Transformation of alpha-crystallin polypeptide chains with aging

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Newly synthesized α-crystallin (NSa) appears relatively homogeneous with an apparent average molecular weight ($M_w$) of $7.4 \times 10^5 \pm 3$ per cent. It is composed primarily of polypeptide chains $A_2$ and $B_2$. Only trace amounts of $B_1$ and small amounts of $A_1$ can be detected. Both $A_2$ and $B_2$ are produced rapidly through post-translational events. Low molecular weight α-crystallin (LMWa) arises from NSa and is composed of 4 polypeptide chains, $B_1$, $B_2$, $A_1$, and $A_2$, with $B_1$ representing approximately 11 per cent of the total polypeptide population and $A_2$ approximately 24 per cent. Little change can be detected in the LMMa polypeptide composition isolated from three-month-old calf lens cortex or nucleus or from two-year-old steer lens nucleus. High molecular weight α-crystallin (HMWa) from calf lens periphery has a polypeptide-chain profile similar to that of LMMa. However, HMWa from calf lens nucleus shows changes in its polypeptide profile which become more pronounced in steer nucleus HMWa. Of particular interest is the appearance of two components with MW's of 17,000 and 13,000 and a splitting of the $A_2$ urea polyacrylamide electrophoresis band. HMWa from steer nucleus was also found to contain a substantial amount of atypical A-chains with masked SH groups. It is suggested that age-dependent changes in the polypeptide-chain composition of α-crystallin affect both its size and physical homogeneity.

Key words: alpha-crystallin, polypeptide chains $A_1$, $A_2$, $B_1$, and $B_2$, newly synthesized, low molecular weight, high molecular weight.

The encapsulated avascular lens is an excellent tissue for the study of aging. A single layer of epithelial cells on the anterior side of the tissue differentiates in the equatorial region into fiber cells with a large increase in the volume of the cell and in the production of proteins. The fiber cells are displaced toward the center of the lens with the production of new cellular components. Pycnosis of the older fiber cells occurs with dedifferentiation to lens fibers. This process continues throughout life although the rate of production of new fibers decreases markedly with age. Thus the oldest regions of the lens are in the center and represent the embryonic and fetal nuclei. The material produced most recently is found in the outer periphery or cortex. Since no protein synthesis occurs in the inner third of the calf lens the protein that was initially synthesized in this...
region is either still present in the older lens or altered by post-translational reactions.

The structural lens protein \( \alpha \)-crystallin is of considerable interest because of its possible involvement in the development of central senile opacities.\(^3\) \( \alpha \)-Crystallin is not a homogeneous protein but has been shown to be composed of a number of populations of macromolecules.\(^3\)\(^-\)\(^6\) Depending upon the method of fractionation, the populations can be defined with somewhat different boundaries. The two major populations of this protein are low molecular weight \( \alpha \)-crystallin (LMWa) which is defined as that population with a molecular weight range of approximately 0.7 to 1.0 \( \times \) 10\(^6\) and high molecular weight \( \alpha \)-crystallin (HMWa), the population of macromolecules with molecular weights greater than 15 \( \times \) 10\(^6\). It has been demonstrated that with aging of the bovine lens the proportion of macromolecules in the high molecular weight population increases more than 5-fold, so that in eight-year-old bovine lens nuclei, 80 per cent of the \( \alpha \)-crystallin is in this form. However, this change occurs primarily in the nuclear region, i.e., the inner 30 per cent of the lens. In the cortex little transformation to high molecular weight can be observed.\(^6\)

Another population of \( \alpha \)-crystallin has been isolated from calf lenses incubated in organ culture in the presence of labeled amino acids.\(^7\) This material, designated highly labeled protein, was found to have a specific activity 8 to 10 times greater than any other isolated lens protein fraction and had an apparent molecular weight of approximately 7 \( \times \) 10\(^5\), at the low end of the molecular weight spectrum of \( \alpha \)-crystallin. It is probable that this \( \alpha \)-crystallin represents newly formed material and it has therefore been designated as newly synthesized \( \alpha \)-crystallin (NSa) in this communication.

