It can further be seen that all specific activities are much less with age which is in contrast with the observed constancy in the $\beta$ crystallin group of the bovine lenses.

In future investigations, additional fractionations must be performed to determine which protein fraction is most affected by aging. The relatively high incorporation of glutamic acid in the last fraction (0.5 N NaOH) in both bovine and human lenses is intriguing but the cause remains obscure. It could be assumed that it could be either an incorporation of glutamic acid in the adenylic ring of soluble RNA, or if in the alkaline condition used, the amino acyl tRNA are stable and it would be interesting to measure incorporation at this level.

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Key words: human lens, bovine lens, aging, soluble protein, glutamic acid incorporation, amino acids.

REFERENCES

The nuclear envelope in the crystalline lens fiber cell. CLIFFORD V. HARDING and STANLEY R. SUSAN.

Rabbit lenses which have been fixed, dehydrated, and dried by a critical-point drying method, can be fractured through the cytoplasm of the differentiating lens fibers, exposing the cell nuclei. The fracture, under these conditions, causes a complete separation of the two membranes of the nuclear envelope from one another, thus exposing entire membrane surfaces (those which line the perinuclear space). These surfaces are not seen in their entirety in typical freeze-fracture or freeze-etch preparations, and consequently have not been described previously. The exposed membrane surfaces which line the perinuclear space have numerous convex structures of approximately 1,000 Å, and some larger more irregularly shaped structures. These appear to be fragments of the nuclear pore complexes. Differences in these structures between young fibers and those nearing completion of differentiation is suggested.

The development of new procedures for the preparation of cells and tissues is making it possible to see some of their internal structures with the scanning electron microscope. These procedures involve splitting or fracturing the tissue after fixation. This has been done, for example, in water immediately after fixation; in air after critical-point drying; in liquid nitrogen; or in plastic, following the usual procedures of embedding for TEM.

Splits can occur between cells, exposing cell surfaces, or through cells, exposing parts of the cell's interior.

Lenses which are fractured in the dried state, may tend to split between adjacent cell membranes of the mature fibers, or through the cytoplasm of the actively differentiating, elongating superficial fibers. In the latter case, the nuclei of these fiber cells are exposed. The present report is concerned with the surface of the cell nucleus in the newly forming lens fibers, as seen with the scanning electron microscope (SEM), and the transmission electron microscope (TEM).

Materials and methods. Lenses from adult New...
Zealand white rabbits (approximately two to three kilograms) were prepared by fixing in phosphate buffered 2.5 per cent glutaraldehyde followed by phosphate buffered 1 per cent OsO₄, or by fixing in neutral formalin alone. Neutral formalin-fixed lenses were cryo-fractured after dehydration while in 100 per cent ethanol frozen by liquid nitrogen. After fracturing, the lenses were thawed in ethanol and critical point dried in Freon 13. Lenses fixed in glutaraldehyde and OsO₄ were dehydrated and critical point dried in Freon 13 before fracturing. These lenses were split at room temperature by initiating a fracture with a razor blade. The fragments were mounted on specimen holders so that the exposed bow regions faced up. They were coated with gold and examined with a Philips-500 scanning electron microscope. Some of the dried, fractured pieces of lens tissue were then immersed in propylene oxide and infiltrated with Epon and subsequently sectioned and prepared for TEM. Altogether, preparations from 28 lenses were used. All specimens except that shown in Fig. 2, B were critical-point dried before fracturing.

Results. Fig. 1, A shows the equatorial region of a lens, split from pole to pole, exposing the bow region. A fracture has occurred through the cytoplasm of the newly-differentiating fibers, exposing the cell nuclei. Fig. 1, B shows the surface of a lens fiber cell nucleus. The surface of the nucleus does not contain obvious "holes" or "pores." It does, however, have numerous small punctate elevations. The smallest of these are circular elevations of approximately 1,000 A in diameter. Larger, roughly circular elevations of up to approximately 1,500 A, are also evident. In order to determine the nature of these nuclear surface elevations, specimens which were dried and fractured according to the exact procedures used in the preparation of lenses for SEM were embedded in Epon and thin sections were made to show the ultrastructure of the exposed nuclear surface with TEM.

Fig. 1, C shows a cross-section through a fiber cell nucleus which had been partially exposed by the fracturing procedure. At the unexposed nuclear surface, there is a double-membraned nuclear envelope. At the exposed surface, however, there is a single membrane. Fig. 2, A shows a nucleus near the fracture surface. The cytoplasm on one side of this nucleus has been partially pulled away during the preparation procedure. This has resulted in a separation of the two membranes of the nuclear envelope. Occasional structures, of approximately 800 A are found between the two membranes in the perinuclear space. These have approximately the same dimensions as the structures on the nuclear surface seen with SEM.

Fig. 2, B shows a preparation which split through the nucleus, as a result of cryo-fracture, producing a cross-section of the nucleus. What appears to be the perinuclear space is evident. Occasional places where this space is obliterated (arrows) may represent portions of the nuclear pore complexes.

The nuclear surface, as seen in Fig. 1, B is characteristic of a majority of the nuclei. In some cases, however, the exposed nuclear surface is characterized by the smaller punctate structures, with the larger ones being virtually absent (Fig. 2, C).

