THE ROLE OF COLLAGEN IN THE ULTRASONIC PROPERTIES OF TISSUE

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Ultrasonic absorption and velocity measurements were made in collagen suspensions, which preserved molecular integrity, and in collagenous tissues. Collagen was chosen for this study because 1) it is the most abundant protein in the human body and 2) there is evidence to suggest that the echographic visualizability of a tissue or organ is determined primarily by its collagen content. These data are used to determine the role of collagen, and protein in general, in the determination of the ultrasonic propagation properties of tissues.

The absorption measurements were made in concentrations ranging from 0.07 to 0.7%, by weight, as a function of frequency at 10°C and 20°C. Velocity measurements were made at 8.87 MHz using a pulse technique. Absorption measurements at nine frequencies in the range from 8.87 to 56.4 MHz were also made with the pulse technique. The resonant cavity method was used for absorption measurements in the range from 0.5 to 1.5 MHz. Absorption measurements in tissues were made in the frequency range from 0.5 to 7 MHz at 37°C using the transient thermoelectric technique. In order to conduct these measurements, a detailed analysis of the measuring procedure was necessary to be able to determine the effects of beam width and thermocouple wire size on the measured ultrasonic absorption coefficient. Ultrasonic velocity measurements at 100 MHz and 22°C were also performed in collagen threads removed from mouse tail tendons using an acoustic microscope. This investigation represents the first comprehensive study of the ultrasonic

propagation properties of molecular collagen and collagenous tissues over an extended frequency range.

The results of these measurements show that collagen exhibits a magnitude of absorption and velocity per unit concentration much greater than that exhibited by the globular proteins. However, it is characterized by a frequency and concentration dependence which is not unlike that of the globular proteins. The frequency dependence of ultrasonic absorption in six tissues, viz., liver, kidney, brain, heart, testicle, and tendon, was found to be much the same as that for the attenuation of those tissues. However, the magnitude of the absorption, revealed by the transient thermoelectric technique, is approximately one third that of the attenuation generally reported. Velocity measurements in collagen fibers, which form tendon and are interspersed throughout nearly all tissues and organs, yield values some 10-20% greater than those obtained for soft tissues, and this may, at least in part, be responsible for variation in ultrasonic visualizability of these tissues. Finally, the absorption and velocity due each to structural and globular proteins, as measured in solution or suspension, when combined linearly in the proportion typical of that for tissues, yields the ultrasonic absorption and velocity actually measured, suggesting that tissue appears ultrasonically as a composite material whose acoustic properties are determined by the ultrasonic properties of its globular protein and collagen constituents.

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Chapter 1

INTRODUCTION

The ultrasonic propagation parameters of biological materials are those measurable quantities which characterize the behavior of acoustic signals propagating within living systems. These properties generally include ultrasonic attenuation and/or absorption, and ultrasonic velocity, which, with the density of the medium, determines the characteristic acoustic impedance of that medium under free-field conditions. The ultrasonic attenuation includes in addition to the absorption of the ultrasonic signal which is degraded to heat, losses due to other mechanisms by which energy is extracted from the propagating wave or is redirected by virtue of the inhomogeneous nature of the medium (Dunn, 1976). The distinction between absorption and attenuation was not universally recognized and considered important until approximately 10-15 years ago. For the purposes of this study, the two are distinguished as attenuation being composed of the absorption process plus scattering. The ultrasonic velocity and characteristic acoustic impedance embody within them both the inertial and restoring force parameters of the propagation medium.

The importance for having available details of the ultrasonic propagation properties of biological materials are twofold. First it can be expected that such available information for a wide range of biomaterials (including pathological states) will contribute to the development of more quantitative ultrasonic medical diagnostic methods employing more than only time of arrival and amplitude of signals backscattered, as in the current most sophisticated procedures (Wells, 1977). Such developments are hampered by the absence of information regarding the basic ultrasonic properties of tissues and has left open the question of the choice of

parameters to be most useful and appropriate in addition to the clinical benefits to be achieved. Second, the physical mechanisms by which ultrasound is propagated within tissues is poorly understood owing to the complexity of the processes involved, and the measurement ambiguities present in the literature, making data interpretation in some cases quite difficult. Attenuation and velocity are most often measured, with ultrasonic absorption in biological tissues receiving little attention (Goss et al., 1978c). Thus the distinction between attenuation and absorption is also not often appreciated.

Pohlman (1939) was the first to measure the ultrasonic attenuation in tissue, and found some differences among body tissues and organs, noting a factor of two difference between fat and muscle tissue. Carstensen et al. (1953) in studies concerning the acoustic properties of blood, identified the role of macromolecular constituents in the absorption process by showing that the absorption of sound is directly proportional to protein concentration whether in solution or within cells. The structural level important in determining the attenuation properties of tissue was investigated by Pauly and Schwan, though reported much later (1971), wherein it was found that two-thirds of the attenuation was attributed to protein, and one-third to tissue architecture. Ultrasonic velocity measurements in various tissues and species were made by Frucht (1953) wherein velocity differences were found to be related to tissue water content and tissue morphology and physiological function. Similar research was also performed concerning the attenuation, as well as the velocity parameter, in a wide variety of tissues (Dussik and Fritch, 1955; 1956), where the importance of tissue constituents and physiological function in the ultrasonic properties exhibited was greatly expanded upon. This study also included the first measurements of attenuation and velocity in highly collagenous tissues, which were found to exhibit ultrasonic properties different

from those found in soft tissues normally studied, viz., parenchymal tissues, with velocity 9-10% greater, and attenuation more than 200% greater, than those values characteristic of soft tissues.

Evidence suggesting that collagen may exhibit unusual acoustic properties have been reported by a number of investigators. Kanagy and Robinson (1956) showed that the sound velocity in leather varies substantially with chemical and physical structure, and follows modifications of the fibrous order produced by strain and aging. Fry et al.(1972) reported that human female post-menopausal breast tissue, and pre-menopausal breast tissue with fibrosing adenosis both exhibited increased reflectivity as witnessed by echographic visualization. Both types of breast tissue are characterized by a proliferation of connective tissue replacing the normal glandular breast tissue. In a later study by Kossoff et al. (1973), concerning the velocity of ultrasound in human female breast tissue, it was concluded that the post-menopausal breast averaged 3-4% lower ultrasonic velocity than that of the pre-menopausal, with the difference again attributed to the proliferation of fatty tissue interlaced with an increased amount of connective tissue.

Variations in collagen content in various tissues could account for the observation by Fry et al. (1971) that pig liver, with well developed connective tissue between lobules, returns high amplitude echoes in echographic visualization, whereas cat liver, in which connective tissue is poorly developed, are of low amplitude. Volume scattering from liver indicates differences which may also be related to pathology. Scattered power from cirrhotic liver was found to be significantly greater than that from normal (Waag, et al., 1975). Both cirrhotic and fibrotic livers also have a greater acoustic impedance than normal livers, with an average increase of about 8% (Yamakawa et al., 1964). This is consistent

with the finding of Boyett and Sullivan (1970) that there may be a three-fold increase in collagen content in cirrhotic liver.

Fields and Dunn (1973) have suggested that it is the elastic properties of most soft tissues, determined primarily by the content of collagen and other structural proteins, which define acoustic contrast during echographic visualization. This hypothesis is based on the fact that the static or low-frequency elastic modulus of collagenous fibers is at least 1000 times greater than those of soft tissues. Since the acoustic velocity (and therefore impedance) is proportional to the square root of the elastic modulus, collagenous tissues are considered to represent a greater impedance mismatch than would be the case for a soft-tissue interface of similar elastic modulii, thereby increasing acoustic reflectivity at parenchymal collagenous tissue boundaries.

In order to describe the transmission of ultrasonic energy into the brain via the meninges, from physiological saline, Johnston and Dunn (1976) proposed a three layer transmission model (Kinsler and Frey, 1962), which required the meninges, a very high collagen content material, which comprised the second layer, to have a velocity some 300 m/sec greater than the two outer media, resulting in a theoretical velocity determination in the collagenous meninges to be on the order of 1800 m/sec.

The role of collagen in the determination of the ultrasonic properties of biological tissues has become a topic of interest. O'Brien (1976; 1977) has shown that correlation relationships between collagen, and other tissue constituents, and the ultrasonic attenuation and velocity exhibited by those tissues exists. O'Donnell et al. (1978a), in investigations of ultrasonic attenuation in myocardial infarcts, suggest that the ultrasonic attenuation increases with increasing collagen concentration in tissues. Thus, while relationships regarding

the role of tissue constituents, especially collagen and other proteins have been identified, little has been done to make these relationships quantitative.

The present study was undertaken to determine the magnitude of ultrasonic velocity and absorption in collagen suspensions in which molecular integrity is preserved, and in collagenous tissues. An additional goal of this study was to determine the role of collagen, and protein in general, in the determination of ultrasonic absorption and velocity in biological tissues. Since the parameter most often used to characterize the loss coefficient in tissues is ultrasonic attenuation, rather than absorption, it was necessary to conduct ultrasonic absorption measurements in a number of various tissues and organs using the transient thermoelectric technique in the frequency range from 0.5 to 7 MHz. In order to conduct these measurements, a detailed analysis of the measuring procedure was necessary to be able to determine the effects of beam width and thermocouple wire size on the measured ultrasonic absorption coefficient. This investigation then represents the first comprehensive study of the ultrasonic properties of molecular collagen and collagenous tissues, over an extended frequency range.

The hypothesis upon which this study is based stems from the observation that the primary mechanism for ultrasonic absorption in tissues occurs at the macromolecular level (Carstensen et al., 1953; Schwan, 1959; Pauly and Schwan, 1971). Each of the constituents of tissue, such as protein, lipid and water might then be expected to contribute to the absorption properties of the tissue which they comprise. Lipids are not considered to greatly contribute to the ultrasonic absorption of most tissues, however, since this type of constituent comprises only a small fraction of the wet weight of most tissues (Snyder et al., 1975), and exhibits a relatively small attenuation coefficient (Pohlman, 1939;

Dussik and Fritch, 1956; Schwan et al., 1953; Schwan et al., 1954). Water comprises 70-80% of most biological tissues (Snyder et al., 1975), but also exhibits a relatively small absorption coefficient, with the attenuation coefficient in parenchymal tissue being approximately 30 times greater than that found in water. Proteins however, in sufficient concentration in aqueous solution exhibit absorption magnitudes in the same range as those of tissue attenuation (Kremkau and Carstensen, 1972; Goss and Dunn, 1974). In addition, absorption of sound in blood has been determined to be directly proportional to protein concentration (Carstensen et al., 1953). Thus, the concentration of protein in tissue appears to be of primary importance in the determination of the ultrasonic properties of tissues.

The protein constituents of tissues can be divided into the globular and structural proteins. These two proteinaceous forms possess very different macromolecular structures which may produce substantial differences in ultrasonic properties at the macromolecular level. Since all biological tissues are composed of globular as well as structural proteins in varying amounts, variations in the ultrasonic properties among tissues may be related to the proportion of protein that is structural, and that is globular. Though these constituents should play a major role in the absorption processes in tissues, little attention has been given this question due to the absence of ultrasonic absorption data in tissues, and to the lack of either velocity or absorption data on the most common structural protein, collagen. The results of this study show that collagen is a protein which exhibits a magnitude of absorption and velocity per unit concentration much greater than that found for globular proteins, but is characterized by a frequency and concentration dependence which is not unlike the globular proteins. Furthermore, the absorption and velocity due each to structural and globular

protein, when linearly combined in the correct proportion typical of biological tissues yields the ultrasonic absorption and velocity measured in biological tissues.

The thesis is organized as follows. Chapter 2 contains a general discussion of absorption mechanisms in tissues and solutions of biomacromolecules and a brief review of the present understanding of the ultrasonic properties of biological tissues. Also included in Chapter 2 is an analysis, using data recently compiled by Goss et al. (1978c), of the dependence of ultrasonic attenuation and velocity on tissue constituents. Chapter 3 deals with the physical and chemical properties of collagen. Chapter 4 is concerned with the various experimental procedures procedures utilized in this study in data procurement, and in preparation of specimens. This chapter also includes the results of the study concerning possible errors encountered when using the transient thermoelectric technique (Goss et al., 1977). Presentation and discussion of ultrasonic measurements in both collagen suspensions and biological tissues, and the relationship determined to be present between ultrasonic properties and tissue constituents from these measurements is the subject of Chapter 5.

Chapter 2

The Ultrasonic Propagation Properties of Biological Tissues: Theory and Measured Values

2.1 General

Despite the widespread use of ultrasonic energy for medical therapy and diagnosis, the precise mechanism(s) by which ultrasound interacts with living systems is not completely understood. The class of mechanisms most commonly invoked to describe ultrasonic absorption in tissues is that of a relaxation phenomena, generally characterized by a broad distribution of relaxation frequencies (Carstensen and Schwan, 1959b; Pauly and Schwan, 1971; Hussey, 1975; Dunn and O'Brien, 1976; O'Brien and Dunn, 1972b; Dunn and O'Brien, 1978). The suggestion that relaxation mechanisms may play a significant role in producing the observed tissue response stems largely from the linear frequency dependence of observed ultrasonic attenuation in all of the soft tissues thus far investigated, excepting lung, (Pauly and Schwan, 1971; Chivers and Parry, 1978) and from the results of ultrasonic investigations in protein solutions considered as models for tissues. From so-called "classical" absorption mechanisms, where viscosity (Stokes, 1845) and heat conduction (Kirchhoff, 1868) of the fluid being irradiated lead to a time lag between the instantaneously changed pressure and the responding density in a sound wave, a quadratic dependence of the absorption coefficient upon frequency is expected instead of the linear dependence of attenuation first observed by Pohlman (1939), and later by many others (Hueter, 1948; Goldman and Hueter, 1956; Hueter, 1958; Johnston et al., 1978; Goss et al., 1978a; 1978b). Hysteresis (Mason, 1950) and relative viscous motion (Fry, 1952;

Carstensen and Schwan, 1959b) may also describe such a frequency dependence, but they do not account for the velocity dispersion observed in aqueous hemoglobin solutions (Carstensen and Schwan, 1959a), and this seems to be important since the primary mechanism for the absorption of sound in tissue appears to occur at the macromolecular level (Carstensen et al., 1953; Pauly and Schwan, 1971). Such velocity dispersion, to be expected if relaxation is important in the absorption process, has only recently been observed in biological tissue (brain), (Kremkau et al., 1976).

The importance of macromolecular absorption to the ultrasonic properties of biological tissues has led to an abundance of research concerning the specific ultrasonic propagation properties of many biologically important molecular species, reviews of which are found elsewhere (O'Brien and Dunn, 1972b; Hussey, 1975; Dunn and O'Brien, 1976; Johnston et al., 1978; Dunn and O'Brien, 1978). The investigation of such simpler systems have been conducted with the expectation that mechanistic details of these media would be applicable to the more complex macromolecular subsystems which form tissues and organs, though the validity of such rationale remains to be established unequivocally. Ambiguities exist not only in the system studied, but also in the loss parameter measured and reported. The most reported loss parameter in biological tissues is ultrasonic attenuation, which includes absorption of the wave in the body of the medium (eventually degraded to heat), as well as all other mechanisms by which energy is removed from the wave, or redirected at media interfaces. Measurements in biopolymer solutions largely yield ultrasonic absorption alone, since sites at which ultrasonic scattering can take place are not present in these media. In tissues however, losses due to other mechanisms than absorption are present. The degree to which attenuation and absorption may be distinguished depends on the particular experimental

method employed for measurement, to the extent that various methods include varying degrees of loss artifact which would contribute to the measured attenuation coefficient (Goss et al., 1978a; Goss et al., 1978b). Hence, it is even sometimes difficult to compare attenuation measurements made on the same type of tissue under similar ambient conditions. Such ambiguity in measurement, in addition to ambiguities brought about by insufficient tissue specimen characterization which has proliferated the literature (Goss, et al., 1978b, Goss, et al., 1978c), has hampered progress toward a full understanding of the processes by which ultrasound is propagated in biological media.

None the less, progress has been made in determining the dependence of ultrasonic attenuation and velocity on major tissue constituents, viz., water, protein, fat, and collagen (Frucht, 1953; Carstensen et al., 1953; Pauly and Schwan, 1971; O'Brien, 1976; O'Brien, 1977; Johnston et al., 1978). Such studies reveal important insights relating to the contribution that each of these components makes towards the ultrasonic properties exhibited by a particular tissue, and may eventually aid in the determination of the mechanism by which ultrasonic propagation occurs in living systems.

This chapter contains a brief synopsis of the theoretical aspects of ultrasonic propagation in fluid-like media, a short review of the literature concerning the measured ultrasonic properties of biological tissues, and a discussion of the dependencies of these properties upon tissue constituents.

2.2 Theory

2.2.1 Classical Absorption Mechanisms

The absorption of energy from sound waves in fluids is associated with a time lag in the condensation, s, defined as the fractional change in density, $s = (\rho-\rho_o)/\rho$, where ρ_o and ρ are the undisturbed and instantaneous densities, respectively,

relative to the varying acoustic pressure. From a macroscopic point of view, viscosity and heat conduction, the so called classical mechanisms exhibit such a time lag between pressure and instantaneous density in a sound wave since instantaneous responses of these parameters are only idealizations not always acceptable in describing the state of the fluid. In liquids, exclusive of liquid metals, viscous losses are predominant while in gases, viscosity and heat conduction are approximately equally important.

When acoustic pressure and condensation are in phase, no absorption of acoustic energy occurs, and the acoustic pressure may be given by (the equation of state of the fluid)

$$p = \rho_o c^2 s \tag{2-1a}$$

or

$$p = K_o s \tag{2-1b}$$

where ρ_o is the undisturbed density, c is the sound velocity in the fluid medium, s is the condensation, and K_o is the adiabatic bulk modulus, a measure of the volume elasticity of the fluid. Stokes (1845) modified (2-1a) to include the fact that the stress is also dependent upon the state of strain and this leads to a modified equation of state as

$$p = \rho_o c^2 s + R \frac{\partial s}{\partial t} \tag{2-2}$$

where R is a constant, the nature and magnitude of which is determined by the specific loss mechanism. If (2-2) is combined with the continuity equation in Eulerian (spatial) coordinates

$$s = - \frac{\partial \xi}{\partial x} \tag{2-3}$$

which describes the change in density which must occur in order that mass per unit volume be conserved during molecular displacements due to the passage of the acoustic wave, where ξ is the particle displacement, and the dynamical force equation

$$\frac{-\partial p}{\partial x} = \rho_o \frac{\partial^2 \xi}{\partial t^2} \tag{2-4}$$

to eliminate s and p, the result is

$$\frac{\partial^2 \xi}{\partial t^2} = c^2 \frac{\partial^2 \xi}{\partial x^2} + \frac{R}{\rho_o} \frac{\partial^3 \xi}{\partial x^2 \partial t} \tag{2-5}$$

Equation (2-5) is the one-dimensional wave equation with an additional term to account for absorption of energy by the medium due to its possessing a finite viscosity.

The solution of (2-5) is assumed to be of the form

$$\xi = \xi_o e^{j(\omega t - k^* x)} \tag{2-6}$$

where $k^* = (k - j\alpha)$ is the complex wave number and k and α are the real wave number and absorption coefficient, respectively. Substituting (2-6) into (2-5) and solving for α and v, the phase velocity, yields

$$\alpha = \frac{\omega}{c\sqrt{2}} \left[\frac{1}{1-\omega^2\tau^2} - \frac{1}{1+\omega^2\tau^2} \right]^{\frac{1}{2}} \tag{2-7}$$

and

$$v = \frac{\omega}{k} = c \left[\frac{2\ (1 + \omega^2\tau^2)}{1 + (1 + \omega^2\tau^2)^{\frac{1}{2}}} \right]^{\frac{1}{2}} \tag{2-8}$$

where τ represents a critical time interval referred to as a relaxation time, as

expressed by

$$\tau = \frac{R}{\rho_o c^2} \tag{2-9}$$

Equation (2-5) can also be derived directly from the hydrodynamic equations, where R would be given by (Kinsler & Frey, 1962)

$$R = \chi + 2\eta_s \tag{2-10}$$

where χ is a longitudinal coefficient of viscosity and η_s is the shear coefficient of viscosity. Since no direct method existed for measuring χ, Stokes (1845) used the theoretical value of the bulk or volume viscosity η_v, given by

$$\eta_v = \chi + \frac{2}{3}\eta_s \tag{2-11}$$

to yield a theoretical value for χ, by assuming that fluids show no viscous reaction to a uniform compression from all directions, i.e., $\eta_v = 0$. Under this assumption

$$R = \frac{4}{3}\eta_s \tag{2-12}$$

and

$$\tau = \frac{4}{3}\frac{\eta_s}{\rho_o c^2} = \frac{4}{3}\frac{\eta_s}{K_o} \tag{2-13}$$

Usually, except at high frequencies and in very viscous liquids, $\omega\tau << 1$, and the absorption coefficient due to shear viscosity may be approximated from (2-7) as

$$\alpha_{sv} = \frac{2\omega^2\eta_s}{3\rho_o c^3} \tag{2-14}$$

where c is, to the same degree of approximation from (2-8)

$$c = \left[\frac{K_o}{\rho_o}\right]^{\frac{1}{2}} \tag{2-15}$$

The other classical acoustic absorption mechanism is heat conduction. Kirchhoff (1868) showed that the relaxation time for this process is given by

$$\tau = \frac{\kappa}{\rho_o c^2 C_p} \tag{2-16}$$

where κ is the thermal conductivity of the fluid, and C_p is its heat capacity at constant pressure. This time lag between pressure and density arises from the finite time required for heat to flow from regions of compression to regions of rarefaction in the acoustic wave. The acoustic absorption associated with this phenomenon is expressed in the coefficient (Kirchhoff, 1868).

$$\alpha_{hc} = \frac{(\gamma-1)}{2c}\,\omega^2\tau \tag{2-17}$$

where γ is the ratio of the heat capacity at constant pressure to that at constant volume (C_p/C_v).

The sum of the acoustic absorption coefficient due to shear viscosity and heat conduction is known as the classical absorption coefficient, and is given by

$$\alpha_{class} = \alpha_{sv} + \alpha_{hc} = \frac{\omega^2}{2\rho_o c^3}\left[\frac{4\eta_s}{3} + \frac{\kappa(\gamma-1)}{C_p}\right] \tag{2-18}$$

The classical absorption coefficient divided by the square of the frequency (classical frequency-free absorption) is seen to be a constant for a given pressure and temperature for a specific fluid,

$$\frac{\alpha_{class}}{f^2} = \frac{2\pi^2}{\rho_o c^3}\left[\frac{4\eta_s}{3} + \frac{\kappa(\gamma-1)}{C_p}\right] \tag{2-19}$$

2.2.2 Relaxational Absorption Mechanisms

Liquids, in general, and biological materials in particular, do not exhibit the ultrasonic absorption predicted by classical theory; the observed absorption being greater and having completely different frequency dependencies than that suggested by the classical mechanisms. Relaxation processes are usually invoked to describe this behavior.

At the molecular level, where the acoustic propagation medium cannot be considered continuous, the average kinetic energy of the molecules may be perturbed by the passage of the wave. If more than one allowable energy state exists for the molecules, the acoustic disturbance can perturb the equilibria, with some molecules extracting energy from, and others releasing energy to, the medium. In such processes, the energy necessary for the molecules to overcome potential energy barriers and to populate higher energy states is supplied by the perturbing sound wave. The magnitude of the absorption that occurs depends upon the time required for the energy level transition to occur, compared to the period of the sound wave, and the energy difference between the states. A single relaxation process occurs when the acoustic wave perturbation involves only two energy states. For this set of conditions the frequency-free absorption can be expressed as

$$\frac{\alpha}{f^2} = \frac{A_A}{1+\omega^2\tau^2} + B \qquad (2\text{-}20)$$

where the first term represents the single relaxation process, for which τ is the relaxation time, and B represents the absorption due to the classical mechanisms.

For systems possessing many perturbable energy states, it may be necessary to consider that many relaxation processes occur, and (2-20) may be summed over all n processes yielding

$$\frac{\alpha}{f^2} = \sum_n \frac{A_n}{1+\omega^2\tau_n^2} + B \qquad (2\text{-}21)$$

Structural relaxation phenomena (Litovitz and Davis, 1965) occurs when the molecular association of the internal structure of the liquid and the prevailing molecular arrangement is so perturbed by the compressional phase of the sound wave to produce a closer packed short range lattice structure. The degree to which the change occurs may be dependent upon the shape of the molecules, the forces of mutual attraction, and the amplitude of the perturbing wave.

The acoustically induced structural arrangements are accompanied by volume changes which suggests that the liquid possess a volume viscosity, the quantity assumed to be zero in classical absorption theory. Since these structural changes are a result of time dependent molecular energy shifts, a relaxation mechanism results. Generally, a structural relaxation mechanism is characterized by a distribution of relaxation times (Litovitz and Davis, 1965), and exhibits a measurable velocity dispersion (Lamb, 1965).

Water is believed to exhibit structural relaxation (Hall, 1948). Macromolecules is aqueous solution provide additional structuring of the medium, as water molecules form hydration layers about the solute molecules, providing the possibility that the presence the acoustic wave induces formation and disruption of these structural arrangements. Such solvent-solute interactions have been proposed to explain the absorption in excess of that due to the solvent alone in hemoglobin (Carstensen, 1960) and amino acid solutions (Burke, et al., 1965).

Thermal relaxation processes in liquids occur when the temperature gradient produced by the passage of an acoustic disturbance through the liquid perturbs molecular rotational and vibrational degrees of freedom. The temperature rises during the compressional phase of the acoustic wave and the internal degrees of

freedom of a fraction of the molecules have their energies altered by the transferred wave energy. The time lag between the periodic temperature variations and the transfer of energy to the internal modes results in energy becoming extracted from the wave process. Thermal relaxation is generally a single relaxation mechanism, with negligible velocity dispersion (Lamb, 1965), and is mot often associated with non-associated non-polar liquids such as benzene. Thermal relaxation is not expected to contribute appreciably to the absorption mechanism in biological materials.

Chemical equilibrium may be perturbed either by the pressure or temperature variations in the acoustic wave. The theory of chemical relaxation is presented by Eigen and DeMaeyer (1973), Hammes (1968), Stuehr and Yeager (1965), Andreae (1965), and Stuehr (1973). It is shown that for a single step reaction $A + B \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} AB$ in dilute solutions, the relaxation time , is given by

$$\tau = \frac{1}{k_{21}+k_{12}(\bar{c}_A+\bar{c}_B)} \tag{2-22}$$

where k_{21} is the rate constant for dissociation, k_{12} is the rate constant for association, and $\bar{c}_A$ and $\bar{c}_B$ are equilibrium concentrations for A and B, respectively. The absorption parameter A_A of (2-20) which may represent the low frequency ($\omega\tau<<1$) absorption due to the perturbation of a chemical process, is given by (Applegate et al., 1968)

$$A_A = \frac{2\pi^2\tau\rho_o c}{RT}\left[\frac{\bar{V}K_E\bar{c}_B(\bar{c}_A+\bar{c}_B)}{K_E(\bar{c}_a+\bar{c}_b)+(K_E\bar{c}_B+1)^2}\right]\Delta U^2 \tag{2-23}$$

where ρ_o, c and τ were defined previously, K_E is the equilibrium constant $\frac{k_{12}}{k_{21}}$, $\bar{V}$ is the volume per mole of the solution, R is the gas constant, T is the absolute temperature, and ΔU is the change in internal energy,

$$\Delta U = \Delta V - \frac{\beta \bar{V}}{C_p} \Delta H \qquad (2\text{-}24)$$

Here, ΔV and ΔH are the standard volume and enthalpy changes, respectively β is the coefficient of thermal expansion and C_p is the heat capacity at constant pressure. If $\Delta V >> \frac{\beta \bar{V} \Delta H}{C_p}$ in (2-23), as is the case for dilute aqueous solutions of some electrolytes, pressure variations due to the acoustic wave perturb the chemical reaction from equilibrium, whereas for non-aqueous solutions the opposite is true, and the temperature gradient which accompanies the acoustic disturbance primarily acts to perturb the chemical equilibrium.

An important contribution to the observed ultrasonic absorption behavior observed in aqueous protein solutions in the pH range 2-4 and 10-14 has been the perturbation of proton-transfer equilibria (Applegate et al., 1968; Hussey and Edmonds, 1971; Zana and Tondre, 1971; 1972; O'Brien and Dunn, 1972a; Zana and Lang, 1970; Slutsky et al., 1977). Here, it is postulated that the pressure variations of the propagating acoustic wave perturbs the proton from the solvent (water) to the solute, and vice versa. The energy required to drive the proton between differing energy states is derived from the acoustic wave process and represents increased absorption. For example, proteins, which are usually comprised of a number of α-amino acids, always contain a large number of residues bearing carboxylic, phenolic, or amine groups on their side chains. In solution, these groups can accept or release protons, with the energy required supplied by the passing acoustic wave.

While the relaxational absorption processes mentioned above are considered most important in biological solutions, their direct contribution to ultrasonic absorption processes in biological tissues is unclear. Hueter (1958) suggested

that different organizational levels of animal tissue, from the molecular to the tissue macrostructure, may contribute to the observed absorption behavior, with low frequency behavior attributed to the gross tissue-matrix, and the high frequency behavior dependent on the molecular constituents of the tissue. Hueter regarded such behavior as indicative of viscoelastic relaxation.

From classical mechanics, deformation of solid materials is assumed to involve, for infinitesimal deformations, linear stress-strain behavior. For liquids, using classical hydrodynamic theory, such deformational behavior may be characterized by a constant viscosity. Viscoelastic materials exhibit both elastic and viscous effects upon mechanical stressing, a phenomena not completely described by classical mechanics.

In developing a description of the classical absorption effects, the stress-strain relations governing acoustic behavior were assumed to be independent of time until dissapative effects were considered, when a time dependent condensation term was added to the original equation of state (see (2-2)). When considering viscoelastic materials, the time dependence of the compressional stress must be included, and the equation of state takes the form

$$p + \tau_v \frac{\partial p}{\partial t} = K_o (s_t + \tau_p \frac{\partial s}{\partial t}) \qquad (2\text{-}25)$$

where p and s_t are the stress and strain, respectively, and τ_v is the time constant of the pressure change under the perturbing force of the volume change, whereas τ_p is the corresponding time constant for the volume to change when the perturbing force is pressure (Litovitz and Davis, 1965). For long temporal events, (2-25) reduces to the original equation of state (2-2), while at high frequencies the time dependent terms dominate and lead to a high frequency compressional modulus

$$\left(\frac{\partial p}{\partial s_t}\right)_{\infty} = K_{\infty} = \frac{\tau_p}{\tau_v} K_o \tag{2-26a}$$

for which it is seen that the two relaxation times are related by the equation

$$\tau_p = \frac{K_{\infty}}{K_o} \tau_v \tag{2-26b}$$

The application of a sinusoidal pressure to the medium (as with the application of an acoustic wave) leads to a complex modulus of compression, K^*, given by

$$K^* = \frac{p}{s_t} = K_o + \frac{K_r \omega^2 \tau_v^2}{1+\omega^2 \tau_v^2} + j \frac{K_r \omega \tau_v}{1+\omega^2 \tau_v^2} \tag{2-27}$$

where $K_r = K_{\infty} - K_o$ is the relaxational modulus. The imagninary part of the complex modulus is proportional to the frequency dependent volume viscosity

$$\eta_v(\omega) = \frac{K_r \tau_v}{1+\omega^2 \tau_v^2} \tag{2-28}$$

which at low frequencies reduces to

$$\eta_v = K_r \tau_v \tag{2-29}$$

Similar to (2-25), the equation for the shear stress may be expressed as

$$T' + \tau_s \frac{\partial T'}{\partial t} = G_{\infty} \tau_s \frac{\partial S'}{\partial t} \tag{2-30}$$

where T' is the shear stress, S' is the shear strain, τ_s is the shear relaxation time constant, and G_{∞} is the high frequency shear rigidity. Here, no time independent strain term is included, since a viscoelastic material is unable to support a dc shear strain. As before, the application of a sinusoidal pressure leads to a complex shear modulus, G^*, which is given by

$$G^* = \frac{T'}{S'} = \frac{G_\infty \omega^2 \tau_s^2}{1+\omega^2 \tau_s^2} + j\omega \frac{G_\infty \tau_s}{1+\omega^2 \tau_s^2} \tag{2-31}$$

where the frequency dependent shear viscosity is given by

$$\eta_s(\omega) = \frac{G_\infty}{1+\omega^2 \tau_s^2} \tag{2-32}$$

which at low frequencies reduces to

$$\eta_s(\omega) = G_\infty \tau_s \tag{2-33}$$

The one dimensional longitudinal wave equation for a harmonic disturbance is

$$\frac{\partial^2 \xi}{\partial t^2} = c^{*2} \frac{\partial^2 \xi}{\partial x^2} \tag{2-34}$$

where the complex velocity of propagation c^* is,

$$c^* = \left[\frac{K^* + 4/3\, G^*}{\rho_o}\right]^{\frac{1}{2}} \tag{2-35}$$

taking into account the complex compressional and shear moduli of the viscoelastic medium. Assuming the solution to (2-34) is of the form of (2-6), the absorption per wavelength and the velocity become,

$$\alpha\lambda = \pi \left[\frac{\frac{K_r \omega \tau_v}{1+\omega^2 \tau_v^2} + \frac{\frac{4}{3} G_\infty \omega \tau_s}{1+\omega^2 \tau_s^2}}{K_o + \frac{K_r \omega^2 \tau_v^2}{1+\omega^2 \tau_v^2} + \frac{\frac{4}{3} G_\infty \omega^2 \tau_s^2}{1+\omega^2 \tau_s^2}}\right] \tag{2-36}$$

$$c = \frac{1}{\rho_o} \left[K_o + \frac{K_r \omega^2 \tau_v^2}{1+\omega^2 \tau_v^2} + \frac{\frac{4}{3} G_\infty \tau_s^2 \omega^2}{1+\omega^2 \tau_s^2}\right] \tag{2-37}$$

Using the low frequency approximation $\omega\tau_v << 1$ and $\omega\tau_s << 1$, (2-36) and (2-37) yield

$$\frac{\alpha}{f^2} = \frac{2\pi^2}{K_o c} (K_r \tau_v + 4/3\ G_\infty \tau_s) \tag{2-38}$$

$$c = \left(\frac{K_o}{\rho_o}\right)^{\frac{1}{2}} \tag{2-39}$$

and substituting (2-29) and (2-33), (2-38) becomes

$$\frac{\alpha}{f^2} = \frac{2\pi^2}{K_o} (\eta_v + 4/3\ \eta_s) \tag{2-40}$$

Thus, the absorption coefficient obtained herein (2-40) is the same as that obtained using the Stokes (1845) analysis. Also, the velocities of (2-39) and (2-15) are identical for the low frequency approximation.

The absorption coefficient for water, the major constituent of biological tissues, is greater than one would expect from consideration of the viscosity and thermal conductivity losses. The measured ultrasonic frequency-free absorption $(\frac{\alpha}{f^2})$ at 20°C is about 25×10^{-17} sec^2/cm, while calculation of the frequency-free absorption value due to viscosity and thermal conductivity is 8.5×10^{-17} sec^2/cm and 0.02×10^{-17} sec^2/cm, respectively. This excess absorption has been attributed by Hall (1948) to a structural relaxation mechanism involving the transition of water molecules from a bonded, "icelike," structure to another "crystalline" structure of smaller specific volume. The relaxation time associated with this process was determined to be on the order of 10^{-12} seconds (Hall, 1948), far above the range of observation for current ultrasonic techniques. Relaxational behavior in the 0.5 to 100 MHz frequency range has been observed in biological tissues however, suggesting that a number of processes may be contributing to the

observed absorption in biological media.

2.2.3 Hysteresis

Hysteresis absorption has been proposed as an absorption mechanism in very viscous fluids at high frequencies (Mason et al., 1947); Litovitz and Lyon, 1954), and is characterized by an absorption coefficient which is proportional to frequency, and a phase velocity independent of frequency. At present however, the origin of such a mechanism in biological materials has not been identified.

2.2.4 Relative Motion

Relative motion produced by an acoustic field between structure elements and the viscous suspending media, arising from differences in density of each, produce frictionlike forces between the structures and the fluid, resulting in absorption of energy from the field. An approximation analysis employing the concepts of frictional force constants and equivalent masses yields a relaxation-type expression for the absorption coefficient with the modification that the relaxation frequency and amplitude are frequency dependent (Fry, 1952; Fry and Dunn, 1962; Brandt et al., 1937). Epstein (1941) considered the problem of absorption of sound in a medium containing spherical particles with diameters small compared to the acoustic wavelength. The absorption coefficient calculated therein yields approximately the same expression as may be obtained when the assumption is made that the structural elements are stiff in shear compared with the imbedding medium. This latter analysis indicates that the latter form of expression also applies to non-spherical elements and is given by

$$\alpha = \frac{C_c}{2v} \frac{(1-\rho_o/\rho_e)^2}{\rho_o \rho_e} \omega_o \frac{(\omega/\omega_o)^2}{1+(\omega/\omega_o)^2} \frac{M}{M_e} \qquad (2\text{-}41)$$

where $\omega_o = \frac{R_f}{M}$, $\omega = 2\pi f$, v is the acoustic phase velocity, ρ_o and ρ_e are the densities of the imbedding media and the structure elements, respectively, and C_c is the volume concentration of structure elements. Here, R_f and M_e are the frictional force constant and the effective mass of the element, respectively, and for spherical particles are given by the following relation

$$R_f = 6\pi a_s \eta \left[1 + \left(\frac{\rho_o}{\eta}\right)^{\frac{1}{2}} a_s \omega^{\frac{1}{2}} \right] \tag{2-42}$$

$$M_e = M + m \left[\frac{1}{2} + \frac{9}{4\left(\frac{\rho_o}{2\eta}\right)^{\frac{1}{2}} a_s \omega^{\frac{1}{2}}} \right] \tag{2-43}$$

where η is the shear viscosity of the imbedding medium, a_s is the radius and M the mass of an element, and m the mass of fluid displaced by a particle. Values for R_f and M_e for non-spherical elements can be estimated from (2-42) and (2-43) by inserting an average value for the radius if the element does not deviate considerably from a spherical shape.

Carstensen and Schwan (1959b) have shown that relative motion measurably contributes to the total absorption in suspensions of erythrocytes in saline (subsequent work by Ahuja (1972, 1974)). It was also shown that (Carstensen and Schwan, 1959b) compared with a single relaxation process, the relative absorption mechanism yields a frequency dependence more indicative of the linear frequency dependence of absorption observed in tissues (Fry, 1952). This mechanism was considered not to apply in the case of concentrated cells, where the virtual absence of suspending medium did not allow such relative motion (Carstensen and Schwan, 1959b).

2.3 Ultrasonic Propagation Properties of Tissues

2.3.1 General

While theoretical aspects of the processes by which ultrasound propagates through biological tissue have not yet been fully elucidated, some insight can be obtained through the examination of the ultrasonic data as a function of such parameters as ultrasonic frequency, temperature, tissue constituents, morphology, and specimen preparation.

In the first measurement of ultrasonic properties of tissues, Pohlman (1939) determined the ultrasonic attenuation in human fat and muscle, using a radiation pressure technique, as a function of ultrasonic frequency (800 kHz and 2.4 MHz), and obtained a nearly linear dependence of attenuation on frequency, a dependence later identified in a number of other investigations and reviews (Hueter, 1948; Dussik and Fritch, 1955, 1956; Goldman and Hueter, 1956; Pauly and Schwan, 1971; Kessler, 1973; Johnston et al., 1978; Chivers and Parry, 1978; Goss et al., 1978a).

Ludwig (1950) measured ultrasonic velocity in a number of tissue types, using a pulse method at 1.25 and 2.5 MHz, but while differences in velocity among various tissues and organs were noted, anisotropy in velocity in muscle was not observed, a phenomenon observed earlier for attenuation measurements (Hueter, 1948; Colombati and Petralia, 1950). Fiber anisotropy in the ultrasonic velocity in biological materials however was observed by Goldman and Richards (1954) in dog thigh muscle, where velocities of 1576 m/sec and 1592 m/sec were obtained, perpendicular to and parallel to the muscle fiber, respectively. Similar behavior was also observed by Dussik and Fritch (1955, 1956) for attenuation measurements in beef and human muscle, where the value parallel to muscle fibers was nearly twice that found for the value perpendicular to the muscle.

Few studies have been concerned with the temperature dependence of ultrasonic attenuation and velocity, as the Goss et al., (1978c) compilation was able to identify only eight such studies in soft tissues, with an average increase in velocity going from 25° to 37°C, assuming linearity, of about 1% (Kikuchi et al., 1972; Bakke et al., 1975; Robinson and Lele, 1972; Rivara and Sanna, 1962; Bradley and Sacerio, 1972; Buschmann et al., 1970; Wladimiroff et al., 1975; Danckwerts, 1974).. The temperature dependence of attenuation is more difficult to assess. Hueter (1948), in measurements of attenuation in mammalian soft tissues, reported no temperature dependence between 20°C and 35°C, while Dussik and Fritch (1956) observed that attenuation in human fat is 10-15% higher at room temperature than at 37°C. Hasegawa et al. (1966) observed a negative coeffieicnt of temperature for attenuation in brain tissue (about 4 %/°C). A positive temperature coefficient (about 5%/°C) for absorption was observed in in vivo mouse spinal cord (Dunn, 1962), and was also observed for attenuation in bone (Kishimoto, 1958), though much less pronounced (1%/°C). More recently, attenuation measurements as a function of temperature in heart tissue (O'Donnell et al., 1977a) and liver homogenate (Danckwerts, 1974) have again exhibited a slightly negative temperature coefficient.

Dunn and Brady (1974) have observed a relatively complex behavior of the frequency-free absorption coefficient with frequency and temperature, which may be described as a family of curves whose maxima decrease in magnitude, and occur at ever higher temperatures, as frequency increases. From this, one would expect that the temperature dependence of attenuation or absorption observed would depend upon the frequency of measurement. This behavior however, would not explain the negative temperature coefficients observed in the literature in the 1-5 MHz frequency range, since a positive temperature coefficient has also been observed

over the same frequency range (Dunn and Brady, 1974; Robinson and Lele, 1972).

The importance of tissue constituents in the determination of the ultrasonic properties exhibited by a particular tissue or organ was first demonstrated by Carstensen et al. (1953), where it was observed that the acoustic properties of blood are determined largely by the protein content and that the absorption coefficient is directly proportional to the protein concentration whether in solution or within the cell. Frucht (1953), in studies of ultrasonic velocity in a variety of tissues and organs of a number of species found that increased water content of subcutaneous fat corresponded to increased velocity of sound, while removing connective tissue decreased the observed velocity. Frucht (1953) also qualitatively investigated the effect of blood content of the organ on the ultrasonic velocity, and found that bleeding the animal before the organ was removed for measurement increased the velocity by about 0.5%. Dussik and Fritch (1955,1956) conducted attenuation and velocity measurements in a variety of tissues, including measurements of connective tissues such as cartilage, ligaments and tendon, and observed that it may be possible to characterize tissues according to their ultrasonic propagation properties and biological function. This proposal, later expanded upon by Dunn (1975), arises from the fact that tissues can be grouped in apparent teleogic fashion with the attenuation and velocity increasing as tissues take on more and more structural function, and water content decreases.

A detailed study conducted in 1956, but published some 15 years later (Pauly and Schwan, 1971), examined the origins of ultrasonic attenuation at successive levels of tissue structure. The results,similar to the conclusion of the earlier study identifying the major contributor to absorption in blood as protein (Carstensen and Schwan 1953), demonstrated that nearly two-thirds of the ultrasonic attenuation in beef liver lies at the macromolecular level, with the remaining one-third

due to structural features of the tissue.

