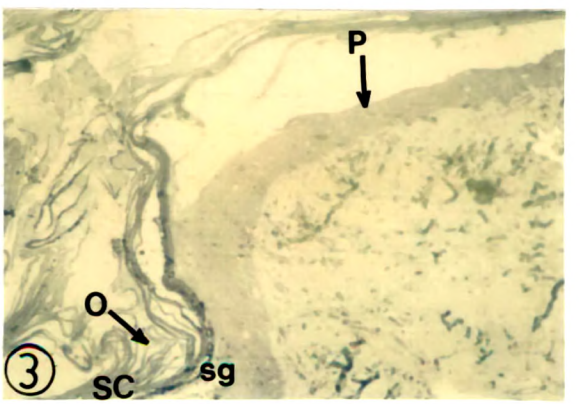
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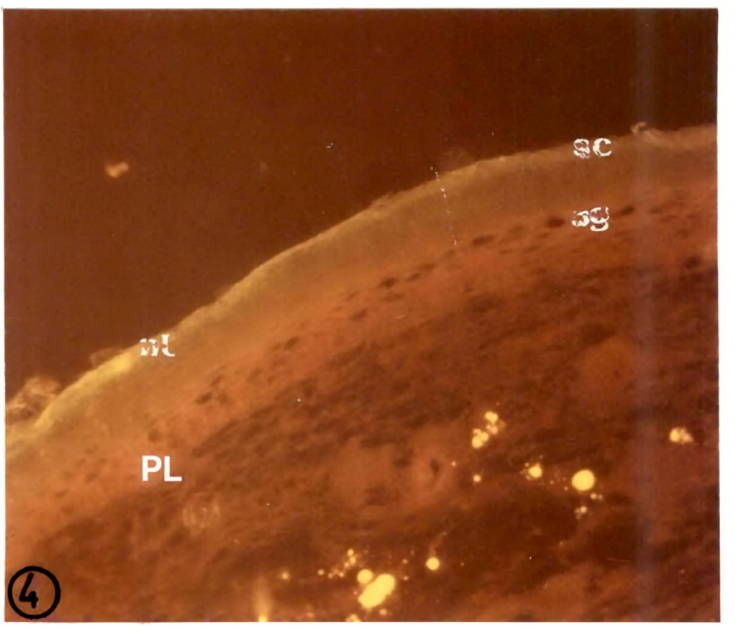
Fig. 4-6 : Localization of neutral lipids using nile red fluorescent probe. Neutral lipids show bright yellow fluorescence, while phospholipids show orange-red fluorescence.

Fig. 4 : 4 day old tail epidermis. Neutral lipids visible in stratum corneum. X 180.

Fig. 5 : 7 day old tail epidermis. Neutral lipids localized in lower epidermis, while phospholipids present in the stratum corneum. X 180.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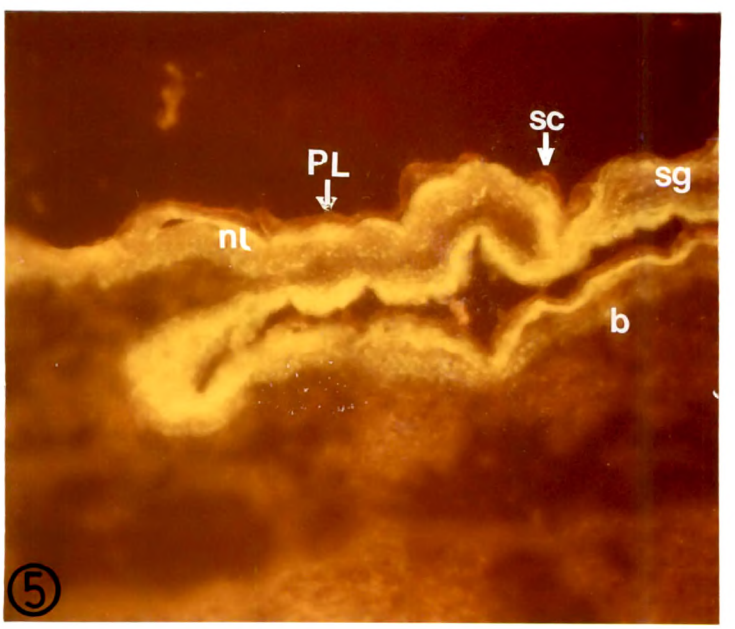
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Fluorescence Studies :

