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Repair and Reconstruction of the Mouse Lens after Perforating Injury

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Purpose: To investigate the process of repair in the epithelial cells and the reconstruction in lens fibers of a mouse lens that has developed opacity because of a large-scale perforating injury.

Methods: Lenses of 4-week-old mice were perforated with a needle through the cornea to induce the development of traumatic cataracts. Over the period from the first day to the fifth month after injury, eyeballs were extracted progressively from the mice and changes in the epithelial cells of these lenses were observed morphologically as well as histochemically.

Results: Following the injury, the epithelial cells of the lenses extended toward the center of the injury, while undergoing repeated proliferation and stratification. After a month, the epithelial cells completely covered the entire injury. Later, a basal lamina and collagen fibers developed among the epithelial cells that had proliferated, the intracellular space enlarged, but the number of cells decreased. Histochemically, a strong actin-positive finding was observed in the epithelial cells in the growth phase. On the other hand, an investigation by means of the TUNEL method revealed epithelial cell death and a decrease in cell number. Maximal cell death was observed in the second month. During this period, lens fibers regenerated, and the clear areas of the cortex increased.

Conclusion: Although the lenses exhibited opacity over a large area, the epithelial cells eventually fully covered the injured area. Once the repair was completed, the number of epithelial cells decreased. At the same time, the lenses were found to have developed increased clarity, leading to reconstruction. The epithelial recovery and the residual posterior suture may be the key to the reconstruction of the lenses. **Jpn J Ophthalmol 2003;47:338–346** © 2003 Japanese Ophthalmological Society

Key Words: Lens epithelium, lens reconstruction, lens repair, mouse lens, traumatic cataract.

Introduction

Perforating wounds and foreign bodies that enter the eye cause traumatic cataracts. Their clinical manifestations vary widely from simple adhesion of iris pigment on the lens surface to anterior/posterior subcapsular cataracts and lens rupture. It has been reported that pathological cataract-causing factors include rapid deterioration of the lens epithelium and denaturation of the cortical fibers.[1] These reports described the mechanisms of repair from experiments in frogs,[2] mice,[3,4] and rabbits[5] in which the lens was injured with a needle and then underwent repair by means of active growth of the lens epithelium. Fagerholm et al[6] pointed out that the speed of sealing from the external environment was a critical factor in determining whether or not cataracts developed. This sealing speed seems to be an important factor in determining the development of traumatic cataract. Recently, Wakasugi et al,[7] who examined the mouse lens, reported that, when aqueous humor entered the anterior chamber from the injury, the position of its retention differed depending on the size of the injured area, which in turn caused a difference in the sites where either an anterior or posterior subcapsular cataract developed. They classified the relationship between the aqueous humor and the development of cataract. Uga,[8] who investigated the mouse lens after small area injury, reported on the mechanism of epithelial repair function in the lens. However, there seems to be little known about the mechanism of recovery from lens opacity arising from wide-scale injury.

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In the present study, the mouse lens was injured over a large area in order to induce opacity in the anterior capsular area, and the progressive repair function in the epithelium was studied morphologically and histochemically. The mechanism of the reconstruction of the opaque lens was examined over a long period.

Materials and Methods

One hundred and sixty eyes from 80 female 4-week-old ddY mice were used in this study. These animals were raised under the conditions of a 12-hour light-dark/ cycle and the provision of food and water ad libitum. All the animals were treated in accordance with the ARVO resolution regarding animals used in research. They were subjected to general anesthesia by intraperitoneal injection of sodium pentobarbital at the dose of 30 mg/kg. The anterior surface of the lens was injured by pricking a needle (0.6 mm in diameter) in the 2-mm section from the needle tip, via the cornea, from the surface of the eyeball to a depth of 2 mm. After injury, a small amount of antibiotic ointment was administered. Eyeballs were progressively enucleated under sodium pentobarbital anesthesia at 1, 7, 14 days, and 1, 2, 3, 5 months after injury. The control group consisted of 40 age-matched ddY mice.

Extirpated eyeballs were examined by four methods: For light and electron microscopy, some eyes were fixed with 4% glutaraldehyde-0.1 M phosphate buffer for several days. During this fixation, the lenses were cut in half and observed under a dissecting microscope. After being washing in the same buffer solution, the samples were postfixed in 1% osmic acid-phosphate buffer, dehydrated by a series of ethanol and embedded in epoxy resin. Thick sections were stained with toluidine blue and observed under a light microscope. Ultrathin sections were double-stained with uranyl acetate and lead citrate, then observed under an electron microscope.

For flat preparations of the lens epithelium, some eyes were fixed in alcohol acetate for 2 days, and were preserved in 70% alcohol. After placing a lens in water, the lens epithelium was peeled off under a dissecting microscope, placed on a glass slide, and dried naturally. These specimens were stained with hematoxylin, dehydrated, embedded in balsam and observed under a light microscope.

