CHAPTER - 6

BARRIER PROPERTIES OF BUFFALO EPIDERMIS : LIPID HISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES

The mammalian stratum corneum (SC), the outermost layer of the skin is a unique structural composite which forms the primary barrier between the body and its external environment. Previously the bulk of SC was considered to represent the permeability barrier (Scheuplein and Blank, 1971). Recent morphological and experimental evidences lead to a more sophisticated view of the SC, showing corneocyte cells composed primarily of the protein keratin surrounded by a three dimensional, multilamellar lipid domain, in a manner reminiscent of 'bricks and mortar' (Michaels et al., 1975; Elias, 1983; Wertz and Downing, 1982).

These intercellular lipid layers found between SC cells originate from the lamellar bodies (LB) (Matoltsy, 1965), which are found in the upper stratum spinosum and stratum granulosum cells. These membrane bound organelles contain several stacks of disks which gives rise to a lamellar pattern (Martinez and Peters, 1971). At the SC-SG interface these LBs are exocytosed into the intercellular space. These small disks, are processed further into continuous broad layers that lie parallel to the surface of horny cells (Elias and Friend, 1975; Lavker, 1976). These highly nonpolar lipid lamellae account for the barrier to water in the epidermis (Elias, 1983).

In contrast to terrestrial mammalian skin, epidermis of aquatic mammals like the cataceans, elaborate two types of lipid structures: (1) LB are elaborated in great abundance in all the suprabasal epidermal layers forming intercellular lipid bilayers in the SC interstices and (2) Non-membrane bound lipid droplets appear and persist in all the epidermal layers, including the outermost SC. The lipokeratinocytes of cetaceans

have been considered as an adaptation to the specific challenge offered by the marine environment, including the constant state of hydration.

Stratum corneum of terrestrial mammals is essentially non-hydrated, but the water content of corneocytes influences the plasticity and functional properties of SC (Blank, 1952; Scheuplein and Blank, 1971). The epidermal lipids are postulated to have a role in water holding properties of SC (Imokawa and Michihiro, 1985; Imokawa et al., 1991). In this light, buffaloes that inhabit tropical countries, and which have a peculiar habit of wallowing in water for prolonged periods during the day as a behavioural mode of thermoregulation, may prove to be an interesting model to study SC organization vis. a vis. hydration.

Morphologically, an expanded conformation, a wide and deep belly and long extreemities make the surface of buffalo proportionately large, thus heat loss becomes more efficient. The dark skin is a defence mechanism against adverse effects of ultraviolet rays, but it makes the animal sensitive to direct sunlight.

As seen earlier, structurally, the Indian Surti buffalo showed thicker skin and also various layers of the skin were much thicker compared to that of an ox and the Egyptian buffaloes (Bagi, 1974). Buffaloes have sparse hair density. The sparseness of the hair and the skin thickness is both an advantage and a disadvantage for heat tolerance, because though heat dissipation is facilitated, the absorbance of energy from direct sunlight is enhanced (IInd World Buffalo Congress, 1988). The density of sweat glands is lower in the skin, compared to other cattle (Shafie, 1985; Bagi,

1974). Sweating activity is apocrine and a fatty secretion is seen in these animals (Yamane and Ono, 1936).

Earlier studies have shown that the epidermis of the buffalo is papillomatous with brown diffused pigment even in the cytoplasm of the upper cells. Pigmentation is intense on the dorsal surface of the body. In buffaloes the arteries branch markedly and supply to sebaceous glands. These glands are located near the middle of hair follicle. Glands are more lobulated and developed. The hair follicle is encircled by 2-3 sebaceous glands in buffaloes (Hafez et al., 1955). The epidermis protrudes deeply in the form of papillae into the subepithelium to make the corneum thicker, almost twice as much as cattle. Epidermis is richly supplied with blood cappilaries to promote heat loss through skin efficiently (Badreldin and Ghany, 1954).

Thus, the paucity of sweat, sebaceous glands and hair follicles afford a very poor impedence to water loss. Moreover, as mentioned earlier, the effects of prolonged hydration on the skin during behavioural thermoregulation are also worth investigating. The present investigations were aimed at providing answers to some of these questions.