Lipids : Nile red fluorescence studies showed a normal pattern of distribution of lipids in 4 day old tail skin i.e. neutral lipids (NL) in the outer SC and phospholipids (PL) in the rest of the epidermis (Fig. 5).

In 7 day old tail skin, the pattern of lipid distribution was exactly the opposite. More of NL was seen in the whole of the epidermis while SC showed increased staining for PL (Fig. 6).

The adult tail skin showed more of NL in the parakeratotic zone, while the orthokeratotic zone showed a normal lipid profile (Fig. 7).

Psoriatic skin showed an increased staining for PL throughout the epidermis with neutral lipid droplets in the stratum corneum (Refer Fig. 4, Chapter 1).

Similar observations were seen with Fat red 7B staining.

Nucleic Acids : 4 day old tail skin showed an equal distribution of RNA and DNA in the spinuous and granular layer, while the basal layer showed DNA (Fig. 7).

In 7 day old tail skin an increase in DNA staining was seen both in basal and spinuous layers, while RNA was more in granular layer (Fig. 8).

In adult tail skin, a slight increase in the amount of DNA was observed in orthokeratotic zone. A normal pattern of DNA and RNA distribution was seen in parakeratotic regions, but there is an increased staining for DNA

Fig. 6 : Adult tail epidermis. Predominance of neutral lipids in parakeratotic zone compared to orthokeratotic zone is evident. X 180.

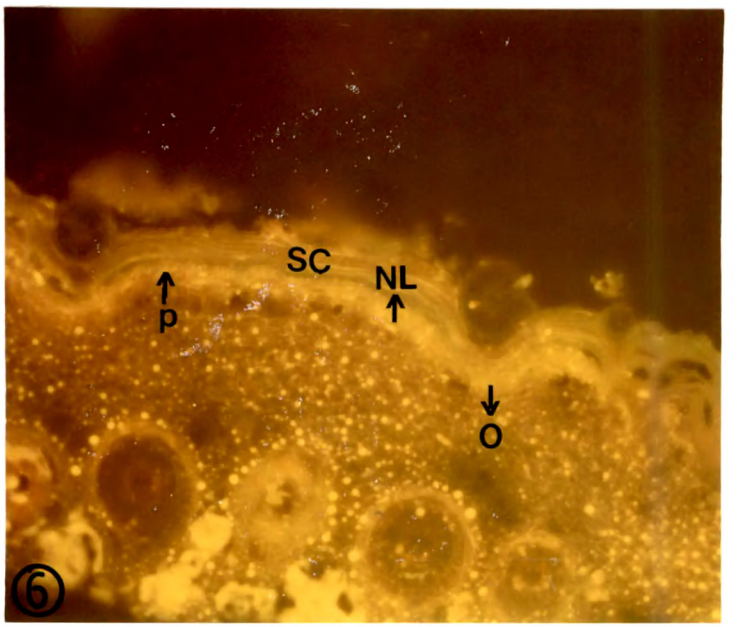
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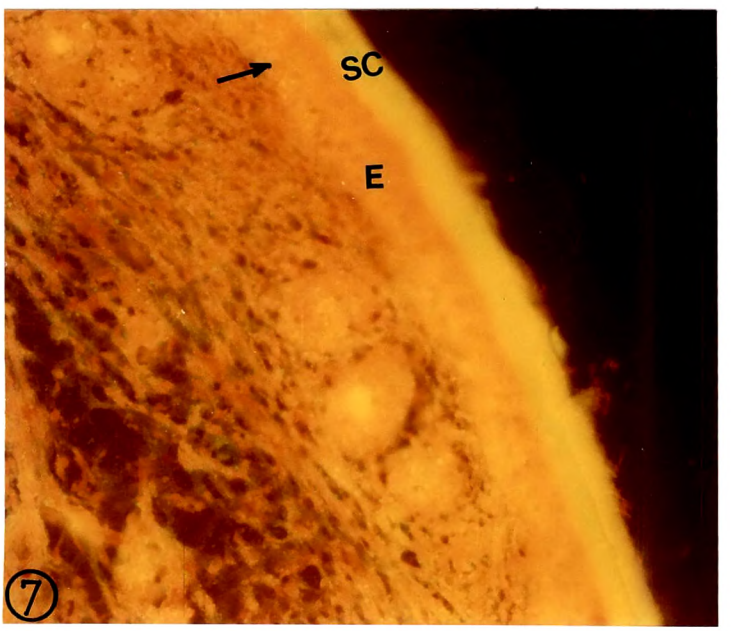
Fig. 7-10 : Localization of nucleic acids using fluorescent probe - Acridine orange. DNA gives bright yellow fluorescence. RNA gives flame red fluorescence.

