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Aging effect on rabbit's lens fibers; A scanning electron microscopic study

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ABSTRACT

Introduction: The eye lens is a minute organ with complex structure that plays an indispensible role in the process of vision. It is composed mainly of modified epithelial cells that form a unique type of fibers. This study was performed to highlight the morphological variations of lens fibers in different age groups in rabbits' lenses. These variations in fibers' structure may have a great impact on the optical properties of the lens. **Material and methods**: Fifteen white male rabbits of different ages ranging from one month to four and half years were equally divided into three groups; young, adult and aged. Their lenses were dissected and processed for scanning electron microscopy. Gross lenses' diameters and A-P axis lengths were assessed using digitalized gross photomicrographs from dissecting microscope. Also, diameter of lens fibers was measured digitally for comparative purposes among the groups. Statistical analysis for significance of obtained data was performed using analysis of variance and student-T test. **Results**: The average equatorial diameter was 6.1% and 14.5% larger in adult and aged lenses than young ones. The average A-P axis length was 14.1% in adult and 21.7% in aged lenses more than in young lenses as well. Lens fibers exhibited some variations in the pattern of lateral interdigitations that became more branched with folding. Fiber diameter demonstrated an increase from young to adult lenses then a decrease in aged lens was noticed. **Conclusion**: Lens fibers undergo some morphological variations by age progression in the form of compaction in addition to changes in the appearance of ball and socket interdigitations. These changes can be correlated to some age-related optical disturbances as senile prespyopia and cataract. **Recommendation**: Age-related changes in the morphology of lens fibers should be considered in any experimental study including the lens to avoid interpretation bias and get more reliable results.

Key words: Lens fibers, lateral interdigitations, aging, compaction, scatter and scanning electron microscopy.

INTRODUCTION

The lens of the eye is a transparent cellular structure that focuses light on the retina. The tissue of the eye lens is unique as it must be strong, flexible and completely transparent. Its transparency is mainly due to its internal structure, biochemistry of its constituents, lens epithelial cells and capsule. Additionally, the lens is devoid of any blood vessels, capillaries or nerves **(Krestić, 2004)**. The lens is normally surrounded by a transparent elastic capsule, which is a very thick basal lamina of a single layer of cuboidal lens epithelium underneath. These cells are found at the anterior, intermediate and equatorial zone of the lens **(Maisel *et al*., 1981)**. At the equator, these epithelial cells continue to divide giving rise to highly elongated cells with ribbon-like appearance referred to as lens fibers. They keep growing in anterior and posterior directions. At areas where lens fibers converge and meet end-to-end, the anterior and posterior sutures are formed **(Kessel and Kardon, 1979)**.

Fiber cell differentiation, in addition to cellular elongation, is characterized by the synthesis of certain crystallin proteins, and the degradation of all membrane-bound organelles, including nuclei **(Piatigorsky, 1981)**. This results in the formation of an organelle-free zone near the center of the lens (within the cells directly in the light path).The mechanism by which organelles are disassembled is not entirely known, but it is documented that the endoplasmic reticulum, mitochondria, and nuclei are rapidly and synchronously degraded **(Bassnett, 1995)**.

Some investigators postulated that an apoptosis-like mechanism might be involved (**Bassnett and Mataic, 1997).** However, **Zandy and Bassnett (2007)** suggested that a specific lens protease -VEIDase- may be involved after being activated by the proteasome.

Because there is no cell turnover in the lens, all cells are retained within the tissue, those nearest the center being the oldest (nucleus) and those nearest the surface being the youngest (cortex). Therefore, the central lens fibers undergo considerable age-related compaction **(Al-Ghoul and Costello, 1997)**. In certain pathological conditions, however, organelles persist in the central fiber cells. For example, the failure of fiber cells to properly degrade their nuclei is a common feature of human congenital cataract **(Zimmerman and Font, 1966)**.

This study was performed to highlight the morphological variations of rabbits' lens fibers in different age groups. These variations in fiber structure may have a great impact on the optical properties of the lens in physiological and pathological situations.

MATERIAL AND METHODS

Animal groups

Fifteen New Zealand white male rabbits of different ages were purchased from the breeding farm of Ministry of Agriculture, Cairo, to be studied in this work. Since rabbits reach sexual maturity between four and six months of age **(Lawson 1998)**, animals were divided into three groups, each of five as follows:

Group I (Young): rabbits of about 1-2 months of age (400-750 gm).

Group II (Adults): rabbits of about 6-18 months of age (1250-2000 gm).

Group III (Aged): rabbits of 3-4.5 years of age (3000-3750 gm).

Animals were housed in the animal house of Faculty of Medicine, Cairo University at ordinary room temperature, exposed to natural daily light-dark cycles, fed with standard laboratory diet and given tap water to drink *ad-libitum*. They were acclimatized for one week before starting this study.

Scanning electron microscopic study

In each group, rats were sacrificed on the due date by decapitation. Immediately, both eye balls were enucleated from the orbits and lenses were dissected then fixed in 10% buffered formol saline for 24 hr at room temperature. Lenses were then washed in 0.1M phosphate buffer and further fixed for 3-5 days in 2.5% glutaraldehyde in phosphate buffer of pH 7.2 at room temperature with fresh fixative changes daily. These fixation conditions were recommended by **Kuszak *et al.* (1989)** to result in negligible osmotic stress in rabbit lenses. After overnight rinsing in the phosphate buffer, the fixed intact lenses were photographed using a digital microscopic camera (Nicon, Japan) and a dissecting microscope (Zeiss, Germany) with 20X objective lens. For each lens, a surface view and a side view Photomicrographs were taken to be used for further morphometry.

Each lens was then split along the anterior suture plan to two halves. The lens pieces were post fixed in 1% aqueous osmium tetroxide at 4 ˚C overnight, washed in phosphate buffer, and then dehydrated in graded ethanol series. After overnight dehydration in 100% ethanol, specimens were dried in Freon 23 (Dupont, Wilmington, DE, U.S.A.) in a Blazers CPD 020 (Blazers, Hudson, NH, U.S.A.), secured on aluminum stubs with silver plaster, sputter coated with gold and examined in a JEOL JSM 35 c scanning electron microscope (JEOL U.S.A. Peabody, Ma, U.S.A.) at 15 kV. Electron micrographs were taken using a series of magnifications ranging from 20X to 4000X for each specimen. The final processing and photography was performed in the service laboratory, National Research Center, Cairo.

