POLARIZATION OPTICAL STUDIES OF HYPERKERATOSIS, PARAKERATOSIS AND DYSKERATOSIS*

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Previous polarization optical studies of adult and embryonic human epidermis have been devoted to the elucidation of the morphology of its fibrous structure. In unstained vertical sections of human abdominal skin, the most intense double refraction occurs in the stratum corneum and stratum lucidum. In these layers birefringent material is oriented parallel to the surface plane; below this region the birefringent fibres are oriented perpendicularly to the surface (1).

Similar observations have been made by other authors (2, 3), among them Mercer, who has compared the appearance of the epidermis under the polarization and electron microscopes. He noted that the fibrils (tonofibrils) in the lower layers of the epidermis were weakly birefringent. At the level of the appearance of keratohyalin there was a sudden rise in birefringence associated with a change in orientation of the fibres from a vertical to a horizontal direction.

In tissue cultures of stripped epidermis, Matoltsy has shown that in 3 to 5 days' old explants the originally vertically oriented birefringent material becomes reoriented in a plane parallel to the surface (4). On the 7th day, a definite keratogenous zone became visible, while all the birefringent epidermal fibres were still oriented parallel to the skin surface. The significance of these findings will be discussed later.

Studies of the development of the birefringent fibrillar system of the human epidermis have yet to be carried out, but similar observations of the chick embryo have been made (5) and it has been shown that around the 16th day of embryonic life the periderm or epitrichium reveals a very weak birefringence. However, moderate birefringence appears during the next 2 days of embryonic life when the true cornified layer is formed. Between the 18th and 21st days of embryonic life, the epidermis reaches its final state; at this stage the cornified layer shows intense double refraction. It is of interest that in the chick embryo, the long axis of the cells of the periderm and of the embryonic horny layer is parallel to the skin surface and the early keratin fibres are also oriented in the same plane.

There is only one polarization optical study of pathologic epidermis. Nieuwmeijer examined the tonofibrils in bullous dermatoses by this method and found differences between the tonofibrillar systems in pemphigus and dermatitis herpetiformis (6). In pemphigus, in the areas of acantholysis, the tonofibrils were greatly decreased, whereas in dermatitis herpetiformis the tonofibrils were pushed aside by the fluid in the bullae.

Recently, I used polarization optical methods to investigate the anomaly of keratin formation in psoriasis (7). It was found that the birefringent tonofibrillar system persisted throughout the psoriatic epidermis and in the parakeratotic scale, but the mature keratin fibres failed to develop into a coherent horny layer. These changes were reflected in the psoriatic nail (8) where immature keratinization was evident from the extensive parakeratosis combined with persistence of a low degree of double refraction in much of the fibrous structure of the nail plate and in the adherent hyponychium. These abnormalities were associated with alterations in the non-fibrous components of the nail and epidermis, manifested histochemically by cytoplasmic metachromasia in the parakeratotic cells and by an increased uptake of the Gram stain in the affected areas.

In the present work the defects in the fibrous structure of the epidermis were investigated in conditions characterized by the formation of anomalous horny layers.

METHOD AND MATERIALS

For the polarization optical investigations we used a Reichert monocular microscope fitted with polaroid discs as substage polarizer and tube analyzer. Photographic records were made with a Retina IIC camera, used in conjunction with a Kodak photomicrographic unit. The sign of birefringence was determined by means of a Zeiss first
order red retardation plate, together with a cap analyzer (9). As in our previous studies (7, 8), birefringence referred to the optic axis of the keratin and pre-keratin fibres (tonofibrils) in the histologic material under examination. With this equipment, unstained skin sections were examined. Biopsy specimens were fixed in 10 per cent formalin, embedded in paraffin and sectioned to 3 μm thickness. The sections were then deparaffinized, cleared in xylene and mounted in synthetic resin. From each specimen sections were also stained with hematoxylin and eosin, buffered thionin (10) and by a modified Gram-Weigert method (11).

Previous routine staining technics had established in the specimens histologic characteristics associated with hyperkeratosis, parakeratosis or dyskeratosis. The sections were classified as follows:

2. Hyperkeratosis with acanthosis: lichen simplex chronicus.
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Fig. 3 and 4. Keratotic basal cell carcinoma showing horn cyst and orientation of tonofibrils parallel to the surface. Magnification X200, X400.

5. Hyperkeratosis, parakeratosis and acanthosis: Verruca plantaris.
7. Parakeratosis: a) psoriasis, b) seborrheic dermatitis, c) keratotic basal cell carcinoma.

RESULTS

In simple hyperkeratosis, the normal pattern of epidermal birefringence was observed, though the stratum corneum containing highly bire-
fringent mature keratin fibres was increased in thickness.

