Changes in corneal ultrastructure resulting from early lens removal in the developing chick embryo

Keith M. Zinn

Lenses were removed from chick embryos at 4 days of incubation and the corneas were studied at various time intervals postoperatively through 17 days of incubation. Approximately 2 to 4 hours postoperatively a wave of mesenchymal tissue, presumably perilimbic in origin, moved in to cover the posterior surface of the cornea. This retrocorneal sheet of tissue grew in thickness and differentiated along lines similar to that of the sclera. At 17 days of incubation, the collagen fibrils within this band of tissue had cross-sectional diameters that were 1.8 to 3.0 times greater than those of fibrils within normal corneal stromas. These changes were found only within the lentectomy group. The corneal stroma, Descemet's membrane, and the mesothelium (endothelium) failed to develop in the lentectomized eyes. Failure of vitreous body enlargement, a concomitant feature of lens removal at 4 days of incubation (Coulombre and Coulombre, 1966), was explored as a causal mechanism. Eyes of chick embryos were intubated at 4 days of incubation, allowing vitreous to escape, and the corneas were studied at various time intervals postoperatively through 17 days of incubation. At 17 days of incubation, the collagen fibrils within the stromas of the intubated group had cross-sectional diameters that did not differ significantly from those in normal stromas. In the corneas of the intubated eyes, Descemet's membrane and the mesothelium were present, having a normal cytoarchitecture, thereby negating lack of vitreous body enlargement as a causal factor. Other possible mechanisms to explain the aberration in corneal development following early lens removal are discussed.

Key words: chick embryo, lens extraction, cornea, embryology, morphogenesis, collagen fibrils, corneal stroma, Descemet's membrane, corneal endothelium, corneal epithelium, collagen, vitreous body, ultrastructure, lentectomy.
layer or lamella, are disposed approximately at right angles to those fibrils in neighboring layers. Within each fiber or lamella, the fibrils are arranged in almost strictly parallel array to form a lattice. This is evident in sections cut parallel to or at right angles to the longitudinal axes of the fibrils. It has been suggested that this architectural arrangement of the collagen fibrils is necessary for the transparency of the stroma.

How is this ordered architecture achieved during development? At 4 days of incubation, the chick cornea consists of an anterior epithelial layer (3 cells thick), a middle or postepithelial zone which is acellular, and a single posterior mesothelial layer of cells (future endothelium) (Fig. 1). A detailed account of the histogenesis of the chick cornea, with the use of the light microscope, has been given by Meyer and O'Rahilly. Electron microscopic studies have been carried out by Jakus, Ohkura, Miyashita, Poul亅quent and associates, and Brini and associates.

According to Brini and associates, the postepithelial zone consists of “fibrilles collagene immature” which are about 100 Å in diameter and have a periodicity of 200 to 220 Å. These fibrils are arranged in loosely packed bundles running parallel to the corneal surface. Beginning at 5 days of incubation the postepithelial layer is invaded by perilimbic mesenchyme (Fig. 1). The main axes of the invading cells are oriented parallel to the corneal surface. At 7 to 8 days of incubation, these mesenchyme cells (fibroblasts) are asso-

Fig. 1. Normal embryonic development of chick cornea at 3, 4, and 5 to 6 days of incubation.
Changes in corneal ultrastructure

What are the mechanisms that govern corneal development during embryonic life? It is known that the processes of growth and differentiation of the cornea continue to depend upon the lens of the developing eye and upon the optic cup long after the initial induction of the anterior corneal epithelium from the head ectoderm. However, there is still little information concerning which details of corneal morphogenesis are dependent upon the influence of the lens. For instance, is the remarkable degree of uniformity in fibrillar diameter, or the highly regular geometric arrangement of the fibrils, attributable to a direct or an indirect lens influence, or are these features independent of the lens? Is the invasion of perilimbic mesenchyme into the postepithelial zone dependent upon the lens influence? What effect, if any, does the lens have upon the morphogenesis of the endothelium and Descemet's membrane? How are the anterior epithelium and the sub-