Morphometric and statistical analysis:

Using Leica Qwin 500 LTD image analyzer, measurement of the equatorial diameter and A-P axis of each lens was carried out using soft copies of the previously shot photomicrographs. The percentage of increase from young lens dimensions was estimated for each group. Scanning

photomicrographs were also used to estimate the average diameter of the fibers in each group putting into consideration that each magnification required a separate calibration.

Data were presented as means ± standard deviation. Comparison of data among different groups was carried out by one way analysis of variance (ANOVA). Comparison between percentages was calculated using Qui-squared test. Results were considered significant when probability (p) was ≤ 0.05, highly significant when (p) ≤0.01 and very highly significant when p ≤0.001 **(Mould, 1989)**.

RESULTS

Lens Dimensions

The rabbits' eye lenses appeared in a crystal clear elliptical shape. The overall lens dimension measurements done digitally on the photomicrographs revealed that the average equatorial diameter of young rabbits' lenses was 8.691 mm and the average A-P axis length was 6.456 mm. In comparison, adult rabbit lenses had an average equatorial diameter of 9.221 mm and an A-P axis length of 7.364 mm. The same parameters measured for the aged rabbits showed an average equatorial diameter of 9.955 mm and an A-P axis length of 7.858 mm. Thus, the equatorial diameter increased by 6.1 % in adulthood stage then reached 14.5 % in old age in comparison to the young group. On the other hand, the A-P axis length increased by 14.1 % then 21.7 % in adulthood and old age stages respectively. These results are represented in **Plate 1** and **Table 1**.

Plate 1: Photomicrographs of intact fixed lenses from one of the young (A&B), adult (C&D) and aged (E&F) rabbits demonstrating variation of the equatorial diameter and A-P axis lengths that increase by age using a dissecting microscope (X20) and Leica Qwin 500 LTD image analyzer.

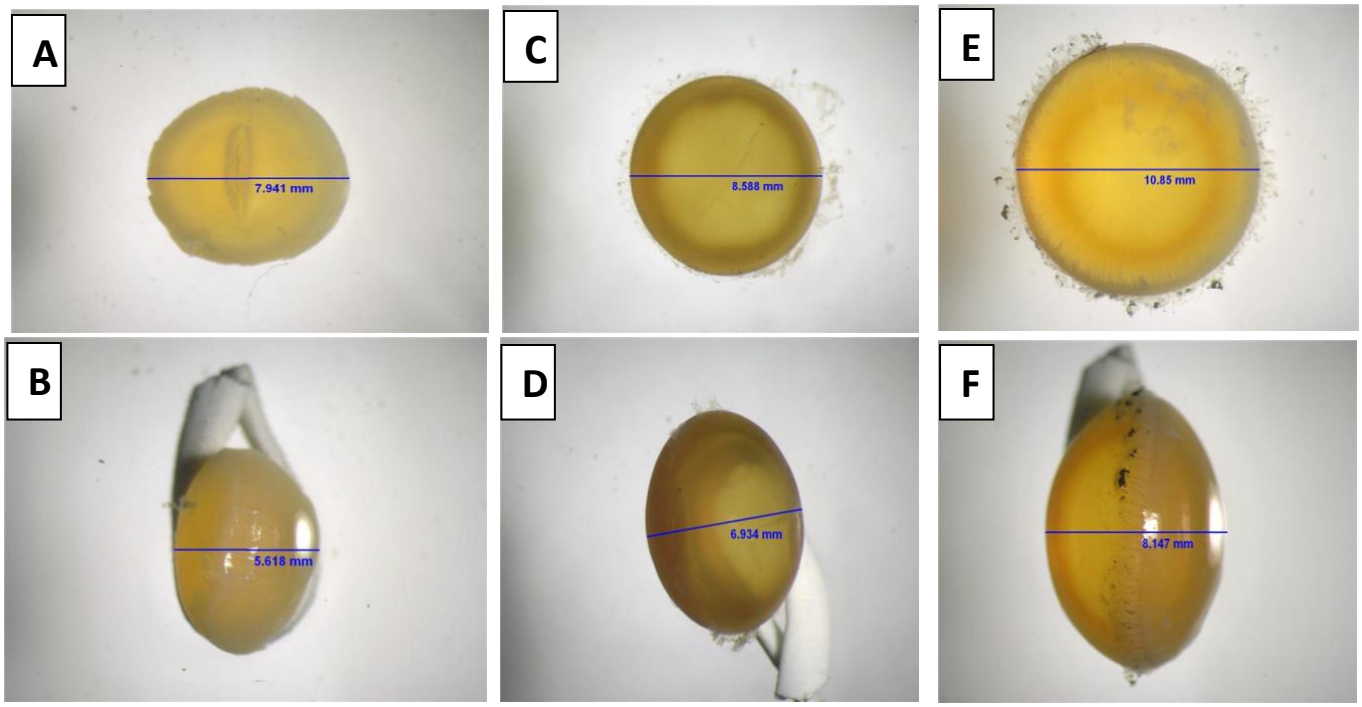


Table 1: Average gross lens dimensions in the three studied groups.

Age Group	Young group	Adult group	Aged group
Average equatorial diameter (mm)	8.691±0.55	9.221±0.5	9.955±0.5*
Average A-P axis length (mm)	6.456±0.87	7.364±0.48	7.858±0.36*
Increase of Average equatorial diameter from young group (%)		6.1*	14.5*
Increase of Average A-P axis length from infantile group (%)		14.1*	21.7*

Values are represented as mean ± SD (n=10).
[Significance was considered P≤0.05]
*=Significant change compared to young group.

