RETROCORNEAL MEMBRANES*†

II. FACTORS INFLUENCING THEIR GROWTH

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DURING our recent studies on the origin of retrocorneal membranes, from which it was concluded that these structures are errant stromal scar tissue, we became increasingly aware that certain conditions of the cornea, other than mere perforation of the Descemet's and endothelial layers, had to be satisfied before these membranes would develop. Three such conditions presented themselves:

(1) That the corneal stroma be in a relatively healthy state and capable of regenerating excess scar tissue;

(2) That the perforation in Descemet's membrane be wide enough to permit the protrusion of this scar tissue into the anterior chamber;

(3) That the endothelium be damaged or absent from the wound margins allowing a sufficient period of time for errant stromal scar tissue to form before complete endothelial repair is accomplished.

It was felt that if these primary conditions were indeed necessary, preventive measures could perhaps be taken before and during penetrating corneal surgery to reduce the incidence of post-operative retrocorneal membranes.

Therefore, observations of human and rabbit specimens, and experimentation on the latter were undertaken to test the validity of each of the suggested conditions.

Method

The histological appearance of serial paraffin sections of human corneal buttons removed at keratoplasty at the Queen Victoria Hospital, East Grinstead, and rabbit corneas in which perforations in Descemet's membrane and endothelium had been produced experimentally, were used as follows:

Condition 1

26 human corneal buttons with perforations in Descemet's membrane and the endothelium caused by previous penetrating keratoplasty in thirteen cases, accidental mechanical trauma in two, and degenerative changes in eleven, were carefully examined, and the histological condition of the stroma was related to the extent of any retrocorneal membrane present, and to the observable effects of Conditions 2 and 3.

Condition 2

The human specimens, above, were examined and the dimensions of the perforations in Descemet's layer related to the extent of any retrocorneal membrane and to Conditions 1 and 3.

Similar observations were made upon sixteen rabbit corneas, in which Condition 1 was

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maximum and Condition 3 was minimized as far as possible, and in which Condition 2 had been produced to varying degrees by:

(A) GRAFTING.—Twelve healthy corneas were grafted—eight autografts and four homografts. In one half of each group great care was taken to achieve good apposition between the Descemet's membranes of host and graft, while in the remainder apposition was poor.

(B) PERFORATING INJURIES.—Four healthy corneas were pierced with a scalpel and the wounds lightly sutured.

Condition 3

(C) GROWTH RATES.—To compare the relative growth rates of damaged, regenerating endothelium and advancing retrocorneal membranes, the backs of twelve healthy rabbit corneas were scratched from 12 to 6 o'clock by a hypodermic needle inserted at the limbus. The scratches were as fine as possible to cause minimum damage to the endothelium and at the same time deep enough to cut through Descemet's membrane. Care was taken not to lose the aqueous humour so as to preserve an internal environment as near normal as possible from the beginning of the experiment. The animals were killed from 1 to 7 days post-operatively and their corneas sectioned and examined.

(D) REMOVAL OF ENDOTHELIUM.—Four healthy rabbit corneas were carefully grafted with fresh autografts from which the entire endothelium had been removed by a relatively non-traumatic method, described elsewhere (Rycroft, 1965).

Results

Condition 1

Of the thirteen human regrant corneal buttons, two buttons removed after accidental perforating injury, and eleven with disease-damaged Descemet's and endothelial layers, twelve, two, and three respectively possessed retrocorneal membranes. In all cases, other than the two accidental perforations, the button was removed some time after the occurrence of the causative injury and thus time, as a factor influencing the size of the membranes, was eliminated.

To determine the relationship between the dimensions of the membranes and Condition 1 alone, only specimens in which Conditions 2 and 3 were clearly evident were chosen. These comprised:

(a) Three regrant buttons which had been originally grafted for keratoconus. In each the host stroma was virtually normal, and had regenerated either a total (one specimen) or a very large post-graft membrane.

(b) Eight regrant buttons originally grafted for various types of keratitis. The host tissue of four of these was very slightly scarred, and large but not total post-graft membranes had developed. The host stroma of three of the remaining four specimens was extensively scarred, and had produced in each a small marginal retrocorneal membrane. The last specimen, which had received five previous grafts, was so scarred as to be almost unrecognizable as corneal tissue histologically; it showed no evidence of a post-graft membrane.

