Clinical and laboratory studies

Effects of topical ammonium lactate on cutaneous atrophy from a potent topical corticosteroid

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Background: Topical corticosteroids produce atrophic changes in skin, including thinning of the epidermis and decrease in dermal ground substance. We observed that 12% ammonium lactate produced an increase in the thickness of epidermis and increased amounts of dermal glycosaminoglycans.

Objective: Our purpose was to determine whether 12% ammonium lactate could minimize cutaneous atrophy produced by a potent topical corticosteroid.

Methods: Clobetasol propionate, 12% ammonium lactate, and both agents were repetitively applied under occlusive patches as well as in open patches on the forearms of human volunteers for 3 to 4 weeks. Biopsy specimens were analyzed for thickness of the epidermis and dermal glycosaminoglycans by image analysis.

Results: Twelve percent ammonium lactate produced a significant sparing of atrophy in both the epidermis and dermis without any influence on the bioavailability or antiinflammatory properties of the corticosteroid.

Conclusion: Twelve percent ammonium lactate may be useful in mitigating the adverse effects of corticosteroid on skin.

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Since the introduction of topical hydrocortisone in 1952, major emphasis has been placed on developing more potent formulations. Through a variety of substitutions and additions to the phenanthrene ring,1,2 "ultrapotent" steroids are now available, which, when applied topically, can penetrate human skin and resolve dermatoses almost as effectively as intralesional therapy. Unfortunately, the use of particularly potent formulations can be associated with undesirable side effects including telangiectasia, epidermal and dermal atrophy, striae, steroid-induced dermatitis (e.g. rosacea), and inhibition of the pituitary-adrenal axis.3-9

Of increasing concern is the potential for cutaneous toxicity from prolonged use of topical corticosteroids. Telangiectasia, thinning, shininess with loss of skin markings, and increased transparency can result from topical steroid therapy.3,5,6 Histologic examination shows that the viable epidermis becomes markedly attenuated with flattening of the dermoepidermal interface. The stratum corneum may also be reduced to a few cell layers.7-14 Basal cells change from a primarily columnar organization to a round or cuboidal form and frequent dyskeratotic or "dying" cells are observed in this region.14 The most profound dermal change involves rearrangement of the collagen and elastin fibrous networks.7,13-17 This is largely the result of an attenuation in synthetic activity of the fibroblast, the consequence of which is a marked diminution in ground substance.14,18-21 Because ground substance occupies the spaces between collagen bundles and elastic fibers, loss of this component causes compaction of the papillary and reticular dermis and a rearrangement of the geometry of the dermal fibrous network.14 More recently, it has also been shown that steroids have a cytotoxic effect on dermal mast cells.22,23

Attempts at circumventing these unwanted cutaneous side effects have primarily involved modifications in frequency of application and/or changes in concentration of a particular steroid, as well as increasing use of hydrocortisone and other nonfluorinated, less potent steroid derivations.24 To date,
efforts to develop a steroid with potent antiinflammatory properties that is also devoid of atrophogenic side effects have been unsuccessful. Because steroid-induced cutaneous atrophy involves attenuation of both basal keratinocyte proliferation and fibroblast synthetic activity, the possibility of pharmacologic intervention with retinoids, which stimulate both basal keratinocyte proliferation and fibroblast activity, has been attempted as a means of blunting the atrophogenicity. In murine skin, retinoic acid prevented skin thinning by dexamethasone as determined by the skinfold thickening assay. In addition, retinoic acid did not appear to affect the ability of dexamethasone or fluocinolone acetonide to suppress inflammation induced by croton oil or 12-O-tetradecanoylphorbol-13-acetate (TPA). Before these studies in mice, there have been no systematic investigations on the clinical and morphologic effects that retinoids or other compounds have on human skin, when used in combination with ultrapotent steroids. However, there are studies that have shown that retinoids can reverse wound healing retardation induced by a variety of antiinflammatory agents including corticosteroids.

