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Intra and Extracellular Cementing Substances

H. P. BADEN, M.D., L. D. LEE, Ph.D. and J. KUBILUS, Ph.D.*

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Synopsis: The STRATUM CORNEUM consists of flattened compacted cornified CELLS which are filled with cross-linked FIBROUS PROTEINS. The association of the fibrous proteins with a SPECIFIC LIPID gives rise to the barrier characteristics of the epidermis. Stratum corneum cells are attached to one another by desmosomes and an intercellular cementing substance. The latter material has been rather poorly documented and described. Recent studies concerning diseases associated with hyperkeratosis which employed a keratolytic gel, have suggested that solubilization of this material can result in the loss of adherence of cells to one another. The solubilized material appears to have unique properties, which will be characterized.

Introduction

The epidermis is a complex tissue, which by means of a variety of mechanisms, acts as a protective barrier for the body. Our understanding of how it operates at the molecular level is gradually expanding, but some aspects have proved more difficult to investigate. This paper will deal with those factors responsible for maintaining the integrity of the tissue, these being the cement materials. This term is used in a very broad sense, since a number of the structural components appear to play some role. We can divide these substances into materials which hold a single cell together and those which hold groups of cells together.

A major component of the epidermal cell is the α fibrous protein, which appears as filaments in electronmicrographs of the skin (Fig. 1). These filaments are first observed in the basal layer and go through a series of changes as the cells ascend into the stratum corneum. It is thought that these 70-80 Å filaments extend across the cell from one wall to another and hook on to attachment plates of desmosomes. Since it has been estimated that the basic fibrous protein has a length which is only a fraction of the width of a cell, the filaments must result from an aggregation of fibrous proteins. The fibrous pro-

*Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114.

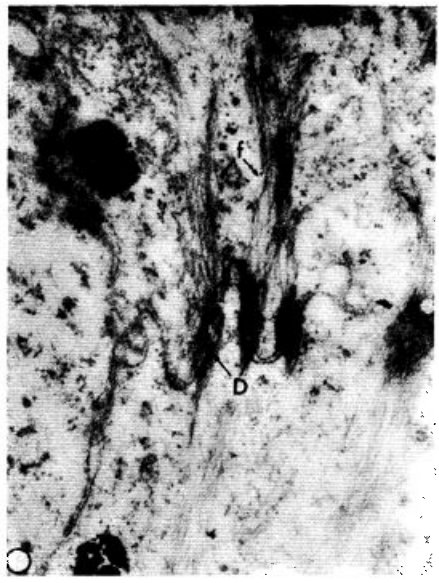

Figure 1. Electromicrograph of cell in stratum spinosum: (D) its desmosome and (F) shows filaments inserting into it

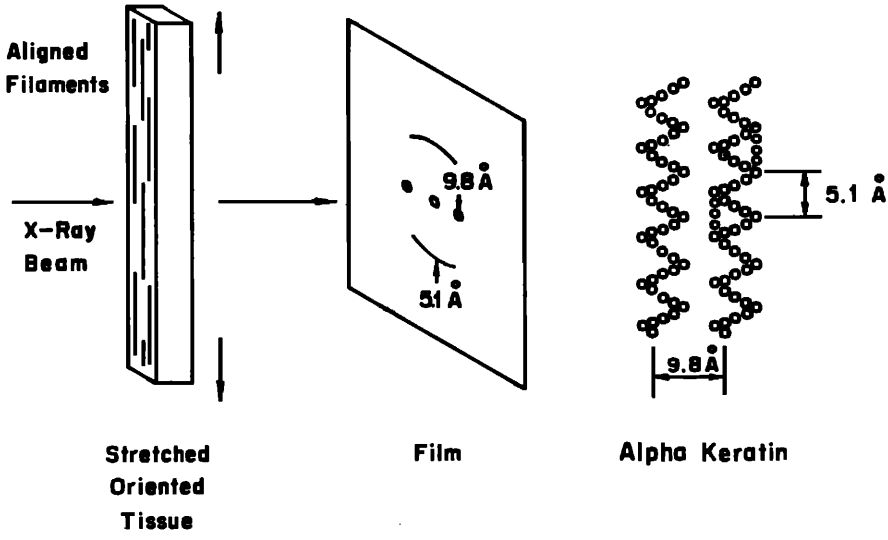

Figure 2. α helix of keratin showing X-ray diffraction pattern

tein has been shown to be a helical molecule, similar, but not identical to the classical α helix (Fig. 2).

