Speed of Sound in Mammalian Tendon Threads Using Various Reference Media

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Abstract—**Acoustic microscopy has now made it possible to characterize biological materials on the microscopic level ultrasonically. The importance of the environmental conditions of the material being examined on its acoustic properties is demonstrated. Mammalian tendon threads (on the order of 150 μm in diameter) were examined with the scanning laser acoustic microscope while varying the media in which they were bathed. A relation between the osmolarity of the reference solution in which the tendon is bathed and the speed of sound of the thread under examination is suggested.**

INTRODUCTION

IT HAS BEEN SUGGESTED [1], [2] that the elastic properties of tissue, which are determined largely by collagen and other structural proteins, are responsible for the acoustic contrast during echographic visualization. Support for this proposal comes from the fact that collagenous fibers have a low-frequency elastic modulus that is about 1000 times greater than most soft tissue. Regressive relations suggest that, to a first approximation, tissue collagen content is related to the tissue's ultrasonic velocity and attenuation coefficient at a frequency of 1 MHz. It has also been suggested [3] that the tissue collagen content is related to acoustic scattering, where scattering is defined as the difference between the attenuation and absorption coefficients of the ultrasonic wave.

The present study examines the speed of sound of a collagen thread as a function of its environment, utilizing acoustic microscopy as the measuring method. This report is part of a continuing study to understand the role of collagen and its effect upon the ultrasonic propagation properties of biological materials [4]–[6]. Collagen content varies according to the type of animal and tendon. Achilles tendon from numerous animals (cattle, dogs, rabbits, rats, guinea pig, and humans) has a collagen content that ranges from 60 to 95 percent dry weight and 22 to 35 percent wet weight [7], [8]. Mouse (LAF_1/J) tail tendon has a collagen content around 56 percent wet weight based upon a hydroxyproline content assey [5]. It is assumed that the ultrasonic propagation properties of collagen are essentially those measured in tendon.

Manuscript received April 1984; revised July 1984. This work was supported in part by the National Science Foundation under Grant ENG 76-22450, the National Institutes of Health under Grants GM 24994 and CA 36029, and the Campus Research Board of the University of Illinois at Urbana-Champaign.

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METHODS

A scanning laser acoustic microscope (SLAM) operating at a frequency of 100 MHz was utilized to determine the ultrasonic speed of the tendon threads. Details of the operation and theory of velocity measurement appear elsewhere [4]. Briefly, the speed is determined for a specimen by measuring the lateral fringe shift of the interference lines in the image compared to that of the reference (for bathing) solution surrounding the specimen from the following equation:

$$C_x = \frac{C_0}{\sin \theta_0} \sin \left\{ \tan^{-1} \left[\frac{1}{1/\tan \theta_0 - (N \lambda_0 / T \sin \theta_0)} \right] \right\} \quad (1)$$

where C_x and C_0 are the ultrasonic speeds of the specimen and the bathing solution, respectively; N is the measured lateral fringe shift; θ_0 is the known acoustic wavelength in the bathing solution; T is the thickness of the specimen; and θ_0 is the angle at which the acoustic wave travels through the reference solution relative to the ultrasonic microscope stage.

Note that for N equal to zero in (1), $C_x = C_0$, there is no lateral shift in the interference lines and the sound velocity of the specimen and surrounding medium is equal. The velocity measurement would then be independent of specimen thickness. The measurement of specimen thickness represents one of the largest sources of error in the velocity determination, and under the conditions of $N = 0$ the specimen thickness is not required. Another potential source of error is the 2π ambiguity in measuring the fring shift [9]. However, for these measurements the individual fringe lines were clearly separated and continuous, and they did not shift horizontally too rapidly.