SEM results

Examination of the lenses of all groups demonstrated presence of a thin capsule on the outer surface of the lens covering the underlying single layer of lens epithelium. Inner to the epithelium, large number of concentric layers of densely packed lens fibers could be seen and representing the superficial and deep cortical fibers. At the center of the lens, a group of straight fibers were passing along the antero-posterior axis representing the embryonic nucleus **(Plate 2)**. Fibers appeared as tightly joined parallel ribbon-like structures with minimal intercellular spaces in between. Each fiber had the shape of a polygon or squashed hexagon with two wide parallel sides and four other smaller ones. The lens fibers interconnect at these smaller sides to form planar sheets. The corners of each fiber demonstrated processes from the cell membrane of variable shapes along the length of the lens fiber **(Plate 3)**.

In the superficial layers of the cortex, two types of interlocking or lateral interdigitations were identified. The first emerged from the angle formed by the two narrow faces and at the end of the elongated hexagonal-shaped lens fiber. It appeared as a ball-shaped structure on top of a narrow stalk and occurs along the length of the fiber. This ball fit into a complimentary shaped socket formed at the edge of the broad and narrow faces between opposed lens fibers in successive growth shells **(Plate 4)**.

The second type of interlocking device found looked like a flap or a tongue that emanates from the angle formed by a broad and a narrow faces along the length of the fibers. These flaps were also alternating with the sockets of the ball-shaped interdigitations of the first type to attach to oneanother by ball and socket joints at their sides **(Plate 4)**.

In this work, fibers were found to display some morphological differences in the studied groups. In the Young rabbits (group I), fibers appeared of small uniform diameter along their length. The balls of the lateral interdigitations were short, small, showed no pedicles and displayed less regular pattern. The intercellular spaces were wider than expected with frequent un-locking of the adjacent fibers **(Plate 5**A**)**. Adult rabbits (group II) lens fibers were of larger diameter

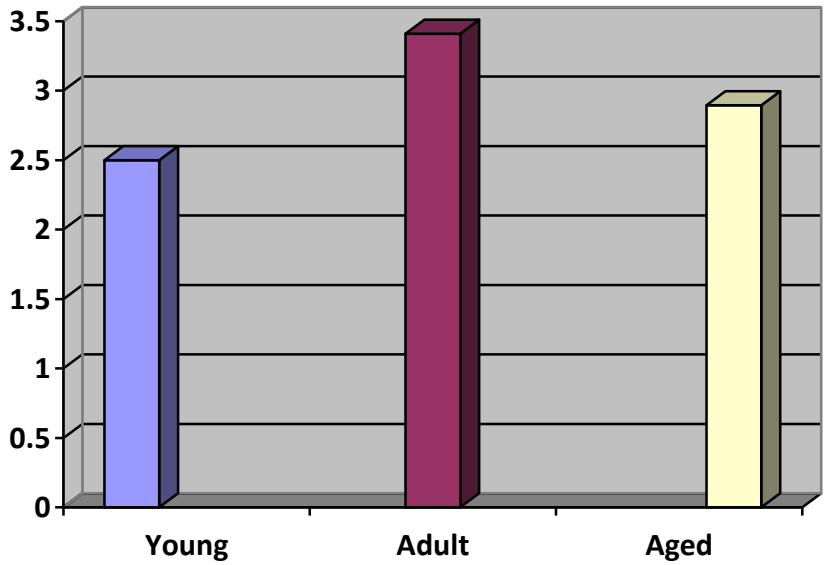
and frequent rod-shaped short toungue-like processes were detected on the broad faces as well as the narrow faces. Their distribution was more regular in the form of straight lines parallel to the linear holes. Typical balls of lateral interdigitations with globular head and long pedicle were observed **(Plate 5**B**)**. Aged rabbits (group III) lens fibers showed a wavy lateral cell membrane with secondary branching of the balls and irregular diameter. The flaps were more globular in shape and were also present in zigzag-like lines accompanied by linearly arranged sockets **(Plate 6)**.

Morphometric and statistical results

Statistical analysis of the gross lens dimensions revealed a significant increase in both measured parameters, namely average diameter and A-P axis length in adult and aged groups in comparison to the young group ($P \leq 0.05$).

Morphometric measurement of the fiber diameter revealed that the average value for the three groups were 2.499 µm in young, 3.409 µm in group II and 2.894 µm in group III respectively. Statistical analysis revealed that the average diameter was significantly high in group II in comparison to groups I & III ($P \leq 0.05$). Moreover, comparison between group I and group III revealed also a significant increase in aged fibers' diameter in comparison to young ones ($P \leq 0.05$). Data are represented in **Figure 1** and **Plate 7**.

Fig. 1: A histogram representing the morphometry of average lens fibers' diameter in the three studied groups.



