

Evaluation of acoustic wave propagation velocities in the ocular lens and vitreous tissues of pigs, dogs, and rabbits

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Objective—To evaluate propagation velocity of acoustic waves through the lens and vitreous body of pigs, dogs, and rabbits and determine whether there were associations between acoustic wave speed and age, temperature, and time after enucleation.

Sample Population—9 pig, 40 dog, and 20 rabbit lenses and 16 pig, 17 dog, and 23 rabbit vitreous bodies.

Procedure—Acoustic wave velocities through the ocular structures were measured by use of the substitution technique.

Results—Mean sound wave velocities in lenses of pigs, dogs, and rabbits were 1,681, 1,707, and 1,731 m/s, respectively, at 36°C. Mean sound wave velocities in the vitreous body of pigs, dogs, and rabbits were 1,535, 1,535, and 1,534 m/s, respectively, at 38°C. The sound wave speed through the vitreous humor, but not the lens, increased linearly with temperature. An association between wave speed and age was observed in the rabbit tissues. Time after enucleation did not affect the velocity of sound in the lens or vitreous body. The sound wave speed conversion factors for lenses, calculated with respect to human ocular tissue at 36°C, were 1.024, 1.040, and 1.055 for pig, dog, and rabbit lenses, respectively.

Conclusions and Clinical Relevance—Conversion factors for the speed of sound through lens tissues are needed to avoid underestimation of the thickness of the lens and axial length of the eye in dogs during comparative A-mode ultrasound examinations. These findings are important for accurate calculation of intraocular lens power required to achieve emmetropia in veterinary patients after surgical lens extraction. (*Am J Vet Res* 2006;67:288–295)

Ophthalmologic applications of quantitative echography have long interested veterinary ophthalmologists. Data pertaining to measurements of the

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speed of sound waves in the eyes of humans and other mammals at various room and body temperatures have been published.¹⁻³ Measurements from those studies were obtained on the premise that the backscattering ocular tissues were homogeneous in composition and neglected the effect of disturbance of sound wave propagation by individual ocular structures. However, accurate determination of the distance (d) between the sound-reflecting surfaces in the eye requires that the velocity of sound (c) in the various tissues be precisely known in addition to the time (t) required for wave passage between the surfaces. These quantities are related⁴ according to the equation $d = c \times t$. Because the mean lens thickness comprises 32% to 35% of the length of the eye^a in dogs and 30% of the length of the eye in rabbits,⁵ an important source of error in axial length calculations may be introduced if a greater speed of transmission through the lens, compared with that in other ocular tissues, is not accounted for. The velocity of acoustic waves in ocular tissues of newly slaughtered cattle has been reported.⁶ More recently, impedance⁷ and attenuation⁸ of sound waves in the eyes of humans and pigs, as well as the distribution of acoustic variables over the cross-sectional area of the porcine lens, were examined.⁹

Accurate determination of axial length and intraocular distances is important for estimating the dioptric power of canine lens implants used in cataract surgery. Additionally, biometry values are frequently used for construction of schematic eyes in optics. A schematic eye is an internally consistent mathematical model, the construction of which requires knowledge of the exact axial location of all optical interfaces (anterior corneal surface, posterior corneal surface, anterior lens surface, posterior lens surface, and retina). Schematic eyes can be used to investigate the optical behavior of the system, including prediction of image size on the retina.¹⁰ A mean ultrasound wave speed of 1,710 m/s through 2 canine lenses has been reported.¹¹ The knowledge that there is variability in the speed of sound waves through healthy human lenses¹² prompted us to reexamine published values for these variables in dogs. Rabbit lenses and vitreous bodies were evaluated because rabbits are frequently used for research in comparative ophthalmology. Data on the velocity of sound waves in rabbit ocular tissues are sparse and may be outdated.¹³ Because of the relative abundance of data pertaining to acoustic wave speed in the eyes of pigs,^{7,8,14,15} the speed of ultrasound waves through porcine ocular tissues was determined

IOL Intraocular lens

as a means of evaluating accuracy of the measurement method used.

The primary objectives of the present study were to measure the speed of sound waves in the lens and vitreous body of dogs, rabbits, and pigs and to evaluate the effects of age, temperature, and time after enucleation on the acoustic variables in these tissues. Results may be useful in the derivation of conversion factors to correct pulse-echo distance measurements in animals that are made on the basis of sound wave velocities through human ocular tissues.

