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CELLULAR MECHANISMS OF A NONPENETRATING LESION
IN THE CORNEA: A MORPHOLOGICAL STUDY OF THE RABBIT

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By

Charles Allyn Uniacke, B.S., O.D., M.S.

* * * * *

The Ohio State University
1973

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Table of Contents

Acknowledgements ................................................................. ii
Vita .............................................................................................. iii
List of Figures ............................................................................... vii
Introduction ................................................................................ 1

Chapter

I. Historical Review ................................................................. 6
II. Objectives .............................................................................. 23
III. Methods ............................................................................... 25
      A. Animal Preparation ...................................................... 25
      B. Histological Procedures .............................................. 26
IV. Results ................................................................................ 29
      A. Biomicroscopy Observations ...................................... 29
      B. Morphological Observations ....................................... 32
         1. Normal Cornea ....................................................... 32
         2. Observations of 15 minutes healing ......................... 33
         3. Observations of 30 minutes healing ......................... 34
         4. Observations of 3 hours healing .............................. 34
         5. Observations of 4 hours healing ............................. 35
         6. Observations of 5 hours healing ............................. 36
         7. Observations of 6 hours healing ............................. 38
Table of Contents (con't.)

8. Observations of 12 hours healing ... 39
9. Observations of 24 hours healing ... 39
10. Observations of 48 hours healing ... 39
11. Observations of 1 week healing ... 40
12. Observations of 2 weeks healing ... 40

V. Discussion ................................................. 41
VI. Summary .................................................. 54

Appendix
A. Standard Methods of Histological Preparation 55
B. Standard Methods of Histological Preparation 56
C. Standard Methods of Histological Preparation 57
D. Histological Observations of Corneal Healing 58

Bibliography .................................................. 88
List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Surgical Technique</td>
<td>66</td>
</tr>
<tr>
<td>2.</td>
<td>Top: Slitlamp picture of lesion immediately after surgery</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Bottom: Slitlamp picture of lesion after 48 hours healing</td>
<td>68</td>
</tr>
<tr>
<td>3.</td>
<td>Photomicrograph of lesion 15 minutes after surgery</td>
<td>70</td>
</tr>
<tr>
<td>4.</td>
<td>Photomicrograph of lesion 30 minutes after surgery</td>
<td>70</td>
</tr>
<tr>
<td>5.</td>
<td>Photomicrograph of lesion 3 hours after surgery</td>
<td>72</td>
</tr>
<tr>
<td>6.</td>
<td>Photomicrograph of lesion 3 hours after surgery</td>
<td>72</td>
</tr>
<tr>
<td>7.</td>
<td>Photomicrograph of lesion 3 hours after surgery</td>
<td>74</td>
</tr>
<tr>
<td>8.</td>
<td>Photomicrograph of lesion 3 hours after surgery</td>
<td>74</td>
</tr>
<tr>
<td>9.</td>
<td>Photomicrograph of lesion 4 hours after surgery</td>
<td>76</td>
</tr>
<tr>
<td>10.</td>
<td>Photomicrograph of bulbar conjunctiva</td>
<td>76</td>
</tr>
<tr>
<td>11.</td>
<td>Photomicrograph of bulbar conjunctiva</td>
<td>78</td>
</tr>
<tr>
<td>12.</td>
<td>Photomicrograph of bulbar conjunctiva</td>
<td>78</td>
</tr>
<tr>
<td>13.</td>
<td>Photomicrograph of lesion 5 hours after surgery</td>
<td>80</td>
</tr>
<tr>
<td>14.</td>
<td>Photomicrograph of lesion 6 hours after surgery</td>
<td>80</td>
</tr>
<tr>
<td>15.</td>
<td>Photomicrograph of lesion 12 hours after surgery</td>
<td>82</td>
</tr>
<tr>
<td>16.</td>
<td>Photomicrograph of lesion 12 hours after surgery</td>
<td>82</td>
</tr>
</tbody>
</table>
List of Figures (con't.)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.</td>
<td>Photomicrograph of lesion 24 hours after surgery</td>
<td>84</td>
</tr>
<tr>
<td>18.</td>
<td>Photomicrograph of lesion 1 week after surgery</td>
<td>84</td>
</tr>
<tr>
<td>19.</td>
<td>Photomicrograph of lesion 2 weeks after surgery</td>
<td>86</td>
</tr>
</tbody>
</table>
The normal cornea is avascular and possesses a high degree of tissue compactness. As a transparent tissue that maintains a high degree of deturgescence, it provides an excellent medium for the morphological study of healing without the complicating factors of vascularity. The cornea provides a number of advantages for the study of healing in its avascularity, compactness, well-defined histochemistry and microscopic anatomy, and its exposed surface, which permits it to be wounded without damage to adjacent tissues or even, if desired, adjacent layers. It, nonetheless, presents disadvantages. It is an unusually highly specialized tissue with mucopolysaccharides that are not found elsewhere in the body.

Inasmuch as the corneal stroma is avascular and relatively acellular, it is an especially valuable tissue for studies of the roles of connective tissue cells, of the cellular elements which invade the cornea in wound healing, or of the development of blood vessels (pannus). When connective tissue is traumatized, healing may occur by two methods: (1) fibrous scar formation in which the tissue defect is repaired with non-specialized connective tissue, and (2) scar restitution in which repair is by tis-
sue characteristic of that injured. Classically, healing is described as occurring in three stages. The first period or lag phase lasts from one to four days and is characterized by the infiltration of inflammatory cells, removal of necrotic debris, and appearance of fibroblasts about the wound site. The second period or fibroblastic phase lasts from day one to day ten and features the deposition of collagen fibers. The final period or cicatricial phase extends from day ten to the completion of healing.

Of the three recognized phases of wound healing, perhaps the most important is the initial phase. All the events in the later phases occur because of what occurs in the lag phase. This is determined by the cell types present, as classified morphologically.

The discussion of epithelial healing has revolved around the relative roles of cellular migration versus cell division. Investigators (1,2,3,4,5) widely believe that healing takes place by migration with little or no increase in mitotic activity. There is little evidence to suggest that there is a burst in mitotic activity which helps epithelial healing (6,7).

Of greater interest, however, is the healing of the corneal stroma, especially since it is relatively acellular. In order for healing to occur, there must be either changes in cellular morphology or invasion of cells capable of repair work. The fibro-
blast is the primary cell involved in stromal replacement, with macrophages involved in phagocytosis.

Retterer (8) and Salzer (9) believed in the transformation of epithelial cells into fibroblasts. Various investigators (10, 11,12,13,14,15) have supported the concept of transformation of stromal cells to fibroblasts, but there has been little morphological evidence to support their belief. Others (12,13) support the idea that migrating macrophages transform into fibroblasts.

Another class of cells of interest is the invading blood cells. There is some disagreement as to which cells can actually be found at the site of the injury and how quickly they got there and via what route.

Weimar (16) and others (17,18,19) found polymorphonuclear leukocytes (PMNL's) at the injury. The cells began to enter the cornea about five hours after the injury, reaching the wound after 24 hours. Dunnington and Smelser (20) found few PMNL's at the wound. Pullinger and Mann (19) found the cells reaching the lesion only through the cornea, while Robb and Kuwabara (17,18) found that the cells reached the wound via the tears and by migration through the corneal stroma.

A variety of techniques has been used for studying the morphology of corneal healing. Many of these techniques are not the best for maintaining good morphology. Some investigators
have used frozen sections for studying cell types. Freezing is quite likely to destroy normal cellular morphology, making identification of all types more difficult. Others have used routine paraffin sections. No investigators made use of a double embedding technique for use with this tissue.

Many of the injuries that were created were of a rather destructive nature. Freezing, or freezing combined with a lesion, or burning the cornea was frequently used. Many of the simpler lesions were perforating lesions, which have the complication of the fibrin plug and the loss of aqueous. The nonpenetrating lesions were either in the central cornea or in the periphery. No investigators utilized a nonperforating lesion extending from limbus to limbus. This procedure may help to determine whether there is a difference in healing between central and peripheral wounds.

The stains most frequently used were Giemsa, and Hematoxylin and Eosin. Autoradiographic techniques using tritiated thymidine were also frequently employed. Tritiated thymidine does not allow the actual study of cell morphology, but only labels those cells which incorporate thymidine, which include epithelial and endothelial cells. This unnecessarily complicates identification of all types, and it does not allow the study of cellular transformation.

It has been suggested that there is disagreement concerning
the origin and fate of cells present in the wound, calling for additional careful morphological studies. It is the purpose of this investigation to study the initial phases of healing in the rabbit cornea following limbus to limbus nonperforating lesion and to determine, with morphological methods, the origin and the fate of the cell population which is present in the wound during the healing process.
The topic of wound healing involves the interpretation of a variety of microtechniques related to histology, histochemistry, protein synthesis, enzymology, and biochemistry. The cornea provides an excellent medium in which to study the interrelationships of various factors related to wound healing in an accessible, observable location where the reparative processes are uncomplicated by the regeneration of blood vessels. Wound healing may be influenced by means of topically applied medications in both a treated and a controlled area of the two eyes of the same animal.

Wound healing may be defined as an interrelated pattern of systemic and local events which follows tissue injury and leads to the replacement of lost tissue (epimorphosis), and, in some species, to the restoration of a lost part (morphallaxes). Connective tissue heals by fibrous scar formation or scar restitution. Either of these will lead to a loss of compactness in the cornea and then to a loss of transparency, with a resulting reduction in vision. The exact course of events in corneal healing depends upon the severity of the injury and the extent to which the various elements of the cornea are involved. The different layers of the normal cornea heal in different ways, i.e., undergo different
morphological changes. Much of the activity occurring during wound healing is taking place during the so-called lag phase. This initial period of three days, formerly considered to be a relatively inert one, is now regarded as an important link in the healing process, for it is during this time that the "building blocks" of repair are laid down (21).

