Studies on the Corneal and Trabecular Endothelium

I. Cement Substance of the Corneal Endothelium*

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The corneal endothelium appears to be a very interesting tissue from the morphological as well as physiological point of view. It forms a limiting layer separating the corneal stroma from the aqueous humour, and is therefore said by many authors to have a great physiological importance in the regulation of the water-balance of the corneal stroma. Thus it appears to be of foremost importance for the normal function of the eye, and also continues at the periphery of the cornea as the endothelium of the trabecular meshwork, which seems to be of similar functional importance.

In studying the functional anatomy of the angle of the anterior chamber, it is clear that the function of the trabecular endothelium cannot be understood without better knowledge of the corneal endothelium itself, both tissues being closely related ontogenetically. As the corneal endothelium is easier to study than the trabecular endothelium, this tissue is the subject of the present paper.

The cytology of the corneal endothelium has been studied in many classical papers. One of the as yet unsolved problems is that of the nature of the intercellular substance, in view of the recent work of Jancso (1955) and McGovern (1955, 1956) on the vascular endothelium and the mesothelium of the peritoneal cavity.

Material and Methods

Fresh corneae of the cat, dog, rabbit, and man were used. Three methods of silver impregnation of the cement substance were tested: the Ranvier classical method, and the new methods of Jancso (1955) and McGovern (1955, 1956). As the last gave the best results it has been used as a basic method.

Fresh corneae were put into a 0.4 per cent. solution of silver nitrate for about 50 seconds and immediately, without any washing, transferred for 4 minutes into a fresh mixture of 1 vol. 3 per cent. solution of cobalt bromide to 1 vol. 1 per cent. ammonium bromide. Some specimens were then washed in distilled water and fixed, according to

* Received for publication September 30, 1957.
the original McGovern method, by the Carnoy mixture. Other specimens were fixed after the silver impregnation in a 4 per cent. formol solution and cut on a freezing microtome. Both variations proved to be of value; the second being used with greater success to prepare thin sections for photographic records. Some sections were stained in addition by a 1:1,000 solution of toluidine blue. The study of metachromatism by this method was much impaired by the simultaneous intensive metachromatic staining of the corneal stroma. The sections were mounted in Canada balsam or in Apáthy's syrup. The full stereoscopic system gives better evidence of the spatial relations of the substances studied. In some cases, the phase-contrast method and the replica method were of great assistance.

Findings

For the corneal endothelium cells, the description by Kolmer and Lauber (1936) is recommended. Several excellent papers have been published on the subject and the morphology of these cells is well known.

In its simplest form the cement substance of the corneal endothelium corresponds to the classical descriptions. The endothelial elements, mostly hexagonal in shape and of uniform size, are bordered by comparatively narrow, somewhat tortuous lines intensively impregnated with silver. This picture is very similar to that given by staining with Heidenhain's haematoxylin; with the latter the border lines are somewhat narrower than those demonstrated by silver methods. The cement substance forms coarse granules of irregular shape and size. Towards the periphery of the first cat's cornea examined, the border lines grew larger as they approached the corneal margin. The grains of the cement substances appeared swollen and the free inner surfaces of the endothelial cells became narrow in their turn. In the periphery of the cornea—at the limbus region—the granules of cement substance swelled to great balls of a granular substance mostly covering the whole inner surface of the cells. Swollen grains and balls of the cement substance, the colour of which was usually some shade of bluish-grey, took up silver on their surface only. In the central portion of the posterior surface of the cornea, where the surface of the endothelial cells was to a large extent free, as the cement lines were narrow, we got the impression of some other kind of substance covering the remaining free surface of the cells with a thin, smooth layer (Fig. 1, opposite).

This substance did not take up silver, only here and there some fine brownish grains were seen on the surface of the cells. Again, this brownish substance grew towards the periphery of the cornea, and differed quite conspicuously from the bluish-grey cement substance (Fig. 2, opposite).

