# 20 Glycerol — Just a Moisturizer? Biological and Biophysical Effects

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## 20.1 INTRODUCTION

### 20.1.1 Glycerol

Gylcerol was discovered in 1779 by the Swedish chemist Scheele and is among the most effective humectant polyols such as sorbitol and mannitol. It is a versatile chemical, and moisturization is due to its high degree of hydroxyl groups, which bind and retain water. Glycerol is found in baby care products and in embalming fluids used by morticians, in glues and explosives; in throat lozenges and in suppositories. Glycerol is a colorless, viscous liquid, and stable under most conditions. Glycerin is nontoxic, easily digested, and is environmentally safe. It has a pleasant taste and odor, which makes it an ideal ingredient in food and cosmetic applications.[1]

Moisturizing agents like glycerol have deeper effects than simply increasing the hydration of the stratum corneum (SC) structural elements. In the last years an increasing number of studies have been accomplished showing new properties of glycerol. Beside the moisturizing benefit attributed to its humectant action, glycerol prevents the SC phase transition. Furthermore, it shows a keratolytic effect by desmosome degradation, influences the protective function of the skin against irritation and penetration of substances through the SC, plasticizes the SC, reduces tissue scattering, stabilizes skin collagen, and accelerates healing processes. Even a virucidal effect of glycerol was reported. The aim of this chapter is to discuss well-known properties of glycerol and to show new aspects in research.



### 20.1.2 Dry Skin

Skin xerosis is related to changes in SC ceramide levels and a disturbance in their structure, as well as to an abnormalitiy in desmosome processing. The consequence of aberrant desquamation is the retention of corneodesmosomes in the superficial layers of the SC.[2] The intercorneocyte linkages are not broken and the peripheral cell does not detach during desquamation. Large clumps of cells accumulate.[3] The resultant incomplete desquamation leads to the appearance of scaly, xerotic, and eczematous skin. A causative factor in reduced corneodesmosomal degradation is the reduction in proteolytic enzyme activity, which again may be caused by intrinsic or extrinsic factors.[4]

Desquamatory proteases and other enzymes mediate their action in the lipid-rich intercellular space and need free water to be active.[3] Disturbed SC lipid structure results in reduced SC hydration and retention of corneocytes on the skin surface. Subsequently skin xerosis becomes evident due to reduced desmosome degradation.[5] The occurrence of dry skin associated with cold, dry weather may result from an extensive, elevated level of skin lipids in the solid state. Thereby, the material that maintains a higher proportion of lipid in the liquid crystalline state may be an effective moisturizer.[6]

An alteration in the generation of natural moisturizing factor (NMF) also may contribute to dry skin formation. Routine soap washing declines NMF levels at the skin surface due to leaching of NMF from superficial SC.[7] Furthermore, aged skin intrinsically has lower NMF levels than younger skin with decreased number of keratohyalin granules and filaggrin in senile xerosis.[8] Dramatic decrease in the environmental humidity reduces total free amino acid generation (and thus the level of NMF and the capacity of the SC to maintain hydration), and subsequently, induces skin surface dryness in the SC.[9]

Dry skin is further characterized by structural changes in corneocyte envelope (CE) as a result of reduced transglutaminase activity. The enzyme is responsible for the transformation of a soft or fragile envelope into a rigid one. Fragile corneocyte envelopes predominate in dry skin.[9]

Abnormalities in lipid lamellar structure or corneodesmolysis are apparent in scaling disorders like X-linked Ichthyosis, atopic dry skin, or in winter xerosis.[2,10] Susceptibility to dry skin also shows a tendency to increase with age.[3] Exposure to dry environment or extreme shifts in external humidity produces important alterations in underlying skin. Dry environment stimulates epidermal hyperplasia and early markers of inflammation. Shift in external humidity induces a profound defect in

permeability barrier function. The clinical effect of these changes ranges from xerosis to aggravation of pre-existing skin diseases.

## 20.2 SKIN MOISTURIZATION

### 20.2.1 Glycerol and Skin Moisturization

Hydration is a key function of the SC. The determinants of SC water content are believed to include the water permeability of the epidermis, the water retaining properties of the SC and the rate of evaporative water loss from the skin surface.[11] The water-retaining capacity of the SC is highly dependent on the phenoptype of the corneocytes, their spatial arrangement, the precise composition and physical packing of extracellular lipids, and the presence of highly hygroscopic compounds between and within the corneocytes.[9]

The SC moisturization is essential for a normal skin physiology. The skin itself preserves water through occlusion (water permeability barrier) and cellular humectancy (NMF). The highly developed lipid lamellae surrounding the corneocytes are a major structural element designed to keep water within the SC.[3] All these lipids are synthesized by the differentiating keratinocytes and form the lipid lamellae during cornification. Lipids help to retain NMF between the corneocytes to allow maximum moisturization of the outer layers of the SC. Effective moisturization helps again to maintain the barrier of the SC. Lipids also influence the activity of certain enzymes within the tissue.[12] Although other lipid species are present in the SC (small amount of phospholipids, glycosylceramides, and cholesterol sulphate), the major lipids are ceramides, cholesterol, and fatty acids. SC lipids are known to be influenced by genetic variation, ageing, dietary influences, seasonal effects, and environmental factors.[12]

The NMF, a mixture of amino acids, derivates of amino acids, and specific salts is a very efficient humectant due to its highly water-soluble and hygroscopic components, which allow absorption of atmospheric humidity and water.[12] Biologically, NMF allows the outermost layers of the SC to remain hydrated despite the desiccating action of the environment. Beside a structural effect due to SC plasticization, NMF also plays a critical role in facilitating key biochemical events.[9] Hydrolytic processes in the SC can only function in an aqueous or semiaqueous environment; an environment effectively maintained by the water-retaining capacity of NMF.[3] NMF is exclusively found in the SC. It is generated by a humidity-regulated proteolytic hydrolysis of filaggrin.[13] This humectant-generating pathway is activated above the stratum disjunctum, as external humidity declines, while the putative aspartate protease, cathepsin B is down-regulated in the lower SC (stratum compactum). Thus, the SC has developed an effective adjustment to environmental conditions in order to be optimally moisturized. This mechanism would not be expected to generate humectants either in the lower SC or under elevated external humidity.