During studies on the effects of daily topical application of buffered ammonium lactate to normal human skin, we observed increases in viable epidermal thickness and ground substance in the dermis (unpublished observations). This prompted us to consider whether, in human skin, ammonium lactate could alleviate cutaneous atrophy produced by an ultrapotent steroid. We have found that buffered ammonium lactate in combination with clobetasol-17-propionate has a significant sparing effect on epidermal and dermal atrophy without inhibiting the bioavailability or antiinflammatory activity of this steroid. These observations suggest an unexpected use for this agent, which is used to treat xerosis.

MATERIAL AND METHODS
Assessment of the bioavailability of the corticosteroid

Ten healthy subjects (five men and five women) between the ages of 19 and 60 years participated in this study. After the forearms were washed with a nonmedicated soap (Ivory) and patted dry with a soft towel, three circular sites, each measuring 1.2 cm in diameter, were outlined in ink on the volar aspect of both forearms. On the morning of the first study date, two randomly assigned sites on each forearm received 20 μl of 12% buffered ammonium lactate (Lac-Hydrin, Westwood-Squibb Pharmaceuticals Inc., Buffalo, N.Y.). The subjects returned 6 to 8 hours later on the same day and received 20 μl of 0.05% clobetasol-17-propionate ointment (Temovate, Glaxo Inc., Research Triangle Park, N.C.) to a third site and to one of the sites previously treated with ammonium lactate. One forearm was then occluded with plastic wrap; the sites on the opposite arm were covered with circular plastic chambers in which a 1 cm hole was punched out from the center. The chambers were applied over each site and taped at the edges to prevent clothing from rubbing against or touching the skin surface. Sixteen hours after the application of the steroid, the protective chambers and plastic wrap were removed and both forearms were washed again with soap and water and blotted dry with a towel.

The degree of blanching at each test site was graded 30 minutes after the products were washed from the skin (16 to 17 hours after application) and again 24 hours after the last application of the test products. Grading of the degree of blanching was done in a blinded fashion under standardized lighting by the same investigator who was unaware of the test product assignment and who did not participate in the application of the test products. The following grading scale was used:

0 = No vasoconstriction
1 = Minimal visible blanching
2 = Definite blanching with well-defined borders
3 = More intense blanching
4 = Intense blanching, spreading beyond the application site

To evaluate potential effects on the bioavailability of corticosteroids after long-term use of 12% ammonium lactate, 12 volunteers were treated twice daily with 0.5 gm of 12% ammonium lactate applied to one forearm and 0.5 gm of the vehicle applied to the opposite forearm. After 3 weeks of application, 20 μl of 0.1% betamethasone dipropionate ointment, 0.025% fluocinolone acetonide, or 0.5% clobetasol propionate were applied to designated 1.5 cm circular areas of the forearm. Each subject was treated with clobetasol propionate, six with fluocinolone acetonide and six with betamethasone dipropionate. Duplicate applications were made in each subject. Sites were covered with a plastic chamber (Hilltop Research Inc., Miamiville, Ohio) in which four circular holes are punched out to allow evaporation of water and to prevent occlusion as well as to protect the site. Seventeen hours later, the chambers were removed and the sites were washed with soap and water. Vasoconstriction was graded 1 hour later, i.e., 17 hours after application of the corticosteroid and again at 24 hours after application.

Assessment of the antiinflammatory activity of the corticosteroid

Twelve healthy subjects (seven men, five women) between the ages of 19 and 51 years participated in this investigation. After the forearms were washed with a bland
soap (Ivory) and patted dry with a soft towel, four circular sites were outlined (two on the volar aspect of each forearm) with ink. The dermatitis was induced by the application of 10 µl of a 1:50 dilution of poison oak-ivy oleoresin to a 0.6 cm disk of filter paper. The filter paper disk was then applied to the skin and taped with impermeable plastic tape. The plastic tape was further secured with surgical tape. This patch was left in place for 6 hours. At the end of 6 hours, the patch was removed and the area washed with soap and water and then dried. The dermatitis was induced on a Friday. The volunteers returned to the laboratory on the following Monday, at which time the test sites were examined and graded. Those persons studied had equal responses in all four sites.

