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ELEMENTS OF TISSUE CHARACTERIZATION

Part I. Ultrasonic Propagation Properties

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Tissues can be characterized ultrasonically by their attenuation, absorption, and velocity, all of which correlate well with the presence of the major tissue components of water, and protein, particularly, collagen. This correlation is examined in solutions of biologically important molecules and in a number of tissues and organs. It is shown that tissues can be grouped according to similar ultrasonic propagation properties, physiological functions, and concentration of elementary constituents. The role of collagen in determining ultrasonic properties of normal and pathological tissues is discussed.

Key words: Absorption; amino acids; attenuation; frequency; mammalian tissues; polypeptides; proteins; tissue characterization; ultrasonics; velocity.

1. Introduction

The ultrasonic propagation properties of biological materials include the behavior of those measurable acoustic parameters, as functions of the state and acoustic variables, which characterize the fate of acoustic signals propagating within the biological environment. The ultrasonic attenuation includes not only the absorption of the ultrasonic signal, which is degraded to heat, but also 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 media. The ultrasonic velocity and the characteristic acoustic impedance, which can be determined with the addition of density information, embody within them both the inertial and restoring parameters of the particular materials. Thus, knowledge of the ultrasonic velocity and loss terms may provide a basis for developing tissue signatures for various biological materials.

The paper will deal with the ultrasonic propagation properties of tissues beginning with the elemental constituents. As water, soft tissues, and organs have very much the same densities and compressibilities, it is instructive to begin a review with properties of aqueous media.

2. Biological Molecules in Solution

A. Water

The measured ultrasonic absorption in water is proportional to the square of the frequency, over the range 10^{-2} to 10^{-3} MHz, with the frequency-free absorption coefficient, α/f^2, having a constant value of 15.7×10^{-17} s^2/cm at 37 °C. The magnitude of this absorption is greater than one would expect from consideration of the so-called classical absorption due to viscosity and thermal conductivity. This absorption in excess of the classical value has been attributed by Hall [1][1] to a structural relaxation mechanism involving a transition between two possible quasi-crystalline states for water. More recent experimental results [2] are consistent with the hypothesis that water undergoes a structural relaxation characterized by a time constant of 10^{-12} seconds, and supports the idea that water is a mixture of two or more states and that the relaxation processes consist of the independent jumping of molecules from one state to another.

The velocity of sound propagation in pure water exhibits a maximum at 75 °C due to the existence of a minimum in the product of density and adiabatic compressibility at that temperature [3]. Similar behavior is exhibited by dilute aqueous solutions, although the temperature of maximum velocity may be decreased since the solutes modify the structural arrangements of water. The velocity in water has been measured most precisely by McSkimin [4], Greenspan and Tschiegg [5], and DelGrosso and Mader [6].

B. Amino Acids

Since it has been observed that proteins play a dominant role in the absorption properties of tis-

[1]Figures in brackets indicate literature references at the end of this paper.

sues, aqueous solutions of amino acids and polypeptides require attention, for completeness. When dissolved in water without additional ionic constituents to influence the state of charge of the amino acid, the solution exhibits a magnitude of the frequency-free absorption parameter, α/f^2, which varies little with frequency in the megahertz range, and differs only slightly from that of the solvent, water [7-12]. Amino acids in aqueous solution at neutral pH may be considered according to their action of structure making or structure breaking in the solvent. Herein, the amino acids are present as doubly charged molecules (zwitterions) and are susceptible to dissociation and recombination reactions upon a change of their environment. Hydrogen-bonding sites are located on both the amino and carboxyl groups, while the side chain may be acidic, nonpolar, or basic. Thus, the potential for breaking or making structure, in the vicinity of the solute molecules are considerable. Hammes and Pace [7] suggested that the predominate ultrasonic absorption mechanism in aqueous solutions of glycine, diglycine, and triglycine is that which involves solute-solvent (water) interaction. When the pH of an amino acid aqueous solution is within the range of 2 to 4 or 11 to 13, the relaxational behavior can be described by a single relaxation frequency. A number of amino acids have been investigated as a function of pH, viz., glycine [9,13-16], serine and threonine [8], glutamic acid, aspartic acid and alanine [14], and arginine and lysine [10,15]. Absorption maxima have been observed within the pH ranges 2 to 4, and 11 to 13, with such peaks being described quantitatively by assuming that the proton-transfer reaction dominates the absorption.

