Experimental traumatic cataract

I. A quantitative microradiographic study

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Traumatic cataract was induced in rat and rabbit. The progression of the posterior subcapsular cataract and the wound healing were followed by quantitative microradiography. This method makes it possible to determine the dry mass content and to calculate the refractive indices in the lens. In the injured rat lens a reduced dry mass concentration was determined in both the wound region and the posterior subcapsular region immediately after trauma. During the first week a subcapsular opacity was observed to enlarge. This opacification always corresponded to a reduction in the subcapsular concentration of dry mass. This reduced content of dry material, mainly consisting of protein, is interpreted as a hydration of the subcapsular cortex. In the rabbit lens a much larger injury had to be made in order to cause any posterior cataract. The same reduction in the content of dry material was determined in regions corresponding to opacities but the reduction was less pronounced than in the rat lens. The border zones between different concentrations of dry mass are suggested to be the main sources of scattered light in the traumatic cataract.

Key words: experimental traumatic cataract, rat, rabbit, quantitative microradiography, dry mass concentration, posterior subcapsular cataract

Traumatic cataract is a common and feared complication following accidental injuries to the eye. Most cases suffering lens injuries will develop cataract. Clinical examination of this type of cataract in humans reveals a local opacification in the area of the lesion followed by a subcapsular opacity at the posterior pole. The latter opacification often develops fast and may soon cover the entire subcapsular region, although the anterior cortex stays clear much longer.

No satisfactory explanation has been given for the early opacification of the posterior subcapsular cortex following damage restricted to the anterior lens segment. In human subcapsular and traumatic cataracts, a marked reduction of dry mass concentration has previously been found in a zone under the capsule.

One way to find an explanation for the mechanism of opacification after lens trauma is to study the process experimentally. The present study has been undertaken in order to determine quantitative changes in the dry mass content following lens injury. The refractive index within the lens is directly proportional to the content of dry mass, which mainly consists of protein. Traumatic cataract was induced in the rat and the rabbit lens, and the progression was followed bio-
Fig. 1. Development of opacification in the rat lens after trauma. The front view of the lens illustrates the relative size of the initial trauma. An opacity in the wound region is seen immediately (A). The opacification develops early at the posterior pole (B) and engages later the entire subcapsular region (C and D). The end result could also be a partially resorbed lens (E). The development of opacification can cease at any stage, with small opacities remaining in the cortex (F to H).

Fig. 2. Development of opacification in the rabbit lens after trauma. The front view of the lens illustrates the relative size of the initial trauma. A local opacity is immediately seen in the wound region (A). Unlike the rat lens the posterior pole is not engaged early during cataract development. Characteristic is the thin zone of increased light scattering in the cortex (B). This zone seems to emerge from the deep wound region (B) and may later be observed in the posterior region (C). At late stages the entire cortex may become more or less opaque (D). Cessation of cataract development leaves minor cortical opacities or a thin zonular opacity (E to G).

Microscopically and by quantitative micro-radiography.

Material

Eighteen Sprague-Dawley male rats weighing approximately 250 gm and 10 rabbits (New Zealand) weighing about 2 kg were used.

The rats were anesthetized with sodium pentobarbital intraperitoneally at a dosage of 50 mg/kg and given 0.5% tropicamide topically in both eyes. One eye was injured with a spring-suspended sharp needle which penetrated the cornea and about 0.5 mm of the lens close to the anterior pole. The diameter of the needle was 0.4 mm. The eyes
were examined biomicroscopically in a Zeiss slit lamp before lens injury, during the first hour after injury, and then daily during the first week, weekly during the following month, and then at monthly intervals. Three rats were killed at each of the following intervals after injury: 5 min, 1 hr, 24 hr, 1 week, 1 month, and 1 year. The rabbits were anesthetized with sodium pentobarbital intravenously at a dosage of 40 mg/kg and the pupils were dilated by 0.5% tropicamide topically. Paracentesis was performed in one eye with a knife-needle. Two superficial incisions were made into the lens. Each incision was 4 mm long and about 0.5 mm deep. These incisions formed a cross with its center at the anterior pole. The eyes were inspected in the same manner and at the same intervals as those of the rats. Two rabbits were killed at each of the following intervals after injury: 5 min, 1 hr, 1 day, 1 week, and 1 month.