Each site then received 20 µl of either A (12% buffered ammonium lactate), B (0.05% clobetasol-17-propionate ointment), A + B (ammonium lactate in the morning and clobetasol propionate in the afternoon, six subjects), B + A (clobetasol propionate in the morning and ammonium lactate in the afternoon, six subjects), or C (white petrolatum). Test product applications were randomized. Test products were gently rubbed into the sites with a glass rod and the sites were then covered with an occlusive bandage to prevent rubbing against clothing. Treatments were continued for 5 consecutive days.

On the last day of treatment, the sites were visually graded by the investigator who was unaware of product assignment and who did not participate in product application or treatment. The following grading scale was used:

0 = Complete clearing
1 = Minimally elevated or palpable border with faint erythema
2 = More elevated lesion with diffuse edema or palpable infiltration and moderate erythema
3 = Uniformly raised lesions with intense edema, erythema, and scaling crusting
4 = Marked edema, intense erythema, and vesiculation

Treatment of normal skin with ammonium lactate and clobetasol propionate, separately and in combination

Open applications. Six healthy men (20 to 34 years of age) served as volunteers. One site on the ventral forearm received treatment B (0.02 ml) daily for 4 weeks. Another site on the ventral forearm received treatment A (0.02 ml) daily for 4 weeks. A third site received A + B daily for 4 weeks. At the end of the 4-week treatment period one 3 mm punch biopsy specimen was obtained from each of the treatment sites with the subjects under local anesthesia. Untreated control and vehicle biopsy specimens were secured at the end of 4 weeks.

Occlusive applications. Six healthy men (20 to 34 years of age) served as volunteers. Treatment B (0.02 ml) was applied occlusively three times weekly (Monday, Wednesday, and Friday) for 3 weeks continuously to a designated area of the ventral forearm. The cream was placed in 15 mm Duhring chambers, which were then fastened to the skin with porous tape. Treatment A (0.02 ml) was applied in a similar manner to a separate area of the ventral forearm. To another site on the ventral forearm, three subjects received treatment B + A to the same site, which was then occluded. This treatment was repeated three times weekly (Monday, Wednesday, and Friday) as described earlier. The other three subjects had the combination treatment in the reverse order in which they received treatment A + B. Similar application schedules as already described were maintained. At the end of the 3-week treatment period one 3-mm punch biopsy specimen was obtained with the subjects under local anesthesia from each of the three treatment sites. Untreated control and vehicle biopsy specimens were secured at the end of 3 weeks.

Light microscopy. Each biopsy specimen was fixed in 10% formalin and was processed for conventional paraffin section histologic examination. Paraffin sections were stained with hematoxylin and eosin and Hale's colloidal iron for acid mucopolysaccharides. These sections were used for all histologic and histogeometric analysis.

Histometric analysis. For estimation of epidermal thickness, care was taken to cut the sections perpendicular to the surface. Histometric measurements of the viable epidermal area were made on both hematoxylin-eosin- and Hale's colloidal iron-stained sections by Magiscan computer-assisted image analysis. Measurements were made from at least four sections per specimen with each section separated by 50 µm. For estimation of ground substance, histometric measurements were made on Hale's colloidal iron-stained sections with a Vickers M-85 microspectrophotometer. Hale's stainable material represents, for the most part, glycosaminoglycans (GAGs) and is commonly used as an indicator of changes in ground substance. Ground substance is the most labile structural component of the dermis and thus changes in this material serve as a marker of dermal atrophy. An average of 10 measurements were taken along the papillary dermis. Each measurement encompassed a 50 µm circular area and the value represents the amount of light emitted at a wavelength specific for the blue material detected by the histochemical stain.

