RETROCORNEAL MEMBRANES*†

I. THEIR ORIGIN AND STRUCTURE

BY

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IN a recent communication, Rycroft (1965) gave some indication of the importance of retrocorneal membranes particularly from the standpoint of a post-operative complication of penetrating corneal surgery. Since then extensive studies of these membranes have been undertaken of both human and rabbit specimens, and we have found them to occur even more commonly than was originally suggested by Rycroft. This has been confirmed by other surgeons.

Until the description by Werb (1962), relatively little interest had been shown in retrocorneal membranes, but a review of the literature, particularly concerning corneal wound healing, reveals that they have been observed previously and that there are differences of opinion regarding their origin and structure. As there has been no correlation of the isolated reports, the structures here called retrocorneal membranes have previously been known by a variety of names, *e.g.* stromal overgrowths (Duke-Elder, 1954); fusiform scars (Morton, Ormsby, and Basu, 1958); laminated membranes (Chi, Teng, and Katzin, 1958); fibrous tissue plugs (Stocker, 1953); post-graft membranes (Werb, 1962). The last was suggested by Werb because these membranes are most commonly noticed clinically after penetrating keratoplasty when they are seen to lie behind the graft. Since, however, structurally identical membranes can follow almost any perforation in the Descemet's and endothelial layers, the more general name of retrocorneal membrane (Leigh, 1960; Hales and Spencer, 1963) is used here to include those resulting from all causes and to distinguish them from anterior graft membranes (Rycroft, 1965).

Furthermore, in order to avoid confusion and to stress that the development of retrocorneal membranes may follow three primary initial causes, the following classification is suggested:

- (1) Post-graft (Fig. 1, overleaf), after penetrating keratoplasty;
- (2) Post-traumatic (Fig. 2, overleaf), after mechanical injury, either surgical (other than keratoplasty) or accidental;
- (3) Post-disease (Fig. 3, overleaf), after degenerative changes.

The macroscopic and biomicroscopical appearances, position, and effects of the post-graft membrane in humans have been admirably described by Werb (1962), and hence the main subject of the present paper is the report on the findings of an extensive histological study of human and rabbit retrocorneal membranes with a view to clarifying their origin and structure.

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FIG. 1.—Total, "old", post-graft membrane after full-thickness autograft. Rabbit. Masson's trichrome. $\times 36$.

FIG. 2.—Large, "old", post-traumatic membrane after retrocorneal scratch. Rabbit. Masson's trichrome. $\times 92$.

FIG. 3.—Large, "old", post-disease membrane after herpes simplex. Human. Masson's trichrome. $\times 92$.

Materials and Method

Material

(1) Twelve human regrant corneal buttons with post-graft membranes, two human graft buttons with post-traumatic membranes after accidental perforating injury, and three with

post-disease membranes, all obtained from the Corneo-Plastic Unit at the Queen Victoria Hospital, East Grinstead.

(2) Sixteen rabbit corneae with post-graft membranes and fifteen with post-traumatic membranes, of which four developed after surgical perforating injury (piercing with a scalpel) and eleven after the endothelial and Descemet's layers had been damaged by a needle inserted at the limbus and drawn across the back of the cornea.

By killing the rabbits at various times post-operatively retrocorneal membranes at different stages of development were obtained.

Method.—Paraffin and, in some cases, fresh-frozen cryostat sections were prepared. The stain mainly used was Masson's trichrome, since this is particularly good for collagen staining and gives good differentiation of the normal corneal membranes, but haematoxylin and eosin, periodic acid-Schiff, and van Gieson's stain were also employed.

Results

(1) The observations that structures identical histologically to post-graft membranes were present in some human corneal specimens that had suffered perforation of the Descemet's and endothelial layers by penetrating trauma other than by grafting, and by degenerative changes after herpes simplex and other keratitis, were strengthened by the results of experimental injuries to fifteen rabbit corneas. Four of these were caused by piercing with a scalpel, and eleven by scratching the posterior corneal surface. In all cases errant fibrous tissue, which was continuous with the injured corneal stroma, grew through the resulting gap in Descemet's membrane to form post-corneal fibrous membranes to various degrees. Hence the use of the general term "retrocorneal membranes" which is to include all such fibrous structures as appear, after injury, between the normal Descemet's membrane and endothelium of a cornea. The terms post-graft, post-traumatic, and post-disease are used more specifically and indicate the type of initial injury to the Descemet's and endothelial layers and hence to the overall cause of the membrane.

(2) Structure of Retrocorneal Membranes

Histologically no difference between the three types of retrocorneal membranes is distinguishable and three stages in their growth and development can be recognized, which may be termed:

- (i) The juvenile;
- (ii) The mature;
- (iii) The old.

