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Response of the Mouse Lens to Varying Sizes of Injured Area

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Purpose: To examine the response of the lens to varying sizes of perforating injury.

Methods: Four-week-old mice were used. Injuries consisted of pricking in the central region of the lens by transcorneal insertion of needles of two different sizes. After injury, the eyeballs were removed sequentially at various intervals up to 30 days and examined morphologically.

Results: The mouse lens showed three patterns of reaction; retained transparency, posterior opacity, and anterior opacity. (1) When the lens remained clear, the injury was small in area. The damaged portion of the lens was repaired by epithelial proliferation. (2) When opacity occurred abruptly at the posterior cortical area, epithelial damage was mild and lens fiber damage was relatively severe. Evans blue dye moved toward the posterior polar region along the cortical fiber arrangement. (3) When opacity developed abruptly at the anterior cortex, the size of epithelial damage and the damage to lens fibers were extensive. The posterior cortex remained transparent. Evans blue dye remained in the anterior polar region just beneath the injured part.

Conclusion: It was found that the size of the injured area is a determinant of repair or opacity, and the site of opacity is dependent on the severity of epithelial damage and the location of the liquefied area. **Jpn J Ophthalmol 2002;46:391–400**

Key Words: Anterior opacity, epithelial repair, injury size, liquefaction, posterior opacity.

Introduction

Traumatic cataract accounts for 5–10% of all traumatic ocular cases.[1] In general, traumatic cataracts are classified as contusion cataracts caused by a strong force, such as blunt trauma to the eyeball, or as perforating cataracts that arise from trauma to the lens caused by perforation of the cornea and sclera by sharp objects made of metal, wood, or glass.

The incidence of perforating cataract accounts for the greater part of traumatic cataract cases.[1] The mode of onset of perforating cataract varies with the depth and the degree of injury. Moreover, there is a diversity of form, including anterior subcapsular cataract, posterior subcapsular cataract, and lens rupture, with the anterior subcapsular cataract being the most frequently occurring type.

Although various factors, such as the regenerative capacity of epithelial cells and functional recovery of the posterior suture, are known to be significant responses to the swelling of lens fibers,[2] and the rapid deterioration and trauma of the lens epithelial cells

