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In vitro percutaneous absorption of alpha hydroxy acids in human skin

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Synopsis

Alpha hydroxy acids (AHAs) are used in many cosmetic products as exfoliants, moisturizers, and emollients. The activity of AHAs on skin is likely influenced by their ability to be absorbed into the different layers of skin. The absorption of a homologous series of AHAs was measured through human skin by using *in vitro* diffusion cell techniques. The [^{14}C] radiolabeled compounds were applied to the skin in an oil-in-water emulsion vehicle. The absorption of the AHAs was measured at pH 3.0, to simulate the pH of the most acidic cosmetic formulations, and at pH 7.0, to observe the effect of complete ionization of AHAs on skin penetration. Much greater absorption of the AHAs was seen at pH 3.0. We also observed substantial absorption into the various skin layers (stratum corneum, viable epidermis and dermis) as well as the receptor fluid. Total absorption of glycolic acid and lactic acid was similar (27–30%). Absorption of the longer-chain AHAs decreased to 21.0% and 19.3%, for 2-hydroxyoctanoic and 2-hydroxydecanoic acids, respectively. At the end of the 24-h studies, these longer-chain AHAs did not form a depot in the skin. The stratum corneum was shown to have a pH gradient with an average pH near 7 at the viable epidermal layer. Therefore, the AHAs ionize to polar molecules as they enter and diffuse through the stratum corneum.

INTRODUCTION

Alpha hydroxy acids (AHAs) are widely used in cosmetic products. The small, short-chained compounds glycolic acid and lactic acid are most widely used, but longer-chain AHAs have been found to increase stratum corneum extensibility and flexibility (1) and have been used in some products. The effects of AHAs on skin structure are noticed in the stratum corneum (2), the viable epidermis (3), and even deeper in the dermal layer (3).

The mechanism of AHA action is still unknown. Van Scot *et al.* have suggested that AHAs reduce stratum corneum corneocyte cohesion by interference with ionic bonding (4). But structural changes in the epidermis and dermis suggest that effects on the stratum corneum could originate from AHA activity in these deeper layers. Understanding the extent of absorption of AHAs is important, particularly with regard to the

localization in the various layers of skin. Also, the effect of AHA chemical structure on absorption was examined by studying the absorption of a homologous series of compounds.

The percutaneous absorption of five AHAs was measured through viable excised human skin in diffusion cells: glycolic acid (GA), lactic acid, 2-hydroxyhexanoic acid (2-hydroxycaproic acid), 2-hydroxyoctanoic acid (2-hydroxycaprylic acid), and 2-hydroxydecanoic acid (2-hydroxycapric acid). AHA absorption was assessed by determining levels of absorbed material in skin layers and in the receptor fluid beneath the skin.

MATERIALS AND METHODS

MATERIALS

[1-^{14}C]Glycolic acid (specific activity, 55 mCi/mmol; 99% purity) was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO), and [1-^{14}C]DL-lactic acid (specific activity, 50 mCi/mmol; 98% purity) was obtained from Sigma Chemical Co. (St. Louis, MO).

[1-^{14}C]2-Hydroxyhexanoic acid (specific activity, 17.6 mCi/mmol; 96% purity), [1-^{14}C]2-hydroxyoctanoic acid (specific activity, 19.2 mCi/mmol; 97% purity), and [1-^{14}C]2-hydroxydecanoic acid (specific activity, 16.4; 92% purity) were synthesized by Research Triangle Institute (Research Triangle Park, NC). [^{3}H]Water (specific activity, 55.5 mCi/mmol; 97% purity) was purchased from New England Nuclear Corp. (Boston, MA). Nonlabeled glycolic acid, lactic acid, 2-hydroxyoctanoic acid, and 2-hydroxydecanoic acid were obtained from Sigma Chemical Co. Nonlabeled 2-hydroxyhexanoic acid was obtained from Aldrich Chemical Co. (Milwaukee, WI). Commercial product 1 (5% GA, pH 2.5) and commercial product 2 (10% GA, pH 3.5) were obtained from a local cosmetics supplier.