2.3.2 Constituent Dependencies of Ultrasonic Attenuation and Velocity

Recent interest in the role of collagen in the ultrasonic properties stem largely from the observation that it is the elastic properties of most soft tissues, determined primarily by the content of collagen and other structural proteins, which define acoustic contrast during echographic visualization (Fields and Dunn, 1973; Kessler et al., 1974a). Much earlier, however, studies on articular tissue (Dussik and Fritch, 1955, 1956; Dussik et al., 1958) showed that tissues with higher collagen content for the most part exhibited greater values of ultrasonic attenuation and velocity than soft tissues containing lesser amounts of collagen. The ultrasonic attenuation in myocardial infarcts has also been observed to increase with collagen concentration (O'Donnell et al., 1978a).

It thus seems clear that the compositon of tissue plays an important role in the determination of the ultrasonic properties of biological materials. The relative importance of each tissue constituent, however, on a quantitative basis, has received little attention. O'Brien (1976) compared the ultrasonic attenuation and velocity properties of various tissues to their water, protein, and collagen contents. On the basis of these comparisons, numerical relationships were determined relating average amounts of tissue constituents to the ultrasonic propagation properties (O'Brien, 1977; Johnston, et al., 1978), for attenuation values taken at 1 MHz. The following relations were obtained (O'Brien, 1977).

For collagen, to a first approximation,

$$A = 0.11\ C^{0.51} \tag{2-44}$$

where A is the ultrasonic attenuation in cm^{-1} and C is the wet weight percentage

of collagen. The best fit parameter, r^2, is 0.93 (unity represents a perfect fit). Similarly, to a first approximation, the ultrasonic velocity as a function of collagen content for nine materials (excluding bone, lung, and skin) is

$$V = 1588 + 32 \ln C \tag{2-45}$$

where V is the ultrasonic velocity in m/sec and $r^2 = 0.84$. Also, to a first approximation, the wet weight percentage of total protein, P, (Johnston et al., 1978) is

$$A = 0.004 P^{1.26} \tag{2-46}$$

where P is the wet weight percentage of protein for 16 biological materials, excluding skin. Thus, to a first approximation there appears to be quantitative relationships between the amount of tissue constituents and the ultrasonic attenuation and velocity.

A recent compilation of empirical ultrasonic properties of mammalian tissues (Goss et al., 1978c) has enabled similar analyses over a larger data base than that previously used. Care was exercised to include in Table 2-1 only those attenuation and velocity data that was described in the literature as fresh tissue. As a result, some tissues which have been ultrasonically characterized do not appear in the tabulation of the ultrasonic propagation properties of tissue in this study used for the determination of constituent dependency.

It is evident from the data in Table 2-1 that no single frequency can be chosen that would fully represent all of the literature measurements listed. Since the attenuation coefficient is generally considered to be a linear function of frequency, one may normalize attenuation by simply dividing by the frequency of measurement, thus making full use of the data base. To examine the assumption

TABLE 2-1

Literature Values of Ultrasonic Attenuation and Velocity in Fresh Biological Tissues

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Blood Vessel	20	10	0.65-0.77	0.065-0.077	1492-1534	Greenleaf et al. (1974)
	NR	6	1.7	0.28	NR	McCutcheon & Freund (1976)
Brain (Human)	37	NR	NR	NR	1525	Willocks et al. (1964)
		0.5	0.1	0.2	NR	Kikuchi et al. (1957)
		1.0	0.2	0.2		
		2.25	0.5	0.22		
		5.0	1.3	0.26		
		1	0.02	0.02		Kremkau et al. (1976)
		3	0.06	0.02		
		5	0.11	0.02		
		2.2	0.032	0.014		
		1	0.052	0.052		
		3	0.16	0.053		
		5	0.30	0.061		
		2.2	0.11	0.05		
		1	0.05	0.05		
		3	0.18	0.06		
		5	0.37	0.074		
		3	0.16	0.053		
		5	0.32	0.064		
		2.2	0.1	0.045		
		3	0.16	0.053		
		5	0.28	0.056		

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Brain (Human)	37	2.2	0.1	0.045	NR	Kremkau et al. (1976)
	30-40	0.97	0.02-0.05	0.021-0.051		Oka & Yosioka (1976)
	NR	NR	NR	NR	1560	Kikuchi et al. (1964)
	37	1	0.05	0.05	NR	Kremkau et al. (1976)
		3	0.13	0.043		
		5	0.25	0.05		
		2.2	0.08	0.036		
	30	0.57	0.01	0.017		Oka (1977)
		0.97	0.06	0.062		
		1.7	0.14	0.082		
		2.9	0.14	0.048		
	5	1.0	0.08	0.08	1543	Kremkau et al. (1976)
	15	1.0	0.07	0.07	1537	
	25	1.0	0.07	0.07	1542	
	41	1.0	0.07	0.07	1558	
	5	5.0	1.1	0.22	NR	
	15	5.0	0.64	0.128		
	25	5.0	0.52	0.104		
	41	5.0	0.5	0.10		
	30	0.57	0.03	0.05		Oka (1977)
		0.97	0.07	0.072		
		1.7	0.14	0.082		
		2.9	0.28	0.096		
	37	1	0.06	0.06		Kremkau et al. (1976)
		3	0.17	0.057		
		5	0.29	0.058		
		2.2	0.13	0.059		
	22	NR	NR	NR	1530	Schiefer et al. (1963)
	NR				1460	Abe et al. (1966)

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Brain (Human)	37	1	0.052	0.052	1546 ± 1	Kremkau et al. (1976)
		3	0.159	0.053	1550 ± 1	
		5	0.30	0.060	1554 ± 1	
		2.2	0.098	0.044	NR	
	30-40	0.97	0.06	0.062		Oka & Yosioka (1976)
Brain (Calf)	24	1.8	NR	NR	1569	Frucht (1953)
	15-18	0.87	0.085	0.098	NR	Colombati & Petralia (1950)
		1.7	0.14	0.082		
		3.4	0.335	0.098		
	Rm	4	0.44	0.11		Bamber et al. (1977)
		5	0.56	0.112		
		6	0.68	0.113		
	NR	NR	NR	NR	1540	Tanaka (1969)
Brain (cat)	37	2.5	0.122	0.049	NR	Hueter (1958)
	25	1.0	0.097	0.097		Hasegawa et al. (1966)
		1.0	0.11	0.11		
	27	0.0	0.10	0.10		
	28	1.0	0.12	0.12		
		1.0	0.093	0.093		
	29	1.0	0.084	0.084		
	37	4.2	0.28-0.5	0.067-0.12	1555-1564	Robinson & Lele (1972)
	22.5	5	0.52	0.104	NR	Ishikawa (1964)
	25	1	0.097	0.097		Hasegawa et al. (1966)
		1	0.11	0.11		
	27	1.0	0.10	0.10		
	28	1.0	0.12	0.12		
		1.0	0.093	0.093		
	29	1.0	0.084	0.084		
	37	4.2	0.28-0.5	0.067-0.12	1555-1564	Robinson & Lele (1972)
	22.5	5	0.52	0.104	NR	Ishikawa (1964)

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Brain (cat)	25	1	0.097	0.097	NR	Hasegawa et al. (1966)
		1	0.11	0.11		
	27	1	0.098	0.098		
	28	1	0.120	0.120		
		1	0.092	0.092		
	29	1	0.085	0.085		
	22.5	5	0.52	0.104		Ishikawa & Nonaka (1964)
	25	4.2	NR	NR	1558	Robinson & Lele (1972)
	30	4.2			1562	
	37				1575	
	40				1560	
	47				1580	
	51				1571	
	38				1568	
	42				1564	
	47				1573	
	52				1578	
	57				1578	
	62				1564	
	67				1565	
	25	1.5	0.201	0.065	NR	Hueter (1958)
		4.5	0.461	0.102		
		1.5	0.109	0.073		
		4.5	0.610	0.136		
		7.5	0.863	0.115		
	37	2.7	0.18-0.32	0.067-0.119	1570	Robinson & Lele (1972)
Brain (Dog)		0.97	0.054	0.056	NR	Yosioka et al. (1969)
	30	0.97	0.052	0.054		Yosioka et al. (1966)
	NR	0.97	0.13	0.134		Oka (1977)

TISSUE	TEMP (C^o)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Brain (Dog)	40	0.58	0.037	0.064	NR	Aoyama (1973)
		0.97	0.056	0.058		
		1.7	0.11	0.065		
		2.9	0.215	0.074		
		4.8	0.388	0.081		
	NR	0.97	0.13±0.01	0.13		Oka (1977)
	25	2.5	NR	NR	1515	Ludwig (1950)
Brain (Horse)	24	1.8			1559	Frucht (1953)
Brain (Pig)	NR	1.25-2.5	0.2	0.16-0.08	NR	Ballantine et al. (1950)
		2.5	0.19	0.076		Hueter & Bolt (1951)
		2.5	0.41	0.164		
		1.25-2.5	0.5	0.4-0.2		Ballantine et al. (1950
	25	2.5	NR	NR	1506	Ludwig (1950)
	24	1.8			1565	Frucht (1953)
	NR	0.8	0.025	0.031	NR	Guttner et al. (1952)
		2.4	0.075	0.031		
		0.3-0.6	0.055F	0.055		Esche (1952)
		2	0.29	0.145		Baboux et al. (1975)
		4	0.58	0.145		
		7	1.6	0.228		
		9	1.7	0.188		
Brain (Rabbit)		5	NR	NR	1508	Nakajimi et al. (1960)
Brain (Rat)	37	3	0.41	0.137	NR	Pond (1968)
Breast (Human)	30 & 37	2	NR	NR	1468-1510	Kossoff et al (1973)
Breast (Lactating)	30 & 37	2			1550	
	40	0.97	0.22	0.227	NR	Oka (1977)
		0.97	0.22	0.227		Nakaima et al. (1976)
	Rm	NR	NR	NR	1485-1530	Greenleaf et al. (1977)

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Cartilage (Beef)	23	1	0.58	0.58	1665	Dussik & Fritch (1956)
		3	1.44	0.48	NR	
		5	2.19	0.438		
Cerebrospinal Fluid (Human	24.4	2	NR	NR	1515	Van Venrooij (1971)
	25.0	2			1509	
	21.8	2			1499	
	NR	1	0.001	0.001	NR	Balantine et al. (1954)
Eye (Human) Aqueous Humor	37	NR	NR	NR	1540	Nover & Glanschneider (1965)
		2.5			1534.1	Tschewnenko (1956)
Cornea		NR			1610	Nover & Glanschneider (1965)
		2.5			1639	Tschewnenko (1965
Lens		10-17	0.09F	0.09	NR	Lizzi et al. (1976)
		4	NR	NR	1638.4	Jansson & Kock (1962)
		15			1659	Coleman et al. (1975)
	34.1	4			1641	Jansson & Kock Mark (1961)
	37	NR			1645	Nover & Glanschneider (1965)
	20	5			1638	Araki (1961)
	29	2.5			1638.2	Tschwenenko (1965)
	37	2.5			1647	
		4			1641	Jansson & Kock (1962)
	NR	12	15	1.25	NR	Nover & Glanschneider (1965)

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Sclera	20	5	NR	NR	1613	Araki (1961)
	37	NR			1650	Nover & Glanschneider (1965)
		2.5			1650	Tschwenenko (1965)
Vitreous		4			1531.7	Jansson & Kock (1962)
	35.2	4			1530	Jansson & Sundmark (1961)
	20	5			1514	Araki (1961)
	37	NR			1540	Nover & Glanschneider (1965)
		4			1532.6	Jansson & Kock (1962)
	29	2.5			1519.1	Tschwenenko (1965)
Eye (Beef) Aqueous Humor	26-31	5			1497	Begui (1954)
		30	0.335	0.011	NR	
	22	4	NR	NR	1495	Oksala & Lehtinen (1958a)
	20	4	0.16-0.33	0.04-0.08	NR	Oksala & Lehtinen (1958b)
	NR	5	NR	NR	1470	Yamamoto et al. (1961)
Cornea	22	4			1550	Oksala & Lehtinen (1958a)
Lens	26-31	5			1616	Begui (1954)
	28	3.25	0.634	0.195	NR	
	22	4	NR	NR	1650	Oksala & Lehtinen (1958a)
	Rm	4			1640	Oksala & Lehtinen (1957)

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Lens	20	4	1.7-2.1	0.42-0.52	NR	Oksala & Lehtinen (1958)
	22	10	2.3	0.23		Filipczynski et al. (1967)
		13.5	3.1	0.23		
Sclera	22	4	NR	NR	1630	Oksala & Lehtinen (1958a)
	NR	5			1660	Yamamoto et al. (1961)
Vitreous	26-31	5			1516	Begui (1954)
	Rm	4			1495	Oksala & Lehtinen (1957)
	22	4			1495	Oksala & Lehtinen (1958a)
	20	4	0.17-0.37	0.04-0.09	NR	Oksala & Lehtinen (1958b)
	22	10	0.1	0.01		Filipczynski et al. (1967)
		18	0.17	0.0094		
		30	0.29	0.0097		
	NR	5	NR	NR	1470	Yamamoto et al. (1961)
Eye (Pig)						
Aqueous Humor	20	5			1498	Araki (1961)
Cornea	20	5			1546	
	NR	5			1580	Yamamoto et al. (1961)
Lens	23, 30, 37	4			1642 + T (°C)	Jansson & Sundmark (1961)
	20	5			1627	Araki (1961)
	NR	5			1530	Yamamoto et al. (1961)
Sclera	20	5			1609	Araki (1961)

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Vitreous	23, 30, 37	4	NR	NR	1469 + 1.8T (°C)	Jansson & Sundmark (1961)
Eye (Rabbit) Lens	NR	5			1540	Nakajimi et al. (1960)
Vitreous		5			1472	
Fat (Human)		0.8	0.051	0.064	NR	Pohlman (1939)
	24	1.8	NR	NR	1476 ± 2	Frucht (1953)
	37	1	0.07	0.07	1479	Dussik & Fritch (1956)
		3	0.18	0.06	NR	
		5	0.27	0.054		
Fat(Beef)	24	1.8	NR	NR	1465	Frucht (1953)
	NR	3			1540	Rich et al. (1966)
Fat (Horse)	24	1.8			1443	Frucht (1953)
	37	1.6	0.07	0.044	NR	Schwan et al. (1953)
		2.5	0.17	0.068		
		4	0.3	0.075		
		6	0.56	0.093		
		7	0.8	0.11		
		1	0.04	0.04	1400	Schwan et al. (1954)
		2	0.10	0.05	NR	
		4	0.30	0.075		
		6	0.55	0.091		
Heart (Human)	25	4	0.8-1.3	0.2-0.325		Nover & Glanschneider (1965)
Heart (Beef)	24	1.8	NR	NR	1570	Frucht (1953)
	35	1.5	0.34	0.227	NR	Hueter (1948)
		2.4	0.452	0.188		
		4.5	0.8	0.178		
	NR	0.3-0.6	0.185F	0.185		Esche (1952)
Heart (Dog)	20-23	2	0.1	0.05		O'Donnell et al. (1978b)
		4	0.2	0.05		

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Heart (Dog)	20-23	6	0.4	0.067	NR	O'Donnell et al. (1978b)
		8	0.45	0.056		
	19.5	2-10	0.072F	0.072		O'Donnell et al. (1977a)
	35		0.061F	0.061		
		2	0.10	0.05		O'Donnell et at. (1978b)
		4	0.19	0.047		
		6	0.33	0.055		
		8	0.48	0.06		
		10	0.65	0.065		
	26	4-12	NR	NR	1572	Goldman & Richards (1954)
Kidney (Human)	22-31	96	10	0.104	NR	Kessler (1973)
		222	51.8	0.23		
	25	4	0.7-1.2	0.175-0.3		Nover & Glaschneider (1965)
Kidney (Beef)	24	1.8	NR	NR	1572	Frucht (1953)
	35	0.8	0.1	0.125	NR	Hueter (1948)
		1.5	0.19	0.127		
		2.4	0.27	0.11		
		4.5	0.5	0.11		
	NR	0.3-0.6	0.11F	0.11		Esche (1952)
Kidney (Dog)	26	4-12	NR	NR	1559	Goldman & Richards (1954)
	24	1.8	NR		1557	Frucht (1953)
Kidney (Pig)	25	2.5			1558	Ludwig (1950)
	24	1.8			1560	Frucht (1953)
	21-22	2.1-2.3	0.184	0.088-0.080	1579	Marcus (1973)
		2.1-2.3	0.161	0.077-0.070	1579	
	NR	5	NR	NR	1566	Nakajimi et al. (1960)

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Kidney (Pig)	25	2	0.08	0.04	NR	Frizzell (1975)
Liver (Human)	37	1.5	0.203	0.135	1540	Mountford & Wells (1972)
	24	1.8	NR	NR	1569.5	Frucht (1953)
	NR	1	0.149	0.149	NR	Dussik & Fritch (1955)
		3	0.249	0.083		
		5	0.35	0.07		
	40	0.97	0.14	0.14		Oka (1977)
		0.57	0.034	0.06		Nakaima et al. (1976)
		0.97	0.14	0.14		
		1.7	0.15	0.088		
		2.9	0.22	0.076		
Liver (Beef)	24	1.8	NR	NR	1578	Frucht (1953)
	35	1.5	0.2	0.13	NR	Hueter (1948)
		2.4	0.245	0.102		
		4.5	0.57	0.127		
	NR	10	1.37	0.137		Hueter & Cohen (1953)
		23	3.0	0.130		
	25	1.5	0.265	0.177		Hueter (1958)
		4.5	0.576	0.128		
		0.59	0.067	0.114	NR	Frizzell (1975)
		1.0	0.091	0.091		
		1.8	0.11	0.061		
		3.3	0.21	0.064		
		5.5	0.36	0.065		
	Rm	4	0.51	0.127		Bamber et al. (1977)
		5	0.63	0.126		
		6	0.71	0.118		
Liver (Dog)	36	1.5	0.201	0.134		Hueter (1958)
		4.5	0.461	0.102		
	25	1.5	0.173	0.115		
		4.5	0.449	0.10		

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Liver (Dog)	30	0.57	0.03	0.05	NR	Yosioka et al. (1966)
		0.97	0.06	0.061		
		1.7	0.07	0.041		
		2.9	0.15	0.052		
		4.5	0.4	0.089		
	26	4-12	NR	NR	1581	Goldman & Richards (1954)
	40	0.97	0.06	0.062	NR	Oka (1977)
		0.57	0.04	0.07		Nakaima et al. (1976)
		0.97	0.05	0.052		
		1.7	0.09	0.053		
		2.9	0.21	0.072		
		4.8	0.50	0.104		
Liver (Horse)	24	1.8	NR	NR	1580	Frucht (1953)
Liver (Guinea Pig)		1.8			1575-1589	
Liver (Pig)	25	2.5			1553	Ludwig (1950)
	24	1.8			1588	Frucht (1953)
	Rm	4	0.48	0.12	NR	Bamber et al. (1977)
		5	0.703	0.141		
		6	0.97	0.162		
Liver (Rabbit)	24	1.8	NR	NR	1607	Frucht (1953)
		1.8			1599	
	26	4-12			1575	Goldman & Richards (1954)
	25	2	0.14	0.07	NR	Frizzell (1975)
Milk (Human)	30	2	NR	NR	1540	Kossoff et al. (1973)
Milk (Beef)	23.5-27	0.6	0.029	0.048	NR	Hueter et al. (1953)
		1.0	0.05	0.05		
		5.0	0.23	0.046		
		10.0	0.46	0.046		
		21.0	0.92	0.044		
		40.0	2.07	0.051		

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Milk (Beef)	23.5 -27	5-11	0.045F	0.045	NR	Lizzi & Laviola (1976)
Muscle (Human)	37	5	NR	NR	1515-1587	Ludwig & Struthers (1949)
		1.5	0.11	0.073	NR	Hueter (1958)
		4.5	0.591	0.131		
		5	NR	NR	1500-1610	Ludwig & Struthers (1949)
		1.5	0.063	0.042	NR	Hueter (1958)
		4.5	0.518	0.115		
		5	NR	NR	1504-1563	Ludwig & Struthers (1949)
	40	0.97	0.15	0.154	NR	Oka (1977)
		0.57	0.10	0.175		Nakaima et al. (1976)
		0.97	0.15	0.155		
		1.7	0.31	0.182		
		2.9	0.10	0.034		
	NR	0.8	0.596	0.745		Pohlman (1939)
Muscle (Human or Beef)	23	1	0.22	0.22		Dussik & Fritch (1956)
	26-28	1	0.16	0.16		
		3	0.48	0.16		
		5	0.71	0.142		
		1	0.08	0.08		
		3	0.30	0.1		
		5	0.40	0.08		
		1	0.1	0.1		
	25	2.5	NR	NR	1540	Ludwig (1950)
Muscle (Beef)	24	1.8			1581	Frucht (1953)
	15-18	0.3	0.09	0.3	NR	Colombati & Petralia (1950)
		0.87	0.18	0.207		
		1.7	0.25	0.147		

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Muscle (Beef)	15-18	3.4	0.60	0.176	NR	Colombati & Petralia (1950)
		0.3	0.075	0.25		
		0.87	0.055	0.063		
		3.4	0.25	0.073		
	20	5	NR	NR	1540	Ludwig & Struthers (1949)
	NR	3	0.17	0.057	NR	McNeely & Noordergraaf (1977)
	21-24	2.1	0.253-0.403	0.120-0.192	1579-1600	Marcus (1973)
		2.1	0.219-0.863	0.104-0.411	1579-1603	
		2.1	0.195-0.242	0.093-0.155	NR	
		2.3	1.15	0.5	1401-1579	
		2.3	0.207	0.09	NR	
	25	1.5	0.098	0.065		Hueter (1958)
		4.5	0.483	0.107		
Muscle (Cat)	37	1.5	0.075	0.05		
		4.5	0.230	0.051		
		1.5	0.106	0.071		
		4.5	0.322	0.071		
Muscle (Dog)	40	0.97	0.09-0.1	0.093-0.103		Oka (1977)
		0.57	0.08	0.140		Nakaima et al. (1976)
		0.97	0.1	0.103		
		1.7	0.15	0.088		
		2.9	0.28	0.096		
	26	4-12	NR	NR	1592	Goldman & Richards (1954)
		4-12			1576	
Muscle (Horse)	24	1.8			1598	Frucht (1953)
Muscle (Pig)		1.8			1580	
Muscle (Rabbit)	26	4-12			1603	Goldman & Richards (1954)
		4-12			1587	

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Muscle (Rat)	23	3.1	0.42	0.135	1608	Bhagat et al. (1976)
		5.35	0.52	0.097	NR	
		7.68	1.0	0.130		
Skin (Human)	40	0.97	0.24	0.247		Oka (1977)
	23	1	0.4	0.4	1498	Dussik & Fritch (1956)
		3	0.85	0.283	NR	
		5	1.1	0.22		
	NR	10	NR	NR	1518	Daly & Wheeler (1971)
Skin (Dog)	40	0.97	0.16	0.165	NR	Oka (1977)
		0.57	0.14	0.246		Nakaima et al. (1976)
		0.97	0.16	0.165		
		1.7	0.36	0.212		
		2.9	0.71	0.245		
		4.8	0.75	0.156		
	37	5	NR	NR	1720	Goans et al. (1977)
Spleen (Human)	18.2	1.6	0.09	0.056	NR	Chivers & Hill (1975
		2.25	0.15	0.067		
		4	0.47	0.117		
		5.6	0.7	0.125		
Spleen (Beef)	24	1.8	NR	NR	1578	Frucht (1953)
	Rm	4	0.36	0.09	NR	Bamber et al. (1977)
		5	0.50	0.1		
		6	0.67	0.11		
Spleen (Dog)	26	4-12	NR	NR	1570	Goldmand & Richards (1954)
Spleen (Horse)	24	1.8			1594	Frucht (1953)
Spleen (Pig)	25	2.5			1515	Ludwig (1950)
	24	1.8			1578	Frucht (1953)
Tendon (Beef)	23	1	0.54	0.54	1750	Dussik & Fritch (1956)
		3	1.25	0.625	NR	
		5	1.94	0.388		
		1	0.58	0.58		

TISSUE	TEMP (C°)	FREQ. (MHz)	ATTENUATION (cm^{-1})	A/F ($cm^{-1}MHz^{-1}$)	VELOCITY (m/sec)	REFERENCE
Tongue (Beef)	35	1.5	0.032	0.021	NR	Hueter (1948)
		2.4	0.037	0.015		
		4.5	0.075	0.017		
		1.5	0.063	0.042		
		2.4	0.13	0.054		
		4.5	0.24	0.053		
Uterus	37	5	NR	NR	1633	Bakke & Gytre (1974)
	23	1	0.22	0.22	1625	Dussik et al. (1958)

of linear frequency dependence of attenuation, the data of Table 2-1 for five tissue types was fit using a linear regression analysis, to a power function of frequency, the results of which are shown in Table 2-2. It appears that for all five tissues, attenuation closely approximates a linear function of frequency, at least to within about ± 10%. Since velocity dispersion in tissue is generally considered to be small, the frequency dependence of velocity is assumed to be insignificant for this purpose.

TABLE 2-2

Frequency Dependence of Attenuation

TISSUE	NUMBER OF DATA POINTS	FREQUENCY DEPENDENCE OF ATTENUATION	BEST FIT PARAMETER
Brain	106	$A = 0.07F^{1.14}$	$R = 0.82$
Heart	22	$A = 0.126F^{1.07}$	$R = 0.98$
Kidney	13	$A = 0.104F^{1.09}$	$R = 0.97$
Liver	43	$A = 0.083F^{1.13}$	$R = 0.93$
Tendon	4	$A = 0.557F^{0.763}$	$R = 0.998$
CARTILAGE	3	$A = .580\ F^{.826}$	$R = 1.000$
FAT	13	$A = .051\ F^{1.269}$	$R = .972$
MUSCLE	45	$A = .138\ F^{.734}$	$R = .702$
SKIN	10	$A = .233\ F^{.937}$	$R = .980$
SPLEEN	7	$A = .043\ F^{1.573}$	$R = .991$

In Table 2-3 average values of ultrasonic velocity and frequency normalized attenuation taken from the data of Table 2-1, and the standard deviation associated with each averaging process are shown for each tissue. Also shown are the number of data points averaged for each tissue. Large standard deviations are thought to reflect differences not only in measurement method, which is most likely responsible for some variation in the observed parameters (Goss et al., 1978b) but also variation in temperature and specimen preparation (although all were reported to be fresh).

Tissue constituent values used in this analysis, shown in Table 2-4, are representative of mammalian tissue. Water, total protein, collagen, and lipid content were taken from a recent compilation by Snyder et al. (1975), and Chvapil (1967), except where otherwise indicated. All values are in terms of wet weight percentage constituent in adult tissues. Most tissues, such as liver, kidney, muscle, and heart contain total protein concentration in the 15-20% range, but contain comparatively little collagen, generally less than 1%. Tendon is the most notable exception since of the 35-40% total protein contained, approximately 30% is collagen. Water content for most soft tissues lie in the 70-80% range, compared to only 60% in connective tissues. Lipid concentrations are greatest in brain tissue, where the quantity is that found in other soft tissues, with only negligible amounts of connective tissue. Connective tissues are thus much different constitutionally than soft tissues, which may be expected to lead to unique ultrasonic properties.

Figure 2-1 through 2-4 show the results of plotting the attenuation values of Table 2-3 as a function of the four constituent quantities, viz., total protein, collagen, lipid, and water. Also shown in each figure are the results of linear regression fit of the data to linear and power functions, and the quality

TABLE 2-3

Average Values of Attenuation and Velocity in Tissue

TISSUE	A/F ($cm^{-1}MHz^{-1}$) + S.D.	NUMBER OF DATA POINTS	VELOCITY + S.D.	NUMBER OF DATA POINTS
Blood Vessel	0.17 ± 0.1	2	1501	1
Brain	0.09 ± 0.06	104	1551 ± 25	34
Breast	0.227	2	1509 ± 33	5
Cartilage	0.5 ± 0.07	3	1665	1
Cerebrospinal Fluid	0.001	1	1508 ± 8	3
Eye				
Aqueous Humor	0.04 ± 0.03	3	1506 ± 26	6
Cornea	NR	R	1584 ± 40	5
Lens	0.42 ± 0.39	7	1633 ± 40	17
Sclera	NR	NR	1635 ± 21	6
Vitreous Humor	0.032 ± 0.03	5	1510 ± 23	13
Fat	0.07 ± 0.02	13	1467 ± 46	6
Heart	0.11 ± 0.08	17	1571 ± 1	2
Kidney	0.12 ± 0.07	14	1566 ± 9	8
Liver	0.099 ± 0.04	43	1578 ± 18	12
Milk (whole)	0.047 ± 0.002	7	1540	1
Muscle (skeletal)	0.146 ± 0.12	49	1566 ± 49	22
Skin	0.235 ± 0.07	11	1579 ± 122	3
Spleen	0.095 ± 0.03	7	1567 ± 30	5
Tendon	0.45 ± 0.067	6	1750	1
Tongue	0.034 ± 0.018	6	NR	NR
Uterus	0.22	1	1629 ± 4	2

TABLE 2-4

Principal Constituents (% Wet Weight) of Biological Tissues.
Total Protein, Lipid and Water Content after Snyder et al., 1975;
Collagen Content Computed from Dry Weight Percentages (Chvapil, 1967)
by Assuming Appropriate Water Content

Tissue	% Total Protein	% Collagen	% Lipid	% Water
Blood Vessel	24(23-27)	5.7(5-6.5)	1.8(1.5-1.9)	70
Brain	10(8-12)	0.16 (0.05-0.28)	11 (9-17)	77.4 (76-78)
Breast	20	-----	3	50-75
Cartilage	16(10-18)	15.5(14-17)	1.3	72(55-58)
Cerebrospinal Fluid	0.028 (0.012-0.043)	-----	-----	99
Eye (Aqueous Humor)	0.005 (0.005-0.016)	-----	-----	98.1
(Cornea)	20	13.5	-----	78
(Lens)	35.5	-----	1.7-2.3	68
(Sclera)	26-27	22-24	0.62	68-75
(Vitreous Humor)	0.013 (0.011-0.016)	0.01-0.05	-----	99
Fat	5	-----	80	15(10.0-21)
Heart	16.5(14-19)	1.7(1.4-2.0)	2.6(2.7-17)	72(63-83)
Kidney	17 (14.7-19.3)	0.865 (0.43-1.3)	5(1.8-7.2)	76(71-81)
Liver	18(16-22)	0.4(0.1-0.7)	6.9 (1.1-11.5)	71 (63.6-73.9)
Milk	3.5	-----	3.5	87.4
Muscle (Skeletal)	17.2(13-20)	0.6(0.4-0.8)	2.2(2.2-9.4)	79 (68.9-80.3)
Skin	33(32-34)	30	0.3-19	62(53.7-72.5)
Spleen	19.5(18.8-20.2)	0.6	1.6(0.85-3	77(72-79)
Tendon	35-40	32	1	63
Testis	12	-----	3	81
Tongue	16-18	-----	15-24	60-72
Uterus	20	17	1.4(0.9-2.2)	79

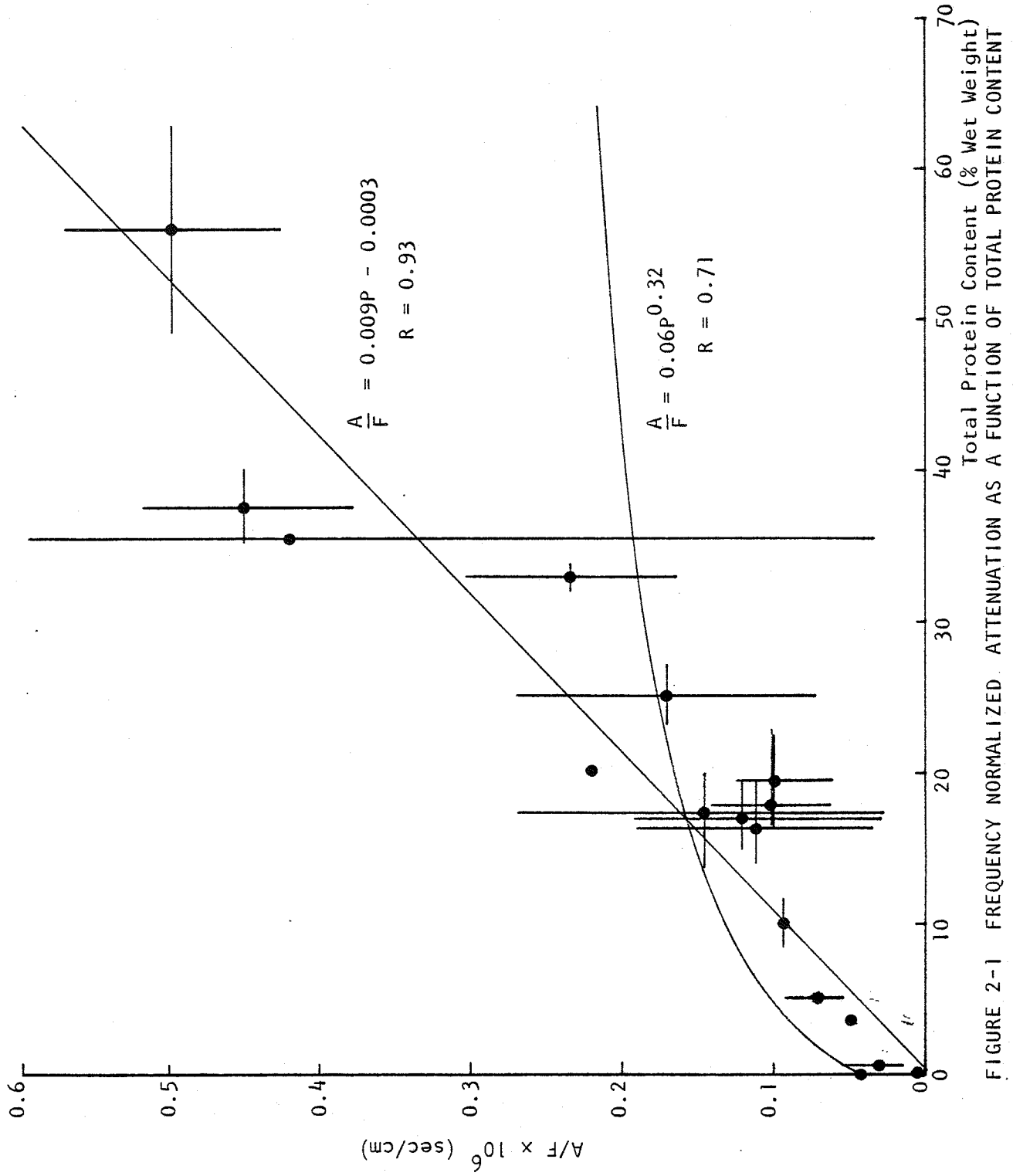

FIGURE 2-1 FREQUENCY NORMALIZED ATTENUATION AS A FUNCTION OF TOTAL PROTEIN CONTENT

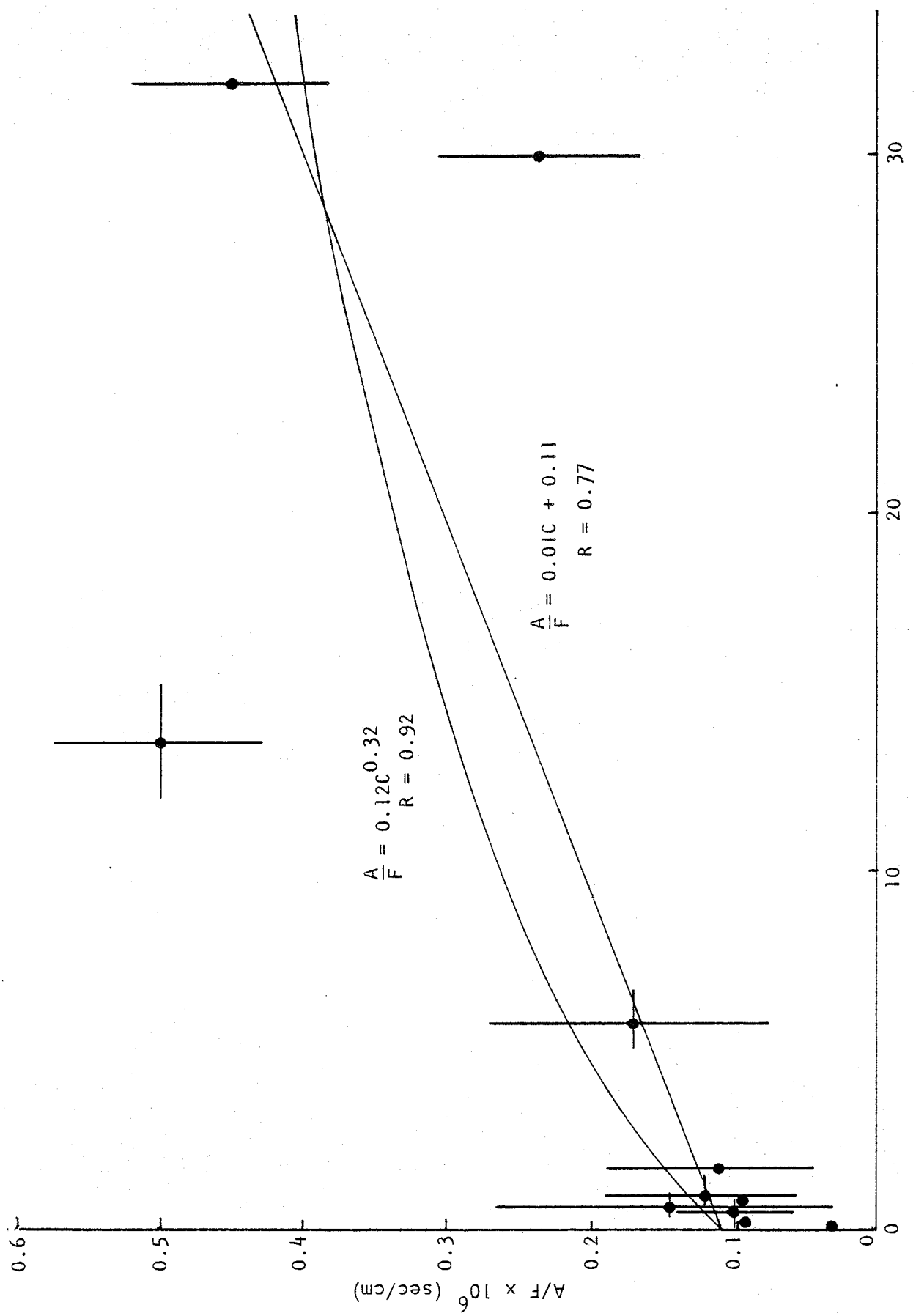

FIGURE 2-2 FREQUENCY NORMALIZED ATTENUATION AS A FUNCTION OF COLLAGEN CONTENT

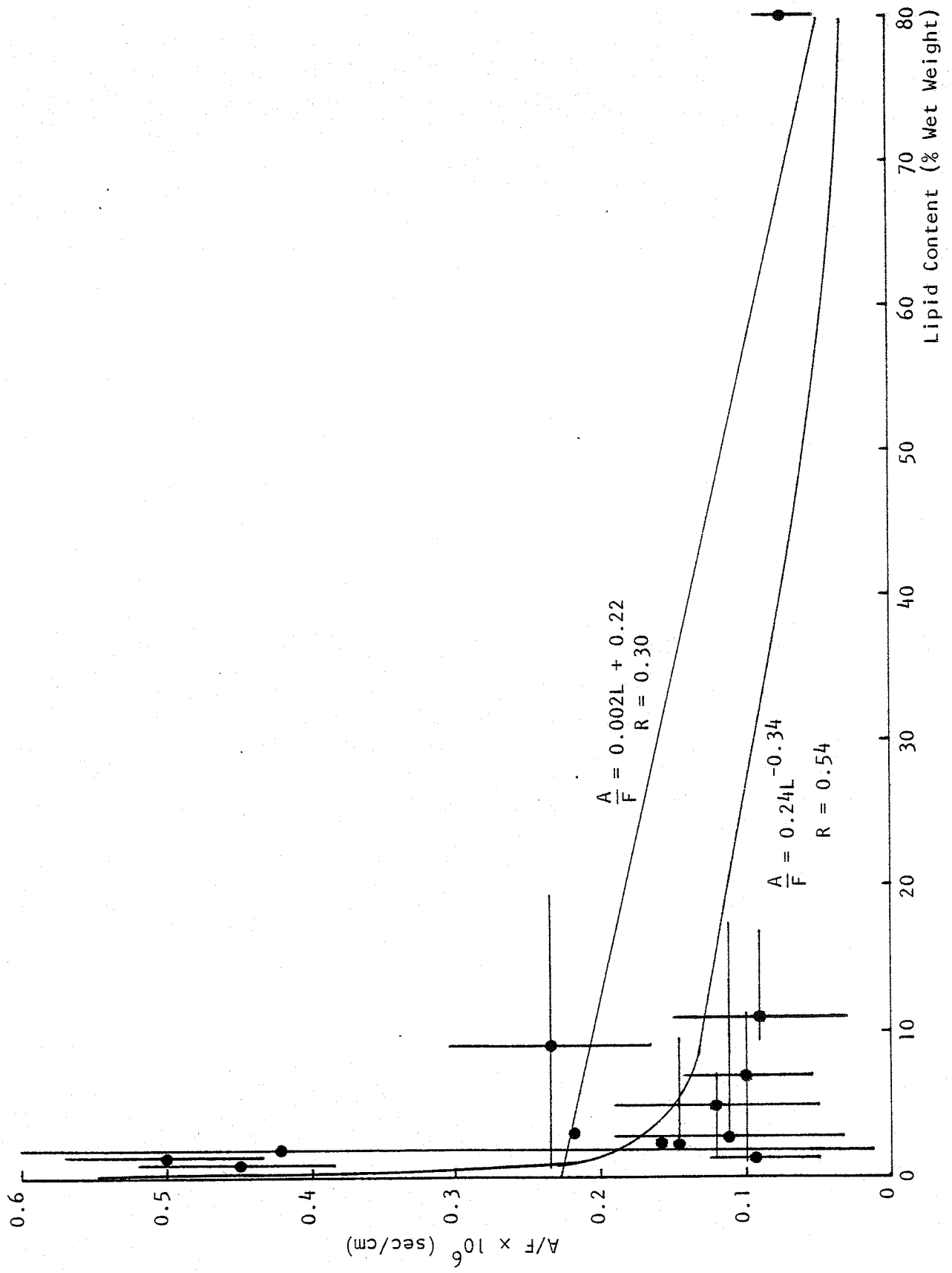

FIGURE 2-3 FREQUENCY NORMALIZED ATTENUATION AS A FUNCTION OF LIPID CONTENT

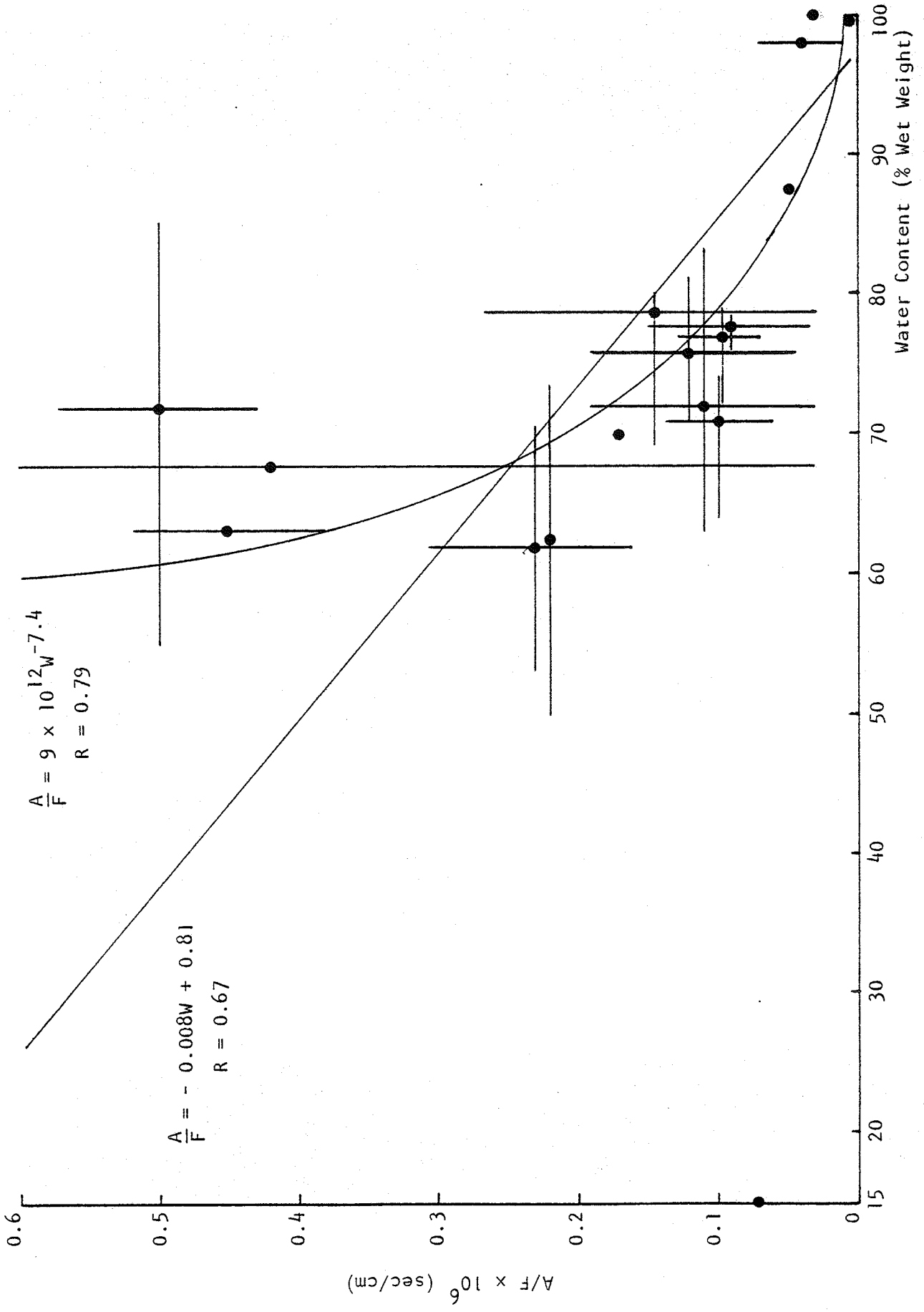

FIGURE 2-4 FREQUENCY NORMALIZED ATTENUATION AS A FUNCTION OF WATER CONTENT

of fit, with R = 1 representing a perfect fit. The expressions best describing the dependence of ultrasonic attenuation upon tissue constituents, from this analysis, exhibit correlation coefficients which are all within a 95% statistical confidence level, unless otherwise indicated. In these expressions, F is the ultrasonic frequency in megahertz. For total protein, with the fit parameter R = 0.93,

$$\frac{A}{F} = 0.009\ P - 0.003 \tag{2-47}$$

where P is the wet weight percentage of total protein; for collagen (R = 0.924)

$$\frac{A}{F} = 0.12\ C^{0.32} \tag{2-48}$$

where C is the wet weight percentage of collagen; for lipid (R = 0.5)

$$\frac{A}{F} = 0.24\ L^{-0.34} \tag{2-49}$$

where L is the wet weight percentage of lipid; for water (R = 0.79)

$$\frac{A}{F} = 9.00 \times 10^{12}\ W^{-7.4} \tag{2-50}$$

Each of these expressions appears to indicate a significant dependence of attenuation upon tissue constituents. The linear dependence of attenuation on total protein is similar to the dependence of attenuation of blood upon protein content (Carstensen et al., 1953), as well as that found for the concentration dependence ($C^{1.2}$) of absorption in a globular protein in aqueous solution, bovine serum albumin, over the concentration range from 5-40% (Goss and Dunn, 1974). The collagen dependence, $C^{0.32}$ is slightly less than that determined by O'Brien (1977), where a $C^{0.52}$ dependence was determined. It is interesting to note that

although collagen concentrations found in tissue are generally much smaller than the amount of lipids, correlation is much greater between attenuation and collagen than between lipid concentration and attenuation, though the analysis in each involved a similar number of data points. The heightened influence on attenuation of collagen over lipids may be explained by considering that the attenuation per unit concentration of collagen is much greater than that per unit concentration of lipid, which is generally thought to be the case (Pohlman, 1939; Dussik and Fritch, 1956; Schwan et al., 1953; Schwan et al., 1954). The strong dependence of attenuation upon water content ($W^{-7.4}$; $R = 0.79$) supports the view brought forward by Dussik and Fritch (1956) and later by Dunn (1975), that the water content of tissues strongly affects the ultrasonic properties exhibited by them.