RESULTS
Effects of ammonium lactate on the bioavailability of clobetasol-17-propionate

The vasoconstriction scores for ammonium lactate, clobetasol propionate, and the combination of ammonium lactate and clobetasol propionate at both time points (16 and 24 hours) are listed in Table I. No blanching was observed in sites treated with ammonium lactate alone. Treatment with amm-
Table I. Effect of 12% ammonium lactate on bioavailability and antiinflammatory activity of topical clobetasol propionate

<table>
<thead>
<tr>
<th>Vasoconstriction*</th>
<th>16 hr</th>
<th>24 hr</th>
<th>16 hr</th>
<th>24 hr</th>
<th>Rhus dermatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>2.0 ± 0.5</td>
<td>2.1 ± 0.6</td>
<td>2.4 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td>1.3 ± 0.9†</td>
</tr>
<tr>
<td>AL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.8 ± 1.1</td>
</tr>
<tr>
<td>CP + AL</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.5</td>
<td>2.5 ± 0.8</td>
<td>2.1 ± 0.6</td>
<td>1.2 ± 0.7†</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.9 ± 1.0</td>
</tr>
</tbody>
</table>

Data expressed as a grading scale. CP, Clobetasol propionate; AL, 12% Buffered ammonium lactate; CP + AL, combination clobetasol propionate/12% buffered ammonium lactate.

*Mean ± standard deviation in 10 subjects.
†Significantly different from petrolatum and ammonium lactate (p = 0.001 ANOVA), not significantly different from each other.

Table II. Effect of long-term use of 12% ammonium lactate on the bioavailability corticosteroids

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>12% Ammonium lactate</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clobetasol propionate</td>
<td>2.3 ± 0.5</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td>Betamethasone dipropionate</td>
<td>2.0 ± 0.5</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Fluocinolone acetamide</td>
<td>1.0 ± 0.6</td>
<td>1.0 ± 0.5</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation.

Ammonium lactate did not influence the vasoconstriction by clobetasol propionate ointment in either the open or occluded test sites (Table I). The individual scores for both forearms were compared by means of paired t tests and no significant differences were found in the degree of blanching by prior treatment with ammonium lactate.

The results of vasoconstriction after 3 weeks of treatment with 12% ammonium lactate are found in Table II. No difference in vasoconstriction was seen on sites treated with ammonium lactate and its vehicle. In this experiment the bioavailability of an ultrapotent corticosteroid (clobetasol propionate), a potent agent (betamethasone dipropionate), and a midpotency steroid (fluocinolone acetamide) was evaluated. No difference was found for any of these agents of differing antiinflammatory capacity.

Effects of ammonium lactate on the antiinflammatory activity of clobetasol-17-propionate

Experimentally induced Rhus dermatitis sites treated with petrolatum showed a mean score of 2.9 ± 1.0, whereas those treated with ammonium lactate showed a mean of 2.8 ± 1.1, a nonsignificant difference. These scores are listed in Table I. There were no discernible differences between sites treated with clobetasol propionate (B) and with clobetasol propionate plus ammonium lactate regardless of the sequence or order of application (A + B or B + A). Mean scores were 1.3 ± 0.9 and 1.2 ± 0.7, respectively. The results confirmed that ammonium lactate had no noticeable effect above that of petrolatum. Clobetasol propionate alone or with ammonium lactate produced a highly significant effect (p = 0.0001) in terms of enhancing the resolution of the Rhus dermatitis compared with treatment with petrolatum alone or ammonium lactate alone.

Histologic effects of ammonium lactate on normal skin and clobetasol-17-propionate-treated skin

Normal skin. Biopsy specimens of subjects treated topically with ammonium lactate either daily in an “open” manner for 4 weeks or under occlusion for 3 weeks revealed an increase in viable epidermal thickness (Figs. 1, a; 2, b; 3, b). In most instances the undulating nature of the dermoepidermal interface was maintained. The granular layer was prominent (Fig. 1, a) compared with that of control specimens (Fig. 1, b). In several subjects Hale’s stainable (GAG-like) material was noted in the intercellular spaces between spinous and granular cells after ammonium lactate treatment (Fig. 2, a); this material was not observed in sections obtained from control or vehicle-treated skin (Fig. 2, a). The “basket weave” architecture of the stratum corneum, characteristic of formalin-fixed normal human skin (Figs. 1, b; 2, a), was maintained after ammonium lactate treat-
Buffered ammonium lactate retards steroid-induced atrophy

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Viable epidermal thickness increased from $67 \pm 11 \mu m$ in the control subjects to $79 \pm 14 \mu m$ after open application (Table III) and from $62 \pm 10$ to $74 \pm 17 \mu m$ after occlusive application (Table IV) of ammonium lactate. Despite an average 19% increase in viable epidermal thickness individual differences were noted. A few showed minimal changes whereas other increased by 50% (Tables III and IV).

