Aging and the Optical Quality of the Rat Crystalline Lens

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Split laser beams of varying separation were directed through the crystalline lenses of rats of young, old, and intermediate age. The variation in back vertex distance was determined from photographs of the focal effects of each lens. The results indicate that negative spherical aberration of the rat lens increases with age. In addition, local reversals in the slopes of the negative spherical aberration curves are larger and more frequent in the older lenses. The increase in negative spherical aberration is likely due to tighter packing of central lens fibers and a consequent increase in central refractive index although change in lens shape may contribute as well. Invest Ophthalmol Vis Sci 24:1162–1166, 1983

The crystalline lens of the vertebrate eye is an ectodermal structure made up of a constantly increasing number of epithelial cells. The peripheral subcapsular site of the cell division that takes place produces a lens of centrally increasing density and refractive index. This variation in refractive index is of substantial optical benefit to the eye in that it helps limit the spherical aberration that would be present in significant quantities if the lens were a homogeneous body, having a single refractive index. Control of lens spherical aberration has been demonstrated, to varying levels of success, in a spectrum of vertebrate species; regardless of whether the lens is the only refractive element of the eye, as in the case of the spherical fish lens, or whether the lens shares the refractive burden of the eye with the cornea. The relatively large subcpherical lens of the rat eye, presumably required to enhance visual sensitivity for a nocturnal life style, has been shown to be overcorrected (Fig. 1) for spherical aberration, i.e., negative spherical aberration.

There has been little attention paid to the effect of age on spherical aberration of the lens. Points of specific interest include the question of whether a continuously variable refractive index distribution is maintained with continued lens growth, whether change in lens shape with age plays a role in control of this aberration, and whether age-related changes in lens proteins result in a deterioration of the refractive quality of the lens. In the context of the last point, it is noted that Palmer and Sivak found small inclusions of discontinuous refractive index in a human lens from an individual of advanced years.

The present study compares optical quality of rat lenses of various ages by measuring spherical aberration.

Materials and Methods

The crystalline lenses of 15 albino rats were used in the study. All 15 were a Wistar-derived WF inbred strain of 5, 15, and 27 months age, with five animals in each age group.

The ocular globes were removed after each animal was killed with CO₂. Lenses were removed carefully from the globe and placed on a thin metal plate with a central opening of either 4.7 or 5 mm diameter, depending on the size and age of the animal. The rear lens periphery was sparingly glued to the plate using Histoacryl® (B. Braun, Melsungen AG), a tissue cement. Lens and plate were then oriented vertically in a small plastic container with front and rear clear glass plates such that the anterior surface of the lens faced the front. The container was filled with Mammalian Tissue Culture Medium (M-199, Bio-Lab Ltd., Jerusalem), including 10% dialized fetal calf serum, and placed in front of a low power (1 mW) helium-neon laser. A 125-mm positive lens and a parallel mirror beamsplitter condensed and split the beam into multiple parallel beams, the separation of which could be altered by changing the angle of incidence to the beamsplitter. The refractive effects of each lens on pairs of beams of various separations were photographed from above with a single lens re-
flex camera and macro lens with bellows. The position of the container was altered vertically and horizontally until a centrally directed beam could pass undeviated through the lens. A photograph of a millimeter rule placed in the path of the beams was used as a control for magnification. The distance between the posterior pole of the lens and the focal point of any two beams (back vertex distance) was measured from projections of the photographic negatives.

For the purpose of interpreting the graphical data presented below, change in back vertex distance to the left along the optic axis, as beam separation is increased, indicates positive (uncorrected) spherical aberration while change in the opposite direction indicates negative (overcorrected) spherical aberration.

The use of a helium-neon laser means that back vertex measurements reflect long wavelength focal values. Focal distances for wavelengths from the blue end of the spectrum would be somewhat shorter, the difference indicating the longitudinal chromatic aberration of the rat lens.

Cold cataracts were produced in lenses of one Wistar rat of each age at a later stage of the study (see Discussion) for the purpose of defining a labile region between the nucleus and cortex. These lenses were frozen on a freezing microtome, hemisected and then thawed. The lenses were sectioned equatorially (perpendicular to the direction of the incident beams) and placed on millimeter square paper to be photographed.

**Results**

The graphical presentation of the data (Figs. 2–4) clearly indicates that the negative spherical aberration of the rat lens increases with age. The mean variation in back vertex distance between the most central point measured (usually 0.5–0.6 mm from the axis) and the most peripheral (1.6–10.18 mm from the axis) is 0.24 (±0.11) mm in young lenses, 0.60 (±0.37) mm in lenses of intermediate age and 0.72 (±0.36) mm in older lenses (Fig. 5). The changes in back vertex distance in the young Wistar rats are similar to the findings of Campbell and Hughes who used lenses of DA rats, 120–140 days of age. To add the perspective of dioptric power, change in back vertex distance can be converted to change in back vertex power using the paraxial formula:

$$Fv_i = \frac{n}{fv_i}$$

where back vertex power (Fv) equals 1.332 (n, the index of refraction of the medium bathing the lens for monochromatic light of 632.8 nm) over back vertex distance (fv). Paraxial rays with back vertex distances ranging from 3.0 to 4.0 mm (Figs. 2–4) would vary in power from 444 to 333 diopters.

