CHAPTER - 3

PIGEON APTERIA AS A MODEL FOR 'DRY AND SCALY SKIN' : EFFECT OF GLYCEROL - A SKIN CONDITIONER

The important functions of skin are to protect the body from infections, the internal organs from dessication, thermoregulation and to prevent transepidermal water loss (TEWL).

In mammals, the barrier to TEWL is formed by the extrusion of the lamellar body contents into the stratum corneum-stratum granulosum (SC-SG) interface. These lamellar bodies are enriched in phospholipids, free sterols and glycosphingolipids (Grayson et al., 1983). These polar lipid contents are converted to nonpolar species like cholesterol, cholesterol esters and free fatty acids, by a battery of enzymes which are also secreted by the lamellar bodies into SC-SG interface. These lipids are then sequestered as lipid bilayers into the SC interstices (Elias et al., 1977; Elias, 1981). These lipid bilayers are thought to play an important role in preventing water loss through SC (Imokawa and Hattori, 1985).

In contrast to the mammalian epidermis, the avian epidermal permeability barrier is formed by the extrusion of free lipid droplets from the avian keratinocytes, into the lower corneocyte intercellular domains. Thus, the avian skin performs a dual function of producing keratin as well as lipid material. They are therefore termed as 'sebokeratocytes' (Wrench et al., 1980). Some of these nonmembrane bound lipids are retained within the corneocytes (Landmann, 1980; Lucas, 1980; Menon et al., 1981).

Menon et al. (1986) observed that these lipid droplets are directly extruded at the lower corneocyte level. The lipid composition of avian corneocytes largely differs from the mammalian corneocytes. The avian SC, shows persistence of glycosphingolipids and triglycerides which are not

seen in the mammalian epidermis. These intercellular lipids, from a barrier to TEWL (Landmann, 1980). Because of the paucity of keratin filaments, the corneocytes of the avian epidermis are closely bound together. Desquamation of SC is not uniform, which gives a dry and scaly appearance to the skin of pigeons. The TEWL has been found to be much higher in pigeons, compared to mammals (Menon et al., 1986). The avian skin therefore seemed to be an ideal model to study dry and scaly skin conditions (Lachyanker, 1987).

In human subjects, humectants are applied topically to retain water in SC. Humectants like glycerol, when topically applied, not only retains water, but also reduces TEWL (Bisett and McBride, 1984). This study was focussed on the effect of glycerol on the dry and scaly skin of the pigeon apteria, as a model system.

MATERIALS AND METHODS

99% pure glycerol (Loba Chemicals) was diluted to 45% and 70% with double distilled water. 0.5 ml of this diluted glycerol was topically applied on the pigeon apteria, for 7 days, with one application daily. Control animal was treated with an application of plain double distilled water for a similar span of time. On 7th day the animal was decapitated and the whole thickness of the skin was cut into fine pieces of approximately 1 mm and fixed in 2% paraformaldehyde/glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 1 hour at room temperature. The tissues were washed in the cacodylate buffer, dehydrated in ascending series or ethanol (percentage) and embedded in Epon-epoxy mixture (McNutt and Crain 1981). Ultrathin

sections were cut and double stained with uranyl acetate and lead citrate and observed under TEM.

Fresh frozen sections at -20°C (12 μ) of the whole skin were also taken to study the distribution of neutral lipids in treated and untreated pigeon apteria, using Nile red (Chapter 1) and Fat red 7B (Chapter 1).

RESULTS

Topical application of 45% glycerol on the dry and scaly skin (apteria) (Fig. 1) of pigeon, for 7 days results in the smoothening of the skin surface. The SC appears smooth and this condition is retained for 10 ± 2 days after glycerol treatment is withdrawn (Fig. 2).

