Direct ultrasonic velocity measurements of mammalian collagen threads[a]

S. A. Goss[b] and W. D. O'Brien, Jr.

Bioacoustics Research Laboratory, University of Illinois, Urbana, Illinois 61801
(Received 9 August 1978; revised 9 October 1978)

Direct ultrasonic velocity measurements have been made in fresh collagen threads from mammalian tendon at a frequency of 100 MHz using the scanning laser acoustic microscope. Tendon is of interest since it contains an unusually large amount of collagen, a structural protein thought to be important to the echographic visualizability of normal and pathologic tissues in medical ultrasound. Results indicate that ultrasonic velocity is appreciably higher than in soft tissues, lending support to the view that tendon, as well as collagen, is markedly distinguished ultrasonically from other biological tissues.

PACS numbers: 43.80.Cs, 43.80.Ev, 43.35.Cg

INTRODUCTION

Collagen is the most abundant single protein in the human body and the most common protein in the entire animal kingdom. Closely associated with the connective tissue of vertebrates, collagen comprises between 25%–33% of the total protein in the human body, and therefore about six percent of the total body weight.[1] There is some evidence to suggest that it is the elastic properties of soft tissues, determined primarily by the content of collagen and other structural proteins, which define acoustic contrast during echographic visualization.[2,3] This hypothesis is based on the fact that the static or low-frequency elastic modulus (Young's) of collageneous fibers is at least 1000 times greater than those of the parenchymal tissues.[2] Since the ultrasonic velocity is proportional to the square root of the elastic modulus, collageneous tissues are thought to introduce a greater impedance mismatch than would be the case for a tissue interface of similar elastic modulus, thereby increasing the acoustic reflectivity of soft (parenchymal) tissue–collageneous tissue boundaries.

While a correlation[4] suggests that tissues with higher collagen content appear to exhibit ultrasonic velocities greater than those of lesser amounts of collagen, the quantitative determination of ultrasonic velocity in tissues which predominately comprise collagen fibers has received little attention. Acoustic microscopy has been used in this study to examine ultrasonic propagation properties in collagen fibers at 100 MHz. Specifically, quantitative determination of the ultrasonic velocity and qualitative evaluation of ultrasonic attenuation have been made.

I. METHOD

A scanning laser acoustic microscope (Sonomicroscope 100®, Sonoscan Inc., Bensenville, Illinois), operating at a frequency of 100 MHz, is employed in both the examination and quantitative velocity determination of the collagen specimens. The operational details of this instrument may be found elsewhere,[5–9] and are only summarized here. The specimen is placed on a sonically activated fused silica stage and is covered with a mirrored coverslip which also provides for a small amount of light transmission. Mechanical perturbations of the coverslip surface due to the acoustic energy transmitted through the specimen are detected by a focused, scanning laser beam probe. These disturbances occur at the acoustic frequency of 100 MHz and are proportional to the acoustic amplitude in each region. The laser light transmitted through the coverslip and specimen allows for the formation of an optical image electronically in perfect register with the acoustic image.

Velocity determinations in small tissue components are made by detecting variations in the acoustic index of refraction using the acoustic interferometry mode.[7,8] Here, the displacement of acoustic interference fringe lines, measured graphically, is related to regional variations in acoustic index of refraction, as described in detail in the Appendix. The velocity of sound in the specimen can be found from

$$c_x = \frac{c_0}{\sin\theta_0}\sin\left[\tan^{-1}\left(\frac{1}{(1/\tan\theta_0) - (N\lambda_0/T\sin\theta_0)}\right)\right], \quad (1)$$

where c_0 is the velocity of sound in the medium surrounding the specimen, θ_0 is the angle from the normal of acoustic beam in the medium surrounding the specimen, N is the normalized lateral fringe shift, T is the thickness of the specimen, and λ_0 is the wavelength of sound in the medium surrounding the specimen. The medium surrounding the collagen specimens is 0.9% saline, for which c_0 is 1507 m/s at 22 °C. Velocity variations on the order of 1%–2% may be discerned using this technique, assuming a specimen thickness of approximately 100 μm and an achievable accuracy of the fringe displacement measurement of 0.1 fringe. Specimen thickness introduced perhaps the greatest single source of error, as this could only be measured to within ± 10% using either a calibrated light microscope, the unperturbed fringe spacing, or a grid cut onto the coverslip to "calibrate" the acoustic micrograph.