C. Polypeptides

When the amino acids are formed into polypeptide chains, the absorption increases dramatically, and the mechanisms believed to be responsible for the absorption in tissues may begin to appear. In general, aqueous solutions of polypeptides at neutral pH exhibit an ultrasonic absorption behavior greatly increased over those of amino acids in solution or of water, and with a somewhat lesser than the square-of-frequency dependence. The absorption in aqueous polypeptide solutions may involve any or all of the following four possible mechanisms, viz., proton transfer, helix-coil transition, solvation, and relaxation of the shear viscosity. Major interest has tended to be focused on the proton-transfer reactions [17-20] and helix-coil transitions [21-24].

D. Proteins

Continuing with increasing complexity of the biological media, the polypeptides may be considered to be arranged in a particular, ubiquitous way to form the globular proteinaceous state. Hemoglobin and serum albumin solutions have received considerable attention, partly because of the availabilities of the materials. Special consideration is given to other macromolecular species such as nucleic acids and polysaccharides.

When biological macromolecules, such as the globular protein hemoglobin are in aqueous solution, a certain amount of the solvent becomes an inherent part of the molecule since the polymer possesses ionic and polar groups which associate with water molecules. In addition, proteins contain a number of nonpolar side chains such that, within the vicinity of the macromolecules, some water structuring occurs. Thus, it is possible that the structure of liquid water, the hydration layer, increases in the neighborhood of the biological macromolecule. It is considered, therefore, that as an acoustic wave propagates through an aqueous solution of biopolymers, it perturbs the hydration layer manifesting absorption of energy by a structural relaxation process. The role of molecular conformation of the biopolymer has also been considered as the origin of the observed ultrasonic absorption. Figure 1 shows the excess frequency-free absorption per unit concentration for several biomacromolecules and supports the view that structuring contributes to the ultrasonic absorption spectra [25]. Both dextran [26], a carbohydrate, and polyethylene glycol [27], a synthetic polymer, assume random coil configurations in aqueous solution and exhibit absorption magnitudes similar to that of gelatin. Hemoglobin has a quaternary structure, bovine serum albumin and ovalbumin have tertiary structure, polyglutamic acid [28], a synthetic polyamino acid, has secondary structure, and the double helix, DNA, has a rigid rod conformation [29]. However, hemoglobin in 5 molar aqueous guanidine hydrochloride solution exists as a random coil [30] and yet exhibits ultrasonic absorption spectra similar to that of hemoglobin in aqueous solutions [31]. The importance of molecular spatial arrangement is thus an unsettled question, though the polypeptide structure appears to be of considerable significance.

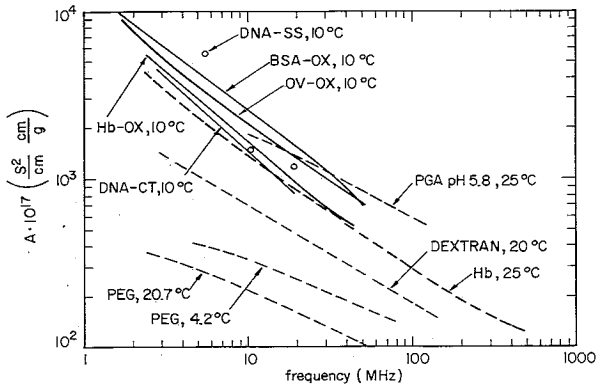

Fig. 1. Excess frequency-free ultrasonic absorption per unit concentration [25].