Similar analyses of ultrasonic velocity dependence on tissue constituents is shown in Figures 2-5 thru 2-8, where expressions describing this dependence obtained by linear regression analysis are also again shown. These expressions are generally characterized by poorer best fit parameters, indicating that the behavior here may be more complex than that which may be expressed as a simple linear or power fit. Still, a better than 95% confidence level exists for all of these correlation coefficients, which describe the quality of fit of these expressions. The expression which was found to describe the dependence of ultrasonic velocity as a function of total protein content of the tissue is ($R = 0.76$).

$$V = 3.5\ P + 1502 \qquad (2\text{-}51)$$

while for collagen ($R = 0.72$)

$$V = 3.9\ C + 1549 \qquad (2\text{-}52)$$

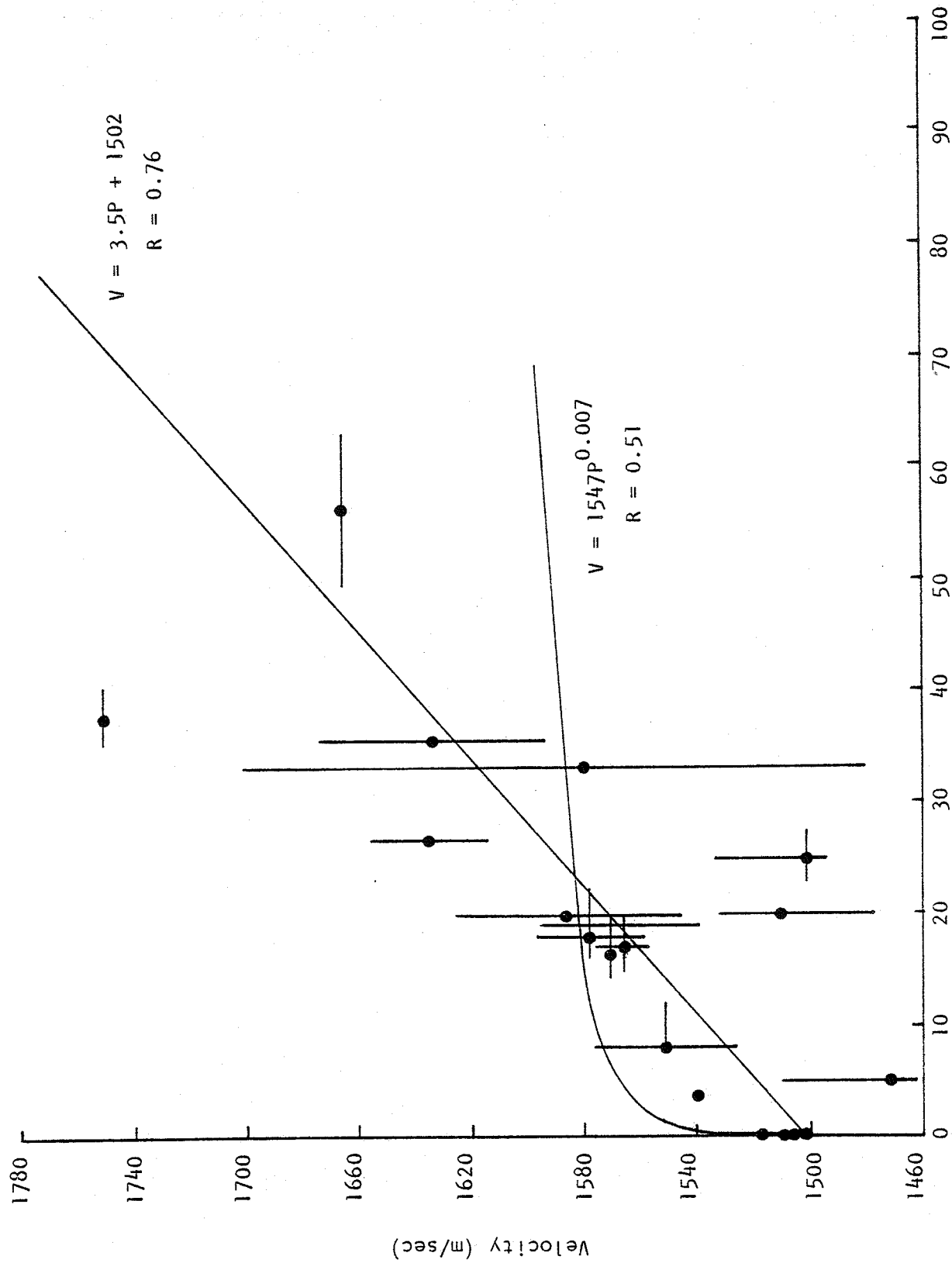

FIGURE 2-5 ULTRASONIC VELOCITY AS A FUNCTION OF TOTAL PROTEIN CONTENT

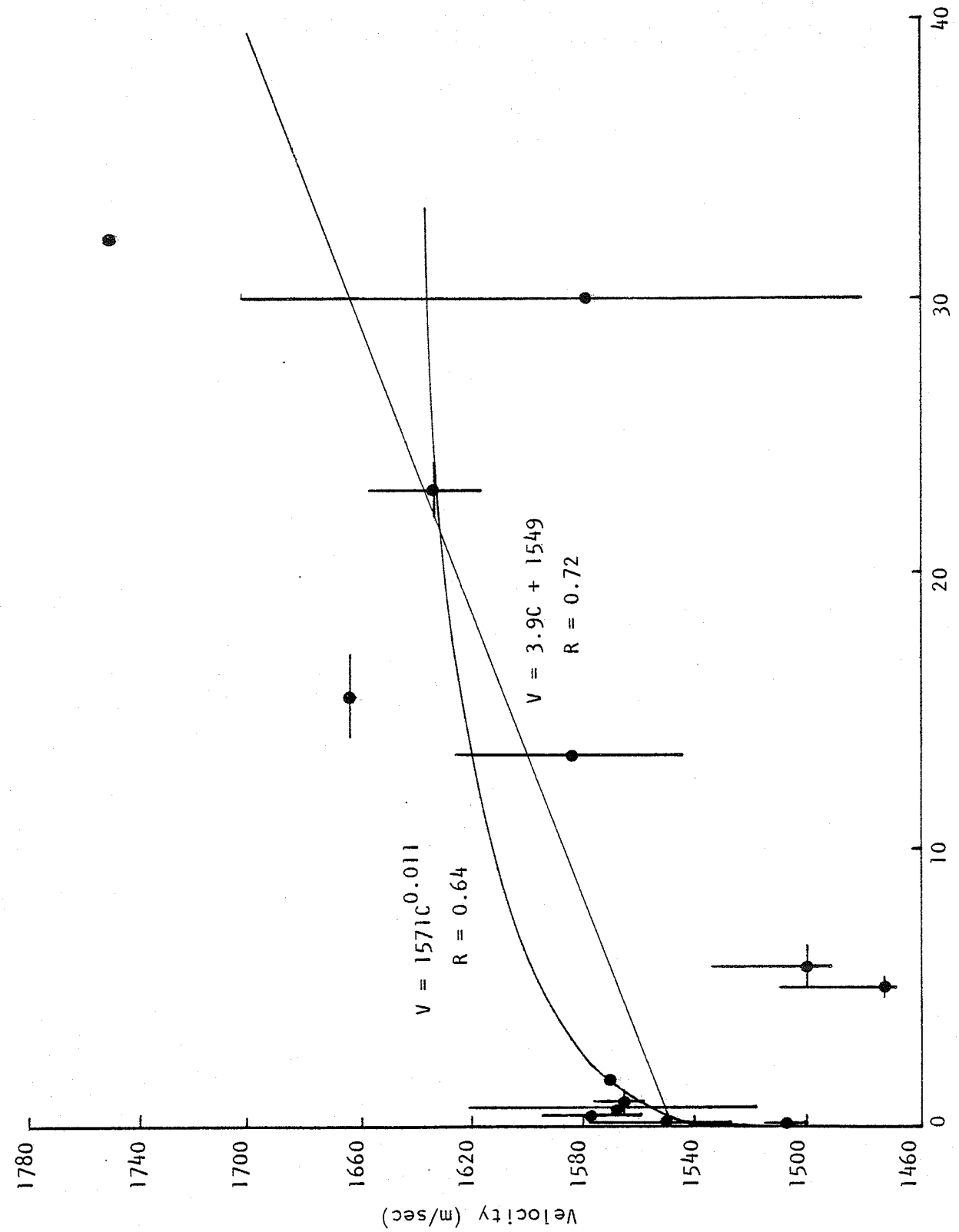

FIGURE 2-6 ULTRASONIC VELOCITY AS A FUNCTION OF COLLAGEN CONTENT

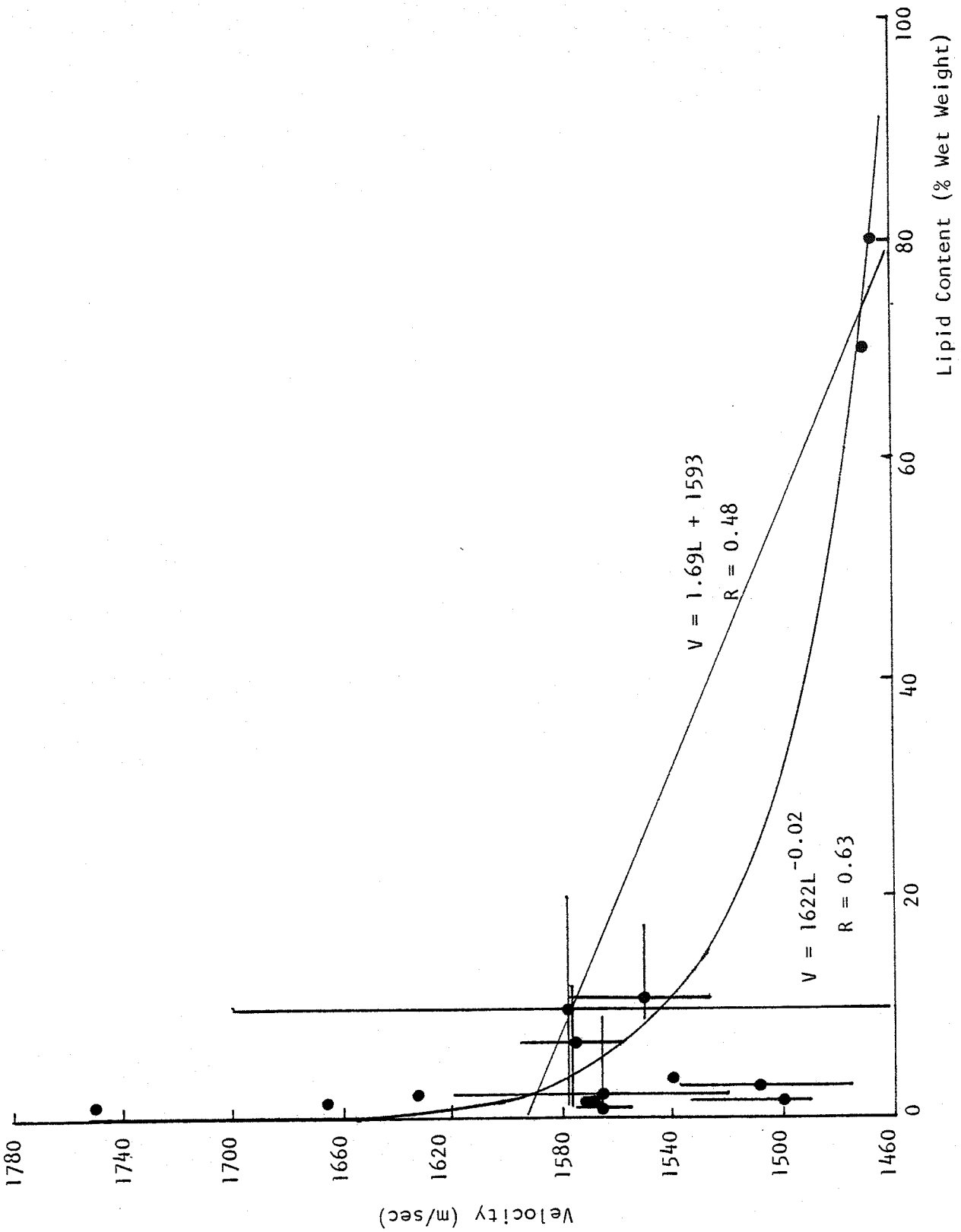

FIGURE 2-7 ULTRASONIC VELOCITY AS A FUNCTION OF LIPID CONTENT

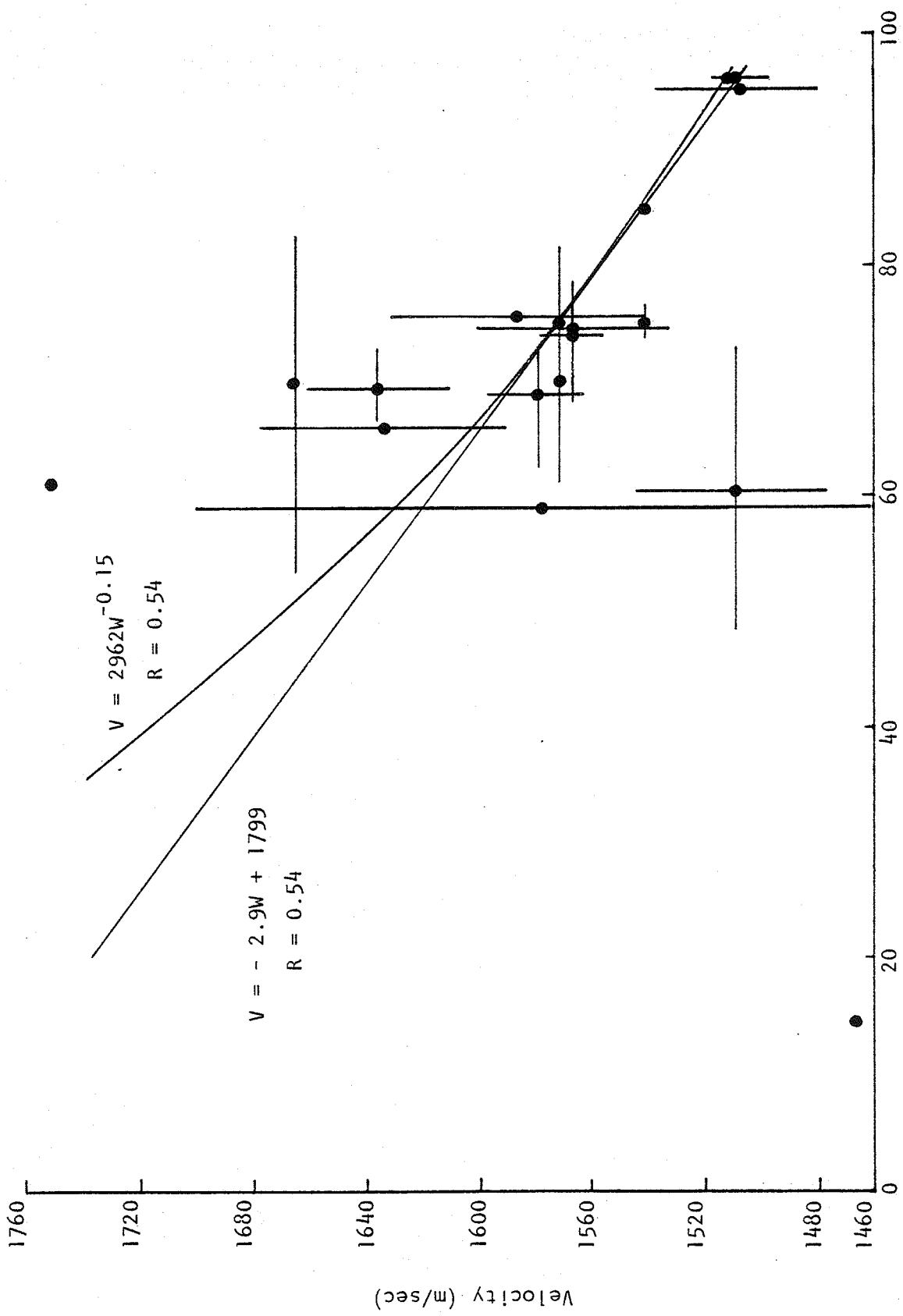

FIGURE 2-8 ULTRASONIC VELOCITY AS A FUNCTION OF WATER CONTENT

For the lipid dependence of velocity (R = 0.63)

$$V = 1622\ L^{-0.023} \tag{2-53}$$

while for water content (R = 0.54)

$$V = -2.9\ W + 1799 \tag{2-54}$$

In each of these equations P, C, L, and W are defined as before, viz., the wet weight percentage of constituent.

The relatively strong influence of total protein, collagen, and water is evident from viewing the data in this manner. Velocity increases with increasing protein and collagen concentration, while increasing water content of a tissue tends to reduce the observed ultrasonic velocity. The expression for collagen dependence of ultrasonic velocity is linear, in contrast to that obtained previously (O'Brien, 1977), in which a logarithmic relation was determined. Nevertheless, the relatively small collagen content of tissues appears to influence the characteristic ultrasonic velocity of these tissues in a significant manner, lending at least qualitative support to the idea first proposed by Fields and Dunn (1973) that enhanced echographic visualizability may be due to a greater acoustic impedance mismatch at soft tissue-collagen interfaces, as suggested by the large difference in the static elastic modulus characteristic of each of these biomaterials. It is thus possible to demonstrate quantitatively, that a relation exists between collagen and the other tissue constituents and the ultrasonic attenuation and velocity exhibited by the tissues as a whole.

Chapter 3

Physical and Chemical Structure of Collagen and Collagenous Tissue

3.1 General

The acoustic properties of any material are determined by its molecular constituents and physical structure (Herzfeld and Litovitz, 1959). The structural complexity of a polymer is related to the diversity of bonds which constrain the constituent atoms (Bernal, 1958). It is, in fact, the many types of intra- and intermolecular bonds constituting protein molecules and their reactions which sets these macrocolecules apart from the most complicated synthetic polymers. This type of highly ordered structure is characteristic of collagenous tissue. The purpose of this chapter is to review briefly the structural hierarchy of collagen, from the molecular level to the fibrous state.

3.2 Molecular Structure

Collagen is made up on 18 amino acids appearing in various proportions according to species (Hannig and Nordwig, 1967; Fietzek and Kuhn, 1976). The following general remarks can be made for most vertebrates. First the hydrophilic nature of collagen is clear, since the combined content of acidic, basic, and hydroxylated amino acid residues exceeds by far the content of lipophilic residues (Eastoe, 1967). This fact suggests that collagenous material should be soluble or have increased volume in polar liquids with higher solubility parameters. Indeed, the best solvents for collagen are aqueous solutions of weak or strong electrolytes. Second, glycine, the simplest amino acid, makes up 35% of the total and is thought to be uniformly distributed throughout the chain structure

(Hannig and Nordwig, 1967). The possible free rotation of the chain suggesting that collagen ought to be quite flexible is mitigated by chain-chain bonding at a higher structural level, restricting rotation at these glycyl residues.

Finally, 25% of the residues are composed of the amino acids proline and hydroxyproline (Hannig and Nordwig, 1967). Since no other protein contains a significant amount of hydroxyproline, the concentration of this residue serves as a measure of total collagen content (Gould, 1967). The proline rings introduces severe restriction to rotation, such that at least 25% of the total amino acids exist as rigid chain links (Eastoe, 1967). The local configuration of these individual chains is dependent upon the stereochemical nature of constituent amino acids, which determine in addition to the bond lengths and bond angles of the chain backbone, the possibility of rotation about skeletal bonds. In polypeptides, rotation about these skeletal bonds favors the formation of inter-or intramolecular hydrogen bonds (Bamford et al., 1956). The chain rigidity introduced by the large amount of proline and hydroxyproline however restricts formation of intrachain hydrogen bonding (von Hippel, 1967), but the great amount of glycine in each chain provides for sterically permissable hydrogen bonding to a large extent. Therefore, collagen chains are helical macromolecules exhibiting a strong preference for interchain, rather than intrachain hydrogen bonding.

The fundamental monomeric unit of collagen is called tropocollagen, after Gross, et al.,(1954), who applied the term to what was first a hypothetical repeating building unit of fibrous collagen, but was soon found to be identical to the collagen monomer which was then in the process of being characterized. The tropocollagen molecule is about 2900$\mathring{A}$ long, and 14$\mathring{A}$ in diameter in dilute solution, and exhibits a molecular weight of about 300,000 (Boedtker and Doty, 1956; Ramachandran, 1967; Glimcher and Krane, 1968). This may be compared with the typical globular protein, which may be spherical with a 50-60 $\mathring{A}$ diameter, and a molecular weight of about 60,000. Each tropocollagen

molecule may be thought of as a "cable" consisting of three polypeptide chains with each chain being in a tight left-hand helix, and the three chains then formed into a right-hand super-helix (Ramachandran and Kartha, 1954; Rich and Crick, 1955; Ramachandran, 1967; Glimcher and Krane, 1968). The pitch of the helix is determined by the limited flexibility of the proline and hydroxyproline residues. The tight triple-stranded structure is possible only because of the high glycine content, as every third amino acid side chain in each strand is essentially within the interior of the molecule, and only glycine with its small volume, can fit into this structure.

Each of the three consitituent polypeptide chains, known as alpha chains, has a molecular weight of 100,000; two having almost identical structures (alpha-1 chains), while the third structure is somewhat different (alpha-2 chains). Two alpha chains can combine by covalent cross-linking to form the so called beta chain, while a formation of three chains is called a gamma chain. The three strands of the helix are therefore tied together by primary, as well as secondary valence bonds, even though the former probably do not make a substantial contribution to the stability of the isolated helix (Bornstein and Piez, 1966).

Different combinations of the alpha chains in the tropocollagen molecule, as well as subtle variations in the amino acid sequence of each chain, have been identified from various connective tissue structures, giving rise to four distinct forms of collagen. The collagen type longest known, and most extensively investigated, is collagen type I which represents the main constituent of skin, tendon, and the proteinaceous matter of bone. Its molecule is composed to two alpha-1 (I) chains and one alpha-2 chain (Traub and Piez, 1971). Cartilage collagen has been designated type II, and contains three identical alpha-1 (II) chains (Miller, 1973). Three identical alpha-1 (III) chains are also found in collagen type III, first

encountered during investigations on the collagen from skin and arterial walls (Miller, et al., 1971; Chung and Miller, 1974) and which occurs in increased proportion in fetal tissues (Epstein, 1974). Basement membrane collagen has been termed type IV, and its molecule likewise appears to contain three alpha-chains (Kefalides, 1973). The complete amino acid sequence has only been determined for the alpha-1 (I) chain (Fietzek and Kuhn, 1975), and the sequence of half the residues of the alpha-2 chain, with alpha-1 (II) and alpha-1 (III) remaining unknown (Fietzek and Kuhn, 1976).

3.3 Fibril and Fiber Structure

The manner in which tropocollagen molecules are organized into long cross-striated fibrils which eventually combine to form collagen fibers, remains unsettled. The electron microscopic appearance of the fibril is that of cylindrical structures of uniform diameter, but varying with development and tissue source from 100Å to 2000Å or more (Harkness, 1968). Their precise length is unknown, but believed to be large, possibly of the order of millimeters. Parallel arrangement of a number of fibrils form collagen fibers, having diameters ranging down to 0.2 μm most often being 1 and 12 μm (Viidik, 1973). When present in parallel-fibered structures such as tendon or ligament, the fibers are assembled into primary bundles, about 100 microns in diameter, the dimensions of which vary with the anatomical structure.

Since it has been demonstrated in studies on native and reconstituted aggregates of collagen that the length of the molecule is 4.4 times that of the period of the native fibril, the older idea of parallel alignment of the molecules with displacement of adjacent molecules by multiples of one-quarter the length of the tropocollagen molecule (Gross et al., 1954) has been, at least partly,

abandoned (Viidik, 1973). This idea also included end-to-end attachment of molecules. It has been suggested that the difference between native period and molecule lengths may be explained by a combination of overlaps and gaps between the molecules in addition to the normal quarter-length-stagger. Grant et al. (1965) have proposed another model emphasizing cross-linking and intertwining of the molecules. The idea that the tropocollagen molecule consists of five segments, four of these having the length of the period (D), seen in the native fibril, and the fifth being shorter (0.4D), has received some support, since it includes the concept of quarter-length-staggering of the four D segments and leaves a gap of 0.6D between contiguous molecules, which is compatible with the observed steric arrangement (Smith, 1968). Woodhead-Galloway et al. (1975) have proposed still another molecular arrangement of the fibril consisting of only two segments, and includes two adjacent gaps in the fibril, rather than the quarter-length-stagger generally accepted.

Collagen fibers never function alone, but as a integrated part of a tissue. Most often this is connective tissue, which can be divided into cells, composed mainly of fibrocytes, and intercellular substance. The intercellular substance is constituted of fibers, not only collagenous, but elastic and reticular as well, along with ground substance, the composition and amount of which is dependent on the particular type of connective tissue. Although in dense connective tissue, such as tendon, the amount of ground substance is small, it is a fairly dominating component in loose connective tissue. Ground substance is generally regarded as a reticular structure containing tissue fluid, which to some extent is derived from blood plasma, and which plays a fundamental role in tissue nutrition (Muir, 1964).

3.4 Structure of Collagenous Tissue

Collagen is present in most mammalian organs and tissues, comprising 25 to 33% of the tissue total protein, and therefore about 6% of the total body weight, making it the most abundant protein in the body (White et al., 1968). The geometrical arrangement and concentration of collagen within any given tissue depends to a large extent on the function of that tissue. The greatest concentration of collagen is present in connective and supportive tissues, the morphology and function of which have been described in detail (Bloom and Fawcett, 1968; Hall, 1965; Elden, 1968).

The main function of tendon is the transmission of the tension developed by muscle to the skeletal system, and is comprised of a parallel collagenous fiber structure, also found in joint ligaments and the diaphragm. Tendons contain about 30% collagen on a wet weight basis, with about 70% of the dry weight of tendon which is collagen (Harkness, 1968). The fibrils are packed closely and parallel to each other to form fibers which eventually form bundles bound by loose connective tissue. The fibrils are virtually all oriented along the direction of the long axis of the tendon the direction of normal physiological stress. The fiber bundles are surrounded by sheaths, the peritendineum internum or endotenon, which fuse with each other at acute angles, as do the fibers (Viidik, 1973). Fibrocytes are present in long parallel rows between the bundles. The assembly of fiber bundles which forms the tendon is covered by another sheath, the peritendineum externum or epitenon, which is covered by loose areolar tissue, the paratenon, and contains a synovial-like fluid.

Such fiber structure forms an almost purely elastic connection of high elastic modulus. The experimental determination of the elastic modulus of tendon has been treated by a number of investigators. In whole human toe extensor

tendon, the static or low-frequency Young's modulus was determined to be 12 x 10^{10} dynes/cm^2 (Van Broklin and Ellis, 1965) while the value in rat tail tendon is 0.8 x 10^{10} dynes/cm^2 (Rigby et al., 1959). Using an ultrasonic technique at a frequency 20 kHz, Mason (1965) obtained a value of 2.6 x 10^{10} dynes/cm^2 in kangaroo tail tendon, which was greater than twice that found in static measurements of this specimen. This investigator also determined that over the 1% to 3% extension range, the dynamic and static moduli increased at the same rate. More recently, results from measurements of the inelastic light scattering from rat tail tendon collagen yield a value of Young's modulus from 14.7 - 21.5 x 10^{10} dynes/cm^2 depending upon the water content of the collagen at frequencies in the 9-11 GHz range (Harley et al., 1977).

The ultimate strength of tendon subjected to longitudinal tension has generally been found to lie in the range 5-10 kg/mm^2 (Harkness, 1968), with tendons of smaller animals generally tending to be weaker than that of larger animals by as much as a factor of three (Yamada, 1965). Yamada (1970) noted that the ultimate tensile strength recorded was inversely proportional to the thickness of the specimen.

Two-dimensional collagen meshworks are found in the periosteum, periochondrium, and membrane fibrosa of joint capsules, as well as in the dura mater, sclera, and some organ capsules, while three-dimensional arrangements are found in skin and within organs. In the former, the collagen fibers are arranged in fibrous sheets, with occasional perpendicular fibers extending into other surrounding tissues to anchor the sheet in place while in the latter, perpendicular fibers are more abundant and form a fibrous tissue matrix. Thus, the presence and role of collagen tissues and organs is quite variable. In parenchymatous organ tissue, collagen forms only a small part of the organ substance, but together with the other

components of connective tissue, functions as structural supporting tissue. The surface of every muscle, as well as each muscle fiber bundle, is covered by a strong connective tissue sheath. Such collagenous fibers are present in eye muscle, as well as in muscles which bind soft tissue structures. Collagenous fibers in skin run predominatntly parallel to the surface, but perpendicular fiber bundles are found quite frequently and tend to increase in amount with skin thickness. The sclera is a solid collagen connective tissue membrane in which collagen bundles run in all directions. The sclera, as well as cornea, contain primarily collagen.

The absolute quantity of collagen in a specific organ as well as the whole body, is a power function of the weight or body. This relation may be expressed as (Chvapil, 1967)

$$W_c = AW_o^B \tag{3-1}$$

where W_c is the weight of the total collagen protein content in the organ, W_o is the weight of the organ, and B ranges from about 1.3 to 1.5, indicating that the collageneous framework of the organ increases more quickly than the organ as a whole.

The mechanical function of collagen, reflected in its role as a supporting entity, sets this protein apart from globular proteins, the other proteinaceous component of tissue. The elastic modulus of connective tissue for example is some 1000 times greater than that in soft tissues comprised predominantly of globular proteins (Burton, 1967). The molecular density of collagen in the native state, 1.16-1.33 gm/cm^3 (Hulmes et al., 1977); Dweltz, 1962) is also greater than the density of soft tissue, which is usually assumed to be similar to that of water. Unlike most proteins, collagen exhibits age-dependent variability in its

physical and chemical characteristics (Jackson and Bentley, 1960). The unique chemical and mechanical properties of collagen among proteins, and the varied structural arrangements with which it endows tissues, suggests that collagen is of potential importance in determining the acoustic properties of tissues.

Chapter 4

Experimental Method

4.1 Introduction

The ultrasonic propagation properties of collagen, in macromolecular suspension and in fibrous states in tissues and organs, were determined using several specialized measuring techniques.

Two systems were employed in the measurement of ultrasonic absorption and velocity in collagen suspensions. The first, termed the high frequency system operates over a frequency range from about 9-62 MHz, and is an automated version of the pulse technique introduced by Pellam and Galt (1946). Pulse techniques eliminate standing waves and minimize localized heating effects prevalent in some continuous wave methods (Herzfeld and Litovitz, 1959). A number of investigators have since used such pulse methods in the ultrasonic characterization of a number of biological molecular species of interest (Fry and Dunn, 1962; O'Brien and Dunn, 1972b; Hussey, 1975; Dunn and O'Brien, 1976; and Dunn and O'Brien, 1978).

Despite the advantages of the high frequency system over continuous wave methods previously eluded to, diffraction effects (Del Grosso, 1964; 1965; Seki et al., 1956) limit the lowest frequency of measurement to about 3 MHz, and requires that the diameter of the sample chamber be large compared to the diameter of the transducer. Under such conditions, the minimum sample volume is on the order of 500 ml (Goss et al., 1978d).

A second measuring technique employed in the measurement of the ultrasonic absorption in collagen suspensions, termed the resonant cavity system, permits measurements in the 0.5-3 MHz frequency range, and requires sample volumesone-tenth

of that necessary in the high frequency system. This system utilizes an acoustic resonant cavity of the type described by Eggers and Funck (1975), whose mechanical Q is proportional to the ultrasonic absorption per wavelength of the liquid filling the cavity. The small sample volume required for this system (about 50 ml) is particularly attractive for measuring a wide range of biomacromolecular solutions and suspensions, especially those which have not been treated due to economic considerations. While relatively new, this technique has found application in the ultrasonic characterization of a number of liquids (Eggers and Funck, 1973; Pethrick, 1972; Slutsky et al., 1977; Grybauskas et al. 1978).

Ultrasonic absorption measurements in biological tissues and organs were made using the transient thermoelectric technique (Fry and Fry, 1954a; 1954b; Goss et al., 1977), which is well suited for determining acoustic absorption in small volumes of highly absorbing liquid or liquid-like media, in vivo as well as in vitro, and may be the only method applicable to tissues, that measures absorption rather than attenuation (Goss et al., 1978d). Thus, the technique allows the determination of that portion of attenuation due to absorption; the remainder being attributed to scattering and other such loss mechanisms present in the attenuation coefficient. The technique depends on the measurement of the time rate of change of temperature when a small tissue imbedded thermocouple is irradiated with an ultrasonic beam of known duration and intensity. The frequency range over which measurements were made in the present study extended from 0.5 to 7 MHz, however the transient thermoelectric technique has been used at frequencies as low as 0.26 MHz (Dunn and Brady, 1974), and with a few modifications as high as 2 GHz in fluid media (Dunn and Breyer, 1962).

Ultrasonic velocity measurements in tissue can be made simply by measuring the time of flight of an acoustic pulse over a known path length in the specimen

(Wladimiroff et al., 1975; Buschmann et al., 1970). However, errors may be encountered in time of flight measurements of the acoustic signal in inhomogeneous media such as tissue. Since a short duration acoustic pulse contains a broad spectrum of components, the frequency dependent effects of velocity dispersion, attenuation, and multiple phase shifts at tissue interfaces can distort and delay the signal (Lange, 1966). Techniques which rely on the detection of the leading edge of the received acoustic pulse are prone to the greatest inaccuracies under such distortions.

Nearly an order of magnitude improvement of accuracy in the velocity measurement can be realized by the use of an acoustic interferometer employing cw excitation (Goldman and Richards, 1954; Hueter, 1958; Goss et al., 1978d). As used in the present study, a standing wave is created within the tissue sample by "sandwiching" the specimen between a transmitting and a receiving transducer. Measurement of the acoustic wavelength by changing either the path length or the frequency yields the ultrasonic velocity of the specimen, if one or the other is held constant, and is known. This method has received wide application in the measurement of ultrasonic propagation speed in a variety of biological materials (Frucht, 1953; Kikuchi et al., 1972; Gilmore et al., 1969).

A commercial ultrasonic microscope was employed in the 100 MHz measurement of ultrasonic velocity in single collagen threads separated from mouse tail tendon. The measuring technique involves the detection of variations in the acoustic index of refraction between the specimen and the bathing medium surrounding the sample for which the velocity is known (Kessler, 1978; Goss and O'Brien, 1978). While still a relatively new technique, acoustic microscopy has proven to be a useful tool in the ultrasonic characterization of micron regions within tissues (Kessler, 1973; O'Brien and Kessler, 1975; Kessler et al., 1974b; Goss and O'Brien, 1978),

and is well suited to the measurements of interest to the present study.

All of the techniques described briefly above will be discussed in detail in this chapter with regard to instrumentation, experimental procedure, and the error and uncertainty encountered when employing these techniques. Finally, sample preparation and characterization will be described, to define completely the specimen undergoing measurement.

4.2 High Frequency System

4.2.1 General

Pulse techniques for the measurement of ultrasonic absorption and velocity in liquids were first introduced by Pellam and Galt (1946) and Pinkerton (1947, 1949). Their system consisted of one piezoelectric transducer coaxially aligned with an acoustic reflector, with variable distance between the two. By recording the attenuation change in electronic circuitry necessary to compensate for the increase or decrease in amplitude of the received acoustic signal as the path length is varied, the ultrasonic attenuation can be determined. Sound velocity measurements can be made by measuring the time required for the pulse to traverse a known distance.

The pulse technique employed in this study is essentially an automated version of the Pellam and Galt system, using two transducers; one to transmit and one to receive the acoustic signal. The transmitting transducer (an X-cut quartz plate or cast piezoelectric material such as barium titinate) fundamental or odd harmonic fundamental thickness mode of vibration is chosen as the carrier frequency. The amplitude of the sinusoidal voltage applied to a piezoelectric transducer is directly proportional to the acoustic pressure amplitude at the transducer surface. After the pulse of sound is transmitted through the medium under investigation, it

falls incident upon the receiving transducer, whose resonant frequency is matched to that of the transmitting transducer. A portion of the pulse of acoustic energy is reflected back to the transmitting transducer, and a portion received and converted to electrical energy. After being amplified and detected the received signal takes the form of the envelope of the original pulse, which is linearly proportional to the received acoustic pressure amplitude.

As the transmitting transducer is moved away from the fixed receiving transducer, the total attenuation of the signal increases, decreasing the amplitude of the pulse envelope. The decrease appears in an exponential manner, assuming the amplitude of a plane, progressive, sinusoidal wave decays exponentially as it propagates through a lossy, homogeneous, infinitely extended medium according to $P(x) = P_o e^{-Ax}$, where P_o is the pressure amplitude at $x = 0$, A is the attenuation coefficient, and x is the acoustic path length traversed by the pulse. Logarithmic conversion of a dc signal obtained by detecting the peak of the pulse envelope, and the recording of this logarithmically converted signal as a function of acoustic path length yields a straight line whose slope is proportional to the attenuation coefficient of the transmitting medium. The attenuation coefficient is synonymous with the absorption coefficient in those liquid or liquid-like media for which it is certain that structural components are of such dimensions that scattering of sound out of the main beam is negligible.

Effects due to diffraction, caused by waves having traveled different distances from various points of the transmitting transducer surface, were minimized by making the lateral dimensions of the measuring chamber at least twice as large as the diameter of the transducer (Del Grosso, 1965). Those remaining diffraction effects contributing to the measured absorption are corrected for by the method of Del Grosso (1964). The manner in which diffraction corrections are made has been

described in detail by Goss (1974), where it was shown that for dilute protein solutions (about 5%), the diffraction correction could represent up to 35% of the total measured absorption at about 3 MHz, while at 15 MHz, where the attenuation due to diffraction is a smaller fraction of the total liquid absorption, the correction drops to approximately 2%.

Speed of sound is measured with the high frequency system by comparing the phase of the received pulse (envelope) with a coherent reference signal as the transducers are moved relative to each other at constant velocity. This comparison results in a periodic interference signal which may be used to evaluate the phase velocity of the sound wave. The diffraction correction for velocity measurements are typically less than 0.003% (O'Brien, 1970); a correction much smaller than the accuracy of the instrumentation system.