Fig. 2. Method for lens removal from chick eye at 4 days of incubation.

associated with larger collagen fibrils which become more numerous and more highly ordered as time goes on. Brini and associates\(^a\) state that at least some of the collagen fibrils attain their adult diameter (200 Å) and periodicity (640 Å) by 8 days of incubation. Once the adult collagen fibrils are formed, according to Karmazsin,\(^b\) there is no change in their diameter or in their periodicity during the rest of embryonic development. Collagen synthesis in the stroma, as a whole, continues through the twentieth day of incubation.\(^c\)

Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933007/ on 10/16/2017
epithelial zone (Bowman's membrane) affected by lens removal early in embryonic development?

The aim of this study is to examine these and related questions concerning corneal development as well as to describe some of the alterations in corneal cytoarchitecture that result when the lenses are removed from the eyes of chick embryos at 4 days of incubation.

Materials and methods

White Leghorn chick embryos which had developed in a forced-draft incubator (37.5° to 38.5° C.) were used. The embryonic age is given in days from the onset of incubation. There were 5 experimental groups: In Group I, lentectomy was performed at 4 days of incubation (67 cases). In Group II, the controls, lentectomy was performed at 4 days of incubation immediately followed by reinsertion of the lens into its former location in the eye—23 cases. All of the above operations were carried out on the right eye as described by Coulombre and Coulombre except that the incision was midglobal (equatorial) instead of perilimbal (Fig. 2). Group III was the normal group (unoperated upon—26 cases). In Group IV were the intubated eyes (15 cases), in which a glass capillary tube 3.0 mm. long, with an outside diameter of 0.4 mm. and an inside diameter of 0.25 mm., was introduced through an incision in the wall of a 4 day eye in such a way that one end rested in the vitreous body and the other in the amniotic cavity as described by Coulombre.
Fig. 4. Electron micrograph of chick cornea at 4 days of incubation, 4 hours post lens removal. Ep, Anterior epithelium; PE, post epithelial layer; RM, retrocorneal mesenchyme. (Magnification ×11,750.)
Group V consisted of the controls for intubated eyes (12 cases), in which a solid glass capillary rod 3.0 mm. long, with an outside diameter of 0.4 mm. was inserted into the wall of a 4 day eye as described for Group IV. All intubations were performed on right eyes only.

The embryos were killed at the following intervals: 0, 1, 2, 3, 4, 6, 8, and 16 hours postoperatively on the fourth day of incubation as well as at 5, 6, 7, 8, 11, 14, and 17 days of incubation. In each case, the globes were enucleated and immediately immersed in 2.3 per cent glutaraldehyde in 0.1M phosphate buffer, pH 7.3, at room temperature, for several hours. Intact globes from 4 and 5 day embryos, and corneal tissue with an attached rim of sclera from the eyes of the older embryos, were postfixed with 1 per cent osmium tetroxide in 0.1M phosphate buffer, dehydrated in graded series of alcohols, transferred to propylene oxide and embedded in Araldite.19 Ultrathin sections were cut with a Porter-Blum ultramicrotome, using glass knives, and placed on collodion-coated No. 200 mesh copper grids. The grids were then stained with uranyl acetate and lead citrate20 and viewed with an RCA-3D electron microscope. One- to two-micron thick sections were also taken, stained with 1 per cent toluidine blue 21 and studied under the light microscope. All sections were cut in a plane normal to the cornea and sclera.

Measurements of fibril diameters were made from micrographs taken at the same tap number

Fig. 5. Globe diameters vs. days of incubation in chick embryos. Upper curve, normal; middle curve, controls where lenses were removed at 4 days of incubation and immediately reinserted into the globes; lower curve, lenses were removed at 4 days of incubation.
on the same electron microscope and printed at the same enlargement. A calibration grid* consisting of a replica of a carbon diffraction grating having 28,800 lines per inch was used to calibrate the measurements. Fibril diameters were measured on photographic prints using a filar ocular micrometer mounted on a stereomicroscope. Randomly selected fibrils viewed on end were measured using the narrowest cross-sectional diameter of each fibril.

