REVIEW ARTICLE

Telomere-dependent senescent phenotype of lens epithelial cells as a biological marker of aging and cataractogenesis: the role of oxidative stress intensity and specific mechanism of phospholipid hydroperoxide toxicity in lens and aqueous

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ABSTRACT

Cataract formation represents a serious problem in the elderly and has a large impact on healthcare budget. Aging and cataract formation are relatively complex phenomena, both *in vivo* and *in vitro*. Telomeres are special structures at the end of chromosomes. They shorten during each round of replication, and this has been characterized as a mitotic counting mechanism. Our review analysis in this work shows that the rate of telomere shortening in human lens epithelial cells during aging and cataract formation is modulated by oxidative stress as well as by differences in antioxidative defense capacity of the normal and cataractous crystalline lenses. Presented in this review studies suggest that telomere shortening in human lens cells and increased oxidative stress are the result of the peroxidative damage to the lens cell membranes and biomolecules induced in the lack of reductive detoxification of phospholipid hydroperoxides as the triggering mechanism of cataractogenesis. Lipid peroxidation (LPO) is a causative factor of cataract. The increased concentrations of primary molecular LPO products (diene conjugates, lipid hydroperoxides) and end fluorescent LPO products were detected in the lipid moieties of the aqueous humor samples obtained from patients with senile and complicated cataracts when compared to normal donors. The progressive accumulation of oxidative damage may act as an important mechanism for organism aging and cataractogenesis. The oxidative stress form and intensity might determine the lens senescence rate and cataract type, making efforts in the cataract prevention challenge more complex. The analyzed challenge in this work is that the reduction in telomere shortening rate and damages in telomeric DNA make an important contribution to the anticataract and life-extension effect of carnosine administered systemically in the formulations stabilizing a dipeptide from the enzymatic hydrolysis with carnosinase, or topically administered to the eye with carnosine ophthalmic prodrug *N*-acetylcarnosine and lubricant formulations thereof including corneal absorption promoters. Telomere length in the human crystalline lens cells is a reflection of aging, cataractogenesis, and lifespan in biogerontological studies. 'In the perspective of every person lies a lens through which we may better understand ourselves.' Ellen J. Langer.

INTRODUCTION

Cataract formation represents a serious problem in the elderly and has a large impact on healthcare budget.

Aging and cataract formation are relatively complex phenomena, both *in vivo* and *in vitro*. Free radicals and oxidative stress have been suggested for a long time to be involved in or even to be causal for both the crystalline lens aging process and cataractogenesis. Telomeres are special structures at the end of chromosomes. They shorten during each round of replication, and this has been characterized as a mitotic counting mechanism. Our review analysis in this work shows that the rate of telomere shortening in human lens epithelial cells during aging and cataract formation is modulated by oxidative stress as well as by differences in antioxidative defense capacity of the normal and cataractous crystalline lenses. The oxidative stress form and intensity might determine the lens senescence rate and cataract type, making efforts in the cataract prevention challenge more complex (*Figure 1*) [1,2].

OXIDATIVE STRESS INTENSITY IN LENS AND AQUEOUS DEPENDING ON AGE-RELATED CATARACT TYPE AND PATHOGENESIS

It is well established that a major factor involved in the development of cataract is oxidative insult [3–20]. Oxidative stress associated with the formation of lipid peroxides is suggested to contribute to pathological processes in aging and systemic diseases, such as diabetes, atherosclerosis, chronic renal failure, inflammation, and retinal degenerative diseases known as statistically significant risk factors for cataract [21–25].

The observation that lipid peroxides are elevated in the lens membranes of some patients with cataract has drawn attention to these toxic oxidants [26–30]. Lipid peroxides can cause cataract, producing damages to both cell membrane and cytosol regions [25,27,28,31–34]. At the membrane, lipid hydroperoxides induce changes in permeability [35–38], refashion the microviscosity (order) of its lipid–protein environment [39–41], cause
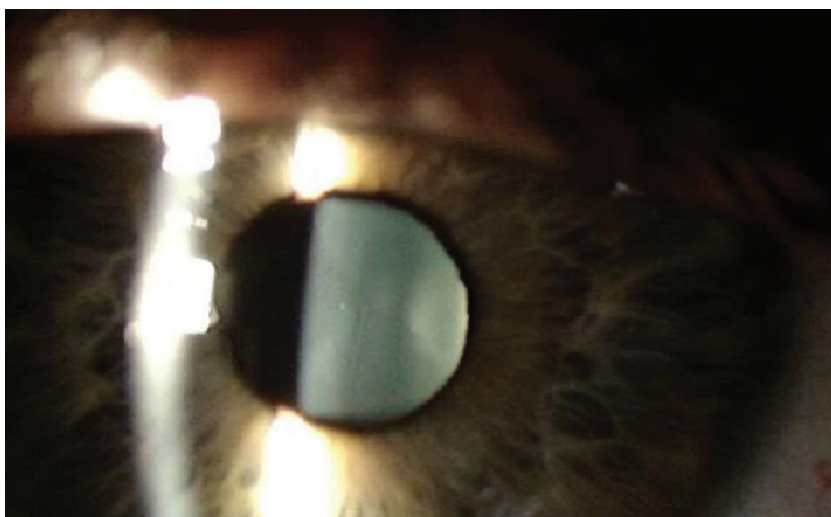

LOW RESOLUTION COLOUR FIG

Figure 1 Recent clinical research is providing strong evidence that cataracts may be prevented by administering *N*-acetylcarnosine lubricant eye drops [1]. How to protect against cataracts is an important concern as we live longer than ever before.

an uncoupling of the membrane-bound enzyme Na, K-ATPase and oxidative inhibition of Ca^{2+}-ATPase in several tissues including the lens [42,43]. Within the cell, lipid peroxides can damage DNA [44], induce a drop in total glutathione and dramatic change in the redox ratio of glutathione, lead to the appearance of new fluorophores and large protein aggregates with low solubility (clouding matrix) in the lens matter [20,45–48].

The aqueous humor contains about 4 μg/mL of high density lipoproteins, which evidently take part in the renewal of lipid compositions of the lens [49,50]. The oxidative modification of lipoproteins in the presence of trace amounts of transition metals (copper or iron) is variously associated with lipid peroxidation (LPO), an increase in net negative charge, hydrolysis of phospholipid and fragmentation of apoprotein B (for review see Ref. [51]) and the oxidized lipid moieties of lipoprotein particles can be implicated to the lens toxicity triggering cataractogenesis [52,53]. Supposedly, this event is controlled because of the ability of reduced glutathione (GSH) of the lens to detoxify hydroperoxides via glutathione peroxidase (GPX) [54,55].

TELOMERE ATTRITION AND TELOMERASE ACTIVITY

Normal human somatic cells undergo a finite number of cell divisions and ultimately enter a nondividing state called replicative senescence. It has been proposed that telomere shortening is the molecular clock that triggers senescence [56].

Telomeres are the special structures of chromosome ends that provide stability and allow the complete replication of the ends (*Figure 2*). Telomere DNA is the repetitive sequence at the end of linear chromosomes, which shortens progressively with cell division and limits the replicative potential of normal human somatic cells. Telomeres are composed of repetitive nucleotide sequences and associated proteins that protect chromosomes from degradation and recombination. Vertebrate telomeric DNA consists of a conserved hexameric sequence (5′-TTAGGG-3′) arranged in tandem repeats [57–61]. Normal somatic cells lose 50–200 bp of their telomeres per cell division because of the 'end-replication problem' and eventually become senescent when their telomeres reach a critically shortened length [62–64]. Telomere length is maintained by a balance between processes that lengthen and those that shorten telomeres. Evidence from various organisms suggests that several factors influence telomere length regulation, such as oxidative stress, telomere binding proteins, telomere capping proteins, telomerase, and DNA replication enzymes. Understanding how these factors interact to coordinate the regulation of telomere length will allow a more complete understanding of telomere function in the cell.

Telomere plays important roles in maintaining chromosome stability by protecting chromosome ends from degradation and end-to-end fusion [65]. The DNA replication mechanism in most human somatic cells is unable to fully replicate the very end of a linear DNA molecule. The shortening of telomere has been suggested
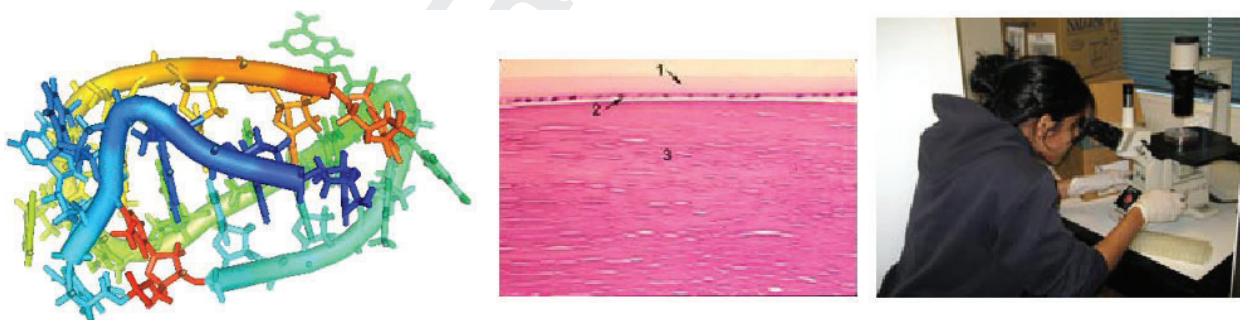
Figure 2 Telomeric structure in human lens epithelial cells (2) forming a loop that caps the end of telomeres. The lens is a transparent soft **15** biconvex structure composed of crystallins. The adult lens measures about 9 mm in diameter and is 3.5 mm thick. It is completely enveloped by the thickest basement membrane in the body, the capsule (#1 in photomicrograph), which is 10–20 μm thick of hyaline material containing type IV collagen. There is a layer of large cuboidal epithelial cells, (the lens epithelium) beneath the anterior capsule (#2 in photomicrograph). In the center (#3 in photomicrograph), tightly packed cells have lost their nuclei and become packed by special transparent proteins (crystallins) to form so-called lens fibers. New lens cells are added to the margin of the lens throughout life from the lens epithelium, but the cells at the cortex and nucleus (center) of the lens do not undergo turnover or replacement and are therefore the oldest cells in the body of an adult. The lens is avascular and nourished by diffusion from the aqueous and vitreous.

to be the mechanism that limits the number of divisions a cell can undergo [66,67]. Because of this end-replication problem, telomere shortens at each round of cell division in the absence of telomerase, and other mechanisms that compensate the telomere loss. Normal human somatic cells can only divide for a finite number of times, which is referred to as replicative senescence.

