AGE CHANGES IN THE HUMAN LENS

B. S. Kasavina and V. M. Akkuev

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Quantitative and qualitative changes in the proteins and lipids of the lens and also in its cortex and nucleus were detected. Four crystallins, differing sharply in molecular weight, and not homogeneous, were identified. The cholesterol fractions in the crystallins were studied and the character of the connection between their protein and lipid part elucidated.

Changes in the protein composition of the lens during ontogenetic development have been described [2, 3, 5, 6, 8, 10]. Conflicting results have been obtained regarding the cholesterol content in the lens [7, 9].

The object of the present investigation was to study quantitative changes in the content of crystallins and cholesterol in the lipid–protein complexes of the totally transparent lens and its cortex and nucleus depending on age, and to examine the character of the connection between its protein and lipid parts.

EXPERIMENTAL METHOD

The experimental material consisted of 63 transparent lenses taken intracapsularly not later than 12 h after death of persons aged from 21 to 115 years dying suddenly or accidentally. To isolate crystallins from the water-soluble proteins of the lens, a slightly modified version of method [4] was used; namely that to determine the homogeneity of the fractions isolated by gel filtration, polyacrylamide gel was used instead of agar. Densitometry of the gel columns containing crystalline was carried out on a modified MF-4 microphotometer with automatic recorder. Total protein of the homogenates, supernatants, and crystallins was determined by Lowry's method [9]. The solutions of crystallins obtained were dried lyophilically and used for determination of their cholesterol fractions [1]. The difference between the total cholesterol and the loosely-bound component constituted the firmly-bound cholesterol, and when expressed as a percentage of the total cholesterol it gave the coefficient of firmness of its bonding with proteins.

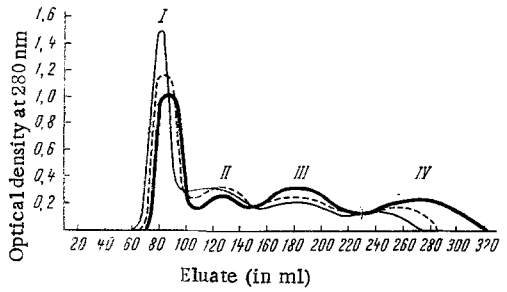

Fig. 1. Gel filtration of water-soluble proteins of the transparent lens from persons of different ages: I) α_1 crystallin; II) α_2 crystallin; III) β crystallin; IV) γ crystallin; thick continuous line age 21; broken line age 64; thin continuous line age 83 years.

EXPERIMENTAL RESULTS

The results of gel filtration and distribution of the crystallins of the water-soluble proteins of the lens by molecular weight are shown in Fig. 1. The water-soluble proteins of total extracts of the transparent lenses consist of four components: subfractions α_1 and α_2 of high-molecular-weight α crystallins, and the low-molecular-weight β and γ crystallins, distributed in accordance with molecular weight. The first fraction eluted from the gel column consisted of high-molecular-weight crystallins with a smaller elution volume, followed by the low-molecular-weight components with a large

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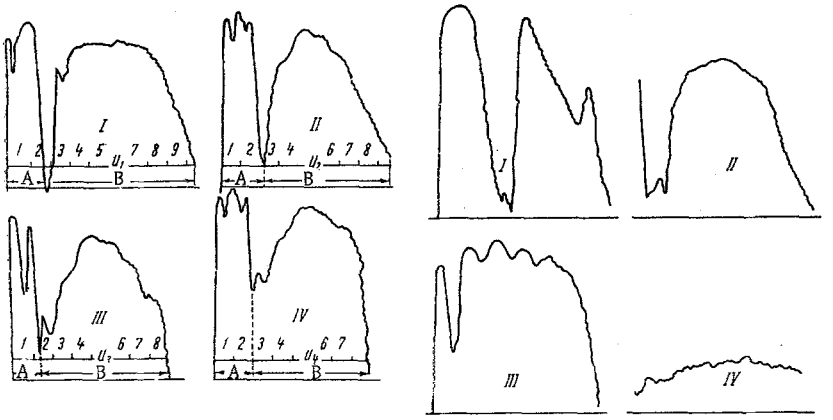

Fig. 2

Fig. 3

Fig. 2. Vertical microelectrophoresis of water-soluble proteins of transparent lens from persons of different ages in polyacrylamide gel (densitometry). I: electrophoresis mobility; A) high-molecular-weight crystallins; B) low-molecular-weight crystallins; I) age 52 years; II) 64 years; III) 76 years; IV) 90 years.

Fig. 3. Vertical microelectrophoresis of crystallins from the human lens in polyacrylamide gel (densitometry). I) α_1 crystallin; II) α_2 crystallin; III) β crystallin; IV) γ crystallin.