Tendon thickness measurements introduce the greatest error in terms of accurately determining C_x from (1). The uncertainty in determining the tendon thickness is within ± 20 μm based upon two procedures; viz., with a specially constructed stage using an optical micrometer and with the SLAM using a calibrated image as a reference. The latter procedure is under the experimental conditions in which the lateral fringe shift is also determined; here, the peak shift was taken in the center of the thread. Since the fringe displacement measurement can be made to within ± 0.1 of a fringe shift, the margin of error in the tendon thread velocity measurement is within $\pm$ four percent.

The tendon threads examined were surgically removed

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TABLE I
ULTRASONIC SPEED IN AQUEOUS SOLUTIONS OF SODIUM CHLORIDE AND GLYCERIN AS MEASURED WITH THE SCANNING LASER ACOUSTIC MICROSCOPE

Concentration (g solute/100 ml volume)	Speed Range (m/s)
NaCl	
0.9	1468-1518
5	1511-1565
13	1597-1647
15	1617-1667
20	1665-1715
25	1711-1761
30	1755-1805
32	1772-1822
Glycerin	
50	1656-1706
60	1686-1736
70	1716-1766
80	1755-1805
90	1795-1845
100	1825-1875
110	1853-1903
120	1875-1925
125	1900-1950

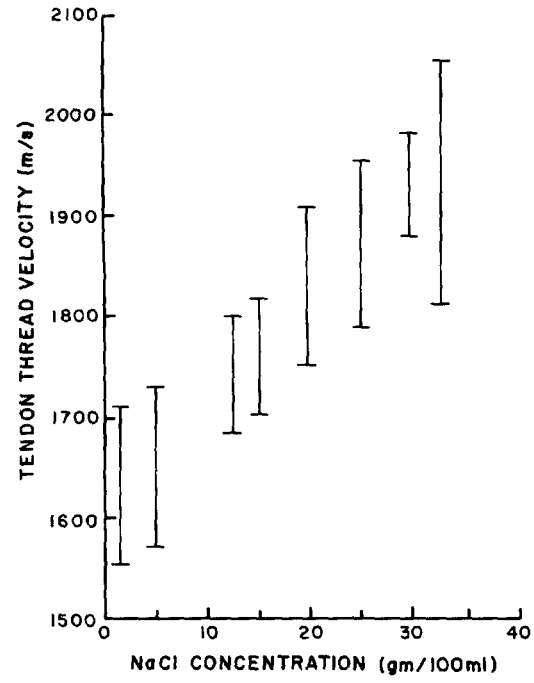

Fig. 1. Tendon thread velocity as a function of sodium chloride concentration.

from the tails of female LAF_1/J mice that were four to five months old. The mice were sacrificed by cervical dislocation, and the tails were removed, skinned, and immediately placed in the particular solution in which they were examined. The tendon was removed from the tail, and single tendon fibers (on the order of 150 μm in diameter) were isolated while bathing in the medium. All measurements were made at room temperature (20-22°C) within one hour after the mice were sacrificed. It was determined that no measurable change in the velocity of the tendon thread occured between five minutes and 24 hours for the tendon thread sitting in the appropriate bathing medium, thus suggesting that the threads had reached equilibrium with the solution rapidly. Also, it had been shown that drying the tendon thread in air and rehydrating it did not affect significantly either the thread diameter or its propagation speed in normal saline [4].

Aqueous solutions of sodium chloride and glycerin were used as reference media. The velocity of each medium as a function of concentration (Table I) was determined with the acoustic microscope, and it compared favorably with the literature [10]. Additionally, the speed of sound was determined for aqueous solutions of sodium chloride over the applicable concentration range with a modified Pellam and Galt pulse technique [11], which has an accuracy within 1 m/s. Aqueous glycerin solutions were too lossy to utilize this pulse technique. Based upon previous work [4], it was expected that the collagen thread velocity would fall within the range of velocity values represented by these solutions.

