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The effect of lipids, with and without humectant, on skin xerosis

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Synopsis

Specialized lipids found in the stratum corneum, namely ceramides, have been shown to have beneficial skin properties due to their lipid bilayer-forming potential in the presence of cholesterol and fatty acids. We were interested in determining whether other bilayer-forming lipids, such as phospholipids, could deliver similar benefits and how these benefits compare with common moisturizer ingredients such as petrolatum and glycerol. We investigated a bilayer-forming mixture of phospholipids, cholesterol, and fatty acid for its effectiveness in treating soap-induced winter xerosis in double-blind, fully randomized clinical trials in which dryness on the dorsal aspect of the hands was visually assessed using a seven-point grading scale. Sixty-six healthy Caucasian women aged over 25 underwent a one-week soap "dry-down" and were treated for two weeks with twice-daily product applications in balanced treatment groups (N = 11). Treatments were compared statistically using repeated Wilcoxon rank sum tests (critical significance level of 5%). Examination of improvements in xerosis *in vivo* established that emulsions of phospholipids, cholesterol, and stearic acid alone, or thickened solutions of glycerol alone, did little to alleviate skin xerosis. When lipids and glycerol were combined, however, the emulsions acted in a synergistic way and reduced skin xerosis significantly and rapidly. In contrast, when phospholipids were replaced with petrolatum in the emulsions, the rate of xerosis alleviation was reduced, which implies that the bilayer-forming capabilities of phospholipids may be important in delivering optimal skin benefit. These studies demonstrate that lipids with bilayer-forming capabilities such as phospholipids can rapidly alleviate skin xerosis when combined with glycerol.

INTRODUCTION

Stratum corneum moisturization is essential for the normal functioning of the skin. The degree of moisturization depends directly on the inherent humetancy properties of the stratum corneum and on its water barrier function (for review, see reference 1). The structure of stratum corneum and its lipid content, probably both intercellular and covalently bound to corneocytes, affect barrier function (2–4). The lipids consist mainly of ceramides, cholesterol, and fatty acids; the total and respective levels of these are

influenced by diet, age, race, environment (and seasons), and certain skin conditions (5–7). In psoriasis, for example, stratum corneum cholesterol levels are raised and fatty acids and ceramides decreased, compared with normal subjects (8). The composition of covalently bound lipids in psoriatic stratum corneum also differs from that of healthy stratum corneum (9). In atopic dermatitis, stratum corneum ceramide levels, particularly ceramide one-linoleate levels, are particularly low (10,11). Changes in stratum corneum lipid levels are also associated with several hereditary disorders (12), the best-described of which is recessive X-linked ichthyosis (RXLI), in which there is a specific abnormality in sterol metabolism (12), which leads to excessively high levels of cholesterol sulphate in the stratum corneum.

Deterioration in skin condition due to seasonal changes in the weather affects far more people than all the above conditions combined, and it is only recently that we have begun to understand the reasons for this situation. Seasonal changes in lipid levels and types occur, with reductions in their levels in winter compared with those in summer (7). Bathing habits also influence the levels of lipids in the superficial layers of the stratum corneum. As a result, in soap-induced winter xerosis, the total levels of stratum corneum ceramides are decreased while those of fatty acids are increased in comparison with normal skin (13–15). The orderly bilayer architecture of these lipids is also disrupted in the superficial layers of the stratum corneum (14,15), which probably contributes to the increased transepidermal water loss known to occur in these conditions (16). All of these conditions are manifested as dry, flaking skin, which may be more or less severe. The underlying factor that causes the changes in skin structure, function, and appearance in skin xerosis is the failure of the normal desquamatory process, which itself depends upon the specific degradation of intercorneocyte cohesive factors (1,15). For instance, it is known that certain proteases in the stratum corneum are responsible for the normal orderly degradation of desmosomes (17,18). It has also been reported that enzymic activity, and thereby desmosome degradation, occurs only above a certain water content in the stratum corneum (19,20). When stratum corneum lipid structure is disturbed, the resulting reduction in stratum corneum hydration leads to the retention of corneocytes on the skin's surface and the manifestation of skin xerosis due to reduced desmosome degradation. If, therefore, stratum corneum moisturization and water barrier function can be restored by the topical use of suitable lipids and humectants, the desquamatory process may be normalized and xerotic skin conditions may be treated more effectively than they are at present.

One compound that is widely recognized for its importance in skin care products is glycerol (21). Products containing glycerol have been shown to be very effective in the treatment of skin xerosis (22), and the action of glycerol has been explained in terms of its occlusive (23), humectant (23,24), and lipid-phase modulating (25,26) properties, all of which translate into moisturization and barrier improvements for the stratum corneum. More recently we have demonstrated that glycerol aids the enzyme lysis of desmosomes in the stratum corneum (19,20). Other occlusive agents also assist to maintain stratum corneum barrier function and improve skin condition. Recently, petrolatum has been shown to penetrate the stratum corneum and interact with stratum corneum lipids (27). Also, stratum corneum ceramides themselves applied topically, particularly in combination with cholesterol and fatty acids, have also been shown to be effective in restoring barrier function in mice with perturbed epidermal barrier function induced by solvents (28), as well as in treating skin xerosis in humans (29,30). The

maintenance of a multilamellar lipid bilayer between the corneocytes has been identified as a key to these benefits (31).