MATERIALS AND METHODS

Samples of dorsal portion of skin (near tail region) were obtained from a local butcher, immediately after the animal was killed. The whole thickness of the skin was cut into fine pieces of approximately 1 cu. mm. and fixed in 2 M paraformaldehyde-glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 1 hour at room temperature and then transferred to fresh

buffer and kept overnight at + 4°C. The tissues were then washed with cacodylate buffer and postfixed in 1% osmium tetroxide (containing 1% potassium ferrocyanide) and processed regularly for electron microscopic studies (McNutt and Crain, 1981) (Chapter 1). Ultrathin sections were cut and double stained with uranyl acetate and lead citrate and observed under TEM.

Several inorganic ions, including lead, lanthanum, barium, silver and thallium have been tested as possible tracers to demonstrate the fluid accessible channels in functional epithelia at the ultrastructural level (Simson and Dom, 1983). Lanthanum has been successfully used as a tracer for diffusion studies (Weihe et al., 1977) and also to study the intercellular junctions and the permeability barrier in human sebaceous glands (Kitson and Lennep, 1984). It has also been used as a probe for cell permeability in the rat heart (Harper et al., 1990). Thus, for comparing the permeability barrier in the rat skin (a non-hydrated skin model) and the buffalo skin, $LaNO_3$ was used as a tracer to study the epidermal barrier at the ultrastructural level. Rat pup skin was chosen as a control for the permeability studies.

Tissues of 1 cu.mm. whole thickness skin were incubated with 1% $LaNO_3$ in water (double distilled) at 37°C for 1 hour at room temperature, fixed in glutaraldehyde - paraformaldehyde fixative and processed routinely as mentioned above for electron microscopy.

For histochemical studies fresh frozen sections at -28°C (12 m thick) of whole skin were also taken to study the distribution of neutral lipids

using Fat red 7B (Sigma) stain (Pearse, 1968) (Chapter 1) and also with nile red (a fluorescent probe for neutral lipids) for confirmation (Greenspan et al., 1985).

RESULTS

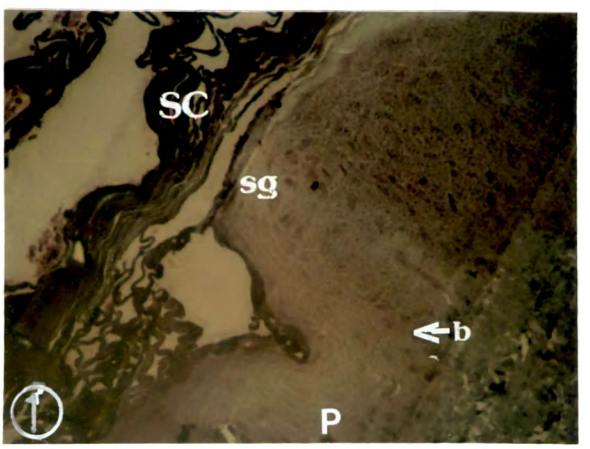
Histochemical studies with Fat red 7B and Nile red showed negligible amount of neutral lipids (NL) in the inner layers of the SC. The outer 3-4 layers showed the presence of NL. The rest of the epidermis showed predominance of phospholipids (PL), though the basal layer did show traces of NL (Fig. 1).

Light microscopic, histochemical observations showed a very thick stratum corneum (SC); consisting of around 15-20 layers. The epidermis is papillomatous. The granular layer is 3-4 layers thick but in the papillomatous epidermis, it is about 6-10 layers thick. The basal layer consists of a single layer of columnar cells with large amount of melanin pigments. There is an infiltration of melanin pigments in the cytoplasm of the upper cells i.e. the stratum spinosum and to some extent in the granular cells (Fig. 2).

Ultrastructural studies revealed that the SC-SG interface is not as convoluted like in rats or mice. Both the spinous as well as the granular layer show large numbers of desmosomes (Fig. 3,5). The keratin filaments and the keratohyalin granules are not as large and distinct as in case of rats/mice (Fig. 4,5).

