Bleb dystrophy of the cornea: Histochemistry and ultrastructure

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SUMMARY Corneal epithelial biopsies from two patients affected with asymptomatic bleb dystrophy were examined by light and electron microscopy. The basis of this disorder appears to be the deposition of a neutral mucopolysaccharide-protein complex as a continuous layer between the basement membrane and Bowman’s layer. This material, although homogeneous in light microscopy, has a fine granular ultrastructure. It is friable, and in view of the apparent integrity of the basement membrane/hemidesmosome system it is suggested that the recurrent epithelial erosions which can occur in this disorder result from shearing of this layer. Fissures in the bleb material contain cells which may play a role in its degradation.

A recently described group of superficial corneal disorders includes the fingerprint, net, bleb (Bron and Brown, 1971) and microcystic dystrophies (Cogan et al., 1964). Brown and Bron (1976) have shown that these dystrophies may be associated with recurrent epithelial breakdown. Thus, they were present in 59% of their patients with the recurrent erosion syndrome. Conversely the same authors (1971) had noted previously that 38% of patients with these superficial dystrophies were also afflicted with symptoms of recurrent epithelial dehiscence. These studies naturally suggest that this group of superficial corneal dystrophies is an important precursor of defective epithelial adherence.

The common ultrastructural basis of these disorders (Cogan et al., 1974; Rodrigues et al., 1974; Broderick et al., 1974) is the deposition of an abnormal or excessive fibrillo-granular protein which accumulates deep to the basal cells, and in the respective cases of fingerprint and microcystic dystrophies is found at the midepithelial level as finger-like protrusions or in continuous sheets.

The bleb form of superficial corneal dystrophy was present in 28% of patients with the recurrent erosion syndrome who were examined by Brown and Bron (1976). Although bleb dystrophy may be clinically interrelated with the other forms, its histopathology is unknown. Two recent patients with asymptomatic bleb dystrophy provided an opportunity to investigate its pathogenesis.

Case reports

Case 1. A 50-year-old White male patient who was otherwise healthy complained of irritation and blurred vision affecting his right eye for the previous week. There had been no preceding ocular symptomatology nor was there a significant family history of eye disease.

Examination showed corrected vision OD = 20/80, OS = 20/20. A dendritic ulcer of the herpes simplex type was present in the epithelium of the right cornea. Profuse subepithelial blebs were present in clusters in the parapupillary zones of both corneas. Their distribution in the left cornea is illustrated in Fig. 1. The blebs, which varied in size, were on the average about 50 μ wide. With the direct ophthalmoscope the blebs had an orange-skin appearance when set against the fundal reflex. Viewed by indirect slit-lamp illumination from the iris the blebs appeared as transparent bubbles with an unreversed lighting effect (Fig. 2), a phenomenon which, as Brown (1971) noted, indicates a refractive index higher than that of the surrounding tissue. In focal illumination the blebs were seen as black dots set in a relucant background. There were no demarcation or other lines in either cornea. In the left eye it was possible to determine that there was no interference with the precorneal tear film and that
of the ablated epithelium; hence a 2 mm Elliot trephine was used to demarcate two biopsies of the adjacent apparently healthy epithelium and underlying blebs. Corneal healing was complete within four days, and there has been no further evidence of corneal ulceration for the past nine months.

Case 2. A 65-year-old White woman, requiring cosmetic blepharoplasty for senile skin changes, was found to have bilateral bleb dystrophy. She had never experienced symptoms suggestive of the recurrent erosion syndrome, nor was there a family history of this disorder. The blebs, although more extensive than seen in case 1, were in all other respects essentially similar. Corneal sensation and lachrymal and lid secretions were quantitatively normal. The ophthalmoscopic appearance of the blebs on the left side is shown in Fig. 3. With the patient's informed consent a 2 mm biopsy of the corneal epithelium and underlying blebs was undertaken from each eye at the times of blepharoplasty. The corneal epithelial defects healed within 24 hours; there was no residual scarring. There have been no ocular symptoms for the past six months of surveillance.

