The ontogeny and specificity of human lens proteins

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The ontogeny and specificity of human lens proteins were studied by immunologic and starch gel electrophoretic methods. Nine antigens were detected in noncataractous lenses by immunoelectrophoresis, while 16 components were formed on starch gel analysis. Of the different antigens, only one was specific for man and anthropoid apes. This antigen which reflected a high degree of species specificity was first detected in the lenses of 6-month-old fetuses after the appearance of the other proteins. Starch gel studies showed that alpha and gamma crystallins are the predominant proteins in the earlier stages of lens development. Furthermore, at least two of these gamma crystallins are markedly decreased in senile cataractous lenses.

The protein composition of the adult human lens has been analyzed by many investigators. Immunologic studies have showed the presence of at least 5 to 8 antigens, one of which was found to be specific for man when compared with nonprimate mammals.1-3 Francois and Rabae,4 using agar microelectrophoresis at high tension, found at least 14 components in a neonatal lens, some of which were markedly decreased or absent in senile cataractous lenses. Changes in the protein composition of the human lens have also been reported by Tapaszto.5

The present study was directed toward analyzing the ontogeny and species specific properties of human lens proteins with the use of both immunologic and two dimensional zone electrophoretic methods. Comparisons were also made of the protein composition of normal adult and senile cataractous lenses.

Materials and methods

Antigens. Lenses were dissected from human fetuses obtained from Receiving Hospital, Detroit (Table I). Only lenses which showed no evidence of opacity were used. They were cleanly dissected from surrounding tissue and iris, and, with capsule intact, homogenized in 0.9 per cent NaCl, with 250 mg. wet weight tissue per milliliter. The total homogenate was centrifuged at 10,000 g and 4° C. for 20 minutes. Protein determinations were made on the supernatant with the method described by Lowry and co-workers.6 Noncataractous young adult lenses were obtained from the Wayne County Morgue, within 12 hours of death. Cataractous human lenses were collected immediately after operation from the Department of Ophthalmology, Receiving Hospital, Detroit.

The lenses of the following adult species were dissected immediately after sacrificing the animal. All procedures involving lens dissection and homogenization were carried out at a temperature of 4 to 10° C.

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Table I. Growth in length during the fetal period*

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<tr>
<th>Age (lunar months)</th>
<th>Crown-rump length (cm.)</th>
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Hominoidae (apes and man)
Man (Homo sapiens); adult 35 to 40 years, neonates, and 3 to 10 month fetuses
Chimpanzee (Pan troglodytes)
Gibbon (Hylobates lar)

Cercopithecoidae (old world monkeys)
Rhesus (Macaca mulatta)
Pig-tail (Macaca nemestrina)
Cynomolgous monkey (Macaca irus)
Green monkey (Cercopithecus aethiops)
Baboon (Papio)

Cebioidea (new world monkeys)
Squirrel monkey (Saimiri sciureus)
Marmoset (Leontocerus oedipus and L. nigricollis)
Spider monkey (Ateles geoffroyi)
Capuchin (Cebus)
Woolly (Lagothrix lagotricha)
Owl (Aotes trivirgatus)
Saki (Chiropotes satanas)

Prosimi
Lorisiformes
Slow loris (Nycticebus coucang)
Potto (Perodicticus potto)
Galago (Galago crassicaudatus and Galago senegalensis)

Tupaiformes
Tree shrew (Tupaia glis)
Order Insectivora
Elephant shrew (Petromus and Rhynchochyon)
Hedgehog (Erinaceus)

Order Carnivora
Cat (Felis cattus)
Dog (Canis familiaris)

Order Artiodactyla
Hog (Sus scrofa)
Beef (Bos taurus)

Order Rodentia
Ground squirrel (Citellus tridecemlineatus, tridecemlineatus)
Rat (Rattus norvegicus)
Mouse (Mus musculus)

Order Lagomorpha
Rabbit (Sylvilagus floridanus)

Antisera. Antisera were prepared in adult chinchilla rabbits against late human fetal lenses (7 to 10 months, 270 to 340 mm. crown-rump length) and will be referred to as AHL1 and AHL2. Antisera were also prepared against newborn and cataractous human lenses. A total of 6 antisera were used. The procedure of antibody production was as follows: 3 ml. of lens protein containing 25 mg. soluble protein per milliliter was emulsified with 2 c.c. of Freund's adjuvant and injected at five separate sites into each of several rabbits. Injections were repeated at intervals of 10 days, and the rabbits were bled when adequate antibody titers were obtained. Usually each rabbit was injected at least three times.

All antisera were tested against a variety of human nonlens tissues and serum. In a few instances, cross-reactions were obtained against human liver. The antisera were then rendered specific by repeated absorption with lyophilized human liver.