4.2.2 Instrumentation

The high frequency system basically consists of the measuring tank housing the necessary mechanical driving and supporting apparatus required to move the transducers relative to one another, and the associated electronic instrumentation. The transducers are a pair of closely matched, X-cut quartz, one inch in diameter with a 1 or 5 MHz fundamental thickness mode of vibration. Displacement of the transducers is achieved using a piston cylinder arrangement shown in Figure 4-1, in which the lower receiving transducer is fixed to the bottom of a cylinder, while the transmitting transducer is mounted on the lower face of a piston moving in the cylinder. Except for the transducer faces, which are gold on chromium plated, all other components of the system which make contact with the liquid under investigation are constructed of #316 stainless steel. The rod assembly is driven by a linear actuator unit (Duff-Norton Ball Screw Jactuator),

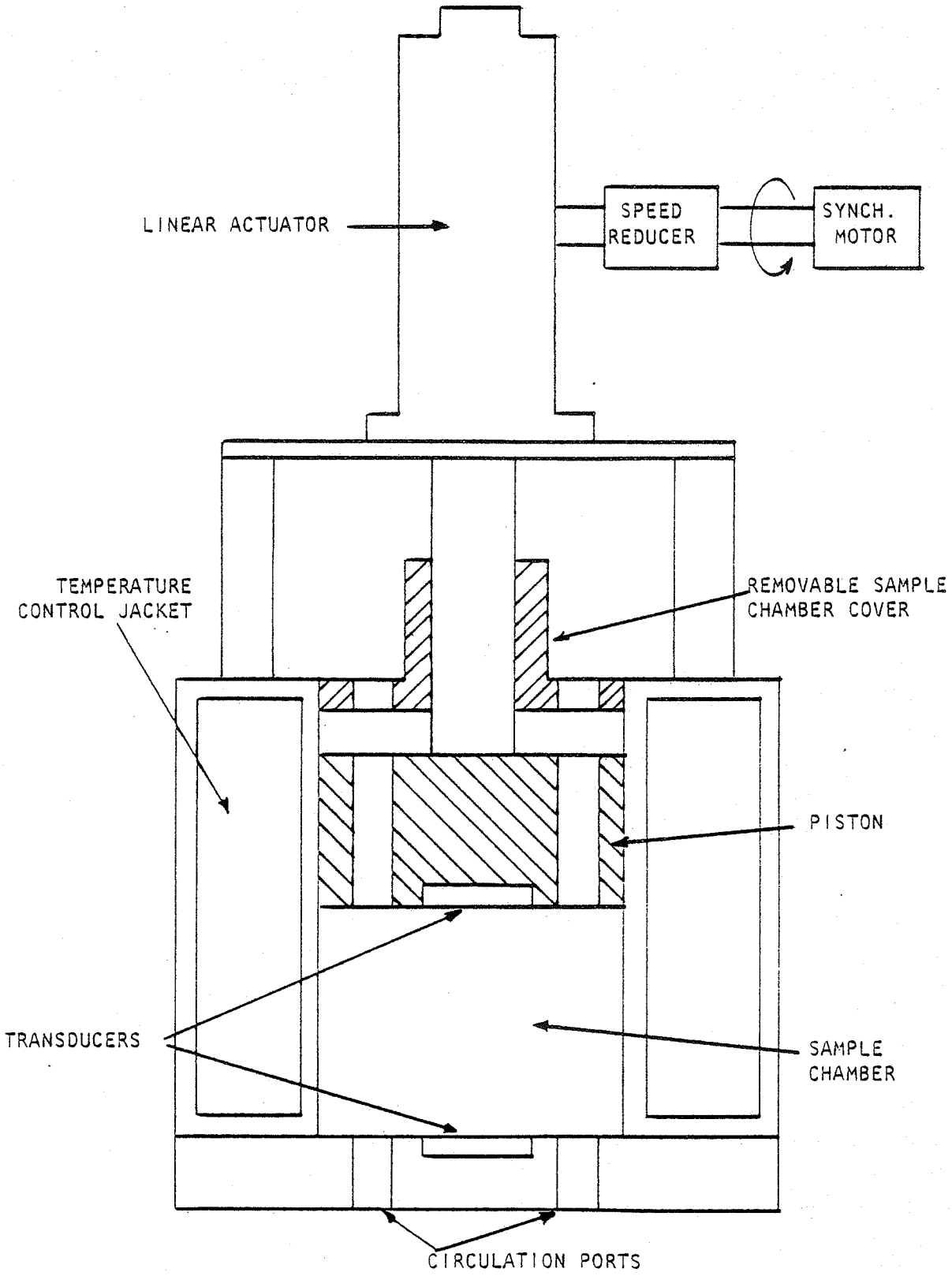

FIGURE 4-1 DIAGRAM OF MECHANICAL SYSTEM OF HIGH FREQUENCY TECHNIQUE

which transforms rotary motion into linear displacement. The actuator is driven by a Bodine, 1/25 horsepower, synchronous, reversible motor through an Apcor multiratio speed reducer allowing reduction ratios from 1:1 to 1:200 in ten increments which, in terms of linear piston velocity is equivalent to speeds of 0.0793750 cm/sec to 0.000396875 cm/sec. Further details of the mechanical apparatus associated with the high frequency system are described by Hawley (1966), O'Brien (1970), and Goss (1974).

Temperature control of the measuring liquid, to $\pm$ 0.1 ^{o}C, is accomplished by circulating fluid from a controlled reservoir through a jacket surrounding the chamber. To avoid thermal and concentration gradients in the test liquid during measurement, the sample is continuously circulated by a variable speed centrifugal type pump.

Figure 4-2 is a block diagram depicting the electronic instrumentation associated with the high frequency system. A pulsed rf signal is produced by mixing the sinusoidal output of a Hewlett-Packard Model 8660B frequency synthesizer (Model 8331 RF Section) capable of generating frequencies from 10 kHz to 110 MHz, with that of a General Radio Type 1217-C unit pulse generator through a double-balanced mixer (Hewlett-Packard Model 10514A). The resulting pulsed rf signal is then amplified with an Electronic Navigation Industries Model 310L wideband amplifier, capable of achieving a flat 50 dB gain over the frequency range from 250 kHz to 110 MHz. The signal is then impedance matched to the transmitting transducer by a L-type network (Everitt and Anner, 1956).

After propagating through the sample liquid, the acoustic signal is received by the receiving transducer and the resulting electrical signal (the envelope of the pulse) fed through a preamplifier (Arulab Preamplifier Model PA-620-L) before being applied to a Hewlett-Packard Model 85531/8522A spectrum analyzer (with

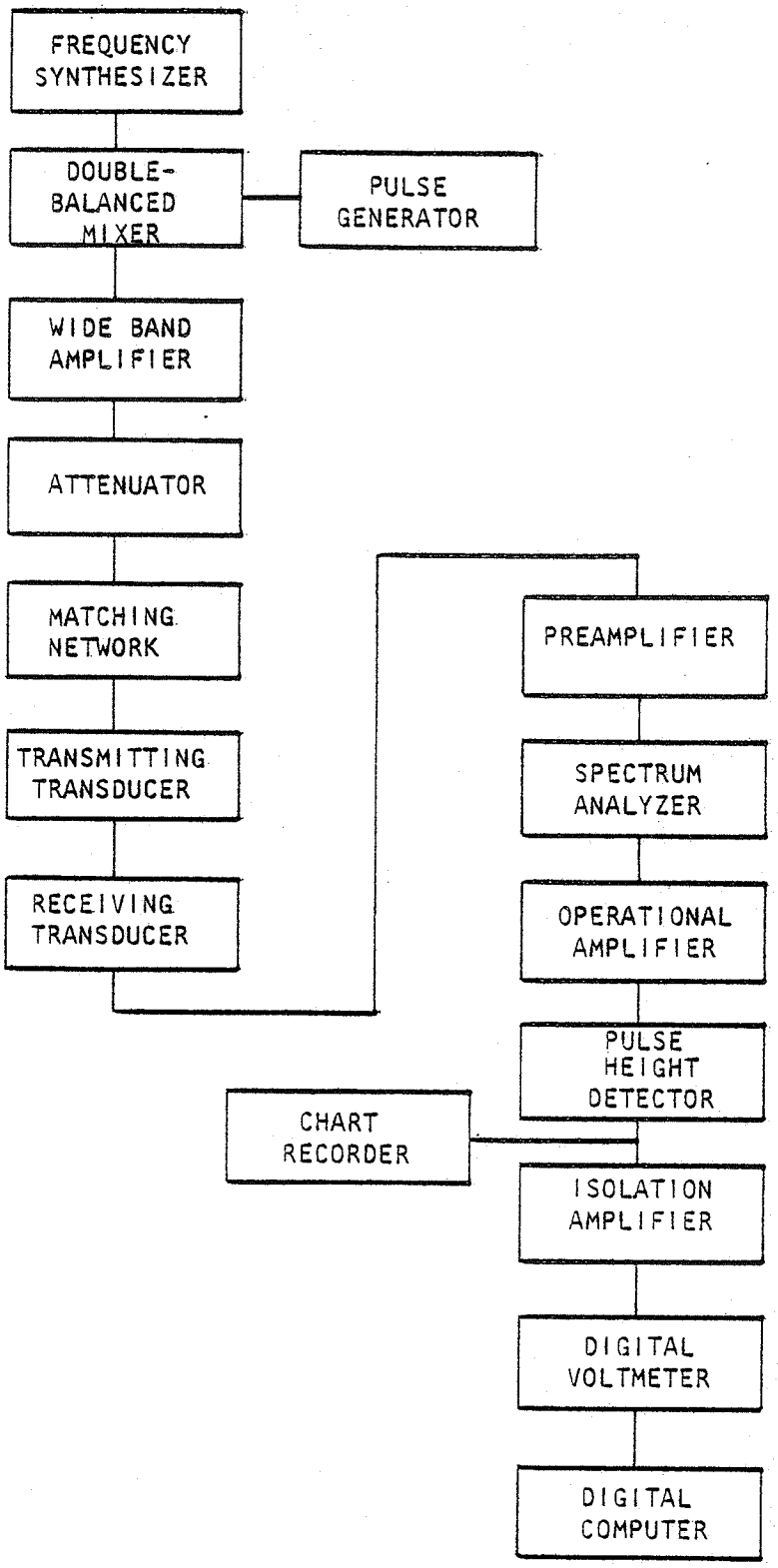

FIGURE4-2 BLOCK DIAGRAM OF HIGH FREQUENCY SYSTEM ELECTRONICS

bandwidth of 300 kHz and frequency range of 0-100 MHz). The analyzer serves both as an amplifier and tuned detector, and is operated in the fixed frequency, linear mode with the output taken as the vertical output of the instrument. A proper input signal for the pulse amplitude monitor (Matec Model 1235A) is attained by passing the output of the analyzer through an operational amplifier (Analog Devices #106D), with variable gain, which serves to invert the received analyzer signal for application to the pulse amplitude monitor. A Tektronix Type 453 dc to 50 MHz oscilliscope serves to monitor the detected pulse through intensity modulation of the z axis.

The output of the pulse amplitude monitor is a dc voltage whose amplitude is proportional to the common logarithm of the positive peak of the input signal. As the transducers are displaced at a constant velocity in either direction, the dc signal, when applied to a Sargent Model SR recorder, is portrayed as a straight line whose slope is proportional to the attenuation coefficient. The numerical value of this slope is conveniently determined by using a digital voltmeter (Systron Donner plug-in Model 1926) and an analog conversion system to transform the dc output of the pulse amplitude monitor to a series of digital data points which may be processed by a digital computer (Digital Equipment Corporation PDP-8) to calculate the attenuation coefficient (Goss, 1974). The computer samples the voltmeter at 2 sec intervals for 80 seconds as the transducers are moved away or toward one another, yielding a total of 40 data points per run. Since the piston velocity is known, it is possible to associate each dc voltage "read" by the computer to the corresponding transducer separation distance. The data points are fitted to a straight line of best fit using a least squares analysis, from which the slope and attenuation coefficient are obtained. All necessary calculations, including the diffraction correction and absorption in excess of that of the

solvent calculation are done on-line by the computer (Goss, 1974).

Velocity measurements are accomplished by algebraically adding the continous wave signal from the frequency synthesizer to the input of the spectrum analyzer. The addition produces an interference pattern which exhibits a peak or null when both signals (when of equal amplitude) are in or out of phase, respectively. Movement of the transducers at a constant velocity then results in a full wave rectified sinusoidal video signal whose period represents one acoustic wavelength, λ, in the liquid under investigation. Since the piston velocity, r_p, is known, the acoustic period T is then $T = \lambda / r_p$ and the measurement of this time yields the speed of sound, c, in the test liquid, as

$$c = T\ f\ r_p \qquad (4\text{-}1)$$

where f is the frequency of measurement, and c, T, and r_p are given above. In the measurement procedure used in this study, the period T is averaged over 100 periods by detecting the peak height of the video signal and feeding this signal into a time interval driver. If the driver signal is then differentiated and zero detected, the signal takes the form of a square wave with period T, resulting in a pulse whenever the detected signal passes through a minimum (every λ / r_p seconds). A Systron Donner plug-in Model 1926A time interval counter then records the time for the piston to travel 100 acoustic wavelengths. Averaging in this way tends to minimize the effects of local signal variations which tend to skew results.

4.2.3 Measurement Error

The measuring system error inherent in the determination of the ultrasonic absorption and velocity using the high frequency pulse technique can be discussed

quantitatively by comparing system obtained values with previously published and accepted values for a liquid such as water having received appreciable attention. At 20°C, comparison of the velocity obtained for distilled water measured in the current system over four trials to that of Greenspan and Tschiegg (1959) yields a difference of less than $\pm$ 0.05%. Similarly, comparison of the frequency-free absorption coefficient (defined as the ultrasonic absorption coefficient divided by the square of the frequency) obtained by this system over four trials at 20°C was within $\pm$ 3% of the accepted value (Pinkerton, 1949).

4.3 Resonant Cavity System

4.3.1 General

The resonant cavity system used in this study is based on the technique originally developed by Eggers (1968) in which two X-cut quartz disks form the end walls of a cylindrical cavity in which the specimen solution is placed. One transducer serves as a transmitter, while the other is the receiver. When the transmitting transducer is excited with a sinusoidal voltage, standing waves in the specimen solution result at particular frequencies f_n that obey the resonance equation (Eggers, 1968).

$$\rho_q \, c_q \tan \frac{\pi f_n}{f_q} = \rho_s \, c_s \begin{bmatrix} \tan \\ -\cot an \end{bmatrix} \frac{\pi}{2} \, \frac{f_n}{f_s} \qquad (4\text{-}2)$$

where ρ_q and c_q are the density and speed of sound in the quartz transducers, and ρ_s and c_s are the corresponding quantities in the solution. The fundamental frequency of the transducer is f_q, and of the liquid filled cavity, f_s. At the resonant frequencies f_n, the receiving transducer registers pronounced voltage peaks, whose frequency separation depends on the sound velocity of the specimen solution, and the half-power (3 dB) bandwidth Δf of which is related to the

attenuation per wavelength, $\alpha\lambda$. In fact, the attenuation coefficient of the specimen solution may be calculated from the quality factor Q of the liquid filled cavity, defined as the frequency, f_n, divided by the 3 dB bandwidth Δf_n of the resonance. This quality factor is a function of the mechanical clamping as well as the attenuation per wavelength $\alpha\lambda$ of the ultrasonic energy, and is expressed as,

$$Q = \frac{f_n}{\Delta f_n} = \frac{\pi}{\alpha\lambda} \tag{4-3}$$

The measured Q however, includes losses associated with attenuation in the solvent, as well as those arising from diffraction, wall effects, imperfect reflections at the quartz surface, etc., besides the desired excess absorption due to the solute. Assuming all of these energy losses are additive (Eggers and Funck, 1973), the measured quality factor Q is given by

$$\frac{1}{Q_{meas}} = \frac{1}{Q_{solute}} + \frac{1}{Q_{extra}} \tag{4-4}$$

where Q_{solute} is the quality factor due to sound absorption in the solute, and Q_{extra} includes solvent and other cell losses previously discussed. The excess solute absorption can be obtained by means of a reference measurement in the same cell at the same frequencies with a suitable reference liquid, having equal or very similar sound velocity to insure the same sound field pattern for both measurements. The excess absorption per wavelength in the specimen solution is then obtained from

$$(\alpha\lambda)_{excess} = \pi\left(\frac{\Delta f_s - \Delta f_r}{f_n}\right) \tag{4-5}$$

where Δf_s and Δf_r are the corresponding 3 dB bandwidths of the n^{th} resonant peak in the sample and reference liquid, respectively.

It has been shown (Eggers, 1968; Eggers and Funck, 1973), that the velocity of sound v_f in an unknown medium may be related to the velocity of sound in some reference medium v_r by the expression

$$\frac{v_f}{v_r} = \frac{D_f}{D_r} \quad \frac{1 + 2(D_f Z_f - D_r Z_r)}{f_q Z_q} \tag{4-6}$$

where Z_f and Z_r are respectively, the acoustic impedance of the unknown and reference medium, and D_f and D_r are the respective separations in frequency units between adjacent resonances for the liquid and reference media and f_q and Z_q, respectively, are the fundamental frequency of the quartz and its acoustic impedance. Equation 4-6 may be approximated by a simpler expression if the unknown medium exhibits acoustical properties similar to that of the reference liquid (Eggers, 1968) as

$$\frac{\delta c_f}{c_f} = \frac{\delta f_n}{f_n} \tag{4-7}$$

where δc_f is the velocity difference between the unknown and reference media, δf_n is the difference between corresponding resonances, and c_f is the velocity of the reference media at a frequency f_n. In general however, velocity measurements are more difficult to perform than absorption measurements due to temperature drift and other instabilities (Eggers, 1973) and velocity measurements using this method were not employed in this study.

In general, the transducer separation distance, the character of the ultrasonic field, cavity shape, mechanical and electrical loading of the transducer, in addition to the acoustical properties of the filling liquid, all play a

predominant role in determining the performance of an ultrasonic resonator (Eggers and Funck, 1976). Residual resonator losses such as those due to diffraction, transducer, and wall losses must be minimized so that the quality factor Q is predominately determined by the sample liquid absorption. Improvements of Q values for liquid-filled resonators by applying a slight static internal pressure to the cavity have been reported recently (Eggers and Funck, 1975; Eggers, et al., 1976). The reasons for this improvement seem to be related to the focusing of the sound beam due to the pressure-effected concavity of the transducers reducing diffraction and side wall effects, and to the elimination of affects of gaseous inclusions within the cavity. The resonant cavity system of the present study utilizes this overpressure technique to measure ultrasonic absorption in liquids in the 0.5-1.5 MHz frequency range.

4.3.2 Instrumentation

The experimental arrangement of the resonant cavity system, shown in Figure 4-3, utilizes a Hewlett-Packard Model 8660B/8331 frequency synthesizer (capable of generating frequencies from 10 kHz to 110 MHz with a frequency resolution of $\pm$ 1 Hz) to supply continuous wave excitation to the transmitting transducer of the resonant cell. The response of the cell to this excitation is measured by the receiving transducer, whose output is then applied to a Hewlett-Packard Model 85541/8522A spectrum analyzer, acting as a tuned receiver, enabling the amplitude of each mechanical resonance to be monitored.

Bandwidth measurements of mechanical resonances are accomplished by adjusting the frequency of the synthesizer to that which will peak the amplitude of the detected signal monitored by the spectrum analyzer. Once this peak or center frequency has been determined, the excitation frequency is then adjusted to those

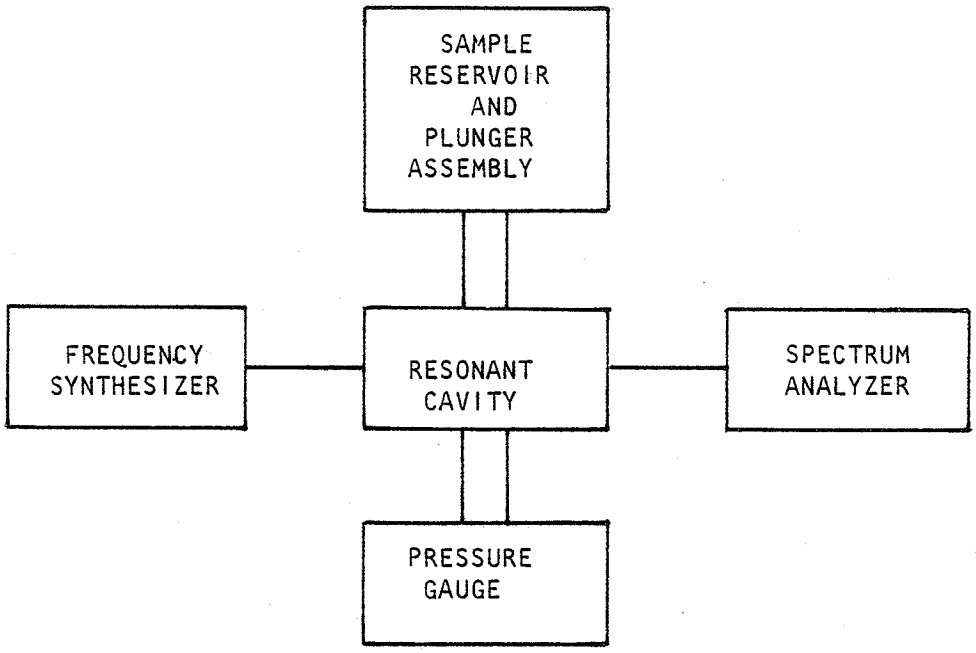

FIGURE 4-3 BLOCK DIAGRAM OF RESONANT CAVITY SYSTEM

frequencies on either side of the center or peak frequency which will reduce by 3 dB the amplitude exhibited on the spectrum analyzer CRT. The numerical difference between these two frequencies yields the bandwidth, which when plotted as a function of center frequency, is related to the absorption coefficient of the liquid filling the resonant cavity.

The measuring cavity itself, shown in cross-section in Figure 4-4, consists of two matched 2 MHz optically flat X-cut quarts disks, 2 inches in diameter. The transducers are separated a total of 1.09 cm by a plexiglass ring. Transducer parallelism is affected by "sandwiching" the transducer-ring assembly between two brass plates held together by four precision threaded stainless steel rods (64 threads/inch), which provide adjustment of parallelism to about $\pm$ 0.002 in. Four neoprene "O" rings between the transducer and brass supporting cylinders and plexiglass spacing ring, respectively, allow for transducer alignment while maintaining a pressure and liquid seal for the measuring chamber.

To avoid the introduction of air bubbles into the cell, charging is affected by filling from a port located at the cell bottom through a cylindrical reservoir adjacent to the cell, and connected to it with Tygon tubing. Excess pressure is introduced by applying a piston to the adjacent reservoir (usually about 10 psi), and measured with a standard pressure guage connected with Tygon tubing to the cell via a port located at the top of the resonant cavity. Pressure in the measuring chamber can be maintained with this arrangement for days to within 1%.

Temperature control to about $\pm$ 0.1^{o}C is accomplished by wrapping plastic tubing circulating fluid from a controlled bath, around the measuring chamber. The apparatus is then insulated from the outside environment using a 2-3 inch layer of styrofoam chips, which completely surround the measuring cell.

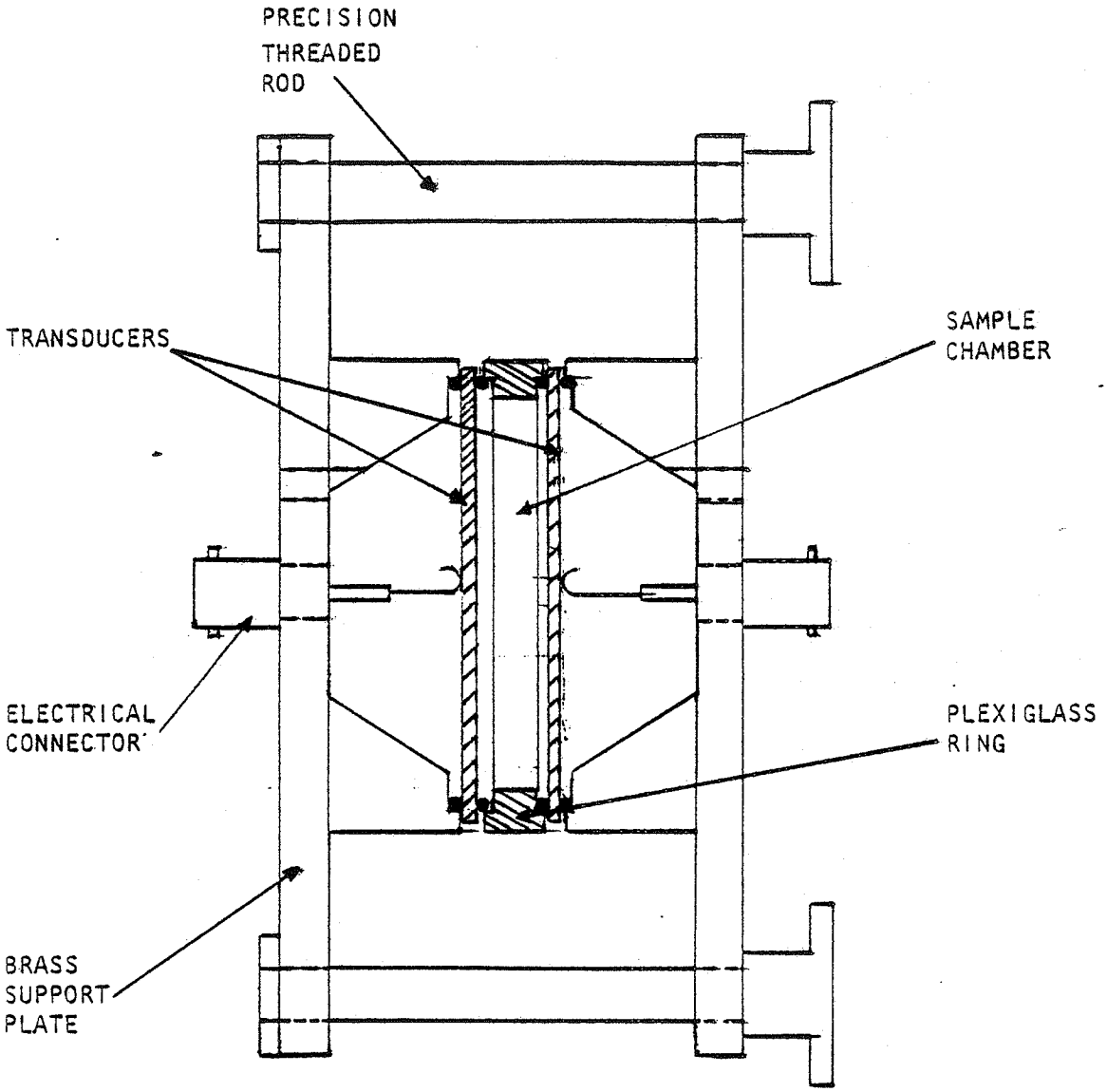

FIGURE 4-4 CROSS-SECTION OF RESONANT CAVITY

4.3.3 Measurement Error

The measurement error inherent in the resonant cavity technique was assessed quantitatively by comparing present measurements to those published in the literature. For this purpose, measurements in aqueous solutions of the globular protein bovine serum albumin (10% concentration, 20°C) obtained using the resonance technique (Frizzell, 1976) was within ± 10-15% of accepted published values (Kessler, 1968; O'Brien, 1970; Goss and Dunn, 1974).

4.4 Transient Thermoelectric Technique

4.4.1 General

The transient thermoelectric technique, as described by Fry and Fry (1954a; 1954b), offers a phase insensitive method of determining ultrasonic absorption in biological materials. While the importance of various phase insensitive methods has been demonstrated by a number of investigators (Marcus and Carstensen, 1975; Busse et al., 1977), the transient thermoelectric technique is currently the only technique, applicable to tissue, which yields the ultrasonic absorption rather than attenuation (Goss et al., 1978d). This method involves first imbedding a small thermocouple in a tissue sample, then irradiating the ensemble with a rectangular ultrasonic pulse of known intensity. The absorption coefficient, α, of the imbedding medium is then given as (Fry and Fry, 1954a),

$$\alpha = \frac{\rho C}{2I} \left(\frac{dT}{dt}\right)_o \qquad (4\text{-}8)$$

where $\left(\frac{dT}{dt}\right)_o$ is the initial time rate of change of temperature as measured by the thermocouple junction (°C/sec), I is the sound intensity at the site of this junction (W/cm^2), and ρC is the product of the surrounding medium's density and heat capacity per unit mass at constant pressure ($Joules/cm^3/°C$).

A typical thermoelectric emf response of a thermocouple embedded in silicone oil from exposure to a one second pulse of ultrasound (1 MHz, 10 W/cm^2) is illustrated in Figure 4-5. The response has an initial fast rise which results from conversion of acoustic energy into heat by the viscous forces acting between the thermocouple wire and the sample. This portion of the response approaches equilibrium very rapidly with a magnitude that depends mainly upon the thermocouple wire radius, the viscous properties of the sample medium, the sound intensity, and the acoustic frequency. The fast rise is followed by a relatively linear rise (in the absence of thermal conduction processes) that is the result of absorption of ultrasound in the surrounding medium. From a determination of the slope of the linear portion of the thermoelectric emf response as a function of time, and using equation 4-8, the absorption coefficient may be calculated.

4.4.2 Instrumentation

A block diagram of the electronic instrumentation associated with the transient thermoelectric technique is shown in Figure 4-6. The output of a crystal controlled oscillator is gated to the prescribed pulse duration (1 sec) and the subsequent retangular rf pulse then applied either to a tuned 50 W or 2 kW power amplifier, through a small preamplifier. The amplified rf pulse is then impedance matched to the ultrasonic transducer using an LC network. The voltage applied to the transducer is sampled through a capacitive voltage divider and fed back to the power amplifier through a stabilizing network. This negative feedback loop insures that the shape of the rectangular rf pulse will not be distorted during the amplification process.

The transducers used in this study employ X-cut quartz as the vibrating element, operating either at fundamental or odd-harmonic frequencies. Both focused

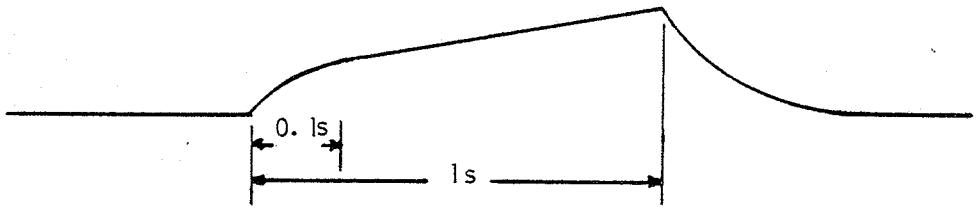

FIGURE 4-5 THERMOELECTRIC EMF RESPONSE OF AN ULTRASONICALLY IRRADIATED THERMOCOUPLE (1 Second Pulse)

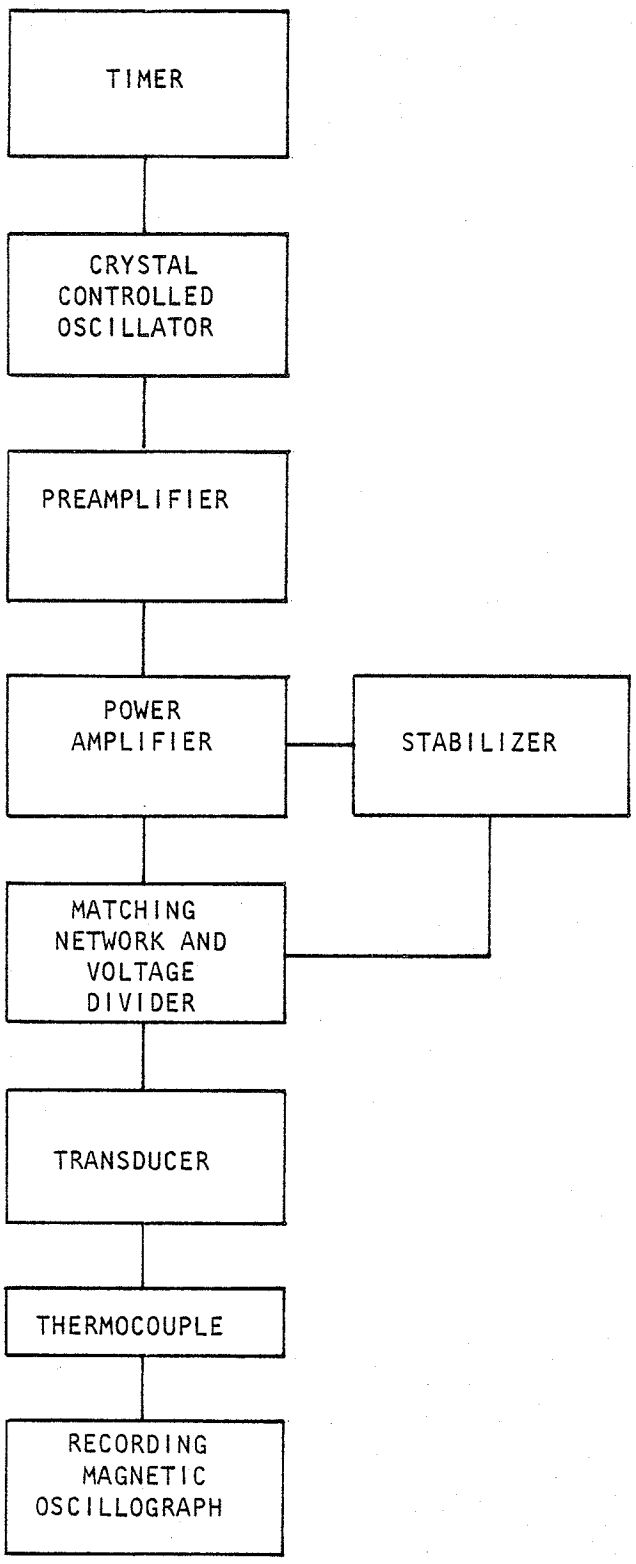

FIGURE 4-6 BLOCK DIAGRAM OF TRANSIENT THERMOELECTRIC TECHNIQUE

and unfocused transducers were used to obtain the ultrasonic intensity and beam width required for the implementation of this measuring technique. The theory and construction of such transducers are described in detail elsewhere (Dunn, 1956; Fry and Dunn, 1962; Brady, 1971; Dunn et al., 1969).

The heat generated by the action of the ultrasonic energy within the measuring specimen is detected using a chromel-constantan thermocouple junction fabricated in the laboratory. The fabrication process involves etching 0.003 in. commercially available wires to such a diameter as to allow the formation of a lapped (0.5-1 mm) soldered junction no more than 0.0005 in. in diameter. Chromel-constantan junctions typically possess a sensitivity of about 60 $\mu v/^{o}C$, and for these particular junction diameters, a dc resistance in the range from 15-25 ohms. The thermocouple emf response is recorded on a Hathaway Model 10 recording Magnetic Oscillograph, which employs a galvanometer (sensitivity = 0.5-0.8 $\mu a/cm$) to convert electrical output of the junction to an optical deflection proportional to the change in temperature which is then photographically recorded (Kodak Linograph Paper).

Since the acoustic intensity of the irradiating beam is required for the calculation of the absorption coefficient by this technique, a primary intensity calibration technique, involving radiation pressure on a sphere is applied (Dunn, 1956; Fry and Dunn, 1962; Dunn et al., 1977). Each specimen-thermocouple combination was used to beam plot the ultrasonic field of the transducer prior to each measurement, in order that errors due to incorrect placement of the specimen imbedded junction could be minimized. Calibration, as well as absorption measurements were performed in a temperature controlled bath ($37 \pm 0.1^{o}C$) containing degassed, 0.9% saline.

The tissue specimen is held stationary in the ultrasonic field using a

stainless steel and lucite frame (in which the opening is much larger than the transducer beam width) to which the tissue specimen was supported using surgical silk. In the case of mouse testicle measurements this scheme was not convenient and two small (2-3 mm) lucite cups, attached from either end of the frame, were used to hold the tissue specimen in place. Thermocouples were imbedded in the tissue using a 30 gauge hypodermic needle, which served to guide the small wire straight through the tissue, facilitating inserting of the thermocouple junction within the tissue.

4.4.3 Discussion

The temperature measured by use of the thermocouple junction is only a true measure of the ultrasonic absorption of the surrounding medium if the ultrasonic beam width is broad enough to minimize the effects of heat conducted away from the junction by the medium, and the size of the thermocouple is reduced to a point where the heat generated by the relative motion of the viscous medium relative to the thermocouple wire when subjected to ultrasound may be neglected. While theoretical consideration was given to these phenomena by Fry and Fry (1954a), the current study has found the description of the diffusion effect to be inadequate for small beam widths and experimental confirmation of both (Fry and Fry, 1954a) to be incomplete. The current work has resulted in the development and experimental verification of a model that gives a more complete description of both effects and has provided a guide for selecting the appropriate beam width and wire size necessary to minimize measurement errors (Goss et al., 1977).

In developing the theory for the thermoelectric effect, Fry and Fry (1954a) estimated the effect of viscous relative motion heating on absorption measurements by this technique assuming plane waves, for 1) the direction of ultrasonic

propagation along the axis of the wire and 2) the direction of propagation at right angles to the wire. Since the latter orientation occurs most often, and results in less viscous heating (Fry and Fry, 1954b), discussion is restricted to that case only. The expression derived by Fry and Fry (1954a) for the amount of heat generated per second per unit length of wire by the viscous forces, Q_v, is a complex function of the physical properties of the tissue and the thermocouple wire, as well as the ultrasonic frequency and intensity, given as

$$Q_v = \frac{IK'M'\omega}{\rho c} \quad \frac{\left[1 - \frac{1 + K}{(\frac{M}{M'} + K)}\right]^2}{1 + \left[\frac{K'}{\frac{M}{M'} + K}\right]^2} \tag{4-9}$$

where I is the acoustic intensity (W/cm^2), ρ is the density of the imbedding medium (gm/cm^3), ω is the angular frequency (radians/sec), c is the ultrasonic phase velocity (cm/sec), M is the mass per unit length of the thermocouple wire (gm/cm), M' is the mass of specimen displaced by a unit length of wire (gm/cm),

$$K = 1 + \frac{\sqrt{2}}{\phi} \tag{4-9a}$$

and

$$K' = \frac{\sqrt{2}}{\phi} + \frac{1}{2\phi^2} \tag{4-9b}$$

The parameter ϕ is defined as

$$\phi = \frac{r_o}{2}\left(\frac{\omega}{\nu}\right)^{\frac{1}{2}} \tag{4-9c}$$

where r_o is the radius of the thermocouple junction (cm), and ν is the coefficient

of shear viscosity of the imbedding medium (P). For values of $\phi \geq 4$, applicable to Dow Corning 710 silicone fluid and to tissue, the heat generation rate per unit length of wire per second is given by Equation (4-9).

Using the expression for Q_v, the temperature change resulting from this heat source, and the time required for it to reach a specified fraction of its equilibrium value was estimated. Two conclusions resulted from this analysis: 1) the magnitude of the temperature rise resulting from viscous force action between the wires and the imbedding medium is of the same order as the temperature rise resulting from absorption, for wire sizes and pulse durations of practical interest, and 2) this temperature change can be separated from that caused by absorption because of its rapid approach to equilibrium (50% of its theoretical equilibrium value in 0.1 seconds of a 1 second pulse). However, since the equilibrium value for the viscous temperature rise is never actually reached, this heating will always contribute, at least to some degree, to the rate of temperature rise at the thermocouple junction.

Heat diffusing away from the region of the thermocouple due to insufficient ultrasonic beam width may also appreciably affect the rate of temperature rise at the location of the thermocouple junction. Since an accurate measure of the time course of temperature rise is necessary for a reliable determination of absorption by this technique, it is apparent that the effect of beam width must be considered to that thermal gradients close to the junction are avoided. Fry and Fry (1954a) investigated this effect, and derived an expression relating the fractional uncertainty in the measurement of temperature at the junction to the half-power beam width of the irradiating beam as

$$\frac{\Delta T_m - \Delta T_{mHC}}{\Delta T_m} = \frac{K}{\rho C}\left[\frac{f''(r)}{f(r)}\right]_{r=0} t \qquad (4\text{-}10)$$

where ΔT_m is the temperature rise in the absorbing medium without heat conduction present, ΔT_{mHC} is the temperature rise with heat conduction, K is the heat conductivity coefficient of the absorbing medium, t is the duration of the irradiation, and ρC is the same as in Equation 4-8. The transverse ultrasonic beam profile close to the axis of the beam was represented by $f(r) = 1 - 2(\frac{r}{B})^2$, where B is the half-power beam width in centimeters, and r is the transverse distance from the axis of the beam. The quantity in the brackets of (4-10) reduces to $\frac{4}{b^2}$ so that Equation 4-10 reduces to

$$\frac{\Delta T_m - \Delta T_{mHc}}{\Delta T_m} = \frac{K}{\rho C}\,\frac{4}{B^2}\,t \qquad (4\text{-}11)$$

If the exposure duration is taken as 1 s, and ΔT_m is considered proportion to the absorption coefficient α in the absence of heat conduction and ΔT_{mHC} is proportional to the absorption with heat conduction present, α_{HC}, Equation 4-11 can be rearranged to give

$$\alpha_{HC} = \alpha\left[1 - \frac{4K}{\rho C}\,\frac{1}{B^2}\right] \qquad (4\text{-}12)$$

However, as stated by Fry and Fry (1954a), this relation accurately describes the effect of beam width on the measured absorption only when these effects are small, i.e., for large beam widths. For small beam widths, it is necessary to develop a more complete description of these effects.

In order to select the appropriate beam width and wire size necessary to minimize errors in the transient thermoelectric technique, the effects of viscous heating at the wire surface and heat conduction in the medium have been

incorporated into a model that yields, with the aid of a computer computation, the temperature at the thermocouple junction as a function of time (Goss et al., 1977). The ultrasonic beam width was represented by a model consisting of ten concentric cylinders each with a different average ultrasonic intensity (Pond, 1970; Lerner et al., 1973; Frizzell et al., 1977). These cylinders defined the heat generation, as a function of time and space, due to the absorption by the surrounding medium. The heat generation due to viscous effects at the wire surface was also represented by ten concentric cylinders. Since the thermocouple is oriented perpendicular to the axis of the sound beam, each cylinder has a radius equal to that of the wire and a length equal to the diameter of the corresponding cylinder, in units of sound intensity, representing the ultrasonic beam.

The temperature at the center of a cylinder is given by (Carslaw and Jaeger, 1959)

$$T(t) = \frac{\dot{q}}{\rho C} \int_o^t \left[1 - \exp\left(- \frac{A^2}{4Dt'}\right) \right] \operatorname{erf}\left[\frac{B}{(dDt)^{\frac{1}{2}}} \right] dt' \qquad (4\text{-}13)$$

where 2A and 2B are respectively, the diameter and length of the cylindrical heat source; $\dot{q}$ is the time rate of heat generation per unit volume, and ρ, C, and D are, respectively, the density, the heat capacity, and the thermal diffusivity of the medium. The time dependent temperature at the focus without the thermocouple wire can be calculated by superimposing the contribution from each of the ten cylinders representing the ultrasonic beam and noting that the heat generation rate $\dot{q}$ is related to the acoustic intensity assigned each cylinder by

$$\dot{q} = 2\alpha I \qquad (4\text{-}14)$$

where α is the sound pressure absorption coefficient and I is the ultrasonic intensity for that cylinder. To this can be added the temperature rise due to the

viscous heating at the wire surface, computed by the same technique, where its heat generation rate, $\dot{q}_v$, is given by the relation

$$\dot{q}_v = \frac{Q_v}{\pi r_o^2} \tag{4-15}$$

where Q_v is the heat generation rate per unit length, and r_o is the wire radius. The viscous heat generation at the wire boundary is thus modeled as a uniform cylindrical source of heat generation with a radius equal to that of the wire. This is a good approximation for small wire sizes, since for $B>>r_o$ both surface generation and uniform $\dot{q}$ generation reduce essentially to a line source of heat with Q_v heat generation per unit length. Thus, with the appropriate choice of the cylinders for a given ultrasonic field, this model can be used to determine the temperature rise as a function of time with viscous and heat conduction effects included.

