

## In vivo Studies Concerning a pH Gradient in Human Stratum Corneum and Upper Epidermis\*

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Human skin has an acid mantle of pH 4–6, contrasting with the almost neutral pH of the interior body and implying the existence of a pH gradient over the horny layer that might influence a variety of epidermal processes. In an attempt to characterize the pH gradient, we applied a glass electrode to the volar surface of the forearm before and after consecutive strippings with sello-tape. Before stripping, the surface pH (mean  $\pm$  SD) was  $4.5 \pm 0.2$  in men ( $n=7$ ) and  $5.3 \pm 0.5$  in women ( $n=7$ ), the values gradually increasing to pH  $6.9 \pm 0.4$  in men and  $6.8 \pm 0.5$  in women after about 100–120 tape strippings, which completely removed the *stratum corneum*. When plotted against the number of strippings, the pH values usually conformed to a sigmoid curve with inflection (50% change) after about 60 strippings, at a level corresponding histologically to the lower third of *stratum corneum*. Similar gradients were found also in skin of the abdomen and calf. Stripping with cyanoacrylate resin produced a similar gradient, even though this form of stripping was 10 times more effective. The healing process after tape stripping was studied by determining pH and transepidermal water loss in 5 persons over a period of 14 days. The importance of the re-established pH gradient is discussed in relation to the many pH-dependent enzymes operating in *stratum corneum*. **Key words:** keratinization; desquamation; stripping; transepidermal water loss; epidermal repair.

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Human *stratum corneum* is a multilayer barrier composed of dead keratinocytes (corneocytes) and specialized intercellular lipids rendering the skin poorly permeable to water and other polar compounds. The horny layer also assists in maintaining a constant internal milieu with a pH of 7.4 in viable epidermis that contrasts with the pH of 4–6 found on most parts of human skin. The "acid mantle" of human skin, first described by Schade & Marchionini in 1928 (1), was originally thought of as a thin film composed of fatty acids, amino acids, and other organic acids deposited on the skin surface. However, the term "acid mantle" is a misnomer because it implies something that can readily be removed from the skin, for example by cleansing. In fact, the pH values are individually quite constant and unaffected by delipidization with organic solvents (2), and the acidity extends well below the lipid film, suggesting that compounds within the horny layer contribute to the low surface pH (3). Exactly how deep the acidity persists in *stratum corneum* has never been

established, but most investigators agree that total removal of the horny layer will raise the pH to ca. 7 (see ref. 4). Considering the many pH-dependent processes involved in cornification and desquamation, we felt that a putative pH gradient over human *stratum corneum* called for more detailed characterization.

Tape stripping of the skin as a means of studying chemical and physical properties of different layers of *stratum corneum* has been described (3, 5–8). We decided to use this method combined with repeated measurements of surface pH to study the acidity of *stratum corneum*, even though stripping constitutes a standardized trauma eliciting characteristic skin reactions, some of which might even perturb the pH gradient. A more direct approach, such as insertion of microelectrodes into the skin, is not feasible owing to the toughness and extreme thinness of non-palmoplantar *stratum corneum*.

The aim of the present study was to characterize the pH gradient in healthy forearm epidermis of man and woman and to investigate how normal surface pH is restored after removal of the horny layer.

### MATERIALS AND METHODS

#### Study design

Twenty healthy persons, 12 men and 8 women, aged 25–49 years, took part in the study after giving their informed consent. They were asked not to apply detergents or ointments to the forearm the day of the experiment. They rested for 10 min before the experiment, which was carried out at room temperature 20–22°C and relative humidity ca. 50%. The study was approved by the Linköping University Ethics Committee.

The surface pH was measured with a flat glass electrode (Radiometer) connected to a pH meter (PHM62 standard pH meter, Radiometer Copenhagen). The instrument was calibrated as recommended by the manufacturer. The tip of the electrode was gently moistened with distilled water and pressed firmly against the skin for ca. 20 s before recording the pH. Duplicate recordings on the same spot were usually performed.