The study of healing involves such subdisciplines as the histochemical nature of healing, affects of drugs on healing, the time course of events, the morphology of the healing sequence, and in the cornea the development or lack of development of blood vessels (i.e. pannus) and lymphatics, depending upon the wound severity and location.

The fundamental biological phenomenon of new vessel formation has important physiological and pathological ramifications. The theories which have been advanced to explain the initiation of new vessel formation in the cornea generally fall under two main headings: (1) that in disease, a substance is elaborated in the cornea which stimulates the limbal vessels to grow towards the site of its maximum concentration, i.e., a process combining growth stimulation and positive chemotaxis, and (2) that the limbal vessels are normally prevented from entering the cornea either because of its chemical content, growth inhibiting substances, or the compactness of its tissue. Vascularization would then arise
through destruction of the antagonizing substance or through a reduction in corneal compactness.

From experimental evidence, it has been believed that the general biological phenomenon of directed tissue growth, as seen, for example, in the development of axons in embryonic neuroblasts, may be controlled by rising and falling gradients of chemical concentration. To this class of cell movement the term "chemotropism" has been applied. The same may apply to proliferating vascular buds. The application of this principle to the problem of corneal vascularization has been particularly emphasized by Campbell and Michaelson (22), who suggested, from their studies on standardized burns in rabbits' corneas, that corneal new vessel formation involves a factor released at the site of the lesion, whence it diffuses to stimulate and direct new vessel growth from the limbal plexus. Campbell and Ferguson (23) showed that corneal vascularization occurs more readily in scorbutic than in normal guinea pigs. They believed this indicated the ability of corneal tissue deficient in ascorbic acid to meet the increased metabolic demands of repair, leading to an accumulation of metabolites which might constitute the factor postulated by Campbell and Michaelson (22).

Histamine has often been considered to be the growth-stimulating factor. Julianelle and Lamb (24) have demonstrated that the intra-corneal injection of egg albumin and bacterial nucleoproteins
can give rise to corneal cloudiness followed by vascularization. If such a diffusing vascularizing substance exists, it never seems to extend beyond the limbus to stimulate proliferation in the immediately adjacent conjunctival or scleral vessels. Such a theory does not help explain the related problem of why the cornea is avascular and normally, even with injury, remains that way.

Local anoxia or the sequential accumulation of acid metabolites might be the stimulus. In the cornea, Bessey and Wolbach (25) and Johnson and Eckardt (26) attributed the marked ingrowth of capillaries, which follows riboflavine deficiency, to a breakdown in the corneal oxidative system. Swindle (27) believed that vessel ingrowth is attributable to an increase in the concentration of hydrogen ions. That oxygen-lack or an excess of carbon dioxide in the tissue is generally a potent factor in the production of neovascularization is supported by a considerable amount of experimental evidence.

The presence of such influences is implicit in the work of Michaelson (28) upon the development and anatomy of the normal retinal vessels. He found that the formation of capillaries is pre-eminently a function of the retinal veins.

More recently, Zauberman et al. (29) have found that certain biogenic amines when supplied from plastic tubes inserted in rabbits' corneas act as vaso-stimulators. Anoxia has also been suggested as a stimulus for new vessel formation. This would suggest
that hyperbaric oxygen, if supplied to such a cornea, should inhibit or retard neovascularization, but this has not been found by Lazar et al. (30).

The hypothesis on the inhibition of new vessel formation by some factor in the corneal tissue was first advanced by Meyer and Chaffer (31). They suggested that polysaccharide was intimately connected with corneal transparency and the absence of blood vessels.

Cogan (32) originated the thesis that the degree of compactness of the corneal tissue is the determining factor in the initiation of neovascularization. He noted that tissues such as cartilage and fingernails cannot be vascularized due to having no invadeable intercellular substance and that such might be applicable to the cornea. He observed that engorgement of the limbal vessels was followed by the formation of saccular aneurysms which burst, and new vessels then proliferated into the resulting hemorrhage between the corneal lamellae. The one event which consistently preceded these vascular changes was swelling of the corneal stroma. Ashton and Cook (33) found that a reduction in the corneal compactness in the region of the limbus is a necessary stage in the development of corneal vascularization. They did not believe that an increase in corneal thickness was the stimulus to new vessel formation, but regarded it, merely, as a removal of compactness which normally obstructs vessel growth. Langham (34) also found that increased hydration of the corneal area adjacent to peri-
limbal capillaries was an essential prerequisite to invasion by blood vessels.

Smith (35) has advanced a hypothesis that mast cells normally inhibit corneal vascularization in some way. Their massive destruction results in a physical or biochemical change in stability of the system.

Collin (36,37,38,39) has found two lymphatic vessels within the corneal stroma following extensive vascularization of the cornea. He believed that the vessels are lined with endothelial cells and that they represent a proliferation of the conjunctival lymphatic system.

One method utilized as a measure of tissue repair is the study of tensile strength. It has been utilized to study the strength of sutured wounds and is a valuable aid to testing the effects of various drugs.

Richards et al. (40) used tensile strength to study the effect of sutures on wound healing. Gasset and Dohlman (41) utilized tensile strength to make a systematic study of the rate of healing of central and peripheral corneal wounds. They found that in the absence of epithelium that the wounds were unable to build any measureable strength.

Aquavella et al. (42) found a reduction in tensile strength of the cornea when the cornea was treated with cortisone. They also found that the epithelium healed faster without cortisone treatment.
A recent technique for studying the healing of corneal injuries is the use of pressure-decay curves. Cole and Leaver (43) connected the eye to a pressure transducer, raised the pressure in the eye to a predetermined level, and recorded the rate of pressure decay. The rate returned to normal by eight days in rats with standardized incisions.

Drugs and their affects on healing have long been of interest. Two drugs or classes of drugs which have received a great deal of attention are IDU and cortisone.

IDU is an antimetabolite that suppresses the formation of DNA. Payrau and Dohlman (44) found that it has no effect on epithelial healing, but that it retards stroma healing, as measured by tensile strength. Hanna (45) found that IDU did not alter the incorporation of thymidine-tritium into the DNA of dividing corneal epithelial cells. It also did not alter the incorporation of thymidine-tritium into the cells of the stroma near the wound edge.

One of the physiologic effects of cortisone is its suppressing influence on the response of mesenchymal tissue to trauma or other injury. Ashton and Cook (46) compared the healing in control series with cortisone treated series. They made no attempt to describe the minute histological developments, but they did note that cortisone retards epithelial regeneration, polymorphonuclear and macrophage infiltration, fibroblastic activity, and
endothelial regeneration. Palmerton (47) also found that cortisone inhibits healing as shown by testing tensile strength of incisions.

Steroids were tested for their affect on corneal healing by Basu (48). The process of wound healing in central and peripheral lesions treated with steroids was compared clinically and histologically with the normal healing process. The rate of healing was the same for central and peripheral injuries, both treated and untreated.

The process of wound repair, when studied at the cellular level, reveals a remarkable series of changes in the enzymology, physiology, and morphology of connective tissue cells in the wound area within a few hours after injury. Many of these changes take place during the so-called lag or latent phase. Anseth (49) has found that glucose-aminoglycans normally present in the corneal stroma disappear from the wound and surrounding area, but galactosaminoglycans are formed or deposited in the wound. Galactosaminoglycans could represent one of the chondroitin sulphates or dermatan sulphate.

Kaufman et al. (50) used tetrazolium stains to demonstrate dehydrogenase enzymes in tissue sections. They feel that an enzyme profile of a given group of cells may be an indication of the viability of the tissue. The metabolism of corneal stroma is oxidative, and healing is a process which requires oxidative energy. Healing tissue was found to have a higher concentration of oxidative enzymes than
normal cornea.

Bracher (51) utilized radioautographic techniques to investigate protein, RNA, DNA, and sulfated mucopolysaccharide synthesis. Protein and RNA synthesis, found after one hour to 24 hours, seemed to be associated with the development of large and numerous nucleoli in the keratocytes undergoing transformation to fibroblasts. DNA synthesis took place shortly before and during the period in which an increase in the number of fibroblasts was observed. Sodium sulfate was incorporated into the mucopolysaccharides of connective tissue. One hour after injection of $^{35}S$-sulphate, the labeled material was found throughout the fibroblast.

Weimar and Haraguchi (52) used 5-nucleotidase activity in cells to study the invading blood cells. Normal corneal cells do not show 5-nucleotidase activity. At 6 and 12 hours after injury, increasing numbers of polymorphonuclear cells were present throughout the stroma. Mononuclear leukocytes that were negative also began to appear about this time. At 24 hours some inflammatory reactions were well developed while in others they were less defined. 5-nucleotidase activity was greater in fibroblasts at wound edge than in stromal cells away from the wound. Corneal stromal fibrocytes were observed to be particularly active in phagocytosing PMNL's between 24 and 48 hours after injury. Weimar (52) concluded that there is some characteristic or action of the wound, itself,
that influences all cells within its range to increase their enzyme activities even during the lag phase.

Probably the easiest area of the cornea to study morphologically is the epithelium. It is normally 5 or 6 cell layers thick and the most metabolically active of the corneal layers.

The method of repair in epithelial wounds of the cornea has revolved around the roles played by cell migration versus cell division. Arey and Covode (6) believed that cell migration is the primary means of corneal repair and cell division the secondary. Shortly after wounding, the mitotic rate fell below normal with recovery to normal by 96 hours. However, the wound was completely covered in 12 hours.

