ULTRASONIC CHARACTERIZATION OF BIOLOGICAL MEDIA

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MASTER OF PHILOSOPHY

ANIL   KUMAR   SINGH

SCHOOL   OF   ENVIRONMENTAL   SCIENCES
JAWAHARLAL   NEHRU   UNIVERSITY
NEW   DELHI-110067,   INDIA
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SCHOOL OF ENVIRONMENTAL SCIENCES
JAWAHARLAL NEHRU UNIVERSITY
NEW DELHI - 110 067
INDIA
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CERTRIFICATE

The research work embodied in this dissertation entitled

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carried out by **Shri Anil Kumar Singh** for the award of the

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far, in part or full, for any other degree or diploma to any

university.

ANIL KUMAR SINGH
Candidate

Dr. J. BEHARI 24/7/91
(Supervisor)

Prof. C.K.VARSHEY
(Dean)

Prof. C. K. VARSHNEY
Dean
School of Environmental Sciences
Jawaharlal Nehru University
New Delhi-110067.

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CHAPTER I

INTRODUCTION

I.   Preliminary Remarks

Ultrasound vibration means frequency which lies beyond the upper audible limit of the human ear, that limit is taken as 20 Kc. In addition to sound wave proper, by which is meant usually longitudinal waves, as well as surface waves and Transverse vibrations provided that the frequencies are above 20 Kc.

The history of medical application of ultrasound began with the observation of Prof. Paul Langain who was the first to observe interaction of radiation with living organism. This observation coupled with the observation of finding that ultrasound can penetrate rather well through, soft tissues led to considerable interest, commencing in 1930s in its possible use for a wide variety of   therapetical purposes.   The interest has continued, a quite wide spread applications has developed particularly in Physical medicine, but it is only relatively recently that we have marked the beginning of some scientific understanding of the phenomena that are involved.

II. Tissue Ultrasound Interaction Cross-section:

Wave matter interactions, absorption and scattering are described in terms of fundamental interaction cross-section for the particles of the medium that interact with the wave. But in biological medium we consider macroscopic cross-section or interaction cross-section per unit volume of the tissue concerned. More specially there are three such macroscopic cross-sections: the attenuation cross-section per unit volume, the absorption cross-section per unit volume, and the scattering cross-section per unit volume.

For better understanding of these parameters let us consider a medium composed entirely of two types of homogeneity. Those which absorb and those which scatter the acoustic energy. There are n_i scattering inhomogeneities per unit volume, each of scattering cross-section σsi, and n_j absorbing inhomogeneities per unit volume, each of absorbing cross-section σaj. Such individual cross-sections are defined as the ratio of the total power absorbed or scattered, by a given inhomogeneity, to the incident intensity. They have dimension of L^2 (Area) and are equal to the cross-section area of a incident plane wave that contains the same amount of power as was either scattered or absorbed, For a beam of approximately constant cross-

sectional area S, total power W and of uniform density, the power scattered by one inhomogenity would be σsi W/S and power absorbed by one inhomogenity would be σ_{ij} W/S. Thus the power scattered and absorbed per unit volume will be given respectively by Σ_i n_{si} σ_{si} W/S and Σ_j n_{aj} σaj W/S.

The quantities Σn_{si} σsi and Σn_{aj} σaj W/S may be redefined (in the absence of multiple scattering) as the scattering cross-section per unit volume μ_s, and the absorption cross-section per unit volume μ_a respectively.

Now the scattering and absorption per unit path length will be μ_sW and μ_aW respectively. Therefore for a thin target of thickness x, the total power scattered will be

$$Ws = \mu s\ W\ x \qquad (1)$$

and the total power absorbed will be

$$Ws = \mu_a W\ x \qquad (2)$$

Therefore, the total power that has interacted with the medium:

$$W = (\mu_s + \mu_a)\ W\ x \qquad (3)$$

The quality $(\mu_s + \mu_a)$ must be the total interaction cross-section per unit volume of the medium and will be denoted by μ.

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$$\mu \quad = \quad \mu_s + \mu_a \qquad\qquad (4)$$

When considering a target of finite thickness equation (3) can be integrated. Considering the initial conditions we get $W = W_o$ (The incident power) at $x = 0$.

Therefore,

$$W \quad = \quad W_o \, e^{-(\mu s + \mu a)x} \qquad\qquad (5)$$

$$\mu \quad = \quad -1/x \, \log_e (W/W_o) \qquad\qquad (6)$$

$$\mu \quad = \quad -10/x \, \log_{10} (W/W_o) \qquad\qquad (7)$$

$$\alpha \quad = \quad -20/x \, \log (P/P_o) \qquad\qquad (8)$$

where α is the amplitude attenuation coefficient and P/P_o is the ratio of signal amplitudes. Here, μ_s and μ_a are expressed in units of $dBcm^{-1}$.

III. Mechanism for Absorption of Ultrasonic Longitudinal wave:

Acoustic absorption, solutions, solute interaction, molecular structure, molecular weight etc. have been studied in detail (Kremkau, 1984-1988, Dunn et.al., 1984, Barnes, 1985) during last few years.

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A series of reviews have been published on the subject like absorption and dispersion of ultrasound in biological medium (Carstensen, 1979, Dunn et.al., 1969, Dunn and O. Brien, 1978, Fry and Dunn 1962, Johnston et.al. 1979, Well 1975).

Attenuation of acoustic Energy during propagation is a complex phenomena. Two mechanism are primarily responsible for attenuation (Chivens 1977, Nicholas 1982). These are (a) the scatterer of the energy out of the acoustic path way and (b) absorption, in which acoustic energy is transformerd into thermal energy. The two mechanism, interact in biological system to produce a net acoustic attenuation which has an approximately linear frequency dependence over the frequency range of typical ultrasound equipment.

A noteworthy factor in biological tissue is that the energy loss associated with the scatterer mechanism is small compared to absorption (Nicholas 1982). Yet when observing a back scattered signal in the reflectance mode, the observed signal energy is dependent upon the scatterer mechanism.

IV. **Semi-Solid Media (Visco-Elasticity)** :

For a perfectly viscous (Newtonian) liquid, the stress is proportional to the rate of change of strain but is

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independent of strain itself. For a perfectly Elastic solid, stress is always proportional to strain. All real materials, however, exhibit some combination of these properties and thus are viscoelastic in behaviour.

At low frequency of oscillation of shear waves, all the driving energy is dissipated in viscous flow of different layers of liquid over each other. If the frequency of oscillation is increased until it is fast for any molecular diffusion to occur during the shear strain, the liquid appears to posses shear rigidity and energy is stored elastically. The change from viscous behavior to Elastic behavior with increasing frequency, the intermediate between these to extremes is called the "visco-Elastic Relaxation".

An ultrasonic longitudinal wave contains both shear and compressional components, thus its propagation may be discussed in terms of both shear and compressional elastic moduli and relaxation time.

<u>Inhomogenous Medium</u>:- The inhomogeneity may occur in inertial or elastic or a combination of these two properties in tissue. In case of inertial inhomogeneity, viscous damping results from the relative translational and rotational motion that occurs between a suspended structure

and the embedding medium. If the density of the inhomogeneity is uniform, it will simply attempt to move back and forth along the axis of sound propagation. All this leads to acoustic damping and absorption takes place. In addition to it, the inhomogeneity causes scattering. An additional dampering of acoustic energy is caused due to heat conduction (between suspending medium and inhomogeneity) caused by the alternate compression and expansion of the sound fields. It has been found (O'Donnella Miller, 79) that thermal losses appear to be dominated (in 1 to 10 MHz frequency range) by various relative motion losses over a wide range of postulated sizes of inhomogeneity in specific tissues.

It is important to note that if we consider the frequency dependence of absorption due to simple relaxation process, the absorption peak is very sharp. On the contrary, the absorption peak is much broader if we take the viscous relative motion into account (Heuter, 1958). The reason for this is that in viscous relative motion the "relaxation" frequency is itself a function of frequency.

A heterogeneous population of inhomogeneities undoubtedly exists in soft tissues. The resultant contribution of viscous relative motion losses due to

absorption coefficient would be obtained by a summation (or integration) over a range of particle sizes, shapes and densities.

V.   Frequency Analysis

The shape of the signal provides information, for instance, from its amplitude or rate of decay. The spectrum of a signal is often examined to identify useful characteristics such as its high frequency content. The spectrum analyser employs a bank of fixed filters of which each is designed to pass components in a specific part of the frequency range examined. The ultrasonic signal is fed simultaneously to all the filters and the spectrum is obtained immediately. This is called real-time frequency analysis.

Finally digital computational technique can be employed to analyse signals. The signal is captured by a device that rapidly samples the amplitude at regular intervals along the waveforms, for example, five samples per cycle. The samples are stored as numbers in the complex memory. A programme then acts on the data and mathematically derives the frequency spectrum in a computer is very slow, it may take minutes to produce a spectrum.

For this reason, high speed programmes have been written that can do the job about 50 times faster. The name Fast Fourier Transform or FFT is applied to these programme.

Gain of Signals (Amplification, overall gain):

In case of a linear amplifier (i.e. applying the same amplification to large and small signals) the large echoes may begin to saturate even if the gain is not high enough to register the weak echoes. For this logarithmic response amplifier is used which amplify weak echoes more than large ones.

Logarithmic amplifiers are difficult to calibrate since different signal levels are amplified by different amounts.

Because of attention in tissue, the echoes received from superficial structures are much larger than these from interfaces deep within the body. To compensate, deep echoes must be amplified more than superficial.

This is done by swept gain of TGC (Time Gain Control). The alternate terms of TGC are Time-Varied-gain, distance attenuation compensation, Time control, sensitive time gain control (STC).

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This things we can do with our delayed sweep available with oscilloscope.

VI. Tissue Characterization

The aim of any development in tissue characterization is to assist the clinician by increasing accuracy in differentiating region of the human body. The differentiation may be one between normality and abnormality or it may be concerned with changes over a period of time associated with irreversible processes such as tumour growth or tumour response to radiation.

The Term tissue characterization may be defined in its purest form as the identification of one or more physical parameters of a small volume of the tissue that are sufficiently well correlated with the type or condition of the tissue. Thus the measurements of tissue physical parameters may alone be used as an effective index of the type or condition of that volume of tissue.

In order to obtain consistent measurement of tissue parameters, the classical transmit plane wave assumption can no longer be used, and the diffraction effects related to the finite aperture of the transducer are of importance. Diffraction effects are produced by the variation with range

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in the relative transmit times of the energy arriving at a given field point from the different source points. It can be shown that the transducer appears as radiating a direct plane wave in its geometrical shadow, puts an 'edge' wave coming from the transducer boundary. The interference between the two waves gives rise to the observed transient field pattern. When finite size transducer are excited by short electrical pulses, the transient pressure waveform and its spectrum are then quite dependent of the field point location. This means that diffraction acts as a filter varies with the field point position.

This approach is consistent with the theory of linear time variant system (i.e. wave equation is linear and time invariant for a constant sound velocity). In Pulse-Echo mode, the relation between the round trip time and the scattered depth allows the diffraction effect to be described as a time varying filter.

Sound speed is one of the most important parameter for ultrasound tissue characterization. So we have reported in chapter III A the measurement of velocity of ultrasound in liver and kidney samples of a goat at different temperatures. In this chapter we also have studied the

effect of fixation on velocity of ultrasound at corresponding temperatures.

In chapter III B we have reported measurement of attenuation in egg (white transparent liquid) and egg (yolk) by (Kremkau method) in transmission mode.

In chapter IV we have discussed theoretical back ground for the effect of diffraction on frequency-dependent attenuation.

In the same chapter IV we have discussed methods for measurement, and experimental results are presented.

Diffraction of Ultrasound:

Pure reflection hardly occurs in acoustics because the wavelength of sound are large enough in comparison to the size of the sample. Thus we receive a substantial amount of sound energy by diffraction, that is, the bending or spreading out of a sound wave after it intersects an aperture. The primary factor in diffraction of course is not the dimension of the wavelength alone or that of intercepting object or aperture but the ratio of the wavelength to object.

Pulse Echo and Diffraction:

In this method wide band pulses are used to interrogate biological media in order to increase the information content of the back scattered ultrasound. Such pulses offer improvements in range resolution and open the possibility of characterizing the scattered distribution and propagation medium by an analysis of the shape or frequency content of the scattered wave. Details of this methods are discussed in Chapter IV.

