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Barrier recovery is impeded at neutral pH, independent of ionic effects: implications for extracellular lipid processing

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Abstract Epidermal permeability barrier homeostasis requires the postsecretory processing of polar lipid precursors into nonpolar lipid products within the stratum corneum (SC) interstices by a family of lipid hydrolases. A specific requirement for β-glucocerebrosidase (β-GlcCer'ase), which exhibits a distinct acidic pH optimum, is particularly well documented. Therefore, we sought to determine whether the recovery of the barrier after acute insults requires acidification of the SC. We examined permeability barrier recovery by assessing changes in transepidermal water loss (TEWL), SC membrane ultrastructure utilizing ruthenium tetroxide (RuO_4) postfixation, and β-GlcCer'ase activity by in situ zymography at an acidic vs neutral pH. Barrier recovery proceeded normally when acetone-treated skin was exposed to solutions buffered to an acidic pH. In contrast, the initiation of barrier recovery was slowed when treated skin was exposed to neutral or alkaline pH, regardless of buffer composition. In addition, enhancement of the alkaline buffer-induced delay in barrier recovery occurred with Ca^{2+} and K^+ inclusion in the buffer. Moreover, the pH-dependent alteration in barrier recovery appeared to occur through a mechanism that was independent of Ca^{2+}- or K^+-controlled lamellar body secretion, since both the formation and secretion of lamellar bodies proceeded comparably at pH 5.5 and pH 7.4. In contrast, exposure to pH 7.4 (but not pH 5.5) resulted in both the persistence of immature, extracellular lamellar membrane structures, and a marked decrease in the in situ activity of β-GlcCer'ase. These results suggest first that an acidic extracellular pH is necessary for the initiation of barrier recovery, and second that the delay in barrier recovery is a consequence of inhibition of postsecretory lipid processing.

Key words Barrier function · pH · Stratum corneum · Lamellar body · Lipid content · Ultrastructure

Abbreviations β-*GlcCer'ase* β-glucocerebrosidase · *CBE* conduritol B-epoxide · *HEPES* *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid · *4-MUG* 4-methylumbelliferyl-β-D-glucoside · *PBS* phosphate-buffered saline · *PIPES* piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) · *SC* stratum corneum · *TEWL* transepidermal water loss · *Tris* tris(hydroxymethyl) aminomethane

Introduction

Formation of the cutaneous permeability barrier requires both the secretion of the lipid and the hydrolytic enzyme contents of lamellar bodies and the subsequent postsecretory processing of polar lipids into their nonpolar lipid products. Whereas lamellar body secretion is regulated by changes in extracellular ions [16, 17], lipid processing appears to be mediated by a set of hydrolytic enzymes, which produce structural transformations within the stratum corneum (SC) interstices [4, 6], leading to barrier formation [4, 12, 21]. To date, two lipid processing enzymes have been shown to be required for the membrane transformations that result in barrier competence, β-glucocerebrosidase (β-GlcCer'ase) [10, 11], and an as-yet-uncharacterized secretory phospholipase A_2 [4, 19]. Whereas the pH optimum of the SC secretory phospholipase A_2 isoform is not known, epidermal β-GlcCer'ase exhibits a distinct acidic pH optimum [9, 10, 26]. Likewise, two other potential processing enzymes, steroid sulfatase and acid sphingomyelinase, also are concentrated in the SC interstices [5, 20], but their dependence on variations in the pH of the SC are not yet known.

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Whereas the epidermal surface has been known for more than a century to be acidic [3, 8, 28], the importance of the acidic pH of the SC for barrier homeostasis is suggested by: (a) the worsening of barrier function associated with alkalization of the skin [25]; (b) the exacerbation of experimentally induced contact dermatitis at alkaline pH [27]; and (c) the association of an alkaline skin pH with diaper dermatitis [1]. Since these observations suggest that the pH of the SC may influence barrier homeostasis, we tested the effects of pH on barrier recovery following acute barrier perturbations in hairless mice. We found that barrier recovery proceeds normally at an acidic pH, while recovery is delayed at neutral pH [7], independently of the ionic environment. Moreover, while lamellar body secretion appears to be unimpeded at neutral pH, postsecretory processing into mature lamellar membrane structures is impeded, and the in situ activity of β-GlcCer'ase is inhibited. Together, these results suggest that extracellular pH influences barrier homeostasis by modulating the processing of lipids in the SC interstices.

Methods

Materials

Conduritol-B-epoxide (CBE) was obtained from Toronto Chemical Co. (Toronto, Canada); 4-methyl umbelliferyl-β-D-glucoside (4-MUG) was obtained from Fluka Chemical Co. (Buchs, Switzerland); ruthenium and osmium tetroxide were from Polysciences (Warrington, Pa.). All other chemicals were from Sigma (St. Louis, Mo.).

