COMMUNICATIONS

CLINICAL AND EXPERIMENTAL STUDY OF THE BASAL MEMBRANE OF THE CORNEAL EPITHELIUM IN KERATITIS BULLOSA*

BY

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The existence of a basal structure separating the different epithelia (glandular, cutaneous) from their stroma, is well known. The histopathology of this basal membrane has been especially well studied at the dermo-epidermic junction, where it is seen as a dense layer, intensely coloured by periodic acid–Schiff (Dupré, 1953; Lever, 1954). Percival and Hannay (1949) noted that it forms an intracutaneous barrier, preventing the diffusion of certain large particles. The authors showed that a large molecular stain injected near a subpapillary bulla diffuses easily through the derma, but does not appear in the liquid of the bulla.

Mariani (1955) described in detail the embryology of the cutaneous basal membrane and alterations in certain bullous cutaneous disorders (pemphigus, pemphigoid, herpes, epidermolysis bullosa).

Prunieras (1954) discussed the physiological and pathological importance of the cutaneous basal membrane and the pathological alterations in eczema and Duhring-Brocq dermatitis.

Concerning the pathology of the cornea, the first reference to the presence of a layer separating the corneal epithelium from Bowman’s membrane was made by Fuchs (1915), who described two layers between the epithelium and Bowman’s membrane in a case of Grönew’s dystrophy. One layer, staining red with Giemsa and grey-blue with Weigert’s stain, is connected with Bowman’s membrane and departing from it is composed of pseudopod-like prolongations between the basal cells of the epithelium. Loewenstein (1940), taking the analysis of Fuchs’s case and the histological examination of three cases of his own as a basis, concluded that this hyalinoid, acidophilic, structureless layer is nothing but a part of Bowman’s membrane showing a different histological reaction.

Busacca (1949) was the first to demonstrate the independence of this membrane from Bowman’s membrane, as well as its analogy with other basal membranes. In the course of three different histological examinations (in

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cases of pterygium, pannus, and keratitis bullosa) using Mallory's stain, he noted that the basal membrane stained in the same way as other basal membranes, but differently from Bowman's membrane, which gave a histological reaction characteristic of collagen tissue.

According to Redslob (1949), the basal membrane regenerates in the same way as the epithelium. Under electron-microscopic examination it is seen as a more or less homogeneous layer, with a spongeliike appearance due to small round or oval vacuoles (Sebruyns, 1950).

The introduction of the Hotchkiss-MacManus stain has given a new impulse to the histological examination of the basal membrane, which stains very clearly by this method and is well differentiated from Bowman's membrane. The investigations of Teng and Katzin (1953) using this stain may be considered as fundamental. They examined the basal membrane in man, in the cat, in the rabbit, in the monkey and in the calf. It is well visible in the eyes of the calf, and less so in the cat and the rabbit, but is very thin in the cornea of the monkey. The thickness varies with the individual, and the pathological condition varies from that of an epithelial cell to a third of Bowman's membrane. The basal membrane extends across the limbal region, where it is thicker, up to the bulbar conjunctiva. It is found at all ages from birth onwards.

Vidal (1951), investigating the chemical composition of the basal membrane, showed that it was made up of three layers: a middle layer containing glycoproteins, which may be depolymerized by mucinase, and the anterior and posterior layers containing fibres of reticulin (scleroprotein).

According to histochemical examinations by LaTessa, Teng, and Katzin (1954), the basal membrane contains plasmalogen (a phospholoid substance) as well as reticular fibres. On the basis of the action of solvents, these authors attributed to the lipid layer of the basal membrane a role in the adhesion of the epithelium. The non-lipid fraction is probably composed of muco- or glycoproteins. The basal membrane can be destroyed by proteolytic enzymes (trypsin and chymotrypsin).

Calmettes, Deodati, Planel, and Bec (1956), in their work on the histology, histochemistry, and origin of the basal membrane, described the membrane in the cornea of the horse, donkey, guinea-pig, and rat. They found that the membrane contained muco- or glycoproteins as well as lipids, but they could find neither acid mucopolysaccharides (the basal membrane not being metachromatic with toluidine blue) nor ribonucleic acid. Contrary to the statements of LaTessa and others (1954), they were able to demonstrate, using Divry's technique, the presence of reticulin, whereas the staining of elastic fibres gave negative results.

The origin of the basal membrane has not been elucidated with certainty. Some authors suggest an epithelial origin, representing a cuticular formation of the basal cells of the corneal epithelium (Redslob, 1949; Busacca, 1949;
Vidal, 1951). They base this opinion on the difference in staining between the basal membrane and Bowman’s membrane, as well as on the fact that the basal membrane regenerates with the corneal epithelium.