Results

**Group I: lentectomy.** From 0 to 2 hours post lentectomy no change was seen in the corneal or perilimbic zones. However, 2 to 4 hours postoperatively there were a few scattered fibroblasts that appeared to be migrating from the perilimbic zone over the posterior surface of the cornea (Figs. 3 and 4). The original mesothelial cells that lined the posterior surface of the cornea, prior to lentectomy, could not be distinguished ultrastructurally from the fibroblastic cells that had invaded the retrocorneal zone. Thus, it was not clear whether the invading cells only covered, or actually mingled with, the cells of the corneal mesothelial layer (future endothelium). From 4 to 8 hours post lentectomy the fibroblasts had increased in numbers to form a sheet of tissue, approximately 5 to 8 cells thick, which completely

*E. Fuller Company, Schenectady, N. Y.
covered the posterior surface of the cornea (i.e., retrocorneal sheet of tissue). No invasion of mesenchyme was seen within the postepithelial zone, which remained acellular. From the fifth to sixth day of incubation the sheet of retrocorneal mesenchyme had increased to about 20 cells in thickness with an occasional mesenchymal cell at the periphery of the postepithelial zone. No distinct mesothelium (endothelium) was observed. From the seventh to the fourteenth day of incubation (3 to 10 days postoperatively) the cells within the retrocorneal sheet of tissue became associated with increasing numbers of collagen fibrils. These fibrils ran in bundles parallel

Table I. Chick embryos at 17 days of incubation

<table>
<thead>
<tr>
<th>Group</th>
<th>Corneal diameter (mm ± S.D.)</th>
<th>Globe diameter (mm ± S.D.)</th>
<th>Collagen fibril diameter (Å ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub-epith. zone</td>
<td>Stroma</td>
<td>Retrocorneal tissue</td>
</tr>
<tr>
<td>I. 4 Day lentectomy*</td>
<td>0.69 ± 0.16</td>
<td>4.89 ± 0.39</td>
<td>211 ± 9</td>
</tr>
<tr>
<td>II. 4 Day lentectomy (control)</td>
<td>3.80 ± 0.34</td>
<td>8.83 ± 0.27</td>
<td>215 ± 12</td>
</tr>
<tr>
<td>III. Normal</td>
<td>4.24 ± 0.19</td>
<td>9.90 ± 0.24</td>
<td>212 ± 10</td>
</tr>
<tr>
<td>IV. 4 Day intubation</td>
<td>1.45 ± 0.15</td>
<td>4.86 ± 0.33</td>
<td>216 ± 18</td>
</tr>
<tr>
<td>V. 4 Day intubation (control)</td>
<td>3.67 ± 0.27</td>
<td>8.61 ± 0.51</td>
<td>207 ± 10</td>
</tr>
</tbody>
</table>

* Days = days of incubation.

Fig. 7. Subepithelial zone of the normal chick cornea at 17 days of incubation. Collagen fibrils 210 Å in diameter. SEZ, Subepithelial zone; BM, basement membrane; Ep, anterior corneal epithelium. (Magnification x60,000.)
Changes in corneal ultrastructure

Changes in corneal ultrastructure to the corneal surface. The cross-sectional diameters of the fibrils varied widely, ranging from 300 to 600 A., whereas in the original postepithelial zone, which remained essentially acellular, the average cross-sectional diameter of the collagen fibrils was about 210 A., with a range of 190 to 281 A. The number of collagen fibrils (per unit area of ultrathin sections) within the postepithelial zone increased with time.