Telomerase is a specialized reverse transcriptase that extends telomeres of eukaryotic chromosomes (*Figure 3*) [68–70]. Telomerase is a ribonucleoprotein polymerase that specifically elongates telomeres. In human cells, telomere length is not maintained, and telomerase is not active in some tissues. The catalytic core of human telomerase is composed of an RNA subunit known as 'hTER' (human telomerase RNA) and a protein subunit named as 'hTERT' (human telomerase reverse transcriptase) [69–72]. Expression of hTERT is a key step of regulation of telomerase in human cells and plays a crucial role in cellular immortalization and cancer development [64,73–76].

Telomerase is a ribonucleoprotein complex that adds TTAGGG repeats onto the 3′ ends of chromosomal DNA termini, thereby compensating for their shortening (incomplete replication) [77,78]. Telomerase is absent or found at extremely low levels in most normal somatic cells but is present in normal proliferating stem and germ cells such as those in the basal layer of the epidermis [79], adult rat seminal vesicle [80], human adult testis [62], ovaries [62], and the majority of cancers [62,81,82]. Corneal epithelial and endothelial cells and the retinal pigment epithelium have previously been shown to be telomerase negative [60,83]. Cells that lack telomerase activity, such as retinal pigment epithelial cells (RPE) and fibroblasts, have a finite number of population doublings (PDs) in culture before reaching senescence. A previous study has shown that there was an age-related difference in telomere lengths in peripheral blood leukocytes [84]. Telomere lengths also decrease with PDs in cultured cells lacking telomerase activity [85]. Transfection of RPE and foreskin fibroblast cells with a telomerase expression vector prevents telomere loss and allows these cells to escape the normal conversion to proliferative senescence. In addition, these transfected cells have unlimited PDs [86,87], indicating an important role for telomerase in determination of proliferative potential [56]. It is now more than a dozen years since the enzyme telomerase was discovered, and since that time, key studies have characterized the structural components of the enzyme and the associated telomeric proteins. These discoveries have led to the proposal that telomerase expression can be used as a helpful marker for diagnostic and prognostic purposes in humans.

Several studies indicate that various genes are implicated in regulation of telomerase activity directly or indirectly. These genes include *c-myc, mad 1, bcl-2, p53, p21*^{WAF1}*,RB, PKC*, and protein phosphatase 2A [88,89]. However, how telomerase affects the expression of other genes is still unclear. Recent studies suggest that, besides its major role in telomere maintenance, telomerase may also interact with nontelomeric DNA [90] and other molecules such as signaling protein p53 [88]. The previous work demonstrates that hTERT can regulate expression of other genes including *c-jun,c-fos, p53* and bcl-Xs and also prevent camptothecin-induced apoptosis in rabbit lens epithelial cells [91]. It is conceivable that through interactions with other molecules, telomerase may exert different functions besides its major role in maintaining the stability of telomere structures.

Besides telomere maintenance, the previous studies have demonstrated that telomerase has other functions beyond telomere synthesis. First, overexpression of hTERT in rabbit lens epithelial cells not only yields telomerase activity but also distinctly suppresses camptothecin-induced apoptosis. The suppression of induced apoptosis by hTERT is associated with its repression of expression of the apoptotic genes including p53 and Bcl-XS but not linked to telomere synthesis [91]. Second, when introduced into human lens epithelial cells, regardless of the presence or absence of the endogenous telomerase activity, hTERT regulates the retinoblastoma (Rb)/E2F pathway to accelerate the cell growth rate [92].

OXIDATIVE STRESS, THE REPLICATIVE LIFESPAN OF CELLS, TELOMERE INTEGRITY, AND CELLULAR SENESCENCE

The replicative lifespan of primary human cells is telomere dependent; however, its heterogeneity is not understood.

The free-radical theory of aging cited later proposes that reactive oxygen species (ROS) are major driving forces of aging and are also critically involved in cellular senescence. Besides the mitochondrial respiratory chain, alternative sources of ROS have been described, which might contribute to cellular senescence. The free-radical theory of aging considers molecular damage caused by the presence and action of ROS as a major cause for

Telomeric G-strand elongation by the telomerase

Figure 3 Telomerase Structure at Work. The telomerase enzyme complex consists of an RNA component and a protein component. The protein component's catalytic subunit acts as a reverse transcriptase (green), using telomerase RNA (blue/white) as a template for the addition of telomeric repeat sequences to the telomere DNA strand (red).

aging processes in most if not all species. The role of ROS as mediators of senescence and determinants of lifespan has been addressed by genetic studies in several model organisms (for review, see ref. [93]). Thus, reducing the level of antioxidant enzymes, such as superoxide dismutase (SOD), leads to a consistent reduction of the lifespan

in many species, including the mouse [94]. Accordingly, extending the antioxidative capacity, for example by overexpression of SOD/catalase [95] has been shown to extend lifespan in an otherwise short-lived strain of the fruitfly *D. melanogaster*, whereas ectopic overproduction of mitochondrial catalase can prolong the lifespan of mice [96]. To determine the role of ROS in mammalian longevity, the authors generated transgenic mice that overexpress human catalase localized to the peroxisome, the nucleus, or mitochondria (MCAT). Median and maximum life spans were maximally increased (averages of 5 months and 5.5 months, respectively) in MCAT animals. Cardiac pathology and cataract development were delayed, oxidative damage was reduced, H_2O_2 production and H_2O_2-induced aconitase inactivation were attenuated, and the development of mitochondrial deletions was reduced. These results support the free-radical theory of aging and reinforce the importance of mitochondria as a source of these radicals [96].

However, there are also examples where overexpression of antioxidant enzymes did not extend the lifespan; thus, a large cohort study demonstrated that overexpression of CuZnSOD does not increase lifespan of the mouse [97]; similarly, catalase-transgenic mice are not long-lived but rather display enhanced sensitivity to oxidative stress [98]. Whereas these data support the concept that ROS-induced damage contributes to aging processes, at least in some specific genetic backgrounds, the role of the individual antioxidant enzymes in this process remains to be determined.

Concerning human aging, many questions about molecular mechanisms have been addressed using *in vitro* senescence models derived from normal diploid human cells. The proliferative potential of human primary cells in culture is limited, and extended passaging of such cells leads to a state of terminal growth arrest, referred to as replicative senescence. While the erosion of telomeres, because of the lack of telomerase activity (for recent review, see [99]), has been recognized as a primary cause of replicative cellular senescence, a variety of other events have been identified that trigger premature senescence. Most notably, oxidative stress was found to induce premature senescence in human fibroblasts [100,101], endothelial cells [102,103], and a variety of other cell types (reviewed in ref. [104]).

The mitochondrial theory of aging [105] suggests a critical role for mitochondrial dysfunction and subsequently increased ROS production as an inducer of aging and premature senescence. Accordingly, replicative

senescence of human diploid fibroblasts (HDF) has been associated with mitochondrial dysfunction, and mitochondrial ROS were identified as important players in the senescence response of HDF [106–108]. However, mitochondrial dysfunction does not seem to be uniformly responsible for senescence in all cell types. This indicates mitochondrial production of ROS as one of the causes of replicative senescence. By sorting early senescent (SES) cells from young proliferating fibroblast cultures, it has been shown that SES cells have higher ROS levels, dysfunctional mitochondria, shorter telomeres, and telomeric gamma-H2A.X foci [108]. The authors propose that mitochondrial ROS is a major determinant of telomere-dependent senescence at the single-cell level that is responsible for cell-to-cell variation in replicative lifespan [108].

In particular, we review the concept that intracellular ROS function as signaling molecules and that oxidants play a central role as mediators of cellular senescence. The fact that telomere length correlates with the final inhibition of proliferation under conditions of varied oxidative stress, while the PD level does not, suggests that telomere shortening provides the signal for cell cycle exit in senescence. In postmitotic cells, no further telomere shortening occurs [101]. However, the sensitivity of terminal restriction fragments to S1 nuclease increases, indicating the accumulation of single-strand breaks in telomeres of nondividing fibroblasts. This effect is found under both normoxic and hyperoxic culture, although it is more pronounced under conditions of higher oxidative stress. It might be speculated that accumulation of single-strand breaks and the resultant loss of distal single-stranded fragments during replication could be a major cause of telomere shortening, possibly more important than incomplete replication per se [101]. To determine whether increased oxidative stress may contribute to the senescent phenotype, cells were treated with tert-butyl hydroperoxide (tBHP), which is known to increase oxidative stress by decreasing the intracellular glutathione levels. It has been documented that mild tBHP stress induces a phenotype of premature senescence in a subpopulation of the treated cells, which closely resembles the phenotype of naturally senescent human umbilical vein endothelial cells, including growth arrest, senescence-associated beta-gal activity, and apoptotic cell death. These results establish a model of premature senescence for human endothelial cells, suitable to analyze mechanisms of age-associated cell death [102]. Furthermore, telomere-shortening rate and cell replicative lifespans can be greatly modified by DNA-

damaging oxidative stress [109–112] via a telomere-specific repair deficiency, which causes stress-dependent accumulation of single-strand breaks [113] and accelerates telomere shortening during DNA replication [114]. This had led to the suggestion that telomere reduction is not strictly programmed, with telomere length acting as a mere cell-division counting device, but instead that telomeres act as sentinels for cumulative oxidative and/or environmental stress triggering division arrest when the damage burden (detected through telomere length) becomes too great [109]. It was hypothesized that accelerated telomere shortening is because of preferential accumulation of oxidative damage in telomeres [113]. The data suggest that metabolic time-dependent single-strand degradation is a major cause of telomere shortening. This is supporting the idea that telomere shortening plays an important role in triggering cellular senescence [114]. Telomeres in most human cells shorten with each round of DNA replication, because they lack the enzyme telomerase. This is not, however, the only determinant of the rate of loss of telomeric DNA. Oxidative damage is repaired less well in telomeric DNA than elsewhere in the chromosome, and oxidative stress accelerates telomere loss, whereas antioxidants decelerate it. We share an assumption here that oxidative stress is an important modulator of telomere loss and that telomere-driven replicative senescence is primarily a stress response [109].