The pH gradient was studied on the middle part of the volar side of the forearm, on the right side of the abdomen, and on the medial aspect of the right calf, avoiding hairy skin. Any hairs present were cut off with scissors before measuring pH. Consecutive layers of *stratum corneum* were removed with tape (4 cm<sup>2</sup> of 3M Scotch Ruban adhesive tape pressed against the skin for a few seconds, then swiftly pulled away) or cyanoacrylate resin (a drop of "Bison Super-lim" applied to the flat end of a metal pin,  $\varnothing$  1 cm, held firmly against the skin for 1 min, then promptly lifted) repeatedly until a glistening surface appeared and pain developed. This usually required 100–120 tape strippings or 10–12 cyanoacrylate strippings. pH measurements were repeated ca. 10 times during the stripping process. The cyanoacrylate strips were removed from the metal pin by immersion in acetone, and the protein content was determined by a modified biuret technique (9).

In some experiments a 4-mm punch biopsy specimen was taken before and on 3 occasions during tape stripping of a larger area (14 cm<sup>2</sup>) of the forearm. The biopsies were taken from a part of the stripping area not involved in pH measurement after infiltrating the skin with 2 ml of lidocain. The specimens were fixed in formalin, processed for light microscopy and stained with haematoxylin and eosin.

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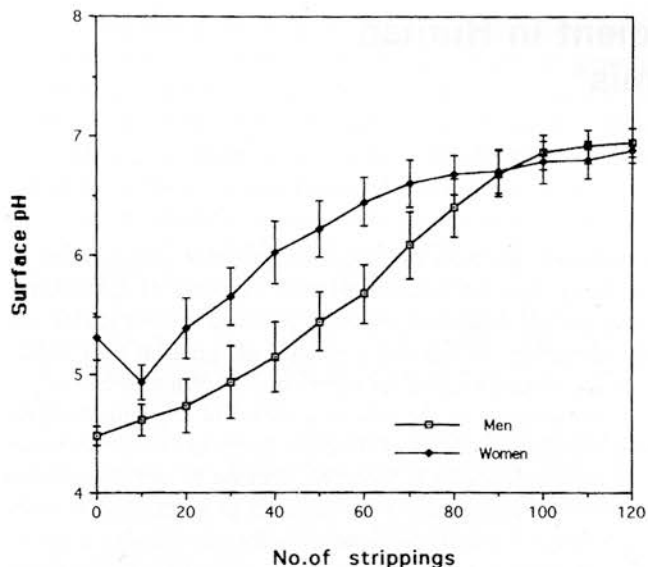


Fig. 1. Change in skin surface pH as a result of tape stripping of male ( $n=7$ ) and female ( $n=7$ ) forearm skin. Mean  $\pm$  SE values.

*Stratum corneum* repair was studied on the forearm of 2 men and 3 women during 2 weeks after 100–120 strippings with adhesive tape. Assessment involved monitoring surface pH (see above) and transepidermal water loss (TEWL) using the Evaporimeter (Servomed, Stockholm, Sweden).

#### Statistics

Statistical analysis was performed with the aid of a personal computer (Macintosh) equipped with a statistical software package (Stat View 512+ and Cricket Graph). Significance of difference was tested using the unpaired *t*-test.

## RESULTS

### Skin surface pH before stripping

The mean  $\pm$  SD pH values on forearm skin differed significantly ( $p < 0.001$ ) in men ( $4.5 \pm 0.2$ ;  $n=7$ ) and women ( $5.3 \pm 0.5$ ;  $n=7$ ), corroborating previous observations of a more acidic skin surface in men (10). Similar pH values were recorded on male abdominal ( $4.7 \pm 0.2$ ;  $n=4$ ) and calf ( $4.5 \pm 0.2$ ;  $n=4$ ) skin. Repeated analyses in 2 men confirmed that the individual forearm pH values remained constant over time and were unaffected by cleansing with water or acetone (data not shown).

