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The influence of hydroxy acids on the rheological properties of stratum corneum

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Synopsis

The influences of lactic acid, sodium lactate, and hydroxy acids on the rheological properties of stratum corneum were investigated in relation to their hygroscopicity and sorption characteristics.

The uptake of water was greater by sodium lactate than by lactic acid at all relative humidities examined and it enhanced the water content in stratum corneum. However, stratum corneum was plasticized markedly by lactic acid and not by sodium lactate. The pliability of stratum corneum was closely related to the sorption of lactic acid; the higher the amount of sorbed lactic acid, the more pliable stratum corneum became.

In addition to lactic acid other non-hygroscopic hydroxy acids, viz., mandelic acid and leucic acid, also had plasticizing effects. It was also found that an α-hydroxy acid is more effective than a β-type in plasticizing stratum corneum while simple carboxylic acids and alcohols had no effect. These hydroxy acids appear to soften stratum corneum by adsorbing to the polar groups of keratin chains and reducing interactions between them without increasing the water content in stratum corneum.

INTRODUCTION

The importance of water in plasticizing stratum corneum is well known, and it is also known that the extensibility of stratum corneum depends on its content of water and hygroscopic substances (1–3). Therefore, many hydrating emollients and moisturizers have been used to soften the thickened stratum corneum and to alleviate dry skin. Lactic acid and sodium lactate, which are natural constituents of stratum corneum, are especially useful for the treatment of xerosis (4) and for the improvement of skin dryness and flakiness (5,6).

However, the action of lactic acid on stratum corneum has rarely been studied, nor have the differences of hygroscopicity and platicizing ability between lactic acid and lactate been clarified quantitatively.

The purpose of this study is to compare the effect of lactic acid with that of sodium lactate on the rheological properties of stratum corneum in relation to their hygroscopicities and sorptions to stratum corneum, and also to clarify the mechanism of action of lactic acid and other hydroxy acids.

MATERIALS AND METHODS

Stratum corneum was removed from human abdominal skin using the heat-trypsinization method described by Kligman and Christophers (7). The ingredients used were reagent grade and hydroxy acids were D-, L-mixtures.

The mechanical properties of the strips of stratum corneum (20 × 5 mm) were measured by a dynamic measuring system. The measurements consisted of the application of a fixed amplitude sinusoidal extension (5 μm amplitude, 30 Hz) on one end of a stratum corneum strip extended by 1% strain. A cyclic stress response was measured by a load cell attached to the other end of the stratum corneum. The ratio of stress to strain leads to the complex modulus, E*. This was divided into two components which are the real part (E′) or component in-phrase with the applied cyclic strain and the imaginary part (E″) or 90 degrees out of phase component. That is, one can write

$$E^* = E' + iE''$$

where i is $\sqrt{-1}$ and E′ and E″ are dynamic elastic modulus and dynamic loss modulus, respectively. It can be shown that $\tan \delta = E''/E'$, where δ is the lag of stress behind strain (8). The tan δ parameter is indicative of a change in the viscous component. It has also been shown that tan δ increases with increases of pliability in stratum corneum or α-keratin fibers (9,10).

The sorption isotherm of water vapor by stratum corneum was examined with a Cahn Electrobalance. The dry weight of a 10-mm diameter sample was measured in an evacuated system. Then water vapor was introduced into the system. As the vapor pressure approached a predetermined value and after equilibrium was reached, the hydrated weight was measured.

In order to study the effects of lactic acid and sodium lactate on the rheological properties of stratum corneum and its water sorption, a sample of stratum corneum was immersed in a 1 mol/l solution of the chemical (ratio of solution/stratum corneum 10 ml/mg) for 1 h and then dried at 25°C and 50% relative humidity (RH) for 24 h to eliminate the effects of humidity variations before rheological measurements. Samples used as controls were soaked in distilled water for 1 h and dried as above.

Hygroscopicities of lactic acid and sodium lactate, which are shown as the water uptake in mg by 100 mg of dry samples, were measured by the Karl-Fisher method. The samples of these materials were equilibrated over saturated salt solutions chosen to give a specific RH ($MgCl_2$:33% RH, $Mg(NO_3)_2$:53% RH, NaCl:76% RH, KCl:84% RH). The water uptakes of other nonvolatile hydroxy acids were measured by equilibrating in an atmosphere of constant relative humidity, weighing and comparing these results with the dry weights.

