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Dynamic Light Scattering of Diabetic Vitreopathy

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ABSTRACT

Background: Diabetes induces pathology throughout the body via nonenzymatic glycation of proteins. Vitreous, which is replete with type II collagen, undergoes significant changes in diabetes. The resultant diabetic vitreopathy plays an important role in diabetic retinopathy. Detecting these molecular changes could provide insight into diabetic ...

... geneity and larger particle sizes in vi... subjects with diabetes as compared to age-matched controls.

Conclusions: DLS can detect and quantify the early molecular effects that cause vit... lagen fibrils to cross-link and aggregate. This could provide valuable insight into o... systemic effects of hyperglycemia, because the molecular changes in diabetic vi... could serve as an index of such effects throughout the body. In addition to the diag... plications, this methodology could provide a rapid, reproducible way to monitor the... to therapy with novel agents intended to prevent the complications of diabetes on ... lar level.

INTRODUCTION

THE EFFECTS OF DIABETES ON THE RETINA, called diabetic retinopathy, result in the leading cause of blindness in Americans between 20 and 74 years of age.[1] Diabetic retinopathy is characterized by pathological changes in the retinal vasculature that interfere wit... mal function of the retinal cells. Alth... siderable progress has been made in f... our understanding of the mecha... which hyperglycemia alters the ret... has been little progress in our abilit... nose this condition during early ...

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fact, there is a prevailing concept that until the retina has microhemorrhages, microaneurysms, and other ophthalmoscopically visible manifestations of diabetes effects, patients with diabetes do not have diabetic retinopathy. It must be appreciated, however, that once these abnormalities are present in the retina, the disease has attained a level of histopathology. This stage is preceded by a variably protracted period of physiopathology that we are currently unable to adequately evaluate in a clinical setting. This limitation greatly hampers attempts to develop new ways to prevent vision loss from advancing diabetic retinopathy. Thus, future progress depends on new approaches and methodologies that can detect changes induced by diabetes during the early period of physiopathology, prior to the onset of histopathology.

Although vitreous is the largest structure within the eye, comprising some 80% of ocular volume, our knowledge of its structure is perhaps the least of all ocular tissues. Historically, investigations of vitreous structure have been hampered by the fundamental difficulty that any attempts to define vitreous morphology are in point of fact attempts to "visualize" a tissue that is invisible by design (Fig. 1).[2] Composed primarily (98%) of water, vitreous is a viscoelastic gel as a result of its major structural components: collagen (primarily type II, but also type IX and a hybrid of types V and XI) and the glycosaminoglycans hyaluronan (HA). An understanding of the pathological changes in vitreous structure and function induced by any disease requires information about the molecular effects of the disease on these two critical molecules.[3] For example, consider that in aging, there is dissociation of collagen from the previously intercalated HA molecules and resultant cross-linking of collagen fibrils into larger and larger bundles of collagen fibrils that ultimately attain visible proportions as fibers (Fig. 2). These events likely play an important role in age-related vitreous degeneration (ARVD), which contributes to posterior vitreous detachment (PVD).[2] Anomalous PVD is an important cause of retinal tears and detachments in many patients.[2,3] Investigations are currently underway to elucidate the nature of normal vitreous collagen and HA interaction and determine how this changes with aging.[3] In time, a better understanding of this phenomenon will result, enabling the development of methods by which to therapeutically or preventatively intervene.

FIG. 1. Vitreous from a 9-month-old child (courtesy of the New England Eye Bank, Boston, MA). The sclera, choroid, and retina were dissected off the vitreous that remains attached to the anterior segment of the eye. In youth, the vitreous is a solid gel, whose function is to fill the center of the eye with a clear viscoelastic structure that inhibits cell migration and proliferation. This insures minimal light scattering and the unhindered transmission of light to the retina for photoreception. (Reprinted from Sebag J: The Vitreous—Structure, Function, and Pathobiology. Springer-Verlag, New York, 1989.)

