In Vivo Measurement of Thickness or of Speed of Sound in Biological Tissue Structures

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Abstract—**Though the wave reflected from thin biological tissue structures is affected by interference, the wave transmitted through thin tissue is not. This implies the possibility of *in vivo* measurement of thickness or of sound speed in thin tissue structures. Spectroscopy facilitates the measurement. Measurement of these quantities are presented for plastic plates, and for *in vivo* thickness measurement of the mitral valve and *in vitro* measurement of the aortic and tricuspid valves of dog hearts. The values obtained for the thickness of the valves from *in vivo* and from *in vitro* acoustic measurements by this method and from micrometer measurement of the excised tissues compare favorably.**

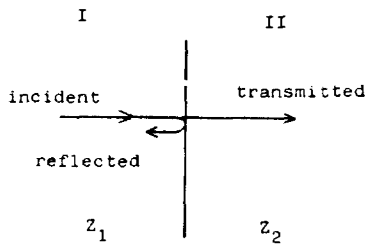

Fig. 1. Acoustic model for the extended media; Z_1, Z_2 are the acoustic impedances of Media I and II, respectively.

I. Introduction

IMPROVEMENTS in ultrasonic pulse echo diagnostic equipment have made it possible to observe abnormal echoes from the interior of some parenchymal organs and of heart muscle with cardiac myopathy and it may be possible to characterize tissues by using such echoes [1], [2]. However, these echoes are from tissue structures which are thinner than the length value of the range resolution. There are, of course, many other tissues of interest which also can be thinner than the range resolution length such as valves, blood vessel walls, stones, and neoplasms.

In the usual ultrasonic diagnostic equipment using the pulse echo method, only information of the amplitude of the echoes, obtained by envelope detection, and the time of arrival are used. However, in such a method the front and the back surface echoes from thin tissue sections are not separable and, therefore, cannot be used to obtain time of flight details in order to provide specimen thickness. A measuring method, in which sound speed or specimen thickness of thin biological tissue layers can be estimated from the echo spectrum, is described and used to obtain *in vivo* tissue details.

II. Theoretical Analysis of Ultrasonic Reflection from, and Transmission Through, Thin Biological Tissue Layers

In order to study the echo properties of thin biological tissues, it is necessary to know details of the reflectivity and the transmissivity of the sections in general. The wave-train-length of the conventional transducer used in the ultrasonic diagnosis is about 4 to 5 wavelengths. For example, the wave-train-length at the frequency of 3 MHz is about 2.5 mm in soft tissues, for which the sound speed is nearly that of water, viz., about 1.5×10^5 cm/s. If the thickness of a tissue section of interest is less than about a half of the wave-train-length, in other words, the range resolution, the front and the back surface echoes are not separable by the methods available as interference occurs.

We begin with a review of the reflectivity and the transmissivity in thick and thin layers. Planar interface and normal incidence are assumed. The reflection coefficient R and the transmission coefficient T, expressed in decibels, for the extended media in Fig. 1 are well-known for continuous wave (CW) operation [3], [4] as

$$R = 10 \log \left(\frac{Z_2/Z_1 - 1}{Z_2/Z_1 + 1}\right)^2 \tag{1}$$

$$T = 10 \log \frac{4Z_2/Z_1}{(Z_2/Z_1 + 1)^2}, \tag{2}$$

where Z_1 and Z_2 are the characteristic acoustic impedances of Media I and II, respectively. Note that the reflection and the transmission coefficients of the extended media in Fig. 1 are determined only by the acoustic impedances of these media. However, for an acoustic model for a thin layer interposed between two extended media, shown in Fig. 2 where only the situation where $Z_1 = Z_3$ is considered, the reflection and the transmission coefficients are determined not only by the acoustic impedances but also by the phase constant, the attenuation constant, and the length of the inserted medium. An equivalent electrical circuit of Fig. 2 and notations used are shown in Fig. 3 and in Table I, respectively. P_0, P_1, P_2 are (effective)

Manuscript received September 16, 1982; revised March 30, 1983. This work was supported in 1981 and 1982 by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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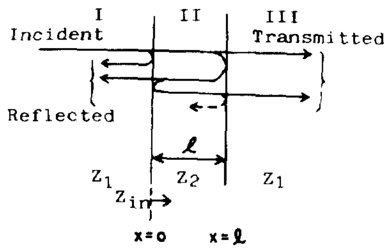

Fig. 2. Acoustic model for a thin layer interposed between two extended media. Z_1, Z_2 are the acoustic impedances. Z_{in} is the input impedance at $x = 0$. l is the thickness of Medium II. The reflected wave is mainly composed of the wave from the front surface of Medium II and one round trip wave in Medium II and it is affected by the interference. The waves of more than two round trips are negligible. The transmitted wave is mainly composed of the direct wave because the waves of three or more than five transits are negligible and is, therefore, not affected by the interference.