The sorptions of lactic acid and sodium lactate by the stratum corneum were determined using ^{14}C-labeled lactic acid. The pH of lactic acid solution was adjusted with sodium hydroxide. Samples of stratum corneum measuring 10 × 10 mm were immersed in 1 mol/l solutions (ratio of solution/stratum corneum 3 ml/mg) for given times at 25°C.

The samples were then removed from the solutions and any excess solution adhering to the stratum corneum was carefully blotted away.

The radioactivity of ^{14}C-labeled lactic acid and/or lactate sorbed by the stratum corneum was determined after digesting the sample in 0.5 ml of 0.1 N NaOH at 60°C for 1 h. After cooling the sample solution, 10 ml of scintillator (PPO 5g and POPOP 0.1 g in 670 ml toluene and 330 ml Triton X-100) was added. The radioactivity was determined with a liquid scintillating system.

RESULTS

HYGROSCOPICITY

Table I shows the effect of RH on the hygroscopicities of lactic acid and sodium lactate at 25°C. In each case, water uptake increased exponentially with increasing RH, but the lactate took up more water than the free acid at all RHs. At 84% RH, sodium lactate adsorbed 170% of its weight in water. The value was more than two times that for lactic acid.

SORPTION ISOTHERM OF WATER VAPOR

Figure 1 shows the effect of sodium lactate treatment on the water uptake by stratum corneum at 25°C. As can be seen, both the treated stratum corneum and the control picked up water in proportion to the amount of moisture present in the atmosphere. At lower RHs the water uptakes with treated stratum corneum were essentially unchanged from those of controls. Above 60% RH, however, larger amounts of water were sorbed by the treated corneum in contrast to the control.

On the other hand, with the stratum corneum treated with lactic acid, the water uptake was the same as with the control (Figure 2). It appears that lactic acid treatment had little effect on the hygroscopicity of stratum corneum.

MECHANICAL PROPERTIES

Dynamic elastic modulus and dynamic loss modulus measurements on stratum corneum did not show good reproducibility. They varied widely because stratum corneum thickness was not constant and was very difficult to measure at all points. However, it appeared that tan δ hardly depended on the sample thickness and was reproducible.

Figure 3 shows the effects of sodium lactate and lactic acid treatments on the rheological properties of stratum corneum. With the sodium lactate-treated sample, the values of

Table I
Hygroscopicities of Lactic Acid and Sodium Lactate at 25°C (H_2O mg/100 mg)

	Relative Humidity (%)			
	33	53	76	84
Lactic Acid	9.1	21.4	52.2	79.9
Sodium Lactate	19.9	56.2	135.8	171.1