RESULTS AND DISCUSSION

Figs. 1 and 2 demonstrate the relationship of the tendon thread velocity as a function of aqueous solutions of sodium chloride and glycerin, respectively. The velocity ranges shown represent the extreme for at least ten thread measurements and, in some cases, as many as fifty thread measurements. Mean value, standard deviation, range of the ultrasonic speed, and number of threads for each of the different bathing media are listed in Table II.

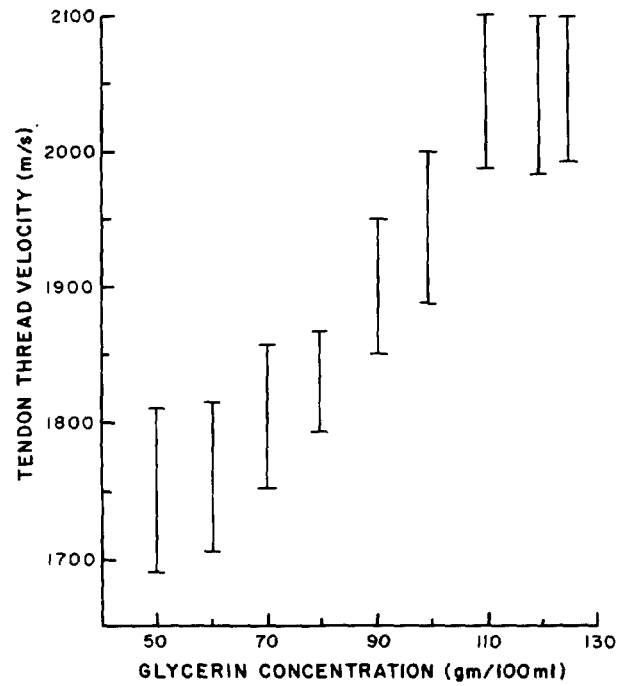

Fig. 2. Tendon thread velocity as a function of glycerin concentration.

Fig. 3 compares the velocity of the tendon thread with that of the respective reference media. The data suggest that if higher solute concentrations were possible, the thread and bathing medium velocities could not be made

TABLE II
ULTRASONIC SPEED OF TENDEN THREADS AS A FUNCTION OF SODIUM CHLORIDE AND GLYCERIN BATHING MEDIA CONCENTRATIONS

Concentration (g/100 ml)	Mean Speed (m/s)	Standard Deviation (m/s)	Speed Range (m/s)	Number of Threads
		NaCl		
0.9	1631	36	1550-1710	50
5	1650	31	1575-1730	25
13	1745	27	1690-1800	22
15	1760	23	1700-1820	17
20	1827	26	1750-1910	23
25	1875	25	1790-1950	18
30	1932	22	1880-1980	12
32	1940	45	1830-2050	14
		Glycerin		
50	1755	27	1700-1810	20
60	1760	30	1712-1812	13
70	1800	35	1751-1855	17
80	1831	21	1792-1862	15
90	1904	24	1850-1950	11
100	1943	31	1885-2000	10
110	2041	28	1985-2100	13
120	2039	30	1980-2100	15
125	2043	33	1990-2100	12