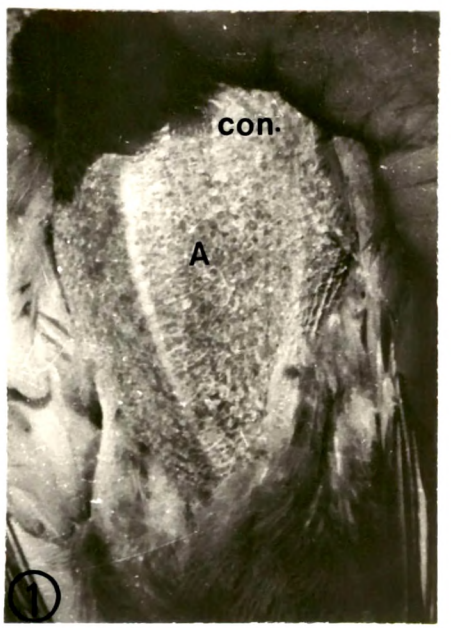
1 μ sections stained with toluidine blue shows swelling of SC (Fig. 7) unlike the control sections, which show highly attenuated corneocytes. The corneocytes also show greater accumulation of lipid droplets compared to the control samples.

Histochemical studies of the glycerol treated pigeon skin, showed a remarkable increase in the staining of the neutral lipids in the SC (Fig. 4) compared to the controls (Fig. 3).

Electron microscopic observations of the control apterial sections, showed number of non-membrane bound lipid bodies (B) in the transitional layers (Fig. 8). These are membrane bound organelles, which show partial lamellar lipid bilayers and partial translucent lipid droplets. These MGB's (LBs), during the course of differentiation lose their membrane and

Fig. 1 : Morphology of a typical, dry and scaly apteria of pigeon (control).

Fig. 2 : Pigeon apteria after topical glycerol treatment. Note the smoothened skin surface after glycerol conditioning (Experimental).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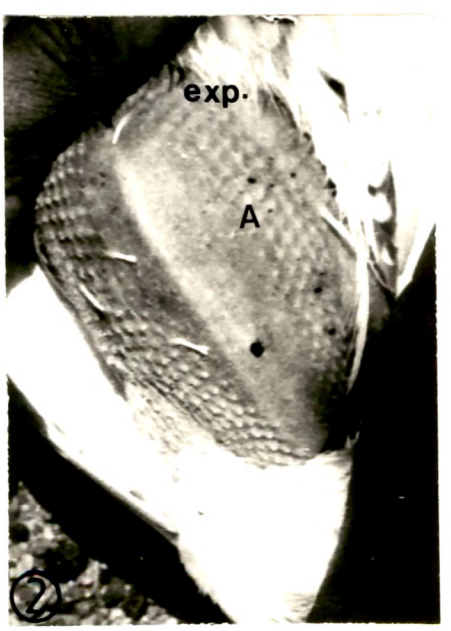
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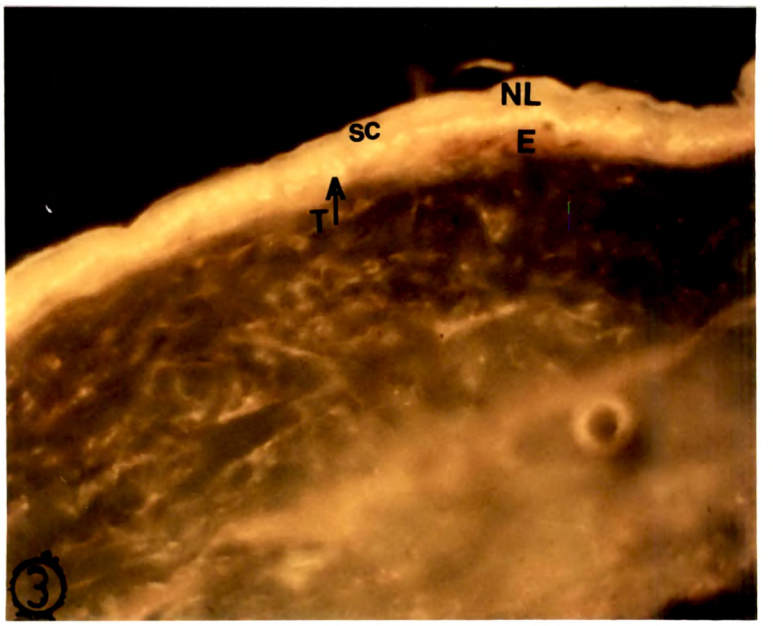
Fig. 3 : Histochemical localization of neutral lipids using nile red in untreated (control) epidermis of pigeon apteria. Note the bright yellow fluorescence in the stratum corneum and the transitional layers. X 50.