Fig. 7 : 4 day old tail epidermis, showing equal distribution of DNA and RNA. Basal layer shows DNA fluorescence (arrow) X 180.

Fig. 8 : 7 day old tail epidermis. DNA localization seen in basal and spinuous layer (arrow). RNA is seen in granular layer. X 180.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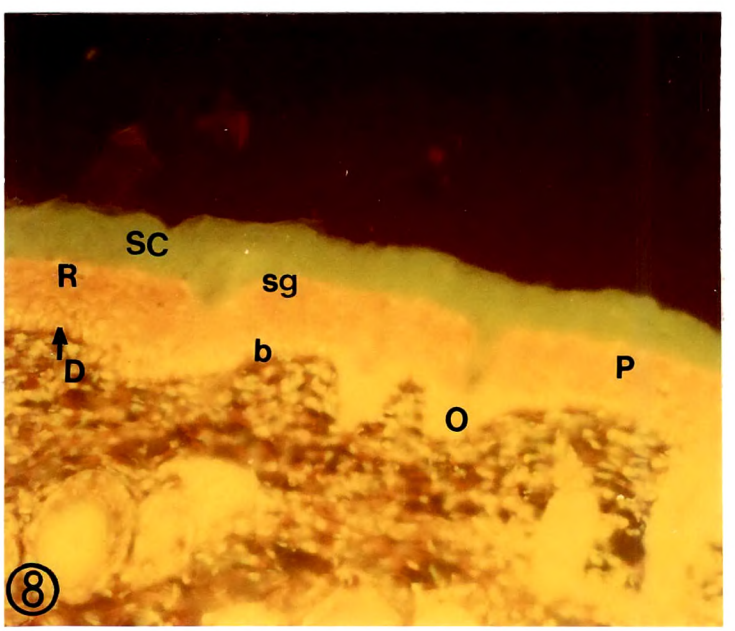
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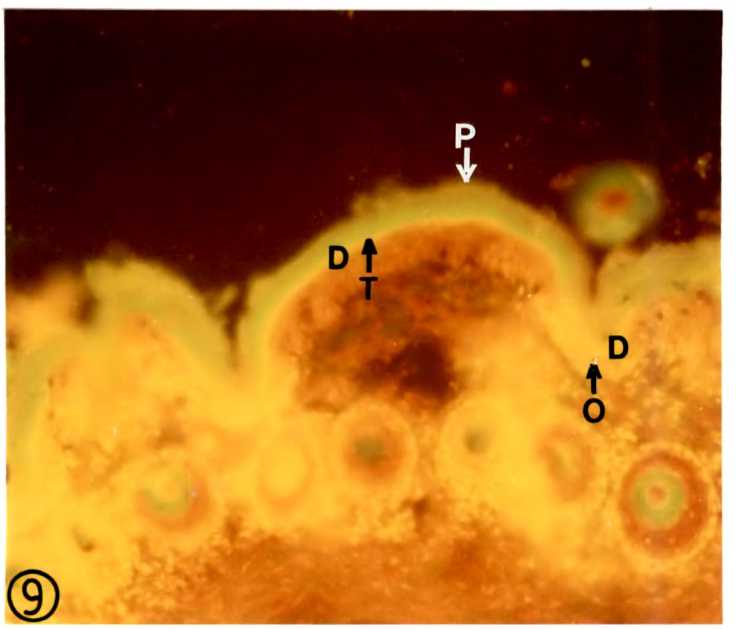
Fig. 9 : Adult tail epidermis. Increased DNA staining in orthokeratotic zone. Parakeratotic zone shows more of DNA in transitional layer. X 180.