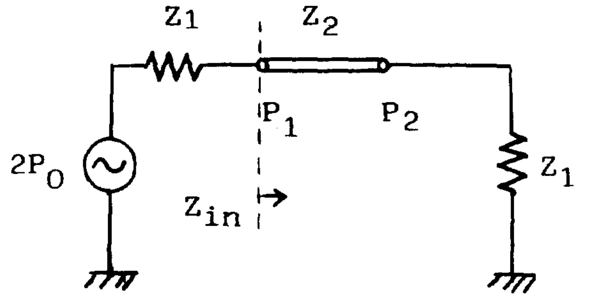

Fig. 3. Equivalent circuit of media represented in Fig. 2. Media I and III are represented by lumped elements and Medium II by a distributed element. P_0, P_1, P_2 are acoustic pressure values of the incident wave, the wave at the boundary of Media I and II and the wave transmitted through Medium II, respectively.

acoustic pressure values of the incident wave, the wave at the boundary of Media I and II and the wave transmitted through Medium II, respectively. It is assumed that CW principles are applicable in this case. The ratio Z_{in}/Z_1, where Z_{in} is the input impedance at the boundary between Media I and II, is [3], [4]

$$Z_{in}/Z_1 = d\left[\frac{1 + d \tanh \gamma l}{d + \tanh \gamma l}\right], \tag{3}$$

where

$$d = Z_2/Z_1, \tag{4}$$

γ is the propagation constant of Medium II, and l is the thickness (length) of Medium II.

The sound intensity reflection coefficient R' in Fig. 2 is given by the square of the absolute value of the sound pressure reflection coefficient as [3], [4],

$$R' = \left|\frac{Z_{in}/Z_1 - 1}{Z_{in}/Z_1 + 1}\right|^2, \tag{5}$$

and the reflection coefficient R, expressed in decibels, is

$$R = 10 \log \left|\frac{Z_{in}/Z_1 - 1}{Z_{in}/Z_1 + 1}\right|^2. \tag{6}$$

TABLE I
Notations Used in Fig. 2

	I	II	III
Acoustic Impedance	Z_1	Z_2	$Z_3 = Z_1$
Attenuation Constant	—	α	—
Phase Constant	—	β	—
Thickness	—	l	—

The transmission coefficient T is determined by forming the sound pressure ratio

$$P_2/P_0 = 2[2 \cosh \gamma l + (d + 1/d) \sinh \gamma l]^{-1} \tag{7}$$

where P_0, P_2 are, respectively, the pressure values of incident and transmitted waves.

Considering the equivalent circuit shown in Fig. 3, the sound intensity transmission coefficient T' is

$$T' = \frac{|P_2|^2/Z_1}{|2P_0|^2/4Z_1} = \left|\frac{P_2}{P_0}\right|^2. \tag{8}$$

Then, the transmission coefficient T, expressed in decibels, is

$$T = 10 \log T'$$

$$= 10 \log\left[4\left\{\left[2 \cosh \alpha l \cos \beta l + \left(d + \frac{1}{d}\right) \sinh \alpha l \cdot \cos \beta l\right]^2 + \left[2 \sinh \alpha l \sin \beta l + \left(d + \frac{1}{d}\right) \cosh \alpha l \cdot \sin \beta l\right]^2\right\}^{-1}\right]. \tag{9}$$

III. Calculation

It is assumed that fibrotic tissues are distributed focally among normal tissues [5]. In Fig. 2 Media I and III represent normal tissues and Medium II represents a layer of fibrotic tissue. The parameters for these media are listed in Table II [6], [7]. The calculated reflection and the transmission coefficients are shown in Figs. 4 and 5. As the trace in Fig. 4 is obtained for 3.5 MHz, the wavelength l_0 in Medium II is thus 0.494 mm.