Materials and Methods

The study was conducted in accordance with the guidelines of the Association for Research in Vision and Ophthalmology and the Institutional Animal Care and Use Committee of the University of Wisconsin. Eyes from young pigs were obtained from a local slaughterhouse, and eyes from rabbits and dogs were collected from animals that were euthanized for projects unrelated to this study. Nine pig lenses, 40 dog lenses, and 20 rabbit lenses were evaluated, along with 16 pig vitreous bodies, 17 dog vitreous bodies, and 23 rabbit vitreous bodies. In instances in which both lenses from a single dog or rabbit were used, mean values of the velocities in both samples were calculated and those values were used for further statistical evaluation. Thus, sound wave velocity through lens tissue was determined for 17 rabbits and 23 dogs. In pig eyes, the vitreous body from each eye was measured separately. Because the vitreous body volume in dogs and rabbits is small, the vitreous bodies of both eyes from a single animal in those species were pooled prior to measurement.

We were unable to obtain adequate volumes of aqueous humor for determination of ultrasonic sound speed in that tissue; however, it can be presumed that concentrations of most constituents in the aqueous humor are sufficiently low that they exert no measurable effect on the propagation speed of ultrasound waves.¹⁴ Moreover, there is no significant difference in the composition of aqueous humor among different species of mammals that have been evaluated.¹⁶ The following formula was derived for acoustic wave velocity through a stock solution similar in composition to human aqueous humor:

$$c_{\text{aqueous humor}} = 1,457.72 + 2.12 \times T \text{ (m/s)}$$

where c is the sound wave velocity in units of meters per second and T is temperature.

The values measured for sound wave velocity through aqueous humor do not differ significantly from the speed of waves through the vitreous body.¹⁴ Therefore, the same velocities are generally applicable to the aqueous and vitreous humor in ocular biometric studies.

Ocular tissues were inspected macroscopically after dissection and were discarded if pathologic changes, such as cataract or liquefaction of the vitreous body, were observed. The cornea and sclera were gently removed, with care taken to preserve the uvea. The choroid and retina were incised vertically and horizontally, resulting in a cross-shaped suspension for the lens. The lens was positioned by attaching the surrounding uveal tissue between 2 concentric disks with a small central opening that was several millimeters larger than the diameter of the lens. This exposed the entire lens surface in the center of the disk, enabling its placement in the path of the acoustic beam between the transducer faces (Figure 1). The vitreous body sample was drawn into a 1.3-mL syringe and injected into a sample holder consisting of a plastic cylinder of known thickness and diameter and lined by two 25- μm -thick membranes.^b

Measurements were made within 12 hours of the time of death of the animal. The eyes were enucleated within 1 hour of the time of death and immediately transferred into a balanced salt solution^c and stored at 4°C until determinations were conducted.

The substitution technique was used for measuring sound wave velocities.^{17,18} This method yields accurate estimates of sound wave speed even when there are small inconsistencies in the sample thickness, provided the sample is being measured in a medium in which the speed of sound is similar to that of the sample.¹⁹ Saline (0.9% NaCl) solution was initially chosen as the measuring medium but caused corrosion of the measuring instruments, so distilled, degassed water was used. To exclude the possibility that the change in measurement medium influenced the speed of sound waves through the tissue, the velocities through 25 rabbit lenses in saline solution and 25 different rabbit lenses in distilled, degassed water were determined. Changes in the speed of sound waves were measured for water and tissue temperatures ranging from 32° to 40°C in 2° increments. The temperature in the water bath was controlled by means of an immersion circulator,^d which maintained a constant temperature in the water tank. Temperature was recorded by use of a mercury thermometer.^e The temperature was monitored after each measurement and was regulated to remain within $\pm 0.5^\circ\text{C}$ of the desired temperature. Each tissue sample was raised to all of the temperatures in the measurement protocol. Before the first measurement and with every temperature increase, samples were placed in the water tank for 5 minutes to permit thermal equilibration. Given the small volume of the specimen relative to the water bath, it was assumed that the temperature of the specimen equilibrated with the surrounding water temperature in the time allotted.

An unfocused 6.4-mm-diameter, single-element transducer^f was driven with a signal generator^g that produced a 10-MHz center frequency and 2-cycle tone burst pulse. The sample was placed in contact with the transmitting transducer, and waves

