INVESTIGATION OF THE STRUCTURE OF THE CORNIFIED EPITHELIUM OF THE HUMAN SKIN*

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Plates 53 and 54

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The structure of cornified tissues, such as hair, wool, nail, and cornified epithelium of the skin, has been investigated largely by polarization optical, x-ray diffraction, or electron microscope methods. Under the polarization microscope all these tissues show a positive double refraction which is considered due to regular arrays of asymmetric keratin particles (12). X-ray diffraction studies reveal the presence of an α-type of keratin, built up of parallel-oriented and somewhat contracted polypeptide chains, showing a characteristic axial periodicity of 5.1 A (1, 2, 4, 5, 9, 10). By means of the electron microscope long protofibrils were demonstrated in cortical cells of wool, measuring about 110 A in width and 2000 A in length (7). These protofibrils were considered as the smallest submicroscopic units of keratin, building up the microscopic tonofibrillae (10).

Although x-ray analysis of the hair, nail, and cornified epithelium of the skin shows that their structures are built up of similar α-type of keratins, differences occur in the direction of principal orientation of molecular chains in these various cornified tissues. In the cortex of the hair, the long axis of molecular chains was found to run parallel to the longitudinal axis of the hair, coinciding with the direction of growth (1, 2, 12). In the nail, the chain molecules were observed to lie perpendicular to the longitudinal axis of the nail plate, hence perpendicular to the direction of growth (5). In the only stratified squamous epithelium heretofore regarded as suitable for x-ray study, *i.e.*, snout of the cow and horse burr (4, 9), the chain molecules run parallel to the surface plane and perpendicular to the direction of growth. Accordingly it appears that while similar molecular chains of keratin are formed in all these epithelial elements their ultimate orientation is determined by different mechanisms in various cornified tissues.

* This paper is dedicated to Professor W. J. Schmidt in commemoration of his 70th birthday.

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The purpose of the present investigation was to obtain information about the submicroscopic structure of thickened cornified epithelium such as the human callus, which has not yet been adequately studied. The callus, in comparison to the hair or nail, is more complex, since it contains various gross structural differentiations, such as the surface ridges and grooves. It is also perforated by numerous sweat ducts. The individual cornified cells are laid down in apparent relationship to these gross structures, hence differently oriented components might be expected in various regions of this keratinous tissue. In order to explore the structure of the callus by optical means, it appeared necessary to prepare serial sections in three principal planes. By determination of the sign of double refraction and optic axes in various regions of such sections, observations were made concerning the structure of the callus and conclusions were drawn about the orientation of epidermal keratin. In the present paper the results of this study are described.

Material and Methods

In this study selected small pieces of human callus were used which originated from the sole of the foot. All pieces showed regular parallel straight grooves and ridges on the surface (Fig. 1). The callus pieces were first softened in distilled water for 24 to 48 hour periods at 5°C. and subsequently pieces 3 to 4 mm. square and 1 to 2 mm. in thickness were cut out and mounted on blocks by freezing. Serial sections 20 to 30 μ thick were cut at -20°C. in the Linderstrøm-Lang freezing cabinet in each of the following planes.

1. Parallel to the surface plane of the callus.
2. Perpendicular to the surface plane and perpendicular to the direction of grooves.
3. Perpendicular to the surface plane and parallel to the direction of grooves.

The sections were dried on slides at room temperature without being mounted or covered.

For the polarization optical studies a Leitz research type of polarizing microscope was used. The sign of birefringence was determined by means of a first order red retardation plate, used in the usual manner as described in numerous treatments of polarization microscopy (3, 13). In either instance, birefringence was referred to the optic axis of a component structure or to a characteristic structural feature of the callus. The optic axes of the component structures of the callus were determined in the conventional manner (3, 13).