It is seen that the reflection coefficient undulates in magnitude as the length of Medium II increases, with maxima appearing for lengths of an odd number of quarter wavelengths and dips appearing for lengths of an even number of quarter wavelengths. With increase of the thickness of Medium II, the reflection coefficient converges to the value given by (1), viz., $R = -29.6$ dB. Thus, it is emphasized that the reflection coefficient is not completely determined by (1), but by the phase relationship of the incident and the reflected waves in Medium II. The reflection coefficient thus depends upon the thickness, the speed and the attenuation in Medium II and the frequency of the wave, in addition to the ratio Z_2/Z_1, of the two acoustic impedances involved. In other words, the wave reflected from a thin medium contains information not only of the ratio of both acoustic impedances, Z_2/Z_1, but also the thickness, sound speed, and attenuation of the medium. The trace of the reflection coefficient versus frequency is shown

TABLE II
ACOUSTIC CONSTANTS [4][1]

		Normal Tissue Media I, II	Fibrotic Tissue Medium II
Acoustic Impedance	(Kg/m^2s)	$Z_1 = 1.717 \times 10^6$	$Z_2 = 1.834 \times 10^6$
Speed of Sound	(m/s)	$c_1 = 1620$	$c_2 = 1730$
Attenuation Constant [5)]	(dB/m)	–	$\alpha' = \begin{cases} 805 \text{ (@3.5 MHz)} \\ 230 \times f \times 10^{-6} \end{cases}$
Wavelength (@3.5 MHz)	(m)	–	$l_0 = 0.494 \times 10^{-3}$
Density	(Kg/m^3)	$\rho = 1.06 \times 10^3$	$\rho = 1.06 \times 10^3$

[1] $\alpha = \dfrac{\alpha'}{8.686}$; $\beta = 2\pi f/c_2$.

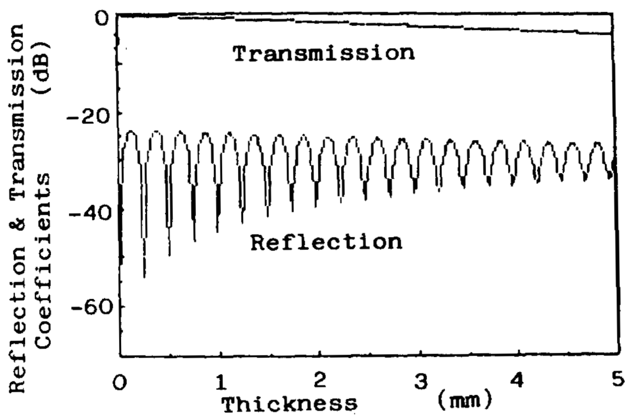

Fig. 4. Calculated reflection and transmission coefficients versus the thickness of Medium II. The wavelength and frequency are 0.494 mm and 3.5 MHz, respectively.

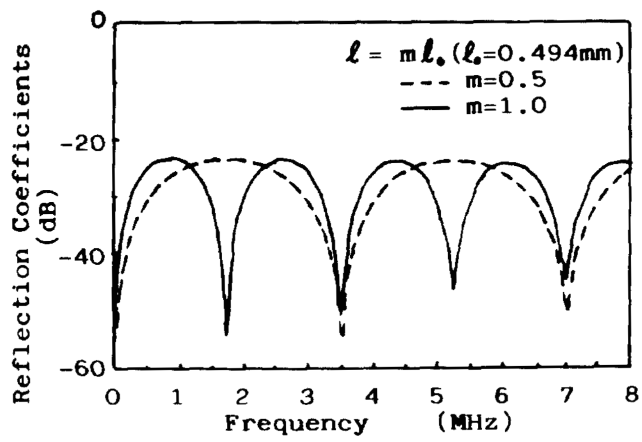

Fig. 5. Calculated reflection coefficient versus frequency. l and l_0 are the thickness of Medium II and the wavelength in Medium II at 3.5 MHz, respectively. Solid and dashed traces are examples when the thickness l of Medium II is 0.494 mm and 0.247 mm, respectively.

in Fig. 5 where it is again seen that maxima appear at the odd numbers of quarter wavelengths and that dips occur at the even number of quarter wavelengths. l is the thickness of Medium II and l_0 is the wavelength (0.494 mm) at 3.5 MHz.

Note that the transmission coefficient varies monotonically with the thickness of Medium II (Fig. 4). Interference phenomena does produce variations in the wave transmitted, but

TABLE III
REFLECTION COEFFICIENT VERSUS ACOUSTIC IMPEDENCES

Acoustic Impedance I II III	Reflection Coefficient Odd × $\frac{1}{4}$ Wavelength	Even × $\frac{1}{4}$ Wavelength
(a) $Z_1 < Z_2 > Z_3$		
(b) $Z_1 > Z_2 < Z_3$	Maxima	Dips
(c) $Z_1 > Z_2 > Z_3$		
(d) $Z_1 < Z_2 < Z_3$	Dips	Maxima

this effect is small enough to be ignored in the present case where the impedance ratio Z_2/Z_1 is nearly unity. The transmission coefficient is the power ratio expressed in decibels, i.e.,

$$T' = \{\exp(-\alpha l)\}^2,$$

where α is expressed in Np/m.

$$T = 10 \log T' = -20\, \alpha l \log_{10} e$$

and for α expressed in Np/m, (9) can be represented as

$$T = -\alpha' l. \tag{10}$$

In other words, the wave transmitted is not affected by interference. The transmission coefficient contains only information of the thickness and the attenuation of the thin interposed layer.