Within figure 1, bovine serum albumin and hemoglobin have a molecular weight of 68,000 while that of ovalbumin is 46,000. β-lactoglobin [32] (not on graph) with a molecular weight of 35,000, would fall in the range determined by hemoglobin, bovine serum albumin, and ovalbumin, while lysozyme [32] (not on graph) with a molecular weight of 14,600, would appear between hemoglobin at 25 °C and dextran. Two random coil polymers, dextran and polyethylene glycol, have been studied as a function of molecular weight. For dextran solutions, the frequency-free absorption per unit concentration increases with increasing molecular weight to a molecular weight of about 10,000, which corresponds to approximately 100 monomer units, beyond which it is independent of molecular

weight [33]. Aqueous polyethylene glycol solutions show similar absorption behavior in that beyond a molecular weight of about 4500, which also corresponds to a chain length of about 100 monomer units, the absorption is independent of molecular weight [27]. Thus, ultrasonic absorption depends to some degree upon molecular weight. Possibly beyond about 100 monomer units, the macromolecule assumes random coil characteristics.

It is uncertain whether the absorption mechanism(s) responsible for energy loss in biological polymer solutions (usually of concentrations less than 10 percent by weight) are the same as those responsible for the absorption properties of tissue [34]. Kremkau and Carstensen [11] have suggested that more highly concentrated macromolecular solutions, promoting solute-solute (intermolecular) interactions, may better approximate the tissue environment, and from the point of view of biological effects, may be the most important level at which the ultrasound interacts. In the case of dilute solutions, where the absorption is considered to vary linearly with polymer concentration, the mechanism of absorption is attributed to processes involving the interaction between the solvent (usually water) and the solute. In other than dilute solutions, the absorption is found to increase nonlinearly, and it has been suggested that this phenomenon is due to intermolecular interaction processes [34-36]. It has recently been shown [37], from absorption measurements of bovine serum albumin, to concentrations of 40 g/100 ml, over the frequency range 3.4 to 15 MHz, and at 20 °C, that the absorption dependence upon concentration does not possess a linear region. Here, the excess ultrasonic absorption can be described adequately by a concentration dependence to the 1.2 power. It thus appears essential to consider intermolecular interaction over the entire range.

3. Tissues

The nearly linear frequency dependence of attenuation in liver, kidney, brain, muscle, fat, and other parenchyma, are considered in the approximate frequency range of 100 kHz to 100 MHz, and the recent findings with regard to the temperature dependence are discussed. Several tissues and organs, such as bone, lung, refractive media of the eye, and collagenous tissues, are singled out for special detailed consideration. It is shown that classification of tissues according to certain ultrasonic propagation properties can be carried out with regard to water and collagen content, and with regard to certain teleological considerations. Pulmonary tissue is an exception here.

A. Frequency Dependence

The dependence of the ultrasonic absorption coefficient upon the acoustic frequency has been studied by numerous investigators. Goldman and Hueter [38] prepared an early compilation of ultrasonic velocity and absorption data in mammalian soft tissues. Therein it is seen that the velocity, excluding lung, is very nearly that of dilute salt solutions and varies only slightly among those tissues. Fatty tissues are exceptions in having a velocity about 10 percent less than the others. Figure 2, taken from their paper, is a graphical representation of the acoustic amplitude absorption coefficient per wavelength for several mammalian tissues in the frequency range of approximately 200 kHz to 10 MHz. As they attempted to include all measurements available at that time, by numerous investigators employing different experimental techniques, the scatter of the data exhibited by the bands, or broad shaded regions is thus not wholly surprising since many neglect to give either complete specifications of their experimental procedure or a description of the state of the specimen used. For example, it is not possible, in many cases, to determine from the literature the temperatures employed by the investigators reporting the data. It is, however, possible to discern several relatively simple relationships. For example, the absorption per cycle, α/f, is generally constant over the frequency range considered. For fat, α/f increases slightly in the frequency range from 1 to 10 MHz. The experimental results for striated muscle and liver appear to exhibit a minimum in the neighborhood of 2 MHz. Kessler [39] has shown that for kidney the linear dependence of the attenuation on frequency exists to about 100 MHz, after which a square law (or greater) dependence exists. Fry [40] has considered a viscous mechanism for the absorption of ultrasound in tissue, in which it is shown that the viscous forces acting between a suitably chosen distribution of suspended particles (or structural elements) and a suspending liquid can account for the experimentally observed linear relationship be-