Buschke (1,2) described the orientation of the epithelial cells for different types of wounds such as pin pricks and linear lesions. The healing of pin pricks started with a radial orientation of the surrounding epithelium after a lag period of one hour. The cells also developed long pseudopodia. The epithelial movement was inhibited by various agents, such as anoxia and various metabolic poisons. In the early stages of healing of lesions, the immediately adjoining cells become tangentially oriented, with only the more distant epithelial cells orienting radially towards the lesion. They suggested that in some phases of the healing process, the syncytial nature of the corneal epithelium was of
importance, and that forces act on it as on a surface coat. The cells in the larger wounds demonstrated a mushroomlike spreading and flattening of marginal cells of the wound.

The work so far has not totally excluded the possibility of mitosis being of importance in epithelial wound healing. Friedenwald and Buschke (3,4) and Hanna and O'Brien (5) have given more conclusive evidence that mitosis plays a little role in epithelial healing. Friedenwald and Buschke found that two to three hours after the injury the surrounding cell nuclei change from original tangential orientation into a radial one. During this same time period, the floor of the lesion was covered. A sharp decline in mitosis occurred during the first few hours, with a return to a normal count after four hours. With no mitosis, a normal number of epithelial cells may be reached by reduction in rate of desquamation.

Khodadoust et al. (7) found that the epithelial healing process was directed primarily toward reestablishing continuity of epithelial covering of the cornea. This was accomplished by epithelial slide and a burst of mitotic activity assisted by a temporary pause in the normal process of exfoliation. When the epithelium was removed, the basement membrane was usually left intact and the epithelium reattached itself to this membrane after 6-7 days of healing. Prior to this, the epithelium was easily lifted
from the wound. By three weeks, the epithelium was completely normal. When the basement membrane was also removed, it took 6-7 weeks before normal adhesion of the epithelium.

Dunnington and Weimar (55) investigated the influence of the presence or absence of epithelium on stromal healing. PMN invasion of the stroma was approximately equal in presence or absence of stromal cell transformation. Stromal cells were noted to change into fibroblasts, although no intermediate stages were shown or described. Epithelium was found to exert a profound effect upon fibroblast formation. In its absence, fibroblast formation was decreased and did not reach a normal rate until epithelium had grown across the wound. Any removal of epithelium caused alteration in appearance of corneal stromal cells which was an important source of fibroblasts. The investigators hypothesized that acetylcholine, which is highly present in epithelium, may be the source of an essential substance for transformation of stromal cells.

The relative lack of cells (only 5% cells by volume) for stromal repair work or for replacement of stromal cells has led to a number of hypothesis as to the origin of fibroblasts, the primary cell involved in stromal replacement. It has been concluded that stromal cells are replaced from: (1) a transformation of epithelial or endothelial cells into stromal cells, (2) undamaged corneal cells, (3) wandering cells from outside of the cornea, and (4) a combination
of these sources.

Retterer (8) studied incisions in guinea pigs' corneas and concluded that the new stromal cells originated from transformed epithelial cells and a multiplication of the stromal cells by mitotic division. Salzer (9) concluded that the epithelial cells, which migrated into a wound made from the external surface of the cornea, are converted into stromal cells. He was unable to find stromal cells in the process of multiplication and offered this as evidence against the participation of stromal cells in repair.

Grillo (10) found that the fibroblasts of wound repair arose predominantly by proliferation of locally resident connective tissue cells, rather than from precursor cells recruited via the vascular system. The work was done on incisions in the trunk of guinea pigs.

Hoffman and Messier (11) developed a method for studying corneas in vitro. The first cells to appear at the margin of the explant were a mixture of epithelial and stromal cells or keratoblasts. These stromal cells resembled fibrocytes, which with environmental changes, changed to large macrophages.

Maumenee and Kornblueth (12,13), after freezing rabbit corneas, found that keratoblasts could be derived from two sources for nonpenetrating wounds of the cornea. Macrophages or fixed-tissue histiocytes could be stimulated by injury to migrate, or they were derived
from activated normal corneal cells. No morphological evidence was presented for this conclusion, however.

Fibroblasts cannot migrate rapidly enough from the limbal tissues to provide the source of the fibroblasts found at the wound edge of central corneal wounds. The fastest rate of migration observed for fibroblasts in vivo is 0.29 mm./day (56), while monocytes can migrate at a rate of 0.35 mm./hr. (57,58).

Weimar (14,15) reported corneal stromal cells at wound edge undergoing morphologic changes to fibroblastlike cells during first 24 hours. The cells increased in size and in number of nucleoli. By 48 hours the cells had transformed from oval to long and narrow. This transformation only occurred in a narrow border about 200 μ wide on either side of the wound. No mast cells were found. The only other cell type at the wound, excluding PMNL's, was the monocyte. Fibroblasts and lymphocytes never entered unless blood vessels invaded the cornea. She also showed the stages of transformation of monocytes to fibroblasts.

Wolter (59) found that migrating stromal cells were clearly distinguishable from invading fibroblasts. He found no evidence that invading leukocytes or lymphocytes developed from stromal or epithelial cells. He concluded stromal cells represented a special type of fibroblasts, which did not change into nondifferentiated wandering fibroblasts. The invading fibroblasts never became quite
like stromal cells either.

Baum (60), using tritiated thymidine, showed that substantial numbers of fibroblasts were derived from sources other than transformation of central stromal keratocytes. He suggested three sources for unlabeled fibroblasts in the wound area: (1) tears, (2) peripheral keratocytes, and (3) aqueous. All but the aqueous were eliminated as possible sources. Wandering cells were found "linked" to fibroblasts.

Kitano and Goldman (61) reported that the fibroblasts were exclusively derived from local keratocytes. They did not find any transformation of monocytes into fibroblasts.

The first detectable changes in the tissues following wounding are those of the inflammatory process. It is generally agreed that the stimulus which initiates inflammation is derived from injured cells. Among the early events is the polymorphonuclear invasion of the wounded area. In this study, the polymorphonuclear cells will be called heterophiles rather than polymorphonuclear leukocytes, which are found in humans, but not in rabbits. Weimar (16) found that polymorphonuclear leukocytes began to invade cornea during the fifth hour after wounding, with it continuing until 24 to 36 hours after the injury.

Robb and Kuwabara (17,18), using staining techniques and autoradiographic techniques, found increasing numbers of PMNL's in blood vessels, which by one hour had entered surrounding tissue. At five
hours, the cells had reached the tear film. The leukocytes moved into corneal stroma from the wound defect with none found within or between corneal epithelial cells lining the wound. No evidence was found for central wounds that inflammatory cells other than PMNL's were migrating toward the wound.

Pullinger and Mann (19) found PMNL's reached the center of lesion within 24 hours. The cells were streamlined and in single file. Within 48 hours, a second cellular invasion occurred by wandering cells, which they called polyblasts. They found no fibroblasts or lymphocytes. Dunnington and Smelser (20), however, found few PMNL's at either 24 or 48 hours stages, and these were the pseudoacidophiles, typical of rabbits. By the seventh day the cells had the appearance of neutrophils. In an earlier paper, Dunnington (62) found PMNL invasion after three days of healing.

The electron microscope has been used by LaTessa and Ross (63) to study corneal healing. PMNL's were first noticed at wound site 24 hours after injury and mononucleated cells were noted by 36 hours. The electron microscope showed no morphological evidence to indicate the presence, or transformation of cells into fibroblasts.

Aurell (64), Binder and Binder (65), Morton et al. (66), von Sallman et al. (67), and Faure et al. (68) investigated the healing of Descemet's membrane and endothelium. Mitotic and amitotic cell division, combined with cellular migration and enlargement of the
endothelial cells, was the method of endothelial repair following injury. Intact descemet's membrane, after being removed, was seen after 21 days healing. It eventually obtained 50% of its original thickness. Binder and Binder (65) and Faure (68) also noted cells with large nuclei in the endothelium. Binder (65) believed they could be macrophages derived from endothelial cells by metaplasia. Binder also believed that the lymphocytes might also originate from endothelial cells.
OBJECTIVES

Knowledge of corneal healing is important, in view of the potential for ocular hazards, such as abrasions from contact lenses, broken lenses, and foreign bodies. Previous investigations have gathered information on corneal healing and affects of vascularization or drugs. The literature contains a number of observations and conclusions concerning origin and fate of cell types seen in corneal lesions. There is confusion concerning the types of cells present, how they arrived at the lesion, and when they began to arrive. This may be explained by the use of techniques inappropriate for careful morphological studies, such as the use of frozen sections, squashed sections, slides with many artifacts, and nonstandardized lesions. Additional morphological studies are needed to resolve the discrepancies between experiments.

This investigation proposes to study, primarily, the initial or lag phase of wound healing, since the progress in this stage determines how healing proceeds. It is during this phase where much of the disagreement between investigators occurs. Using careful morphological techniques, so that a minimum of artifacts is created and using standardized lesions, this investigation
proposes to study the origin and the fate of the cell population present in the wound.
METHODS

The following is a description of the techniques employed for the morphological study of avascular corneal healing of non-penetrating wounds.

Animal preparation

Young, healthy, male and female albino rabbits were used in this study. All animals weighed between 3.0 and 5.0 kilograms. Prior to their use in this study, the animals had been maintained in good physical health in the animal laboratory of The Ohio State University. Both eyes of every animal were examined with a biomicroscope before their use in order to verify that they were free of pathology or other obvious defects.

Surgery was performed under a general anesthesia containing a mixture of 5% pentobarbital sodium (Diabutol) and 20% ethyl carbamete (Urethane) in saline. Induction of Stage III anesthesia was quickly accomplished and lasted for 3-4 hours. The level of anesthesia created was such that there was no flexure reflex.

The surgery consisted of using a scalpel for creating a nonpenetrating wound running from 1 mm. outside the superior
limbus at 12:00 to 1 mm. outside the inferior limbus at 6:00. Surgery was performed with the aid of a biomicroscope and high intensity light source (Figure 1). An attempt was made to limit the depth of the cut to the anterior 1/3 of the corneal stroma.