Experimental protocols

To produce marked barrier perturbation, flanks of male hairless mice (hr/hr; Simonsen Laboratories, Gilroy, Calif.; <3 months of age), previously anesthetized with intraperitoneal injection of chloral hydrate, were treated with acetone-soaked cotton balls by gently rubbing the skin for several minutes, until the transepidermal water loss (TEWL) rates exceeded 5 mg/cm² per h (normal TEWL rates for unperturbed epidermal barrier are <0.1 mg/cm² per h). Each group comprised three or four animals. To produce moderate barrier perturbation, the same protocol was used to give a TEWL level of about 1 mg/cm² per h (single experiment, three animals).

Immediately after acetone treatment, the skin was warmed to approximately 35°C and the TEWL was measured using an electrolytic water analyzer (Meeco, Warrington, Pa.). The device's sampling cup, covered with Parafilm, was flushed with 99.9% pure nitrogen gas at 100 ml/min. After removal of the Parafilm, the cup was placed on the previously treated flank. TEWL was measured immediately after acetone treatment, and at various times after barrier disruption, up to 24 h, as shown. To minimize hydration effects, TEWL was recorded only after levels had reached their lowest point (i.e. 10–15 min). The opposite, nonimmersed flank served as either an untreated, or an equivalently treated air-exposed control.

After barrier disruption with acetone, one flank was submerged in isotonic sucrose or Ca^{2+}- and Mg^{2+}-free sodium phosphate-buffered saline (PBS), or with the indicated buffers, for the time intervals shown, at either pH 5.5 or 7.4. Additional animals were exposed to isotonic Ca^{2+}- and K^+-containing PBS (see legend to Fig. 4), isotonic *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES), isotonic piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES), and isotonic sodium citrate buffers over a range of pH values (i.e. 5.5, 6.5, 7.4, 8.5) after equivalent degrees of barrier disruption. While anesthetized, the animals were suspended on a mesh netting to avoid contact with the Petri dish [16]. The solutions were maintained at 35°C for the entire procedure. After 2 h, and either 4 or 5 h, the animals were removed, excess fluid was gently blotted off the skin surface, and TEWL was remeasured. The percentage recovery of TEWL was calculated by comparing TEWL measurements prior to immersion (immediately after barrier perturbation with acetone) with measurements after 2, 4 or 5, and 24 h. The pH had not changed in any of the solutions at the end of the immersion experiments. TEWL recovery rates after barrier disruption and immersion in isotonic sucrose, with air exposure alone, and with normal, unperturbed skin immersed in neutral vs acidic pH buffers for 2.5 h served as additional controls. Free Ca^{2+} concentrations in the solutions were measured using a Ca^{2+} electrode.

Transmission electron microscopy

Full-thickness skin samples were obtained from euthanized animals, minced to <0.5 mm³, and fixed in modified Karnovsky's fixative overnight. All samples were then divided and postfixed in the dark in either 0.5% ruthenium tetroxide or 2% aqueous osmium tetroxide, both containing 1.5% potassium ferrocyanide [12]. Thin sections were examined, with or without further contrast with lead citrate, in a Zeiss 10A electron microscope operated at 60 kV.

In situ zymography

To assess the effect of pH on β-GlcCer'ase activity in situ, we developed an in situ enzyme assay method. Briefly, fresh human skin sections were obtained from surgical margins, immediately placed into keratinocyte growth medium, and snap-frozen in 10.24% polyvinyl alchohol/4.26% polyethylene glycol medium (OCT, Miles Laboratories, Elkhart, Ind.) within 30 min of excision, sectioned (20 μm), and mounted onto poly-L-lysine-coated slides. Similar patterns were seen in mouse and human skin, but localization was better in human samples because of the increased number of cell layers in the epidermis and corneum [15]. Unfixed sections were equilibrated in the appropriate buffer, i.e. citrate/phosphate buffer (pH 5.5) or HEPES/Tris buffer (pH 7.4), for 30 min at 22–25°C. The pH of the buffer solutions was measured at the beginning and end of each incubation. Sections were incubated with substrate solution, containing 0.5 m*M* 4-MUG in the buffer solution for 30 min, covered with glass coverslips, sealed, and incubated for 16 h at 4°C (less diffusion of signal occurs at 4°C), and viewed on an inverted Zeiss (Thorn-wood, N.Y.) laser scanning confocal microscope (objective 40×, aperture 1.2, brightness set to maximal and contrast adjusted to 329 arbitrary units, pinhole 22). Scans were subjected to signal analysis, representing relative rates of 4-MU release, using Zeiss imaging software. Controls included both inhibitor-treated samples, i.e. 100 μ*M* CBE [9, 10] at both pH values, and substrate-excluded samples.

Statistical analysis

All TEWL results are presented as the mean ± SEM. A paired two-tailed Student's *t*-test was used to compare rates of recovery in the various experimental groups, based upon 100% abnormality in each animal.