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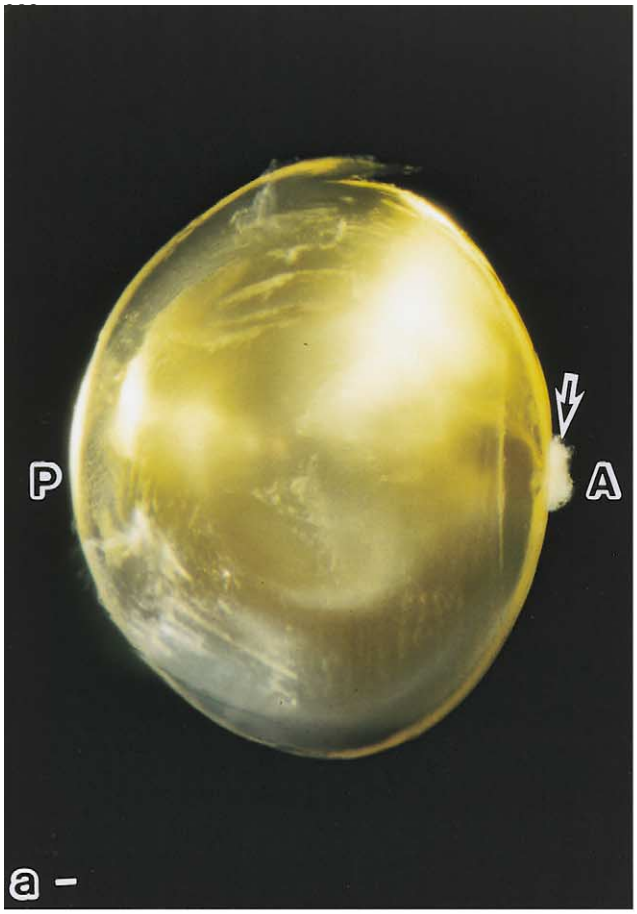
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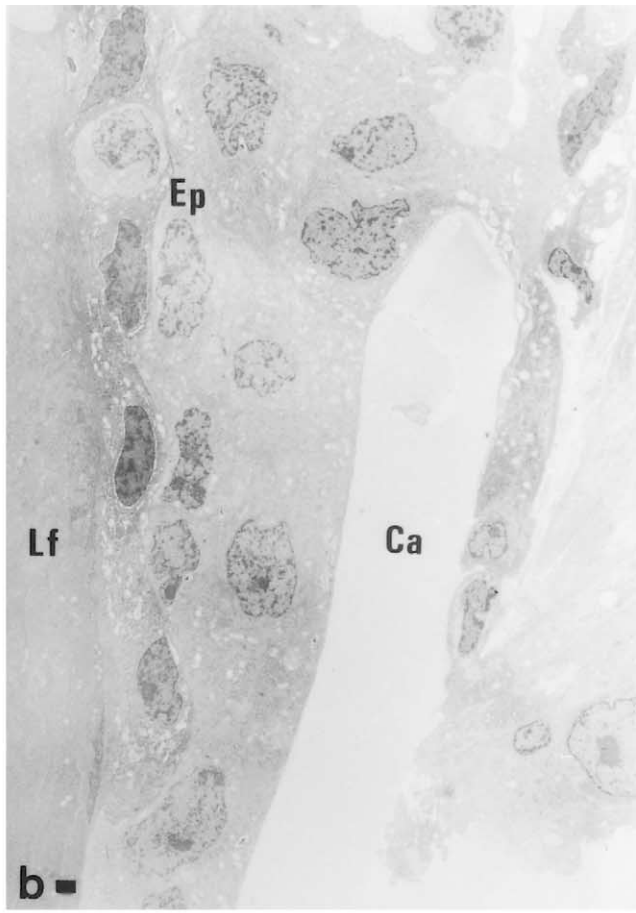
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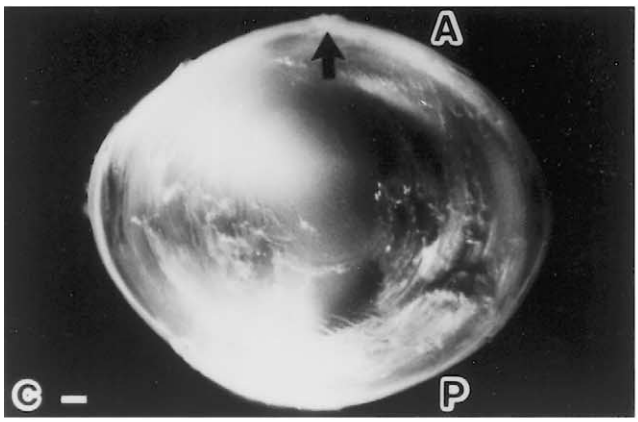
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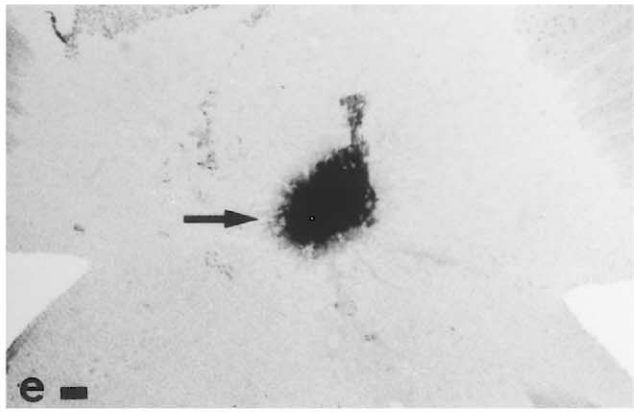
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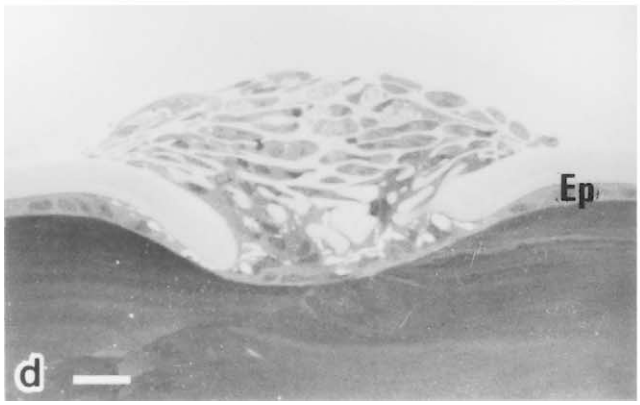
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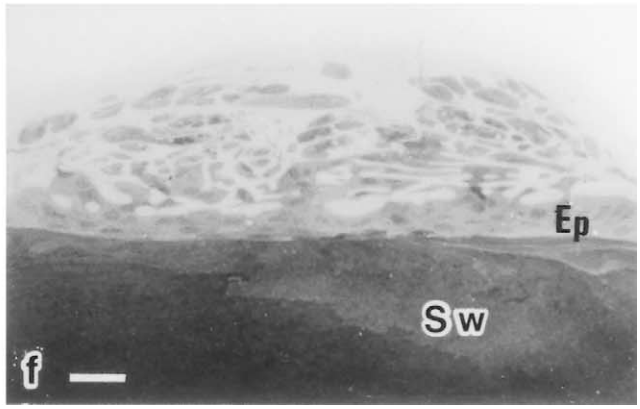
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in human and animal models, the precise formative mechanism of traumatic cataract remains obscure.[3–5]

In previous studies, we injured the mouse lens through the cornea with a needle and investigated the subsequent response of the lens to the trauma. These results revealed that a lateral injury of the lens had the effect of accelerating progression of cataract, that the lens recovered from a shallow injury,[6] and that a deep injury extending to the perinuclear zone caused rapid development of opacity.[7]

In the present study, we conducted a morphological investigation to determine the effect on the lens of differences in the size of the trauma at the anterior pole of the lens.

Materials and Methods

One hundred and twenty eyes from 60 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 inserting needles of two different diameters (diameter of the 2-mm section from the needle tip: either 0.3 mm or 0.6 mm needle) via the cornea, from the surface of the eyeball to a depth of 2 mm. The needle with a 0.6-mm diameter was used mainly to evoke the response of the lens to large area injury. After injury, a small amount of antibiotic ointment was administered. Eyeballs were progressively enucleated under sodium pentobarbital anesthesia at 4, 8, and 24 hours and 5, 7, 20, and 30 days after injury. The control group consisted of 20 age-matched ddY mice.

Extirpated unilateral eyeballs were fixed in 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 dissection, the injured areas were roughly divided into three types according to the extent of injury, and light microscopic observations were made to confirm the division into small, intermediate, and large injury area samples. These samples were postfixed in 1% osmic acid—phosphate buffer, followed by an ethanol series and embedding 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.

The contralateral 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 slide glass, and dried naturally. These specimens were stained with hematoxylin, dehydrated, and embedded in balsam and observed under a light microscope.