Striking increases in ground substance, as detected by Hale’s stainable material, were noted after treatment with ammonium lactate in both the open and occluded treatment regimens (Fig. 2, a and b). Microspectrophotometry showed a 49% and 51% increase in Hale’s stainable material after open and occlusive treatment with ammonium lactate, respectively (Table V). Vascular profiles were also more prominent after ammonium lactate treatment (Figs. 1 and 2, a and b). No increases in the cellularity of the ammonium lactate–treated skin were noted when compared with control skin (Fig. 1). No evidence of irritation was seen; there was no inflammatory infiltrate or any evidence of cell injury in the epidermis.

Clobetasol propionate–treated skin. The epidermis was markedly thinned in all subjects after 4 weeks of daily treatment with clobetasol propionate as well as after 3 weeks of occlusion (Figs. 2, c and 3, c). This thinning was accompanied by a flat dermoeipidermal interface. The stratum corneum was also dramatically thinned (Fig. 2, c), appearing as a wispy layer of horny cells, compared with the “basket-weave” structure characteristic for control and ammonium lactate–treated skin (Figs. 1 and 2, a and b). With image analysis, viable epidermal thickness was reduced from $67 \pm 11$ to $32 \pm 8 \mu m$ (Tables IV and V), and from $62 \pm 10$ to $34 \pm 5 \mu m$ (Tables III and V), after “open” and occlusive steroid treatments, respectively. These represent decreases of 51% and 46%, respectively, and are highly significant ($p = 0.01$). As in previous studies, epidermal cytologic changes were mostly confined to the basal layer, where a shift in basal cell polarity from columnar to cuboidal was routinely observed (Fig. 3, a and c). In addition, focal areas of basal cell atypia were noted. Furthermore, a marked reduction in melanosome distribution was observed (Fig. 4, c), which is consistent with previous reports of steroid-inhibited melanogenesis. A marked compaction of the papillary dermis was noted after both methods of steroid delivery (Fig. 2, c). This compaction resulted from the loss of ground substance between collagen and elastin bundles, as evidenced by a lack of Hale’s stainable material after steroid treatment (Fig. 2, c). All subjects had attenuated amounts of GAGs, but occlusion resulted in the most dramatic diminution in this product (Table V). Microspectrophotometric analysis revealed a 47% decrease in Hale’s stainable material after open steroid treatment versus a 79% decrease after occlusive treatment (Table V). In addition to these changes in the dermal structural elements, there was a marked reduction in vascular profiles after steroid treatment (Fig. 2, c).

Ammonium lactate/clobetasol combination. The combination of ammonium lactate with clobetasol propionate resulted in an overall reduction in corticosteroid-induced changes. Although the viable epidermis was still thinner than controls, overall epidermal architecture more closely resembled control...
and ammonium lactate-treated epidermis than steroid-treated epidermis (Figs. 2 and 3). Basal cells appeared more columnar and less disorganized than the steroid treated specimens (Fig. 3, c and d). In most subjects the dermoepidermal interface was undulating rather than flat (Fig. 3, c and d) and the horny layer was thicker and had a "basket-weave" appearance (Fig. 2, c and d). Pigment distribution within keratinocytes did not appear to be altered after combination therapy (Fig. 3, d), whereas steroid treatment resulted in a marked absence of pigment (Fig. 3, c). When the combination treatment was administered in an open fashion, the viable epidermis decreased from an average thickness of 67 ± 11 to 43 ± 8 μm, a 35% decrease (Table V). Compared with clobetasol propionate, the combination therapy
Buffered ammonium lactate retards steroid-induced atrophy