Fig. 1 : Histological features of buffelo epidermis, showing a thick layer of stratum corneum and stratum granulosum. Granular layer is seen to be more thick in the papillomatous epidermis. X 315.

Fig. 2 : Histochemical localization of neutral lipids in buffalo epidermis. Only the outermost 3-4 layers of stratum corneum show traces of neutral lipids (arrow). X 250.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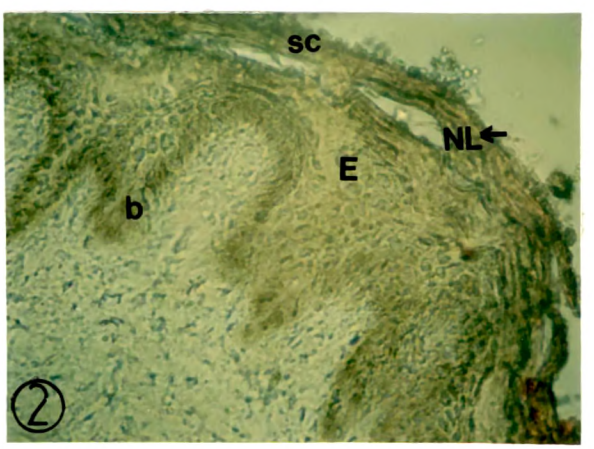
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Fig. 3-6 : Electron micrographs of buffalo epidermis.

Fig. 3 : A view of stratum spinosum cell, showing presence of lamellar bodies (X). Number of desmosomes is seen to be more. X 25,000.

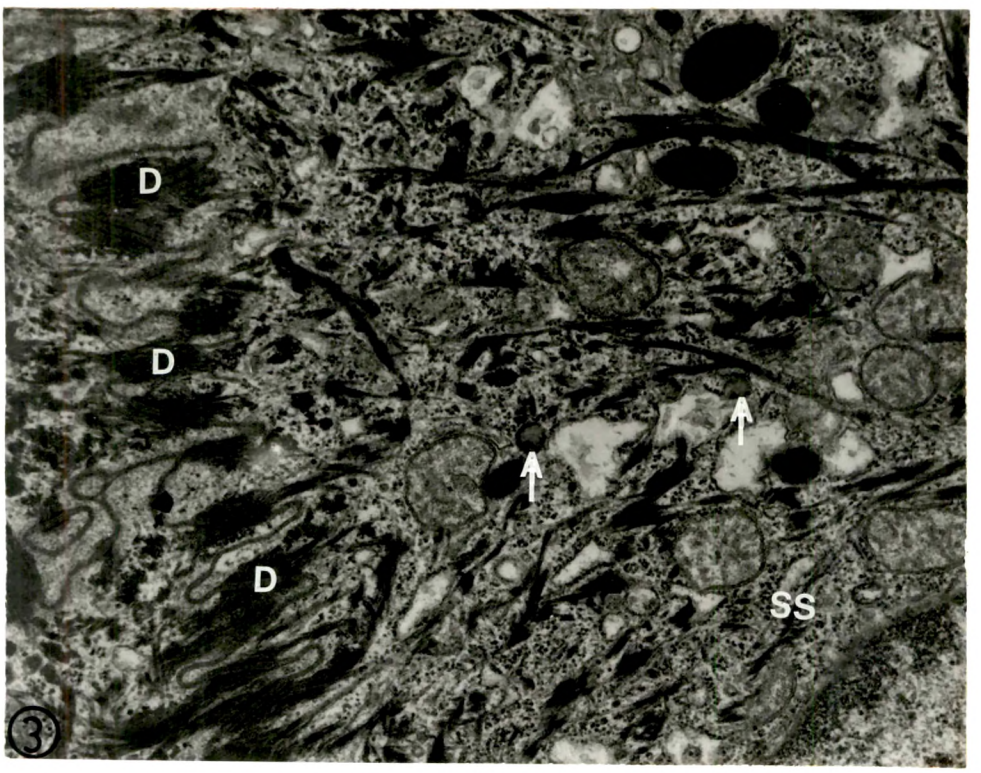
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Fig. 4 : Low magnification view of granular layer, with numerous lamellar bodies (arrow), and indistinct keratohyalin granules. X 16,000.