OIL-IN-WATER EMULSION FORMULATIONS

Percutaneous absorption of glycolic acid was studied by using two oil-in-water emulsion formulations (Formulations A and B). The composition of Formulation A is given in Table I. It contained two non-ionic emulsifying agents: polyethylene glycol (PEG) 100 stearate (2%) and PEG-4 lauryl ether (Laureth-4) (1%). Formulation B had the same composition as Formulation A, except that 1% ammonium laureth sulfate (ALS), an ionic surfactant, was used in place of the Laureth-4. Formulation A was the vehicle used in most of the percutaneous absorption studies.

Emulsions containing 5% AHAs were prepared by dissolving the acid in either the pH 3 or pH 7 buffer, readjusting the buffer to the proper pH, and then mixing with the other ingredients in phase B. Phases A and B were heated separately to 75–80°C, and then phase B was added to phase A and mixed at high shear in an Omni-Mixer Homogenizer (Omni International, Warrenton, VA) for 1 h. Mixing was continued at a lower shear until the temperature of the emulsion reached room temperature. Phase C, the preservative, was then added, and the emulsion was stirred for an additional 30 min. For the 0.5% emulsions, a stock emulsion (containing no AHA) was prepared, and then

Table I
Composition of the Oil-in-Water Emulsions for 5% Alpha Hydroxy Acids

Formulation A	Grams per 100 grams emulsion
Phase A	
Polyoxyethylene (100) glycerol stearate (ICI Surfactants, Wilmington, DE)	2.0
Mineral oil (light) (Penreco, Karns City, PA)	10.0
Cetearyl alcohol (Henkel Corp., Hoboken, NJ)	3.0
Phase B	
Laureth-4 (Lipo Chemicals, Paterson, NJ)	1.0
Propylene glycol (Aldrich Chemical Co., Milwaukee, WI)	5.0
Alpha hydroxy acid	5.0
Phthalate-HCl buffer*	
Potassium phosphate-NaOH**	73.0
Phase C Preservative	
Methyl-p-hydroxybenzoate (Pfaltz & Bauer, Inc., Stamford, CT)	0.5
Propyl-p-hydroxybenzoate (Pfaltz & Bauer, Inc., Stamford, CT)	0.5

* Phthalate-HCl buffer (pH = 3): 50 ml of 0.1 M potassium biphthalate + 22.3 ml of 0.1 M HCl diluted with water to 100 ml.
** Potassium phosphate-NaOH buffer (pH = 7): 50 ml of 0.1 M potassium phosphate + 29.1 ml 0.1 M NaOH diluted with water to 100 ml.

appropriate amounts of AHA were added to aliquots of the stock emulsion to give the desired concentration of AHA.

PERCUTANEOUS ABSORPTION EXPERIMENTS

Skin absorption studies were conducted by using human skin freshly obtained from abdominoplasty procedures. The skin was placed in a saline solution at the clinic and kept in cool packing as it was transported to the laboratory and transferred to Hepes buffered Hanks' balanced salt solution (HHBSS). Subcutaneous fat was removed from the skin, and the surface was gently cleaned with a 10% soap solution and rinsed with distilled water. The skin was mounted on a Styrofoam block and cut with a Padgett dermatome (Padgett Instruments, Dermatome Division, Kansas City, MO) to a thickness of 200–340 μm. Skin discs were prepared with a punch and placed epidermis-side up in Teflon flow-through diffusion cells (5). Prior to assembly, the flow-through diffusion cell system was disinfected with 70% ethanol and rinsed with receptor fluid. The diffusion cells were maintained at 35°C in an aluminum holding block heated by a circulating water bath; this maintained the surface temperature of the stratum corneum at 32°C. The skin was perfused with HHBSS, pH 7.4, receptor fluid at a flow rate of 1.5 ml/h to maintain the viability of the skin in the diffusion cells for the duration of the 24-h study (6).