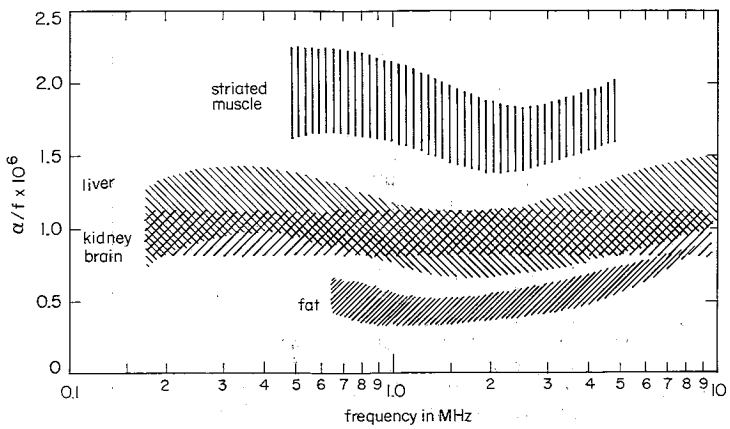

Fig. 2. Acoustic amplitude absorption coefficient (in dB/cm) per wavelength versus frequency for several mammalian tissues [38].

tween acoustic absorption coefficient and ultrasonic frequency. The frequency band over which linearity obtains (in the model) is determined by the limits of the distribution of values for the parameters chosen to describe the structural elements. Below the linear range the theory predicts a quadratic dependence, in agreement with experiment.

Figure 3 is another way of presenting the data which may be suggestive for determination of mechanisms of absorption [41]. Here, the logarithm of the absorption coefficient is plotted as a function of the logarithm of the ultrasonic frequency, and the slopes of the resulting curves are examined (the slope is the exponent of frequency upon which the magnitude of the absorption coefficient depends). Figure 3 shows data for several materials of increasing biological complexity, exhibiting correspondingly increasing complexity in absorptive behavior. The urea solution exhibits a slope of 2, indicative of classical viscous absorption for which α/f^2 is a constant. Homogenized milk, a suspension of fat particles and hydrated casein complexes, exhibits a slope of nearly unity from approximately 1 to 40 MHz. Behavior of this type cannot be explained in terms of simple viscosity or scattering theories. The curves of the absorption coefficients of egg albumin, brain tissue, liver, and striated muscle (not shown in fig. 3) exhibit slopes between 1 and 2 in the neighborhood of 1 MHz and approach a slope of 2 at higher frequencies. Hueter [41] has suggested that this type of frequency dependence can be described for specific muscle preparations by a double relaxation process in which the bulk (volume) viscosity of the tissue possesses a relaxation frequency near 40 kHz, and the shear viscosity possesses a relaxation near 400 kHz. Although it is conceded that this is an oversimplification of a complicated process, it will be shown below that the temperature dependence of the acoustic absorption coefficient lends support to this view.