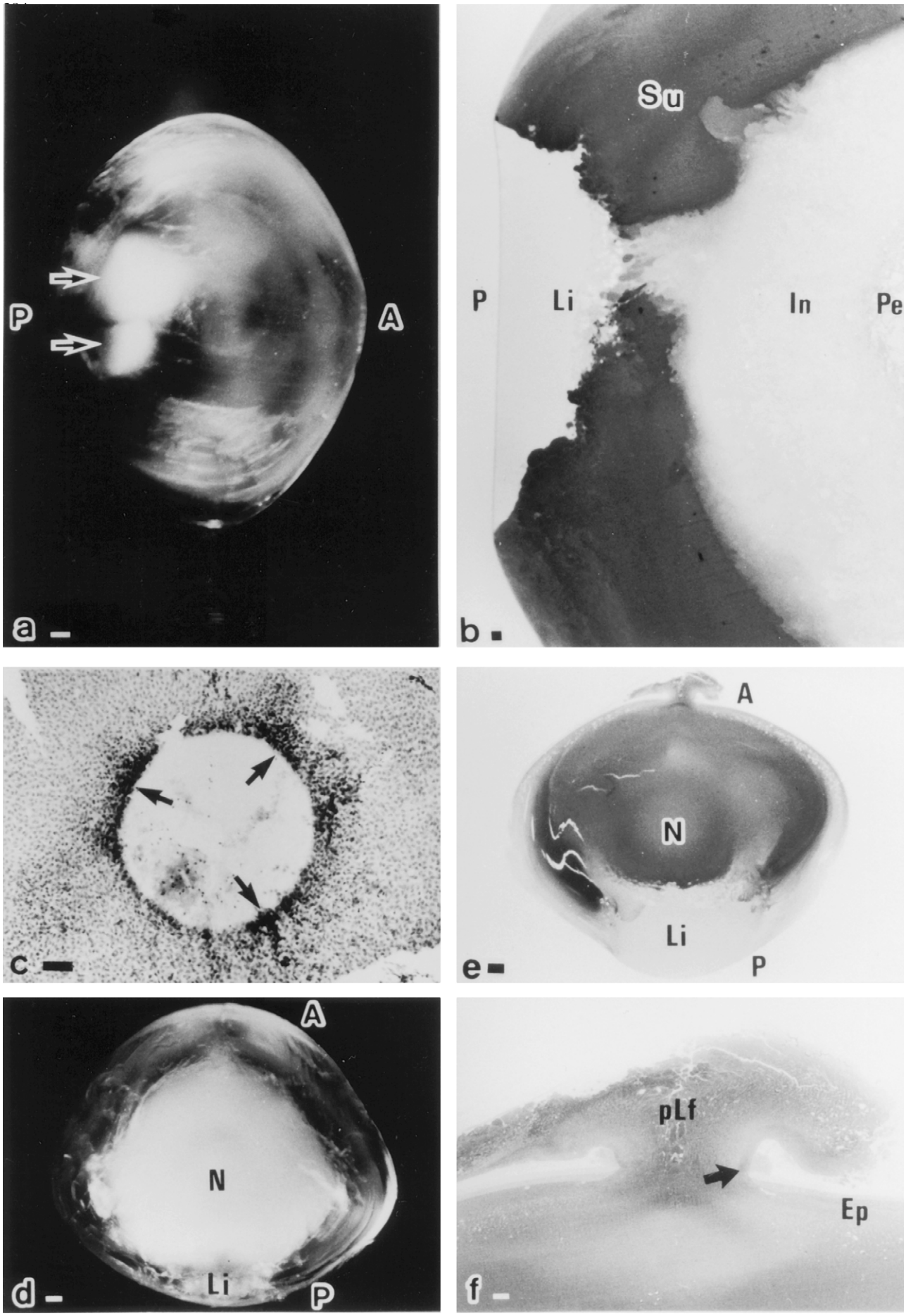
To determine the intralenticular movement of the resulting liquefaction from the injured part to the posterior part of the lens, 20% Evans blue (MW = 960) dye was injected into the lens with a 31-gauge needle and Ito syringe in several tracer experiments.

Results

The type of response of the lens to injury can be classified into three broad categories, depending on the degree of injury and the location of opacity.

1. Small area injury: When an injury was small in area, ranging from 80–100 μm, 17 of the 20 lenses subjected to small area injury stayed clear for a long time after the trauma. The remaining three lenses developed posterior opacity. At 24 hours after injury, damaged lens fibers formed a small opaque area on the anterior surface of the lens (Figure 1a). Five days after injury, marked proliferation of the epithelial cells was observed around the area of trauma (Figure 1b). Seven days after injury, the opaque area over the trauma became flat and small (Figure 1c). Histological examination revealed that the wounded area consisted of aggregations of cell groups and multilayered epithelial cells over the trauma region (Figure 1d). One month after in-