Fig. 5 : Granular cell (high magnification), showing distinct lamellae (arrow) in the lamellar bodies (X). Desmosomes are more in number compared to rat epidermis. X 40,000.

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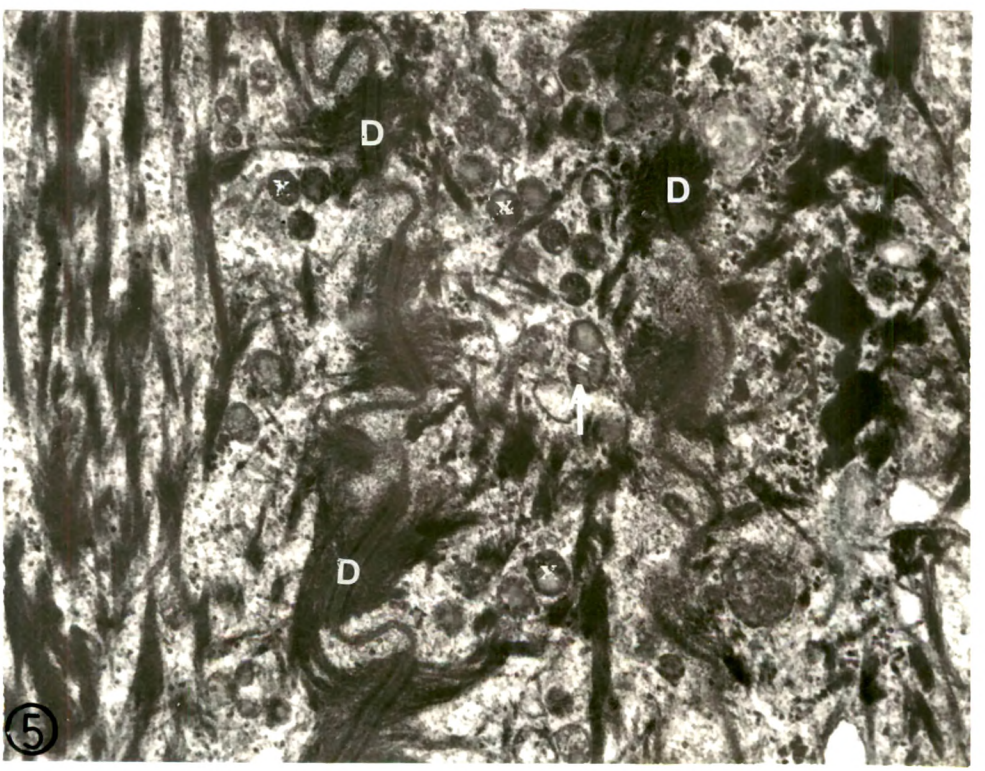
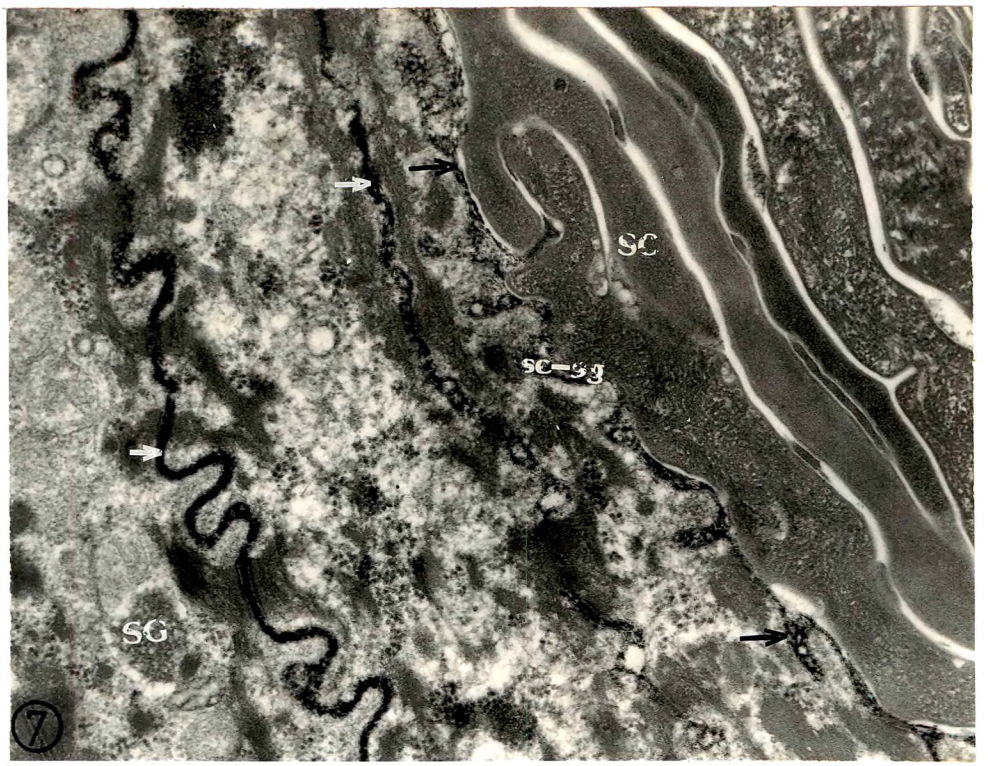