The above theory will be applied to a practical use of the pulse echo method. Two typical characteristic features of the usual ultrasonic pulse echo equipment are: 1) the ultrasonic wave-train-length is approximately 4 to 5 wavelengths, and 2) the ultrasonic wave has a finite frequency bandwidth. The above theory should be applicable to the study of reflection and transmission phenomena in a thin layer for which the thickness is less than about a half of the wave-train-length. Thus, the echo pattern contains information about the layer of interest in addition to the attenuation in the propagation path. The information available from reflection observations of such a layer of interest are the impedance ratio Z_2/Z_1, when the layer is thicker than a half of the wave-train-length, or the impedance ratio Z_2/Z_1, the speed of sound, the layer thickness and attenuation in the layer when it is thinner than a half of the wave-train-length. The above model is one in which a thin layer of another material is sandwiched between two extended media. Table III shows the relationship of the positions of the maxima and dips for the possible cases for different relative values of the media impedances. The present paper deals only with the case (a), where $Z_3 = Z_1$.

IV. Simulation Experiment

The following elementary experiment was carried out. A thin plastic plate immersed in water was chosen as a reflector sample with and without an obstacle between the sample and a 13-mm diameter, 2.25 MHz focal type ultrasonic transducer. The focal distance, and the focal zone, of the transducer are about 7 cm and between about 4 and 8 cm, respectively. The sample *A* and the obstacles *B*, *C*, and *D* were

A: plastic plate (1.00 mm in thickness),
B: plastic plate (0.52 mm in thickness),
C: silicone rubber plate (2.35 mm in thickness),
D: glass plate (1.08 mm in thickness).

Each plate was placed at the distance of 4 cm from ultrasonic transducer in water. The pulse repetition rate was 2 kHz and the spectrum was obtained from the 3-4 μs gated analog signal. The spectrum of the echo from a plate, minus that from the front surface of an aluminum block, was taken as equal to the reflection coefficient of the plate. Consequently, the positions of maxima and dips were found to be as follows:

A: maxima (1.80 MHz, 3.00 MHz), dip (2.42 MHz),
B: no maximum, dip (2.16 MHz),
C: no maximum, no dip.
The front and back surface echoes are separable, no interference phenomena present.
D: no maximum, dip (2.66 MHz).

The acoustic impedances of *A*, *B*, *C*, and *D* are approximately 3, 3, 1.5, and 17 ($\times 10^6$ kg/m^2 s), respectively. Fig. 6 shows the echo spectrum of sample *A* (double peaks) and the reference spectrum which exhibits the characteristics of the equipment (one peak). Fig. 7 shows the subtraction spectrum composed of the spectrum of sample *A* minus the reference spectrum which is equal to the reflection coefficient of sample *A*. Fig. 8 shows the subtracted spectrum, when the obstacle *B* is inserted, and is equal to the reflection coefficient of *A* plus the two way transmission coefficient of *B*. The positions of the maxima and the dips do not move. When two pieces of sample *C* were inserted, the positions of the two maxima moved, in the direction of lower frequencies, by 0.1 MHz because of the greater attenuation of *C* over *B*. However, the position of the dip did not move. Finally, Fig. 9 shows that when *D* was inserted the spectrum changed remarkably.

Thus, if there are thin layers in the propagation path between a medium of interest and the transducer, maxima or dips are not produced in the intervening path. It thus becomes known that the medium of interest is a thin layer, if a subtraction of an echo spectrum minus the reference spectrum has maxima and/or dips. It is even more interesting that the thickness or the sound speed of the interposed medium of interest can be

