Ultrasound in Med. & Biol. Vol. 11, No. 2, pp. 307–318, 1985
Printed in the U.S.A.

0301-5629/85 $3.00 + .00
Pergamon Press Ltd.

●*Original Contribution*

DETERMINATION OF THE NONLINEARITY PARAMETER *B/A* OF BIOLOGICAL MEDIA

W. K. LAW,† L. A. FRIZZELL and F. DUNN
Bioacoustics Research Laboratory, University of Illinois, 1406 W. Green Street, Urbana, IL 61801, U.S.A.

(*Received in final form* 11 *June* 1984)

The nonlinearity parameter *B/A* has been determined for various biological solutions and soft tissues using the thermodynamic and finite amplitude methods. Agreement between the two methods is better than 10% for the tissues and 1% for the solutions. Fat has a *B/A* value around 11, the highest among soft tissues studied. Other soft tissues including liver, muscle, brain, and heart muscle have *B/A* values close to 7. Based on the observed linear relation between the *B/A* value and solute concentration in protein solutions, and also the lack of dependence of the *B/A* value on solute molecular weight in dextran solutions, it is postulated that the nonlinearity in these solutions is due to solute–solvent interactions. The general trend of increasing *B/A* value with specimen structural hierarchy suggests that the nonlinearity of biological materials is related to this feature. These observations suggest the use of the nonlinearity parameter *B/A* in tissue characterization, particularly since structural alteration often attends the pathological state.

1. INTRODUCTION

Ultrasound continues to be used extensively in the diagnosis of diseases and in therapeutic applications. Because of the analytical complexity of dealing with nonlinear phenomena, it has long been considered useful and acceptable in the biomedical applications of ultrasound to treat acoustic propagation phenomena as obeying linear relationships (Kinsler *et al.*, 1982). However, experimental evidence is accumulating to indicate that such treatment may not always be appropriate (Carstensen *et al.*, 1980, 1981; Goss and Fry, 1981; Muir and Carstensen, 1980).

The possibility of production of nonlinear phenomena in human tissue by ultrasonic diagnostic and therapeutic instruments has emerged as a worthwhile question (Muir, 1980; Duck and Starritt, 1983). In the diagnostic use of ultrasound, understanding of nonlinear phenomena in mammalian tissue may lead to improved accuracy and provide more precise information on the state of the tissue. Imaging a function of the nonlinearity parameter has been suggested (Ichida *et al.*, 1983). In the therapeutic area, the detailed understanding may lead to better control of the heat deposition of ultrasonic energy (Carstensen *et al.*, 1981; Carstensen *et al.*, 1982). Furthermore, for both applications it is important to determine whether the nonlinear phenomena produce harmful effects to the patient. In order to estimate the amount of acoustic nonlinearity which may occur in a biological medium, the nonlinear characteristics of the medium itself must first be determined. It is for this reason the project of determination of the nonlinearity parameter *B/A* of biological media was initiated.

During the course of the investigation, the nonlinearity parameter was found to change with the protein concentration and with the structure of the tissue, as shown in previous reports (Law *et al.*, 1981; Dunn *et al.*, 1981, 1982, 1984). On the other hand, the nonlinearity parameter was found to be insensitive to the molecular weight of the solute in solutions. In general, the behavior of the nonlinearity parameter *B/A* was found to be quite different from the more frequently measured acoustic parameters, namely, acoustic velocity and acoustic absorption coefficient. For example, molecular weight of the solute in the dextran solutions strongly affects the absorption coefficient of the solution but scarcely influences the *B/A* value (Law *et al.*, 1981). In another example, the sound velocity in fatty tissue is lower than in non-fatty tissue, yet the *B/A* value in fat is much higher than that of non-fatty tissues (Dunn *et al.*,

† Current address: General Electric Company, Medical Systems Operations, 3920 Security Park Drive, Rancho Cordova, CA 95670, U.S.A.

1984). The unique properties of the B/A parameter raised an interesting possibility of using the nonlinearity parameter as a means for the characterization of tissue.

In the initial studies an acoustical method was employed for determining the nonlinearity parameter which is believed to have the potential for measurements *in vivo*. This paper reports the efforts to confirm the results reported in the previous papers using a completely independent method, the thermodynamic technique (Beyer, 1960). Also, more extensive measurements using the finite amplitude technique are reported.

2. METHODS OF MEASUREMENT

2.1. *Finite amplitude method*

The finite amplitude method of B/A measurement has been described previously (Beyer, 1960). Briefly, the B/A value of a medium is related to the second harmonic generated in the medium. A receiving transducer having the same size as the sound source, coaxial and parallel to it, is used to determine the amplitude of the second harmonic signal generated in the medium. In a small source amplitude approximation, for a medium with near linear frequency dependence of absorption, the relationship can be written as

$$p_2(z) = p_1^2(0)\frac{(B/A + 2)\pi f z}{2\rho_0 c_0^3} e^{(\alpha_1+\alpha_2/z)z}F(z) \qquad (1)$$

where z is the axial distance between the receiving transducer and the sound source, ρ_0 is the equilibrium density of the medium, c_0 is the infinitesimal amplitude wave velocity, f is the frequency of the fundamental component, $p_1(0)$ is the acoustic pressure of the sound source averaged over its effective surface area, and $p_2(z)$ is the acoustic pressure of the second harmonic component, averaged over the surface of the receiving transducer. The attenuation coefficients at the fundamental and second harmonic frequencies are α_1 and α_2, respectively, and $F(z)$ is a diffraction correction term derived for the finite amplitude generation of the second harmonic from an ideal piston source (Ingenito and Williams, 1971). Equation (1) is accurate to ±2% for $(\alpha_1 - 2\alpha_2)\, z < 1/2$.

In order to eliminate the necessity for measuring the attenuation coefficients, and thereby eliminating the errors associated with such measurements, an extrapolation scheme was utilized in the measurements (Law *et al.*, 1981). The amplitude of the second harmonic signal was measured at several distances away from the sound source. These data were accurately fit, on a semi-log plot by a straight line, allowing extrapolation to the point $z = 0$, so that the exponential term in eqn (1) was eliminated and $F(z)$ was a constant.