Although the skin produces ceramides as the main bilayer-forming lipid in the stratum corneum, we were interested to determine whether other bilayer-forming lipids could mimic their behavior. Phospholipids are the main bilayer-forming lipids found in plasma membranes of living cells. However, they also occur in low levels in the lower layer of the stratum corneum but are usually hydrolyzed in the outer cellular layers. Nevertheless, as they are capable of forming lamellar lipid phases, they may be, therefore, of use for skin care treatments. We were interested to determine if a mixture of phospholipids, cholesterol, and fatty acids would have beneficial effects in the treatment of skin xerosis, and if these benefits could be enhanced in the presence of glycerol.

MATERIALS AND METHODS

IN VITRO ELECTRON MICROSCOPY STUDIES

Preparation of stratum corneum. Fresh skin (Buckshire Corporation, NJ) was washed with ethanol (70% v/v), cut into 3-cm-wide strips, and dermatomed (0.3-mm thick). The skin was then placed dermis side down onto trypsin solution (0.2% w/v) in sterile, calcium- and magnesium-free, phosphate-buffered saline (PBS), and incubated at 4°C for 18 hours. The epidermis was separated from the dermis and floated epidermis side down on fresh trypsin solution and incubated at 37°C for two hours to release epidermal cells. This procedure was conducted two more times with fresh trypsin and followed by rinsing in PBS. The resulting isolated stratum corneum was floated onto a nylon mesh and desiccated. The isolated stratum corneum was delipidized by extraction in propan-2-ol (0.1 g SC/100 ml solvent) in a sealed vial at 42°C for one hour and dried. This procedure allowed reproducible extraction of the free intercellular lipids but not the covalently bound stratum corneum lipids.

Product treatments. The delipidized stratum corneum was treated with the following three solutions:

1. Chloroform-methanol (2:1 v/v) containing 24 mg/ml of a mixture of phospholipid, cholesterol, and stearic acid (1:2:1 by weight).
2. Chloroform-methanol (2:1 v/v) containing 24 mg/ml of a mixture of petrolatum, cholesterol, and stearic acid (1:2:1 by weight).
3. Chloroform/methanol solution (2:1 v/v).

In each case, a known weight of solution was dispensed onto the surface of the stratum corneum at a loading of approximately 300 $\mu g/cm^2$ and gently rubbed into the stratum corneum. The treated stratum corneum pieces were sandwiched in a nylon mesh to maintain the orientation, and immediately prepared for electron microscopy as described below.

Electron microscopy of stratum corneum. The stratum corneum samples were pre-fixed in 0.2 M sodium cacodylate-buffered glutaraldehyde solution (2.5% v/v, pH 7.2) for approximately 18 hours and then cut into 1-mm-wide strips. The strips were rinsed in cold buffer (0.2 M sodium cacodylate) for 20 minutes followed by a two-hour soak in fresh cold buffer. This step was followed by two further 20-minute rinses in cold buffer. The strips were then post-fixed in ruthenium tetroxide solution (0.2% in 0.2 M sodium

cacodylate buffer) for 15 minutes. The cold buffer rinse and soak procedure above was then repeated. The stratum corneum was dehydrated by soaking in acetone solutions (25%, 50%, 70%, 95%, 100%, 100%, 100%) for 15 minutes each. The stratum corneum was then infiltrated with Spurr resin:acetone solutions (50:50 v/v for 18 hours; 70:30 for 3 hours; 90:10 for 30 minutes; 100:0 for 30 minutes; 100:0 for 18 hours). After cutting the stratum corneum from the nylon mesh, the stratum corneum was embedded in fresh Spurr resin and cured at 60°C for approximately 72 hours. Sections were cut (500–600 angstroms), stained with uranyl acetate and lead citrate, and viewed using the JEOL 1200EX electron microscope.

IN VIVO DRY SKIN STUDIES

Panelist screening criteria. Each study group was comprised of Caucasian women aged over 25, who are susceptible to dry skin and could therefore be expected to show the clearest response to moisturization treatment. None of the panelists took part in any other study for the duration of these studies. All of the panelists were healthy and free of any medical or physiological condition that might affect the assessment of skin or its reaction to the treatment plan. The panelists were not regularly taking drugs or medication and were not nursing or knowingly pregnant. All panelists gave written and witnessed informed consent.

Experimental design. These were double-blind, fully randomized clinical trials. All treatments and assessments were conducted on the dorsal aspect of the hands. The same protocol was used in each trial and consisted of the following three phases: (1) seven-day "dry-down"; (2) 14 days of treatment; (3) five days of regression.