Glycerol is known to diffuse into the SC[14] and retains water in the skin. The water–glycerol mixture than hydrates and plasticizes the skin to prevent dehydration and the resultant physical damage in a stressful environment. Whether glycerol in the viable epidermis can also affect the generation of new SC is not known. Alterations of the course of corneocyte synthesis might result in an SC more resistant to dehydration.[15] Batt and Davis stated, that glycerol acts due to its physical effects on the status of water in the outer layers of the SC.[14] Glycerol may interact with the SC lipid structures or proteins, altering their water-binding and hydrophilic properties.[14] Skin-moisturizing effects depend on the amount of absorbed humectant and their physicochemical properties in SC.[16] It has been reported that the excellent skin moisturization effect of glycerol is due to the high accumulation of glycerol in SC.[16,17] Glycerol forms a persisting deposit/reservoir in the depth of the SC within the lipids without disruption of liquid crystallinity and lamellar structure.[17,18] Ultrastructurally, high-glycerol (25, 40%) caused changes in human skin consisting of intracellular expansion of corneocytes and intercellular expansion between corneocytes (bulking).[18] The expansion was evident throughout the full thickness of the SC. The "bulking" is believed to enhance the resilience of skin exposed to

harsh climatic conditions (enhance barrier characteristics of the SC, which, in turn, leads to a new effective moisturization of the skin). On the other side, undiluted glycerol leads to a dehydration of the skin upon osmotic actions and produced ultrastructurally only minimal or superficial changes in the appearance of the SC.[18]

### 20.2.2 Glycerol and Skin Hydration

The smallest polyols [ethan-1,2-diol, glycerol and polyethylene glycols (PEGs)] are miscible with water in all proportions, that is, they have an infinite solubility in water. Cohen et al. stated that the higher the solubility of polyols, the higher the ability to absorb water.[19]

Bissett et al. investigated the effect of glycerol formulations on lower legs with dry skin. The effectiveness of glycerol was dose dependent with a maximal benefit at 20 to 40%. An important factor is the total quantity of applied glycerol.[15]

Gloor et al. observed the concentration dependency of the hydrating effect of glycerol. An increase in the dose of glycerol from 5 to 10% in an oil-in-water-emulsion improved the SC hydration and protective effect against the dehydration by tensides.[20]

Fluhr et al. presented similar results.[21] Four different vehicles (water in oil and oil in water emulsion) and two different glycerol concentrations (5 and 10%) were tested. 10% glycerol was more efficient than 5%, independent of the basic formulation. However, the o/w emulsion seemed to be more effective than the w/o formulation.[21]

Okamoto et al. investigated the skin-moisturizing effect of glycerol depending on the absorbed amount in SC and the concentration profile. The skin-moisturizing effect increased linearly with the amount of absorbed humectant in the SC and was dependent on the hygroscopicity of the humectants. A repeated application twice daily for 10 days leads to an accumulation of glycerol in SC.[16]

### 20.2.3 Glycerol and Hygroscopicity

Humectancy or hygroscopicity is the tendency of a substance to absorb water from the surroundings.[22] Pure glycerol for example, absorbs its own weight in water over 3 days.[11] The connection between *in vitro* humectancy and *in vivo* moisturization is not a simple correlation. Glycerol, which had the lowest humectant activity *in vitro*, from the set glycerol, diglycerol, and triglycerol, was the best eliminating the signs of skin dryness (erythema, SC hydration) in a guinea pig model.[22] The widespread concept has to be challenged that if a material is capable of absorbing water either from the environment or from the skin tissue, then it is a clinically useful moisturizer.

Froebe observed that glycerol behaves as a humectant at high humidity (92% relative humidity), but not at very low humidity (6% relative humidity).[23]

### 20.2.4 Gylcerol and Evaporation

Changes in transepidermal water loss (TEWL) following glycerol treatment are an instrumental evidence for skin moisturization induced by glycerol.[17] Thereby, single application tests can be predictive of long-term results with humectant-based moisturizers. Electrical measurements of skin conditions correlate well with skin grades.[24] Topical applied water produced only a transient benefit due to the rapid evaporation. Glycerol applied under controlled ambient conditions (relative humidity: not exceeding 65%, room temperature: 20°C) reduced the magnitude of the natural water flux from the skin surface and the rate of evaporation of water from applied aqueous solution.[14,17] TEWL values were significantly and persistently reduced as well as the skin surface profile roughness after treatment with glycerol compared to water treated areas.[14,17] This might be one explanation why seasonal changes caused by low relative humidity can be prevented by glycerol.[25,26]

## 20.3 PREVENTION OF THE SC PHASE TRANSITION

### 20.3.1 Skin Barrier Organization — Role of Lipids

Structure of SC and its lipid content affect the permeability barrier function. Visualization studies revealed that the penetration route across the SC resides in the intercellular tortuous pathway between the corneocytes. This implies that SC lipids play a key role in the skin barrier function.[27] Another major controlling element in barrier homeostasis seems to be the epidermal Calcium ion.[28]