A 20-min skin barrier integrity check, using [^{3}H]water, was conducted prior to the application of the AHA test formulations to ensure that the permeability of the human skin was in the normal range and that the skin was not damaged (7). Cells in which the percent of the applied dose of [^{3}H]water absorbed through the skin was greater than the historical limit of 0.35% were discarded.

The AHA test formulations were previously prepared to give an average dose of 0.55 μCi of [^{14}C] radiolabeled AHA per cell. The emulsion was applied to the skin at 3 mg/cm^2 of exposed skin in the diffusion cells (exposed skin = 0.64 cm^2). At the end of each experiment the skin surface was washed three times with 0.3 ml of a 10% soap solution and rinsed three times with 0.3 ml of distilled water to remove unabsorbed material remaining on the surface of the skin. The skin was removed from the diffusion cell and tape-stripped with Scotch Magic™ cellophane tape (3M Commercial Office Supply Division, St. Paul, MN) ten times to remove the stratum corneum.

The remaining epidermis was separated from the dermis with heat. The skin was wrapped in Saran Wrap plastic wrap (DowBrands L.P., Indianapolis, IN) and submerged in a 60°C water bath for 40 s. The skin was unwrapped and the epidermis was then slowly peeled from the dermis. The epidermis and dermis were cut into thin strips with a razor and digested with tissue solubilizer.

ALPHA HYDROXY ACID ANALYSIS

The absorbed radioactivity in the 6-h receptor fluid fractions and the skin layers was measured by liquid scintillation counting (Minaxiβ Tri-Carb® 4000 Series liquid scintillation counter, Packard Instrument Co., Downers Grove, IL) using Ultima Gold™ (Packard Instrument Co., Meriden, CT) liquid scintillation cocktail.

BARRIER INTEGRITY DETERMINATIONS

The barrier integrity of hairless guinea pig skin following 24-h exposure to glycolic acid formulations was assessed by measuring the steady-state rate of penetration of [^{3}H]water and then calculating a permeability constant (Kp). Skin from 4- to 6-month-old male hairless guinea pigs [strain Crl:AF/HA (hr/hr)Br] (Charles River Laboratories, Wilmington, MA) was dermatomed to a thickness of 200–300 μm and assembled into flow-through diffusion cells. Glycolic acid formulations were applied to the surface of the skin (3 mg/cm^2), while some diffusion cells containing skin were left untreated (control skin). After 24 h, the surface of the skin (including untreated control skin) was washed three times with 0.3 ml of a 10% soap solution, rinsed three times with distilled water, and blotted dry with a cotton-tipped applicator. [^{3}H]water (2.34 to 2.88 μCi) was applied in excess (800 μl) to the surface of the skin, the diffusion cell was covered, and effluent from the flow cell was collected every half hour until a steady-state rate of permeation was established (about 4 to 4.5 h). Permeability constants were calculated by dividing the rate by the initial concentration of [^{3}H]water.

SKIN SURFACE pH MEASUREMENTS

The pH profile of human skin in flow-through diffusion cells was determined 24 h after

application of an O/W Emulsion (Formulation A, without AHA) at pH 3.0. The O/W emulsion was applied to the surface of the skin (3 mg/cm^2), and after 24 h the skin was washed, rinsed, and dried in a manner described previously. The skin was removed from the diffusion cell, and the pH of the skin surface was measured on a Corning pH meter model 320 (Corning Inc., Science Products Division, Corning, NY) using an MI-404 Flat Membrane pH Electrode with an MI-402 Micro-Reference Electrode (Microelectrodes, Inc., Bedford, NH). The layers of the stratum corneum were removed by stripping 15 times with cellophane tape. After each tape strip, the pH of the skin surface was measured.

STATISTICAL ANALYSIS

Total absorption values represent the combined absorption values for receptor fluid and skin (stratum corneum, viable epidermis, and papillary dermis) and were compared by the Student's *t*-test or a one-way analysis of variance (ANOVA, SigmaStat™ Statistical Software, Jandel Scientific Software, San Rafael, CA). The permeability constant (Kp) determinations were compared statistically by performing a Student's *t*-test, and an ANOVA. The Student-Newman-Keuls test was used as the method for multiple pairwise comparisons at a significance level of $p < 0.05$ (SigmaStat™ Statistical Software).