Elevated levels of glucose affect tissues throughout the body by altering protein structure and function through the phenomenon of nonenzymatic glycation.[4] One of the most ubiquitous and important proteins altered by this process is collagen. Hyperglycemic effects on collagen underlie the basement membrane pathology in blood vessels throughout the body, including the retina. At present there are no methods by which to evaluate glycation effects on collagen in the retinal vasculature. An alternative approach that could provide insight into this process would be to evaluate glycation effects upon collagen in another ocular tissue, eg, vitreous. In recent years, vitreous has come to be recognized as an important contributor to advanced diabetic retinopathy. In fact, one investigator has proposed the use of

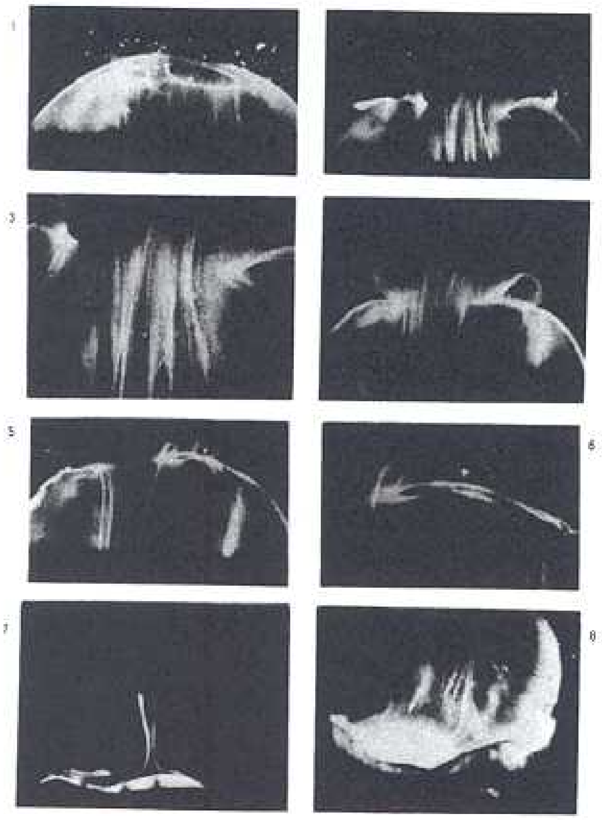

FIG. 2. Vitreous structure in the adult human. Dark-field slit microscopy of dissected vitreous from middle-aged adults demonstrates a prominent fibrous structure. The visible fibers result from aggregation of collagen fibrils that are no longer separated by hyaluronan molecules. (Reprinted with permission from Sebag J: Age-related changes in human vitreous structure. Graef Arch Clin Exp Ophthalmol 225:89–93, 1987.; Sebag J, Balazs EA: Morphology and ultrastructure of human vitreous fibers. Invest Ophthalmol Vis Sci 30:1867–1871, 1989.)

the term proliferative diabetic vitreo-retinopathy to refer to the advanced stages of diabetic retinopathy, and has even developed a classification system to grade these advanced stages.[5] Recently it was discovered[6] that diabetes has structural effects on vitreous that can precede its effects on the retina. The term diabetic vitreopathy has been coined to refer to this phenomenon.[7]

Studies have determined that there are elevated levels of glucose in the vitreous of diabetic patients.[8] Not unexpectedly, other studies[9] detected nonenzymatic glycation of human vitreous collagen in diabetes. It is believed that these effects underlie the structural abnormalities in diabetic vitreopathy,[6,7] similar to the effects of hyperglycemia on collagen elsewhere in the body.[4] Interestingly, these alterations appear to be quite similar to those observed in vitreous during aging,[3,6,10] consistent with the concept that diabetes induces accelerated aging in target tissues and organs.[4] In particular, the glycation of vitreous collagen appears to result in cross-linking and aggregation of adjacent collagen fibrils that is virtually indistinguishable from age-related vitreous degeneration (Figs. 2 and 3). Methodologies, such as Fourier-transform Raman spectroscopy,[11] are being tested as a means to detect and quantify the early molecular effects of hyperglycemia on vitreous and other structures in the eye as well as elsewhere in the body. Considering that the aforementioned effects of diabetes on vitreous collagen induce cross-linking and aggregation of fibrils into larger than normal fibers (Fig. 3), it has been of interest to apply the methodology of dynamic light scattering (DLS) to assess the particle sizes found in diabetic vitreous and compare these to nondiabetic controls.