Results

Neutral pH buffers retard the initiation of barrier recovery

To test whether extracellular pH influenced barrier recovery, we compared the kinetics of recovery in acetone-treated mouse skin exposed to physiologic (pH 7.4) or acidic (pH 5.5) buffer solutions. Barrier repair proceeded significantly more quickly ($P < 0.01$ at 2 and 4 h, $P < 0.05$ at 24 h) in skin sites exposed to pH 5.5 HEPES buffer than in sites exposed to pH 7.4 (Fig. 1). Skin sites immersed in acidic buffer demonstrated a rate of barrier recovery comparable to either skin exposed to air or immersed in isotonic sucrose alone (data not shown; see references 16, 17). Skin sites immersed in PIPES at pH 7.4 or pH 5.5 or in phosphate demonstrated identical curves at 2 and 4 h, suggesting that this phenomenon was independent of the type of pH buffer used. Specifically, for HEPES buffer, the delay was 25%, 40%, and 8%, for PIPES buffer, 20%, 33% and <5%, and for phosphate, 32%, 38% and 10% at two, five and 24 h, respectively. Since the kinetics of recovery in each of the buffers were similar after 2 h, regardless of pH (i.e. the slopes for neutral pH and acidic pH were parallel), the delay at neutral pH appears to occur primarily during the initial phase of barrier recovery, i.e. in the first 2 h. Furthermore, when skin sites first exposed to phosphate buffer at pH 7.4 were returned subsequently to pH 5.5, barrier recovery normalized so that between 2 h and 5 h the recovery rates (i.e. slope of the recovery curve) were no longer significantly different from the rate of recovery of skin exposed to the pH 5.5 buffers alone (Fig. 2; compare Fig. 1).

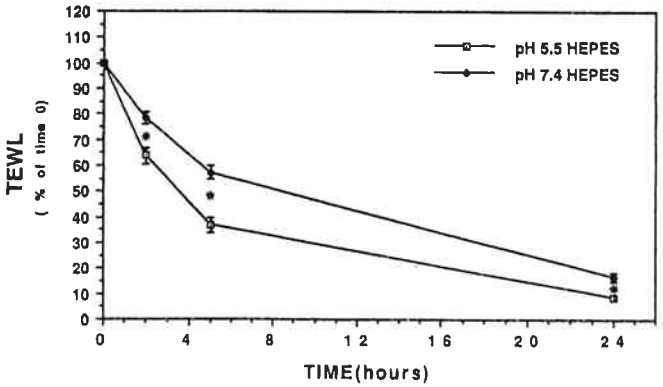

Fig. 1 Barrier repair is inhibited by neutral pH buffers. Transepidermal water loss (TEWL) rates were measured in animals exposed to bathing solutions of acidic or neutral pH. The epidermal barrier was perturbed to ≥ 5 mg/cm² per h with gentle wiping of acetone, and the animals' flanks were immersed in 10 m*M* HEPES, buffered to either pH 7.4 or 5.5. **P* > 0.01 (< 0.05 at 24 h). Values are means ± SEM (three or four animals per group)

The rapid normalization of recovery rates after 2 h, despite ongoing exposure to neutral pH, might have been a consequence of the restoration of a partial barrier, allowing the SC to regenerate an acidic pH. To test this hypothesis, we next measured barrier recovery in animals with a lesser initial barrier abnormality (i.e. ≈ 1 mg/cm² per h vs ≤ 5 mg/cm² per h, as in Fig. 1). The epidermal permeability barrier recovery was no different in skin exposed to pH 5.5 or pH 7.4 buffers when the barrier was only moderately disrupted (data not shown). Together, these experiments demonstrate that exposure of markedly permeabilized epidermis to neutral pH buffers results in a significant delay in the initiation of barrier recovery. This delay appears to be: (a) related to the extent of the initial insult; (b) not attributable to toxicity because it is rapidly and

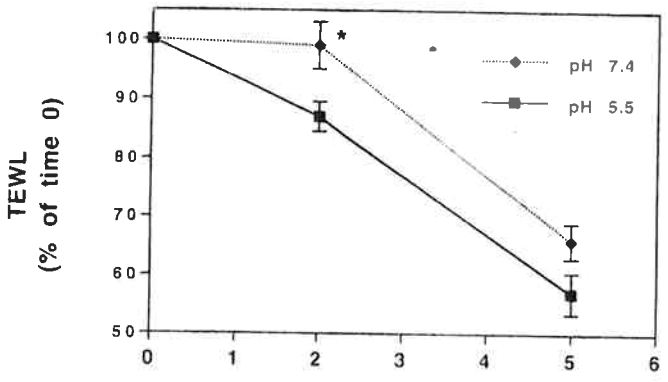

Fig. 2 Secondary exposure to acidic buffer reverses the initial neutral pH-induced delay of barrier recovery. TEWL rates were measured in animals after the epidermal permeability barrier had been perturbed to ≥ 5 mg/cm² per h with acetone wipes. The flank skin of one set of animals was immersed in solutions buffered to pH 7.4 with 10 m*M* HEPES for 2 h, then switched to either fresh pH 7.4 buffer (not shown; cf. Fig. 1), or pH 5.5 (also buffered with 10 m*M* HEPES) (◆) for the indicated times. The control animals (■) were exposed to pH 5.5 throughout the experiment. **P* < 0.05. Values are as means ± SEM (three animals per group)