RESULTS

The *in vitro* percutaneous absorption of glycolic acid was measured from an oil-in-water (O/W) emulsion (Formulation A) at a concentration of 5% at pH 3.0 and 7.0. Greater glycolic acid absorption was observed in all locations with the emulsion adjusted to pH 3.0. Total absorption of glycolic acid in 24 h decreased from 27.2% at pH 3.0 to 3.5% with the pH 7.0 emulsion (Table II). Significant amounts of glycolic acid were found in the receptor fluid at pH 3.0 (2.6%), but larger amounts were found in the skin layers (24.6%). Glycolic acid was not only located in the surface layer (the stratum corneum), but greater amounts were found in the deeper skin layers (the viable epidermis and dermis).

In order to study the effects of surfactants on the percutaneous absorption of glycolic

Table II
Percent Applied Dose Absorbed of 5% AHA in Formulation A

	5% Glycolic acid		5% Lactic acid		5% 2-OH-hexanoic acid	
Location	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7
Receptor fluid	2.6 ± 0.7[a]	0.8 ± 0.3	3.6 ± 1.2[b]	0.4 ± 0.1	32.9 ± 2.6[a,b]	1.0 ± 0.2
Stratum corneum	5.8 ± 2.8	1.2 ± 0.4	6.3 ± 1.4	3.2 ± 0.8	3.4 ± 0.4	2.8 ± 0.3
Viable epidermis	6.6 ± 2.5	0.8 ± 0.3	6.6 ± 0.9	3.2 ± 0.8	2.8 ± 1.4	3.7 ± 1.3
Dermis	12.2 ± 1.4[a]	0.6 ± 0.2	13.9 ± 2.3[b]	2.9 ± 1.3	4.0 ± 1.8[a,b]	2.0 ± 0.3
Total in skin	24.6 ± 4.0[a]	2.6 ± 0.6	26.8 ± 4.5	9.4 ± 2.1	10.2 ± 3.3[a]	8.4 ± 1.0
Total absorption	27.2 ± 3.3	3.5 ± 0.9	30.4 ± 3.3	9.7 ± 2.0	43.1 ± 5.9	9.4 ± 1.1

Values are the mean ± SEM of two to five determinations in each of three subjects. Values obtained at pH 3.0 in each location with similar superscripts are significantly different from each other (ANOVA, $p < 0.05$).

acid, a second emulsion (Formulation B) was prepared with 1% ammonium laureth sulfate (ALS). ALS is an ionic surfactant contained in some AHA rinse-off formulations. Total absorption of glycolic acid was unchanged at either pH (Table III). However, the absorbed glycolic acid was distributed differently in the skin and receptor fluid at the end of the 24-h studies. A greater amount of the absorbed material was found in the receptor fluid with the use of Formulation B, with approximately 12% of the applied dose completely penetrating skin at pH 3.0.

The effects of Formulations A and B on the barrier properties of hairless guinea pig skin were compared with the effects of two commercial AHA products. A [^{3}H]water permeability constant was determined after 24-h exposure to each of the formulations (Figure 1). The average of Kp values for all formulations was higher than the control (no emulsion) value. However, a one-way analysis of variance showed that none of the formulations were significantly different from each other ($p < 0.05$). Formulation A was utilized as the vehicle for the additional AHA absorption studies.

Variability in GA absorption (Formulation B) through the skin from the five human donors (Figure 2) was observed. The skin from all donors was within our historically normal limits of [^{3}H]water absorption ($\leq$0.35% of the applied dose) as assessed by the 20-min test prior to application of the GA formulations (7). Glycolic acid absorption through donor skin varied from 24% to 44% of the applied dose. A high correlation was observed between the water and glycolic acid absorption values ($r^2 = 0.92$) from each donor, indicating that the variability in glycolic acid absorption was associated with the normal variability in the barrier properties of human skin.