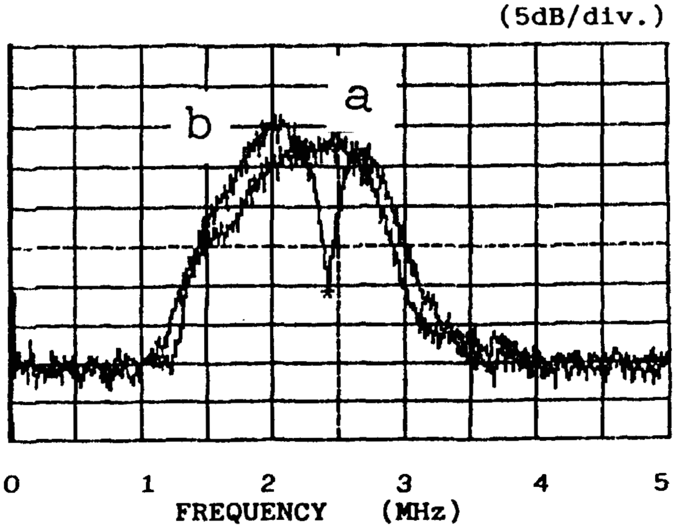

Fig. 6. Frequency spectra of the echoes (a) from extended medium (one peak; reference spectrum) (b) from sample *A* of 1.00 mm plastic plate (double peaks).

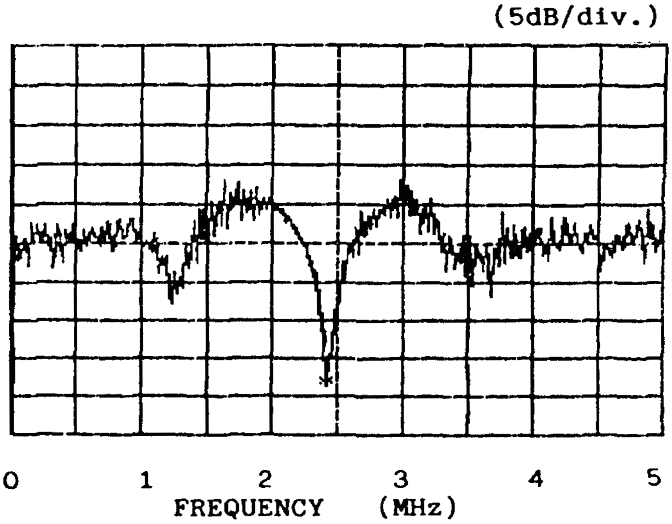

Fig. 7. Subtraction of the spectra in Fig. 6: (b – a).

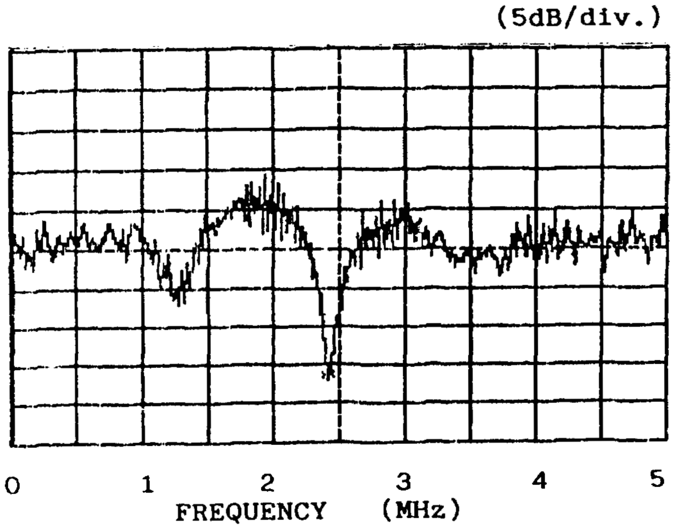

Fig. 8. Subtracted spectrum when obstacle *B* is inserted.

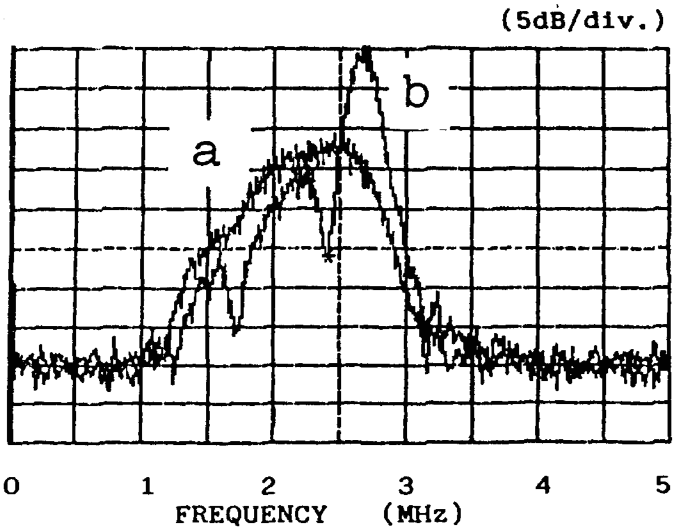

Fig. 9. Frequency spectra of two echoes: (a) from extended medium (reference); (b) from sample A when obstacle D is inserted.

calculated using the frequencies at which the dips and/or the maxima occur. However, since the maxima can move by virtue of the attenuation in the propagation path, dips may be preferable for use in estimating the thickness or the speed of sound.