During the dry-down phase all panelists washed the back of their hands between two and four times a day with soap to induce dryness. The treatment phase consisted of twice-daily product application (am and pm) with continued soap washing (two times per day). During the regression phase, panelists ceased all product application, but continued with the twice-daily soap washing. In addition, all panelists abstained from using any other moisturizer on or near the hand during all phases. In each study group, 75 panelists who underwent the dry-down had their hands visually assessed for dryness on Day 1 of the study, using a seven-point hand dryness scale (see Table I for grading scale). The grading was carried out using an Optivisor® with a number 10 lens (mag. × 3.5) and an Anglepoise® lamp with a 60-watt daylight bulb. The 66 panelists that best met the dryness criterion of grade-5 dryness score on Day 1 were selected to continue with the treatment and regression phases. Each panelist was randomly assigned to a pair of treatments, and one treatment was tested on each hand. The treatments were allocated such that each treatment was tested on 11 hands of 11 panelists, balanced for right and left hands. Treatment products were packed for individual panelist use, in containers fitted with dispensers that delivered 0.5-ml aliquots, with each panelist receiving approximately 100 g of each of the two formulations assigned to her. Panelists were shown how to dispense and apply the product (0.5 ml per application, two times per day). To enable comparison between studies, two control treatments were included in each study. These were an untreated control (negative control) and a standard commercial moisturizer (positive control).

The skin condition of the backs of the hands was visually assessed as described above on Days 3, 5, 8, 10, and 12 of the treatment phase, and on Days 15, 17, and 19 of the

Table I
Skin Dryness Grading Scale

Grade	Description
1	Normal, moist skin. Dermatoglyphics present as bunched or stacked triangles, plump and evenly spaced. Skin tone even and uniform with a slight shine.
2	Individual dermatoglyphics more visible due to whitening borders. Isolated small flakes of dry skin and slight wrinkling may appear.
3	Broad white borders to dermatoglyphics, in which corners or small portions have started to lift and peel. Small dry skin flakes give a "light powdery" appearance. Skin puckering may occur, as well as small red dots.
4	Entire sides of dermatoglyphics lift and peel back to create large dry skin flakes and rough appearance. Redness more evident.
5	Almost all dermatoglyphics lift and demonstrate flaking, with flakes anchored at one end to produce an alligator skin appearance. Uneven, rough appearance is obvious. Uniform redness looks like mild sunburn.
6	Dermatoglyphics disappear completely to produce flaking, cracking, and a dry, powdery appearance, with deep furrows and redness below, more like a moderate sunburn. Skin looks abraded.
7	Intense flaking and scaling give a chalky or crusty appearance, with cracking, fissuring, extreme redness, and possibly open abrasions and bleeding.

regression phase. All assessments were made prior to the morning product application (treatment phase) and at least one hour after washing.

Product treatment comparisons. The treatment effects of the particular formulations that will be compared in this paper contained the following actives:

1. Glycerol (1%)
2. Phospholipid, cholesterol, and stearic acid (4%, 1:2:1)
3. Phospholipid, cholesterol, and stearic acid (4%, 1:2:1) and glycerol (1%)
4. Phospholipid, cholesterol, and stearic acid (4%, 1:2:1) and glycerol (5%)
5. Vaseline® Petroleum Jelly, cholesterol, and stearic acid (4%, 1:2:1) and glycerol (5%).

The above products were prepared as aqueous, thickened (xanthan gum, 1%) and preserved (DMDM hydantoin, 0.2%) lotions adjusted to pH 7 (sodium hydroxide, hydrochloric acid).

Statistical analysis. All comparisons were performed using repeated Wilcoxon rank sum tests. The critical significance level was adjusted to maintain an overall level of 5%.

RESULTS

ELECTRON MICROSCOPY OF STRATUM CORNEUM

In the electron micrographs of normal stratum corneum that has not undergone the delipidization treatment described earlier, the typical lamellar lipid structure can be seen in the intercellular spaces of the stratum corneum (Figure 1A). In comparison, the intercellular lipids are clearly removed in the delipidized stratum corneum treated only with the vehicle (Figure 1B). Comparison of the effect of the two lipid treatments on delipidized stratum corneum indicates that phospholipid-cholesterol-stearic acid treat-