Unfractionated calf \( \alpha \)-crystallin had previously been shown to contain four polypeptide chains designated \( A_1, A_2, B_1, \) and \( B_2 \)\(^8\)\(^-\)\(^10\) held together by noncovalent forces. The more basic B-chains have MW's of 22,500 and the \( A \)-chains MW's of 19,500.\(^4\) Palmer and Papacostantinou\(^11\) as well as Schoenmakers and Bloemendal\(^12\) have shown that \( \alpha \)-crystallin isolated from pre-natal lens contains only three subunits. It has been found that the \( A_1 \) chain is not synthesized but is the result of a post-translational reaction.\(^11\)\(^,\)\(^12\) Furthermore, it has been reported that in lens epithelial cells only \( A_2 \) and \( B_2 \) polypeptide chains could be detected.\(^14\)\(^,\)\(^15\)

In the present communication a study of the effect of aging upon the polypeptide chains of \( \alpha \)-crystallin is reported. It is suggested that NSa initially contains only \( A_2 \) and \( B_2 \) polypeptide chains and it is shown that not only \( A_1 \) but \( B_1 \) as well is produced by post-translational events. It is also reported that whereas no significant change occurs with aging in LMWa, HMWa shows marked changes in polypeptide-chain composition and sulfhydryl reactivity. It is proposed that the transformations in polypeptide-chain composition may be related to the size and heterogeneity of the \( \alpha \)-crystallin population.

Methods

The newly synthesized protein fraction from calf lens was prepared by a modification of the method of Spector, Wandel, and Li\(^9\)\(^-\) NEC-445 L-amino acid-C\(^14\) mixture, 1.5 \( \mu \)Ci per milliliter (New England Nuclear, Boston, Mass.), was used in the incubation medium in the place of C\(^14\)-histidine and the incubation was continued for 16 hours. For the preparation of the various molecular weight forms of \( \alpha \)-crystallin, lenses of different ages were used. Calf lenses were obtained from animals three months of age and steer lenses from two-year-old animals. The peripheral region of the lens was defined as the outer 70 per cent (wet weight) and was obtained by removing the central region of the lens with a trephine. The nuclear region represented the inner 30 per cent (wet weight) and was obtained by removing the capsule and placing the lenses in 2 mM phosphate buffer, pH 7.6, and stirring at 4° C. until the outer 70 per cent (wet weight) was removed.

\( \alpha \)-Crystallins were prepared from lens homogenate utilizing the DEAE cellulose-gel filtration procedure of Spector and co-workers.\(^4\) LMWa represented the second peak from Bio-Gel A-5m (exclusion limit 5 \( \times \) 10\(^6\) daltons). High molecular...
weight α-crystallins were prepared using Bio-Gel A-15m (exclusion limit $15 \times 10^6$ daltons) and represented the first peak. Iodoacetic acid was recrystallized from light petroleum ether (boiling point, 32 to 50° C.). Acrylamide and bis-acrylamide were recrystallized from acetone. All other materials were reagent grade and used without further purification.

Polyacrylamide gel electrophoresis was carried out with 10 per cent gels and a modified, pH 8.9, Tris-glycine buffer system of Ornstein and columns were used at 3 ma. per tube. When gels were to be sectioned for counting 0.6 by 9 cm. columns were used at 6 ma. per tube. The gels were stained with either Amido Black or Fast Green as described by Maurer. The stained gels were scanned at 630 nm. using a modified Zeiss PMQ spectrophotometer. For measuring radioactivity, the stained gels were sliced into 1.56 mm. sections. Corresponding sections from gels run simultaneously were combined and placed in counting vials. The gel sections were dissolved in 1.0 ml. of 30 per cent hydrogen peroxide by heating at 60° C. overnight. Twenty milliliters of Aquasol (New England Nuclear, Boston, Mass.) were added to each sample and they were then counted using a Packard Tri-Carb scintillation counter.

Molecular weights were determined by the sodium dodecylsulfate (SDS) polyacrylamide electrophoresis method of Shapiro, Vinuela, and Maizel as modified by Weber and Osborn. Pepsin, MW 35,000, was used as the marker protein in all experiments. Parallel tubes containing trypsin, α-chymotrypsin, and β-lactoglobulin were run occasionally.

Affinity chromatography of α-crystallin was carried out on a p-aminophenylmercuric acetate Bio-Gel A-5m column (APMA) as described by Stauffer and co-workers.

Protein samples (2 mg.) were hydrolyzed in sealed, heavy-walled pyrex tubes with constant boiling HCl after flushing the tubes with N₂ and evacuating to less than 0.05 mm. Hg. The hydrolysates were dried under vacuum and analyzed with a Beckman-Spinco Model 121 automatic amino-acid analyzer. Cysteine was determined as carboxymethylcysteine using the procedure of Crestfield, Moore, and Stein as modified by Augustyn and Spector.