METHODOLOGY

Dynamic Light Scattering (DLS)

DLS is a well-known laboratory technique that is frequently used to measure average size or size distribution of microscopic particles (3 nm–3 μm in diameter) suspended in a fluid medium. New-generation DLS instrumentation, developed at NASA to conduct fluid physics experiments on-board the space shuttle and space station orbiters, is used in this study. These have been extensively described elsewhere[12–14] and therefore the methodology will only be summarized. The input beam from a semiconduction laser (670-nm wavelength) at 50 μW power was projected into the eye and the scattered signal was collected by the DLS probe for a duration of 10 seconds. The signal was then detected by an avalanche photodiode detector system (EG&G Canada, Model PCS2). A time correlation function (TCF; see Fig. 4) was constructed using a digital correlator card (Brookhaven Instrument, BI9000). The slope of the TCF provides a measure of particle size in the vitreous.[15,16]

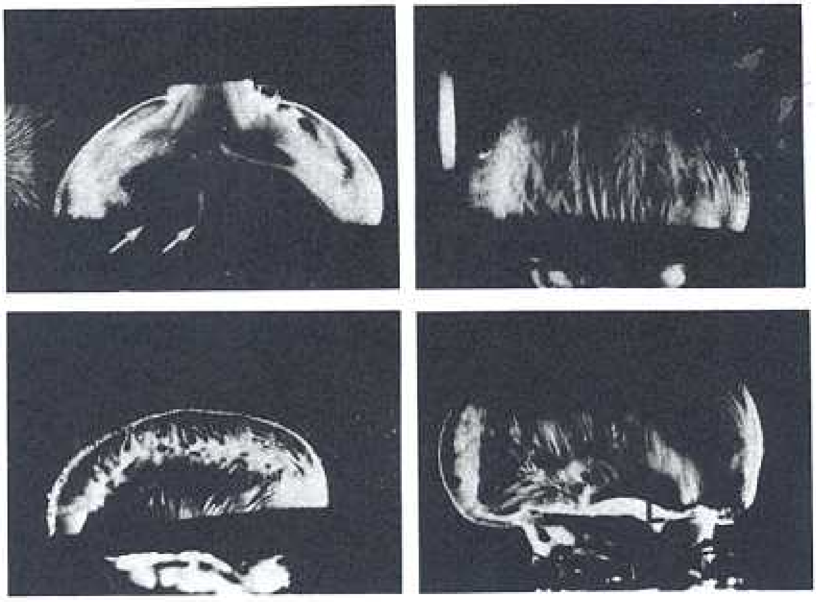

FIG. 3. Vitreous structure in a child with diabetes. Dark-field slit microscopy of dissected eyes from a 9-year-old girl with a 5 year history of type I diabetes and no clinical evidence of diabetic retinopathy demonstrates a fibrous structure that is indistinguishable from age-related vitreous degeneration. (Reprinted with permission from Sebag J: Abnormalities of human vitreous structure in diabetes. Graef Arch Clin Exp Ophthalmol 231:257–260, 1993.)

