Dependence of the ultrasonic properties of biological tissue on constituent proteins

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The absorption and speed of ultrasound in mammalian tissues are discussed in relation to the fractional protein content. To a first approximation, biological tissues are shown to correspond to composite materials whose ultrasonic propagation properties are determined by the individual ultrasound properties of their globular and structural protein contents.

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INTRODUCTION

The study described herein was undertaken to determine the role of proteins, in general, and collagen, in particular, in the observed absorption and speed of ultrasound in biological tissues. It had earlier been observed that ultrasonic absorption in tissues occurs at the macromolecular level,[1,2] though an additional contribution to attenuation arises from tissue structural features.[3] Each of the molecular constituents of tissue, e.g., protein, lipid, water, etc., might then be expected to contribute to the absorption properties of an integrated tissue they comprise, and to do so in some proportion to their content. Thus, lipids would not be considered to contribute greatly to the ultrasonic absorption of most tissues, since they comprise only a small fraction of the wet weight.[4] Though water comprises 70–80% of most biological tissues,[4] it exhibits a relatively small intrinsic absorption coefficient, being a few percent of the attenuation coefficient of parenchymal tissues in the low megahertz frequency region.[5] Proteins, however, in sufficient concentration in aqueous solution have been found to exhibit magnitudes of absorption coefficient in the same range as those of tissue attenuation.[6,7] Similar results have also been reported for ultrasonic velocity.[8] Furthermore, the absorption coefficient and velocity of high-frequency sound in blood and in protein solutions have been shown to be dependent on the protein concentration.[1,5,7,8] Additionally, some degree of correlation appears to exist between tissue constituent contents, when taken singly, and velocity and attenuation of ultrasound.[9–11]

The protein constituents of tissues can be considered, for the purpose of this study, to comprise the globular and structural forms, each exhibiting greatly differing chemical roles, macromolecular structures, and ultrasonic properties, which can be expected to contribute in substantially different ways to the overall ultrasonic properties of tissues and organs. While a number of globular proteins have been characterized ultrasonically,[12–15] structural proteins have received little attention in this regard, probably due to the difficulties involved in the preparation of suitable suspensions. The first such data has recently become available for dilute acetic acid suspensions of collagen (pH = 3, 20 °C), a major structural protein.[16] Suspension concentrations were limited to the approximate range 0.07%–3.7% by weight due to the extraordinary viscosity (280 poise at 1%,[17]) of even these dilute suspensions. While the ultrasonic characteristics of collagen suspensions are thus limited to a rather restricted concentration range, these results indicate that collagen exhibits magnitudes of absorption coefficient and velocity, each per unit concentration, much greater than those exhibited by the globular proteins, though the frequency and concentration dependencies are not unlike those for the globular proteins.

Recent ultrasonic absorption measurements on six tissues, at 37 C over the frequency range 0.5–7 MHz, showed the absorption coefficient to have approximately the same frequency dependence as the attenuation coefficient, obtained from the general literature, but to have a magnitude approximately one-third that of the attenuation.[18] These new data make possible a comparison between attenuation in protein solutions and in collagen suspensions (attenuation due almost totally to absorption) and attenuation absorption in tissues.

Since all biological tissues are composed of varying amounts of globular and structural proteins, the differences in the ultrasonic properties observed among tissues may be related to the respective local concentrations of these molecular species. An aim of this study was to explore the roles of globular and structural proteins in several tissues and to determine the nature of the influence each exerts upon the inherent ultrasonic velocity and absorption coefficient of these biological structures.

I. ULTRASONIC VELOCITY AND ABSORPTION IN TISSUES AND IN CONSTITUENT PROTEIN PREPARATIONS

To examine the influence of ultrasonic properties of globular and structural proteins on those of tissues, the

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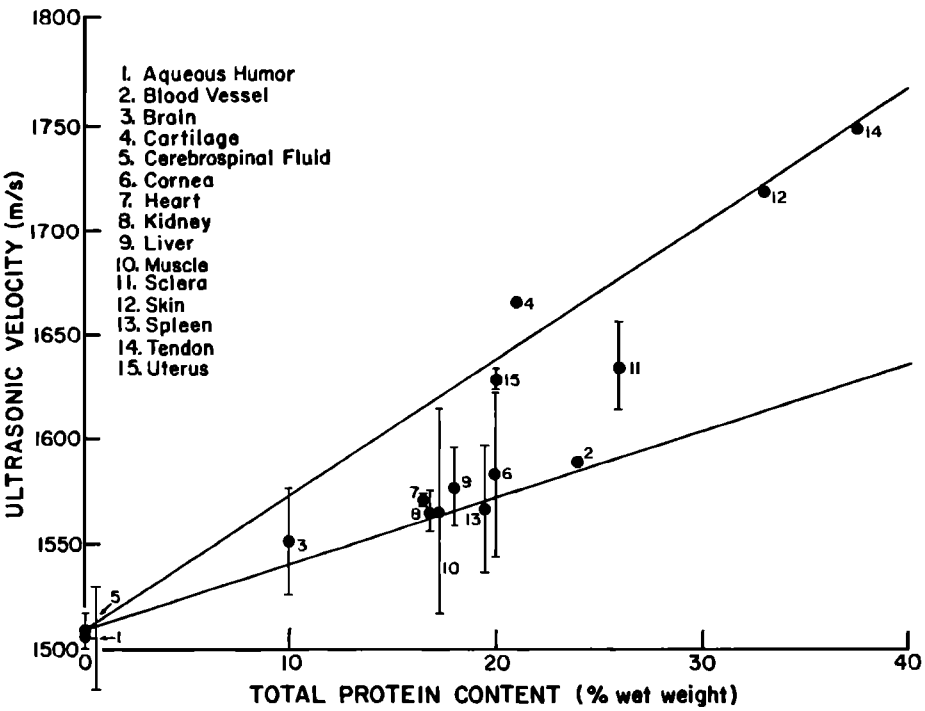