Methods

Two of the epithelial biopsies (one from each patient) were fixed in 10% formalin, dehydrated, and then infiltrated with paraffin wax. Paraffin sections were treated with a variety of tinctorial and histochemical techniques which will be mentioned alongside the results obtained. The other two specimens were prepared for transmission electron microscopy (TEM), the examination being under-
taken with an RCA Emu 3G instrument. The disc from case 1 was placed in 24% cold glutaraldehyde prior to osmification, whereas the specimen from case 2 was fixed in cold Karnovsky’s (1965) fluid and then treated with a solution of 2% osmic acid and 2% ruthenium red in accordance with Luft’s (1965) method for mucopolysaccharides. Both specimens were ultimately embedded in epoxy resin.

Orientational sections from the specimens embedded in resin were stained with toluidine blue and viewed by light microscopy, while suitably thin sections were stained with lead citrate and uranyl acetate. Skin obtained at blepharoplasty from case 2 was formalin-fixed and embedded in formalin. Subsequent sections were stained routinely.

Observations

LIGHT MICROSCOPY
Skin biopsies show predictable age changes, but there are no abnormal accumulations of periodic acid-Schiff (PAS) positive material in the region of the basement membrane, which appears normal.

The four corneal biopsies consist of corneal epithelium, basement membrane, and a subepithelial deposit of ‘hyaline’ material.

The corneal epithelium is invaginated on its deep surface by mounds of a homogeneous material which appears pink in haematoxylin and eosin preparations but is pale blue after staining with toluidine blue (Fig. 4). Occasional mounds show fissures in which flattened cells with elongated processes are found (Fig. 5). The bleb material bordering these fissures is often less intensely stained than elsewhere. The basement membrane of the cornea appears intact and of normal thickness. Corneal epithelium overlying the mounds is healthy apart from the presence of a few darkly stained cells in the basal layer.

The table summarises the results of various histological and histochemical methods on the basement membrane and subepithelial bleb material.

PAS positivity of the bleb material is not affected by pretreatment of the sections with either diastase or hyaluronidase (at pH 2 and pH 4). Both basement membrane and the subepithelial ‘hyaline’ material are monorefringent in polarised light.

<table>
<thead>
<tr>
<th>Method</th>
<th>Basement membrane</th>
<th>Subepithelial bleb material</th>
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<tbody>
<tr>
<td>Milon reaction</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Toluidine Blue</td>
<td>Lavender</td>
<td>Orthochromatic Positive</td>
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<tr>
<td>PAS</td>
<td>metachromasia</td>
<td>Positive</td>
</tr>
<tr>
<td>Sudan black B</td>
<td>Strongly positive</td>
<td>Negative</td>
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<tr>
<td>Gomori reticulin stain</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Congo Red (dichroism) amyloid stain</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Thioflavin T (fluorescence) amyloid stain</td>
<td>Negative</td>
<td>Negative</td>
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TRANSMISSION ELECTRON MICROSCOPY
Apart from the intracytoplasmic vesicles in some of the basal cells, which may well be artifactual, and the presence of rare electron-dense acantholytic cells in this layer, the corneal epithelium does not exhibit any cytologic abnormality. The basal cells lie on a normal basement membrane, which has its usual complement of hemidesmosomes (Fig. 6). The bleb material is composed of fine granules measuring about 70 Å. In sections from the biopsy treated with ruthenium red the bleb granules exhibit no increase in electron density. Irregularly banded fibrils which are orientated perpendicularly form an incomplete seam beneath the basement membrane. No spatial relationship exists between the bleb configuration and the subepithelial disposition of these fibrils. In places where the plane of biopsy cleavage has been deep enough it can be determined that the bleb material is not confined to the mounds, but forms a continuous subepithelial layer. Near the edges of the specimen occasional spherical accumulations of bleb material appear to lie entirely within the epithelium. These accumulations are not surrounded by basement membrane and have presumably been displaced from the subepithelial layer by the trauma of biopsy.