In some places, the agglomerations of this grey substance were seen to grow to both sides of the originally narrow border lines, which were frequently well preserved in their primary condition. The covering substance on the inner surfaces of the endothelial cells forms a subject of sufficient importance to be treated separately.
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Fig. 1.—Corneal endothelium of the cat; central region of cornea. The cell-boundaries are narrow, and clearly composed of small grains of an argyrophil substance. McGovern's method. ×550.

Fig. 2.—More peripheral region of the same cornea as in Fig. 1. The cement substance of the cell-boundaries appears increased and its grains swollen. This material was impregnated immediately after killing the animal by an electric shock. McGovern's method. ×550.
In the endothelium of a freshly enucleated human eye from a patient with an orbital tumour, the cement substance was also enlarged, but this enlargement had taken place throughout the whole posterior surface of the cornea. The vision of the eye was well preserved, but the exposure of the cornea to the air in the lower parts had resulted in a small infiltration at the limbus. Thus it seems possible that the enlargement of the cement substance was due to the slight irritation of the corneal tissue. Irregularities of the border lines were observed (Fig. 3, opposite) as well as the loop formations described by Jancsó and other authors.

In many places doubled and complicated lines were seen, confirming the findings of McGovern, who saw the lines of the cement substance running across the cement surface, not only along the cell boundaries. This observation is perhaps explained by other facts, which will be discussed elsewhere.

At the periphery of the cornea, especially that of man, the endothelial cells often become irregular in shape, elongate in the meridional direction, and form small fan-shaped groups (Fig. 4, opposite).

Similar groups were observed in my previous studies of the relief of the posterior corneal surface. The comparison of the results of silver impregnation with those obtained by the replica method were interesting, as the latter did not demonstrate clearly the granular cement substance and the inner surface of the endothelium cells appeared much smoother. This is further evidence of the existence, in addition to the cement substance, of another kind of amorphous substance covering and smoothing the posterior surface of the endothelial cells as well as the surface of the granular cement substance.

**Discussion**

The cement substance lining the cell boundaries of the corneal endothelium appears to be very similar to that described by McGovern in blood vessels. The present findings support his descriptions of the behaviour of the cement substance, which is more variable than is generally admitted. This variability of the cement substance cannot be considered as a *post-mortem* change, as it occurred in a certain region of the cornea, whereas at the same time other regions were seen with narrow border lines only. Moreover the human cornea was impregnated immediately after the enucleation. The observations of Jancsó (1955) and other authors also tend to confirm this opinion. While there can be no doubt about this fact, it is questionable whether the part played in this process by the mast cells suggested by McGovern is generally valid. I can find nothing in the literature about the presence of mast cells in the neighbourhood of the corneal endothelium, nor did I find any in a study of the metachromatism of the covering substance. Yet there is certainly some spreading factor which brings about the swelling and perhaps the dissolution of the cement substance by depolymerization, and this substance is probably a
Fig. 3—Irregularities of cell-boundaries of peripheral corneal endothelium in a human cornea. McGovern's method. ×1,000.

Fig. 4.—Human cornea: extreme periphery of corneal endothelium and its transition to the trabecular meshwork. Mosaic pattern of peripheral endothelium. Increased amorphous substance covering surface of endothelium in zone of transition (T) in thin, argyrophil layers. McGovern's method. ×550.
product of the endothelial elements themselves. This is also the opinion of Wolf (1956) in his study of the behaviour and renovation of the cement substance of the vascular endothelium. It seems very probable that various factors are capable of reacting with this substance as well as of modifying the secretion of the endothelial cells. The covering substance appears somewhat different from the cement substance; McGovern and Wolf have also seen a thin covering layer of this kind; that it represents only a modified cement substance (as is supposed by McGovern) appears doubtful to me because of the different behaviour of the two substances. Nevertheless, it seems possible that on some occasions the swollen cement substance could cover the whole free surface of the endothelial cells and—perhaps—unite with the covering substance itself.

Summary

The study of the cement substances of the corneal endothelium revealed some new aspects of this substance and its variability. In the opinion of the author the endothelium probably has an active role in the secretion of the cement substance as well as in the production of the spreading factor which seems to play a part in the dissolution and renovation of the cement substance and its adaptation to various influences.

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doi: 10.1136/bjo.42.9.529

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