(c) The two discs removed after accidental perforation by a nail showed inflammation but no scarring. Each had been excised shortly after wounding and thus insufficient time for retrocorneal membrane development had elapsed before the tissues were fixed. However, each possessed a juvenile membrane (Sherrard

and Rycroft, 1967), and there is little doubt that these would have grown and matured had time permitted.

(d) Ten original corneal buttons with perforations in Descemet's layers caused by a variety of means, *e.g.* herpes, abscess, ulcers, thermal and chemical burns, aphakic dystrophy. Three of these showed only slight stromal scarring, and in each small, discontinuous retrocorneal membranes had developed in association with each perforation. The stroma of the remaining seven discs was heavily scarred, and none possessed a retrocorneal membrane.

It is feasible that the variations in the dimensions of the retrocorneal membranes in the foregoing examples are related to the variations in the state of the corneal stroma, since Conditions 2 and 3 are optimum.

The greater occurrence of post-graft as opposed to post-disease membranes (Sherrard and Rycroft, 1967) is probably explained by the fact that recipient corneas are treated before grafting so that they may be in the most healthy condition possible, and then at operation much of the scarred stroma is removed. These two procedures combine to promote the existence of Condition 1. Conversely, post-disease membranes are rare because a disease capable of perforating Descemet's membrane will usually result in dense scarring or destruction of the adjacent stroma, thus minimizing Condition 1.

Condition 2

In order to relate the size of retrocorneal membranes to the width of the perforation in Descemet's membrane, specimens in which Conditions 1 and 3 were satisfied were alone examined. These comprised seven human regrant buttons of which six exhibited poor alignment of host and graft entailing a large space between the Descemet's membranes. A large post-graft membrane was present in each. The apposition of host and graft in the remaining specimen was good, leaving only a small gap in Descemet's layer, and associated with it was a very small, marginal post-graft membrane. In two of these specimens both situations are seen. On one side apposition of host and graft Descemet's layer is poor, and a large retrocorneal membrane takes root in the resultant gap, while on the other side apposition is good, giving rise to a very small post-graft membrane.

As only a small number of specimens were available, others were produced experimentally by producing gaps in Descemet's membrane of healthy rabbit corneas in the two ways described under Method A and B. The animals were killed at various times post-operatively and their corneas examined histologically. The times of killing and the resulting condition of Descemet's membrane and the occurrence and relative sizes of retrocorneal membranes are set out in the Table (overleaf).

In these experiments Condition 1 was constant and maximum, and the endothelium traumatized as little as possible, except in Specimen 11. The Table shows that where enough time (*i.e.* more than 7 days) was allowed post-operatively for membranes to reach maturity, and the resulting gap in Descemet's layer was small (*e.g.* specimens 1, 3, 6, 10, 14, and 15), very small, thin, retrocorneal membranes ensued. Conversely, where the perforation in Descemet's layer is wide (*e.g.* specimens 2, 4, 5, 9, 11, and 13), larger, thick, retrocorneal membranes resulted.

Thus it seems that the size, in particular thickness, of a retrocorneal membrane

TABLE

Rabbit No.	Cause of Injury	Extent of Perforation of Descemet's Layer		Condition of Endothelial Repair	Post-operative Age (days)	Resulting Retrocorneal Membrane
		Attempted	Achieved			
1	Autograft	Narrow	Narrow	Complete	64	Very thin, marginal (old)
2		Wide	Wide	Complete	64	Thick, total (old)
3		Narrow	Narrow	Complete	52	Very thin, marginal (old)
4		Wide	Very wide—much recoil	Complete	35	Very thick, marginal (old)
5		Wide	Wide	Complete	35	Thick, marginal (old)
6		Narrow	Narrow	Partially covers retrocorneal membrane	14	Very thin, marginal (mature)
7		Narrow	Wide	Marginal mitoses	7	Thick (juvenile)
8		Wide	Very wide	Marginal mitoses	7	Small, but very thick (juvenile)
9	Homograft	Wide	Wide	Partially covers retrocorneal membrane	35	Very thick, marginal (mature)
10		Narrow	Narrow	Covers most of retrocorneal membrane	21	Thin, marginal (mature)
11		Wide, damaged by freezing	Very wide, with numerous secondary perforations	Complete	21	Massive, total (old)
12		Narrow	Wide	Marginal mitoses	7	Thin, marginal (juvenile)
13	Piercing	Wide	Very wide	Complete	35	Thick, almost total (old)
14		Narrow	Narrow	Complete	35	Thin, marginal (old)
15		Narrow	Narrow	Complete	28	Thin, marginal (old)
16		Wide	Very wide	No indications of repair	7	Large, globular (juvenile)

