EXAMINATION OF MAMMALIAN TENDON WITH ACOUSTIC MICROSCOPY USING VARIOUS REFERENCE MEDIA

BY

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Thesis

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Chapter 1 - Introduction

A. Introduction

Traditionally, biological systems and materials have been studied using radiographic imaging on the macroscopic level or optical and electron microscopy on the cellular and subcellular levels. Biochemical studies provided complementary information as to what was occurring in the biological system at the molecular level. The application of ultrasound as a diagnostic imaging technique for biological systems led to the realization of the necessity for basic research focusing on the mechanical properties of biological materials and systems.

Ultrasonic waves are produced by either a piezoelectric or ceramic source. These waves, when directed toward a specimen, may be either reflected or transmitted at the interface between the specimen and the medium coupling the specimen to the ultrasonic source. Either the reflected or transmitted wave may be processed into an ultrasonic image.

Ultrasonic imaging has seen extensive clinical application in obstetrics. Typical information available from ultrasonic imaging might include, fetal position, fetal age, and information on any fetal abnormalities in development. In addition, ultrasonic examination has been used in gynecology, cardiology, and abdominal studies[1].

The mechanical properties of a biological material are largely responsible for the contrast of an ultrasonic image. Characterization of a material, by measurement of its' acoustical properties, allows classification of the material in accordance with these properties. In particular, the following parameters are important:

1. Velocity of propagation of sound through a material,
2. Attenuation of sound in the material,
3. Characteristic acoustic impedance of the material, and
4. Bulk modulus of elasticity of the material.

All of these properties, to some degree, will determine the quality of the image that is obtained in an ultrasonic examination.

The velocity of sound is the transit time for a sound wave to travel through a material. The attenuation is a measure of the difference of the sound wave amplitude as it travels from one point to another in a material. Attenuation is the sum total of loss due to heating and loss due to redirection of the sound wave by scattering, reflection, and refraction. The velocity of propagation can be considered to be independent of frequency while attenuation has an approximately linear dependence on frequency.

The characteristic acoustic impedance and bulk modulus of elasticity are both quantities which depend upon the material density (mass/volume) and velocity of propagation. The specific acoustic impedance of a material is defined as the ratio of the acoustic pressure (excess pressure due to the sound wave) to the particle velocity, the velocity at which an elemental volume of the specimen is displaced from its resting position by the sound wave. In general this is a complex quantity. The real part of the specific acoustic impedance is termed the characteristic acoustic impedance (Z) and is the product of the density (ρ) and the velocity of propagation (c):

$$Z = \rho c$$

The bulk modulus of elasticity is a measure of the difficulty of compressing a given material from its original volume to a smaller volume. In terms of the

density and velocity, the bulk modulus of the elasticity (B) is:

$$B = \rho c^2$$

Fields and Dunn[2] suggested that it was the collagen content of tissues which was responsible for the tissues' echographic visualizability, collagen being a major structural protein of the connective tissue. Support for this theory came from the fact that collagenous fibers have a low frequency elastic constant which is a magnitude of three times greater than most soft tissue. In addition, O'Brien[3] has shown that to a first approximation, mathematical relations can be derived relating collagen content of a tissue to the tissue's ultrasonic velocity and attenuation coefficient. Collagen is the primary structural protein of the body, comprising more than one-third of the total body protein[4]. The collagen fibers bind separate cells into tissue and tissues into organ systems. By understanding the mechanical properties of collagen it should be possible to gain a basic understanding of the diagnostic image produced by an ultrasonic scan.

This particular study examines the ultrasonic properties (velocity and characteristic acoustic impedance) of a collagen thread through the use of acoustic microscopy. This was not possible until recently because the resolution required to make these measurements on a single thread was not available. The scanning laser acoustic microscope (Sonomicroscope 100 ®, Sonoscan Inc., Bensenville, IL) utilizing an ultrasonic frequency of 100 MHz is able to achieve a resolution of 20 μm in biological media. The specimens examined were mammalian tendon threads (diameter ≈150 μm). Tendon is approximately 30% collagen wet weight and 70% dry weight[5]. The collagen fibers which make-up tendon lie in a parallel arrangement next to each other along the length of the tendon. Because

of the high collagen content and general orientation of the collagen fibers in tendon, it is reasonable to assume, that, to a first approximation, the ultrasonic properties of collagen are essentially the properties measured in tendon.

A previous study[6] examined the collagen fibers bathed in physiological saline (0.9%). Acoustic microscopy relies on the acoustic properties of the medium in which the specimen is bathed for determining the properties of the specimen. For this reason, the present study varied the bathing media to check the consistency of the previously published results. Another goal was to obtain velocity and impedance matches between the bathing media and the collagen thread. A match implies that the medium velocity of characteristic acoustic impedance is equal to that of the bathing media and problems inherent in the microscopic measuring technique be reduced. These problems will be discussed later. Thirdly, it is generally believed that single isolated tendon threads are acoustically homogeneous, i.e., the acoustic velocity and density are homogeneous throughout the thread. This assumption is examined as well. Finally, the present study examines what effect, if any, the surrounding media may have on the acoustic properties of the specimen.

Chapter 2 - The Acoustic Microscope

A. General Theory of Operation

The acoustic microscope provides information about the elastic properties of a tissue. Its general operation is pictured schematically in Figure 1. The transducer sonicates the specimen imparting a particle displacement to it. The particle displacement in any particular region of the specimen is proportional to the amplitude of the sound field in that particular region. A plastic coverslip which has a thin gold film on one side is placed on top of the specimen. The coverslip is partially transparent to light. The coverslip also is displaced by a magnitude proportional to the sound field in any particular region. A laser beam scans the coverslip. Part of the laser light passes through the coverslip and specimen to a photodetector below the stage. The light which passes through the tissue is processed into an optical image. Because of the gold film on the coverslip, the remaining light is reflected. Since there is a particle displacement in the coverslip, and since the particle displacement amplitude varies with the sound wave amplitude in any particular region of the coverslip, the angle at which the light beam is reflected in a particular region is proportional to the particle displacement and, therefore, the sound wave amplitude in that region. The reflected light falls on a knife edge which partially covers a second photodetector. Depending on the angle of reflection, more or less of the light will be shadowed from the photodetector by the knife edge. By this means, the acoustic image is produced on a television monitor. This particular image is an acoustic transmission image. In addition there is an interference mode of operation. The interference mode is created by combining a reference signal with the acoustic signal which has been transmitted through the specimen. This causes constructive

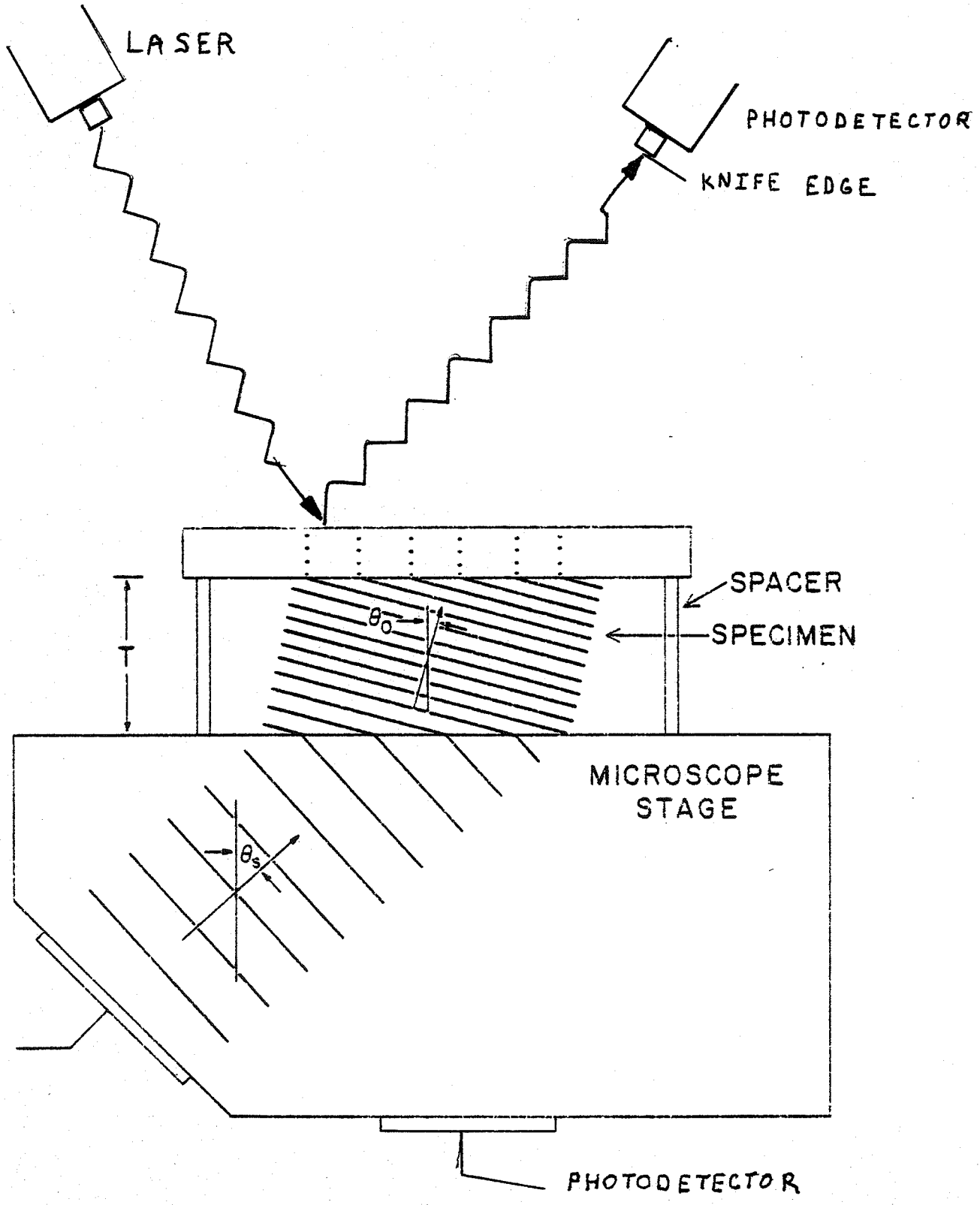


Fig. 1- Schematic of Microscope Stage

and destructive interference, creating a set of interference lines on the television monitor. The shift of these lines in any particular region of the specimen as compared with the surrounding medium is proportional to the delay in the sound due to different sound velocities in the different regions. With this mode, localized sound velocity measurements can be made. Photographs of each mode are shown in Figure 2 for collagen threads in normal saline.

