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ULTRASONIC ATTENUATION AND BACKSCATTERING BY MAMMALIAN ORGANS AS A FUNCTION OF TIME AFTER EXCISION

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Abstract—Ultrasonic attenuation and backscattering were measured for bovine brain, spleen and liver and for porcine liver as a function of time after excision for times up to 120 hr. The attenuation coefficient exhibits insignificant changes while the mean backscattering amplitude decreases substantially during the period such specimens are likely to be used. The changes in the two parameters are believed to reflect, in large measure, their origins, viz., the molecular level for attenuation and macrostructural level for backscattering.

INTRODUCTION

Current and continuing clinical interest in ultrasound has established the need for complete specification of the acoustic characteristics of selected tissues and organs and of their pathological states (Linzer, 1976). Instrumentation, precision in clinical diagnoses, and patient safety may well be beneficiaries of collations of acoustic parameters such as absorption, attenuation, scattering, velocity, impedance, elastic constants, etc., as functions of frequency, temperature, age, treatment, pathological state, etc. A few research programs have been concerned with providing such details, and much useful information has become available (Dunn *et al.*, 1969). However, recent commentaries on the availability of such information from the literature (Chivers and Hill, 1975a, b; Wells, 1975) suggest far more concentrated efforts will have to be mounted to amass the quantity of data required for the desired developments to materialize. Much of this is likely to be accomplished with animal tissues *in vivo* and/or fresh *in vitro* preparations. However, it may always be the goal to have final values from human tissues, in particular for those of pathological interest, and this may mean that specimens obtained during autopsy will come to be relied upon greatly. Two major questions arise herein with regard to the usefulness of such material. First, controversy over the stability of some of the acoustic parameters of interest has long existed (Dunn, 1965). An early observation (Hueter, 1958) that substantial reduction in ultrasonic attenuation by liver occurs with progressive autolysis, appears now (possibly) to have been overstated (Frizzell, 1976). Five day aging of human brain is reported to reduce attenuation 20% (Kremkau *et al.*, 1976). Second, the relationship of acoustic parameters obtained from autopsied material to that obtained *in vivo* can be questioned. For some purposes cessation of blood flow may render the tissue of interest unusable (Lomonaco *et al.*, 1975) though von Gierke found no significant change in the attenuation coefficient between the living and dead states (Dunn, 1965).

The present study was undertaken to examine ultrasonic attenuation and backscattering with the view toward documenting any changes occurring from time of death for some 100 hr, for several unstable (friable) tissues. It was a goal of the study that conclusions be drawn regarding the value, for specific ultrasonic measurements, of human tissues obtained at autopsy. The brief study (Crosby, 1974) of the effect upon ultrasonic attenuation in excised beef spleen, as a function of time for different tissue handling methods and storage regimes, aided the planning of this investigation.

METHODS

The method employed for the attenuation measurements has been described previously (Papadakis *et al.*, 1973; Chivers and Hill, 1975a). Briefly, a pulse of ultrasound, emitted by an appropriate transducer, is reflected from a plane surface situated normal to the direction of sound propagation. The reflected pulse, which is isolated by a time-gating circuit, is received by the same transducer, amplified, and fed to a spectrum analyzer which displays its Fourier transform. The attenuation due to a double traverse of the tissue specimen cut to have plane parallel surfaces, is the difference between the received echo signal with and without the sample interposed between transducer and plane reflector, provided a logarithmic spectral display of amplitude is employed.

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The pulse durations chosen allowed the frequency range of interest to be covered by the smallest number of transducers. Thus, three transducers, having norminal frequencies of 2.5, 5 and 6 MHz, respectively, cover the frequency range 1.5 to 7 MHz and in this study attention was devoted to the range 4 to 6 MHz. The gate length was chosen such that its spectrum would not affect that of the echo pulse.

The 10 mm diameter transducer had a 6 dB beam width of 2.5 mm at, and was positioned 45 mm from, the center of the specimen. The reference plane surface was placed 70 mm from the transducer to reduce the diffraction correction to approximately $0.02\,\mathrm{dB\,cm^{-1}}$, i.e. a negligible amount. The methodology was tested and verified by making measurements with materials of known acoustic properties, viz., castor oil and cotton seed oil (Dunn and Breyer, 1962). This procedure also provided the accuracy in the attenuation coefficient obtained by this method as $\pm 0.5\,\mathrm{dB\,cm^{-1}}$.

A small computer, linked to the system, facilitated data collection and analysis.