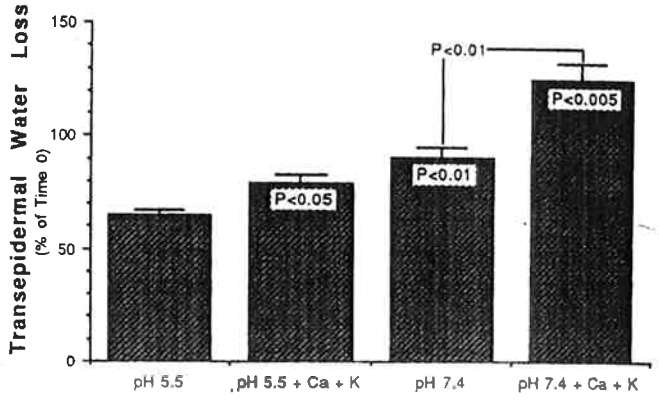

Fig. 3 pH influences barrier recovery independent of ions. The effects of maximally effective concentrations of Ca^{2+} (1.0 m*M*) and K^+ (40 m*M*) added to acidic (pH 5.5) or neutral (pH 7.4) citrate buffer 100 m*M* are shown. The epidermal permeability barrier first was disrupted as described in the legend to Fig. 1, and the animals' flanks were then immersed in 100 m*M* citrate buffer with or without the ions for 0–4 h. TEWL rates were normalized to 100% at 0 h, and the results are presented as percentage recovery 4 h after barrier perturbation (means ± SEM, three or four animals per group). The *P*-values shown are in relation to the value citrate buffer (pH 5.5) or as indicated

completely reversed, and it occurs with neutral, or near-physiologic, pH buffer; and (c) not due to effects of the buffers on preformed SC extracellular membranes (see below).

Neutral pH inhibits barrier recovery independent of extracellular ions

Since both neutral pH (shown above) and elevated extracellular Ca^{2+} [16, 17] delay barrier recovery, we next sought to determine whether the pH effect occurs independently, or through a common mechanism. Ca^{2+} and K^+ appear to mediate barrier repair by regulating lamellar body secretion, and the maximal inhibiting concentrations (extracellular) are 1.0 m*M* Ca^{2+} and 40 m*M* K^+, respectively [16, 17]. If neutral pH blocks barrier recovery by inhibiting lamellar body secretion by the same mechanism, then a combination of neutral pH with increased extracellular Ca^{2+}/K^+ should produce no greater inhibition of recovery than elevated Ca^{2+}/K^+ alone. Conversely, if these maneuvers each block by different mechanisms, then a combination of neutral pH with raised extracellular Ca^{2+}/K^+ should inhibit barrier recovery to a greater degree than either condition alone. Therefore, we disrupted the epidermal permeability barrier with acetone (TEWL $\geq$ 5 mg/cm^2 per h), as above, and immersed the animals' flanks for up to 4 h in each of the solutions as detailed in the legend to Fig. 3. The extracellular pH was buffered with 1.0 m*M* citrate, an organic buffer with a relatively low affinity for Ca^{2+} at this concentration (the stability of extracellular free Ca^{2+} in this buffer during experiments was verified using a Ca^{2+} electrode).

The most rapid recovery occurred in pH 5.5 citrate buffer, with no added Ca^{2+} or K^+, when both secretion and