CHAPTER II

REVIEW

Sound velocity is one of the most important ultrasound characterization perameter. Density and compressibility of the medium determine sound velocity. Many scientists have measured the velocity (Buyer & Letcher 1969, Goss et at. 1979, Mc Skimin 1964 and Matheson 1971). The earliest researcher used absolute methods (Pellam & Galt 1946), Time of flight (TOF) using time delay of an oscilloscope (Ludwing 1950). Pulse Echo superposition method (Beyer & Lecter 1969), Pulse echo overlap method (Papadakis 1964). The workers who measured velocity by absolute method are Greenspan & Tschiegg (1957), Goldman and Richands (1954) Del Grosso and Mader (1972), Behari and Singh, (1981) etc.

Relative methods are used by Kossoff et al (1973), Nasoni et al (1979), Foster et al (1984), to name a few.Effects of fixation on velocity are summarized by Bamber et al (1979) Nassiri et al 1979, Bamber & Hill (1979).

The effect of fixation on velocity are summarized by Bamber et.al (1979) Nassiri et.al (1979), Bamber and Hill (1979) In recent work Berger et.al (1990) deals with the estimation of slope of attemation in human breast tissue.

They accomplished this in reflection mode with short time Fourier analysis. They also took account of diffraction effect, tissue depth and specular reflections. According to him a population of 49 normal women shows large inter-individual variations of the attenuation co-efficient. A multi liner regression allows correlation of this variation with the duration of the wo-man's genital life and pregnaries.

In diagnostic ultra sound applications, acoustics signals are transmitted into the body and the reflection from interfaces between regions of different acoustic impedances are observed. The reflected signals are then processed to determine some desired characteristics of the medium, such as locations and reflecting strengths of the interfaces for Imaging (Taylor and Goreliek 1981) or its attenuation properties for tissue charcterization (Kuc, 1980). When an ultrasound Pulse is transmitted into a medium the power spectra of reflected singals vary as function of range from the transducer. These spectral changes are caused by diffraction effects, due to finite-sized apeture and by the properties of medium (Kuc 1984). When the properties of medium are to be determined from the back-scattered signals, the diffraction effects, tend to mask these properties, especially in near field (Kuc &

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Donald 1984). The change in the spectral properties of signals reflected from near and far limits in the range of a medium can be used to characterizes the medium (Kuc, "Estimating the acoustic attenuation from reflected ultrasound singals). Kuc compared averaged power spectra at different depths for a series of ecographic A - lines. According to him spectral difference will indicate the incremental changes that the spectra undergo due to propagation through the media and due to diffraction.

It was during 1972 (Rale and Namery), that it was recognized that the frequency-dependent attenuation of tissues can be indicative of the presence or absence of disease states in a tissue or organ. Accordingly frequency dependent attenuation in tissues has been calculated from measurements of reflected, transmitted or backscattered ultrasound and used to study abnormalities in the eye (Lizzi & coleman 1977).

As attenuation in the tissue is frequency dependent (Ophir & Maklad, 1978, Flax, et.al 1983, Hottier and Bernatets 1984). the amount of energy received will be function of both frequency and depth. Attenuation acts as a time-varying frequency filter. For this reason, most of the alogrithms are based on the calculation of the power spectra

of the windowed r.f. signal (Laugier et.al, 1985). Short time Fourier analysis allows the use of the totality of data point and provides a represenatation of energy density vs time and frequency (Berger & Perrin 1980).

However, the major problem encountered while measuring attenuation is diffraction. It has been shown that diffraction also acts as a time varying frequency filter and so yields a biased attenuation slope value. The diffraction filtering effect has already been stated by different investigators (Fink and Cordoso 1983; clooster mans and Jhijssen 1983; O.Donnell 1983; Fink et.al 1984, and Regula & Kuc 1984). The effect is due to the finite aperture of the probe, and makes the spectral content of the acoustic wave incident on a scatterer dependent on the location of the scatterer in the field. The major point is that any estimation of the slope of attenuation in the region of interest, if not corrected for diffraction effect, yields a value which depends on the location of the region of interest in the acoustic field of the transduer. It has been shown (Cordoso & Fink 1982); (Cloostermons and Thijssen 1983, O'Donnel 1983) that the diffraction effect and attenuation effect were separable in the frequency domain and that the diffraction effect could be represented by a

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mean diffraction filter, completely characterized by the geometical specifications of the probe (diameter and focal distance). This property leads to easy computation or experimental calibration of the diffraction filter, and makes possible the correction of the diffraction effect by inverse filtering. Due to diffraction effects associated with a finite aperture the beam patterns for each frequency within the band width of the incident pulse are different. In the far field of any aperture the beam patterns is simply related to the spatial Fourier transform of the aperture, where the spatial frequency variable is proportional to the ultrasonic frequency (O' DONNELL 1983).

The frequency content of the signal depends on the scattering properties of the tissue. Besides diffraction, time varying frequency filter, there is a scattering, time - varying frequency function related to the degree of inhomogencity in real tissue. Such an effect, due to the presence in the tissue of any large scattererer (i.e., no longer considered as a point like scatterer), may introduce a bias into the attenuation slope estimation.

Scattering cross-section of the tissue varies as a function of depth. Secondly, the case in which the frequency dependence of the scatter cross-section changes. Again this

will not appreciably affect the total signal attenuation, yet it would produce a spectral shift in the back scattered signal. Keeping these facts in mind characterizations of attenuation of ultrasound in materials by the measurement of spectral shifts was first reported by Seberian and Merkulova (1967).

During the past years different methods for the in vivo measurement of attenuation have been proposed. Kuc (1978) compared averaged power spectra at different depths for a series of ecographic A-lines. Another method has been proposed by ophir (1978) who compares the averaged intensities of back - scattered signal from two mono-chromatically illuminated parallel c-planes. Jones (1976) established the attenuation by comparing averaged power spectra in small time windows at two different depth for a series of A-lines. Also Leeman et. al. (1981) used homomorphic filtering to estimate changes in the interrogation pulse-shape with depth and therefore provide a measure of the frequency dependent attenuation.

Ultrasound attenuation coefficient has been used to detect large number of abnormalities in tissues, and hence has broader applicability in the field of medicine and biology. Durinckx et al (1986, 1988) indicated that

ultrasound attenuation is correlated with liver function tests during their study of adults with and without liver disease. Importance of fat in the estimation of accoustic attenuation in liver (Duerinekx 1988), pre and postnatal reduction in ultrasound attenuation (Chiang et al 1991) have been studying in detail during last few years.

In this way we conclude that attenuation of ultrasound in tissues is an important parameter in many respect. As a measure of loss per propagation distance, the attenuation coefficient at any frequency governs the penetration depth of ultrasound, and thereby acts as a limiting factor for imaging deep organs. The magnitude and frequency dependence of attenuation is a complicated function of the composition (collagen, fat, water) and bio-chemical environement of an organ (Dunne et al 1969, Kremkau and carstensen, 1972, Goss et al 1980), thus forming a potential basis for tissue characterization. In laboratory studies of myocardial in farcts (Donnell et al, 1979) and carbon tetrachloride liver toxicity (Parker and Tuthill, 1986) changes in attenuation has been measured as a function of time as gross changes in tissue composition and function occured. Clinical measurements of attenuation are more difficult to obtain because of the presence of overlying tissues and the lack of simple transmission paths through unifrom regions of the

target tissue. Nonethless, many strategies exists for estimating attenuation using back scattered signals (Parker and Waag 1983, Parker et al 1984, Leeman etal 1984). The most widely considered class of estimators assume that attenuation increases linearly with frequency, then derived an attenuation slope parameter ß, which presumbly describes the tissues (Lizzi and coleman 1977, Kuc and schwatz, 1979, Kuc 1985). Unfortunately, the frequency dependence of attenuation departs from linear concept in a variety of normal and diseased tissue (parker and Tuthill, 1986, Naryana et al , 1984, Goss et 1979), and small deviation from the linear with - frequency assumption will produce significent error in estimation (Narayana and ophir 1983).

It is well known that the attenuation of ultrasound passing through lossy medium is frequency dependent (rak 1978 and Red wood 1963)

$$\alpha = \alpha_0 \ f^n$$

Most of the soft tissues and body liquids exhibit a value of n close to unity. The value of n begin to increase to-wards 2 for soft tissues at higher frequencies. The value n=1.2 for liver is generally stated by worker (Chiver 1978.

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The function dependence can be modeled either by a power law of in type $\alpha = \alpha 0 \, f^n$ or by an exponental of type $\alpha = \alpha^1_0 \, e^{n1f}$, Where α_0, n^1 and α_0^1, n are characteristics of attenuating medium (Bamber & Hill 1981) α is the attenuation coefficient of the material and f is the frequency. Since the attenuation experienced at higher frequency is generally larger than at low frequencies, a wide band ultrasonic pulse which propagates through such medium will be distorted. This results in a central frequency down shift of the spectrum. For materials with a linear frequency dependence this frequency shift is proportional to the integrated attenuation (KUC 1976) and to the square of the band width of the pulse (Ophir & Jaeger 1982).

The frequency dependence of attenuation, expressed in dB/cm/MHz has been systematically estimated with two alogrithms, both based on a short Time Fourier analysis (Fink, Hother 1983 ad Hottier Bernatets 1984). There are several problems in attenuation estimation the first problem is the dependence of the precision of the estimation on the depth of the tissue, the variance of the estimation is related to tissue thickness and a minimum tissue depth with be necessary in order to achieve a reasonable estimate.

Next problem is due to interference between scatterers. This effect necessitates an averaging of the information of many r.f. A-Lines. Both effects increase the minimum tissue volume which must be investigated and lead to a statistical approach.

In addition to these well known characteristics, a third problem is due to the specular reflectors that can be found in tissue.

The last problem is due to the inhomogencity of the sound field. The so called diffraction effects must be corrected for.

Taking the above problem under strict supervision we can apply this method for in vivo measurement also.

In this dissertation method based an the short-Time Fourier analysis of ecographic data, providing a local unbiased estimation of the attenuation slope. The objective of the short time Fourier analysis is to permit tracking of the depth dependence of the local tissue roundtrip transfer function. It can be shown that depth dependence spectral of centre of mass or centiode of power distribution allows simple determination of the frequency dependent attenuation we have further repeated the experiment at different Temp.

i.e. 29.5 33.5 and 36.5 in order to estimator Temperature and frequency dependence attenuation.

A. MEASUREMENT OF SPEED AND ATTENUATION OF ULTRASOUND IN BIOLOGICAL MEDIA

Measurement of Speed of Propagation

Introduction

We used here `Relative Method' for measurement of velocity of ultrasound in different biological media (tissues and eggs). We have also examined the effect of fixation on velocity on liver and kidney.

In this method measurements are made of variants of general pulse transit time or time of flight (TOF) techniques. This method is suitable for solid tissues whose thickness measurement is difficult. In these measurements we have used 0.9% saline as reference medium. First of all we measured the velocity of ultrasound at known temperature in 0.9% saline.

If c_w be the velocity of ultrasound in water, the sample thickness Δx, and time shift is ΔT, then the velocity of ultrasound in the unknown medium c_t is given by :

$$\frac{1}{c_t} = \frac{1}{c_w} - \frac{\Delta T}{\Delta X}$$

Samples : We have taken five samples of different thickness for investigation.

Liver and kidney (goat): Measurements are performed on liver and kidney obtained from freshly scarificed animals. Samples were immediately kept in 0.9% saline and refrigerated for six hours.

Liver and kidney samples were kept in 10% formaline for 48 hours and then cut into rectagular slices of different thickness.

Eggs : Fresh eggs obtaned from market and kept in refrigerator for six hours. Its white transparent liquid was extracted.

Eggs yolk also separated from the eggs and we also measured ultrasound velocity through this.