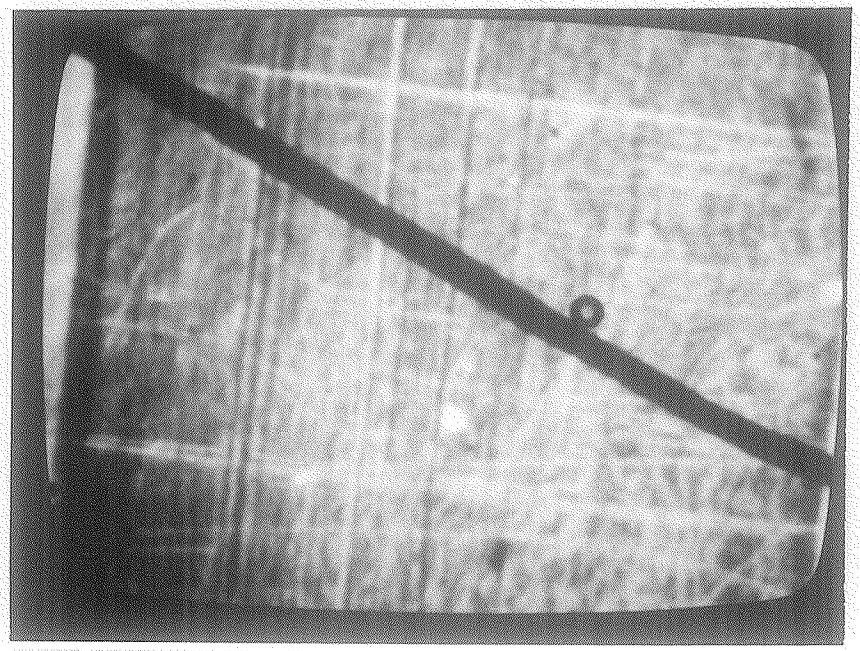
B. Ultrasonic Velocity Measurements

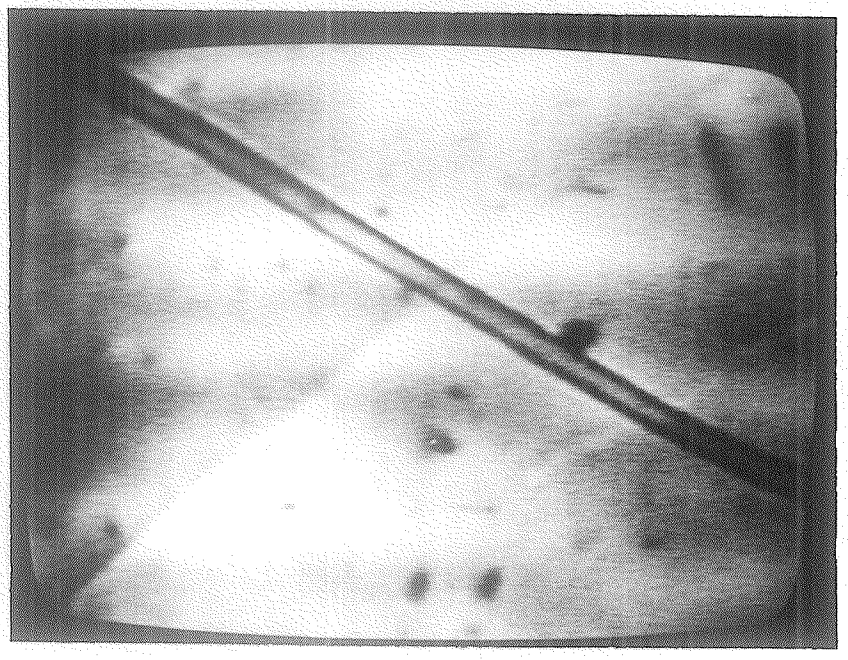
The following has been described elsewhere[6], and is provided here, with greater detail, to explain the method used in obtaining ultrasonic velocity measurements using the Sonomicroscope 100. Reference to Figure 3 will assist in understanding the text which follows.

The stage has a known acoustic velocity of c_s. The angle at which the sound enters the stage is θ_s, and the distance between wavefronts is λ_s, one wavelength of the acoustic wave in the stage. On the stage is a specimen with an unknown ultrasonic velocity (c_x) which is bathed in a liquid medium of known ultrasonic velocity (c_o). At the interface between the stage and the liquid-specimen the following is true according to Snell's Law:

$$\frac{\sin\theta_s}{c_s} = \frac{\sin\theta_x}{c_x} = \frac{\sin\theta_o}{c_o} \qquad (1)$$

where θ_x and θ_o are the angles of transmission of sound in the specimen and liquid bath, respectively. Note that though there is some reflection of sound at this interface, in the figure it is not represented, as it has no real importance to this discussion. Note also that since the frequency of the acoustic wave does not change as it passes through various media, the wavelength does by





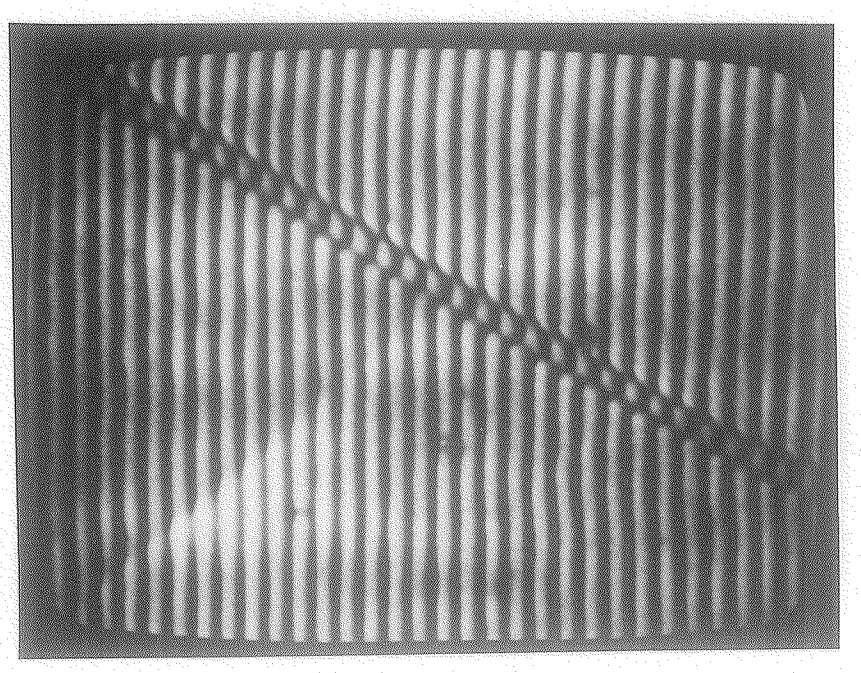


Figure 2. Acoustic Microscope Operational Modes

a) Optical

b) Acoustic Transmission

c) Acoustic Interference

Specimen is mouse tendon bathed in physiological saline

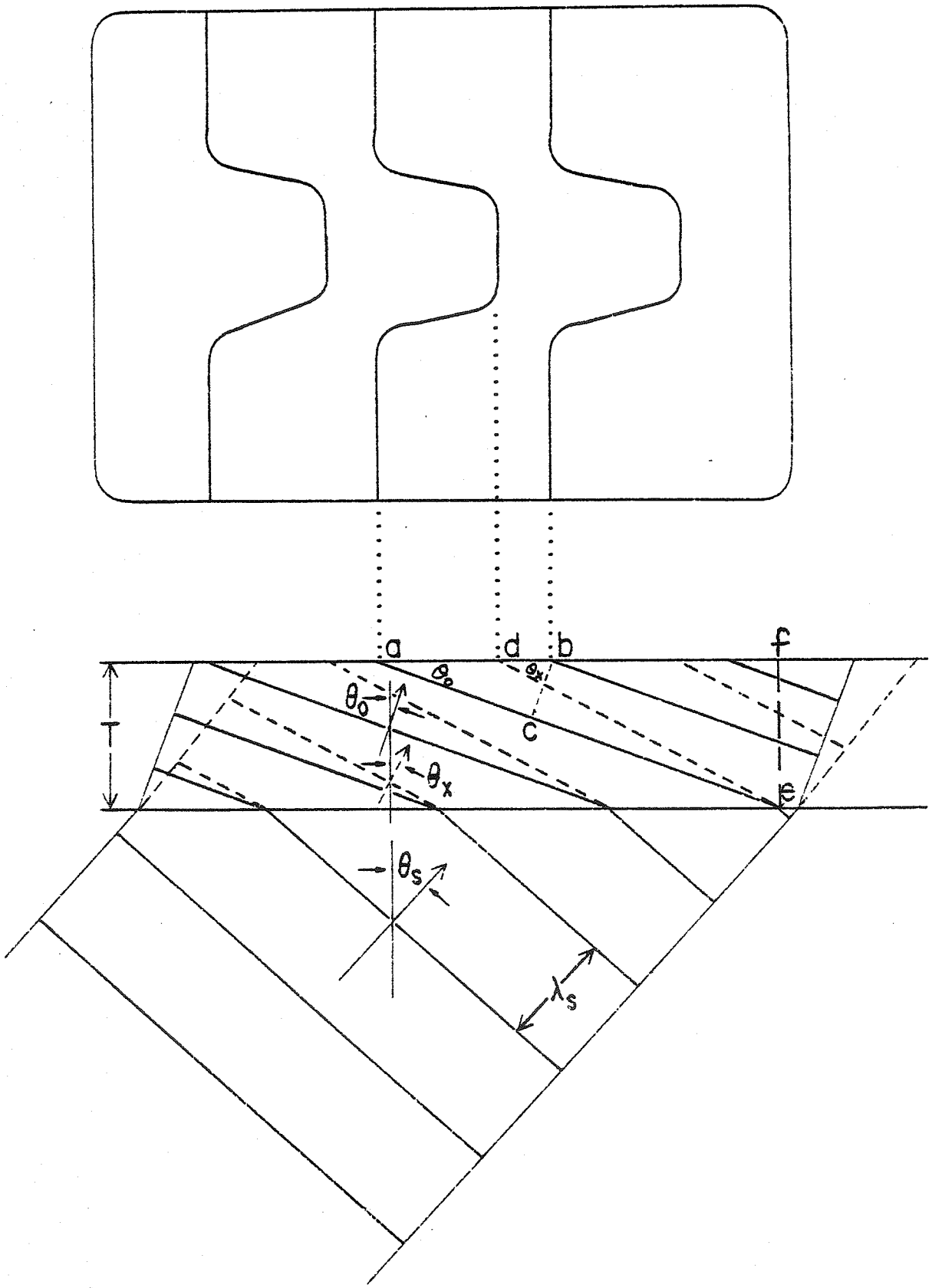


Fig. 3 Schematic showing relation of acoustic wave geometry to interferogram.

the following relation:

$$f = \frac{c}{\lambda}$$

where f is the acoustic frequency, c is the ultrasonic velocity and λ is the wavelength of the acoustic wave in the material. Using this relation we find that the wavelength of sound in the liquid bath and the specimen, and therefore, the distance between wavefronts in each, is λ_o and λ_x, respectively. The sound propagates through the liquid and the specimen and it incident on the coverslip at angles of θ_o and θ_x, respectively. There is again reflection and transmission at this interface, which is not shown since unrelated to the discussion.