Where the hyperkeratosis was associated with acanthosis, especially in lesions with pseudo-epitheliomatous hyperplasia, as in a case of chronic stasis dermatitis, this normal pattern was exaggerated. In particular, the keratogenous zone was broadened and very sharply defined; the birefringent tonofibrils were increased in number, but their orientation was identical with those in the normal epidermis (Fig. 1).

In chronic radiation dermatitis, when hyperkeratosis was associated with epidermal atrophy, there was a paucity of tonofibrils and these were oriented parallel to the skin surface (Fig. 2). A similar orientation of the tonofibrils was seen in an example of keratotic basal cell carcinoma with epidermal atrophy and in leukoplakia involving the muco-cutaneous junction of the lip (Figs. 3, 4, 5).*

* It has been shown that in sections of the buccal mucosa and in sections of the cow's nose
In psoriasis, as previously described (7), the prekeratin fibrils persisted throughout the epidermis and in the parakeratotic scale. The birefringent fibres were oriented perpendicularly or at an angle to the surface plane. Mature keratin fibres occurred in isolated groups, oriented parallel to the surface. This pattern is apparently specific for psoriasis; in other pathologic material no comparable distribution was found. Thus in seborrheic dermatitis, with marked parakeratosis, the normal orientation of the doubly refractile epidermal fibres was retained, though in parakeratotic areas the birefringence was less intense than in the normal horny layer.

Dyskeratosis, defined as a faulty keratinization of individual epidermal cells (12), is reflected under the polarization microscope. In Darier's disease, the villous epidermal projections showed normal tonofibrils. The corps ronds contained birefringent fibres of low intensity, oriented parallel to the long axis of the cells. In the abnormal horny layer, the grains appeared to be

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**Fig. 7.** Plantar wart showing peripheral area with alternating columns of highly birefringent and poorly birefringent fibres, following the areas of fully cornified and parakeratotic cells and oriented in the long axis of these cells. A well-defined keratogenous zone can be seen. Magnification X200.

The plantar wart showed a peculiar pattern of birefringence. The central area, occupied by a mass of compressed cornified cells displayed intense birefringence with orientation of the component fibres parallel to the surface. In the periphery, the alternating columns of parakeratinic and fully cornified cells showed varying degrees of double refraction. In effect, the orthokeratotic areas revealed a more intense birefringence than the parakeratotic zones. The orientation of the keratin fibres followed the direction of the long axis of the cells within the modified horny layer, and thus varied with the papillomatous elevations and depressions. The keratogenous zone was clearly demarcated and the tonofibrils somewhat increased in number, in a manner similar to that seen in pseudoepitheliomatosus hyperplasia (Figs. 6, 7, 8).

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epidermis (which histologically resemble one another with respect to their differentiated epithelium), the tonofibrils are oriented parallel to the surface.

**Fig. 8.** Plantar wart demonstrating details of the keratogenous zone and tonofibrils. Magnification X400.
A detailed discussion of the Gram staining of some of these lesions and its possible significance is published in another paper (13). Cytoplasmic Gram positivity was associated not only with parakeratotic cells, but also with areas of dyskeratosis, especially where the dyskeratotic cells were exfoliated from the epidermal tissue. Under oil immersion it could be seen that this cytoplasmic uptake of the Gram stain was localized to granules; no evidence was obtained that Gram positivity was related to the fibrous structure of the cell.

DISCUSSION

From these observations, certain pathological patterns of epidermal birefringence emerge. Where the whole epidermis shows simple hyperplasia, there is an exaggeration of the normal pattern with two significant features; an increase in the number of tonofibrils and a wide, well-defined keratogenous zone. Preliminary measurements with a micrometer eye-piece suggest that the width of the keratogenous zone and the abundance of tonofibrils are related to the actual amount of hyperkeratosis. When hyperkeratosis occurs in the absence of these features, it is only a relative and not an absolute hyperkeratosis. In this case the horny layer forms a greater proportion of the epidermis, but is not thickened and even may be decreased in width.

Two other characteristic patterns have been consistently observed. One is the peculiar structure of the psoriatic epidermis which has already been described in detail. The other is the “de-differentiation” of the epidermal fibrous system in chronic epidermal atrophy and in certain premalignant dermatoses, as manifested by the orientation of the tonofibrils parallel to the surface. It is of interest to recall that this pattern is identical with that found in skin explants (4), in the epidermis of chick embryos (5) and in normal keratinizing mucous membranes.