As a result, the positions of dips, from which the thickness or the sound speed of the reflector sample A is estimated, are not affected by any obstacle, if the ratio of the obstacle impedance to that of the transmission medium is less than about 3. The positions of maxima could be affected by some obstacles.

In practice, thickness or sound speed is calculated using the value of the dip frequency as follows: 1) When the thickness of sample A is known, the sound speed c can be calculated by

$$c = f\lambda = (2/n) fl, \tag{11}$$

where f is frequency, λ is wavelength and the integer n is the number of dips between 0 MHz and the dip frequency of interest (the dip at $f = 0$ is not counted). In Fig. 7 the dip at 2.42 MHz can be known to be the second dip counted from $f = 0$, viz., $n = 2$. Then c is calculated ($l = 1.0 \times 10^{-3}$ m, $f = 2.42 \times 10^6$ Hz, $n = 2$) to be 2420 m/s. 2) When the speed of sound c is known, the thickness l is given by

$$l = (n/2)\, c/f. \tag{12}$$

Thus the theory has been demonstrated.

V. *In Vivo* and *In Vitro* Experiment

A. Experiment

The thicknesses of mitral, aortic and tricuspid valves of two dogs were measured according to the following procedures: 1) The *in vivo* echo from a mitral valve of desired phase was obtained by the ECG synchronous method [8], the echo was digitized at a 60 MHz sampling frequency for storage, and a frequency spectrum of the echo was obtained. 2) The heart was excised and placed in a 25°C water bath to obtain the *in vitro* echoes from valves for spectrum analysis. In 1) and 2), the subtraction method was applied in which the echo spectrum minus a reference spectrum (logarithmic scale) was obtained.

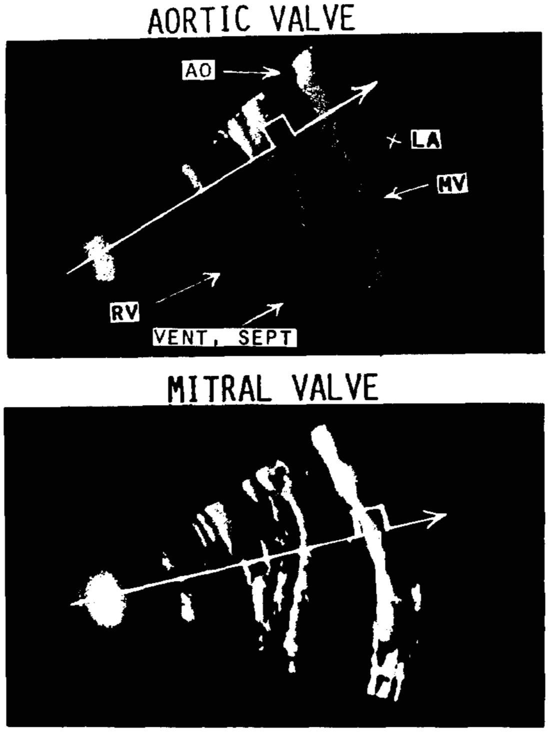

Fig. 10. Echograms of the dog heart AO: Aorta, LA; Left atrium, RV; Right ventricle, MV; Mitral valve, Vent. sept; Ventricular septum. The echo from each valve is obtained by 3-4 μs gating.

3) The heart was then dissected and each valve was surgically removed from the heart and the thickness measured with a micrometer, in order to compare with the thickness data obtained by the spectroscopic method. The measurement system utilizing the spectroscopic method is mainly composed of single element transducers, a single transducer sector scan type ultrasonic diagnosis instrument (Aloka), a digitizer, a spectrum analyzer, and a controller. In the experiment, a 13-mm diameter, 3.5 MHz transducer having a focal zone between about 5 and 7 cm was used (Aerotec). An analog signal method was chosen for spectrum analyzing because of ease. The valves studied were a mitral valve in 1) and mitral, aortic and tricuspid valves in 2) and 3).