The relationship between the wavefronts in the liquid and sepcimen to the interference lines on the television monitor is as shown in Figure 2. Assume that the specimen thickness is T. The normalized lateral fringe shift (N) is defined as:

$$N = \frac{\text{line segment ad}}{\text{line segment ab}} \qquad (2)$$

This quantity can be restated in terms of the parameters of the acoustic waves of the stage, liquid bath, and specimen. Note that the angle cab is θ_o, the angle between the wavefront of the acoustic wave travelling through the liquid bath and the stage. Using the definition of sine on the triangle abc results in:

$$\sin\theta_o = \frac{cb}{ab}$$

where $cb = \lambda_o$, the distance between wavefronts in the liquid bath. Rearranging:

$$ab = \frac{\lambda_o}{\sin\theta_o} \qquad (3)$$

Also:

$$ad = af - df \tag{4}$$

The line segment af can be obtained by using the definition of tangent on the triangle aef. Note that the angle fae is equal to the angle cab, or θ_o. Therefore:

$$\tan\theta_o = \frac{ef}{af}$$

but ef = T. Rearranging:

$$af = \frac{T}{\tan\theta_o} \tag{5}$$

The angle fde is equal to θ_x, the angle between the specimen wavefront and the stage. Using the definition of tangent on the triangle def as before yields:

$$df = \frac{T}{\tan\theta_x} \tag{6}$$

Substituting equations (5) and (6) into equation (4):

$$ad = \frac{T}{\tan\theta_o} - \frac{T}{\tan\theta_x} \tag{7}$$

and substituting equations (3) and (7) into equation (2):

$$N = \frac{\sin\theta_o}{\lambda_o}\left|\frac{T}{\tan\theta_o} - \frac{T}{\tan\theta_x}\right|$$

Rearranging the equation:

$$\theta_x = \tan^{-1}\left|\frac{1}{\frac{1}{\tan\theta_o} - \frac{N\lambda_o}{T\sin\theta_o}}\right| \tag{8}$$

Rearranging equation (1):

$$c_x = \frac{c_o}{\sin_o} \quad \sin\theta_x$$

and substituting for θ_x from equation (8):

$$c_x = \frac{c_o}{\sin\theta_o} \cdot \sin\left\{\tan^{-1}\left|\frac{1}{\frac{1}{\tan\theta_o} - \frac{N\lambda_o}{T\sin\theta_o}}\right|\right\} \tag{9}$$

The velocity of the unknown specimen is now in terms of known or measurable quantities.

Note that if N is equal to zero in equation (9), $c_x = c_o$. This means that if there is no lateral shift in the interference lines, then the velocity of the specimen and surrounding medium are equal. Note also that in this case the velocity measurement is independent of specimen thickness. The measurement of specimen thickness represents perhaps the largest single error in velocity measurement with the acoustic microscope. If a reference medium has a known ultrasonic velocity and the medium is such that there is no detectable shift in the interference lines, then the error due to the precision of the thickness measurement of the specimen is reduced.

C. Theory of Ultrasonic Impedance Measurements

The contrast in an acoustic image, as mentioned before, is due to a mismatch in the acoustic impedances of the materials being studied. In the case of the acoustic transmission image of the microscope the impedance mismatch is between the specimen and the surrounding medium. If the surrounding medium is changed such that the characteristic acoustic impedances of the medium and specimen are

are closer to each other, then a reduction in the contrast of the acoustic image would be observed. If a perfect impedance match were obtained, then there should be no contrast between medium and the specimen. This assumes that the specimen is homogeneous with respect to the acoustic parameters, ultrasonic velocity, and density.

Chapter 3 - Experimental Procedures

A. General Procedures

The tendon threads examined in this experiment were surgically removed from the tails of four to five month old female LAF_1/J mice (Jackson Labs). The mice were sacrificed by cervical dislocation and the tails removed, skinned, and immediately placed into either normal saline or the medium in which they were to be examined. The tendon was removed from the tails and single tendon fibers (on the order of 150 μm in diameter) were isolated while bathed in the medium. A single tendon fiber was placed on the acoustic microscope slide in the orientation shown in Figure 4. The microscope slide is anologous to a glass slide used in optical microscopy in that it is essentially transparent to the sound wave as glass is to light. The dark line across the slide acted as a reference so that the area examined under the acoustic microscopy was the same area in which the thickness was determined using light microscopy.

The thickness of the specimen was determined by assuming that the thread was a cylinder and measuring the diameter using a calibrated ocular micrometer. Since this assumption is not perfectly true, a range of diameter measurements are made along the length of the marked region on the slide.

The specimen was then placed on the stage of the acoustic microscope and a doughnut shaped spacer placed around it to prevent crushing by the coverslip which was placed over the specimen. The area of the thread crossed by the reference line was then located with the optical image monitor and optical, acoustic transmission, and acoustic interference images were observed. Photographs of each image were taken as a permanent record. During the entire procedure, care was taken so as not to allow the specimen to dry out. However, Goss and O'Brien[6]

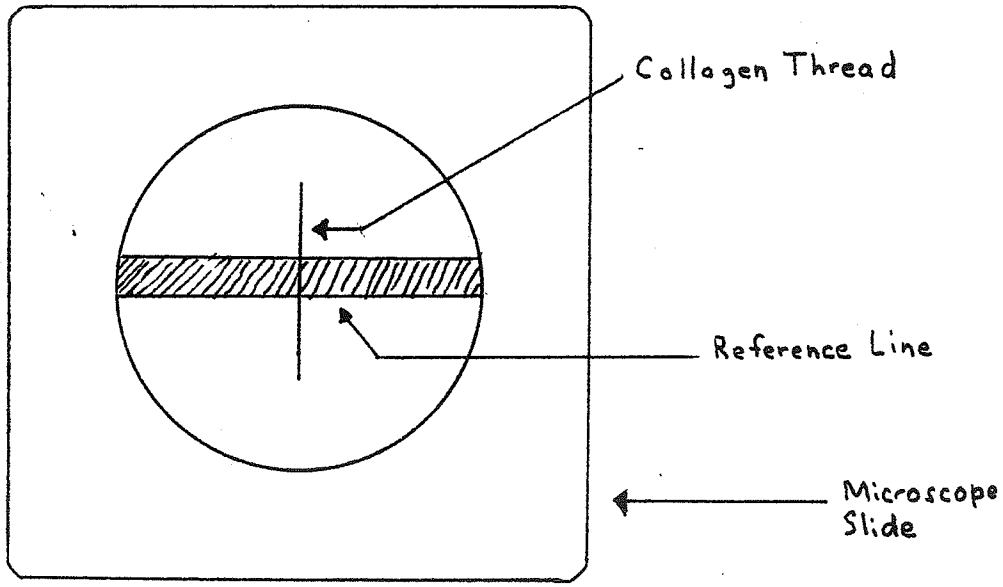


Figure 4. Schematic of microscope slide showing orientation of collagen thread with respect to the reference line

have shown that drying and rehydrating has no significant effect on the collagen thread velocity.

B. Discussion of Media Used

Two different media at various concentrations were used as the reference media for microscopic examination. Aqueous solutions of sodium chloride were initially used since the previous study[6] utilized normal saline (0.9%). In addition, aqueous solutions of glycerin were used because of their high ultrasonic velocity (pure glycerin ≈ 1926 m/s). The velocity and characteristic acoustic impedance of the solutions were changed by varying the concentration of solute (either sodium chloride or glycerin). For sodium chloride the concentration varied from 0.9% to 31% (saturated). The glycerin concentration was varied from 50% to 125%. For both the sodium chloride and glycerine, N% implies that the solution consists of N grams of solute per 100 ml of total solution.

C. Measurement of Reference Media Velocity and Characteristic Impedance

The sound velocity of each reference media was determined in the following way. A primary standard media was made of a saturated sodium chloride solution. The velocity of propagation of this solution was determined by an independent measuring system known as the high frequency system. Details of this system are described elsewhere[7]. This primary standard was used to determine the ultrasonic velocity of a piece of polyethylene, of known thickness, with the acoustic microscope. The polyethylene became a secondary standard and was used to determine the ultrasonic velocity of each of the reference media. The characteristic acoustic impedance was determined by finding the density of each solution in a handbook[8] and calculating the impedence using the measured velocity. Graphs of solution velocities and impedances appear in Figures 5, 6, and 7.