2.2. *Thermodynamic method*

For the thermodynamic method of measurement, the nonlinearity parameter B/A, through thermodynamic expansion, is expressed as the sum of two terms (Beyer, 1960), a pressure derivative term $(B/A)_P$ which is the dominant part of the expression, and a temperature derivative term $(B/A)_T$. Mathematically one may write

$$\frac{B}{A} = 2\rho_0 c_0\left(\frac{\partial c}{\partial p}\right)_T + \frac{2c_0 T\beta}{C_p}\left(\frac{\partial c}{\partial T}\right)_p = \left(\frac{B}{A}\right)_P + \left(\frac{B}{A}\right)_T \qquad (2)$$

where ρ_0 is the equilibrium density, c_0 the infinitesimal wave velocity, $(\partial c/\partial p)_T$ the change of sound speed with pressure at constant temperature, β the volume coefficient of thermal expansion, T the temperature in Kelvin, C_p the heat capacity at constant pressure, and $(\partial c/\partial T)_p$ the change of sound speed with temperature at constant pressure. Thus the values of B/A were determined by measuring the change of sound velocity with pressure and temperature. As described previously, the β and C_p values of water can be used to substitute for the actual values of the samples and still provide satisfactory accuracy in the measurements (Law *et al.*, 1983).

Figure 1 is a schematic diagram of the apparatus showing the velocimeter completely enclosed in a water filled pressure vessel, a hydraulic pump for changing the hydrostatic pressure inside the pressure vessel through a nickel bellows, a temperature controlled water bath for maintaining the velocimeter at the desired temperature, and electronic instrumentation for the precise measurement of the propagation time of an acoustic pulse travelling through the sample in the velocimeter.

Figure 2 is a detailed diagram of the velocimeter showing the sample chamber with parallel quartz crystal transducers resonant at 3 MHz mounted in housings at each end of the chamber. The sample chamber is comprised of two coaxial hollow cylinders of unequal lengths, joined together by a flange and five screws. The design allows the placement of soft tissue samples inside the chamber without disassembling the transducer housing. The length of the sample chamber at 25°C is 3 ± 0.001 in, and the diameter of the bore is 2 in. The thermal coefficient of expansion, the elastic tension moduli, and Poisson's ratio of the 304 stainless steel used to make the chamber are, respectively, 17.3×10^{-6} °C^{-1}, 28×10^6 lb in^{-2}, and 0.29, as specified by the manufacturer. From these data, the lengths of the sample chamber at various temperatures and hydrostatic pressures can be determined. Rubber diaphragms covering openings

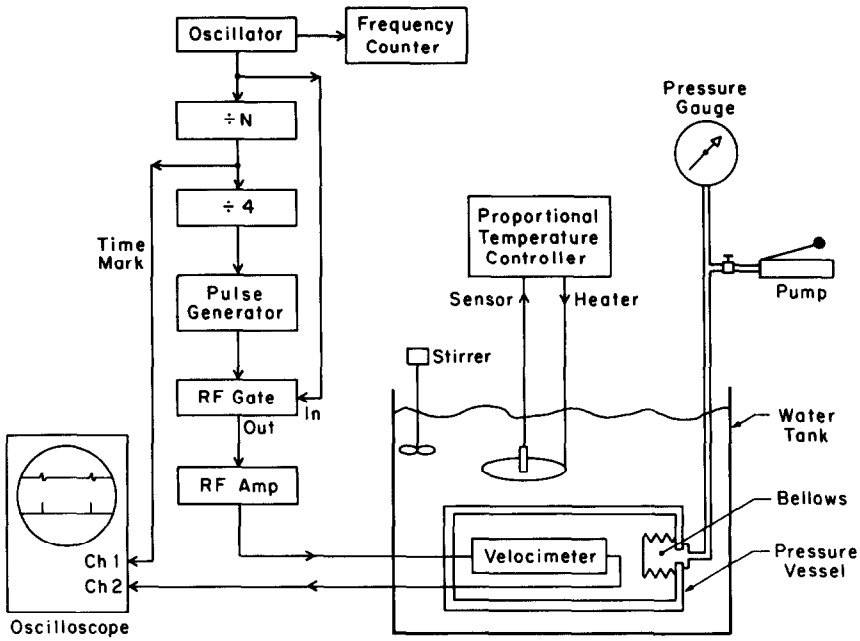

Fig. 1. Schematic diagram of the thermodynamic method for B/A measurement.

to the sample chamber and the transducer housings transmit the hydrostatic pressure from outside of the velocimeter to the inside and also equalize the hydrostatic pressure in front of, and behind, the quartz crystals to eliminate stress on the crystals from the applied pressure.

The crystal transducers are ¾ in in diameter, fine ground on both faces, with complete gold on chromium plating on the ground side and over a concentric circle ½ in in diameter on the other side. The crystals are edge clamped to the sample chamber by the transducer housings and sealed with a gasket between the housing and the crystal. The transducer housings are filled with silicone oil and lined with rubber absorbers with a 45° angle to the axis of the crystals to eliminate reflections at the back face of the crystals.

The pressure vessel is made of ¼ in thick 304 stainless steel. Two threaded end caps close the pressure vessel. One end cap contains the bellows

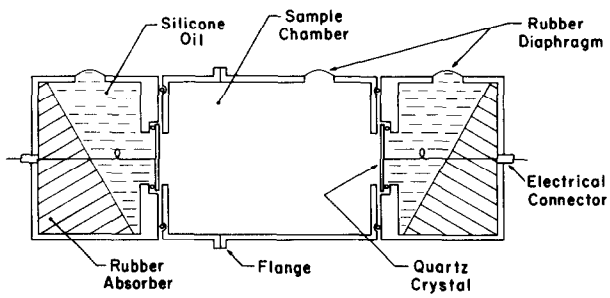

Fig. 2. Schematic diagram of the velocimeter.

and the other contains the electrical feed-through connectors for the source and receiving crystals. The velocimeter is also attached to this cap so that the cap and velocimeter can be removed as a complete assembly. The cap is tapered conically at the center so that when oriented vertically, air bubbles collect when the cap is screwed into the pressure vessel. The fill hole is plugged by a small screw and O-ring after the cap is put in position.

The hydrostatic pressure generation system consists of a hydraulic hand pump connected to the pressure vessel and a pressure gauge. The pressure gauge is a bourdon tube type with a range of 0–3000 psi and a full scale accuracy of ±0.1%. The pressure is transmitted to the pressure vessel through a bellows which has a spring rate of 80 lb in^{-1} and an effective area of 4.45 in^2. At 2000 psi, the pressure differential across the bellows is computed to be 2 lb in^{-2} which is subtracted from the gauge reading to obtain the true pressure applied to the velocimeter.

The pressure vessel, with velocimeter, is submersed in the water bath which is temperature controlled by a proportional temperature controller. All measurements are made at 30°C. The temperature of the bath is monitored by a platinum resistance thermometer which has a resolution of 0.001°C and an accuracy of ±0.01°C. The temperature fluctuation of the bath is less than ±0.02°C during a 6 h period. The stability of the sound velocity in the velocimeter is used as an indicator of thermal equilibrium.