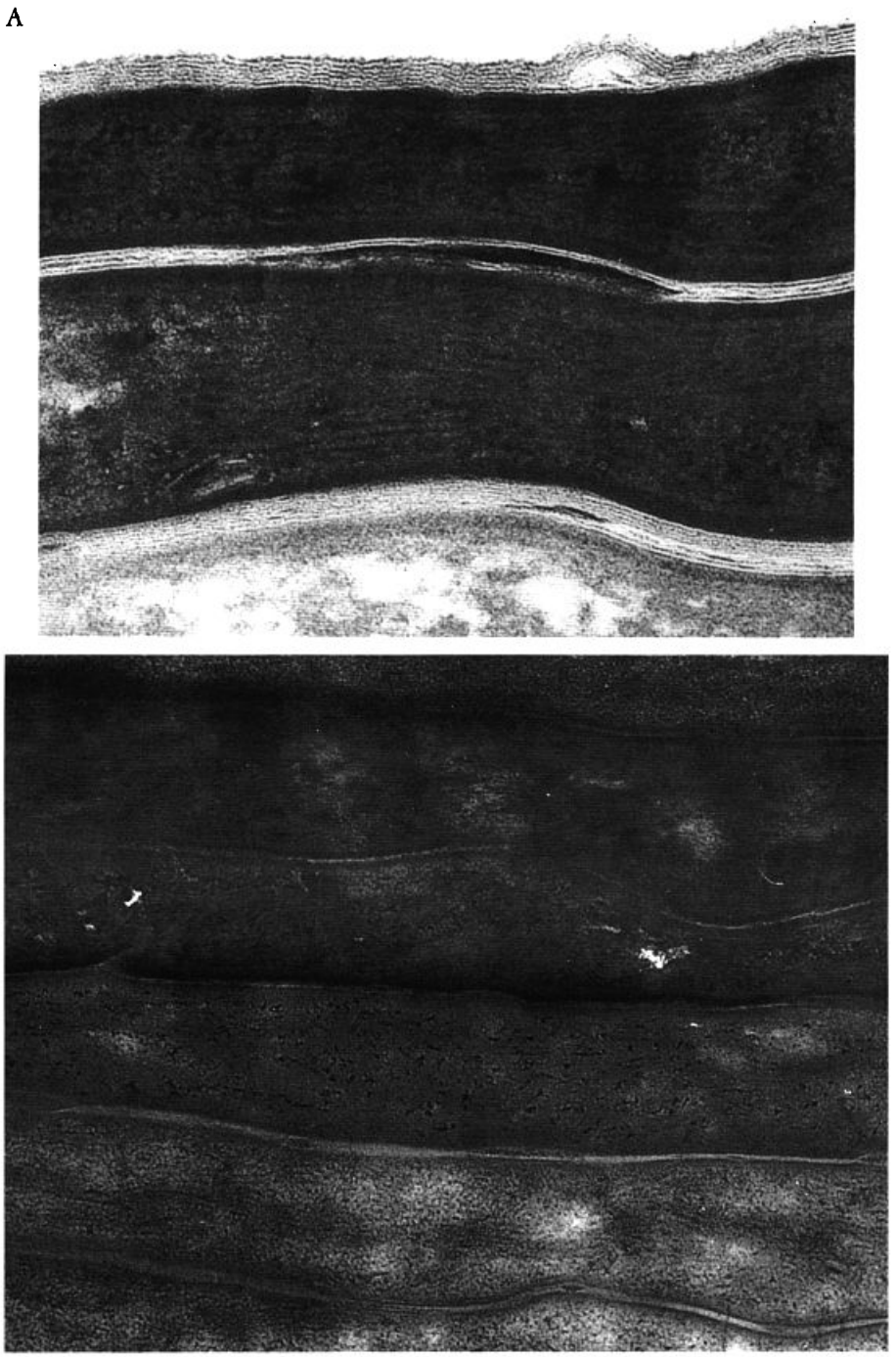
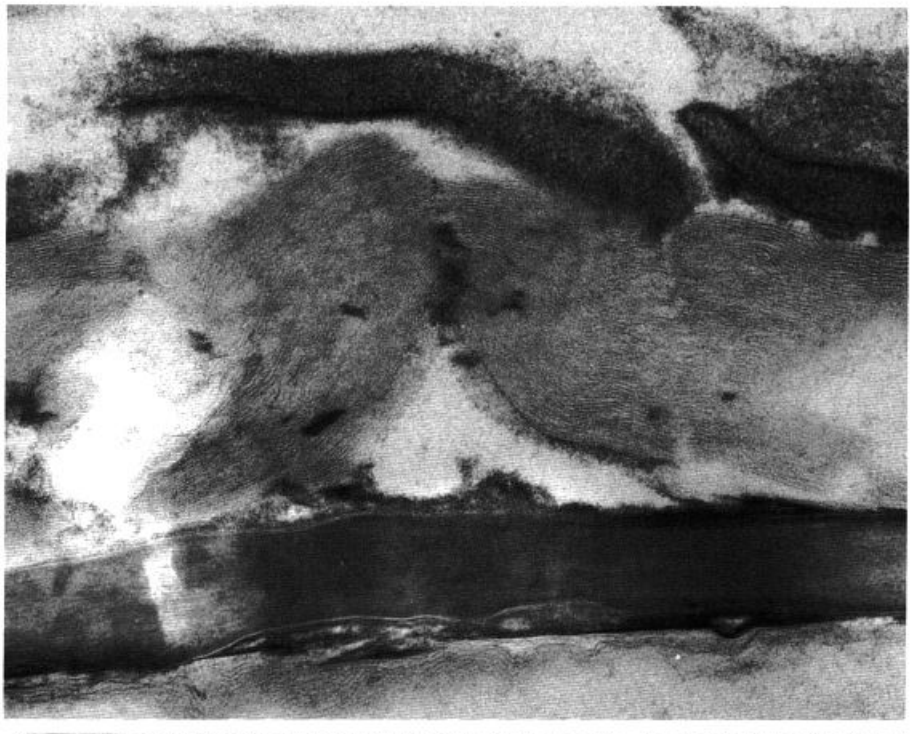