This model was used first to determine the importance of the imbedding medium physical properties and thermocouple wire size when considering experimental error introduced by viscous heating at the surface of the thermocouple wire. Using a large beam width (1 cm), for which the effects due to heat diffusion may be considered to be negligible (as shown below), the theoretical determination of the rate of temperature rise at 0.5 s after the initiation of a 1 s ultrasonic pulse is used to assess the relative proportion of heating due to absorption of sound energy by the imbedding medium, to that due to viscous heating. For the case with no wire present, and the beam width is large, the resulting rate of rise is just that calculated from Equation 4-8. When non-zero diameter wire sizes and small beam widths are considered, a relative value of absorption is obtained by calculating the product of the ratio of the theoretical slope calculated above to the slope (4-8), and the true absorption coefficient of the medium. This theory is now

applied to biological tissues and to Dow Corning 710 silicone fluid, a convenient tissue model because of its acoustic absorption and impedance. For tissues, the value for the absorption used in this calculation is chosen to provide the best agreement with experimental data; however, the absorption coefficient in silicone fluid was determined using the resonant cavity technique (Section 4.3 of this chapter).

Table 4-1 lists theoretical and experimental results for Dow Corning 710 silicone fluid using two viscosities, 2.4 and 4.0 P, at 37 and 30°C, respectively (Dow Corning, 1958), and two finite wire sizes, 76 and 13 μm in diameter. An ultrasonic beam width of approximately 1 cm was used for the experimental measurements at 0.5 MHz, the same as the values used in the theoretical calculations. The thermal constants used for the above calculations are listed in Table 4-2. Good agreement, usually within the standard deviation of measurement, exists between theory and experiment. It is apparent, for even the smallest thermocouple examined, that the absorption coefficients measured by this method could be as much as 50% greater than the actual value. Thus, further reduction of thermocouple size in low absorbing, high viscosity materials is necessary to avoid significant measurement errors.

Errors are reduced to tolerable (less than about 10%) levels for materials of lower viscosities. A comparison between theoretical and experimental results was also performed in biological tissue, which exhibits a shear viscosity at least a factor of 20 below that of the silicone oil (Frizzell et al., 1976). The results for mouse teste, shown in Table 4-3, for various estimated viscosities (since only a range of viscosity data is presently available for soft tissues), indicate agreement to within 10% of the theoretical absorption coefficient at 0.5 MHz using 13 μm diameter thermocouple wire, while the larger 76 μm thermocouple junction show

TABLE 4-1

Theoretical and Experimental Effect of Thermocouple Wire Size upon Measured Ultrasonic Absorption in Dow Corning 710 Silicone Oil at 0.5 MHz

Wire Diameter (μm)	Viscosity (P)	Temperature (°C)	Absorption Coefficient* Theory (cm^{-1})	Absorption Coefficient* Experiment (cm^{-1}) mean + S.D.
0	4.0	30	0.010**	-
	2.4	37	0.008**	-
13	4.0	30	0.015	0.020 ±0.0019
	2.4	37	0.013	0.013 ±0.0031
76	4.0	30	0.060	0.053 ±0.0069
	2.4	37	0.047	0.042 ±0.0026

*Theoretical and experimental results obtained using 1 cm ultrasonic beam width (half-power).

TABLE 4-2

Thermal Properties at 37°C

Material	Density (gm/cm^3)	Diffusivity (cm^2/sec)	Heat Capacity cal/°C gm
Dow Corning 710	1.089	8.8×10^{-4}	0.363
Soft Tissues	1.05	1.4×10^{-3}	0.86

TABLE 4-3

Theoretical and Experimental Effect of Thermocouple Wire Size upon Measured Ultrasonic Absorption in Fresh Mouse Teste at 0.5 MHz

Wire Diameter (μm)	Estimated Viscosity (P)	Temperature (°C)	Absorption Coefficient* Theory (cm^{-1})	Experiment (cm^{-1})
0		37	.007**	
13	0.01	37	.0075	.0078
	0.05		.0076	
	0.10		.0078	
76	0.01	37	.0084	.01
	0.05		.0087	
	0.10		.0105	

*Theoretical and experimental results obtained using 1 cm ultrasonic beam width (half-power).
**Chosen for best fit to experimental data.

agreement only to about 60%. Again, good agreement exists between theory and experiment for the range of viscosities used. It should be pointed out that these effects are more significant in teste than in other tissues due to its relatively low absorption coefficient.

Additional calculations were performed to illustrate better the dependence of the error, due to viscous heating, on the wire size and the absorption of the

measured material. The results of the analysis are shown in Figure 4-7 where the percentage error due to viscous heating is plotted as a function of the actual or true absorption coefficient for various wire sizes, assuming a viscosity of 0.05 P and an ultrasonic frequency of 0.5 MHz. It is apparent that for the tissue properties used (Table 4-2), a 13 μm diameter thermocouple wire results in viscous heating errors from 0.3 to 8% over the range of absorption coefficients normally attributed to soft tissues, vis., 0.1 to 0.007 cm^{-1}. Further inspection of Figure 4-7 indicates that the error due to viscous heating in tissue increases linearly with the thermocouple wire radius. While these data describe the errors quite accurately, it is intended that they be used as guides as to the appropriate thermocouple wire size to keep measurement errors below specified limits, rather than as corrections applied to erroneous measurements.

The effect of beam width upon the measured absorption coefficient was also examined theoretically with the approach described earlier, as well as experimentally. The experimental procedure involved varying the aperature of a 3 MHz focused transducer to obtain various beam widths at the focus. The absorption coefficient in liver, teste, and Dow Corning 710 fluid was then measured as a function of beam width, again, using (4-8) where $(\frac{dT}{dt})_o$ was taken as the slope of the temperature-time curve at 0.5 s after the initiation of a 1 s ultrasonic pulse. The model developed here was then used to determine the effect of beam width on the slope of the temperature-time curve at 0.5 s after the sound is turned on, i.e., the effect on the measured absorption coefficient.

Figure 4-8 shows the results of measurements of ultrasonic absorption coefficient in liver tissue, as a function of the reciprocal of the beam width, made with a 76 μm copper constantan thermocouple. Plotted in the same figure are the results from: Curve 1--Fry theory, diffusion effect only; Curve 2--model developed

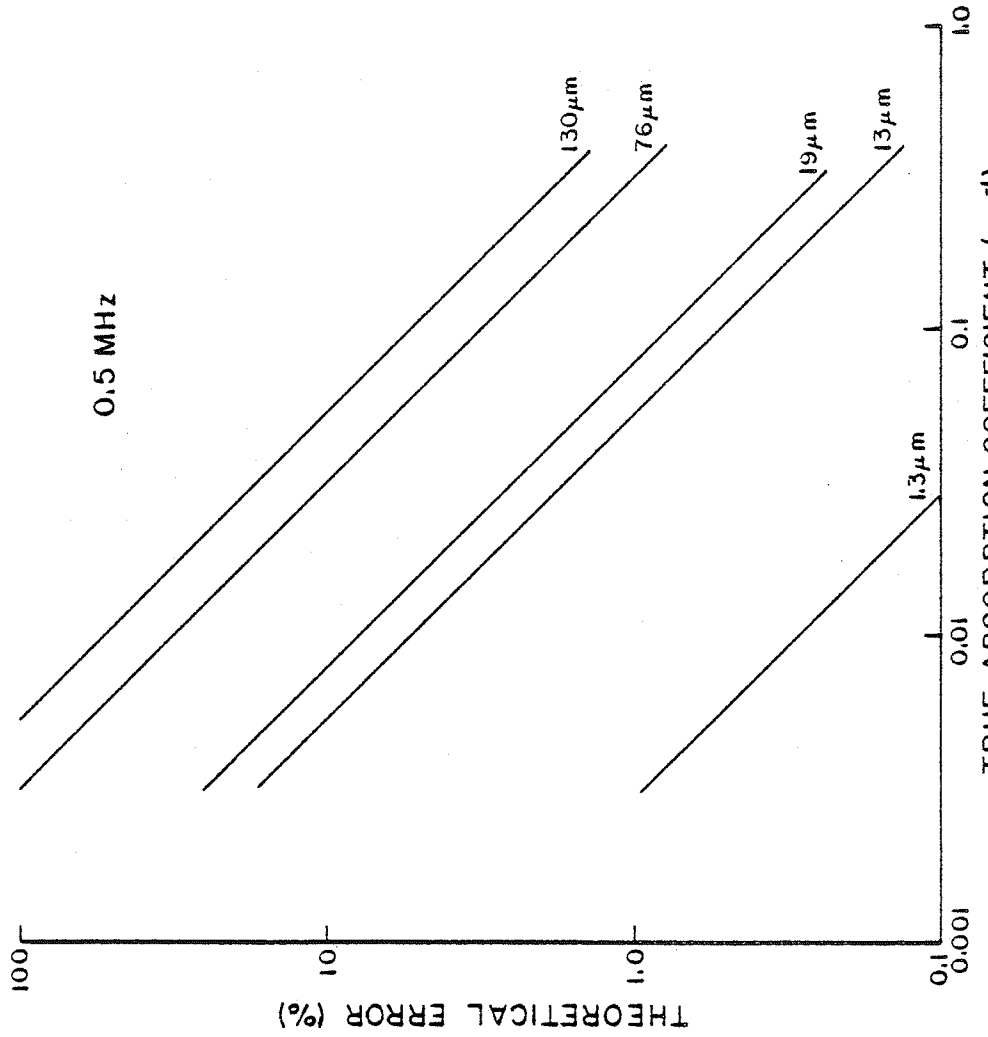

FIGURE 4-7 PERCENTAGE ERROR DUE TO VISCOUS HEATING AS A FUNCTION OF THE TRUE ABSORPTION COEFFICIENT FOR VARIOUS WIRE DIAMETERS (0.05P, 0.5 MHz)

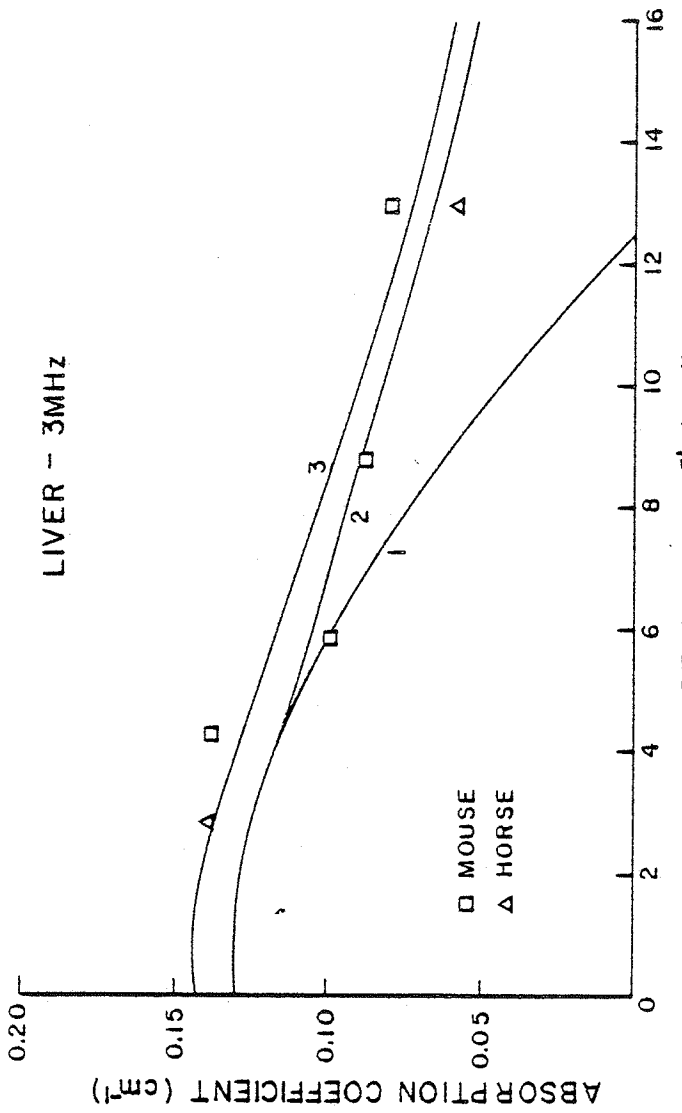

FIGURE 4-8 THEORETICAL AND MEASURED ABSORPTION COEFFICIENTS IN FRESH MOUSE AND HORSE LIVER VERSUS RECIPROCAL OF BEAM WIDTH AT 3 MHz, 37°C. (see text for explanation of curves 1, 2, and 3)

in this study, diffusion effect with the added heating due to the presence of 76 μm diameter thermocouple wire. The good agreement between Curves 2 and 3 and the disagreement of Curve 1 for smaller beam widths is evident.

Figure 4-9 shows similar comparisons for mouse teste at 7 MHz where good agreement between the shape of the Curves 2 and 3 and the experimental data points is seen.

Figure 4-10 shows the results of absorption measurements on Dow Corning 710 silicone fluid made with the thermocouple wire etched to 13 μm near the junction and lap soldered (the 76 μm junctions were butt soldered). Curves 1 and 2 are the same as in Figures (4-8) and (4-9), but Curve 3 represents theoretical results with a 13 μm diameter thermocouple wire and Curve 4 the theoretical results with a 76 μm diameter wire. As shown in Tables 4-1 and 4-2, the effect of viscous heating in the high viscosity silicone oil is dramatic when compared to tissue. However, the effect at 3 MHz is much smaller than that at 0.5 MHz because the absorption in the oil is considerably greater. The theoretical results were calculated using a value for the absorption coefficient of 0.23 cm^{-1} based on an independent measurement by the resonant cavity technique. Note that the measured values are approximately 20% low when compared to the predicted results in Curve 3. It is believed this results from ultrasonic streaming in the oil which reduces the measured temperature rise. Although the measurements are low, the same trend, as a function of reciprocal beam width, is seen for experimental and theoretical results.

The following observations resulting from the above analysis of beam width and wire size on the measured absorption coefficient by the transient thermoelectric technique can be made: 1) A thermocouple wire 13 μm in diameter contributes to viscous heating which is about 0.3 to 8% of the heating due to absorption, over

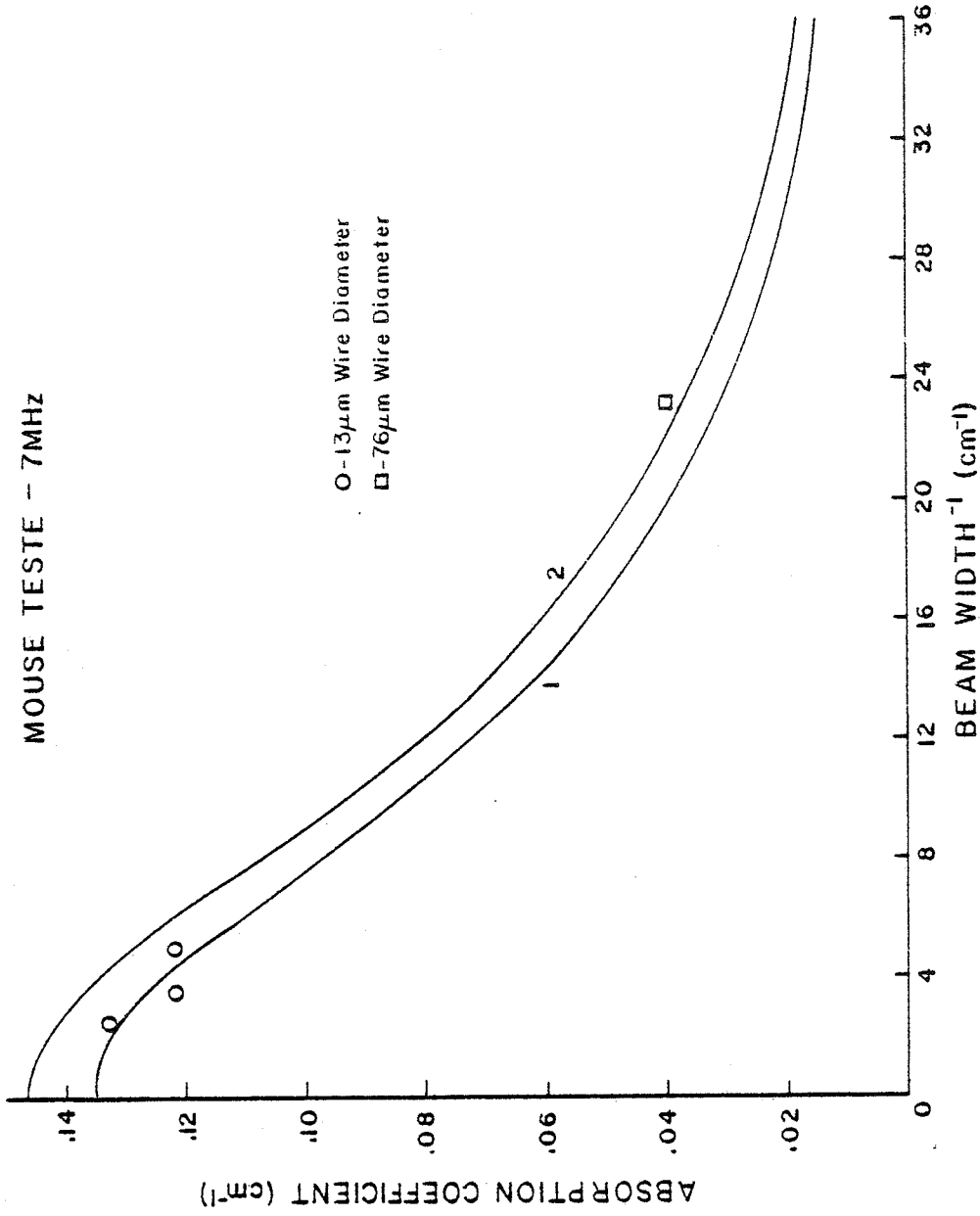

FIGURE 4-9 THEORETICAL AND MEASURED ABSORPTION COEFFICIENT IN MOUSE TESTE VERSUS RECIPROCAL BEAM WIDTH AT 7 MHz, 37°C. (see text for explanation of curves 1, 2, and 3)

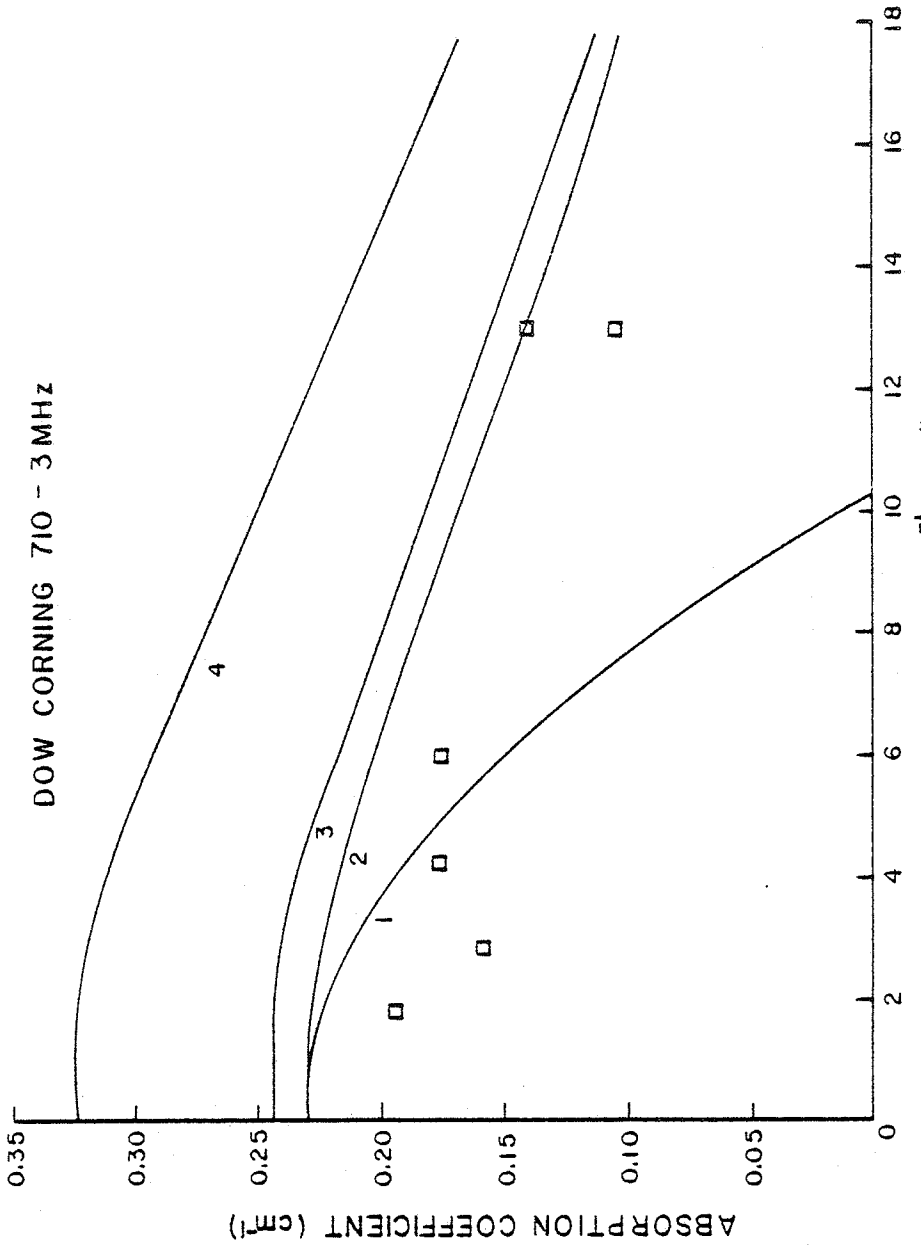

FIGURE 4-10 THEORETICAL AND MEASURED ABSORPTION COEFFICIENT IN DOW CORNING 710 FLUID VERSUS RECIPROCAL BEAM WIDTH AT 3 MHz, 37°C. (see text for explanation of curves 1, 2, 3, and 4)

the absorption range applicable for most tissues, at 0.5 MHz. 2) Viscous heating is less dependent upon frequency than is the bulk heating due to absorption in all tissues examined thus far, resulting in smaller errors due to this effect at higher frequencies. 3) Acceptable measurement errors (less than about 10%) are achieved only when using half-power beam widths larger than about 0.3 cm as the irradiating source (13 μm dia. thermocouple). This is important when considering the use of strong focused transducers to obtain intensity levels necessary to achieve a measurable temperature rise, since the beam width of a focused transducer varies approximately inversely with frequency. The use of focused transducers would thus lead to diffusion errors increasing with frequency. Each of these, among others mentioned by Fry and Fry (1954a) must be considered when meaningful and accurate measurements using the transient thermoelectric technique are desired.

4.5 Acoustic Interferometer

4.5.1 General

Ultrasonic velocity measurements in biological tissues were made using a fixed path acoustic interferometer, a device first suggested by Hubbard (1934), with further consideration given by Ballou and Hubbard (1944), and by Borgnis (1957). Basically, the technique involves the employment of two transducers, acting as transmitter and receiver, respectively, and separated by a known distance, with the specimen between the two. The transmitting transducer generates plane cw compressional waves in the specimen. A standing-wave pattern is thus set up between the parallel faces of the two transducers which reacts on the receiving transducer and influences the electrical impedance presented at its terminals. As the frequency of the signal exciting the transmitter is varied, the transducer impedance will pass through a series of maxima and minima, the former occurring

when the distance between the two transducers is an integral number of half-wavelengths. Thus, if the frequencies of two adjacent impedance maxima are f_1 and f_2, and the transducers are spaced a distance d apart, then the velocity in the intervening specimen, c, is given by

$$c = 2d(f_2 - f_1) \tag{4-16}$$

Such a method allows the measurement of ultrasonic velocity using acoustic interferometry where transducer movement is not possible or desirable.

4.5.2 Instrumentation

A block diagram depicting the instrumentation associated with the fixed path acoustic interferometer as used in this study is shown in Figure 4-11. A Hewlett-Packard Model 8660B frequency synthesizer (operating at a frequency of 1 MHz, with a frequency resolution of $\pm$ 1 Hz) provides the sinusoidal excitation to the transmitting transducer, and also displays the excitation frequency. The transducers are commercial PZT broadband transducers (Panametrics Model V305 and V308) having center frequencies of 2.25 and 5.0 MHz, and a bandwidth greater than 50%. The variation in transducer impedance is monitored by a Hewlett-Packard Model 85531/8522A spectrum analyzer (300 kHz bandwidth) which acts as a tuned receiver. Transducer separation is measured with the aid of a micrometer assembly with a least count of $\pm$ 0.001 in. and which also serves to maintain parallelism between the transducers by a dovetail bearing. It is necessary to add to the measured transducer separation distance the thin matching layer covering the faces of both transducers.

Application of this technique involves placing the specimen between the two transducers, and immersing the entire ensemble in 0.9% saline, to assure adequate acoustic coupling. The transducer separation is then determined from the micrometer

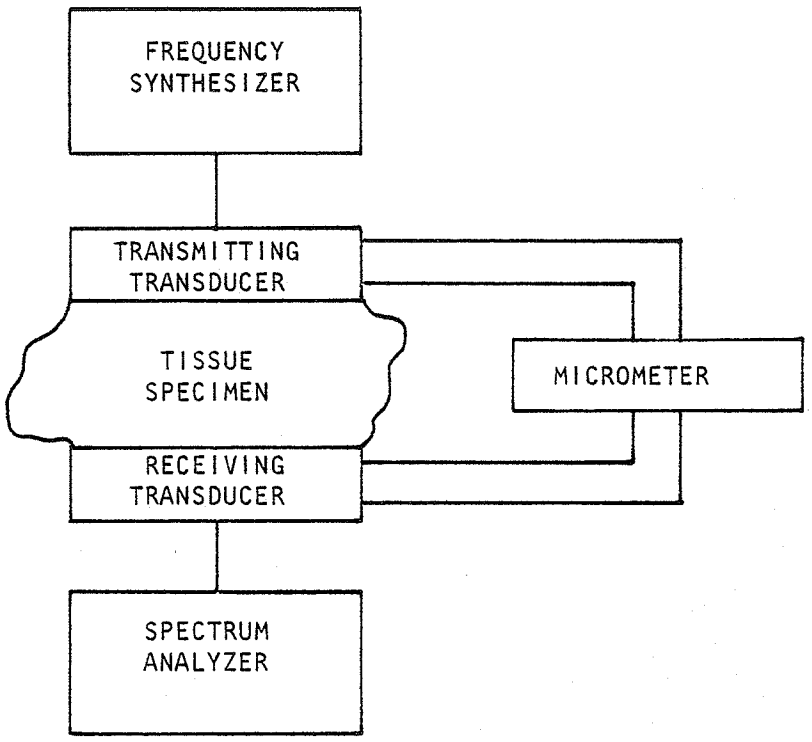

FIGURE 4-11 BLOCK DIAGRAM OF ACOUSTIC INTERFEROMETER

reading, and the matching layer correction made. The frequency span required for the signal at the spectrum analyzer to pass through ten consecutive peaks is then determined, allowing the calculation of ultrasonic velocity from (4-16). All measurements using this technique were made at room temperature (23°C) with additional temperature control.

4.5.3 Error

This technique was used to determine the ultrasonic velocity in distilled water in order to evaluate the measurement error. Results of these measurements compared to those accepted values found in the literature (Greenspan and Tschiegg, 1959) agree to within $\pm$ 0.3%.

4.6 Acoustic Microscopy

A scanning laser acoustic microscope (Sonomicroscope 100 ^{R} manufactured by Sonoscan, Inc., Bensenville, IL) operating at a frequency of 100 MHz was employed in both the examination and quantitative velocity determination in collagen threads from mammalian tendon. The operational details of this instrument have been discussed in detail elsewhere (Korpel et al., 1971; Kessler et al, 1972; Kessler et al., 1974b; Kessler, 1974; Kessler, 1978), 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 allows 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 and are proportional to the acoustic amplitude in each region. The laser light transmitted through the coverslip and specimen allows the formation of an optical image in synchronization with the acoustic image.

Velocity determinations in small tissue components (50-100 μm) are made by detecting variations in the acoustic index of refraction, using the acoustic microscope in the acoustic interferometry mode (Kessler et al., 1972; Kessler et al., 1974b; Kessler, 1978). In principle, the intersection of two plane, coherent sound beams at a detector plane will result in an interference pattern depicted as a series of regularly spaced fringe lines. If an object whose velocity of sound differs from that of the surrounding medium interupts one of the beams, localized shifting of the fringe lines will result. The displacement of a fringe, measured graphically, is related to regional variations in the acoustic index of refraction. The velocity of sound in the specimen is given by (Kessler, 1978; Goss and O'Brien, 1978)

$$c_o = \frac{c_o}{\sin\theta_o} \sin\left[\tan^{-1}\left\{\frac{1}{\frac{1}{\tan\theta_o} - \frac{NL_o}{\Delta T \sin\theta_o}}\right\}\right] \qquad (4\text{-}17)$$

where c_o is the velocity of sound in the medium surrounding the specimen, θ_o is the angle from the normal of the acoustic beam in the medium surrounding the specimen, N is the normalized lateral fringe shift, ΔT is the thickness of the specimen, and L_o is the wavelength of sound in the medium surrounding the specimen. Typically, the medium surrounding the specimen is 0.9% saline, for which c_o is 1507 m/s (25°C) (Greenspan and Tschiegg, 1959), L_o is 1.5×10^{-3} cm, and θ_o is 10.16°, as determined from

$$\frac{\sin\theta_o}{c_o} = \frac{\sin\theta_q}{c_q} \qquad (4\text{-}18)$$

where $\theta_q = 45^\circ$ and $c_q = 5750$ m/sec (Kinsler and Frey, 1962) are the sound beam angles from the normal and the speed of sound, respectively, is fused silica.

Velocity variations under these conditions on the order of 1-2% may be discerned using this technique, assuming a specimen thickness of approximately 100 μm

and minimum measureable fringe displacement of 0.1 fringes. In practice, the specimen thickness introduces the greatest single source of error, as this parameter can often only be measured to within ± 10%, using either a calibration grid machined onto the coverslip, a calibrated light microscope, or the unperturbed fringe spacing to "calibrate" the screen of the acoustic microscope to determine this dimension.

4.7 Specimen Preparation

4.7.1 Collagen Suspensions

Calf skin collagen (freeze-dried, M_w = 360,000) was used in the preparation of collagen suspensions employed in this study, and the procedure for its suspension was furnished by Devro Corporation, Somerville, New Jersey, where this material is used in the manufacture of edible sausage casings. Amino acid analysis of Devro collagen (Lees, 1977; Edwards, 1978) has indicated purity to greater than 90%, using standard hyroxyproline assay procedures for collagen. Since collagen is generally insoluble in water, another medium, 3% (0.5 M) acetic acid has been suggested as a suitable suspending liquid that does not disturb the molecular integrity of the specimen, but does provide a uniform collagen suspension (Tsuzuki, 1975), and is employed in this study. The acetic acid solution was prepared by adding 45 ml of glacial acetic acid (Allied Chemical Co., Morristown, NJ) to the appropriate amount of singly deionized and distilled water so that the volume totaled 1.5l. A weighed amount of collagen (depending on the specimen concentration desired) was then introduced to the suspending medium in a Waring Model 16 Blender, and agitated at the lowest speed for no more than 10 seconds at one time for a total of 50-60 seconds. This pulse operation of the blender was performed to avoid any appreciable heating of the collagen suspension due to the

agitation process. The specimen suspension was then slowly stirred on a magnetic stirrer (Cole-Parmer Model 9" x 9") for 3-4 days, while refrigerated at 7-8°C, to allow the production of a uniform suspension. At this point the specimen liquid is placed in the ultrasonic measuring tank, allowed to reach thermal equilibrium, and the ultrasonic measurements made. The solution is then removed from the tank and a small volume (25-50 ml) removed for more exact concentration determination.

The concentration of collagen by weight, of the test liquid, was determined after each ultrasonic measurement by evaporating over air until dry (usually 48 hours) and placing in a vacuum desicator for 24 hours before weighing on an Ainsworth Type 10 Analytical Balance (with precision of $\pm$ 0.1 mg). It has been found that uncertainty in the solute concentration of solutions measured may be determined to better than 0.3% (Goss, 1974).

4.7.2 Mammalian Tissues

Mammalian tissue used in the ultrasonic absorption and velocity determinations were obtained immediately after death and stored at room temperature in physiological saline until measured, usually within 1-2 hours after excision. Beef tendon, liver, and kidney were obtained from previously bled animals approximately two years of age from the University of Illinois College of Agriculture slaughterhouse as were pig liver specimens, while mouse (LAF_1/J, Jackson Labs) testicle and liver, and cat brain, liver, kidney, and heart were surgically removed from healthy animals maintained in the laboratory. Horse testicle was surgically removed from an autopsy specimen, less than 24 hours after death. Care was taken to avoid stretching, crushing, or otherwise mechanically deforming tissue specimens prior to or during measurement.

4.7.3 Collagen Threads

Tail tendons were surgically removed within minutes post mortem from ten month old female, and 12 month old male LAF/J mice (Jackson Labs), and placed immediately in 0.9% saline solution at room temperature (22°C). Single tendon fibers on the order of 42-115 microns in diameter were then isolated, placed on the acoustic microscope stage, and again bathed with saline solution. Care was taken to assure that the specimen did not dry out. A doughnut shaped spacer was placed between the specimen and coverslip to prevent the coverslip from compressing or crushing the small fiber. Acoustic measurements were performed at room temperature, usually with 1-2 hours post mortem.

Chapter 5

Results and Discussion

5.1 Collagen Suspensions

5.1.1 General

The ultrasonic absorption and velocity in dilute collagen suspensions was measured as a function of frequency and solute concentration at 10° and $20^{\circ}C$. Velocity measurements were performed at 8.87 MHz using the high frequency system, as were absorption measurements at nine frequencies in the range from 8.87 to 56.4 MHz. Absorption measurements in the range from 0.5 to 1.5 MHz were performed using the resonant cavity technique. Suspension concentrations ranged from about 0.07 to 0.7% by weight due to the extraordinary viscosity (280 Poise at 1%, Tsuzuki, (1975)) of even these dilute suspensions.

The absorption data is presented in terms of the excess frequency-free absorption coefficient $\frac{\Delta\alpha}{f^2}$, defined as

$$\frac{\Delta\alpha}{f^2} = \frac{\text{total suspension absorption - solvent absorption}}{(\text{frequency})^2} \qquad (5\text{-}1a)$$

$$= \frac{\alpha_{\text{suspension}} - \alpha_{\text{solvent}}}{f^2} \qquad (5\text{-}1b)$$

In this equation, $\alpha_{\text{suspension}}$ has been corrected for diffraction effects, and in case of this study, α_{solvent} is the absorption characteristic of the 3% acetic acid used to suspend the collagen. Absorption data is usually expressed in this manner, because $\Delta\alpha/f^2$ is a constant for pure Stokes liquids (liquids for which the only mechanisms for absorption are due to the classical ones of shear viscosity and thermal conductivity, and also because the absorption coefficient of the

solution is usually greater than that of the solvent, and is generally the quantity of interest. The excess-frequency free absorption per unit concentration of solute, sometimes referred to as the specific absorption, is expressed as

$$\alpha_{sp} = \frac{\Delta\alpha}{C_s f^2} \qquad (5\text{-}2)$$

where C_s is the concentration by weight (gm/100 cc) of solute in the solvent.

The ultrasonic absorption and velocity of the collagen "solvent" (3% aqueous acetic acid solution) was determined using the same experimental systems used in the collagen suspension measurements. The velocity of sound in this solvent was determined to be 1460 $\pm$ 9 m/sec and 1494 $\pm$ 2 m/sec ($\pm$ standard deviation) at 10°C and 20°C respectively. This is about 1% greater than the corresponding values for water (1448 m/sec at 10°C; 1483 m/sec at 20°C) determined by Greenspan and Tschiegg (1959). The total absorption of the 3% acetic acid solution, determined in this study, is shown in Figure 5-1 as a function of ultrasonic frequency, at 10°C and 20°C. Compared to water, the absorption in 3% acetic acid is somewhat greater in magnitude, and exhibits a frequency dependence slightly less than the f^2 dependence characteristic of water. The best linear regression exponential fit to this acetic acid absorption data yields an approximately $f^{1.9}$ frequency dependence at both temperatures (R = 0.91 and 0.86 at 10°C and 20°C, respectively, determined with 97.5% confidence level). Figure 5-2 compares the results of the present study (best fit straight lines) to data for aqueous acetic solutions of Stumpf and Crum (1966), and of Jackopin and Yeager (1972). Since neither of these studies dealt specifically with 0.5 M (3%) aqueous acetic acid solutions at 20°C, and since data from these studies for comparison here had to be interpolated from graphical presentations of the data, the comparison is not complete. Still, it is seen that at least at the higher frequencies, the presently obtained values are

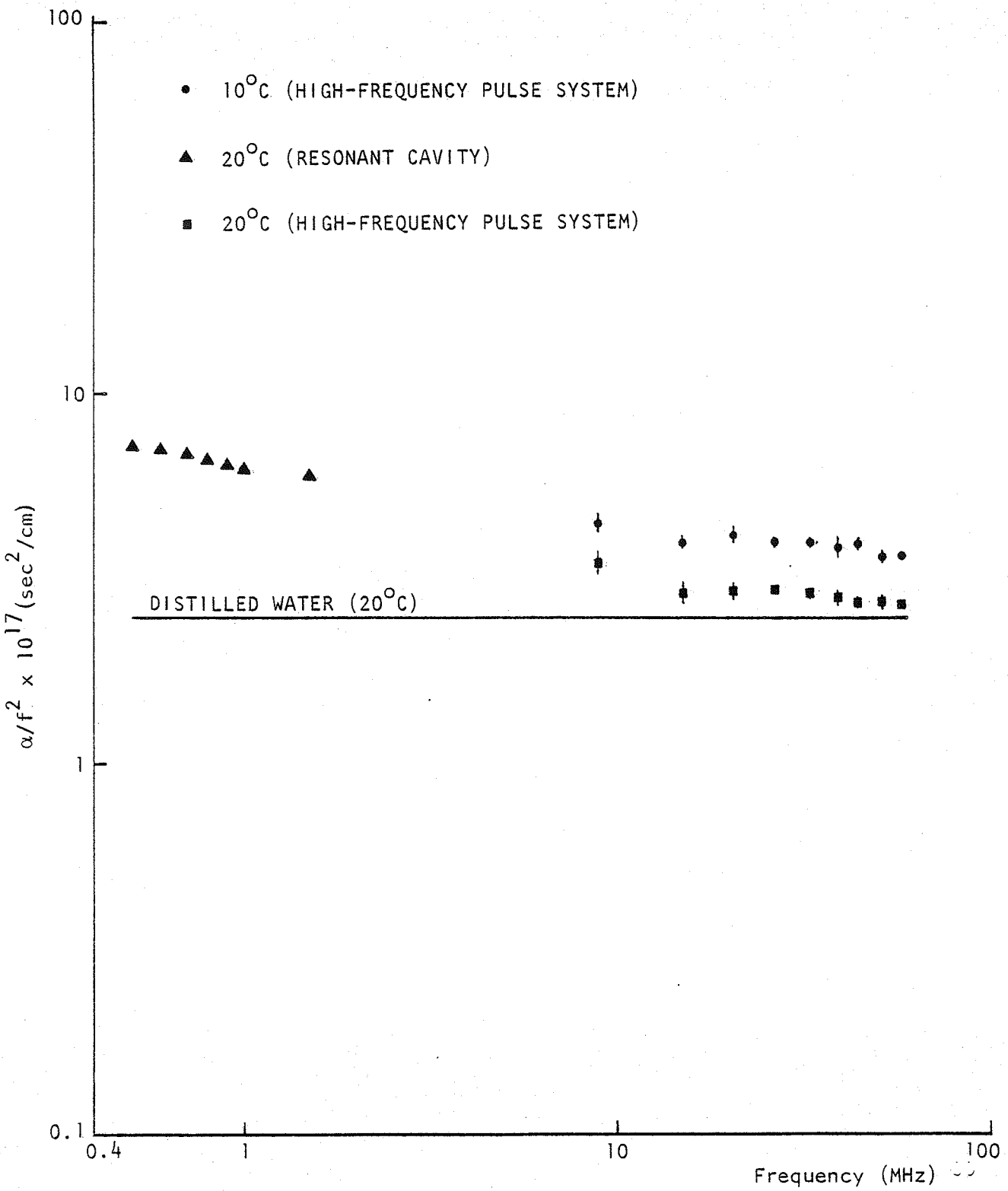

FIGURE 5-1 FREQUENCY-FREE ABSORPTION AS A FUNCTION OF FREQUENCY IN 3% (0.5M) AQUEOUS ACETIC ACID AT 10°C AND 20°C. (value for water shown for reference)

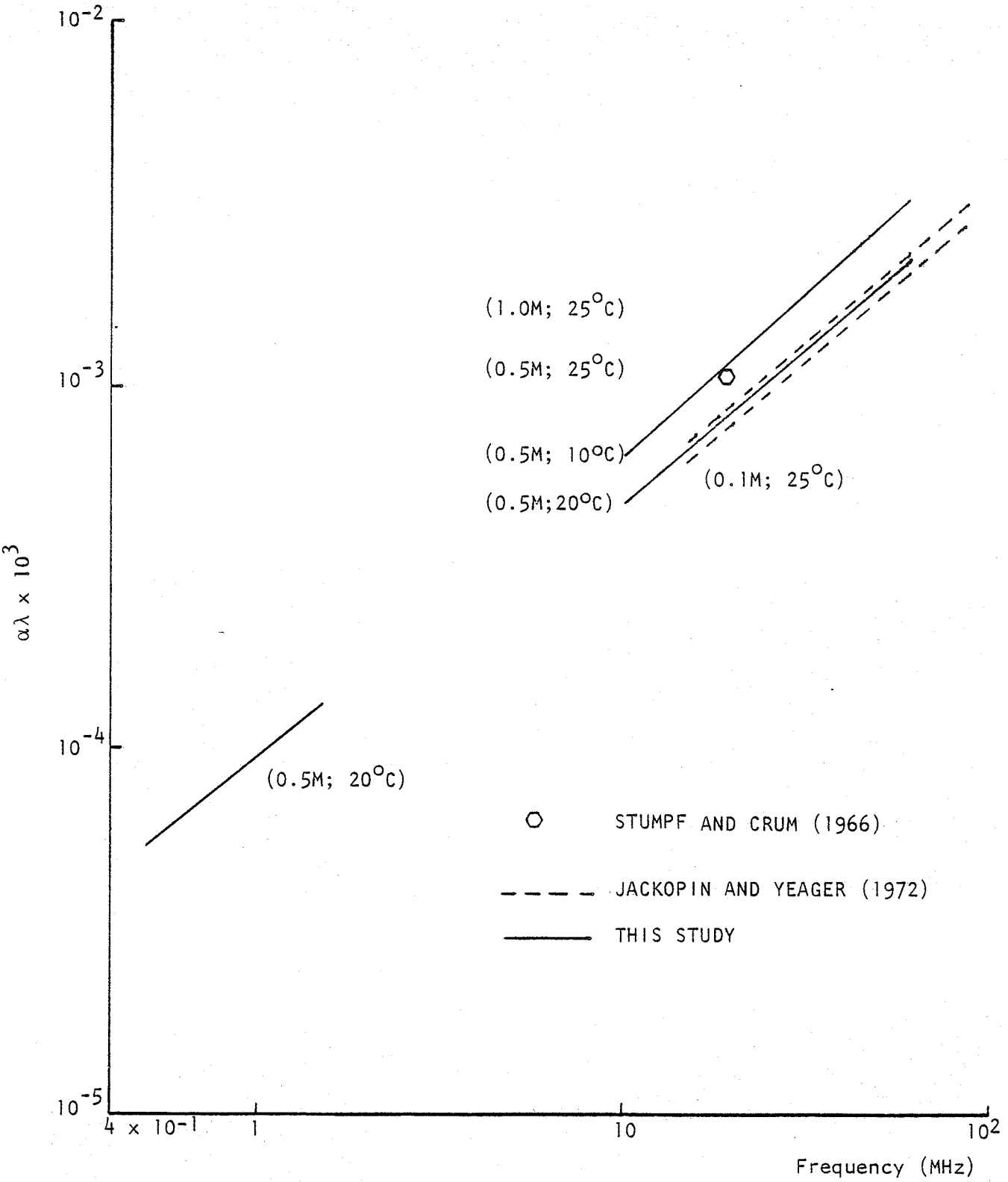

FIGURE 5-2 ABSORPTION PER WAVELENGTH IN AQUEOUS ACETIC ACID SOLUTIONS VERSUS FREQUENCY

within about 10-15% of those obtained by other investigators. Best fit values of the frequency-free absorption coefficient are used in the computation of the excess absorption due to the collagen solute alone, computed using (5-1).