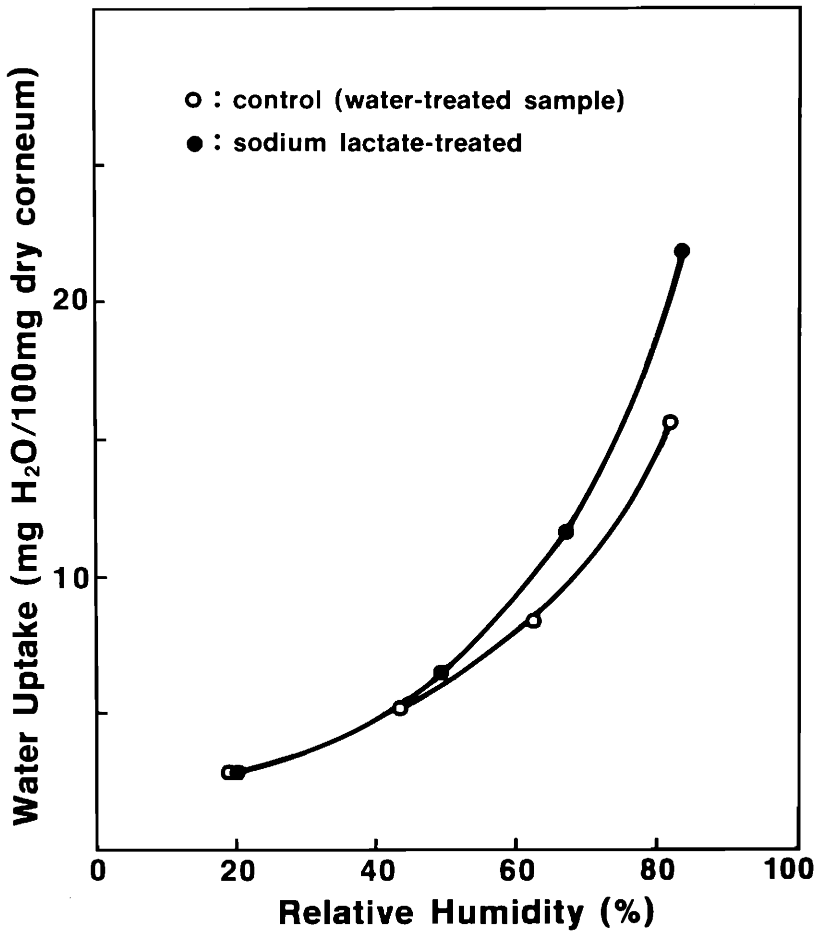

Figure 1. Hydration data showing the effect of sodium lactate treatment on the uptake of water by human stratum corneum at 25°C.

tan δ were higher than those of controls above 60% RH, though they were little changed from controls at lower RHs. The change of tan δ with RH corresponded to water uptake by stratum corneum. On the other hand, the lactic acid-treated stratum corneum had higher tan δ values than the sodium lactate-treated sample and the control at all relative humidities. This shows that lactic acid treatment plasticized the stratum corneum more than sodium lactate at every RH even though it did not increase the water content in stratum corneum.

SORPTION OF LACTIC ACID AND PLIABILITY

Figure 4 shows the effect of immersion time on the sorption of lactic acid from a 1 mol/l solution at 25°C. The amount of lactic acid sorbed by the stratum corneum increased with increasing immersion time and did not reach a saturation value during the time examined. Changes of stratum corneum mechanical properties are also shown in Figure 4. Tan δ increased with immersion time. The pliability of stratum corneum