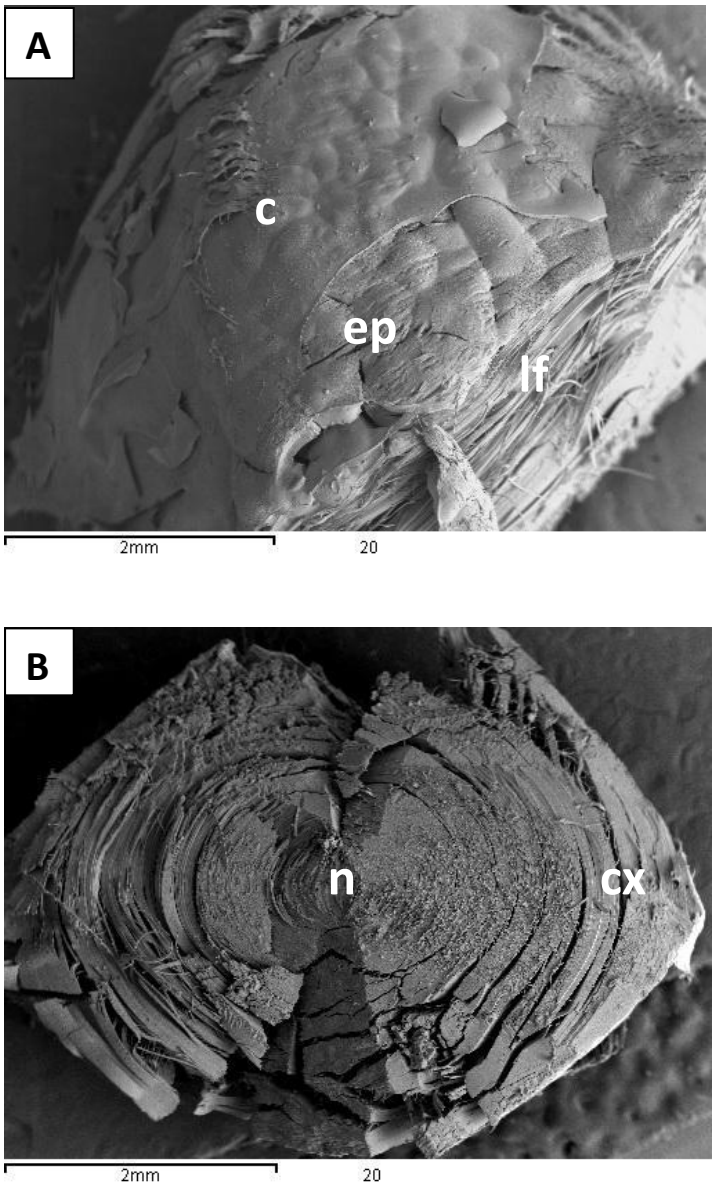


Plate 2: Scanning electromicrographs showing:

A- a surface view of an infantile lens (group I) demonstrating the outer capsule (c) covering a single layer of epithelium (ep) followed by lens fibers (lf) (X20).

B- A side view of the same lens showing a group of straight fibers were passing along the antero-posterior axis representing the embryonic nucleus (n) followed by concentric layers of fibers representing the foetal and adult nuclei followed by the cortex(cx) (X20).

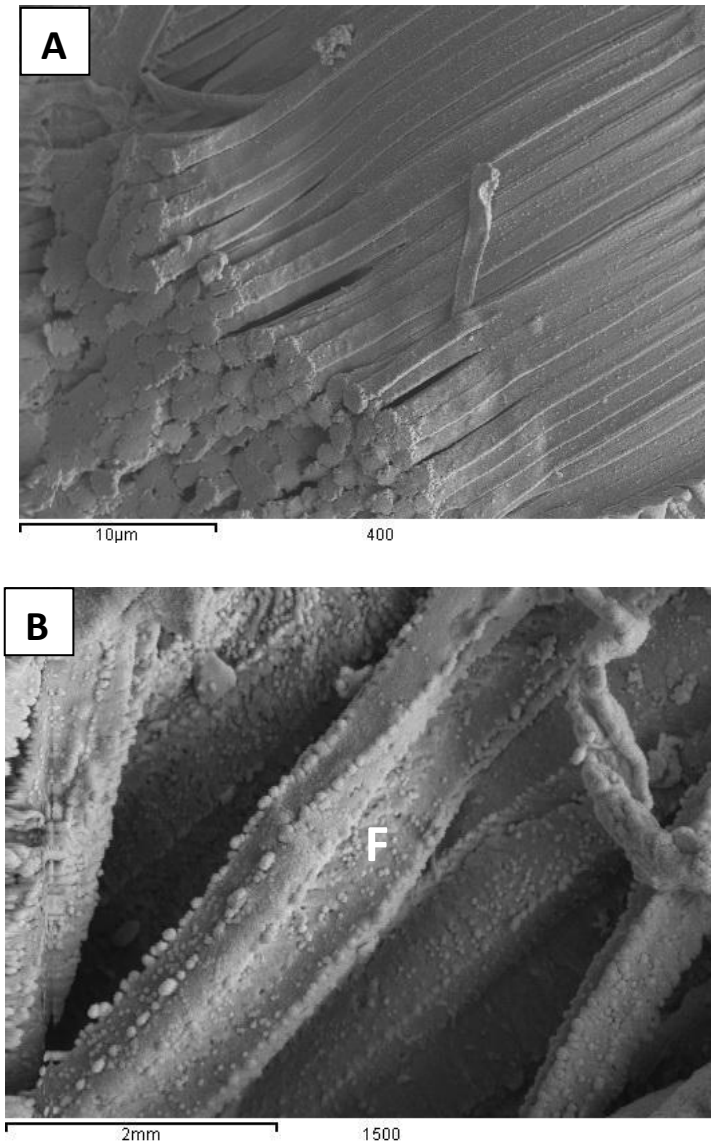


Plate 3: Scanning electromicrographs showing:

A- Lens fibers appear as tightly joined parallel ribbon-like structures with minimal intercellular spaces in between. (X400).

B- Fibers (F) had the shape of a polygon or squashed hexagon with two wide parallel sides and four other smaller ones. The corners of each fiber demonstrated processes from the cell membrane of variable shapes along the length of the fibers (X1500).