Apparatus required : Pair of transducers (4 MHZ), Cathode ray oscilloscope, Pulser and Water bath with temperature controlling device.
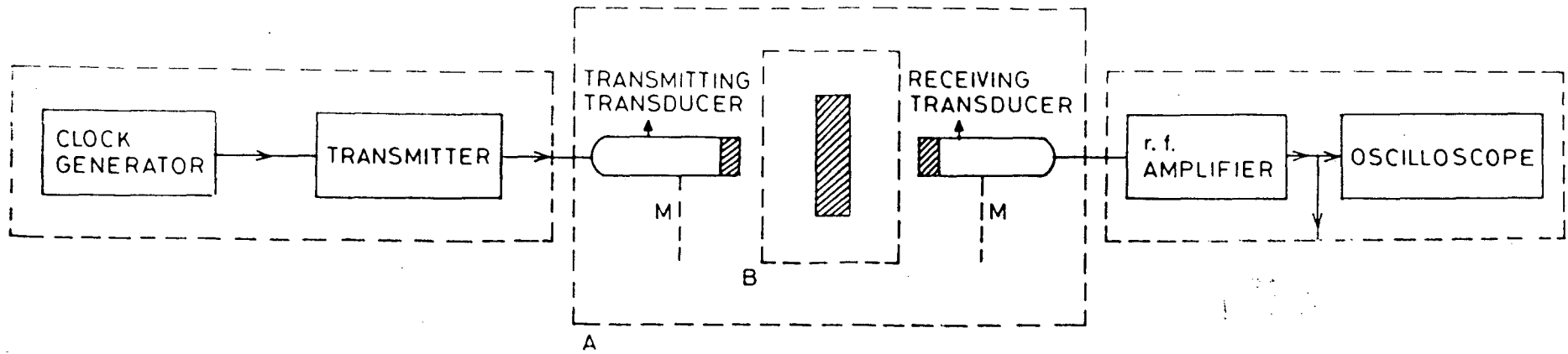
A : Anechoic Ultrasonic chamber filled with degassed water
    at room temperature as shown in Fig. 1

B : Temperature controlled water bath with acoustic windows
    and sample holder as shown in Fig. 1

M M : Movable stands for transmitting and receiving transducer

Fig. A Experimental set up for measurement of transmission parameters of soft tissues

Ultrasonic speed determination :

In transmission technique for determining the ultrasonic speed, the sample is introduced between a source and a receiver (Insertion technique). The measurement of the sample thickness and of the shift in arrival times of the acoustic pulse allow computation of the ultrasonic speed, c_t in the sample using the relation.(1)

Measuremnts are made in a thermostat water tank filled with 0.9% normal saline. The temperature of the liquid was measured with a high sensitive thermometer. The temperature was allowed to stabilized. This temperature stability means that variation of the ultrasound speed in the saline due to fluctuation in temperature were less and we may nglect it. The speed of sound in the saline, measured at different temperature are shown in figure (A) :

As we have mentioned earlier we used insertion technique in which the sample holder was kept between the pair of transducers. Sample hold is a cubator of known thickness. The sample was cut such that. its size was exactly equal to. thickness (Δx) of the sample holder.

The two transducers are co-axially aligned and their alignment was checked with a laser beam.

Results:

Table

Table III.1.A

Average Speed of propagation at different temperature

(MS^{-1})

Temp	Liver (fresh) (Mean)	SD	Liver (fixed) (Mean)	SD
30.5°C	1592.8	2.04	1569.56	2.12
33.5°C	1596.8	1.5	1574.48	2.03
36.5°C	1602.9	4.9	1576.5	1.29
39.5°C	1603.3	6.63	1583.2	2.82

Rabbit

Temp	Liver (fresh) (Mean)	SD	Liver (fixed) (Mean)	SD
24°C	1599	1		Chivers (1978)
26°C	1575	9.4		

Table III.2.A

Temp	Kidney (fresh) (Mean)	SD	Kidney (fixed) (Mean)	SD
30.5°C	1568.6	2.67	1546.2	1.96
33.5°C	1573	1.04	1551.3	1.99
36.5°C	1574.5	2.87	1552.22	1.4
39.5°C	1578	1.82	1559.2	1.80

Temp	Rabbit			
24°C	1560-1571			Chivers (1978)
26°C				

Table III.3.A

Velocity of ultrasound in egg (White)

Five samples of different thickness are taken

Frequency	Temp.	C_w M/S	TOF in water	TOF in sample	T	x	C_t M/S
4 MHZ	20°C	1490	89.0	88.0	1.0	5.6	1530.7
			98.2	97.4	0.8	4.7	1529
			99.6	98.9	0.7	4.0	1530
			100.0	99.6	0.4	2.3	1529.6
			105.3	104.95	0.35	2.0	1530
4 MHZ	22°C	1500	90.0	89	1.0	6.2	1537.2
			91.5	90.75	0.75	4.7	1536.7
			92.3	91.6	0.7	4.0	1540.4
			92.5	91.9	0.6	3.7	1537
			92.5	92.15	0.35	2.3	1535
4 MHZ	24°C	1508	92.5	91.5	1.0	6.2	1545.6
			92.5	91.75	0.75	4.7	1543.4
			92.5	91.9	0.6	3.7	1545.2
			92.5	92.1	0.4	2.6	1545.8
			92.5	92.15	0.35	2.3	1543.8

Table III.4.A

Egg White

	Average Velocity	Standard Deviation
$20^{\circ}C$	1529.8	0.56
$22^{\circ}C$	1537.3	1.75
$24^{\circ}C$	1544.8	9.75

Table III.5.A

Egg yolk

$20^{\circ}C$	1509	0.873
$22^{\circ}C$	1507.3	0.36
$24^{\circ}C$:		

At this temperature no significant Time shift (between 0.9 sline and egg yolk) was observed. The speed of propagation at $24^{\circ}C$ in egg yolk coincides with the speed of ultrasound in 0.9% sline.

Results are discussed in Chapter IIIB where we have reported attenuation of ultrasound in egg white and egg yolk.

PROPAGATION SPEED VS TEMPERATURE AND EFFECTS OF FIXATION
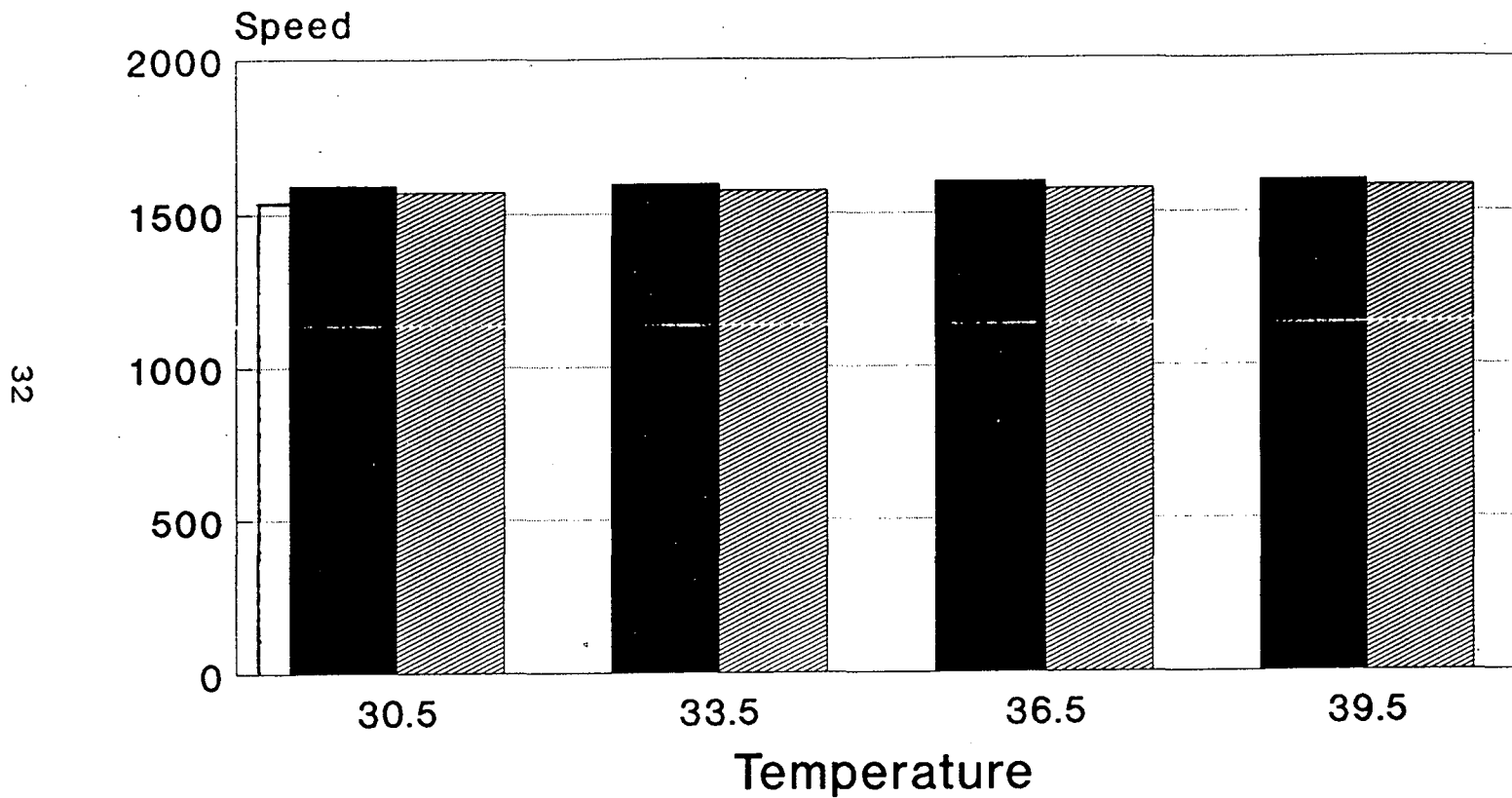
Velocity of Ultrasound in Eggs VS Temprature
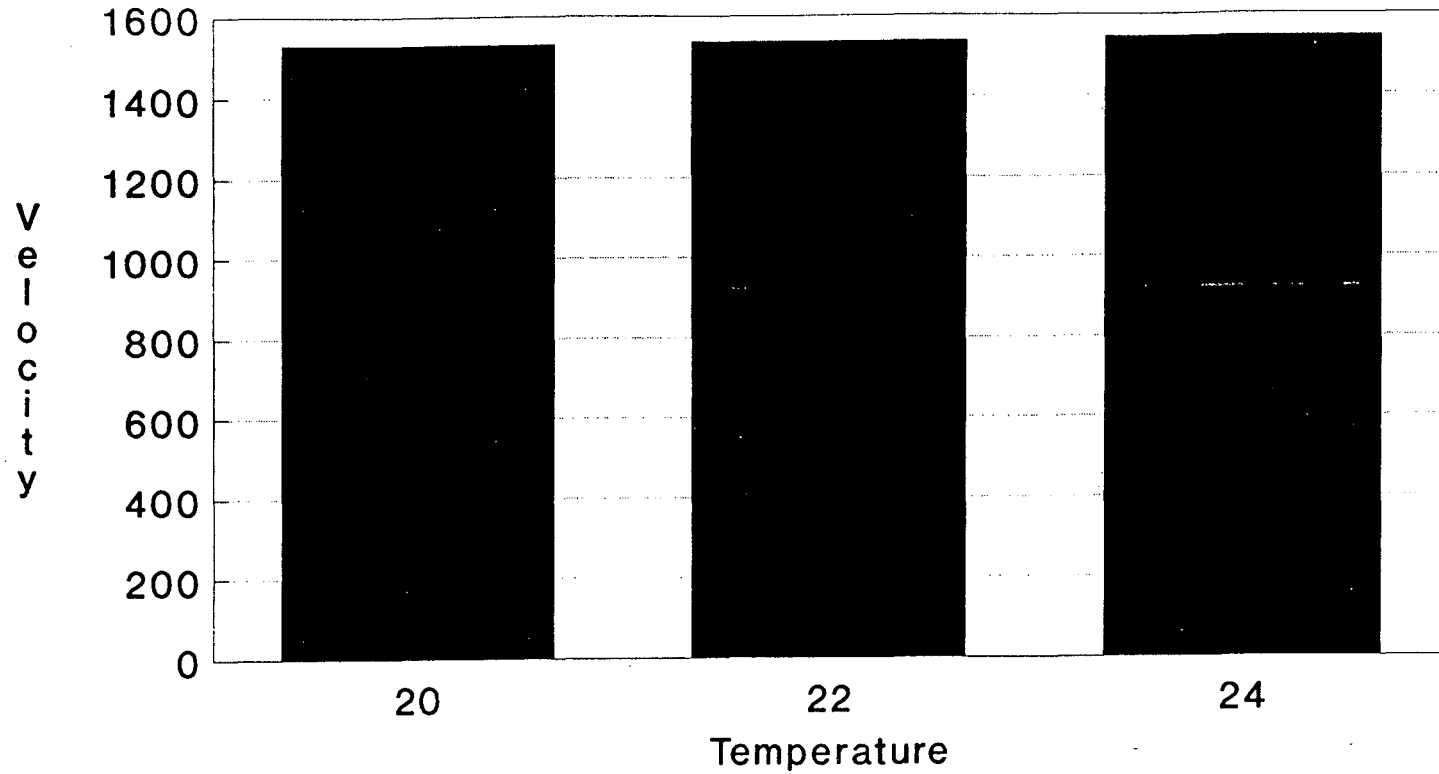
Series 1   Egg white

First we put saline in the cubator and time of light (TOF) was noted down. Then in the same cubator saline was replaced by the tissue (or bioloical liquid). Again we noted down the TOF. The difference between these two t_o - t = ΔT was noted down. Here t_o and t_1 be the TOF in water and the tissue (or biological liquid) respectively.