It is noted that the variation in back vertex distance is more significant between young and old and young and intermediate lenses than between old and intermediate lenses. However, the results plotted for the old lenses are less monotonic than either young or intermediate lenses in that zonular shifts toward positive spherical aberration or sudden jumps in negative aberration are larger and more common. Some local variation between positive and negative spherical aberration is also found in psychophysical studies of the human eye and in the study of the excised human lens.

**Discussion**

As noted in the introduction, spherical aberration of the eye can be controlled through peripheral flattening of the cornea, asphericity of the surfaces of the lens and variation in lens refractive index. Since the
Fig. 2. Variation of back vertex distance with eccentricity for lenses of young rats.

Fig. 3. As Figure 2 for lenses of rats of intermediate age.

Fig. 4. As Figure 2 for lenses of older rats.
present work deals only with the lens, potential corneal changes with age need not be considered. In any case, the lens of the rat eye is of much greater refractive importance than the cornea and corneal neutralization of the lens spherical aberration is probably not possible. Furthermore, the subspherical shape of the rat lens is reminiscent of the spherical fish lens; a lens in which control of spherical aberration is obviously a result of refractive index variation alone.

To test the possibility that the change noted may be due to flattening of the lens with age, four eyes in each age category were rapidly frozen in liquid nitrogen and sectioned horizontally on a freezing microtome. Measurements of equatorial and axial lens diameter were made from photographs of central portions of the eye following removal of thin sections. The measurements indicate that the lens becomes slightly more spherical with age; the average equatorial diameter being 13.7% greater than the axial diameter in young rats and 11.6% greater in old ones. Since this change is likely to have the effect of decreasing the magnitude of the negative spherical aberration, we have assumed that the shift toward more negative spherical aberration represents an increase in the disparity between the refractive indices of the lens centre and the lens periphery due to tighter packing of central fibers. It is pertinent to note that Howcroft and Parker found little or no change in asphericity of adult human lenses of various age. However, study of external lens shape does not test the possibility of a change in shape of one or more of the inner lens shells and this factor cannot be ruled out.

It should be noted that the rat eye is believed to be incapable of accommodation, and, therefore, lens shape should not be affected by the absence of zonular tension on the excised lens. In addition, the rat lens has a rigid consistency and high refractive index relative to the lenses of other terrestrial vertebrates. At the same time, great care was taken during the removal and mounting procedure to avoid altering lens shape.

As mentioned earlier, localized shifts in the direction of positive spherical aberration appear to be larger and more common in the older lenses, although they can be seen in young lenses as well (Figs. 2–4). Their intermediate eccentric location suggests that such shifts may correspond to the location of a perinuclear zone. Although there is little doubt that the nucleus occupies a larger proportion of the lens with age, the identification of the nucleus of the lens varies with the interpretations and definition employed by individual workers (for example, embryonic nucleus vs. adult nucleus). However, the rat lens, in common with fish lenses, exhibits an irreversible turbidity in the region between the nucleus and cortex when the lens is subject to cold, and it is thought that this region is the least stable in terms of either protein or supramolecular organization. Cold-induced turbidity was produced in lenses from one Wistar rat of each age (see Materials and Methods) to see if the turbid zone could be related to age variations in spherical aberration (Fig. 6). In the young lens, the turbid zone consists of an annular ring with a clear central (nuclear) area of about 1.0 mm diameter and a turbid ring of about 0.2 mm thickness. In lenses of intermediate age, the turbid ring has a clear central area of about 1.5 mm while the thickness of the ring has increased to approximately 0.7 mm. In old lenses both the central and intermediate areas remain turbid, leaving a narrow (0.2 mm) clear peripheral zone. Only in the case of young lenses does there appear to be a possible relationship with zonular variations in spherical aberration. Here, lenses of three of the five eyes studied show such variations at positions 0.5 mm from the axis (Fig. 2). In the older lenses and lenses of intermediate age such variations tend to be found at 0.75 and 1.25 mm from the axis, respectively. This indicates that such zonular variation is probably not directly related to the growth of the circumscribed nuclear area.
A final note concerns the possibility that spherical aberration of the eye contributes to the uncertainty experienced by workers attempting to allocate a specific refractive state to the rat eye. The present results demonstrate that age of the animal is an important factor to consider when comparing separate studies of the rat eye, as well as studies involving the eyes of other mammals.

Key words: age, lens, spherical aberration, rat

Acknowledgment

The authors are grateful for the help and encouragement of Professor D. Gershon

References


Fig. 6. Hemisected lenses of young (top), intermediate age (middle) and old (bottom) rats, showing variation of cold cataracts with age.