

Membrane cholesterol and phospholipid in consecutive concentric sections of human lenses

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Abstract Lens membrane preparations have been shown to have a remarkable rigidity which increases in the inner nuclear region of the lens and has been correlated with the cholesterol (C)/phospholipid (PL) ratio. However, the distribution of these lipids in single lenses had not been determined. Utilizing a new technique for isolating consecutive layers of a human lens, lipid composition and contents of seven pairs of normal lenses from subjects ranging from 54 to 77 years old have been analyzed. It was found that the PL content remains relatively constant at 22–24 $\mu\text{g}/\text{mg}$ through all but the nuclear 10–15% of the lens dry weight where it drops precipitously to about 7 $\mu\text{g}/\text{mg}$. The C distribution is more complex; the C content is at a low level of 14 $\mu\text{g}/\text{mg}$ in the outer cortical 15–20%, rises to 25 $\mu\text{g}/\text{mg}$ in the inner cortical 40–60% of the total lens weight, and drops to 12 $\mu\text{g}/\text{mg}$ upon reaching the nucleus. Thus, the continuous increase in the lens C/PL ratio is due to the increase in C in the cortex and the large decrease in PL in the nucleus. Analyses of phospholipid and fatty acid composition in the different regions of the lens indicate significant differences. However, the abundance of mono-unsaturated fatty acids contributing to the rigidity of the membrane has only minor variation. The lens has a remarkably low overall lipid content of 4% and only 2% in the nuclear region. Calculation of the surface area of the nuclear fiber cell suggests that less than one-third of the membrane is made of PL bilayer. Thus, a mosaic of PL and C patches or some other type of structure involving membrane fusion must be present. Conversion of the % dry weight occupied by the concentric fiber fractions to their location on the lens axis in mm indicates that the nuclear 15% dry weight of the tissue occupies more than 50% of the axial length. This region contains the embryonic lens and the primary lens fibers. Similarly, the metabolically active outer 20% of the dry weight accounts for less than 10% of the visual axial length and contains cells undergoing terminal differentiation. Cataractous lenses have lipid distributions similar to those of the normal lenses suggesting that membrane lipid is either not involved in cataract formation or that the primary insult is localized in an undetectable small number of fiber cells. — Li, L.-K., L. So, and A. Spector. Membrane cholesterol and phospholipid in consecutive concentric sections of human lenses. *J. Lipid Res.* 1985. 26: 600–609.

Supplementary key words lipid distribution • membrane rigidity • cholesterol/phospholipid • lipid bilayer • visual axis • lens cortex • lens nucleus

The mammalian lens is an ideal organ for the study of aging. It is avascular and composed primarily of enucle-

ated fiber cells which are produced all through life from a single layer of epithelium underneath the anterior surface of the capsule. The newly formed fibers in the periphery (the outer cortex) displace the already formed fibers toward the center of the tissue (the inner nucleus). Since there is no loss of fibers, the lens weight increases continuously throughout life.

Previous studies from this laboratory indicate that the rotation of fluorophores incorporated into lens fiber membrane are markedly hindered as compared with other tissues. Furthermore, the rotation is restricted to a significantly greater extent in the inner (nuclear) than in the outer (cortical) fiber membrane preparations (1). These observations suggest that the lens membrane is very rigid and that there is a cortex to nucleus increase in membrane rigidity. This exceptionally high rigidity can be explained by the high cholesterol (C) content of the lens fiber membranes inasmuch as the surface viscosity or rigidity of lipid membranes in aqueous buffer is directly proportional to the C content (2). It is also relevant to note that a number of laboratories have shown that the cholesterol/phospholipid (PL) ratio of the lens fiber membrane is one of the largest found in biological systems and increases in the nuclear region (3–6).

A question that arises from the observed cortex to nucleus increase in lens C/PL ratio concerns the extent to which the changes are due to the absolute increase in cholesterol or to relatively stable cholesterol levels concomitant with a decreasing phospholipid content. The fibers are not homogeneous in terms of composition, metabolism, or age. It is well established that only in the epithelium or outer cortex of the mature lens is there significant metabolic activity (7–9). There are numerous reports of age-dependent changes that occur in the lens proteins and in the pattern of protein synthesis (9–13).

Abbreviations: C, cholesterol; PL, phospholipid; TLC, thin-layer chromatography; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; SPM, sphingomyelin; FA, fatty acid; GLC, gas-liquid chromatography.

Differences in the lipids from the total cortex and nucleus of individual bovine lenses have been reported (3, 14). However, such work has not been done on the human lens lipids.

In the present studies, a technique has been developed which removes successive concentric layers of fibers from single lenses. Analyses of lipid from these fractions showed a surprising, distinctive regional distribution of C and PL corresponding to fiber cells in different stages of development and aging. Such differences could not have been detected if only total cortex and nucleus were analyzed.

MATERIALS AND METHODS

Preparation of successive fiber fractions and lipid extracts

Normal lenses were obtained from the Eye Bank for Sight Restoration, Inc., (New York, NY) within 24 hours of death and stored at -70°C before use. Cataractous lenses were received from the Harkness Eye Institute of the College of Physicians & Surgeons, Columbia University, New York, NY and were processed within 4 to 6 hr after surgery to avoid postoperative changes. Concentric fractions of individual lenses were obtained by rolling the decapsulated lens on a piece of parafilm cooled underneath by a metal block in ice. This method allows removal of relatively small amounts of the lens surface material. The procedure was performed in a Plexiglas box filled with argon to prevent lipid oxidation. From 54-year or older lenses, seven to nine successive fractions of 20–30 mg wet tissue were isolated. The separated lens fiber material was washed off the parafilm with 0.2–0.3 ml of water for each fraction.