Aliquots of each antiserum were also absorbed with lyophilized hog or beef lens, until no cross-reaction was obtained with nonprimate lenses.

Immunologic methods. The technique of immuno-electrophoresis has been previously described. Double diffusion tests in agar after the method of Ouchterlony were also used. Twelve milliliters of 1.5 per cent agar prepared with 0.9 per cent NaCl was pipetted on glass plates, 5 by 4 inches in size. Wells were cut in the gel with a cork borer of 4 mm. diameter. Each well can hold 0.05 ml. of reagent.

Combined filter paper-starch gel electrophoresis. In this procedure, the lens proteins are first separated by paper electrophoresis and then by starch gel electrophoresis in a tris discontinuous buffer system. For the initial paper separation, 0.1 ml. of lens extract containing 3 to 8.5 mg. of protein is applied in a straight line across the width of a strip of Whatman 3MM filter paper (39 cm. long and 3.9 cm. wide). Electrophoresis is carried out for 16 hours in 0.2M sodium acetate buffer (0.0534M sodium barbital and 0.046M sodium acetate adjusted to pH 8.6 with acetic acid). After completion of the initial separation, a strip of the filter paper, 13.5 cm. long and 1.5 cm. wide (representing the length of the protein separation), is then cut out and placed in a slot cut at the one end of a starch gel block. The starch gel is made with 12.5 Gm. of Connaught hydrolyzed starch per 100 ml. of Sigma tris buffer (18.3 Gm. Sigma, 1.121 and 2.1 Gm. citric acid in 2 L. of distilled water). The electrode trays, boric buffer is used (5 Gm. sodium hydroxide and 57 Gm. boric acid in 2 L. of distilled water). The starch gel electrophoresis is at 6 volts per centimeter and lasts for 4 to 5 hours. It is terminated when the brown line, which forms during the electrophoresis in the tris dis-
continuous buffer system, has migrated 10 cm. beyond the slit in which the filter paper strip had been inserted. The starch gel is then bisected, stained, and decolorized.

Results

The immunoelectrophoretic patterns obtained when an antiserum to late fetal lens (AHLi) was tested against human lenses of different ages is shown in Fig. 1. Six precipitin bands were formed by lens extracts of 3 and 4-month-old fetuses (50 to 100 mm. crown-rump length), 7 by 5-month-old lenses (150 to 180 mm. crown-rump length), and 9 by lenses of 6 month and older fetuses (200 to 340 mm. crown-rump length) as well as noncataractous adult lenses. A significant feature of the 3 month fetal lens pattern is the prominence

![Fig. 1. Immunoelectrophoretic patterns of fetal lens tested with antiserum AHLi prepared against 7 to 10-month-old fetal lenses. a, 3 to 4 month fetal lens extract; b, 5 month lens extract; and c, 6 month lens extract. Precipitin arc 1 was formed only by the lens of 6-month-old fetuses. Lens preparations were made on a basis of wet weight (all 250 mg. per milliliter).](image)

![Fig. 2. Precipitin reaction of antiserum AHLi absorbed with 4 to 5 month fetal lenses and tested against 6 month fetal lens. The antiserum placed in the wells between a and b and b and c has not yet been completely absorbed. The antiserum, however, placed in the well below c, forms only one precipitin reaction (arc 1). This antiserum did not cross-react with the lens of 5 month fetuses or nonprimate mammals.](image)
of the most cathodal precipitin bands (representing gamma crystallins). Precipitin band 1, which is placed further toward the anode than the two alpha crystallin bands, was first detected in lenses of 6 month and older fetuses, but not seen in

![Fig. 3](image)

Fig. 3. The precipitin pattern formed by lens antiserum (AHLs) absorbed with lyophilized 5 month fetal lens and tested against various tissue extracts of a 9-month-old fetus. Li, human liver; Le, lens; Ki, kidney; Br, brain. Protein concentrations of all extracts adjusted to 25 mg. soluble protein per milliliter.

the patterns obtained with younger lenses. The alpha crystallin bands were identified by eluting the protein from the appropriate spots of a two dimensional starch gel analysis of adult lens, and subjecting the eluent to electrophoresis. The precipitin bands were identical in position to those indicated in Fig. 1. Furthermore, they gave precipitin reactions which merged with the alpha crystallin bands of the total extract when tested according to the technique of Wadsworth and Hanson. Antiserum (AHLs), after absorption with the lens of 4 and 5-month-old fetuses, formed only precipitin band 1, when tested against the lenses of 6 month or older fetuses (Fig. 2). Normal adult as well as cataractous lenses gave this reaction, but not any fetal lens younger than 6 months. This absorbed antiserum did not react against extracts of nonlens tissues prepared from 9-month-old fetuses or adult man (Fig. 3).