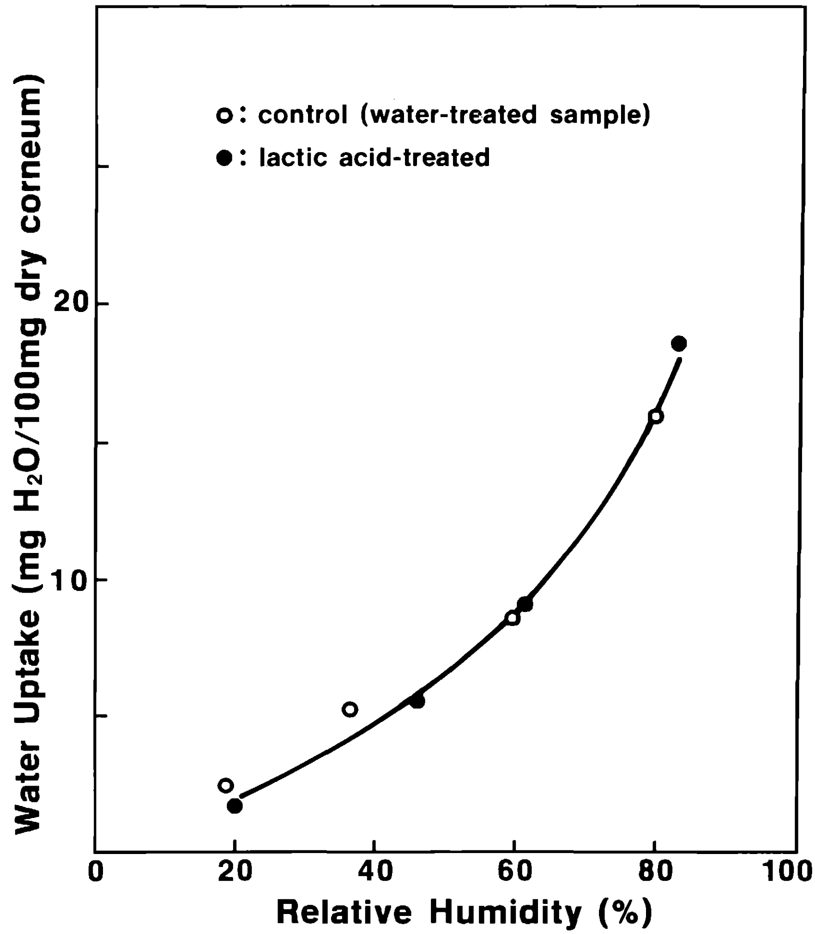

Figure 2. Hydration data showing the effect of lactic acid treatment on the uptake of water by human stratum corneum at 25°C.

was closely related to the sorption of lactic acid. Hence, the higher the amount of sorbed lactic acid, the more pliable the stratum corneum became.

The effects of pH on the sorption of lactic acid and the corresponding tan δ values for treated stratum corneum are shown in Table II. Stratum corneum was immersed for one hour in solutions having a final concentration of lactic acid plus sodium lactate of 1 mol/l. The sorption of lactic acid to solvent-damaged stratum corneum has been investigated by Middleton (5). Our experimental results agree with his results in that the sorption of lactic acid decreases as the pH of solution increases, i.e., lactic acid is sorbed more easily than sodium lactate by stratum corneum. Also the tan δ for treated stratum corneum decreased when the pH of the treatment solution increased. It was therefore shown that stratum corneum was plasticized by lactic acid more than by sodium lactate.

The influence of rinsing in water on the desorption of lactic acid and tan δ was investigated for the stratum corneum which had been treated with a 1 mol/l lactic acid solution. As can be seen in Figure 5, the residual amount of lactic acid was appreciably

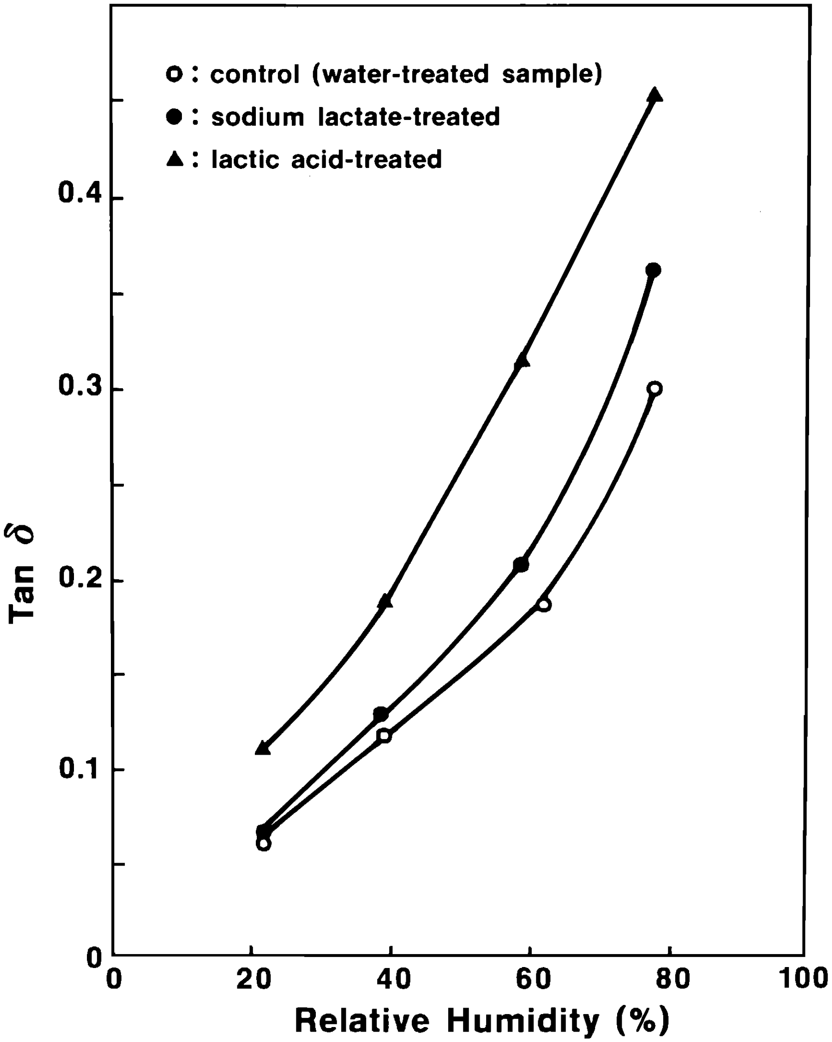

Figure 3. Plot of tan δ as a function of relative humidity for human stratum corneum treated with lactic acid or sodium lactate at 25°C.