Figure 1. Sagittal section of the mouse lens at 24 hours after small area injury. (**a**) A small opaque area (arrow) is seen on the anterior surface (A) of the lens. P: posterior side. Bar = 50 μm. (**b**) Electron micrograph of the wounded area of the lens at 5 days after injury. Proliferated epithelial cells (Ep) are seen on the outer surface of the capsule (Ca). Lf: lens fibers. Bar = 1 μm. (**c**) Sagittal section of the lens at 7 days after injury. The wound becomes small and flat (arrow). A: anterior side, P: posterior side. Bar = 100 μm. (**d**) Photomicrograph of the sectioned specimen at 20 days after injury. The wound was covered with proliferated epithelial cells (Ep). Bar = 50 μm. (**e**) Whole mount flat preparation of the epithelium at 1 month after injury. The epithelial cells proliferating at the wound (arrow) were intensely stained. Bar = 100 μm. (**f**) Photomicrograph of the sectioned specimens at 1 month after injury. The epithelial cells (Ep) form a continuous layer. Some cells at the surface area of the proliferated epithelial cells had disappeared. Sw: swollen cells. Bar = 50 μm.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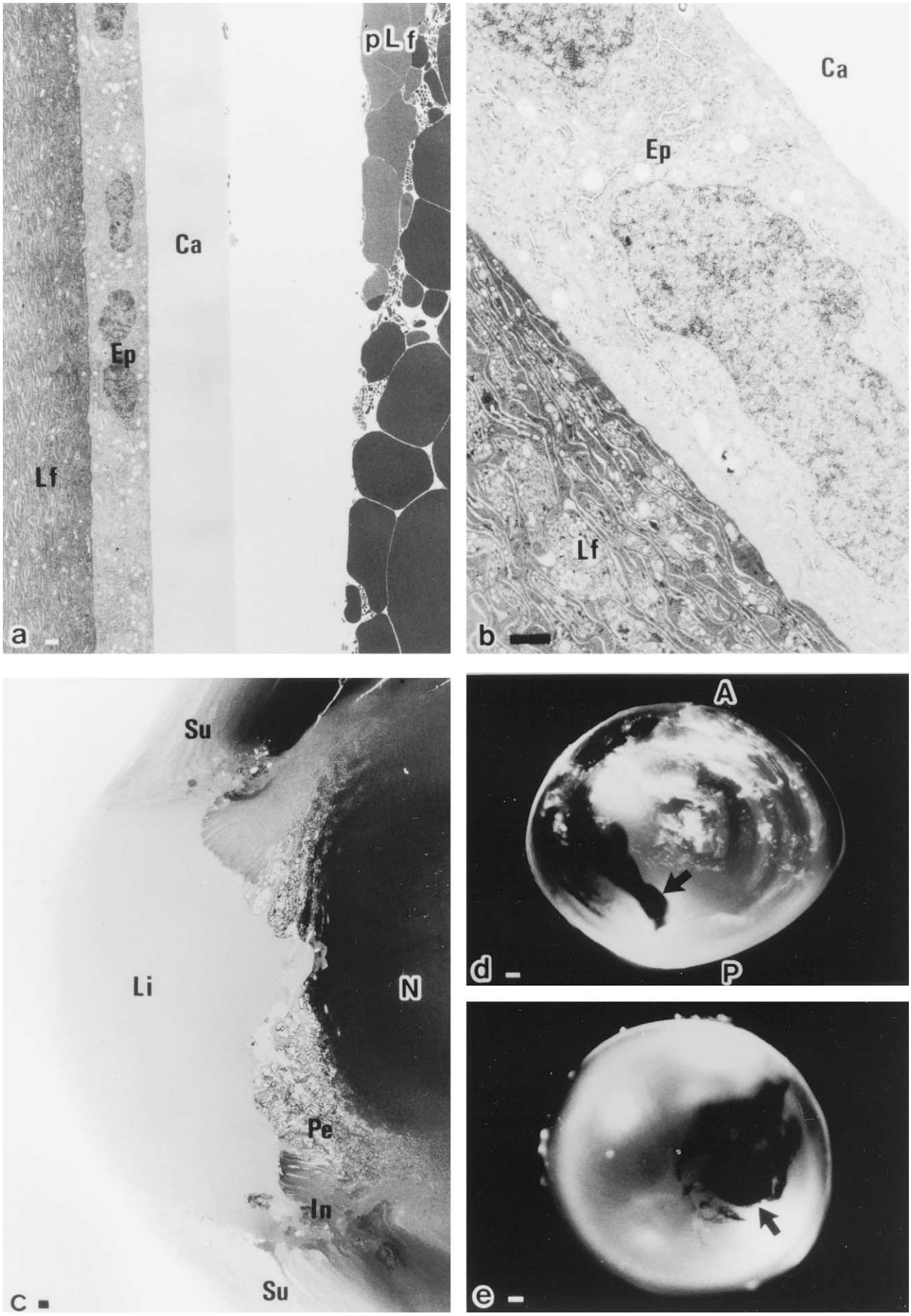
jury, flat preparations of the lens epithelium showed cell clumps, which were stained deeply with hematoxylin in the trauma region (Figure 1e). In histological specimens, the proliferated epithelial cells over the wound had become elongated, and the cells often had disappeared at the surface area (Figure 1f).