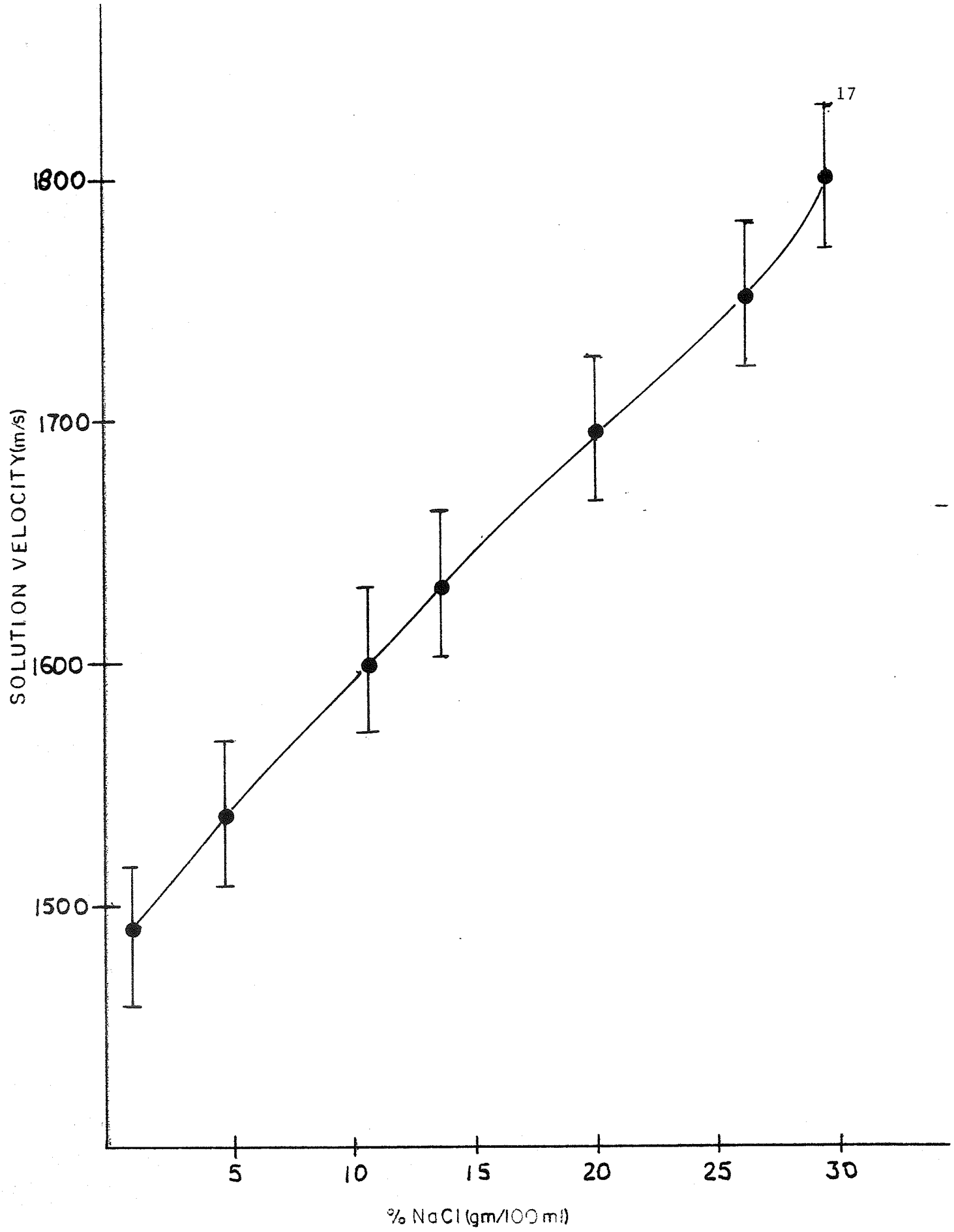


Figure 5. A plot of velocity vs. concentration for sodium chloride

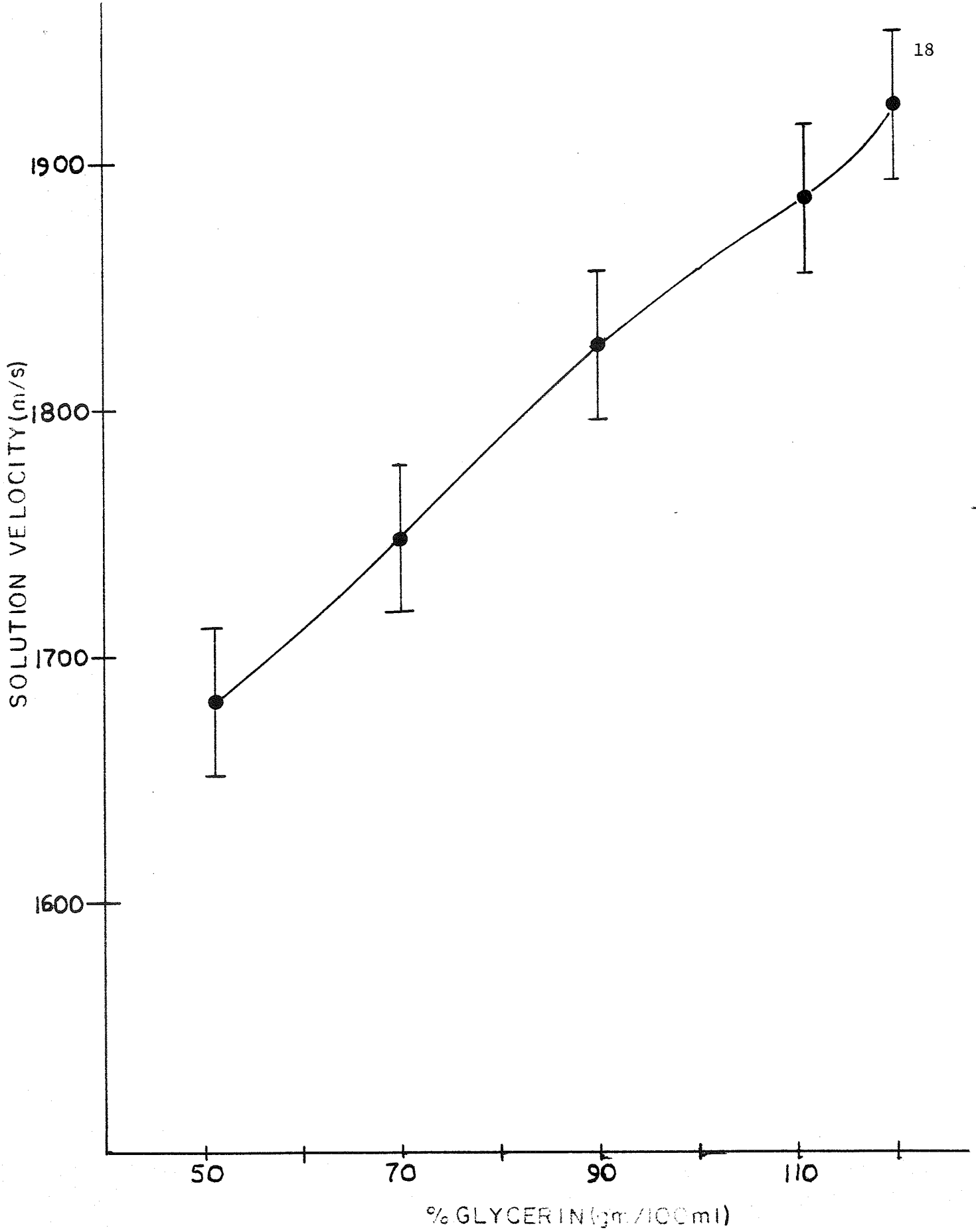
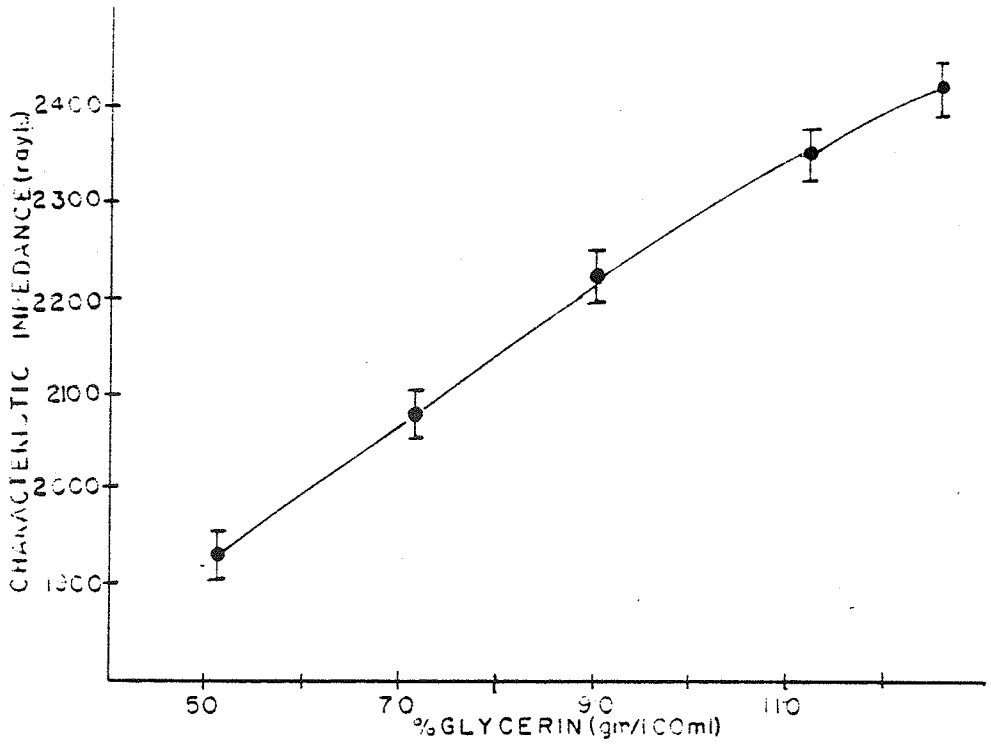


Figure 6. A plot of velocity vs. glycerin concentration



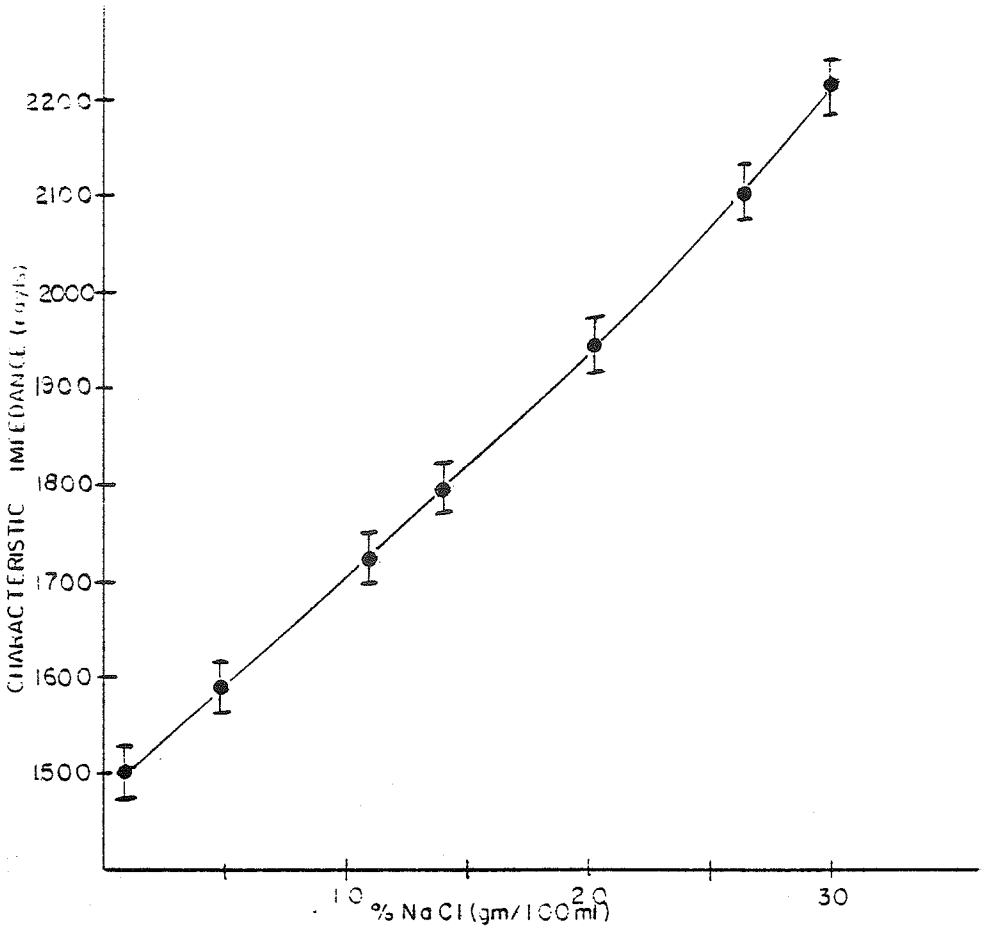


Figure 7. Plots of characteristic acoustic impedance vs. sodium chloride and glycerin concentrations

Chapter 4 - Results and Discussion

A. Results

Two particular studies were done. The first study involved taking a single thread and subjecting it to a number of different reference media which had the same range of velocities as that determined by Goss and O'Brien[6] for collagen threads in normal saline. Table I summarizes the results for two threads examined in this way. The range of velocities for these two threads is 1705-2027 m/s. The mean velocity is 1881 m/s and the standard deviation is 50 m/s.