Equilibrium centrifugation was carried out as described previously using the high-speed equilibrium method of Yphantis. A filled Epon double-sector cell equipped with sapphire windows and a J rotor was used in a Beckman Model E analytic ultracentrifuge equipped with Rayleigh interference optics. Photographs were taken with Kodak spectrographic plates and were examined with a Nikon Shadowgraph Model 6C. A partial specific volume of 0.73 was determined from the amino-acid composition.

Table I. Molecular weights of NS activity-crystallin fractions

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Molecular weights (± 3%)</th>
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<tr>
<td>24</td>
<td>800,000</td>
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<tr>
<td>Run A</td>
<td>710,000</td>
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<tr>
<td>27</td>
<td>730,000</td>
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<tr>
<td>36</td>
<td>760,000</td>
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<tr>
<td>Run B</td>
<td>730,000</td>
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<td>41</td>
<td>730,000</td>
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Results

This study of the effect of aging on α-crystallin was begun with an examination of the characteristics of NS activity. In order to obtain a greater incorporation of labeled amino acid into the protein than was observed previously a concentration of approximately 1.5 μCi of labeled amino acids per milliliter of incubation medium was employed during a 16 hour incubation of calf lenses. The resulting twofold increase in the specific activity of the isolated protein provided a better indicator of the purity of the preparation than was previously possible. The protein was prepared by the usual procedure of DEAE cellulose chromatography, followed by agarose A-5m fractionation. It was found that after the usual purification with an agarose A-5m column, considerable low specific activity protein was still present. Therefore, an additional two passes through this column were employed. Fig. 1, A represents the third pass through the column of the high specific activity fractions from the second agarose run. While considerable purification was observed, some material of lower specific activity was still apparent on the ascending side of the peak. Fractions 24 through 28 were therefore pooled and after passage through the same column gave the profile shown in Fig. 1, B. It should be noted that the volumes of the fractions in this experiment were decreased from 7.3 ml. to 4.8 ml. Within experimental error most of the material in the peak now appears to have a constant specific activity although the ascending side continues to have some contamination.
Fig. 1. Chromatography of NSα on a 3.5 by 42 cm. column of agarose A-5m. (A) Profile of third pass of NSα through the column. The solid bar indicates fractions pooled; (B) Profile of pooled fractions from (A) after rechromatography. Arrows indicate fractions which were used for ultracentrifugation studies. -•-•-• Elution monitored at 280 nm. -O-O-O Specific activity of the peak fractions.

with lower specific activity protein. Based on other experiments, it is probable that this contamination is contributed by LMWα.

In order to determine the molecular weight of the NSα, fractions were taken from both peaks shown in Fig. 1 and subjected to sedimentation equilibrium experiments under the conditions indicated in the Methods section. In all cases, a linear relationship was observed between the log of the protein concentration and the square of the radius from the center of rotation. Such observations suggest that within the limits of experimental error each fraction appeared to be homogeneous. The results obtained with the different fractions are shown in Table I. These determinations indicate a fairly consistent molecular weight. Although only fractions with relatively constant specific activity with respect to each other were examined, the somewhat high molecular weights obtained with fractions on the ascending side may again reflect some contamination with LMWα. The average molecular weight of all fractions is approximately $7.4 \times 10^5 \pm 3$ per cent. While this value is somewhat higher than the previously reported $M_x$ value of $6.8 \pm 0.2 \times 10^5$ it should be noted that an $M_x$ value of $7.4 \times 10^5$ was also observed earlier. On the basis of the experimental results, the NSα appears to be reasonably homogeneous and on the low side of the total α-crystallin molecular weight range.