5.1.2 Concentration Dependence

The concentration dependence of the ultrasonic absorption in proteins is deemed important since absorption is influenced to some extent by the relation between protein and its environment (Carstensen, 1960). A linear dependence of absorption on concentration is usually attributed to processes involving the interaction between the solvent and solute only, and is limited to appropriately low concentrations. A non-linear absorption-concentration dependence may be attributed to intermolecular interaction processes (Carstensen, 1960).

The concentration dependence of the excess frequency-free absorption in collagen suspensions at $10^{o}C$ and at $20^{o}C$ is shown in Figures 5-3 and 5-4, respectively. At $10^{o}C$, the concentration dependence was measured at nine ultrasonic frequencies from 9 to 56 MHz while at $20^{o}C$, data was taken at sixteen measurement frequencies, from 0.5 to 56.4 MHz of which thirteen were considered reliable. Data obtained at $20^{o}C$ at 44.4, 50.5, and 56.4 MHz are not shown in Figure 5-4 due to the wide scatter of data at these frequencies arising from the relatively small differences in the magnitude of the absorption of the suspension and solvent. For all of the other frequencies, however, at both temperatures it would appear that upon simple examination of Figures 5-3 and 5-4 that a nearly linear dependence of absorption on collagen concentration is demonstrated. To examine more closely this relationship, data at each frequency was fit to a power function of the form $\frac{\Delta\alpha}{f^2} = \alpha_f C^x$ where $\Delta\alpha/f^2$ is the excess frequency-free absorption coefficient, α_f is a frequency dependent constant, C is the collagen concentration in gm/100 cc and x is the

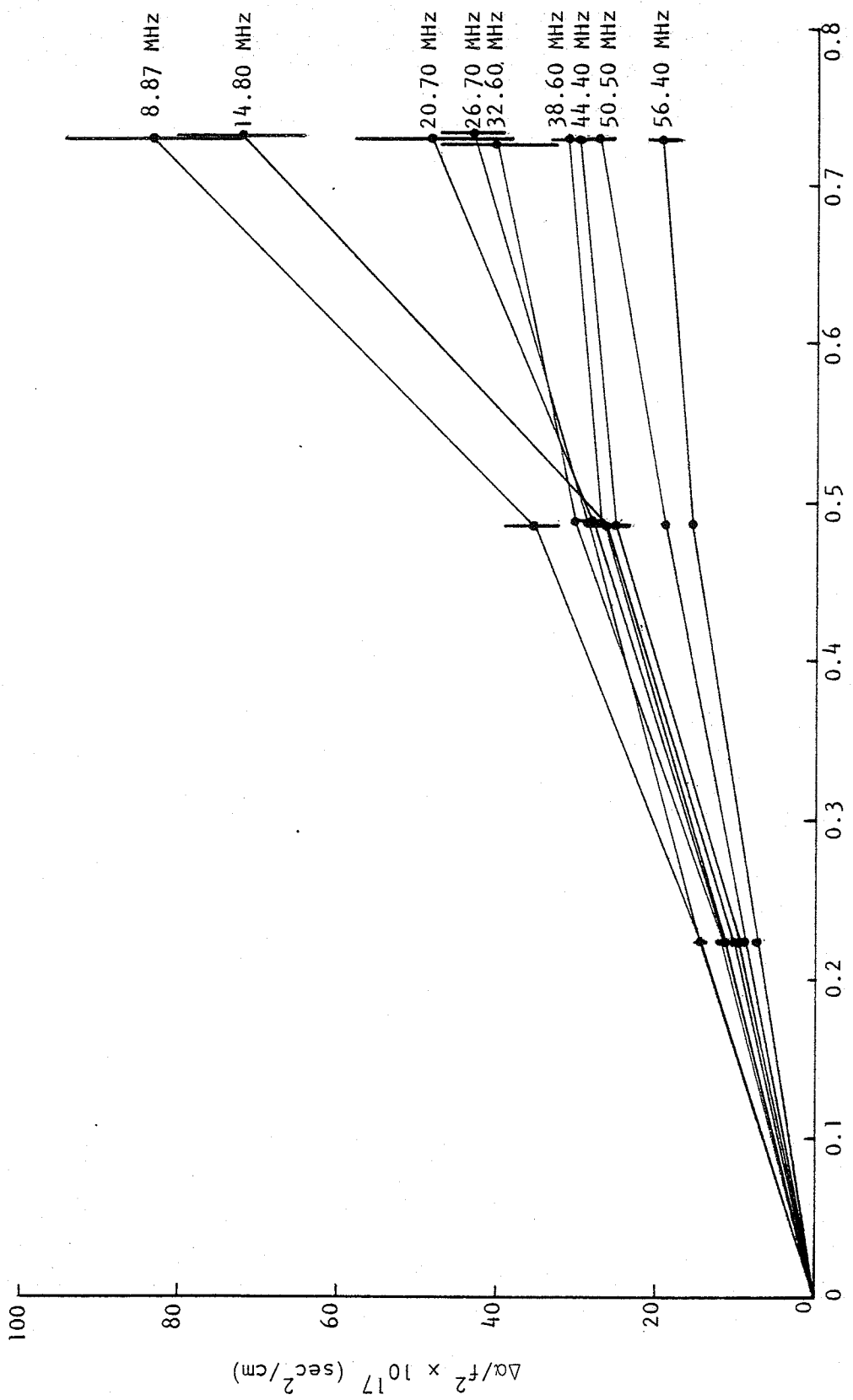

FIGURE 5-3 FREQUENCY-FREE ABSORPTION VERSUS COLLAGEN CONCENTRATION AT 10°C (bars indicate ± standard deviation)

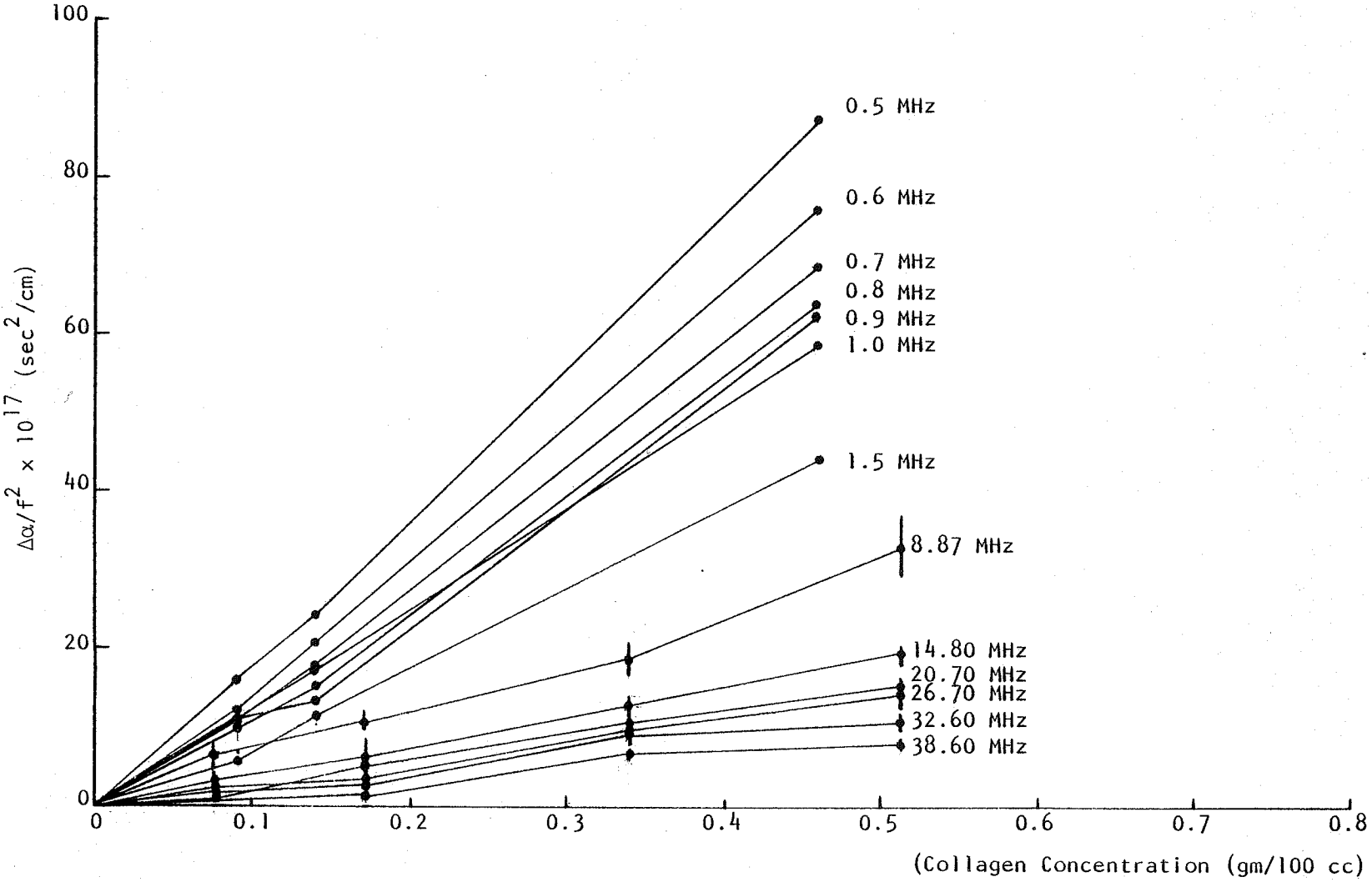

FIGURE 5-4 FREQUENCY-FREE ABSORPTION VERSUS COLLAGEN CONCENTRATION AT 20°C (bars indicate ± standard deviation)

exponent describing the concentration dependence, with x = 1 indicating a linear dependence. The results of these computations are shown in Table 5-1 for all of the measurement frequencies, at both temperatures. Also shown is the quality of fit parameter, R, to describe the degree to which the data fit the derived mathematical function, with R = 1 signifying a perfect fit to the data. It is seen that the mean exponent at 10°C and at 20°C are nearly identical, ranging from 1.12 to 1.14. The mean value here at 20°C excludes data at 44.4, 50.5, and 56.4 MHz for reasons previously described. Thus, it appears that the concentration dependence of absorption in collagen can be assumed to be slightly greater than linear, and appears to be independent to temperature, and frequency.

Such linear or nearly-linear behavior of the absorption coefficient with varying protein concentration has been observed by a number of investigators, predominately in blood proteins. Carstensen and Schwan (1959a) reported that the ultrasonic absorption in hemoglobin solutions is linear to approximately 15 gm/100 cc. This finding was further documented in uncrystallized and twice crystallized hemoglobin (O'Brien and Dunn, 1972b), though the concentration at which the absorption deviated from linearity was found to be dependent on the purity of the sample.

More recently, a non-linear dependence of absorption on concentration has been reported in ovalbumin (O'Brien, 1970), and in the synthetic polymer polyethylene glycol (Kessler et al., 1970). Thus, the linear concentration dependence of absorption has been considered to prevail for differing molecular strcutures, particularly when close inspection over a relatively broad concentration range has not been of paramount importance. A non-linear dependence of absorption was reported for bovine serum albumin in aqueous solution, for measurements of absorption in the concentration range from 5 to 40% by weight (Goss and Dunn, 1974),

TABLE 5-1

Concentration Dependence of Absorption in Collagen Suspensions

(less than 1%)

Best Power Fit Concentration Exponents

(A_x proportional to C^x)

Frequency	10°C		20°C	
	Exponent	Fit	Exponent	Fit
0.5	-----	-----	1.05	0.999
0.6	-----	-----	1.14	0.999
0.7	-----	-----	1.14	0.999
0.8	-----	-----	1.16	0.998
0.9	-----	-----	1.12	0.997
1.0	-----	-----	1.05	0.999
1.5	-----	-----	1.25	0.997
8.87	1.5	0.989	0.856	0.986
14.80	1.66	0.982	0.907	0.993
20.70	1.25	0.999	1.60	0.976
26.70	0.971	0.999	0.968	0.960
32.60	1.12	0.986	0.983	0.949
38.60	0.961	0.974	1.35	0.936
44.40	1.00	0.980	-----	-----
50.50	0.897	0.999	-----	-----
56.40	0.866	0.987	-----	-----

MEAN: $C^{1.14 \pm 0.28}$ MEAN: $C^{1.12 \pm 0.19}$

wherein a concentration dependence to the 1.2 power at 20°C, over the frequency range from 3.4 to 15 MHz was found. Also, the concentration dependence of absorption in solutions of the peptide antibiotic bacitracin (Slutsky et al., 1977) yields the value of the 1.2 power, with deviation from apparent linearity occurring at only about 2%. The large disparity in molecular weight between bacitratin (M_w = 1400) and bovine serum albumin (M_w = 68,000), as well as differences in molecular structure, calls to question the role of concentration of specific molecular species in the absorption process.

The results of the present study indicate a nonlinear concentration dependence in the concentration range for about 0.07 to 0.7%, though examination of greater concentration could readily define more clearly the degree of this nonlinearity. For example, an additional absorption measurement at 0.5 MHz at 37°C using the transient thermoelectric technique (α = 0.005 cm^{-1}) yields the following expressions when fit to lower concentration data already presented. At 0.5 MHz, where C is the wet weight percentage of collagen,

$$\frac{\Delta\alpha}{f^2} = 325.7\ C^{1.31} \tag{5-3}$$

and at 1 MHz

$$\frac{\Delta\alpha}{f^2} = 189.1\ C^{1.24} \tag{5-4}$$

assuming that the absorption is proportional to the frequency to the 1.5 power, as shown in the next section of this chapter.

It is not suprising to find that a non-linearity may indeed be present in this concentration range for collagen, merely due to the size of the collagen molecule, which has been reported to be cylindrical in shape, 2900Å long x 14Å

diameter in solution, exhibiting a molecular weight of about 360,000 (Boedtker and Doty, 1956). Thus, one might expect greater intermolecular interaction due to this increased molecular volume, which is some 7 times greater than that in hemoglobin. For example, the center to center intermolecular distance becomes equal to the molecular diameter in hemoglobin at a concentration of about 90% (concentration at which molecules are forced to touch). For the collagen molecule, however, assuming the molecule in solution acts as a 2900Å diameter sphere, the "touching concentration" occurs at about 0.0025%, which is 30 to 300 times smaller than the concentrations used in this study. It thus seems clear that intermolecular interaction occurs throughout the concentration range investigated ultrasonically herein, and could be responsible for the observed nonlinearity in the dependence of absorption in collagen concentrations.

The collagen concentration dependence of velocity is shown in Figure 5-5 for both measurement temperatures. The data indicate a linear dependence of velocity on collagen concentration, the slope and intercept of which appear to be dependent to some degree upon temperature. At 20°C, the function describing this dependence is

$$V = 7.5C + 1494 \qquad (5\text{-}5)$$

where V is the ultrasonic velocity, and C is the collagen concentration (gm/100 cc), with the fit parameter R = 0.98, while at 10°C, the equation best describing the data is

$$V = 9.9C + 1460 \qquad (5\text{-}6)$$

with R = 0.96.

In addition to the dependence of the speed of sound of the suspension upon

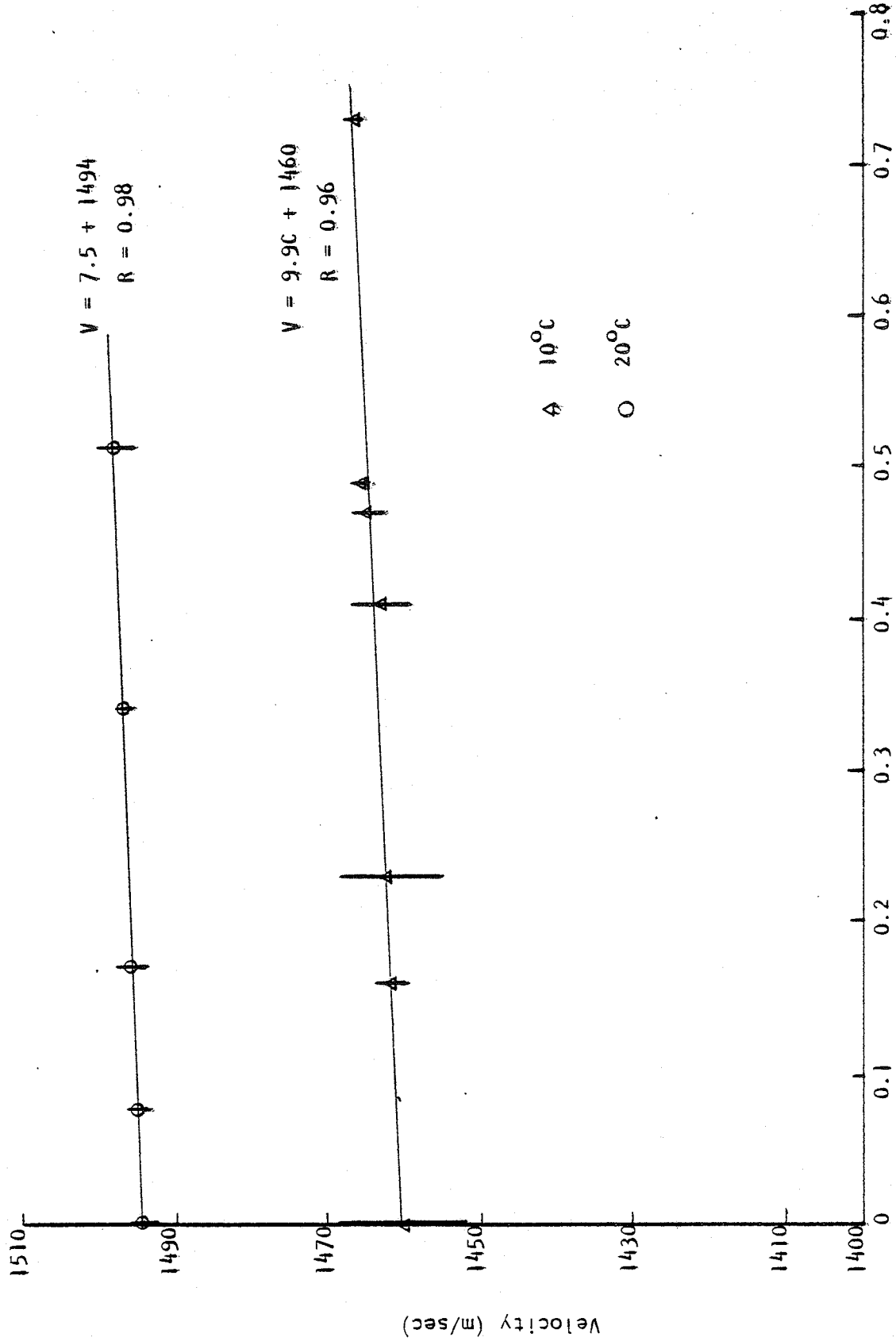

FIGURE 5-5 ULTRASONIC VELOCITY AS A FUNCTION OF COLLAGEN CONCENTRATION AT 10°C AND 20°C
(bars indicate ± standard deviation)

collagen concentration, there appears to be a slight dependence upon temperature also, though confidence in this dependence may be mitigated by the limited number of data points able to be measured over the restricted concentration range. The velocity-concentration slopes for collagen suspensions are, however, at least twice as great as found in aqueous solutions of globular protein (Goss, 1974). which yields the equation

$$V\ 3.5C + 1475 \qquad (5\text{-}7)$$

with R = 0.99. Similar analysis of the data for hemoglobin of O'Brien (1970) and Carstensen and Schwan (1959a) yield slopes of 3.1 to 3.9, respectively, at 10°C and 25°C. Another globular protein, ovalbumin, exhibits a similar concentration dependence of velocity (O'Brien, 1970). It thus appears that collagen, a structural protein, exhibits a speed of sound per unit concentration in suspension at least twice that found in globular protein solutions. The implications of these observations will be discussed later in this chapter with regard to the ultrasonic properties of biological tissues.

5.1.3 Frequency Dependence

The frequency dependence of excess frequency-free absorption in collagen suspensions of various concentrations is shown in Figure 5-6 in terms of the best fit straight line to the absorption data at each concentration and temperature, over the frequency range from 8.87 MHz to 56.4 MHz, and 0.5 MHz to 1.5 MHz. Broken lines represent the data at 10°C, and solid lines the data at 20°C. In order to obtain a useful and uniform measure of the frequency dependence in collagen suspensions, the nearly linear concentration dependence of absorption is utilized to provide an average frequency dependence over all concentrations by plotting $\Delta\alpha/Cf^2$ (where C is the collagen concentration (gm/100 cc) as a function

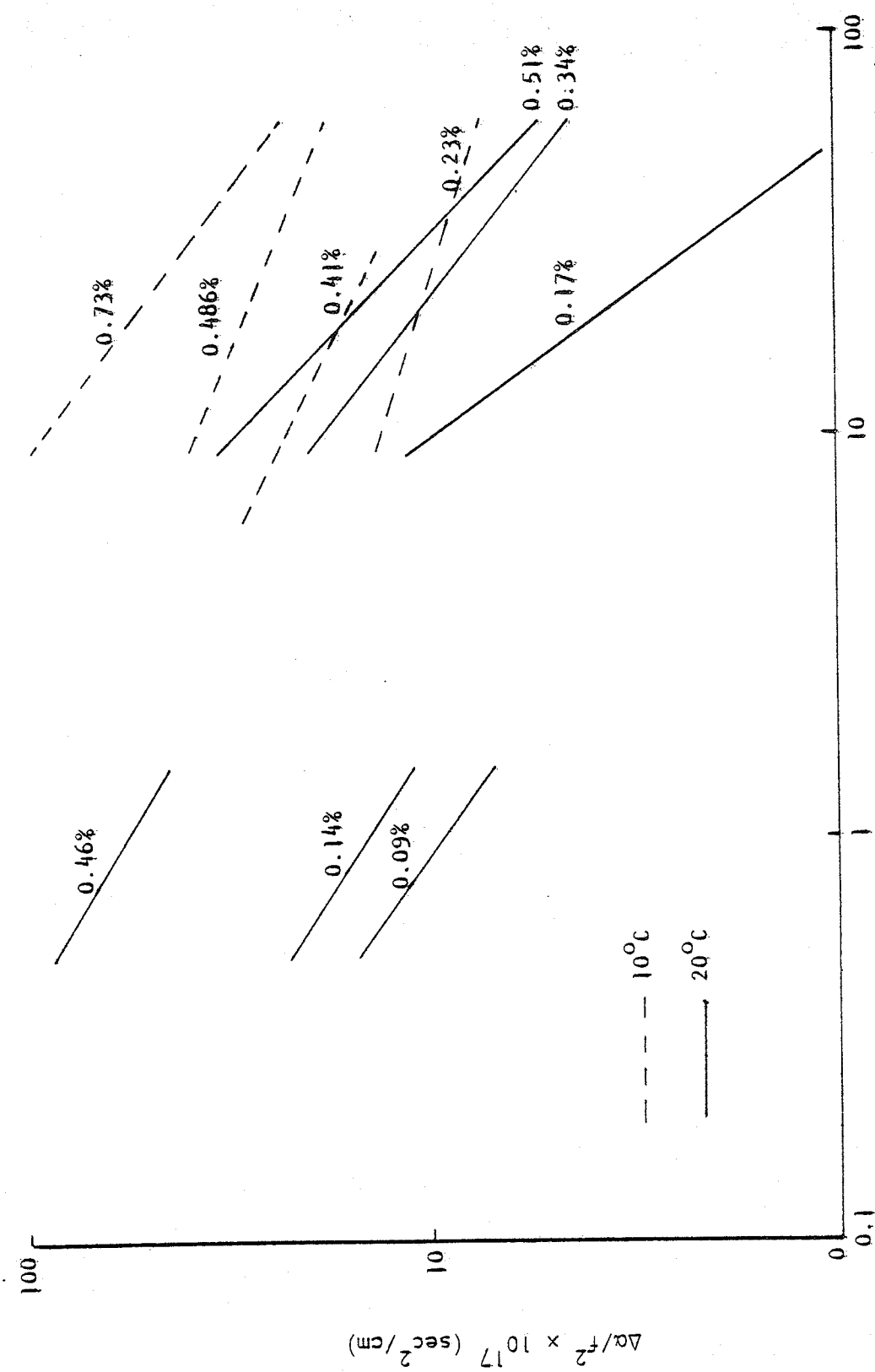

FIGURE 5-6 FREQUENCY DEPENDENCE OF EXCESS FREQUENCY-FREE ABSORPTION IN COLLAGEN SUSPENSIONS OF VARIOUS CONCENTRATIONS AT 10°C and 20°C

of frequency. This plot, shown in Figure 5-7, is comprised of the average value ($\pm$ the standard deviation over all concentrations) of the excess frequency-free absorption per unit collagen concentration as a function of frequency. From this data, a frequency dependence of excess frequency-free absorption of $f^{-0.56}$ and $f^{-0.53}$ was determined at 10°C and 20°C, respectively. The parameters describing the quality of fit of these dependencies were 0.89 (9 data points at 10°C) and 0.97 (16 data points at 20°C).

The frequency dependence obtained here for collagen is not unlike that found in other proteins. Goss and Dunn (1974) determined the frequency dependence of excess frequency-free absorption in aqueous solutions of bovine serum albumin to be the frequency raised to the -0.55 power. Similar frequency dependencies have been determined for hemoglobin (O'Brien and Dunn, 1972b; Edmonds et al., 1970), ovalbumin (O'Brien, 1970) and DNA (O'Brien, 1970). Each of these macromolecules however, possesses a very different spatial configurations in solution. Serum albumin and ovalbumin (molecular weights 68,000 and 46,000 respectively), like most globular protein molecules are constructed of a strongly bonded or tightly folded chains (Foster, 1960), forming a rigid compact globule at neutral pH. Hemoglobin (molecular weight 68,000) consists of four polypeptide chains which interlink together to form a characteristic globular aggregate. The nucleic acid (molecular weight 10^6 - 10^9) DNA in aqueous solution exists as a double helix, i.e., two chains wound tightly together and held by hydrogen bonding between adjacent bases, which form a rod-like structure. Collagen, as previously described takes the form of a triple helix "cable," of molecular weight 360,000. It appears then that structural diversity among intact proteins may not effect the characteristic frequency dependence of ultrasonic absorption for the proteins studied thus far. It is interesting to note, however, that the same

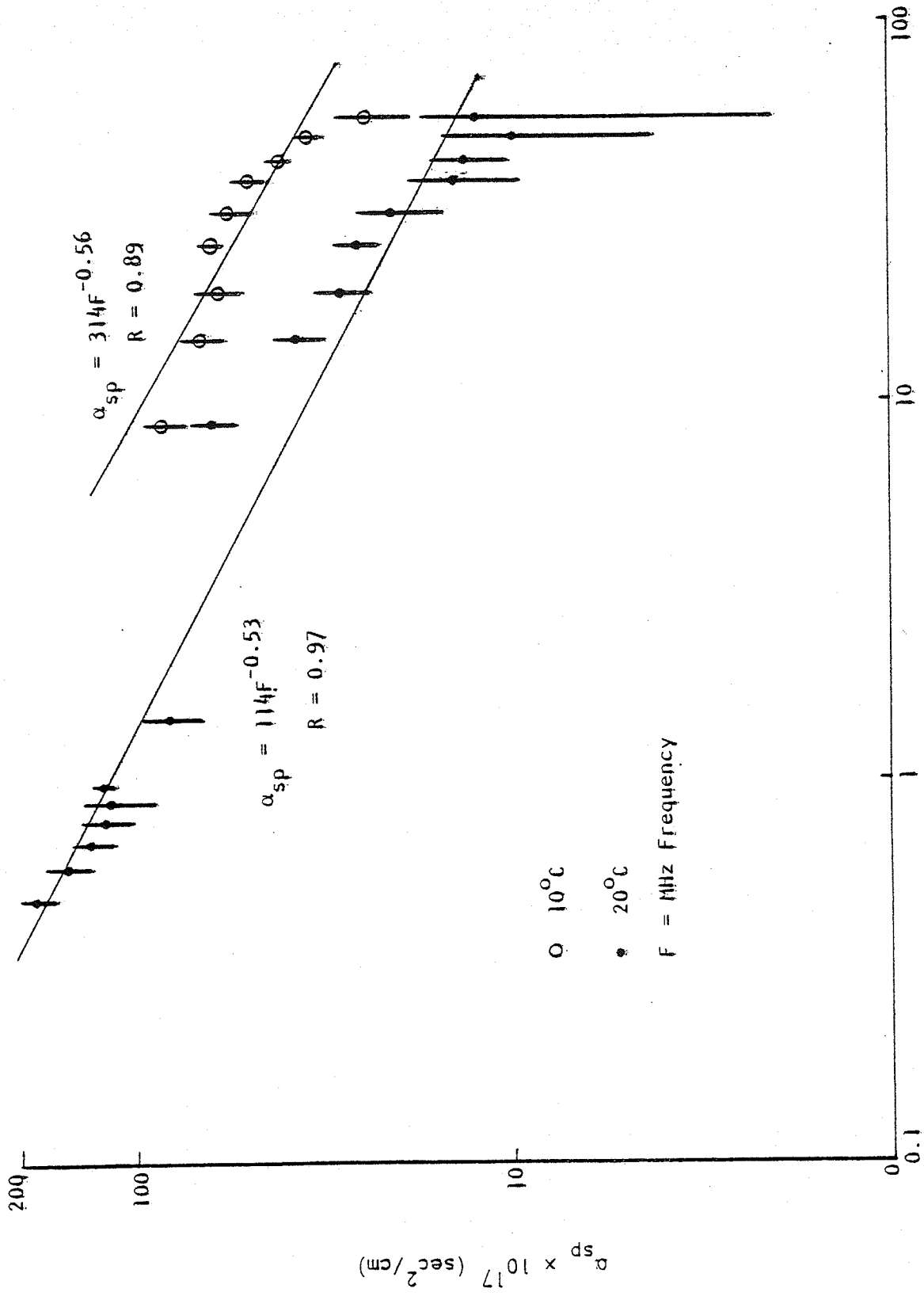

FIGURE 5-7 SPECIFIC ABSORPTION IN COLLAGEN SUSPENSIONS VERSUS FREQUENCY AT 10°C and 20°C (bars indicate the standard deviation over all concentrations)

frequency dependence of absorption found for these proteins has been determined for dextran, a branched polysaccharide having litte in common with the structure of proteins (Hawley and Dunn, 1969). It is not suprising then to find that collagen, even taking into account its rather unique and complex structure, exhibits a frequency dependence of absorption not unlike other proteins. The common frequency dependence found for nearly all biopolymers possessing higher order structuring indicates that a common absorption mechanism may be responsible for these observations.

Of particular interest to the present study is the magnitude of the ultrasonic absorption spectrum exhibited by collagen in its macromolecular form. In Figure 5-8 the ultrasonic absorption per wavelength per unit concentration is shown as a function of frequency for collagen, and a number of other biological polymers including hemoglobin, serum albumin, DNA, and the denatured form of collagen, viz., gelatin. Two curves are shown for hemoglobin and for serum albumin, since a non-linear absorption dependence on concentration has been determined for these biopolymers. Although similar non-linear dependence of absorption on concentration has been determined for collagen, for the purposes at hand it may be approximated as linear (to within $\pm$ 15-20%) for the concentration range dealt with here (less than 1% by weight). Figure 5-8 suggests that the molecular structure, as well as the degree of concentration, has much to do with the ultrasonic absorption characteristics exhibited by a particular biopolymer. For dilute solutions, collagen and DNA are seen to exhibit the greatest absorption per wavelength per unit concentration. At 10 MHz, the absorption parameters for collagen and DNA are 130% greater than for dilute hemoglobin and 60% greater than for dilute serum albumin. Collagen and DNA also possess the greatest degree of structuring, with collagen consisting of a three-stranded triple helix, and DNA, the two-stranded double

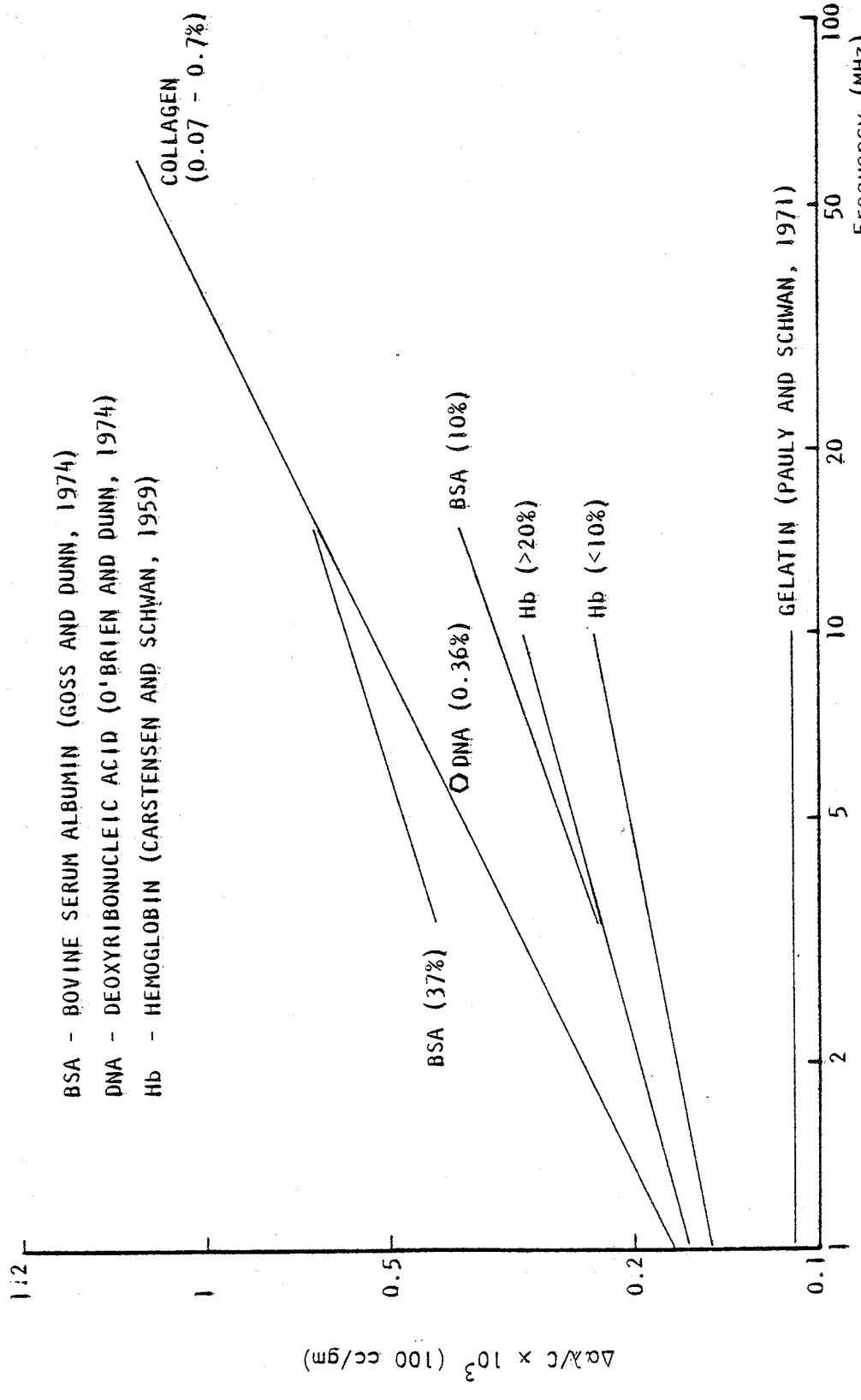

FIGURE 5-8 ULTRASONIC ABSORPTION PER WAVELENGTH PER UNIT CONCENTRATION FOR A NUMBER OF BIOLOGICAL POLYMERS

helix. The complete breakdown of the helical structure of collagen results in the formation of gelatin, which assumes a random-coil configuration in solution. It is clear from Figure 5-8 that the breakdown of the helical collagen structure and the fragmentation of the respective chains results in a severe alteration in both the magnitude and frequency dependence of the absorption parameter. By increasing the concentration of globular proteins, it is clear that the absorption per unit concentration can be significantly increased to a level greater than that characteristic even of collagen. Thus, increasing the concentration by a factor of four (10% to about 40%) increases the absorption per unit concentration by nearly a factor of two at 10 MHz, such that concentrated serum albumin exhibits an absorption per wavelength per unit concentration on the order of a dilute collagen suspension. Such behavior has been attributed to some form of macromolecular interaction (Kremkau and Carstensen, 1972; Kremkau, 1972; Kremkau et al., 1973), where the absorption per unit concentration increases as the level of macromolecular organization increases.

5.2 Biological Tissues

5.2.1 Frequency Dependence of Ultrasonic Absorption

Ultrasonic absorption measurements using the transient thermoelectric technique were made in six biological tissues of varying tissue macrostructure, including collagenous tissue, as a function of frequency in the range from 0.5 MHz to 7 MHz at the temperature of 37°C. Tissues were obtained from a number of species. Brain, kidney, and heart specimens were obtained from cats; liver tissue was obtained from beef, cat, mouse, and pig; tendon was obtained from both cat and beef, and testicle was obtained from mouse. Among the various parenchymal tissues, little species variation of the absorption parameter was determined.

However, in highly collagenous tissue such as tendon, some species difference was found, and this was restricted to the magnitude and not the frequency dependence of the absorption parameter. Therefore, species differences will not be considered further in this section.

The results of the ultrasonic absorption measurements as a function of frequency, shown in Figures 5-9 to 5-14 provide a means by which the frequency dependence of the various tissue types may be compared. Also included in these figures are the best least squares linear regression power fit describing the frequency dependence of absorption (where F is the frequency in MHz), and the correlation coefficient R which describes the quality of that fit (to better than 90% confidence level). The results of these measurements reveal a nearly linear frequency dependence of absorption for all of the six tissues studied, with the power to which the frequency is raised, always falling in the range from 1.0 to 1.18. As was the case for the dependence of the attenuation coefficient upon frequency discussed in Chapter 2, the frequency dependence of absorption, summarized in Figure 5-15, exhibits little variation with tissue type, even though differences in water, total protein, and collagen content vary as 20, 15, and 30%, respectively, among the six tissues treated. It appears as though the frequency dependence of attenuation or of absorption is insensitive to the constituent differences exhibited by these particular tissues. This behavior may be related to the similar frequency dependence observed in molecular collagen and globular proteins, previously described.