Each lens fraction was suspended in a final volume of about 0.5 ml of water and homogenized in a 12-ml polypropylene tube (Fisher Scientific, Springfield, NJ) with a Ross microemulsifier (Hauppauge, NY) at a setting of 3.5 for 20 sec under argon. After the removal of aliquots for dry wt and protein determinations, the remainder was extracted with spectral grade chloroform-methanol 2:1 (Mallinkrodt, St. Louis, MO) for lipid in the same tube (15). The lipid extracts were dried in a Savant Vac concentrator (Hicksville, NY) and kept at -20°C until use. Solvent blanks and extractions containing known amounts of cholesterol, protein, phospholipid, and fatty acid were run. Recoveries of approximately 90% were obtained in all cases.

Dry weight and protein determination

The dry weight was determined by heating 10–20 μl of lens homogenates in the microbalance aluminum pan (Cahn Instruments, Cerritos, CA) at 110°C for 4–6 hr.

Some of the samples were hydrolyzed in 6 N HCl and analyzed in a Beckman amino acid analyzer (13).

Cholesterol and phospholipid determination

Cholesterol was measured by the cholesterol oxidase (EC1.1.3.6; *Brevibacterium* sp.) assay (cholesterol kit, Boehringer, Indianapolis, IN) in Triton X (16). Total PL was determined as phosphorus after digestion of samples with HClO_4 (17). Individual phospholipids were identified by thin-layer chromatography (TLC) with standards from Supelco (Bellefonte, PA), on silica gel 60 TLC plates (EM Darmstadt, Cincinnati, OH). The plates were washed in the first solvent, heated to 110°C for 30 min and then developed with two solvents in one-dimension (18). The first solvent was chloroform-methanol-acetic acid-water 35:15:6:1 (v/v); the second was hexane-isopropyl ether-acetic acid 65:35:2 (v/v). After charring the plates with a solution of 10% cupric sulfate in 8% phosphoric acid (19), the individual lipids were quantitated by densitometry using an LKB-soft laser densitometer (LKB Instruments, Paramus, NJ). The detector response was calibrated with known amounts of standards. For the same quantity of phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), and sphingomyelin (SPM), the areas detected were within $\pm 7\%$. No color yield correction was made for the small amounts of lyso-PE and lyso-PS because of difficulty in resolving lyso-PS, lyso-PE, and PC in the TLC.

Fatty acid (FA) composition

Lipid extracts were hydrolyzed according to a modified procedure of Aveldaño and Horrocks (20). To each sample (50 μg of PL), 1 ml of 5% H_2SO_4 in acetonitrile-water 9:1 and 10 μg of tricosanoic acid as internal standard (Supelco) were added and heated at 110°C for 1 hr. Degassing and drying were carried out with the Savant Vac concentrator using argon to fill the evacuated sample chamber. Free fatty acids were separated into the upper phase after the addition of 1 ml of methanol saturated with hexane and 0.5 ml of water and dried. FA methyl esters were prepared by heating FA with 3 N methanolic HCl (Supelco) at 80°C for 20 min, and they were analyzed by gas-liquid chromatography (GLC) (4) with an HP 5080 Gas Chromatogram (Hewlett-Packard, Paramus, NJ). A 2-mm glass column packed with Chromosorb 200 (Supelco) was eluted with N_2 carrier gas at 30 ml/min using a temperature gradient from 145°C to 200°C in 12 min. The identity of the FA methyl esters was established by comparison with standard methyl esters obtained from Supelco.

Location of the successive fiber fractions on the lens visual axis

The position of a concentric fiber layer on the axis of a 60-year-old human lens was determined as follows.

Based on Philipson's radiogram of lens sections (21), the protein concentration or dry wt was estimated to be approximately 20% in the cortex and increased to 40% in the nucleus. The volume in ml, V , occupied by a given protein solution (either the total lens or a concentric fiber fraction) can be expressed as:

$$V = W/(C \cdot \rho) \quad \text{Eq. 1}$$

where W = dry wt in gm, C = weight fraction or percent protein concentration, and ρ = the density of solution in g/ml. Using an average dry weight of 65 mg and a protein concentration of 25% for an average 60-year-old lens, a volume of 0.236 ml is obtained using equation 1 and $\rho = 1.10$ g/ml extrapolated from Crouch and Kupke (22). This value is in good agreement with the volume of 0.221 ml calculated from the axes of six 60–63-year-old lenses and the volume for an oblate ellipsoid; i.e.,

$$V = \pi a^2 b / 6 \quad \text{Eq. 2}$$

where, $a = 10 \pm 1.0$ mm and $b = 6.5 \pm 0.7$ mm are the measured long and short axes, respectively. Combining equations 1 and 2, the total lens dry weight can be expressed as

$$W_t = \pi a^2 b \cdot C \cdot \rho / 6 \quad \text{Eq. 3}$$

Similarly, by assuming a constant protein concentration throughout the lens, the dry wt of the remaining lens is derived as

$$W_r = \pi a_r^2 b_r \cdot C \cdot \rho / 6 \quad \text{Eq. 4}$$

where a_r , b_r are the axes of the remaining lens. Since the weight of the fraction or fractions removed is determined experimentally and expressed as $\Sigma\%$ of total lens dry wt, we obtain

$$\Sigma\% \cdot W_t = W_t - W_r \quad \text{Eq. 5}$$

Substituting equations 3 and 4 into equation 5, we obtain

$$a^2 b (1 - \Sigma\%) = a_r^2 b_r \quad \text{(Eq. 6)}$$

Assuming that the shape of the remaining lens is similar to that of the original, i.e.,

$$a/b = a_r/b_r \quad \text{Eq. 7}$$

and substituting a or b of equation 6 with equation 7, we obtain

$$a_r = a(1 - \Sigma\%)^{1/3} \text{ or } b_r = b(1 - \Sigma\%)^{1/3} \quad \text{Eq. 8}$$

Using equation 8, the x-ordinate in Fig. 1 is converted from percent dry wt to that of $a_r/a = b_r/b$, the normalized axial lengths occupied by the remaining lens as shown in Fig. 2.