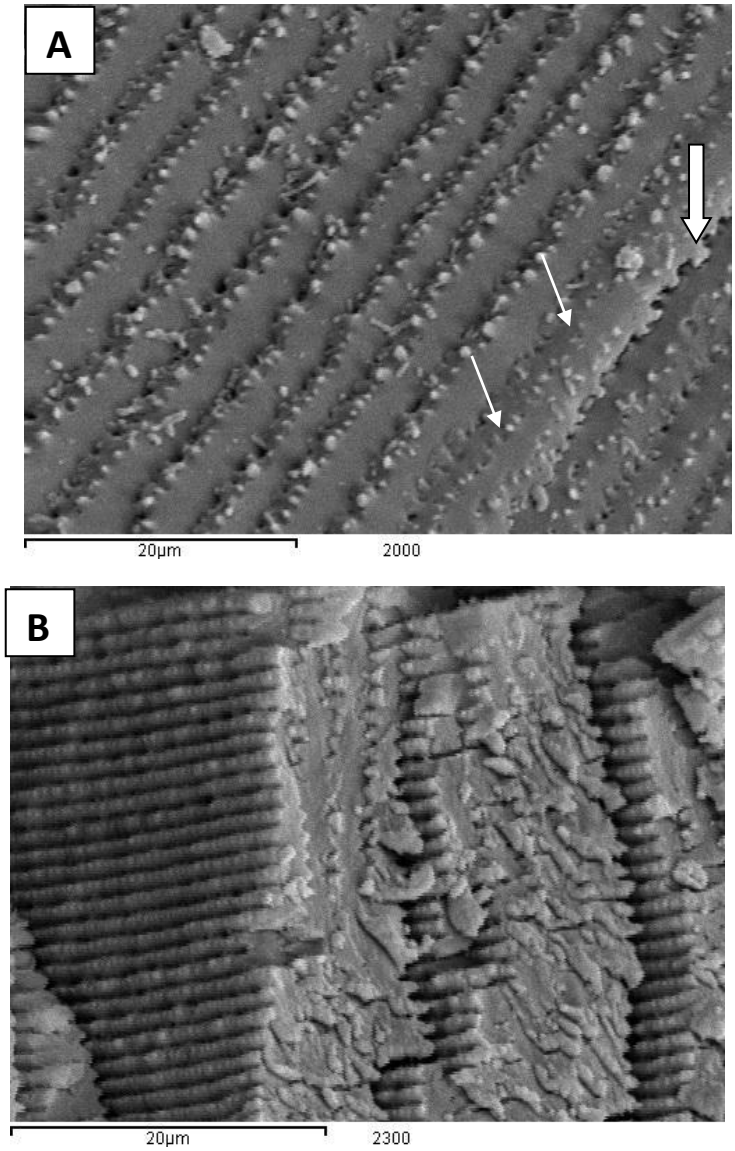
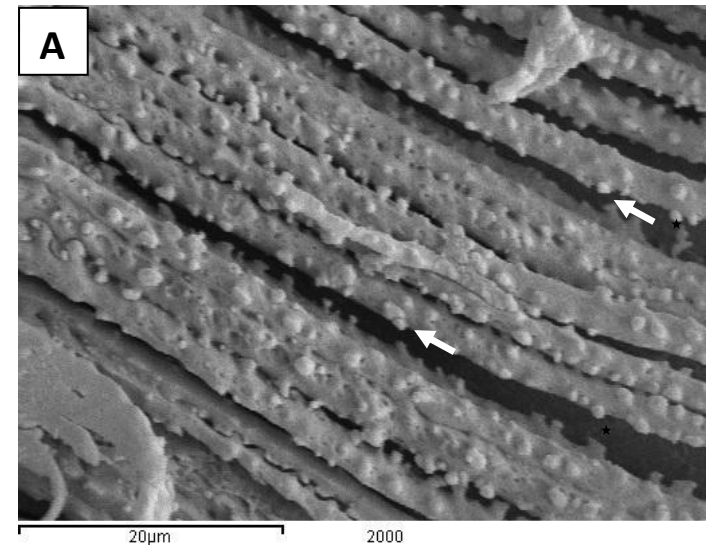


Plate 4: Scanning electromicrographs showing:

A- locked lens fibers appear as tightly joined parallel ribbon-like structures with lateral interdigitations in the form of ball and socket (thick arrow) and tongue-like flaps (thin arrows) (X2000).

B- Tightly packed fibers forming successive layers or shells (X2300).



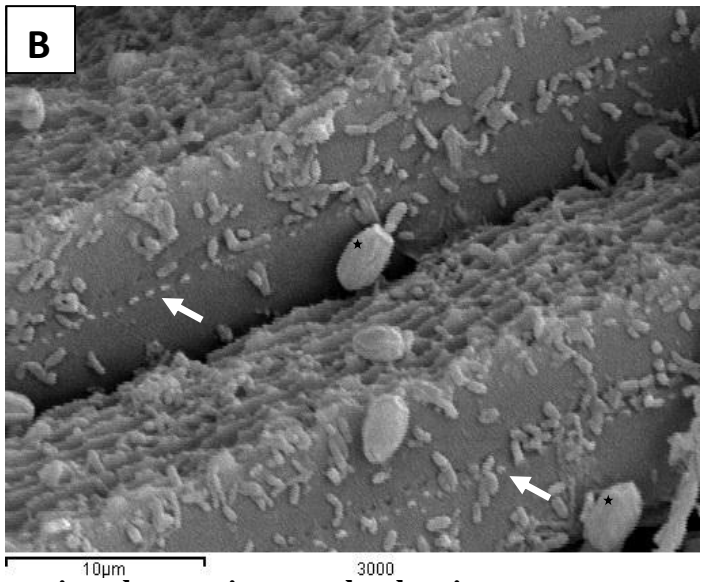


Plate 5: Scanning electromicrographs showing:

A- In the young rabbits (group I), fibers appeared of small uniform diameter all through their length with short, small non-pediculated balls of the lateral interdigitation (arrows) and displayed less regular pattern. The intercellular spaces were wider than expected (stars) with frequent un-locking of the adjacent fibers (X2000).

B- Adult rabbits (group II) lens with larger fiber diameter and frequent tongues (arrows) in straight lines on the broad faces as well as narrow faces parallel to the linear holes. Typical balls (stars) of lateral interdigitations with globular head and long pedicle were observed (X3000).