FIG. 1. Dependence of ultrasonic velocity at 1 MHz on tissue proteins. Tissue measurements (•) are compared with the globular protein-free curve (upper curve) and the collagen-free curve (lower curve), both taken from Eq. (1). Standard deviations are indicated for tissues for which several literature values were available. The ranges of protein content are given in Table I.

ultrasonic absorption and velocity are considered, to a first approximation, to result from a combination of the individual properties of each type of these two constituent proteins. Figure 1 shows the ultrasonic velocities of various biological materials[19,20] as a function of the wet weight percentage of total protein. The average temperature of measurement among these studies was 27 °C. Here the total protein content of tissue is assumed to be in the form of globular protein and collagen, concentrations of which are shown in Table I for each of the various tissues. A least-squares analysis of the data of 15 tissues yields the plane of best fit for the dependence of velocity on globular and structural protein content as

$$V = MP + NC + V_0 \tag{1a}$$

$$= 3.2P + 6.5C + V_0, \tag{1b}$$

where V is the ultrasonic velocity of the specimen tissue, P and C are, respectively, the wet weight percentage of globular protein and of collagen present in the tissue, M and N are, respectively, the dependence of velocity on concentration of globular protein and of collagen, and V_0 is that velocity which results when P and C are zero. Equation (1) provides an acceptable fit to the data as exhibited by the relatively small rms error to signal ratio, viz., 1.1%, and an intercept of 1508 m/s which agrees well with the ultrasonic velocity in physiological saline at 27 °C, viz., 1513 m/s. [The rms error to signal ratio is defined as

$$r = 100\left[\sum_{m=1}^{N} [V(P_m, C_m) - V_m]^2 \Big/ \left(\sum_{m=1}^{N} V_m^2\right)^{-1}\right]^{1/2},$$

where $V(P_m, C_m)$ is the velocity given by Eq. (1), V_m is the measured velocity, and N is the number of tissues.] The upper and lower solid lines of Fig. 1 describe the dependence of the velocity given by Eq. (1) if $P = 0$ and $C = 0$, respectively. The dependence of ultrasonic velocity on globular protein content obtained from these data ($M = 3.2$ at 27 °C) is reasonably similar to that found for other globular proteins, such as bovine serum albumin[21] (3.5 at 20 °C), hemoglobin[8] (3.8 at 25 °C), and blood plasma[22] (4.0 at 20 °C; 3.4 at 37 °C). Though the concentration range and nature of macromolecular collagen suspensions for which velocity data are available allow a less complete comparison, the slope for the dependence of velocity on tissue collagen ($N = 6.5$ at 27 °C) is similar to that found for dilute acetic acid suspensions of collagen[18] (7.5 at 20 °C). Further, the ratio of the dependencies of the ultrasonic velocity upon collagen concentration in suspension and globular protein concentration in solution (considered as that for bovine serum albumin), viz., 2.1, agree well with that given by Eq. (1), i.e., $N/M = 2.0$.

Thus, it is suggested here that a tissue predominantly comprised of globular protein will exhibit an ultrasonic velocity near the collagen-free curve, with a tissue wholly constituted of globular protein falling on that curve. On the other hand, tissues providing structural function such as tendon, where a substantial fraction of protein is in the form of collagen, would be expected to fall well above the collagen-free curve, due to the available evidence suggesting increased velocity per unit concentration of collagen over blood proteins. These views appear to be borne out as liver, heart, kidney, muscle, and spleen, all with approximately the same total protein content (approximately 16.5%–19.5%) of which very little is collagen (less than 6% of the total protein fraction), have nearly the same velocities, and appear near the collagen-free curve. The total protein content of tendon is 37.5%, with 85% of this being in the form of collagen. The data for beef tendon[19] and for collagen fibers, separated from mouse tail tendon,[20] fall very nearly on the globular protein-free curve. Cartilage also contains a substantial amount of colla-

TABLE I. Principal constituents (% wet weight) of biological tissues.[a]