In the dry wt (volume) to linear axis conversions shown above, we assumed that the protein concentration remains constant at 25% with a density of 1.10 g/ml and

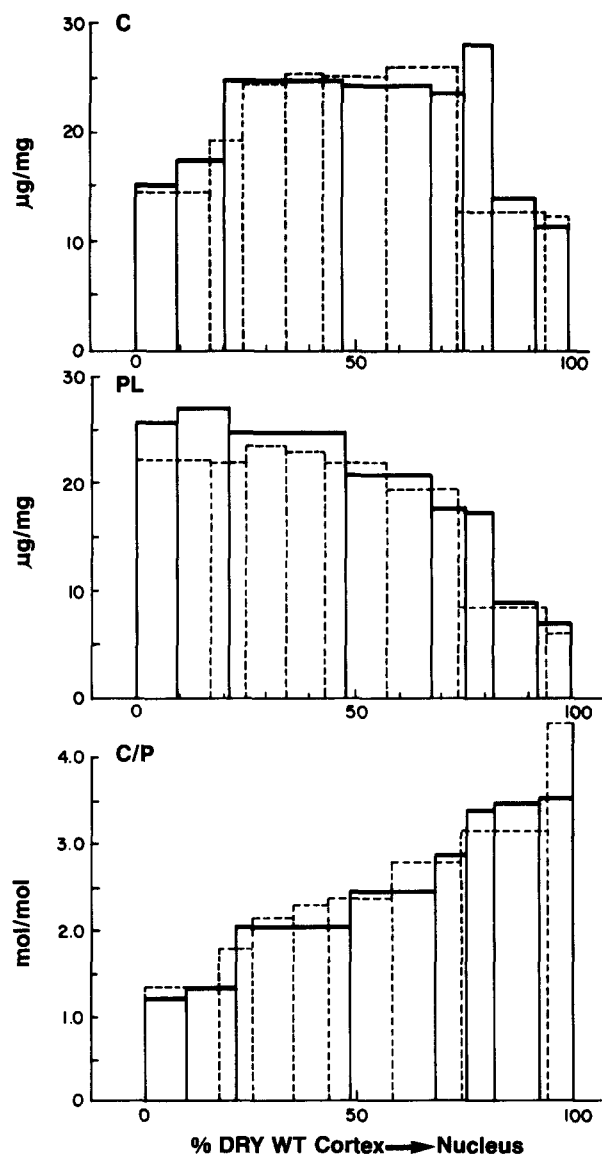


Fig. 1 Distribution of cholesterol (C), phospholipid (PL), and mol C/PL (C/P) ratio in a pair of 63-year-old normal lenses; —, left and ---- right lens. The lipid contents of each fiber fraction are given in $\mu\text{g}/\text{mg}$ dry wt. The distribution of the fiber fractions on the cortex to nucleus axis is expressed as the % total lens dry wt occupied by each fraction. The average total dry wt of the 63-year-old lenses is 67 mg. The 0% represents the outer surface of the intact lens, while the 100% represents the nuclear center. The mol C/PL ratio (C/P) in the lowest panel is derived from the corresponding C and PL data by taking 800 as the averaged molecular weight of PL.

that the shape or the ratio of the axes of the remaining lens is the same as the original. How much of an error does this introduce?

Depending on the condition of the lens, the shape of the remaining lens in about a third of the cases approached that of a sphere. This usually occurred after several concentric fiber fractions had been scraped off and less than a third of the total wet tissue remained. In such cases, the