B. Results

The positions of the mitral and aortic valves were recognized by the echograms of Fig. 10 in which the echo from each valve was obtained by 3-4 μs gating. Figs. 11 and 12 show the spectra of each echo from those valves. The upper trace in these figures show the spectrum of each echo and the lower trace shows the result of subtracting away the reference spectrum of Fig. 13. The result of subtracting involves the reflection coefficient (relative value) at the area of interest and attenuation in the path. In order to calculate the thickness of the valves of interest, use was made of the value of sound speed, assumed to be 1530 m/s, and the dip frequency of the subtracted spectrum. Table IV shows the values obtained for the thicknesses. For some spectra, dips did not appear or the shape or positions of the dips were not clear. The judgment that a dip is appropriate for measurement use is determined by their periodic distribution, viz., their positions must be at

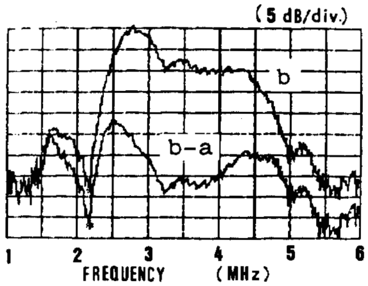

Fig. 11. Spectrum (b) of the *in vivo* echo from the mitral valve in Fig. 10 and subtraction (b − a); (a) is reference spectrum shown in Fig. 13.

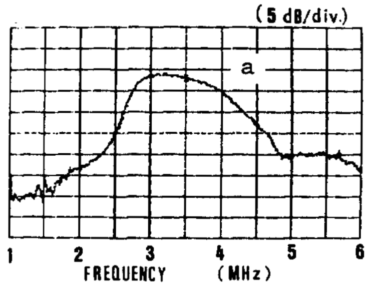

Fig. 13. Reference spectrum (a) obtained from the echo from extended medium (aluminum block).

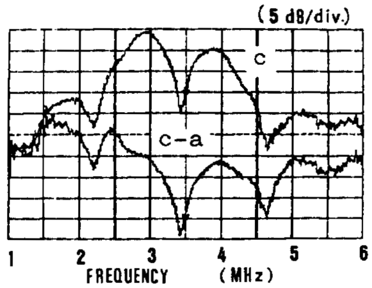

Fig. 12. Spectrum (c) of the *in vitro* echo from the aortic valve in Fig. 10 and subtraction (c − a).

TABLE IV
THICKNESS IN MILLIMETERS OF EACH VALVE

Example 1			(mm)
	Spectroscopic Method Middle	Apex	Micrometer Middle (T)
Mitral Valve Leaflet	0.683 (Fig. 11) 0.635	0.317 (*in vivo*) (*in vitro*)	0.6–0.7
Aortic Valve Cusp	0.657 (Fig. 12)	(*in vitro*)	0.6–0.7
Tricuspid Valve Leaflet	0.281	(excised)	0.25–0.3
Example 2			
	Spectroscopic Method Middle		Micrometer Middle (T)
Mitral Valve Leaflet	0.494 0.513	(*in vitro*) (*in vitro*)	0.45–0.54
Aortic Valve Cusp	0.543	(*in vitro*)	>0.4
Tricuspid Valve Leaflet	0.271	(excised)	0.15–0.26

even numbers of a quarter wavelength. Using this criteria, Figs. 11 and 12 can be understood as follows: Fig. 11 [2.24 MHz (λ), around 3.5 MHz (1.5 λ), 5.12 MHz (2 λ)], Fig. 12 [2.21 MHz (λ), 3.41 MHz (1.5 λ), 4.64 MHz (2 λ)], where λ is the wavelength of ultrasound at each frequency. Determination of an accurate value for the sound speed was attempted from a direct measurement of the thickness of the excised valves using a micrometer. However, only the thickest part of the valve could be measured by the micrometer so that if the valve was rough, the values of T in Fig. 14 and Table IV are rough. Also, since each valve was necessarily somewhat compressed by the discs of the micrometer, the values measured should be smaller than the true value. It is seen that from the data obtained by the method and the micrometer measured values shown in Table IV, that the sets of data compare rather favorably.

C. Discussion

1) *Assumption of Sound Speed*: Table IV shows the thicknesses estimated from an assumed sound speed of 1530 m/s. If the sound speed is different from this value by as much as 100 m/s, an error of about 6 percent is introduced. As it is impossible to measure a thin-tissue layer, such as a valve, which is thinner than the length value of the range resolution, by current ultrasonic tomography, the several percent uncertainty of the *in vivo* measurement method is considered acceptable.