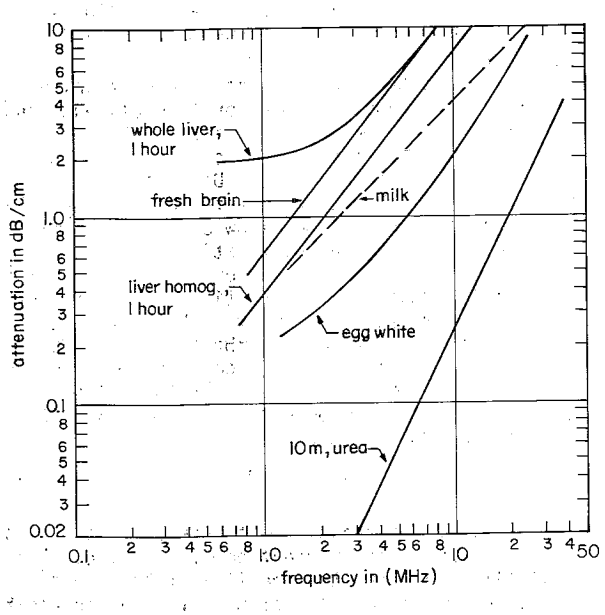

Fig. 3. Acoustic amplitude absorption coefficient versus frequency for materials of different biological complexity [41].

B. Temperature Dependence

Details of the absorption coefficient as a function of temperature and frequency have recently become available. Figure 4 shows observations on mammalian central nervous system, the only tissue for which such data are available.

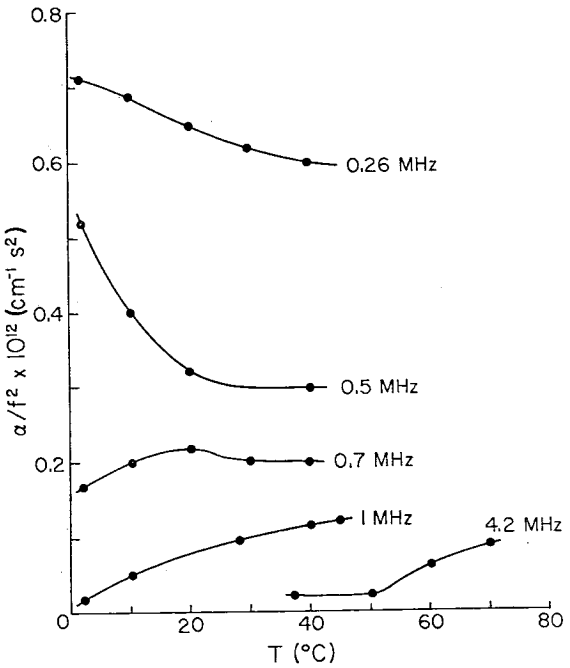

Fig. 4. Frequency and temperature dependence of ultrasonic absorption in mammalian central nervous tissue [43].

The curves for 0.26, 0.5, 0.7, and 1 MHz represent in vivo measurements in the spinal cords of neonatal mice (essential poikilotherms) [42-44] and those for 4.2 MHz are in vitro measurements in brains of adult cats (homeotherms) [45]. The relatively complex behavior of the frequency-free absorption coefficient with frequency and temperature suggests a family of curves whose maxima decrease in magnitude, and occur at ever higher temperatures, as frequency increases, supporting the suggestion mentioned above. It is not known whether other soft tissues exhibit similar behavior but Kishimoto [46] has observed a positive temperature coefficient for the absorption coefficient of bone in the frequency range 1.4 to 4.5 MHz. These data illustrate the necessity for complete specification of the state of specimens when reporting experimental results.

C. Absorption and Velocity in Bone

Bone is a tissue possessing acoustic propagation properties greatly different from those of the soft tissues discussed previously. An early study of specially prepared skull bone, in the frequency range 0.6 to 3.5 MHz (25 to 35 °C), yielded a quadratic dependence of the absorption coefficient upon frequency with a transition to a linear dependence beyond about 2 MHz [47]. An average value found for the acoustic amplitude

absorption coefficient per unit path length in skull bone, in the neighborhood of 1 MHz, was of the order of 1 cm^{-1}, approximately an order of magnitude greater than that of soft tissues of the same temperature. However, recent observations have called these values into question, and Adler and Cook [48] have obtained absorption measurements of 1.5 cm^{-1} and 2.2 cm^{-1} in freshly frozen dog tibia at room temperature at, respectively, 3 and 5 MHz. Reports of measurements of the longitudinal speed of sound in bone are largely in agreement that it is approximately twice that of soft tissues [46,48-50]. Anisotropy of elastic properties and variations in density of bone present special problems for measurement and for interpretation of results [48-50].