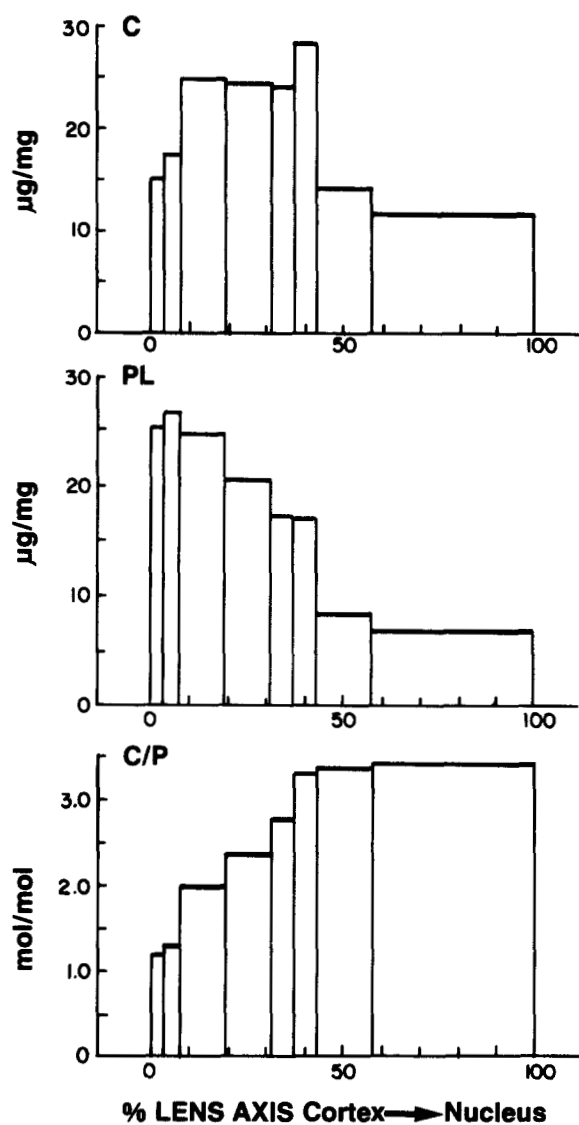


Fig. 2 Distribution of C, PL, and mol C/PL ratio (C/P) in the left lens shown in Fig. 1. The distribution of the concentric fractions is given as the % of total cortex to nucleus axial length occupied by each fraction. The original lens is approximated as an ellipsoid with long and short axis of $a = 10$ and $b = 6.5$ mm, respectively. The remaining lens, after the removal of successive fractions, has dimensions of a_r and b_r as the axes. The distributions of the fraction in percent of the long and short axis; i.e., a_r/a and b_r/b , are identical and were calculated from equation 8, $a_r/a = b_r/b = (1 - \Sigma\%)^{1/3}$ where $\Sigma\%$ is the sum of % dry wt occupied by the successive preceding fractions and obtained from Fig. 1.

diameter of the remaining lens nucleus, D , can be calculated from the expression for the volume of a sphere, $V_s = \pi D^3/6$, which is also obtained from equation 1 using the dry wt and protein concentration of the sphere. The calculated diameters are given in Table 1 taking 65 mg as the total dry wt for a 60-year-old lens. Value for protein concentration of 40% is also included. This is the highest protein concentration reported for the nuclear region of the human lens.

At a protein concentration of 25%, the nuclear sphere of 15% total dry wt occupies 62.8 and 40.8%, respectively, of the short and long lens axial length. The same nuclear 15% dry wt is calculated to occupy 53% of the axis according to equation 8; i.e., when the shape of the remaining lens remained the same as the original (Fig. 2). A larger proportion of the short, visual axis is taken up by assuming a spherical shape. The change in protein concentration from 25 to 40% produces approximately 15% variation in the calculated axis value. However, note that even at 40% protein concentration, the nuclear 5% dry wt occupies approximately 36% of the axial length.

RESULTS

Distribution of C and PL in normal lenses

Seven pairs of normal lenses from subjects ranging in age from 54–77 years were examined. For each lens, consecutive layers of fiber cell material were removed; this resulted in the isolation of seven to nine fractions. The dry weight and C and PL content of each fraction were determined. Typical results are shown in Fig. 1 for a pair of normal lenses from a 63-year-old individual. The distribution of C content in μg per mg dry weight of each concentric fraction is reported as a function of the location of the fraction in % of the total lens dry weight. The surface of the lens is represented as 0% and the center of the lens as 100%. Three distinct regions were observed; the outer cortex, approximately 20–25% of the total lens dry weight where the C content increased from 14 to about 18 $\mu\text{g}/\text{mg}$, the inner cortex, 50–60% of the total lens where the C reached a maximum level of about 25 $\mu\text{g}/\text{mg}$, and the inner nucleus, 15–20% of the lens having the lowest C level of about 13 $\mu\text{g}/\text{mg}$.

TABLE 1. Diameter of remaining nuclear spheres^a containing 5 and 15% of the original lens mass

Diameter (D)	15% Wt Remaining		5% Wt Remaining	
	Protein Conc.		Protein Conc.	
	25%	40%	25%	40%
in mm	4.08	3.44	2.82	2.38
as % of $b^b = 6.5$ mm	62.8	52.9	43.4	36.6
as % of $a^c = 10$ mm	40.8	34.4	28.2	23.8

^aThe diameter, D , of the remaining lens sphere is calculated from the volume of a sphere, $V_s = \pi D^3/6$. V_s is also derived from equation 1; $V_s = W_r/(C \cdot \rho)$, where W_r is the dry weight of the sphere obtained from the remaining % wt multiplied by the total lens wt (65 mg) and ρ is the density of the remaining lens, equal to 1.10 and 1.15 g/ml at the respective protein concentration C, of 25 and 40%.

^bMeasured short and long axes for 60–63-year-old human lenses.

Unlike C, the PL in approximately the cortical 70% of the lens remained relatively constant ranging from 22 $\mu\text{g}/\text{mg}$ in the most peripheral 10% to 24 $\mu\text{g}/\text{mg}$ in the remaining cortex. In the deeper fibers of this region near the nucleus, the values appeared to decrease somewhat. In the nuclear 15–20% of the total tissue weight, there was a precipitous drop in PL to 6–8 $\mu\text{g}/\text{mg}$. The average values of the seven pairs of lenses are shown in **Table 2**. Because of the difficulty in obtaining exactly the same amount of material for the lens sections, there is some variation in the size of the regions from different lenses. However, as shown in Table 2, the variation in lipid values between lenses after adjusting for the size of the sections is no more than $\pm 10\%$.

The change in the mol ratio of cholesterol/phospholipid caused by the variation in concentration of these constituents is shown in Fig. 1-C/P. There is a gradual increase in the ratio from approximately 1.2 in the outer cortex to values greater than 3.5 in the nucleus. This pattern differs markedly from that obtained with the C or PL data. The increase in the ratio is due to two factors: a cortical increase in C while the PL levels remain relatively constant, and then a greater drop in PL than in C levels in the nuclear region of the lens.