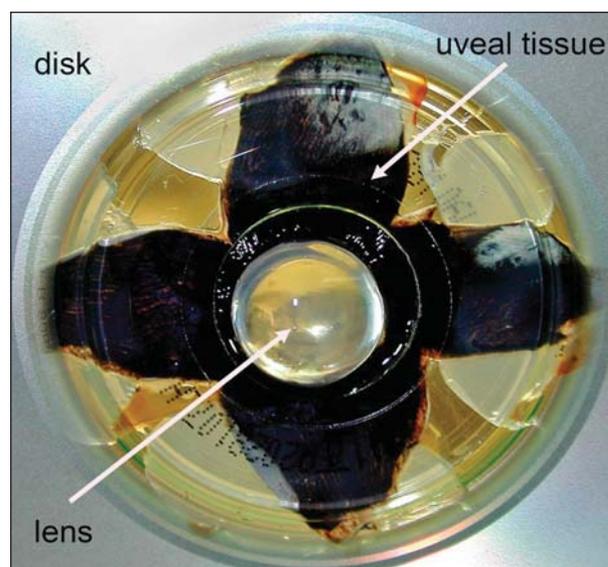


Figure 1—Photograph of a handmade device for fixation of lens tissue during measurement of acoustic wave transmission through ocular tissues. The cornea and sclera were removed, and the choroid and retina were incised vertically and horizontally in the shape of a cross for suspension of the lens. The lens is held by attaching the surrounding uveal tissue between 2 concentric disks with a central opening, several millimeters larger than the diameter of the lens. This apparatus allowed the entire lens surface to be exposed in the center of the disk and enabled its placement in an ultrasound beam path between the transducer faces.

were detected with a 3.2-mm receiver.^h Signals were recorded on a digital oscilloscope.ⁱ After observing the received waveform in water, the time shift of an easily recognized zero crossing in the received signal waveform was measured after the lens or vitreous tissue was introduced into the path of the ultrasound beam. Lens thickness was determined by use of a micrometer system^j (accuracy, ± 0.01 mm) used to control the axial position of the receiving transducer and calibrated by aligning the transducer such that it was in direct contact with the transmitting transducer face, which was in a fixed position. This baseline micrometer reading was recorded. The receiving transducer was moved away, and the lens was inserted between the transducer faces. The receiving transducer was advanced until the lens surface was in firm contact with both transducer faces, causing a slight reduction in the curvature of the lens surface. Compression of the lens was conducted carefully to avoid rupturing the lens capsule. The procedure for measuring the sound wave speed through the vitreous body was similar and was conducted by aligning the transducer and receiver on both sides of the sample holder. The difference in the micrometer readings between this position and the baseline reading (ie, without the sample inserted) provided an estimate of the tissue thickness. Three estimates of the time shift were recorded without disturbing the position of the transmitting transducer. Ignoring the propagation time in the thin layers of the tissue holder, the speed of sound was calculated by use of the following equation:

$$c_t = \frac{c_w}{1 - c_w \Delta t_s / d_t}$$

where c_t is the speed of sound in the ocular tissue (lens or vitreous body); c_w is the speed of sound in distilled, degassed water at the measurement temperature; Δt_s is the time shift; and d_t is the thickness estimate of the tissue (lens or vitreous body). The velocity of sound in distilled water at a given temperature is calculated by use of the following equation:

$$c_w = 1,557 - 0.0245 (74 - T)^2 \text{ m/s.}$$

Table 1—Summary of acoustic wave propagation velocities (c ; mean \pm SD [m/s], as available) measured in vitreous humor and lens tissue in different species in various studies.

Study	Species	Temperature (°C)	Frequency (MHz)	Medium	c_{vitreous}	c_{lens}
Jansson and Kock ¹²	Human	37	4	Distilled H ₂ O	1,532 \pm 0.5 (n = 35)	1,641 \pm 1.2 (35)
Thijssen et al ⁷	Human	20	10	Saline	1,506 \pm 3 (4)	1,620 \pm 3 (4)
De Korte et al ⁸	Human	20	20	Saline	1,514 \pm 3.2 (13)	1,590 \pm 6.4 (13)
Jansson and Sundmark ¹⁴	Porcine	22	4	Distilled H ₂ O	1,510 (30)	1,665 (30)
Rivara and Sanna ¹⁵	Porcine	37	4	Distilled H ₂ O	1,532	1,672/1,675
Thijssen et al ⁷	Porcine	20	10	Saline	1,497 \pm 1 (7)	1,651 \pm 2 (7)
De Korte et al ⁸	Porcine	20	20	Saline	1,501 \pm 5.2 (5)	1,633 \pm 10.7 (10)
Present study	Porcine	32	10	Distilled H ₂ O	1,523 \pm 0.9 (16)	1,681 \pm 10.0 (9)
		34	10	Distilled H ₂ O	1,527 \pm 1.1 (16)	1,680 \pm 8.2 (9)
		36	10	Distilled H ₂ O	1,532 \pm 0.8 (16)	1,681 \pm 6.3 (9)
		38	10	Distilled H ₂ O	1,535 \pm 0.8 (16)	1,682 \pm 7.7 (9)
		40	10	Distilled H ₂ O	1,540 \pm 0.9 (16)	1,683 \pm 6.7 (9)
		32	10	Distilled H ₂ O	1,526	1,710 (2)
		32	10	Distilled H ₂ O	1,523 \pm 1.5 (17)	1,712 \pm 18 (23)
Schiffer et al ¹¹	Canine	34	10	Distilled H ₂ O	1,527 \pm 1.0 (17)	1,709 \pm 18 (23)
		36	10	Distilled H ₂ O	1,532 \pm 0.7 (17)	1,707 \pm 19 (23)
		38	10	Distilled H ₂ O	1,535 \pm 0.7 (17)	1,707 \pm 18 (23)
		40	10	Distilled H ₂ O	1,540 \pm 0.9 (17)	1,706 \pm 18 (22)
		32	10	Distilled H ₂ O	1,522 \pm 1.6 (23)	1,748 \pm 26 (8)
Present study	Rabbit	34	10	Distilled H ₂ O	1,526 \pm 1.3 (23)	1,737 \pm 24 (17)
		36	10	Distilled H ₂ O	1,531 \pm 1.1 (23)	1,731 \pm 21 (17)
		38	10	Distilled H ₂ O	1,534 \pm 1.0 (23)	1,732 \pm 20 (17)
		40	10	Distilled H ₂ O	1,539 \pm 1.0 (23)	1,728 \pm 29 (17)
		20	10	Distilled H ₂ O		1,686 \pm 16 (10)
Osubeni and Hamidzada ³⁶	Camel	20	10	Distilled H ₂ O		1,686 \pm 16 (10)