5.2.2 Magnitude of Ultrasonic Absorption

Nevertheless, the summary of absorption data shown in Figure 5-15 reveals rather pronounced differences in the magnitude of the ultrasonic absorption

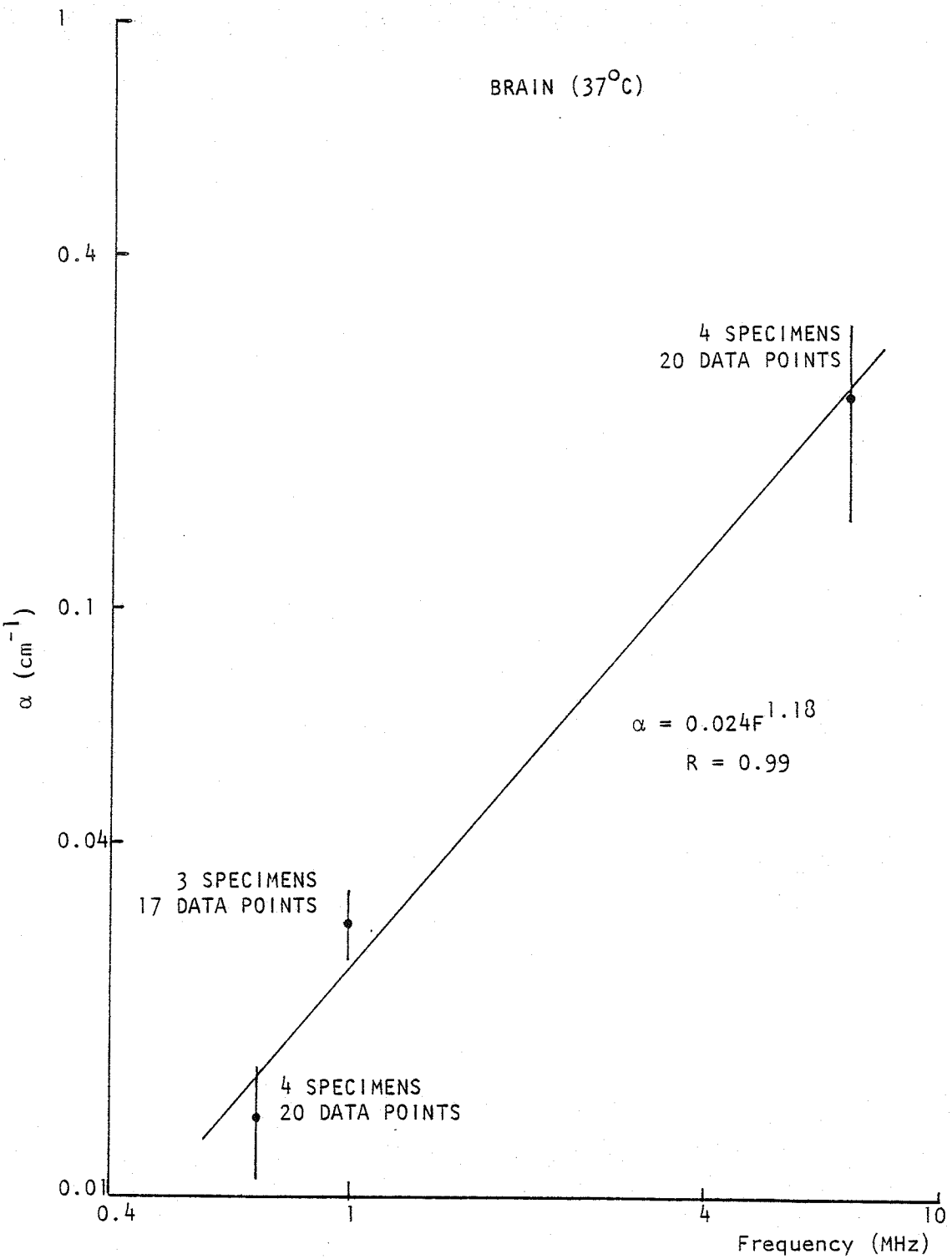

FIGURE 5-9 FREQUENCY DEPENDENCE OF ULTRASONIC ABSORPTION IN BRAIN TISSUE AT 37°C (bars indicate standard deviation)

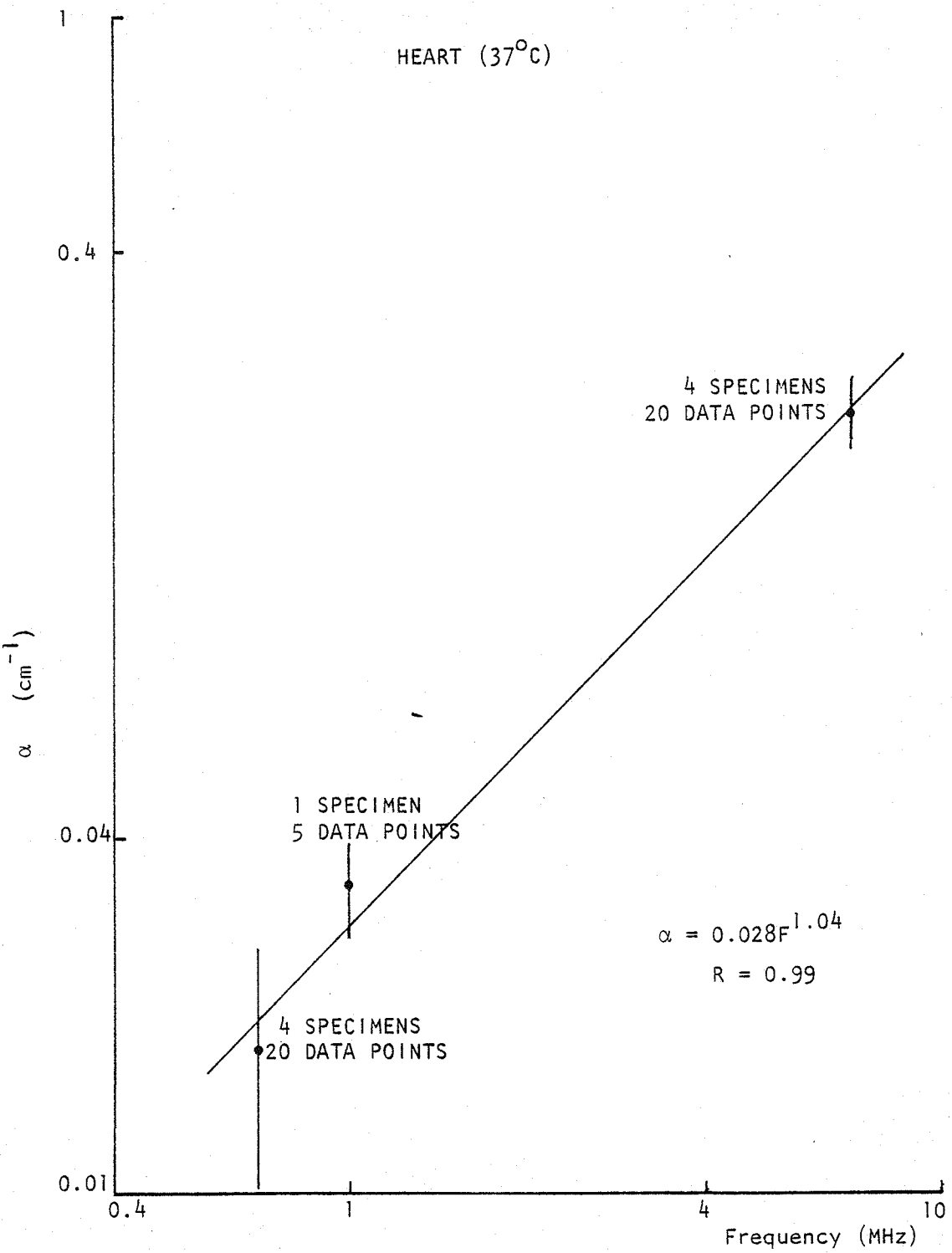

FIGURE 5-10 FREQUENCY DEPENDENCE OF ULTRASONIC ABSORPTION IN HEART TISSUE AT 37°C (bars indicate standard deviation)

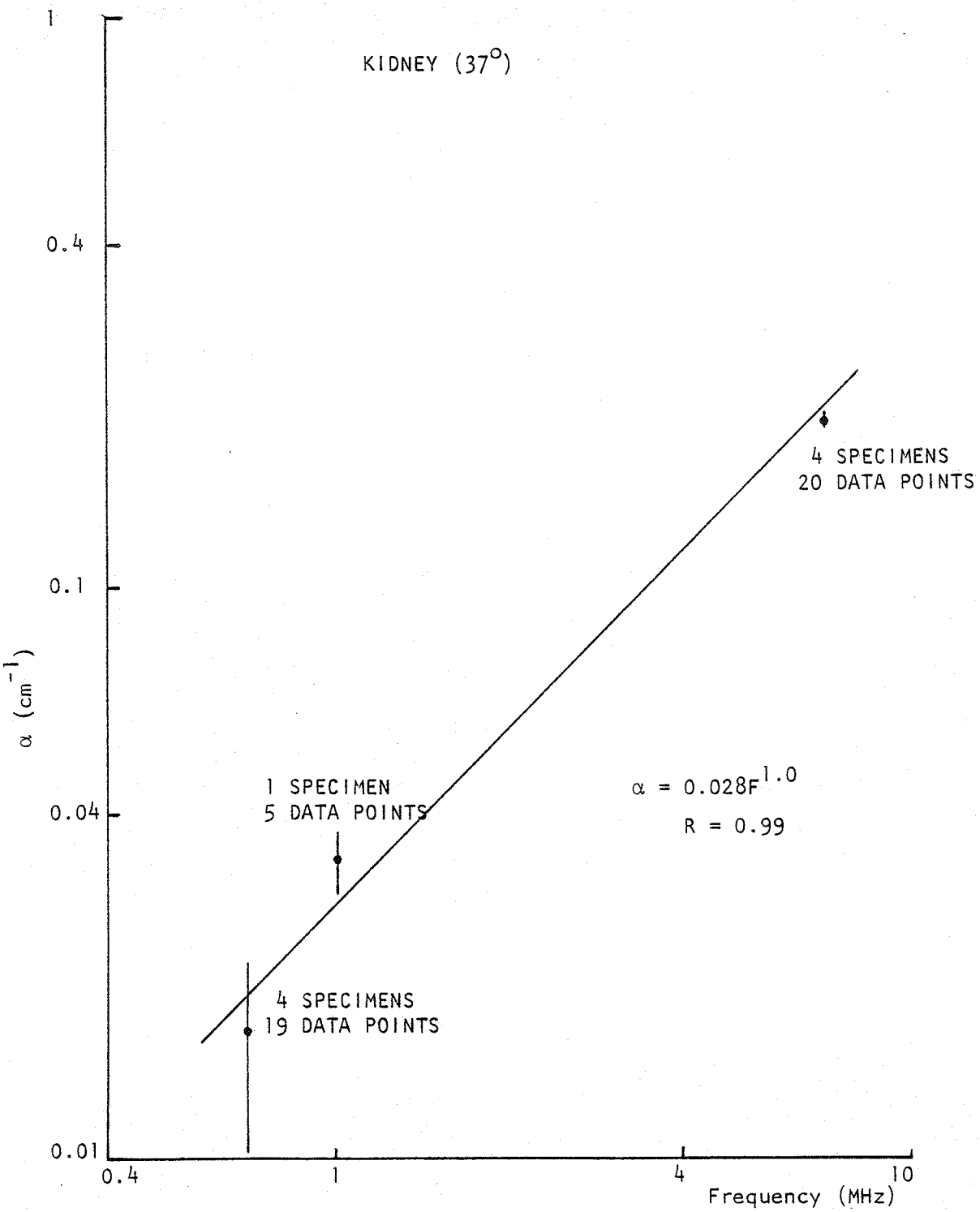

FIGURE 5-11 FREQUENCY DEPENDENCE OF ULTRASONIC ABSORPTION IN KIDNEY TISSUE AT 37°C (bars indicate standard deviation)

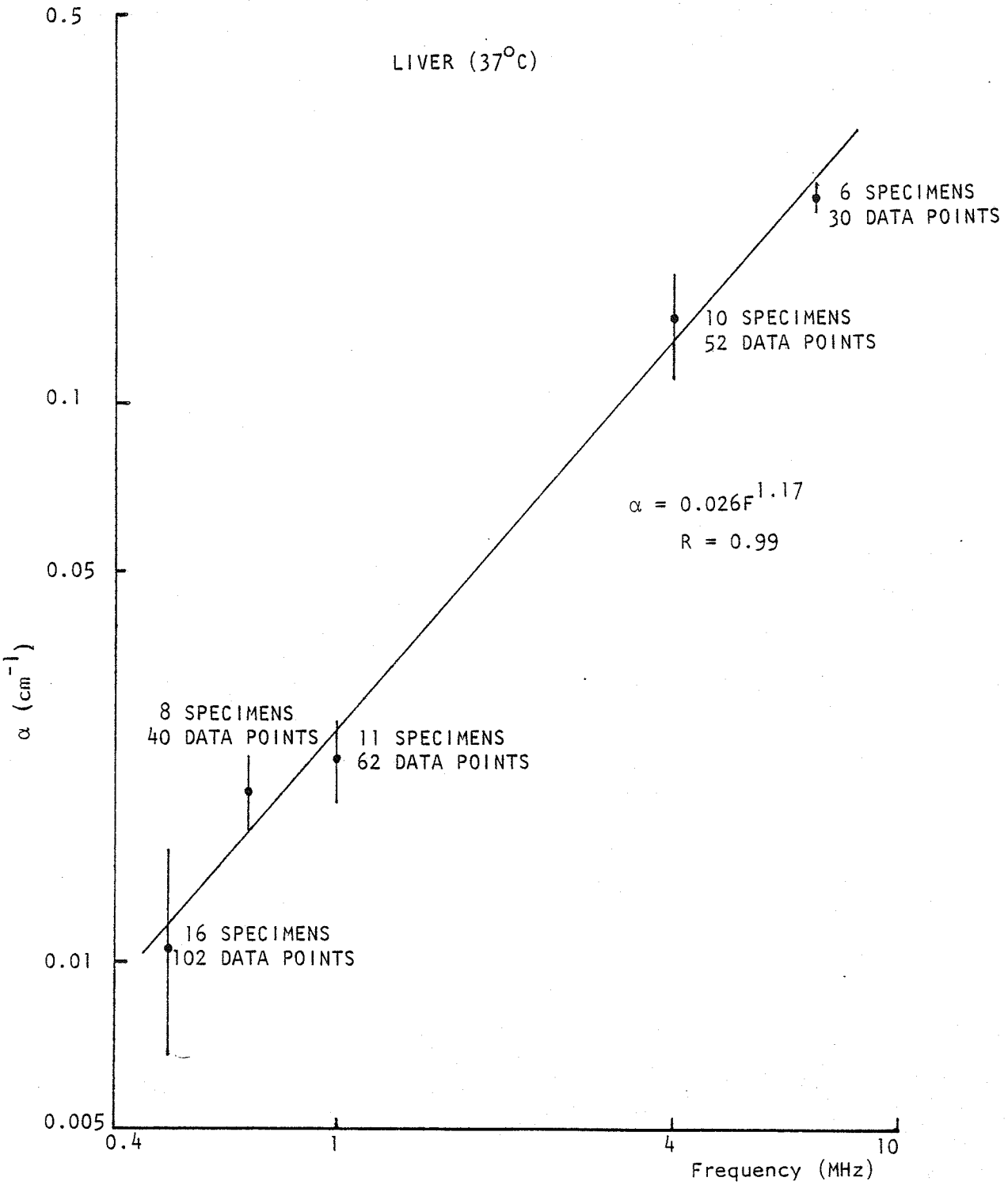

FIGURE 5-12 FREQUENCY DEPENDENCE OF ULTRASONIC ABSORPTION IN LIVER AT 37°C (bars indicate standard deviation)

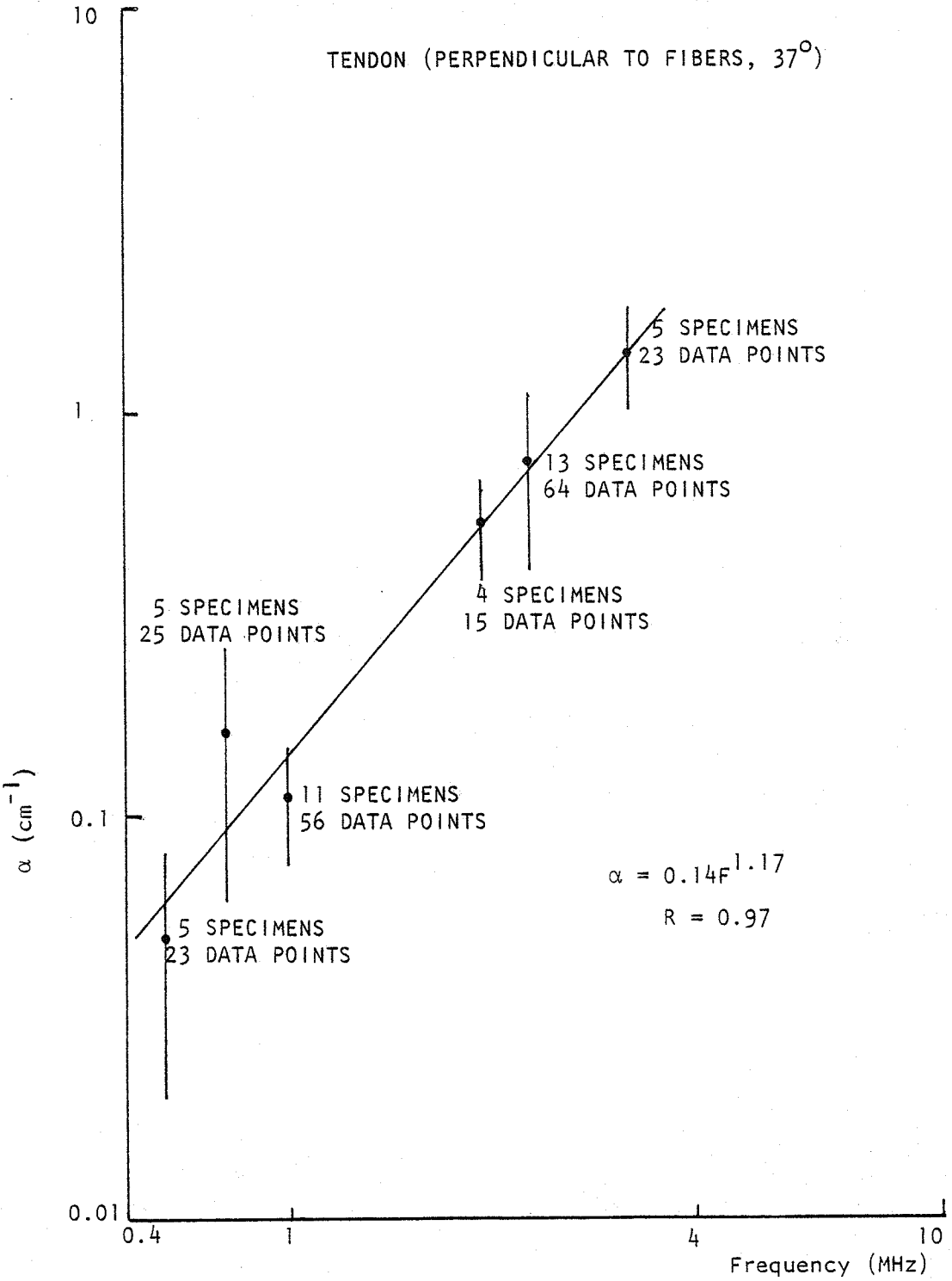

FIGURE 5-13 FREQUENCY DEPENDENCE OF ULTRASONIC ABSORPTION IN TENDON (PERPENDICULAR TO FIBERS) AT 37°C (bars indicate standard deviation)

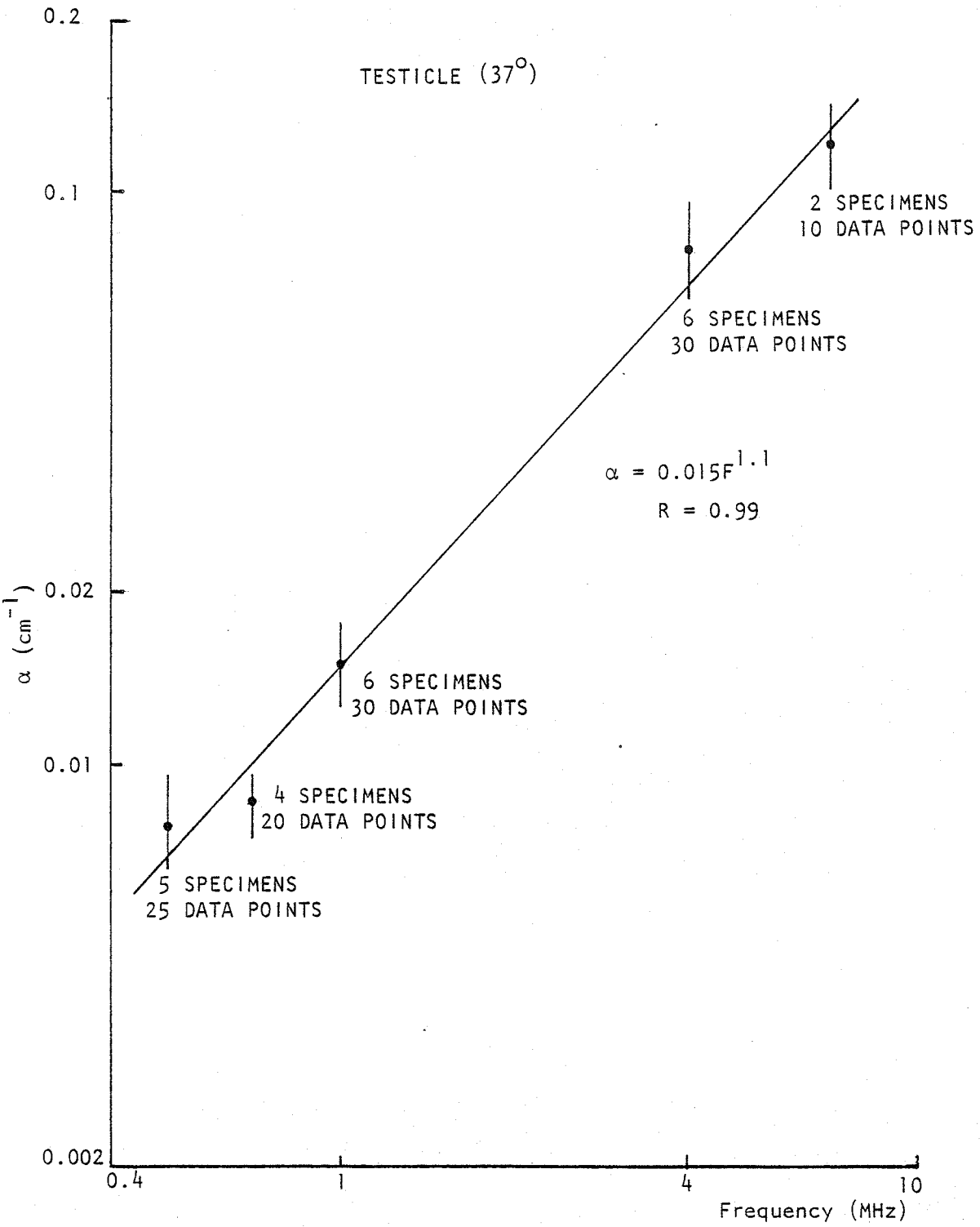

FIGURE 5-14 FREQUENCY DEPENDENCE OF ULTRASONIC ABSORPTION IN TESTICLE (bars indicate standard deviation)

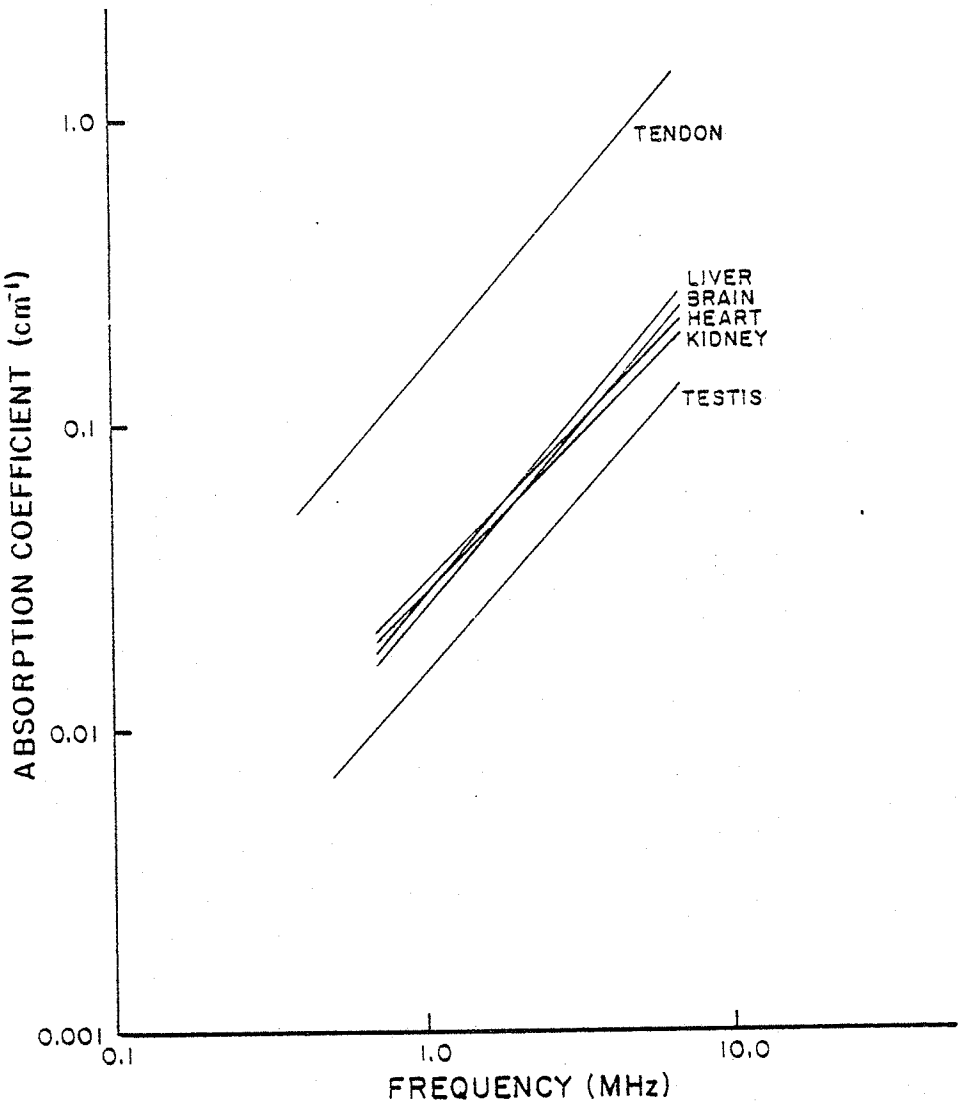

FIGURE 5-15 SUMMARY OF FREQUENCY DEPENDENCE OF ULTRASONIC ABSORPTION IN VARIOUS TISSUES AT 37°C

coefficient among the various tissues investigated. At 1 MHz, kidney, liver, heart, and brain all exhibit roughly the same magnitude of absorption, while tendon and testicle exhibit very different absorption coefficients. It is seen that the absorption coefficient of tendon is four to five times that of liver, while the absorption of testicle is only one-half that of liver. The constitution of these tissues may be invoked in seeking an understanding for this behavior. Referring to Table 2-2, it is seen that heart, liver, and kidney are all 16-18% by weight protein, and roughtly 1-2% collagen. Each of these tissues also is approximately 72-75% water. Tendon, on the other hand, has a total protein content of 35-40%, some 30% of which is in the form of collagen, with water comprising only about 63% of the tissues wet weight. On the basis of the increased absorption per unit concentration in molecules of collagen, reported previously in this chapter, one might expect a greater absorption coefficient than found in other tissues containing less collagen. Testis, on the other hand, contains very little collagen, but does contain an exceptional amount of water (greater than 80%), with only about 12% of the tissue wet weight composed of protein. It appears from this data that the constituent properties greatly contribute to the ultrasonic absorption observed in these biomaterials.

5.2.3 Species Dependence of Ultrasonic Absorption

The dependence of ultrasonic absorption on organ species was investigated for liver tissue of beef, pig, cat, and mouse as well as in tendon from beef and cat, over the frequency range from 0.5 to 7 MHz at 37°C. The results in liver tissue are shown in Figure 5-16 where it is seen that with the possible exception of the data at 0.5 MHz, where some scatter possibly due to specimen variation is seen, little difference in ultrasonic absorption is observed among the four

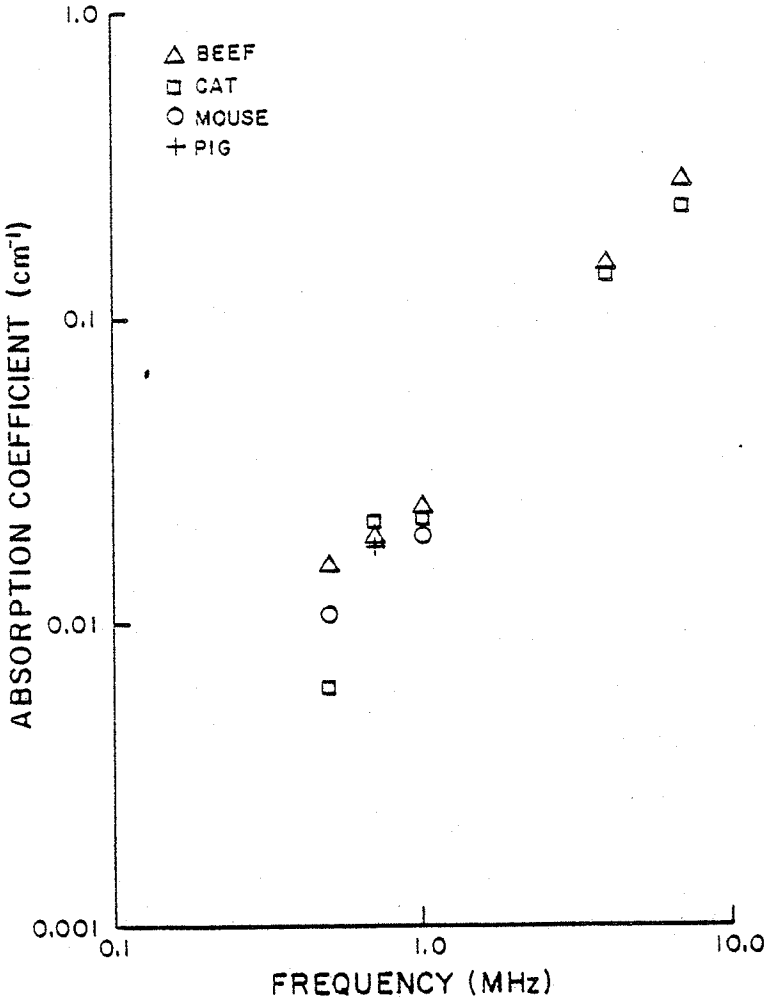

FIGURE 5-16 SPECIES DEPENDENCE OF ULTRASONIC ABSORPTION IN LIVER AT 37°C

species studied, since all variation is within the standard deviation of the mean value. It would appear however, that absorption in beef liver is slightly greater at most frequencies than that either for cat or mouse liver. This might be expected on the basis of the relation between organ weight and connective tissue content eluded to in Chapter 3. Beef liver, for example, is reported to contain nearly three times as much collagen as mouse liver contains (Neuman and Logan, 1950). However, pig liver contains six times the collagen of mouse liver, yet exhibits an absorption coefficient at 0.7 MHz nearly identical to that of cat, beef as well as mouse liver. There appears to be no clear relationship between tissue species and the absorption coefficient determined by connective tissue content. The inability to identify such a relationship may be due to the distribution of the collagen within the organ. Placement of the thermocouple junction at the identical anatomical position within the livers of tissues which vary in size so greatly is extremely difficult, and was not accomplished in this study. Within the organ itself however, the distribution of the concentration of connective tissue changes considerably. There is nearly twice as much collagen at the base of the liver than near the edge of the liver lobe (Harkness and Harkness, 1956). Such variations may well serve to cover any species dependence of absorption, unless collagen assays could be taken at the precise point of measurement, or the collagen distribution within the tissue is more uniform.

Tendon represents a tissue in which collagen is present in an organized grouping of regular parallel fibers. The collagen content also varies with animal species, with the dry weight percentage ranging from 86-95% in cattle and hogs (Mitchell, 1950), 92% in dogs (Eichelberger and Brown, 1945), and 79% in rats (Kao et al., 1960). Tendon thus represents a tissue for which the species dependence of ultrasonic absorption may better be examined. In the present study, lower

limb tendon of cattle and cats were examined with regard to their ultraonic absorption coefficients measured perpendicular to the fiber direction. These results are shown in Figure 5-17, wherein the magnitude of the absorption coefficient of cat tendon is seen to be about 70% below that of beef tendon at 1 MHz, while there is only about a 8% difference in frequency dependence, which is within experimental error. At least a portion of the lesser magnitude of absorption observed in cat tendon is thought to be attributed to the lesser amount of connective tissue present in the tendon of an animal of much smaller body weight (see Chapter 3), requiring less collagen as a primary structural material (Chvapil, 1976). This may be following findings with other physical quantities such as tensile strength, which can vary as much as a factor of two among animals of various species, with the tendons of smaller aniamls being the weaker (per unit cross-sectional area of tendon) (Yamada, 1965). However, another factor in considering the source of the differences in absorption between cat and beef tendon may arise from the gross macrostructure of the tendon and tendon fibers. The fibers of beef tendon are closely and uniformly arranged (and even difficult to separate by gross means), whereas in cat tendon, a number of smaller tendons, each of which are composed of many fibers, are wound around each other (and easily separable). The relatively loose structural arrangement present in cat tendon may in part be responsible for the lesser absorption coefficient observed therein. Thus, tissue morphology in addition to tissue constituency, must be considered in order to gain a complete understanding of the ultrasonic properties of biological tissues.

5.2.4 Dependence of Ultrasonic Absorption on Tissue Constituents

The above discussion suggests that relationships between absorption and

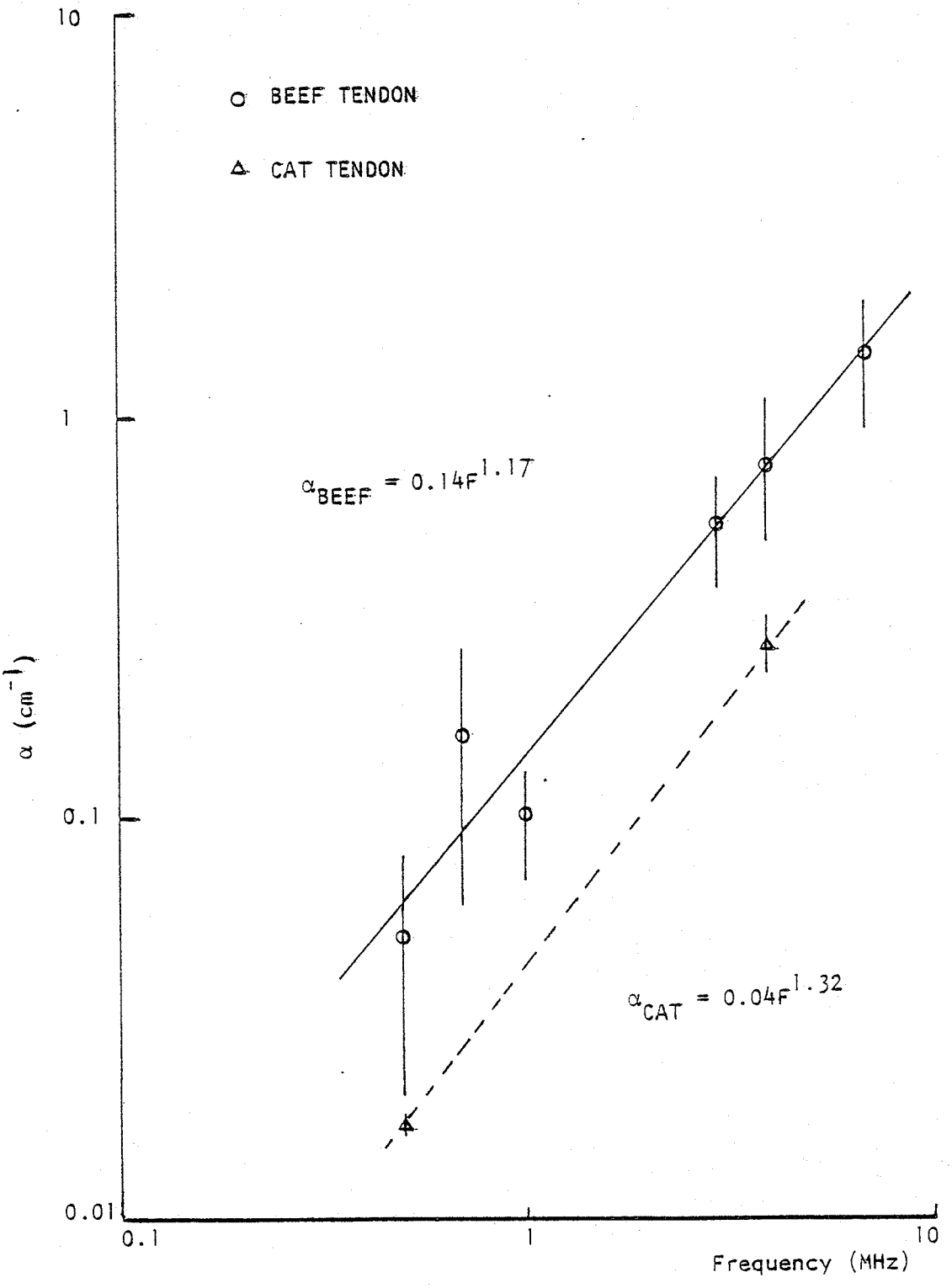

FIGURE 5-17 SPECIES DEPENDENCE OF ULTRASONIC ABSORPTION IN TENDON AT 37°C (bars indicate standard deviation)

tissue constituents, similar to those obtained in Chapter 2 with regard to attenuation and velocity, may exist. Such expressions relate more quantitatively the association between the ultrasonic properties of biological tissues, and particular molecular species of which those tissues are composed. For ultrasonic absorption, this type of correlation was attempted for the six tissue types dealt with above using the 1 MHz intercept of the best fit equations of the absorption as a function of frequency, and the tissue constituent data of Table 2-4 (Snyder et al., 1975). Figure 5-18 through 5-21 show the absorption coefficient so correlated as a function of the total protein, collagen, lipid, and water content, respectively. Again, for each data set, both linear and power fits to the data were obtained, and expression best describing the relationship between absorption and a particular tissue constituent determined, as shown on each curve. For the dependence of ultrasonic absorption $\alpha(cm^{-1})$ on the total protein content of the tissue, the best least squares linear regression fit to the data is

$$\alpha = 0.0048P - 0.045 \qquad (5\text{-}8)$$

where P is the wet weight percentage total protein. The correlation coefficient R which describes the quality of fit for this case is 0.96. The dependence of absorption on the tissue collagen content is

$$\alpha = 0.004C + 0.023 \qquad (5\text{-}9)$$

with R = 0.99, where C is the wet weight percentage of collagen. For total lipid, the expression (R = 0.689)

$$\alpha = 0.074L^{-0.63} \qquad (5\text{-}10)$$

where L is the wet weight percentage total lipid, while for water (R = 0.92)