II. SPECIMEN PREPARATION

Tail tendons were surgically removed within minutes post mortem from 10-month-old female, and 12-month-

[a]Portion of this work was presented at the 95th Meeting of the Acoustical Society of America 16–19 May 1978, Providence, RI [J. Acoust. Soc. Am. 63, S28(A) (1978)].

[b]Present address: Ultrasound Research Laboratory, Indianapolis Center for Advanced Research, Indianapolis, Indiana 46202.

 0001-4966/79/020507-05$00.80 © 1979 Acoustical Society of America

FIG. 1. Acoustic micrograph of a fresh collagen thread. (Sample No. 10–2.)

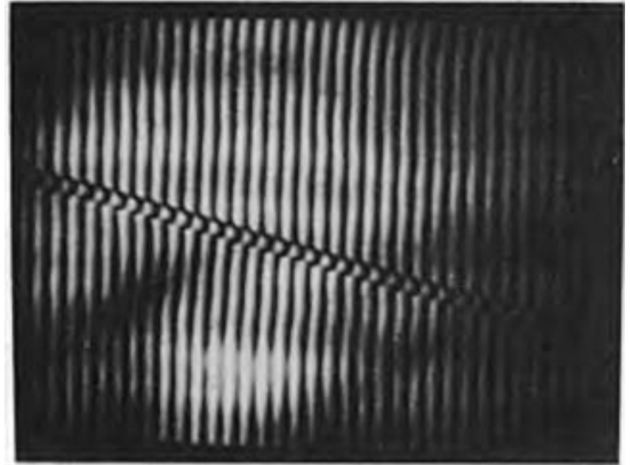

FIG. 3. Interferogram of a fresh collagen thread. (Sample No. 10–2.)

old male LAF_1/J mice (Jackson Labs), and placed immediately in 0.9% saline solution at room temperature (22 °C). Single tendon fibers, sometimes referred to as primary fiber bundles,[10] ranging in size from 42–115 μm in diameter, were isolated, placed on the acoustic microscope stage under no tension, and again bathed with 0.9% saline solution. Care was taken to assure that the specimen did not dry out. A doughnut shaped spacer was placed between the stage and coverslip to prevent the coverslip from compressing or crushing the small fiber. Acoustic measurements were performed at room temperature, usually within 1–2 h post mortem.

III. RESULTS AND DISCUSSION

The acoustic micrographic image of the tendon fibers, shown in Fig. 1, is indicative of seemingly an acoustically homogeneous thread, with few attenuating structures detectable from within the fiber. This is in contrast to the optical image of the same fiber (Fig. 2), which is seen to be opaque. The relatively low acoustic attenuation noted here could be the result of either the specimen size (42–115 μm) or the attenuating properties of the thread or a combination thereof owing to the approximate limit of detectability of 5-dB attenuation as determined by attenuation measurements of water with the microscope. For example, the ultrasonic attenuation at a frequency of 1 MHz is about 1 cm^{-1} in high collagen content tissue,[4] and assuming a linear frequency dependency and extrapolating to 100 MHz yields 100 cm^{-1}. For a 60-μm-thick specimen, this results in a total attenuation around 5 dB. However, for the thicker specimens the total attenuation remained within the limits of detectability, suggesting that the actual attenuation for the collagen thread may be less than 5 dB per 115 μm at 100 MHz. Further studies will be required to define the nature of this observation.

Velocity measurements in fresh mouse tendon threads (Table I) at 100 MHz yield a value of 1733 ± 56 m/s, for eight specimens taken from two different animals. Figure 3 shows a typical interferogram from which the velocity data are obtained. To assure that fluid loss from the specimen during mounting on the stage did not result in abnormally high measured values of velocity, these same specimens were dried in air for 30–127 min, then placed on the microscope stage in 0.9% saline for several minutes and remeasured, yielding a value of 1747

FIG. 2. Optical image of a fresh collagen thread. (Sample No. 10–2.)

TABLE I. Summary of ultrasonic velocity data in mouse tail tendon fibers at 100 MHz. Average value for wet fibers is 1733 ± 56 m/s, while for rehydrated 1747 ± 66 m/s.

Sample #	T_{wet} (μm)	c_{wet} (m/s)	Time Dried (min)	$T_{rehydrated}$ (μm)	$c_{rehydrated}$ (m/s)
10–2	63–65	1716–1723	30	60–75	1740–1810
10–18	70–75	1751–1771	95	75–80	1736–1754
10–25	65–75	1740–1782	122	75–90	1697–1740
10–33	42–50	1798–1867	127	50–70	1757–1880
11–14	90–100	1657–1675	55	75–83	1798–1846
11–25	55–75	1697–1778	60	75–90	1668–1704
11–35	100–105	1699–1710	63	100–115	1647–1670
12–14	90–107	1664–1699	67	70–95	1712–1799

±66 m/s. While the mean velocity of the rehydrated specimens is somewhat greater than that of the fresh samples, the variation (less than 1%) is within the 3%–4% standard deviation among all of the specimens, and is not considered significant.