The sample varients ΔT and Δx are noted down to calculate velocity of ultrasound.

Precautions:

1.   All measurements are made in far field.
2.   The sample thickness was cut with accuracy in the case of tissues.
3.   Temperature fluctuation was minimized.

Effect of Fixation on Velocity of Ultrasound

Chemical fixation are used to preserve tissue specimens for histological and anatomical studies. There are various fixatives in use but we used the most common fixative formaline (10%) in our experiment. The sample was immediately kept in 10% formaline for 48 hours. After 48 hours the sample became more rigid (due to fixation). Due to this increased rigidity cutting of the samples into specimen of uniform thickness became easier. The sample

tissues were cut into slices of known thickness. Here also we applied the same "Relative Method" for computing speed of ultrasound in the tissues under investigation.

Results and Discussions

The results obtained are presented diagramatically (A):

1. We find that between Temperature range $30^{\circ}C$ to $40^{\circ}C$ temperature coefficient is positive.

2. The shape of the curve and appearance of maxima near $39.5^{\circ}C$, seems to be parallel to the behaviour of dilute aqueous salt solution.

3. Examination of data reveals that increasing speed of sound from one tisue to another correlates with an increasing protein content particularly, the structural protein collagen and also with decreasing water content.

4. Variation within a particular tissue such as liver are little influenced by the collagen content and appear to be primarily determined by the water content.

5. Cartensen and Schwan (1959) have measured the dispersion of the velocity of sound in solutions of

human hemoglobin and found that it is very vrey small, but increases with concentration of protein.'

6. The work carried out by Cartensen (1953) on the determination of the acoustic properties of blood and its compnents found that for four types of protein (plasma, red cells, hemoglobin, and albumin) the increase in velocity of sound over that in pure water ($10-40^{\circ}$ C).

7. Cartensen and Schwan (1959) have measured the dispersion of velocity of sound in solutions of human hemoglobin and found that it is very small, but increases with the concentration of protein.

8. We found that the velocity of ultrasound in the biological sample fixed in 10% formaline (liver and kidney) decreases due to fixation. The average percentage decrease is 1.35 (approximately the same) in liver and kidney tissue at 4 MHZ.
Bamber and Hill (1979) obtained 1.54% decrease in velocity due to fixation. Propagation of speed versus frequency for 15 tissue samples in three fresh normal adult human brain are also plotted Kremkau (1981). He obtained fresh tissue speed to be is 10 ms^{-1} higher than fixed.

N.Yang et al (1991) obtained velocity of 8 ms^{-1} less in fixed breast tissue than that unfixed. In our case we have obtained velocity 23 ms^{-1} less in fixed tissued (liver and kidney).

1. We have also quoted the result obtained by Kremakau (propagation speed versus temperature for fresh normal adult Corna radiata at 1 MHZ. A minimum occurs at approximately 15° C (Kremakau

Conclusion:

The speed of sound is very important in tissue characteristic. It is more a fundamental than either the attenuation or scattering characteristics, in the sense that these are influenced to some exent by variations in sound speed. Temperature is an important variables to consider when making measurement of propagation speed. The temperature coefficient dc/dt for non-fatty tissues are positive. The velocity of ultrasound is less in unfixed tissue than that in fresh tissues. This is due to the increased density of tissues as a result of fixation. The sound speed in liver and kidney (Goat) is higher than that in water.

B. ULTRASONIC ATTENUATION MEASUREMENT IN EGGS

Attenuation also play an important part in determining the sound power reaching in a particular tissue. They also play an important role in determining the natural and magnitude of biological effects of ultrasound.

Many workers have measured speed and attenuation in different biological medium. ultrasonic velocity and attenuation in mammalian tissues (chivers & Parry 1978, Fredrick W. Kremkan et al 1981, 1984) were detailed studied. Ultrasound attenuation in biological tissue using a bone transducer were measured (Behari and Ray 1987). Thirty six years ago Gold man (1956) said of their summary that "No Critical discussion is indicated at this time, but it is anticipated that the accumulation of further data on the basis of more prprecise and extensive measurements should permit important generalization on the acoustic characteristic of living mater". At this time we have large member data on the basis of which we can generalise the acoustic charactertic of the living matter.

The precise measmement of attenuation is somewhat complex and data varies accordingly, there fore we must take account of the type of tissues, the animal species, the conditions of the tissue and the temperature. It is

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virtually impossible to separate any biological variation
present from the variations that may arise from above
causes.

Here measurement is based on the transmission pair
technique. (Kremkan 1981, Behari, Singh and Ray Behari
1987). We have white hens eggs as sample. Any attempt to
make the accurate measurement of a given physical or
chemical attribute of eggs encounters the problem of
variability. First of all the chemical composition and
physical properties of egg vary for different breeds of
hens. Secondly, individual eggs vary from one another and
the third but not least, changes take place in any one egg
as its ages. However, there is no evidence from the work
reported here that any of these factors were significant in
compunction with the errors. The work on egg white and
comprehensive article on egg yolk including a summery of its
properties (Powrie 1968) have been dealt in brief.

The detailed published data on speed and absorption of
ultrasound in eggs were carried out by Povey and wolkinson
(1980) at 1.25 MHZ.

Methodology

The measmement is based on transmission technique using two transducers (Kremkau 1981). Two transdueers are submerged in bath containing 0.9% slice. The ultrasonic pulse excites the transmitting transducer. The reciver is placed co-axially to receive it. The receiving channels is fed to the screen of cathode ray oscilloscope. This enables us to see the clear picture of the transmitted pulse, through the or sample under investigations (Figure A).

We took all the readings in the far field of the trensduer (4.MHZ). Because in far field region of the transmitter the receiver transducer will not perturb the original field pattern. We used 0.9% saline as a coupling medium. A highly sensitive thermometer is used for recording temperature of the sample.

The amplitude of the received pulse with the sample and without doubled distilled water was measured. Attenuation coefficient can he calculated from the equation given below.

$$a = \frac{1}{d} \left[(20 \log \frac{A_o}{A_1}) - a_c \right] + a_w \ - - - - \ - \ - -(9\ )$$

Where a is the tissue attenuation coefficient in $dBcm^{-1}$

d is the sample thickness in cm, Aois the received amplitude with doubled distilled water, A as the received amplitude with present, a_c. is the correction for window loss in dB, aw ia attenuation (absorption co-efficient in water in dB cm^{+1}

a_c and a_w corrections were found to be less than 1%. So we neglected both of these. In addition reflection loss resulting from impedance discontinuity between water and tissue is not significant.

Hence, $a = 1/d(20 \log \frac{A^{\circ}}{A_1})$ ---------------- 10

There fore finally we use this equation for measuring attenuation co-efficient. We have also measured velocity of ultrasound in egg white and egg yolk at 20°C, 22°C, with the method as described in chapter III A. The results obtained are given here. We did not measure Ultra sound speed at higher Temperature because the effect of the temperature might induce irreversible changes.

Frequency.	Tem.	velocity
4 MHz	20°C	1530 M/S
	22°C	1538 M/S
	24°C	1544 M/S

Velocity of U.S. in Egg Yolk.

Frequency	Temp	Velocity
4 MHz	20°C	1509 M/S
22°C	22°C	1507 M/S
	24°C	1509 M/S

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Table III.1.B

The results

Egg Yolk

Frequency MHz	A_0 Volts	A_1 Volts	d. Cm.	a. dB cm^{-1}	Averge Att.
4	1.4	1.1	5.5	0.381	0.0385
Tem.,,	1.4	1.115	5.0	0.395	
20°C,,	1.4	1.15	4.5	0.38	
,,	1.4	1.17	4.0	0.39	
,,	1.4	1.25	2.6	0.379	
4	1.4	1.05	5.5	0.454	
24°C,,	1.4	1.08	5.0	0.451	
,,	1.4	1.12	4.5	0.431	0.443
,,	1.4	1.14	4.0	0.446	
,,	1.4	1.23	2.6	0.432	
4	1.4	1.12	5.5	0.352	
20°C,,	1.4	1.128	5.0	0.375	
,,	1.4	1.15	4.5	0.38	
,,	1.4	1.18	4.0	0.377	0.371
,,	1.4	1.25	2.6	0.379	

Attenuation is egg white transprent liquid.

Egg sample are kept in refrigerator for six hours. Its white
tansparent liquid are sepenated from egg yolk. Attenuation
is meagmed at 4 MHz at Temperature 20°C, 22°. and 24°C.

Table III.2.5

Egg White

Tem.	frequency	A_0 (Volts)	A_1 (Volt)	d (in cm.)	a dbcm^{-1}	Averge
20°C	4.0 MHz	1.4	1.35	2.6	0.121	
	4.0 ,,	1.4	1.33	4	0.111	0.116
	4.0 ,,	1.39	1.3	5	0.116	
	4.0 ,,	1.39	1.13	4.5	0.114	
	4.0 ,,	1.39	1.29	5.5	0.118	
	4	1.38	1.34	2.6	0.098	
22°C	4	1.38	1.32	4	0.097	
	4	1.38	1.31	4.5	0.1	
	4	1.38	1.3	5	0.104	
	4	1.38	1.29	5.5	0.107	
	4	1.4	1.36	2.6	0.097	
	4	1.4	1.34	4	0.095	
24°C	4	1.4	1.33	4.5	0.099	0.1
	4	1.4	1.32	5	0.102	
	4	1.4	1.31	5.5	0.105	

Result and Discussion

(a) Egg yolk has a negative temperature coefficient of atsorption.

(b) Attenuation in egg yolk is much higher than that of egg liquid (white Trensparant liquid).

(c) Generally attenuation decreases with Temp. In the case of egg yolk.

(d) No Temp. co-efficient could be expected within the Temperauture range 20-24°C. An attempts to interprete the results on eggs is full of controversy. We first discuss egg white. The work carried out by carstensen

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(1953) on the determination of the acoustic properties of blood and its components found that for four types of protein (Plasma, red cells, hemoglobin and albumin), the increase in velocity of sound over that in pure water (in the range 10°-40°C) averaged roughly 4 ms^{-1} per g protein per 100-cm^3 solution. Assuming a protein content of egg white of $^{-}$10 g. per 100^3cm of solution would yield values for the speed of sound in egg white about 40 ms^{-1} higher than that in pure water at the same temperature. Cartensen and Schwan (1953) have measured the dispersion of velocity of sound in solutions of human, hemoglobin and found that it is very small, but increases with the content of protein. At 11.4 gm/100cm^3 the dispersion was only 0.6 ms^{-1} over the frequency range of 0.3 to 10 MHz.

The original and the most accurate work on absorption of sound in protein solution was carried out by carstensen · ·, $\mathscr{E}$ (1959) between the frequency range 800 KHz to 3 MHz. Carstensen and schwan subsquently extended the range to 10 MHz. His result at 20°C for the average absorption of red cells, hemoglobin and albumin have been plotted, for a concentration of 10 gm protein per 100 cm^3. Cartensen et al (1959) found that larger plasma protein absorbed more

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ultrasound then red cells, hemoglobion, and albumin proteins. However, it is not possible to invoke this reason as the explanation for the difference between our results and those of Cartensen (1959). Indeed, ovalbumin, the constitnent (63%) of egg white protein has a molecular weight of ~44000 (although conalbumin, comprising 12% of egg white does have a molecular weight - of 80,000) For comparision, the molecular weights of hemoglobin and albumin are 64400 and 65600, respectively. Zaretskii et al (1972) have explained the difference in absorption at 21 MHz, the protein's since they also found that the molecular weight alone would not account for this difference.

Egg yolk, a 30% fat, 51% water emulsion is further complicated by the presence of 16% protenis. The attenuation is dominated by a process other than absorption due to proteins. As regards the velocity measurements, no Temerature coefficient of velocity could be detected within the temperature range 20°-25°C.

Winkinson's (1980) work on egg white and yolk at 5,10, and 15 MHz indicates a difference between the sound speed in egg white (thick and thin averaged) and egg yolk of about 30 ms^{-1}. The absolute values of sound in egg yolk found are about 10 ms^{-1} lower than those given by Winkinson (1980).

Conclusion

Egg yolk and egg white have very different ultrasonic characteristics. The Temperature coefficient of the speed of sound in the former could not be detected, where as that of the latter is vary close to that of water. On the other hand the negative Temperature co-efficient of absorption in yolk shows up quite clearly, where as that of the white is small and possibly changes sign near 4 MHz.