The α proteins have been most extensively studied in cow snout epidermis (1, 2, 3). The α protein of the viable epidermis, prekeratin, can be solubilized by urea or buffers of organic acids below pH 2.7. Purification from acid buffers can be achieved by isoelectric precipitation. This material is insoluble in pH range 3-10, but can be maintained in solution at neutral pH by the addition of urea, guanidine, or sodium dodecyl sulfate (SDS). The analysis of amino acid reveals a high content of the acidic amino acids and glycine (Table I). The cystine content is quite low, unlike the α protein of hair and nail. In SDS electrophoresis has shown that a number of components (Fig. 3), and immunologic studies indicate that the A and B families are distinct from one another, but both are necessary to form an α helix. These results are best interpreted as the prekeratin molecule consisting of 3 polypeptides, 2 A chains and 1 B chain. It would appear that a major prekeratin exists with the A, A', B chains and a minor one exists with an A, A', B' chain. No cystine cross-links occur between the polypeptides of prekeratin.

As the cells of the viable epidermis become cornified at the base of the stratum corneum, the α fibrous proteins become cross-linked and can only be solubilized by alkaline buffers that contain a denaturing agent such as urea and a reducing agent (4, 5). This process is irreversible, and the stratum corneum fibrous proteins become cross-linked when the reducing agent is removed. The amino acid composition of the stratum corneum reveals a one-half cystine content of 2 residues/100 residues indicating that no one-half cys-

Table I

Amino Acids	Prekeratin
Lysine	5.1
Histidine	1.0
Arginine	6.1
Aspartic acid	9.1
Threonine	4.0
Serine	11.1
Glutamic acid	14.1
Proline	1.4
Glycine	16.4
Alanine	6.7
Valine	4.0
Methionine	1.3
Isoleucine	3.5
Leucine	9.2
Tyrosine	2.8
Phenylalanine	3.6
Half cystine	0.6

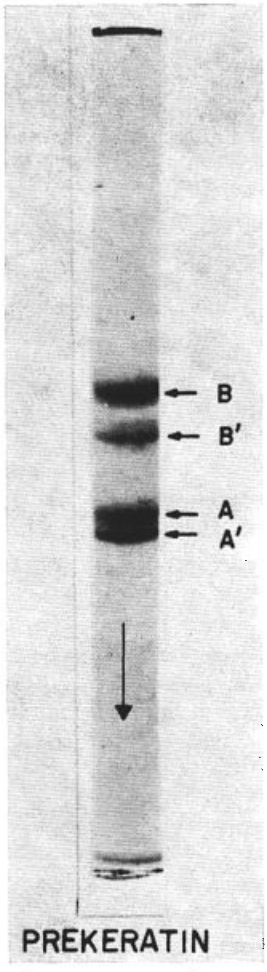

Figure 3. Polyacrylamide SDS electrophoresis of prekeratin. There are two groups of polypeptides

tine rich matrix proteins are present as has been the case with hair and nail (6).

Thus, the system for maintaining the integrity of the epidermal cells in the stratum corneum involves filamentous protein, which is attached to the cell wall, and, which shows interchain disulfide cross-linkage (another structural protein complex, keratohyalin, which is unique to the epidermis is also involved.) This material is newly synthesized in the granular layer and has been thought to coat the filaments and stabilize them. The controversy, which

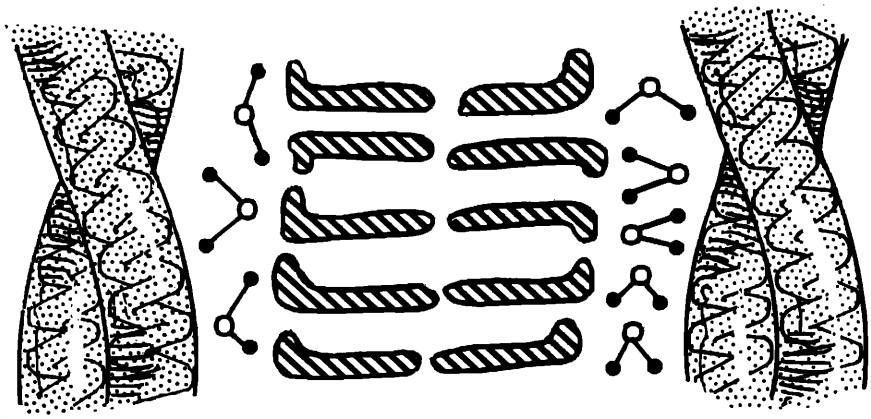

Figure 4. Polar lipid is shown between polypeptide chains with its long axis perpendicular to fibre

exists concerning the chemical nature of this material, probably has as its basis the complex nature of the material and the different methods used by several investigators to isolate it (7, 8). It has been difficult, however, to accept this concept of keratohyalin, since in a number of conditions, including ichthyosis vulgaris, no keratohyalin is formed, yet the filaments and stratum corneum appear to be normally stabilized. We feel that more work is necessary to determine the exact role of keratohyalin.