2. Intermediate area injury: Of the 40 lenses subjected to intermediate area injury, 26 lenses suddenly showed opacity at the posterior cortical area, whereas the other 14 lenses stayed clear. The size of the trauma in this type was 250–300 μm in diameter. Liquefaction occurred in the lenses and opacity appeared at the posterior polar region 8 hours after injury (Figure 2a). Histological examination revealed that the posterior suture had separated and liquefaction had occurred in this area (Figure 2b). Flat preparations of the epithelium at 8 hours after injury revealed an acellular region that had a diameter of 300 μm (Figure 2c). By 24 hours after injury, the liquefied area had enlarged and opacity had extended to the anterior pole (Figure 2d). Histological investigation revealed that lens fibers had degenerated in the region of perinuclear opacity and the liquefied area had enlarged in the posterior subcapsular region (Figure 2e). Higher magnification of the damaged area showed outer protrusion of a relatively large volume of lens fibers and a slight degree of epithelial proliferation (Figure 2f). By electron microscopy, the protruded lens fibers showed a marked swelling (Figure 3a). The lens fibers near the wound assumed a wavy appearance containing small vacuoles (Figure 3b). On the other hand, the posterior cortex of the lens was largely liquefied (Figure 3c). The lens fibers of the perinuclear zone, the intermediate layer, and the superficial layer around the nucleus were atrophic.
3. A tracer-injection experiment revealed that the dye moved towards the posterior side along the superficial and intermediate layers of the lens (Figure 3d). A posterior view of the same lens showed that the dye was diffusely distributed in the posterior polar region (Figure 3e).
4. Large area injury: The 60 lenses subjected to large area injury showed extensive areas of both epithelial and lens fiber injury. Of these 60 lenses, 43 lenses developed anterior opacity, while 17 lenses developed posterior opacity. The size of the trauma in the anterior opacity was 400–800 μm in diameter. Dissecting microscopic observation at 4 hours after injury revealed the occurrence of a small amount of opacity in the anterior subcapsular area (Figure 4a). Histologically, epithelial cell loss as well as cortical cell degeneration (Figure 4b) was observed over a broad area in the vicinity of the injury at 4 hours after injury. Disintegrated epithelial cells and swollen lens fibers were seen by electron microscopy in the vicinity of the wound (Figure 4c). Flat preparations of the epithelium revealed expansion of the epithelial damage in the periphery of the area of epithelial loss within the trauma area (Figure 4d). At 8 hours after trauma, the separating anterior suture was observable, together with damaged lens fibers (Figure 5a). At 24 hours after injury, the lens developed a mature cataract, but opacity did not occur in the posterior cortical region (Figure 5b). This condition of anterior opacity remained unchanged even at 1 week after injury (Figure 5c). Histological investigation of the same specimens revealed lens fiber degeneration in the area consistent with that of the opacity and liquefaction in the anterior subcapsular region (Figure 5d). Higher magnification of the posterior cortical area showed that the superficial layer of the cortical fibers appeared to be relatively normal, though the lens fibers of the intermediate layer had disintegrated (Figure 5e).

Figure 2. Sagittal section of the lens at 8 hours after intermediate area injury. (**a**) Small opaque areas are seen at the posterior cortical area (arrows). A: anterior side, P: posterior side. Bar = 100 μm. (**b**) Photomicrograph of the lens at 8 hours after injury. The posterior polar region is liquefied (Li), and the lens fibers of the superficial layer (Su) are atrophic. The fibers in the intermediate layer (In) and perinuclear zone (Pe) are markedly disintegrated. P: posterior side. Bar = 50 μm. (**c**) Flat preparation of the lens epithelium at 8 hours after injury. Active proliferation is seen at the periphery of the wound (arrows). Bar = 50 μm. (**d**) Sagittal section of the lens at 24 hours after injury. Opaque area is seen at the posterior part of the lens, whereas the anterior (A) cortex remains unchanged. Liquefied area (Li) is seen in the posterior polar region (P). Bar = 100 μm. (**e**) Photomicrograph of the lens at 24 hours after injury. Large liquefied area (Li) is seen at the posterior polar region. The fibers around the lens nucleus (N) are disintegrated. A: anterior side, P: posterior side. Bar = 100 μm. (**f**) Higher magnification of the anterior portion of the same specimen as in (**e**). The lens fibers protrude outside (pLf). The proliferating epithelial cells (Ep) are seen along the protruded fibers (arrow). Bar = 20 μm.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5. A tracer-injection experiment showed that the dye remained restricted to the anterior subcapsular area of the lens (Figure 5f). Another tracer injection disclosed that the dye remained in the anterior subcapsular area, although the lens developed opacity on its anterior side (Figure 5g).

Discussion

Three patterns of reaction were observed depending on the size of injury. For trauma with a small area (80–100 μm), the injured area was covered by proliferated epithelial cells and the lens retained transparency. For intermediate area injury (250–300 μm), epithelial damage was limited to the periphery of the wound. At 8 hours after injury, liquefaction occurred near the posterior suture, and opacity extended from the posterior part to the anterior part, thereby forming a mature cataract. For large area injury (400–800 μm), lens fiber and epithelial cell damage was extensive, liquefaction of the anterior subcapsular area occurred, and opacity extended posteriorly from 4 hours after injury, resulting in development of a mature cataract after 24 hours.

The present study showed that for small area injury, the entire area of the wound was covered with proliferated epithelial cells within a few days, thereby preserving transparency of the lens. Fagerholm and Philipson[5] reported that invasion into the lens by a foreign body was prevented by the covering by epithelial cells within 1 week after trauma. Accordingly, prevention of foreign body invasion from outside the lens during the early period of trauma appears to be an essential factor underlying the retention of lens transparency. Uga[8] has pointed out that proliferating cells covering the injured region decreased in number, produced collagen fibers, and formed scar tissue. The repair mechanism of the epithelial cells observed in the present study is similar to that after direct lens injury with laser without corneal injury.[9,10]

In the present study, in a case with intermediate injury, epithelial cell damage was limited and there was severe injury to the anterior cortical fibers; hence the liquid components resulting from the trauma reached the posterior pole, separated the posterior suture causing liquefaction in this region, resulting in opacity from the posterior part of the perinuclear region. Hatono,[11] who injected a tracer from the anterior region of the lens, reported that the tracer moved along the periphery of the nucleus and proceeded to the posterior pole in mice, but moved directly through the lens nucleus to the posterior pole in cats and dogs. We confirmed a similar movement of the tracer (Evans blue) from the anterior side to the posterior side of the lens in the present study. The movement of the tracer toward the posterior cortex after intermediate area injury may reflect one of the superficial routes of metabolic substances. However, the tracer in the case of a large area injury could not migrate toward the posterior cortex because of extensive damage to the superficial route. Similar mechanisms of posterior opacity onset have been reported in the mouse eye from a deep needle trauma reaching the perinuclear zone[7] and in several hereditary cataract models,[12–14] in which liquefaction components accumulated in the posterior subcapsular region.