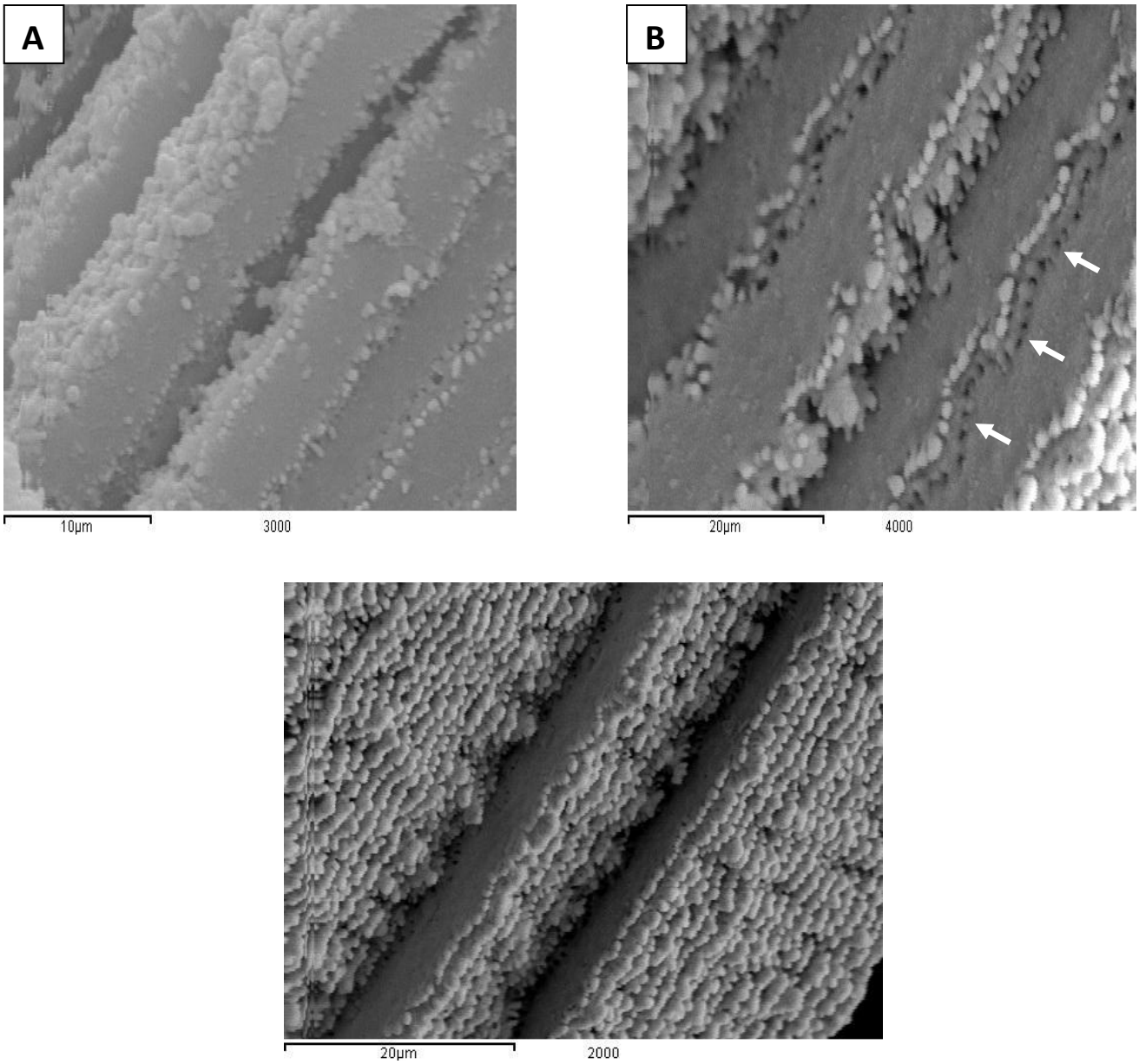


Plate 6: Scanning electromicrographs of aged rabbits (group III) lens fibers showing:

A- a wavy lateral cell membrane (X3000).
B- secondary branching of the balls and irregular diameter of the fibers. The flaps were more globular in shape and were also present in zigzag-like lines accompanied by linearly arranged holes (arrows) (X4000).
C- side view of successive layers of fibers with irregular branching lateral interdigitations (X2000).
D-