RESULTS

Examination of the serial sections with the polarizing microscope showed that optically the callus comprises two types of elongated regions which alternate regularly. One of these regions occurred beneath the areas occupied by the surface grooves (sulci cutis). The approximate length and width of such an area coincided with those of a groove and included the entire thickness of the callus. These regions of the callus were termed "groove areas." The longitudinal axis of a groove area was considered to run parallel to the surface plane of the callus in the direction of the groove and has been selected as the reference axis of callus. The other elongated regions occurred beneath the surface ridges (cristae cutis) and were termed "ridge areas." The ridge areas appeared to be wider than the groove areas and were penetrated by sweat ducts. These different regions of the callus were separately analyzed and revealed the following optical properties under low magnification of the polarizing microscope.

1. In sections which were cut parallel to the surface plane of the callus, the groove areas appeared as intensely birefringent parallel lines running across the entire length of the sections (Fig. 2). Each showed a maximal brightness if its longitudinal axis was set to 45° from the polarizing directions of the Nicol prisms, indicating a predominant axial orientation. The sign of double refraction of groove areas was positive with respect to the longitudinal axis.

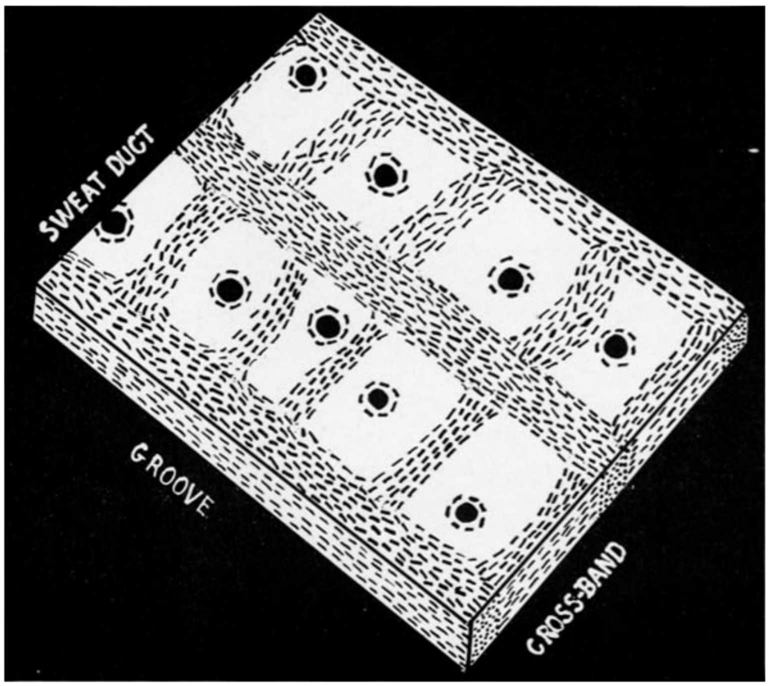
The regions between groove areas corresponded to ridge areas and showed a complex picture. A narrow region around the sweat ducts revealed positive index ellipsoids with long axes oriented circumferentially, indicating an annularly oriented structure. Surrounding this, a random orientation was seen, roughly forming concentric rings. Midway between neighboring sweat ducts intensely birefringent cross-bands were observed connecting groove areas in a more or less transverse direction (Fig. 2). These cross-bands were not observable in ordinary light; they showed up only in polarized light. Each revealed positive double refraction with respect to its longitudinal axis.

2. In sections which were cut perpendicular to both surface plane and direction of grooves, regularly alternating dark and bright bands were seen (Fig. 3). The dark bands corresponded to groove areas and in most sections they appeared isotropic at all angles of rotation indicating that the optic axis lies perpendicular to the plane of sectioning and therefore coincides with the longitudinal axis of the grooves.

The bright bands corresponded to ridge areas and darkened and brightened four times in the course of a 360° rotation of the slide. The sign of double refraction was positive with respect to the surface plane of the callus. Since in these areas neither an intense double refraction nor full darkness occurred at any angle of rotation, this suggested that in this plane of sectioning the structural elements appear in a more or less disordered form.

3. In sections which were cut in a plane perpendicular to the surface plane and parallel to the direction of grooves, different pictures were seen depending on the region through which the cut was made. Those sections which originated from groove areas showed an intense positive axial birefringence (Fig. 4). Those originating from ridge areas showed differing and complex pictures, indicating variable and complicated orientation of the structural elements, as was also seen in the sections cut parallel to the surface plane of the callus.