CHAPTER IV

ESTIMATION OF ATTENUATION SLOPE IN LIVER AND KIDNEY

I. <u>Pulse</u> <u>Echo</u> <u>Signal</u> <u>Analysis</u> <u>as</u> <u>a</u> <u>Function</u> <u>of</u> <u>Temp</u>:

The Pulse-Echo-signal observed from a shoft tissue may be considered as a coherent summation of echoes comming from a distribution of point scatteres (which is a function of Temperature)embeded in an attenuating medium. The electric voltage depends upon electric excitation through a set of transfer function that depends on the transducer design and on the nature of propagating medium and temperature. The goal of quantitative pulse-echo ultrasound is to measure these parameters. The depth varying mean diffraction filter as a function of temperature of the tissue will provide parameters of tissues differentiation. In order to perform this statistical analysis, the electrical pulse-echo response due to a single scatterer embedded in the attenuating medium must be first modelled. Individual scatter: -we assume that the acoustic property of the scatterer is Temperature dependent. Let $\vec{r}_o$ be the position of the scatter. The Fourier transformation of the windowed ecographic signal comming from a single scatterer located as ro gives the value $E(\vec{r}_o, v)$. The round trip Transfer function of this scatterer is defined by the ratio of this

spectrum over the electrical input spectrum $e(v)$, where the symbol $\tilde{}$ denotes Fourier transformation;

$$\tilde{E} \; (\vec{r}, \; v) \; = \; H^1 \; (\vec{r}_o, v) \; \tilde{e} \; (v).$$

The round trip transfer function for one scatterer located at r_o may be written as

$$H^1 \; (\vec{r}_o v), \; I^1(v) \; H \; (\vec{r}_o, v) \; u(v) \; 1 + (\vec{r}_o, v) \; I^R(v)$$

where $u(v)$ is the frequency-dependent scattering coefficient.

The ecographic signal observed as a voltage function $E(t)$ depends on the voltage function $e(t)$ applied to the electric terminals of the transducer, through a set of Transfer function that depend on acousto-electric transfer function, and the geometry of the tranducer and on the propagating medium (velocity, frequency-dependent attenuation, spatial distribution and frequency response of the scatterer).

There are two groups of Transfer function one that is independendent of the scatter position and another depend strongly on scatterer.

The acousto-electric transfer function in Transmission I^T (v) in reception I^R(v) and frequency dependent scattering amplitude u(v) are among the transfer function independent of scatter position. The frequency and temperature dependent scattering characteristics u(v) defined as the ratio of the scattered wave amplitude to the incident wave amplitude at each frequency at a given temperature. For small size "Rayliegh scatter" $U(v) \alpha v^2$ (Rayliegh).

The transfer function associated with the ultrasonic propagation in the medium e.g. diffraction and attenuation depend strongly on the location of the scatterers relative to Transducer. The directivity pattern of a transducer depends on the frequency and a scatterer located at the point $\vec{r}_o$ experiences a frequency -dependent acoustical force.

II. Role of attenuating medium:-

The effect of frequency dependent attenuation α(v) can be taken into account in the radiative pricess by correcting the classical Rayleigh Sommerfield Greens function

$$\frac{e^{ikR}}{2\pi R}$$

In liver, for frequencies in the range 1 to 10 MHz, the attenuation is approximately proportional to the frequency

$$\alpha(f) = \beta f \qquad .$$

Here, we have neglected the velocity dispersion linked with attenuation (the relative velocity dispersion is of the order of 10^{-4}; Donn, et al 1978). In this case Green's function can be replaced by attenuating spherical wave of the form.

$$\frac{e^{[i2\pi v/c - \alpha(v)]R}}{2\pi R}$$

Thus a new radiative transfer function is:

$$H(\vec{r}_0, v) = \iint O(x,y) \; e^{[i2\pi v/c - \alpha(v)]R} . ds$$

where $O(x,y)$, is the aparture function. If we further assume that all the points on the radiating aparture are nearly equidistant we can obtain an important simplification of the above expression (A). Thus by defining $\bar{R}(x,y,z)$ as the average distance from the radiating aparture to (x,y,z) we can separate completely the effect of diffraction and of attenuation in the global transfer function:

$$H(\vec{r}_0, v), = H(\vec{r}_0, v) \; A(\vec{r}_0, v). \text{ where } A(\vec{r}_0, v) = e^{-[\alpha(v)R]}$$

The exact estimation of the frequency dependent attenuation may be obtained from an ideal medium of well separeted scatterers, whose location are exactly known and whose echoes are now overlapping.

Kuc (1979) and Kak (1978) showed that the corrected echographic spectra from two non overlapping scatters remains Gaussian (in case of linear frequency-dependent attenuation, Gaussian shape filters are of practical use) but with a mean frequency translated towards the low frequency (IEEE symposium 1976).

III. Local Transfer Function:

For actual biological medium, the scattered density is important. It makes the determination of transfer function much more complicated. At a given time, response mixes the contribution of several scatterers. However, the selection of non-overlapping echoes becomes practically impossible because of the scatterer density observed in the medium.

In order to generalize the model proposed for an individual scatterer to tissue, we must have to model a tissue as a continuous distribution of scatterers where each differential volume dV has a spherical wave scattering amplitude $U(\vec{r}_o,v)dV$.

If the dispersion is uniform then

$$U(\vec{r}_O, v) = S(\vec{r}_O) \, U(v).$$

The frequency dependence of the scattering Co-efficient is independent of the spatial co-ordinates. So the variables are separated. The echo-comming from the medium is, of course, the coherent summation of the individual echoes.

IV. Short Time Fourier Analysis:

The local transfer function varies slowly with depth and a short-Time Fourier Analysis (STFA) can give a good estimation of the local tissue round trip transfer function versus the round trip time (Fink and Hottier 1982, Hottier et al 1982).

Short time Fourier Analysis (STFA) consists in sampling the signals by a sliding window. For each position of the window, the sampled signal is Fourier transformed and its spectrum is computed. The spectral content of the echographic signal scattered by an identified slice of soft tissue (located at a mean distance d from the probe) may be found by calculating the power spectrum within a time window centred around time = 2d/c, where c is the mean speed of sound. The STFA performs the calculation of the power spectrum through a sliding time window such that

$$R(\tau,v) = \left| \int_W E(t) \ W(t-\tau) e^{-2\pi v t} \, dt \right|^2$$

where $W(t)$ is the time window, $R(\tau,v)$, the power spectrum corresponding to the signal reflected by the slice of tissue located at distance d. Equation (B) gives Time frequency representation (TRF) of the echo signal which is appropriate for measuring the effects of time frequency-dependent attenuation and for studying the Time Varying Filter (TVF) effects on the spectral content of the r.f. signal.

V. Attenuation Slope:-

The spectral running moments of the short term Fourier analysis are simply correlated to attenuation slope.

The running moment of order n is defined as

$$m_n(\tau) = \int f^n |R \ (\vec{r},t)| \, dv.$$

The running spectral centriod and the running variance are

$$f(\tau) = \frac{m_1(\tau)}{m_2(\tau)}$$

$$\sigma^2(\tau) = \frac{m_2(\tau)}{m_0(\tau)} - \frac{(m_1(\tau))^2}{(m_0(\tau))^2}$$

If the diffraction effects are corrected, the only time varying part in the running spectrum is due to attenuation. Hence $R(v,t)$ can be written as

$$R(v,\tau) = R_0(v)e^{-(\alpha(v)c\tau)}$$

And if the attenuation is linear with respect to frequency, we have

$$R(v,\tau) = R_0(v)e^{-(\beta|v|c\tau)}$$

and

$$m_n(\tau) = \int_{v_1}^{v_2} R_0\, e^{(-\beta|v|c\tau)}\, v^n dn$$

By derivation under the integral sign we get

$$\frac{dm_n(\tau)}{d\tau} = -\beta c m_{n+1}(\tau)$$

and

$$\frac{df}{d\tau} = \frac{d}{d\tau}\frac{m_1(\tau)}{m_0(\tau)}$$

$$= \frac{1}{m_o(\tau)} \frac{dm_1(\tau)}{d\tau} - \frac{dm_1(\tau)}{m_o^2(\tau)} \frac{dm_o(\tau)}{d\tau}$$

Hence
$$\frac{df}{d\tau} = -\beta c^2 \frac{m_2}{m_o} - (\frac{m_1}{m_o})^2 \quad \text{or} \quad \frac{df}{d\tau} = -\beta c \sigma^2(\tau)$$

Thus the knowledge of the local spectral centriode shift and of the local spectral variance allows simple estimation of ß (sattenuation slope) without any assumption on the pulse shape (Fink & Cordoso 1983).

SAMPLE: Samples (Liver & Kidney) were obtained from slaughter house. These were obtained fixed in 10% formaline for 48 hours and then were cut into rectangular form. The sample thickness was kept 2.5cm in each case (Liver and Kidney).

Spectral Parameters: (a) Centriod of Power-distribution.

The centriode also called centre of gravity of the terminal points of the set is given by

$$f(\tau) = \Sigma \frac{dBxMHz}{\Sigma dB}$$

Its unit is MHz.

Spectral variance:- It is given by

$$\Sigma \ \frac{dB(MHz)^2}{\Sigma dB} - \frac{\Sigma(dBxMHz)^2}{\Sigma dB}$$

Its unit is $(MHz)^2$.

Scewness: Scewness is the degree of asymetry of the distribution.   A measure of the asymetry is given by the difference (Mean-Mode).

Scewness = Mean-Mode/S.D.

$$= \ \frac{X-Mode}{S}$$

The moment coefficient of skewness

$$a_3 \ = \ \frac{m_3}{\sqrt{m_2}^3}$$

where $m_2 = \overline{(X-\overline{X})^2}$
and $m_3 = \overline{(X-\overline{X})^3}$

VI. Method.:

Measurements are made in a thermostat water tank unit designed specially for this purpose. We used 0.9% sline instead to fill the tank. A transducer (nominal frequency 10 MHz)   is attached on a rotating arm. Single transducer is used in a transmitting and receiving mode . Transducer

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holder can be moved vertical up and down exactly in perpendicular direction. Transducer can be rotated till proper adjustment are made. We fixed the transducer at a position where pulse-echo amplitude is maximum. In this condition, axis of transducer lies at the centre of the sample. The sample (2.5 cm Thickness, Liver & Kidney) was kept fixed on a glass slab in front of the transducer. Reflected pulses are fed to a spectrum analyser, the output of which is fed to the input channels of a Cathode ray oscilloscope (555711C Iwatsu, Japan).

Transducer transmit ultrasonic pulses that impinge upon the sample. A zone around the centre (were ultrasonic pulses falls , upon) was located and the size of the sample choosen such that it was greater than the zone.