Fig. 10 : Psoriatic epidermis showing increased DNA staining throughout the epidermis. RNA is seen to be localized in the upper viable epidermis. X 66.



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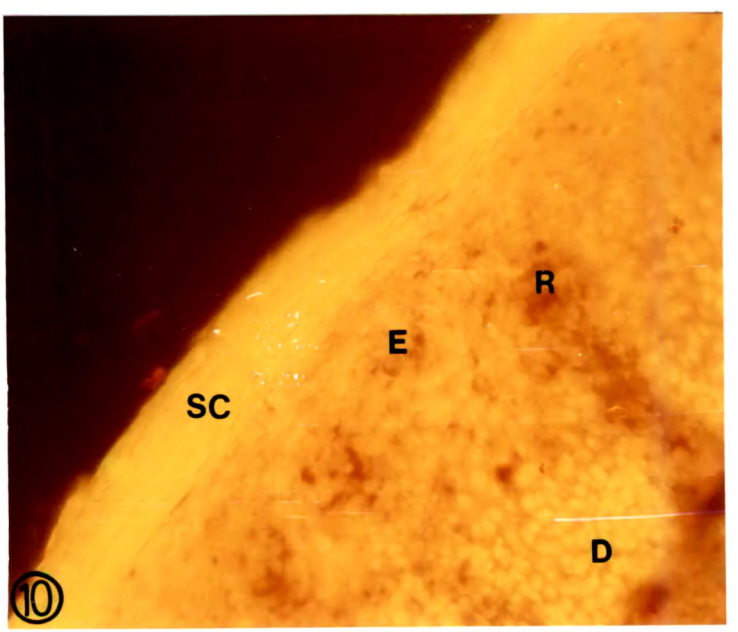

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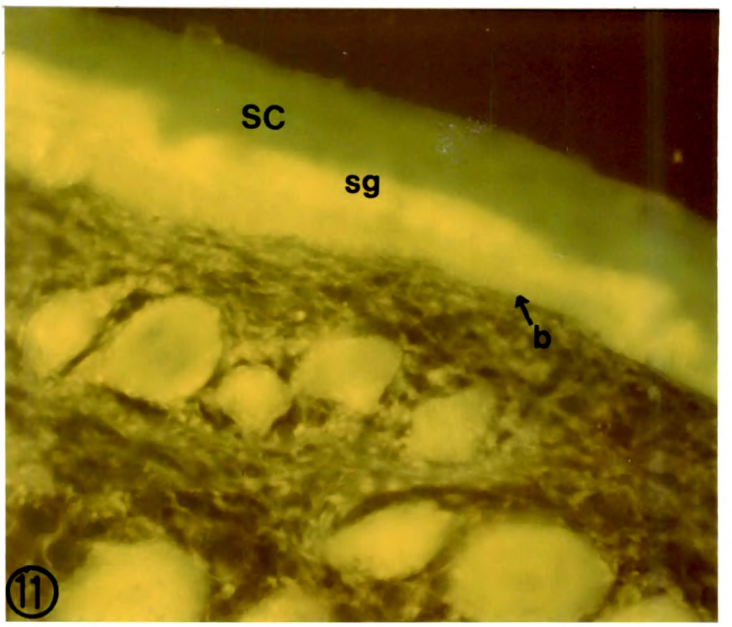
Fig. 11-14 : Calcium localization using fluorescent probe chlorotetracyclin (CTC). Bright yellow fluorescence indicates presence of calcium.