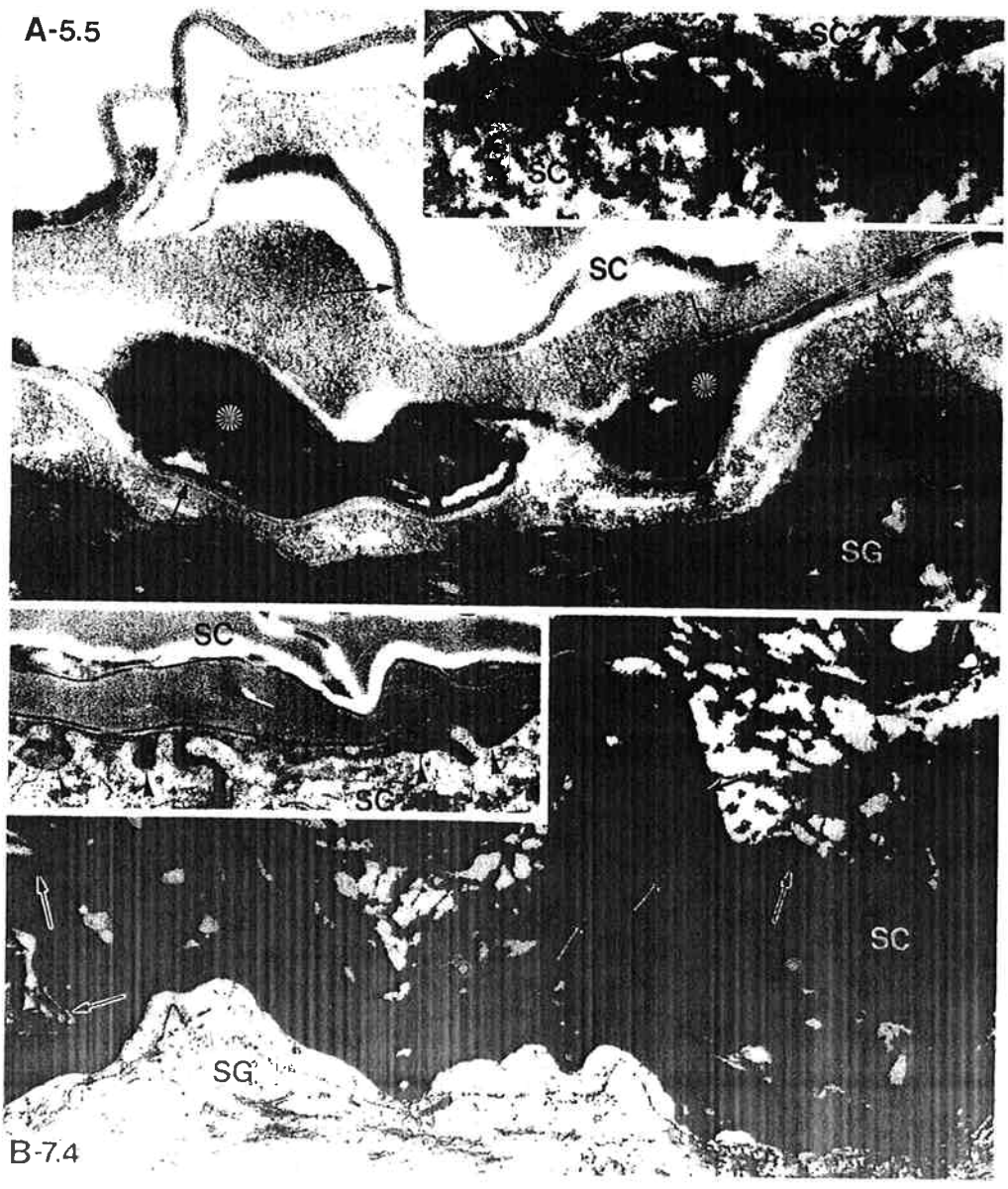

Fig. 4A,B External pH alters extracellular lamellar membrane maturation, not lamellar body secretion. Skin samples were obtained from mice after the epidermal permeability barrier had been perturbed by tape stripping, and the animals were immersed for 2 or 4 h in Ca^{2+}/Mg^{2+}-free PBS, buffered with 10 m*M* HEPES to pH 7.4 or pH 5.5 (the delay in recovery rate was 40% at pH 7.4 in relation to that at pH 5.5 in this experiment). **A** At pH 5.5, as in normal stratum corneum (SC), secreted lamellar body sheets began to transform into lamellar unit structures one cell layer above the stratum granulosum (SG)-SC interface (*arrows*, *arrowheads* in *insert*). **B** At pH 7.4, transformation into mature lamellar membrane structures occurred infrequently, with the predominance, instead, of incompletely processed lamellar body-derived sheets in the lower SC (*arrows*). Lamellar body secretion, however, was not inhibited at pH 7.4 (*insert*, *arrowheads*). Large areas of phase separation (*asterisks*) may indicate sites distended by buffer solutions. **A** *A insert* **B** RuO_4 postfixation; **B** *insert* OsO_4 postfixation; **A** ×115 000, **A** *insert* ×105 000, **B** ×67 000, **B** *insert* ×40 000