The method used in making the back-scattering observations has also been discribed previously (Nicholas and Hill, 1975). Briefly, an approximately circular cylindical tissue specimen was positioned with its long axis normal to and directly in the path of the sound beam, the acoustic coupling medium being water. The transducer was employed as both the transmitter and the receiver. The specimen was rotated about its cylindrical axis such that the amplitude of the backscattered signal could be obtained from all angles. A time gate, centred on the axis of rotation, selected a portion of the echo signal, the total such received signal thus corresponding to the backscattering from a volume of tissue determined by the beam width and the time gate. The signal is processed to provide the amplitude of the volume-backscattered signal, at a particular ultrasonic frequency, as a function of angular rotation. Scattering patterns so obtained appear to be characteristic of the tissue viewed (Nicholas and Hill, 1975).

The 22 mm diameter 1 MHz transducer has a 6 dB beam width of 6.0 mm at, and was positioned 48 mm from the center of the specimen. For the 4 MHz transducer these values are, respectively, 15 mm diameter, 3.5 mm beam width, and 136 mm distance. For both transducers the distance of the transducer face to the center of the specimen was chosen to be a few millimeters into the Fraunhofer diffraction zone.

The tissues, viz., bovine spleen, brain and liver and porcine liver, were obtained at commercial slaughter houses immediately after, and brought to the laboratory within 30 min of slaughter. Thus, the exact times of death and excision were known. Except for the short transit period, the tissues were kept at room temperature for the entire period of observation as this was held to be a "nearly worst possible situation" as regards preservation of tissue morphology. A second such "worst possible situation" was the acoustic coupling fluid, viz., tap water, a small volume of which was in contact with the tissues throughout. These two "situations" were tolerated as it was felt that tissue degeneration would be promoted, making the results more prominent and easier to interpret.

RESULTS

Attenuation

The following results involved one sample of beef brain, three of beef spleen, four of beef liver and three of pig liver. The specimens were slabs having plane, parallel major faces of approximately $50\,\mathrm{cm^2}$ and ranging in thickness from 1.4 to 3.0 cm. It was found necessary to remove gas accumulating within the specimen (associated with autolysis). Acceptable procedures involved manual manipulation, until bubbles ceased appearing on the upper surface, or placing the entire preparation in an environment of reduced pressure, to nearly the vapor pressure of water, until bubbles ceased emerging from the sample volume. Omission of this procedure leads to extremely high values for the measured attenuation, particularly for the more advanced stages of tissue deterioration, e.g. in an extreme case for porcine liver at 5 MHz. the attenuation coefficient was immediately reduced form $14\,\mathrm{dB\,cm^{-1}}$ to $5\,\mathrm{dB\,cm^{-1}}$ upon invoking the second procedure described above. The attenuation coefficient was evaluated for 22 spectral components, in the frequency range of interest, and the two constants b and m computed for the relation

$$A = bf^m \tag{1}$$

where A is the ultrasonic attenuation coefficient in $\mathrm{dB\,cm^{-1}}$, f is frequency, b is the intercept and m is the slope of the regression line (computed by the method of least squares) corresponding to this relationship. The attenuation coefficient at the specific frequencies 4, 5 and 6 MHz were then obtained from equation 1 and these are used to characterize the dependence of the loss factor on time after excision. Measurements were made at numerous times during the working day with the specimens remaining in the sample holders in the apparatus for the full duration of observation, i.e., up to 120 hr after death of the animal, at which time they were putrid and offensive for handling.

The results of these observations largely confirm, though extend appreciably, those of Crosby (1974) and Frizzell (1976). Thus it is found that the *in vitro* change in ultrasonic attenuation, as a function of time after death, is insignificant over the time periods such tissues are likely to be used. Table 1 summarizes these findings and Fig. 1, showing examples of liver at 4 MHz spleen at 5 MHz, and brain at 6 MHz, may be considered typical of the time course of the observed attenuation. The apparent rapid changes in attenuation seen in Fig. 1 are believed to reflect spatial variations in attenuation in the samples and are possibly exaggerated here as tissues were placed in the beam path for measurement, removed and replaced for subsequent measurements with an uncertainty of approximately ± 0.5 mm in the position of the sample container. The equivocal nature of ultrasonic properties of liver, observed by others (Frizzell, 1976), is apparent in Table 1 as possibly exhibiting greater spatial variations than either spleen or brain. An additional factor may be the greater uncertainty as to whether all acoustically troublesome gas is eliminated from the propagation path. The uncertainty in any attenuation measurement, by the method employed herein, was approximately ± 0.5 dB cm^{-1}.