The mechanism of anterior onset of opacity in cases with a large area of injury can be predicted as follows. Because a large number of epithelial cells disappear and there is extensive cortical fiber damage, the liquid components infiltrating directly from the wounded region cause swelling and liquefaction in the anterior cortical area, resulting in separation of the anterior suture accompanied by opacity, which advances to the perinuclear zone, thereby leading to the development of a mature cataract. To our knowledge this type of cataract showing the onset of opacity from the anterior cortex has not been previously reported in the mouse. Opacity arising in the anterior subcapsular region has been reported in the rat[15] and rabbit.[4]

The mechanism of onset of the cataract caused by an ocular perforating injury in the human eye varies in accordance with the depth and severity of injury, but it is said that there are several forms of cataract,

Figure 3. Electron micrograph of the wounded portion of the same specimen as in Figure 2E. (**a**) The protruded lens fibers (pLf) are markedly swollen. Ca: capsule, Ep: epithelial cells, Lf: lens fibers. Bar = 1 μm. (**b**) Higher magnification of the lens fibers and epithelial cells near the wound area. The lens fibers (Lf) form wavy appearance. The epithelial cells (Ep) contain abundant cell organelles. Ca: capsule. Bar = 1 μm. (**c**) Photomicrograph of the posterior portion of the same specimen as in Figure 2E. Liquefied area (Li) is seen in the posterior polar region. The lens fibers in the superficial layer (Su), intermediate layer (In) and perinuclear zone (Pe) are atrophic. N: nucleus. Bar = 10 μm. (**d**) Sagittal section of the lens at 5 days after injection of Evans blue dye. The dye is seen migrating toward the posterior side (P) along the lens fiber arrangement of the superficial and intermediate layers (arrow). A: anterior side. Bar = 100 μm. (**e**) Posterior view of the same specimen as in (**d**). The dye is diffusely distributed at the posterior polar region (arrow). Bar = 100 μm.

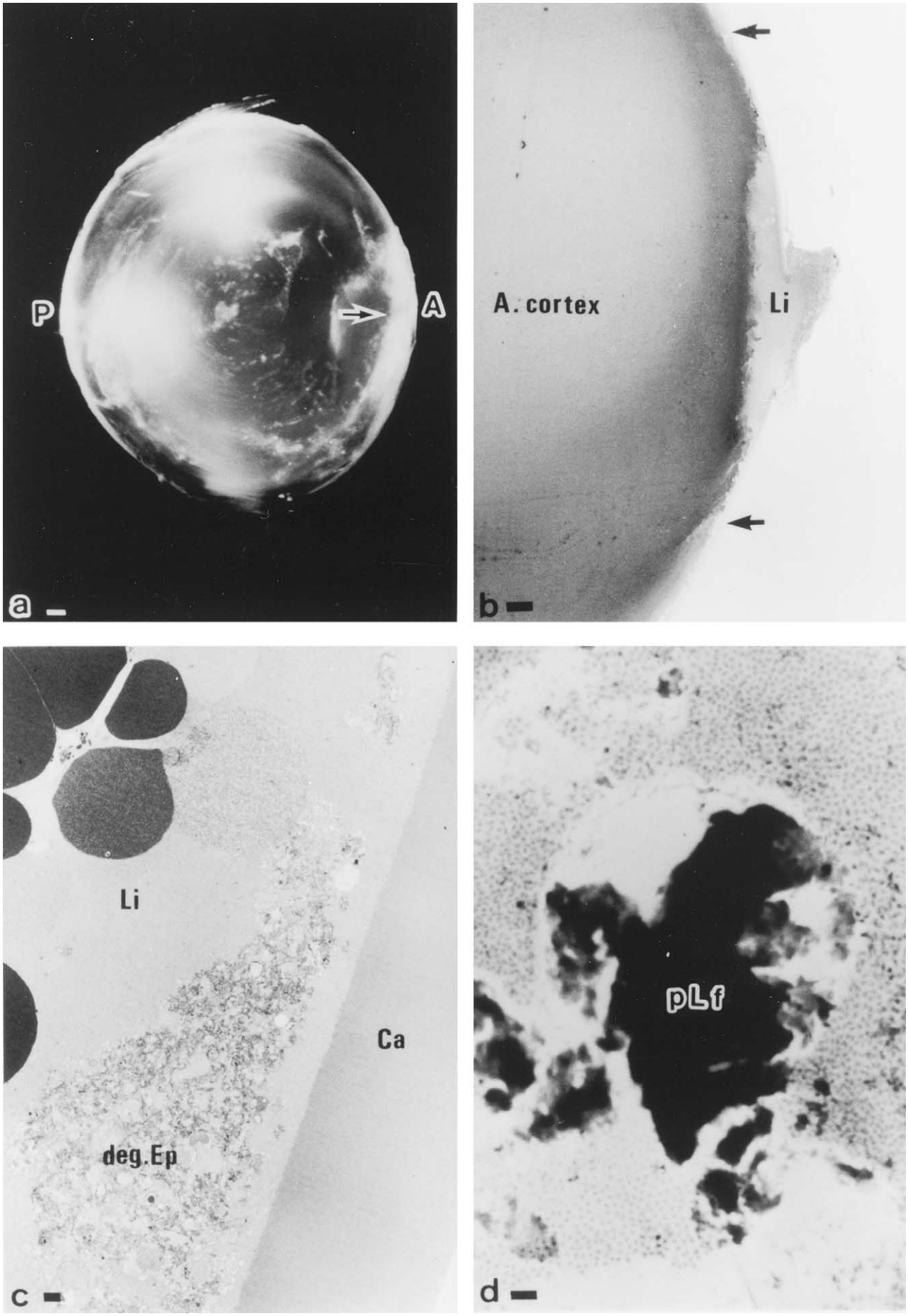
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Figure 4. The lens at 4 hours after large area injury. (**a**) Sagittal section shows pale opaque area just beneath the anterior (A) cortical region (arrow). P: posterior side. Bar = 100 μm. (**b**) Photomicrograph of the anterior polar region of the lens at 4 hours after injury. Liquefied area (Li) occurs just beneath the wound. Epithelial cells are lost between arrows. A: anterior side. Bar = 50 μm. (**c**) Electron micrograph showing epithelial damage and fiber swelling at the periphery of the wound. Ca: capsule, degEp: degenerating epithelial cells, Li: liquefied area. Bar = 1 μm. (**d**) Flat preparation of the lens epithelium at 4 hours after injury. Protruded lens fibers (pLf) are seen in the center of the figure. In addition to circular epithelial cell loss, extended epithelial loss is observed. Bar = 50 μm.