TELOMERES AND TELOMERASE ACTIVITY IN LENS EPITHELIAL CELLS OF NORMAL AND CATARACTOUS LENSES

The adult crystalline lens is lined on its anterior surface by a monolayer of lens epithelial cells (LEC) with diverse replicative potential, including the anterior or central zone, the germinative zone and the equatorial zone. The central LEC are rarely mitotic and generally considered to be quiescent, the germinative LEC have mitotic activity and some of these cells have the potential to divide throughout life and the equatorial LEC terminally differentiate into the lens fiber cells [115–118].

Telomerase activity was found in LEC in all three mammalian species (in canine, feline, and murine groups) investigated in the study [119], confirming that the presence of telomerase activity in these cells is not a canine-specific phenomenon. At least 36 samples in each of the three regions of the lens capsule from normal canine lenses were analyzed by telomeric repeat amplification protocol-enzyme linked immunosorbent assay (TRAP-ELISA) [119]. Telomerase activity was found in all three LEC regions of the canine lenses (central, germinative, and equatorial) with no significant differences in the level of activity between the different regions. Treatment of the normal eyes with topical medications used prior to cataract surgery had no effect on the TRAP-ELISA results. Telomerase activity was absent in canine lens fiber cells, corneal endothelium, corneal epithelium from both the limbal and central regions, and rabbit nonpigmented ciliary epithelium. The results were analyzed according to the age of the animal. Surprisingly, telomerase activity was significantly lower in all three regions analyzed in canine LEC from dogs <1 year of age than in adult dogs. LEC from normal murine and feline lenses also possessed telomerase activity. Similar to the canine samples, the three regions of the feline lens capsules all had similar levels of telomerase activity [119]. Telomerase activity in 23 central capsulotomy specimens collected from naturally occurring canine cataracts were compared to levels in the central lens capsule from normal canine eyes, and surprisingly, significantly higher telomerase activity was found in the cataractous specimens.

Cells that have telomerase activity generally have longer telomeres than cells lacking this activity [83]. As cataractous canine LEC had a higher level of telomerase activity than normal LEC in canine group, it was important to determine whether this was reflected in altered telomere lengths in LEC from cataractous canine lenses. In the normal lens capsule samples, there was some heterogeneity in telomere length within each sample as indicated by the number of bands in each lane. However, all normal lens capsules studied and all regions of the LEC had similar banding patterns ranging 4–20 kb, indicating telomere lengths did not differ between the three regions [119]. The longest telomere in all normal canine samples measured was consistently 20 kb. Telomeres from cataractous canine capsules also had the same lower range but the upper limits were higher than the normal samples varying 20–24 kb [119]. Similar to telomerase activity, pretreatment of normal eyes with topical medications used prior to cataract surgery did not affect telomere lengths. It was not surprising to find telomerase activity in the germinative LEC, as this cell population has the potential to undergo mitosis throughout the life of the host [116,117]. Cells in the equatorial region of the lens, though not highly mitotic, may retain telomerase activity as the germinative cells migrate into this region. However, once the cells terminally differen-

tiate into fiber cells, they lose telomerase activity, as no activity was found in the fiber cells. Although some postmitotic cells have telomerase activity [120], many terminally differentiated normal somatic cells do not [121]. As previous studies have indicated that central LEC are quiescent and rarely undergo mitosis [116,117], the presence of telomerase activity in this cell population was surprising and more difficult to explain. Telomerase-negative cells can divide many times in culture, PDs of 56 and 88 in RPE cells and foreskin fibroblasts, respectively, are possible [56] before telomeres become critically short and cell senescence is triggered. The crystalline lens is exposed to constant nonionizing radiation in the form of ultraviolet light, which causes chromosomal damage by inducing DNA strand breaks [122]. Damage to genomic DNA is more effectively repaired than damage to telomeric DNA and oxidative damage to DNA in cells without telomerase activity can result in critical shortening of telomeres and cell senescence after just one cell division [113]. When cells become senescent, they are unable to re-enter the cell cycle and their phenotype dramatically changes [123], and they begin to elaborate senescence-associated β-galactosidase, proteases, extracellular matrix components and inflammatory cytokines. Conversion of LEC to the senescent phenotype is likely to be deleterious to normal lens physiology and transparency. Therefore, the presence of telomerase activity in the central epithelium might be necessary to prevent ultraviolet light-induced DNA damage and critical telomere shortening.

The loss of telomerase activity upon cell passage and telomere shortening may lead to conversion to the senescent phenotype as evidenced by increasing numbers of cells stained with the senescence marker, β-galactosidase. Although it is tempting to suggest such a causative relationship, other factors may predominate to cause senescence, including nonoptimal culture conditions. Indeed, optimization of culture media and conditions enables LEC to escape crisis and grow for more than 20–30 passages [124].

The basal epithelium of the epidermis, another cell type susceptible to ultraviolet light-induced damage also possesses telomerase activity that is upregulated upon exposure to ultraviolet light [125,126]. The ability of ultraviolet light to up regulate telomerase activity could have some effects on an age-related increase in activity of this enzyme. Dogs <1 year of age had significantly less telomerase activity than adult dogs [119]. This suggests that the LEC of young dogs possess a basal level of telomerase activity that increases as the animal matures. However, telomerase activity is increased in all three

regions of the lens with age even though the germinative and equatorial regions are shielded from ultraviolet light by the iris. It is possible that other local environmental factors, such as H_2O_2 and other peroxide compounds, could contribute to DNA damage in LEC leading to activation of telomerase activity.

The telomere shortening in rodents may play some role at least as a marker of cell aging in rats although perhaps less of a causal one than in humans [127]. To examine telomere shortening directly in lens epithelial cells (LECs) *in situ*, the authors utilized fluorescence *in situ* hybridization (FISH) of a peptide nuclei acid probe to the telomere repeat sequence (TELO-FISH). Although it is not clear whether such moderate telomeric erosion can limit cell division in rodent LECs, the telomeric shortening correlated well with previous studies demonstrating reduced clonal replicative potential and reduced rates of *in vivo* DNA replication in LECs from old rodents and a delay in this attenuation in animals on chronic caloric restriction [127]. Examination of LECs in each of nine young and nine old ad lib fed (AL), and nine old calorically restricted (CR) rat lenses demonstrated that rat LEC telomeres were shortened by 21% in old AL fed rats relative to young controls ($P < 0.01$) and that CR reduced this loss to 12% ($P < 0.05$) [127]. This suggests a possible relationship between telomeric shortening, loss of replicative potential in LECs, and cataract appearance in rodents. Oxidative or other damage to the telomeric DNA appears to act synergistically increasing the probability of capping failure and cell senescence even with only moderate average shortening [112,128,129].

Both telomerase activity and telomere lengths were increased in LEC from canine cataractous lenses compared with those from normal canine lenses. Numerous genes regulate telomerase activity within the cell [130] but the process is quite complex and currently not well understood. Perhaps telomerase is upregulated in cataractogenesis in dogs as a protective response to DNA damage, as it has been shown that there is significant damage to DNA in LEC from human cataractous lenses compared to normal lenses [131].

There has been some time ago a renewed interest in the association between DNA damage to the human lens epithelium and the development of lens opacities in human patients [131]. In approximately 50% of the cataractous samples analyzed, the proportion of cells containing DNA single-strand breaks was significantly higher than in control lenses. It is important that no relationship between age and DNA damage was noted.

These findings are consistent with the hypothesis that in some human patients with cataract, DNA damage in the lens epithelial cell population may be related to the development of lens fiber cell opacity [131]. The possible relationships and effect for these phenomena are suggestive for oxidative damage, telomere shortening and loss of replicative capacity and corresponding to senescent phenotype alteration of gene expression patterns contributing to human age-related cataract formation, in that order. However, this evidence is presently correlative.

In the recent study [132], the authors reported that human telomerase reverse transcriptase (hTERT) displays additional functions beyond telomere synthesis. They have demonstrated that hTERT introduced into bovine lens epithelial cells (LECs) can suppress differentiation. Furthermore, the authors provided the evidence [132] that hTERT can regulate the RAS/RAF/MEK/ERK pathway to mediate the suppression of bovine lens epithelial cell differentiation. This study provides evidence that hTERT regulates both proliferation and differentiation in eukaryotes. The results of another study suggest that hTERT, when overexpressed in human lens epithelial cells, accelerates cell growth rate through regulation of RB/E2F pathway and possibly other genes [92].

Among the human, bovine, and rabbit lenses examined, only the central epithelium from the 6-month rabbit lens displayed telomerase activity [133]. In both transparent and cataractous human lenses, hTERT activity and expression were not detected. However, the template RNA was present in both types of human lenses [133]. The telomeres found in transparent human lenses were approximately 1 kb longer than those in cataractous human lenses. These results suggest the possibility that telomere shortening is associated with human cataractogenesis. The primary cultures and later passages of HLECs also displayed no detectable telomerase activity. Introduction of hTERT cDNA into HLECs followed by G418 selection yielded a stable line of HLECs expressing hTERT. In this line, hTERT has supported normal growth after 48 PDs and also enhanced antiapoptotic activity against oxidative stress [133]. hTERT introduced into HLECs prevents replicative senescence through telomere synthesis. The hTERT-transfected cells with normal growth have a maximum telomere length of approximately 13 kb. Shortening of this telomere length to a certain degree signals cellular senescence, as observed in vector-transfected HLECs, which have a maximum telomere length of approximately 10 kb.

Thus, HLECs use a telomerase-dependent mechanism to maintain their telomere stability [133].

FAILURE TO WITHSTAND OXIDATIVE STRESS INDUCED BY PHOSPOLIPID HYDROPEROXIDES AS A POSSIBLE MECHANISM OF HUMAN LENS CATARACTOGENESIS AND HLEC TELOMERE SHORTENING

The ROS generated in the mitochondrial electron transport chain and exposure to environmental agents such as UV light and ionizing radiation can cause oxidative stress within cells by reacting with macromolecules and causing damage, such as mutations in DNA, destruction of protein structure and function, and peroxidation of lipids [134]. Among these effects of ROS, LPO is perhaps the most damaging to cells, because it is an autocatalytic chain process initiated by the abstraction of electrons from unsaturated fatty acids, and a single ROS can lead to the formation of large amounts of phospholipid hydroperoxides (PL-OOH) and breakdown toxic products such as 4-hydroxy-2-nonenal (4-HNE) [135]. In isolated systems, both PL-OOH [136] and 4-HNE [137,138] have been shown to cause cataract.