Fig. 6 : Lamellar bodies are seen to extrude their contents into the intercellular space at the stratum corneum - stratum granulosum interface, as well as at the upper granular layer level. Note that the corneocyte intercellular space also contains electron lucent lipid mass (arrow), and the lamellar contents seem to occupy a small surface area. X 43,200.



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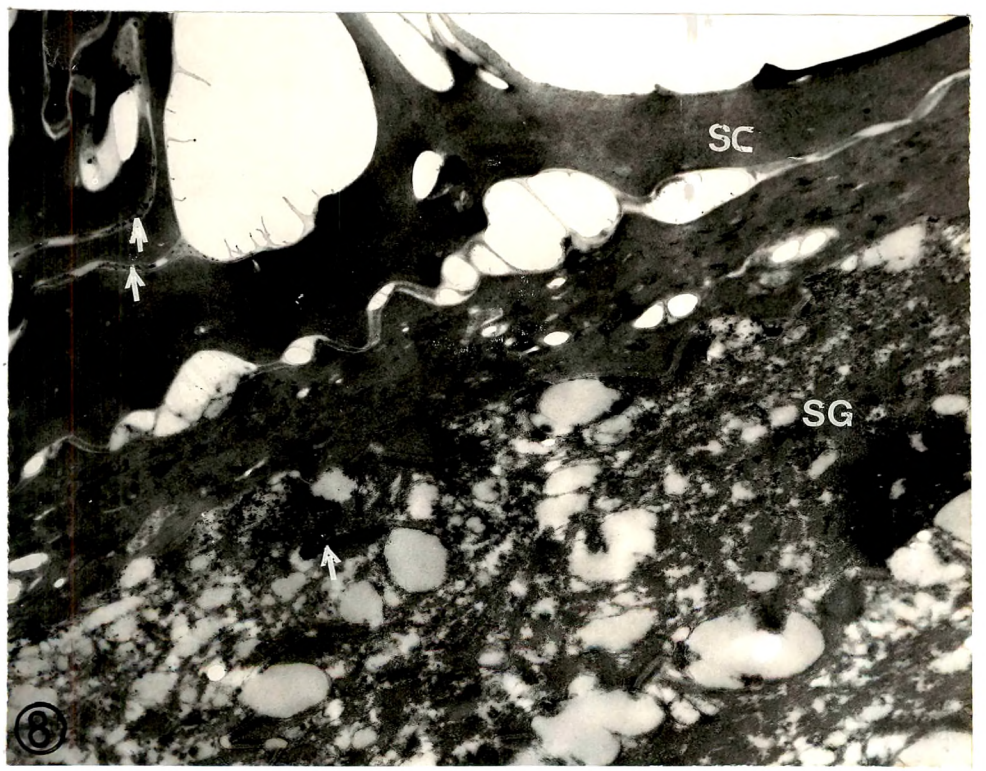

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Fig. 7-10 : Electron micrographs of lanthanum nitrate permeation.