### A pH gradient revealed by stripping

Fig. 1 shows the surface pH in 7 men and 7 women during consecutive tape strippings of forearm skin. The strippings eventually removed the horny layer, resulting in a glistening layer shown to be *stratum granulosum* on histological examination (Fig. 2). Fig. 1 shows that, except for slightly reduced pH values in women after 10 strippings, the surface pH increased with increasing number of strippings in both men and women. At least in males, values conform to a sigmoid curve, starting at pH 4.5 and levelling out at pH 6.9 after about 100 strippings. The inflection of the curve (50% change) in men occurred after about 60 strippings at a point corresponding to the lower third of *stratum corneum* (see Fig. 2). The pH gradient curve is shallower in women than in men, consistent with our naked-eye

observation that tape stripping seems to remove the horny layer rather more quickly in women than in men.

In order for us to exclude the possibility that the very application of tape increases the surface pH, another stripping technique using cyanoacrylate resin was tried in 6 men. Fig. 3 shows that cyanoacrylate produced results similar to those of tape stripping, although *stratum corneum* was removed after only 10–12 instead of 100–120 strippings. The tendency towards a higher pH value after completed cyanoacrylate stripping ( $7.3 \pm 0.3$ ) than after tape stripping ( $6.9 \pm 0.3$ ) may be explained by the fact that cyanoacrylate adheres more readily to wet surfaces and can remove keratinocytes also from *stratum granulosum*.

The mean  $\pm$  SD protein content of the cyanoacrylate strips was  $0.8 \pm 0.6$  mg ( $n=58$ ), corresponding to an area of  $0.8$  cm<sup>2</sup>. There were no consistent differences in protein content between samples obtained at different depths of *stratum corneum*, suggesting that cyanoacrylate removes corneocytes at fairly constant rates throughout the horny layer. The same is also the case with tape stripping (11).

Using tape stripping, we also investigated the pH gradient in abdominal and calf skin of 3 men, and the results were compared with those obtained in male forearm skin (Fig. 4). Although the pH was slightly higher in abdominal than in calf and forearm skin before stripping, similar pH gradients were found in all three regions. A slight shift to the left of the abdominal gradient is consistent with our naked-eye observation that tape stripping seems to remove the horny layer rather more quickly from the abdomen than from the forearm or calf.

### Surface pH and TEWL during *stratum corneum* repair

After *stratum corneum* had been removed from the volar surface of the forearm by tape stripping, the repair process was studied by monitoring surface pH and TEWL in 5 persons (Fig. 5). Directly after stripping, all had a surface pH of ca. 7 and a TEWL of ca.  $140$  g/m<sup>2</sup>/h, which is consistent with total absence of horny layer. Fig. 5a shows the pH values measured first daily and then at 4-day intervals for up to 2 weeks, when clinical healing was almost complete. Considerable inter- and intraindividual variations in the pH values can be seen. In 4 persons, the values dropped rapidly over the first few days, reaching ca. pH 5.5 on days 3–7, followed in some cases by a further fall to pretreatment values over the next week. In one person the normalization of surface pH was more gradual, requiring the whole study period. The individual pH values after 2 weeks were very similar or identical to pretreatment values.

Fig. 5b shows the corresponding TEWL values. Again, the individual time course varied, but in most cases the TEWL seemed to return to normal within 1 week of stripping. Interestingly, the woman in whom the pH showed a slow return to pretreatment values (see Fig. 5a) also showed a delayed normalization of TEWL.