To measure the time of flight of an acoustic pulse travelling through a sample in the velocimeter,

the received acoustic signal, together with the electromagnetic pick-up of the electrical driving signal to the source, is displayed on one channel of an oscilloscope. The second channel displays a series of time marks which are synchronous with the driving signal applied to the source. The interval between time markers is adjustable and measured to ±0.001% with a digital counter. By using the delayed sweep option of the oscilloscope, which allows the expanded display of a small portion of the waveform, the time markers can be aligned to a fixed reference point of the wave packet to the accuracy of $^1/_{100}$ of a cycle. The seventh zero crossing in the driving signal and the received signal are arbitrarily chosen as reference points. The electrical signal driving the source transducer is pulse modulated cw, with a carrier frequency of 3 MHz, a pulse duration of 5 μs, and an interpulse time of 200 μs to allow multiple reflections between the transducers to decay to a negligible amplitude before initiation of the following pulse.

In the computation of B/A using eqn (2), both the absolute velocity in the sample and small changes of velocity with applied pressure and/or temperature are required. The time of flight measurement, described above, provided 1 part in 10^4 resolution for measuring the small change of velocity, but must be corrected for a fixed time delay associated with the electrical circuits and the transducers in order to measure the absolute velocity accurately. Water is used as a reference liquid to determine the delay due to the electronics since its sound velocity has been determined with a high precision (Greenspan and Tschiegg, 1959). Results of measurements indicate that the delay amounts to approximately a 1% correction to the absolute velocity, and does not change with pressure or temperature applied to the velocimeter.

2.3. *Error analysis for the finite amplitude method*

The errors in the finite amplitude measurements of B/A arise mostly in the calibration procedures, which involve the absolute calibration of a source transducer, a similar calibration of another transducer which serves as a secondary standard, and the calibration of a receiver transducer using the secondary standard as a reference. The estimated errors of these procedures are (1) Determination of average source pressure: ±2% random error in selecting the source driving voltage, ±3% random error in the radiation force balance power measurement due to mechanical and thermal disturbances, ±2% systematic error due to the change of buoyancy in the acoustic target resulting from ultrasonic heating, and ±2% systematic error due to the uncertainty in determining the effective area of the transducer. (2) Calibration of the receiver: ±3.6% RMS random error and ±4% systematic error in the absolute calibration of the secondary standard, as described in the previous paragraph, ±2% random error in measuring the output voltage of the receiver, and ±1% error in the alignment of the source and receiver.

There are also errors in alignment, in reading the second harmonic amplitude, in measuring the density and the sound velocity, and in the deviation of the source transducer from a perfect piston source associated with the actual determination of the nonlinearity parameter. These errors are listed below for measurements of liquid and tissue specimens. The errors are greater for tissue than for liquids due to the latters' inhomogeneous, semi-solid, and deformable nature. The estimated errors are, therefore, listed separately for the two different types of materials.

(3) Measurements of Liquid Samples: ±½% random error in density, ±½% random error in sound velocity, ±2% random error in measuring the amplitude of the second harmonic component, and ±2% systematic error due to deviation of the transducer from an ideal piston source. (4) Measurements in Tissues: ±1% random error in the density, ±1% random error in the sound velocity, ±2% random error in measuring the amplitude of the second harmonic component, ±2% random error in measuring the thickness of the samples, and ±2% systematic error due to deviation of the transducer from an ideal piston source.

The nonlinearity parameter is, from eqn (1)

$$\frac{B}{A} + 2 = \frac{2\rho_0 c_0^3}{\pi f} \frac{p_2(z)}{z p_1^2(0)} F^{-1}(z)|_{z \to 0}. \qquad (3)$$

Assuming that all the random errors are independent of each other, the RMS error in $(B/A + 2)$ is ±6.6% for liquid samples, and ±7.1% for tissues. The total systematic error is ±8% for liquid and tissue samples.

2.4. *Errors in the thermodynamic method*

The second term in eqn (2) for computing the B/A value requires knowledge of the specific heat C_p and the thermal coefficient of expansion β of the specimen materials, which are not available in the literature. However, the value of C_p for rabbit liver has been reported (Bowman *et al.*, 1975) to be about 20% less than that of water, and the value of β for a 30% BSA solution was measured to be 13% greater than water (using a calibrated capillary to determine the volume change of a sample inside a volumetric flask as a function of temperature, with correction for the thermal expansion of the flask). Based on these details, it is assumed that the values of β and

C_p for the biological materials studied are not significantly different from that of water, for the purpose of computation of (B/A), since β and C_p are involved only in the $(B/A)_T$ term, which contributes only about 3% to the total B/A value. This approximation introduces approximately a 1% error to the computed B/A value. The errors in the other parameters in the $(B/A)_T$ terms are much smaller than those of β and C_p and are considered negligible. The error in determining the values of $(B/A)_T$ is set at $\pm 2\%$, to allow for variations of β and C_p more drastic than that observed for rabbit liver and BSA solutions.

The more significant contribution of errors comes from the first term, namely, in the measurements of sound velocity, density, and $(\partial c/\partial p)_T$. The error in the velocity at a fixed temperature–pressure point is determined by: (1) the errors in measuring the time-of-flight of the acoustic pulse, (2) the error in determining the length of the sample chamber, i.e. the acoustic path, and (3) the effect of random temperature fluctuations of the temperature controlled bath on the sound velocity. The error in $(\partial c/\partial p)_T$ can be considered as the combination of the errors in two velocity measurements at two different hydrostatic pressures, divided by the difference in hydrostatic pressure between the two measurements. The uncertainty in temperature and pressure is expressed as equivalent speed errors in the analysis. The errors are itemized as follows: (1) Error in density: $\pm$½% for liquids and $\pm 1\%$ for tissues. (2) Errors in velocity measurements: for the solutions and tissues measured, $c_0 = 1600$ m s^{-1}, $(\partial c/\partial T)_p \simeq 1.5$ m s^{-1} °C^{-1} and $(\partial c/\partial p)_T \simeq 0.012$ m s^{-1} psi^{-1}. (a) The random error in the temperature, which is ± 0.02°C, can be expressed as an equivalent speed error of $1.5 \times (\pm 0.02) = \pm 0.03$ m s^{-1}. (b) The random error in hydrostatic pressure, which is ± 5 psi, can be expressed as an equivalent speed error of $0.012 \times (\pm 5) = \pm 0.06$ m s^{-1}. (c) For the time-of-flight measurement there is a $\pm 0.008\%$ random error in aligning the time mark to a fixed reference point of the wave packet, and a $\pm 0.001\%$ random error in the accuracy of the frequency counter. The RMS error in the time-of-flight is therefore $\pm 0.009\%$, or ± 0.14m s^{-1} when expressed as an equivalent speed error. (d) There is a $\pm 0.03\%$ systematic error in determining the length of the sample chamber, i.e. the acoustic path length. However, the error cancels out in the measurement of $(\partial c/\partial p)_T$ and is significant only in the measurement of the absolute velocity. One may consider the $\pm 0.03\%$ error to be much smaller than the other errors and negligible. (3) Computed indeterminancy in $(\partial c/\partial p)_T$: the RMS error in the measurement of sound velocity is the combination of items (a), (b), and (c), namely, $\pm(0.03^2 + 0.06^2 + 0.14^2)^{1/2} = \pm 0.16$m s^{-1}. The RMS error in $(\partial c/\partial p)_T$ is the combination of errors in two velocity measurements, divided by the interval, namely, $\pm[(0.16)^2 + (0.16)^2]^{1/2}/2000 = \pm 0.00011$ m s^{-1} psi^{-1} $= \pm 0.9\%$.