Biochemical studies of the cornified epithelium of the skin (11) show that in addition to keratin it contains a relatively high amount of lipid material (7 to 9 per cent). Since some lipids are birefringent and might therefore contribute to the anisotropy of the callus, it appeared important to repeat the observations on sections which had been extracted by lipid solvents. For this purpose a few sections of each series were extracted for 24 to 48 hours with ethanol and subsequently with ether. Since no change in strength and sign of double refraction was noted after extraction, it was concluded that the birefringence of the callus is attributable to the keratin component and that lipids do not contribute to the observed optical properties of the callus.



TEXT-FIG. 1. Schematic picture of oriented areas of the human callus. Broken lines indicate the direction of orientation of keratin in various regions of the callus.

INTERPRETATION AND DISCUSSION OF RESULTS

It has been amply demonstrated that mammalian keratins are characterized by positive birefringence and that their optic axes correspond to the orientation of the molecular chains (2, 5, 6, 9, 12). Although x-ray diffraction data are not available on callus, thereby precluding positive identification of the birefringent elements with α-keratin polypeptide chains (8), the likelihood of this identity permits deductions about the orientation of the submicroscopic structure of callus as follows:—

Since the optic axis of a groove area is parallel to the longitudinal axis and shows a positive sign of double refraction with respect to this axis, it may be concluded that in groove areas keratin is oriented parallel to the surface plane of the callus and to the direction of the grooves.

In ridge areas a more complicated structure prevails, mainly as a consequence of random orientation of individual cornified cells. The cross-bands, connecting adjacent groove areas, appeared as linearly oriented components of ridge areas. Since they revealed positive double refraction with respect to their longitudinal axis, it might be concluded that the preferential orientation of keratin in cross-bands is in a transverse direction, being at right angles to that in groove areas. The circumferential positive index ellipsoid about the sweat ducts seems to originate from circumferentially oriented single cornified cells. Whereas the orientation of keratin in the single cornified cells appears linear and parallel to the longitudinal axis of the cell, the array of circumferentially oriented cells gave rise to a continuous annularly oriented keratin structure.

In Text-fig. 1 the oriented regions of the callus are schematically reproduced and the direction of orientation of keratin is indicated by broken lines. It can be seen that the cross-bands of ridge areas together with groove areas form a fairly regular lattice-like pattern, while around the sweat ducts an annular structure occurs. The lattice-like pattern demonstrated in the present studies comprises an additional structural modification of the keratin in the submicroscopic dimension. It is proposed that functionally the lattice structure increases the mechanical strength of the stratified squamous epithelium as well as assuring isodimensional mechanical and physical responses to mechanical stress. It is further postulated that the annular structure around the sweat ducts is an important support for the wall of the sweat ducts, perhaps assuring their patency.

SUMMARY

To explore the submicroscopic structure of the human callus by the polarization optical method, serial sections were prepared in three principal planes and the sign of double refraction as well as optic axes of various regions of the sections was determined. It was concluded that in areas under the grooves keratin is oriented parallel to the surface plane of the callus and to the direction of the grooves. In component structures of ridge areas such as in sweat duct areas a circumferential, in cross-band areas a linear, and in other parts a random structure was seen. The cross-bands of ridge areas in association with groove areas revealed a regular lattice-like submicroscopic pattern which was considered as an important mechanical system of the cornified epithelium.

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EXPLANATION OF PLATES

PLATE 53

FIG. 1. Human callus of the sole of the foot showing regularly arranged grooves and ridges on the surface. *A*, low magnification; *B*, high magnification.

FIG. 2. A section of human callus which was cut parallel to the surface plane. Parallel birefringent lines correspond to groove areas; the birefringent cross-bands are component structures of ridge areas. *PO*, polarizer axis; *AN*, analyzer axis.