The absorption of lactic acid and 2-hydroxyhexanoic acid was determined from an O/W emulsion at a concentration of 5% (Table II). The pH-related differences in total absorption observed for these compounds were substantial but were less than the difference seen for GA. Total AHA absorption at pH 3.0 did not differ significantly among the three AHAs (ANOVA, $p = 0.087$). However, at pH 3.0, receptor fluid levels of 2-hydroxyhexanoic acid were significantly higher than those of the other 2 AHAs, and skin levels of 2-hydroxyhexanoic acid were significantly lower than the values for GA.

The longer-chain AHAs were tested at 0.5% concentration because of solubility limitations and to simulate product usage (Table IV). 2-Hydroxyhexanoic acid absorption studies were repeated at this lower concentration for comparison of absorption with

Table III
Percent Applied Dose Absorbed of 5% Glycolic Acid in Formulation B

	Percent applied dose absorbed	
Location	pH 3.0	pH 7.0
Receptor fluid	12.2 ± 5.3	1.4 ± 0.7
Stratum corneum	2.4 ± 1.3	0.1 ± 0.0
Viable epidermis	11.6 ± 2.5	0.4 ± 0.2
Dermis	8.6 ± 2.0	0.4 ± 0.1
Total in skin	22.6 ± 3.2	0.9 ± 0.0
Total absorption	34.8 ± 3.9	2.3 ± 0.8

Values are the mean ± SEM of two to six determinations from five donors (pH 3.0) and three determinations from three donors (pH 7.0).

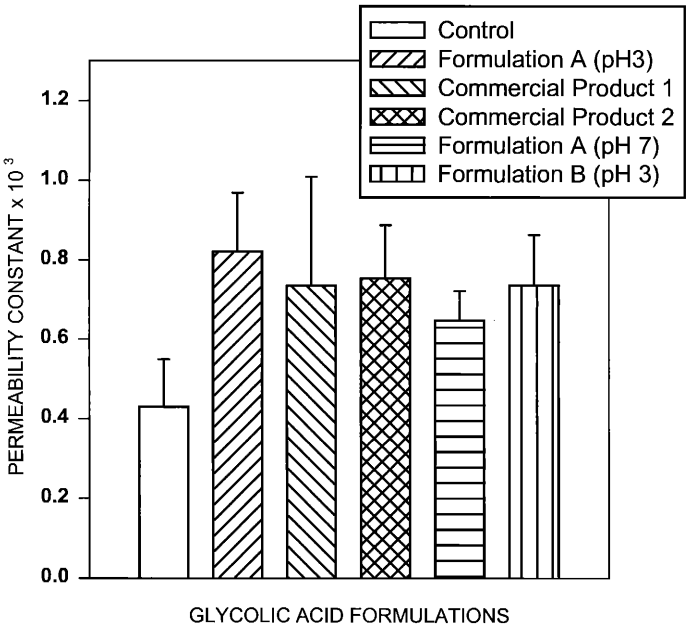

Figure 1. The effect of various glycolic acid formulations on the barrier properties of hairless guinea pig skin. The values are the mean ± SEM of three to four determinations in each of two to five animals. A one-way ANOVA indicated that none of the formulations were significantly different from each other ($p < 0.05$).

chemical dose. The receptor fluid percentage absorbed is significantly lower at the 0.5% dose level, but the skin and total absorption percentages are not statistically different (*t*-test, $p < 0.05$). There was no significant difference between 0.5% 2-hydroxyhexanoic, 2-hydroxyoctanoic, and 2-hydroxydecanoic acids with regard to total absorption (ANOVA, $p = 0.19$) or receptor fluid levels (ANOVA, $p = 0.28$). However, 2-hydroxyhexanoic acid totals in skin values were significantly higher than corresponding values for the other AHAs.