Velocity measurements with the scanning laser acoustic microscope on dental[11] and myocardial[12] tissues have been in agreement with independent, lower frequency measurements. In addition velocity measurements with the microscope used in this study on soft polyethylene sheets are in agreement with handbook values.[13]

While these collagen thread velocity measurements are about 10–20% greater than those obtained for parenchymal tissues at much lower frequencies, they are commensurate with the value of velocity in beef tendon (whole, measured parallel to fiber direction) reported by Dussik and Fritch, of 1750 m/s at 1 MHz.[14] O'Brien[4] has shown that the following relation exists between ultrasonic velocity v, and the wet weight percentage of tissue collagen C, for nine tissues in the 1–10-MHz frequency range

$$v = 1588 + 32 \ln C \qquad (2)$$

and, for tendon collagen (30% wet weight[15,16]) this expression yields about 1700 m/s which is within 2% of the measurements reported herein. Similar measurements of ultrasonic velocity in liver tissue at 100 MHz with the acoustic microscope[17] have identified little difference from that reported at much lower frequencies,[18] indicating that if a velocity dispersion does exist over the 1–100 MHz frequency range in this tissue, it is much smaller than the differences identified in the present study between parenchymal tissues and collageneous fibers. Since networks of similar collageneous fibers are interspersed in varying amounts throughout all body tissues and organs, such velocity differences may be responsible, at least in part, for variations in the ultrasonic impedance, and thus the subsequent visualizability, of tissues and organs. Tissue density, however, also contributes to the acoustic impedance. Results from the literature[19,20] indicate that the density of molecular collagen in the native state is 1.16–1.33 g/cm^3, which is appreciably greater than that found in soft tissues, which is usually assumed to be similar to that of water. It appears then that the density, as well as the ultrasonic velocity is greater than that found in soft tissues, and suggests that the echographic visualizability of tissue is dependent, to some degree, on the amount and arrangement of collagen within a particular tissue.[2]

ACKNOWLEDGMENTS

The authors acknowledge gratefully the partial support of this work by grants from the National Science Foundation (ENG 76-22450), the National Institute of General Medical Science (GM 12281) and the Campus Research Board of the University of Illinois at Urbana-Champaign.

APPENDIX

The following derivation is based upon unpublished results of L. W. Kessler and P. R. Palermo (Sonoscan, Inc., Bensenville, Illinois) and is provided to clarify the phenomenon and process responsible for obtaining acoustic velocity measurements.

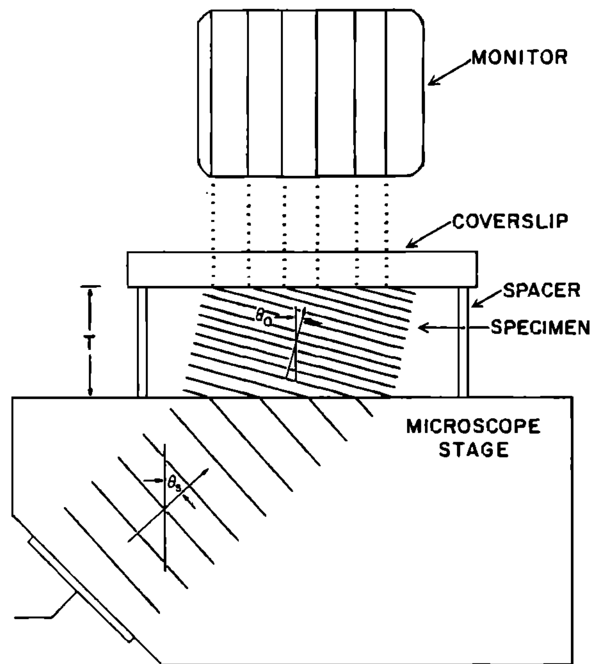

FIG. A1. Schematic representation of acoustic, constant phase wavefronts and their relationship to the interference pattern displayed on the Sonomicroscope 100 ® monitor.