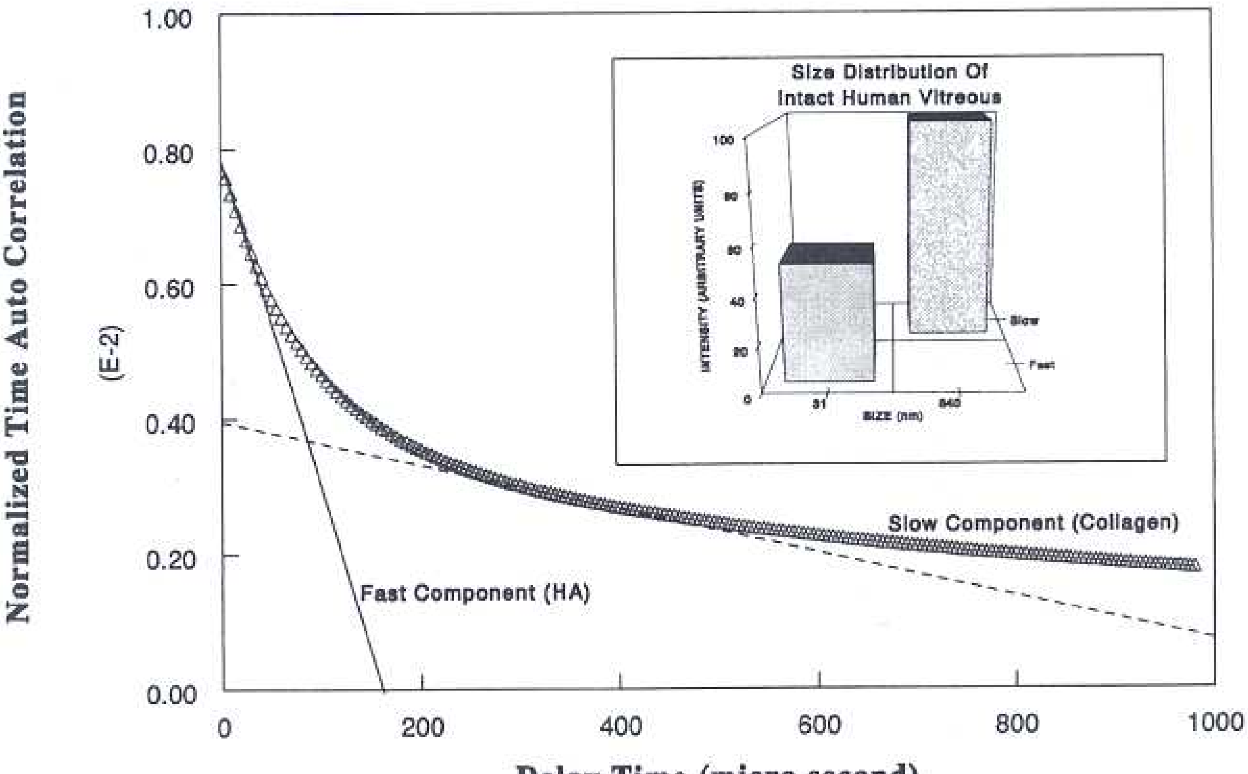

FIG. 4. Dynamic Light Scattering of Vitreous. The correlation function in this graph demonstrates the two components that make up the DLS curve. In vitreous, the "slow" component (open triangles) primarily arises from collagen. Tangent lines determined by an algorithm that fits the curve using a double exponential model indicate the different values corresponding to the fast (left end of the abscissa) and slow (right end of abscissa) components of the DLS curve. The data can be used to determine the distribution of particle sizes, represented as the bar graph in the inset to the upper right, where the left bar is collagen and the bar to the right is the particle size distribution determined from the "fast" component of the time relaxation curve, arising primarily from hyaluronan.

Vitreous specimens

Specimens for DLS consisted of 2 pairs of human eyes obtained at autopsy and studies were performed within 36 hours of death. One donor was a 70-year-old woman with no diabetes while the other was a 72-year-old woman with type 2 diabetes of unknown duration. At room temperature, the sclera, choroid, and retina were dissected off the vitreous in 2 different manners. For DLS measurements across the anterior vitreous, a "window" approximately 3 × 3 mm in size was dissected at the level of the equator. This enabled the DLS laser to scan across the anterior and central vitreous. In preparations for axial DLS measurements the cornea, iris, and lens were removed, leaving the posterior lens capsule intact.

