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The Contribution of the Resistant Cell Membranes to the Properties of Keratinized Tissues

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Synopsis—Electron microscopy of hard (hair) and soft (epidermis) keratin suggests that modified cell membranes are cemented by a continuous layer in the former and by a patchy layer in the latter. These differences are related directly to the desquamating nature of epidermis and the persistent behavior of hair and nails. This intercellular "membrane complex" is more resistant to chemical attack than intracellular keratin but easily dissolved by tryptic or peptic digestion.

Introduction

The keratinized tissues are cellular tissues, i.e., they consist almost entirely of cells filled with keratin and with a very small amount of intercellular binding material. They are to be contrasted with the connective tissues where the intercellular material enormously preponderates and the properties of the tissue are effectively those of the *intercellular* fibers and colloidal matrix. To take one property as an example, the physical strength of a cellular tissue is the strength of the complex of the component cells and their adhesive connections. Thus in a keratinized tissue the physical strength depends on: (a) the strength of the intracellular keratinized protein, (b) that of the cell membranes

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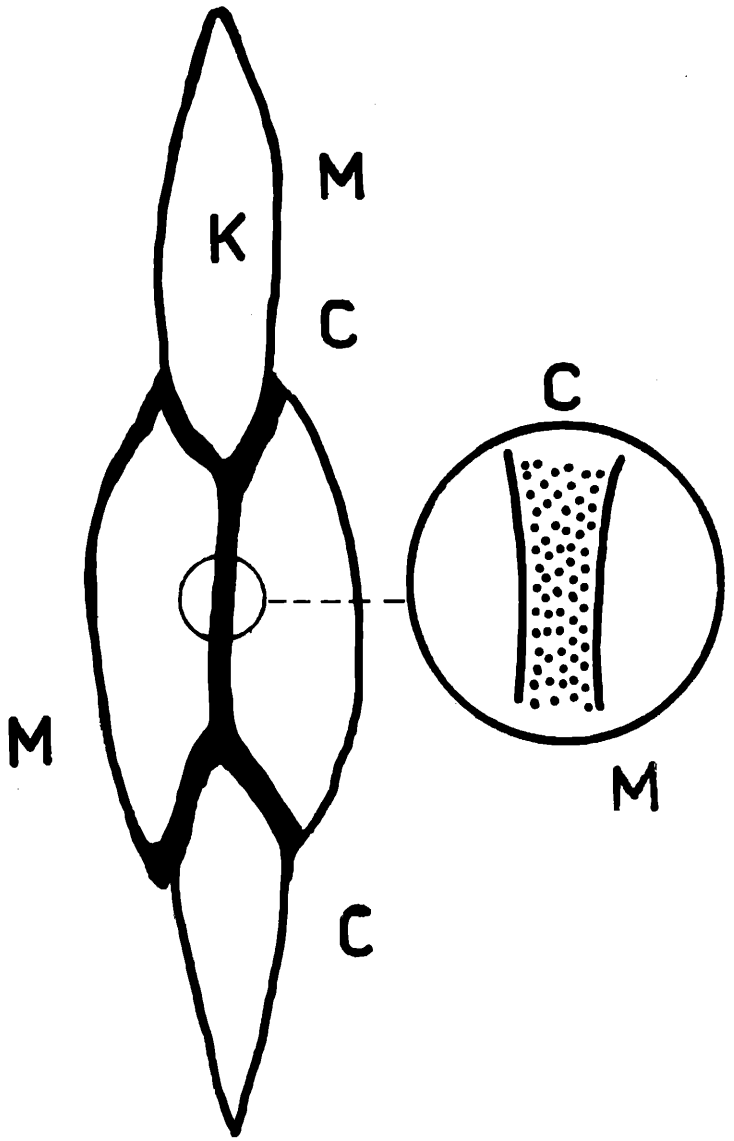

Figure 1. The relation between the keratinized cell contents (*K*), the resistant cell membranes (*M*) and the intercellular bonding material (*C*) in a keratinized tissue (here the hair cortex). Inset: the cell membrane-intercellular cement complex

enclosing the protein and (c) that of the intercellular cement, and these components are partly in parallel and partly in series (Fig. 1) (1). As with a chain this structure is no stronger than its weakest link.