Antiseptic conditions for surgery were maintained by using Zepharin chloride to wash the rabbit's face, front and back paws, and especially around the eyes. This was done to prevent the development of any corneal or adnexal disease processes which might complicate the normal morphology of corneal healing.

After the injury was made, visual inspection of the cornea and conjunctiva was made with and without a biomicroscope. This was done once an hour for the shorter healing tissues for the first six hours of healing, and then once every six hours.

The animals were housed in antiseptic cages that had been autoclaved, treated with Zepharin chloride, and sprayed with Staphene. A filter cap was placed over each cage to prevent airborne pathogens from entering the cages. No rabbit developed any apparent pathology during the duration of the experiment.

**Histological Procedures**

The nonpenetrating (with one exception) corneal injuries were allowed to heal for the following periods of time: 15 minutes, 30 minutes, 3, 4, 5, 6, 12, 24, and 48 hours, 1 week
and 2 weeks. Twenty rabbits were used. At the end of the healing period, the animal was sacrificed using an overdose of sodium pentobarbital. Prior to sacrifice, the injury was examined for the final time with the biomicroscope in order to make clinical observations and to eliminate from the experiment any lesion that may have become contaminated due to disease.

After termination, the eyes were removed and were fixed either in 10% buffered formaldehyde or in Zenker-formalin solution, which is a fast fixer used for fixing cellular detail. After 24 hours of fixation, the eyes were washed with running water and then placed in 70% ethanol. After being washed with several changes of ethanol, they were left in the ethanol over night. The next day the posterior half of the eye was removed along with the lens and vitreous. The cornea and remaining tissue were sectioned along a line perpendicular to the vertical nonpenetrating wound, extending from 3:00 to 9:00.

A double embedding process was utilized for mounting the cornea (Appendix A). The cornea, as a dense connective tissue, was difficult to section. The goal was to maintain good corneal integrity and to minimize artifacts.

Serial sections 5u thick were made using an AO rotary microtome. These sections were mounted on slides using albumin.

Slides from each time period were then stained with 0.5% Toluidine blue (Appendix B). Toluidine blue is an excellent stain for observing metachromasia, nuclear detail, and cell morphology.
A second sample of slides was stained by Masson's Trichrome Method, which was modified by the use of Harris' hematoxylin solution (Appendix C). This technique stains nuclei black; cytoplasm, keratin, and intercellular fibers red; and collagen blue.

The permanent slides were then examined for cellular morphology, and summaries of observations were written. Photomicrographs were taken as evidence of the morphological changes that were noted as healing time increased.
RESULTS

**Biomicroscopy Observations**

Before the lesion was created, each eye was examined for obvious pathology or previous corneal lesions. Each cornea examined was fairly irregular in shape, but it was anticipated that this would have no influence on the morphology of avascular corneal healing. Of the 40 corneas examined, none were found with any obvious previous scar formation, pannus, edema, or active pathology.

The conjunctiva of each animal was also examined initially. Each eye was found to have a slight amount of localized injection, primarily at the superior and inferior limbus. Animals were rejected from the study if they were found to have more than the typically slight amount of limbal injection.

When making the nonpenetrating, limbus to limbus, lesion, the incision was started 1 mm. outside the superior limbus and terminated at the optical axis of the cornea. Termination was necessary because of the rolling of the eyeball. The nonpenetrating lesion was completed by starting 1 mm. outside the inferior limbus and then trying to meet the superior lesion at the center. The lesion was easily visible through the biomicroscope. This technique, sometimes resulted in
a double lesion in the central cornea.

As soon as the lesions were completed for each eye, the eyes were examined with a biomicroscope (Figure 2). The appearance and depth of the lesion could then be determined.

In one eye, a penetrating lesion was accidently created. While this study did not deal with penetrating lesions, it was decided to follow it through for five hours of healing. As soon as the penetration was created, the aqueous was lost with collapse of the anterior chamber. The initial slit lamp appearance showed slight edema, with an obvious penetrating lesion, although the edges of lesion were in apposition. The length of the penetration was estimated to be 3-4 mm. Within 1 to 2 hours a fibrin plug had formed in the lesion. By making use of the Tyndall phenomenon, it was possible to see a slight aqueous flare. The anterior chamber had completely reformed by the end of 3 hours healing time. The animal was sacrificed after 5 hours of healing. By this time there was a reduction in the slight aqueous flare. No other penetrating lesions were created.

The typical slit lamp observation just after the lesion was created showed a slight increase in conjunctival injection in the region of the lesion. The lesion, itself, was observed as a gray line in both direct and indirect viewing. Using the technique of sclerotic scatter, a very slight edema could be observed at the margins of the lesion. Optic sections using the slit lamp showed
the lesions to vary slightly in their depth from about 1/8 to 1/3 the depth of the stroma (approximately .05 to .1 mm.).

At one hour healing time, a slight increase in conjunctival injection was found both superiorly and inferiorly, but especially inferiorly. There was no further increase in the amount of conjunctival injection after one hour, and by 12 hours the amount of injection had decreased to the presurgical condition. No blood vessels or lymphatics were found to grow into the cornea from the limbus for any lesion.

Slight edema was noticed at the margin of the lesion until 12 to 24 hours of healing time had elapsed. After this time, edema was no longer apparent. The tears of the rabbit normally have floating debris easily visible with the biomicroscope. At one hour healing time, the debris in the tears had increased over the amount present in the presurgical tear film. In some rabbits, after 15 minutes healing time, there appeared to be red blood cells throughout the extent of the lesion. These collected from the slight bleeding due to cutting the conjunctiva.

Some eyes showed a complete loss of epithelium in the area of the lesion. The loss usually occurred below the optic axis and covered an area approximately 2 mm. by 2 mm., centered on the lesion. This did not happen with all lesions, possibly due to better technique in creating the lesions. At five hours the tears reached their maximal quantity of debris, but returned to normal by 12 hours.
While normal tears are known to have cells present, no cells of any type were seen in the tears of these rabbits. No cells were seen in the stroma either.

The lesion was partially covered by epithelium after 5-6 hours and was usually completely filled with epithelium after 12 hours. Slitlamp observation was not sufficient for indicating the method of epithelial repair. The lesion was observable even after two weeks healing. After five hours of healing no further changes were found in the lesion by slit lamp observation.

Morphological Observations

Normal Cornea

The normal cornea of the rabbit consists of epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. The epithelium is a stratified, squamous, nonkeratinized epithelium. The basal layer consists of columnar cells over which there are 3 to 5 rows of polyhedral and squamous cells. Two to four mitotic figures could be seen in each limbal to limbal cross-section.

At the limbus, the layers of the conjunctival epithelium increases to 10 to 15 cells with most of this increase occurring mainly in the wing cells. Goblet cells are not present in the limbal epithelium, but are found in the periphery of the bulbar conjunctiva. The organization of the epithelium is similar to that of the cornea.
The stroma of the conjunctiva contains blood vessels, fibroblasts, which are the principal cells seen, mast cells, lymphocytes, and occasional heterophiles. The connective tissue of the conjunctival stroma has a varying degree of compactness. It is densest near the end of Bowman's layer. The connective tissue is a loose, irregular connective tissue composed almost entirely of loosely arranged bundles of delicate collagen fibers.

**Observations of 15 minutes healing**

The earliest period in time for which histological observations were made after initiation of the lesion was 15 minutes. The slides showed lesions that extended in depth to about 1/4 the thickness of the cornea into the anterior stroma. At the margins of the lesions were epithelial and keratocytic cell fragments (Figure 3). The epithelial and stromal margins of the lesion were quite irregular due to collagen strands and slight edema. The toluidine blue stain showed a loss of metachromasia in the stroma within an area approximately 50 to 100 microns surrounding the lesion. A slight increase in stromal sagittal thickness at the lesion site only was also observed.

Keratocytes, which are normally present, were present throughout the stroma. There were no apparent changes in the cells close to the lesion when compared to those in normal stroma (the nuclei stained quite dark demonstrating dense chromatin which is the normal pattern for stromal keratocytes). There was no apparent migration
toward lesion, since there was no change in the nuclei or orientation of the cells. There was no mitosis of these cells.

No heterophiles were found at the lesions or in the peripheral vasculature of the conjunctiva. No apparent changes in the corneal epithelium had occurred. There was no thinning or sliding and no increase in the rate of mitosis.

Observations of 30 minutes healing

The 30 minute lesions had the same histological appearance as described for the 15 minute lesions (Figure 4). The only apparent epithelial changes were a pyknosis of cell nuclei immediately adjacent to the wound margins. There were no keratocyte changes and no heterophiles in the lesion or in the peripheral vasculature.

Observations of 3 hours healing

The earliest morphological indications of healing were found in the lesions that had completed three hours of healing time. The epithelium at the lesion was thinner due to an apparent sliding of cells toward the wound. The fault began to narrow and to fill in with epithelial cells having basophilic cytoplasm. No increase in epithelial mitosis was observed anywhere across the epithelium. Some areas of the lesion had epithelial cells within the lesion. The cells appeared to migrate toward the bottom of the lesions lining the walls of the faults first. An area in one lesion had a rather
extensive loss of epithelium.

Heterophiles were found on the walls of the wedged-shaped fault in the stroma (Figure 5). They also appeared in the interfibrillar spaces of the adjacent stroma (Figures 6, 7, 8). The density of these cells was not large. They seem to be concentrated toward the bottom of the lesion and within 100 microns of the edge of the lesion. These cells could also be seen in greater numbers in the peripheral vasculature, in the connective tissue of the conjunctiva, and in the conjunctival epithelium. They appeared to be migrating from the blood vessels to the epithelium of the conjunctiva. They appeared to be more concentrated in the central parts of the lesion. No heterophiles were observed between the area of the lesions and the limbus. This suggests that these cells reach the lesions via the tear film.