bears some relationship to the extent of the perforation in Descemet's layer produced by trauma to healthy corneas. One could say that the extent of the perforation in Descemet's layer influences the initial quantity of errant stromal tissue which can protrude into the anterior chamber.

Condition 3

That the endothelium must be unhealthy or incomplete in the region of the perforation in Descemet's membrane became evident from some unexpected results of the foregoing experiments on rabbit eyes and observations of human material, but primarily from the poor results achieved in preliminary deep-freeze storage experiments on rabbit corneas (not published). In these, donor corneas were stored at -79°C . for various times and then grafted into healthy rabbit eyes and, although Condition 2 was minimized as far as possible, large post-graft membranes resulted, many of which were total, even in specimens where the gap between host and graft Descemet's layer was comparatively small, but in these the membranes were thin. Histological examination of similarly frozen, but ungrafted, corneas revealed the stroma and epithelium as relatively normal, while the endothelium was absent or very degenerate. The variation in thickness of the post-graft membranes was apparently governed by Condition 2, and it was therefore the mere presence of the extensive membranes that led to the suspicion that endothelial well-being was necessary for their prevention. Furthermore, in other specimens it was confirmed that only old retrocorneal membranes possess a complete endothelial covering, mature growing membranes have no endothelium at the growing edge, and juvenile stages are quite naked (Sherrard and Rycroft, 1967).

In order to obtain some data concerning the relative growth rates of damaged, regenerating endothelium and the advancing retrocorneal membrane, the experiments described under Method C were performed, and it was seen that mitotic figures in the endothelial cells at the wound margins are visible 24 hours after injury, while no indication of a retrocorneal membrane is distinguishable. At 48 hours, endothelial regeneration is advancing and now the precursors—accumulation of stromal corpuscles showing some mitotic figures at the wound site—of retrocorneal membranes appear; 2 days later the groups of cells show a laminated arrangement, and from this time onwards they secrete collagen to form retrocorneal membranes proper.

Unfortunately, owing to the tendency of Descemet's membrane in the rabbit to recoil and contract on cutting, only one specimen demonstrated the situation after a very small injury to this and the endothelial layer. This was a 4-day-old specimen and it showed the membrane precursor completely covered by endothelium. Many of the grafted specimens in which Condition 1 is maximum, and the gap in Descemet's membrane and thus in the endothelium is small, similarly indicate that in a wound of this nature endothelial repair is completed before or just after the juvenile membrane appears, and in the longer term post-operative cases it is seen that large retrocorneal membranes do not develop. On the other hand, where the injury is extensive, necessitating a considerable amount of endothelial regeneration in order to cover the defect, large retrocorneal membranes eventually develop. These phenomena are seen respectively in specimens 1, 3, and 10, and 2, 4, and 11 in the Table; they suggest that complete endothelialization of a wounded area prevents the growth of a retrocorneal membrane.

Clearly, in the cases so far considered, Conditions 2 and 3 are almost inseparable, the area of stroma denuded of endothelium being approximately equal to the size of the perforation in Descemet's membrane. In order to confirm that Conditions 2

and 3 are indeed separate entities as regards retrocorneal membrane growth, Experiments D were conducted in which Condition 1 was maximum, 2 was minimized, and 3 was greatly exaggerated. Thus an area far greater than any gap in Descemet's layer was denuded of endothelium and the advance of regenerating endothelium was greatly retarded. The results were dramatic: in two cases thick total membranes and in two very large and almost total membranes developed; they very strongly indicate that, provided Conditions 1 and 2 are satisfied, extensive retrocorneal membranes will form in the absence of an endothelium. Condition 2 apparently influences the thickness, and Condition 3 the extent, of a retrocorneal membrane. These results lend much weight to the hypothesis that damage to the endothelium greatly increases the possibility of retrocorneal membrane development.