In the SC lipids form two crystalline lamellar phases.[27] The mixture of both phases produces the optimal barrier to water loss from SC. The balance between the liquid crystalline and the solid crystal phases is determined by the degree of fatty acid unsaturation, the amount of water, and probably by other yet undiscovered factors. A pure liquid crystal system, produced by an all-unsaturated fatty acid mixture, allows a rapid water loss through the bilayers with a moderate barrier action. The solid system produced with an all-saturated fatty acid mixture causes an extreme water loss due to breaks in the solid crystal phase.[6,23] Studies with mixtures prepared with isolated ceramides revealed that cholesterol and ceramides are very important for the formation of the lamellar phases, and the presence of ceramide 1 is crucial for the formation of the long-periodicity phase.[27] The occurrence of dry skin associated with cold, dry weather for example, may result from an extensive, elevated level of skin lipids in the solid state. Therefore, a material that maintains a higher proportion of lipid in the liquid crystalline state may be an effective moisturizer.[6]

### 20.3.2 Glycerol and SC Phase Transition

Froebe reported the prevention of SC phase transition *in vitro* by glycerol. Glycerol 10% in a SC lipids mixture inhibited the transition from liquid to solid crystals even when water content was reduced by low humidity (6%). At high humidity, but not at low humidity glycerol acts as a humectant. Therefore, glycerol might act as a skin moisturizer and skin conditioner by inhibiting lipid phase transition from liquid to solid state in dry atmosphere.[23] It is hypothesized that glycerol maintains the fluidity of the lipid membrane through interaction with polar head groups of the lipid bilayers rather than by penetrating the alkyl chains.[23,29] In sum, glycerol seems to enable the skin lipids to preserve its normal structure even when underhydrated.

## 20.4 KERATOLYTIC EFFECT BY DESMOSOME DEGRADATION

### 20.4.1 Desmosomal Degradation

Desmosomes are critical structural elements for the cell–cell adhesion complex between adjacent keratinocytes. They are dynamic cell components, whose composition and structure are critical for normal epidermal function, tissue morphogenesis, and differentiation.[30,31] Regulation of desmosomal assembly and disassembly appears to include both internal and external mechanisms.[30] Calcium plays a key role in maintaining desmosomal integrity, while signal transduction between desmosomes and adherent junctions appears important to regulate their assembly and disassembly.[30] Corneodesmosomes are the main cohesive force within the SC.[32] Other components that contribute to the SC cohesion are the van der Waal's forces holding together the lipid lamellae and the corneocyte interdigitation.[32]

The cohesive forces holding the corneocytes together are progressively degraded to allow a regulated cell shedding at the surface of the skin, a process known as desquamation.[3] Thereby, the enzymatic degradation of inter-corneocyte linking structures, or a reduction in intercorneocyte forces, must occur in a carefully controlled manner in order to maintain the integrity, and thus epidermal barrier function.[3] The desquamatory enzymes are believed to be extracellular. The most important

enzymes are the stratum corneum chymotryptic (SCCE) and stratum corneum tryptic enzyme (SCTE) as well as Cathepsin D.[33,34] Their activity is pH dependant. SC lipid phase behavior will influence enzymatic activity. This indicates that the maintenance of the water content of the SC is vital for the normal orderly process of cell loss from the surface of the skin. The SC desquamatory proteases are critically influenced by water activity within the tissue, and desmoglein 1, desmocollin 1, and corneodesmosin degradation are all reduced at low environmental humidity.[35–37] In sum, insufficient SC moisturization and water content leads to defective desquamation.[12,37]

### 20.4.2 Glycerol and Desmosomal Degredation

Desmosomal degradation has been shown to be a humidity-dependent event. The degradation is significantly reduced at low relative humidity. Rawlings et al. demonstrated that at high (80% relative humidity) but not at low relative humidity (44% relative humidity) glycerol further enhanced desmosomal degradation. This enhanced desmosomal degradation was confirmed by decreases in levels of immunoreactive desmoglein 1, a marker of desmosome integrity. Measurements of the mechanical strength of SC sheets using an extensometer indicated a reduction in the intercellular forces following glycerol treatment. One possible explanation for the effects of high humidity and glycerol on desmosomal structure is that they influence the activity of desquamatory enzymes due to SC water regulating key proteases involved in the protein degradation. Beside the humectant properties of glycerol, the lipid-phase modulating and occlusive properties may also contribute to the improvements in SC desquamatory enzyme activity crucial to desquamatory process.[37] The increase in desmosome digestion following glycerol treatment may be important in subjects, for example, with skin xerosis. The enhanced desquamation seems to be an initial effect to detach nonphysiological scales. In the second step glycerol seems to strengthen the SC integrity.

## 20.5 PROTECTION AGAINST IRRITATION

### 20.5.1 Protection against Irritation

Bettinger et al. performed a standardized washing procedure after pretreatment with 10% glycerol-containing o/w emulsion compared to vehicle. The glycerol-containing emulsion inhibited the dehydration of washing in contrast to vehicle.[38] Grunewald et al. investigated different barrier creams including glycerol (10%) applied in an oil-in-water emulsion regarding their efficacy against repetitive washing with Sodium Lauryl Sulfate (SLS). Glycerol-containing o/w emulsion led to a protection against the barrier-damaging and irritating action of SLS. Especially the protection against skin dehydration was remarkable due to the hygroscopic effect of glycerol.[39] Bettinger et al. induced a skin damage by either tape stripping or acetone treatment and applied glycerol (70%) or tap water in an occlusive way.[40] After 5 h they compared the barrier function using biophysical tests. Glycerol associated with occlusion led to a faster reconstitution of the protective skin barrier compared to water. The reactions against DMSO, NaOH, and SLS were significantly diminished in glycerol-treated areas. Glycerol sustains the transepidermal water flow, at least partially, despite an occlusive film and leads to a faster reconstitution of the skin barrier.[40]