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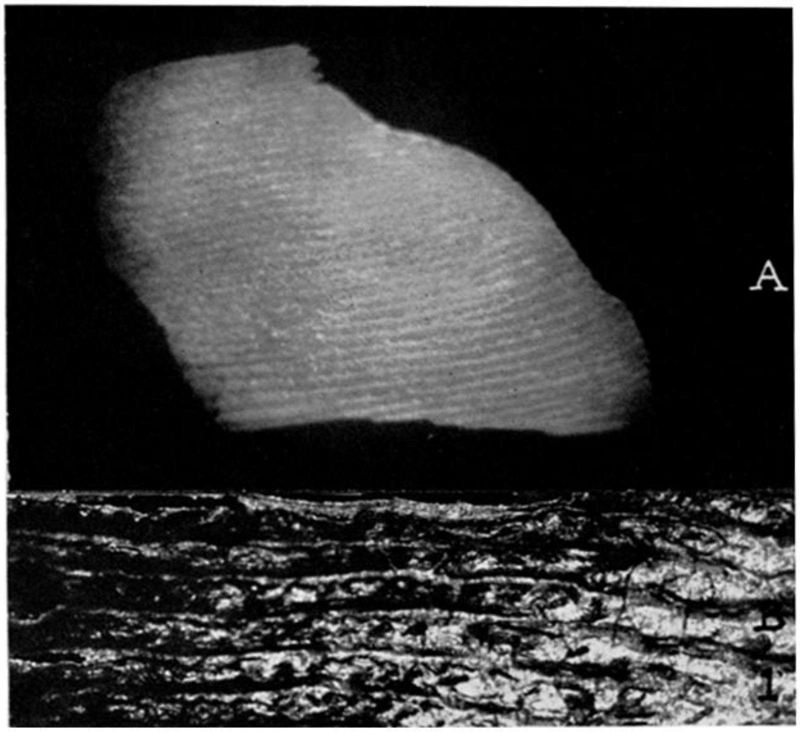
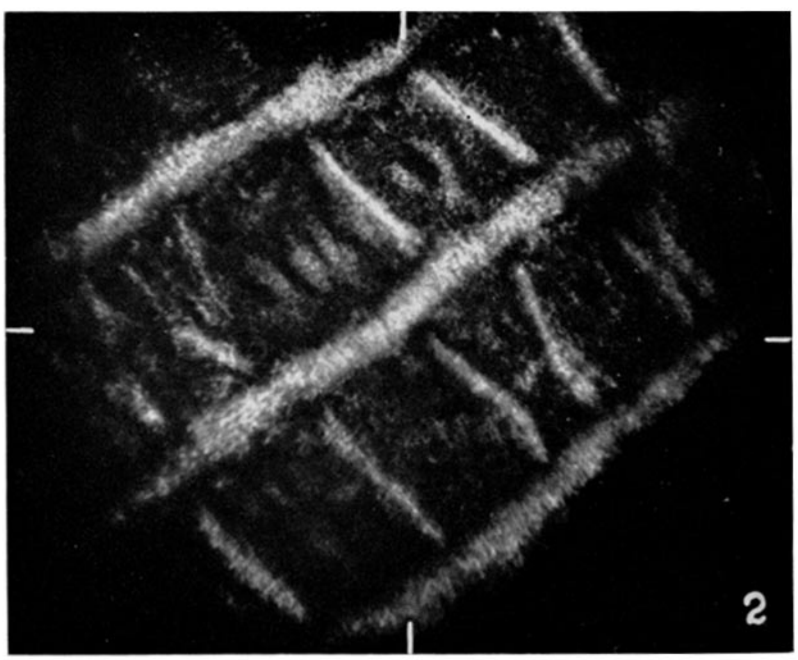