A final unique feature of the keratinization process is the deposition of a lipid material between the filaments (9). X-ray diffraction studies have indicated that a polar lipid, with its long axis perpendicular to that of the filaments, appears as cornification procedes (Fig. 4). It is likely that this protein lipid complex functions as the barrier. Extraction of the stratum corneum with lipid solvents removes the lipid, and at the same time, the barrier function of the stratum corneum is lost.

What has been described may be called the intracellular cement materials and probably is the major barrier of the stratum corneum. Our preliminary work with human and animal epidermis indicates that, what has been found in bovine snout epidermis, is generally applicable to both. As research continues in this area, new facts will be added to complete the picture.

Information on intercellular cement is far less complete. In the viable epidermis, no irreversible linkage between cells can be present, since cells move up from the basal layer to the stratum corneum. The desmosomes of the epidermis are clearly important in holding cells together, and when these are disturbed, as in certain diseases, acantholysis or cell separation and blistering occurs (10). As a cell rises in the epidermis, these attachments must constantly be broken and reformed. The mechanism for this process has not been clarified.

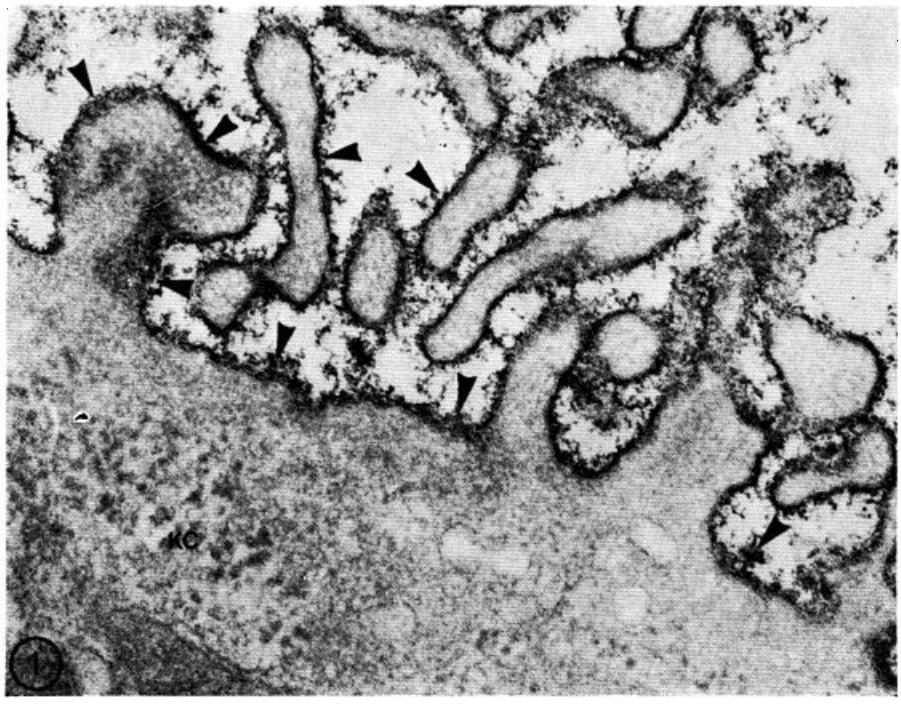

Figure 5. The glycocalyx is shown between epidermal cells in culture. The material is stained with Ruthensum red and has fuzzy appearance

A carbohydrate material called glycocalyx (11) has been described as coating keratinocytes in the viable epidermis (Fig. 5). This material may be the antigen which reacts with the antibody found in the sera of patients with pemphigus. The role that this material plays in holding cells together remains to be demonstrated.

In the stratum corneum, a firm attachment between cells is formed. This is in part, a result of the stacking (which has been observed), which permits careful overlapping of cell and maximum use of cell surfaces (12). The thickness of the stratum corneum is almost certainly related to the capacity of cells to stick together. Eventually, at the skin surface, loss of cell adhesion occurs and desquamation results. By inference from what has been observed in certain forms of ichthyosis, cell separation is easier to achieve at a higher water content of the stratum corneum. Thus, the common type of winter dry skin frequently ameliorates when the individual is exposed to a high humidity environment.