Fig. 4 : Histochemical localization of neutral lipids in glycerol treated (experimental) epidermis, of pigeon apteria using nile red. Note an increase in neutral lipid staining in the stratum corneum and transitional layers compared to control. X 50.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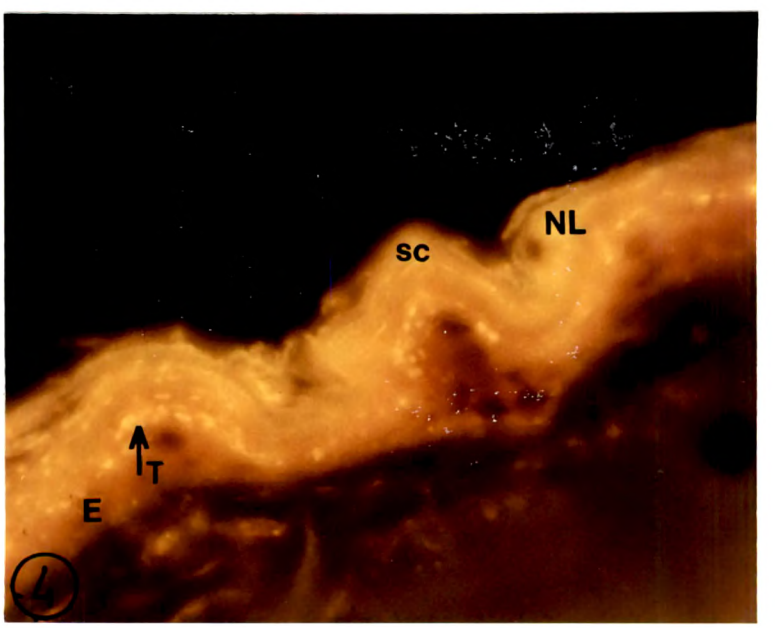
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Fig. 7 : Photomicrograph of glycerol treated epidermis showing swelling of stratum corneum. X 50.

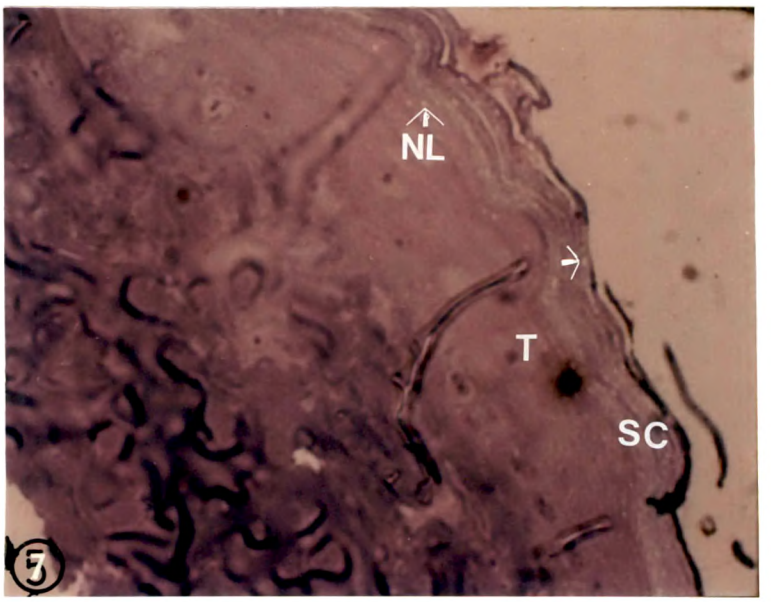
NL
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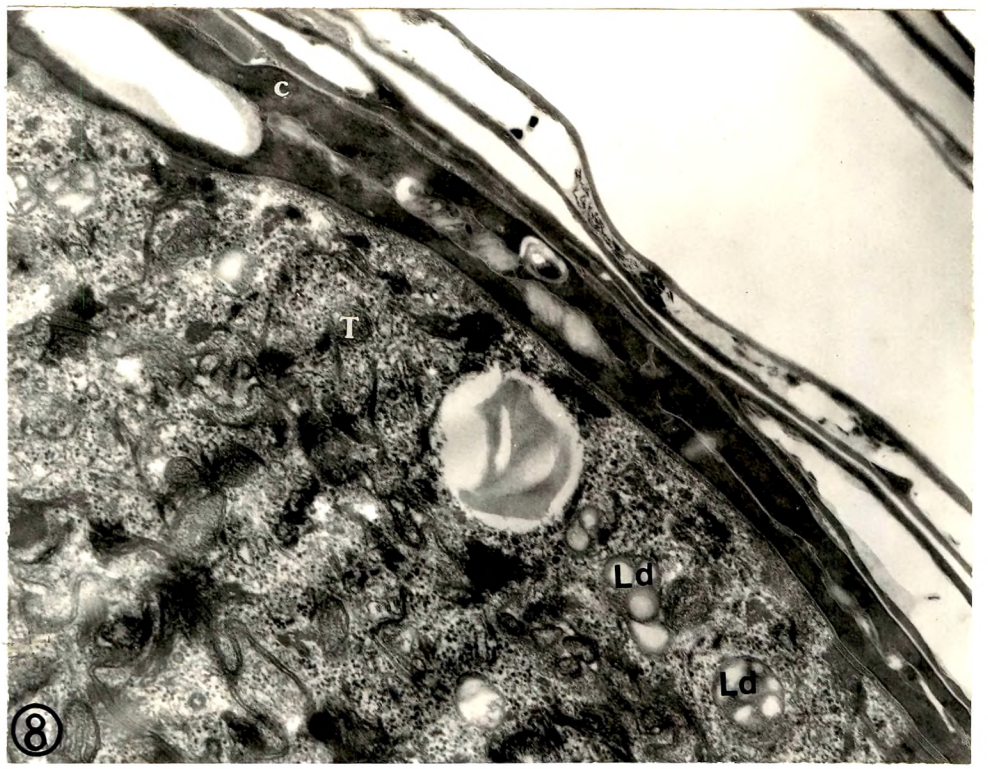
Fig. 8 : Electron micrograph of untreated pigeon apterium, showing attenuated corneocytes and the fusion of lipid droplets in the transitional layer, which are then extruded out into the intercellular space at the SC-SG interface. X 27,000.