## DISCUSSION

Previous investigators have examined regional, environmental and disease-related variations in skin surface pH and have reported raised values in the groin, in skin washed with alkaline

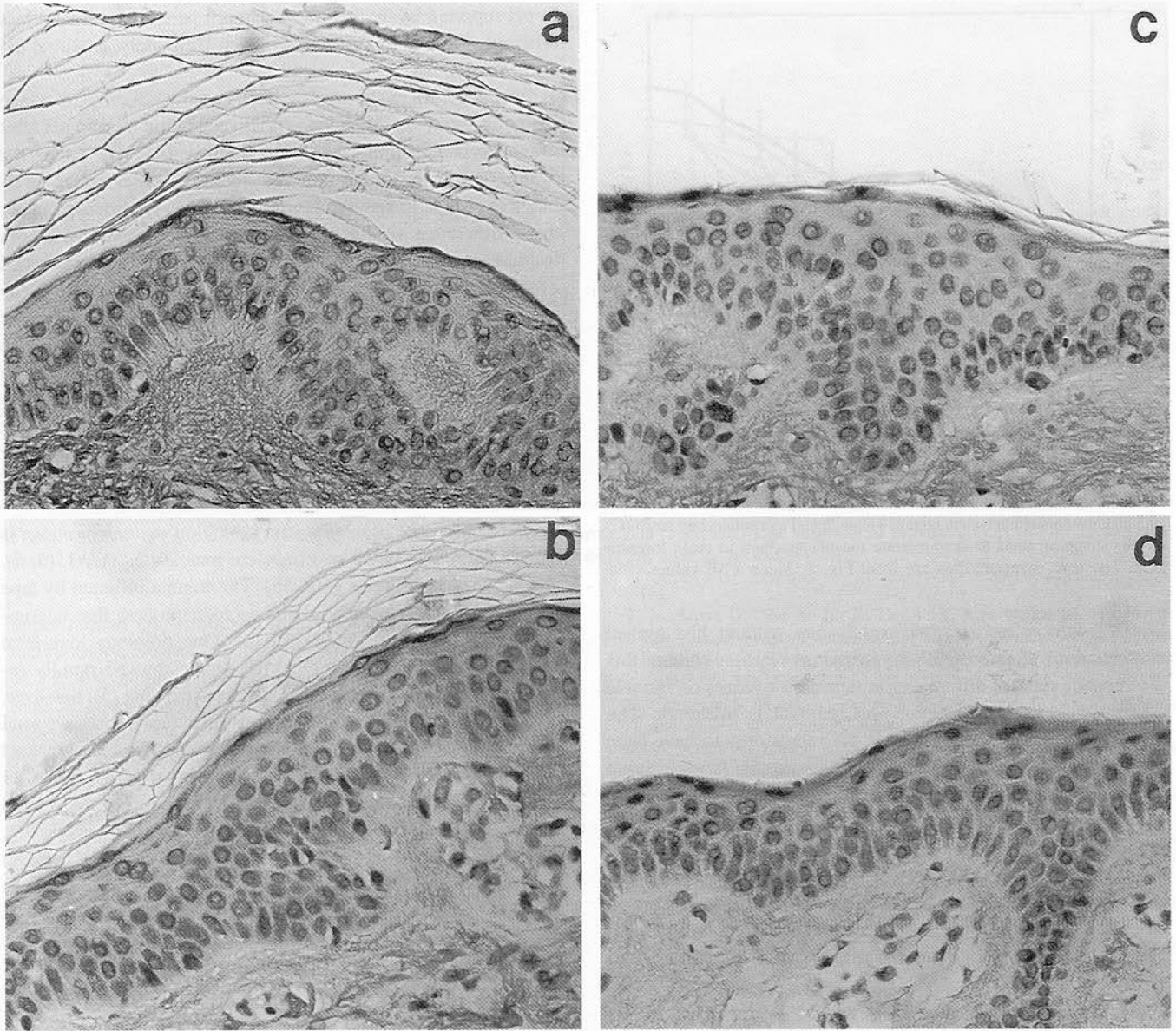


Fig. 2. Micrographs of skin obtained (a) before, and after (b) 10, (c) 50, and (d) 100 tape strippings of male forearm skin. Stratum corneum is artifactually expanded in (a) and (b) due to maximal hydration. Original magnification  $\times 86$ . See Methods.