The random error in determining the parameter $(B/A)_P$ is the combination of errors in density and $\partial c/\partial p)$, namely, $(0.5^2 + 0.9^2)^{1/2} = \pm 1\%$ for liquids, and $(1^2 + 0.9^2)^{1/2} = \pm 1.3\%$ for tissues. Adding the $\pm 2\%$ uncertainty in estimating the value of $(B/A)_T$, the error in (B/A) is $\pm 3\%$ for liquids, and $\pm 3.3\%$ for tissues.

There are other possible sources of error in the thermodynamic determination of B/A of tissues. Firstly, due to the semi-solid, deformable nature of the tissue, the length of the sample could not be measured accurately. The length of the sample was not precisely 3 in long, but was usually longer by about 0.04 in. The extra length was provided deliberately so that the sample could be compressed slightly by the two transducers at the specimen ends to provide an intimate surface contact between the sample and the transducer elements. It is difficult to estimate the error caused by this compression, since the amount of compression itself was not unique. However, since there was space in the sample chamber to allow the tissue to extend laterally when compressed longitudinally, it is felt that the compression did not change the acoustic properties of the tissue sample in any significant way.

Another source of error comes from the inhomogeneity of the tissue. Gross inhomogeneity, most notably the presence of blood vessels (and biliary vessels in the case of liver), ventricles in the brain, etc., can cause an acoustic pulse to arrive at the receiver at slightly different times, either due to a difference in velocity in parts of the tissue, or due to an alteration of the sound path through scattering and diffraction. Again, the amount of uncertainty is difficult to determine because the extent and location of inhomogeneities are generally unknown. One general indication of gross inhomogeneity is the presence of a detected acoustic signal outside the packet of received waves, indicating arrivals of sound sooner or later than the main packet, though no such occurrences were observed. It is therefore concluded that either the inhomogeneity affects only a small portion of the arriving acoustic energy, so that the effect is not detectable, or that the time difference between different arrivals is smaller than one wave period so that the arriving waves are not distinguishable from each other. In the first case, inhomogeneity can be confidently neglected. In the second case, the uncertainty in the time of flight is less than one wave

period, or equivalent to less than ±1% in the sound velocity.

There is also a possibility that a distribution of B/A values exists in the tissue samples. In that case, one is observing an average value of the distribution of B/A. Whether such a distribution exists and in what manner the B/A values are averaged remains a question at present.

In view of the uncertainty in sample length and in the effect of inhomogeneity on velocity, the error estimate in the thermodynamic determination of B/A in tissue is increased from the original estimate of 3.4 to 5%.

2.5. *Sample preparation*

Liquid samples were prepared in a manner similar to that described previously (Dunn *et al.*, 1981). However, for dextran solutions, there was a long time lapse between the measurements using the finite amplitude method and the thermodynamic method. The solutions were frozen at −5°C during this period and then warmed to room temperature and stirred thoroughly before use. Freshly prepared solutions were also measured and did not indicate any difference in property as compared to the stored-frozen samples.

Tissue samples were obtained fresh from the slaughter house, stored at 5°C and studied within 24 h. The samples were cut with a 5 in wide microtome blade into a roughly cylindrical shape with parallel end surfaces, about 3 in long and 2 in in diameter, to conform to the shape of the sample chamber. After placing the sample in the sample chamber, the chamber was filled with 0.9% saline solution to occupy all spaces.

3. RESULTS AND DISCUSSIONS

3.1. *Finite amplitude method*

The nonlinearity parameter B/A was measured at 3 MHz and 30°C using the finite amplitude method for 15 different materials, including several organic liquids, aqueous protein solutions, and whole and homogenized tissues, which can be divided into three categories: standard liquids, tissue models, and tissues.

3.1.1. *Measurement of standard liquids.* Among the standard liquids, water is probably the most extensively studied, and one of the most important biological constituents. Figure 3 shows the results of a measurement of second harmonic data in water, plotted as $\ln p_2(z)/zp_0^2$ vs z. Measurements begin at $z = 0.8$ cm since multiple reflections produced interference at shorter distances. A theoretical curve, based on eqn (1) and using the literature values of the absorption coefficient, speed of sound, and density, but with a B/A value adjusted to fit the extrapolated intercept of the experimental data, is also plotted in the figure for comparison. The theoretical curve agrees to within ±0.5% with the expressions obtained by other investigators (Ingenito and Williams, 1971; Cobb, 1983), since the expressions are similar for a medium of less absorption. Note that the theoretical curve between $z = 0$ and 1 cm is also an extrapolation since the theory is no longer valid in this region. The published reports of the B/A of water at 30°C, determined by the thermodynamic technique, give a value of 5.2 (Beyer, 1960). As seen in Fig. 3, a B/A value of 5.3 is obtained. A least-square linear regression fit to the experimental data results in a curve with a slightly less negative slope compared to the theoretical curve. If the experimental data were fitted to a straight line with the same slope as the theoretical curve, the resulting B/A value would be 2% greater. Since the effect of absorption in water was negligibly small when compared to the effect of diffraction, the error can be the result of either an inadequacy in the diffraction theory or a departure of the sound source from the assumed perfect piston operation. Since the

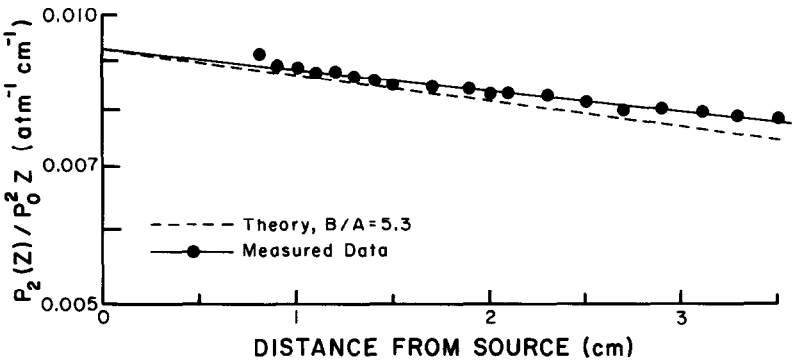

Fig. 3. Comparison of measured second harmonic component with theory, for degassed water; using $\alpha_1 = 0.002$ cm^{-1} and $\alpha_2 = 0.008$ cm^{-1}.