D. Refractive Media of the Eye

Begui [51] has studied the acoustic properties of the refractive media of the eye *in vitro*. He determined the ultrasonic absorption coefficient of the aqueous and vitreous humors at 30 MHz and that of the lens at 3 MHz. The specimens were obtained from excised fresh calf eyes. At 30 MHz and 27.5 °C, the aqueous and vitreous humors both exhibit an acoustic amplitude absorption coefficient of 0.35 cm^{-1}. Since this is approximately 50 percent greater than the absorption coefficient of dilute salt solutions, it suggests that the absorption coefficients of the humoral media of the calf eye possess a viscous-type dependence upon frequency; that is, the absorption coefficient probably increases as the square of the frequency. The lens of the calf eye exhibits a value of 0.7 cm^{-1} for the acoustic amplitude absorption coefficient at 3 MHz and 28 °C. Since the lens contains a relatively high concentration of protein, it is reasonable to assume, in the absence of further information, that the frequency dependence of the absorption coefficient of the lens resembles that of other soft tissue for which the absorption appears to be dominated by the protein content, *i.e.*, it is probable that the absorption coefficient per unit path length of the lens varies approximately with the first power of the frequency. Some investigators currently using ultrasonic methods for diagnosing disorders of the human eye feel that the lens absorption value given by Begui is larger than that for the human lens *in vivo*. The possible discrepancy may result because of species differences. Indeed, Begui observed that the viscosity of the intraocular fluid of calf eyes is greater than the values normally stated for the fluid media of human eyes. Further, the specimens used by Begui were first stored (at temperatures in the neighborhood of 0 to 5 °C) and were used for measurement purposes within a time interval of 10 days. Begui obtained for the speed of sound in refractive media of the eye 1497 m/s for the aqueous humor, 1516 for the vitreous humor, and 1616 for the lens.

E. Pulmonary Tissue

Two earlier studies [52,53] showed that ultrasonic attenuation in freshly excised dog lung was unusually high, that the speed of sound was considerably less than that of water, and that both of these quantities had a strong dependence on pulmonary inflation and acoustic frequency. It was also shown that a pathological condition involving an accumulation of liquid-like matter within the pulmonary architecture, had the effect of appreciably reducing both the attenuation and the velocity. Two recent studies have provided details of the frequency and inflation dependencies. Dunn [54] has shown that for excised dog lung of inflation to a fraction of residual air, such that the specimen density is 0.4 g/cm^3, the attenuation increases exponentially from 4 cm^{-1} at 1 MHz to 12 cm^{-1} at 5 MHz. In this same frequency range, the speed of sound increased linearly from 0.66×10^5 cm/s to 1.2×10^5 cm/s. These findings are in general agreement with those of Bauld and Schwan [55] who also showed that, for fixed inflated specimens, the energy reflected at the lung-liquid interface ever increases the gaseous inflation allowing for lesser amounts of energy to enter the lung.

4. Role of Collagen

Collagen is the most abundant single protein in the human body and the most common protein in the animal kingdom. It is closely associated in connective tissue of vertebrates and comprises between one-quarter and one-third of the total protein in the human body, being about six percent of the total body weight [56]. However, more than the prevalence of collage in the body, there is some evidence to suggest that its contribution to the elastic properties of most soft tissues, together with other structural proteins, determines acoustic contrast during echographic visualization [57,58]. 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 ultrasonic velocity is proportional to the square root of the elastic modulus, collagenous tissues are thought to introduce a greater impedance mismatch than would be the case for a soft tissue interface, thereby increasing the acoustic reflectivity. The increased deposition of collagen and the concomitant increase in attenuation seen in many pathological conditions is a basis for ultrasonic differential diagnosis.