Fig. 3. Higher magnification light micrographs depict viable epidermal (E) changes in same person after treatment with 12% ammonium lactate (b), clobetasol propionate (c), and combination ammonium lactate/clobetasol propionate (d) for 4 weeks in open fashion. Pigment distribution within keratinocytes (arrows) did not appear altered after either ammonium lactate (b) and/or ammonium lactate/clobetasol propionate combination (d) when compared with control specimens (a). However, clobetasol propionate treatment resulted in ablation of epidermal melanization. Note change in basal cells (B) to primarily cuboidal form after clobetasol propionate treatment (e), whereas combination ammonium lactate/clobetasol propionate (d) maintained more typical columnar basal cell (B) shape. (X400.)

resulted in a 16% thicker viable epidermis, which was significant (p = 0.05). A much greater sparing of the viable epidermis was observed when the combination was administered under occlusion. Viable epidermal thickness decreased from an average of 62 ± 10 to only 52 ± 7 μm (Table IV), a 15% decrease. Compared with clobetasol propionate, occlusive combination therapy resulted in a viable epidermis that was 67% thicker, which was significant (p = 0.01). Two subjects had a slightly thicker viable epidermis after 3 weeks of occlusive combination therapy and one subject had a less than 5% decrease in viable epidermal thickness (Table IV).

Dermal sparing was also evident after combination ammonium lactate/clobetasol propionate treatment: There was an overall increase in the amount of GAGs detected in the combination specimens when compared with the steroid-treated material (Fig. 2, c and d). However, more variability was encountered between subjects as well as between methods of treatment (open vs occlusion) (Table V). Vascular profiles were more prominent after combination treatment and little, if any, cellular infiltrate was noted in the dermis after the combination therapy (Fig. 2, d).

DISCUSSION

In this study we have shown that concomitant use of 12% buffered ammonium lactate with a potent topical steroid results in a significant sparing of at-
Table III. Changes in viable epidermal thickness after 4 weeks of daily treatment (“open”) with 12% buffered ammonium lactate (AL), clobetasol propionate (CP), and the combination ammonium lactate/clobetasol propionate (AL + CP)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control</th>
<th>AL</th>
<th>CP</th>
<th>AL + CP</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>59</td>
<td>81</td>
<td>26</td>
<td>36</td>
</tr>
<tr>
<td>B</td>
<td>63</td>
<td>94</td>
<td>29</td>
<td>40</td>
</tr>
<tr>
<td>C</td>
<td>70</td>
<td>78</td>
<td>32</td>
<td>41</td>
</tr>
<tr>
<td>D</td>
<td>73</td>
<td>83</td>
<td>47</td>
<td>57</td>
</tr>
<tr>
<td>E</td>
<td>83</td>
<td>85</td>
<td>34</td>
<td>43</td>
</tr>
<tr>
<td>F</td>
<td>54</td>
<td>53</td>
<td>27</td>
<td>40</td>
</tr>
<tr>
<td>Mean</td>
<td>67</td>
<td>79*</td>
<td>32*</td>
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</tr>
<tr>
<td>SD</td>
<td>11</td>
<td>14</td>
<td>8</td>
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</tr>
</tbody>
</table>

*p < 0.01 compared with control.  †p < 0.01 compared with CP.

Table IV. Changes in viable epidermal thickness after 3 weeks of occlusive treatment with 12% buffered ammonium lactate (AL), clobetasol propionate (CP), and the combination ammonium lactate/clobetasol propionate (AL + CP)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control</th>
<th>AL</th>
<th>CP</th>
<th>AL + CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55</td>
<td>59</td>
<td>34</td>
<td>59</td>
</tr>
<tr>
<td>B</td>
<td>52</td>
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<tr>
<td>F</td>
<td>62</td>
<td>60</td>
<td>33</td>
<td>41</td>
</tr>
<tr>
<td>Mean</td>
<td>62</td>
<td>74*</td>
<td>34†</td>
<td>52*‡</td>
</tr>
<tr>
<td>SD</td>
<td>10</td>
<td>17</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
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*p < 0.05 compared with control.  †p < 0.01 compared with CP.  ‡p < 0.05 compared with CP.