Location of the concentric fiber fractions on lens axis

The data in Fig. 1 are expressed as the % lens dry wt occupied by each concentric fiber fraction on the cortex to nucleus axis. The % dry weight can be converted to a unit of length in mm according to the derivation given in the Methods section. The results obtained with the data from the left lens in Fig. 1 are illustrated in Fig. 2. As a consequence of the cubic to linear transformation, the distribution of the fractions on the basis of dry wt becomes considerably different from that of the axial length. For example, the cortical 48% of the dry wt containing the outermost three fractions represents only approximately 20% of the axial length. It is interesting to note that the outer 20% of the dry wt, where the C content begins to increase, occupies less than 10% of the lens axial length

and appears to be correlated with the region containing elongating as well as some mature fiber cells (23, 24). In contrast, the nuclear 18% dry wt (the innermost two fractions) now surprisingly corresponds to about 55% of the total axial length and has a disproportionate influence upon the optical properties of the lens. Such data explains Philipson's observation that while more than 50% of the axial length of a 60 year-old-lens has a protein concentration of 40% (21), the observed protein concentration for the whole lens is about 25%. The nuclear C/PL ratio of greater than 3.0, therefore, represents a large proportion of the fiber membrane lipid composition in the optical path length of the lens than would be expected.

Lipids of the capsule epithelium

Analyses of the capsule epithelia from three pairs of lenses of 63–67 years of age showed that, on a per lens basis, each capsule epithelium contained $15 \pm 2.5 \mu\text{g}$ of C and $27.5 \pm 1.5 \mu\text{g}$ of PL. The average mol C/PL ratio varied from 0.86 to 1.36. Since the preparations were occasionally contaminated with some lens fibers that had a higher C/PL ratio, the lower ratio of 0.86 may more closely approximate the true value for the epithelium.

Changes in phospholipid composition in individual normal human lenses

To determine the PL composition of the concentric fiber fractions, lipid extracts (15) of these fractions were subjected to one-dimensional TLC developed with two solvents. A typical PL pattern of a 63-year-old normal lens is shown in **Fig. 3A**. The dominant lenticular PL is sphingomyelin (SPM). On the basis of comparison with standards of known R_f , at least two SPMs, palmitoyl (shown in Fig. 3A) and nervonyl (not shown) sphingosylphosphocholine, were identified. To quantitate the changes in the PL, the TLC plate was scanned with an LKB densitometer and the integrated areas were normalized against the total area. A number of major changes were observed. There was a relative decrease in PS and PE and a corresponding increase in the SPM in going from the outer periphery to the inner core of the lens. As shown in **Fig. 4**, the relative abundance of these PLs was plotted as a function of the % dry wt as in Fig. 1 to indicate the abrupt changes in certain fiber layers. Analyses of the lyso-PL were not made because lyso-PL was present in small amounts and lyso-PE and lyso-PS could not be separated from each other or PC (Fig. 3A). It is interesting to note that while the total PL content did not change significantly in the outer and inner cortex, the composition did change markedly. The change in PL composition occurred in the same region in which the C content increased to its maximum value. The outer cortical 20% of the lens dry mass occupying less than 10% of the axial length appeared to correspond to the region where fiber

TABLE 2. Regional distributions of C, PL contents in 54–77-year-old human lenses^a

	Outer Cortex ^b (15–20% wt)	Inner Cortex ^b (40–60% wt)	Nucleus ^b (10–15% wt)
C ($\mu\text{g}/\text{mg}$)	14 ± 1.6	25 ± 2.4	12 ± 1.3
PL ($\mu\text{g}/\text{mg}$)	22 ± 2.3	24 ± 2.1	7 ± 1.8
C/PL (mol/mol)	1.2 ± 0.2	2.2 ± 0.3	3.4 ± 0.5

^aValues are given as means \pm SD of the seven pairs of lenses.

^bVariation in the dry weight of the lens regions is caused by the variation in the size of the sections from different lenses. The three regions usually contain respectively 2–3, 4–5, and 1–2 successive fractions of a lens. Only those fractions having $\frac{1}{3}$ or more of their dry weight distributed in one region were included in estimating the averages.