On the seventeenth day of incubation (thirteenth day postoperatively) the mean global and mean corneal diameters were much smaller than those of the normal 17 day embryo (Table 1) and appeared to have followed the pattern of development as depicted in Figs. 5 and 6, respectively. Also, the cornea at 17 days of incubation was opaque as compared to the normal. Electron microscopic examination of the anterior corneal epithelium and its basement membrane showed no significant morphological differences from normal 17 day corneas, although the subepithelial zone in the lentectomy group was slightly less organized (Figs. 7 and 8). The average diameter of the collagen fibrils in this zone was 211 ± 9 A., which was very similar to the normal (Table 1).

The retrocorneal sheet of tissue (Figs. 9 and 10) in the lentectomy cases differed ultrastructurally from normal corneal stroma (Fig. 11) in the following ways. The total number of lamellae, the average thickness of the lamellae, and the number of collagen fibrils per lamella were less in the retrocorneal sheet of tissue than in the normal stroma. Although these tissues had been treated similarly, the collagen fibrils within the retrocorneal sheet of tissue stained more deeply with uranyl acetate and lead citrate stains than did collagen fibrils within the stromas of the control and normal groups (II and III). The collagen fibrils within the retrocorneal

![Fig. 8. Subepithelial zone of the chick cornea at 17 days of incubation. Lens was removed at 4 days of incubation. Collagen fibrils 210 A. in diameter. SEZ, Subepithelial zone; Bm, basement membrane; Ep, anterior corneal epithelium. (Magnification x60,000.)](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933007/ on 10/16/2017)
Fig. 9. Retrocorneal band of tissue (corneal stromal zone) at 17 days of incubation with lens removed at 4 days of incubation. Pattern B. The asterisk indicates that no electron microscopically resolvable structures were seen. CF, collagen fibrils. (Magnification 11,500×.)

sheet of tissue ran parallel to each other within a given bundle and appeared to be oriented with their main axes parallel to the corneal surface. The fibroblasts in the retrocorneal sheet, like the keratocytes in the normal stroma, also had their long axes running parallel to the corneal surface.

The retrocorneal sheet appeared to have two distinct, yet coexisting, architectural patterns that differed from the normal and control stromas. In some regions, designated as pattern A, the fibroblasts were more tightly packed together than in the normal. An occasional bundle of collagen fibrils was present in the extracellular space between neighboring fibroblasts (Fig. 10). In other regions, referred to as pattern B, the fibroblasts were more widely spaced than in pattern A, yet they were closer to each other than in normal stromas. There were occasional bundles of collagen fibrils extracellularly as well as zones, several μ in area, within the ul-
trathin sections which had no electron microscopically resolvable structures present (Fig. 9). In both patterns A and B the fibroblasts appeared to be more numerous per unit area than the keratocytes in the stromas of the normal and the control groups. In addition, blood vessels were observed in the retrocorneal tissue with the use of the light and the electron microscopes.

Most noteworthy was the marked difference in collagen fibril diameters among the various experimental groups (Figs. 12 and 13). The normal and control groups had mean collagen fibril diameters of about 210 and 238 Å, respectively (Table I), whereas the collagen fibrils within the retrocorneal band of tissue (lentectomy group) had values ranging from 320 to 680 Å (Fig. 14). Within any one retrocorneal sheet, the collagen fibril diameters were fairly uniform, not varying more than ±40 Å from the mean. However, as noted above, there was a considerable variation...
Fig. 11. Normal chick corneal stroma at 17 days of incubation. K, Keratocytes. (Magnification 11,500x.)

Fig. 12. Normal chick corneal stroma at 17 days of incubation. CF, Collagen fibrils. (Magnification 60,000x.)
in fibril diameter within the lentectomy group from one animal to another. The periodicity of the collagen fibrils within the retrocorneal sheet of tissue as well as in the stromas of normal and control groups was approximately 640A.