Experimental setup

In eyes prepared by dissecting an equatorial window, DLS measurements were made at multiple points 0.5 mm apart, spanning the equatorial vitreous from one side to the other. DLS measurements along the optical (anteroposterior) axis were similarly made at 0.5-mm intervals, beginning at the posterior lens capsule back to the posterior vitreous cortex. The vitreous was scanned using a DLS probe in conjunction with a micropositioning assembly as shown in Figure 5. The translation stages (Newport, model 423) with motorized actuators (Newport, model 850B) control motion in the X, Y, and Z directions. A motion controller (Newport, model MM1000DC) receives a series of commands from the Pentium processor based computer/correlator (EPS, 7600 series) via RS232 cable and drives the actuators for precise positioning of the probe. The correlator software (Brookhaven Instrument, BI9000) is modified to achieve online control of the probe position without leaving the program environment.

DLS measurement of artificial vitreous solutions at room temperature (not presented) showed no deterioration in the results over a 24-hour period of time.

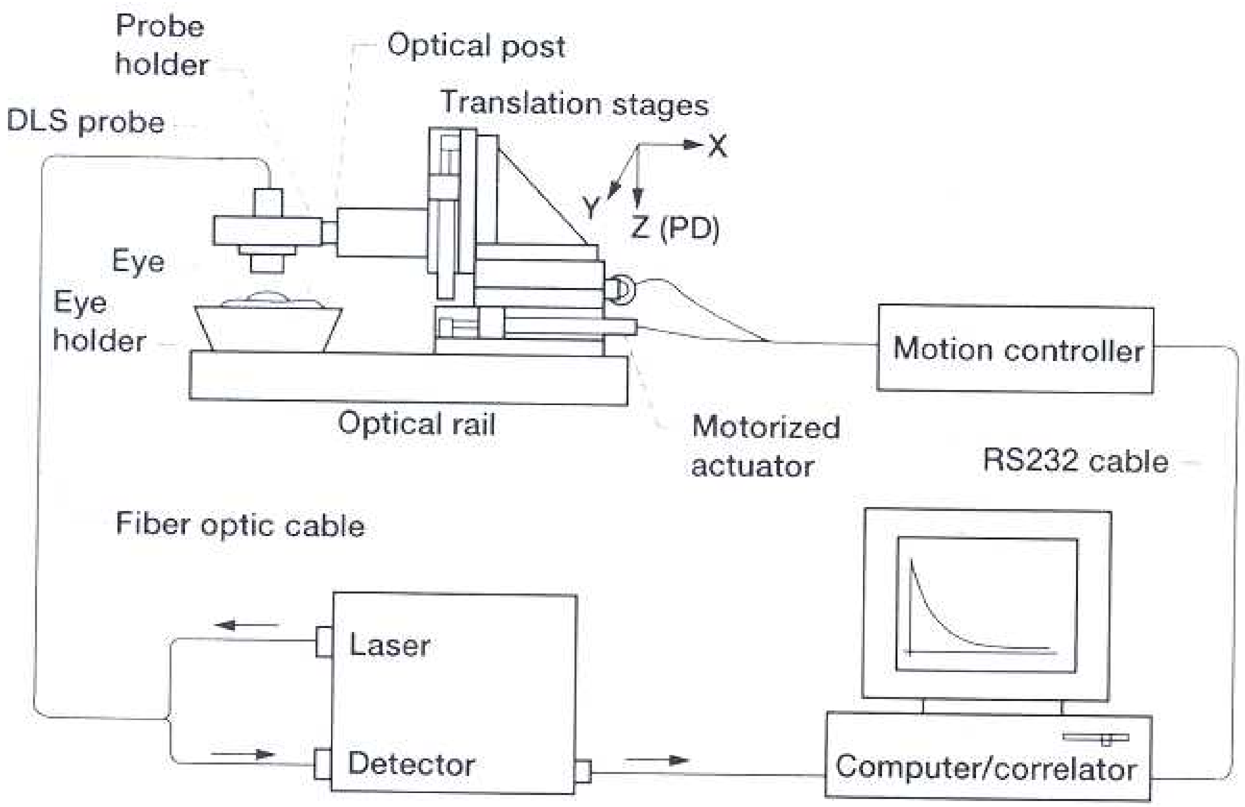

FIG. 5. Experimental Setup for DLS of Human Vitreous. Vitreous was scanned using a DLS probe in conjunction with a micropositioning assembly as described in the methodology. The slope of the TCF provides a measure of particle size in the vitreous.