Calmettes and others (1956) stated that the basal membrane had its origin in the underlying connective tissue, under the influence of epithelio-connective tissue interaction. According to these authors, in the animal species lacking Bowman’s membrane, the basal membrane is seen as a condensation of the stromal fibres. A similar aspect is seen in the nasal mucous membrane of man (Planel and Dupré, 1953; Bec, 1955). Using Sudan stain, which gives a strongly positive reaction for the basal membrane, the latter appears as a superficial condensation of Bowman’s membrane.

We find little in the literature about the physiological importance of the basal membrane of the corneal epithelium, despite the fact that the histochemical and histological examinations mentioned above establish this importance beyond a doubt. As well as the already cited publication of Busacca (1949), Moro (1957a) found an irregular and finely serpiginous corneal basal membrane in gargoyleism. In some places it was very thin, with interruptions, and an accentuation of the laminated structure. Moro (1957b, c) also described a dissociation and hyperplasia of the corneal basal membrane at the site of the dyskeratotic lesions of the epithelium in Bowen’s disease.

In the “crocodile-shagreen” degeneration of the cornea (Moro and Amidei, 1953), the course of the basal membrane becomes wavy, the lamination accentuated, and the thickness of the membrane irregular.

Offret and Haye (1957) noted, in a case of epithelioma of the cornea, that rupture of the basal membrane of the epithelium preceded destruction of Bowman’s membrane, and that this rupture could be considered as a sign of malignancy. These authors did not find alteration of the basal membrane in corneal herpes.

If we consider the chemical and histological structure of the basal membrane of the corneal epithelium, it seems evident to us that it plays an important role in the adhesion of the corneal epithelium to the underlying tissue, and this we have tried to demonstrate experimentally. The starting point of our experiment was, on the one hand, the observations made by LaTessa and others (1954) concerning the destructive effect of trypsin and chymotrypsin, and on the other hand, the results of the examination by Buschke (1951) indicating the importance of the proteolytic processes of this tissue in the cohesion of the corneal epithelial cells. Also of interest are the experiments of Herrmann and Hickman (1948), indicating that the proteolytic enzymes provoke loosening of the epithelium. They also noted the similarity between this loosening of the corneal epithelium and the vesicular alteration of the skin.

We employed the experimental method of Cogan (1940); this author induced keratitis bullosa by modifying the osmotic pressure which exists on
the one hand at the surface of the corneal epithelium and on the other on the side attached to Bowman’s membrane. To provoke a difference in the osmotic tension, we replaced, by Cogan’s method, the aqueous humour of an enucleated rabbit globe with a hypertonic solution of 10 per cent. NaCl, and then placed it in distilled water. We were able by this method, to provoke a typical but discrete keratitis bullosa.

In another series of experiments, we placed the globe in a solution of trypsin ("Trypure" Novo) for several hours before similar treatment. Comparing the two series of experiments we established the following results: few and small bullae were found in the globes not treated with trypsin (Fig. 1), and the number and size of the bullae were appreciably increased after previous treatment with trypsin (Fig. 2).

![Fig. 1.—Rabbit’s eye, placed in distilled water after injection of 10 per cent. NaCl into the anterior chamber. Note the small epithelial bullae (arrow) at the left edge of the opaque lens. (Some bubbles of air are seen at the top of the anterior chamber.)](image1)

![Fig. 2.—Rabbit’s eye, put into a solution of trypsin before injection of 10 per cent. NaCl into the anterior chamber and subsequently placed in distilled water. Large epithelial bullae are easily distinguished.](image2)

Sometimes we even observed complete bullous detachment of the corneal epithelium. Histological examination clearly shows the subepithelial situation of the bullae as well as a loose basal membrane, stretched and unraveled between the stroma and the epithelium (Fig. 3).

![Fig. 3.—Histological appearance of the cornea of the same rabbit as in Fig. 2. Note bullous detachment of epithelium which is still connected to stroma by remnants of stretched basal membrane.](image3)
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In man, the basal membrane of the corneal epithelium is normally seen as a dense, more or less homogeneous layer at the limit between the epithelium and Bowman's membrane (Fig. 4).

To support our experimental results we examined histologically some cases of bullous keratitis and Fuchs's epithelial dystrophy, that is, two corneal disorders characterized above all by vesicular formations.

Observation 1

A married woman born in 1878 had an operation for cataract of the right eye in 1942 and of the left eye in 1944. Before this she had already experienced attacks of lacrimation accompanied by violent pain.