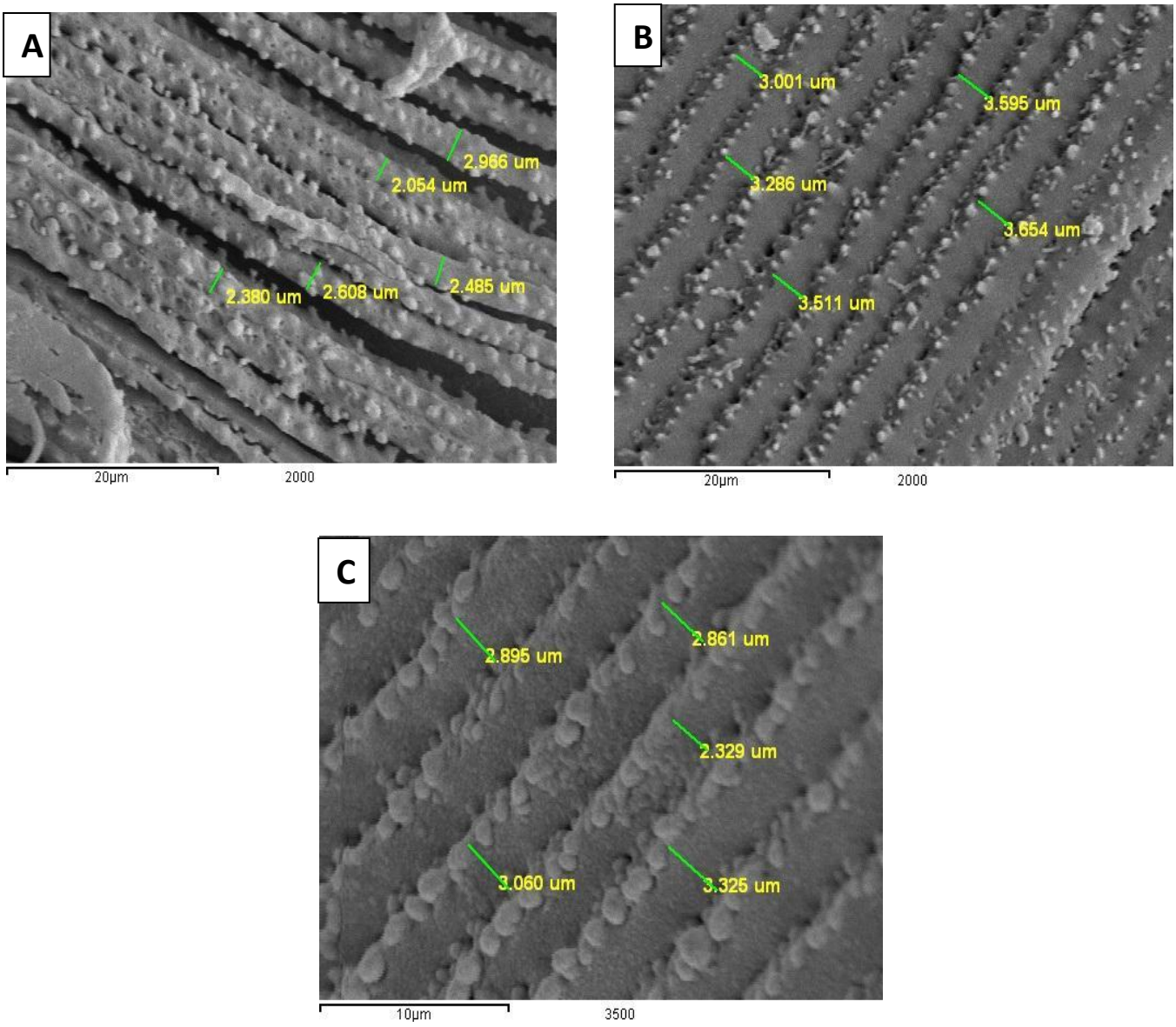


Plate 7: Computer-assessed morphometry of the average lens fiber diameter in the three studied groups, young (A), adult (B) and aged (C) from scanning electromicrographs (A& B X2000, C X3500).

DISCUSSION

Rabbit lenses, in the present work, demonstrated an age-related increase in both equatorial and A-P axial dimensions. Specifically, average equatorial diameter was 6.1% and 14.5% larger in adult and aged lenses than young ones. The average A-P axis length was 14.1% in adult and 21.7% in aged lenses more than in young ones as well. This increase was larger than anticipated with respect to the earlier work of **Kuszak and Al-Ghoul (2002).** The lens dimensions measurements in the three groups showed that rabbit lens, like all vertebrate lenses, grow throughout life and this is in accordance with **Lovicu and Robinson (2004)**. This increase is consistent with earlier published data showing that between 2–4 years of age rabbit lenses have not yet reached a growth plateau as **Van Heyningen** mentioned in his work **(1977)**. It is likely that the increase in overall lens thickness (A-P axis) is an important component that has an impact on transmission of light through the lens. Furthermore, the change in size of the lens may have an influence on pathogenesis of some ocular diseases **(Brown and Hungerford, 1982)**.

The present study demonstrated the presence of a thin capsule on top of the simple cubical epithelial layer. This capsule is clear in vivo and represents a very thick basal lamina of lens epithelium. **Krestić (2004)** mentioned that it is made of glycoproteins synthesized in thin layers by the lens epithelium. These glycoproteins condense to form elastic network of short interlacing thick microfilaments that isolate the lens from the rest of the body. It is impermeable to antigens, macrophages and plays an important role in lens metabolism. The capsule is highly elastic and ultimately is an essential feature to facilitate accommodation process. By aging, this elasticity is progressively lost and significantly contributes to the development of senile prespyopia. Furthermore, the zonular fibers which are inserted into the lens capsule just anterior and posterior to the lens equator, usually become more distant from the equator and this may also contribute to development of senile prespyopia **(Chylack, 1994 and Cavallotti and Cerulli, 2008)**.

The innermost part of the lens is formed of the nucleus which contains the old fibers. Central fibers pass directly along the A-P axis of the lens forming the embryonic nucleus. These fibers originally developed from the epithelial layer of the posterior capsule in very early stages of embryogenesis. These fibers act like a corner stone for newly formed fibers to circulate around it in concentric layers similar to onion leaves **(Kuszak and Costello, 2002)**. By aging, these fibers are packed more densely due to their reduced movement during accommodation and are thus more tightly interconnected. The increased compaction is employed to provide more space for newly formed fibers to lie on. This process is accompanied by variable degrees of water loss and formation of more strong interlocking devices between the nuclear fibers **(Al-Ghoul and Costello, 1997)**.

The interlocking devices take the shapes of balls on a short stalk, tongue-like flaps, and fingerlike processes that fit into complementarily shaped sockets, prints, and fingerprints, respectively, of adjoining fibers. In the present work, the sockets were demonstrated however, the corresponding balls showed age variations in the form of irregularity and folding of the cell membrane. This was in agreement with **Kuszak *et al.* (1980)** who added that gap junctions comprising more than 50% of the fiber-cell membrane may serve as ultrastructural interlocking devices as well. The interlocking devices and gap junctions are probably necessary to maintain fiber order which is a critical requirement for lens transparency **(Hogan *et al.*, 1971)**. With increased maturation, the uniform morphology of the fibers and their interlocking devices is lost. The highly repetitive ordered alignment of young uniformly shaped fiber cells acts to

minimize large-particle scatter **(Al-khudari *et al.*, 2007)**.

In the current study, only the sockets were demonstrated as complementary counter parts to the balls while the prints and finger prints couldn't be identified because they needed larger magnifications than the maximum used here (X4000 only).

The short clumsy irregular balls present on lateral sides of young lenses realized in the present work seemed to link the adjacent fibers less strongly than in adults. This could explain the wide unlocked areas frequently met with in that group in comparison to the other two. However, no similar comment was met within the previous related studies we could go through.

The fold formation of the lens fibers realized in this study demarcated the onset of compaction which is thought to be an ongoing process affecting fibers at all stages of lens development, growth and aging **(Kuszak and Al-Ghoul, 2002)**. Additionally, ultrastructural investigations have shown that compaction alters the membrane architecture of fibers, affecting both the overall membrane topology **(Freel *et al.*, 2003)** and the distribution of intramembrane particles and junctions **(Vrensen *et al.*, 1992)**. This component of compaction has an influence on both the packing density and arrangement of fibers, and also produces accordion-like folds that reduce overall fiber length. In the present investigation, similar changes were noted in aged rabbit lenses.