To determine the histochemical reaction of actin, some eyes were fixed in 4% paraformaldehyde-0.05 M phosphate buffer for 1 day. After washing with sucrose-PBS (0.01 M phosphate buffer, pH 7.2, 0.9% NaCl, 7% sucrose) overnight, the samples were sectioned at 7μm thickness by a microtome for sectioning frozen samples, stained with BODIPY®-phalloidin (Molecular Probes, Eugene, OR, USA) for 1 hour at room temperature, and observed under a confocal laser microscope.

For detection of cell death, terminal deoxynucleotidyl transferase (TdT)-mediated -dUTP-biotin nick end labeling (TUNEL) method was performed using the ApopTag In Situ Apoptosis Detection Kit (Intergen, Purchase, NY, USA). Briefly, some frozen samples were treated with proteinase K in a concentration of 2 μg/mL and reacted with terminal deoxynucleotidyl transferase for incorporation of dUTP-biotin to nick end. The incorporated dUTP-biotin was reacted with peroxidase-labeled avidin and visualized with 0.2 mg/mL diaminobenzidine tetrahydrochloride (DAB)(Dojin Chemical, Kumamoto), and 0.005% hydrogen peroxide.

Results

Lens opacity developed anteriorly in 121 of the 160 eyes; opacity developed posteriorly in 26 eyes; and clarity persisted in the remaining eyes. Of these 3 groups of eyes, only those lenses that developed anterior opacity were used as test materials. The opaque lenses with anterior opacity were easily reproducible.

On the first day after injury, mature cataracts had already developed in the lenses. Sagittal sections revealed that the opaque sites had slightly deviated toward the anterior side of the lenses, and there was a considerable amount of transparent posterior cortex (Figure 1a). Epithelial cells of the injured lens regressed toward the lateral side of lens (Figure 1b). Electron microscopically, two layers of cells were noted at the edge of the epithelium, of which the inner layer consisted of small cells, suggesting a leading edge cell (Figure 1c). Histochemically, the cytoplasm of the cells at the edge of the epithelium was filled with an increased amount of actin (Figure 1d). In the control cells, a large amount of actin was found at the apex and base of the epithelial cells, with a small amount existing in the middle (Figure 1e). Electron microscopical examination showed that more intracellular filaments were found at the apex of the cells (Figure 1f). These filaments were comparable to a strongly positive area for actin.

On the fourteenth day after injury, histological observation showed swelling and stratification at the edge of the epithelial cells (Figure 2a). From the electron microscopic observations, the proliferated cells contained well-developed rough endoplasmic reticulum around an elliptical nucleus, but relatively few mitochondria were present (Figure 2b). When the cytoplasm was observed at high magnification, intracellular filaments were found to exist in a large number (Figure 2c). Histochemically, actin

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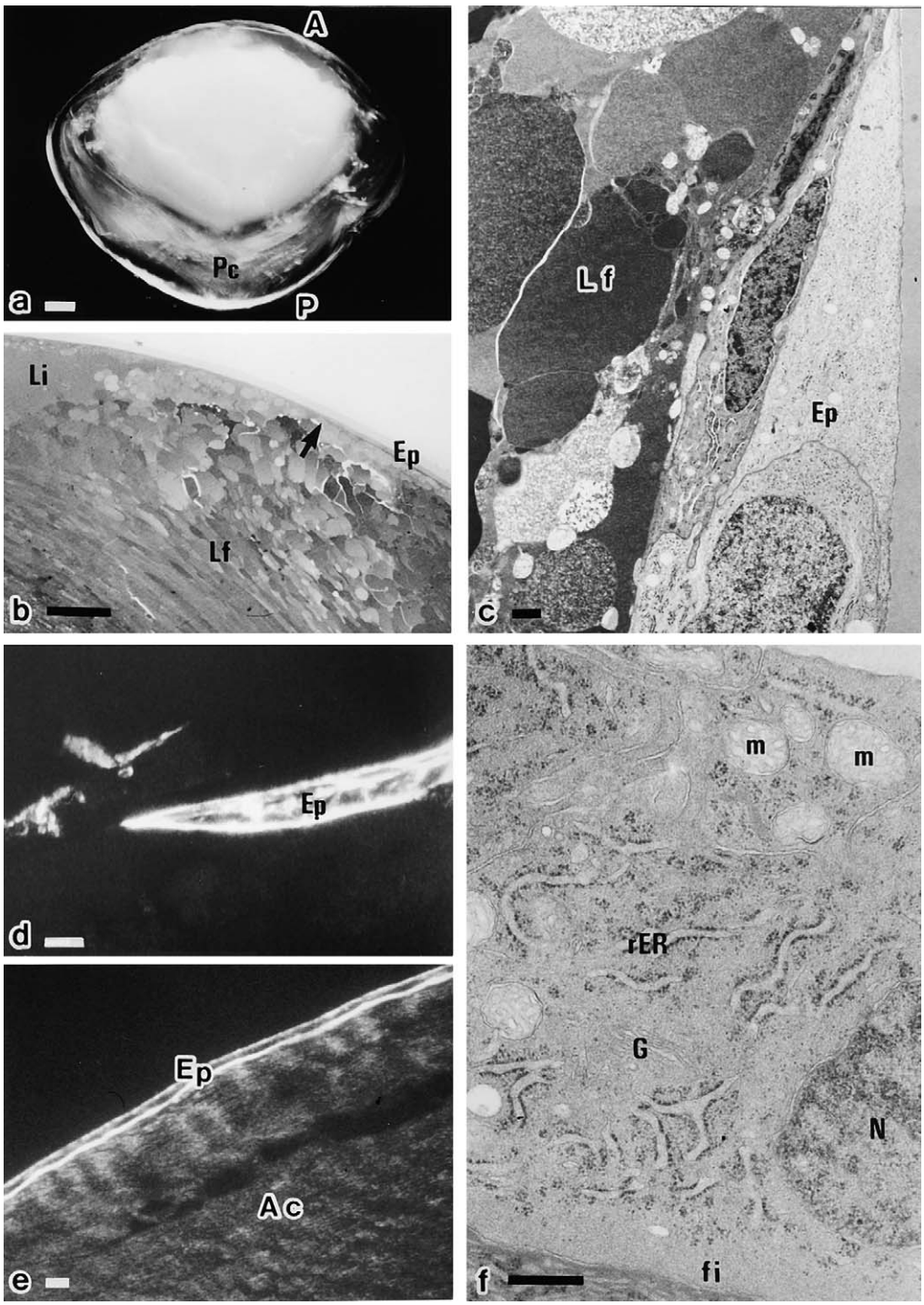

