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MEASUREMENT OF ACOUSTIC NONLINEAR PARAMETERS AND SOUND SPEED IN EXCISED HUMAN LIVERS

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ABSTRACT

In this study we used a pressure jump method to measure acoustic nonlinearity parameter B/A in excised normal and diseased liver tissues. This method follows the time course of phase change of a transmitted sound signal as the hydrostatic pressure on the medium is altered. From the transient and steady-state phase changes due to the pressure release, isentropic and isothermal components of B/A are determined. Sound speed, c, in each liver sample is measured and a portion of liver sample is analyzed for fat and water content. A quantitative relationship between the acoustic parameters and composition is observed. Conversely, this relationship is used to determine tissue composition from the measurement of B/A and c parameters.

INTRODUCTION

The hypothesis underlying the application of ultrasound for tissue characterization is that pathologic changes alter the physical and the chemical properties of a tissue medium and the changes can be detected and quantified by ultrasound. Several ultrasonic parameters have been used in the past to characterize tissues and these studies have been extensively reviewed [4,7,9]. The objective of the majority of these studies was to use sound parameter as numerical indices of tissue state. However, the problem of determining tissue composition from the acoustic indices has received little attention. This paper addresses this point of view and proposes the use of sound speed and acoustic nonlinearity parameter, B/A, to determine tissue composition. This possibility has also been independently proposed by Apfel [1] recently.

Hypothesis

Sound speed in human liver decreases with increasing fat and water content [2]. In rat liver the sound speed, c, decreases at the rate of 2.3 m/sec per unit increase in the weight percent of fat concentration [13]. On the other hand, acoustic nonlinearity, B/A, in breast fat is about 1.4 times greater than parenchymal tissue [11]. Also pig fat is highly nonlinear to sound propagation [8]. These results clearly indicate a close relationship between sound parameters c and B/A and tissue composition, in particular the relative amounts of water and fat. Thus, it may be hypothesized that from the measurement of sound speed and B/A it should be feasible to determine tissue composition. To test this hypothesis we studied excised human livers. Sound speed and B/A measurements were made on livers containing variable amounts of fat and water by using the methods summarized below and described earlier [11,12].

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Experimental

Fresh human liver, obtained within an hour after postmortem examination, was enclosed in a specially designed pressure chamber. The sample was degassed by alternately compressing and releasing the pressure inside the chamber. B/A measurements were made by following the time course of phase change of a transmitted sound signal as the hydrostatic pressure on the medium was reduced quickly from 950 psi. The instantaneous and the equilibrium phase changes during the pressure release yield the coefficients of change in sound speed with pressure and these values in turn were used to determine isentropic and isothermal values of B/A [11]. Sound speed was measured at 20°C by measuring the transmission time of a shock excited pulse. Sound speed at higher temperature was determined by measuring the change in phase of received continuous wave ultrasound as the temperature was raised to a known temperature. From the phase change, time delay was determined and used to calculate sound speed at the new temperature [11]. Acoustic measurements were made in the temperature range of 30°-37°C and in total the 16 samples of livers were studied.

A portion of each sample used for acoustic measurements was analyzed for its composition and histology. For histologic analysis the sample was fixed in formalin, hematoxylin and eosin stained paraffin sections and examined by a pathologist. Chemical analysis involved measurement of percentage weights of water and fat. Water content was determined by heating a known weight of tissue at 70°-80°C in an oven for about 2-3 hours. The loss in water, as indicated by the sample acquiring constant weight, was used to determine percent water content. Lipids were extracted from the tissue samples by 1:1 mixture of isopropanol and diethyl ether [5]. Aliquots of extracts were analyzed for triglycerides, cholesterol, and phospholipids by standard clinical nonenzymic method described by Ellefson and Caraway [6]. The net sum of these three components was taken as the total fat content.