For the second study, a number of threads were examined at each of a number of concentrations of sodium chloride (0.83% → 30%) and glycerine (50% - 125%). Each thread was examined with only one reference medium. The velocity results for the threads examined in this way are summarized in Table II. The range of velocities vs. concentration is plotted for the sodium chloride and glycerine solutions in Figures 8 and 9, respectively. Figures 10 and 11 are plots of velocity difference (C THREAD - C REFERENCE) vs. concentration. A plot of collagen velocity vs. solution velocity is found in Figure 12. Finally, a plot of collagen velocity vs. osmolarity is found in Figure 13.

B. Error Analysis

While there are several possible errors which could affect the accuracy of velocity measurements made by acoustic microscopy, these are reduced to a minimum by proper calibration of the instrumentation. Examples of these include calibration of the acoustic microscope's center frequency and calibration of the optical microscope's ocular micrometer. Random error affecting the precision of the measurements is the more significant problem with acoustic microscopy. These

TABLE I. Summary of Ultrasonic Velocity Data of Two Mouse Tail Tendon Fibers at 100 MHz Using Various Media for Reference Velocity

SAMPLE NO.	MEDIUM %(g/100 ml volume)	C MEDIUM m/s	T (μm)	C THREAD (m/s)
83-124	75.0Gl[1]	1740-1790	114-134	1847-1947
	65.9Gl	1709-1759		1800-1900
	54.8Gl	1670-1720		1859-1959
	45.3Gl	1636-1686		1715-1815
	31.1Na[2]	1764-1814		1832-1932
	30.0Na	1755-1805		1867-1967
	29.1Na	1747-1797		1881-1981
	27.9Na	1737-1787		1857-1957
84.13F	31.1Na	1764-1814	159-189	1837-1847
	65.9Gl	1709-1759		1882-1892
	95.3Gl	1806-1856		1905-2005
	75.0Gl	1740-1790		1838-1938
	27.9Na	1737-1787		1841-1941
	29.1Na	1747-1797		1832-1932
	30.0Na	1755-1805		1837-1937
	31.1Na	1764-1814		1813-1913
	65.9Gl	1709-1759		1860-1960
	75.0Gl	1740-1790		1809-1909
	85.3Gl	1774-1824		1847-1946
	95.3Gl	1806-1856		1927-2027

1. Gl = glycerin
2. Na = sodium choride

TABLE II. Summary of Velocity Data at 100 MHz for Mouse Tail Tendon Fibers in Various Reference Media

SPECIMEN	MEDIUM %(g/100 ml volume)	C MEDIUM m/s	T (μm)	C THREAD (m/s)
91-5	0.83Na[2]	1468-1518	130-150	1593-1673
91-11			144-214	1522-1652
91-14			112-128	1624-1712
91-17			102-118	1632-1706
91-20			113-119	1633-1703
91-23			106-134	1544-1632
91-26			97-105	1613-1693
95-10			139-154	1579-1657
95-13			115-147	1557-1647
95-16			101-157	1577-1721
95-28			111-115	1636-1706
95-31			183-199	1553-1623
95-34			163-174	1530-1600
91-24	4.8Na	1511-1565	149-183	1592-1678
91-32			145-161	1615-1689
91-35			105-113	1675-1695
92-5			134-182	1634-1754
96-2	10.8Na	1574-1624	116-136	1681-1765
96-5			105-117	1724-1804
96-8			170-176	1682-1752
96-11			121-161	1668-1778
96-17			158-172	1637-1707
96-23	13.8Na	1605-1655	97-133	1757-1887
96-26			120-146	1716-1806
96-29			96-148	1710-1890
96-32			118-150	1741-1839
96-35			108-136	1762-1864

SAMPLE NO.	MEDIUM %(g/100 ml volume)	C MEDIUM m/s	T (μm)	C THREAD (m/s)
97-5	26.4Na	1724-1774	117-149	1883-2003
97-11			155-203	1823-1925
97-15			114-155	1870-2622
97-17			138-152	1952-2036
97-20	29.8Na	1774-1824	81-97	1901-1999
97-23			165-175	1858-1928
97-29			158-184	1860-1950
97-32			114-140	1960-2040
103-2	50.9Gl[2]	1656-1706	167-171	1728-1798
103-5			142-158	1618-1794
103-8			150-196	1698-1748
103-11			146-166	1870-1978
103-14	90.1Gl	1790-1840	102-112	1906-1980
103-17			127-143	1853-1931
103-20			158-169	1894-1999
103-23			119-169	1870-1878
103-26	125.7Gl	1898-1948	99-127	2017-2119
103-29			177-153	2033-2129

1. Na = sodium choride
2. Gl = glycerin

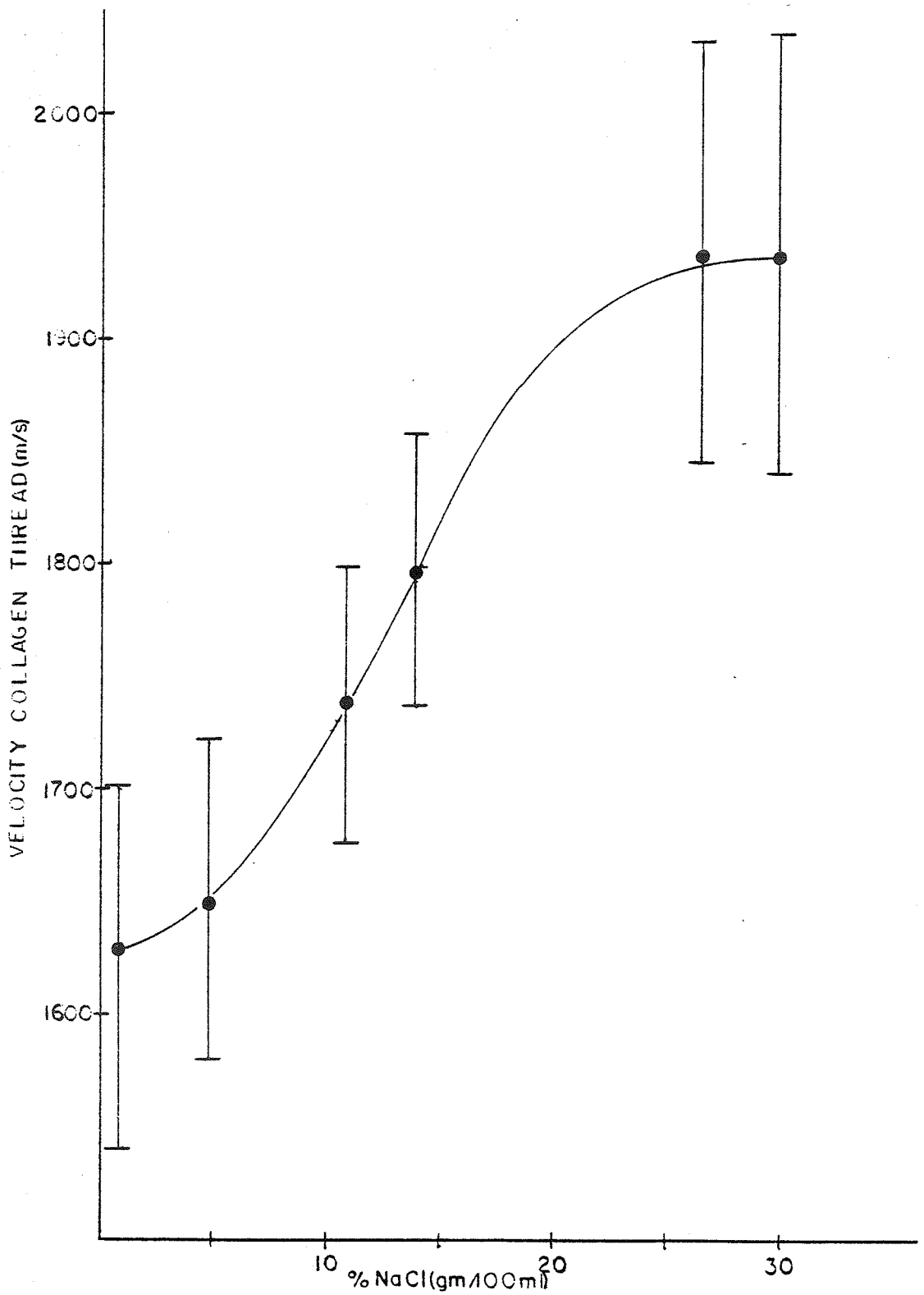


Figure 8. Collagen thread velocity as a function of sodium chloride concentration