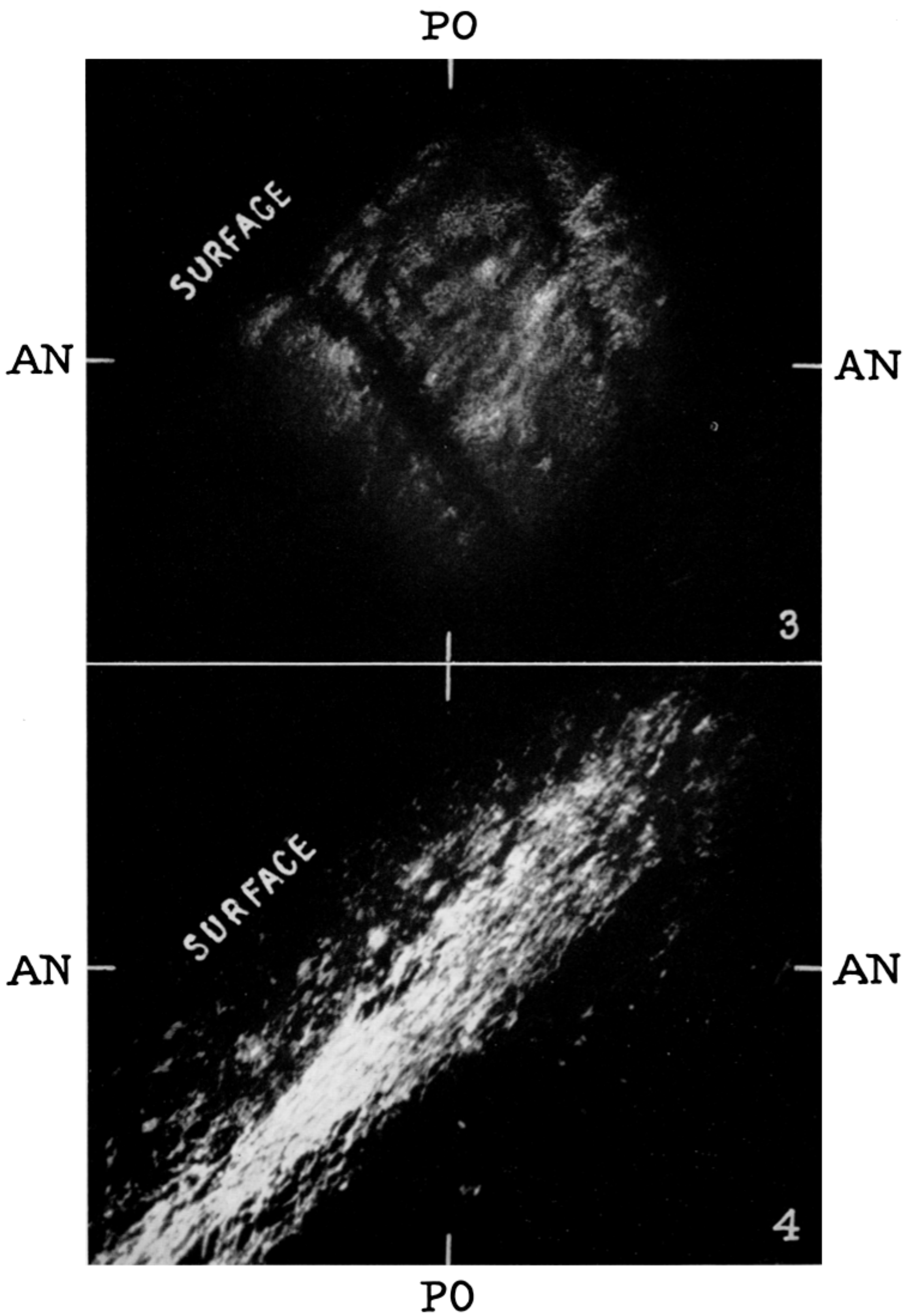


(Matoltsy and Odland: Structure of cornified epithelium)

PLATE 54

FIG. 3. A section of human callus which was cut perpendicular to the surface plane and perpendicular to the direction of grooves. Dark bands correspond to groove areas and show continuous darkness at any angle of rotation. The birefringent bands are the ridge areas. *PO*, polarizer axis; *AN*, analyzer axis.

FIG. 4. A section of human callus which was cut perpendicular to the surface plane of the callus and parallel to the direction of grooves. The cut passed through a groove area and the entire section appears birefringent. *PO*, polarizer axis; *AN*, analyzer axis.



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