Data are from published studies and the present study.
Saline = Saline (0.9% NaCl) solution.

After the sound wave velocities in the lens and vitreous body of dogs were derived, conversion factors for correcting distances measured on scanners that were calibrated for human tissue were calculated by dividing the speed of sound through the animal ocular tissue by the speed of sound through the human tissue.

Statistical analysis—A 2-sample independent t test was used to compare the velocities of sound waves through rabbit lenses measured in saline solution and distilled water. In dogs and rabbits in which both eyes were used, the mean value of wave speeds through both lenses was calculated. Mean values for the 3 repeated measurements at each temperature point and the intratissue SD were determined, and the intratissue mean values were used to calculate an inter-tissue mean value for each temperature point and the inter-tissue SD. Data were analyzed by use of a linear mixed-effects model with a random animal effect and a first-order autoregressive correlation structure for the within-animal observations, which means that an observation at a given time point depends only on the observation before that point. Model assumptions were checked with a residual versus fitted values plot and a qq plot. Regression analysis was performed to determine whether there was a linear relationship between mean sound wave velocities in the vitreous body and temperature. Analysis of covariance was performed to compare slopes of the regression curves of c_{vitreous} versus temperature. Values of $P < 0.05$ were considered significant.

Results

There was no significant difference in the velocity of ultrasound waves through rabbit lenses at 38°C in saline solution (n = 25 lenses; mean velocity, 1,722 \pm 22.6 m/s), compared with the velocity in distilled water (25; 1,727 \pm 21.3 m/s; $P = 0.467$). Thus, to minimize corrosive effects on equipment, distilled, degassed water was used as the medium for all subsequent mea-

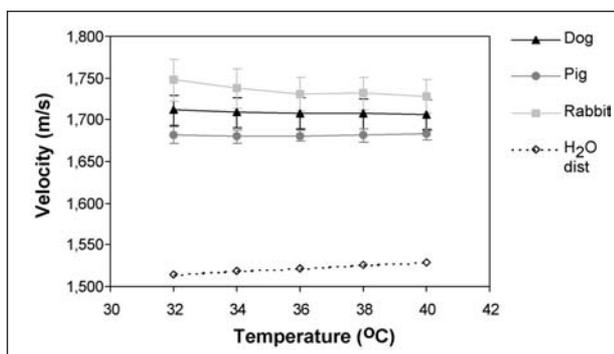


Figure 2—Acoustic wave propagation velocities (mean \pm SD) through lenses of different species at various temperatures.

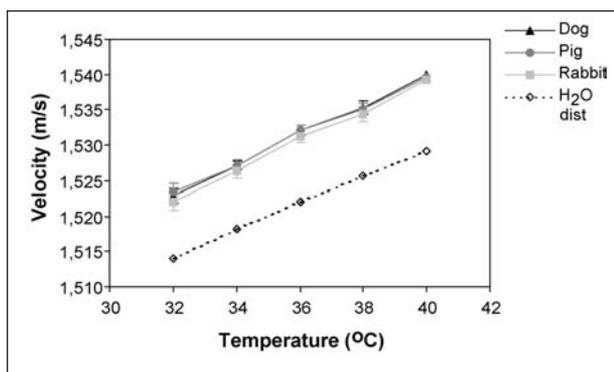


Figure 3—Acoustic wave propagation velocities (mean \pm SD) through vitreous body tissue of different species at various temperatures.