All anthropoid apes formed precipitin arc 1 when tested against the unabsorbed or absorbed antiserum (AHLs) (Fig. 4). No reaction, however, was obtained with nonprimate mammals, while an equivocal band was found with prosimian lenses.

![Fig. 4](image)

Fig. 4. Immunoelectrophoretic patterns of squirrel monkey (a), rhesus (b), and beef lens (c) tested with AHLs. Precipitin arc 1 was formed only with the monkey lenses. All preparations were made with 250 mg. wet weight of tissue per milliliter of saline.
After antiserum AHL was absorbed with beef or hog lens, only one precipitin arc was formed against 6 month or older human lens. This reaction was located in the same position and was identical immunologically to precipitin arc 1. Of the 6 different human lens antisera (including one prepared against human cataract), 4 detected the antigen corresponding to precipitin arc 1.

Antiserum AHLi, after absorption with beef lens, was tested against various primate lenses with the double diffusion technique in agar (Fig. 5). Only one precipitin band was formed. Late human fetal, normal adult, and cataractous lenses, as well as chimpanzee lens extracts, formed reactions of identity, but each gave faint spurs against gibbon lens. All hominoids (human, chimpanzee, and gibbon) formed spurs against old world monkeys (cercopithecoids), and the latter gave spurs against most new world monkeys (ceboïds). With different preparations of owl monkey lens, some gave reactions of identity with old world monkey lens, while others showed partial identity. The reactions of prosimian lenses (potto, loris, galago, and tarsius) were faintly detectable or equivocal. No reaction was obtained with tree shrew and nonprimate mammalian lenses. Of the various antisera which detected precipitin

Fig. 5. Precipitin reactions of antihuman lens serum (AHLi). Protein concentrations at 40 mg. per milliliter. The spur formed by human lens against the gibbon is too faint to be seen in the photograph. h, human; c, chimpanzee; gi, gibbon; r, rhesus; s, squirrel monkey; o, owl monkey.

Fig. 6. Immuno-electrophoretic pattern of senile cataractous lens (a) and normal adult lens (b) tested with antiserum AHL. Both preparations were made with 250 mg. wet weight of tissue per milliliter of saline. The arrow indicates the precipitin reactions which appear to be lacking in the cataractous lens. The dotted line indicates precipitin arc 1, which is not clearly seen in the photograph.
band 1, 2 gave reactions as described above, while 2 antisera did not distinguish the reactions of the different Anthropoidea.

The precipitin reactions of normal and cataractous adult human lenses tested against antiserum AHL₂ are shown in Fig. 6. (The most anodal migrating precipitin band 1 is too faint to be seen in this photograph and is indicated by the dotted arc.) The cataractous lens lacks 2 gamma crystallin precipitin reactions which are present in the normal lens (indicated by arrow). When antiserum AHL₂ was absorbed with cataractous lens, and tested

**Fig. 7.** Immunoelectrophoretic pattern of senile cataractous (a) and normal adult lens (b) tested with antiserum AHL₅, absorbed with cataractous lens. No reaction was formed against the cataractous lens, but precipitin bands were formed with the normal lens. Both preparations were made with 250 mg. wet weight of tissue per milliliter of saline.

**Fig. 8.** Starch gel patterns of fetal lens extracts. The horizontal arrow indicates the direction of the original separation in paper. The vertical arrow indicates the direction of separation in starch gel. All preparations were made with 250 mg. wet weight of lens per milliliter of saline. The total protein applied for the different preparations (in increasing age) was 3.2, 5.6, 6.3, and 7.1 mg., respectively.
against normal lens, 2 precipitin reactions were formed in the region corresponding to antigens of beta and gamma crystallin mobility. The absorbed antiserum did not react with the cataractous lens (Fig. 7).

The ontogeny of human lens proteins as reflected by zone electrophoresis with paper followed by starch gel analysis is shown in Figs. 7 and 8. In 4 to 5-month-old lenses, a total of five components were detected, mainly in the position of alpha and gamma crystallins. In the newborn lens at least thirteen proteins are recognized, two alpha crystallins, five beta, and four gamma crystallins. In the lens of 5 to 6-month-old fetuses, a slow migrating gamma crystallin spot 1 is prominent, while only one alpha crystallin is present. The second alpha crystallin spot is seen as a faint staining reaction in the lens of 7 to 8 month fetuses, but clearly evident in the newborn lens pattern. In the lens pattern of 5 to 6 month fetuses, gamma and alpha crystallins are readily seen, while beta crystallin proteins are now more clearly recognizable.

Comparing the fetal, newborn, and adult lens patterns, it can be seen that component 3 is first clearly defined in the adult lens. This protein is specific for primate lenses.

The starch gel pattern of cataractous lens is deficient in gamma crystallin component 1, while component 2 is present in a decreased amount. These are the proteins which are prominent in fetal life.