Tissue	% total protein	% collagen	% lipid	% water
Blood vessel	24(23–27)[b]	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	21(18–24)	15.5(14–17)	1.3	72(55–85)
Cerebrospinal fluid	0.028 (0.012–0.043)			99
Eye				
(aqueous humor)	(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	37.5(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

[a] Data after Snyder *et al.*[4] and Chvapil.[25] Collagen content was computed from dry weight percentages assuming appropriate water content.
[b] Example, mean value 24%, total range of values 23%–27%.

gen and its velocity also falls near the globular protein-free curve.[19] Velocity measurements in pig skin[23] yield the value of 1720 m/s, which appears near the globular protein-free curve, as skin contains about 33% protein, of which about 91% is in the form of collagen. It thus appears that the ultrasonic velocity of tissues is governed in a predictable way by the ultrasonic properties of their constituent protein contents.

The ultrasonic absorption coefficient of tissues appears to be governed similarly, though a least-squares analysis analogous to that performed for velocity is forestalled until adequate data become available for an appropriate variety of tissues. Figure 2 shows the ultrasonic absorption coefficient at 1 MHz as a function of protein content for several biological tissues. Also shown are the empirical relations describing the ultrasonic absorption of globular protein solutions (considered as bovine serum albumin) at 20 °C as a function of concentration in the range 5%–40% by weight, viz., $\alpha = 0.00053C^{1.2}$, where C is the concentration in g/100 cc, (very nearly the same as the wet weight percentage of globular protein) and the dependence of absorption on collagen concentration in suspension[18] (pH = 3) in the range from 0.07% to 3.7%, viz., $\alpha = 0.0019C^{1.2}$. The absorption coefficients measured for tissues appear to fall between these curves. Tendon, which has a substantial collagen content, is seen to exhibit an absorption coefficient well above the globular protein solution

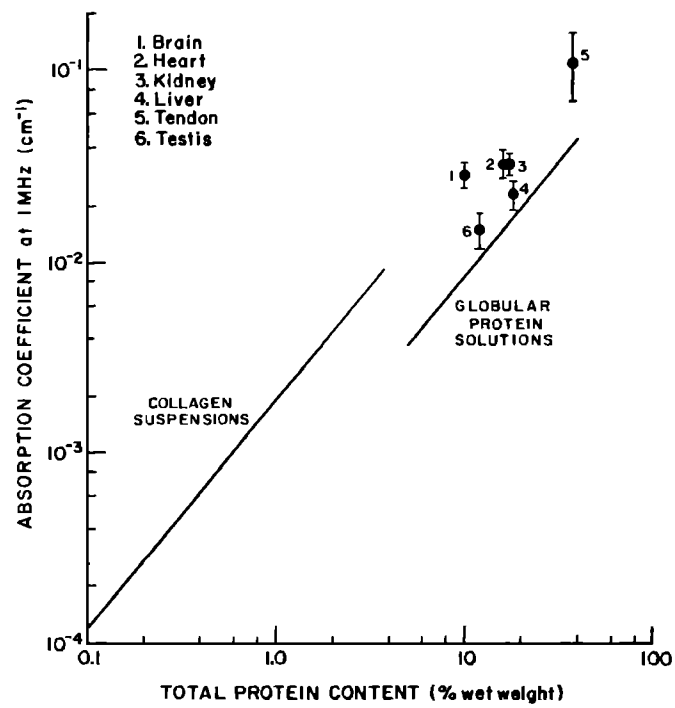

FIG. 2. Dependence of ultrasonic absorption, at 1 MHz on tissue proteins. Tissue measurements[18] (•) appear to fall between the curves defining the ultrasonic absorption of globular protein solutions and of collagen suspensions. The error bars represent the standard deviation. The ranges of protein content are given in Table I.

curve. Tissues such as liver, kidney, and heart all have approximately the same quantitative (mainly globular protein) content, and exhibit very similar absorption properties appearing near the globular protein solution curve. Brain and testis possess the least amount of protein, approximately 10% and 12%, respectively. While brain exhibits a greater absorption coefficient than might be expected on the basis of its protein content, the results for all other tissues, including testis, agree well with the hypothesis that the absorption coefficient is determined by the globular and structural protein contents.

II. CONCLUSIONS

From the above discussion it can be argued that protein constituents of tissues determine quantitatively their ultrasonic properties. For parenchymal tissues, for which the collagen content is relatively small, the absorption and velocity appear to be governed by the major macromolecular component of most tissues, i.e., the globular proteins. Since the absorption and velocity per unit concentration associated with the presence of collagen are, respectively, approximately four and two times that for the globular proteins, even relatively small changes in collagen content can measurably affect these ultrasonic properties. These phenomena begin to provide an understanding of the echographic "texture" characteristics of tissue structure.[24] For the highly collagenous stromal tissues, gross changes in absorption and velocity are observed to occur due to a lessening of the globular protein content, and to an increasing of the total content of all protein in the form of collagen. Thus, biological tissues may be considered, to a first approximation, as composite materials whose ultrasonic propagation properties are governed by the individual ultrasonic properties of collagen and globular proteins.

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