Figure 1. Electron micrographs of stratum corneum samples (ruthenium tetroxide fixation, uranyl acetate lead citrate stain; magnification: 1 mm = 2.5 angstroms). A: Normal stratum corneum. B: Delipidized stratum corneum treated. C: Delipidized stratum corneum treated with phospholipid, cholesterol, and stearic acid. D: Delipidized stratum corneum treated with petrolatum, cholesterol, and stearic acid. See text for details.

C



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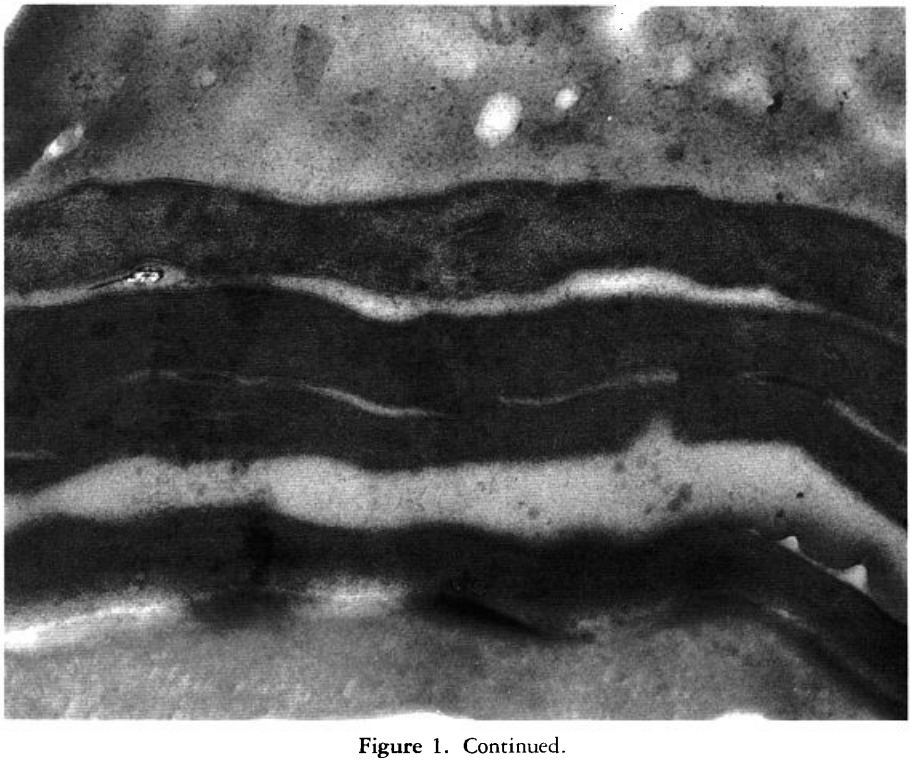

Figure 1. Continued.

ment (Figure 1C) is able to partially reform the lamellar lipid-like structure whereas the petrolatum-cholesterol-stearic acid treatment cannot (Figure 1D). Thus, it is apparent that the phospholipid-based lipid system, because of its bilayer-forming properties, may offer a potential advantage over the non-bilayer-forming petrolatum-based lipid system if such combinations are incorporated into moisturizers.

IN VIVO STUDIES

It is first noted that the respective treatment effects for the negative control (no treatment) as well as for the positive control (standard commercial moisturizer) did not differ significantly from study to study. This allowed for valid statistical comparisons of the different product treatments using absolute dryness scores irrespective of the study group a particular treatment was used in.

Figures 2 and 3 show that 1% glycerol (treatment 1) and 4% phospholipid-cholesterol-stearic acid mixture (treatment 2) alone are ineffective in alleviating skin xerosis compared to the no-treatment control over this particular time course. However, as seen in Figure 4, the results for the combined treatment (treatment 3:4% phospholipid-cholesterol-stearic acid, plus 1% glycerol) show a marked improvement in skin xerosis