There were no visible changes in the keratocytes throughout the stroma. No other cell types were present at the lesion.

Observations of 4 hours healing

The lesions that had completed four hours of healing presented nearly the same appearance as the three hour lesions. Individual differences between four hour lesions existed. The difference between lesions was the extent to which epithelial cell sliding had taken place. Some had numerous epithelial cells lining and filling the lesion, others had only a few cells lining the lesion. No increase
in epithelial mitosis rate was observed.

All the lesions had heterophiles present both in the lesion and in the surrounding stroma (Figure 9). The greatest concentration of these cells was in the part of the wounds in the central cornea. Some of these cells had migrated from the edges of the fault into the interfibrillar spaces of the stroma at the edge of the wound. None of these cells had migrated more than 400 microns away from the lesions toward the limbus. The direction of migration of heterophiles could be identified because the cytoplasm and granules precede the nucleus, thus, the cytoplasm indicated direction of movement of the cells.

The blood vessels of the bulbar conjunctiva had numerous heterophiles in their lumina. This was true even for vessels some distance from the limbus, well into the conjunctival fornix. The cells were scattered throughout the conjunctival connective tissue and some were observed in the epithelium of the bulbar conjunctiva (Figures 10,11, 12). Many of the cells appeared to be migrating toward the conjunctival epithelium. None were observed to be migrating from the limbus toward the lesions.

The keratocytes throughout the stroma remained normal in appearance.

Observations of 5 hours healing

Some of the corneas which had completed five hours of healing
had lesions which were completely filled with epithelium, others were nearly filled with epithelium. This difference appeared to be due to lesion size. However, the epithelium immediately over the lesions was thinner than normal. The epithelium had a normal rate of mitosis, as compared with normal corneal epithelium.

Heterophiles were still observable in the conjunctiva; although, for some lesions they appeared to be less numerous than for the lesions with four hours of healing. These cells were still present in the lesions, and some had penetrated approximately 400 microns into the stroma (Figure 13). No cells were found to be migrating from the limbus toward the lesions. There were some changes present in the stromal cells adjacent to the wound. There was an enlargement of stromal cell nuclei and a redistribution of chromatin from the typical dense pattern to a pale nucleus with finely divided chromatin with prominent nucleoli. The cytoplasm remained clear. No migration of fibroblasts from the limbus was observed.

The single penetrating lesion was examined histologically after five hours of healing. A fibrin plug completely filled the wound. There were some epithelial cells in the lesion. More mitotic figures were noted in the epithelium than had been noted for other lesions.

The lesion had larger numbers of heterophiles than did other lesions with five hours of healing. Heterophiles were present in the conjunctiva. Some heterophiles were found in the aqueous and in the blood vessels of the iris root. None were found to be migrating
from the limbus to the wound, i.e. none were present in the intervening space between the limbus and the area of the lesion.

Changes in stromal cells equivalent to that previously noted for lesions with five hours healing were also found.

**Observations of 6 hours healing**

The lesions that had six hours of healing were all filled with epithelial cells, although the epithelium over the lesion was not of normal thickness (Figure 14).

There was a decrease in total numbers of heterophiles present in the wounds and in the bulbar conjunctiva, relative to lesions with 4 or 5 hours healing time. Some of the heterophiles had migrated from the lesion toward the limbus. Dead heterophiles could be observed in the lesion and in the stroma. Dead heterophiles could be recognized by their nuclei. Normal heterophiles have three-lobed nuclei, dead heterophiles have lost the lobes and appear to have three separate nuclei. No lymphocytes or monocytes were present, either in the lesions, in the migration path, or in the conjunctival connective tissue.

The change of the stromal cells was more evident in these lesions than in the five hour lesions.
Observations of 12 hours healing

One lesion after 12 hours healing showed a large loss of epithelium which was being repaired by sliding cells (Figure 15). The rest of the slides showed the epithelium completely filling the wounds. It was now of normal thickness, even over the wounds (Figure 16).

A few heterophiles could still be found in the lesions, the stroma near the lesions, and in the periphery. They were quite reduced in number from the peak times of 4-5 hours.

There were the same changes in the keratocytes in the vicinity of lesions. The nuclear material did not have the typical dense chromatin pattern, but was now finely divided and did not stain as darkly.

Observations of 24 hours healing

Lesions after 24 hours of healing had the same appearance as lesions after 12 hours of healing. The epithelium filled the lesions. One or two heterophiles were in the stroma adjacent to the lesions and in the periphery (Figure 17). The stromal cells within about 400 microns of the lesions had the typical nuclear pattern of fibroblasts.

Observations of 48 hours healing

At 48 hours, heterophiles were no longer present in the conjunctiva, with only one survivor present in the lesions. None were
present in the stroma.

Definite fibroblastic activity existed in the immediate area of the lesions. One fibroblast was observed to be mitosing. No fibroblasts were found migrating from the limbus toward the lesions.

Observations of 1 week healing

At one week there were no heterophiles at the lesions or in the bulbar conjunctiva (Figure 18). Fibroblasts were present in the lesions, especially in the deepest stromal extent of the lesions.

Observations of 2 weeks healing

More fibroblasts were noted in the lesions with two weeks of healing. There are no heterophiles or other cell types present in the lesions except for epithelial cells (Figure 19).
DISCUSSION

Both the clinical and histological characteristics of corneal healing have been studied in the past. There has been disagreement concerning the cell types present in the wounds, the times at which they first appear, and their source and manner of approaching the lesions. Some of the differences could be due to species difference or to the method of creating the wound, site, and location of the wound (Appendix D). Many of the investigators used frozen sections or squashed sections for their study, but these techniques have the problem of destroying cellular morphology, making cell identification difficult or impossible. Many of these difficulties have been eliminated in this investigation by improved tissue fixation techniques and improved imbedding and sectioning techniques, which permitted study of lesions with a minimum of artifacts. A limbus to limbus nonpenetrating lesion was created so that differences between peripheral and central corneal healing, if any, could be studied. The incision created minimum destruction of corneal epithelium and stroma.

The results of the slitlamp investigation were inconclusive concerning corneal healing. The resolution available was insufficient
for seeing cells unless a major migration of cells through the stroma had occurred. Such a migration did not occur, however.

The slit lamp was useful only for demonstrating the depth of the lesion, whether corneal clouding had occurred and its location, or if there had been a loss of corneal epithelium. It was also useful for confirming that no vascular invasion of the cornea had taken place at any time during the healing study.

No morphological changes in the corneal epithelium were observable after 15 minutes of healing. Pyknotic nuclei in cells adjacent to the lesion were observed after 30 minutes of healing. Buschke (1,2) reports a sloughing of epithelial cells after these cells were damaged by pin pricks, but no time period was given for the loss of these cells. Brach H (51) found pyknotic nuclei present in the area of an incision 1 1/2 hours after the incision was made.

After three hours of healing, sliding of cells into the lesions was observed. There was a general thinning of the epithelial layers in the areas immediately adjacent to the lesions. The epithelium, while in the process of sliding, described a wedged-shaped appearance. Khoudadoust et al. (7) described a tongue of epithelial cells sliding in over the denuded area after several hours of healing.

Most investigators (7,17,18,19,51) agree that epithelial sliding is the method by which the epithelium repairs itself. Some investi-
gators (1,2,3,4,5) have not specifically stated that epithelial sliding takes place, but they did note a filling in of the lesion with epithelium, without specifically mentioning how the lesion became filled. The experimental results presented in this dissertation support the evidence of epithelial sliding.

The other method of epithelial repair would be an increase in mitotic rate of the epithelial cells. No such increase was found during the duration of this experiment with the possible exception of the single penetrating lesion that was created. Arey and Covode (6) believed that increase in mitosis may play a secondary role in epithelial healing. However, a number of experimenters (3,4,5) have given convincing evidence that mitosis plays little role in epithelial healing.

Robb and Kuwabara (17), Newell (69), Friedenwald and Buschke (3,4) found a lag time of one hour before epithelial sliding began. Khodadoust et al. (7) found a lag time of several hours, and Maumenee and Kornblueth (13), and Robb and Kuwabara (18) found a lag time of 20-24 hours. The length of the lag time appeared to be related to the severity and type of the wound. Pin pricks had a short lag time, while burns or freezing had a much longer lag time.

The basal cells were the leading cells in the epithelial slide. They had a more compacted appearance than their typical columnar shape in normal epithelium. Buschke (1,2) found that large
lesions in the early stages of healing showed the immediately ad-
joining epithelial cells became tangentially oriented toward
the lesion. The cells demonstrated a mushroomlike spreading and
flattening of margin cells of the wound. The orientation of cells
in this study was not observed because cross-sections of tissue
were used, rather than the flat, squashed sections used by Buschke.

The earliest signs of epithelium being present in the les-
ions was after three hours healing. The lesions that had four
to five hours of healing had large numbers of epithelial cells
within the lesions. There were individual differences between
lesions in relation to the number of cells within them, but these
differences appeared to be related to the extensiveness of the
lesion. The epithelium migrated into the lesions from both sides,
one to two cell layers thick. The rate of sliding was sometimes
greater from one side of the lesion than for the other. Since
the lesion was centered on the cornea, the nearness to the limbus
was equal for both sides; therefore, this cannot explain the dif-
fERENCE. No differences could be seen between the two sides to
explain the difference. By six hours, the lesions were completely
filled with epithelium, although the epithelium over the lesion
was only 2-3 cell layers thick. After 12 hours of healing, the
epithelium had a smooth specular surface over most lesions.