The enzymatic detoxification of lipid-derived hydroperoxides, in which GSH-dependent selenoperoxidase of the lens is thought to play a key role, leads to reduction of hydroperoxides (LOOH) to alcohols at the expense of GSH (Eqn 1) which regeneration to the reduced form is catalyzed by GSSG reductase (Eqn 2), utilizing NADPH as a cofactor [139,140].

$$LOOH + 2GSH \rightarrow LOH + GSSG + H_2O \qquad (1)$$

$$NADPH + H^+ + GSSG \rightarrow NADP^+ + 2GSH \qquad (2)$$
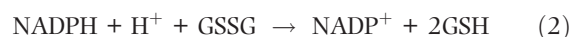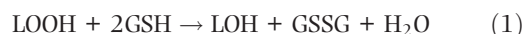
Studies with phospholipid membrane vesicles (liposomes), which lacked cholesterol and lipoproteins, have indicated that classical GPX does not act directly on phospholipid hydroperoxides either membrane bound or detergent dispersed [141]. The phospholipid hydroperoxides present in the human erythrocyte ghost natural membrane rich in cholesterol comparably to the lens membranes [142] are also unreactive with GPX unless first hydrolyzed by phospholipase A_2 [143]. At last, cholesterol hydroperoxides were found to be totally resistant to GPX, even after extraction from the membrane [143]. These findings raised a question of how the lens might detoxify phospholipid hydroperoxides which

mediate oxidative stress implicated in development of maturity onset cataract. The ability of the lens to catalyze directly *in situ* reduction of phospholipid hydroperoxides in model membranes and the lens GPX ability to remove certain organic (including liberated fatty acid) hydroperoxides were evaluated in our previous study [136].

Lipid and phospholipid contents in aqueous humor samples obtained from human eyes were reported (*Figure 4*)[52]. Typical UV absorption spectra of lipids have their maximum in the 206-nm regions related to absorption of isolated double bonds of hydrocarbon phospholipid chains (*Figure 5*). Lipid extract from normal aqueous humor exhibits a modest shoulder of absorption at about 230 nm and a certain absorption peak around 280 nm in the UV region. However, lipid extract of patients with cataract usually exhibits the stronger absorption shoulder at 230 nm, characteristic of conjugated double bonds in the fatty acids (primary LPO products) and an absorption peak around 280 nm (characteristic of ketones, secondary molecular LPO products) (*Figure 5*, upper curve). When the level of secondary products not removed into the water-soluble phase was not significantly increased in UV absorption spectra of lipid extracts of aqueous humor from the cataractous patients (Table I), the distinct increase of the

contents of the end fluorescent LPO products expressed in terms of arbitrary fluorescence units/µg phospholipids (PL) was about 2.4-fold and 4.2-fold higher correspondingly for immature or mature cataracts when compared to normal lenses (Table I). In the samples of aqueous humor aspirated from donor eyes with normal lens, one in about 42 PL molecules contains a conjugated diene, while in case of immature cataract (IDLC 10–64%) [144,145], the average amount of conjugated dienes reaches one in 19 aqueous PL molecules, whereas in mature cataract (IDLC 64–100%) this estimation averages one conjugated diene per 11 PL molecules. Net concentrations of primary (diene conjugates, phospholipid hydroperoxides) and end (fluorescent) LPO products were found to be increased in aqueous humor of the anterior chamber in cases of cataract irrespective of its genesis comparatively to donor (control) eyes and in line with the maturity stage measured by degree of clouding (*Table I*).

With an increase in the relative area of opacity of the lens from 0.1 to 0.8–1.0 relative units, the rate of H_2O_2 decomposition in the medium surrounding lenses fell from 200–300 to 65–80 nmol/lens per h (Table II). We found that the ability to metabolize H_2O_2 is not diminished in early cataracts. This argues that disturbance of the redox balance in the lens on this stage of cataract is
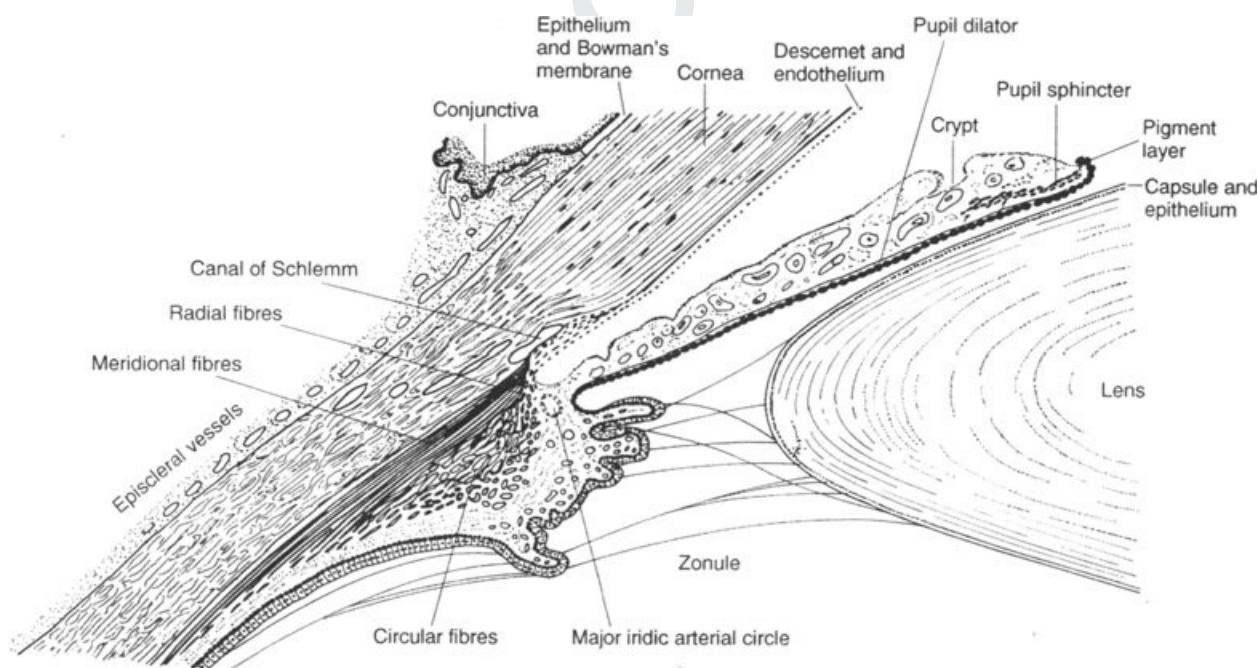
Figure 4 Scheme of the lens suspended in the anterior chamber of the eye washed by aqueous humor possessing the crystalline lens metabolism.
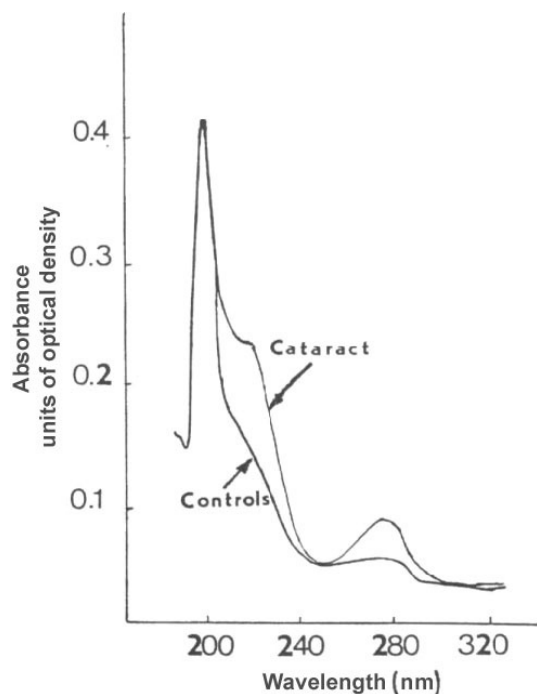
Figure 5 UV absorption spectra of lipid extracts (methanol-heptane 5:1, v/v) from the aqueous humor samples, obtained from human eyes: normal (control, lower curve) and mature cataract (upper curve).

not severe. We know that the principal enzymes of antioxidant protection of tissues, utilizing H_2O_2 as their substrate, are catalase and GPX. Addition of 3-amino-1H-1,2,4-triazole, a specific catalase inhibitor, to the incubation medium of the lens in a concentration of 10 mM did not lead to any significant decrease in the rate

of H_2O_2 decomposition. This suggests that the main cause of the rapid destruction of H_2O_2 by normal lenses was not catalase, but rather the GPX + GSH system, and that, consequently, activity of this system actually falls during cataract development. As will be clear from the results, addition of exogenous GSH to the incubation medium of the lens caused a marked increase in the rate of H_2O_2 decomposition (Table II). Meanwhile, after repeated addition of the same quantity of H_2O_2 (10^{-4} M) to the lens, the rate of its decomposition (190 ± 20 nmol/lens per h) was lower (however, not significantly, $P > 0.1$, $n = 3$) than the rate of decomposition of the first portion of H_2O_2 (245 ± 40 nmol/lens per h). This evidently indicates that the human lenses treated with a single pulse of 0.1 mM H_2O_2 can tolerate this exposure without apparent damage even at the stage of immature cataract thus to retain their peroxide-metabolizing activity, at least for a short period of time, probably because of an active glutathione redox cycle. Activity of the protective antiperoxide systems controlling the oxidation–reduction balance of the lens is thus determined by the possibility of maintaining high concentrations of reduced glutathione in the cells of the lens. It can accordingly be concluded that the effectiveness of peroxide-metabolizing systems in lenses affected by mature cataract is disturbed. This disturbance may be based both on a decrease in activity of any of the enzymes controlling the redox balance and also on a deficiency of cofactors for enzymic and nonenzymic antioxidative reactions (glutathione). The main enzymes of the antioxidative system in the lens are catalase, SOD, glutathione reductase, and GPX [146]. It was found that

Table I Lipid peroxidation (LPO) products in aqueous humor from the eyes of patients with cataract.