Figure 1a. Sagittal section of the mouse lens at 1 day after large area injury. Opaque area is seen at the anterior two-thirds of the lens. The posterior cortex (Pc) containing the equatorial region is undamaged. A: anterior side, P: posterior side. Bar = 200μm. **1b.** Photomicrograph of the sectioned specimen at 1 day after injury. The epithelial (Ep) edge faded by injury is indicated by the arrow. Lens fibers (Lf) facing the liquefied area (Li) are swollen. Bar = 50μm. **1c.** Electron micrograph showing the epithelial (Ep) edge at 1 day after injury. A slender cell attaches to the posterior surface of the tapering cell at the edge. Swollen lens fibers (Lf) are seen. Bar = 1μm. **1d.** Confocal micrograph showing actin reaction at the epithelial edge (Ep) at 1 day after injury. Bar = 20μm. **1e.** Confocal micrograph showing actin reaction in the epithelial cells (Ep) of the control lens. Strong positive reaction is seen at the cortical side of the cell rather than at the capsular side. Ac: anterior cortex. Bar = 20μm. **1f.** Electron micrograph showing the cytoplasm of an epithelial cell of a control lens. A thick layer of filaments (fi) is present at the cortical side. G: Golgi apparatus, m: mitochondria, N: nucleus, rER: rough endoplasmic reticulum. Bar = 0.5μm.

was found abundantly in the surroundings of the stratified cells, but was found in only a small amount in the central part (Figure 2d).

In the first month after injury, sagittal sections of the lenses still showed mature cataracts. The nuclei had shifted anteriorly to a slight extent (Figure 2e). Flat preparation of the epithelial cells showed that stratified epithelial cells had completely covered the injured site (Figure 2f). Histologically, single-layer epithelial cells became stratified towards the central portion consisting of 7 to 8 layers (Figure 3a). Electron microscopically, the proliferated cells were found to consist of dark and bright cells (Figure 3b). Higher magnification of the cytoplasm showed many filaments with a somewhat sparse course (Figure 3c). Histochemically, strong positivity for actin was found in both the surface and deep layers of the proliferated epithelial cells (Figure 3d). When the extent of cellular death was investigated by means of the TUNEL method, no positive cells were observed in the control epithelial cells at this stage (Figure 3e). On the other hand, there were a few cells with weak positivity in the proliferated cells (Figure 3f).

In the second month after injury, opacity decreased in the sagittal lens sections, and the degree of clarity of the cortex was found to have increased in the area ranging from the equator to the anterior cortex (Figure 4a). Histologically, the cells were entangled with one another to form a network structure at the base of proliferated cells. In the superficial layer of these cells, spindle-shaped cells were distributed either alone or in contact with one another (Figure 4b). A network structure at the base of proliferated cells was also confirmed by electron microscopic observation. In the intercellular spaces a basal laminar structure and cell debris were observed (Figure 4c). At higher magnification of the cytoplasm a large number of filaments were observed (Figure 4d). Among the proliferated cells, those that had undergone cellular death were observed by electron microscopy. These cells contained some characteristic granular substances (Figure 4e). A histological study of actin showed that many cells still were actin-positive (Figure 4f). A study of cell deaths by means of the TUNEL method detected a relatively large number of positive cells (Figure 4g).

In the fifth month after injury, only the perinuclear zone was opaque, and most parts of the lens were replaced by a transparent cortex (Figure 5a). Histologically, there was only an extremely small number of proliferated epithelial cells, and the stratified portion had also decreased (Figure 5b). Electron microscopic observation showed flattened epithelial cells and the capsule were retained around these cells (Figure 5c). Histochemically, actin-positive cells were relatively few in number (Figure 5d). The number of TUNEL positive cells had decreased remarkably (Figure 5e). Electron microscopic examination showed that atrophic lens fibers, forming membranous whirls at their ends in the deep anterior cortex, were compressed by newly elongating lens fibers immediately beneath the epithelium (Figure 5f).