Table 2—Regression formulas and R^2 and P values of sound wave speed in dog, pig, and rabbit vitreous tissues.

Species	Regression formula	R^2	Lack of fit test
Canine	$c_{\text{vitreous}}(\text{m/s}) = 1,455 + 2.12 \times T (\text{°C})$	0.971	$P = 0.022$
Porcine	$c_{\text{vitreous}}(\text{m/s}) = 1,459 + 2.00 \times T (\text{°C})$	0.978	$P = 0.005$
Rabbit	$c_{\text{vitreous}}(\text{m/s}) = 1,454 + 2.12 \times T (\text{°C})$	0.957	$P = 0.010$

T = Temperature (°C).

measurements. Data from published studies and data collected in the present study were compared (Table 1).

Absolute values for the speed of sound waves through lens tissue differed significantly among the species examined. Velocities for sound waves through the lens and vitreous body of humans at 37°C are 1,641 and 1,532 m/s, respectively.¹² Conversion factors calculated from data in the present study were 1.024, 1.040, and 1.055 for pig, dog, and rabbit lens tissues, respectively, and 1.002, 1.002, and 1.001 for pig, dog, and rabbit vitreous tissues, respectively. Data from the present study indicated that there was no linear correlation between temperature and the velocity of ultrasound waves through lens tissue in the species or temperature ranges examined. Velocities of sound waves through dog lenses at 36°, 38°, and 40°C were significantly ($P < 0.001$) different from the velocity at 32°C, but temperature had no effect on the speed of sound waves through rabbit and pig lenses (Figure 2). In contrast, the velocity of sound in the vitreous tissue increased significantly and linearly with increasing

temperature (Figure 3). The regression formulas as well as the R^2 and P values for sound wave speed in dog, pig, and rabbit vitreous tissues were summarized (Table 2). There were no significant ($P = 0.4$) differences among slopes of the 3 regression curves.

Values of sound wave velocity through dog and rabbit lenses and vitreous tissue at 36° and 38°C, respectively, were plotted against time after enucleation, and there was no significant effect of time after enucleation on the speed of sound waves in any tissue examined (Figure 4).

Scatterplots were created for the speed of sound against age for dog and rabbit lenses and vitreous tissues at 36° and 38°C, respectively (Figure 5); no significant effect of age on sound wave velocity through dog ($P = 0.403$) or rabbit ($P = 0.372$) vitreous tissues was detected, and age had no effect on the speed of sound waves through dog lenses ($P = 0.887$). The speed of sound waves through rabbit lenses increased ($P = 0.032$) by 3.57 m/s per month of age.

Discussion

Several techniques for determination of the velocity of sound waves in ocular tissues have been described,^{6,14} including a double transmission technique.^{9,20,21} It is known⁹ that the speed of sound waves in the lens is not constant but slows gradually from the center of the structure to the periphery. It follows that the mean velocity through the lens depends on the path of the ultrasound beam. We used a through-transmission technique in which a separate receiving transducer measures the transmitted pulse. The tissue was brought into direct contact with the transducer and receiver, and compression of the lens tissue and damage to the lens capsule were avoided. Thus, values reported in the current study were spatial means over the beam path. The same technique was also used for measuring sound wave speed in the vitreous body, except that the sample was housed in a receptacle lined by thin membranes, which do not appreciably influence transit time.

The temperature range used in our measurements corresponded to physiologic body temperatures of the species examined. The steady-state isothermal contours for a human eye with a blood temperature of 38.5°C are 34.5°C for the aqueous humor, 35.6°C for the center of the lens, 37.4°C for the vitreous body directly behind the lens, and 38.4°C in the posterior portion of the vitreous body.²² Similarly, a temperature profile and the corneal surface temperature in rabbit eyes have been determined.²³ Measurements were taken for air temperatures close to 23°C and rabbit rectal temperatures of approximately 39°C. The anterior lens surface temperature was approximately 36°C, and the posterior lens surface temperature was approximately 38°C. Thus, velocities through the lens at 36°C and through the vitreous body at 38°C correspond best to the in situ condition in eyes of the species examined. The speeds of sound waves at these temperatures were chosen for illustration of the relationships between velocity and time after enucleation and velocity versus age and to calculate conversion factors with respect to human ocular tissue.