Methods

After atraumatic extraction, the lenses from both species were immediately prepared for quantitative microradiography. The lenses were frozen in isopentane, which had been precooled in liquid nitrogen to about —140° C. After freeze-sectioning to a thickness of about 10 µm the sections were freeze-dried. Each section used, and a reference system were mounted in close contact to a fine-grained photographic emulsion and exposed to soft X-rays generated at 3 kV. The microradiograms were evaluated densitometrically. The dry mass concentration could be determined in volumes smaller than 100 µm^3. In all the wounded and control lenses, densitometrical evaluation of one microradiogram from a central section was performed. At each time interval, three wounded rat lenses with corresponding control lenses and two wounded rabbit lenses with control lenses were analyzed. In all the lenses the dry mass of the subcapsular cortex at the posterior pole was determined. The determinations were always performed close to the capsule and in apparently normal cortical tissue at a depth of 0.4 mm. The X-ray absorption was also measured in other areas corresponding to a lens opacity and the adjacent clear region. The mean dry mass content and a 99% confidence interval were calculated in each point from 20 determinations.

Results

The development of traumatic cataract in rat lenses (Fig. 1) and in rabbit lenses (Fig. 2) was different. The injury had to be much larger in the rabbit than in the rat in order to cause a progressive cataract. No pathological opacities were seen in the control lenses from both species.

Rat lenses

0 to 1 hr following injury. When the needle was retracted after penetrating the lens, an immediate protrusion of lens material was seen. A subcapsular opacity extending about 0.5 mm from the wound was formed during the first 1 to 2 min. The initially fairly clear protruding lens mass became more opaque within the first hour after injury. The local anterior subcapsular opacity did not change in size after the first 5 min. Within the first hour, a thin posterior subcapsular opacity was observed in 10 of the 18 lenses. Mi-
Fig. 4. Anterior subcapsular cortex of a normal (A) and a wounded (B) rat lens 5 min after trauma. A thin zone of dry mass reduction is seen underneath the epithelium (e) adjacent to the wound in (B), in contrast to the even X-ray absorption in the control lens (A). Capsule is missing in both A and B. Dry mass determination: A, 1, 0.20 ± 0.01 g cm\(^{-3}\); 2, 0.19 ± 0.01 g cm\(^{-3}\); B, 1, 0.06 ± 0.02 g cm\(^{-3}\); 2, 0.20 ± 0.02 g cm\(^{-3}\). (Micro-radiographs; calibration bar 100 \(\mu\)m.)

croradiographic analyses of lenses taken 5 min and 1 hr after injury revealed lens fibers protruding between the rolled up edges of the lens capsule at the wound (Fig. 3). A reduced dry mass content was determined subcapsularly adjacent to the wound (Fig. 4). At the posterior pole swollen lens fibers with a reduced dry mass concentration were found in four of six wounded lenses. These changes were confined to a thin area in this subcapsular region (Fig. 5).

24 hr after trauma. The posterior subcapsular opacity was observed more frequently (10 out of 12 lenses) and was then more distinct. In the micro-radiograms the protruding lens fibers were swollen, and a reduced dry mass concentration could be observed within the wound area. In two of the three wounded lenses analyzed at this stage, the dry mass content was reduced by more than 50% at the posterior pole.

1 week after trauma. The protrusion of lens material at the wound was less pronounced. The wound area was opaque. The posterior opacity engaged the entire posterior part and appeared more dense than previously, with a rough surface.