Discussion

In this study mature cataracts tended to develop routinely with an anterior orientation if the injury of the lens epithelial cells extended over an area of 100–200μm in diameter. In this case, the more closely the epithelial cells approached the side of the lens, the greater was the number of cells that were lost. Epithelial cells were found to cover the injury gradually, when cell stratification to form 2–3 layers occurred in the first week and 5–6 layers in the second week. By about a month after injury, the central part of the injury was completely covered with epithelial cells that were stratified into 7–8 layers. Histochemically, a strong actin-positive finding was obtained from the epithelial cells in the repair period.

It has been reported that actin in the epithelium of the lens plays a role in accommodation.[9] It has been pointed out that actin served as the basic engine in the crawling or gliding locomotion of the cell movements at the time of repair.[10] In the observation of this study, cells were found to move while they were growing, and a strongly actin-positive finding was observed in these cells. There are reports that leading edge cells play a significant role in the repair of injury in the stratified squamous epithelium of cornea, when the leading edge cells are coordinated in cell movement.[11,12] However, it is still unknown whether or not these leading edge cells are involved in the cell movement at the time of lens repair and reconstruction.

This study revealed that, once the injured area had been covered, the proliferated epithelial cells produced type-I and type-IV collagen among the cells,[8] thus increasing the intercellular spaces in the second post-injury month. Histochemically, the actin-positive finding persisted in the proliferated cells, but when cellular deaths were examined by the TUNEL method, positive findings were still found frequently in isolated epithelial cells. This indicates that there is a decrease in actin-positive findings and a decline in the reactions, with a peak in the first month, but that positive cells detected by the TUNEL method showed an increase in the second month and a subsequent rapid decrease in numbers. These observations indicate that epithelial cells play the major roles of cell growth and cell movement during the period of injury repair and reconstruction, but once the injury has been covered, the

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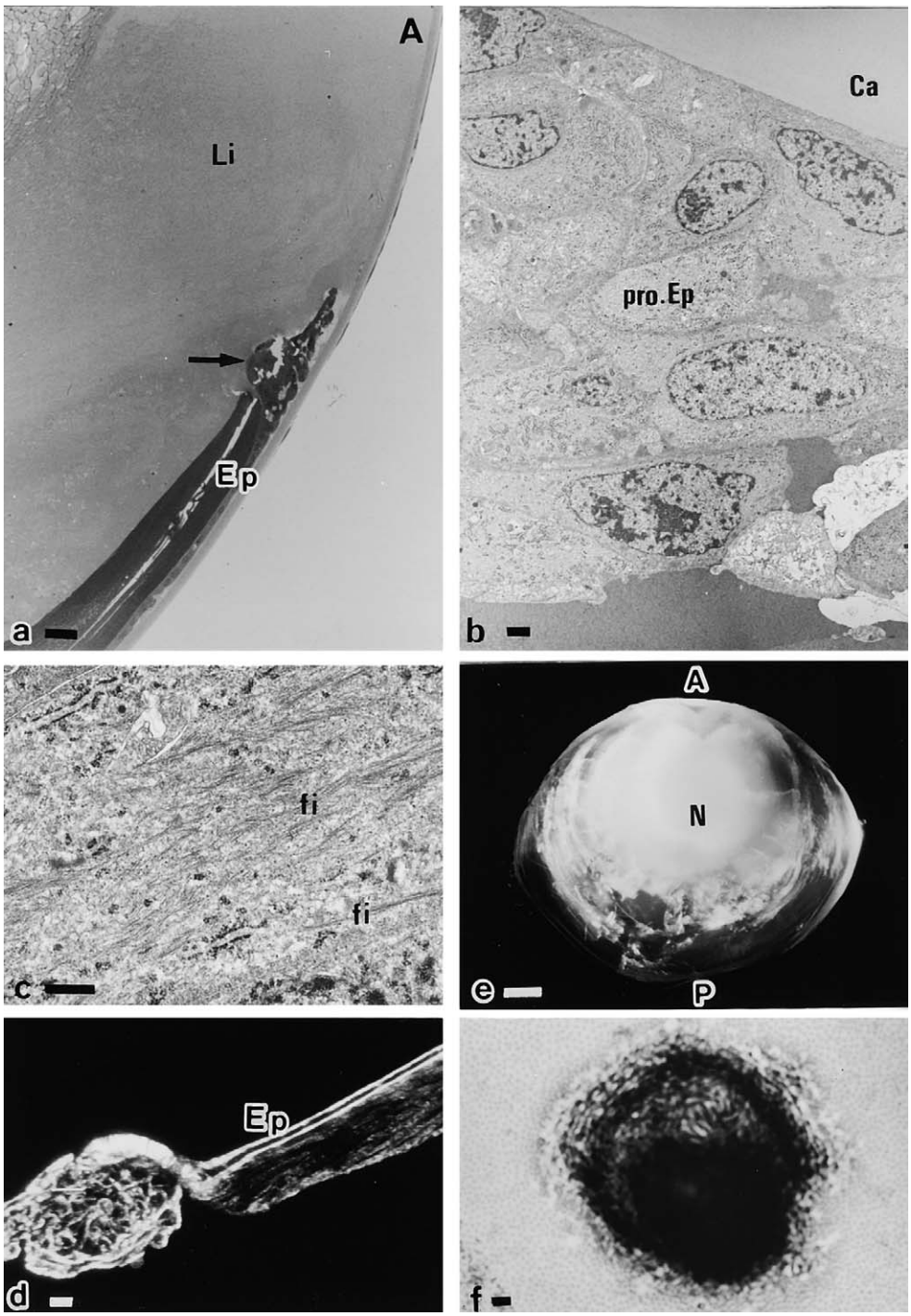