Figure A1 schematically depicts the acoustic, constant phase wavefronts and their relationship toward the interference pattern displayed on the monitor when the Sonomicroscope 100® (Sonoscan, Inc.) is operated in the interference mode. The wavefronts, which are separated by a distance of one wavelength, are shown traveling from the transducer toward the microscope stage surface at an angle θ_s relative to the stage surface normal. The acoustic energy is incident upon the stage surface-specimen boundary with part of the energy transmitted and refracted according to Snells' Law and part reflected (not included for clarity). The transmitted energy, shown traveling through a homogeneous material such as water, is incident upon the lower surface of the coverslip. Again, the transmitted signal which is highly attenuated in the coverslip and the reflected signal are not shown. Conceptually, if a superimposed reference wavefront at the same frequency as the transmitted energy is incident normally to the coverslip surface, a stationary ripple pattern is produced due to constructive and destructive interference. The spacing of this ripple pattern is dependent upon the velocity of sound in the specimen, as well as that in the fused silica microscope stage, and the angle θ_s. Such an interference pattern could be detected and displayed on a monitor, as shown in Fig. A1. If the specimen velocity is reduced, the fringe lines of the ripple pattern would move leftward, i.e., in Fig. A1, θ_0 would decrease. The reference wavefront, in practice, is not generated within the microscope stage assembly, but rather is generated electronically following detection of the scanning laser.

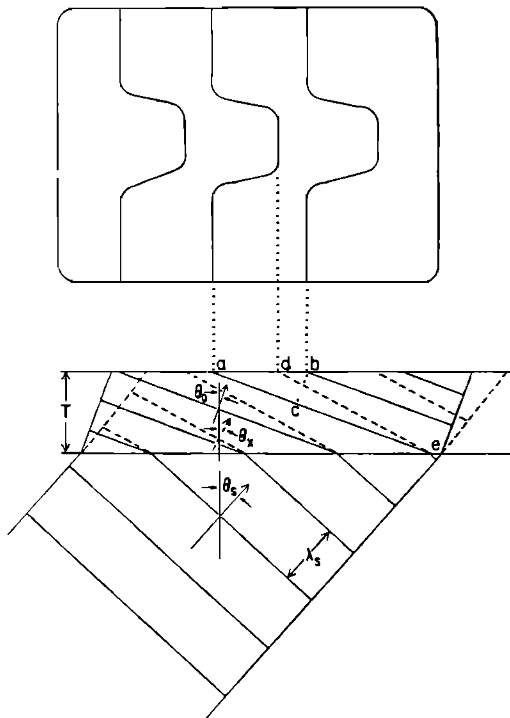

FIG. A2. Detailed, schematic representation of acoustic, constant phase wavefronts necessary in the derivation of Eq. (A6).

This phenomenon permits the quantitative determination of ultrasonic velocity and is further described in Fig. A2 wherein the acoustic energy is incident upon the stage surface-specimen interface at the angle θ_s. Two separate but superimposed transmitted wavefronts are shown, one into a known medium at an angle θ_0 and the other into an unknown medium at an angle θ_x, the relationship being described by Snells' Law, viz.,

$$\frac{\sin\theta_s}{c_s}=\frac{\sin\theta_0}{c_0}=\frac{\sin\theta_x}{c_x} \tag{A1}$$

or, as a single frequency prevails,

$$\frac{\sin\theta_s}{\lambda_s}=\frac{\sin\theta_0}{\lambda_0}=\frac{\sin\theta_x}{\lambda_x}\ . \tag{A2}$$

In Fig. A2, the unknown medium is shown to have an ultrasonic velocity greater than the known medium, and both have the same thickness T. The normalized lateral fringe shift is defined as the distance the fringe shifts (in this case to the right) from the known fringe to the unknown fringe, divided by the distance between two adjacent known fringes, that is,

$$N=\frac{\text{line segment } ad}{\text{line segment } ab}\ . \tag{A3}$$

From triangle abc, where line segment bc is perpendicular to and at the distance λ_0 from the wavefront ae, it can be seen that

$$\text{line segment } ab=\frac{\lambda_0}{\sin\theta_0} \tag{A4}$$

and, similarily, from triangle ade

$$\text{line segment } ad=\frac{T}{\tan\theta_0}-\frac{T}{\tan\theta_x} \tag{A5}$$

yielding

$$N=\frac{T\sin\theta_0}{\lambda_0}\left[\frac{1}{\tan\theta_0}-\frac{1}{\tan\theta_x}\right]\ . \tag{A6}$$

Therefore, the ultrasonic velocity c_x in the unknown specimen is determined by first calculating θ_x from Eq. (A6), and substituting this value into Eq. (A1).