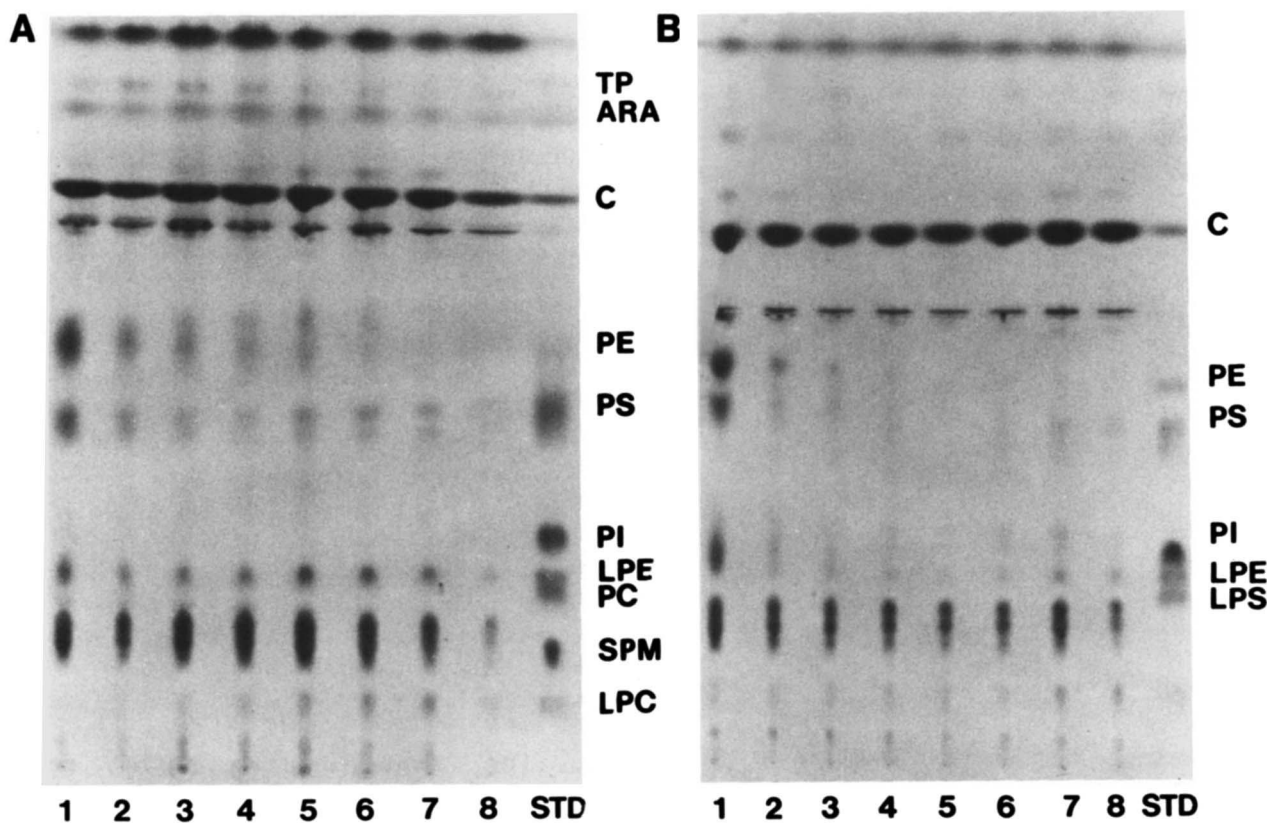


Fig. 3 Thin-layer chromatographs of lipids isolated from the same fractions as in Fig. 2 of a 63-year-old normal lens (A) and those of a 65-year-old cataractous lens (B). The numbers 1–8 denote the successive fractions isolated from the outer cortical to the nuclear region of the lenses. The same fractions from the left lens of the pair of 63-year-old lenses used in Fig. 2 were analyzed by TLC. Lipid standards used are: TP, tripalmitin; ARA, arachidic acid; C, cholesterol; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; LPE, lyso-PE; LPS, lyso-PS; PC, phosphatidylcholine; SPM, palmitoylsphingosylphosphocholine; LPC, lyso-PC. The plate used for Fig. 3A was developed to 11 cm, while that for Fig. 3B was developed to 9 cm with the first solvent. Both were developed to 15 cm with the second solvent.

elongation is completed (24). The nuclear fraction appeared to have a distinct PL composition containing a higher lyso-PC (Fig. 3A) level and a lower SPM level than that of deep cortex. The PL composition in the nucleus was similar to that reported by Broekhuysse for a 66-year-old lens (3).

Distribution of lipids in cataracts

The characteristic distribution of C and PL contents in a 65-year-old cataract obtained from the Eye Institute at Columbia University is shown in Fig. 5. The lens had extensive cortical and nuclear opacities and is classified as C⁺⁺, N⁺⁺ according to the CCRG convention (25). The distribution of overall lipids was quite similar to that for normal lenses shown in Fig. 1. Very low C and PL contents were found in the nucleus and there was a progressive increase in C content in the outer cortical 50% of the lens. As expected, several less severe cataracts of C⁺⁺, N⁺⁺ classification showed the same lipid distribution depicted in Fig. 5.

Analyses of the PL composition by TLC (Fig. 3B) show that changes in the major lipids are comparable to those observed with normal lenses; i.e., the decreases in PE and PS and the corresponding increase in SPM as fibers were displaced toward the nucleus. Even with the extensive nuclear involvement of this cataract (N⁺⁺), the unique PL composition of the nuclear fibers having a high LPC and a lower SPM content than the deep cortex remains apparent. The appearance of minor component(s) with *R_f* values similar to phosphatidylinositol (PI) in the inner cortex and nucleus may be characteristic of cataracts (compare Fig. 3A with 3B).

Changes in fatty acid composition in normal lens

In addition to the C/PL ratio, the fatty acid acyl group also contributes to the rigidity of the membrane lipid bilayer. Saturated fatty acids having a higher melting point increase the rigidity of the membrane. Fatty acid methyl esters obtained by transesterification of the lipid extracts from lens regions were analyzed by GLC and the

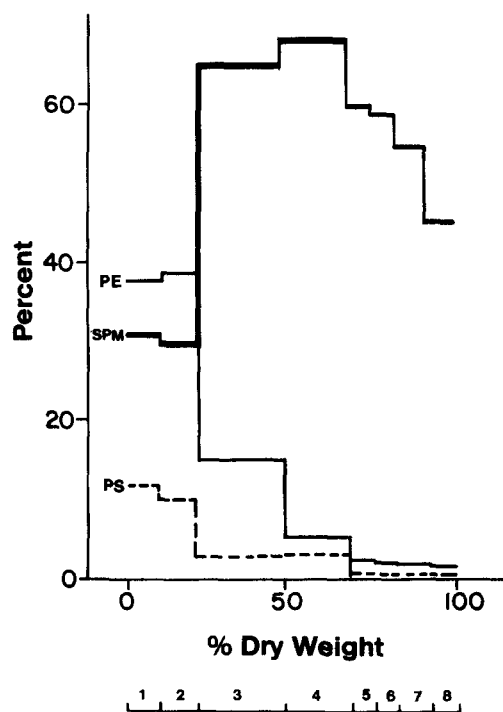


Fig. 4 Changes in PL composition in the normal 63-year-old lens used in Fig. 3. The distribution of the % PE (—), SPM (—) and PS (---) in the successive fractions 1-8 is expressed as the % total dry wt occupied by each fraction as in Fig. 1.

data for a 57-year-old normal lens are shown in Table 3. To obtain sufficient amount of lipid for the analysis, the lens, after the removal of capsule epithelium, was divided by dry wt into three successive fiber fractions of 45% outer cortex, 32% inner cortex, and 18% nucleus, respectively. The GLC data indicated that three major fatty acids, palmitic (16:0), oleic (18:1), and nervonic (24:1) accounted for more than 90% of the total. Among the minor components, only stearic acid (18:0) could be observed with reasonable reproducibility. The decrease in the relative abundance of 18:1 found in deeper regions of the lens conforms with the observed disappearance of PE and PS in the inner fractions. The concomitant increase in 24:1 is apparently due to the relative increase of SPM containing N-nervonyl sphingosylphosphocholine, an observation similar to that reported for bovine lens (3). The lowest 24:1 value was obtained with the lipid extract from the capsule epithelium that was shown by TLC to contain the lowest relative level of SPM (not shown).