Dark-field slit microscopy

The dissection of the sclera, choroid, and retina off the entire vitreous that is left attached to the anterior segment and illuminated from the side with a slit-lamp beam creates a horizontal optical section through the vitreous. This has been previously described extensively.[3,6,10]

RESULTS

DLS measurements of particle size distribution demonstrated that there are 2 groups of molecular species in vitreous, one underlying the "fast" component of the correlation function and the other accounting for the "slow" component (Fig. 4). The slow component in the DLS measurements from specimens of nondiabetic subjects with equatorial windows showed a fairly consistent particle size determination across the anterior vitreous (Fig. 5). However, in vitreous from patients with diabetes, there was a different pattern of DLS results. The central vitreous had evidence of heterogeneity in particle sizes with generally larger particle sizes within the vitreous from diabetic subjects as compared to nondiabetic controls (Fig. 5). DLS measurements along the optical (anteroposterior) axis similarly showed larger particle sizes in the vitreous from diabetic subjects as compared to age-matched controls.

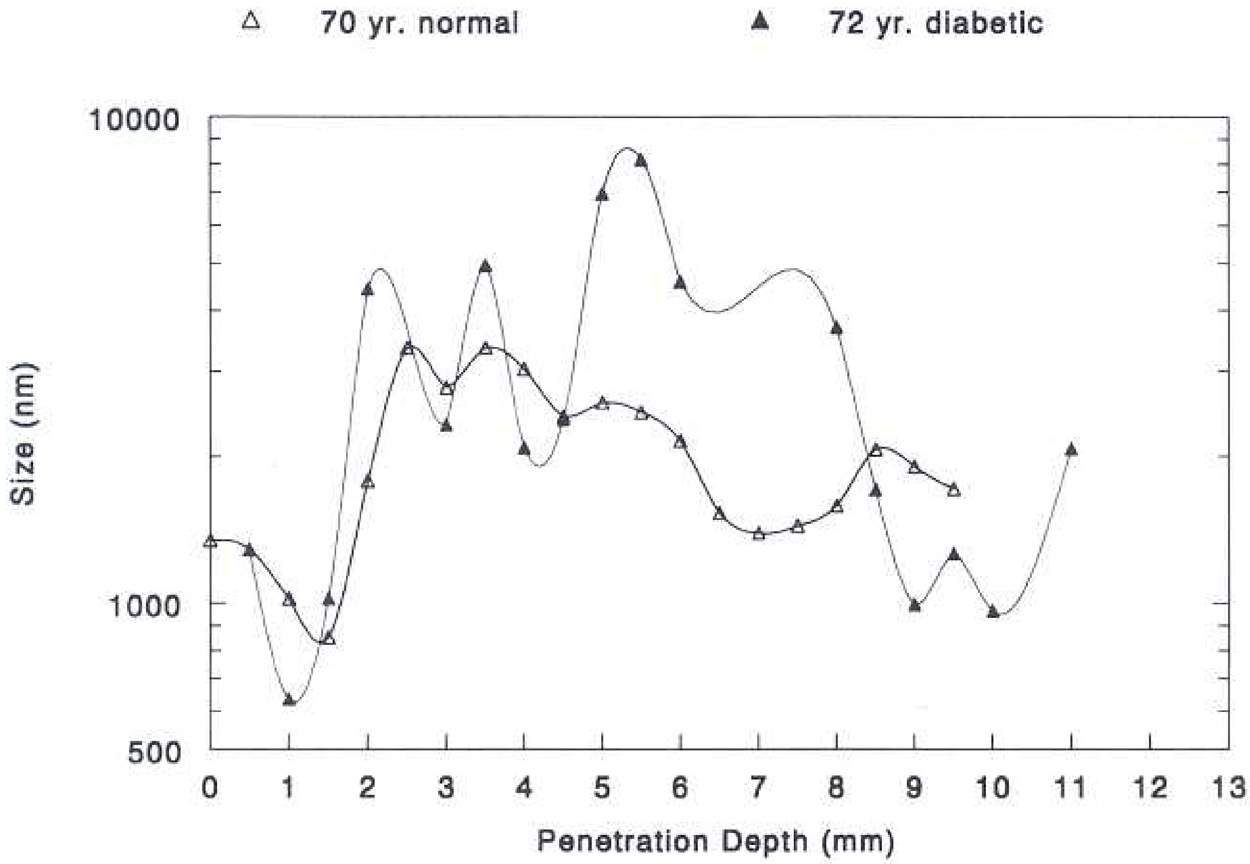

FIG. 6. Dynamic Light Scattering (DLS) of Vitreous in Diabetes. The abscissa represents the distance from the equatorial surface into the vitreous at which the DLS measurements were made, in steps that were 0.5 mm apart. The ordinate indicates the average particle size calculated from the "slow component" of the time relaxation curves (see fig. 4) DLS measurements. In the 72-year-old patient with diabetes (closed circles) there were larger and more varied particle sizes than in the 70-year-old non-diabetic (open triangles).

DISCUSSION

It is reasonable to assume that HA is the major contributor to the fast component and vitreous collagen is the predominant molecule influencing the slow component of the DLS curve. There is considerable optical anisotropy in the slow component data that is increased in specimens from diabetic subjects. These findings are likely the result of glycation effects on collagen with cross-linking and aggregation of adjacent collagen fibrils into larger bundles of parallel fibers. This optical anisotropy in diabetic vitreous is the fundamental abnormality that underlies diabetic vitreopathy.[7] It results from glycation of vitreous collagen[9] and resultant cross-linking of collagen molecules into bundles of parallel collagen fibrils.[6] The glycation and even the cross-linking could result not only from diabetes effects on type II collagen, the predominant forms in vitreous, but also from effects on type IX and the hybrid type V/XI.[17] As the latter comprises the central core of a vitreous collagen fibril, the type II collagen coating this core and especially the type IX collagen on the surface of the fibril would seem to be particularly important targets for glycation and participants in the process of cross-linking between adjacent collagen fibrils. This not only causes structural changes within vitreous col-