Fluhr et al. investigated the influence of glycerol on the recovery of damaged SC barrier function. The skin of the test sites was initially damaged by tape stripping and treated with glycerol (99.8%) (glycerol, glycerol and occlusion, occlusion alone, untreated field) for three days. Glycerol alone and glycerol with occlusion improved barrier function. Occlusion alone did not result in changes in barrier repair and SC hydration, which was controversially discussed in literature earlier.[41–45] Occlusion and glycerol together were capable to enhance moisturizing properties of the system, but not to influence the water flux through deeper layers of the SC and therefore barrier repair induced by glycerol itself. Glycerol, by absorbing water, can stimulate a water flux creating a stimulus for barrier repair. The observed effects were based on the modulation of barrier repair and were not biased by

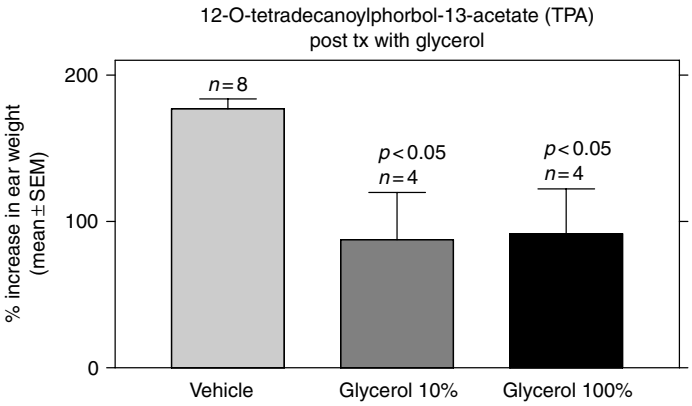


**FIGURE 20.1** Both applications (10% aqueous solution and 100%) reduced the TPA-induced ear swelling significantly compared to water (vehicle) by about 50%.

the humectant effect of glycerol.[46] In the second step, an irritation was induced by repetitive washing using 2% SLS solution for 4 days (3 times daily).[46] After that the test areas were also treated with glycerol (25 and 50% glycerol, 33,3% DAC base cream, 41.7 and 16.7% water). The treatment was performed for 3 days, 3 times per day. Even 7 days after the end of the treatment with glycerol an increased SC hydration and a reduced transepidermal water loss was observed. Especially, TEWL has a great importance for repair mechanism of the epidermis after barrier damage.[46] It has been shown that glycerol protects against irritation caused by washing procedure.[38,46,47]

Unpublished data on a TPA (12-O-tetradecanoylphorbol-13-acetate) irritation model[48] could show that glycerol pretreatment prevented ear swelling in a nondose dependent way (Figure 20.1). Irritant contact dermatitis was induced by a single topical application of 10 ml of 0.03% (wt/vol. in acetone) TPA on the inner and outer surfaces of the left ears of male mice. The right ears were treated with vehicle alone (acetone). At 18 h, when TPA-induced inflammation is maximal, ear thickness was measured with a digital caliper (Mitutoyo Corp., Tokyo, Japan). Ear swelling, measured by thickness and weight, was calculated according to the following equation:

$$\text{Ear swelling } (\%) = 100 \times (a - b)/b$$

where $a$ is the thickness or weight of treated left ear and $b$ is the thickness or weight of control right ear. Both applications (10% aqueous solution and 100%) reduced the TPA-induced ear swelling in hairless mice significantly in comparison to water (vehicle) by about 50%.*

### 20.5.2 Penetration Enhancing Effect of Glycerol

Bettinger et al. described a penetration-enhancing effect of glycerol. A significant increase in hexyl nicotinate penetration on a glycerol-treated site was observed.[40] The explanation for the effect includes the interaction of glycerol with intercellular lipids, the inhibition of the lipid transformation by glycerol, the desmolytic effect of glycerol, and the hydrating effect of glycerol.[40]

### 20.5.3 Accelerating the Healing Processes

The restorative properties of high-glycerin therapeutic moisturizers are hypothesized to be related to a glycerol reservoir within the SC. This provides a mechanism for enhancing barrier characteristics

*This study was performed in collaboration with A.J. Sagiv, School of Pharmacy, Cell Pharmacology Unit, University of Jerusalem, Israel.

of the SC, which, in turn, leads to a new effective moisturization of the skin. The suggested role of glycerol in normalization of barrier function is essential in the healing of dry skin and in wound healing.[18]

### 20.5.4 Protection against X-ray and 365 nm Ultraviolet Light

It has been reported that glycerol protects bacterial cells and transforming DNA against both x-rays and 365 nm ultraviolet light. The mechanism whereby glycerol acts is unknown.[49]

## 20.6 INFLUENCE ON PHYSICAL PROPERTIES OF THE SKIN

### 20.6.1 Mechanical Properties of the Skin — the Role of the Epidermis

The epidermis plays a role in skin mechanics. Thereby, hydrophilic as well as hydrophobic substances affect mechanical properties of the skin. Changes in skin mechanics can be the result of either a direct influence of a substance on the intercellular matrix, or an epiphenomenona, for example, a physiological shift of water between the tissues aimed to maintain physiological homeostasis.[50] The hydration level of the SC affects its mechanical properties. Increased hydration of the SC influences its extensibility and elasticity.[51–54] Examples from human diseases such as ichthyotic and xerotic disorders indicate that thickening of the SC due to hyperkeratosis and increased corneocyte cohesion is responsible for a marked decrease in the flexibility of the entire SC.[55]