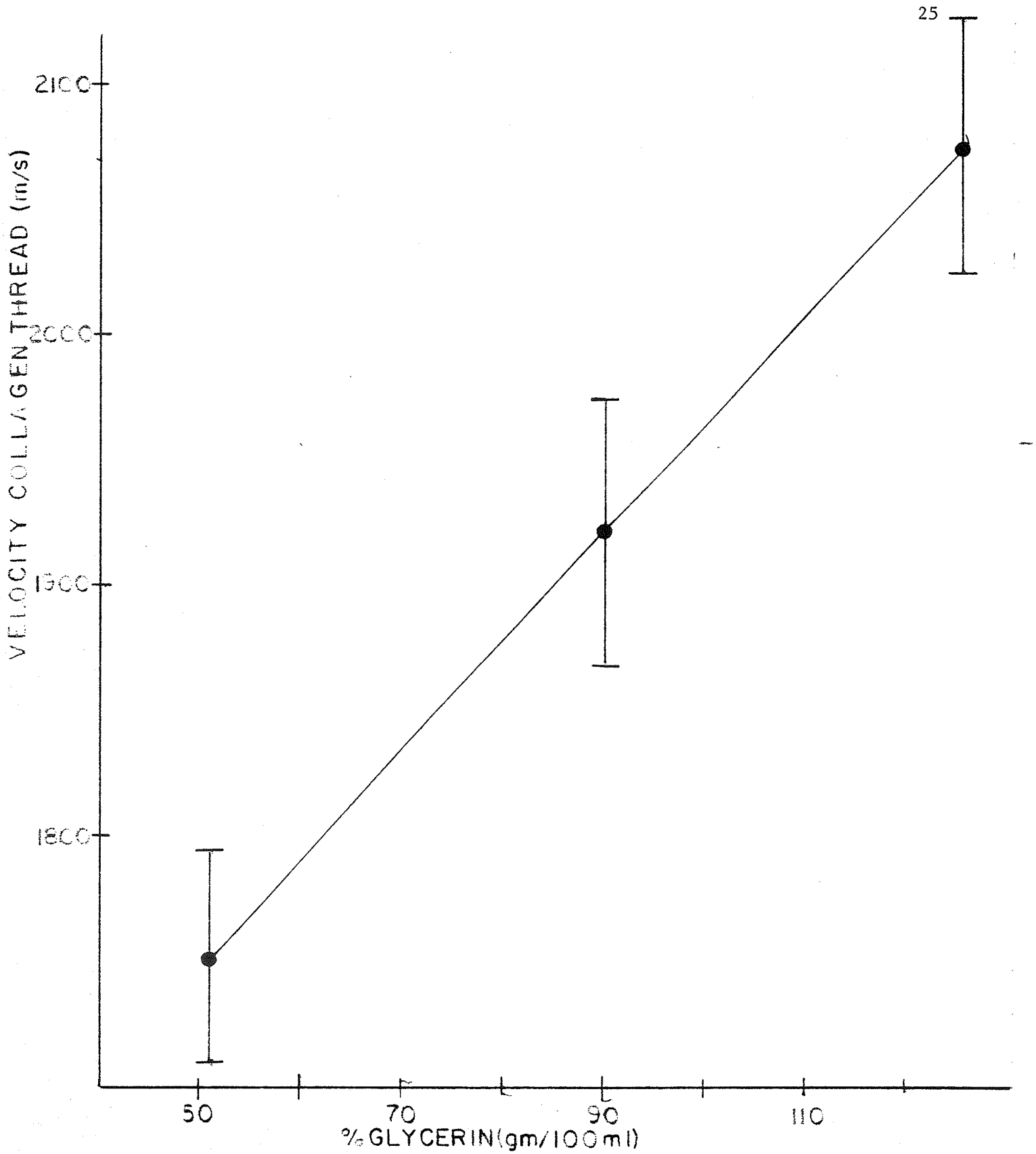


Figure 9. Collagen thread velocity as a function of glycerin concentration

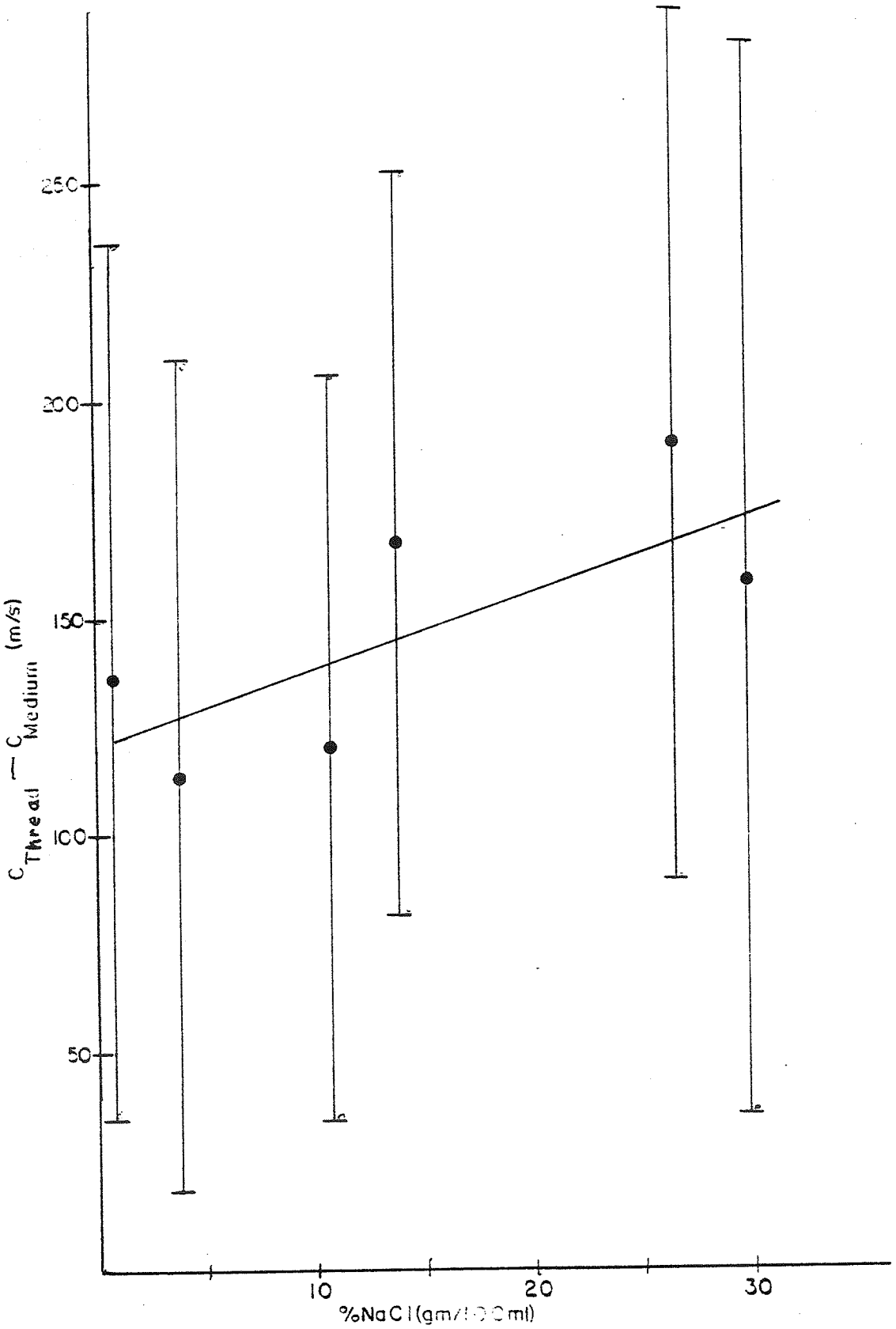


Figure 10. Velocity difference (C THREAD - C MEDIUM) as a function of sodium chloride concentration

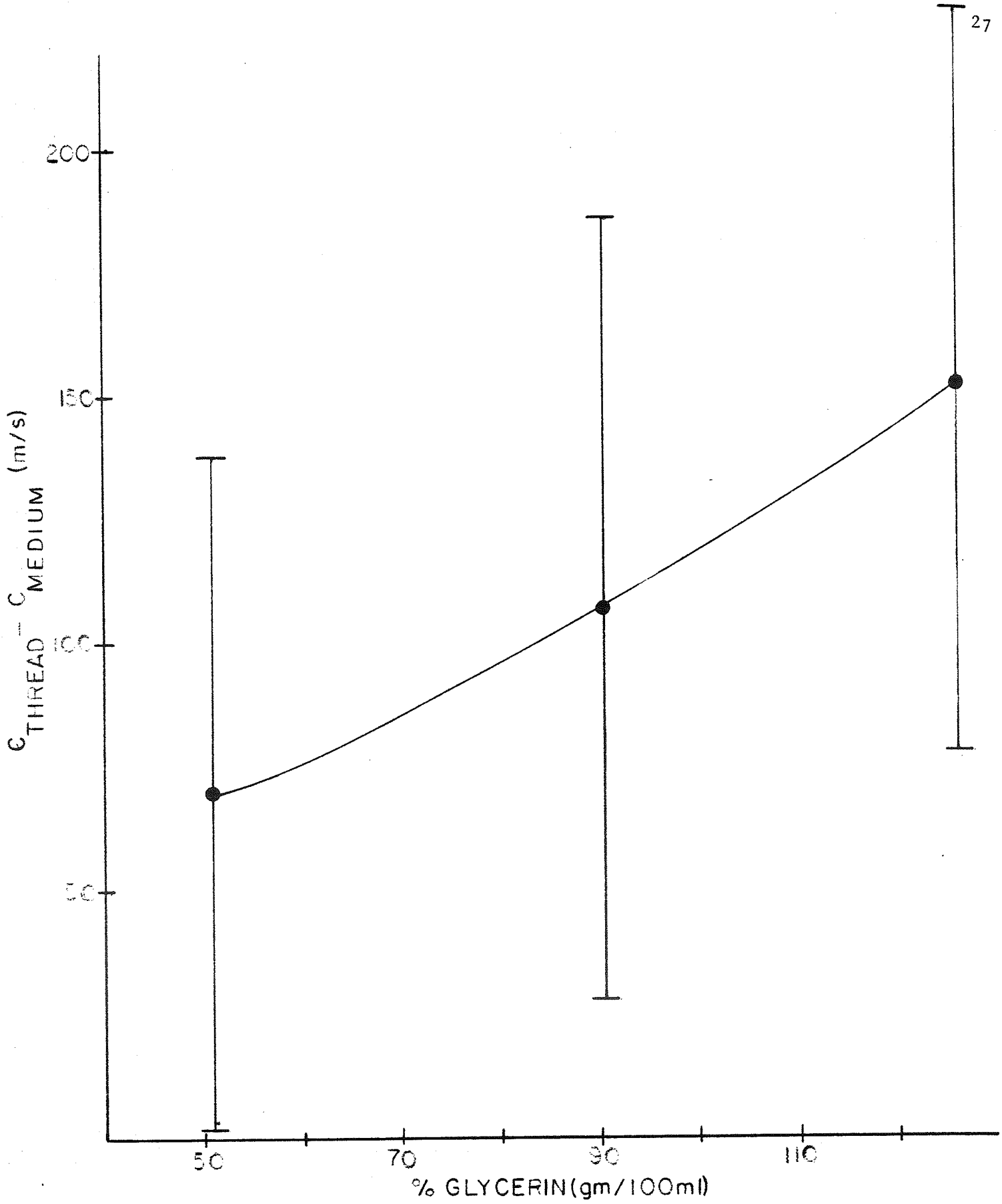


Figure 11. Velocity difference (C THREAD - C MEDIUM) as a function of glycerin concentration