Table V. Summary of histogeometric changes*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VET</th>
<th>GAGs</th>
<th>VET</th>
<th>GAGs</th>
<th>VET</th>
<th>GAGs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td>+19</td>
<td>+49</td>
<td>-51</td>
<td>-47</td>
<td>-35</td>
<td>-17</td>
</tr>
<tr>
<td>Occlusion</td>
<td>+19</td>
<td>+51</td>
<td>-46</td>
<td>-79</td>
<td>-15</td>
<td>-56</td>
</tr>
</tbody>
</table>

GAGs, Glycosaminoglycans; VET, viable epidermal thickness; for other abbreviations see Table III.

*p < 0.01 compared with control.
†p < 0.01 compared with CP.
‡p < 0.05 compared with CP.

ropathy in both the epidermis and dermis. This effect was observed whether the combination therapy was applied in an “open” manner or under occlusion. Furthermore, the order in which the combination was applied (i.e., steroid followed by ammonium lactate or vice versa) did not significantly alter the steroid-sparing effect. Treatment of the skin with ammonium lactate had no effect on the bioavailability or antiinflammatory activity of clobetasol propionate, indicating that the sparing phenomenon was not a result of dilution or inactivation of the steroid preparation by ammonium lactate, but rather that ammonium lactate had interfered with the atrophogenicity of clobetasol propionate. This antagonism may result from the ability of topical ammonium lactate to elicit a hyperplastic response in the epidermis and dermis, which, in turn, counteracts the inhibitory effects that steroids exert on both keratinocyte proliferation and fibroblast synthetic capacity. Whether this is specific for ammonium lactate or is a general property of α-hydroxy acids is not presently known.

Changes in normal skin after topical treatment with ammonium lactate are in many ways similar to those noted during wound healing and in the rebound period after steroid-induced atrophy and in retinoic acid treatment of photoaged skin. Increases in overall viable epidermal thickness as well as in the number of granular layers suggest that ammonium lactate may stimulate epidermal proliferation. It is unlikely that this hyperplastic response represents a nonspecific response to injury or irritation because no signs of irritation were seen either clinically or histologically even when 12% ammonium lactate was applied under an occlusive dressing for a 3-week period. The appearance of Hale’s stainable (GAG-like) material in the intercellular spaces between spinous and granular cells after ammonium lactate treatment has also been noted in normal and photoaged human skin treated.
with retinoids. In the present study ammonium lactate resulted in a 19% increase in epidermal thickness. Although this increase is not as dramatic as noted after topical retinoid therapy (40% thickening), in the present study ammonium lactate was only administered for 4 weeks compared with a 3- to 6-month exposure to retinoic acid. Treatment of normal skin with ammonium lactate did not result in either clinical or histologic evidence of irritation or dermatitis. Furthermore, there were no reports of subject discomfort during or after treatment with ammonium lactate. This lack of clinical side effects was confirmed by the absence of any histologic signs of inflammation or irritation after 4 weeks of "open" treatment or 3 weeks of occlusive delivery of ammonium lactate. This is in contradistinction to the irritation, scaling, and dryness experienced by many patients after short-term therapy with retinoic acid. Retinoic acid markedly thins the stratum corneum, and can be associated with dermal inflammation. In the present study, little histologic alteration was noted in the organization of the stratum corneum after ammonium lactate treatment, which may explain why clinical or histologic irritation was not in evidence after use of this compound. α-Hydroxy acids have been reported to exert their therapeutic effects in ichthyosis and dry skin by decreasing corneocyte cohesiveness. This action has been postulated to occur at the lower, newly formed levels of the stratum corneum rather than the more mature upper layers. Maintenance of a normal-appearing stratum corneum after ammonium lactate treatment of clinically "normal" skin suggests that the "keratolytic" effects of this compound may be restricted to those conditions of hyperkeratosis where the stratum corneum is abnormally thickened.

REFERENCES

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