### 20.6.2 Plasticizing and Smoothing Effect of Glycerol

Batt and Fairhurst investigated the changes in SC, which occurred after application of water, occlusion (4 h), or glycerol. The hyperhydration resulting from complete suppression of TEWL by occlusion induced topographical changes on the skin surface. A general flattening of the skin surface was observed consistent with the swelling of the SC due to hydration of the tissue. Treatment with aqueous glycerol over 4 h induced a significant, long-lasting reduction in surface profile roughness for at least 20 h comparable to those observed after occlusion.[17]

In 1988, Batt et al. again observed the changes in physical properties of the SC following treatment with glycerol or water. The results showed that treatment with water produced a rapid but short-lived response characterized by a reduction in TEWL and in electrical impedance, smoothing of the skin surface profile, and an increase in the coefficient of friction. Application of glycerol-containing solutions (5 and 15%) and products (o/w cream 10% glycerol, o/w lotion 15% glycerol), in contrast, increased and extended the observed effects.[14]

Overgaard et al. investigated the short-term influence of tap water and glycerol on skin mechanics (hysteresis, distensibility, elasticity and resilient distensibility). The substances were applied on the forearms of healthy volunteers in an occlusive way for 10 min. Immediately and 10 min after removal of the test substances measurements were performed. Glycerol created a significant change of hysteresis and distensibility. Water compared to glycerol appeared to have a short-term effect on hysteresis and distensibility, marked by a pronounced increase and a fast return to baseline. Glycerol had a slower increase and a more prolonged effect suggesting that the outermost layers of the skin have been altered more substantially. It might be possible that glycerol attracts water by osmosis from the deeper layer of the epidermis.[50] The rapid onset of changes favors a more direct action but may be due to the ability of each substance to penetrate the SC.[50,56]

Pederson et al. studied the influence of water and glycerol on skin mechanics. Both substances were applied on the forearm and changes in hysteresis and distensibility were quantified.[50] They showed, in contrast to Overgaard et al., that glycerol induced a more rapid onset on the hysteresis (after 3 min) than water. The glycerol effect was detectable until the end of testing (15 min). Distensibility showed a transient increase induced by glycerol, while no changes were seen with water. Altogether, the onset of action for both substances, water and glycerol, was rapid. Therefore the effects were supposed to take place in the outermost layers of the epidermis.[50] The immediate effect of glycerol may be related to the reservoir formation, rather than to a more profound effect to the epidermis.[50,57]

Rigal and Leveque demonstrated in a long-term study a pronounced effect of 10% glycerol (o/w emulsion) regarding mechanical properties of the skin, which persisted up to one week after the treatment (treatment of three weeks).[58]

### 20.6.3 Reduction of Tissue Scattering

Skin is a highly complex structure consisting of many inhomogenities. Much of the light scattering in biological tissues is due to its variation in polarization, which can be characterized by variations in the index of refraction. Cellular and intercellular components contribute to the scattering properties of the skin.

Vargas et al. applied glycerol to rat and hamster skin and observed an alteration in optical properties.[59] The transmittance increased and a decrease in diffuse reflectance occurred after an application of glycerol on the dermal site of the skin *in vitro*. *In vivo* injection of glycerol allowed a better visualization of structures in the dermis. It was hypothesized that glycerol reduced random scattering primarily by localized dehydration and better index matching with collagen. Glycerol has a refractive index of about 1.47 which is similar to that of collagen.[60] Furthermore, the application of glycerol causes cells in the skin to shrink. A reduction in diameter with no change in refractive index or volume fraction would result in a decrease in scattering contribution from these cells. The complete mechanism that causes reduction in scattering is not fully understood at this time.[59]

### 20.6.4 Stabilization of Collagen

Native collagen binds glycerol preferentially whereas denatured collagen neither binds nor repels glycerol. The surface interaction of native collagen with glycerol is energetically more favorable than its interaction with water. Glycerol stabilizes the triple-helical structure of solubilized calf skin collagen and may lead to the inhibition of the *in vitro* self-association of monomers into fibrils.[61]

## 20.7 NEW ASPECTS IN RESEARCH

### 20.7.1 Aquaporin-3

The aquaporins are a family of small, integral membrane proteins that function as plasma membrane transporters of water and in some cases small polar solutes. There are at least 10 distinct aquaporins in mammals with specific expression patterns in epithelial, endothelial, and other tissues. Studies in aquaporin-null mice indicated a key role for aquaporins in the urinary concentrating mechanism, fluid secretion of glands, brain swelling, skin moisture, hearing and vision, and gastrointestinal absorption.[62]

The mode of action of glycerol both on SC hydration and epidermal barrier function seems to be related to the aquaporin-3 (AQP3) channel. The basal layer of epidermal keratinocytes contains AQP3, a small membrane protein that functions as a facilitated transporter of water and glycerol.[11] Glycerol is transported very slowly into the epidermis and thus its transport rate is sensitive to the intrinsic glycerol permeability of the basal keratinocyte layer.

