of adequate compensatory sympathetic activity, induce further progressive increases in manifest myopia. In this respect if a longitudinal study were to show a correlation between refractive correction of young myopes and associated changes in TA levels, then the control of simple myopia with topical beta adrenergceptor drugs becomes an interesting possibility. It is proposed that by further defining the autonomic innervation characteristics of tonic accommodation, this paper provides a useful model for testing the above hypothesis.

**Key words:** tonic accommodation, laser optometer, ciliary muscle innervation, Isoprenaline, Tropicamide

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The Comparison of Human Lens Crystallins Using Three Monoclonal Antibodies

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Three monoclonal antibodies against lens crystallin have been used to study the accumulation of specific polypeptides during development of the human lens. One antibody which recognizes an antigen common to three polypeptides with molecular weights close to 31,000 reacted equally well to the human lens cortex and nucleus and had a similar binding activity to proteins isolated from embryonic and older human lenses. This suggests that the antigen accumulates to the same degree in human lenses during development. The second monoclonal antibody to the 24,000 molecular weight gamma-crystallin showed increased binding at the older ages indicating the increased accumulation of this protein during lens development. The third monoclonal antibody to the 27,000 molecular weight beta-crystallin showed increased binding at the older ages indicating the increased accumulation of this protein during lens development. The third monoclonal antibody to a beta-crystallin of 27,000 molecular weight showed little if any reactivity at the embryonic age and revealed a clear difference between the binding with cortical and nuclear protein at older ages. It appears that the 27,000 molecular weight beta-crystallin may not be synthesized in the embryonic human lens. Invest Ophthalmol Vis Sci 26:1028–1031, 1985

One of the areas of active investigation in human lens research has been the determination of the distribution of the crystallins in the normal and cataractous lens.1–4 The lens undergoes alterations in some of the crystallins during differentiation and aging. These alterations are important in understanding the differences between normal aging and the process of cataract formation. Recently the low molecular weight crystallins have been characterized. A gamma-crystallin with a molecular weight of 21,000 has been described which is the main gamma-crystallin in the young lens, but which is only a minor component in the older lens. In contrast, a gamma-crystallin having a molecular weight of 24,000 (γ24) becomes the main constituent in the older lenses.5,6 γ24, measured using a monoclonal antibody D4C, represents only about 3% of the total soluble protein in the fetal lens but is about 25% in the adult lens.6
A monoclonal antibody (BC7) to a β-crystallin (β27) of 27,000 molecular weight has been described. This antibody has been shown to cross-react with human, rat, and mouse lens soluble protein. This polypeptide appears to be developmentally regulated since it is absent in embryonic mouse and rat lenses. A third monoclonal antibody (AE4) that was made against human beta-crystallin reacts to a human antigen shared by three polypeptides with molecular weights around 31,000.

By using the three monoclonal antibodies D4C, BC7, and AE4, we have observed differences in the developmental pattern of three human lens antigens. We present data on the binding activity of each antibody to embryonic and adult human lenses. In addition, we compare the distribution of these antigens in the cortex and nucleus of human lenses.

Materials and Methods. Sixteen-week-old embryonic, 2-month-old, 20-year-old, and 72-year-old normal human lenses were obtained from the National Diabetes Research Interchange. The lenses were frozen on dry ice and the nucleus was dissected from the cortical material. For the embryonic and 2-month-old lenses, the separated fractions of nucleus to cortex had a wet weight ratio of 1:1; whereas in the 20-year-old and 72-year-old lenses, the ratio was 1:2. The lenses were homogenized and centrifuged exactly as described previously. Western blotting and immunoblot were performed on adult human lens homogenates using SDS polyacrylamide gel electrophoresis to check the reactivity of the AE4 and BC7 monoclonal antibodies. For the D4C antibody, G-75 gel exclusion chromatography was used to separate the three major gamma-crytallins from the human lens. These proteins were run on a polyacrylamide gel under nondenaturing conditions using the Laemmli method but without SDS. A western blot and an immunoblot were then performed using the D4C antibody.

All hybridoma cell lines were maintained in Dulbecco's MEM medium with 10% fetal bovine serum. Enzyme linked immunosorbent assays (ELISA) were performed using a direct binding method. Fifty microliters of the soluble lens protein was coated in each well of a microtiter plate (Dynatech #220-25, Dynatech Labs, Alexandria, VA). The homogenates were diluted in phosphate buffered saline to give a working range of 0.1 μg/ml to 0.5 mg/ml protein. The plates were washed with 1% bovine serum albumin in phosphate-buffered saline and then incubated with 50 μl of the appropriate undiluted tissue culture supernatant (containing the antibody) for 18 hr. A sheep antirat IgG linked to β-galactosidase was added after washing the plate. One hour later, o-nitrophenyl β-galactopyranoside was added and the amount of product in each well was quantitated spectrophotometrically at 414 nm with a reference wave length of 590 nm. Controls for nonspecific binding of the monoclonal antibody, for nonspecific binding of the second antibody, and for nonspecific reaction of the antibodies with bovine serum albumin were done and all values were adjusted for these controls. The maximum absorbance values obtained for each antibody were assigned a value of 100% binding for the ELISA system in order to compare the samples from different ages. All data points were done in triplicate.

Results. The specificity of the three monoclonal antibodies is illustrated in Figure 1. The AE4 antibody was made against human β2 crystallins and reacted with an antigen only shared by three crystallin polypeptides with molecular weights around 31,000. The D4C antibody was made against human gamma-crystallin. D4C antibody reacted with the γ24 protein fraction only and did not cross react with alpha- or beta-crystallins. The BC7 antibody reacted only with a polypeptide at 27,000 molecular weight. The reactive polypeptides for each of these monoclonals were different and distinct from one another.
weight polypeptides accumulate in the human lens at about the same rate during the first 20 years of life. Thus, these polypeptides comprise about the same percent of the total soluble protein from the fetal lens and from older lenses.

The \( \gamma_24 \) antigen can be isolated from other proteins in the lens using gel exclusion chromatography. A competitive inhibition ELISA can thus be used to quantitate the amount of \( \gamma_24 \) in the lens. Consistent with the quantitation data, the direct binding assay using D4C antibody showed considerably more binding at older ages than at the fetal age. \( \gamma_24 \) antigen recognized by the D4C antibody also does not show a difference in cortical or nuclear accumulation but is more concentrated in the older lens than in the fetal one. This suggests that the \( \gamma_24 \) protein is present at embryonic ages and becomes a larger percentage of the total soluble protein in the lens during aging.

The BC7 antibody gave clear differences between the cortex and the nucleus as well as differences in the development of the lens. Little, if any, immunoreactive \( \beta_{27} \) polypeptide is present in the fetal lens. This correlates well with data from the mouse and rat. In the older lenses, a difference can be measured between nuclear and cortical protein suggesting the polypeptide accumulates after embryogenesis of the lens. Thus, the \( \beta_{27} \) polypeptide may not be synthesized by the embryonic human lens but may only be important to the older lens especially in the cortical areas.

Monoclonal antibodies are powerful tools to study lens crystallins. Use of these monoclonal antibodies provides an opportunity to examine human lens development more closely. Although this report represents only an initial comparison of these monoclonal antibodies, these antibodies should be of assistance in trying to understand the interactions of the proteins and polypeptides in the young lens and the changes in these interactions during the development of the lens.

**Key words:** human lens, monoclonal antibodies, lens crystallins, \( \beta \)-crystallins, low molecular weight proteins, Elisa, \( \gamma \)-crystallin

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