Skin from two human skin donors was assembled in diffusion cells, treated with an O/W emulsion (Formulation A, without AHA), at pH 3, and maintained in the cells for 24 h. The skin was removed from the cells, and the pH of the skin surface was determined initially and following each of 15 tape strippings (Figure 3). Initial skin surface pH values were approximately 5.3 for the two donors. The pH of the stratum corneum increased gradually to 6.5 and 7.3 for the two donors as the stratum corneum layers were completely removed.

DISCUSSION

The percutaneous absorption of GA is dependent on the pH of the formulation since the ionized molecule is more polar and therefore less readily absorbed. The effect of pH on the ionization of GA (pKa = 3.8) can be calculated from the Henderson-Hasselbach equation (Figure 4). At pH 3.0, the GA remains mostly un-ionized (87%) and even at pH 3.8, 50% of the compound is in the un-ionized form. We have evaluated GA and

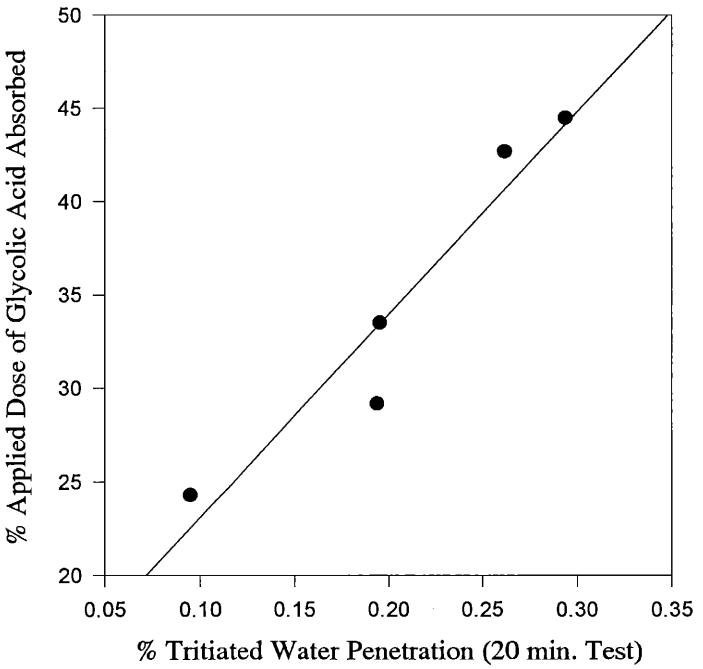

Figure 2. Human skin variability: correlation between barrier integrity and glycolic acid absorption. The values are the mean of two to six determinations in each of five subjects.

Table IV
Percent Applied Dose Absorbed of 0.5% AHA in Formulation A

	0.5% 2-OH-hexanoic acid	0.5% 2-OH-octanoic acid	0.5% 2-OH-decanoic acid
Location	pH3	pH3	pH3
Receptor fluid	10.1 ± 2.7	15.4 ± 3.1	8.8 ± 2.5
Stratum corneum	3.2 ± 0.9	1.4 ± 0.3	2.6 ± 0.6
Epidermis	8.4 ± 1.1[a]	2.8 ± 0.4[a,b]	5.8 ± 0.9[b]
Dermis	6.7 ± 0.7[a,b]	1.4 ± 0.2[a]	2.1 ± 0.3[b]
Total in skin	18.3 ± 2.6[a,b]	5.5 ± 0.9[a]	10.5 ± 1.0[b]
Total absorption	28.4 ± 3.9	21.0 ± 2.5	19.3 ± 3.1

Values are the mean ± SEM of two to five determinations in each of three subjects. Values in each location with similar superscripts are significantly different from each other (ANOVA, $p < 0.05$).

other AHAs at pH 3.0 to simulate the acidic pH of some commercial cosmetic products containing these ingredients. The effect of pH is clearly seen in Table II on both receptor fluid and skin levels of the three lower-chain AHAs. The magnitude of reduction in absorption at pH 7.0 differed among the AHAs in some locations. Less of a pH difference was seen in the skin levels obtained with lactic acid and 2-hydroxyhexanoic acid. Even at pH 7.0, between 9% and 10% of the applied lactic and 2-hydroxyhexanoic acid was absorbed.