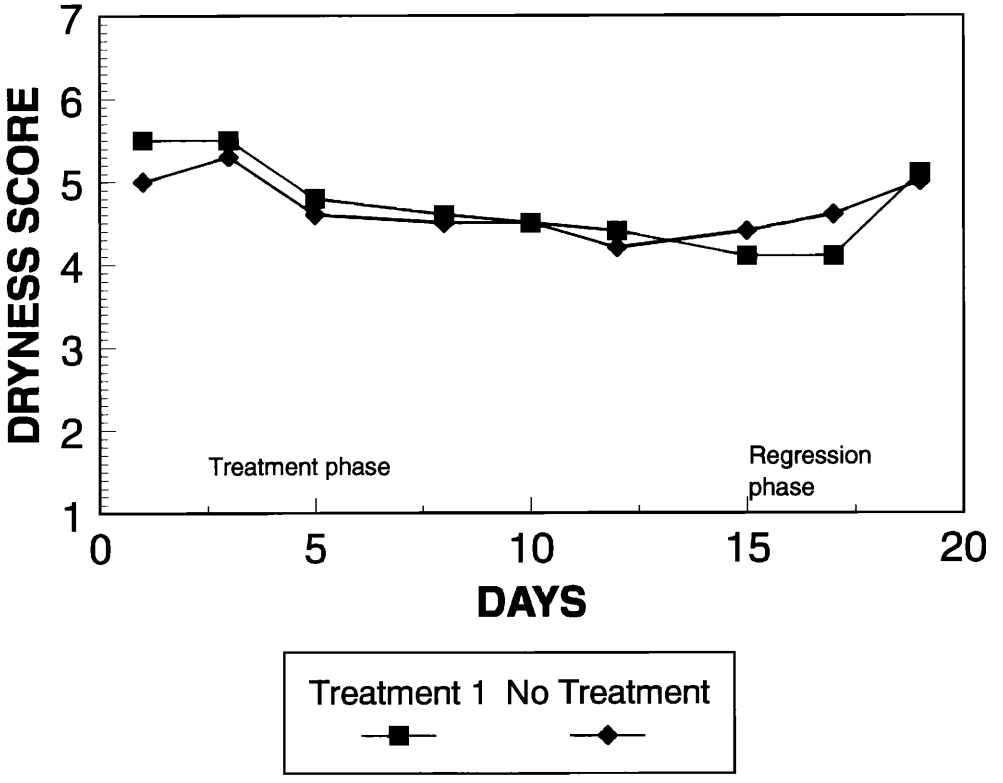

Figure 2. Moisturization efficacy test comparing the effect of 1% glycerol (treatment 1) to a no-treatment control.

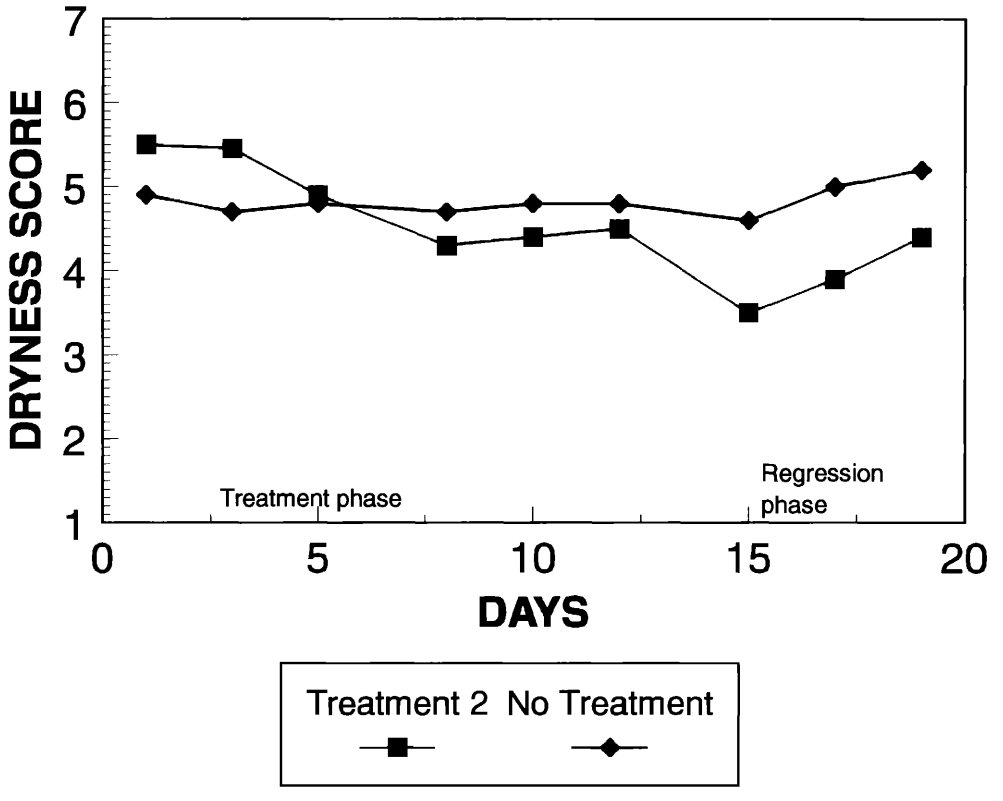

Figure 3. moisturization efficacy test comparing the effect of a lotion containing 1% phospholipid, 2% cholesterol, and 1% stearic acid (treatment 2) to a no-treatment control.

alleviation in contrast to those for the two individual treatments shown in Figures 2 and 3.