Fig. 9-11 : Electron micrographs of glycerol treated pigeon apterium.

Fig. 9 : Transitional layer, showing an increase in the number of lamellar bodies (arrow) compared to control. X 10,000.



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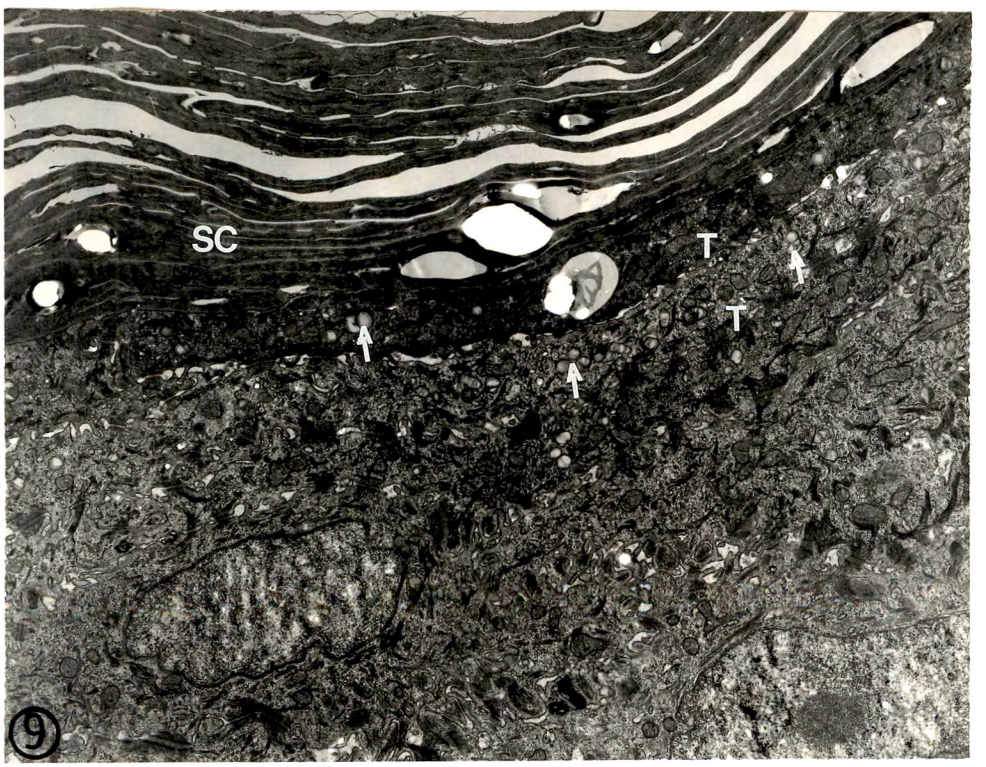