LPO products	State of the lenses/degree of lens clouding %		
	Control ($n = 10$) (0.0–10)	Immature cataract ($n = 12$) (10–64)	Mature cataract ($n = 16$) (64–100)
Diene conjugates			
OD$_{232}$/mg phospholipids	5.86 ± 0.44^a	$7.93 \pm 0.65^*$	$9.48 \pm 0.34^{**}$
OD$_{232}$/OD$_{206}$	0.307 ± 0.024	$0.425 \pm 0.035^{***}$	$0.518 \pm 0.020^{**}$
Mole/100 mole phospholipids	2.37 ± 1.14	5.40 ± 1.70	$9.44 \pm 0.89^{**}$
Triene conjugates, ketone, and aldehyde products			
OD$_{274}$/mg phospholipids	2.33 ± 0.13	2.39 ± 0.23	2.86 ± 0.40
OD$_{274}$/OD$_{206}$	0.122 ± 0.007	0.133 ± 0.012	0.163 ± 0.022
Fluorescence products			
Fl/µg phospholipids	17.2 ± 6.4	$42.0 \pm 7.3^*$	$71.7 \pm 20.2^*$

^{a}Mean ± S.D. (n, number of the examined samples). Significant differences with control: $^*P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.02$. The development of quantitative morphometric criteria for evaluation of the lens opacities was presented [144,145].

activity of catalase, and also of GPX (utilizing H_2O_2) in human lenses with cataract did not differ from that in control (Table III). Meanwhile activity of SOD, GPX, the latter able to catalyze reduction of organic hydroperoxide (including hydroperoxides of lipids), in lenses with mature cataract was sharply reduced when compared to normal lenses (Table III). Kinetic studies showed that lipid hydroperoxide approached saturation at concentration of approximately 1 mM, and the apparent Km value was 0.434 mM (*Figure 6*). At the stage of mature

cataract, glutathione lipoperoxidase activity was significantly inhibited (Table III, *Figure 6*; maximum enzymic rate Vmax is decreased); however, Km was unchanged. This indicates that during cataract formation a deficiency of GPX activity occurs because of the noncompetitive inhibition of enzyme.

Human normal or cataractous lenses of different etiology or degrees of opacity were exposed at 37 °C for 1 h to linoleic acid 9-monohydroperoxide (LOOH) or PLOOH in the contents of 0.5 mg/mL liposomes suspended in the lens incubation medium. Reducibility of LOOH or membrane PLOOH was studied by spectrophotometric (conjugated diene) or iodometric assays of the residual LOOH (PLOOH) levels during the incubation of lenses (or without a lens in a background study), and MDA [4] levels were determined as thiobarbituric acid (TBA)-reactive substances (TBARS) [136]. The starting level of LOOH in the incubation medium was of 0.5 mM and 1 μmol PLOOH per 112 μmol of membrane PL. Incubations with normal human, immature or mature cataractous lenses for 1 h at 37 °C caused a decrease of total liberated fatty acid hydroperoxide (LOOH), correspondingly, to 310 ± 50 nmol/lens (mean ± SEM, *n* = 10) at integral degree of lens clouding (IDLC) of 0–10% (ages 17–64); to 192 ± 85 nmol/lens (*n* = 4) at IDLC of 10–65% (ages 60–89) and to 85 ± 70 nmol/lens (*n* = 4) at IDLC of 66–100% (ages 71–86) in 1.0 mL volume of incubation medium (*Figure 7*). Within the same time interval of incubation and temperature, human transparent or cataractous lenses showed little or no reduction of the liposome membrane PLOOH (*Figure 7b*) but, instead, the assay system detected more absorbance changes of conjugated diene, iodometric and TBARS measurements

Table II Decomposition of H_2O_2 (10^{-4} M) by lenses with different degrees of opacity at room temperature (M ± m).

Experimental conditions	Rate of decomposition of H_2O_2, nmol/lens per h	Relative area of zone of opacity of lens, relative units
Human lenses		
Transparent lens (5)	270 ± 60	0.0–0.1
Immature cataract (11)	245 ± 40	0.1–0.7
Mature cataract (7)	71 ± 7*	0.8–1.0
Mature cataract + 10 μM GSH (3)	806 ± 300**	0.8–1.0
Immature cataract +10 mM		
3-Amino-1H-1,2,4-triazole (3)	220 ± 20	0.1–0.7
Immature cataract + 2nd addition of H_2O_2 (10^{-4} M) (3)	190 ± 20	0.1–0.7
Rabbit lens (5)	400 ± 60	0.0–0.08
Mice normal lens (C57Bl) (5)	24 ± 3	0.0–0.1
Mice cataractous lens (F2(CBA × C57Bl)) (5)	23 ± 3	0.1–0.2

Number of lenses given in parentheses.
*P < 0.01 compared with transparent lens. **P < 0.01 compared with ripe cataract.

Table III Activity of antioxidant enzymes and rate of decomposition of H_2O_2 by transparent human lenses and human lenses affected by cataract (M± m).

| Lens | Lens area of opacity (1) | Lens homogenate | | | | |
		Rate of H_2O_2 decomposition (2)	Catalase (3)	Superoxide dismutase (4)	Glutathione reductase (5)	Glutathione H_2O_2	Peroxidase TBHP (6)
Transparent human (*n* = 10)	0–0.1	270 ± 60	7.0 ± 1.8	76.2 ± 20.1	0.355 ± 0.083	1.3 ± 0.2	0.51 ± 0.04
Immature human cataract (*n* = 9)	0.1–0.7	245 ± 40	6.7 ± 1.1	61.0 ± 14.2	0.111 ± 0.020****	0.98 ± 0.21	0.404 ± 0.049***
Mature human cataract (*n* = 8)	0.8–1.0	71 ± 7**	6.0 ± 3.4	20.1 ± 7.9*****	0.059 ± 0.015*	0.92 ± 0.21	0.195 ± 0.047*

(1) Ratio of area of zone of opacity to total area of lens; (2) in nmol H_2O_2 /h per lens at 20 °C; (3) in μmol H_2O_2 /min per lens at 37 °C; (4) in conventional superoxide dismutase units per lens; (5) in μmol NADPH/min per lens; (6) in μmol NADPH/min per lens with H_2O_2 or tert-butylhydroperoxide (TBHP) as the substrate. *P < 0.001, **P < 0.01,***P < 0.1, ****P < 0.02, *****P < 0.05 (*n* = 15) compared with transparent lens. (4)–(6) Measurements were performed at 37 °C.
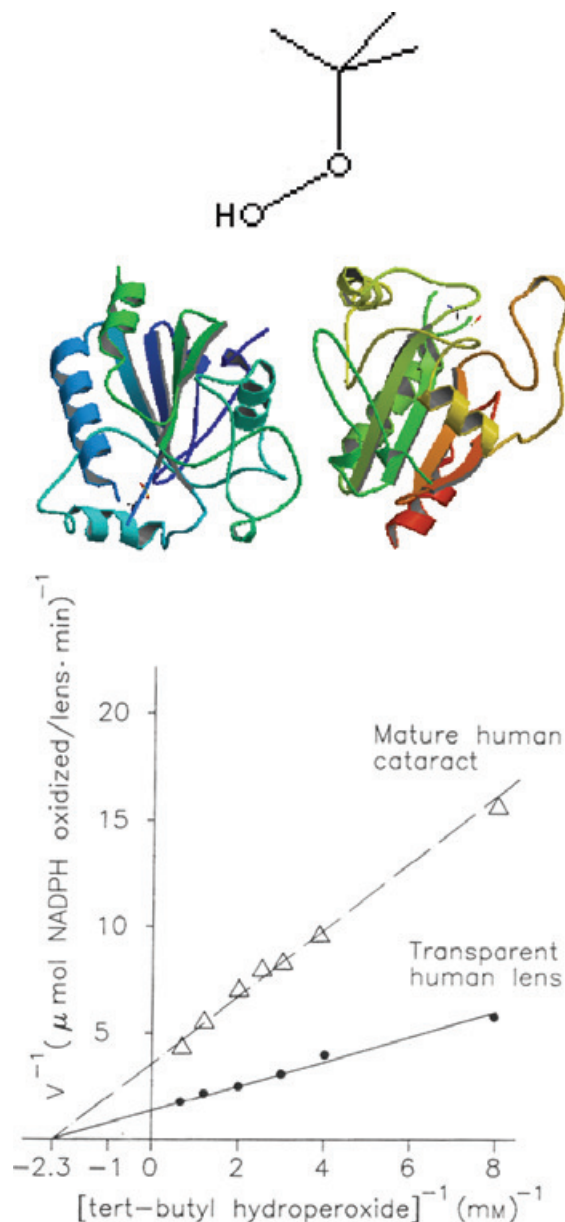
Figure 6 Reciprocal velocity of glutathione peroxidase of normal and cataractous human lenses as a function of reciprocal concentration of tert-butylhydroperoxide. Tert-butylhydroperoxide was varied from 0.13 to 1.52 mM with a constant glutathione concentration of 5 mM. The crossing point with the abscissa axis $(-Km)^{-1}$ indicates the Km value for normal and cataractous human lenses of 0.434 mM. The crossing points with the ordinate axis outline $(Vmax)^{-1}$. At the concentration of tert-butyl hydroperoxide of 0.74 mM, the share of the occupied active centers of GSH peroxidase is calculated as: $f_{ES}= ([S])/([S] + Km) = 0.63$.

in incubations of transparent or immature cataractous human lenses, indicating the PLOOH-dependent propagation of lipid hydroperoxides and that the assay system was fully operational. Incubation with 0.5 mM EDTA produced no net reducing effect on the LOOH(PLOOH) level over that observed in control incubations (LOOH or PLOOH alone). The reducing agent 10 mM $NaBH_4$ showed the significant reactivity (62%) for the measurable LOOH reduction relative to the background decay.