The keratinized tissues are classified as desquamating (or soft) keratins (the epidermis) and the nondesquamating, hard and persistent keratins (nails, hair, etc.). This classification emphasizes an important functional distinction: the constantly growing epidermis maintains its near-constant thickness by disintegration into cellular fragments on its outer face, whereas the persistent nails and hairs are more permanent structures, which must be cut or worn away by use. In considering the coherence of these tissues we have clearly to take into account not only the properties of the protein (keratin) enclosed within the cells but also the properties of the cell membranes and the materials bordering them. Most of what we know concerning these matters comes from the study of hair (or wool), and this work will be summarized first. An account of similar investigations concerning skin will then be described. In each material we are concerned in the first place to establish the fine histology of the tissue and, second, to determine the chemical nature of each of the morphologically distinct components.

Cell Membranes and Intercelluar Material in Hair

That hairs were cellular tissues was appreciated by the earlier histologists, but the fact was often overlooked in the days when it was fashionable to regard a hair as a rod of a more or less uniform polymer, "keratin." A detailed description of the keratinized cells based on electron micrographs was given by the present writer and his colleague, Birbeck (2). The special chemical nature and the important role of the cell membranes was clearly recognized in this work, and since that time numerous other investigators have confirmed and extended these findings (3).

Briefly, the oriented bundles of intracellular keratinized filaments are enveloped by a modified cell membrane, which usually appears thicker than the original plasma membrane of the prekeratinized cells in the hair follicle but is clearly derived from it. These membranes are cemented together by rather uniformly thick sheets of some distinctly different substance, the whole forming what might be termed the "*cell membrane complex.*" The cell membrane complex is effectively a continuous phase, a reticulum extending throughout the cortex of the hair and enclosing the keratinized filaments in its interstices (Figs. 1 and 2). When the hair is stretched, the membrane complex and the intracellular

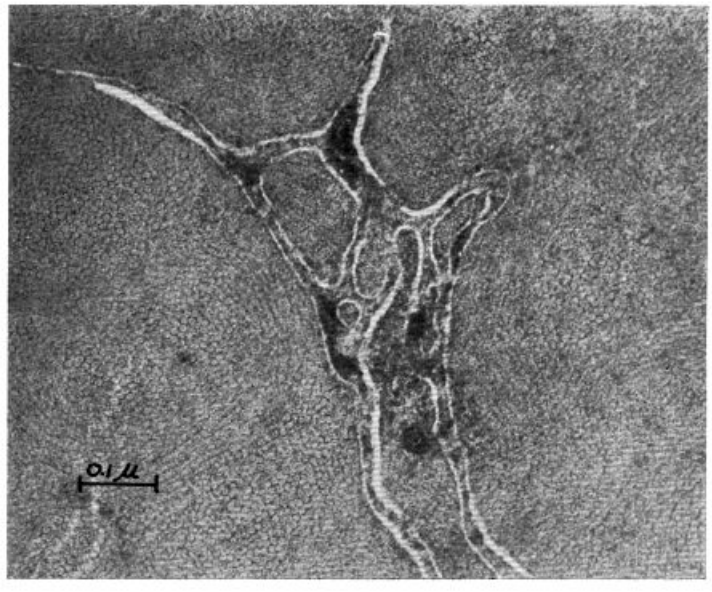

Figure 2. An electron micrograph of a cross section of hair (wool) showing the resistant membranes enclosing the bundles of keratin filaments. The membranes here appear light, being unstained by the method used. (photograph by B. K. Filshie)

filaments extend together, and the properties of both will contribute to the elastic properties of the whole hair. When a hair is treated chemically, the behavior of each of the components must be considered, since their chemical makeup proves to be very different.

The chemical resistance of the membrane complex proves in fact to be complementary to that of the intracellular keratin. That is, reagents or treatments which dissolve keratin do not dissolve the membrane complex; *vice versa*, the membranes may be removed without affecting the keratin. The biological advantages of this arrangement are obvious since the range of chemical environments against which a keratinized tissue offers protection is greatly widened. Keratin, owing its stability to disulfide bond cross linking, is very vulnerable to reducing and oxidizing agents and to alkaline conditions, which rupture this bond. It is precisely toward such conditions that the membrane-complex is resistant (see Table I).