To ascertain the polypeptide-chain composition of this NSα, gel electrophoresis was performed. For SDS gel electrophoresis a pepsin marker was used in all experiments and standard polypeptide markers were run in parallel gels. The results (Fig. 2) demonstrate that the newly formed protein contains polypeptide chains with MW's of 19,500 and 22,500, corresponding to the α-crystallin A and B chains, respectively (Fig. 2, b). The profile of LMWα is added for reference (Fig. 2, a). Urea gel electrophoresis (Fig. 3) indicates that only when a relatively large amount of material was applied to the gel, could a Bi and Aβ peak be detected (Fig. 3, c). To examine the relative amounts of the subunits more critically, the NSα and typical LMWα from calf periphery were run simultaneously on 11 urea polyacrylamide gels, stained with Fast Green, and then scanned at 630 nm. All 11 gels gave similar profiles. The summation of the scans of these gels is shown in Fig. 4, again confirming the markedly lower Bi and Aβ content in the NSα. The relative
amounts of polypeptide in each peak are reported in Table II. The data indicate that approximately ⅔ as much B₁ and ⅓ as much A₁ are present in this NSα preparation as compared to LMWa. In some experiments slightly less B₁ and A₁ were observed. The ratio of B to A remains constant in all preparations.

Such observations suggest that B₁ and A₁ may arise rapidly in a post-translational process and may not be initially present in NSα. In order to evaluate this point, NSα obtained from the third pass through the agarose A-5m column (Fig. 1, a) was applied to 11 urea polyacrylamide gels. Following electrophoresis and staining, the gels were scanned and then sectioned. Since the scans of all 11 gels were essentially identical, comparable sections from all gels were pooled and prepared for counting. In Fig. 5, the composite scan and radioactivity profile are shown. Good separation of the four polypeptide chains was again obtained and essentially no radioactivity was observed in the slices representing B₁ and A₁, while considerable activity was associated with the A₂ and B₂.
peaks. It should be noted that the profiles for absorption and radioactivity coincided with each other. In Table III, the data obtained from this experiment are summarized. The total radioactivity detected in the B₁ peak is 22 counts per minute giving a specific activity of 29 counts per minute per A₆₃₀. This small value is less than 1/2 the specific activity observed for B₂, suggesting that there is essentially no incorporation into B₁. A similar conclusion can be drawn from the A-chain data where A₂ has a specific activity of 830 counts per minute per A₆₃₀ and A₁, a specific activity of 8 counts per minute per A₆₃₀. It is interesting to note that while the A₁ polypeptide represents 9 per cent of the total NS protein, only about 0.1 per cent of the radioactivity is present in this fraction. Such data suggest that while the process of converting A₂ to A₁ occurs at a significant rate, it is a post-translational process and thus although appreciable A₁ is present, it contains only a small amount of radioactivity.

It can be concluded from the data presented above that NSα contains essentially 2 polypeptide chains, A₂ and B₂, and that with time transformation of A₂ to A₁ and B₂ to B₁ occurs. The appearance of LMWA apparently requires the presence of B₁ and A₁ polypeptides. Analysis of LMW α-crystallin isolated from a number of sections of the periphery and nucleus indicates that such fractions have relatively constant ratios of B₁ to A₁ and A₂ to A₁.* Such results imply that the B₁ and A₁ components are formed rapidly and after attaining a given concentration do not increase further. No other changes can be observed in LMWA from calf lens periphery.

On the basis of such results, it was of

interest to examine the effect of aging upon the subunit profile of HMWa as well as LMWa. In Fig. 6, the results obtained with urea gel electrophoresis are shown. Figs. 6, A and 6, B illustrate characteristic profiles obtained with NSa and LMWa from calf periphery. The profile of LMWa from two-year-old steer nucleus shows little change, although a band which is infrequently observed between A2 and A1 in protein isolated from calf nucleus is now somewhat more distinct and consistent (Fig. 6, c). HMWa and LMWa from calf periphery have the same profiles (Figs. 6, b and d). However, unlike LMWa, the HMWa changes markedly with aging. HMWa from calf nucleus has two additional bands in the B region (Fig. 6, e). While the A1 band is normal, A1 now appears to have become widened and somewhat diffused in contrast to the band observed in the HMWa from calf periphery (Fig. 6, d). There are also a few minor components which appear to move more rapidly than A1. With the steer nucleus HMWa, this general picture becomes even more pronounced (Fig. 6, f). The A1 region appears to have split into a number of distinct components and pronounced bands with mobilities greater than A1 can be noted. A number of weaker bands are also present between B1 and A2 and a distinct band appears between B2 and B1.