Human lenses with different degrees of opacity and transparent rabbit or mice lenses were incubated in the tissue medium containing 0.5 mg/mL liposome suspension as the oxidation substrate for 3 h at room temperature, and the kinetics of LPO reaction were estimated by measuring MDA, liposomal-conjugated dienes and trienes making appropriate corrections for liposome autooxidation (*Figure 7c–e*). In the absence of the lens, virtually no oxidation of liposomes took place during 180 min (*Figure 7c–e*, curve 5). In the presence of the lens, a marked increase in concentration of different molecular LPO products was demonstrated for the proper time of incubation. The rate of MDA accumulation during the first 30 min of incubation was significantly higher in the presence of transparent human lenses (and also of lenses at the initial stage of cataract) than in the presence of lenses with a mature cataract and the final MDA level within 3 h of incubation was 2.5- to 4.5-fold higher for transparent lens than for the lens with a ripe cataract, integrally indicating that human mature cataractous lenses peroxidized liposomes less than the transparent lenses probably because of the lens epithelial cells mitochondria function .

Mitochondria are important organelles for cellular respiration and energy supply through ATP generation. Mitochondria are also the major cellular source for generation of ROS and at the same time the main targets of ROS-induced oxidative damage. Mitochondrial dysfunction and oxidative stress have been implicated in cellular senescence, cataractogenesis, and the aging process [30,147]. The relationships between ROS generation, lipid compositional changes, antioxidant power, and mitochondrial membrane potential were determined in a human lens epithelial cell line, HLE-B3 [30]. By specifically uncoupling the mitochondrial proton gradient, the authors determined that the mitochondria are most likely the major source of ROS generation. ROS generation correlated inversely with mitochondrial membrane potential and the amount of cardiolipin, factors likely to contribute to loss of cell viability. The results support the idea that hyperoxic damage to HLE-B3 cells derives from enhanced generation of ROS from the mitochondrial electron transport chain resulting in the oxidation of cardiolipin. With extended hyperoxic
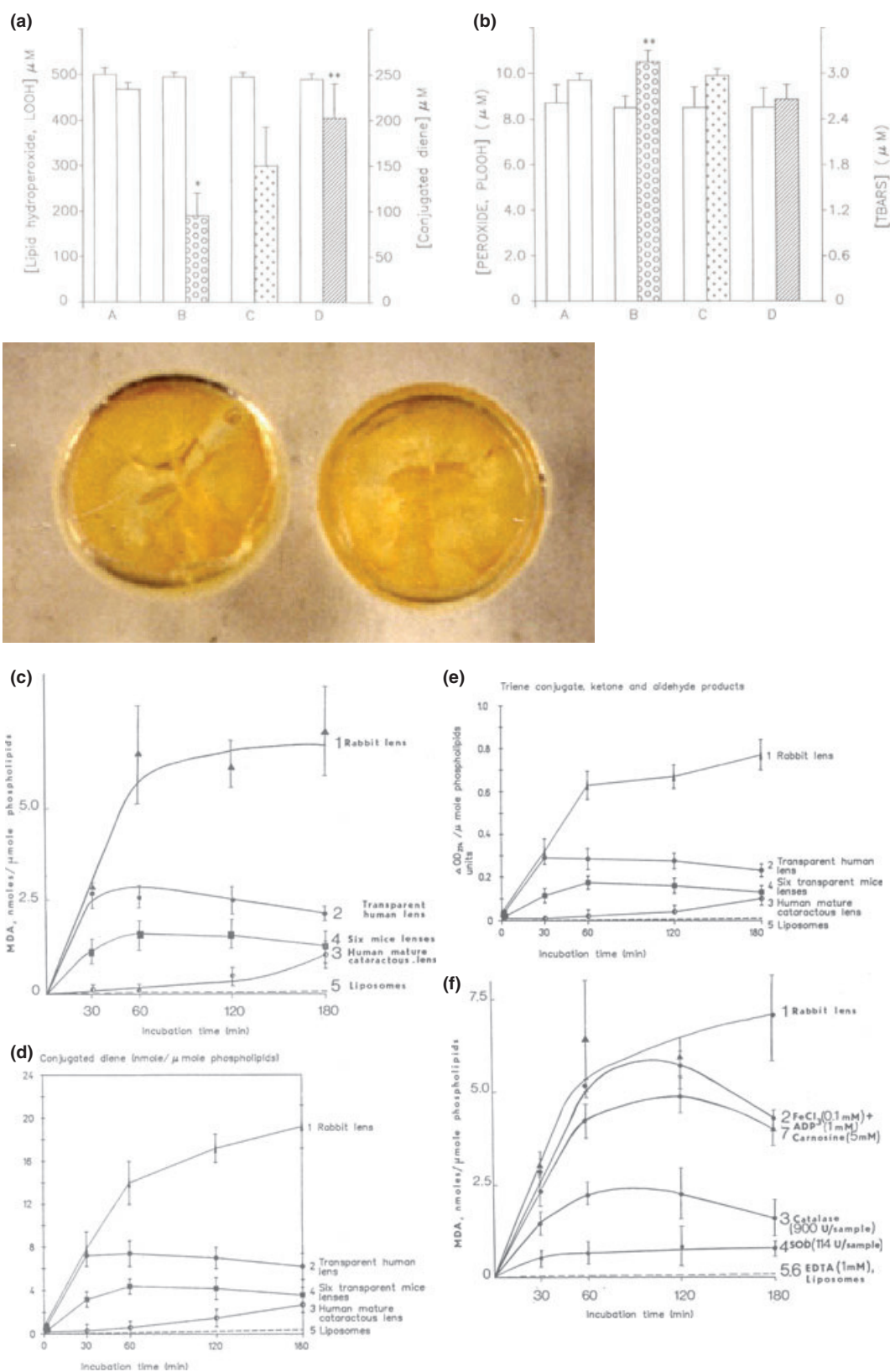
Figure 7 Reactivity of hydroperoxides with human normal and cataractous lenses measured by iodometric, conjugated diene and TBARS [20] assays. The lenses were treated with either (a) 0.5 mM linoleic acid 9-monohydroperoxide (LOOH) or (b) membrane phospholipid hydroperoxide (PLOOH) (1 μmol PLOOH per 112 μmol of membrane phospholipid in 0.5 mg/mL liposomes) suspended in the incubation medium of the lens (Hanks' balanced salt solution, pH 7.0) and incubated for 1 h at 37 °C. At the end of incubation, samples were aspirated and the content of lipid hydroperoxide was determined. Initials levels of peroxide in the reaction mixture and at the end of incubation are shown. (a) A, background incubation of LOOH without lens; B, LOOH incubation with transparent human lens (IDLC 0–10%, ages 17–64, $n = 10$); C, LOOH incubation with immature cataractous human lens (IDLC 10–65%, ages 60–89, $n = 4$); D, LOOH incubation with mature human cataract (IDLC 66–100%, ages 71–86, $n = 4$). Significant differences from transparent lens **$P < 0.05$ and from the background incubation *$P < 0.01$ are indicated. (b) A, background incubation of PLOOH in liposomes without lens; B, PLOOH with transparent human lens (ODLC 0–10%, ages 20–45, $n = 3$); C, PLOOH incubation with immature human cataractous lens (IDLC 10–65%, ages 55–70, $n = 3$); D, PLOOH incubation with mature human cataractous lens (IDLC 66–100%, ages 76–89, $n = 3$). Significant difference from the initial level of PLOOH in the reaction mixture ** $P < 0.05$ is indicated. Normal lenses obtained from the cases of incidental death and cardiovascular shock were included in the group of transparent lenses. The lenses obtained during surgery from the patients with senile (9 lenses) and complicated (5 lenses) cataracts were incubated in the C and D groups on the basis of IDLC measurements. Data columns shown with error bars are means ± SEM of 3–10 lenses. The accumulation of lipid peroxides: TBA-reactive substances (c), conjugated diene (d); triene conjugates, ketone, and aldehyde products (274 nm absorbing material) (e) in the liposomes (0.5 mg/mL) incubated alone (control) or in the lens-containing medium B at room temperature for 3 h. Samples were taken at zero time and at varying time intervals as indicated in the figures. The above fixed aliquots of samples (50–500 μL) were directly used for the measurement of TBA-reactive substances. Also, a similar amount of incubated sample was partitioned through chloroform as described in the lipid extraction procedure and after dissolution in 2–3 mL of methanol-heptane mixture (5 : 1,v/v) was used for detection of conjugated diene and triene conjugate/ketones (274 nm absorbing material). (1) Transparent rabbit lenses (IDLC 0–8%), Mean ± SEM, $n = 5$; (2) transparent human lenses (IDLC 0–10%), $n = 4$, initial cataract (IDLC 10–40%),$n = 3$; (3) human mature cataractous lenses (IDLC 64–100%), $n = 5$; (4) six normal mice lenses (IDLC 0–10%), $n = 3$; (5) (control) liposomes, $n = 5$. Experimental details are given in the text. (f) Effect of various oxygen radical scavengers on lipid peroxide formation in liposomes added to the incubation medium of the normal rabbit lens (IDLC 0–8%). In a total volume of 3.0 mL, the incubation mixture of the lens-containing medium B, 0.5 mg/mL liposome suspension and the appropriate concentrations of scavenger as indicated. Mean values for the MDA concentrations are given for a representative experiment, with the error bars indicating the standard deviation obtained for the group of 3–5 lenses.

insult, the oxidants overwhelm the antioxidant defense system and eventually cell death ensues [30].

The level of MDA accumulation after incubation for 3 h of rabbit lenses was 3.5- and 5.3-fold higher than that of normal human or mouse lenses. The larger normal lenses (rabbit or human) are metabolically active and generate the ROS and lipid peroxides more rapidly than cataractous lenses with the exhausted pool of reductants or tiny mouse lenses [52]. *Figure 7c, 7f* documents in some cases, a small decrease in the liposomal MDA concentration after incubation for 2 h. This might be connected with MDA utilization by the lens itself (interaction of MDA with amino group, or its lowering by lenticular aldehyde dehydrogenase [148]). A considerable reduction of the accumulation rate of the liposomal LPO products was found after the addition of catalase (900 U/sample) (*Figure 7f*, curve 3). This suggests a role of H_2O_2 in promotion of LPO by the lens. Addition of SOD (114 U/sample) to the incubation medium of the lens led to a marked reduction of the liposomal MDA level (*Figure 7f*, curve 4), suggesting the ability of the lens to generate O_2^- in the surrounding medium. Addition of the ADP-Fe complex to the incubation medium of the lens decreased accumulation of

TBA-reactive material in liposomes by 33–50%, indicating a decomposition of the accumulated TBA-reactive material. The LPO is resulted from free-radical oxidation reactions induced by active oxygen forms with participation of transition metal ions in the free catalytically active state. This is supported by the virtually total inhibition of TBA-reactivity in the liposomes after the addition of the chelating agent 1 mM EDTA eliminating the free and accessible transition metal ions from the peroxidizing system (*Figure 7f*, curve 5). In the presence of 5 mM L-carnosine in the rabbit lens/liposome-containing medium, a decrease in the TBA-reactivity by approx. 25% for 2 h of incubation was revealed (*Figure 7f*, curve 7). The specific inhibitory effect of L-carnosine in the incubation system detects OH· or lipid peroxy radicals (LOO·) generated in the medium surrounding the lens in organ culture [149].