The spectrum analyser analyses the signal in different components, and noted the signal amplitude at different frequencies on the Cathode ray oscilloscope. We noted backscattered signal amplitude between 3.6 MHZ to 10 MHZ. The same set of reading is accomplished at different sample depths e.g.78 μs, 88μs, 98 μs, 108 μs, 118 μs, 128 μs and 138 μs.
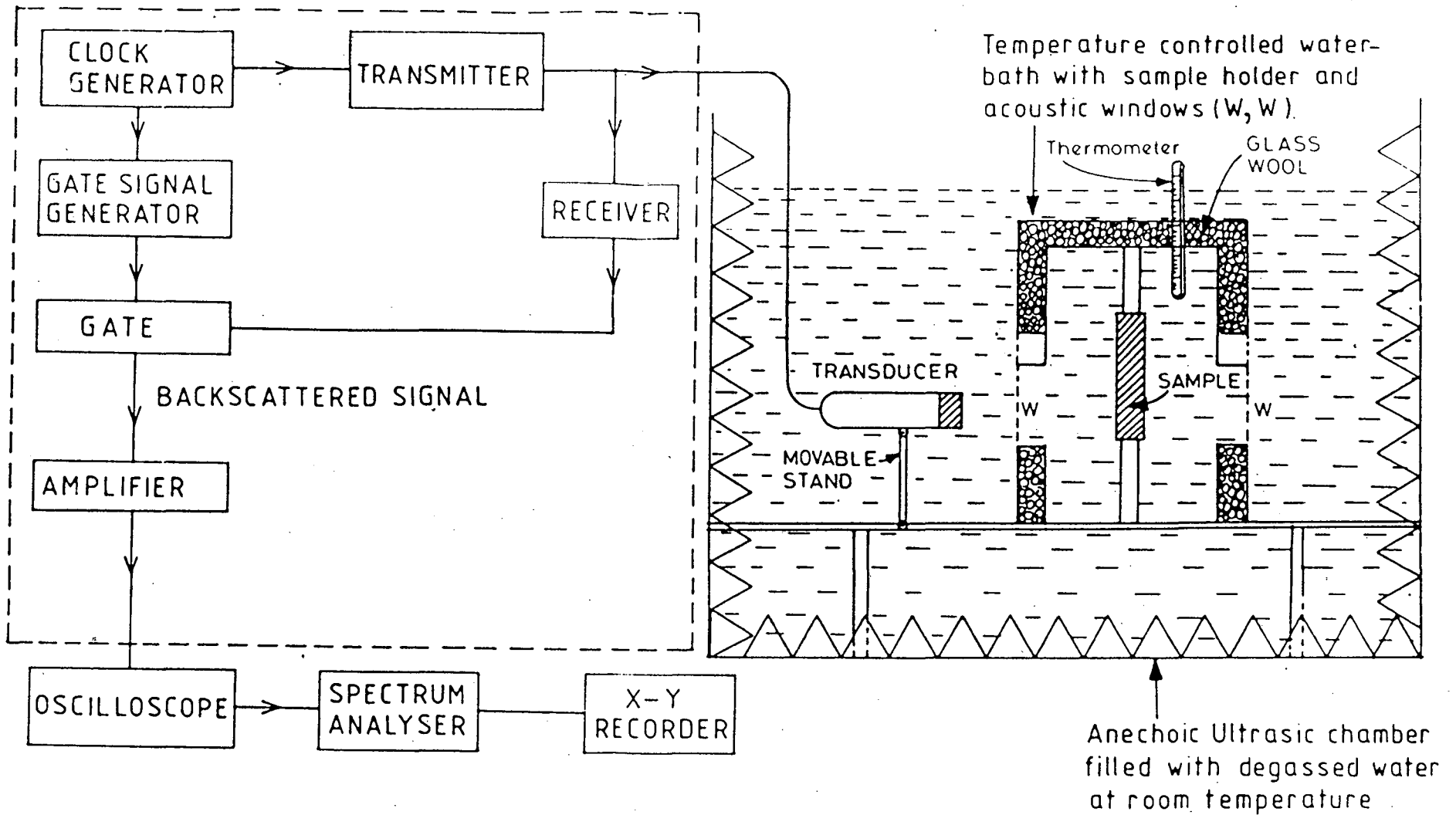
Fig(B) Experimental set up for measurement of Backscattering from soft tissues

Diagram labels:

CLOCK GENERATOR

TRANSMITTER

GATE SIGNAL GENERATOR

RECEIVER

GATE

BACKSCATTERED SIGNAL

AMPLIFIER

OSCILLOSCOPE

SPECTRUM ANALYSER

X-Y RECORDER

Temperature controlled water-bath with sample holder and acoustic windows (W, W).

Thermometer

GLASS WOOL

TRANSDUCER

SAMPLE

MOVABLE STAND

W

W

Anechoic Ultrasic chamber filled with degassed water at room temperature

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Steps for calculation:-

    (a)   Third order smoothing for signal voltages from samples.

    (b)   Coverting them into dB

          20 log (D ÷ H)

Where D is the first and H is the last signal voltages. We have normalized with the sample voltages itself.

    (c)   Finding out the centriode and spectral variance of the centriode.

    (d)   Slope of centriode Vs depth (time in μs) depth. curve is plotted using linear regression equation

          Y = A + BX

    (e)   Plots: Estimation of attenuation coefficient using the relationships :

$$\frac{df}{d\tau} = -2\beta c\ \sigma^2(\tau)$$

Where $f(\tau)$ be the centriode of power distribution, ß is the attenuation slope in dB/cm/MHz, C be the speed of ultrasound in the medium under investigation, df/dτ is the slope of centriode and $\sigma^2(\tau)$ be the variance of spectral centriode.

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Table IV.1.

Liver 30.5°C

Smooth voltages at different frequencies at particular depth.

CHAPTER IV

MHZ	148 μs	138 μs	128 μs	108 μs	98 μs	88 μs	78 μs
4	0.3	0.39	0.42	0.45	0.4	0.51	0.54
4.4	0.52	0.62	0.67	0.72	0.77	0.81	0.84
4.8	0.82	1.0	0.99	1.08	1.13	1.18	1.2
5.2	1.13	1.27	1.31	1.39	1.49	1.55	1.55
5.6	1.35	1.45	1.5	1.61	1.71	1.77	1.77
6.0	1.42	1.48	1.55	1.67	1.77	1.81	1.82
6.4	1.34	1.34	1.45	1.54	1.64	1.7	1.71
6.8	1.12	1.12	1.22	1.31	1.41	1.47	1.5
7.2	0.85	0.87	0.93	1.02	1.12	1.2	1.25
7.6	0.65	0.65	0.68	0.76	0.87	0.95	1.25
8.0	0.49	0.49	0.5	0.57	0.71	0.77	0.85
8.4	0.38	0.4	0.43	0.47	0.63	0.7	0.77
8.8	0.34	0.38	0.4	0.45	0.62	0.69	0.78
9.2	0.32	0.35	0.38	0.2	0.61	0.7	0.79
9.6	0.27	0.29	0.31	0.34	0.5	0.61	0.64

Smooth Voltage

Liver goat at 30.5°C.

Liver 30.5 (Deg. Centigrade)

Normalized Power Spectrum

Table IV.2

MHZ	138 μs	128 μs	108 μs	98 μs	88 μs	78 μs
4.0	5.1	4.60	4.08	3.52	2.92	2.27
4.4	4.16	3.85	3.04	2.82	2.2	1.52
4.8	3.3	3.16	2.78	2.4	1.63	1.72
5.2	2.75	2.74	2.4	1.8	1.28	1.01
5.6	2.35	2.35	2.05	1.53	0.915	0.62
6.0	2.15	2.1	1.91	1.4	0.76	0.36
6.4	2.11	2.06	1.75	1.2	0.68	0.00
6.8	2.53	2.36	2.00	1.36	0.74	0.00
7.2	3.35	2.99	2.39	1.58	0.78	0.00
7.6	5.68	3.29	2.53	1.36	0.39	0.00
8	4.78	3.9	3.22	1.31	0.17	0.00
8.4	6.1	5.3	4.39	1.84	1.07	0.44
8.8	7.2	6.14	5.22	2.43	1.41	0.96
9.2	7.85	6.79	5.6	2.362	1.49	0.78
9.6	7.49	7.08	5.35	2.00	1.2	0.62
dB	4.46	3.92	3.27	1.93	1.17	1.03
N =	15	15	15	15	15	10

Spectral Analysis :

Centriod :

78 μs	88 μs	98 μs	108 μs	128 μs	128 μs
7.00	6.94	6.89	6.39	6.0	5.59

Variance of (centriod) power distribution

3.14	3.24	3.29	3.26	3.42	3.37

t	f(t)
78	7.00
88	6.94
98	6.89
108	6.39
118	6.0
128	5.59

$$\frac{df(t)}{dt} = -0.0288$$

Velocity of ultrasound in goat liver (fixed) at 30.5°C
1570 M/S

78 μs (7 MHZ) β	88 μs (6.94 MHZ) $\alpha = \beta f$	98 μs (6.89 MHZ) $\alpha = \beta f^{r2}$
0.13	0.912	1.346
0.111	0.77	1.135
0.091	0.628	0.924

Variation of ░▒▓░░ Centroid with Depth (in Micro Second) Liver 30.5 (Deg.C.)
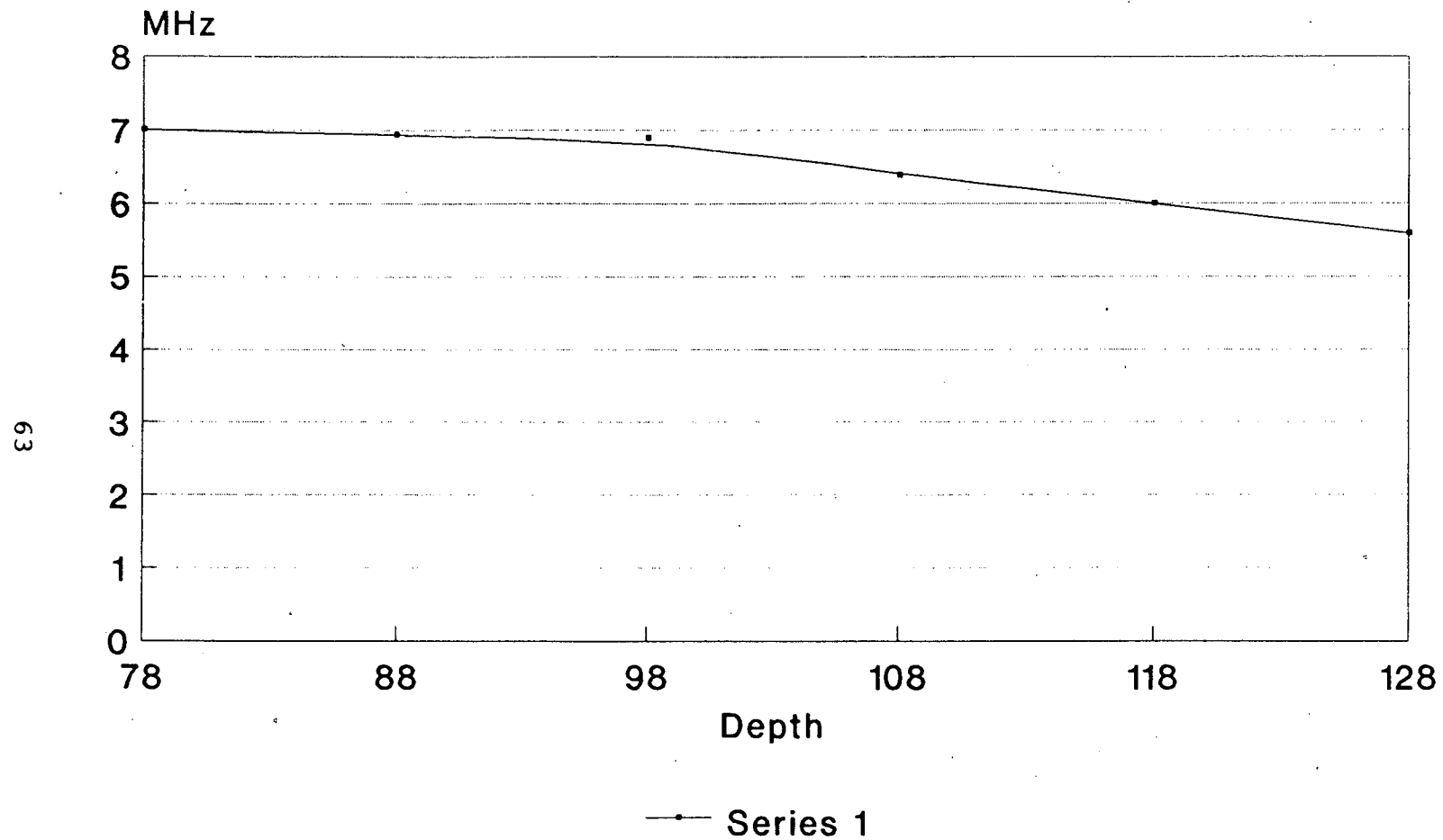
NORMALIZED POWER SPECTRUM (Liver Fixed) 30.5 (Deg. Centigrade)
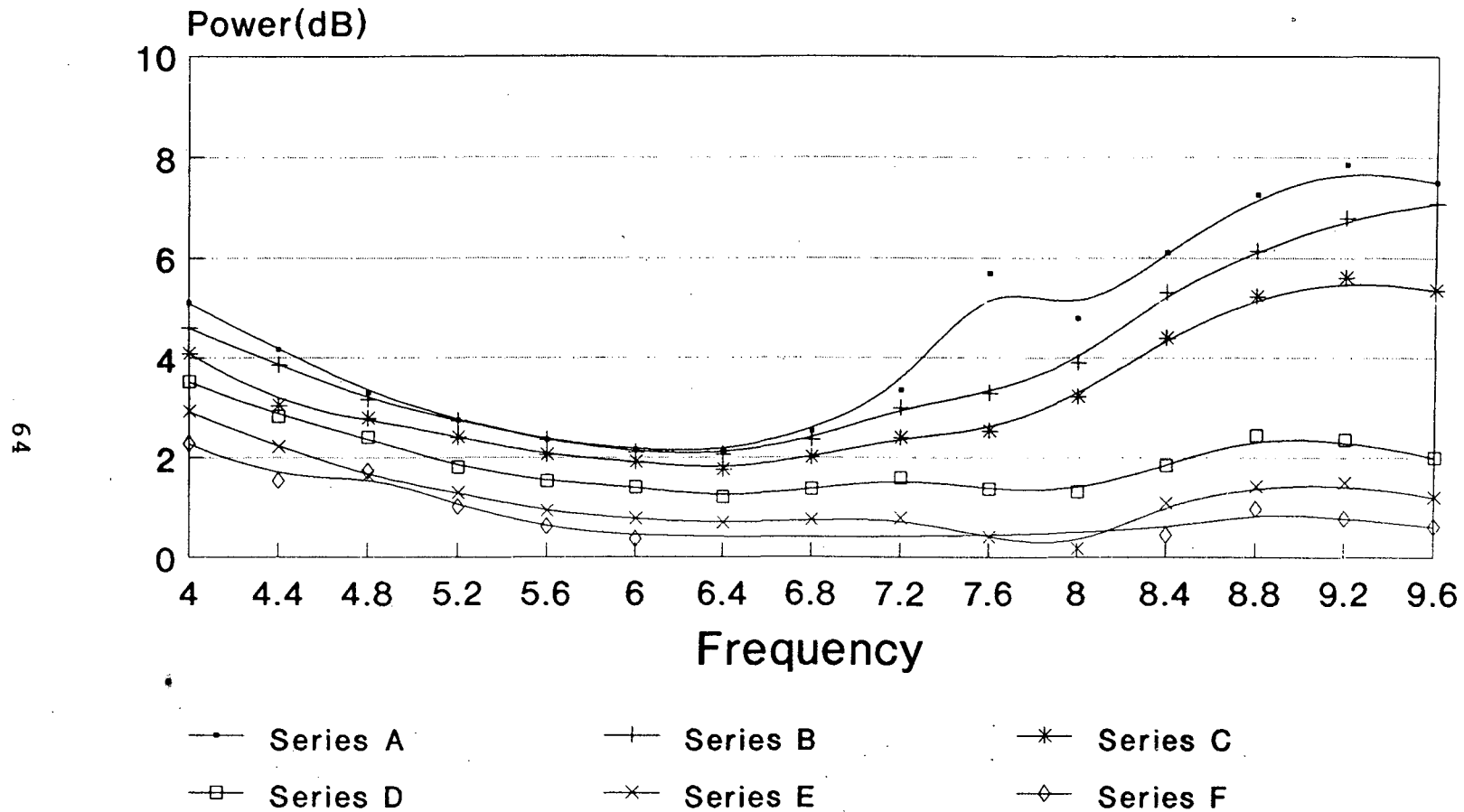
Table IV.3
LIVER 33.5°C