**Group II: control-lentectomy.** Control corneas were examined with the electron microscope at various intervals, from several hours to 13 days of incubation post-operatively. No morphological differences were observed when they were compared with normal corneas at comparable ages. More specifically, there was no retrocorneal invasion of perilimbic mesenchyme in the lentectomy control group (II) as had been observed in the lentectomy group. Descemet's membrane and the posterior epithelium were intact and normal. All the corneas in this group were transparent.

It should be noted that the control groups (II, V) had smaller mean corneal and mean global diameters than embryos at 17 days of incubation (Table I). This difference was statistically significant (P < 0.05). At 4 days of incubation, the global incisions did not heal immediately. This permitted the escape of vitreous substance from the eye for a variable period of time (from 6 to 36 hours). This was probably responsible for the smaller corneal and global diameters observed in the controls (II, V).

**Group III: normal.** At 17 days of incubation, the chick cornea was transparent, with a diameter of 4.24 ± 0.19 (S.D.) mm. (Table I). The thickness of the central corneas as measured in histological sections was approximately 300 microns. The anterior epithelium of 5 to 6 cell layers rested on a basement membrane. The subepithelial zone was about 4 microns thick and contained collagen fibrils whose long axes ran parallel to the corneal surface. These fibrils were arranged in small lamellae 3 to 5 fibrils thick (Fig. 7), and had a cross-sectional diameter of 212 ± 10 A. Posterior to this zone was the stroma which consisted of many lamellae, each considerably thicker than the lamellae in the subepithelial zone and each composed of bundles of collagen fibrils running parallel to each other and parallel to the corneal surface. The cross-sectional diameter of these collagen fibrils was 210 ± 9 A throughout the entire stroma. Interspersed in the stromal matrix were keratocytes whose long axes ran parallel to the collagen fibrils as well as to the corneal surface. Descemet's membrane and the endothelium conformed to the detailed

![Fig. 13. Retrocorneal band of tissue at 17 days of incubation with lens removed at 4 days of incubation. CF, Collagen fibrils. (Magnification 60,000×.)](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933007/)
Group IV: intubated and Group V: control-intubated. Because the vitreous body fails to enlarge following lentectomy,17 experiments with intubated globes were carried out to determine what effect, if any, vitreous body enlargement had on the development of corneal fine structure. Intubated globes (IV, V) were studied immediately after intubation, at 4 days of incubation, and at 13 days postoperatively.

On gross examination, at 17 days of incubation, the intubated globes were found to be smaller in mean corneal and global diameters (Table I) than the 17 day normals. However, the cross-sectional diameters of the collagen fibrils within the subepithelial and stromal zones were similar to 17 day normals (Table I).

Discussion

In order to interpret the data relating to lentectomy at 4 days of incubation, it is important to understand not only the degree of development of the normal eye at that time, but also the consequences of lentectomy. First, the cornea at this stage is comprised of 3 layers, namely, an anterior epithelium, underlaid by an acellular postepithelial zone which, in turn, is covered posteriorly by a single layer of mesothelial cells. Lens extraction was performed therefore at a stage prior to the mesenchymal invasion of the postepithelial zone from the perilimbic areas (Fig. 1). Second, it has been shown15-18 that corneal growth and differentiation are dependent upon the presence of the lens during the embryonic period. More specifically, Coulombre and Coulombre18...
have shown the importance of the lens to corneal development through at least the fifth day of incubation. Third, it has been shown\(^{10}\) that the lens plays a vital role in accumulation of the vitreous during development which in turn is necessary for the growth of the outer coats of the eye including the cornea. Finally, if lentectomy is complete, there is no regeneration of the lens from the remaining ocular tissues of the chick embryo.\(^{22}\)