Table 1. Values of B/A of standard liquids, determined by the finite amplitude method

Material	Velocity (cm s^{-1})	Density (g cm^{-3})	B/A This study	B/A† Literature
Water	1.509×10^5	1.00	5.5 ± 0.3	5.2
50% methanol water mixture	1.483×10^5	0.915	8.5	8.3
Ethylene glycol	1.644×10^5	1.11	9.93	9.7
Glycerol	1.87×10^5	1.26	9.4	9.0

† Beyer (1974).

accuracy of the theory has been verified experimentally (Ingenito and Williams, 1971; Cobb, 1983), it appears that the error was most likely caused by the sound source which produced diffraction effects slightly less than those of a perfect piston source.

The average of seven measurements made over a two year period produced an average B/A of 5.5 for water, with a standard deviation of ± 0.3.However, more recent measurements gave a B/A value of 5.3 consistently. To investigate the possible effect of the quality of water on the B/A value, tap water was used in a B/A measurement and found to be 2% less than that of well-degassed water. However, the 2% difference was well within the margin of error of the finite amplitude measurement system. The difference between the earlier measurements and the more recent ones was attributed to calibration of transducers; there being three separate calibrations throughout the two years of measurement. Each of the calibrations resulted in a slightly different calibration number due to unavoidable variations in experimental conditions, uncertainties in instrument readings and, perhaps to a lesser extent, changes with time of the ceramic transducer assembly properties.

The agreement between the finite amplitude method and literature values of B/A was good for the other liquids studied and shown in Table 1. The measurements of B/A values of the standard liquids are consistently 2–4% greater than the B/A values reported in the literature, indicating the possibility of a systematic error in the transducer calibrations or in the deviation of the transducers from an ideal piston source. However, the discrepancies are within the estimated 8% error for the calibration process.

3.1.2. *Measurements of tissue models.* The B/A value for aqueous bovine serum albumin (BSA) solutions of 8–40% concentration are shown in Fig. 4. The B/A value for water is included as the zero concentration value. The error bar on the water datum point indicates the standard deviation of the seven measurements. Each of the other points represents the result of one measurement. The results of an initial measurement has been reported previously (Law *et al.*, 1981). The measurements for BSA were repeated approximately two years after the initial measurements. For both sets of measurements, the values of B/A were found to increase approximately linearly with concentration. However, the values of B/A in the more recent measurements were below those of the earlier measurements. The two sets of measurements had similar slopes, but the earlier values were greater by approximately 10%, and the data points were more scattered. The discrepancy is probably due to a combination of two factors. First, there may have been differences in the calibrations between the two sets of measurements, as is exemplified in the different values of B/A obtained for water. Second, a drift was observed in the output of the internal calibrator of the spectrum analyzer with

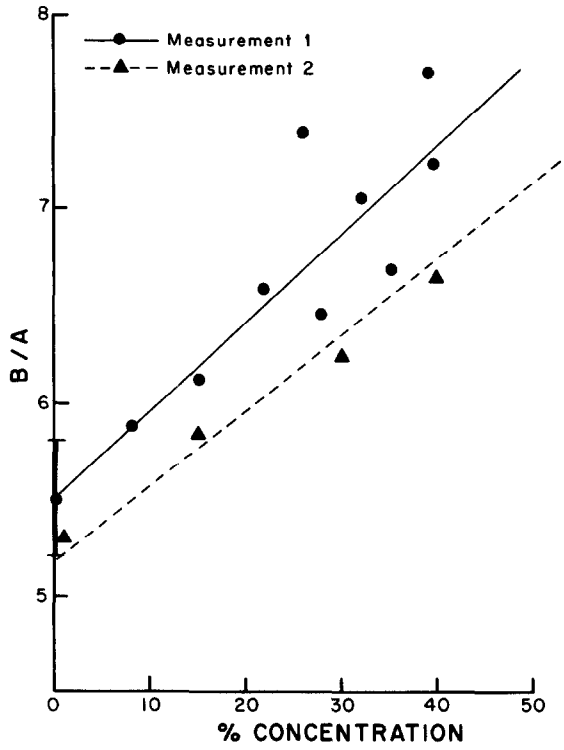

Fig. 4. B/A *vs* solute concentration for BSA solutions, determined by the finite amplitude method.

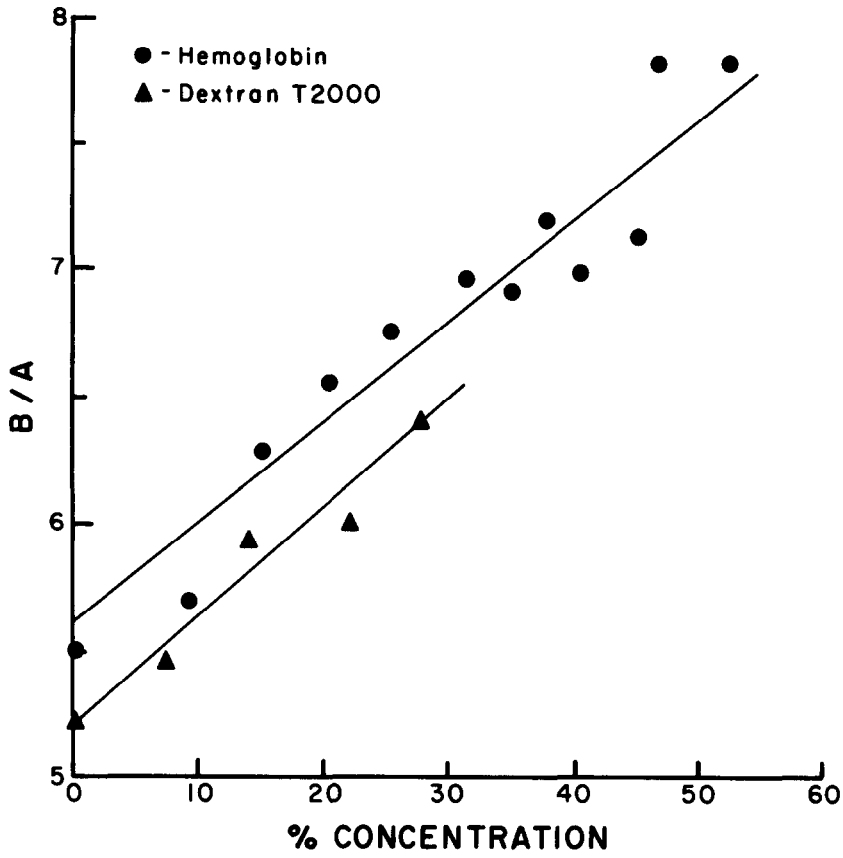

Fig. 5. *B/A vs* solute concentration for hemoglobin and dextran 2000 solutions.