Figure 5 shows the effectiveness of 5% glycerol combined with 4% of either of two different ternary lipid mixtures. It can be seen that when phospholipid is used in the lipid mixture instead of an equivalent amount of petrolatum (treatment 4: phospholipid-cholesterol-stearic acid, plus 5% glycerol, vs treatment 5: petrolatum-cholesterol-stearic acid plus 5% glycerol), a more rapid response ($p < 0.05$ by Day 8) in skin xerosis alleviation is observed, although both treatments were effective in alleviating dry skin over the larger time frame of the study.

DISCUSSION

Occlusive agents and humectants have been used widely in skin care products for the treatment of skin xerosis over many years. Because of a lack of understanding of the pathophysiology of skin xerosis, improvements in skin care treatments have been empirical. Recently, however, several investigators have reported specific abnormalities in stratum corneum structure and composition in winter xerosis (13–15). These studies have shown that skin xerosis is related to changes in stratum corneum ceramide levels and a disturbance in their structure, as well as to an abnormality in desmosome pro-

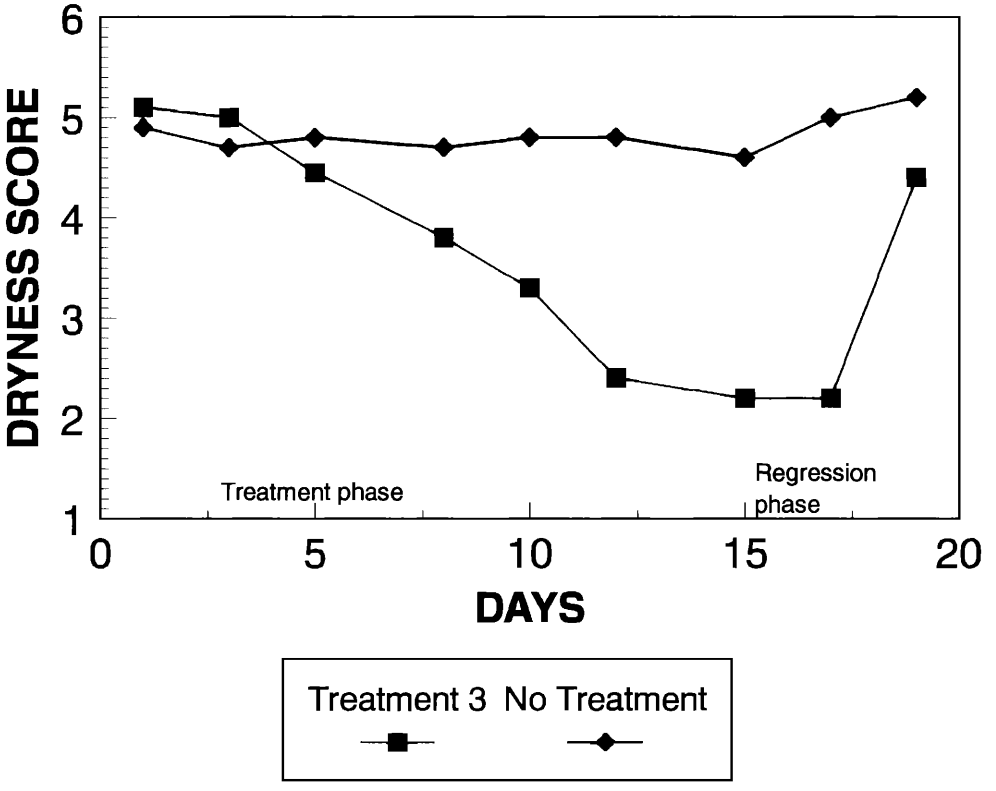

Figure 4. Moisturization efficacy test comparing the effect of a lotion containing 1% phospholipid, 2% cholesterol, and 1% stearic acid, plus 1% glycerol (treatment 3) to a no-treatment control.

cessing (14,15). Thus, perturbation of stratum corneum lipid architecture and composition appears to lead to a reduction in the activity of proteases responsible for desmosome degradation (17,18), and the resultant faulty desquamation leads to the appearance of skin xerosis.

Barrier lipids, occlusive agents, and humectants are, therefore, being investigated for their interactions with, and effects on, stratum corneum components and processes (19,20,25,26,27,31). Ceramides and ceramide-like lipids (pseudoceramides) in combination with cholesterol and fatty acids have been shown to be very effective (28–31) for the treatment of experimentally induced skin xerosis. Petrolatum has been shown to mix with the stratum corneum lipids, which may explain its beneficial effects on skin (27). Also, glycerol has been shown to fluidize stratum corneum lipids and prevent their crystallization in low-humidity conditions (25,26). This property probably also influences the effect of glycerol on the proteolysis stratum corneum desmosomes (19,20).