Fig. 11 : 4 day old rat tail epidermis. Low calcium in basal layer (arrow), while granular layer shows more fluorescence. X 180.

Fig. 12 : 7 day old tail epidermis. Increased fluorescent evident in granular layers (arrow). X 180.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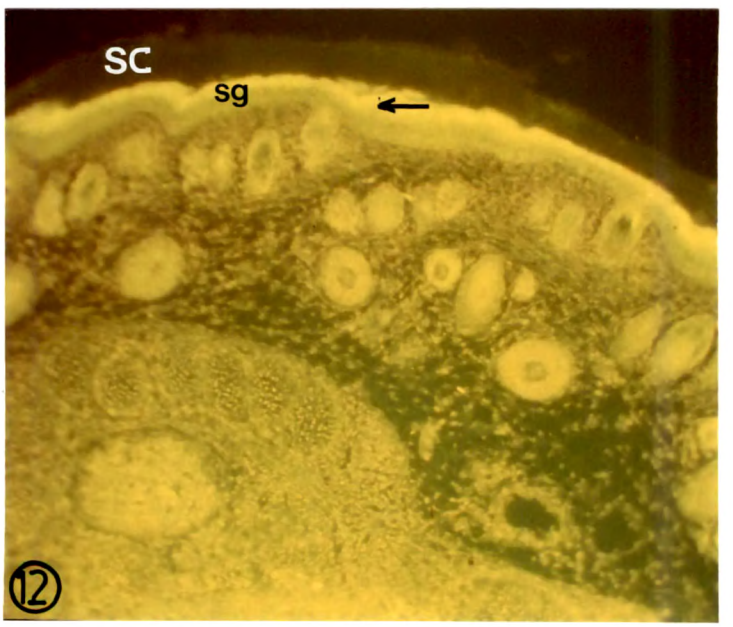
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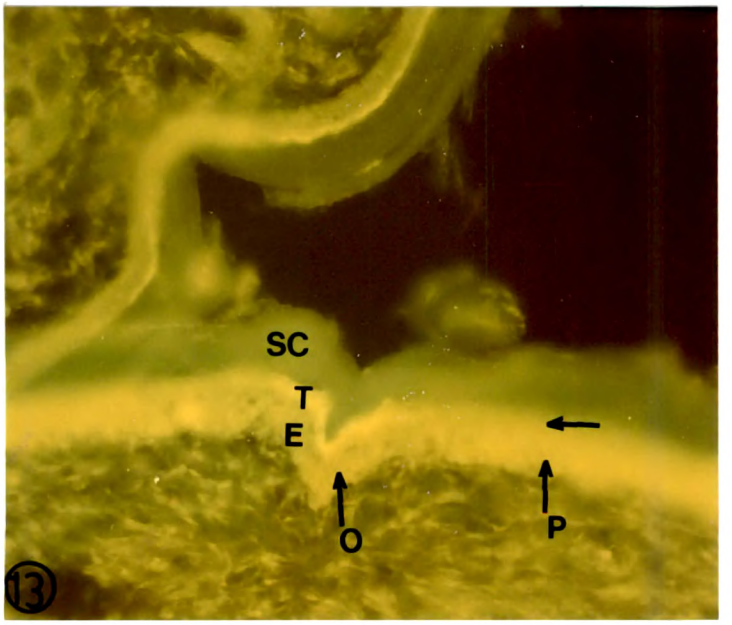
Fig. 13 : Adult tail epidermis. Calcium localization in granular layer of orthokeratic zone. Parakeratotic region shows less fluorescence at stratum corneum - stratum granulosum interface (arrow), while the stratum corneum is totally devoid of calcium. X 180.