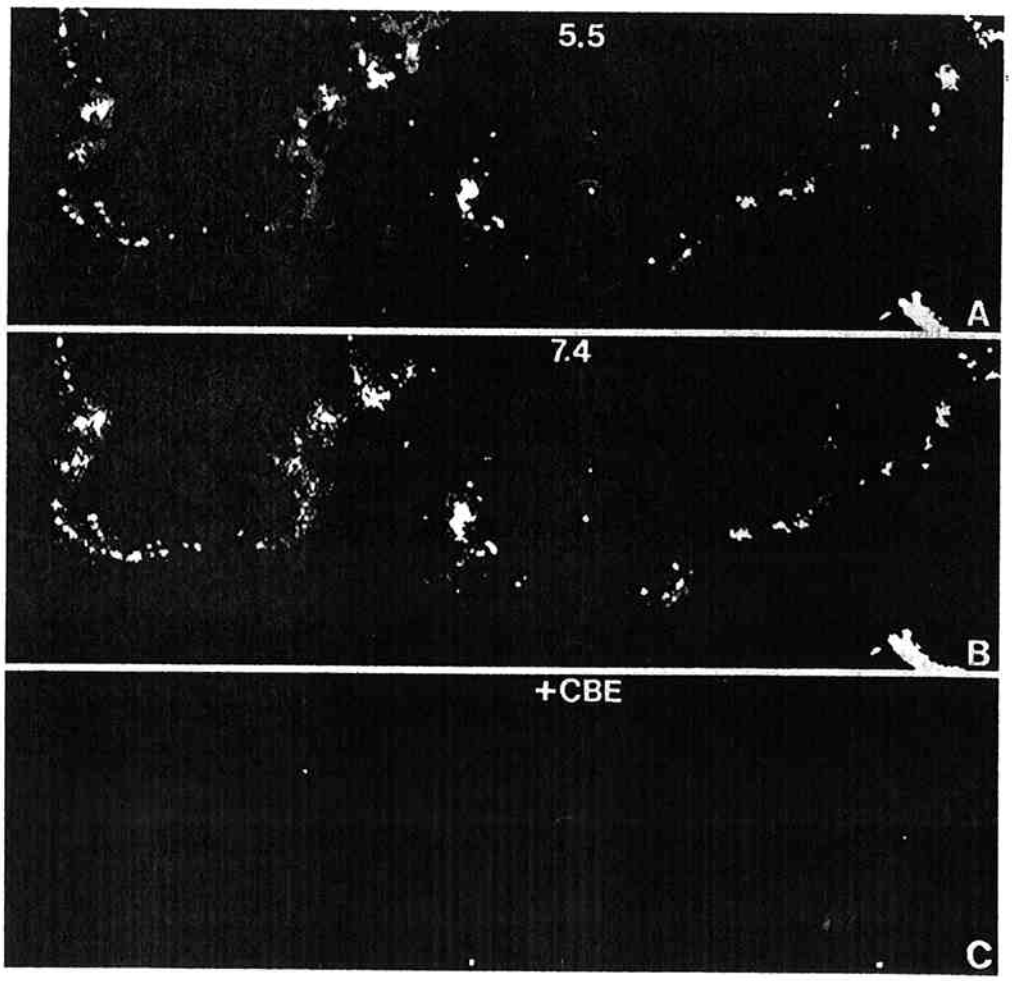

Fig. 5A–C Neutral pH inhibits β-glucocerebrosidase activity in situ. Serial frozen sections of human skin incubated with the 4-methylumbelliferyl-β-D-glucoside substrate at either pH 5.5 (**A**) or 7.4 (**B**). The activity of β-glucocerebrosidase is presented using a pseudocolor scale. The color shift from blue to white (A-to-B) reflects approximately a tenfold decrease in enzyme activity. **C** The specificity of the enzyme reaction is shown in parallel sections incubated with the fluorogenic substrate at pH 5.5 in the presence of 100 μ*M* conduritol-B-epoxide. A–C × 250

extracellular processing should have been unimpeded (Fig. 3; compare Fig. 1). When Ca^{2+} (1.0 m*M* free Ca^{2+}, as measured using a Ca^{2+} electrode) and K^+ (40 m*M*) were added to the acidic citrate buffer, recovery was delayed significantly (see also references 16 and 17). As with the other buffers, pH 7.4 citrate buffer alone inhibited barrier recovery (Fig. 3; $P < 0.01$; compare Fig. 1). Finally, the addition of 1.0 m*M* Ca^{2+}, with or without 40 m*M* K^+ to neutral citrate buffer, produced a further significant delay in barrier recovery in comparison with sites exposed to the neutral pH buffer alone (Fig. 3; $P < 0.01$ vs pH 7.4 alone; data for pH 7.4 + Ca^{2+} alone not shown). Indeed, the combination of neutral pH with raised Ca^{2+} and K^+ extended the interval before barrier recovery began, i.e. the barrier function actually deteriorated between 0 and 4 h after barrier disruption (Fig. 3, compare Fig. 1). These findings are consistent with the hypothesis that extracellular Ca^{2+} and K^+ primarily regulate lipid secretory mechanisms, while extracellular pH may influence postsecretory lipid processing. Moreover, this delay in barrier repair cannot be attributed to buffering of extracellular Ca^{2+}, since such an effect would be expected to decrease free Ca^{2+} concentrations resulting in enhanced lamellar body secretion and more rapid barrier recovery.