Backscattering

The results involve two bovine and one porcine spleen and one bovine and two porcine liver, specimens, each sculpted into a right circular cylinder of approximately 1 cm radius and 6 cm length. The cylinder of tissue was supported by,

Table 1. Initial and terminal values of attenuation coefficients

Tissue		Time after Death (hours)	Attenuation Coefficient (dB cm^{-1}) Frequency (MHz) 4	5	6	Remarks
Bovine Brain		1	3.8	4.9	5.9	
		49	3.5	4.5	5.5	
Bovine Spleen		1.5	3.1	4.3	5.8	Average initial values
	A	49	2.8	3.8	5.0	
	B	52	3.1	4.0	5.0	
	C	52	2.5	3.5	4.3	
Bovine liver		3	4.4	5.5	6.2	Average initial values
	A	122	3.6	5.1	6.7	
	B	122	5.8	6.8	7.8	
	C	122	4.1	5.1	6.2	
	D	122	8.0	7.5	7.0	
Porcine Liver		4.5	4.7	6.3	7.9	Average initial values
	A	79.5	4.8	6.3	7.9	
	B	79.5	4.3	7.2	10.9	
	C	79.5	3.4	4.8	6.4	

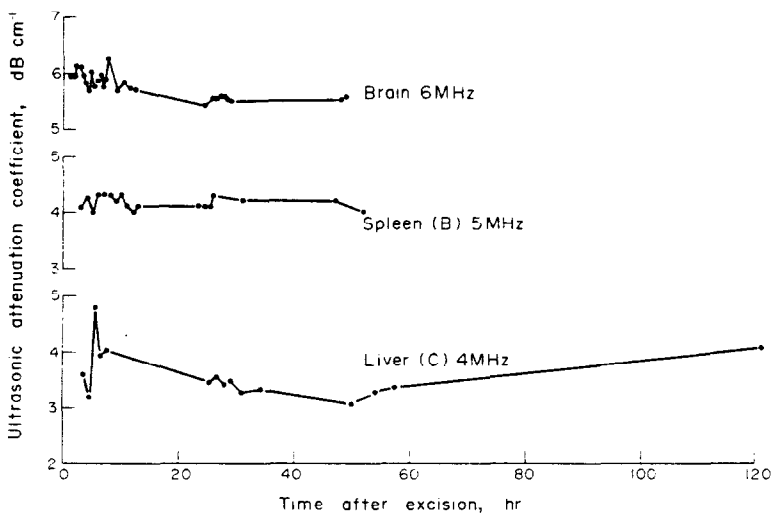

Fig. 1. Examples of ultrasonic attenuation of bovine liver, spleen and brain at, respectively, 4, 5 and 6 MHz, as a function of time after excision.

and encapsulated in, a closely fitting polyethylene cylinder having 76 μm wall thickness. The lower end of the plastic cylinder was heat-sealed closed and a brass disc, 3.2 mm thick having the same radius as the tissue specimen, was placed in the bottom to provide sufficient weight to ensure that the vertical orientation was always maintained. The cylindrically cut tissue specimen was then inserted over the disc to fill the polythylene cylinder, whose upper end was closed off by a brass fixture containing a rim-grooved disc that could be secured to the plastic with a rubber O-ring. The brass fixture also contained a shaft for attaching to a stepping motor capable of rotating the specimen about its long axis. Intimate acoustic coupling from the transducer to the specimen was assured as both were submerged in the water-filled sound tank and all gaseous inclusions were removed from the tissue holder by manual manipulation under water.

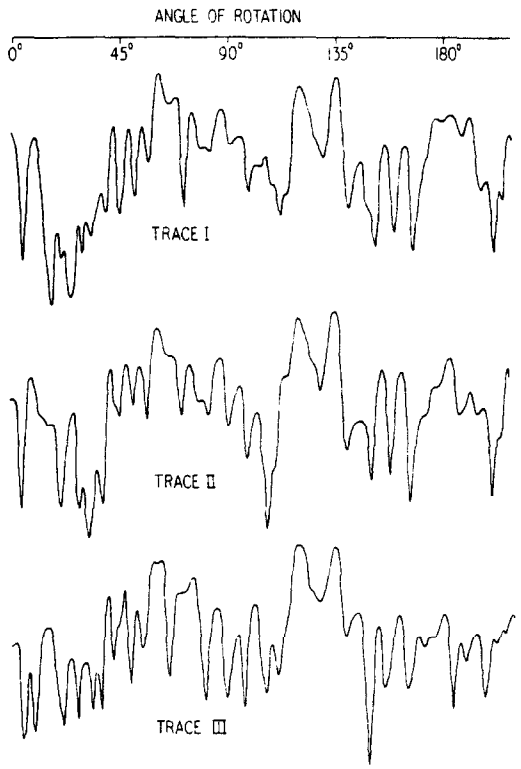

Fig. 2. Examples of 1 MHz ultrasonic backscattered signal amplitude component as a function of angle of rotation (approximately 180°) for a sample (A) of bovine spleen.