RESULTS

Table 1 summarizes the composition and acoustic parameters in various excised livers. Except for fatty livers, all the diseased livers contain larger fraction of water than the normal livers. In all the diseased livers studied, except the one with lymphatic leukemia, sound speed was lower than the normal counterpart. The values of B/A and (B/A)' for normal liver were found to be ~ 30% greater than the values reported for water. These parameters for cirrhotic and tumorous liver (high water content) were smaller than the corresponding values of normal livers. As one would expect on the basis of earlier measurements [8,11], fatty liver was significantly more nonlinear than the normal samples.

DISCUSSION

To determine how sound speed and B/A parameters of various components add, let us consider layers of water, fat, and proteins one on top of the other and enclosed in a container of a fixed area of cross section. If one assumes that the net time taken for a sound wave to travel through the mixture is the sum of the time taken to travel through the individual

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Table 1. Summary of acoustic measurements and tissue composition made on excised normal and diseased human livers

Tissue state	T (°C)	c (m/sec)	B/A	(B/A)'	x_W	x_F
Normal (6)	20 30 37	1567 1584 1592	6.31 6.72 6.75	6.04 6.52 6.61	0.7633	0.0361
Congestive (3)	20 30 37	1550 1572 1581	6.33 6.50 6.88	6.06 6.30 6.64	0.8031	0.0308
Lymphoma Leukemia (1)	20 30 37	1566 1584 1595	5.97 6.29 6.37	5.63 6.15 6.22	0.8072	0.0285
Carcinoma (2)	20 30 37	1553 1569 1579	6.17 6.15 6.34	6.03 6.00 6.11	0.8433[a]	0.0362[a]
Cirrhosis (1)	20 30 37	1546 1594 1562	6.16 6.01 6.07	5.43 5.88 5.88	0.8389	0.0155
Fatty (slight) (2)	20 30 37	1560 1574 1581	6.87 7.11 7.46	6.67 6.98 7.24	0.7615	0.0431
Fatty (marked) (1)	20 30 37	1528 1527 1522	8.36 8.83 9.12	8.29 9.06 9.44	0.6066	0.2736

Numerals in parenthesis in the first column of the table represent number of samples studied; (a) Determined for only one sample.

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components, the resultant sound speed through the mixture is related to its components by the following expression [12]:

$$\frac{1}{c} = \frac{x_F}{c_F} + \frac{x_W}{c_W} + \frac{x_R}{c_R} \qquad (1)$$

$$\text{or } 1/c = 1/c_R + (1/c_W - 1/c_R)\, x_W + (1/c_F - 1/c_R)\, x_F, \qquad (2)$$

where, c and x represent sound speed and volume fractions and the subscript F, W, and R represent the three components fat, water, and protein, respectively. Since B/A is related to the product of sound speed and its differential with respect to pressure (Eq. 6 of [3]), Eq. 1 can be used under the simplifying assumption that the volume fractions do not change with pressure to obtain the following mixture law:

$$(B/A)/\rho c^3 + [(B/A)/\rho c^3]_F\, x_F + [(B/A)/\rho c^3]_W\, x_W + [(B/A)/\rho c^3]_R\, x_R \quad (3)$$

$$\text{or } N = N_R + (N_W - N_R)\, x_W + (N_F - N_R)\, x_F, \quad (4)$$

where the symbol N represents the quantity $(B/A)/\rho c^3$ and the subscripts F, W, and R represent the fat, water, and protein components. It must be noted that Eqs. 1 to 3 assume the ideal mixing conditions, i.e., volume of mixing is zero. If one assumes biological tissues to be ideal mixtures of water, fat, and a residual component consisting largely of proteins and carbohydrates the quantities c and N should be a bivariate function of x_W and x_F according to Eqs. 2 and 5. Therefore, the measured data of sound speed and N was correlated to tissue composition x_W and x_F by multiple linear regression analysis to obtain equations of the type,

$$1/c = (5.088E\text{-}6) + (1.515E\text{-}6)\, x_W + (1.982E\text{-}6)\, x_F \quad (5)$$

$$N = (1.419\text{-}15) + (0.0443E\text{-}15)\, x_W + (3.617E\text{-}15)\, x_F. \quad (6)$$