**Retrocorneal tissue.** With these concepts in mind, let us examine the sequence of events following lentectomy. Several hours postoperatively, at 4 days of incubation,\(^{23}\) or at Hamburger-Hamilton\(^{24}\) stages 16 to 22 (Genic-Galvez\(^{25}\)), there is an invasion of presumably perilimbic mesenchymal elements along the posterior surface of the cornea (Figs. 3 and 4). At 8 hours post lentectomy, this band of cells is about 8 cells thick and continues to increase in thickness, finally differentiating into a band of sclera-like tissue by 17 days of incubation (Fig. 15). Ultrastructurally, the scleral collagen with its surrounding matrix and complement of fibroblasts is similar to the retrocorneal band of mesenchymal tissue (Figs. 9, 10 and 15) in that the collagen fibrils have similar cross-sectional diameters, periodicities, and packing with an orientation parallel to the surface of the globe. Because of these structural similarities, it would be reasonable to assume that the fibroblasts that cover the cornea posteriorly after lentec-
tomy probably come from the perilim bic
mesenchymal pool.

This retrocorneal invasion of presumably
perilimbic mesenchyme appears to be a
specific response to lens removal. Control
eyes (lens removed and immediately rein-
serted into its former location), intubated
eyes, and eyes with perilimbic incisions
only, do not have a subsequent retrocor-
neal mesenchymal invasion. Therefore, it
must be concluded that early in corneal
development the lens in some way keeps
the perilimbic mesenchymal pool of cells
from invading the retrocorneal zone. The
fact that very few mesenchymal cells ap-
ppear in the postepithelial (acellular) zone
of the lentectomized eye suggests that the
lens may also play a role in the mesen-
chymal invasion of the postepithelial zone
during corneal development.

There is no direct cell contact between
the lens and corneal tissues after 3 days of
incubation. This fact when coupled with
the preceding experimental observations
raises the possibility that the lens emits an
influence(s) or substance(s) capable of
diffusing across the space between the
lens and cornea. Furthermore, this lens in-
fluence(s) might, in some way, control the
normal patterns of differentiation of certain
cell population(s) residing within the cor-
nea. If this is so, then removal of the lens
would result in changes in the normal se-
quence of differentiation of the cornea.
However, at the present time, there is no
biochemical evidence to prove or disprove
this hypothesis.

What about the increased diameters of
the collagen fibrils present in the retrocor-
neal zone? Does this observation have a
clinical counterpart? Recently Kenyon
and Maumenee reported the electron mi-
croscopic findings in a patient with con-
genital hereditary corneal dystrophy. These
authors found alterations in the lamellae
as well as enlargement of the stromal col-
gen fibrils to almost twice normal diam-
eter. These stromal changes mirror very
closely those cited for the retrocorneal tis-
sue of the chick embryo implying, possibly,
that similar mechanisms for aberrant de-
velopment may be involved. However, the
similarity between these two systems ap-
pears to end here because in the congeni-
tal hereditary corneal dystrophy the lens,
Descemet's membrane, and the endo-
thelium are present, whereas they are
absent in the experimental lentectomy
cases.

Endothelium and Descemet's mem-
brane. There is a consensus that Desce-
met's membrane originates from, or at
least under the influence of, the posterior
epithelium. In the chick embryo this takes
place on the seventh day of incubation
with the appearance of small bundles of
microfibrils 3 μ thick. At hatching the
membrane is 450 μ thick. The periodicity
and architectural arrangement of the col-
gen fibrils that comprise Descemet's mem-
brane are described by Jakus.

How does this sequence of development
fit in with the experimental observations
described above? It had been observed
that neither endothelium nor Descemet's
membrane developed in any of the globes
that underwent lentectomy at 4 days of
incubation. However, the controls and the
intubated eyes all had normal-looking
endothelial cell layers as well as Desce-
met's membranes when examined at 17
days of incubation. Also, within several
hours after lentectomy, at 4 days of incu-
bation, the single layer of mesothelial cells
(future corneal endothelium) appeared to
be covered posteriorly by the invading
mesenchymal elements (presumably from
the perilim bic pool of cells). It was not
clear, however, from this study if the origi-
nal mesothelial layer maintained its posi-
tion subjacent to the acellular postepi-
thelial zone or whether its cells migrated
within the retrocorneal sheet of mesen-
chyme. Part of this uncertainty was due
to the fact that the mesothelial cells ap-
ppeared to be ultrastructurally identical to
the invading mesenchyme. In the retrocor-
neal sheet of tissue, at 17 days of incuba-
tion, no cells were observed to have a
cuboidal shape typical of the mesothelium.
(endothelium) of the adult, nor was there any indication that Descemet's membrane had formed.