Fig. 8-10 : Lanthanum nitrate permeation in buffalo epidermis.

Fig. 7 : Rat pup epidermis (control) showing lanthanum permeation only till the stratum corneum - stratum granulosum interface (arrow) and not in the corneocyte intercellular domains. X 30,000.

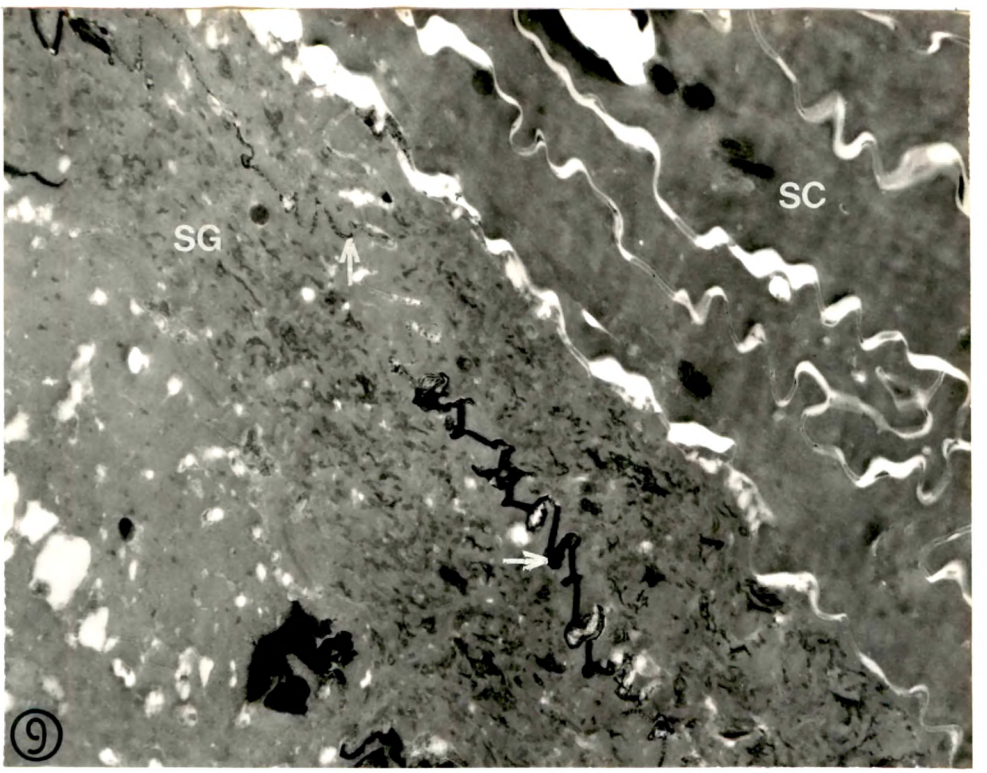
Fig. 8 : Lanthanum permeation even in stratum corneum interstices (arrow). X 20,000.

Fig. 9 : Lanthanum precipitates seen to permeate near the stratum corneum layer (arrow). X 16,000.

Fig. 10 : Lanthanum nitrate seen to permeate through the lipid bilayers in the intercellular space (arrow). X 50,000.

Abbreviations :