It has been mentioned in an earlier communication (Sherrard and Rycroft, 1967) that a characteristic of "mature" and "old" retrocorneal membranes is the presence of a thin, homogeneous layer juxtaposed to the newly-formed endothelium. Except for its being extremely thin in comparison with the normal Descemet's layer, it resembles the latter in all other observable respects. This has been described by numerous authors (Morton, Ormsby, and Basu, 1958; Leigh, 1960; Hales and Spencer, 1963), and confirmed by electron microscopy in our laboratories (Seal, Inman, Rycroft, and Sherrard, in press).

It is possible that it is the secondary Descemet's layer that actually arrests retrocorneal membrane growth and not the endothelium directly. However, there is some evidence that this layer is formed by endothelial action upon the collagen or precollagen in contact with it, and that endothelial cells are essential for the formation of the secondary Descemet's membrane. These suggestions are being tested by further histological, electron microscopical and histochemical observations.

Discussion

The foregoing observations and experiments strongly indicate that the three postulated conditions are indeed necessary for retrocorneal membrane development.

Since it is relatively certain that retrocorneal membranes are of stromal origin and may be considered as "overgrown stromal scars" (Sherrard and Rycroft, 1967), the first condition, that the corneal stroma be in a healthy state, is obvious. However, it is worthy of note that any pathological cornea which is deemed fit for keratoplasty will, in the great majority of cases, be a potential source of a retrocorneal membrane. Thus, Conditions 2 and 3 become more important clinically. This aspect is exaggerated where unscarred corneas are operated upon as, for example, in cases of keratoconus.

In normal circumstances the Conditions 2 and 3 are relative to one another, in that providing the endothelium is not abnormal in either graft or host the size of the wound to be covered by new endothelium is equal to the extent of the gap in Descemet's membrane. Thus it is important to ensure close apposition of Descemet's membrane, either when resuturing a simple perforating injury or when fixing a penetrating graft in place; this will form as complete a barrier as possible to a potential retrocorneal membrane, thus reducing Condition 2, and secondly it will minimize the area lacking an endothelium, thus reducing Condition 3.

The apparent influence of the endothelium (Condition 3) as a separate entity in

relation to retrocorneal membrane development becomes important when considering the surgical handling of a cornea and the age and preservation of donor eyes. In the first case care must be taken not to damage this extremely delicate layer, particularly at the edges of a wound. In the second instance it appears that the freshest possible donor material is still the best, although much has been done to preserve the endothelium in frozen eyes by the injection of glycerol or DMSO into the anterior chamber (Mueller, 1964).

Condition 3 is probably the most important, for Condition 1 is also a condition of satisfactory healing, and it has been shown that some stromal scar tissue will penetrate even small perforations in Descemet's membrane if the endothelium is extensively damaged. It seems that this scar tissue can be prevented from growing into an opaque retrocorneal sheet only by the formation of a continuous endothelial lining which, directly or indirectly, by the formation of a "secondary Descemet's membrane", arrests further growth of errant stromal tissue.

Summary

Three primary factors which apparently promote the growth of retrocorneal membranes have been postulated:

- (i) That the corneal stroma be healthy and capable of regenerating excess collagenous material;
- (ii) That there be a perforation in Descemet's membrane large enough to permit the passage of the excess material into the anterior chamber;
- (iii) That the corneal endothelium be damaged or otherwise unhealthy or incomplete in the region of the wound.

The value of each factor has been tested by comparative observations of human pathological corneal material and of rabbit specimens in which each factor has been diminished and exaggerated in turn.

The results indicate that each postulated factor is valid, and it is proposed that by reducing the second and third conditions—the only two within the control of the surgeon—the occurrence of post-operative retrocorneal membranes may be diminished.

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