In the micro-radiograms, epithelial regeneration was seen underneath the protruding mass at the wound. The posterior areas with reduced dry mass concentration had reached deeper into the cortex and demonstrated distinct but irregular borders toward an ap-
Fig. 6. Posterior region of a rat lens 1 week after trauma. Biomicroscopically a dense subcapsular opacity was observed. Reduced X-ray absorption and corresponding reduced dry mass content can be seen in the subcapsular region and deeper into the cortex. Dry mass determinations: 1, 0.08 ± 0.03 gm • cm⁻³; 2, 0.21 ± 0.03 gm • cm⁻³; 3, 0.12 ± 0.03 gm • cm⁻³; 4, 0.49 ± 0.08 gm • cm⁻³. (Microradiograph; calibration bar 100 μm.)

Parently normal cortex in the three lenses analyzed (Fig. 6).

1 month after trauma. The wound was seen as a white, nonprotruding patch. The subcapsular opacity, earlier found only in the posterior part, had reached the anterior subcapsular region in all but one of the lenses. The microradiographic analyses revealed a marked reduction of dry mass (more than 50%) in the entire subcapsular region in two of the three injured lenses (Fig. 7).

1 year after trauma. An opaque cortical zone covered by a thin zone of subcapsular cortex was seen in two of the three lenses. One of the three lenses was small and totally opaque. The microradiograms revealed a zone of reduced dry mass concentration with an apparently normal subcapsular zone in two of the lenses (Fig. 8) or as in the small lens, a totally vacuolized cortex with a very low dry mass concentration (Fig. 9).

Rabbit lenses

0 to 1 hr after trauma. Biomicroscopic findings at the site of the wound were similar to those seen in the rat lens. The local anterior opacification adjacent to the wound was less pronounced initially but was clearly visible. At first the protruding lens mass was clear but became less transparent during the first hour.

The microradiographic appearance of the wound was similar to that observed in the rat. No changes in the dry mass content were determined at the posterior pole at this stage.

24 hr after trauma. The wound was very opaque and slightly larger than the original injury. The remaining lens including the posterior subcapsular region was always clear.
Fig. 7. Central section parallel to the optic axis of a rat lens 1 month after trauma. A subcapsular zone with reduced dry mass surrounds the lens. Anterior pole to the left. Dry mass determinations: 1, 0.11 ± 0.02 gm · cm⁻³; 2, 0.25 ± 0.03 gm · cm⁻³. (Microradiograph; calibration bar 1 mm.)

Fig. 8. Anterior (A) and the posterior (B) subcapsular cortex in a rat lens 1 year after trauma. Biomicroscopically, a zonular opacity was seen with a thin clear cortex outside. Zones with swollen lens fiber cells and reduced dry mass (between arrows) are shown surrounded by a zone of apparently normal lens fibers. This normal subcapsular zone is thicker at the anterior region (A) than in the posterior (B). (Microradiographs; calibration bar 100 μm.)

In the microradiogram a reduced dry mass content could be determined in the apical end of the protruding lens fibers (Fig. 10). The dry mass content was normal at the posterior pole.

1 week to 1 month after trauma. During this period the wound opacity decreased to a small, slightly protruding white rim. Around this opacity a very thin zone with a reduced transparency was seen inside the clear subcapsular cortex in three of the four lenses, as schematically shown in Fig. 2, B. In two of
Fig. 9. Totally opaque rat lens 1 year after trauma. Extensive changes can be seen in the cortex as irregular masses with swollen and vacuolized lens fibers. Dry mass determinations: 1, 0.02 ± 0.01 gm • cm⁻³; 2, 0.25 ± 0.06 gm • cm⁻³; 3, 0.65 ± 0.03 gm • cm⁻³. (Microradiograph; calibration bar 100 μm.)

these lenses this opacity reached into the posterior lens cortex, and in the third it only reached the equator. This pattern of opacification was more dense in certain sectors of the anterior cortex.

The microradiographic analyses revealed a reduced dry mass in all the areas with visible opacities. At the wound, the epithelium regenerated under the scar tissue (Fig. 11). The anterior subcapsular cortex had a reduced dry mass content in three of the four lenses. In the interface between this zone and the inner apparently normal cortex, very enlarged cells with reduced dry mass content were seen. These vacuole-like cells were situated in the same region as the dense cortical opacities (Fig. 12). After a month the scar tissue was reduced, and the underlying cortex appeared normal (Fig. 13).