Lamellar bodies - LB; Stratum corneum - Stratum granulosum interface - sc-sg; Stratum corneum - SC; Stratum granulosum - SG; Upper granular layer - UGL; Neutral lipids - NL; Stratum spinosum - SS; Desmosomes - D; Papillomatous epidermis - P.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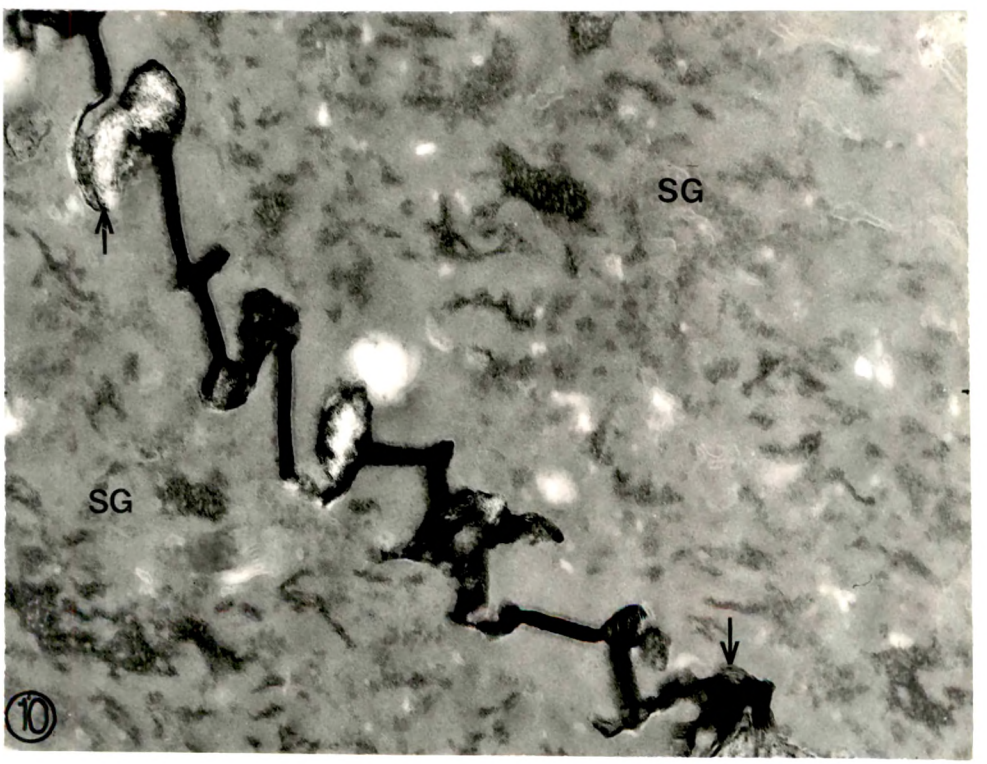
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Many lamellar bodies are seen in the granular cells (Fig. 4,5). These LBs are seen to secrete their contents into the intercellular space (Fig. 6), of the uppermost SG (stratum granulosum) cells and also into the SC-SG interface. Moreover, the surface area occupied by the secreted LB contents seem to be much less compared to the typical terrestrial mammals (rat epidermis). The SC shows no retention of LB contents/or NL droplets (Fig. 6).

Permeability experiments with $LaNO_3$ as a tracer in normal/control epidermis (rat pup, 2 day old), showed precipitates of $LaNO_3$ not permeating past the SC-SG interface (where the lamellar contents are secreted) (Fig. 7). Unlike the rat epidermis, the $LaNO_3$ showed an upward flux into the SC interstices, as it was visualized even in these domains, in the buffalo epidermis (Fig. 8). Lanthanum nitrate precipitates are visible in the corneocyte intercellular domains along with the bilayered mortar lipids (Fig. 10).

DISCUSSION

The thermal exchange between the livestock and the environment occurs through sensible heat loss (non-evaporative); radiation, conduction, convection and through evaporative heat loss (World Buffalo Congress, 1988).

The waterproofing barrier of reptilian and mammalian skin is thought to be intercellular epidermal lipid lamellae in SC (Elias and Friend, 1975; Elias et al., 1977; Scheuplein, 1978; Landmann, 1979, 1980; Elias, 1981; Wertz and Downing, 1982).

In buffaloes, each hair follicle is surrounded by 2-3 sebaceous glands. The sweating activity is apocrine with a fatty secretion (Yamane and Ono, 1936). The sweat and the sebaceous secretions to some extent, solve the problem of preventing excessive loss of water from the body surface. On the other hand, epidermis is richly supplied with blood vessels (capillaries) and the hair growth is sparse, which helps to promote heat loss through the skin efficiently (Badreldin and Ghany, 1954).