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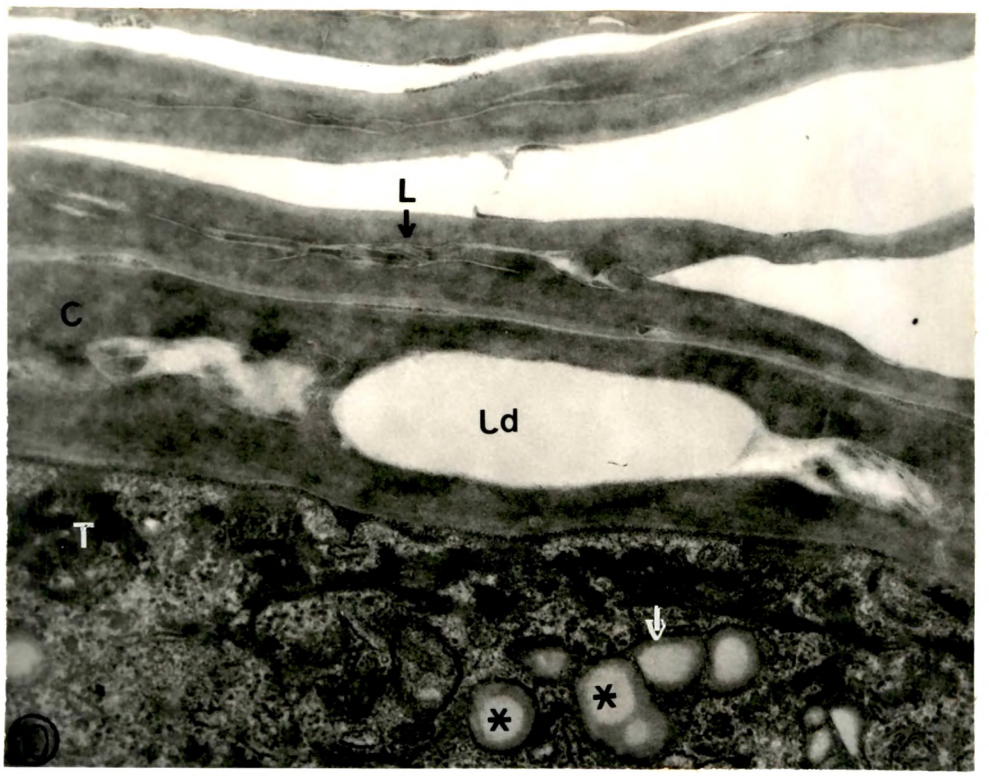
Fig. 10 : Upper corneocytes, showing intracellular retention of lipid droplets and lamellae. X 63,000.

Fig. 11 : Lamellar bodies with a lamellar periphery (arrow) and a translucent core (*). X 40,000.

Abbreviations :