decreased by rinsing, and lactic acid molecules were almost totally desorbed after 1 h rinsing. Concomitantly, the tan δ values decreased with increasing rinsing time and finally reached the non-treated level.

EFFECTS OF α-HYDROXY ACIDS AND SIMILAR COMPOUNDS ON STRATUM CORNEUM PLIABILITY

Plasticizing effects were investigated using α-hydroxy acids with different molecular weights and structurally similar compounds. Figure 6 shows the results of stratum corneum treatment with 10% aqueous α-hydroxy acid solutions (same method as lactic acid treatment) on tan δ values. Tan δ incresaed as the number of carbon atoms in the treatment hydroxy acid increased up to C4. Further increases in plasticizing effect were not apparent in the range of C6 to C8. Hygroscopicities of these α-hydroxy acids are

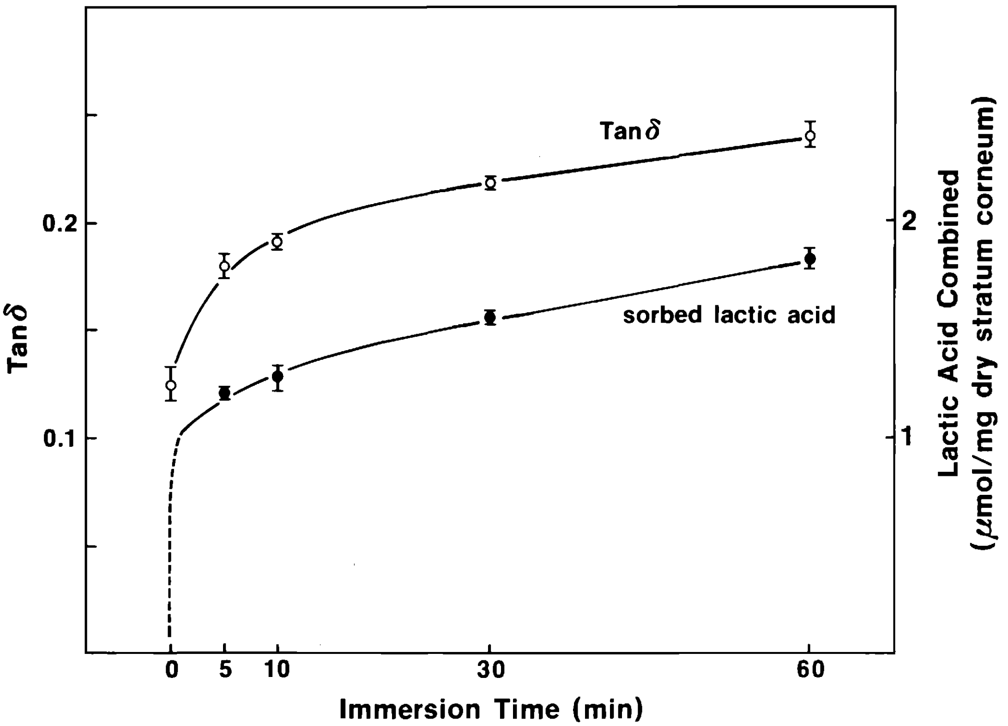

Figure 4. Tan δ (at 50% RH) and lactic acid sorption (at 25°C) as a function of immersion time in 1 M lactic acid solution for human stratum corneum. Results are shown as means and S.E.

shown in Table III. Mandelic acid and leucic acid had low hygroscopicities but nevertheless had noticeable platicizing ability toward stratum corneum.