Mice deficient in AQP3 have threefold reduced SC water content compared to wild-type mice, a reduced skin elasticity, and delayed SC barrier recovery after tape stripping.[63,64] AQP3 null mice express a more than twofold reduced glycerol content in SC and epidermis, without altered serum and dermal glycerol levels. The reduced SC hydration in AQP3 deficient mice could not be corrected by skin occlusion or placement in a humectant atmosphere, indicating that water transport through AQP3 is not a rate-limiting factor on SC hydration. Glycerol applied topically or systemically, but not glycerol analogs, corrected SC hydration defect, reduced skin elasticity and delayed barrier recovery.[11] Analysis of glycerol dynamics indicate an impaired glycerol transport into the epidermis and SC through the relatively glycerol impermeable basal keratinocyte layer resulting in reduced epidermal and SC glycerol content in AQP3-deficient mice.[11] The reduced SC glycerol content has the consequence of a decreased SC hydration due to the water-retaining (humectant) properties of glycerol. Reduced skin elasticity results directly from reduced SC hydration. Another consequence of reduced epidermal cell glycerol content is the delayed restoration of the barrier function after acute barrier disruption.[11]



### 20.7.2 Sebaceous Glands and Glycerol

Fluhr et al. showed that glycerol regulates SC hydration in sebaceous gland deficient (Asebia) mice.[65] The Asebia mice present normal epidermal barrier function and lamellar membranes despite the presence of sebaceous gland hypoplasia. These mice are characterized by evidence of epidermal hyperplasia, mast cell proliferation, and profound abnormalities in SC hydration (20 to 50% normal). Furthermore, the SC showed a significant depletion, but not an elimination, of nonpolar lipids of presumed sebaceous gland origin. The endogenous glycerol levels were profoundly reduced in SC as well as lipase activity in sebaceous gland structures.[65] The abnormal hydration could be corrected by topical glycerol, while sebaceous lipids, including topically-applied glycerolipids, water alone, and other humectants were ineffective. These results demonstrate the requirement for sebaceous-gland-associated lipases in the generation of the hydrating fraction of glycerol in normal skin. Glycerol generation occurs primarily within the pilosebaceous follicle, rather than at the skin surface.[65] In sum, cutaneous sebaceous glands seem to be an important source for the hydrating fraction of glycerol.

### 20.7.3 Glycerol and Corneocyte Surface Area

In an unpublished study we could show in an *ex vivo* assay that topical application of glycerol 9.0% compared to NaCl 2.9%, a $H_2O$ control area and an untreated site significantly reduced the corneocyte surface area. The corneocyte surface area was assessed with the VIC method.[66] The corneocytes were collected *in vivo* using a modified detergent scrub technique.[67–69] A metal ring (diameter: 28 mm) was firmly pressed on the ventral forearm. One milliliter of Triton X-200, 0.5% (RADIM, Italy) in 0.075 M phosphate buffered saline (pH 2.5) was pipetted inside the metal ring. The skin surface was gently scrubbed for 60 sec with a Teflon™ stick. The corneocyte containing detergent solution was pipetted into a 1.5 ml Eppendorf-tube and centrifuged with 2800 rpm for 40 sec in order to concentrate the cells. The 20 $\mu$l of the cell concentrate were extracted from the bottom of the Eppendorf-tube and transferred onto a microscope slide. The liquid aliquot was dried for 5 min. A videomicroscope picture was taken with VMS 70 A Video Microscope (SCALAR, Japan) with 200× amplification. In order to get a better and standardized contrast, the microscope slice was put on a black evaluation sheet of D-Squame™. An area with well-separated corneocytes was selected and approximately 50 corneocytes on two different sites of the specimen were measured. The images were analyzed using NIH Image™ 1.59, USA on a Macintosh-PC with the same threshold for all pictures. The surface area was calculated in pixels.

Twenty-seven healthy volunteers with a mean age of 42 (range 31 to 56) were included in the study. The mean temperature during the study was 21.7°C (range 24 to 32%) and the relative humidity of 27% (Range 24 to 32%). The three aqueous solutions (Glycerol 9.0%, NaCl 2.9%, $H_2O$) were

swiped with a cotton wool tip for 2 min on a surface of 2 × 3 cm. The cotton wool tip was soaked with the solutions at the beginning and after 1 min.

Noninvasive measurements were performed for 2 h soaking the surface area. The SC hydration was measured with a capacitance based Corneometer CM 820, visco-elastic parameters [total extensibility (Uf) and elasticity (Ua/Uf)] were assessed using the suction device Cutometer (all instruments: Courage&Khazaka electronics GmbH, Cologne, Germany). The visco-elastic parameters were assessed as surrogate (indirect) measurements of deeper effect on SC hydration[70] while capacitance assessed the more superficial part of the hydration.[71]

The study could show that glycerol induced a shrinking of superficial corneozytes, which is independent from osmotic effects (Figure 20.2). An equimolar NaCl solution had no significant influence on the corneocytes surface area compared to untreated and $H_2O$ treated corneocytes. Only a mild, but significant increase of SC hydration, measured by capacitance was monitored (Figure 20.3).



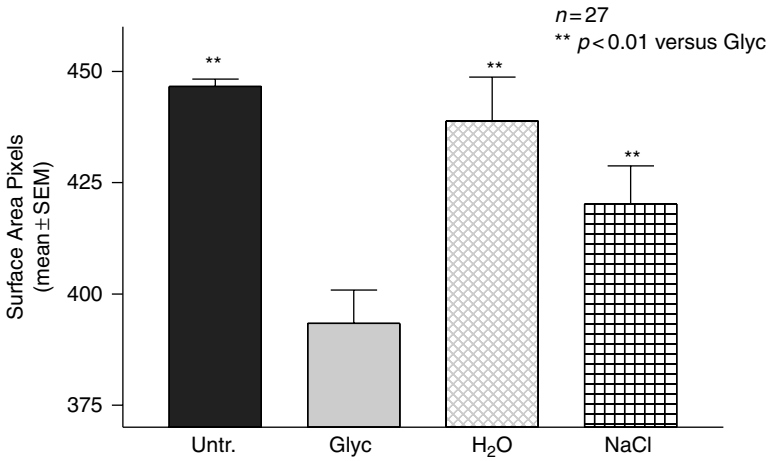


**FIGURE 20.2** Glycerol induced a significant shrinking of superficial corneocytes, which is independent from osmotic effects. An equimolar NaCl solution had no significant influence on the corneocytes surface area compared to untreated and $H_2O$ treated corneocytes.