Discussion. Rabbit lenses which have been fixed, dehydrated, dried in a critical-point drying apparatus, and then fractured (anterior-posterior fracture), show exposed fiber cell nuclei. The exposed surfaces of these nuclei have numerous convex punctate structures, approximately 1,000 A or greater in diameter. There is no evidence of openings in the nuclear surface.

Observations with TEM of the fractured nuclear surfaces show that the fracturing procedure separates the two membranes of the nuclear envelope. The SEM photographs of the exposed nuclear surfaces, therefore, are showing the entire surface of the inner membrane of the nuclear envelope which faces the perinuclear space.

It is conceivable that the punctate structures seen on the nuclear surface of Figs. 1, B and 2, C, are portions of nuclear pore complexes, which remain with the inner membrane, when the two membranes of the nuclear envelope are separated. Variations in size of the punctate structures could depend on the varying degrees to which the nuclear pore complex (and perhaps adhering fragments of the outer membrane) remain with the inner membrane.

Since the two membranes of the nuclear envelope are separated during the fracturing procedure, the "crater" formed when a nucleus is removed from a surface during fracturing, should display the surface of the outer membrane of the nuclear envelope which faces the perinuclear space. It has been found that this surface also has small punctate structures of the same general size and dimensions as those on the exposed nuclear surface.

If the nuclear pore complex is split at the time of fracture, the inner membrane might carry with it portions of the nuclear pore complex which appear as the punctate structures on the exposed nuclear surface. The origin of the punctate structures on the outer membrane, as seen on the crater surface, can be explained the same way. The nucleus in Fig. 2, C is characterized by the almost exclusive presence of the relatively small punctate structures. This fiber cell is relatively old, based on its location deep within the
Fig. 1. A, portion of rabbit lens bow region, in lens which had been fractured from pole to pole. The fracture line passes through the cytoplasm of the nucleated fibers. Exposed nuclei appear light. Craters formed when nuclei were removed during the fracturing procedure appear dark. The anterior part of the lens is upward. Nuclei of the youngest fiber cells are at lower right. SEM, ×300. B, surface detail of single exposed nucleus from relatively young fiber cell. Convex structures, approximately 1,000 Å in diameter, and larger structures (approximately 1,500 Å) characterize the surface. SEM, ×19,000. C, cross-section through nucleus which had been exposed by fracturing procedure. The double-membraned nuclear envelope is evident below. At the exposed nuclear surface (upper portion of photograph) only the inner membrane of the nuclear envelope remains. TEM, ×24,000.
Fig. 2. A, cross-section of nucleus, partially dislodged by fracturing procedure. The two membranes of the nuclear envelope have separated at the left side of the nucleus. Structures approximately 800 Å in diameter appear at nuclear surface. TEM, ×27,000. B, cryo-fractured specimens. Fracture through nucleus showing perinuclear space and nuclear pores (see arrows). SEM, ×8,200. C, nucleus of fiber cell near end of period of differentiation. The relatively small punctate structures (approximately 1,000 Å) are abundant. However, the larger surface structures seen in Fig. 1, B are absent. SEM, ×18,000.
bow region. A few other such observations have been made, which suggest that changes in nuclear surface topography which accompany lens fiber maturation may be conveniently studied with SEM (compare Fig. 2, C with Fig. 1, B). The ocular lens appears to be an excellent object for this kind of study, since the nuclei of fiber cells at all stages of development can be easily exposed in a single specimen, and conveniently prepared for SEM.

In conclusion, it appears that the dry fractured crystalline lens is an excellent object for the study of the nuclear membrane surfaces which face the perinuclear space. Fracture planes which pass between the two membranes of the nuclear envelope exposing entire membrane surfaces (which are not seen in their entirety in freeze-etch preparations) can be reproducibly obtained with the methods described. Also replicas of the nuclear surface can be made for high resolution analysis with the transmission electron microscope. Finally, because of the unique structure of the lens, these membrane surfaces can be studied at various stages of fiber cell differentiation.

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REFERENCES


A study of the distribution and origin of myo-inositol in the cornea of the rabbit. M. V. RILEY.

The concentration of inositol in the cornea of the rabbit is about one millimole per kilogram tissue water, a level eight- to tenfold that of the aqueous humor. The present study shows that its concentration in the stroma is about the same as in the epithelium and endothelium. Uptake of 2-3H inositol by the corneal layers above the level in the medium was demonstrated in vitro, but the low ratios obtained do not exclusively indicate active transport of the compound. Synthesis of inositol from glucose by the cornea was shown and the estimated rate is compatible with the hypothesis that inositol originates in the cornea and diffuses across the endothelium to the aqueous humor.

The ciliary body and lens accumulate myo-inositol in vitro against a concentration gradient by means of transport mechanisms that are highly specific, show Michaelis-Menton kinetics and are dependent upon metabolic activity of the tissues. Active transport mechanisms with similar characteristics have been shown to be responsible for the accumulation of certain amino acids within these tissues and also within the cornea. By analogy, therefore, it might be expected that the high concentration of myo-inositol in the cornea, approximately eight times that of the aqueous humor, is also due to an active transport system similar to those of the lens and ciliary body.

However, whereas the amino acids are about fivefold more concentrated in the epithelium than in the stroma, inositol is found at roughly equal concentrations in these two layers. It has been