room temperature, by as much as ±10% on an especially hot day during spring and summer when the air conditioning system of the building failed. (The observed drift with temperature was within the manufacturer's specification and was not due to malfunctioning of the unit.) Since some of the earlier data for the BSA solutions was obtained during that time of the year, the spectrum analyzer drift could have affected the measurements. The problem had been corrected later by calibrating the spectrum analyzer against a high-precision RF voltmeter and by superior control of the room temperature. The decreased amount of scatter in data points, as is evident in the more recent measurements in BSA, indicates that the problem had been corrected effectively.

Another source of variation may be associated with measurements of the biological materials themselves. To minimize the effects of sample variation, a direct comparison between the finite amplitude method and the thermodynamic method was made on the same sample of BSA solution and has been discussed (Law *et al.*, 1983). This comparison indicated that the more recent finite amplitude measurements agreed well with the thermodynamic measurements when the same sample is used for both measurements.

Measurements of *B/A* were made on a second protein solution, hemoglobin, shown in Fig. 5. The *B/A* values for both BSA and hemoglobin solutions were found to increase approximately linearly with solution concentration.

The *B/A* value for dextran T2000 ($Mw = 1.56 \times 10^5$ Da) is also shown to increase approximately linearly with concentration (Fig. 5), with the rate of

Table 2. Values of *B/A* of dextran solutions of different solute molecular weights, determined by the finite amplitude method

Material	*Mw* (Daltons)	Concentration (gm/100 cc)	Velocity (cm sec^{-1})	Density (g cm^{-3})	*B/A*
Dextrose	180	28.9	1.61×10^5	1.10	6.04
Dextran T150	1.5×10^5	24	1.57×10^5	1.08	5.94
Dextran T2000	2×10^6	26.4	1.56×10^5	1.08	6.2

Table 3. Values of B/A of soft tissues

Material	Velocity (cm s^{-1})	Density (g cm^{-3})	B/A
Beef liver†	1.588×10^5	1.05	8.0
Beef liver†	1.599×10^5	1.05	7.5
Beef liver	1.596×10^5	1.05	7.5
Beef liver‡	1.610×10^5	1.05	8.9
Beef liver‡	1.632×10^5	1.05	6.2
Beef liver‡	1.620×10^5	1.05	7.9
Beef brain	1.548×10^5	1.03	7.6
Beef heart	1.566×10^5	1.05	6.8
Beef heart	1.570×10^5	1.05	7.4
Pig muscle	1.593×10^5	1.07	7.5
Pig muscle	1.607×10^5	1.07	8.1
Pig fatty tissue	1.439×10^5	0.98	11.0
Pig fatty tissue	1.455×10^5	0.93	11.3

† Measured at 23°C.
‡ Samples obtained from the same animal.

increase approximately the same as the BSA and homoglobin solutions. The difference in intercept of the two curves probably is due to differences in calibration of transducers between the two measurements. Thus a linear long chain molecule is not very different from a globular molecule in the capability to increase the solution nonlinearity. Table 2 lists the B/A values for solutions of dextran T2000, T150 and dextrose of molecular weights 2×10^6, 1.5×10^5, and 180 Da, respectively. The B/A values of these solutions are nearly the same when normalized to the same concentration by assuming that a linear relationship exists between B/A and concentration. Thus, the B/A value in the dextran solution appears to be insensitive to molecular weight over four orders of magnitude of molecular weight.

3.1.3. *Measurements of soft tissues.* Six samples of beef liver, two samples of beef heart muscle, one sample of beef brain, two samples of pig muscle, and two samples of pig subcutaneous fat were measured. Three of the six samples of liver came from the same animal. The results of B/A measurements of these tissues, together with velocity and density data, are listed in Table 3.

Beef liver had an average B/A value of approximately 7.7, with a standard deviation of 0.9. It is not clear whether this standard deviation is a result of the variation in individual samples or uncertainties in the experimental technique. However, of the six samples of liver, the two samples that deviated most from the average B/A value, and from each other, came from the same animal. Thus, if the variation is due to differences in samples, the B/A value actually varies within the liver. Alternatively, if it is assumed that the B/A value is approximately the same in different parts of a liver, the variation would have to be attributable to the experimental technique.

Beef liver, beef heart, beef brain, and pig muscle all have B/A values between 6.8 and 8.1. However, pig fat has a much greater B/A value, namely, around 11, which possibly reflects the high fat content in fatty tissues, which may reach 75% of the total weight (Wells, 1977).

3.2. *Results of thermodynamic measurements*

Results of the thermodynamic measurements and the parameters used for computations are summarized in Table 4, all at 30°C. A major purpose of these measurements was to confirm the results of the finite amplitude measurements of B/A and to use the superior accuracy of the thermodynamic method to resolve small differences in the values of B/A of dextran solutions of different solute molecular weights. Measurements were made of degassed and distilled water, BSA solutions, dextrose and dextran solutions, whole and homogenized beef liver and pig fat.

3.2.1. *Comparison of thermodynamic and finite amplitude methods.* Results of the thermodynamic and finite amplitude measurements are listed in Table 5. When only one or two samples are measured, the results are listed individually. The data in Table 5 were accumulated over a two-year period and, consequently, may reflect sample differences and slight variations in the technique of sample preparation. Despite such possible effects, the agreement among the liquid samples is considered excellent, namely, ±4%.

In view of the good agreement between the thermodynamic method and the more recent finite amplitude measurements, the present finite amplitude system and its calibration is judged to be satisfactory for measurements of liquid samples.