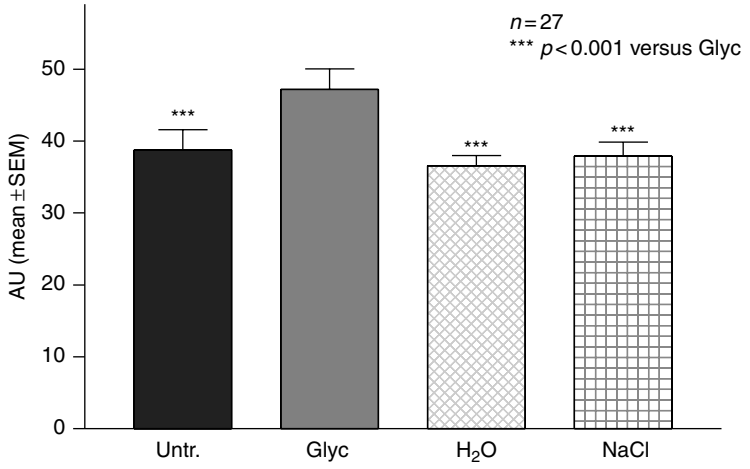


**FIGURE 20.3** Only a mild, but significant increase of stratum corneum hydration, measured by capacitance was monitored.

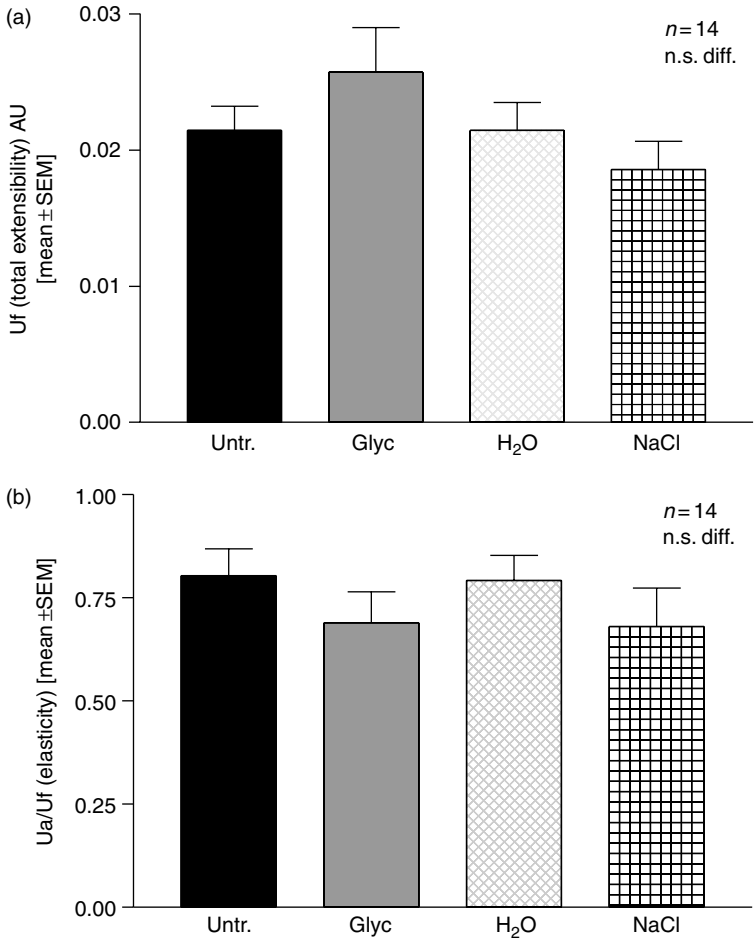


**FIGURE 20.4** Deeper effects of glycerol on stratum corneum hydration could be ruled out by assessing the indirect hydration related mechanical parameters of deeper parts of the epidermis, namely the total extensibility (Uf) [Panel a] and the elasticity (Ua/Uf) [Panel b].



However a deeper effect of glycerol in our short-term model could be ruled out by assessing the indirect hydration related mechanical parameters of deeper parts of the epidermis, namely the total extensibility (Uf) (Figure 20.4[a]) and the elasticity (Ua/Uf) (Figure 20.4[b]). Long term application might induce smaller surface corneocytes resulting in a more compact SC. Subsequently this effect might be an explanation of the preventive properties of glycerol-containing formulation in irritant contact dermatitis.[47,72] The mechanisms responsible for this glycerol-specific effect of corneocyte shrinking is yet to be studied.

### 20.7.4 Glycerol Concentrations and Formulations

The composition of the formulation has been shown to be critical for the delivery of a maximal glycerol benefit.[3] The concentration of glycerol is important. It has been shown that glycerol is an effective moisturizer and skin conditioner when used at levels above 3%.[73] Undiluted glycerin can actually serve to dehydrate skin, based upon osmotic action. Later it was reported that even 1% glycerol has a hydrating effect, at least when applied together with bilayer-forming lipids, phospholipids, cholesterol, and stearic acid.[5] As described by Fluhr et al. 10% glycerol was more