Occasional blebs show horizontal fissures which are partially occupied by unidentified cells (Fig. 7). These cells have numerous thin cytoplasmic processes, in which tonofibrils are a prominent component. They are richly endowed with membrane-bound vesicles containing sparse granular material. In places the vesicles are aligned near

![Fig. 6](image1)

**Fig. 6** Intact basement membrane/hemidesmosome system separates basal cell (E) from the granular bleb material. An incomplete seam of vertically orientated fibrils (F) lies beneath the basement membrane. Bleb dystrophy (lead citrate/uranyl acetate), TEM, ×10000

![Fig. 7](image2)

**Fig. 7** Part of cell embedded in bleb material is surrounded by sharply defined zone in which granules are either sparsely distributed or absent. E, Basal epithelial cell; BM basement membrane, bleb dystrophy (ruthenium red), TEM, ×10000

![Fig. 8](image3)

**Fig. 8** Detail of Fig. 6 to show membrane-cald vacuoles V arranged close to the cell membrane. Some of the vacuoles have opened extracellularly. Bleb dystrophy (ruthenium red), ×71000

the cell membrane, where some of them open on to the surface (Fig. 8). Bleb granules in the vicinity of these cells are frequently absent or else considerably reduced in number, producing a pericellular zone of rarefaction which is sharply demarcated from the main mass of bleb granules. The granules which are present in these rarefied zones have a smudged appearance and tend to be aggregated in a linear manner.
Discussion

The histological substrate of bleb dystrophy, as adduced from the present study, is the accumulation of a continuous layer of fibrillo-granular protein between the basement membrane of the epithelium and Bowman’s layer. The outer surface of this material is moulded in the form of hemispherical mounds or blebs which indent the overlying epithelium without deforming its superficial layers. The epithelial basement membrane together with its hemidesmosomal attachments is normal in form and staining reactions. The bleb material has for the most part a finely granular ultrastructure, but alongside the basement membrane irregularly banded fibrils form a discontinuous seam. These filaments appear to correspond with special fibrils of the dermis described by Palade and Farquhar (1965) which have an anchoring role. Corneal epithelial cells overlying the blebs, although displaced or distorted, in general show little ultrastructural abnormality, but occasional basal cells are acantholytic and electron-dense. Similar cells were noted in the corneal epithelium in fingerprint dystrophy by Brodrick et al. (1974). In the latter condition these cells were relatively common and resulted in microcyst formation, which is not seen in bleb dystrophy unless epithelial erosion has occurred.

Subepithelial granular material in Cogan’s microcystic dystrophy, which is morphologically similar to that seen in the present study of bleb dystrophy, has been described as ‘basement membrane-like’ by Cogan et al. (1974). The present study does not provide evidence for this assertion. It demonstrates on the contrary that the subepithelial material contains only a few fibrils which have formed in the presence of a normal basement membrane/hemidesmosomal system. Moreover, unlike basement membrane, it has no affinity for Gomori’s reticulin stain. Studies with fluorescent antibody conjugates raised against both mesodermal and ectodermal basement membranes and other proteins may resolve this question. The bleb material exhibits PAS positivity, which resists digestion with diastase; these findings together with its lack of ruthenium red reactivity suggest the presence of a neutral polysaccharide-protein complex.

The presence of unidentified cells which lie in rarefied seams of the bleb material is a striking feature present in a minority of the blebs (Figs. 4, 6, and 7). The seams which contain a few granules, the outlines of which are often smudged, are sharply demarcated from the main mass of compact bleb material. Numerous cytoplasmic vesicles are present in these cells (Fig. 7). In some instances the vesicles are aggregated in a row alongside the plasma membrane, where a few of them are open on to the cell surface. It is tempting to speculate that these cells are synthesising a cathepsin which is degrading the bleb granules, but the evidence is circumstantial. It seems wiser to defer any generalisations about cell morphology and the possible secretion of enzyme until the nature of the bleb material is clearer.

A defective basement membrane was noted in traumatic recurrent erosion by Goldman and others (1971) and in spontaneous recurrent erosion by Tripathi and Bron (1972), who also noted a paucity of hemidesmosomes. In contrast, the present study of bleb dystrophy indicates integrity of the basement membrane hemidesmosomal system. Moreover, a similar observation was made in the fingerprint disorders both of dystrophic type by Brodrick et al. (1974) and of that sometimes seen in metaherpetic keratitis by Brodrick and Dark (1976). Surgical removal of the epithelium in all three conditions resulted in scarring of the subepithelial granular material. Thus it is possible that epithelial erosion associated with some of the superficial dystrophies is a sequel to splitting of this abnormal subepithelial layer of granular material without involving defects in the basement membrane or its anchorage system. If this is indeed the primary mechanism in such cases, then surgical treatment, when indicated, should aim at removing this friable material from the anterior surface of Bowman’s layer.

References