In our study we found that the orientation of the keratin and prekeratin fibres was directly related to the long axis of their parent cells. The factors which orient the keratin and trichohyalin fibres in the hair follicle were studied by Mercer. He observed that the cells in which these filaments formed were slowly distorted into spindle shapes as synthesis proceeded; nevertheless, the increase in length of the cells was not sufficient to account for the drawing out of the filaments. Mercer has assumed that, because the filaments

FIG. 10 a and b. Senile keratosis showing amorphous keratin masses, the irregular pattern of the anisotropic epidermal fibres and isolated dyskeratotic cells. Magnification ×200, ×400.

FIG. 11. Cornu cutaneum with packed mature keratin fibres of high intensity birefringence and isolated dyskeratotic cells, displaying a lower degree of birefringence. Magnification ×200.
grow parallel to the long axis of the cell, the slight
flow of cell contents oriented the initial filaments
and that later growth maintained the same
orientation. Furthermore, he believed that lateral
association between adjacent fibrils was a sig-
nificant factor in this process (14).

Such lateral association of fibrils has been
demonstrated, not only in electron micrographs
of the human epidermis (15), but also in electron
micrographs of the protein tonofibrin, which has
been extracted from the epidermis. This protein is
believed to form the substance of the tonofila-
ments (16). Some of these interpretations must
remain tentative in the light of Charles’ recent
electron microscopic studies (17) of the hair
follicle. This author has shown that trichohyalin
granules in the root sheath cells do not elongate
into fibres, but that the nonfibrous trichohyalin
is deposited around individual tonofilaments.
Similarly, keratohyalin is precipitated on the
tonofibrils in the course of epidermal keratiniza-
tion within the granular layer (18).

Despite these conflicting viewpoints, it would
appear reasonable to assume that the orientation
of tonofibrils may be related to changes in cell
shape and perhaps to alterations in the viscosity
of the nonfibrous cell contents.

From Weiss’s experiments (19), two important
points should be mentioned. First, the alignment of fibres may influence the shape of cells; secondly, cell and fibre orientation may be a result of differential adhesion. We are now sufficiently familiar with the methods of adhesion of prickle cells to realize the significance of the latter observation. According to Selby (15), the familiar intercellular bridges or desmosomes surround the cells of the stratum germinativum and there is some electron dense material between the cell membranes within the desmosomes. Yet, in the stratum granulosum the desmosomes lie closer to each other along the cell periphery and the material within the desmosomes is more conspicuous. Selby suggested that the close approximation of desmosomes is a result of an overall decrease in cell volume. This may indeed be an important factor in the altered orientation of tonofibrils within this layer.

At present, we can only form hypotheses concerning the abnormal orientation and lack of maturation of keratin fibres in certain dermatoses. Anomalous cell orientation would seem to be of foremost importance in the peculiar alignment of tonofibrils seen with epidermal "de-differentiation" as in pre-malignant dermatoses. Abnormal cell adhesion may be the primary factor in producing the patterns of epidermal birefringence in dyskeratosis and particularly in Darier's disease. Indeed it has been shown that in Darier's disease the formation of corps ronds occurs because epidermal cells are unable to form prickles (intercellular bridges) on some or all sections of their cell walls (18). In psoriasis, we have certain experimental corroboration for our theory that abnormal cell adhesion and incomplete synthesis or deposition of keratohyalin together lead to the persistence of tonofibrils and the inhibition of mature keratin fibre formation (18).

SUMMARY

Unstained skin sections were studied under the polarization microscope, with relation to the orientation, maturation and degree of birefringence of the anisotropic fibres of the epidermis. In hyperkeratoses with concomitant hyperplasia of the epidermis, there was an exaggeration of the normal pattern of birefringence. Abnormal characteristics of the distribution and orientation of the epidermal birefringent fibres were associated with various dermatoses.

The pattern in psoriasis has been found to be specific for this disease. A peculiar development of the tonofibrils was demonstrated in chronic radiation atrophy and with certain premalignant and malignant conditions; the prekeratin fibres were oriented parallel to the surface, as in embryonic skin and in skin explants. The doubly refractile fibres in the dyskeratotic cells in Darier's disease have been compared with those found in parakeratotic cells.

Various factors which contribute to the formation and orientation of keratin fibres in the normal and pathologic epidermis were discussed.

ADDENDUM

Quite recently the author has had the opportunity to study sections of an intra-epidermal squamous cell carcinoma, revealing the malignant dyskeratotic cells characteristic of Bowen's disease. Under polarized light, in these sections the anisotropic tonofibrils had disappeared in the affected areas except peripherally, where a few remained oriented parallel to the surface. (see Fig: 12 a, b). This finding coincides with observations made by von Albertini (21) in his electron microscope studies of epidermal carcinoma. He showed that tonofibrils were markedly increased with epidermal hyperplasia but that with the loss of cytoplasmic differentiation involved in malignant change, there was first a malformation and later, complete lack of formation of tonofibrils.

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