TABLE 1. Content of Crystallins (in percent) in Water-Soluble Proteins of Human Lens Cortex and Nucleus

Crystallins		In lens cortex (in %)		In lens nucleus (in %)		t	Statistical significance (P)
		$M \pm m$	$\pm\sigma$	$M \pm m$	$\pm\sigma$		
α_1	5	50,6±3,6	8,18	43,2±4,9	11,9	1,23	>0,05
α_2	3	28,8±3,3	7,54	25,2±2,8	6,2	0,80	>0,05
β	5	15,8±1,9	4,18	26,6±8,8	19,7	1,20	>0,05
γ	5	5,9±1,7	3,60	6,1±1,87	3,7	0,08	—

elution volume. The results also showed that, with advancing age, there is a tendency for the peaks of the subfractions of α crystallin to merge, and also some decrease in the elution volumes of the crystallins, which is confirmed by electrophoresis of the lens proteins of persons of different ages in polyacrylamide gel. There is a tendency for the connection between the subfractions of α crystallin and β crystallin to be increased, while at the same time their electrophoretic mobility is reduced (Fig. 2: $I_1 > I_2 > I_3 > I_4$).

High-voltage disk microelectrophoresis of the crystallins isolated by gel filtration showed (Fig. 3) that they are not homogeneous. The first subfraction (α_1) of the high-molecular-weight α crystallin consisted of three and the second of one to two components. The β crystallins consists of five to seven components. Since the γ crystallin has an opposite electrical charge, this protein migrated toward the cathode and thus was not present on the column. A study of the relative content of crystallins in total extracts of the water-soluble proteins of the lens showed that the content of the α_1 subfraction of the high-molecular-weight α crystallin increased from 30% at age 21 years to 54% at age 83 years, while the content of the α_2 subfraction of this crystallin was doubled during the same period (from 12.6 to 26.1%). The relative content of low-molecular-weight crystallins (β and γ) showed a tendency to decrease. For example, the content of β crystallin at ages from 21 to 83 years fell by half (from 30 to 15%), while the content of γ crystallin during this same period fell from 27.4 to 5.1%.

The number of fractions of water-soluble proteins in the cortex and nucleus of the lens and their distribution by molecular weight on gel filtration were found to be identical. No clear subdivision between the subfractions of α crystallin in the lens nucleus could be obtained. The relative content of crystallins in the water-soluble proteins of the lens cortex and nucleus is given in Table 1. Despite the apparent difference in crystallin content in the cortex and nucleus of the lens, this difference is not statistically significant.

TABLE 2. Content of Cholesterol Fractions in Crystallins of the Human Lens and Coefficient of Firmness of Its Bonding with Proteins

Crystallins	Total cholesterol (in mg %)	Loosely-bound cholesterol (in mg %)	Firmly-bound cholesterol (in mg %)	Coefficient of firmness of cholestrol binding with proteins
α_1	2 128±27	1 549±70	579	26,0
α_2	1 493±35	1 214±14	279	18,7
β	323±19	259±8	64	19,0
γ	210±20	168±17	42	20,0

This is because of the wide range of variation in individual content of crystallins, a particularly characteristic feature of β crystallin, for which it may amount to ±8.8%. This evidently depends on the degree of sclerosis of the lens. With age, a gradual accumulation of total cholesterol takes place in the human lens, but this increase only becomes significant after the age of 71-80 years; at ages of more than 90 years, no increase in the content of this lipid is observed. There is a comparatively wide range of variation in the cholesterol content within each age group and between individual decades. The total cholesterol content in the water-soluble lipoproteins of the lens nucleus was found to be 2.4 times higher than in the cortex, and this evidently reflects different conditions of metabolism in these parts of the lens.

To study the quantitative and qualitative relationships between the protein and lipid parts of the water-soluble complexes of the lens more closely, the cholesterol fractions were determined in the structural units of the lens fibers, in the crystallins themselves. So far, in the literature, they are spoken of as "pure" proteins. However, the present experiments showed that crystallins are also found in combination with cholesterol. Data for the quantitative content of cholesterol fractions in the crystallins of the human lens in persons over 40 years old, and the character of the connection between the protein and lipid parts of the crystallin complexes are given in Table 2. The content of individual cholesterol fractions bound with the crystallin proteins shows that the content of this lipid is directly proportional to the molecular weight of the crystallins. In this case, most of the lipids are represented by loosely bound cholesterol. The coefficient of firmness of cholesterol binding with proteins was found to be independent of molecular weight and to have a mean value of 20.9. Consequently, with increase in age there is a quantitative change in the lipid–protein complexes of the human lens, reflecting the qualitative state of the protein components, as well as possible conformational changes in the crystallin molecule. Data relating to aggregation of crystallins with age confirm the likelihood of these changes. The increase in the number of bonds between the crystallins and in their lipid content evidently decreases the number of active groups of the protein molecule and this, in turn, reduces the rate of its self-renewal.

These biochemical changes are manifested as a disturbance of the plasticity of the lens tissue and this is expressed as a decrease in its power of accommodation.

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