The differing GA absorption profiles with Formulations A and B (Tables II and III) illustrate the potential effects of cosmetic vehicles on AHA absorption. Differences in

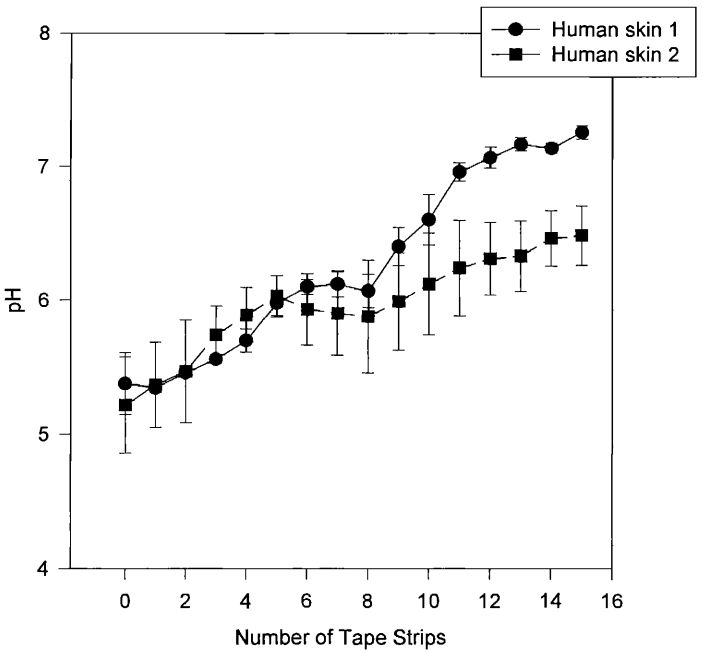

Figure 3. The pH profile of human skin in flow-through diffusion cells 24 h after application of an O/W emulsion, pH 3.0. The values are the mean ± SEM of three determinations in each of two subjects.

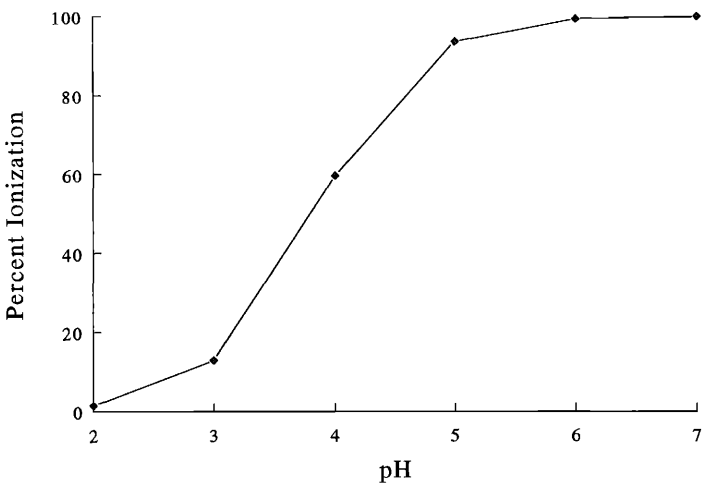

Figure 4. The effect of pH on glycolic acid ionization as determined by the Henderson-Hasselbach equation.

skin response to similar AHA products (based on concentration and pH) may be due to differences in absorption due to vehicle effects. However, neither Formulation A nor Formulation B differed from two commercial products in their effects on hairless guinea pig barrier integrity (Figure 1).

The variability in absorption properties of normal human skin is illustrated in Figure 2. Absorption values obtained from the skin of one or two donors can be misleading,

particularly if skin is damaged in harvesting or is stored before use. The use of a standard compound such as tritiated water to check barrier integrity of the skin aids in the assessment of the accuracy of absorption measurements.