Figure 2a. Photomicrograph of the anterior part of the lens at 2 weeks after injury. Stratification at the epithelial edge (Ep) is more remarkable. A: anterior side, Li: liquefied area. Bar = 20μm. **2b.** Electron micrograph showing stratification at the epithelial edge at 2 weeks after injury. Proliferated epithelial cells (pro.Ep) are intimately in contact with each other. Ca: capsule. Bar = 1μm. **2c.** Higher magnification showing the filaments (fi) in the cytoplasm of the epithelial cells in Figure 2b. Bar = 0.5μm. **2d.** Confocal micrograph of the stratified cells at the epithelial edge (Ep) at the same stage as in Figure 2a. Strong positive reaction of actin is seen at the periphery of the stratified cells. Bar = 20μm. **2e.** Sagittal section of the lens at 1 month after injury. Opaque area becomes slightly smaller. The lens nucleus (N) is dislocated anteriorly. A: anterior side, P: posterior side. Bar = 200μm. **2f.** Flat preparation of the epithelial cells at 1 month after injury. Damaged area is completely covered with proliferated epithelial cells. Bar = 20μm.

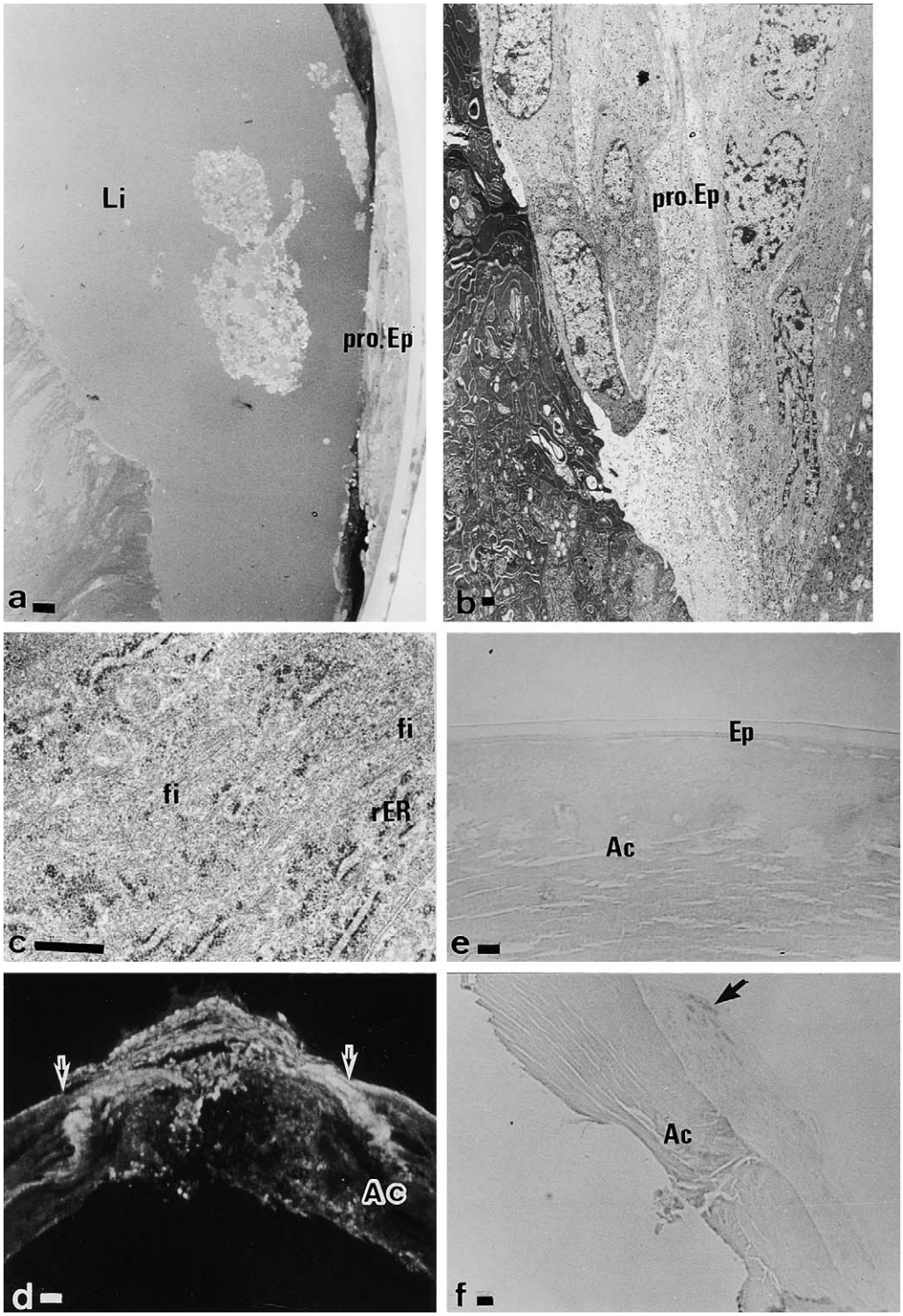

Figure 3a. Photomicrograph of the anterior part of the lens at 1 month after injury. Stratification of proliferated epithelial cells (pro.Ep) becomes markedly thick at the central portion. Liquefied area (Li) is still present just beneath the epithelium. Bar = 20μm. **3b.** Electron micrograph showing stratification of proliferated epithelial cells (pro.Ep) at 1 month after injury. Bar = 1μm. **3c.** Higher magnification of the cytoplasm of the proliferated epithelial cells. Filament bundles (fi) are seen. rER: rough endoplasmic reticulum. Bar = 0.5μm. **3d.** Confocal micrograph of the proliferated epithelial cells at 1 month after injury. Strong positive reaction of actin is seen throughout the area between arrows. Ac: anterior cortex. Bar = 30μm. **3e.** Confocal micrograph of the epithelial cells (Ep) of the control lens. No TUNEL positive cells are seen. Ac: anterior cortex. Bar = 20μm. **3f.** Confocal micrograph of the epithelial cells at 1 month after injury. A few positive cells are seen at the surface of the proliferated epithelium (arrow). Ac: anterior cortex. Bar = 20μm.