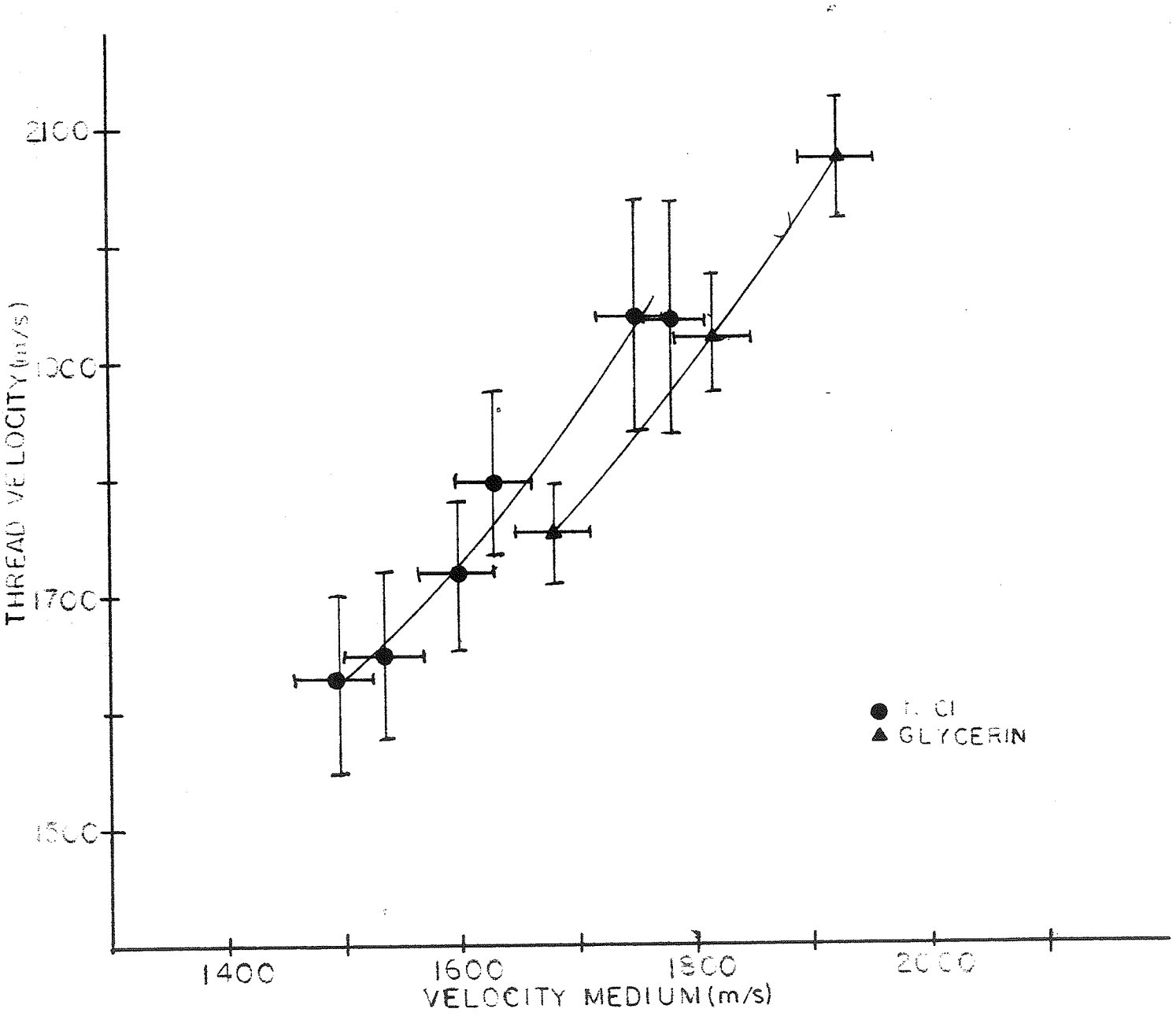


Figure 12. Collagen thread velocity as a function of the reference medium velocity

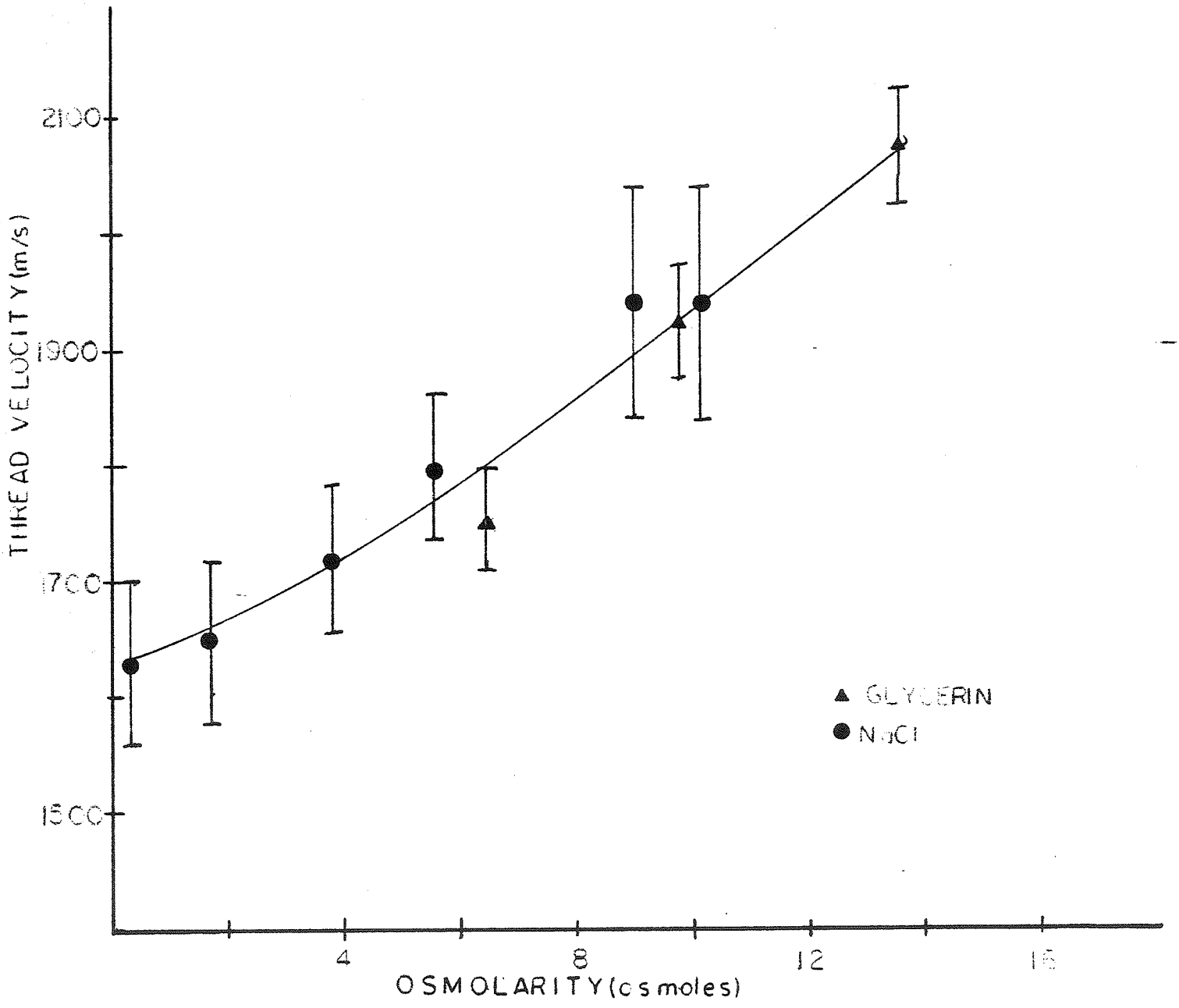


Figure 13. Collagen thread velocity as a function of the reference medium osmolarity

errors involve the subjectivity of the experimenter in making measurements of the normalized lateral fringe shift of the interference lines and the specimen thickness (see Chapter 2B). An example would best illustrate this. First, the velocity of the reference media is determined. Assume that the thickness of the polyethylene is a known value of absolute magnitude equal to 110 μm. The only precision error is then in the measurement of the lateral shift of the interference lines. A range of 0.1 mm in each of the line segments ab and ad results in a range of 0.2 normalized shifts. Using Chapter 2B, equation (9) this translates into a velocity range of 50 m/s. Assuming that the mean value of the reference is 1493 m/s, the range will be 1468-1518 m/s. For the collagen thread, the precision of the diameter must also be taken into account. Assume the diameter range is 20 μm. Again the lateral shift has a range of 0.2. In addition, the velocity of the known medium has range of 50 m/s. Using equation (9) again, the range of the collagen thread may be as much as 150 m/s. For simplicity, the normalized lateral fringe shift was taken to be an absolute value. This reduces the range by about 60 m/s to a range of 60-100 m/sec, depending on the actual diameter range.

C. Discussion

The mean value of the collagen threads presented in the first study (1881 m/s) is 8% higher than the value reported by Goss and O'Brien[6]. Given the experimental precision of the microscope, this value seems to be in fairly good agreement.

However, the second study casts doubt on whether this is actually true. Figures 8 and 9 indicate that there is a trend toward an increase in the collagen thread velocity as the solute concentration (either sodium chloride or glycerin)

is increased. The percentage increase over the total range for sodium chloride is approximately 15%. For glycerin the percentage increase is also about 15%. Graphically (Figure 8 and 9) it can be seen that, even taking into account the precision of each measurement, the trend toward this increase still exists. The velocity difference (C THREAD - C MEDIUM) plots shown in Figures 10 and 11 shed no light on the relation between the solution concentration and the increased velocity. Any number of relations can be drawn from these plots due to the large range of velocity difference values. However, the plot of solution velocity vs. thread velocity (Figure 12) does seem to shed some light. This plot implies that the sodium chloride effect on the collagen velocity was a little bit greater than that of the glycerin. This suggests that the reason for the velocity increase in the collagen thread is not due completely to a velocity increase in the solution but rather, a difference in the media themselves. This hypothesis is supported by the results shown in Figure 13 of collagen velocity vs. osmolarity. This data suggest that the reason for the velocity change is indeed due to the osmolarity of the solution. More data using glycerin solutions at concentrations not yet examined will assist in confirming this hypothesis.