In the present investigation we have demonstrated that a lipid mixture consisting of phospholipid, cholesterol, and stearic acid, when combined with low levels of glycerol, functions synergistically to deliver efficacious skin benefits. Indeed, the synergism is apparent in that neither the lipids alone, nor glycerol alone, at the concentrations used in this study, alleviated skin xerosis significantly. Also, the use of the bilayer-forming phospholipid provided a more rapid reduction in xerosis compared to the non-bilayer-

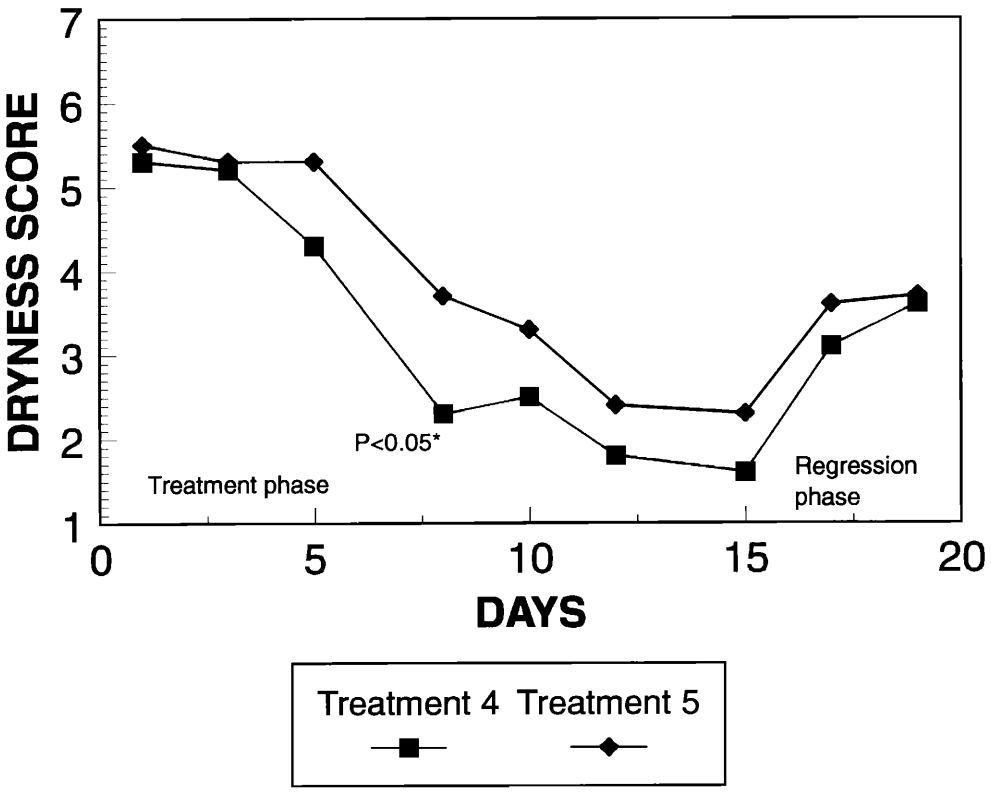

Figure 5. Moisturization efficacy test comparing the effect of a lotion containing 1% phospholipid, 2% cholesterol, and 1% stearic acid, plus 5% glycerol (treatment 4) to a lotion containing 1% petrolatum, 2% cholesterol, and 1% stearic acid, plus 5% glycerol (treatment 5).

forming petrolatum. The improved skin xerosis benefit of the phospholipid-containing lotions appears to be related to the bilayer-forming potential of phospholipids, as supported by the *in vitro* electron microscopy results. It is noted, however, that both the phospholipid and petrolatum-containing lotions were still highly effective in alleviating skin xerosis compared to the no-treatment control.

The reasons for the phospholipid-glycerol synergy are not yet fully understood, but glycerol may modulate the lipid-phase behavior of the topically applied lipid mixture, especially allowing the formation of a lamellar phase that may increase the performance of glycerol, or glycerol may allow a better delivery of the lipids into the stratum corneum. Overall, it is hypothesized that improved stratum corneum barrier function, as well as increased stratum corneum water content, is due to the formation of additional exogenous lipid bilayers that in the presence of glycerol improve the hydration of the stratum corneum and allow normal desmosome degradation to occur. At the low levels of lipids and glycerol used in this study, this effect is apparently synergistic. It is noted, however, that glycerol alone, at higher concentrations than those used in this study, has been found to be effective in treating skin xerosis (23). High concentrations of glycerol are, however, aesthetically unpleasant unless formulated carefully. Higher concentrations of the lipids and glycerol were not examined in this study. To fully under-

stand the reasons for the glycol–lipid synergy, however, physical characterization of the lotions will be required, especially *in situ* on the skin surface after evaporation of excess water.

In conclusion, glycerol-based lotions, with bilayer-forming lipids, phospholipid, cholesterol, and stearic acid, restored xerotic skin conditions to normal more rapidly compared to those with petrolatum, cholesterol, and stearic acid. Such lotions, through their ability to optimally restore stratum corneum moisturization by enhancing barrier function and improving humectancy, should permit or reestablish normal desquamation, thereby facilitating the rapid alleviation of skin xerosis.

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