Table 1 contains ultrasonic attenutation, velocity, water content, total protein content, and collagen content for various tissues [59]. It is apparent that the greater the collagen content, the greater the attenuation. Dependence of attenuation and velocity upon water content are also apparent. These data allow empirical relations to be formed for more quantitative assessment of the role of collagen content of tissues upon their ultrasonic propagation properties. For example, figure 5 represents a summary of table 1 of the ultrasonic attenuation at 1 MHz as a function of the wet weight percentage of collagen for ten tissues. Using linear regression by the method of least squares, a reasonable fit to the data is provided by the relation

$$A = 0.17\ C^{0.3}, \qquad (1)$$

where A is the ultrasonic attenuation in cm^{-1} and C is the wet weight percentage of collagen. The best fit parameter, the coefficient of determination, r^2, yields a value of 0.71 (unity represents a perfect fit). Equation 1 is represented on figure 5 by the solid straight line. Logarithmic, exponential, and linear functions were also analyz-

Table 1. Ultrasonic attenuation and velocity for tissues of various water, protein and collagen content.

Tissue	Attenuation at 1 MHz (cm^{-1})	Velocity (m/s)	Water (%)	Protein (%)	Collagen (%)
Water (20 °C)	0.0003	1483	100		
Amniotic fluid	0.0008	1510	97	0.27	
Aqueous humor	0.10 - 0.017	1497	99	0.005 - 1	
Vitreous humor	0.10 - 0.017	1516	99 - 99.9	0.02 - 0.25	0.014 - 0.067
CSF	0.0012	1499 - 1515	99	0.03	
Plasma	0.01	1571	90 - 95	7	
Testis	0.019 (absorption)		84	12	trace
Blood	0.02	1571	74 - 83		
Milk	0.04	1485	87	3 - 4	
Fat	0.04 - 0.09	1410 - 1479	10 - 19	5 - 7	yes
Spleen	0.06	1520 - 1591	76 - 80	17 - 18	0.5 - 1.2
Liver	0.07 0.13	1550 - 1607	68 - 78	20 - 21	0.1 - 1.3
Kidney	0.09 - 0.13	1558 - 1568	76-83	15 - 17	0.5 - 1.5
Brain	0.09 - 0.13	1510 - 1565	75 - 79	10 10	0.04 - 0.3
Spinal cord	0.09 - 0.12		64 - 80		
Striated muscle	0.18 - 0.25	1568 - 1603	66 - 80	20 - 21	0.7 - 1.2
against grain	0.08 - 0.12	1592 - 1603			
with grain	0.16	1576 - 1587			
Heart	0.25 - 0.38	1572	77 - 78	17	0.4 - 1.6
Tongue			62 - 68	14 - 17	
against grain	0.58	1575			
with grain	0.28	1585			
Lens	0.10 - 0.20	1616	63 - 69	30 - 36	
Articular capsule	0.38				
Integument	0.40	1498	60 - 72		7 - 30
Cartilage	0.58	1665	23 - 34 70	49 - 63	10 - 20
Tendon			63	35	32
against grain	0.54	1750			
with grain	0.58				