The resistance (mechanical and chemical) of keratin has been frequently emphasized; the quite extraordinary chemical resistance of the altered cell membranes, which enclose the keratin as a bag encloses its contents, is not so commonly appreciated. We may well wonder what is

TABLE I

Chemical Resistance of Components of Hair to Various Reagents

Reagent	Intracellular Keratin	Membrane-Complex[a]
Sodium sulfide	Dissolves	Resistant
Sodium hydroxide (pH 11–12)	Dissolves	Resistant
Sodium thioglycolate, pH 11	Dissolves	Insoluble
Thioglycolic acid plus 10 *N* urea (pH 6–11)	Dissolves	Resistant
Peracetic and/or performic acids followed by alkali	Dissolves	Resistant
Hydrogen peroxide and alkali	Dissolves	Resistant
Tryptic and peptic digestion	Resistant	Dissolves

[a] The chemical behavior of medulla (when present) is like that of the membrane-complex.

the nature of a biological material which is resistant to: 10 M urea containing various reducing agents, caustic soda of pH > 12, cuprammonium sulfate, strong sodium sulfide solutions, etc. In fact, no true solvent for these membranes is known; yet their protein basis is revealed by the ease with which they are broken down by proteolyic enzymes.

When wet, the membraneous ghosts remaining after the keratin has been removed from any of the keratinized tissues have a long-range rubber-like extensibility about five times that of the oriented fibrous keratin of hair (100% extensibility).

The chemical basis of the unusual resistance of the membrane complex therefore remains to be established. One of the principal difficulties is to separate it in a pure form for chemical analysis. Relatively pure membrane-complex can be prepared by Alexander and Earlands' method (4) (oxidation by peracetic acid followed by alkaline extraction of the oxidized keratin, cf. Fig. 2). It is contaminated by traces of keratin and by remnants of the cellular apparatus of the cells (nuclei, mitochondria, etc.), the whole accounting for about 10% by weight of the fiber. If it proves to contain disulfide bonds like keratin itself, these must be in some sterically sheltered position where they are not reduced by keratinolytic agents; possibly some unrecognized bond is present.

Cell Membranes and Intercellular Material in the Epidermis

The duplex structure (intracellular keratin filaments plus resistant membrane-complex) is characteristic of all the keratinized tissues (5, 6). The hard keratins are essentially like hair. The epidermis, however, proves to have special features, which are apparent in electron micrographs of the intact tissue and in those of the resistant components separated from the tissue after extraction with a keratinolytic reagent (7).

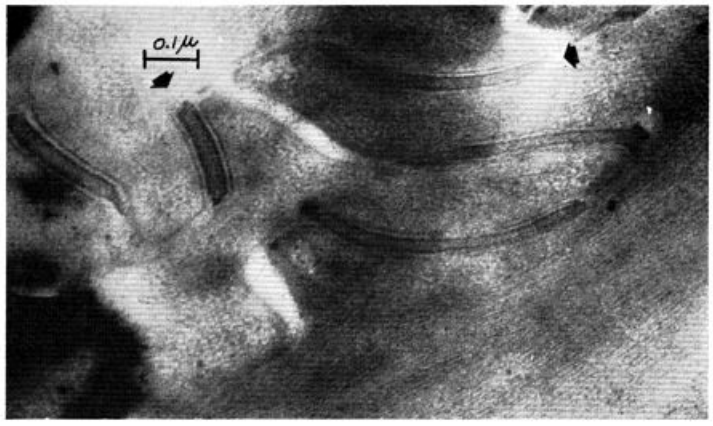

Figure 3. An electron micrograph of a section of the stratum corneum (human) showing the limited development of adhesive patches (at arrows). Compare with the continuous layer of bonding material in hair (Fig. 2)

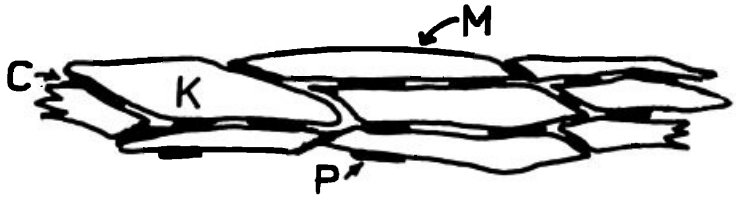

Figure 4. Drawing illustrating the patchy nature (*P*) of the intercellular adhesive (*C*) substance in the upper layers of the stratum corneum (M-cell membranes, K-keratinized contents of cells)