For example, if the known medium is water at 25 °C (c_0 = 1497 m/s) and the acoustic microscope stage is fused silica (c_s = 5968 m/s), θ_0 = 10.22° for θ_s = 45°. Given a normalized lateral fringe shift of 0.5 and a specimen thickness of 100 μm, θ_x is calculated to be (at an ultrasonic frequency of 100 MHz) 11.04°, which yields c_x = 1616 m/s.

[1]A. White, P. Handler, and E. L. Smith, *Principles of Biochemistry* (McGraw–Hill, New York, 1968), p. 871.
[2]S. Fields and F. Dunn, "Correlation of echographic visualizability of tissue with biological composition and physiologal state," J. Acoust. Soc. Am. **54**, 809–812 (1973).
[3]L. W. Kessler, S. I. Fields, and F. Dunn, "Acoustic Microscopy of Mammalian Kidney," J. Clin. Ultrasound. **2**, 317–320 (1974).
[4]W. D. O'Brien, Jr. "The Relationships Between Collagen and Ultrasonic Attenuation and Velocity in Tissue," in *Proc. Ultrasonics International* 77 (IPC Science and Technology Press, Guildford, England, 1977), pp. 194–205.
[5]A. Korpel, L. W. Kessler, and P. R. Palermo, "An Acoustic Microscope Operating at 100 MHz," Nature **232**, 110–111 (1971).
[6]L. W. Kessler, A. Korpel, and P. R. Palermo, "Simultaneous Acoustic and Optical Microscopy in Biological Specimens," Nature **239**, 111–112 (1972).
[7]L. W. Kessler, P. R. Palermo, and A. Korpel, "Practical High Resolution Acoustic Microscope," in *Acoustical Holography*, edited by G. Wade (Plenum, New York, 1972), Vol. 4.
[8]L. W. Kessler, P. R. Palermo, and A. Korpel, "Recent Developments with the Scanning Laser Acoustic Microscope," in *Acoustic Holography*, edited by P. Green (Plenum, New York, 1974), Vol. 5.
[9]L. W. Kessler, "A Review of Progress and Applications in Acoustic Microscopy," J. Acoust. Soc. Am. **55**, 909–918 (1974).
[10]D. H. Elliott, "Structure and Function of Mammalian Tendon," Biol. Rev. Cambridge Phil. Soc. **40**, 392–421 (1965).
[11]L. W. Kessler, "Review of Progress and Applications in Acoustic Microscopy," J. Acoust. Soc. Am. **55**, 909–918 (1974).
[12]D. E. Yuhas and L. W. Kessler, "Acoustic Microscope Analysis of Myocarolium," in *Proc. 2nd International Symposium of Ultrasonic Tissue Characterization*, edited by M. Linzer (U. S. Government Printing Office, Washington, D.C.) (in press).
[13]W. P. Mason, "Acoustic Properties of Solids," in *American Institute of Physics Handbook*, 3rd ed. (McGraw–Hill, New York, 1972).
[14]K. T. Dussik, D. J. Fritch, M. Kyriazidou, and R. S. Sear, "Measurements of Articular Tissue with Ultrasound," Am. J. Phys. Med. **37**, 160–165 (1958).
[15]R. D. Harkness, "Collagen," Sci. Prog. London **54**, 257–274 (1966).
[16]R. D. Harkness, "Mechanical Properties of Collagenous Tissues," in *Treatise on Collagen*, edited by B. S. Gould (Academic, New York, 1968), Vol. 2, Part A, Chap. 6.
[17]W. D. O'Brien, Jr., "Ultrasonic velocity and qualitative

appearance of fresh and fixed liver tissue with acoustic microscopy," J. Acoust. Soc. Am. 63, S1, S28 (1978).
[18]S. A. Goss, R. L. Johnston, and F. Dunn, "Comprehensive Compilation of Empirical Ultrasonic Properties of Mammalian Tissues," J. Acoust. Soc. Am. 64, 423–457 (1978).
[19]N. E. Dweltz, "Structure of Feather Keratin," in *Collagen*, edited by N. Ramanathan (Interscience, New York, 1962), pp. 179–198.
[20]D. J. S. Holmes, A. Miller, S. White, and B. B. J. Doyle, "Interpretation of the Meridional X-ray Diffraction Pattern from Collagen Fibers in terms of the Known Amino Acid Sequence," J. Molec. Biol. 110, 643–666 (1977).