Table 4. Values of B/A determined by the thermodynamic method

Material	ρ (g cm^{-3})	c (m s^{-1})	β ($°C^{-1}$)	C_p (J g^{-1} $°C^{-1}$)	$(\partial c/\partial p)_T$ (m s^{-1} psi^{-1})	$(\partial c/\partial T)$ (m s^{-1} $°C^{-1}$)	$(B/A)_P$	$(B/A)_T$	B/A
Water	0.996	1509	3.67×10^{-4}	4.18	0.0119	1.748	5.17	0.14	5.31
BSA (38.8%)	1.094	1615	3.67×10^{-4}	4.18	0.0130	1.115	6.58	0.10	6.68
Dextrose (25%)	1.092	1602	3.67×10^{-4}	4.18	0.0115	1.345	5.84	0.12	5.96
Dextran T150 (24%)	1.088	1567	3.67×10^{-4}	4.18	0.0119	1.676	5.91	0.14	6.05
Destran T2000 (26%)	1.090	1574	3.67×10^{-4}	4.18	0.0120	1.550	5.90	0.13	6.03
Beef liver	1.05	1588	3.67×10^{-4}	4.18	0.0147	1.15	7.13	0.10	7.23
Pig fat	0.93	1440	3.67×10^{-4}	4.18	0.0285	−2.98	11.08	−0.23	10.9

Table 5. Comparison of B/A values determined by the thermodynamic and finite amplitude methods

Material	(B/A) Thermodynamic	(B/A) Finite amplitude
Water	5.31	5.5 ± 0.5†
Dextrose (25%)	5.96	—
Dextrose (28.9%)	—	6.04
Dextran T150 (24%)	6.05	5.94
Dextran T2000 (26.4%)	6.03	6.2
Beef liver	7.23, 7.0	7.7 ± 0.9‡
Beef liver homogenate	7.0, 6.53	6.8 ± 0.4§
Pig fat	10.9	11.0
		11.3

† Average of seven samples.
‡ Average of five samples.
§ Average of three samples.

The agreement in tissue samples appears to be affected by the nature of the sample. The averaged values of B/A determined by the two methods differs by 9% for liver samples, 2% for pig fat and 1% for liver homogenate. The discrepancies appear to increase with the degree of tissue inhomogeneity. The liver homogenate is the most homogeneous among the three, followed by pig fat, which contains few blood vessels of various sizes and orientations. The standard deviation of the values of B/A of whole liver measured by the finite amplitude method is ± 12%, perhaps an indication of the significance of tissue structure and inhomogeneity.

The thermodynamic method measurements of dextran solutions confirmed the results obtained by the finite amplitude method. The B/A values of dextrose and dextran solutions are relatively insensitive to the molecular weight of the solutes, namely, a possible 2% increase over the range of molecular weights 10^1–10^6, as shown in Fig. 6.

A decrease in the value of B/A for homogenized liver as compared to whole liver is also confirmed by the thermodynamic method. It seems likely that the process of homogenization destroys nonlinearity-producing aspects of liver structure.

4. SUMMARY AND CONCLUDING REMARKS

The nonlinearity parameter B/A has been determined for tissue models (aqueous solutions of biological macromolecules) and for excised tissues using the finite amplitude and thermodynamic methods. The two methods yielded excellent agreement for the liquid samples and clearly acceptable agreement for the soft tissues (± 10%). The discrepancy between the two methods for tissues is most probably due to the combined effects of tissue inhomogeneity and the flexible and deformable nature of tissue which made the measurement of the sample thicknesses less accurate, as compared to the liquids. The formation of gas bubbles, as a result of autolysis, may also contribute to the discrepancy.

The general demeanor of the tissue model results is that B/A increases nearly linearly with solute concentration but, for a fixed concentration, is relatively insensitive to the molecular weight of the solute molecules. Also, as previously reported (Law *et al.*, 1981), whole mammalian blood, whose dry weight consists largely of protein, exhibits a B/A value slightly larger than that of protein solutions of the same dry weight concentration. Excised mammalian liver yields a B/A value significantly greater than blood or protein solutions of the same dry weight content, but homogenization of the tissue reduces this B/A value. Among the soft tissues measured, fat has the highest B/A value.

It is of interest to compare these experimental findings with the theory of intermolecular potential as a physical basis for the nonlinearity in a medium (Hartman, 1979). In these models, it is assumed that each of the molecules is a rigid sphere which moves under the force of an acoustic wave, against the forces which hold the molecules together. It is further assumed that the intermolecular potential is at a minimum at the equilibrium molecular separation, and it is more difficult to force the molecules together than it is to pull them apart. As a result, when a liquid is compressed to a smaller volume, it becomes more difficult to compress.

When *applied to a solution,* this model implies that three kinds of interactions between molecules are possible, e.g. solute–solvent, solute–solute, and solvent–solvent. The solvent–solvent interaction accounts for the baseline value of B/A at zero concen-

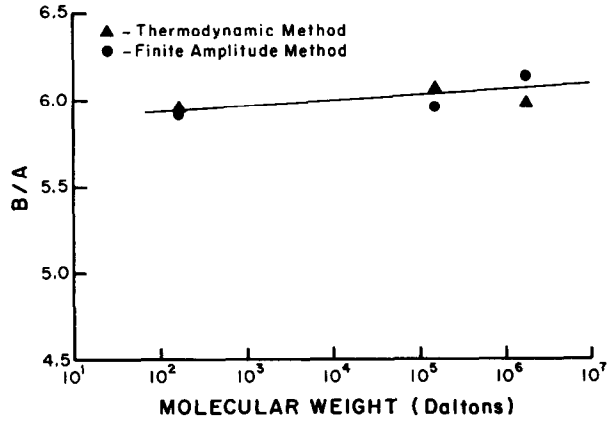

Fig. 6. B/A *vs* solute molecular weight for dextran solutions.

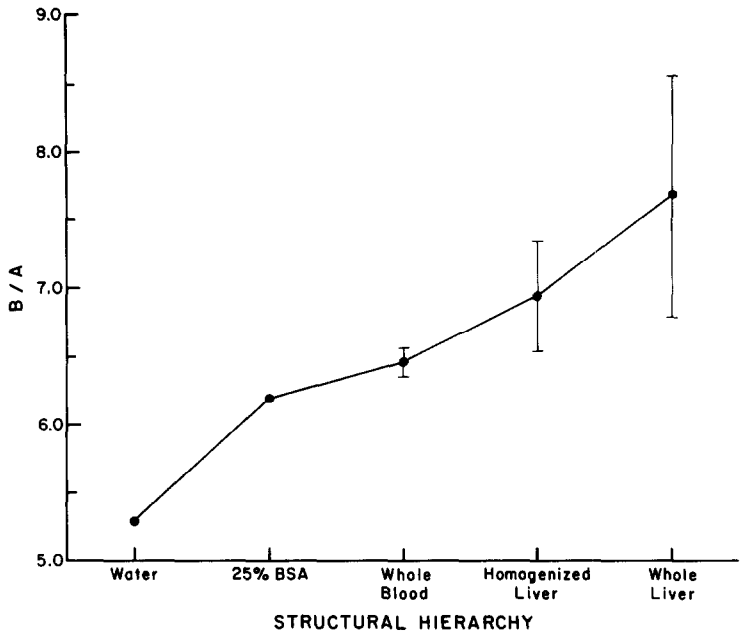

Fig. 7. Schematic representation of B/A *vs* specimen structural hierarchy.