Telomeres emerged recently as important indicators of oxidative stress that accumulates *in vivo* in the human body and have been suggested as an underlying event in many diseases. Shortened telomeres in blood cells during aging can be caused by oxidative stress [101] or decreased telomerase activity [150]. Telomeres are very sensitive indicators of cumulative oxidative stress,

whereas more acute measurements of oxidative stress could be below the detection limit for short-lived oxygen-derived radicals. Accordingly, telomeres have been identified as potential biomarkers for age-associated disease risk, progression, and mortality in many studies. For instance, telomere length in blood cells is a good indicator for the likelihood of vascular dementia after stroke [151,152]. Telomere shortening and increased oxidative stress have been described even for processes such as high psychological stress in caring mothers [153].

Changes in telomerase activity in cell types such as lymphocytes, endothelial cells and tissue stem cells could influence processes relevant for healthy aging. It has been shown recently in a longitudinal study that improvements in nutrition and lifestyle were associated with increases in telomerase activity correlating with decreases in low-density lipoprotein cholesterol and psychological distress [154]. Thus, comprehensive life-style changes could significantly increase telomerase activity, stabilize telomeres, and decrease oxidative stress within tissues and organs.

In this work, we originally propose that the existence of shortened telomeres in the human lens epithelial cells during aging and cataract progression is the result of oxidative attack to the lens by phospholipid hydroperoxides present in the aqueous humor and lens cellular membrane structures in the lack of efficacy of the antioxidant protection in the lens toward these damaging oxidant promoters (*Figure 8*).

HUMAN LENS TELOMERES AS A PROSPECTIVE BIOLOGICAL TARGET FOR THERAPEUTIC CLINICAL EVALUATION WITH CARNOSINE OPHTHALMIC PRODRUG TOOLS

L-Carnosine (β-alanyl-L-histidine) is a naturally occurring dipeptide and abundant in muscle and nervous tissues in many animals, especially long-lived species [155–157]. Carnosine and related dipeptides such as anserine are naturally occurring histidine-containing compounds. They are found in several tissues most notably in muscle where they represent an appreciable fraction of the total water-soluble nitrogen-containing compounds. We suggest it has an important role in cellular homeostasis and maintenance. We have found appreciable levels of L-carnosine in transparent human lenses which are markedly depleted in mature cataracts [158]. The concentration of carnosine in transparent crystalline lenses detected was about 25 μM. At different stages of cataract
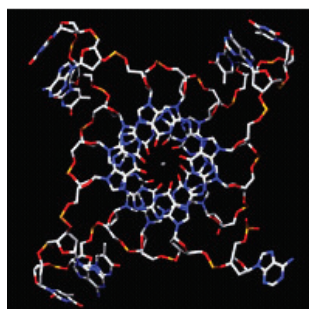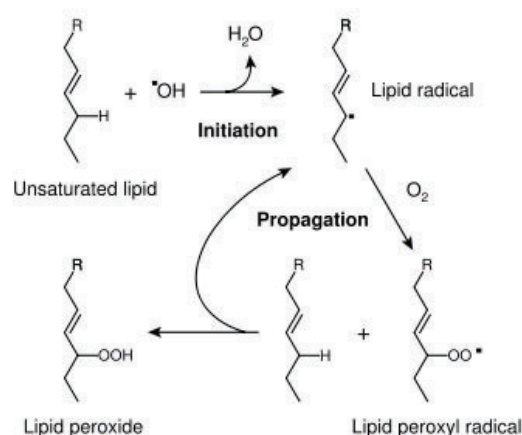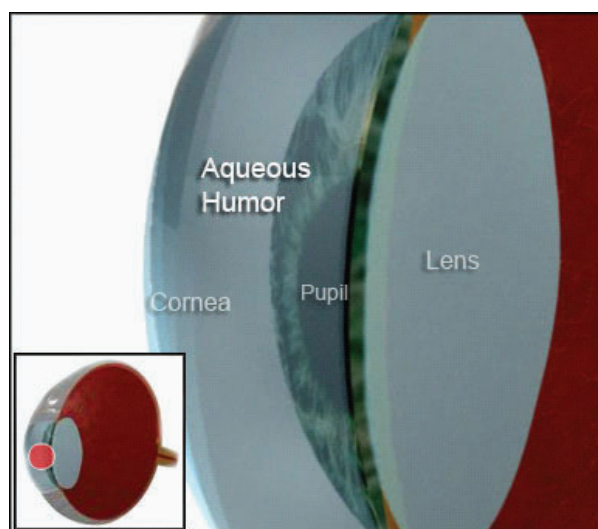

Figure 8 Lipid hydroperoxide mediated damaging mechanism shortening telomeres in the human lens epithelial cells during aging and human cataract formation.

development, the level of carnosine fell, reaching about 5 μM. Research with *N*-acetylcarnosine (NAC), an ophthalmic prodrug of L-carnosine, demonstrates that it is effective not only in preventing cataracts but also in treating them. NAC has been shown to improve vision by

partially reversing the development of the cataract, thus increasing the transmissivity of the lens to light [159]. The biological role of these dipeptides is conjectural but they are believed to act as cytosolic buffering agents. Numerous studies have demonstrated, at both the tissue and organelle level, that they possess strong and specific antioxidant properties. Carnosine and related dipeptides have been shown to prevent peroxidation of model membrane systems leading to the suggestion that they represent water-soluble counterparts to lipid-soluble antioxidants such as alpha-tocopherol in protecting cell membranes from oxidative damage. Other roles ascribed to these dipeptides include actions as neurotransmitters, modulation of enzymic activities and chelation of heavy metals. It has been recently confirmed that carnosine active ingredient possesses several biological functions in the cell, including metal ion-chelating, antioxidation, free-radical scavenging, and the inhibition of nonenzymic glycosylation of proteins (transglycating activities) in ophthalmics [160].

We proposed a deglycation system involving removal, by transglycation of sugar or aldehyde moieties from the Schiff bases by ophthalmic aldehyde scavenger L-carnosine derived from its ocular bioactivating sustained release prodrug 1% NAC lubricant eyedrops containing a mucoadhesive cellulose compound combined with corneal absorption promoters in drug delivery system [160].

Carnosine attenuates the development of senile features when used as a supplement to a standard diet of senescence accelerated mice (SAM) devoid of serum carnosinase activity [156]. Its effect is apparent on physical and behavioral parameters and on average life span. A striking antisenescence effect of carnosine was demonstrated by McFarland and Holliday [161–163]. They showed that HDF grown in 20 mM carnosine had an extended lifespan, both in PDs and in chronological time. The dipeptide L-carnosine had beneficial effects on cultured human fibroblasts. Physiological concentrations in standard media prolong their *in vitro* lifespan and strongly reduce the normal features of senescence. Late passage cells in normal medium were rejuvenated when transferred to medium containing carnosine, and became senescent when carnosine was removed [161]. Neither D-carnosine, (beta-alanyl-D-histidine), homo-carnosine, anserine, nor beta-alanine had the same effects as carnosine on human fibroblasts.

In the recent work [164], the authors studied the effect of carnosine on a human fetal lung fibroblast strain, which was kept in either a continuously prolif-

erating or proliferation-inhibited state. The results indicate that carnosine can reduce telomere shortening rate possibly by protecting telomere from damage. Cells continuously grown in 20 mM carnosine exhibited a slower telomere shortening rate and extended lifespan in PDs. When kept in a long-term nonproliferating state, they accumulated much less damages in the telomeric DNA when cultured in the presence of carnosine. The authors suggest that the reduction in telomere shortening rate and damages in telomeric DNA made an important contribution to the life-extension effect of carnosine [164].

The knowledge recently introduced into the public domain by this group of authors provide a solid evidence that human lens epithelial cells are subjected to the oxidative damage with ROS and phospholipid hydroperoxides during aging and cataractogenesis and that these oxidants might be primarily responsible for telomere shortening in the human lens epithelial cells [165]. The clinical evidence is also presented that carnosine that finds its way into the aqueous humor and the crystalline lens through the time-release from its topically applied to the eye ophthalmic prodrug NAC admixed with carboxymethylcellulose, is able to reduce telomeric attrition in the lens epithelial cells through a diminution in the oxidative stress thus preventing the expression of the senescent phenotype of the lens epithelial cells [165]. In the present review, we are supporting the effects of carnosine on telomere of the human lens epithelial cells during aging and cataract formation based on the established clinical efficacy of L-carnosine ophthalmic prodrug NAC to partially reverse and prevent human cataracts in clinics (*Figure 9*) [1,2,159,160]. The cumulative results from the literature demonstrated that carnosine at physiological concentration might remarkably reduce the rate of telomere shortening in the continuously surviving lens cells subjected to oxidative stress induced by phospholipid hydroperoxides in the lack of efficient antioxidant lens protection .