(i) The juvenile membrane in rabbits is recognizable from the 5th to 7th post-operative days, although in two cases mature characteristics were present on the 7th day. At this stage the membrane is composed mainly of cells (Figs 4 and 5, overleaf) which very closely resemble stromal fibroblasts, seen bordering the entire wound edge, in that they stain similarly, tend to be spindle-shaped in section, and contain a very large nucleus which is spherical or oval, unlike the kidney-shaped nucleus of rabbit corneal endothelium, and contains several distinct nucleoli (Weimar, 1957). Mitoses among these cells are rare, while divisions of endothelial cells bordering the wound area are to be seen, but much less frequently than on the 1st and 2nd post-operative days when they are numerous. The cells of the juvenile membrane plug the gap caused by wounding in Descemet's membrane. In some cases they protrude into

FIG. 4.—Disc-shaped juvenile retrocorneal (post-traumatic) membrane. Rabbit. D = Descemet's membrane. Masson's trichrome. $\times 144$.

FIG. 5.—Globular juvenile retrocorneal (post-graft) membrane. Rabbit. h = host; g = graft. *Note:* The intense black spots in the membrane and graft stroma are melanine granules liberated by damaging the iris at operation. Masson's trichrome. $\times 144$.

the anterior chamber in a globular form (Fig. 5) but more usually they form a disc-shaped layer over the site of injury (Fig. 4). With high magnification and Masson's trichrome staining, very fine green fibres can be seen ramifying between the cells (Fig. 6, opposite) in the older juvenile membranes, and the appearance is that these are secretions of the cells. This suggestion is strengthened by electron micrographs of retrocorneal membranes (Seal, Inman, Rycroft, and Sherrard, in press).

FIG. 6.—Fibrous appearance of juvenile retrocorneal (post-graft) membrane. Rabbit. D = Descemet's layer of graft. Masson's trichrome. $\times 360$.

(ii) The mature membrane (Fig. 7) is histologically very similar to normal corneal stroma. It occurs from the 8th post-operative day onwards, the time at which it reaches its full development being dependent upon several conditions, *e.g.* the extent of the injury, the health of the cornea in general, therapeutic drugs employed, etc. The main constituent of a membrane at this stage is fibrous collagen, arranged in lamellae and with exactly the same staining reactions as those of normal stroma. The lamellae run parallel to the curvature of the cornea, but, unlike normal stroma, their arrangement relative to one another is not regular and this accounts for the opacity of the membrane. Many of the collagen fibres in the membrane are directly continuous with some of those of the corneal stroma.

FIG. 7.—Portion of "mature" retrocorneal (post-graft) membrane. Rabbit. Note: concentration of fibroblasts (f) along "growing edge". D = Descemet's layer of graft. Masson's trichrome. $\times 144$.

The ratio of cells to collagen is approximately the same as in normal stroma, except at the free, growing edge of the membrane where there is a concentration of cells (Fig. 7). The latter are indistinguishable from stromal fibroblasts in sections. The mature retrocorneal membrane is lined with endothelium which extends from both wound margins over the appropriate surface of the membrane, but while it is still active endothelial cells do not cover the growing edge.

In many sections a very thin homogeneous Descemet's membrane-like layer is distinguishable between the endothelial lining and the fibrous collagenous stroma at the root of the membrane. There is usually a narrow space between the retro-

corneal membrane and Descemet's layer of the cornea at this stage.

(iii) The old membrane (Fig. 8) is structurally similar to the mature form but is distinguishable by the absence of a "growing edge". It tends to lie closely apposed

FIG. 8.—Old, fringe retrocorneal (post-graft) membrane. Human.
Note: closeness of membrane to graft Descemet's layer (D); structural similarity to normal corneal stroma (S); absence of terminal concentration of fibroblasts; total endothelial covering (e). Masson's trichrome. $\times 144$.

FIG. 9.—Edge of "old" retrocorneal (post-graft) membrane with secondary Descemet's layer (d). D = Descemet's layer of host. Rabbit. Periodic acid-Schiff. $\times 720$.

to the Descemet's layer of the cornea to which it is bound by a continuous sheet of endothelium. A secondary Descemet's-like layer now envelops the membrane (Fig. 9).

If left long enough, the old membrane frequently becomes vascularized, but this has been observed in humans only.

The structure of a membrane in the old stage, the nature of its cells, and the secondary Descemet's layer have been confirmed by electron microscopy (Seal and others, 1965).