In Fig. 7, the SDS gel electrophoresis patterns of these preparations are shown. A pepsin marker has been added as a reference. NSa and LMWa always show the typical two-band pattern with the slower moving component having a MW of 22,500 and representing the B-chains and the faster band having a MW of 19,500 corresponding to the A-chains (Figs. 7, a, b, and c). While the calf periphery HMWa (Fig. 7, d) shows essentially the same pattern as LMWa, calf nucleus HMWa (Fig. 7, e) shows two additional bands which become more pronounced in the steer nucleus (Fig. 6, f). These bands correspond to components with MW's of 17,000 and 13,000.
Fig. 7. SDS polyacrylamide gel electrophoresis patterns of α-crystallin (a) NSα; (b) LMWα-calf periphery; (c) LMWα-steer nucleus; (d) HMWα-calf periphery; (e) HMWα-calf nucleus; and (f) HMWα-steer nucleus. Running buffer, 0.1 M phosphate, pH 7.0, and 0.2 per cent SDS. The gels were stained with Coomassie Blue.

Examination of the amino-acid composition of NSα and LMWα from calf periphery and HMWα from steer nucleus indicates excellent agreement for almost all amino acids (Table IV). It is, therefore, unlikely that the changes in the subunit profiles can be accounted for by contaminating polypeptides.

Besides the marked difference in the molecular weight and polypeptide-chain profile which distinguished LMWα from steer nucleus HMWα, there is still another characteristic in which these preparations are dissimilar. The reactivity of the SH groups of some of the A-chains of the HMW protein appears to be considerably lower than their counterpart in LMWα. This lack of reactivity was observed during the development of methodology to isolate pure A- and B-chains.21 Utilizing the observation that only the A-chains contain SH groups, p-aminophenylmercuric acetate-Bio-Gel A-5m (APMA) was utilized to bind the SH-containing polypeptides of LMWα in 7 M urea. As shown in Fig. 8, one major peak was observed before the addition of mercaptoethanol and another after its addition. The first major peak was composed primarily of nonsulfhydryl-containing B-chains and the second major peak of A-chains. A very small first peak containing A-chains was also observed. When a similar fractionation was performed with HMWα, a considerably different profile was obtained. A marked loss in the A-chain Peak 3 fraction and a concomitant increase in Peak 1 was noted. Examination of Peaks 1 and 2 indicated that Peak 1 is primarily A-chain and Peak 2 primarily B-chain. In Fig. 9, the urea gels obtained with these fractions are shown. The unfraccionated HMWα is included for reference (Fig. 9, a). Fig. 9, b illustrates the results obtained with the first fraction of Peak 1. Only A-chains are apparent but succeeding fractions show increasing contamination with B-chains. It can also be seen (Fig. 9, c) that the second peak also contains considerable A-chain contamination. When the first fraction of Peak 1 was examined for SH content, it was found that on the basis of carboxymethylation with iodoacetic acid
followed by amino-acid analysis, this material contained approximately 1 SH per MW of 20,000. Such a result would be expected for A-chains. Rerunning these atypical A-chains through the APMA column results in no binding. Such results suggest that a large proportion of the A-chains of HMWα from steer lens nucleus has been masked and is no longer available.
The data reported in this communication suggest that NSα initially contains only two polypeptides, A2 and B2. All additional chains appear to arise as a result of posttranslational reactions. The appearance of altered polypeptides, particularly A1, occurs so rapidly that NSα lacking this polypeptide has not been observed. It is of interest in this regard to note that in epithelial cells only the A2 and B2 chains of α-crystallin could be detected.14,15 The content of A1 and B1, usually found in NSα indicates that A1 is produced much more rapidly than B1. This may be due to the twofold greater concentration of A1 in NSα. Approximately one-third of both A2 and B2 could react with an immobilized SH reagent. Another interesting point is that the new, most rapidly moving band found in HMWα urea gels appears to be associated primarily with the Peak 2 region rather than the atypical Peak 1 or Peak 3 zones.

SDS gel electrophoresis of the total Peak 1 as well as Peaks 2 and 3 from the APMA column appear to support the conclusion obtained with urea gels (Fig. 10). HMWα from steer nucleus was added as a reference (Fig. 10, a). Peak 1 (Fig. 10, b) shows primarily that A-chains with a MW of 22,500 are present. A component of MW 17,000 can also be distinctly observed. Peak 2 shows similar results except that the B-chains are now the major fraction (Fig. 10, c). It is of interest to note the presence in significant amounts of the 13,000 MW component. This component may be the most rapidly moving band found in the urea gel electrophoretic pattern of Peak 2. Peak 3 shows no B-chain macromolecules but a very distinct component with a MW of 17,000 as well as the usual A-chain band.