No significant differences in the total absorption of AHAs at pH 3.0 were observed in either Tables II or IV. However, some differences in absorption were obtained in levels measured in the receptor fluid or skin locations. 2-Hydroxyhexanoic acid levels are much higher in the receptor fluid and lower in skin than other AHAs (Table II). The skin levels of 2-hydroxyoctanoic acid are lower than the other AHAs in Table IV. A different comparison of the AHA absorption values can be made by examining the percent of the absorbed dose remaining in the skin (Figure 5). It appears that there is a tendency toward a decrease in the percentage of the absorbed AHA remaining in skin and stratum corneum (data not shown) with the longer-chain compounds. This is opposite to what might be expected for the more lipophilic compounds, especially 2-hydroxydecanoic acid. The octanol/water (pH 3.0) partition coefficients for 2-hydroxyhexanoic, 2-hydroxyoctanoic, and 2-hydroxydecanoic acids were determined to be 3.7, 30.6, and 71.1, respectively.

However, these acids ionize to polar compounds at physiological pH as they enter and are absorbed through the stratum corneum. The pH of human stratum corneum in the diffusion cells was determined to range from initial surface values of approximately 5.3 up to values ranging from 6.5 to 7.3 at the stratum corneum–viable epidermal interface (Figure 3). These values are in agreement with *in vivo* stratum corneum stripping studies that found a pH gradient in human stratum corneum ranging from pH 4.5 to 5.3 on the skin surface to a pH of about 7 at the viable epidermal layer (8). Therefore, these longer chain AHAs are not expected to form a reservoir in skin based on their lipid solubility properties.

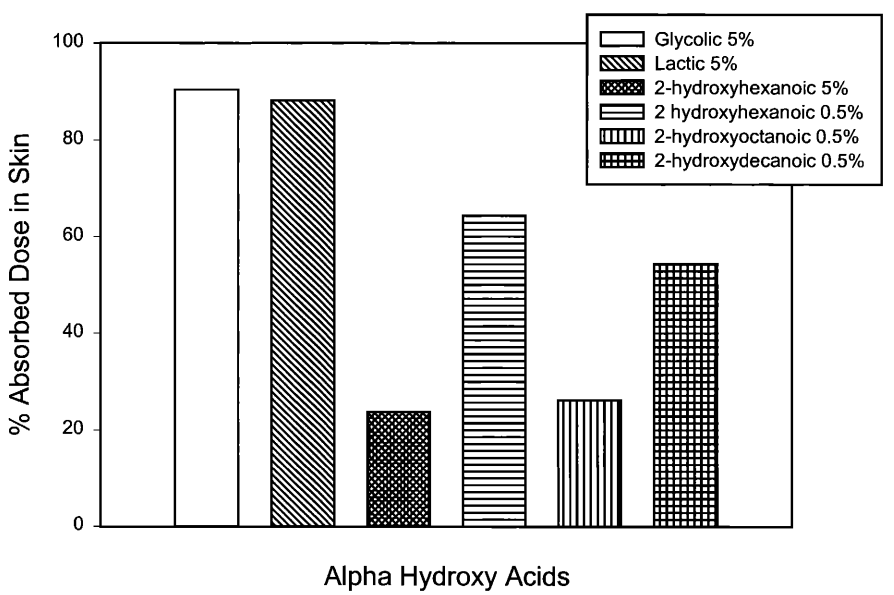

Figure 5. The effect of AHA chain lengths on skin levels. The values are the mean ± SEM of two to five determinations in each of three subjects.

The percutaneous absorption of GA through animal skin has previously been reported from an aqueous solution (9). Absorption values of 0.7% and 0.9% were reported in 8 h through minipig and hairless mouse skin, respectively, from a pH 3.8 aqueous solution. But an infinite dose applied to skin of over 100 µl per cm^2 makes the values not relevant to "in use" conditions.

We have found that AHAs are extensively absorbed into and through human skin from a relevant dose of an O/W emulsion adjusted to pH 3.0. Approximately 27% of the applied dose of glycolic acid was absorbed in 24 h, and there was no significant difference in total absorption when compared with values obtained from longer-chain AHAs.

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