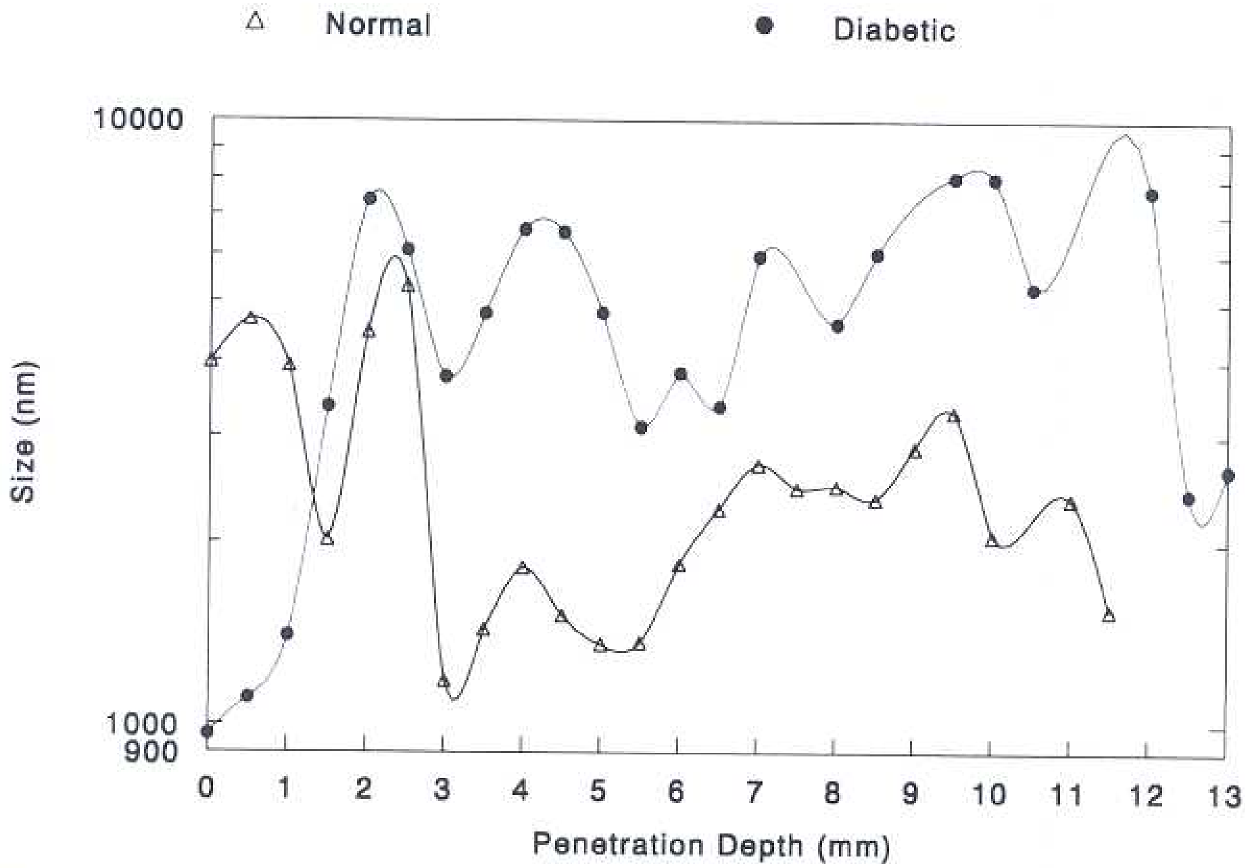

FIG. 7. The fellow eyes of the subjects described in figure 6 were prepared by removing the cornea, iris, and lens from the globe, leaving the posterior capsule of the lens intact. DLS measurements were made along the antero-posterior axis at steps 0.5 mm apart, beginning behind the posterior capsule of the lens. The abscissa represents the distance from the lens capsule. The ordinate shows the particle sizes determined from the "slow component" of the time relaxation curves (see fig. 4). In the 72-year-old patient with diabetes (closed circles) there were larger and more varied particle sizes than in the 70-year-old non-diabetic (open triangles).

lagen fibrils, but also functional abnormalities that play a role in progressive diabetic retinopathy and vision loss.[7]

These preliminary investigations suggest that the noninvasive method of DLS using the probe developed by Ansari[12–14] at NASA can detect the molecular effects of diabetes and hyperglycemia on extracellular matrix components such as collagen.[15] Ubiquitous throughout the body, collagen is an important target of diabetes. The alteration of collagen and other such molecules eventually leads to the histopathology observed in later stages of disease, changes that in the retina are recognized as the classic hallmarks of diabetic retinopathy. Previous studies[18] have indeed found a correlation between vitreous particle sizes and stages of diabetic retinopathy. With the DLS probe used in the study presented herein, however, it appears possible to detect molecular changes in vitreous that may predate changes in the retina, and perhaps other parts of the body.

The ability to identify this phenomenon at such an early stage enables the study of molecular mechanisms of disease. To detect such changes earlier than at the stage of histopathology affords an opportunity to develop methods with which to intervene prior to the development of irreversible changes, thereby preventing pathology. The noninvasive nature of this methodology further offers the possibility to perform repeat measurements and provide a gauge of the response to therapy.

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