including anterior subcapsular cataract, posterior subcapsular cataract and lens degeneration. The results of the present study suggest that the injured area of epithelium, the amount of lens fiber damage, and the site of liquefaction have a substantial influence on the form and course of cataract formation. The size of the trauma area determined whether the lens recovered or developed opacity, and the severity of epithelial trauma determined the site of lens fiber liquefaction which, in turn, determined the site of onset of opacity. Accordingly, these findings indicated that the size of injury to the lens is a significant underlying cause of cataract onset.

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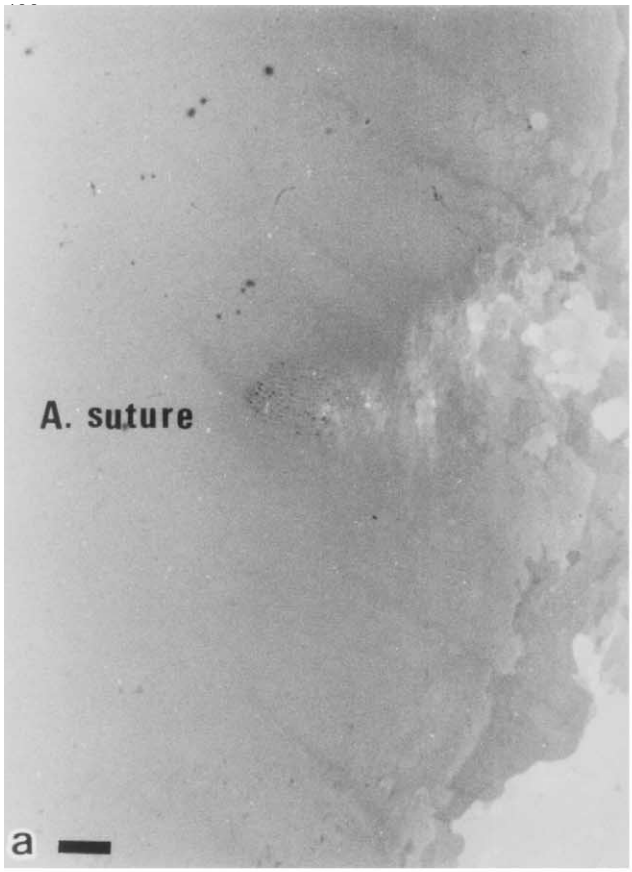
Figure 5. Eight hours after large area injury. (**a**) Photomicrograph of the anterior polar region at 8 hours after injury shows the anterior suture is separated. Bar = 50 μm. (**b**) Sagittal section of the lens at 24 hours after injury. Opacity (Op) occurs at the anterior side (A) of the lens. P: posterior side. Bar = 100 μm. (**c**) Sagittal section of the lens at 7 days after injury. Opaque area (Op) has deviated to the anterior side (A) of the lens. Posterior (P) cortex remains unchanged. Bar = 100 μm. (**d**) Photomicrograph of the same specimen as in (**c**). Disintegration of the lens fibers is consistent in the opaque