The period of time required for the epithelium to, not only
fill the lesion, but also regain normal thickness above the lesion has been found by various investigators \( (3,4,6,7,11,12,13,17,18,19, 48,51,53,54,63,70) \) to vary from two to three hours to two weeks. The difference in healing time appears to be related to the severity of the lesion. Lesions that involved only the epithelium took only two to three hours for complete repair \( (3,4,53,54) \). Lesions such as nonpenetrating, penetrating, burns, and freezing, took longer for the epithelium to fill the wound. Various investigators \( (6,7, 11,12,13,48,51) \) have reported that it took from six hours to two weeks for the epithelium to fill the lesion.

Even though the lesions of this experiment extended from limbus to limbus, no vascularization of the cornea took place for any lesion. While it was not the intent of this study to investigate vascularization, it is interesting that none occurred, since one theory to explain vascularization is the release of a substance during corneal injury that promotes vessel growth \( (22,23,24) \).

While this investigation was interested primarily in the short term aspect of corneal healing, i.e. the lag period, some observations were made concerning stromal healing. No epithelial or endothelial cells were observed to be transforming into stromal cells. The epithelial cells did slide into the lesions, but no intermediate stages between epithelial cell and stromal cell were seen. While Retterer \( (8) \) and Salzer \( (9) \) concluded that epithelial cells trans-
formed into stromal cells, no evidence of such a transformation was observed in this study.

Transformation of stromal cells to fibroblasts was observed in lesions, beginning with lesions that had five to six hours of healing. This was indicated by the changes taking place in stromal cells adjacent (300-400 microns) to the wound. There was an enlargement of stromal cell nuclei and a redistribution of chromatin from the typical dense pattern to one with finely divided chromatin with prominent nucleoli. The cytoplasm remained clear and relatively invisible.

Bracher (51) noted that development of large and numerous nucleoli in the keratocytes undergoing transformation to fibroblasts were demonstrable as early as one hour after wounding. He found the first real fibroblastlike cells at the lesion at 48 hours. His radioautographic technique, combined with his histological technique, did not permit him to show any of the stages of transformation, however.

Weimar (14,15), Robb and Kuwabara (18), Baum (60), Wolter (59), and Kitano and Goldman (61) believed that stromal cells in the vicinity (200-500 microns) of the lesion transformed into fibroblasts for the purpose of wound healing. Weimar (14,15), Baum (60), and Kitano and Goldman (61) described the steps of transformation of keratocytes into fibroblastlike cells, as the development of several large nucleoli within a fusiform nucleus, as compared to the usually dense
chromatin of the nucleus of the keratocyte. Baum (60) and Weimar (14,15) found that only 20-25% of the stromal cells transform to fibroblasts. Weimar (14,15) reported the earliest changes occurring after six hours healing with definite fibroblasts present at 24 to 48 hours. Others (60,61) report that the transformation starts at 48 hours.

Wolter (59) felt that corneal stromal cells represent a special type of fibroblast which was not observed to lose differentiation completely and which was not seen to change into nondifferentiated, wandering fibroblasts. Such observations were not made in this experiment. It is difficult to compare fibroblasts in loose connective tissue with fibroblasts in the corneal stroma, because the compactness of the stromal connective tissue tends to distort cell shapes. Wolter (59) and Weimar (14,15) used squashed flat sections which tend to further distort cellular morphology.

Some investigators (46,48,62) have reported the presence of fibroblasts in lesions after healing times of three to six days. No information was given as to the source of these cells.

Another possible source for fibroblasts at lesions was the migration of fibroblasts to the wound from the conjunctiva. No evidence of fibroblasts being present in the stroma between the limbus and the region of lesion was found in this study. A lack of such evidence would indicate that no fibroblasts migrated to the wound from the limbus. Dunnington and Smelser (20) reported
migration of fibroblasts from the limbus which occurred 24 to 48 hours after the lesion was made. Wolter (59) found such a migration only when severe stromal damage had occurred.

LaTessa and Ross (63), using an electron microscope, and Pullinger and Mann (19) found no evidence of presence of fibroblasts at healing lesions. Pullinger and Mann (19) found fibroblasts only if vascularization of the cornea occurred.

Baum (60) created nonpenetrating lesions of the cornea by cutting through the endothelium, Descemet's membrane, and into the stroma. Although he found no fibroblasts within the aqueous, he felt that the fibroblasts reached the wound via the aqueous. No evidence for this source of fibroblasts was found in this study.

A possible alternate source of fibroblasts for stromal healing would be the transformation of other cell types (other than epithelial, endothelial, or keratocytes) into fibroblasts. For this to occur, these other cells, which can be identified, must reach the lesion and then should show a series of intermediate cell types as they transform into fibroblasts. The only cell types present at the lesion, in this study, were epithelial cells, heterophiles, keratocytes, and fibroblasts. None of these cells, except for the keratocytes, showed any intermediate cell types.

Weimar (15) found that monocytes migrated through the stroma to the site of the lesion in rat corneas. She also found intermediate cell types which suggests that these monocytes were trans-
forming into fibroblasts. No monocytes were observed at the lesions in this study with rabbits, which could suggest a species difference. Pullinger and Mann (56) found that after 48 hours of healing, wandering cells, which could have been monocytes, entered the stroma. They found evidence that these cells were capable of transformation into fibroblasts.

This investigation was primarily interested in the first period of corneal healing known as the latent period, which occurs before the fibroblastic or second period. This first period, typically, extends in time from creation of the lesion until the end of the first day. During this period this investigation has found morphological evidence of epithelial healing, the initial phases of fibroblastic activity, and a cellular invasion. Lesions elsewhere in the body produce an inflammatory reaction, which among other things includes the invasion of the lesion by various migrating blood cells.

Lesions which had completed three hours of healing time were found to have been invaded by heterophiles only. The peak activity occurred for lesions with four to five hours of healing time. The reactions were most obvious for the parts of the lesion nearest the center of the cornea. From six hours to 24 hours the numbers of heterophiles present in the lesions began to decrease, and evidence of dying cells could be observed.
The appearance and decline of these cells was paralleled in the bulbar conjunctiva, as far as the deepest extent of the fornices. The cells were observed beginning three hours after creation of the lesions to be within the lumina of the vessels of the conjunctiva. They were within the loose connective tissue of the conjunctiva, and they could be seen between epithelial cells of the conjunctiva. At the same time they were seen in the epithelium of the cornea, within the lesion itself, and a few within the interfibrillar spaces of the corneal stroma.

No heterophiles were found in the stroma between the limbus and the immediate vicinity of the lesion (400 microns) for any lesion after any healing period. These observations and the fact that monocytes and migrating blood cells can migrate at only a rate of 0.35 mm/hr. (57, 58), which means that it should have taken the heterophiles about 16 hours to move from the limbus to the central part of the cornea, a distance of about 5 mm. in a rabbit, suggest that the heterophiles reach the lesion via the tear film.

Monocytes and lymphocytes were never found at any of the lesions. Lymphocytes were seen within the conjunctiva. Other investigators report these and other cell types being present in the lesion. They disagree as to the types of cells that can be found at a lesion, how quickly they begin to appear, and how they got there.
Dunnington (62) found large mononuclear cells and lymphocytes three days after the lesion was created. Dunnington and Smelser (20) found lymphocytes occasionally after 24 hours, but usually after five days of healing. Wolter (59) also found lymphocytes present at the lesions. The lymphocytes were observed to be migrating through the stroma. Wolter (59) found them migrating in long straight chains, perhaps in Bowman's channels. Pullinger and Mann (19) and Weimar (14) found lymphocytes in the lesions only when blood vessel infiltration had occurred. This investigation found lymphocytes only in the conjunctiva and never at the lesions.

Dunnington (62) found large mononuclear cells after three days. Robb and Kuwabara (17,18) found mononuclear cells in the periphery after 8 hours, Maumenee and Kornblueth (13), Weimar (14,15), Kitano and Goldman (61), and Weimar and Haraguchi (52) also found monocytes present in the lesions occurring from 12-24 hours. These cells invaded the lesion from the limbus, and they were found in rats and rabbits and for both penetrating and nonpenetrating lesions. This investigation found no monocytes at the lesions. The reason for this difference in findings is unknown, but it is not due to species difference or type of lesion. Many of these studies did use frozen sections or squashed sections which tends to distort cellular morphology.

Macrophages were also found at the lesions by some investigators (13,19,46,62). They were found at the lesions within 24 to 48 hours
via migration through the stroma.

This investigation found heterophiles present in the lesions and the conjunctiva starting at three hours, increasing by four to five hours, and gone from the lesions and the conjunctiva by 48 hours. No heterophiles were found between the limbus and the wound. This morphological evidence suggests that they arrived at the lesions only via the tears. A wide variety of peak times for the cell population and of migration routes have been found by other investigators.

Bracher (51) found leukocytes occasionally as early as 1 1/2 hours, increasing until 12 hours, and decreasing until 72 hours. LaTessa and Ross (63), Pullinger and Mann (19), Kitano and Goldman (61), and Ashton and Cook (46) found leukocytes present at the wound within 24-48 hours. Dunnington (62) and Basu (48) found them as late as three to four days after the creation of the wound. Dunnington and Smelser (20) and Maumenee and Kornblueth (13) rarely found polymorphonuclear leukocytes. Weimar (16), Weimar and Haraguchi (52), and Robb and Kuwabara (16,17) observed leukocytes in the lesions as early as six hours, increasing in numbers to 24 hours, and decreasing in numbers after this time.

Many investigators (13,14,48,51,61,62,63) only observed the presence of polymorphonuclear leukocytes at the wound, but they made no observations as to how they got there. It can be hypothesized that the cells migrated to the wound. Dunnington and Smelser (20), Pullinger and Mann (19), Weimar (16), Weimar and Haraguchi
and Ashton and Cook (46) observed that the leukocytes invaded the corneal stroma from the limbus and migrated toward the lesion. Their evidence was the serial progression of groups of leukocytes from the limbus toward the lesion. Pullinger and Mann (19) even found the cells streamlined and in single file.