According to Neifach (Lopashov and Stroeva\textsuperscript{12}), the formation of the mesothelium and Descemet's membrane is "always related to the presence of a cavity under the cornea..." and with obliteration of such a cavity these two structures will not form. The retrocorneal invasion which presumably covers the mesothelial cells posteriorly, thereby obliterating the cavity under the mesothelium, seems to satisfy the conditions set forth by Neifach as stated above. Therefore, one would not expect mesothelial (endothelial) cell differentiation and subsequent formation of Descemet's membrane. Also, the fact that these structures fail to develop only in the lentectomy group would seem to implicate the lens directly or indirectly with the successful morphogenesis of the corneal mesothelium and Descemet's membrane.

**Anterior epithelium and subepithelial zones.** It is clear from the observations cited earlier in the paper that development of the normal cytoarchitecture of the anterior corneal epithelium is not affected by removal of the lens at 4 days of incubation as is the growth of the corneal surface area and diameter (Fig. 6). Therefore, the morphogenesis of the anterior corneal epithelium may be independent of the lens, at least after 4 days of incubation.

The similarity of fibril diameters in the subepithelial zones of the lentectomy, control, and normal groups after 17 days of incubation raises several questions regarding the normal development of this zone. For example, which cells produce the collagen fibrils and what controls their cross-sectional diameters? In the lentectomy cases, at 17 days of incubation, it has been observed that immediately subjacent to the subepithelial zone there are fibroblasts surrounded by collagen fibrils 1.8 to 3.0 times larger than the fibrils within the subepithelial zone. It is rather unlikely that these fibroblasts form collagen fibrils of a particular diameter (approximately 210 Å.) at one period in development, and then switch to the manufacture of fibrils that are 1.8 to 3.0 times larger at another period in development. A more likely possibility would be that these fibroblasts have the ability to synthesize the basic building blocks of collagen, tropocollagen, and the final size of the fibrils depends upon the milieu of the extracellular environment where collagen fibrils are formed.\textsuperscript{22, 26, 28} Therefore, it is possible that the anterior corneal epithelium alone may have the capacity to form the collagen fibrils within the subepithelial zone. One observation in support of this idea is that in the early 4 day embryo, before any fibroblasts invade the cornea or form a mesothelium, there is an acellular zone containing fibrils immediately subjacent to the anterior corneal epithelium. In this case the epithelium is almost certainly the component responsible for the formation of the subepithelial zone.

Whatever the mechanism that is involved, it is clear that, at least after 4 days of incubation, the lens does not control the cross-sectional diameters of the collagen fibrils within the subepithelial zone. Also, it is apparent that the organization of the collagen fibrils at 17 days of incubation in the subepithelial zone of the lentectomized eye is considerably more advanced than in the postepithelial zone at 4 days of incubation. This indicates that even though the lens has been removed, the subepithelial zone was able to undergo a significant amount of development.

The author is greatly indebted to Dr. Alfred J. Coulombre for his many helpful suggestions and criticisms regarding the embryological aspects of this work as well as to Drs. Keith C. Richardson, Milton Brightman, and Thomas Reese for their advice concerning electron microscopy. I would like to thank Mrs. Jane Coulombre for her advice regarding the histological and microsurgical techniques used. Also my thanks to Mr. Edward Moodhe who helped with the photography.

**REFERENCES**