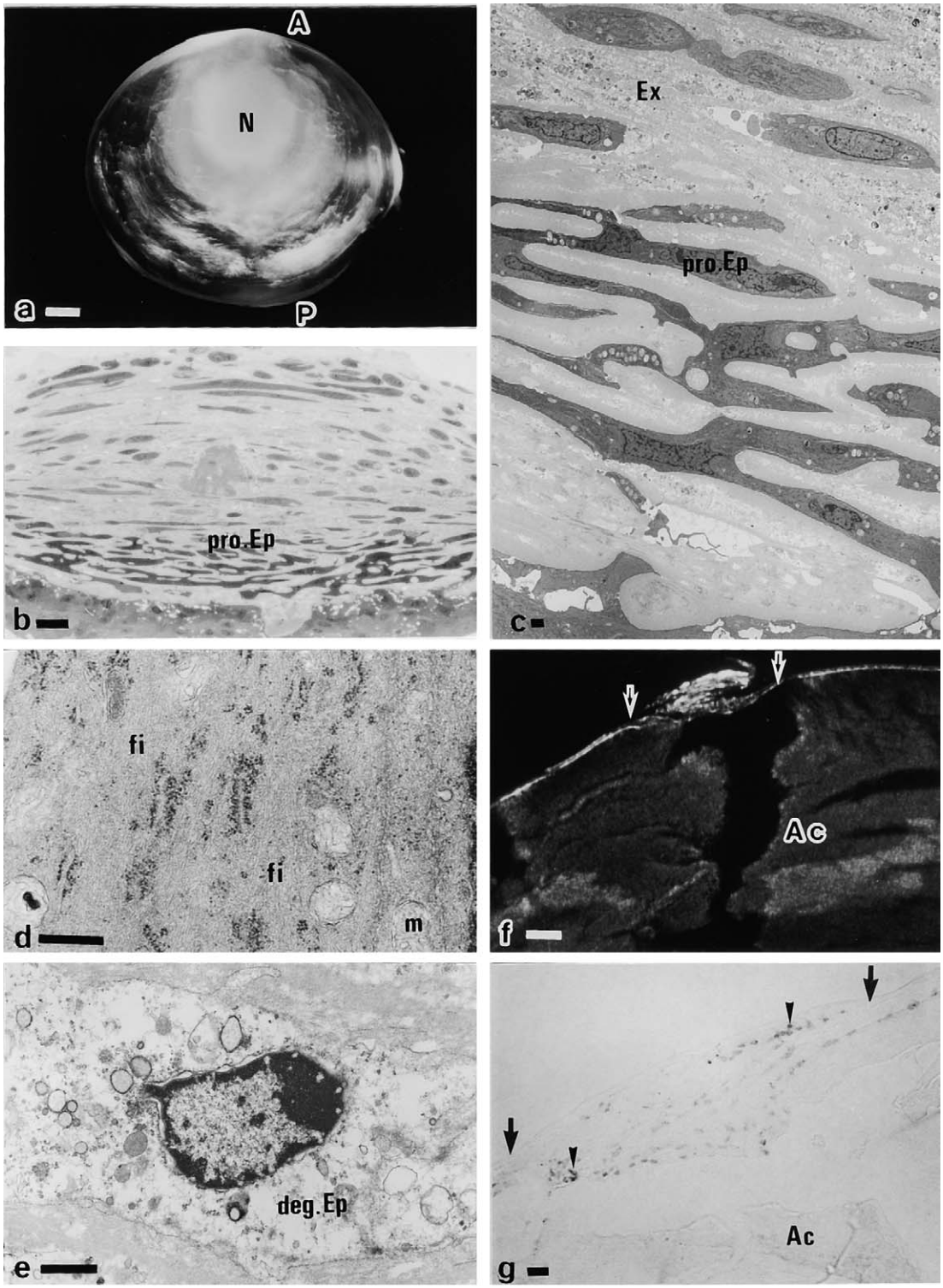

Figure 4a. Sagittal section of the lens at 2 months after injury. Opaque area is clearly reduced. The lens nucleus (N) is dislocated anteriorly. A: anterior side, P: posterior side. Bar = 200μm. **4b.** Photomicrograph of the wounded portion at 2 months after injury. The proliferated epithelial cells (pro.Ep) become slender, and extracellular space is enlarged. Bar = 20μm. **4c.** Electron micrograph showing the proliferated epithelial cells at the wound at 2 months after injury. The cells form a complicated network. The basal lamina and cell debris are seen in the enlarged extracellular space (Ex). pro.Ep: proliferated epithelial cells. Bar = 1μm. **4d.** Higher magnification of the cytoplasm of the proliferated epithelium. The filaments (fi) are abundantly seen. m: mitochondoria. Bar = 0.5μm **4e.** Electron micrograph showing a degenerating cell (deg.Ep) within the proliferated epithelium at 2 months after injury. The cell contains some granular substances. Bar = 1μm. **4f.** Confocal micrograph of the proliferated epithelium at the wound at 2 months after injury. Positive reaction of actin is seen between arrows. Ac: anterior cortex. Bar = 100μm. **4g.** Confocal micrograph showing TUNEL-positive cells (arrowheads) at the wound (between arrows) at 2 months after injury. Ac: anterior cortex. Bar = 20 μm.