The migration of leukocytes from the limbus toward the wound was found for both penetrating and nonpenetrating lesions and for more severe lesions created by burns and freezing. This investigation found heterophiles migrating from the lesions toward the limbus, but none from the limbus toward the lesion.

Robb and Kuwabara (17) found polymorphonuclear leukocytes in the conjunctiva at one hour and in the conjunctival epithelium at five hours. They found an increase in the number of cells at the lesion up to 24 hours, after which the number of cells at the lesion began to decrease. They found no cells in the stroma so they theorized that the cells reached the lesion via the tears. They also found mononuclear cells in peripheral lesions. In a second paper (18), they found polymorphonuclear leukocytes and mononuclear cells reaching the lesion both via the tears and by migration. This study supports their conclusion that heterophiles reach lesions via the tears, but no heterophilic migration from the limbus to the lesion was found in the stromal. No monocytes were found in the lesion or in the stroma at all.
SUMMARY

Epithelial healing occurs by the sliding of cells down the wall of the lesion, filling the lesion, and then regaining initial thickness over the lesion. No indications of increased mitosis of epithelial cells was found.

Heterophiles began to fill the conjunctival vessels after three hours of healing. They migrated into the conjunctival stroma, through the conjunctival epithelium, and then into the tears. They reached the site of the lesion via the tears and then entered the lesion migrating into the corneal stroma (maximum 400 microns).

No cellular migration from the limbus toward the wound of any cell type was observed.

Heterophiles were the only migrating blood cells found at the lesion in this study.

Heterophiles at the site of the lesion reached a maximum population about four to five hours after wounding, then subsided until none were found at 48 hours healing time (the cells were observed to die at the lesion).

Stromal cells, within 400 microns of the lesion, were observed to change morphology and to resemble typical fibroblasts beginning about five to six hours after the lesion was created.
Appendix A

STANDARD METHODS OF HISTOLOGICAL PREPARATION

DOUBLE EMBEDDING TECHNIQUE

1. Fixation 12-24 hours in formalin or formal-Zenker's
2. Wash in running tap water one hour
3. 70% ethanol three changes in 12-24 hours
4. 95% ethanol two changes in 3-6 hours
5. 100% ethanol two changes in 3-6 hours
6. Methyl benzoate three changes 12 hours each
7. 1% parlodion in methyl benzoate 24 hours
8. 2% parlodion in methyl benzoate 24 hours
9. 4% parlodion in methyl benzoate 24 hours
10. Benzene three changes in 3-4 hours
11. Paraffin three changes in 3-4 hours
12. Embedded in paraffin for sectioning at 5μ
Appendix B

STANDARD METHODS OF HISTOLOGICAL PREPARATION

TOLUIDINE BLUE STAINING TECHNIQUE

1. Deparaffinize in three changes xylene 10 minutes
2. 100% ethanol two changes two minutes each
3. 95% ethanol two changes two minutes each
4. 70% ethanol two minutes
5. Lugol's six minutes - used only for specimen fixed in formal-Zenker's to remove mercury
6. 70% ethanol two minutes
7. Distilled $H_2O$ two minutes
8. Toluidine blue 0.5% 15 minutes
9. Distilled $H_2O$ 1 dip
10. 70% ethanol 1 dip
11. 95% ethanol 1 dip
12. 95% ethanol 2 minutes
13. 100% ethanol two changes two minutes each
14. Zylene three changes two minutes each
15. Mount in picolyte
Appendix C

STANDARD METHODS OF HISTOLOGICAL PREPARATION

MASSON'S TRICHRome METHOD

1. Deparaffinize and hydrate to distilled water through Lugol's if tissue fixed in formal-Zenker's - see Appendix B
2. Mordant in Bouin's solution for one hour at 56°C
3. Cool and wash in running water until yellow disappears
4. Rinse in distilled water
5. Harris' Emeatoxylin solution for ten minutes
6. Wash in running water ten minutes
7. Dip in saturated LiCO until blue - two dips
8. Rinse in distilled water
9. Biebrich scarlet-acid fuchsin solution two minutes
10. Rinse in distilled water
11. Phosphomolybdic-phosphotungstic acid solution 10-15 minutes
12. Aniline blue solution five minutes
13. Rinse in distilled water
14. Glacial acetic acid - 1% 3-5 minutes
15. Dehydrate in ethanol, clear in xylene - see Appendix B
16. Mount in picolyte
## APPENDIX D

### HISTOLOGICAL OBSERVATIONS OF CORNEAL HEALING EPITHELIUM

<table>
<thead>
<tr>
<th>Author</th>
<th>Animal</th>
<th>Lesion</th>
<th>Staining</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buschke (1,2)</td>
<td>Rat, Frog</td>
<td>Pin pricks, Linear lesions</td>
<td>Hematoxylin</td>
<td>Flat preparations, sloughing epithelial cells, Radial orientation basal cells as lesion filled</td>
</tr>
<tr>
<td>1947, 1949</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friedenwald</td>
<td>Rat</td>
<td>Pin Pricks</td>
<td>Colchicine</td>
<td>Cellular movements after one hour lag period, mitosis of little help in healing</td>
</tr>
<tr>
<td>(3,4) 1944</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arey (6)</td>
<td>Rat</td>
<td>Trench</td>
<td>Hematoxylin-Eosin</td>
<td>Primary repair lesion by migration, secondary role in repair-increase in mitosis</td>
</tr>
<tr>
<td>1943</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khodadoust (7)</td>
<td>Rabbit</td>
<td>Removal large area epithelium</td>
<td>Hematoxylin-Eosin</td>
<td>Tongue epithelial cells sliding, lag time several hours, normal thickness, three hours</td>
</tr>
<tr>
<td>1968</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maumenee (13)</td>
<td>Rat</td>
<td>Frozen, central peripheral</td>
<td>None given</td>
<td>Migration begins 20-24 hours-single layer, normal thickness by nine days.</td>
</tr>
<tr>
<td>1949</td>
<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robb (17,18)</td>
<td>Rabbit</td>
<td>Central or peripheral nonpenetrating lesions or burns</td>
<td>H+E, Tritiated thymidine</td>
<td>Epithelium begins to slide along wound after one hour, defect filled by 24 hours burns have lag of 20-24 hours</td>
</tr>
<tr>
<td>1962, 1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Animal</td>
<td>Lesion</td>
<td>Staining</td>
<td>Results</td>
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<tr>
<td>------------</td>
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<td>-------------------------------</td>
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<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Hoffman</td>
<td>Rabbit</td>
<td>Frozen or in vitro</td>
<td>None given</td>
<td>Lag time of 30-40 hours</td>
</tr>
<tr>
<td>1949</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pullinger</td>
<td>Rabbit</td>
<td>Mustard Gas</td>
<td>None given</td>
<td>Sliding cells recovered lesion by two weeks</td>
</tr>
<tr>
<td>1943</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hasu</td>
<td>Rabbit</td>
<td>Central, peripheral burn</td>
<td>H + E</td>
<td>By 24 hours proliferation epithelial cells at margin, by fourth day filled lesion</td>
</tr>
<tr>
<td>1958</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bracher</td>
<td>Rabbit</td>
<td>Central, perforating</td>
<td>Tritiated markers</td>
<td>Half of 6 and 12 hours and all 24 hour lesions covered with epithelium</td>
</tr>
<tr>
<td>1967</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigelman</td>
<td>Rat</td>
<td>Pin pricks</td>
<td>None given</td>
<td>Healing complete by 3 hours</td>
</tr>
<tr>
<td>1954</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LaTessa</td>
<td>Rabbit</td>
<td>Central, nonpenetrating</td>
<td>Uranyl acetate</td>
<td>Lag time 1 1/2 hours, lesion filled 24-36 hours</td>
</tr>
<tr>
<td>1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>Rabbit</td>
<td>In vitro, central penetrating</td>
<td>H + E, PAS</td>
<td>Solid plug epithelium by 48 hours</td>
</tr>
<tr>
<td>1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Animal</td>
<td>Lesion</td>
<td>Staining</td>
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</tr>
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<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Weimar¹⁴ 1957</td>
<td>Rat</td>
<td>Incision, Central</td>
<td>Giemsa</td>
<td>Squashed sections, transformation stromal cells forms 24 to 48 hours</td>
</tr>
<tr>
<td>Weimar¹⁵ 1958</td>
<td>Rat</td>
<td>Incision, Central</td>
<td>Giemsa</td>
<td>Squashed sections, transformation stromal cells and monocytes from 24 hours</td>
</tr>
<tr>
<td>Hoffman¹¹ 1949</td>
<td>Rabbit</td>
<td>Frozen or in vitro</td>
<td>None given</td>
<td>Transformation macrophages and invasion fibrocytes from periphery</td>
</tr>
<tr>
<td>Maumenee¹³ 1949</td>
<td>Rat</td>
<td>Frozen-central, peripheral</td>
<td>None given</td>
<td>Transformation macrophages and stromal cells, migration stromal cells from 20-24 hours</td>
</tr>
<tr>
<td>Robb¹⁸ 1964</td>
<td>Rabbit</td>
<td>Central or peripheral nonpenetrating incisions, burns</td>
<td>Tritiated thymidine</td>
<td>Proliferation and transformation stromal cells by 24 to 48 hours</td>
</tr>
<tr>
<td>Author</td>
<td>Animal</td>
<td>Lesion</td>
<td>Staining</td>
<td>Results</td>
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<td>----------------</td>
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<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Pullinger</td>
<td>Rabbit</td>
<td>Mustard gas central</td>
<td>None given</td>
<td>Transformation wandering cells by 24 hours, fibroblast migration if vascularization occurs</td>
</tr>
<tr>
<td>1943</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dunnington</td>
<td>Rabbit</td>
<td>Central freezing with perforating lesion or just lesion</td>
<td>Radio-autograph</td>
<td>Invasion of fibroblasts from periphery from 24 to 48 hours</td>
</tr>
<tr>
<td>1958</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bracher</td>
<td>Rabbit</td>
<td>Central perforating</td>
<td>Tritiated markers</td>
<td>Transformation stromal cells by 48 hours</td>
</tr>
<tr>
<td>1967</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LaTessa</td>
<td>Rabbit</td>
<td>Central Incision nonpenetrating</td>
<td>Uranyl acetate</td>
<td>No evidence for fibroblasts found at any time</td>
</tr>
<tr>
<td>1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wolter</td>
<td>Rabbit</td>
<td>Incision</td>
<td>Hortega</td>
<td>Flat, frozen sections, migrating stromal cells transform, severe lesion get invasion fibroblasts</td>
</tr>
<tr>
<td>1958</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baum</td>
<td>Rabbit</td>
<td>Freezing with lesion from anterior chamber</td>
<td>Tritiated thymidine</td>
<td>Transformation stromal cells by 48 hours and invasion fibroblasts via aqueous</td>
</tr>
<tr>
<td>1971</td>
<td></td>
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<tr>
<td>Author</td>
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<td>Results</td>
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<tr>
<td>------------</td>
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<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Kitano</td>
<td>Rabbit</td>
<td>Central, penetrating</td>
<td>Toluidine Blue</td>
<td>Transformation stromal cells, peak activity 48-72 hours</td>
</tr>
<tr>
<td>1966</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>Rabbit</td>
<td>In vitro, central</td>
<td>Hematoxylin Eosin-PAS</td>
<td>Epithelium intact no transformation stromal cells, epithelium removed transformation stromal cells - 3 days</td>
</tr>
<tr>
<td>1964</td>
<td></td>
<td>penetrating</td>
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## MIGRATING BLOOD CELLS