Hydroxy acids have both OH and COOH groups. In order to study which group is more effective in softening stratum corneum, plasticizing effects of alcohols and acids were examined. The results are shown in Figure 7. Unlike lactic acid, all of the materials tested did not show appreciable plasticization effects.

Furthermore, α-hydroxy acids were compared with the β-type having the same numbers of carbon atoms for their plasticizing effects on stratum corneum. Tan δ values of stratum corneum treated by β-hydroxypropionic acid and β-hydroxybutyric acid were 0.194 and 0.165, respectively. Each value was lower than that obtained with the corresponding α-hydroxy acid. This shows that the α-type hydroxy acid was more effective in plasticizing stratum corneum than the β-type hydroxy acid.

DISCUSSION

The elastic forces in stratum corneum arise from intra- and inter-chain interactions, and the plasticization of stratum corneum is caused by water and urea which break hydrogen bonds in keratin (9). Plasticizers such as water and urea may be adsorbed at polar sites in keratin and mask the centers of force for keratin-keratin intermolecular attraction by selectively solvating the stratum corneum at these points, widening the

Table II
Effect of pH on Lactate Sorption by and Mechanical Properties of Stratum Corneum (at 50% RH) Immersed in 1 M/l Lactic Acid/Lactate Solution at 25°C

pH	Sorption (μmol/mg Dry Corneum)	Tan δ
2.0	1.83	0.240
3.8	0.65	0.170
8.0	0.24	0.155
Control	—	0.125

keratin interspace. Therefore, the larger the number of these molecules present, the more the stratum corneum is plasticized (3).

By our experimental results, it was shown that sodium lactate is slightly sorbed by stratum corneum. It also doesn't have an effective plasticizing effect under 50% RH, where the water content of stratum corneum is the same as in the control. However, it was also shown that the stratum corneum treated with sodium lactate became more pliable than the control above 60% RH, where it took up more water. From these results, the mechanism of plasticization by sodium lactate may be as follows.

Sodium lactate does not have a direct effect on stratum corneum pliability, but it adheres on the surface of stratum corneum and increases hydration by its hygroscopic

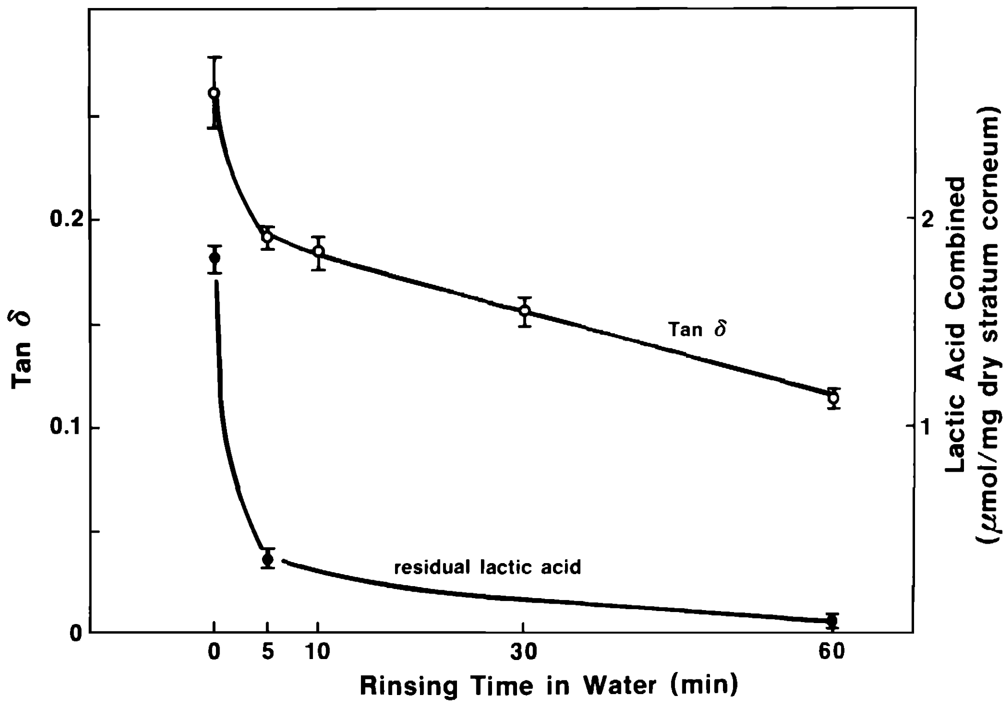

Figure 5. Tan δ (at 50% RH) and lactic acid combined as a function of rinsing time in water for human stratum corneum after immersion in 1 M lactic acid solution at 25°C. Results are shown as means and S.E.