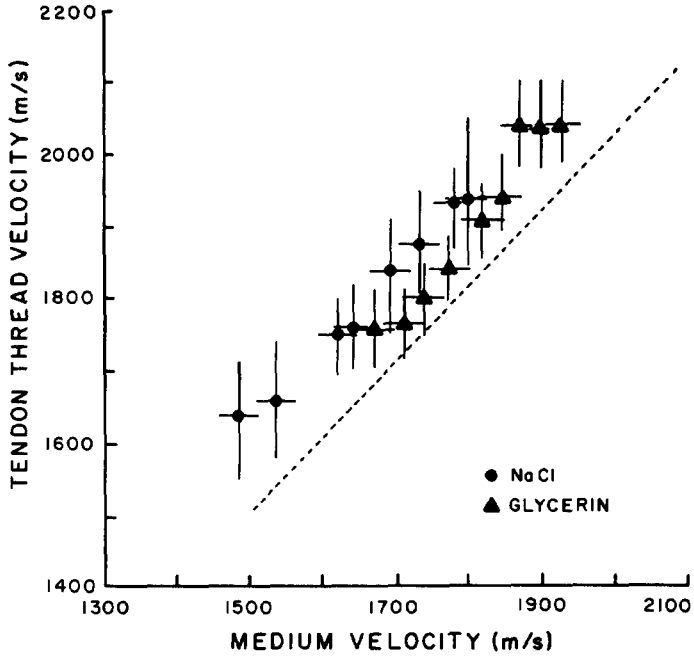

Fig. 3. Tendon thread velocity as a function of reference media velocity. The dotted line is where reference and media velocities are equal.

equal. Higher solute concentrations are not possible because the greatest concentration of the sodium chloride was a saturated solution and the greatest concentration of glycerin was of pure glycerin.

Fig. 4 graphically represents the velocity of the tendon thread as a function of the bathing media osmolarity. A partial dependence of the thread's velocity on the molecular particle concentration is suggested from this figure. Two possible mechanisms based upon thread permeability to the solute molecules are suggested. First, if the tendon

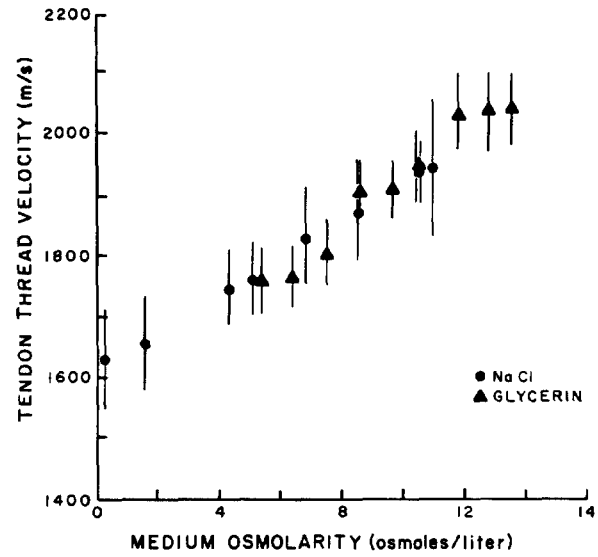

Fig. 4. Tendon thread velocity as a function of reference medium osmolarity.

thread is impermeable to the solute molecule (either sodium chloride or glycerin) and the osmotic concentration of the reference medium is greater than that of the thread, then there will be a movement of water molecules out of the thread. This means that as the concentration of the reference solution is increased, the water content of the thread is decreased. If, on the other hand, the thread is permeable to the solute molecules, these molecules will displace water molecules as they diffuse passively into the thread, also resulting in a decrease in water concentration.

Both of these mechanisms are supported by a previously expressed view [12] that for biological tissues there is a generalized trend toward an increase in ultrasonic velocity as tissue water content decreases. These suggested mechanisms also raise the question of the degree of accuracy for determining the density of biological tissues by using a float/no float test in salt solutions with known specific gravities, as described by others [13]. This particular method involves placing the tissue specimen in a solution with different and gradually increasing concentrations of salt until a concentration is found at which the tissue floats. The tissue density is then considered to be the same as the salt concentration. However, if the propagation speed through the tissue changes in relation to the solution in which it is observed, then perhaps changes in density also occur, and the validity of this method is brought into question.

This study further points out the importance of a complete understanding of tissue preparation procedures and their possible effects upon measuring the properties of ultrasonic propagation.

ACKNOWLEDGMENT

Appreciation is extended to Ms. Valerie M. Maynard for performing some of the pulse technique velocity measurements.

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Charles A. Edwards, photograph and biography not available at the time of publication.

William D. O'Brien, Jr., for a photograph and biography see page 25 of the January 1985 issue of this TRANSACTIONS.