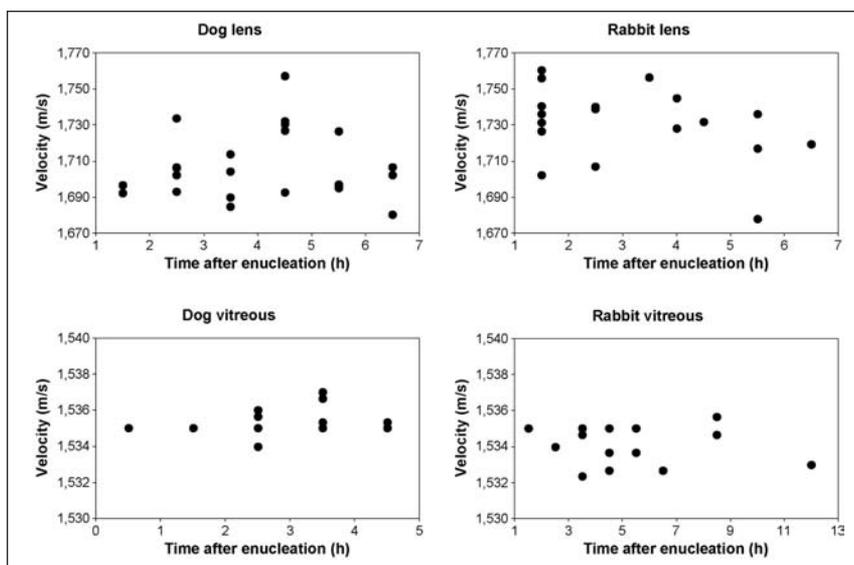


Figure 4—Relationship between sound wave velocities through lens and vitreous body tissue of dogs and rabbits and time after enucleation.

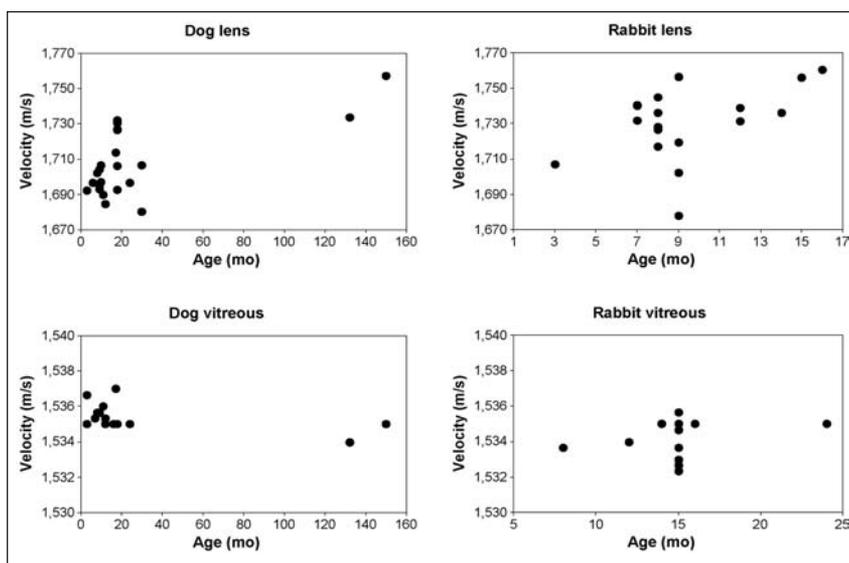


Figure 5—Relationship between sound wave velocities through lens and vitreous body tissue of dogs and rabbits and age.

The ultrasound frequency of 10 MHz corresponds to the standard frequency of most commercially available A-scan probes.

Physiologic saline solution would be a preferred measurement medium because of its isotonicity and because velocities through saline solution approximate those through body tissues. However, distilled, degassed water was used as the measurement medium so that corrosion of the measuring instruments could be avoided. The speed of sound waves in any medium is determined primarily by characteristics of the medium.⁴ An expression for the velocity of sound waves in liquids or body tissues is the following: $c = \sqrt{B/\rho}$, where B is the appropriate elastic modulus (bulk modulus) and ρ is the density. A study²⁴ of the bulk modulus of lenses revealed that, compared with lenses from young humans, the

modulus is 5 times greater in lenses of young rabbits and 21 times greater in lenses of adult cats. The higher speed of sound waves through rabbit lens tissue is consistent with the higher bulk modulus value. Sonic wave velocities in rabbit ocular tissues have been established¹³; values of 1,646 m/s were measured for lens tissue at 35°C and 1,532 m/s for the vitreous body at 37°C. Those values for the velocity through the vitreous body correlated well with values in our study, but we cannot explain the lower speed of sound waves through lens tissue detected in the other study.¹³ The difference in the number of subjects (6 lenses in the earlier study vs 17 in the present study) may play a role; however, the lowest value we measured for sound wave velocity through rabbit lenses was 1,678 m/s, a value that is higher than the mean value in the earlier study. The age range of animals used in both studies was similar and did not explain the difference. Because the sound speed values obtained from pigs and dogs in the present study were well within the range of published values,^{7,8,11,14,15} accuracy of the measurement technique used in our study is supported.