2) *Incident Angle*: One reason that the dip may not appear, or the shape or position of the dip not be clear, is associated with a large incident angle of the reflecting surface. Fig. 15 illustrates this. Here the subtracted spectrum has an invalid dip at 2.67 MHz when the incident angle to the parallel face plastic plate specimen, 0.52 mm in thickness, is 9 deg. Thus, it is a requirement of this method that incidence angle be as near to normal incidence as possible.

3) *Selection of the Tomographic Plane*: When the goal is measurement of biological tissues, as discussed in this paper, it is necessary to select not only the tomographic plane for display, but also the approach that is suitable for the goal of measurement or the shape and direction of an area of interest.

4) *Shape of Transducer*: The 3.5 MHz transducer used in the measurement of dog hearts has a beam width of about 2 mm in the focal zone. The ultrasound beam width depends upon the transducer design. For example, a transducer whose diameter is large, for example 2 to 3 cm, is considered to be convenient to measure a small area because it can have a very narrow beam in the focal zone and produce an acceptable spatial resolution.

5) *Single Element Transducer*: A tissue specimen of interest is not always located at the midangle position of the sector scanned. But as a single element transducer has the same di-

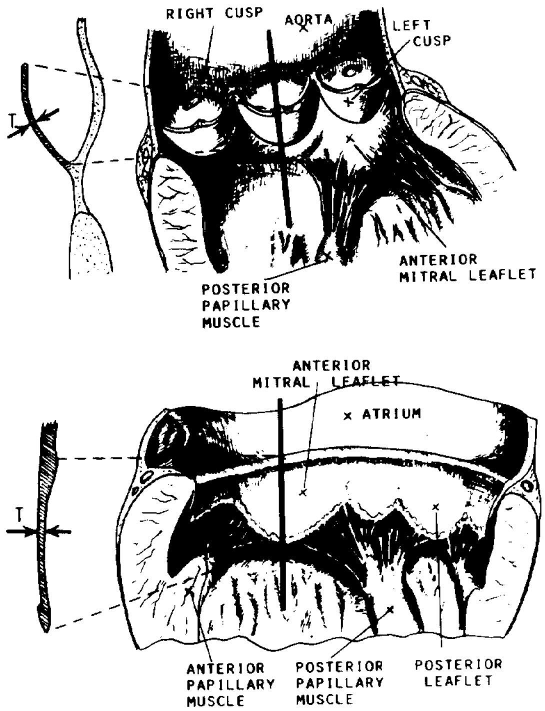

Fig. 14. Sketches of heart: notation T is thickness of aortic or mitral valve.

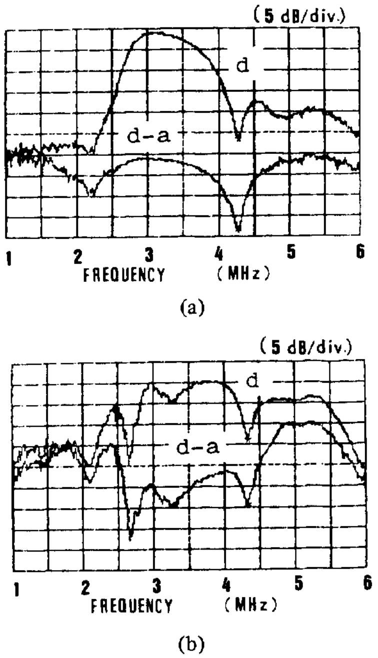

Fig. 15. Spectra (d) of the echoes from a plastic plate (0.52 mm in thickness) and subtraction (d − a) (a) 0 deg incidence angle; (b) 9 deg incidence angle.

rectivity and the same spectrum at all transducer positions in the sector scan, only one reference spectrum for subtraction is needed.

6) *Biplane Cardiotomography*: When an area of interest can be located at the midangle position of the sector scanned, the biplane cardiotomography [9] can make the setting of incident angle easier.

7) *Patient and Disease Variation*: No information is available on the importance and influences of variations from patient to patient, disease to disease, etc., as only two dog hearts have been studied thus far.

VI. Conclusion

Though the experimental procedures carried out did not satisfy completely all the criteria listed in the Section V-C, acceptable measurements of the thickness of thin layer mitral, aortic, and tricuspid valves of dog hearts were obtained. The method is expected to be useful for noninvasive measurements, one of the merits of ultrasonic diagnosis schemes.

Acknowledgment

We acknowledge the valuable assistance of Mr. A. Takahashi of our depeartment. F. Dunn's tenure in Japan was made possible by a Japan Society for the Promotion of Science Fellowship.

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