Trace No.	Time after excision (hr)	Mean backscattering amplitude (dB rel perfect reflector)	Range of mean backscattering amplitude (dB)
I	5	−66	31
II	9	−67	32
III	28.5	−68	30

The time gate of 5 μs was chosen to be entirely within the tissue specimen, and the energy reflected back to the transducer was recorded, for a full revolution of the sample, at 1000 discrete angular positions. Figure 2 shows examples of the data so obtained from which it is seen that the relative mean backscattering amplitude, the number of crossings at the mean (or any other level), and possibly other characteristics may be chosen for observance of change of the tissue with time after excision. As change in only a single one of the characteristics is necessary, and possibly sufficient, to identify change in the acoustic properties of a tissue, the mean backscattering amplitude was selected. (It should be noted that arbitrary selection of the characteristics monitored does not guarantee that the most sensitive one is chosen.)

Figure 3 shows the relative mean backscattering signal amplitude as a function of time after excision for bovine spleen at 1 MHz and porcine liver at 4 MHz. It is seen that backscattering amplitude changes of the order of 100% are manifest after 1 day with the spleen, remaining essentially unchanged for the next 4 days, while the liver sample changed by more than 300% during the first day with continued unidirectional changes on succeding days. Table 2 shows the initial and terminal values for the relative mean backscattering amplitude for all the tissue specimens investigated. Porcine liver

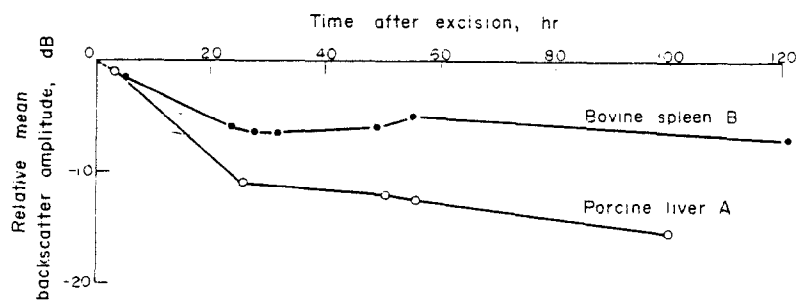

Fig. 3. Examples of relative mean ultrasonic backscattering amplitude as a function of time after excision. The values shown for the bovine spleen and porcine liver are relative to their respective mean backscattering amplitudes at zero time and thus should not be compared absolutely.

Table 2. Initial and terminal relative mean backscattering amplitude values

Tissue		Time after death (hours)	Relative mean Backscattering Amplitude (dB) 1 MHz	4 MHz	Remarks
Bovine Spleen	A	2.5	−0.6		
	A	28.5	−3		
	B	5	−1.5	−2	
	B	121	−7	−7	
Bovine Liver	A	5	+5	+5	Example of effect of
	A	121	+11	+7	not being able to remove all necessary gas
Porcine Spleen	A	2	−0.7		
	A	51	−2		
Porcine Liver	A	3	−2	−1	
	A	100	−20	−15.5	
	B	3	−1.5	0	Example of effect of
	B	50	+15	+11	not degassing

B and bovine liver A are good examples of failure to exclude all accumulating gas before measurement.

CONCLUSIONS

It has been shown that while ultrasonic attenuation of mammalian organs is relatively insensitive to the autolytic processess occurring after excision to at least five days, provided evolving gases are removed, ultrasonic backscattering shows appreciable changes beginning, possibly, at time of excision. Investigators concerned with attenuation measurements may, therefore, feel confident that tissues obtained from autopsies will yield sensible results for the periods they are likely to retain them (without fixation). However, those concerned with backscattering measurements must exhibit caution in their use of autopsy materials lest their measurements yield values not characteristic of *in vivo* organs.

Explanation of the difference in behavior exhibited by ultrasonic attenuation and backscattering, as a function of time after excision, may be sought at the level of biological structure at which their major components originate. Attenuation may be considered to be composed of absorption and scattering processes with the former accounting, for example, for approximately 75% of the loss at 4 MHz in liver (Pauly and Schwan, 1971). Thus absorption processes may be expected to dominate attenuation behavior. Tissue absorption is believed to be associated with macromolecular species, particularly protein interactions (Dunn *et al.*, 1969). Therefore, changes in attenuation would not be expected to become manifest until major volume fractions of macromolecular structures become degraded to much lower molecular weights than are present *in vivo*. Conversely, backscattering is believed to occur at the level of structure commensurate with the wavelength in the tissue of the interrogating radiation (Nicholas and Hill, 1975). Thus, for the ultrasonic radiation employed in this study, tissue structures of the order of a millimeter (e.g. the lobular structure of the liver) may be responsible for the backscattered signal. As it is these relatively large parenchymal structures that disintegrate first with autolytic processes (Weinbren, 1966), backscattering might be expected to exhibit early changes following death. This view is consistent with the observation (Figs. 2 and 3) that, while the mean backscattering amplitude decreases with time after excision, the range of variations of this parameter with rotation does not become appreciably altered. Thus the scattering structures continue to be observed, after death, though the ability to scatter acoustic energy wanes with increasing time.

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