The intercellular bonding material (also called cement) in the hard keratins is a more or less continuous layer about 400–600 A thick, and it is essentially unaltered by the removal of the keratin (Fig. 2). In the keratinized epidermis, in contrast, this layer is discontinuous, being limited to discrete patches which unite the two opposed membranes over only part of their entire surfaces (Figs. 3 and 4). These rounded patches (1–2 μ) in diameter can be pictured as flattened balls of adhesive slipped between the cells. In fact, this seems to be what they are, since they originate within the cells as the contents of closed sacs and open onto their faces, as has been shown recently by Matoltsy and Parrakal (8). These rounded patches can be seen (Fig. 4) as numerous small studs on the surfaces of isolated epidermal cells after special staining (9).

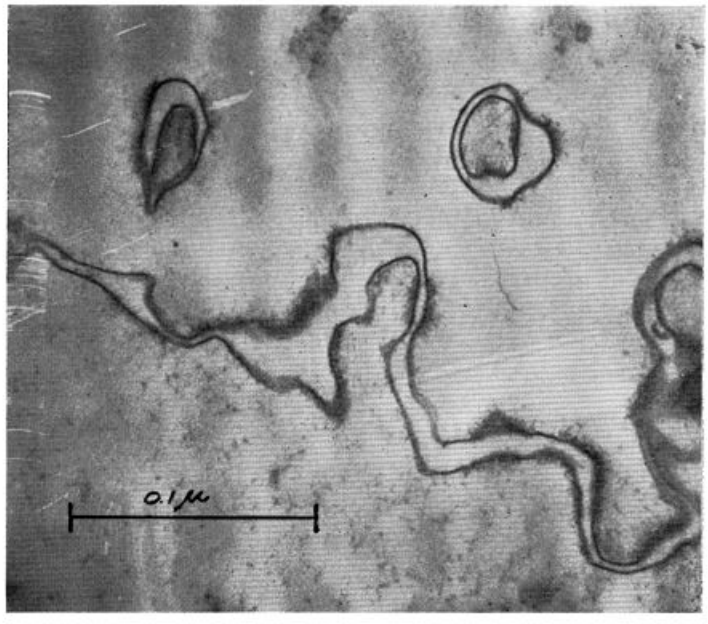

Figure 5. Electron micrograph of resistant membranes isolated from epidermis. Note: the membranes have separated, and the intercellular adhesive patches have been dissolved. Cf. Fig. 3

When membranes are isolated from epidermis by the same methods used to separate them from hair, a further difference emerges. The intercellular adhesive layer in hair is insoluble, and the separated membranes remain adherent, as described above (Fig. 2); the adhesive patches in skin are, however, dissolved, and the membranes separate (Fig. 5).

Similarly, the membranes in hair never separate during the normal lifetime of the tissue; the tissue is nondesquamating. The cells of epidermis of course separate, and the tissue desquamates. Electron micrographs of the superficial layers show that the membranes themselves persist, the breaks occurring in the adhesive spots (the keratinized contents also begin to fray). Thus this characteristic property of epidermis can be traced down to the properties of the adhesive patches between the cells (Fig. 4).

Some Reflections

From the observations described above, it is clear that our understanding of a keratinized tissue will remain incomplete until the chemical nature of the intercellular cement substances and the altered cell mem-

branes are known. The first step is the separation of each of the components in a pure form and in adequate amounts. An adequate analysis should answer the following questions:

(a) What chemical events occur during keratinization to change the labile phospholipid-protein complex of the living cell membrane into the extraordinarily resistant substance found in the hardened tissue?
(b) Similarly, what is the chemical composition of the resistant intercellular bonding substance which unites the altered membranes?
(c) What is the chemical difference between the intercellular material in hair and that in epidermis, which causes one to be permanent and the other to disintegrate?

These separations have been attempted and have not proved entirely satisfactory up to date; they could probably be perfected were effective use to be made of electron microscopy to control the purity of the preparations. Hopefully, we can look forward to having answers to the above questions before too long.

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