and liquefied (Li) areas. The nucleus (N) is dislocated anteriorly. A: anterior side, In: intermediate layer, Pe: perinuclear zone, Su: superficial layer, P: posterior side. Bar = 50 μm. (**e**) Higher magnification of the posterior part of (**d**). The superficial layer of the posterior part appears almost normal. The lens fibers of intermediate layer (Un) are markedly disintegrated. Bar = 50 μm. (**f**) Sagittal section of the lens at 8 hours after injection of Evans blue dye. The dye is seen just beneath the anterior subcapsular region. Bar = 100 μm. (**g**) Sagittal section of the lens at 8 hours after injection of Evans blue dye. The dye is seen in the same region as in (**f**), but opaque area (Op) occurs in the anterior side (A). P: posterior side. Bar = 100 μm.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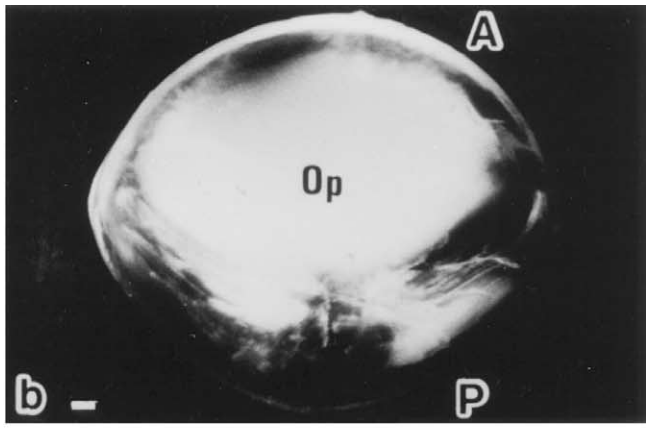
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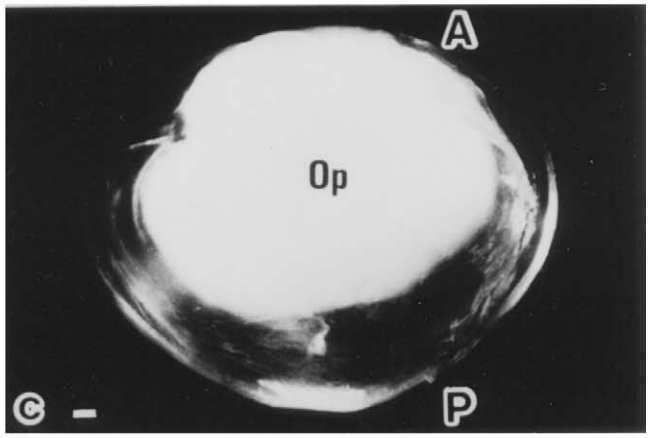
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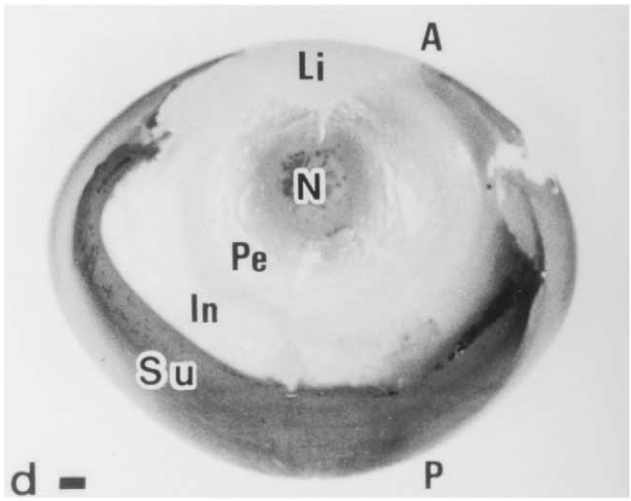
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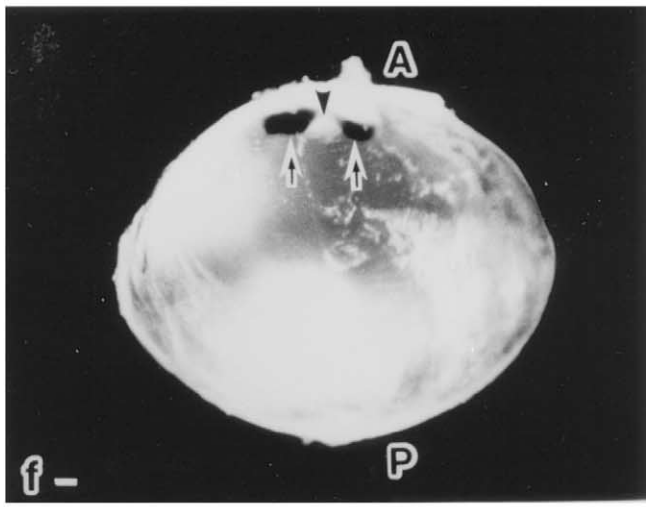
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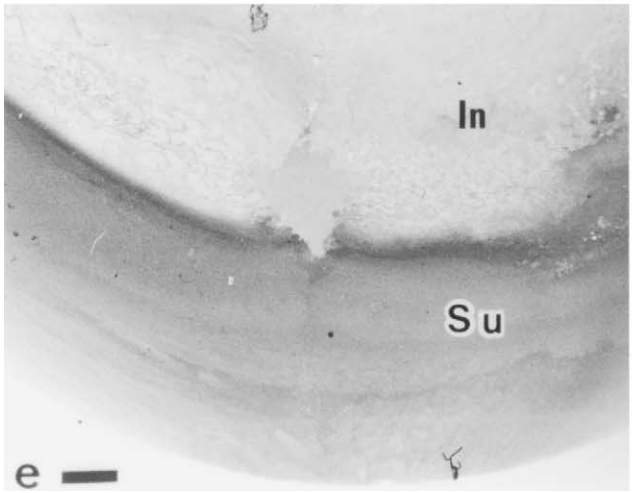
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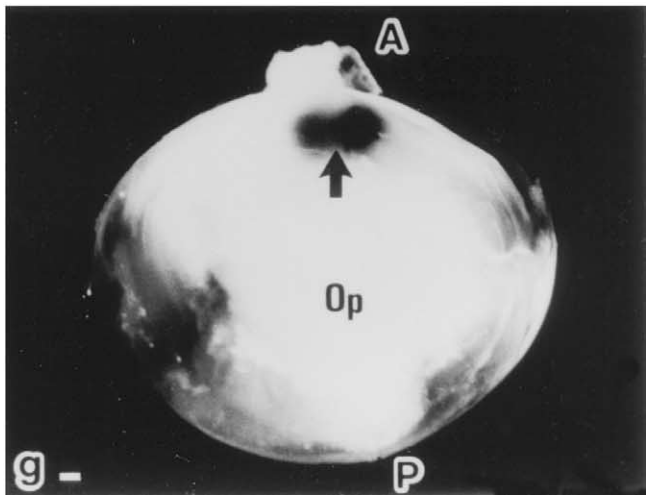
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