Discussion
The data reported in this communication suggest that NSα initially contains only two polypeptides, A2 and B2. All additional chains appear to arise as a result of posttranslational reactions. The appearance of altered polypeptides, particularly A1, occurs so rapidly that NSα lacking this polypeptide has not been observed. It is of interest in this regard to note that in epithelial cells only the A2 and B2 chains of α-crystallin could be detected.14,15 The content of A1 and B1, usually found in NSα indicates that A1 is produced much more rapidly than B1. This may be due to the twofold greater concentration of A1 in NSα. Approximately one-third of both A2 and B2...
and B₂ are converted to their altered form A₁ and B₁. It would appear that this transformation occurs after aggregation of the macromolecule. Dissociation of the α-crystallin aggregates, which contain a minimum of approximately 35 polypeptides, has not been observed under physiologic conditions. The transformation of the initially synthesized polypeptide chains to A₁ and B₁ not only occurs rapidly but appears to be limited to the outer cortex. No further transformation of this type can be observed in the inner cortex, the nucleus of three-month-old bovine lens or two-year-old steer lens. Why does the production of A₁ and B₁ cease in the inner regions of the lens? It is quite clear that α-crystallin produced in old lenses also undergoes this transition. Thus the conditions necessary to produce this transformation are still present in the outer cortex of older lenses. It is unlikely that the environment of the macromolecules of the outer cortex is sufficiently different from that of the inner cortex to explain the cessation of the transformation. It is conceivable that the architecture of the macromolecule is constructed in a manner which allows only one-third of the polypeptide chains to have regions accessible for transformation reactions. Since Bₓ and Aₓ have amino-acid compositions identical to their respective B₂ and A₂ counterparts²¹ the transformation probably involves modification of existing amino-acid residues. Recently, Bloemendal and co-workers²⁵ have suggested that a glutamine in A₂ is deamidated, forming A₁. The relatively similar change in the electrophoretic mobilities of B₁ and A₁, in comparison with B₂ and A₂, suggests that comparable reactions occur with both A₂ and B₂, possibly catalyzed by an enzyme.

NSα appears to be a reasonably homogeneous macromolecule. With the appearance of appreciable amounts of B₁ and A₁ considerable physical heterogeneity and an increase in apparent Mₜ occurs. This population of α-crystallin aggregates has been referred to as LMWa. It is possible that other factors may also influence the physical state of this population since, despite the constancy of its polypeptide chain population, some increase in molecular weight has been noted in our laboratory in fractions taken from the nuclear region of the lens. Similar observations have been reported by Van Kamp, Schats, and Hoenders.¹⁸ In this respect it is of interest to note that when LWMα is deaggregated with 7 M urea and then allowed to reaggregate by dialyzing out the dissociating material, smaller sized aggregates are observed.²⁶ Such results suggest that low molecular weight components may influence the aggregate state of the protein.

The situation with HMWα is somewhat different. This material isolated from calf periphery appears to have the same polypeptide-chain composition as LMWa, from which it probably arises, in spite of the more than 15-fold increase in molecular weight. It is interesting that the outer 70 per cent of the bovine lens does not show a significant increase in this component with aging.⁶ In the nuclear region the HMWα component increases dramatically with aging and marked changes in polypeptide electrophoretic polyacrylamide gel patterns are noted. Such observations indicate that the conversion to HMW occurring in the nuclear region may involve a different process from that noted in the periphery. The appearance of polypeptide chains with lower molecular weights than the A- and B-chains suggests that limited proteolysis may be involved in the conversion to some of the newly observed chains. Such transformations may also affect the molecular weight and heterogeneity of the protein.

Previous work has indicated that calcium may influence the aggregate state of α-crystallin.⁵,²⁷ Furthermore, reaggregation studies in the presence of calcium indicate that only the A-chains which appear to have masked SH groups are aggregated to high molecular-weight species.²⁷ Such an observation would suggest that calcium does not play a role in the formation of
HMWa in the lens periphery where the presence of atypical A-chains is insignificant. It has also been observed that glucose is associated with HMWa but not with LMWa.\textsuperscript{6}

These observations suggest that a variety of factors may influence the transformation with aging of \(\alpha\)-crystallin from a homogeneous relatively small macromolecule to the giant aggregates that may be the source of central senile cataracts. The overall scheme suggested by the data is shown in Fig. 11.

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REFERENCES