CONCLUSION

Telomere is the repetitive DNA sequence at the end of chromosomes, which shortens progressively with cell division and limits the replicative potential of normal human somatic cells [164]. Normal human somatic cells shorten their telomeres during their lifespan leading eventually to dysfunctional telomeres, growth arrest and replicative senescence. Expression of TERT, the catalytic subunit of telomerase, counteracts telomere shortening,
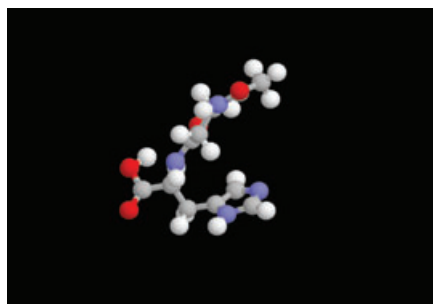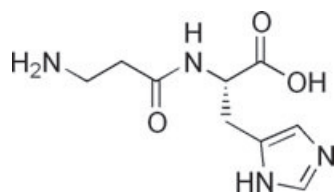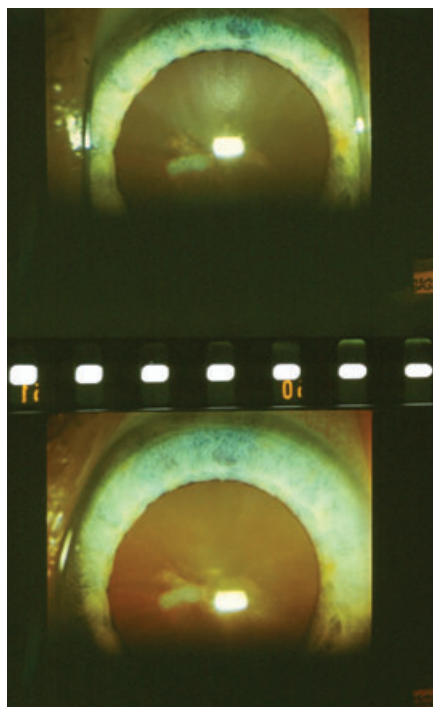
n. Chemical Name: N-Acetyl-L-carnosine

$C_{11}H_{16}N_4O_4$
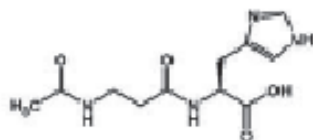
Molecular Weight: 268.27

Chemical Structure:

Figure 9 Telomeres in human lens cells as the therapeutic target of ophthalmic prodrug of L-carnosine. Partial human cataract reversal is observed within 5 months of daily treatment with 1% *N*-acetylcarnosine lubricant eye drops (for details, see [159]).

extends the replicative potential and prevents replicative senescence [56].

Among the human, bovine, and rabbit lenses examined, only the central epithelium from the 6-month rabbit lens displayed telomerase activity. In both transparent and cataractous human lenses, telomerase activity and hTERT expression were not detected [133]. The telomeres found in transparent human lenses were approximately 1 kb longer than those observed in cataractous human lenses. These results suggest the possibility that telomere shortening is associated with cataractogenesis. Presented in this review studies have suggested that telomere shortening in human lens cells and increased oxidative stress are the result of the peroxidative damage to the lens cell membranes and biomolecules induced in the lack of reductive detoxification of phospholipid hydroperoxides as the triggering mechanism of cataractogenesis. LPO is a causative factor of cataract. The increased concentrations of primary molecular LPO products (diene conjugates, lipid hydroperoxides) and end fluorescent LPO products were detected in the lipid moieties of the aqueous humor samples obtained from patients with senile and complicated cataracts when compared to normal donors. The progressive accumulation of oxidative damage may act as an important mechanism for organism aging and cataractogenesis.

We originally suggest in this work that the reduction in telomere shortening rate and damages in telomeric DNA made an important contribution to the anticataract and life-extension effect of carnosine administered systemically in the formulations stabilizing a dipeptide from the enzymatic hydrolysis with carnosinase or topically administered to the eye with carnosine ophthalmic prodrug NAC and lubricant formulations thereof including corneal absorption promoters. Telomere length in the human crystalline lens cells is a reflection of aging, cataractogenesis and human lifespan in biogerontological studies.

ACKNOWLEDGEMENTS

treatment of ophthalmic disorders, including cataracts as well as (PCT International Publication Number WO 2004/064866 PCT/JP2004/000351) protecting the described in the article therapeutic applications. Innovative Vision Products, Inc. is a Pharmaceutical and Nanotechnology Development Company with a focus on innovative chemical entities, drug delivery systems, and unique medical devices to target specific biomedical applications. Over the last decade, IVP has developed a track record in developing these technologies to effectively address the unmet needs of specific diseased populations.

CONFLICT OF INTEREST

Declaration of interest: The authors report the interest in the Intellectual Property and marketing of the described modalities protected with the patents. The authors bear primary responsibility for accuracy of made statements and employment of the described products and for the content and writing of the article.

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Adobe Acrobat Professional or Acrobat Reader (version 7.0 or above) is required to e-annotate PDFs. Acrobat 8 Reader is a free download: http://www.adobe.com/products/acrobat/readstep2.html

Once you have Acrobat Reader 8 on your PC and open the proof, you will see the Commenting Toolbar (if it does not appear automatically go to Tools>Commenting>Commenting Toolbar). The Commenting Toolbar looks like this:
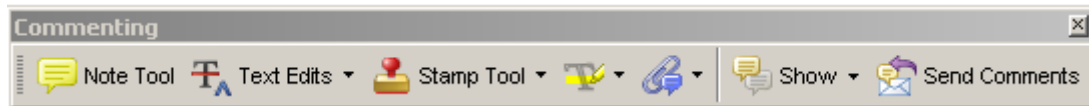

If you experience problems annotating files in Adobe Acrobat Reader 9 then you may need to change a preference setting in order to edit.

In the "Documents" category under "Edit – Preferences", please select the category 'Documents' and change the setting "PDF/A mode:" to "Never".
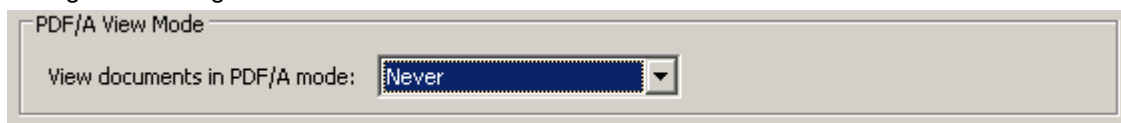
Note Tool — For making notes at specific points in the text

Marks a point on the paper where a note or question needs to be addressed.
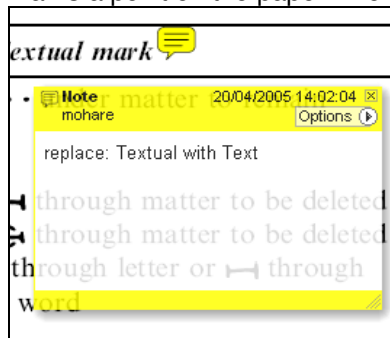
How to use it:

1. Right click into area of either inserted text or relevance to note
2. Select Add Note and a yellow speech bubble symbol and text box will appear
3. Type comment into the text box
4. Click the X in the top right hand corner of the note box to close.

Replacement text tool — For deleting one word/section of text and replacing it

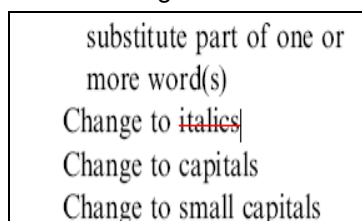Strikes red line through text and opens up a replacement text box.
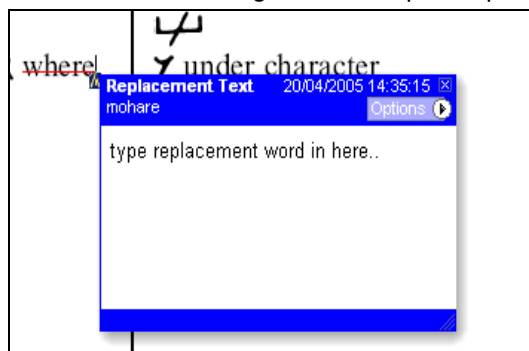
How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Replace Text (Comment) option
5. Type replacement text in blue box
6. Click outside of the blue box to close

Cross out text tool — For deleting text when there is nothing to replace selection

Strikes through text in a red line.

How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Cross Out Text

## Approved tool — For approving a proof and that no corrections at all are required.

How to use it:
1. Click on the Stamp Tool in the toolbar
2. Select the Approved rubber stamp from the 'standard business' selection
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Highlight tool — For highlighting selection that should be changed to bold or italic.

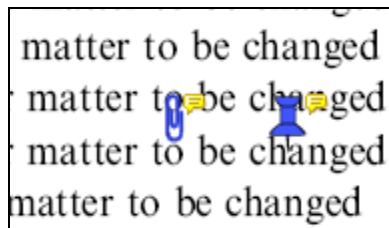Highlights text in yellow and opens up a text box.
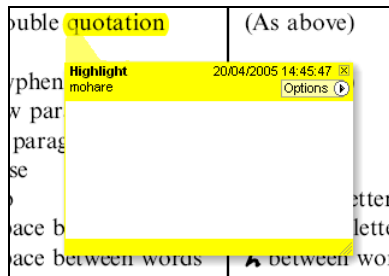
How to use it:
1. Select Highlighter Tool from the commenting toolbar
2. Highlight the desired text
3. Add a note detailing the required change

Attach File Tool — For inserting large amounts of text or replacement figures as a files.

Inserts symbol and speech bubble where a file has been inserted.

How to use it:
1. Click on paperclip icon in the commenting toolbar
2. Click where you want to insert the attachment
3. Select the saved file from your PC/network
4. Select appearance of icon (paperclip, graph, attachment or tag) and close

Pencil tool — For circling parts of figures or making freeform marks

Creates freeform shapes with a pencil tool. Particularly with graphics within the proof it may be useful to use the Drawing Markups toolbar. These tools allow you to draw circles, lines and comment on these marks.
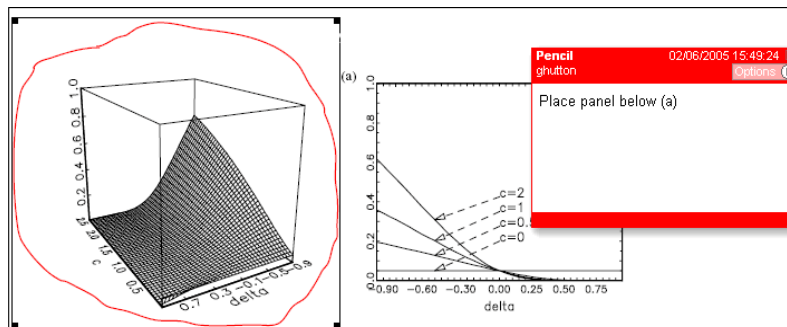
How to use it:
1. Select Tools > Drawing Markups > Pencil Tool
2. Draw with the cursor
3. Multiple pieces of pencil annotation can be grouped together
4. Once finished, move the cursor over the shape until an arrowhead appears and right click
5. Select Open Pop-Up Note and type in a details of required change
6. Click the X in the top right hand corner of the note box to close.

Help

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