Smooth voltages at different frequencies at particular depth.

MHZ	138 μs	128 μs	118 μs	108 μs	98 μs	88 μs	78 μs
4.0	0.27	0.53	0.5	0.47	0.44	0.41	0.36
4.4	0.49	0.83	0.8	0.76	0.71	0.66	0.57
4.8	0.79	1.11	1.17	1.12	1.07	0.98	0.9
5.2	1.1	1.54	1.54	1.48	1.38	1.3	1.21
5.6	1.32	1.76	1.76	1.7	.1.6	1.49	1.41
6.0	1.39	1.81	1.8	1.76	1.66	1.54	1.42
6.4	1.31	1.7	1.69	1.63	1.53	1.44	1.31
6.8	1.09	1.49	1.46	1.4	1.3	1.21	1.09
7.2	0.82	1.24	1.19	1.11	1.01	0.92	0.84
7.6	0.58	1.24	0.94	0.86	0.75	0.67	0.6
8.0	0.42	0.84	0.76	0.7	0.56	0.49	0.44
8.4	0.32	0.76	0.69	0.62	0.46	0.42	0.34
8.8	0.25	0.77	0.68	0.61	0.44	0.39	0.3
9.2	0.23	0.79	0.69	0.61	0.43	0.37	0.26
9.6	0.21	0.68	0.58	0.5	0.3	0.28	0.22

Table IV.4
Normalized Power Spectrum

LIVER 33.5^{O}C

MHZ	78 μs	88 μs	98 μs	108 μs	118 μs	128 μs
4.0	5.86	5.35	4.81	4.24	6.63	2.5
4.4	4.58	4.26	3.81	3.22	2.59	1.31
4.8	3.56	3.4	3.03	2.64	1.87	1.13
5.2	2.92	2.92	2.58	1.97	1.45	0.83
5.6	2.5	2.40	2.2	1.67	1.05	0.57
6.0	2.29	2.25	2.05	1.54	0.89	0.19
6.4	2.26	2.21	1.91	1.35	0.82	0.00
6.8	2.72	2.54	2.17	1.53	0.9	0.00
7.2	3.59	3.24	2.63	1.81	1.0	0.21
7.6	6.6	4.19	3.42	2.23	1.25	0.29
8.0	6.02	5.15	4.44	2.5	1.34	0.4
8.4	7.51	6.67	5.74	3.15	2.36	0.53
8.8	9.77	8.69	7.75	4.19	3.86	1.58
9.2	10.72	9.54	8.47	5.44	4.13	1.06
9.6	10.63	9.25	7.96	3.52	2.92	0.83
dB	5.44	4.8	4.2	2.78	2.00	0.88
$\beta f(t)$	7.17	7.11	7.09	6.84	6.79	6.12
$\sigma^2(t)$	3.15	3.25	3.36	3.54	3.87	4.19

Slope of centriod

$$\frac{df(t)}{dt} = -0.02$$

Velocity of ultrasound at 33.5°C is a Liver (fixed) =

1574 M/S

Attenuation slope ß and

Attenuiation coefficient ßf and $ßf^{1.2}$ are given below.

78 μs (7.16 MHZ) ß	88 μs (7.13 MHZ) α = ßf	98 μs (7.14 MHZ) α = $ßf^{r2}$
0.11	0.787	1.67
0.094	0.667	0.988
0.079	0.563	0.833

Centriod VS Depth
Liver 33.5 (Deg. Centigrade)

89
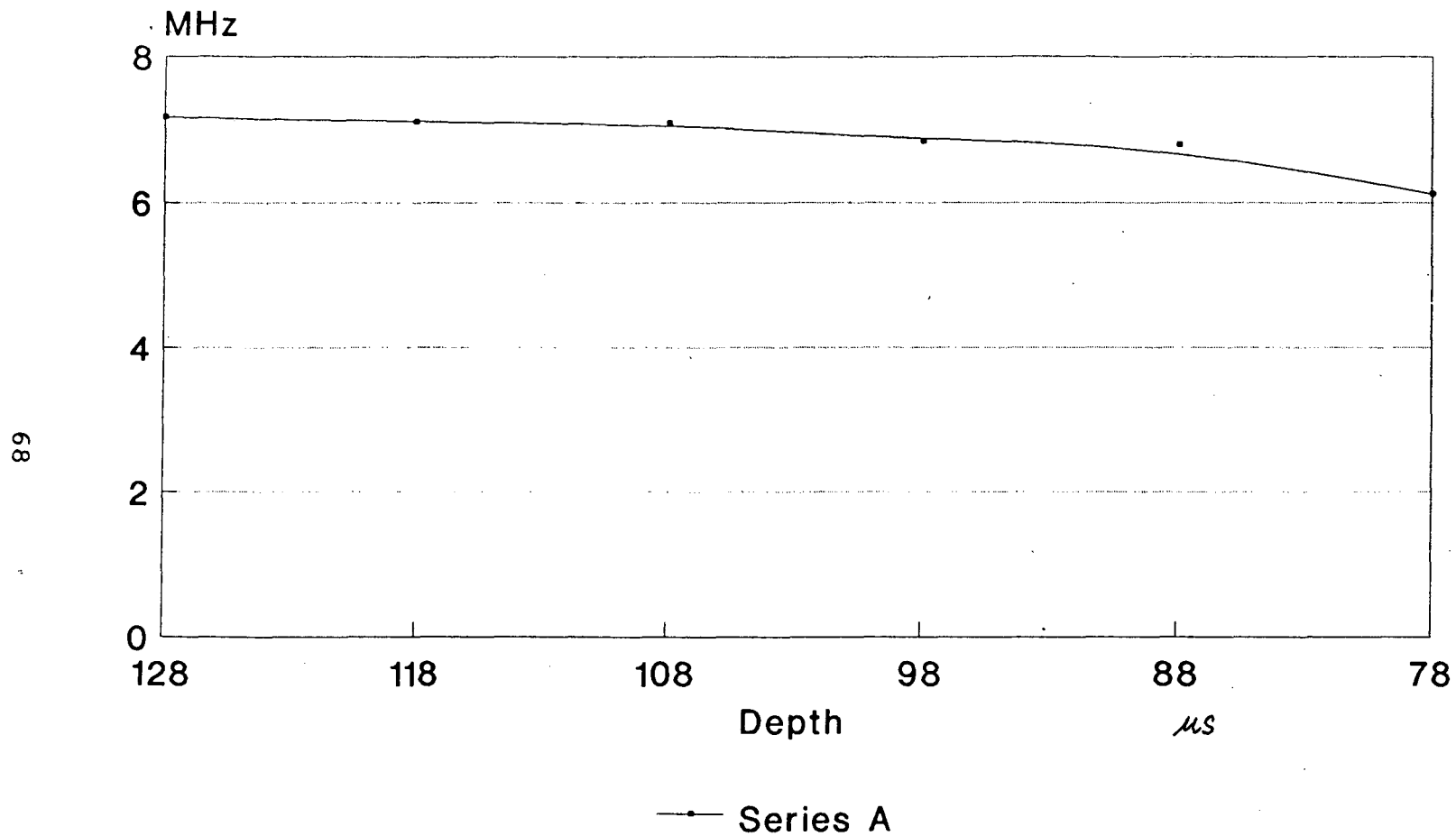
NORMALIZED POWER SPECTRUM (Liver Fixed)
33.5 (Deg. Centigrade)

Power (dB)

Frequency (MHz)

Series A
Series B
Series C
Series D
Series E
Series F

69
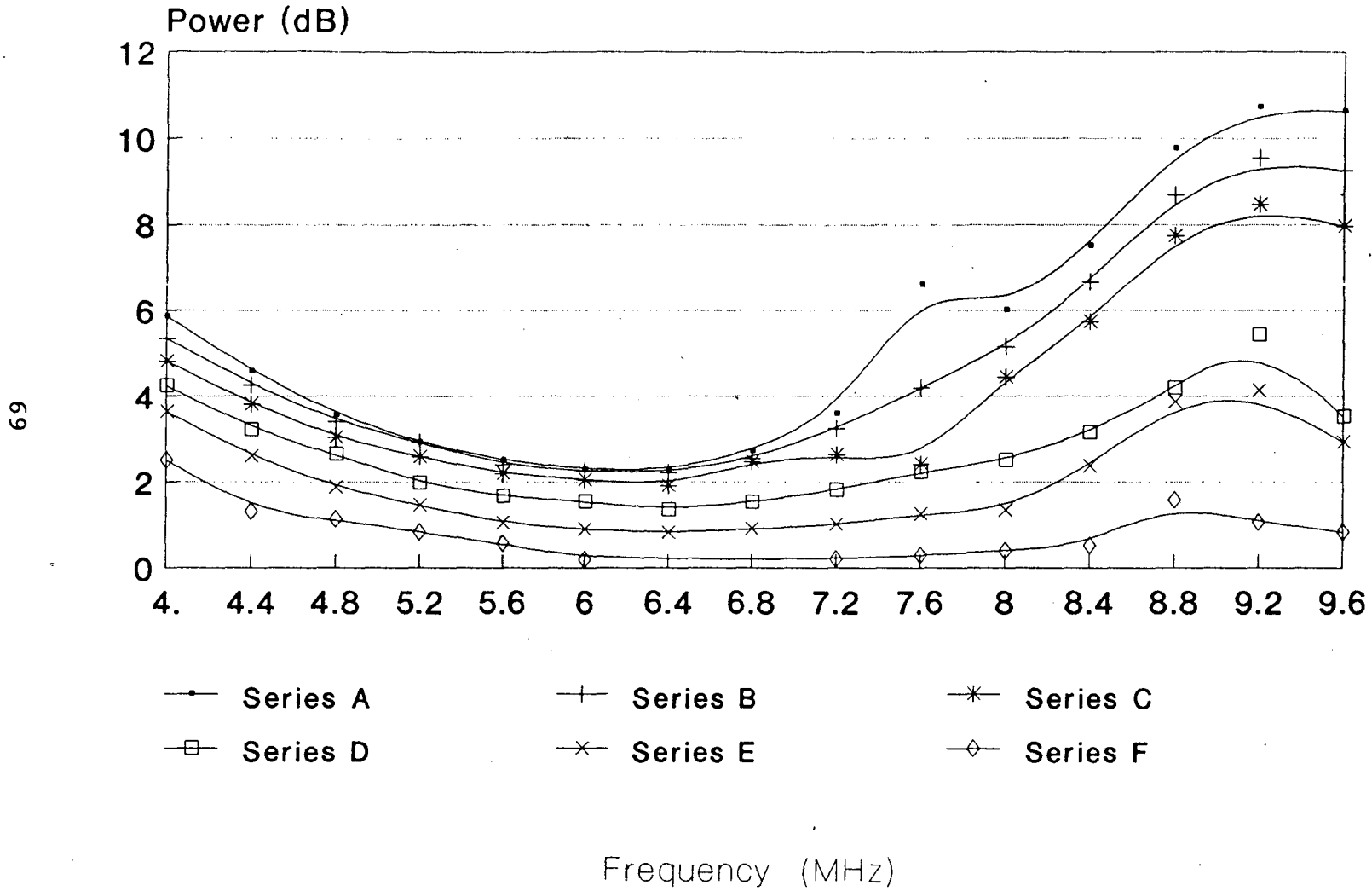
Table IV.5.