tration. The lack of dependence of the B/A value on solute molecular weight suggests that the interaction between molecular subunits within a solute macromolecule does not contribute significantly to the nonlinearity of the solution. This observation is consistent with the assumption of rigid molecules in the theory of intermolecular potential (Hartman, 1979). The solute–solute interaction also appears to contribute insignificantly to the nonlinearity of the medium, since the amount of interaction between solute molecules should increase more than linearly with the number of solute molecules present, thus resulting in a nonlinear increase of B/A with concentration, which is not supported by the experimental observations. However, the observed linear increase of B/A values with concentration is consistent with the theory of solute–solvent interaction. Each interaction between a solute molecule and the solvent can be viewed as a 'nonlinear spring'. The number of such 'nonlinear springs', and hence the overall nonlinearity of the medium, increases linearly with the amount of solute, namely, the concentration of the solution.

The greater value of B/A *for tissue* as compared with protein solutions of the same dry weight content, together with the decrease of B/A value with tissue homogenization, suggest that the structure of a tissue plays an important role in determining its B/A value. One may speculate that the nonlinear interaction exists on two levels: an intermolecular level and an intercellular, or macrostructural, level. The binding or interaction between microstructures again acts as 'nonlinear springs', increasing the B/A value of a tissue above that of the constituent protein solutions. When such structures are destroyed by homogenization, the value of B/A then decreases. This dependence of the B/A value on structure is illustrated in a schematic diagram of B/A vs structural hierarchy in Fig. 7. The values of B/A are shown to increase progressively with increasing hierarchy in structure.

It is felt that with the knowledge of the nonlinearity parameter of tissue presented herein, a better understanding of the nonlinear interaction of ultrasound with biological media can be achieved. To exploit the potential use of the nonlinearity parameter in diagnostics and tissue characterization, an *in vivo* method of B/A measurement should be developed. Measurements of B/A in diseased tissues, which may be structurally different than normal tissues, for example cirrhotic liver, should also be performed. Finally, to understand better the mechanism behind the nonlinear properties of tissue, more sophisticated models of tissues should be investigated, for example using liposomes (Strom-Jensen *et al.*, 1984) as a model for membraneous cells may be profitable.

Acknowledgement—The authors acknowledge gratefully the partial support of this research by grants from the NSF and the NIH.

REFERENCES

Beyer R. T. (1960) Parameter of nonlinearity in fluids. *J. Acoust. Soc. Am.* **32**, 719–721.

Bowman H. F., Cravalho E. G. and Woods M. (1975) Theory, measurement, and application of thermal properties of bioma-

terials. In *Annual Review of Biophysics and Bioengineering* (Edited by L. J. Mullins, W. A. Hagins, L. Stryer and C. Newton), Vol. 4, pp. 43–80. Annual Reviews Inc., Palo Alto.

Carstensen E. L., Law W. K., McKay N. D. and Muir T. G. (1980) Demonstration of nonlinear acoustical effects at biomedical frequencies and intensities. *Ultrasound Med. Biol.* **6,** 359–368.

Carstensen E. L., Becroft S. A., Law W. K. and Barbee D. B. (1981) Finite amplitude effects on the thresholds for lesion production in tissue by unfocused ultrasound. *J. Acoust. Soc. Am.* **70,** 302–309.

Carstensen E. L., McKay N. D. and Delecki D. (1982) Absorption of finite amplitude ultrasound in tissues. *Acoustica* **51,** 116–123.

Cobb W. N. (1983) Finite amplitude method for the determination of the acoustic nonlinearity parameter *B/A*. *J. Acoust. Soc. Am.* **73,** 1525–1532.

Duck F. A. and Starritt H. C. (1983) Acoustic shock generation by ultrasonic imaging equipment. *Br. J. Radiol.* **57,** 231–240.

Dunn F., Law W. K. and Frizzell L. A. (1981) Nonlinear ultrasonic wave propagation in biological materials. *IEEE Ultrasonics Symp.* pp. 527–532.

Dunn F., Law W. K. and Frizzell L. A. (1982) Nonlinear ultrasonic propagation in biological media. *Br. J. Cancer* **45,** Suppl. V, 55–58.

Dunn F., Law W. K. and Frizzell L. A. (1984) The ultrasonic nonlinearity parameter for biological media. *Arch. Acoust.* **9.**

Goss S. A. and Fry F. J. (1981) Nonlinear acoustic behavior in focused ultrasonic fields: observations of intensity dependent absorption in biological tissue. *IEEE Trans. Sonics Ultrason.* SU-28.

Greenspan M. and Tschiegg C. E. (1959) Tables of the speed of sound in water. *J. Acoust. Soc. Am.* **31,** 75–76.

Hartman B. (1979) Potential energy effects on the sound speed in liquids. *J. Acoust. Soc. Am.* **65,** 1392–1396.

Ichida N., Sato T. and Linzer M. (1983) Imaging the nonlinear ultrasonic parameter. *Ultrasonic Imaging* **5,** 295–299.

Ingenito F. and Williams A. O., Jr. (1971) Calculation of second-harmonic generation in a piston beam. *J. Acoust. Soc. Am.* **49,** 319–328.

Kinsler L. E., Austin R. F., Coppens A. B. and Sanders J. V. (1982) *Fundamentals of Acoustics.* Wiley, New York.

Law W. K., Frizzell L. A. and Dunn F. (1981) Ultrasonic determination of the nonlinearity parameter *B/A* for biological media. *J. Acoust. Soc. Am.* **69,** 1210–1212.

Law W. K., Frizzell L. A. and Dunn F. (1983) Comparison of thermodynamic and finite amplitude methods of *B/A* determination in biological materials. *J. Acoust. Soc. Am.* **74,** 1295–1297.

Muir T. G. (1980) Nonlinear effects in acoustical imaging. In *Acoustical Imaging* (Edited by K. T. Wang), Vol. 9. Plenum Press, New York.

Muir T. G. and Carstensen E. L. (1980) Prediction of nonlinear acoustical effects at biomedical frequencies and intensities. *Ultrasound Med. Biol.* **6,** 345–357.

Strom-Jensen P. R., Magin R. L. and Dunn F. (1984) Ultrasonic evidence for structural relaxation in large unilamellar liposomes. *Biochem. Biophys. Acta* **769,** 179–186.

Wells P. N. T. (1977) *Biomedical Ultrasonics,* p. 121. Academic Press, London.