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<th>Staining</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Maumenee¹³</td>
<td>Rat</td>
<td>Frozen-central, peripheral</td>
<td>None given</td>
<td>PMN's rare, monocytes at lesion, macrophages</td>
</tr>
<tr>
<td>1949</td>
<td>Rabbit</td>
<td></td>
<td></td>
<td>Squashed sections, PMN's and monocytes, lymphocytes only when vascularization</td>
</tr>
<tr>
<td>Weimar¹⁴,¹⁵</td>
<td>Rat</td>
<td>Incision, central</td>
<td>Giemsa</td>
<td>PMN invasion of cornea from limbus beginning five hours increasing to 24 to 36 hours</td>
</tr>
<tr>
<td>1957, 1958</td>
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<tr>
<td>Weimar¹⁶</td>
<td>Rat</td>
<td>Incision, central</td>
<td>Cell count</td>
<td>Leukocytes at lesion after 6 hours via tears, mononuclear cells in periphery after 8 hours</td>
</tr>
<tr>
<td>1952</td>
<td></td>
<td>nonpenetrating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robb¹⁷</td>
<td>Rabbit</td>
<td>Central, peripheral</td>
<td>H + E</td>
<td>Leukocytes via tears and migration 24-48 hours, mononuclear cells by migration 24-48 hours</td>
</tr>
<tr>
<td>1962</td>
<td></td>
<td>nonpenetrating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robb¹⁸</td>
<td>Rabbit</td>
<td>Nonpenetrating incision, burn</td>
<td>Tritiated thymidine</td>
<td></td>
</tr>
<tr>
<td>1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pullinger¹⁹</td>
<td>Rabbit</td>
<td>Mustard gas central</td>
<td>None given</td>
<td>Leukocytes migrate single file 24 hours-1 week, polyblasts by 48 hours, lymphocytes only with pannus</td>
</tr>
<tr>
<td>1943</td>
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**MIGRATING BLOOD CELLS (con't.)**

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<thead>
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<th>Animal</th>
<th>Lesion</th>
<th>Staining</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunnington²⁰</td>
<td>Rabbit</td>
<td>Central freezing with perforating lesion or just lesion</td>
<td>Radio-autograph</td>
<td>A few leukocytes migrating from limbus-24 hours, lymphocytes by fifth day</td>
</tr>
<tr>
<td>1958</td>
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</tr>
<tr>
<td>Basu⁴⁸</td>
<td>Rabbit</td>
<td>Central, peripheral burn</td>
<td>H + E</td>
<td>PMN's at lesion by fourth day</td>
</tr>
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<td>1958</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ashton⁴⁶</td>
<td>Rabbit</td>
<td>Perforating, central</td>
<td>H + E</td>
<td>PMN's and macrophages migrating from limbus 24-48 hours</td>
</tr>
<tr>
<td>1951</td>
<td></td>
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</tr>
<tr>
<td>Weimar⁵²</td>
<td>Rats</td>
<td>Penetrating, central</td>
<td>5-nucleotidase</td>
<td>Frozen sections, leukocytes (polymorphonuclear and mononuclear) and monocytes throughout stroma 6-12 hours</td>
</tr>
<tr>
<td>1967</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wolter⁵⁹</td>
<td>Rabbit</td>
<td>Incisions</td>
<td>Hortega</td>
<td>Flat sections, leukocytes and lymphocytes migrating in straight chains in Bowman's channels</td>
</tr>
<tr>
<td>1958</td>
<td></td>
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</tr>
<tr>
<td>Kitano⁶¹</td>
<td>Rabbit</td>
<td>Central perforating</td>
<td>Toluidine Blue</td>
<td>Monocytes and leukocytes by 48-72 hours</td>
</tr>
<tr>
<td>1966</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Animal</td>
<td>Lesion</td>
<td>Staining</td>
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<tr>
<td>Brachcr</td>
<td>Rabbit</td>
<td>Central perforating</td>
<td>Tritiated markers</td>
<td>Leukocytes between incision and limbus by 6 1/2 hours</td>
</tr>
<tr>
<td>1967</td>
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<tr>
<td>LaTessa</td>
<td>Rabbit</td>
<td>Central nonpenetrating</td>
<td>Uranyl acetate</td>
<td>PMNL's and mononuclear cells in lesion by 24-36 hours</td>
</tr>
<tr>
<td>1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dunnington</td>
<td>monkey</td>
<td>Corneal, limbal incisions</td>
<td>Toluidine Blue</td>
<td>PMNL's and large mononuclear cells by third day</td>
</tr>
<tr>
<td>1957</td>
<td>cat</td>
<td></td>
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</table>
Figure 1  Surgery was performed on an anesthetized rabbit using a scapel. The biomicroscope was used during surgery, so that greatest possible control over creation of the lesion could be maintained.
Figure 2  Top: Lesion as seen with slit lamp immediately after surgery. Note gray line indicating the lesion.

Bottom: Lesion as seen with slit lamp after 48 hours healing time. Gray line indicates the lesion.
Figure 3  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing wedge-shaped lesion 15 minutes after surgery.

The walls of the lesion show epithelial cell debris. (260X)

Figure 4  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion 30 minutes after surgery. (670X)
Figure 5  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion three hours after surgery.

Note heterophils on wall of wedge-shaped wound (arrows). (670X)

Figure 6  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion three hours after surgery.

Typical heterophils are seen at base of lesion (a) and in the interfibrillar spaces on both sides of the wound (arrows). (260X)
Figure 7  Meridional section of rabbit's cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion three hours after surgery. Note that the stromal fibers show lack of organization. A sheet of basal cells has advanced into the lesions; typical heterophils appear in the lesion and in the interfibrillar spaces surrounding the wound (arrows). (670X)

Figure 8  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion three hours after surgery. Note that the stromal fibers show lack of organization. A sheet of basal cells has advanced into the lesion; typical heterophils appear in the lesion and in the interfibrillar spaces surrounding the wound (arrows). (670X)
Figure 9  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with Masson's trichrome stain showing the lesion four hours following surgery.

Note the tongue of epithelial cells sliding into the fault (arrow).

Heterophils (h) are seen migrating from the lesion into the interfibrillar spaces. (670X)

Figure 10  Section of rabbit bulbar conjunctiva fixed in Zenker's fluid embedded in paraffin/parlodion and stained with toluidine blue showing increased numbers of heterophils in subepithelial connective tissue and accelerated heterophil migration through the epithelium into the tear film. (260X)
Figure 11  Sections of rabbit bulbar conjunctiva fixed in Zenker's fluid embedded in paraffin/parlodion and stained with toluidine blue showing evidence of accelerated heterophils (arrows) migration through the epithelium into the tear film. (670X)

Figure 12  Sections of rabbit bulbar conjunctiva fixed in Zenker's fluid embedded in paraffin/parlodion and stained with toluidine blue showing evidence of accelerated heterophils (arrows) migration through the epithelium into the tear film. (670X)
Figure 13 Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing the lesion five hours after surgery. Note the heterophils in the lesion and in the interfibrillar spaces. (670X)

Figure 14 Meridional section of corneal epithelium fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion in central cornea at six hours after surgery. The wound has been filled with epithelial cells; heterophils are still seen in the interfibrillar spaces (arrows). (260X)
Figure 15  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing the lesion 12 hours after surgery. Note the tongue-like process of epithelial cells which appear to be sliding into the stromal fault. No heterophils are seen in the lesion. (670X)

Figure 16  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing the lesion 12 hours after surgery. Note that epithelial cells have filled the stromal fault. A few heterophils may be seen in the interfibrillar spaces. (670X)
Figure 17 Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion 24 hours after surgery. The stromal fault is filled with epithelial cells. Very few heterophils are seen near the lesion at this stage. (670X)

Figure 18 Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion one week after surgery. (670X)
Figure 19  Meridional section of rabbit cornea fixed in Zenker's fluid, embedded in paraffin/parlodion and stained with toluidine blue showing lesion two weeks after surgery. (330X)
BIBLIOGRAPHY