All the fiber fractions as well as the capsule epithelium maintained the same level of fatty acid saturation of about 30% reflecting the increase in 24:1 and decrease in 18:1. These results indicate that the average acyl chain composition or viscosity of the hydrocarbon core of lens PL bilayers remains the same throughout the lens.

DISCUSSION

The very low lipid content of lens fibers

Since the sum of C and PL represents more than 95% of the total lipid, the data in Figs. 1 and 5 indicate that the human lens has an average total lipid content of about 40 $\mu\text{g}/\text{mg}$ or 4% of tissue dry wt. This is a very low value as compared with other tissues (26) such as erythrocytes (15%), muscle (20%), and liver (25%), etc. The low lipid content indicates the availability of a small amount of membrane material to envelope the fiber mass. This is reflected in the large dimensions of the fibers, some of which reach a length of 1 cm. Furthermore, the lower lipid content of the nuclear fibers suggests that the fiber cell volume is larger in the nucleus than in the cortex. In human as well as rat lenses, the larger volume appears to be achieved through an increase in the fiber width (23, 24). At the low lipid content of 2% or less, the nuclear fiber membrane lipid is stretched over a large surface area and mechanical breakage could occur readily. Poor preservation of these nuclear membranes for microscopic observation has been noted (24).

The high cholesterol content in fiber lipid membrane

In comparison with other tissues, the C/PL mol ratio is exceedingly high in lens fibers, ranging from 0.8 in the

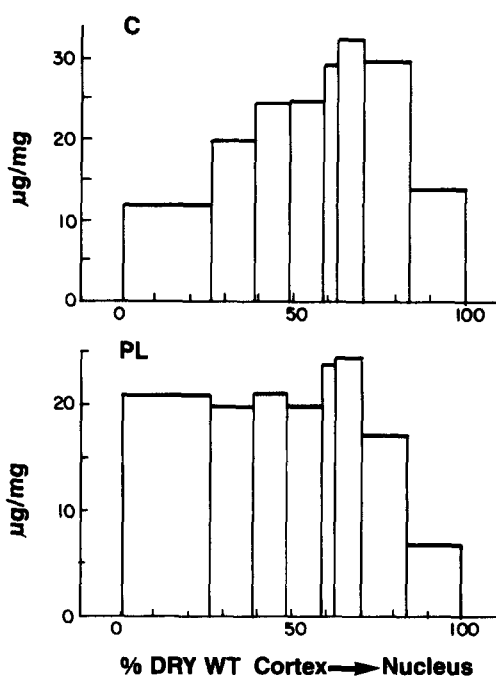


Fig. 5 Distribution of C and PL contents of the concentric fiber fractions from a 65-year-old cataractous lens. The location of the fraction on the lens axis is given in % total dry wt as in Fig. 1.

TABLE 3. Regional fatty acid composition (in %) of a 57-year-old normal lens^a

Section	Fatty Acid			
	16:0	18:0	18:1	24:1
Capsule-epithelium	27.2	4.6	39.2	21.5
Outer cortex (45% dry wt)	26.5	3.7	38.4	24.0
Inner cortex (32% dry wt)	28.6	4.9	30.3	30.2
Nucleus (18% dry wt)	30.9	2.5	23.1	36.1

^aFatty acids were determined as the methyl esters by GLC after hydrolysis of the lens fiber lipid extracts with 5% H₂SO₄ in acetonitrile-water 9:1 and subsequent esterification with methanolic HCl. Values are mean of three to five determinations. SD is 11% except for 18:0 where it is \pm 23%.

capsule epithelium to 3.5 in the nucleus. It has been shown that more than 90% of the cellular C is associated with the plasma rather than internal membrane in the intact cell (27). In the lens, this is probably a low estimate since mature fibers have little or no internal organelles. A C/PL of 1 represents a very rigid plasma membrane. The highest known value of 1.32 is observed with myelin (28). In addition, elevated levels of cholesterol are known to inhibit Na-K ATPase activity in vitro and in vivo (29). When the level is raised to give a C/PL ratio of 1 in erythrocytes, more than 50% of the enzyme activity is lost (30). Even though ATPase activity has been shown to be present in the outer cortex (31), the C/PL ratio of greater than 1 in this region may inhibit the activity to such a degree that transport of lens electrolytes is found almost exclusively in the extracellular space of lens fibers (32). That lens physiology can be approximated by treating the lens as an osmometer with most of the active components residing in the monolayer epithelium has been proposed (8).

The molecular orientation of C has been deduced from dielectric measurements of synthetic membrane in aqueous KCl. The 3'-hydroxyl ring spans the acetyl region of the PL in that the hydrophilic hydroxyl group is intercalated between the PL phosphate and glycerol oxygen, while the bulk of the nonpolar ring as well as the short alkyl chain are in the hydrocarbon core of the PL bilayer (33). Due to the rigid ring structure, increase in C has been demonstrated to result in a linear increase in the *interfacial* viscoelasticity of lipid membrane up to a C/monooleoyl glycerol mol ratio of 4 (2). Lamellar C packing in biological membranes has not yet been demonstrated. The much lower solubility of C compared to PL would severely limit the cellular communication across the nuclear fiber membrane. The tendency for C to aggregate into clusters at a C/PL ratio greater than 0.3 (34) may be the molecular basis for the extensive membrane fusion recently observed with lens fibers (35). This membrane fusion may also explain the observed low lipid content.