(3) Development of Retrocorneal Membranes in Rabbits

From 1 to 4 days post-operatively there is little sign of a developing retrocorneal membrane, except for some mitoses and migration of corneal corpuscles, which now resemble the fibroblasts described by Weimar (1957), through the oedematous stroma in the region of the wound. However, as stated by Chi, Teng, and Katzin (1960), endothelial mitoses are numerous during this time, particularly during the

2nd post-operative day, but unless the injury, or the gap in Descemet's membrane, is extremely small the daughter cells fail to cover it before the 5th or 6th days when the juvenile membrane appears. The latter grows, first by spreading as a sheet of two to four cells in thickness over the injured area of Descemet's membrane, but later by expanding both laterally and in thickness to form the mature stage. The increase in thickness is due mainly to collagen production by the cells which are thus pushed further and further apart by the accumulating collagenous fibres.

The further development of a retrocorneal membrane may be arrested at any time and may thus give rise to a variety of appearances, *e.g.* small fringe membranes (Fig. 8), total retrocorneal sheets (Fig. 1), or, in extreme cases, great corneal masses which fill the anterior chamber and even penetrate the vitreous (Levkoieva, 1947). Arrest is apparently effected by total endothelial overgrowth of a membrane, since juvenile membranes and the growing edges of the mature forms show fibroblastic activity in the absence of an endothelial covering, while in old membranes, which are completely covered by endothelial cells, no fibroblasts are distinguishable, the cells resembling "resting" corneal stromal corpuscles.

Discussion

The origin of the cells present in a juvenile membrane, and hence the membrane itself, is open to some discussion. The opinion of Morton and Ormsby (1958) is that a wound involving Descemet's membrane and endothelium is first overgrown by endothelium which forms a fusiform scar of cellular tissue. Although these authors were unable to determine the exact origin of the scar, *i.e.* whether it was fully endothelial or contained some stromal elements, they suggested that it became converted into stroma-like tissue by hyalinization which proceeded from the underlying stroma. Briefly, they suggest that endothelium is converted to stroma-like material.

Stocker (1953) and Dunnington (1958) held a converse view, and suggested that the wound is first plugged with stromal tissue, the outermost cells of which later become converted into endothelium. This suggestion was based upon observations that, in the early stages (4 days post-operatively—Stocker, 1953), there is no continuity between the cut endothelium and the flat outermost cells of the tissue plug in the wound, but that after 6 days there is to be seen a continuous endothelial covering to both the Descemet's membrane and the stromal plug.

Chi and others (1960) stated that both endothelial and stromal cells participated in the healing process and that the defects appeared to be covered by an irregular mass of cells, most of which had elongated nuclei.

Werb (1962) indicated a belief that post-graft membranes originated entirely from the host stroma.

The observations here reported indicate that at first the wound is plugged by stromal cells alone, which then secrete collagen. The so-formed juvenile membrane may or may not then increase in size according to whether or not it becomes overgrown by endothelium proliferating from the wound edges. This is apparently dependent upon the extent of the original wound and the relative growth rates of the endothelium and juvenile membrane. Initially this hypothesis was based upon the morphology of the cells of the juvenile membrane which are quite dissimilar from endothelial cells but resemble stromal fibroblasts as described by Weimar (1957). However, Binder and Binder (1957) and Chi and others (1960) have shown that

newly-formed and mitosing endothelial cells are polymorphic, and hence it is not sufficient to base an opinion upon morphology alone.

In several specimens our observations are similar to those of Stocker (1953), in that it is seen that Descemet's membrane has greatly retracted and recoiled on cutting, taking the endothelium with it, and tissue plugs or juvenile membranes still form in these cases, even though their site of formation is far removed from all endothelium. Therefore, it seems reasonable to assume that the membrane or plug originates from stromal elements and not from endothelium. Furthermore, in grafting experiments in which the entire donor endothelium was removed (unpublished), larger retrocorneal membranes resulted than in cases in which the endothelium was little damaged, indicating that the lack of endothelium promotes membrane development instead of retarding it as would be expected if endothelial cells were a primary component of retrocorneal membranes. It is also worthy of notice that tissue plugs, scars, retrocorneal membranes, or whatever term is applied to them, develop only when Descemet's membrane is cut, thus disturbing the limiting layer of the stroma. When endothelium alone is injured no scar results, but the defect is covered by a smooth endothelial layer which, in time, appears quite normal (Chi and others, 1960).

We suggest, therefore, that retrocorneal membranes are entirely products of the stroma and may be considered as being errant scar tissue, and that endothelialization of a wounded area prevents, not augments, their development. Preliminary electron microscopic observations in our laboratories lend much weight to this view (Seal and others, in press).

Summary

Retrocorneal membranes of different genesis have been recognized and classified. Three phases of retrocorneal membrane development, marked by definite structural characteristics, have been described in rabbits.

The relative growth rates of regenerating endothelium and errant stromal scar tissue—the retrocorneal membrane—in rabbits have been reported.

The origin and development of retrocorneal membranes have been discussed in the light of the present study and work of other authors.

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