Transitional layer - T; Corneocyte - C; Stratum corneum - SC; Lipid droplet - LD; Retained lamellae - L; Neutral lipid - NL; Apterium - A.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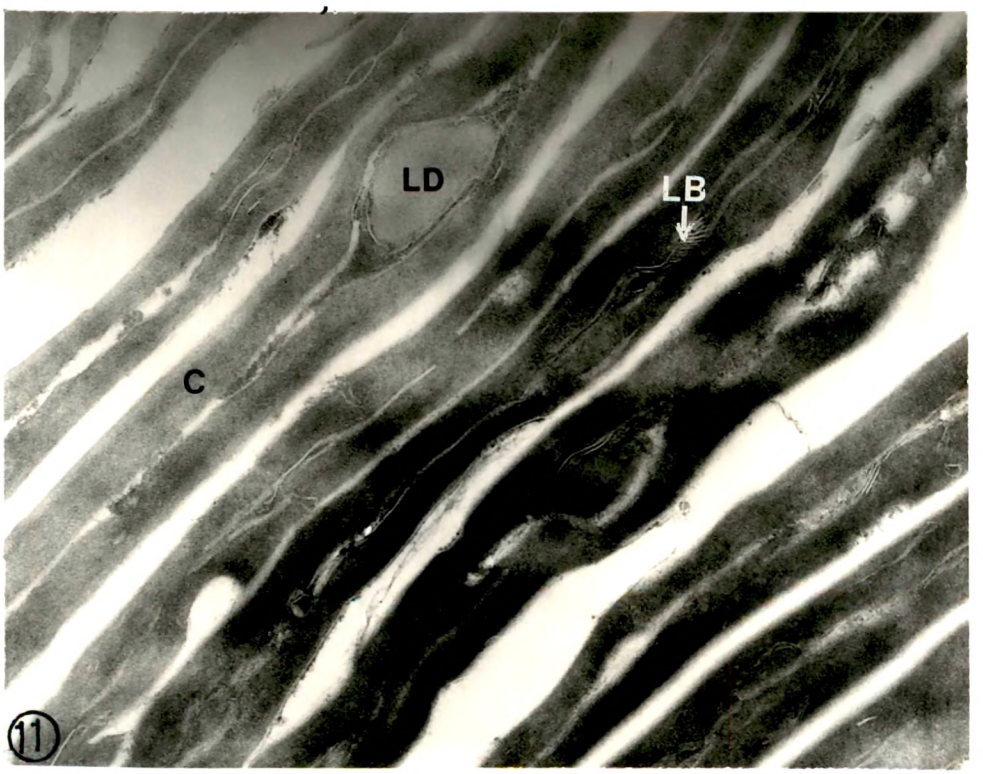
LD
LB
C
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the lipid droplets are directly extruded out of the lower corneocytes into the intercellular space. The corneocytes closely adhere to each other with very less intercellular space. The lower corneocytes are devoid of cell organelles and very less or negligible lipid droplets are retained in the upper corneocyte layers.

Glycerol treated skin, shows comparative increase in the number of the LBs, in the transitional layer (Fig. 9). These bodies are membrane bound and their outer spaces show a lamellar lipid bilayer configuration, while as the core shows translucent lipid droplets (Fig. 10). These LBs lose their membrane and coalesce at the lowermost corneocyte level into large lipid droplets. Unlike control, most of the lipid droplets are retained intracellularly in the upper corneocytes, while little is extruded into the intercellular spaces (Fig. 11).

DISCUSSION

Glycerol is known to diffuse into the SC. The water that is lost trans-epidermally is held by glycerol in the SC, which results in the smoothening of the skin surface by swelling the outer corneal layers (Batt et al., 1988). Batt and Fairhurst (1986), have also shown that glycerol builds up in the skin to form a reservoir and slow release from these pools could account for the persistence of its effect for a longer period.

Removal of the structural lipids by acetone/ether treatment from the SC drastically increases TEWL. This suggests a possible role of the structural lipids in the water holding properties of SC (Imokawa and Hattori, 1985). According to Elias et al. (1979), the intercellular corneocyte lipids form

multiple layers alternating with water. Essential fatty acid deficiency also enhances rate of water loss through SC (Yardley et al., 1981). The effect of glycerol on skin is attributed to the humectant property. Fribery and Osborne (1985), modified the model of Elias, and proposed that free fatty acids in the lipid bilayers of the SC lipids are partially saponified to form a lamellar liquid crystal with water. This balance is not attributed only to the degree of polarization of lipids (i.e. polar to nonpolar), but to the degree of fatty acid unsaturation. These studies propose that glycerol enables the intercellular corneocyte lipids to preserve and maintain the liquid crystal state, thereby reducing water loss.

The findings of glycerol treatment also show an increased number of LBs. These lipids in the LBs are not extruded into the SC-SG interface or in between the transitional and SC interface. Rather, these lipids seem to coalesce to form huge lipid droplets, some of which are extruded directly into the intercorneocyte spaces at the first corneocyte level. But, according to these observations, huge lipid droplets, some even retaining their lamellar configuration, are seen to accumulate in the upper corneocytes. This suggests, a possible role of glycerol to induce lipid synthesis in the skin.

Thus, in avian skin, glycerol probably plays a dual role by decreasing TEWL and possibly inducing lipid synthesis. However, lipid synthesis in human/mammalian dry and scaly skin, after topical application of glycerol remains to be seen.