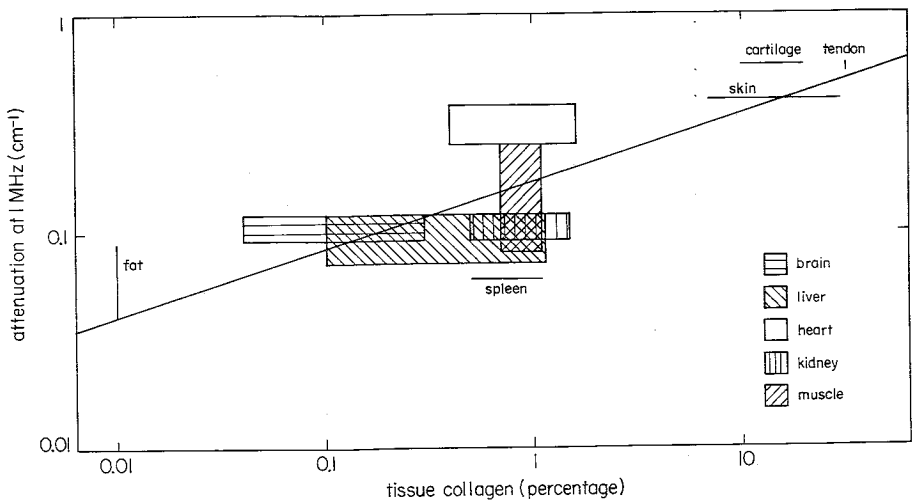

Fig. 5. Attenuation at 1 MHz as a function of the percentage of tissue collagen for 10 tissue types [59].

ed but yielded worse fits than equation 1. Similarly, to a first approximation, the ultrasonic velocity was examined as a function of collagen content for 8 tissues, excluding integument, and yielded the expression

$$C = -1700 + 230 \ln v, \qquad (2)$$

where v is the ultrasonic velocity in meters/second and $r^2 = 0.91$. Again, first approximations for wet weight percentage of total protein, P, yielded

$$A = 0.004\ P^{1.26},\ r^2 = 0.77, \qquad (3)$$

for 16 biological materials excluding integument. Thus to a first approximation there appear to be mathematical relationships which can be developed to relate the amount of tissue constituents to the ultrasonic propagation properties. There are some tissues, such as fat and integument, which may have to be treated separately but, otherwise, this approach suggests that such relationships may aid in developing ultrasonic tissue signatures which can be incorporated into clinical instrumentation.

5. Concluding Remarks

What emerges from all this is summarized in table 2, following Dussik and Dunn [60,61], which is an attempt to characterize tissues according to their ultrasonic propagation properties and biological function. It is seen that tissues can be grouped according to apparent teleology fashion with relatively narrow ranges of attenuation values within each group. The attenuation approximately doubles from group to group in the direction of increasing attenuation, and the speed of sound increases in the same direction. Further, proceeding from group to group in the same direction, tissues of ever-decreasing water content and ever-increasing structural protein content become included. Thus, it is seen that ultrasonic attenuation and velocity may be invoked to characterize tissues according to functional, structural, and teleological criteria. Possibly detailed measurements will allow assignment of resolvably unique values to each tissue structure, including usefully differentiable values for pathological states. Should this be the case, ultrasonic attenuation and impedance values, as functions of state and acoustic parameters, media, etc., should specify uniquely tissues for diagnostic purposes.

Acknowledgment

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Table 2. Average attenuation of tissues by categories.

	Tissue attenuation categories	Attenuation at 1 MHz (cm^{-1})	Tissue	Assumed teleology	General trends
1.	Very low	0.03	serum	ion, metabolic, etc.,	↑ Increasing structural protein content; Increasing speed of sound
		0.01	blood	transport convection	
2.	Low	0.06-0.07	adipose tissue	energy and (water) storage	
3.	Medium	0.08-0.11	nervous tissue	physiological	
		0.11	liver	function	
		0.08-0.16	muscle	parenchymal	
		0.23	heart	tissue	
		0.3	kidney		
4.	High	0.4	integument	structural	
		0.5	tendon	integration	
		0.6	cartilage	stromal tissues	
5.	Very high	1 or more	bone (mineralized)	skeletal framework	Increasing H_2O content ↓
		> 4	pulmonary tissue	gaseous exchange	

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