soaps, and in fungus-infected skin (4, 12, 13). We now present details of a vertical pH gradient in human *stratum corneum* ranging from pH 7 in upper, viable epidermis to pH ca. 5 on the skin surface. An interesting feature of this gradient is its sigmoid shape in men, with the most dramatic pH shift occurring in mid- and lower *stratum corneum* where many pH-dependent enzymes are operating (see below). The gradient was demonstrated on several parts of the body using two different stripping techniques, suggesting that it is not an artefact. However, pH values recorded in a semi-hydrophobic milieu such as *stratum corneum* should be interpreted with caution. In common with other investigators, we do not know whether surface pH actually reflects the hydrogen ion concentrations of intercellular water (probably distributed in the form of micro-lagoons embedded in *stratum corneum* lipids) or if it represents the combined acidity of exposed corneocytes, lipids and water-soluble compounds. Fur-

ther, the influence of our analytical technique on the true pH gradient is obscure. Especially in the later stages of stripping, when most of the *stratum corneum* has been removed, fluid from underlying epidermal layers might diffuse to the surface and spuriously raise the pH values. Accordingly, we are now attempting to establish a new non-invasive technique for demonstration of epidermal pH gradients *in situ*. In the meantime, the results obtained with stripping should be regarded with circumspection.

Tape stripping is commonly used for studying the different layers of *stratum corneum* (2, 3, 5, 6). Depending on the type of adhesive tape used, different amounts of *stratum corneum* are removed at each stripping. In our hands 100–120 strippings were needed fully to remove the *stratum corneum* from skin of the volar aspect of the forearm. This corroborates previous findings in thigh skin (6). Others have reached the “glistening



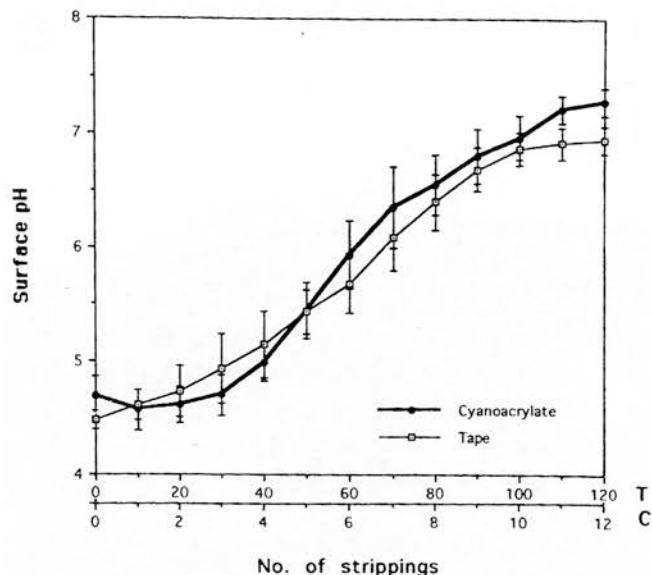


Fig. 3. Comparison between tape (T) ( $n=7$ ) and cyanoacrylate resin (C) ( $n=6$ ) stripping used to demonstrate the pH gradient in male forearm skin. The tape stripping data are from Fig. 1. Mean  $\pm$  SE values.

layer" (often called *stratum granulosum* without histological confirmation) after only 30–70 strippings (3, 5). Whether this discrepancy reflects differences in tape adhesiveness or variable completeness of *stratum corneum* removal is uncertain. The effect of tape stripping on surface pH seems only to have been reported twice previously. Lo et al. (2) state that tape stripping significantly increases the surface pH but give no details about the pH gradient in *stratum corneum*. In contrast, Wilhelm et al. (3) found decreasing pH values (from 5.3 to 4.6) after 30–60 tape strippings of female forearm skin. Although we also found a slight fall in surface pH after the initial 10 strippings in women, our results strongly suggest that additional stripping is always associated with increased pH values. The continued fall