Arrangement and configuration of phospholipid in fiber membrane

The phospholipids of biological membranes are generally believed to exist in the bilayer conformation with the hydrophobic fatty acid acyl group forming the inner core while the phosphate moiety interfaces with the aqueous medium. The viscosity of the hydrocarbon core is close to that of the equivalent normal paraffinic liquids of similar chain length (36). Based on the micrographs of Kuwabara (24), each nuclear fiber can be approximated as an ellipsoid cylinder. A surface area of 0.045 mm² is calculated from $2 \cdot \pi l \sqrt{(a^2 + b^2)/2}$, using $a = 1.2 \mu\text{m}$, $b = 1.625 \mu\text{m}$, and $l = 5 \text{ mm}$ as the cross-sectional semi-axes and length of the cylinder, respectively. From the volume of the cylinder ($\pi a b l$), a protein concentration of 40%, and a corresponding density of 1.15 g/ml, the total protein or dry wt is calculated to be 11.7 ng/fiber from equation 1 in the Methods section. Taking 2% as the lipid content, 3 as the C/PL ratio (Table 2), and the high estimate of 40 Å² as the surface area occupied by each PL molecule having an averaged molecular weight of 800 (37), a value of 0.013 mm² is obtained for the total area covered by the PL in the bilayer configuration. Comparing this value with that of 0.045 mm² estimated for the surface area of the fiber cylinder and considering that the above calculation applies only to ideally smooth surfaces while the fiber membrane is known to be highly convoluted, it is concluded that less than a third of the nuclear fiber membrane is made of bilayer phospholipids. A mosaic of PL bilayer and C patches or mixed C-PL bilayer or some other unknown structure of efficient packing arrangement is needed to satisfy the low lipid content as well as the high C/PL ratio of the fiber membrane. A mixed SPM and C bilayer has been suggested to exist in myelin (38).

Lipids of cataractous fiber membrane

The distribution of C as well as PL content appear to be similar in normal and cataractous lenses. While the appearance of minor PL components have been revealed by TLC, the distribution of the major PLs, SPM, PS, and PE, remains the same in cataract. These results suggest that, if fiber membrane lipid alterations are involved in cataract formation, they are localized in a very few cell layers and not detected. Since the C/PL ratio, a measure of membrane lipid rigidity, is much higher in nuclear than cortical fiber, it is likely that the mechanism of opacification in nuclear fibers differs from that for lens cortex.

Even though the cataracts used in this study had both cortical and nuclear opacities, extensive clear regions remained throughout some of the lenses. If the fiber membrane is assumed to be the site of initial damages leading to opacification, it is puzzling why only a portion of the fibers with the same membrane rigidity laid down at the

same stage of lens development was involved. Likewise, it is intriguing to note that the insult leading to extensive nuclear fiber damage spared a large portion of the less rigid cortical membranes. One possible explanation is that the primary insult occurred prior to the displacement of these fibers to the nucleus, at a time during their initial development when the lipid membrane was less rigid.

Changes in lipid during fiber elongation and aging

In the region corresponding to the cortical 20% of the lens weight or less than 10% of the visual axial length, the PL content was relatively constant (Figs. 1 and 2) while the protein concentration increased from 25 to 40% (21). These results indicate that during the large increase in fiber volume (200-fold or more) the increase in PL is comparable to that of the protein. The continual increase in C concentration in the cortical 40–50% of the lens dry wt suggests that C accumulates at a greater rate than either PL or protein. The region where C content reaches its maximum coincides with the region (cortical 20–25% of lens weight, Fig. 1) where stepwise changes in PL composition occurred (Fig. 4). On an axial length basis, this region occupies approximately the outermost 10% and appears to correspond to the region where fiber reaches its maximum length (24). Thus, lipid changes observed with these fractions may be related to fiber elongation and/or development.

Qualitatively, the averaging of the present data on the distribution of C and PL in individual concentric fractions gives results comparable to those obtained from total cortex and nucleus (3, 4, 14). However, without data on several concentric cortical and nuclear fractions, the increase in C level in the outer cortex, the rising to its maximal value in the inner cortex (40–60% of lens total dry wt), and its subsequent decline in the nucleus, as well as the gradual decrease in PE and PS in the inner cortex, would not have been noted (3). Since very little fatty acid synthesis or turnover is found in the deep cortex, even in very young rat lens (6), the decrease in PE and PS may be attributed to aging effects.

In the nucleus, the decrease in the relative SPM content is compensated by a large increase in lyso-PC in addition to the age-related increase in lyso-PS and lyso-PE as observed in the deep cortex (Fig. 4, lanes 7 and 8). The very low C level in the nucleus and the rather sharp cortex to nucleus drop in C level (Fig. 1) suggest that the observed lipid composition of the nucleus (10–15% of total lens dry wt) may represent the accumulated effects of aging on the distinctive membrane lipid of embryonic lens fibers.

The most surprising conclusion from the volume or dry wt to linear axial length conversion of the lipid distribution data is that the nuclear 10% of the lens dry wt occupies approximately 50% of the optical path length. Thus, a uniform lipid composition for the major portion

of the visual axis is achieved through the lens geometry and the manner in which newly formed fibers are layered over the preceding ones. Homogeneity in protein concentration in this region has been reported (21). Based on in vivo slit-image photography of lenses between the ages of 8 and 82 years, this region of homogeneous lipid and protein has been identified as the fetal nucleus (39). In a later publication, data on the lipids of individual lenses of various ages will be reported to further establish the distinct lipid composition of the lens nucleus as well as to distinguish changes due to fiber development from those due to aging. ■■

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