The possible dependence of the collagen thread velocity on solution osmolarity suggests the following mechanism. First, assume that the thread is impermeable to sodium chloride and glycerin. By increasing the osmolarity of the medium surrounding the collagen thread, an osmotic gradiant is created. To reach an equilibrium state, water must flow from the thread, into the surrounding medium. This would imply that for a thread bathed in physiological saline, the velocity of sound is due to both the water content and the collagen and that when the water is removed, because of the osmotic gradient, the velocity measured is an actual collagen thread velocity. This is reasonable if we look upon removal of

the water as increasing the collagen content of the tendon fiber. It has been shown[3] that as the collagen content is increased for different tissues, there is also an increase in the velocity of sound propagation. This fact is in agreement with the hypothesis suggested.

Another explanation of the relation between collagen velocity and the reference osmolarity is also possible. In this case, the tendon thread is permeable to sodium chloride and glycerin. If this is true, then as the reference solution osmolarity is increased above normal saline, there will be a net diffusion of the solute (sodium chloride or glycerin) into the thread.

The velocity measurement from the thread would then be due to both the solute and the thread. This also is reasonable if the thread is looked at as being in a suspension. An increase in the osmolarity of the bathing solution, is in this case, an increase in the concentration (osmolarity) of the suspension. With an increase in concentration, a velocity increase is also expected.

Figures 14 and 15 are a compilation of acoustic transmission images for collagen threads in each of the media examined. For the sodium chloride solutions, there seems to be little difference in the image contrast. For the glycerine, the image contrast appears to be quite significant. This difference can actually be attributed to the high attenuation of the glycerin solutions which resulted in considerable loss of the acoustic signal.

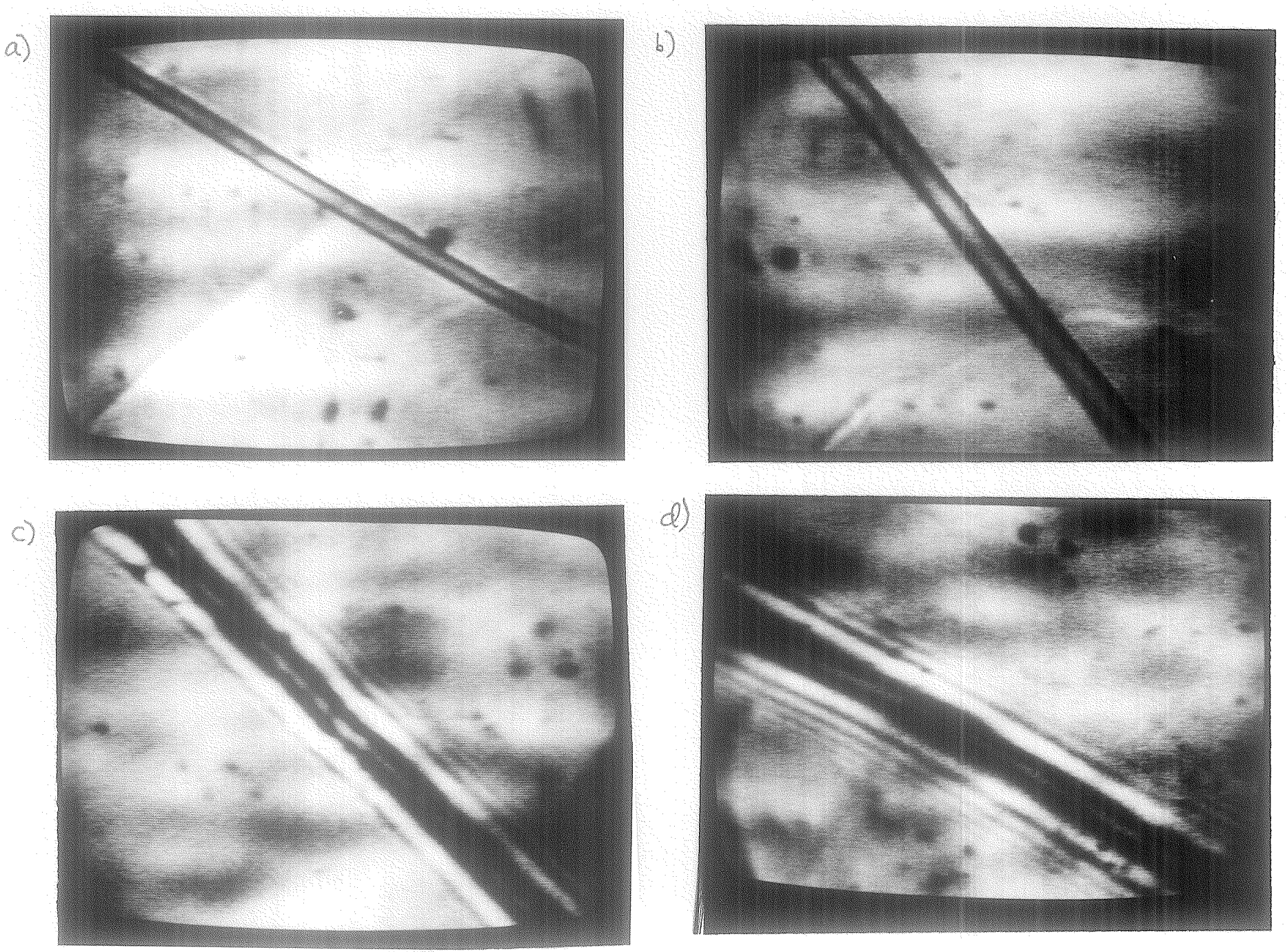


Figure 14. Acoustic transmission images for collagen threads in a) 0.83% NaCl b) 4.8% NaCl c) 13.8% NaCl d) 26.4% NaCl

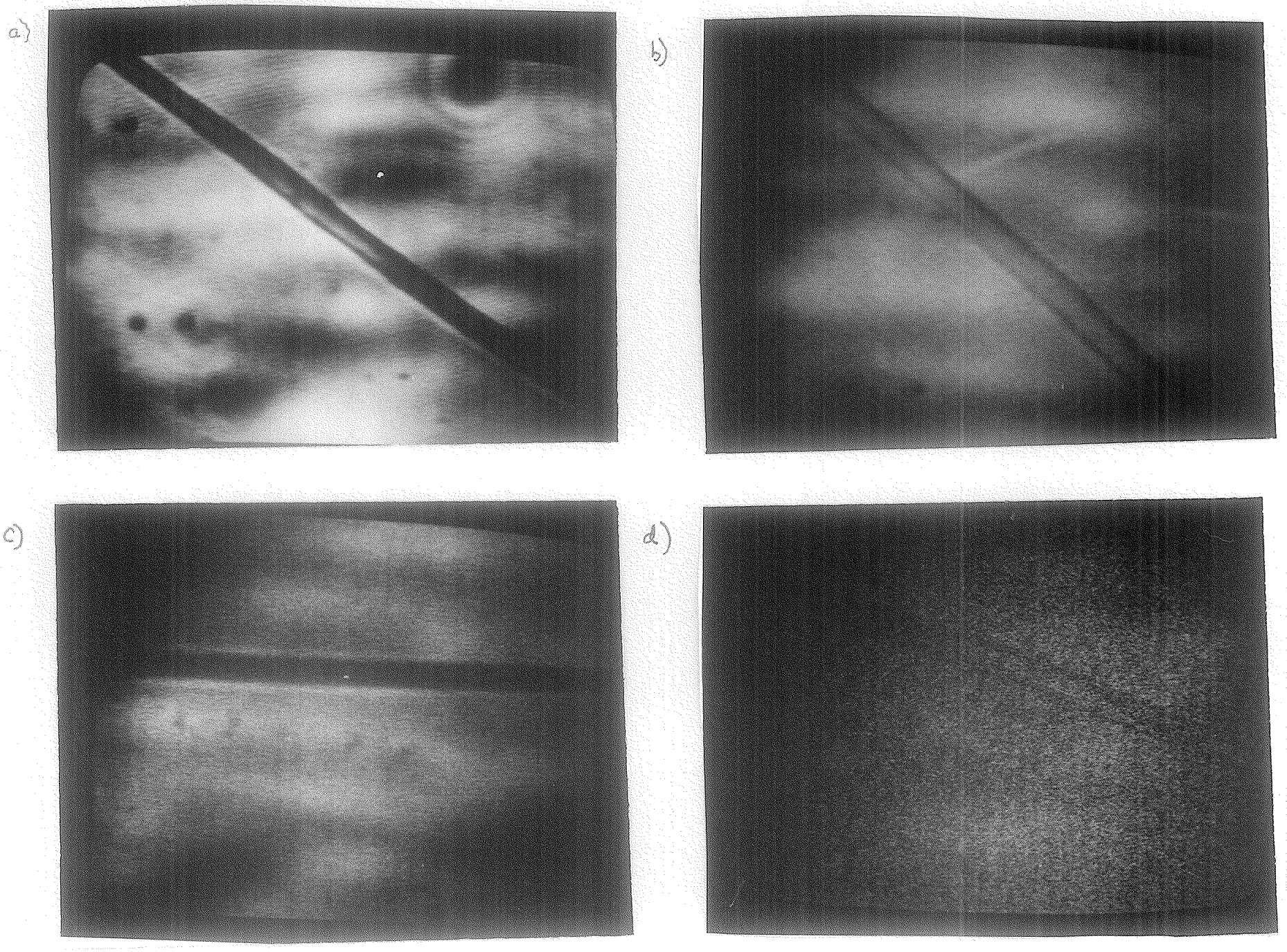


Figure 15. Acoustic transmission for collagen threads in a) 29.8% NaCl b) 50.1% Glycerin c) 90.0% Glycerin d) 125.7% Glycerin

Chapter 5 - Summary and Suggestions

A. Summary and Suggestions

The results of this study suggest that the collagen velocity varies with the osmolarity of the solution used as a reference medium. For this reason, a velocity and characteristic acoustic impedance match were not possible. Two possible mechanisms have been forwarded to explain the results obtained.

The results of this study suggest certain areas where more work is needed. The precision of the velocity measurements of the collagen thread may be increased if the reference media velocities were all determined by using the high frequency system. In addition, more data on the effects of glycerin on the thread velocity might help to clarify some of the trends seen in this study. Finally, a study should be done to determine what effect, if any, the solutions have on collagen thread diameter. If the thread diameter actually become smaller, this would suggest that the thread was impermeable to the solutes and the first possible mechanism presented would be confirmed. However, no change at all would be inconclusive as it may imply only that the thread is too structured to shrink.

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