Recent studies have perhaps indicated new approaches for looking at the cement material of the stratum corneum (13, 14). In studies designed to im-

prove the therapy of ichthyosis, it was discovered that mixtures of propylene glycol in the 40 to 80 per cent range in water, under plastic occlusive dressings, resulted in rapid shedding of the stratum corneum. This appeared to be true for ichthyosis vulgaris and sex-linked ichthyosis. A marked increase in the effectiveness of the treatment resulted from the addition of salicylic acid. A gel containing salicylic acid and propylene glycol, which worked quite effectively has been finally developed. Use of this preparation under occlusive plastic dressings overnight resulted in rapid and dramatic loss of the thickened stratum corneum. Not only could this effect be observed in ichthyosis vulgaris and sex-linked ichthyosis, but in lamellar ichthyosis as well (Fig. 6). One could observe true keratolysis that is separation of the stratum corneum in sheets, which could be removed by rubbing the skin when the dressings were removed.

This action of propylene glycol and salicylic acid is not peculiar to the stratum corneum of ichthyosis, but can also be observed in hyperkeratosis as-

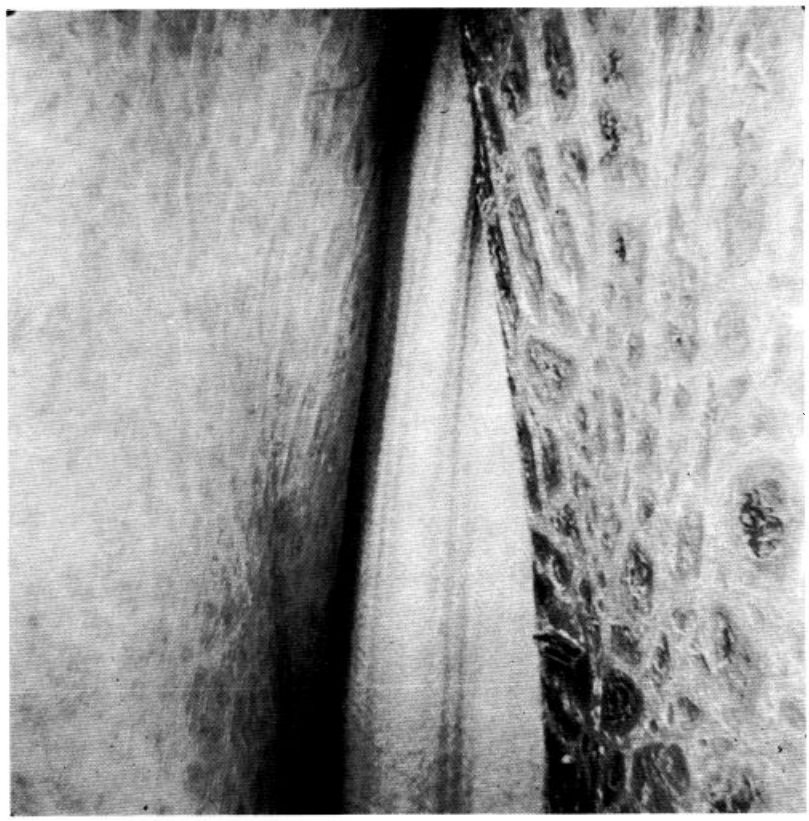

Figure 6. Appearance of skin of individual with lamellar ichthyosis after treatment with salicylic acid propylene glycol gel: untreated on right and treated on left

sociated with psoriasis and eczema (15). It effects the stratum corneum of all body surfaces including the palms and soles. This can also be observed with normal skin, indicating that factors involved in thickening of the stratum corneum may be an exaggeration of normal mechanisms for holding the stratum corneum together.

The nature of the cementing substances in the stratum corneum remains to be demonstrated. Although they are difficult to visualize, still the desmosomes are present and may have been modified to become very resistant cross-links by dessication. In addition, intercellular material has been described as appearing above the granular layer. This material has been poorly defined, but has been proposed by some authors as a cementing substance. It is not known how this material relates to the glycocalyx in the viable layers of the epidermis, and some have suggested that it comes from the membrane coating granules. Finally, as yet unrecognized materials may play a major role.

A firm fact of some significance is that hair and nail must have quite different mechanisms for holding cells together. The propylene glycol solutions and propylene glycol and salicylic acid gel do not cause keratolysis of nail and hair even after prolonged use. If mechanisms similar to stratum corneum were involved in holding cells together, these tissues would not be as resistant.

A reasonable approach to this problem is to treat stratum corneum with agents known to produce keratolysis and to determine the nature of the solubilized products. Recent work has begun in our laboratory, which uses solutions of propylene glycol and salicylic acid. For technical reasons, materials with molecular weights below several thousand are not amenable to investigation in our preliminary study. However, we have identified solubilized polypeptides in the molecular weight range 5,000 to 15,000 using electrophoretic techniques. Identification of their chemical composition is in progress, and these may give clues to importance in cell cement.

Acknowledgments

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