Fig. 14 : Psoriatic epidermis. Low calcium fluorescence in basal layer (arrow), but increased fluorescence is evident in rest of the layers of epidermis including stratum corneum. X 66.

Abbreviations :

Stratum corneum - sc; Stratum granulosum - sg; Basal layer - b; Neutral lipids - NL; Phospholipids - PL; Parakeratotic zone - P; Orthokeratotic zone - O; DNA - D; RNA - R; Transitional layers - T; Upper viable epidermis - E.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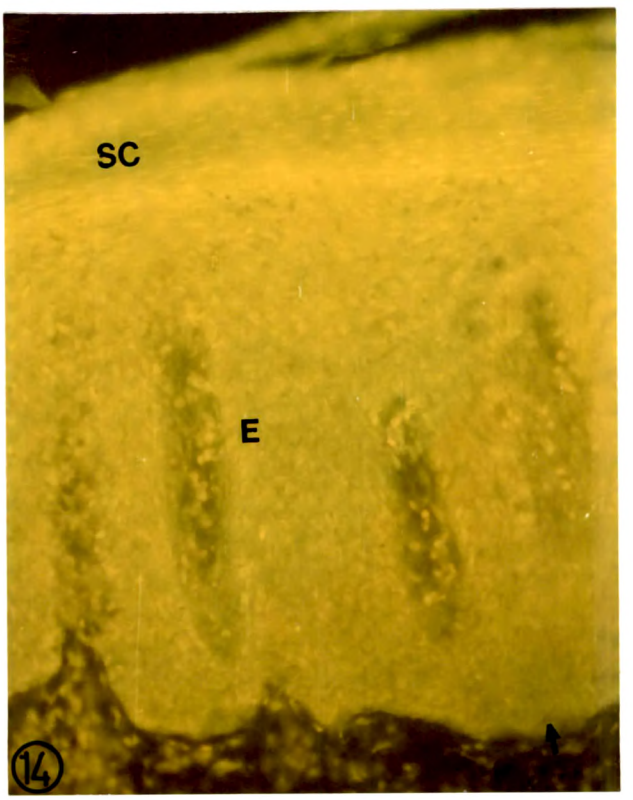
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in the transitional layers and at the SC-SG interface (Fig. 9).

Psoriatic epidermis showed a significant increase in the DNA staining throughout the epidermis, including SC (Fig. 10).

Calcium : 4 day old tail skin, showed a low Ca^{++} fluorescence in the lower epidermis (i.e. basal + spinuous layer), while the granular layer showed high amount of Ca^{++}. SC did not show any trace of Ca^{++} (Fig. 11).

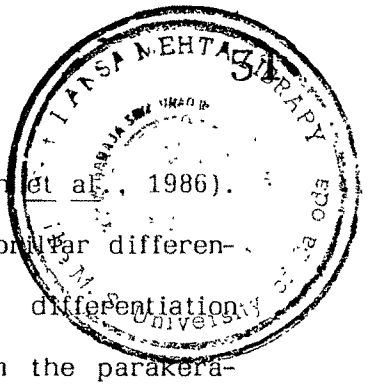
7 day old tail skin showed an increased fluorescence in the upper epidermis i.e. the spinuous and granular layers in both the para- and orthokeratotic areas, while the basal layer showed a normal profile (Fig. 12).

In adult tail skin, normal pattern of Ca^{++} was observed in the orthokeratotic zone, but the parakeratotic zone showed comparatively very low Ca^{++} fluorescence at the SC-SG interface. SC did not show any presence of Ca^{++} in both ortho- and parakeratotic areas (Fig. 13).

Psoriatic tissue on the other hand had low content of Ca^{++} in the basal layers, but the upper spinuous, the transitional layers including SC, showed very high Ca^{++} fluorescence (Fig. 14).

DISCUSSION

At birth the follicles in the tail are closely packed together but growth of the tail during the first week causes separation of the follicles with the formation of the tail scales in the intervening sites (Wrench, 1980).