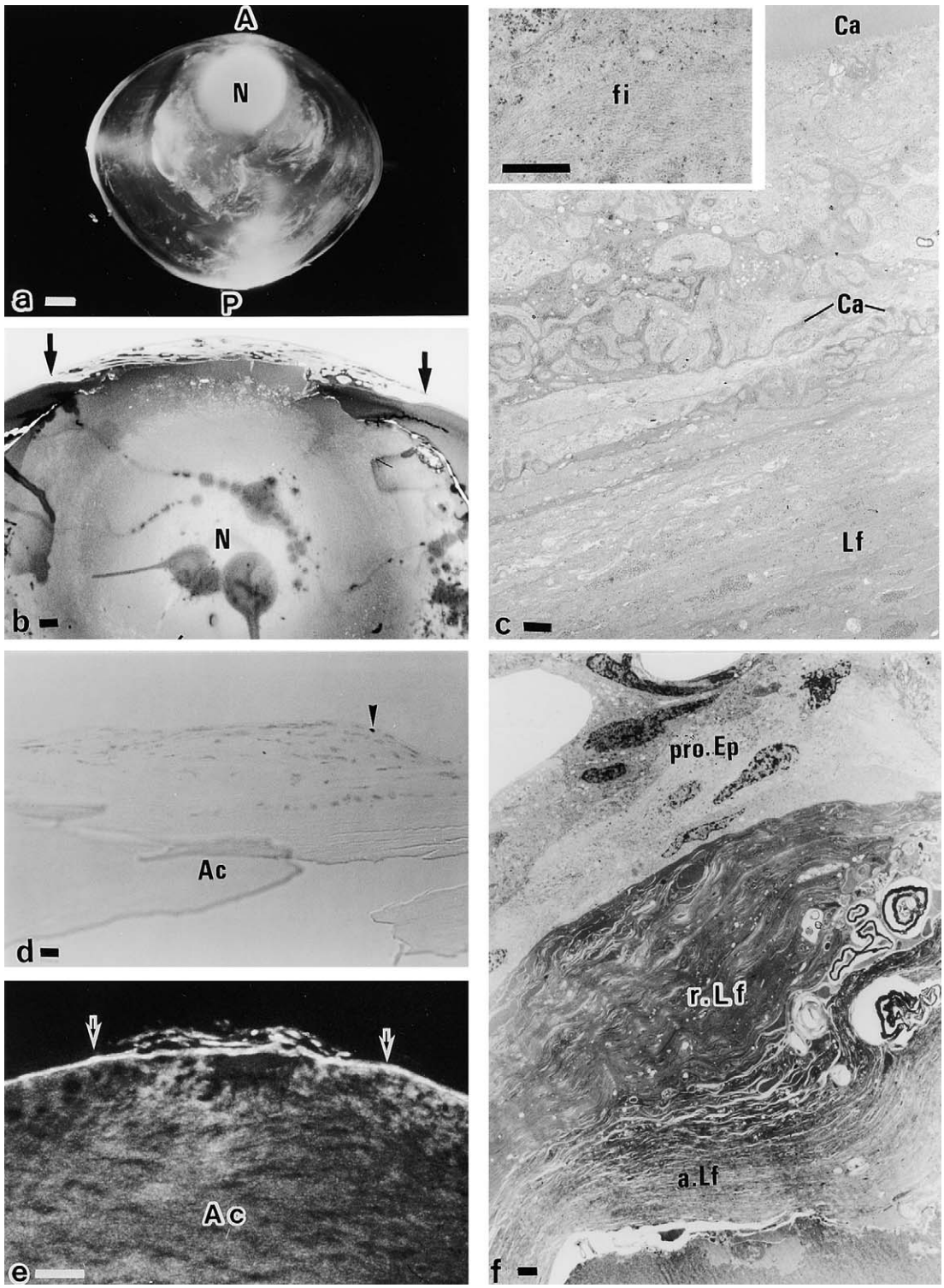

Figure 5a. Sagittal section of the lens at 5 months after injury. Opaque area is confined in the nucleus (N), whereas transparent area of the lens is markedly increased. A: anterior side, P: posterior side. Bar = 200μm. **5b.** Photomicrograph of the anterior part of the lens at 5 months after injury. Proliferated cells on the wound are markedly reduced (between arrows). The lens nucleus (N) is dislocated to nearby the wound. Bar = 50μm. **5c.** Electron micrograph showing complicated capsule (Ca) at the wound at 5 months after injury. Ca: capsule. Lf: lens fiber. Bar = 1μm. Inset: The epithelial cells at the wound contain a small number of filaments (fi) in the cytoplasm. Bar = 0.5μm. **5d.** Confocal micrograph showing positive reaction of actin in a few epithelial cells at the wound (at arrowhead) at 5 months after injury. Ac: anterior cortex. Bar = 20μm. **5e.** Confocal micrograph showing TUNEL-positive cells at the wound (between arrows). Positive reaction is seen in only a few cells. Ac: anterior cortex. Bar = 20μm. **5f.** Electron micrograph of the anterior cortex replacing atrophic lens fibers (a.Lf) with newly elongating lens fibers (r.Lf). The former fibers form membranous whirls at their cell ends. pro.Ep: proliferated epithelial cells. Bar = 1μm.

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epithelial cells show a continuous decrease. The arrangement of the lens is restored to the former single-layer structure, but scar tissue persists.

The mitotic activity of the normal lens epithelium is relatively high in adult rabbits, cats and dogs when compared with the dividing cell number per lens. Among other animal species, monkey lenses have only a small number of dividing cells. On the other hand, when the number of mitoses was calculated per 100,000, mice and rats had the highest activity, whereas dogs and monkeys were lower in dividing activity than other species.[13] The mitotic activity of the human lens may resemble that of the monkey lens. It is considered that the lens epithelium after injury shows high cell activity in all animal species, even including the human lens.