The present results do not minimize the importance of oxidation of lens cytoplasmic and membrane proteins, the development of cross-linked, high molecular weight protein aggregates, or the various ultrastructural fiber alterations that have been documented in both aged normal lenses and in age-related nuclear cataracts **(Garner and Spector, 1980)**. However, the present data underscores the fact that compaction is one of the important changes that precedes and may, in fact, contribute to the development of age-related cataract.

It is well established that the precise arrangement and packing of fibers in regular layers contributes to lens transparency **(Kuszak and Brown, 1994)** and hence, any disruption or modification of this exact fiber arrangement adversely affects lens function (**Kuszak *et al.*, 1994)**. Because age-related compaction alters fiber ultrastructure (impacting both the packing density of fibers and their membrane topology) it is logical to assume that those compaction-induced changes can ultimately contribute to the degradation of the optical properties of the lenses.

It is well known that the nucleus is harder than the more superficial fiber layers in adult mammalian lenses and that this increase in hardness becomes more pronounced with age **(Pau and Krantz, 1992)** as does an increase in nuclear stiffness **(Heys *et al.*, 2004)**. Both factors contribute to the loss of nuclear flexibility **(Fisher, 1971)**. Hence, if the lenses in this study were subjected to a limited amount of osmotic stress, it is apparent that the central fibers would be less susceptible to osmotic pressure than the peripheral fibers. With aging, the response to osmotic factors would be further lessened.

One possible cause for the significant reduction in fiber diameter in aged lenses of the present study is water loss. In fact, Raman spectroscopy of both rabbit and human lenses has shown that the water content decreased dramatically from the superficial cortex to the deep cortex **(Huizinga *et al.*, 1989)**. Unfortunately, there is no clear agreement about water content in the fibers of aged and cataractous lenses. Some analyses showed decreased water content **(Deussen and Pau, 1989)**, some found no change with aging or cataract state **(Heys et al., 2004)** while others showed an increase in r water content with aging **(Siebinga *et al.*, 1991)**and cataract **(Mizuno and Ozaki, 1991)**.

CONCLUSION

Lens fibers undergo some biochemical and morphological variations by age progression in the form of compaction in addition to changes in the appearance of lateral interdigitation system. These changes can be correlated to some age-related optical

disturbances as senile prespyopia and cataract.

RECOMMENDATION

Through the use of scanning electron imaging in the eye lens, many details which cannot otherwise be observed in situ due to transparency, minute dimensions, and diffraction can be both qualitatively and quantitatively imaged provided that appropriate magnifications are used. Age-related changes in the morphology of lens fibers should be considered in any experimental study including the lens to avoid interpretation bias and get more reliable results.

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تأثير التقدم في العمر على ألياف عدسة العين في الأرانب البيضاء: دراسة بإستخدام الميكروسكوب الإلكتروني الماسح

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تعتبر عدسة العين عضو دقيق ذو تركيب معقد ويلعب دورا اساسياً في عملية الإبصار حيث يتكون من خلايا طلائية معدلة لتكون نوع فريد من الألياف و بناءا على ذلك تم تصميم هذه الدراسة لإلقاء مزيدا من الضوء علي التغيرات الشكلية المحتملة في المراحل السنية المختلفة لعدسة عين الأرانب البيضاء حيث أن التباين في تركيب هذه الألياف يمكن أن يكون له تأثير بالغ على الخواص البصرية للعدسة.

المواد والطرق المستخدمة: تم الاستعانة بخمسة عشر من ذكور الأرانب البيضاء و التي تراوحت أعمارهم من شهر إلي أربع سنوات ونصف وتم تقسيمهم سنيا إلي ثلاث مجموعات متساوية في العدد: أرانب حديثة السن، بالغة، متقدمة السن . وبعد تشريح عيونهم و استخراج العدسات منها تم تجهيزها للفحص باستخدام الميكروسكوب الآلكتروني الماسح بالاضافة إلى إجراء قياس لقطر جميع العدسات و سمكها في المحور الأمامي الخلفي عن طريق صور تم الحصول عليها بإستعمال كاميرا رقمية تم تركيبها على ميكروسكوب تشريحي، كما تم أيضا قياس قطر الألياف المكونة للعدسة بغرض مقارنتها في المجموعات الثلاث و أعقب ذلك اجراء تحليل احصائي للنتائج التي تم الوصول اليها للوقوف على مدى دلالتها الاحصائية عن طريق تحليل للمتغيرات و اختبار (الطالب-T).

النتائج: أظهرت النتائج وجود زيادة ذات دلالة احصائية في متوسط قطر العدسات بمقدار 6.1% للعدسات البالغة و 14.5% للعدسات االمتقدمة في السن مقارنة بالعدسات حديثة السن و كذلك كان سمك هذه العدسات لكل من الأرانب البالغة والمتقدمة في العمر أكبر من مثيلاتها صغيرة العمر بمقدار 14.1% و 21.7% علي التوالي. كما وجد أن هناك زيادة واضحة في قطر ألياف العدسات البالغة عنها في المجموعتين الأخرتين، كما بدت بعض التغيرات في شكل الامتدادات الجانبية المتداخلة التي أصبحت أكثر تفرعا مع وجود تموجات أو ثنيات في العدسات المتقدمة في السن.

الاستنتاج: نتستنتج من ذلك إلى أنه مع التقدم في العمر تحدث بعض التغيرات في حجم العدسة و شكل الألياف المكونة لها في صورة انضغاط للألياف بالاضافة إلى تغير ملحوظ في الزوائد الجانبية المتداخلة بين الألياف مما يمكن أن يفسر بعض اضطرابات الابصار المرتبطة بتقدم السن مثل طول النظر و المياه البيضاء.

التوصيات: يوصى بوضع هذه التغيرات المرتبطة بتقدم العمر في أبعاد العدسة و شكل أليافها بعين الاعتبار أثناء تقييم نتائج الأبحاث الخاصة بعدسة العين للحصول على نتائج أكثر دقة و تفادي التقييم غير الدقيق لها.