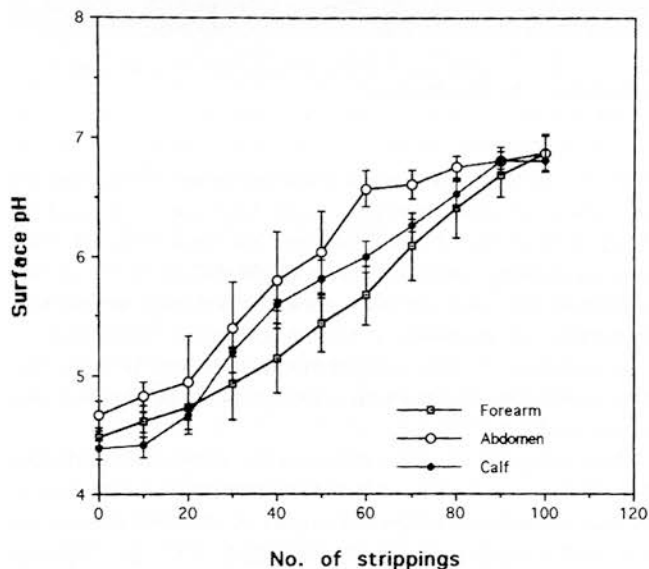


Fig. 4. Change in surface pH during tape stripping of 3 different skin areas in men. The forearm results are from Fig. 1 ( $n=7$ ). The other results are from 3 different persons. Mean  $\pm$  SE values.

in pH reported by Wilhelm et al. might be explained by a less efficient stripping of *stratum corneum*. Ultimately, a neutral pH must be attained.

Stripping with cyanoacrylate resin is more effective than tape stripping, but to our knowledge no investigation on skin pH after cyanoacrylate stripping has been published. In our hands the cyanoacrylate technique required about 10 times fewer strippings than tape to remove *stratum corneum*. Compensating for this difference, similar pH gradients were observed with both techniques. Although the irritative and sensitizing properties of cyanoacrylate limit its use in humans, a major advantage of the technique is that standardized sheets of horny layer can be removed and used for various analyses. In the present study, the protein content of the cyanoacrylate strips was shown to be around 1 mg/cm<sup>2</sup>, with no consistent differences between samples obtained at different depths. We are therefore confident that the number of corneocytes removed at each stripping is fairly constant and that no systematic error will distort the pH gradient.

One of the acute problems after removing *stratum corneum* is barrier failure, reflected in a transient increase in TEWL (5, 6), also demonstrated by us (Fig. 5b). The trauma inflicted by tape stripping of skin normally elicits a repair process that is complete within a couple of weeks (5). One previous long-term study of skin pH during epidermal repair showed rapidly increasing values on the day after adhesive stripping (3); however, the results of that study are difficult to interpret because total removal of the horny layer was not confirmed. We found instead that the surface pH rapidly decreased during the first days after stripping, followed by a slow normalization during the second week when TEWL had already returned to baseline level (Fig. 5a). The parallelism between restoration of surface pH and barrier repair (also exemplified in one case of delayed healing) suggests that a normal pH gradient over *stratum corneum* is important for restoration of epidermal homeostasis.