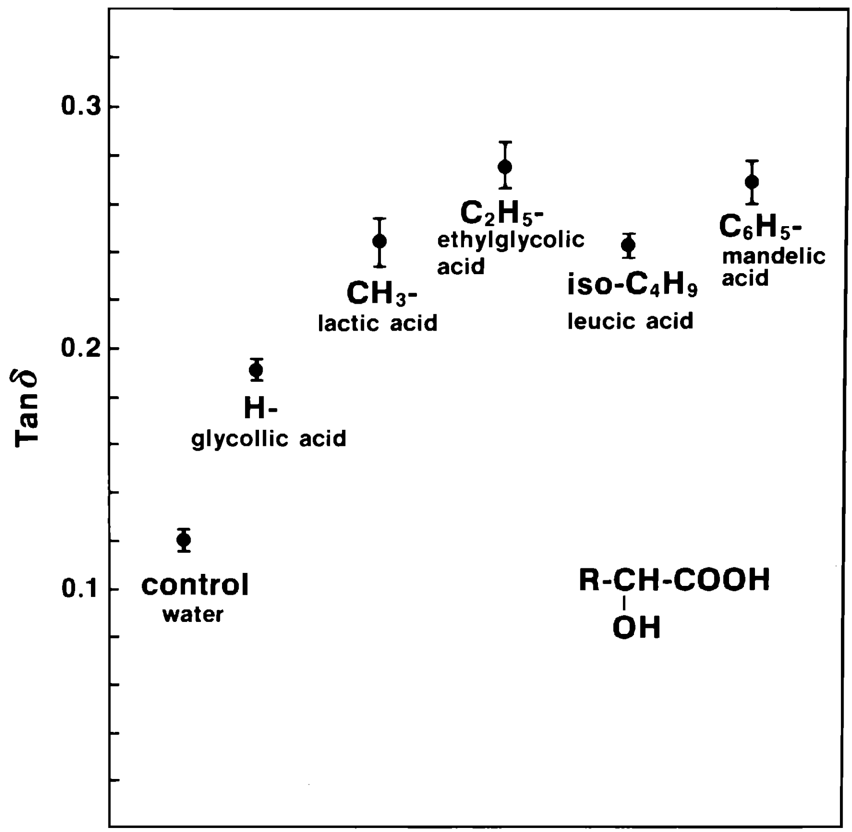

Figure 6. Effects of α-hydroxy acids on the mechanical properties of human stratum corneum at 25°C and 50% RH. Results are shown as means and S.E.

nature. The water molecules adsorbed by lactate plasticize stratum corneum. This was confirmed by the results showing that the water uptake and tan δ were parallel with each other for the stratum corneum treated by sodium lactate. Since the hygroscopicity of sodium lactate is low at the lower RHs and the quantity of sorbed lactate is small, there may be no significant difference in water uptake between treated stratum corneum and the control at the lower RHs.

On the other hand, lactic acid markedly plasticized stratum corneum at every RH even though it did not increase the water content in stratum corneum. Furthermore, the

Table III
Hygroscopicities of α-Hydroxy Acids at 25°C (H_2O mg/100 mg)

	Relative Humidity (%)			
	33	53	76	84
Glycollic Acid	0.5	5.6	73.5	114.5
Ethylglycolic Acid	5.1	11.7	29.3	51.4
Leucic Acid	0.0	0.1	1.3	1.8
Mandelic Acid	0.0	0.1	0.1	0.1