Discussion

A local opacity, cataracta traumatica constrictiva, is consistently present at the site of injury in both rat and rabbit lenses. This opacity invariably seems to be accompanied by a reduction and a variation in the dry mass concentration in the wound area (Figs. 3, 4, 10-13). In the microradiograms, an immediate reduction of the dry weight concentration was determined in a large area around the initial wound as early as 5 min after trauma.

The opacity of the anterior subcapsular region is probably the result of several mechanisms. Rupture of the capsule and the epithelium is followed by a protrusion of cortical lens fibers. However, the passage of aqueous humor through the local epithelial defect and in between the subcapsular lens fibers is probably most important. The lens fibers underneath the epithelium were swollen, and the dry mass concentration was reduced. The reduced concentration of dry material in the subcapsular cortex we interpret as a hydration of the lens fibers, caused by an increased inflow of sodium and water.

In the rat lens a posterior subcapsular opacity appeared within 1 hr after injury. In the microradiograms a reduction of the concentration of dry mass was determined in all regions corresponding to an opacity. The progressive posterior cataract is probably a result of the absence of epithelium in the wound area. The defect in the epithelial barrier enables leakage of aqueous humor into
A reduced dry mass content can be determined in the ends of the protruding lens fibers. Dry mass determinations: 1, \( 0.03 \pm 0.02 \text{ gm} \cdot \text{cm}^{-3} \); 2, \( 0.22 \pm 0.03 \text{ gm} \cdot \text{cm}^{-3} \); 3, \( 0.22 \pm 0.01 \text{ gm} \cdot \text{cm}^{-3} \). (Microradiograph; calibration bar 100 \( \mu \text{m} \).)

The lens. This creates an altered extracellular milieu in the subcapsular cortex. The most vulnerable region is the posterior part where no epithelium is present. In this region the lens fibers normally have a limited but sufficient sodium and potassium transport capacity.\(^{10, 11}\) However, this active transport capacity does not seem to be sufficient to counteract an increased sodium concentration following an anterior lens injury. The early progression of the posterior subcapsular opacity indicates that the epithelium in the wound area has not healed and/or is not functioning sufficiently well.

In the rabbit lens no changes could be detected at the posterior lens region during the first 24 hr. This might be explained by a species difference in the lens size, the arrangement of lens fibers, and ion-pumping capacity of both epithelium and lens fibers. After a week, however, hydration of the posterior subcapsular region could be observed.

The concentration of dry mass is directly proportional to the refractive index, as stated in \( n_{\text{lens}} = 1.333 + 0.0018 \cdot C \), where \( C \) is the dry mass in grams of lens substance per 100 ml.\(^7\) In the lens the dry mass is mainly protein.

Whenever an opacity was visible in the slit-lamp microscope, a border zone between markedly different concentrations of dry mass was found in the corresponding lens region. Such interfaces are often irregular and correspond to steep gradients in refractive index.
Fig. 12. Wound area in a rabbit lens 1 week after trauma. The anterior subcapsular cortex has a reduced dry mass content. A sharp interface is formed between this area and the inner normal cortex. At this interface vacuoles with very low dry mass concentration can be seen. Dry mass determinations: 1, $0.11 \pm 0.01 \text{ g m}^{-3}$; 2, $0.06 \pm 0.03 \text{ g m}^{-3}$; 3, $0.23 \pm 0.02 \text{ g m}^{-3}$. (Microradiograph; calibration bar 100 μm.)

Fig. 13. Wound area in a rabbit lens 1 month after trauma. The epithelium (e) has regenerated under the scar tissue (s), and the cortex underlying the wound has a normal dry mass content. Dry mass determinations: 1, $0.08 \pm 0.03 \text{ g m}^{-3}$; 2, $0.20 \pm 0.02 \text{ g m}^{-3}$. The horizontal lines in the cortex are artificial cracks. (Microradiograph; calibration bar 100 μm.)

We suggest that these changes in dry mass and refractive index are the major sources of light scattering in the lens opacities secondary to lens injury.

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REFERENCES