From a biological point of view, a change in pH of about 2 units over so short a distance as 10–15  $\mu$ m (the thickness of human non-palmoplantar *stratum corneum*) is a dramatic event. It is reasonable to assume that many pH-dependent processes within upper epidermis are greatly affected by this pH gradient. For example, hydrolytic enzymes, some of which are stored in the lamellar bodies of *stratum granulosum* and released to the extracellular space during cornification, will be activated by acidic pH (14). Such hydrolases have been incriminated both in the formation of a competent permeability barrier and in the desquamation process, which requires controlled degradation of desmosomes in upper *stratum corneum* (15). Another example relates to the lipid metabolism which is crucial for epidermal homeostasis. Thus, epidermal cholesterol is esterified by a pH-dependent enzyme, acyl CoA:cholesterol acyltransferase (EC 2.3.1.26), which more than ten-folds its activity when pH is reduced from 7 to 5 (16). Similarly, human skin contains a vitamin A esterifying enzyme, acyl CoA:retinol acyltransferase (EC 2.3.1.76), with a pH optimum of 5.6 (17) that differs from the neutral pH optima observed for corresponding enzymes in other organs and in rodent skin characterized by a neutral surface pH (18). This suggests that in the course of human evolution certain enzymes have become adapted to the low pH in

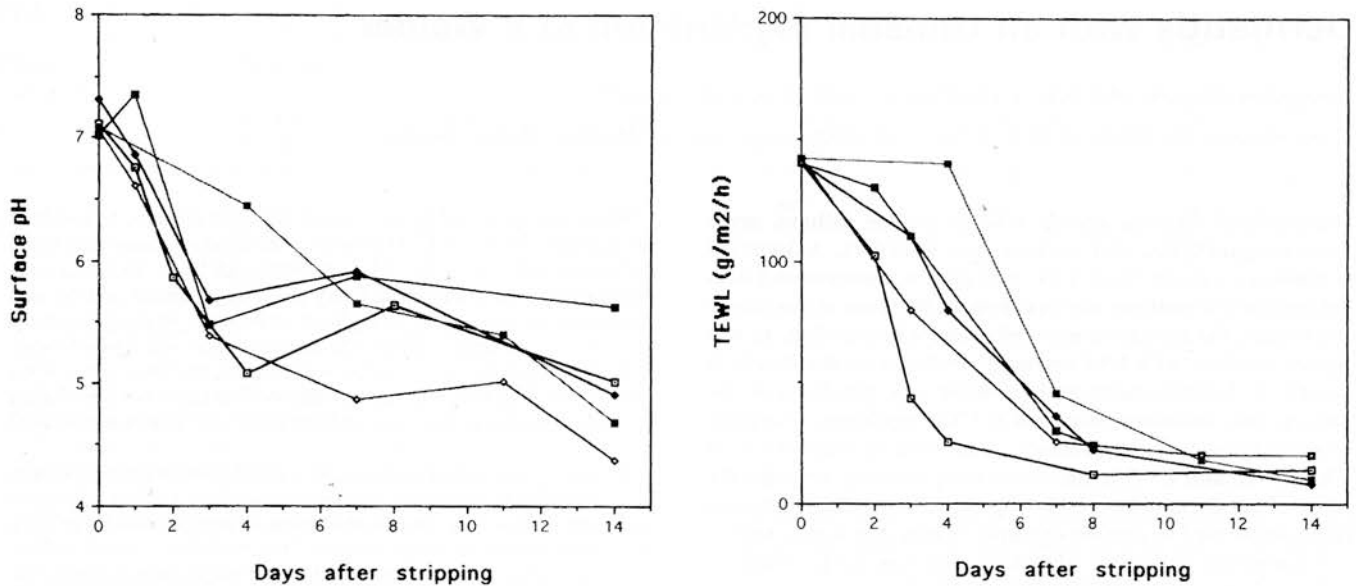


Fig. 5. Changes in (a) pH and (b) transepidermal water loss (TEWL) during skin repair after tape stripping of forearm on day 0. Individual values from 2 women and 3 men are interconnected. The same individual symbols are used in (a) and (b).

upper human epidermis and utilize the pH gradient to control their activity. However, before speculating about the importance of the pH gradient in epidermal homeostasis, the compartmentalization and exact spatial relationship between the pH-dependent enzymes in *stratum corneum* and the surrounding pH values must be elucidated.

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