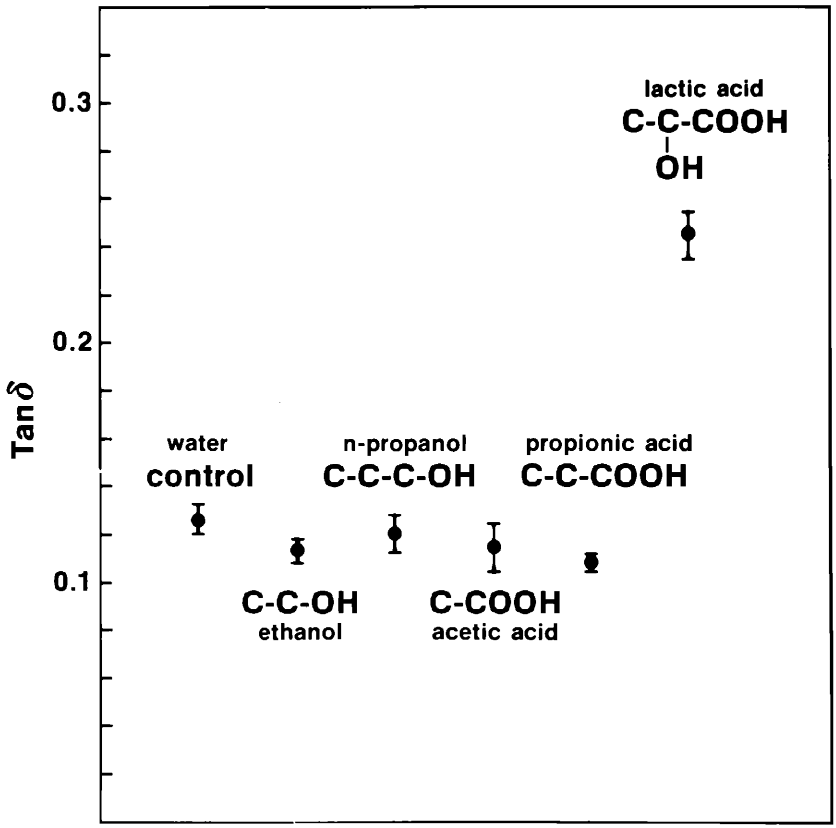

Figure 7. Effects of acids and alcohols on the mechanical properties of human stratum corneum at 25°C and 50% RH. Results are shown as means and S.E.

pliability of stratum corneum was dependent on the quantity of sorbed lactic acid. This can be explained assuming that lactic acid behaves as a plasticizer like water. It may be adsorbed by the polar groups of keratin chains to reduce the interaction between the groups, resulting in the softening of stratum corneum. Therefore, the rheological properties of stratum corneum gradually recovered to the non-treated level concomitant with the desorption of lactic acid. This is similar to the case of water treatment (11). From the observation that not only lactic acid but also other hydroxy acids, especially non-hygroscopic mandelic acid, have plasticizing effects, it may be concluded that water is not necessarily the only material capable of softening stratum corneum. Alpha-hydroxy acid may be incorporated in stratum corneum and break hydrogen bonds in keratin to lower elasticity as with water.

Recently, Alderson *et al.* (12) have found that 2-hydroxycaprylic acid could increase the extensibility of normal guinea-pig footpad stratum corneum without increasing its water content. We too showed that non-hygroscopic α-hydroxy acids soften human stratum corneum by adsorbing to the keratin chain and reducing interactions between them.

A good plasticizer for polymeric materials has: (A) polar groups that interact suitably with polymer chains, (B) low volatility, and (C) a suitable molecular configuration.

These conditions may also be applied to a plasticizer for stratum corneum. Therefore, not only the characteristic of being easily sorbed to stratum corneum but also low plasticizer volatility seems to be an important factor in maintaining stratum corneum flexibility for prolonged periods. Mandelic acid is more effective than glycollic acid and lactic acid in plasticization of stratum corneum, partially due to its lower volatility.

The treatments with alcohols and acids having the same number of carbon atoms as lactic acid had no effect on the rheological properties of stratum corneum. This indicates that both carboxyl and hydroxyl groups are necessary in the molecular structure of a plasticizer for stratum corneum. This is further confirmed by the results that citric, malic, and tartaric acids also have plasticizing effects (unpublished data).

It is still not clear why an α-hydroxy acid has more plasticizing ability than the β-type, but it is assumed that the α-type penetrates more readily into the inter-keratin chains to reduce the interaction between them and has a favorable molecular structure to interact with the keratin chains.

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