Given that the composition of the lens is approximately two-thirds water and one-third protein, species differences in lens sound wave velocities are probably caused by differences in their relative contents of protein and water. In another study,²⁵ water content in the nucleus of human lenses was approximately 65%, whereas in the cortex, it was 69%. Those percentages were higher than values derived from rabbit lenses, in which 50% and 69% of the nucleus and cortex, respectively, are water. That study²⁵ further revealed that, in contrast to cattle, rabbit, and rat lenses, in which the protein content gradually increases to the depth of the nucleus, the nucleus of a clear human lens has a constant and relatively low protein content. The acoustic variables of lens tissue are significantly correlated with lens protein content.²⁶ The velocity of sound waves through the porcine lens is associated linearly with protein concentration, with measured velocities of 1,700 m/s in the center decreasing to 1,500 m/s in the periphery of the structure.⁹

Vitreous gel is a hypocellular, highly hydrated (> 98% water) extracellular matrix. The gel structure is maintained by a sparse network of thin, unbranched collagen fibrils comprising collagen types II and Nr:V, XI, and IX. In the vitreous body of mammals, gly-

cosaminoglycan hyaluronan is a major component filling the space between collagen fibrils.²⁷ The fact that the structure and composition of the vitreous humor are conserved across species is reflected in the sound speed values obtained in the present study. The intra-species SDs were also small. The practical implication of these results is that use of conversion factors is not necessary when measuring the vitreous body thickness of different species of animals by use of an A-scan ultrasound machine programmed for use in human ocular tissues.

The effect of aging on acoustic transmission characteristics of ocular tissues in humans has been investigated and was significant for attenuation of echo amplitudes in the lens and for ultrasound velocity and attenuation of amplitude in scleral tissue.⁸ In our study, there was an increase in sound wave velocity through rabbit lenses with increasing age but there was no effect on the velocity through vitreous tissue in any of the species or in the lenses of dogs. It is known from a study²⁴ of human lenses that the Young modulus of the lens nucleus remains constant before the age of 40 and then increases, probably because of changes in the moisture content of the nucleus. In another report,²⁸ elasticity of the lens increased but the lens density remained nearly constant with age. It follows that the speed of sound waves through lens tissue might also be expected to increase with age. However, in 1 study,²⁹ the speed of sound waves in human lenses did not change with age. In another study,¹² there was no age-associated change in sound wave speed in 7 clear lenses in humans 5 to 68 years of age. In a study³⁰ in humans in which investigators measured lens thickness by use of slit-lamp photography and ultrasonography, it was found that measurements made via both methods were in agreement only when a decrease in sound wave speed of 3 m/s per year was assumed. Nucleosclerosis does not appear to influence the velocity of ultrasound waves through the lens, but cataractous changes substantially affect sound speed, decreasing the velocity of waves with increasing severity of cataracts.¹² In that study, values had a wide range (1,543 to 1,665 m/s). Those findings were corroborated in another study,³¹ in which 50 cataractous lenses were evaluated. The type and location of lenticular opacity determine whether sound wave speed increases or decreases.³² In our study, lenses from old dogs (mean age, 12 years) had nucleosclerosis but no signs of cataract development and the mean speed of sound waves through those lenses was higher than the mean velocity through the other lenses. Thus, there may be an increase in the velocity of sound waves with increasing age in dog lenses, even though our data did not indicate a significant association. This may be because the distribution of ages among our animals was skewed and the number of lenses from old dogs was small. Contrary to findings from a previous study,¹³ an effect of age on sound wave speed in rabbit lenses was detected in the present study, with the speed of sound increasing with increasing age. It was not possible to create a statistically robust model from our data, although linear regression has been a good model for describing the effects of age on acoustic variables in other tissues.³³

In the present study, an effect of age on the speed of sound through vitreous humor was not detected in any of the species examined. This finding was in agreement with results of earlier studies,^{12,13} in which there was no influence of age on sound wave speed in vitreous tissue from humans and rabbits. The age of the animals in which vitreous tissue was evaluated in our study ranged from 8 to 24 months in rabbits and from 3 to 150 months in dogs. The mean age of rabbits may have been too young and the group of 2 eyes from old dogs may have been too small to reveal any influence of age on sound wave speed in vitreous tissue, but it cannot be ruled out that the progressive liquefaction in the vitreous body as a result of aging would influence the velocity of ultrasound waves.