Sound speed and density in the above equations are expressed in cm/sec and gm/ml, respectively. This pair of equations were obtained by using the data measured at 30°C. Similar equations were obtained for temperatures of 20°C and 37°C. The pairs of equations of the type 5 and 6 can be rearranged so that x_W and x_F become independent variables and 1/c and N are the dependent variables, i.e.,

$$x_F = -(3.569E\text{-}1) + (2.810E14)\, N - (8.216E3)/c \quad (7)$$

$$x_W = -(2.891) - (3.676E14)\, N + (6.708E5)/c. \quad (8)$$

Equations 7 and 10 enable us to determine x_F and x_W from the measured values of sound speed, acoustic nonlinearity and the density of the medium. To test the equations we measured B/A, c, and density of egg white and egg yolk of chicken eggs. These values, tabulated in Table 2, were used in regression equations for 30°C and 37°C to predict tissue composition.

Figures 1 and 2 compare the predicted composition with the values quoted in the literature [10]. The predicted composition is within 7 percent of the values quoted in the literature [10]. As one would expect, egg white is found to be rich in water whereas egg yolk is rich in fat.

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Table 2. Sound speed and nonlinearity parameters in egg white and egg yolk of chicken eggs

Medium	T (°C)	ρ (gm/ml)	c (m/sec)	B/A
Egg white	30	1.07	1562	5.81
	37	1.07	1571	5.84
Egg yolk	30	1.02	1505	9.76
	37	1.02	1500	9.95

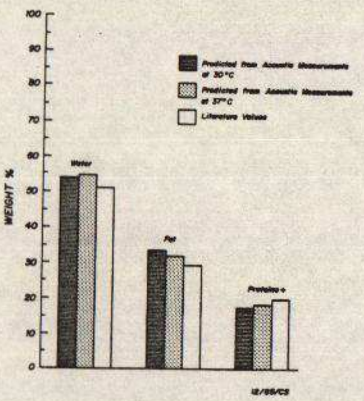

Fig. 1. Comparison between the true and the predicted composition of egg yolk.

We also used the B/A, c, and density data on bovine serum albumin, whole blood, and various solutions of dextran [8] to determine their composition. Fig. 3 summarizes the relationship between the predicted and the true composition. There is a 1:1 relation with a regression coefficient of 0.998 between the predicted and true composition. It must be noted that the minor components as indicated by the triangles in Fig. 3 show negative values, thereby implying the volume of the mixture to be greater than the individual components by 1 to 4 percent. Although not verifiable at the present time, one possible explanation for this artifact could be related to the simplifying assumption that biological tissues are ideal mixtures of their components, which may not be completely true.

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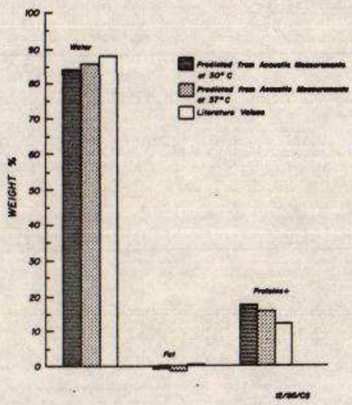

Fig. 2. Comparison between the true and the predicted composition of egg white.

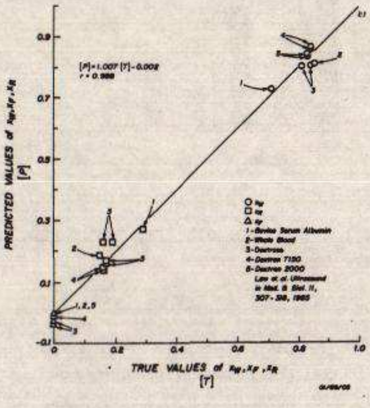

Fig. 3. Comparison between the true and the predicted composition of (1) bovine serum albumin, (2) whole blood, (3) dextrose, (4) dextran T150, (5) dextran 2000.

In conclusion, in this paper we presented data on sound speed and acoustic nonlinearity and proposed a method that can be used in in vitro situations to determine composition of tissues from the acoustic measurements.

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