A regenerating phenomenon was observed in this study. After a large-scale injury was created in the anterior part of the lens, a mature cataract developed, but gradually the opacity of the lens decreased, and instead, the transparent portion increased in size. In this case, opacity developed in a slightly anterior portion of the lens, but the equatorial epithelium, lateral cortex, and posterior cortex remained normal. If there is no obstruction to the differentiation of the equatorial epithelial cells into lens fibers, and if the posterior suture is closed, then once the repair of the injured epithelium has occurred, the opaque portion of the lens is replaced gradually with an increasing number of normal lens fibers, starting from the equator. At this time, opaque lens fibers are not engulfed by macrophages, but are found to decrease due to compression by the surrounding normal lens fibers and due to the atrophy of the opaque lens fibers themselves. It has been reported that regeneration of the lens is also observed in genetic cataract in rats of the SCR strain[14] and in traumatic cataract in humans.[15] However, in the case of SCR rats, reconstruction was only half completed because the epithelial cells of SCR rats are inhibited genetically from differentiation into lens fibers. The results of the present study showed that the lens could be reconstructed, depending on the site of the injury. The reconstructive phenomenon of the human lens after perforating injury may be fundamentally similar to that of the mouse lens indicated by the present study.

In summary, in the present study, mice lenses were injured over a wide area by puncturing the anterior part of the lens with a needle to induce the formation of a mature cataract. One month was required for mice to recover from the injury by means of epithelial cell proliferation. During that period, the presence of actin-positive cells was found to be associated with the movement of injured epithelial cells toward the central portion. Furthermore, a study based on the TUNEL method revealed that the epithelial cells that had proliferated underwent cell death, following a peak in the second month.

Repair and reconstruction of the epithelial cells occurred, and at the same time, lens fibers underwent reconstruction. Opaque lens fibers were compressed and atrophy occurred in these lens fibers. Accordingly, the transparent part of the cortex increased, leading to reconstruction of the lens. A key factor underlying this phenomenon might be the encircling and compressing action of lens fibers from the entire periphery of the lens.

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