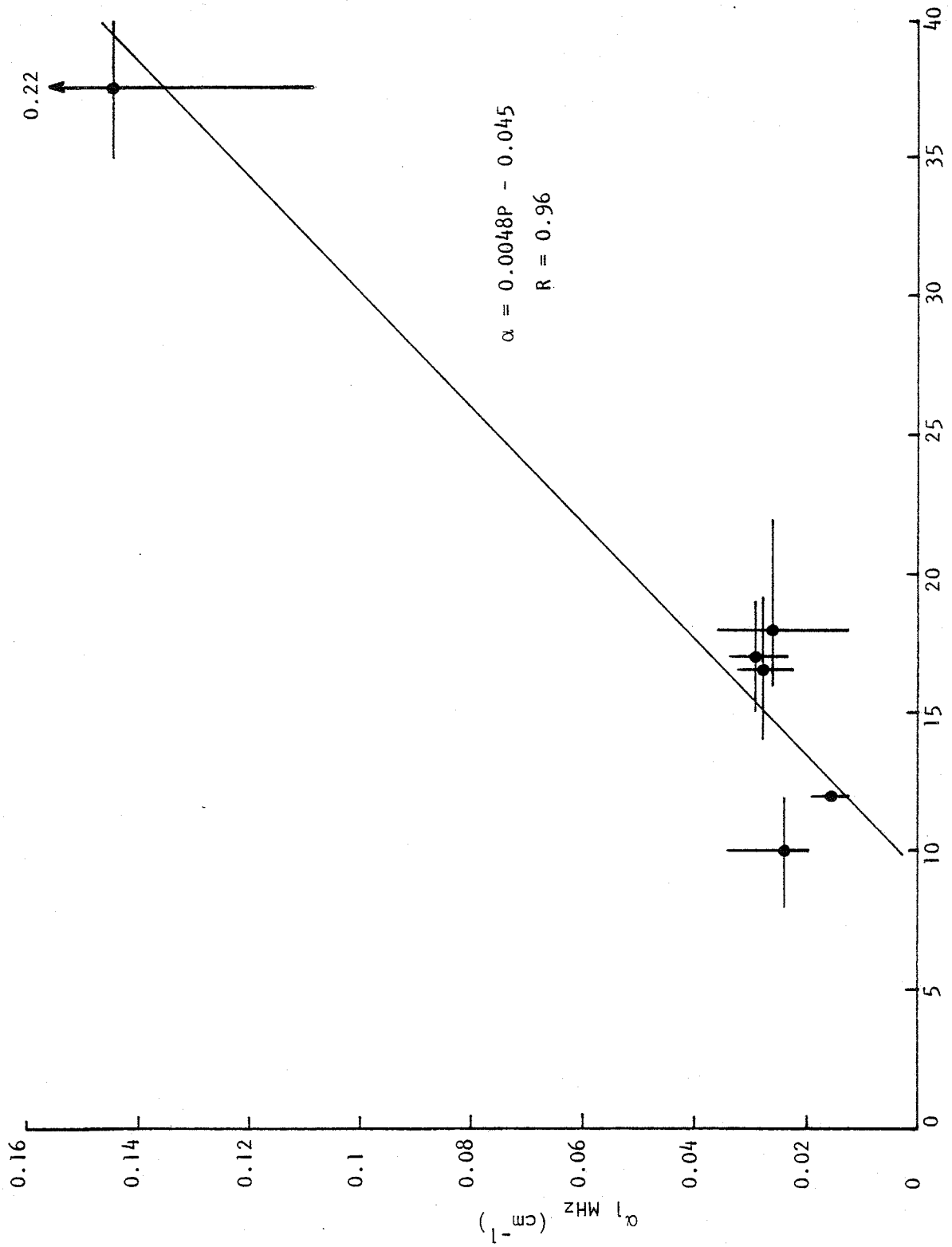

FIGURE 5-18 ULTRASONIC ABSORPTION (at 1 MHz, 37°C) VERSUS TOTAL PROTEIN CONTENT

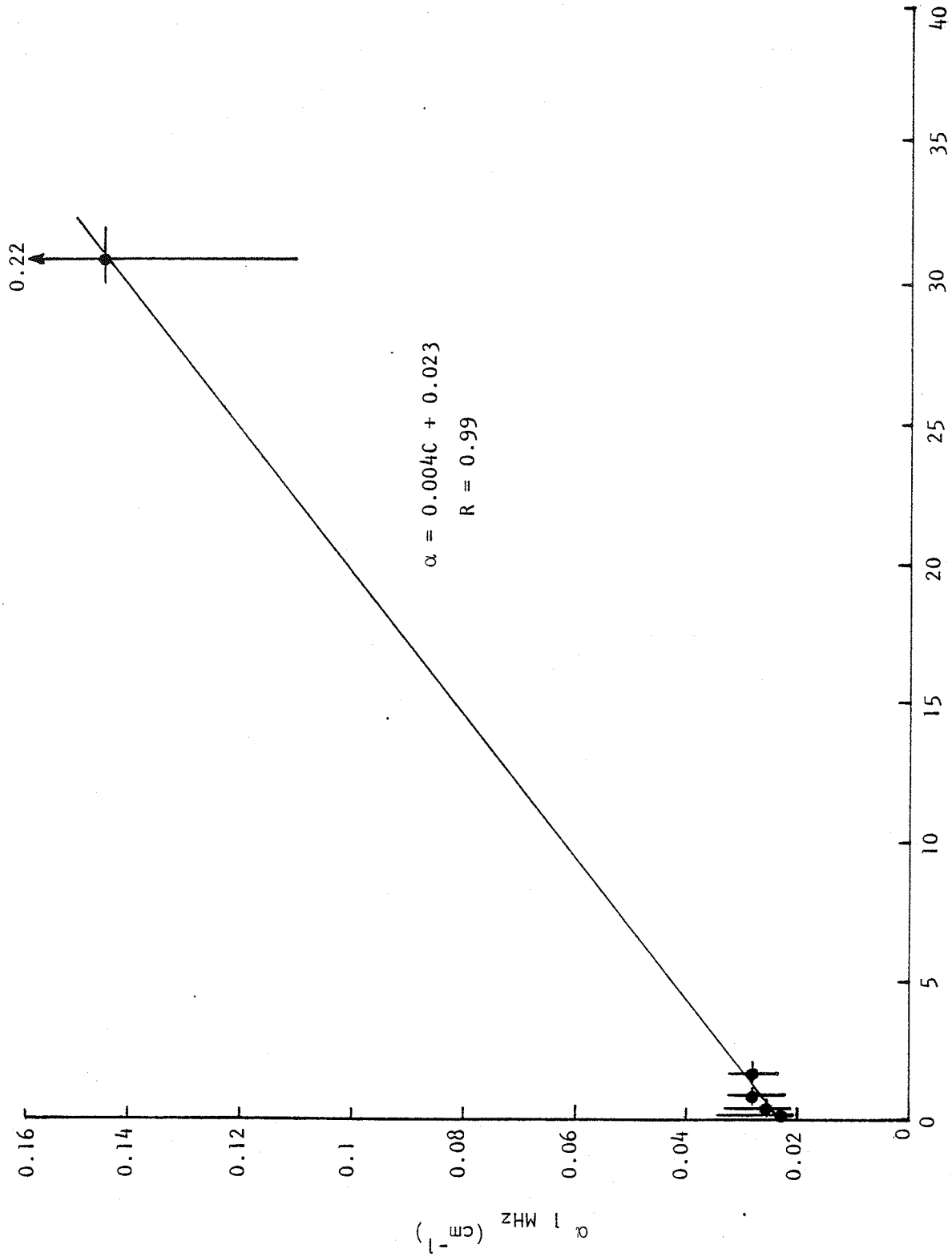

FIGURE 5-19 ULTRASONIC ABSORPTION (at 1 MHz, 37°C) VERSUS COLLAGEN CONTENT

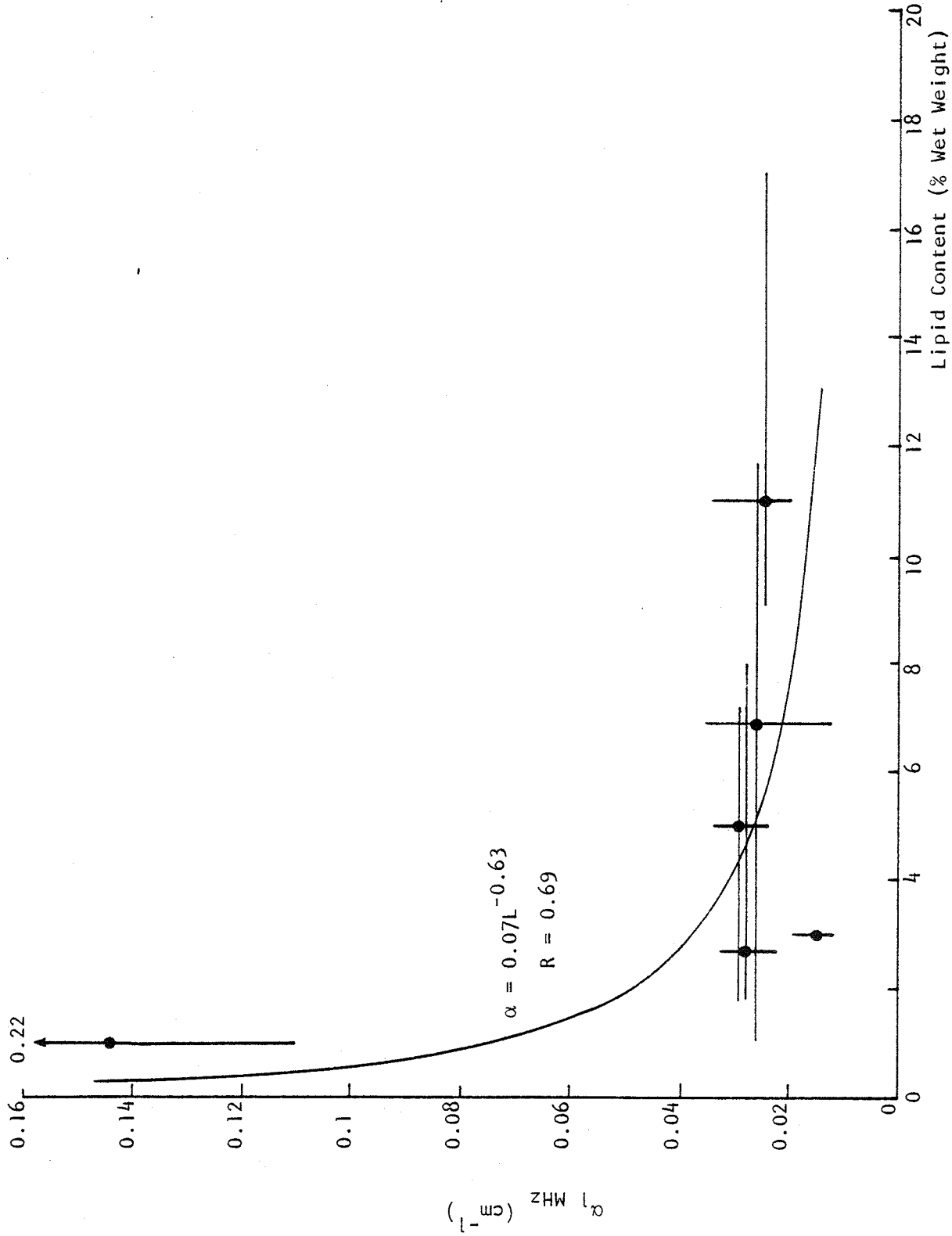

FIGURE 5-20 ULTRASONIC ABSORPTION (at 1 MHz, 37°C) VERSUS LIPID CONTENT

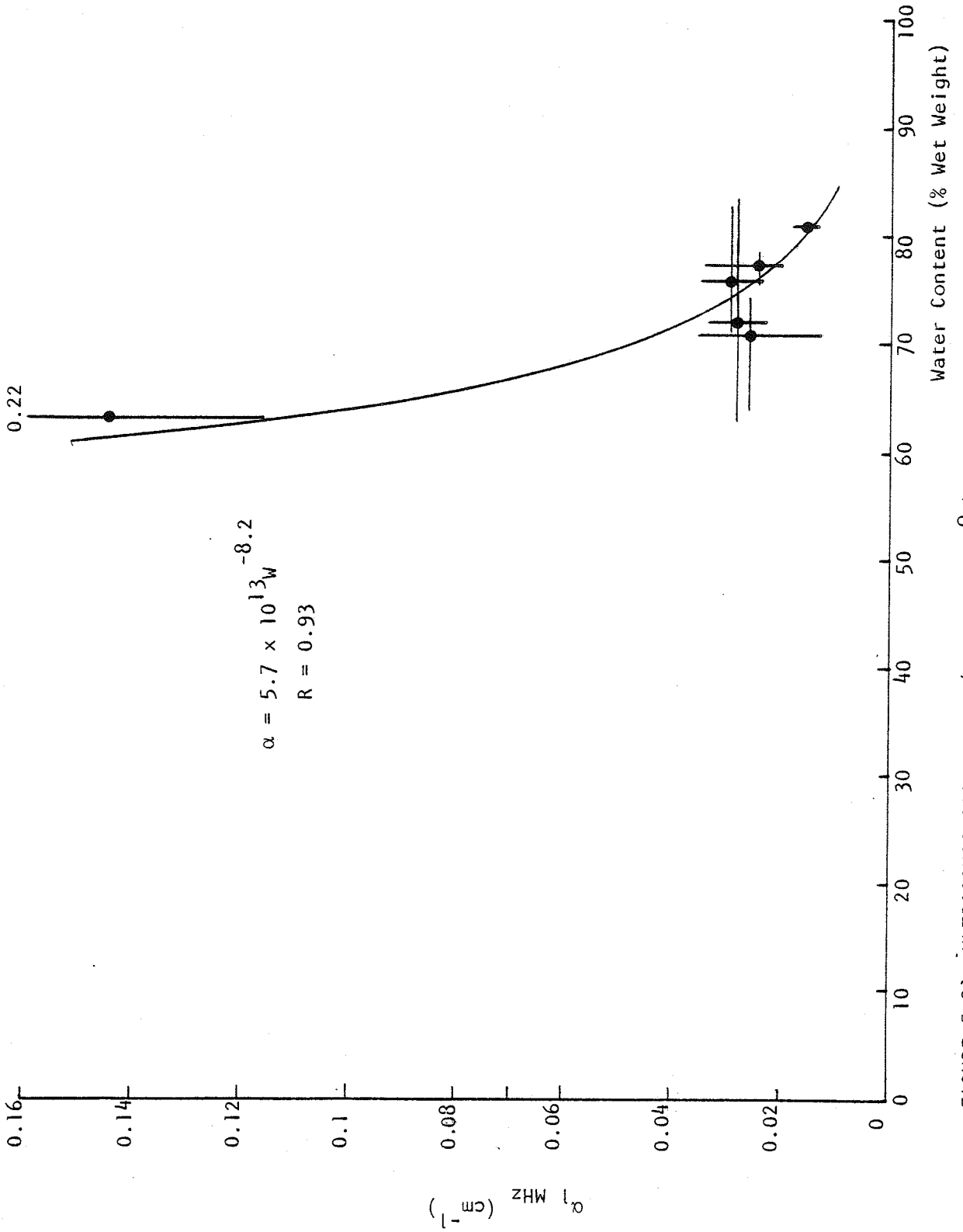

FIGURE 5-21 ULTRASONIC ABSORPTION (at 1 MHz, 37°C) VERSUS WATER CONTENT

$$\alpha = 5.71 \times 10^{13} W^{-8.2} \qquad (5\text{-}11)$$

where W is the percent water content of each tissue.

Again, as was found previously for the ultrasonic attenuation and velocity in tissues, it is clear that there is a relationship between the ultrasonic absorption exhibited by a tissue, and the composition of that tissue. The absorption coefficient is seen to vary linearly with tissue total protein and collagen content. This is somewhat suprising, since intense intermolecular interaction of the type found in concentrated protein solutions results in a non-linear concentration dependence. Such interactions might be expected in soft tissue, where proteins comprise nearly 20% of the wet weight (Snyder et al., 1975). It is felt however, for results over only six tissue types that the concentration to the first power determined here is very similar to the concentration to the 1.2 power determined previsously for globular proteins (Goss and Dunn, 1974) and the concentration to the 1.13 power determined in this study for dilute collagen suspensions.

The water content of tissue appears by Figure (5-21) to have a strong affect on the absorption coefficient. Decreasing the water content from 80% in testicle to 60% in tendon increases the absorption coefficient observed some 860%. However, since the magnitude of ultrasonic absorption in water is only about 1% of that found in tissues at 1 MHz, the increased ultrasonic absorption in tissue must be attributed mainly to the solid constituents of tissue.

Lipids, by this analysis, appear to have very little influence on the ultrasonic absorption coefficient observed, since the expression describing the relationship between the two is only a weak function of lipid concentration, and the correlation coefficient for both linear and power fits to the data are poor, both being less than 0.7. The relatively small lipid content of tissues, in addition to the small attenuation coefficient which has been measured in tissues of

high lipid content such as fat (Dussik and Fritch, 1956; Schwan et al., 1954; Pohlman, 1939) is the likely explanation for this behavior.

The presentation of ultrasonic absorption data as a function of constituent parameters provides a method for differentiating quantitatively between collagenous and non-collagenous tissues. Tendon contains 35-40% protein (Snyder et al., 1975), of which about 30% is collagen (Elliott, 1965; Harkness, 1968). In comparison, parenchymal tissues such as liver, kidney, or brain possess only half the protein of tendon (about 20%), nearly all of which is in the form of globular protein, with the collagen content no more than about 1%. The absorption coefficient of tendon, however, is more than 400% greater than that found in these parenchymal tissues, owing to its distinctive constitutative properties. The prevalence, as well as the relatively high absorption per unit concentration of globular and structural proteins is thought to be responsible for the observed behavior.

5.2.5 Comparison of Ultrasonic Attenuation and Absorption Coefficient

It is interesting to compare the magnitude and frequency dependence of attenuation, as reported from literature values in Chapter 2, and the absorption coefficients obtained in the present study for the six particular tissues.

Attenuation by the definition noted previously in this study includes absorption and other losses such as those due to scattering, the latter of which might be expected to introduce a higher order frequency dependence to the attenuation coefficient. In Figure (5-22), the best fit least squares linear regression curves to the attenuation and absorption data, as described in detail previously, are shown as a function of ultrasonic frequency. From this comparison, it is clear that for these tissues, there is little difference between the frequency dependence

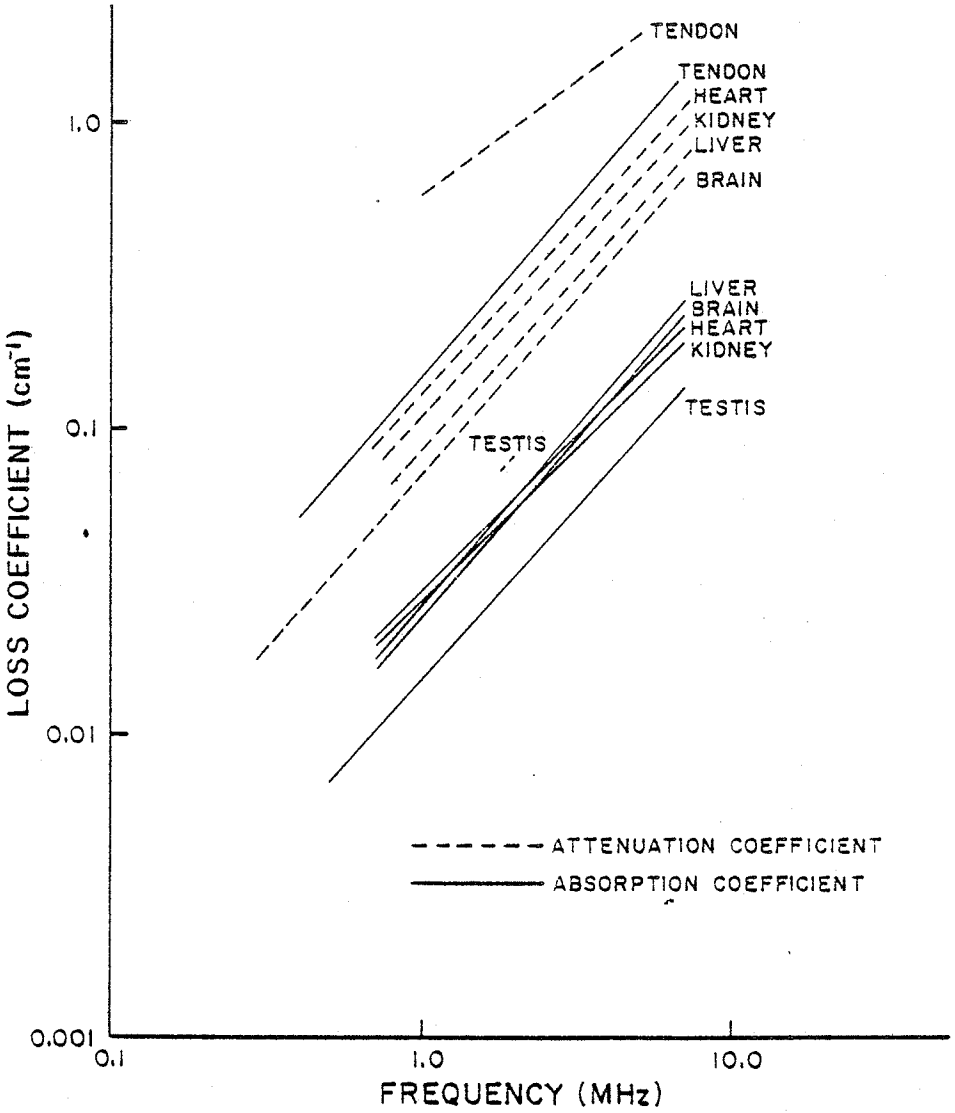

FIGURE 5-22 COMPARISON OF ULTRASONIC ATTENUATION AND ABSORPTION IN VARIOUS TISSUES AS A FUNCTION OF FREQUENCY

of attenuation and of absorption in the 0.5 to 7 MHz frequency range. Tissues for which some disagreement of frequency dependence occurs are tendon and testicle. For the former, the difference in frequency dependence shown is not considered to be significant since the attenuation data for tendon is that for a single study, while the others are numerical averages over a number of studies. For the latter, differences in frequency dependence cannot be determined at this time, due to the lack of attenuation data. For all of the other tissues however, the agreement of the frequency ddpendence for the attenuation and for the absorption suggests that whatever the source of the differences in magnitude between attenuation and absorption values (i.e., scattering, reflection, measurement artifact), that mechanism is also linearly dependent upon frequency.

The magnitude of the attenuation (A) and absorption (α) coefficients are seen to be greatly different however. Though the basic groupings of each loss param eter in terms of tissue constituents can be discerned for both attenuation and absorption (tendon greatest; testis least; with the loss observed in heart, kidney, liver, and brain situated between the two), there is nearly a factor of 3 difference between the attenuation and absorption observed for each tissue type. This difference is more clearly shown in Table 5-2 where the ration α/A is formed for the six tissue types shown in Figure 5-22. Here it is seen that the ratio of attenuation to absorption is nearly constant for all tissues, suggesting that tissue macrostructure or constituents has little to do with this diversity found in the measured loss coefficient. An exception to this consistency occurs for testicle, for which the ratio of absorption to attenuation is notably less than for other tissues. Attenuation measurements in testicle (Frizzell, 1975) were made using a radiation pressure technique, a primary method for the measurement of the second order quantities of intensity and power (Rooney, 1973; O'Brien,

TABLE 5-2

Comparison of Ultrasonic Absorption and Attenuation

Tissue	Absorption α	Attenuation A	α/A
Brain	0.024	0.07	0.34
Heart	0.028	0.126	0.22
Kidney	0.028	0.104	0.27
Liver	0.026	0.083	0.31
Tendon	0.144	0.577	0.25
Testis	0.015	0.035*	0.43

* extrapolated from 2 MHz data (Frizzell, 1975) assuming linear frequency dependence

1978a; Kossoff, 1965; Hill, 1970). This technique offers a phase insensitive, frequency independent technique for power and intensity attenuation measurements in biological tissues. As such it is much less susceptible to error due to phase cancellation artifacts. Such artifacts have been described by Marcus and Carstensen (1975), who compared a piezoelectric receiver (phase-sensitive) with a radiation-force receiver (phase-insensitive), and also by Busse et al. (1977), comparing the piezoelectric receiver with an acoustic-electric receiver. Both studies found that measurements using piezoelectric (phase-preserving) recievers yielded higher apparent attenuation values than those obtained using phase-insensitive receivers. Phase cancellation artifacts are thought to be the source of this error. In the comparison of ultrasonic absorption and attenuation of Table 5-2 testicle represents the only tissue in which the attenuation was

measured by only the radiation force technique, since the other attenuation values represent the averages of measurements obtained using a number of measurement techniques, including pulse transmission (Goss et al., 1978d), pulse reflection (Mountford and Wells, 1972), spectrum analysis (Bamber et al., 1977; Chivers and Hill, 1975) as well as radiation pressure techniques. Measurement technique may thus, at least to some extent, be responsible for the disparity in absorption/attenuation ratios between the various tissues, and even more importantly, serve to explain at least a portion of the observed difference in absorption and attenuation present for each tissue.

To examine the effect of measurement technique on the observed attenuation in tissues, the data of Table 2-1 for fresh liver and brain was separated on the basis of the measurement technique and the mean value for the tissue attenuation coefficient obtained by each method compared. These two tissues were chosen for this comparison because of the availability of data over a variety of measuring techniques. The results of this comparison, shown in Table 5-3 are quite revealing, since nearly a factor of three can be obtained in the measured attenuation coefficient depending upon the method of measurement chosen. Of course, variation in temperature, specimen preparation and other such factors, which are included within all the data, would be expected to produce scatter in the data. For both tissues, spectrum analysis techniques yielded the greatest attenuation coefficient, with pulse transmission techniquesyielding slightly lower values. Radiation pressure techniques,as suggested earlier by the testicle data, yielded the smallest attenuation coefficient, most likely due to the phase-insensitivity characteristic of this technique.

The dependence of the measured attenuation coefficient on the method by which that value was obtained, and the differences between attenuation and absorption

TABLE 5-3

Ultrasonic Attenuation Values Obtained
Using Various Measurement Techniques

Method of Measurement	Brain Tissue	Liver Tissue
Pulse Transmission	0.088 $\pm$ 0.1	0.117 $\pm$ 0.03
Spectrum Analysis	0.149 $\pm$ 0.04	0.132 $\pm$ 0.017
Radiation Pressure	0.053 $\pm$ 0.03	0.077 $\pm$ 0.02

elucidated in this study, suggest that is may no longer be possible to assign a value of ultrasonic attenuation to a particular tissue without also specifying the method by which the value was obtained, and the purpose for which it will be used. For example, the "best" attenuation coefficient for a tissue is generally thought of at present to be one in which measurement artifacts due to phase cancellation, reflection, or other sources are minimized. By this criteria, the "best" attenuation coefficient may be a factor of two below that determined when such artifacts were not taken into account. A measure of the "best" attenuation coefficient might be required in biophysical applications where the attenuation from within a particular tissue is required, where losses due to measurement artifacts are undesirable. In the other applications of the attenuation coefficient in biological tissues, however, as in the attenuation observed using a diagnostic ultrasound instrument, the "best" attenuation coefficient would not encompass all of the losses actually encountered, since such instruments do not account for the type of measurement artifacts described previously. Thus for this example, the

mos\ \`priate "attenuation" coefficient would be only that coefficient actually .th a specific diagnostic instrument, and could only serve as a relative attenuation index rather than a universal ultrasonic property characteristic of any particular tissue.

In the light of the above discussion, the measurement technique which would be most appropriate to describe the attenuation from within tissues is that of radiation pressure. If radiation pressure values of attenuation are compared (Table 5-2) to the absorption values of the present study, the ratios of absorption to attenuation in brain and liver, 0.455 and 0.388 respectively, agrees well with that previously presented for testicle (0.433). On a gross basis then, variation in tissue constituents for these tissues does not seem to be responsible for the observed difference between this measure of attenuation and absorption. There is, however, relatively little difference in collagen content between these three tissues. Collagen is the tissue constituent (other than those in bone) having the greatest velocity per unit concentration known, could be expected to contribute substantially to internal scattering and ultimately to the attenuation. This hypothesis requires however, that radiation pressure attenuation measurements be made in highly collagenous tissue, and no such values could be found in the literature. It is therefore possible that further measurements will define a more specific role of collagen in the explanation of the difference between attenuation and absorption.

5.2.6 Ultrasonic Velocity in Collagen Fibers

While tissues with higher collagen content exhibit ultrasonic velocities greater than those with lesser collagen content (see Chapter 2), the quantitative determination of ultrasonic velocity in tissues comprised predominately of

collagen fibers has received little attention. Dussik et al. (1958) measured the ultrasonic velocity in beef tendon at 1 MHz and found the velocity to be at least 9-10% greater than that reported for less collagenous soft tissues.

This value of velocity in tendon is confirmed by the results of the present study, where using an acoustic interferometer operating at a frequency of 1 MHz (with an accuracy of better the $\pm$ 0.3%), the velocity at 22°C in fresh beef tendon measured perpendicular to the fibers was determined to be 1752 m/sec. Fresh beef and pig liver were also measured with this apparatus at 22°C, to examine the effect that the increased collagen content of pig liver would introduce. Pig liver is about 0.91% collagen, while beef liver is 0.72% collagen, on a wet weight basis. The results of this velocity measurement yields a slightly greater ultrasonic velocity in pig liver (1606 m/sec) than in beef liver (1595 m/sec).

While the evidence above suggests an increased velocity in associated collagen fibers, which form tendon and are interspersed throughout nearly all tissues and organs, the ultrasonic velocity in single collagen threads (50 to 100 microns in diameter), which constitute the fibrous structure of tendons and other tissues, have not previously been measured, possibly due to their exceedingly small specimen size. Such measurements have been made possible by the development of the scanning laser acoustic microscope, which is well suited to the measurement of micron sized samples. Velocity measurements in fresh mouse tendon threads (Table 5-4) at 100 MHz yield a value of 1733 $\pm$ 56 m/sec, for eight specimens taken from two animals at 22°C. To assure that fluid from the specimen did not result in abnormally high measured values of velocity, these same specimens were dried in air for 30 to 127 minutes, then placed on the microscope stage in 0.9% saline for several minutes and remeasured, yielding a value of 1747 $\pm$ 66 m/sec. While the mean velocity of the rehydrated specimens is somewhat greater than the fresh

TABLE 5-4

Summary of Ultrasonic Velocity Data in Mouse Tail Tendon Fibers at 100 MHZ. Average Value for Wet Fibers in 1733 ± 56 m/sec, while for Rehydrated 1747 ± 66 m/sec.

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	1657-1670
12-14	90-107	1664-1699	67	70-95	1712-1799

samples, the variation (less than 1%) is within the 3-4% standard deviation among all of the specimens, and is not considered significant.

While these measurements are about 10-20% greater than those obtained for soft 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/sec at 1 MHz (Dussik et al., 1958). Similar measurements of velocity in liver tissue at 100 MHz with the acoustic microscope (O'Brien, 1878b) have identified little difference in the ultrasonic velocity from that reported at a much lower frequencies (Goss, et al., 1978c), 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 collagenous tissues and fibers. Since networks of similar collagenous 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 ultrasonic visualizability of these tissues and organs.

5.3 Role of Collagen and Other Protein in the Determination of the Ultrasonic Propagation Properties of Biological Tissues

While the empirical relations obtained above for the dependence of ultrasonic absorption and velocity on various tissue constituents suggest that the ultrasonic properties exhibited are determined primarily by these constituents, it is difficult to separate the specific role of each individual constituent. Nevertheless, the ultrasonic properties of each constituent may be considered to combine in some manner to form the ultrasonic properties exhibited by tissues. The ultrasonic properties of collagen in suspension, obtained in the present study, and those of globular proteins in solution obtained elsewhere (Goss and Dunn, 1974), comprise the ultrasonic properties of the major macromolecular constituents of tissue. In order to examine the role of the ultrasonic properties of globular and structural proteins on those of tissues, the ultrasonic absorption and velocity is considered, to a first approximation, to result from the linear combination of the individual ultrasonic properties of each protein.

In Figure 5-23, the ultrasonic velocities of various biological materials are shown as a function of the wet weight percentage of total protein. The lower smooth curve represents the liver concentration dependence of velocity in aqueous solutions of bovine serum albumin in the concentration range from about 5 to 40%

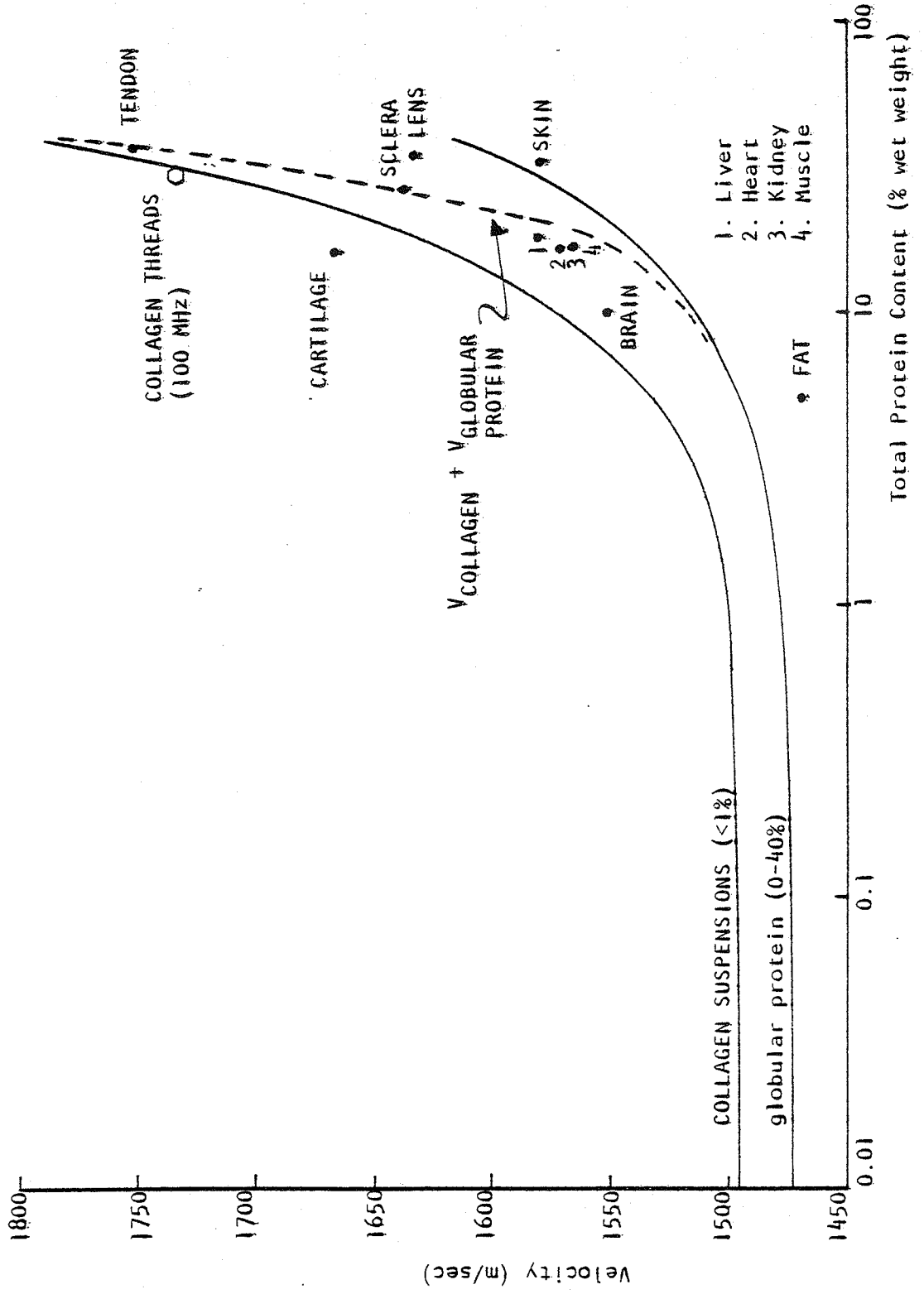

FIGURE 5-23 DEPENDENCE OF ULTRASONIC VELOCITY ON TISSUE PROTEINS

by weight at 20°C (Goss and Dunn, 1974). The upper curve represents the linear dependence of velocity determined in collagen suspensions at 20°C for the present study, in the 0.07 to 0.7% concentration range. When the ultrasonic velocity data for a variety of biological tissues of Chapter 2 are included, the collagen and globular protein curves encompass most of the tissue velocity data. This suggests that if a tissue is predominantly comprised of globular rather than structural protein, the velocity in that tissue would fall nearer to the globular protein curve, with a tissue wholly constituted of globular protein falling on that curve. On the other hand, collagenous tissues such as tendon where the great majority of protein is in structural form would be expected to fall on or near the collagen curve. Closer examination seems to bear out this observation. For example, liver, heart, kidney, and muscle all possess approximately the same total protein (about 17%) content, of which very little is collagen (less than 1%). The velocity in these tissues are also approximately the same, and fall closer to the globular protein curve. Tendon has a total protein content of 37.5%, with 30-32% of the total protein in the form of collagen. The data presented in Section 5.2.6 for beef tendon and for the collagen fibers separated from mouse tail tendon fall precisely on the pure collagen suspension curve. The total protein in cartilage is also comprised predominantly of collagen, and the velocity in this tissue also falls close to the pure collagen curve. Fat contains little protein, and the velocity in such tissue falls far below those of other tissues.

Lens and skin are two tissues which have substantial collagen content, but whose velocity does not fit well with the pattern described above. These tissues are relatively thin however, in terms of the small number of wavelengths present, which tend to increase errors in the velocity measurements. Recent velocity measurements in pig skin (Goans et al., 1977) indicate that the velocity in this

tissue is of the order of 1720 m/sec reported in Chapter 2. This more recent velocity determination would agree well with that predicted by the pure collagen curve. Skin is about 33% protein, of which about 30% of the total protein is collagen. Thus, it appears thae the ultrasonic properties of tissues are governed in some way by the ultrasonic properties of the individual macromolecules which comprise them.

If the empirical equations describing the ultrasonic velocity in the pure collagen and globular proteins are linearly combined, and the collagen and globular protein concentrations characteristic of the various biological tissues used to calculate the resultant velocity for those proportions, the broken line in Figure 5-23 is obtained. Good agreement exists between this method of handling the empirical data and measured values of velocity in biological tissues.

It appears that the ultrasonic absorption coefficient is governed by similar relations. Figure 5-24 describes the ultrasonic absorption coefficient at 1 MHz as a function of total protein content for various biological media. As before, the lower curve represents the empirical relations describing the ultrasonic absorption of globular protein solutions at 20°C as a function of concentration in the range 5 to 40% by weight (Goss and Dunn, 1974). The upper curve is that describing the dependence of absorption on collagen concentration (see equation (5-4)) in the range from 0.07 to 3.7%. Addition of the absorption coefficients of the six tissues measured in this study at 37°C are seen to fall between these curves. Tendon, for which collagen is the major protein, is seen to exhibit an absorption coefficient very near that predicted by the empirical relation describing the absorption in a pure collagen suspension. Tissues such as liver, kidney, and heart all have approximately the same constituents (mainly

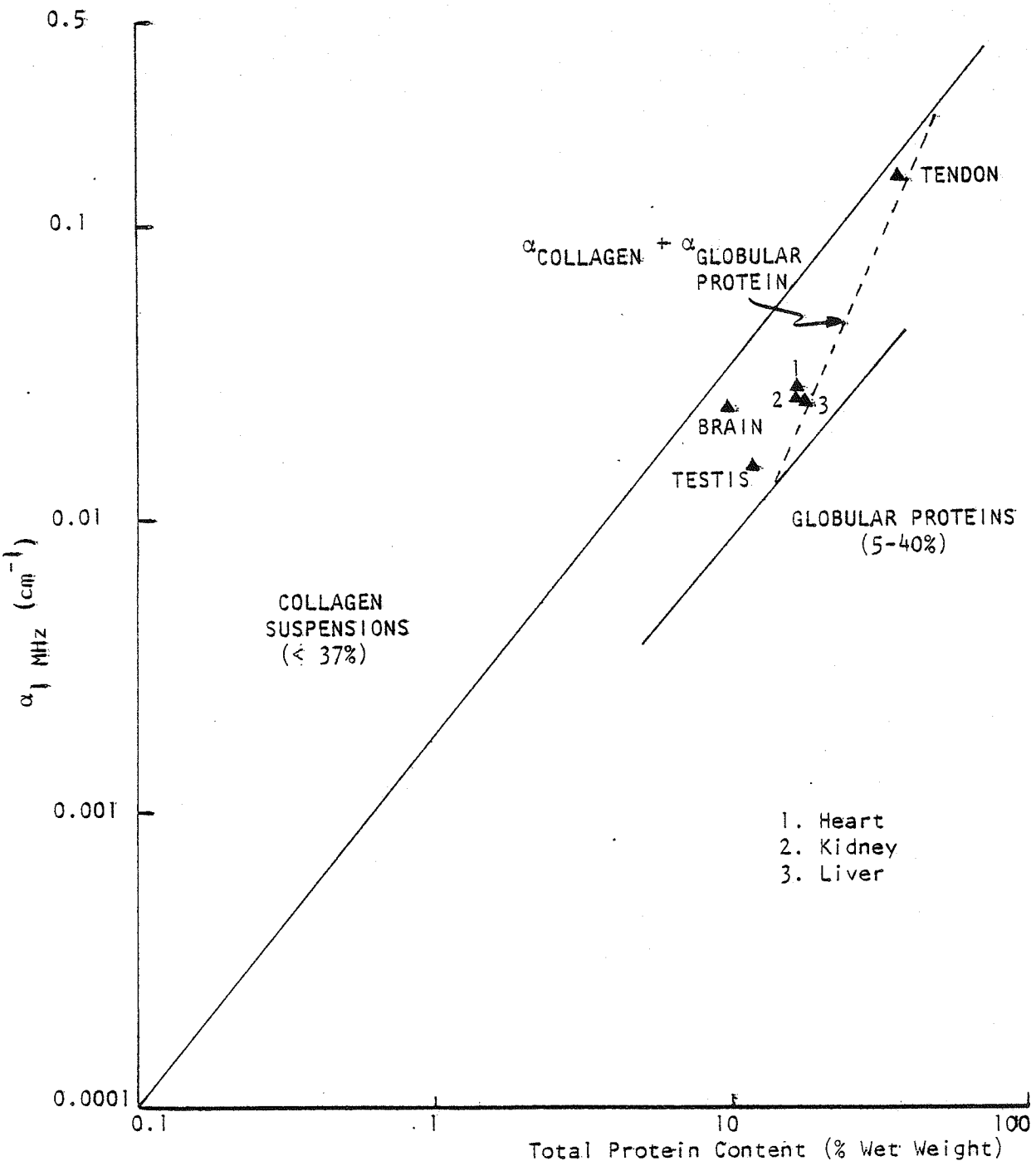

FIGURE 5-24 DEPENDENCE OF ULTRASONIC ABSORPTION AT 1 MHz ON TISSUE PROTEINS

globular protein), and exhibit very similar absorption properties falling near to the pure globular protein curve. Testis and brain possess the least amount of protein (10-12%). Testis is seen to exhibit the lowest absorption coefficient, falling close to the pure globular protein. Brain exhibits an absorption coefficient greater than that which could be predicted on the basis of constituent properties, at least in terms of total protein content. Brain however, has more than twice the lipid content of the other tissues examined, and this replacement of water and protein by lipids may be responsible for this behavior.

The linear combination of the empirical relations describing the ultrasonic properties of molecular collagen and globular protein, for the proportions of these constituents characteristic of various tissues results in absorption coefficients depicted by the broken line shown in Figure 5-24 It is seen that predicted absorption in tissues based on the linear superposition of absorption properties of protein constituents agrees well with the absorption coefficient actually measured in the tissues examined here.

In light of the data obtained in this study, the role of collagen and other proteins in the determination of the ultrasonic properties of biological tissues becomes more quantitatively determined. For most soft tissues, where the overall collagen content is quite small, the absorption and velocity appear to governed largely by the major macromolecular component of most tissues, viz., globular proteins. Even in these tissues, the absorption and velocity per unit concentration in collagen is still about twice that for globular proteins, so that relatively small changes in collagen concentration can measurably affect the ultrasonic properties. Such alteration may well explain the echographic "texture" characteristic of a certain tissue structure. For highly collagenous tissues, gross changes in absorption and velocity are observed due to the absence of globular protein, and the doubling of the total protein, all in the form of collagen.

Thus, biological tissue acts as a composite material whose ultrasonic properties are governed by the individual ultrasonic properties of collagen and globular protein.

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APPENDIX

DATA TABULATION

3% Aqueous Acetic Acid Solution

Temperature = 10°C

Velocity (8.87 MHz) = 1460 ± 9 m/sec

Frequency (MHz)	$\alpha/f^2 \times 10^{17}$ (sec^2/cm)
5.71	44.9
7.98	43.6
8.87	43.3
10.28	42.7
12.64	42.5
14.98	41.5
20.70	40.3
21.80	40.2
26.70	39.5
28.66	39.3
31.00	39.0
32.60	38.8
38.60	38.3
44.44	37.9
50.50	37.5
56.44	37.1
62.44	36.8

3% Aqueous Acetic Acid Solution

Temperature = 20°C

Velocity = 1494 ± 2 m/sec

Frequency (MHz)	$\alpha/f^2 \times 10^{17}$ (sec^2/cm)
8.87	32.8
14.80	31.0
20.70	29.9
26.70	29.5
32.60	28.5
38.60	28.0
44.44	27.6
50.50	27.2
56.44	26.9

Collagen Suspensions

Temperature = 10°C

(C = collagen concentration (gm/100 cc))

	$\Delta\alpha/f^2 \times 10^{17}$ (sec^2/cm)*		
Frequency (MHz)	C = 0.73%	C = 0.49%	C = 0.23%
8.87	83.3 ± 11.2	35.5 ± 3	14 ± 1
14.80	71.9 ± 8.2	25.9 ± 2	9.9 ± 1
20.70	48 ± 9.9	28.2 ± 1.5	11.2 ± 1
26.70	43.1 ± 4.3	28.7 ± 1	14 ± 1
32.60	38.9 ± 7	30.2 ± 1	11 ± 2
38.60	30.8 ± 2	26.6 ± 2	10.6 ± 2
44.44	30.3 ± 1	25.2 ± 1.5	9.9 ± 2
50.50	26.8 ± 2	18.5 ± 1	9.5 ± 1
56.44	18.8 ± 2	15.4 ± 0.3	7.1 ± 0.4

* ± standard deviation

Collagen Suspension
(8.87 MHz)

Temperature = 10°C

Concentration (gm/100 cc)	Velocity* (m/sec)
0.41	1463 ± 5
0.47	1465 ± 2
0.73	1467 ± 1
0.49	1466 ± 1
0.23	1462 ± 7
0.16	1462 ± 2

* ± standard deviation

Collagen Suspensions

Temperature = 20°C

(C = collagen concentration
(gm/100 cc))

	$\Delta\alpha/f^2 \times 10^{17}$ sec^2/cm		
Frequency (MHz)	C = 0.46%	C = 0.14%	C = 0.09%
0.5	87.3	24.4	16.0
0.6	75.7	20.4	11.7
0.7	68.6	17.1	10.7
0.8	63.9	14.8	9.8
0.9	62.2	12.9	10.4
1	58.3	16.8	10.5
1.5	43.8	11.2	5.5

Collagen Suspension

Temperature = 20°C

(C = collagen concentration (gm/100 cc))

	$\Delta\alpha/f^2 \times 10^{17}$ (sec^2/cm)*			
Frequency (MHz)	C = 0.52%	C = 0.34%	C = 0.17%	C = 0.08%
8.87	33.1 ± 4	19.3 ± 2	10.1 ± 2	6.3 ± 2
14.80	18.7 ± 1	12.5 ± 1	5.8 ± 2	3.4 ± 1
20.70	14.7 ± 1	9.3 ± 2	4.6 ± 0.9	0.65 ± 0.6
26.70	13.8 ± 2	10 ± 2	3.2 ± 0.3	2.5 ± 1
32.60	10 ± 2	9.2 ± 2	2.5 ± 1	1.9 ± 0.3
38.60	7.6 ± 1	6.6 ± 3	1 ± 0.2	0.8 ± 0.7
44.44	7 ± 0.5	6.9 ± 2	0.6 ± 0.7	0.2
50.50	5.3 ± 1	5.4 ± 2	0.4 ± 0.7	---
56.44	---	3.2 ± 3	3.5 ± 0.7	1.3 ± 1

* ± standard deviation

Collagen Suspensions
(8.87 MHz)

Temperature = 20°C

Concentration (gm/100 cc)	Velocity* (m/sec)
0.523	1498 ± 2
0.34	1497 ± 1
0.17	1496 ± 2
0.076	1495 ± 2

* ± standard deviation

Biological Tissues
(Cat, Mouse, Pig and Beef)

Temperature = 37°C

	α^* (cm^{-1})					
Tissue	0.5 MHz	0.7 MHz	1 MHz	3 MHz	4 MHz	7 MHz
Brain	------	0.014 ± 0.003	0.029 ± 0.004	-----	-----	0.23 ± 0.09
Heart	------	0.018 ± 0.009	0.033 ± 0.006	-----	-----	0.21 ± 0.03
Kidney	------	0.017 ± 0.007	0.033 ± 0.004	-----	-----	0.20 ± 0.002
Liver	0.010 ± 0.006	0.020 ± 0.003	0.023 ± 0.004	-----	0.14 ± 0.03	0.24 ± 0.02
Tendon	0.050 ± 0.03	0.16 ± 0.1	0.11 ± 0.04	0.53 ± 0.2	0.75 ± 0.4	1.4 ± 0.5
Testis	0.0078 ± 0.002	0.0085 ± 0.001	0.015 ± 0.003	-----	0.079 ± 0.02	0.12 ± 0.02

* ± standard deviation

Biological Tissues

Temperature = 37°C

Species Dependence

Species/ Tissue	α^* (cm^{-1}) 0.5 MHz	0.7 MHz	1 MHz	3 MHz	4 MHz	7 MHz
Beef Liver	0.015 ± 0.01	0.019 ± 0.003	0.024 ± 0.005	-----	0.14 ± 0.01	0.28 ± 0.01
Cat Liver	0.0062 ± 0.002	0.021 ± 0.003	0.022 ± 0.003	-----	0.14 ± 0.04	0.23 ± 0.01
Mouse Liver	0.010 ± 0.004	-----	0.019 ± 0.0008	-----	-----	-----
Pig Liver	-----	0.018 ± 0.0008	-----	-----	-----	-----
Beef Tendon	0.050 ± 0.03	0.16 ± 0.1	0.11 ± 0.04	0.053 ± 0.2	0.75 ± 0.4	1.4 ± 0.5
Cat Tendon	0.02 ± 0.005	-----	-----	-----	0.27 ± 0.03	-----

* ± standard deviation

VITA

Stephen Anthony Goss was born in Chicago, Illinois on November 21, 1949. He received his primary education in Evergreen Park, Illinois, and upon graduation from Evergreen Park Community High School in 1967, entered the University of Illinois at Urbana-Champaign to study electrical engineering. In January, 1972 he graduated with a B.S. and entered the graduate school of the University of Illinois at Urbana-Champaign with a Graduate Research Assistantship in the Bioacoustics Research Laboratory of the Department of Electrical Engineering. In August, 1974 he earned his M.S., his thesis entitled "The Concentration Dependence of Ultrasonic Absorption in Aqueous Solutions of Bovine Serum Albumin," and he remained in this laboratory until completion of his Ph.D. thesis in October, 1978. Mr. Goss is a member of the Sonics and Ultrasonics Group, and of the Biomedical Engineering Group, of the Institute of Electrical and Electronics Engineers, and of the Acoustical Society of America. He is co-author of nine papers in the field of ultrasonic biophysics.