Slit-lamp examination showed bilateral vascular degeneration of the corneal epithelium. Histological examination of a section of the cornea taken during a corneal transplant in the right eye showed thinning and considerable laceration of the epithelium which was greatly desquamated, in some places in large strips. The epithelial cells were vacuolated and degenerate. Bowman's membrane was fully conserved. A slight increase in the number of corneal cells was found between the dissociated corneal laminae. At the sites of epithelial desquamation, the unravelled basal membrane extended between the basal cells of the epithelium and Bowman's membrane in the form of delicate, slightly wavy, laminated filaments (Fig. 5).
Observation 2
A married woman born in 1876 had had a bilateral antiglaucomatous iridectomy performed 20 years before a keratoplasty operation was undertaken. A year earlier, she had had an intracapsular cataract extraction in the left eye, followed a month later by corepraxia on the same eye. Recurrent erosions of the epithelium, parenchymatous oedema, and endothelial and epithelial degeneration of this eye were treated by lamellar keratoplasty.

Histological examination of the corneal epithelium showed that it was greatly desquamated. The lamellar structure of the stroma was indistinct, and in some places the number of cells was greatly increased, being represented especially by invading fibroblasts and leucocytes. At the level of a small epithelial bulla, the dissociation of the basal membrane into two layers was clearly distinguished, one attached to the basal cells of the epithelium, the other to Bowman’s membrane (Fig. 6). Elsewhere, the structure of the basal membrane was loose.

Observation 3
A married woman born in 1890 showed epithelial oedema of Fuchs’s type, with a linear ulcer between 4 and 6 o’clock in the right eye on slit-lamp examination. Similar alterations, with desquamation of the epithelium, were seen in the left eye. The cornea was thickened. A mushroom graft was performed.

Histological examination of the excised cornea showed that the epithelium was greatly altered in its structure, especially in the basal layer, where the cells were shrunk in places and showed vacuoles and empty spaces between them. Abundant verrucosities were present in Descemet’s membrane. The endothelium was only partially preserved. The basal membrane was finely vacuolated. As in the preceding case, it was divided into two layers at the sites of desquamation. It was unravelled, and in places thinned or absent (Fig. 7, opposite).

Observation 4
A married woman born in 1870, showed epithelial degeneration of Fuchs’s type, with bullae formation as well as thickening of the cornea and endothelial lesions on slit-lamp examination of the cornea.
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**FIG. 7.**—Histological aspect of cornea in Fuchs's epithelial degeneration. The epithelium is thickened, oedematous, and partially desquamated; the basal membrane is frayed and, in some places, thinned or absent.

Histological examination of a disc of cornea obtained while performing a mushroom graft showed a rather thickened epithelium. Cells of the basal layer had lost their habitual elongated form and were almost entirely cuboid. The intercellular cement was very loose. Small vacuoles and pyknotic nuclei were surrounded by a very clear zone. In one place dehiscence of the epithelium had occurred and in others the beginning of basal vascular formations. Bowman's membrane was conserved. The rest of the stroma was slightly oedematous. The basal membrane was clearly visible, and distinctly thickened with marked palisade-like structure (Fig. 8).

**FIG. 8.**—Histological appearance of cornea in Fuchs's epithelial degeneration (Observation 4) showing oedematous epithelium with cuboidal basal cells and a thickened basal membrane with palisade-like structure.
Discussion

The chemical and structural complexity of the basal membrane of the corneal epithelium indicates that it plays an important physiological role, which should be related to the adhesion of the epithelium to Bowman’s membrane, as well as to the metabolic processes of the epithelium and the stroma. It follows that the basal membrane participates likewise, primarily or secondarily, in many pathological changes of the cornea. Injury to the membrane is demonstrated by structural or tinctorial modification.

Using Cogan’s method, we provoked keratitis bullosa of the rabbit cornea. We were able to show that trypsin solution favoured formation of epithelial bullae, proving that the proteins and the proteolytic processes of the basal membrane play an important role in the physiological adhesion of the epithelium to the underlying layers.

We examined some cases of bullous epithelial dystrophy of the cornea, especially Fuchs’s type. Staining with periodic acid–Schiff permitted us to show that the basal membrane underwent important structural alteration in these corneal lesions. Indeed, the more or less thinned basal membrane showed a loosening and unravelling sometimes accompanied by vacuolization. In our last case, however, the basal membrane was thickened, showing a very marked palisade-like structure.

It is proposed to extend these researches on the behaviour of the basal membrane of the corneal epithelium in different forms of dystrophy and degeneration of the anterior limiting membrane.

Summary

The author stresses the important role of the basal membrane of the corneal epithelium in the adhesion of this membrane to the underlying layers. In experimental keratitis bullosa of the rabbit, obtained by Cogan’s method, he was able to show that trypsin clearly favoured the formation of epithelial bullae.

Examining several cases of keratitis bullosa, especially of Fuchs’s type, in man, using periodic acid–Schiff stain, he was able to observe important structural alterations of the epithelial basal membrane in them all.

ADDENDUM

Since this manuscript was sent to the editor, a paper on the basal membrane of the corneal epithelium has been published by Offret and Haye (1959). It deals in detail with the histological behaviour of this membrane in different corneal affections, among them keratitis bullosa, but the authors report no personal case of Fuchs’s epithelial degeneration.

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