Neutral pH appears to alter extracellular processing, rather than inhibition lamellar body secretion

Since the effects of ions on the barrier are linked to the regulation of lamellar body secretion [16], we next assessed whether the effects of pH on barrier homeostasis also are attributable to lamellar body secretion, or instead, to inhibition of extracellular processing. Samples taken from acetone-treated skin, exposed to solutions buffered to pH 5.5, demonstrated both unaltered lamellar body secretion and normal formation of extracellular lamellar membranes by 2 h (Fig. 4A). In contrast, skin samples exposed to pH 7.4 solutions for 2 or 4 h (recovery rates 30–40% delayed) exhibited extensive extracellular membrane abnormalities. Rather than the uniformly normal appearing lamellar membrane unit structures seen at pH 5.5 (Fig. 4A) and in normal skin (not shown; see references 12, 21), the extracellular spaces of the lower SC contained numerous foci with loosely packed elongated arrays of partially processed lamellar body-derived sheets (Fig. 4B). These "immature" membrane structures resembled those seen with diminished epidermal β-GlcCer'ase, in which the final stage of transformation and condensation into mature lamellar multilayers does not occur [10, 11]. In contrast, regardless of the buffer and time-point in-

vestigated, both the number and the internal contents of lamellar bodies appeared unaltered (not shown), even when the delay in barrier recovery was maximal. Moreover, secretion and deposition of lamellar body contents at the stratum granulosum-SC interface did not appear to be altered at neutral pH (Fig. 4B, insert).

To further investigate the potential effects of neutral pH on extracellular lipid processing, we assessed epidermal β-GlcCer'ase activity at neutral and acidic pH by an in situ zymography technique. These studies utilized human skin, because the greater number of cell layers in the SC yielded better enzyme localization. As seen in Fig. 5A and B (representing pH 5.5 and 7.4, respectively), enzyme activity in frozen sections appeared to be localized to both the stratum granulosum-SC interface and the lower SC. The color shift in Fig. 5A in comparison with Fig. 5B (from blue to white) indicates a pH-dependent decrease (approximately tenfold) in enzyme activity at the same location. Finally, the specificity of this method is shown in Fig. 5C, which shows that enzyme activity had completely disappeared, even at pH 5.5, when incubations included the specific β-GlcCer'ase inhibitor, CBE (100 μM) [9, 10]. Similar patterns were seen in mouse skin (not shown). These results show that the in situ activity of one key enzyme of extracellular processing, β-GlcCer'ase, is decreased at a pH that delays barrier recovery. Together, these experiments suggest that a raised extracellular pH alters the extracellular processing of extruded lamellar body contents, rather than the formation/secretion of these organelles.

Discussion

Although prior studies have shown repeatedly that the pH of the surface of the SC is acidic [3, 8, 25, 28], the functions of this "acid mantle" remain incompletely understood. The experiments described here suggest one potential physiologic role, i.e. provision of the correct milieu for the extracellular processing of certain secreted polar lipids into their more nonpolar products, as required for barrier function in a terrestrial environment. Barrier recovery is retarded when acetone-treated skin is exposed to either neutral or alkaline pH, regardless of buffer composition. This effect persists for the first 2 h, but recovery rates normalize thereafter (i.e. the slopes of recovery rates are parallel), despite ongoing exposure to neutral pH buffers. One interpretation of these results is that extracellular processing takes place only during the first 2 h after acute insults to the barrier. Our results suggest, instead, that the normalization of recovery rates after 2 h, despite ongoing exposure to a neutral pH buffer, occurs as a result of restoration of a partial barrier. The finding that neutral pH did not delay barrier recovery after lesser insults is consistent with this hypothesis. Such a partial restoration would allow the SC to begin to sequester an acidic internal pH, while excluding the external neutral pH buffers, thereby allowing normal recovery. These experiments did not, however, address the mechanism(s) responsible for maintaining/restoring an acidic pH in the SC [2]. Nevertheless, these results provide further support for the importance of the acid mantle, and perhaps explain also the known "irritancy" of alkaline soaps/detergents [25].

Several lines of evidence suggest that the regulation of barrier recovery by pH is a physiologic and not a toxic effect. First, barrier recovery proceeds normally in acidic, but not at neutral (i.e. physiologic), pH. If the effect of neutral pH were simply toxic, one would expect the non-physiologic acidic pH to inhibit barrier recovery further. Second, the inhibitory effect of neutral pH on barrier recovery is rapidly and completely reversed on switching to an acidic pH. Third, increased extracellular Ca^{2+} impaired barrier recovery of skin exposed to acidic solutions, suggesting that regulatory mechanisms based upon Ca^{2+} influx (i.e. energy-dependent ion pumps) remain intact in skin exposed to acidic pH. Fourth and finally, electron microscopy revealed: (a) no evidence of cytotoxicity, and (b) normal formation and secretion of lamellar bodies in skin sites exposed to either acidic or neutral pH. Thus, the delay in barrier recovery by neutral pH does not reflect a selectively toxic effect.