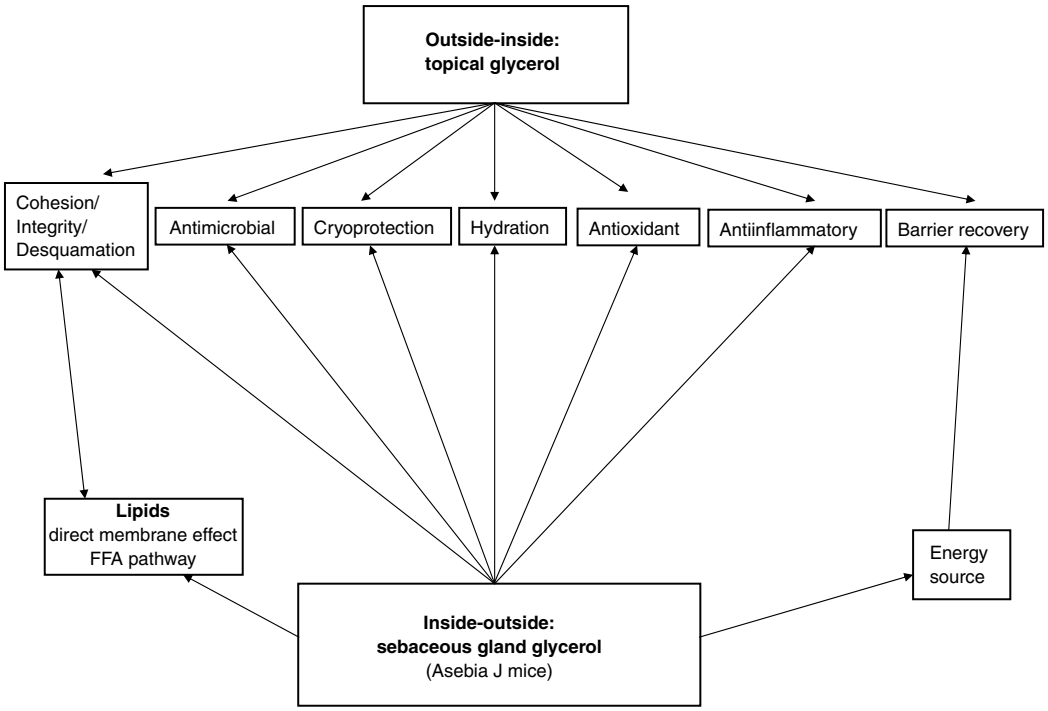


**FIGURE 20.5** Current concept on functions of topical glycerol (outside–inside-concept) as well as the functions of endogenous glycerol (inside–outside-concept).

efficient than 5%, independent of the basic formulation used. And the combination of 5% glycerol and 5% urea was more effective regarding the hydrating and protective properties than 10% urea or 10% glycerol. Explanations for the observed effect included the possibility that urea enhances the penetration of glycerol into deeper layers of the SC and thus improves function of glycerol.[21] Observations performed by Bettinger and Fluhr showed that the glycerin effect was more pronounced when used in an o/w emulsion compared to a w/o emulsion.[21,74]

Treatments with glycerol in water reversed the skin dryness induced on the skin of guinea pigs using 2% SLS solution. When dissolving glycerol in medium chain triglycerides (MCT) oil, no moisturizing effect was detected. Without a certain amount of water, glycerol is probably inactive in the MCT oil. The mere presence of an adequate amount of a humectant-moisturizer in a cosmetic product is not a proof of efficiency.[75]

### 20.7.5 Virucidal Effect

van Baare et al. studied the virucidal action (against herpes simplex virus, poliovirus, and human immunodeficiency virus) of various concentrations of glycerol at different temperatures.[76] Glycerol has a virucidal activity. The virucidal interaction is dependent on its temperature and concentration. Glycerol might influence the enzymatic processes of nucleic acid breakdown.[76]

## 20.8 SUMMARY

Glycerol is a hygroscopic, nonvolatile, and viscous substance that shows special benefit as a humectant in comparison to liquid and crystalline polyols. Glycerol has been used as an effective moisturizer and humectant in cosmetic products and is recognized as an over-the-counter skin protectant.[73]

Glycerol hydrates the SC.[16,20,21] It is a humectant due to absorption of water from the atmosphere[22,23] and reduces the evaporation rate from the skin surface.[14,17,25,26] It has been shown that glycerol forms a persisting reservoir in the depth of SC (bulking).[16–18] Furthermore, it prevents lipid phase transformation.[23] Improvement of SC desquamatory enzyme activity and desquamation itself is also induced.[37] Glycerol protects against irritation caused by washing procedure,[38,46,47] tape stripping,[40,46] or acetone treatment.[40] The influence on the mechanical properties of the skin includes a plasticizing and smoothing effect of glycerol,[14,17] the reduction of tissue scattering,[14,17,59] and the stabilization of collagen.[61] Even a virucidal effect of glycerol was reported.[76] New research indicates that the mode of action of glycerol both on SC hydration and barrier function is related to the AQP3 channel.[11,62–64] Furthermore, cutaneous sebaceous glands seem to be an important source for the hydration fraction of glycerol.[65] The action of glycerol depends on the concentration[5,20,21,73] and the formulation.[21,74,75]

Glycerol is a key molecule in skin physiology in terms of its primary humectant and biosynthetic functions, and the secondary effects of increased SC hydration.[11] It is not surprising that glycerol is effective in treatment of dry skin conditions due to the fact that dry skin in the broadest sense of the words is associated with aberrant corneodesmolysis,[3] barrier lipid disruption,[3] and alterations in the generation of NMF.[7] Understanding the mechanism of action of glycerol also supports the understanding of diseases associated with dry skin, for example, ichthyosis, atopic dermatitis, winter xerosis, and other. Figure 20.5 summarizes the current concept on functions of topical glycerol (outside–inside-concept) as well as the functions of endogenous glycerol (inside–outside-concept). The latter has yet to be confirmed in *in vivo* studies with human volunteers.



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