Discussion

The data recorded in this study show that the human lens contains at least 9 antigens, of which only one is species specific and present in mammal and cata-

![Fig. 9. Starch gel patterns of infant (7 months post natal), normal adult (35 years), and senile cataractous lenses (cat). Component 1 of the normal lens is not detectable in the cataractous pattern, while component 2 is markedly reduced in concentration. All preparations were made with 250 mg. lens per milliliter of saline. The total amount of protein applied for infant lens was 7.5 mg., that of the normal adult lens was 8.2 mg., that of the cataract lens (lower left) 6.0 mg., and for the cataract lens (lower right) 5.0 mg.]
ractous lenses. This antigen is highly negatively charged and migrates further toward the anode at pH 8.6 than the major alpha crystallin proteins. Halbert and Manski similarly detected an antigen specific for human cataractous and rhesus lens, when compared to nonprimate mammalian lenses. This protein, however, remained close to the antigen well in the region of alpha crystallin reactions.

The immunoelectrophoretic studies of Witmer and Buehler, employing antacataract human serum, however, showed that the human specific lens antigen migrated considerably further to the anode than the other lens proteins. Using protamine sulfate precipitation, these authors obtained evidence which suggests that the species specific antigen belongs to the alpha crystallin group of proteins. Another slower migrating antigen was considered to be the organ specific alpha crystallin.

The studies of Heitz similarly showed that the beef lens species specific antigen had greater mobility than the major alpha crystallin protein. It is evident, however, from the precipitin pattern of the 3 month lens (Fig. 1A) and from starch gel studies, that alpha crystallin itself consists of two proteins, while the species specific antigen is a more rapidly migrating antigen. The final characterization of this antigen must await its isolation in pure form and a determination of its molecular size and other properties.

Considering the ontogeny of human lens proteins, it is of interest to compare the data obtained with immunologic and starch gel analyses. Both methods showed that alpha and gamma crystallins appear to be the predominant proteins at early stages of development between 4 to 6 months of fetal life. Although beta crystallins are present at this stage, they increase considerably in relative concentration at later stages of fetal life. It is also striking that, although only 9 antigens were found in the human lens, as many as 16 are present in the starch gel pattern of newborn and adult lenses, respectively. It is possible that some of the latter proteins represent enzymes or other components not specific for the lens, or that they result from protein interactions during the process of electrophoresis.

The species specific human lens antigen was first detected at a late stage of lens formation in 6 month or older fetuses after the appearance of the other proteins. Halbert and co-workers also reported on the first appearance of a new lens antigen in rabbits 10 days post partum. The phenomenon of a more species specific protein appearing late in ontogeny is also reflected in studies on serum gamma globulin. The phylogenetic relationships which could be deduced from this protein are in line with observations previously recorded for some of the serum proteins. Thus, man and chimpanzee showed a closer relationship to each other than to the old world monkeys, which in turn gave better reactions than the new world monkeys.

Immunologic comparisons of normal and mature cataractous lenses showed a deficiency in the latter lenses of at least two of the slower migrating proteins, probably representing beta and gamma crystallins. Indeed, starch gel analyses clearly showed that the mature cataractous lens lacks, or has decreased amounts of, at least two proteins which have the mobility of gamma crystallin. Francois and Rabaey, using agar microelectrophoresis at high tension, similarly reported that there was a marked deficiency of proteins of low mobility in the cataractous lens. On the other hand, alpha crystallin was always present in relatively high concentration, although the amount was decreased when compared to normal lenses. Tapaszto, however, reported that in hypermature cataractous lenses the amount of slowly migrating lens proteins is on the average 20 per cent higher than that in the normal lens. However, since this author analyzed the homogenate of cataractous lenses (not the supernatant), it is difficult to compare these data with the findings of Francois and Rabaey, or the results of our investigation. Indeed Tapaszto refers to the increased
amount of slow-moving protein as semi-soluble albuminoid, a fraction not usually detected when lens extracts are analyzed, since it is removed during centrifugation. Furthermore, it is well known that the albuminoid fraction increases considerably during the cataractous process.

The prominence of gamma crystallins 1 and 2 in early stages of development, and their decrease in the cataractous process, suggests that they correspond to the embryonic protein, as described by Francois and Rabaey. Such a protein is synthesized almost entirely in embryonic life, and in the adult lens it is found mainly in the nucleus. The electrophoretic position of these human lens proteins is similar to that found for embryonic proteins in the nucleus of the galago and beef lenses. The marked decrease of these proteins in the cataract is probably caused by the fact that the pathologic process mainly affects the nucleus.

REFERENCES


Erratum

The title of the article by Drs. Friedman, Kopald, and Smith in the October, 1964, issue of this JOURNAL should have been, "Retinal and choroidal blood flow determined with krypton-85 in anesthetized animals."