We also assessed whether the effect of external pH might be attributable to pH-directed modulations in intracellular Ca^{2+}, thereby altering lamellar body secretion. Intracellular Ca^{2+} is controlled by extracellular pH in smooth muscle cells and in A-431 keratinocytes [14, 22]. Therefore, an alkaline extracellular pH might retard barrier recovery by raising intracellular Ca^{2+}. Although it is not possible to quantitate intracellular Ca^{2+} in stratum granulosum cells with currently available methods, we showed that the effects of pH are likely mediated through a different pathway than the secretory pathway controlled by changes in extracellular Ca^{2+} and K^+. Manipulation of extracellular pH not only alters intracellular Ca^{2+}, but can also influence secretion and endocytosis [13, 23]. However, ultrastructural studies demonstrated normal secretion of lamellar bodies, suggesting that any intracellular ionic changes, that might result from an external neutral pH, do not produce significant alterations in lamellar body secretion. Thus, while extracellular Ca^{2+} and K^+ control barrier homeostasis through their effect on lamellar body secretion [16, 17], it appears likely that the major effect of extracellular pH is on extracellular processing of lipid(s).

Further support for a purely extracellular site of action came from our ultrastructural studies, which revealed not only normal lamellar body contents and ongoing secretion, but also impaired postsecretory processing of lipids in skin exposed to solutions buffered to pH 7.4. Whereas the secreted lipids unfurl at the stratum granulosum-SC interface, they are not processed over the first 4 h into mature lamellar membrane structures. Indeed, the images at pH 7.4 are very similar to those encountered when the acidic pH-dependent lipid-processing enzyme, β-GlcCer'ase, is genetically deleted, for example in (a) a transgenic model of Gaucher's disease [11] or (b) a subset of patients with severe neuropathic, type 2 Gaucher's disease [11, 24], or chemically inhibited [10]. In all of these mod-

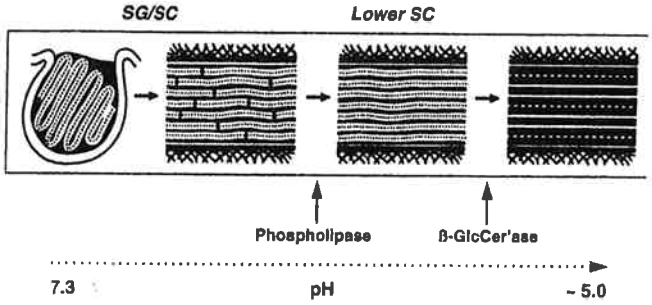

Fig. 6 Diagram of possible orchestration of extracellular lipid processing by stratum corneum pH gradient. Lipid is secreted from granular cells at the stratum granulosum-stratum corneum interface (SG/SC) into an extracellular environment buffered to neutral pH. Initial processing of the lipid bilayers into elongated, fused sheets is performed by enzyme(s) with a neutral pH optimum, such as secretory phospholipase A_2. As the partially processed lipid migrates upward, the extracellular environment becomes progressively more acidic, activating β-GlcCer'ase, and perhaps other enzymes with an acidic pH optimum, allowing the final processing step into mature lamellar multilayers

els, secreted glucosylceramides are not processed to ceramides and accumulate in affected SC [10, 11, 24]. Here, we provide indirect evidence to support the extracellular site of action of the pH buffers. Although the conditions of the zymographic studies did not duplicate the physiological studies, extracellular solutions, buffered to neutral pH, inhibited β-GlcCer'ase activity in situ, consistent with the acidic pH optimum of this enzyme in the epidermis, i.e. pH 5.5 [9]. Yet, direct lipid biochemical evidence of glucosylceramide accumulation at neutral pH is lacking, owing to the apparent loss of polar lipid precursors into the bathing solutions. Nevertheless, the available ultrastructural and zymographic evidence supports the view that the neutral pH effect may alter extracellular processing of polar lipids.

A neutral pH also may adversely effect other putative lipid-processing enzymes with acidic pH optima (e.g. acid sphingomyelinase). Conversely, those processing enzymes with a neutral pH optimum; e.g. secretory phospholipase A_2 [18, 19] may be less affected. Since a marked decrease in pH occurs from the granular cell layer (i.e. neutral pH) to the SC (acidic pH), sequential activation of these processing enzymes could be orchestrated by pH changes (Fig. 6). Furthermore, alkaline skin pH worsens barrier function [25] and exacerbates dry skin [25], contact dermatitis [27] and atopic dermatitis [1]. The present report suggests a mechanism underlying these clinical observations: extracellular pH regulates barrier homeostasis by controlling the postsecretory processing of lipid precursors, degraded by enzymes with an acidic pH optimum. Thus, maintenance of an acidic SC pH, either by preservation of the lipid barrier or by exogenously applied acidic buffers, is important to normal skin barrier function. Conversely, allowing the SC to become alkaline might exacerbate common skin conditions.

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