There was a concern regarding the heating of the same tissue specimen to temperatures over the entire range of temperatures studied, a process that could introduce irreversible and likely cumulative degradation of tissue structures and thereby alter wave propagation speed. However, results of other studies^{18,34} have revealed that although the propagation speed and attenuation coefficient for sound waves in canine liver tissue varied with temperature, the variation was not a function of tissue coagulation. Continuously heating and measuring properties in the same tissue samples over the entire temperature range of the study yielded values for both attenuation and propagation speeds that were similar to those derived from specimens heated to a single target temperature.^{18,34} There were no significant changes in propagation speed estimates before and after temperature increases in the same tissue specimens measured at 37°C.³⁴ Thus, it appears that tissue denaturation does not have an appreciable impact on sound wave propagation speed. On the basis of these results, we used a measurement protocol in which the speed of ultrasound waves through the same tissue sample was measured at 5 temperatures (32°, 34°, 36°, 38°, and 40°C) in order of increasing temperature. Results revealed that there was no relationship between the speed of sound waves through lens tissue and temperature. There were no significant differences in values measured at different temperatures in porcine ocular tissues. The speed of sound waves through dog and rabbit lenses even decreased slightly with increasing temperature, although a significant difference was detected only for the velocity in dog lenses at 32°C, compared with measurements at the other temperatures. This finding was in contrast to other data in the literature; in 1 study,¹⁴ there was an increase in sound wave speed (through lenses) of 1 m/s per °C. Analysis of those data yielded the following regression line for the velocity of ultrasound in the porcine lens: $c_{\text{lens}} = 1,642.61 + 1.00 \times T$ (m/s). It is possible that the greater range (23° to 35°C) of temperatures used in that study permitted detection of the association between sound wave speed and temperature. The investigators also reported that an increase in temperature increased the sound wave speed in vitreous humor in pigs by 1.8 m/s per °C. The derived regression formula was $c_{\text{vitreous}} = 1,468.95 + 1.81 \times T$ (m/s) and correlated well with

the formula derived in the present study. Slopes of the regression curves calculated from our data were not significantly different and paralleled the curve of distilled water.

A relationship between the speed of sound waves and time after enucleation was not observed for ocular tissues from any of the species examined in our study. This finding is in agreement with those from other studies.^{7,12} Most of the samples we used were evaluated within 2 to 4 hours of enucleation. The maximal time that elapsed between the animal's death and measurement procedures was 12 hours.

Data from the present study indicate that conversion factors for the speed of sound waves through lens tissue are needed to accurately perform comparative quantitative A-mode ultrasound studies. For example, an underestimation of approximately 4% results when calculations of lens thickness in dogs are made on the basis of sound wave speeds in human tissues. In contrast, error in estimation of vitreous body depth is approximately 0.2% and thus negligible. Use of values for human lenses in biometric measurements in dogs results in a 1.5% underestimation of the axial length of the dog eye. Practical benefits of these results include the calculation of individual IOL power in cataract patients. For example, in a theoretical formula derived on the basis of geometric optics, multiplication of the measured variables by the appropriate conversion factors yields a mean IOL power of 41.8 diopters, whereas without the conversion factors, a mean IOL power of 43 diopters is calculated; this 3% overestimation of the lens power would result in a myopic refractive state after cataract surgery and IOL implantation. Even small errors in refractive state substantially impact visual acuity in dogs. Reducing focus of the eye by just 2 diopters causes a shift in Snellen acuity from approximately 20/75 to 20/150.³⁵ Because the speed of sound through rabbit lens tissue is faster than in dogs and thus the difference is larger, compared with speeds in human tissues, the error introduced by not using a correction factor would be even greater.

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- b. Saran Wrap, 25 microns, Dow Chemical Co, Midland, Mich.
- c. BSS, Alcon Laboratories, Forth Worth, Tex.
- d. Haake DC10 immersion circulator, Haake, Karlsruhe, Germany.
- e. Standard mercury thermometer, Brooklyn Thermometer Co Inc, Farmingdale, NY.
- f. Transmitting transducer, 10.0-MHz center frequency, 6.4-mm radius, Panametrics V309, Waltham, Mass.
- g. Function generator, Wavetek model 81, San Diego, Calif.
- h. Receiving transducer, 10.0-MHz center frequency, 3.2-mm radius, Aerotech Delta, Krautkramer Inc, Lewistown, Penn.
- i. Digital oscilloscope, LeCroy 9410, Chestnut Ridge, NY.
- j. Micrometer system, Ardel Kinematic Corp, College Point, New York.

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