Parakeratosis becomes apparent on day 9 after birth (Bladon et al., 1986). Parakeratosis without keratohyalin causes a defective tonofibrillar differentiation as well as marked disturbances in the cellular differentiation (Brody, 1962). The absence of keratohyalin granules from the parakeratotic areas of rat tail skin, made it an ideal model for studying psoriasis (Bladon et al., 1984).

The distribution of different classes of lipids within the epidermis, has been reviewed in detail by Yardley and Summerly (1981) and by Yardley (1983). The occurence of hydrocarbons and cholesteryl sulfate in the epidermis and the relationship between this lipids and ichthyosis and desquamation has also been discussed (Elias, 1983; Williams, 1983). The fluorescence and electron microscopic observations of distribution and secretion of lamellar lipids in psoriatic epidermis, has been discussed in Chapter 1.

The rat tail skin, during the process of parakeratosis shows increased NL in lower epidermis. This could be related to an increased rate of mitotic division during this phase, which in turn gives less time for the processing of these NL to PL in lower spinuous layers. In the adult stage, the lipid profile in the parakeratotic region of rat tail skin bears no similarity to the psoriatic skin. This is possibly because parakeratosis in psoriasis is a pathological condition, while as in rat tail skin, it is a normal mode of development.

During the onset of parakeratosis in the tail skin the increased fluorescence for DNA in basal and spinuous layer probably indicates an increase in the

rate of mitosis in the basal and suprabasal layers. For the synthesis of keratins, and different enzymes that are needed for differentiation, mRNA is essential (Fuchs and Hanukoglu, 1986). The granular layer thus shows an increase in RNA, suggesting an active process of protein synthesis, during this phase.

In adult tail skin, the parakeratotic zone shows an increase in DNA staining at the SC-transitional layer interface. The psoriatic epidermis on the other hand shows large amount of DNA throughout the epidermis including the SC. An increase in the rate of mitosis and a very short time for the transition of cells, during psoriasis could lead to an abnormality in the processing of these nucleic acids. Normally there is an active removal of DNA from the corneocytes during differentiation (Kirscher and Furlong, 1967). Retention of DNA in SC, further supports our earlier ultrastructural studies on psoriasis which shows retention of nuclear material even in the upper layers of SC. These observations and reduced RNA staining in psoriatic epidermis further adds support to earlier investigations, where the expression of keratin polypeptides in psoriatic lesion is abnormal (Baden et al., 1978; Bowden et al., 1983; Matoltsy et al., 1983; Levine and Mcleod, 1979).

The role of calcium in keratinization has been discussed earlier in this chapter. With the onset of parakeratosis, there is an increase in Ca^{+2} fluorescence in upper spinuous and SG layers which indicates normal terminal differentiation of the differentiating keratinocytes during this phase (Menon et al., 1985). Adult tail skin showed less Ca^{2+} at the SC-transitional layer interface, but unlike psoriasis the SC shows no retention

of Ca^{2+} in the parakeratotic zone. The increased CTC staining in the suprabasal layers and SC of psoriatic epidermis supports the findings of Menon and Elias (1990), which points out that high concentrations of Ca^{2+} indicates loss of normal calcium gradient that programmes terminal differentiation (parakeratosis). Retention of these cations may further lead to abnormal cohesion, desquamation and permeability barrier in psoriasis.

The above observations hardly show any resemblance of the parakeratotic zone of the tail skin to the psoriatic skin at the light microscopic level. The possible reason as mentioned earlier could be because, parakeratosis is a normal phenomenon in rat skin, while in psoriasis it occurs as a pathological condition. At ultrastructural and cellular or molecular level there may be similarities. Studies on the secretion of lamellar bodies in parakeratosis of rat tail skin, like in psoriasis, and biochemical studies of lipids and epidermal enzymes will further give us an insight into the similarities in the process of parakeratosis, if any, in the rat tail skin and the psoriatic skin.