In normal terrestrial mammals (rats/mice) lamellar contents that are secreted into the intercellular space, are transformed into broad bilayers by membrane fusion process. These uninterrupted, superimposed bilayers, in turn, help in curtailing TEWL (Landmann, 1986). Landmann (1980), compared the lamellar granules in all the three classes viz. reptiles, aves and mammals. Many investigations suggest that quantitative and qualitative alterations in these lamellar lipids are responsible for both normal and abnormal SC barrier phenomenon (Elias, 1983).

Buffalo epidermis shows an abundance of LBs in the spinous as well as in the granular layers. Inspite of their high numbers, these lamellar contents, after secretion, occupy a significantly less surface area in the intercellular space, which may be related to poor barrier properties and increase in TEWL. Light microscopic studies also show less staining of NL in the SC interstices, which further indicates an alteration in the processing of the polar lipids to more nonpolar species (hydrophobic), probably leading to a failure/defect in the formation of broad bilayers in the intercellular interstices. Ultrastructural observations show electron lucent areas in the corneocyte intercellular domains. Continuous sheets of lipid bilayers are not evident, which supports our earlier findings.

An increase in the number of desmosomes in the spinous, granular and the corneal layers of the epidermis, could indicate a compact cell arrangement in the epidermis by increasing cell adhesion in these layers. Elevated corneocyte retention could lead to increased intracorneal cohesion (Marks et al., 1981). The paucity of NL in the SC interstices could cause a weak lipid cohesive force in buffalo corneocytes. Therefore, the increase in the number of desmosomes could be a means, to effectively rivet the corneocytes together (Chapman and Walsh, 1989), thereby increasing the thickness of the SC and in turn probably helping the animal, to tolerate heat to some extent.

Keratohyalin granules are light refractile bodies and their formation begins in the upper spinous cells. During the early stages small aggregates of amorphous particles are formed. Later these grow in size by continuous deposition of amorphous particles on their surfaces (Lavker and Matoltsy, 1971).

These amorphous particles consist largely of proteins like histidine rich protein (HRP), cysteine rich protein (CRP), proline rich protein (PRP) and arginine rich protein (ARP), which are formed presumably by those ribosomes that form a shell around the KHGs. The KHGs coalesce to form large dense masses and tonofilament bundles condense at the cell periphery during the transition of the granular cells. Modifications of tonofilaments and KHGs by proteolysis takes place. Finally the keratohyalin masses disperse, mix with remnants of cell organelles and penetrate the inter-fibrillar spaces of filament bundles (Fuchs and Hanokoglu, 1986; Lavker and Maltoltsy, 1970, 1971).

Observations with buffalo epidermis show indistinct, small keratohyalin granules, thus indicating a possible anomaly in their maturation. Keratohyalin granules contain profillaggrin and filagrin, which are involved in water holding properties of SC. The paucity of KHGs in buffaloes may be related to their habit of soaking themselves in water, which alleviates the need for water holding properties of SC. This adaptation would thus reduce the need of a great water holding capacity for SC. Alternately, marine mammals like cetaceans also do not show any presence of keratohyalin granules which further supports our findings (Menon et al., 1986).

Permeability studies in normal terrestrial animals (as observed in rat epidermis), showed that the upward movement of $LaNO_3$ stops at the SC-SG junction, where the LB secretion occurs, creating a hydrophobic barrier for permeation of water from the viable layers of the epidermis. This occludes the upward / outward flux of the tracer at the SC-SG interface. In barrier defects such as EFAD conditions, etc; the tracer is seen to move up further into the SC interstices (Hou et al., 1989). This is correlated with high TEWL values, that are measured. In buffalo, such readings are not available, but still, the permeation of this electron lucent heavy metal, in the SC intercellular domains may provide a good evidence of a possible high TEWL values in these animals.

The waxy secretion of the sebaceous and sweat glands on the surface of the buffalo skin acts as a water repellent. Squalene, is known to be an excellent water repellent in the otters (Lindholm and Downing, 1980). Cholesterol and wax esters are also found to serve as antiwetting agents in the skin of mink (Colton et al., 1986).

In view of the paucity of sweat glands, and the fact that an increased TEWL for thermoregulation would cause excessive body dehydration; the behavioural thermoregulation of buffaloes seems to be an excellent adaptive strategy in their tropical habitat.