Liver (Goat) 36.5°C

Smooth voltages at different frequencies at particular depth.

MHZ	148 μs	138 μs	128 μs	108 μs	98 μs	88 μs	78 μs
4.0	0.26	0.32	0.37	0.41	0.44	0.47	0.49
4.4	0.44	0.5	0.55	0.59	0.62	0.65	0.67
4.8	0.67	0.73	0.78	0.82	0.85	0.88	0.9
5.2	0.9	0.96	1.02	1.06	1.11	1.14	1.16
5.6	1.04	1.1	1.16	1.21	1.26	1.31	1.34
6.0	1.03	1.09	1.15	1.2	1.25	1.3	1.33
6.4	0.95	1.01	1.07	1.12	1.17	1.22	1.25
6.8	0.71	0.77	0.83	0.82	0.93	0.98	1.02
7.2	0.53	0.59	0.65	0.7	0.75	0.8	0.84
7.6	0.39	0.45	0.51	0.56	0.6	0.64	0.68
8.0	0.29	0.32	0.41	0.46	0.51	0.56	0.6
8.4	0.23	0.29	0.35	0.38	0.45	0.49	0.54
8.8	0.21	0.21	0.29	0.32	0.43	0.46	0.53
9.2	0.19	0.19	0.19	0.28	0.41	0.45	0.51
9.6	0.15	0.15	0.15	0.26	0.33	0.38	0.44

Table IV.6.

Normalized Power Spectrum

MHZ	138 μs	128 μs	108 μs	98 μs	88 μs	78 μs
4.0	1.8	3.06	3.96	4.57	5.14	5.5
4.4	1.11	1.94	2.55	2.98	3.39	3.65
4.8	0.74	1.32	1.75	2.07	2.37	2.56
5.2	0.56	1.09	1.42	1.82	2.05	2.2
5.6	0.49	0.95	1.32	1.67	2.0	2.2
6.0	0.49	0.96	1.33	1.68	2.02	2.22
6.4	0.53	1.03	1.43	1.81	2.17	2.38
6.8	0.7	1.36	1.86	2.34	2.8	3.15
7.2	0.93	1.77	2.42	3.02	3.58	4.0
7.6	1.24	2.33	3.14	3.74	4.3	4.83
8.0	1.63	3.01	4.01	4.9	5.72	6.32
8.4	2.01	3.65	4.36	5.83	6.57	6.92
8.8	-	2.8	3.66	6.22	6.81	8.04
9.2	-	-	3.37	6.68	7.49	8.58
9.6	-	-	4.78	6.85	8.07	9.35
dB	1.02	1.94	2.76	3.75	4.3	4.79
W =	12	13	15	15	15	15

78 μs	88 μs	98 μs	108 μs	128 μs	128 μs
7.16	7.13	7.14	6.92	6.45	6.02
3.02	3.05	3.09	3.07	2.58	2.26

$$\frac{df(t)}{dt} = -.02$$

Velocity of ultrasound in goat liver (fixed) at 36.5°C
    1576 M/S

78 μs (7.16 MHZ) β	88 μs (7.13 MHZ) $\alpha = \beta f$	98 μs (7.14 MHZ) $\alpha = \beta f^{r2}$
0.101	0.721	1.068
0.089	0.638	0.945
0.077	0.55	0.815

Variation of Centroid with Depth (in Micro Second) Liver 36.5 (Deg.C.)

MHz

Depth

—•— Series 1
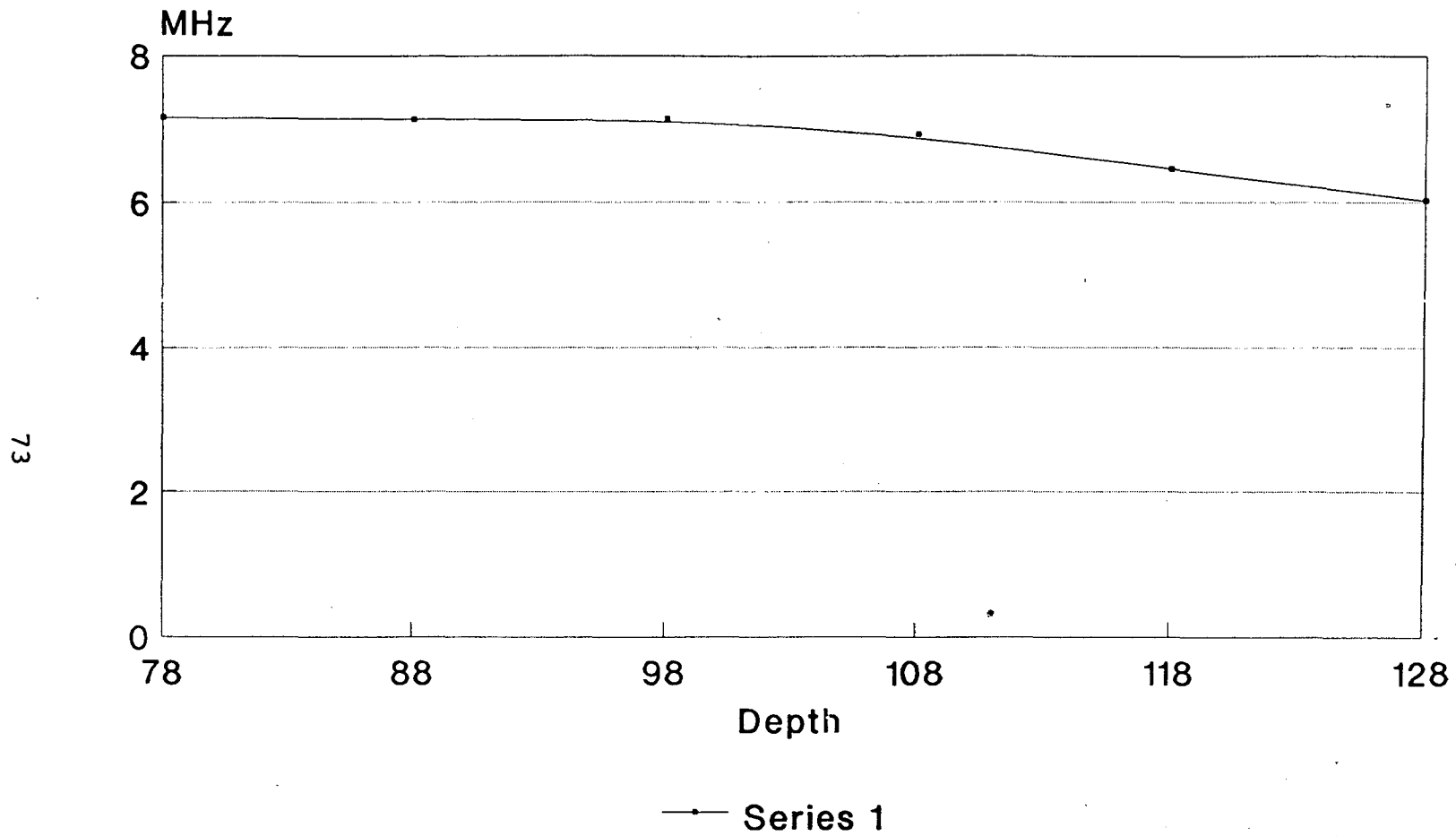
POWER SPECTRUM (Kidney Fixed)
36.5 (Deg. Centigrade)
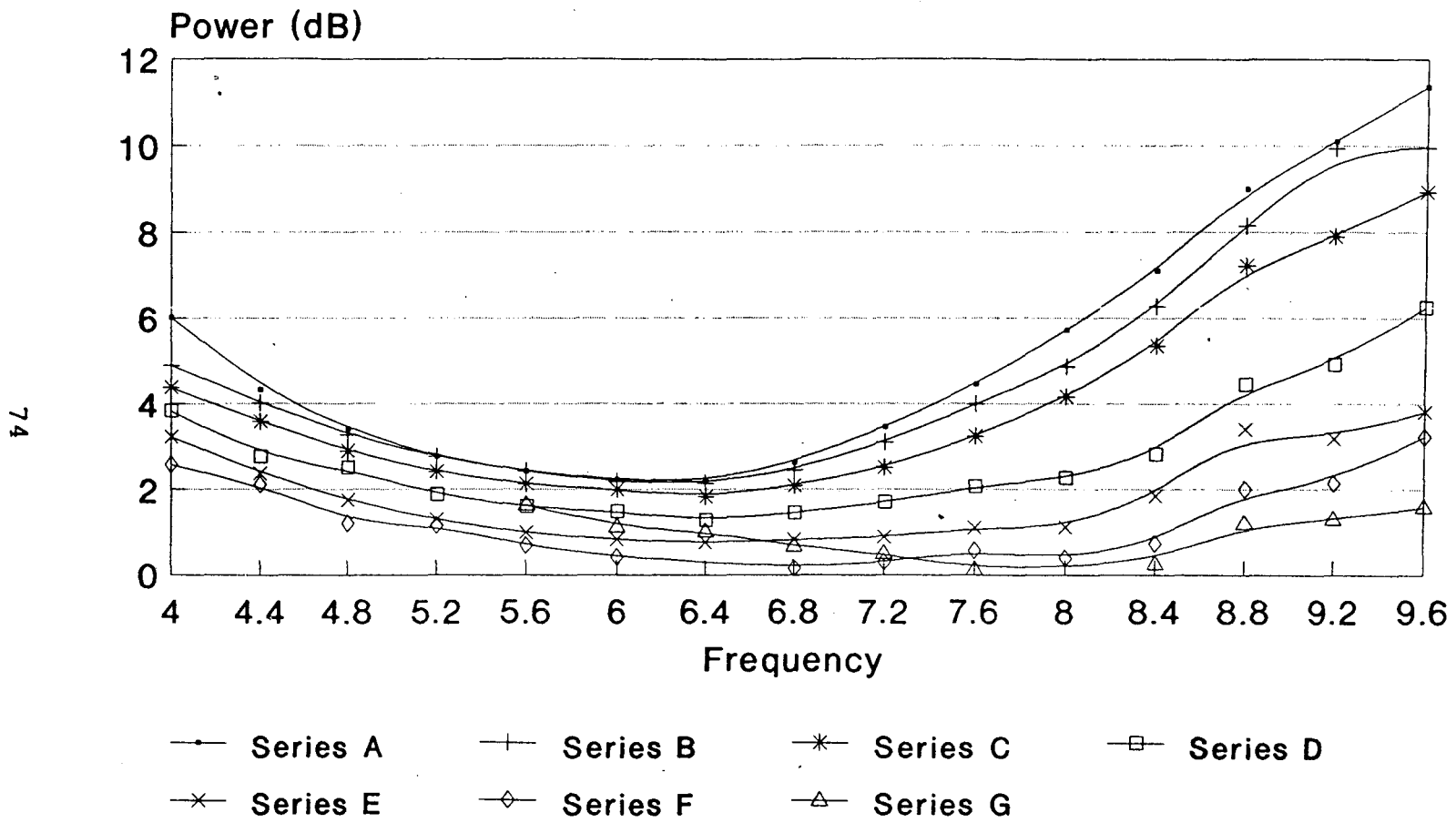
Table IV.7

Kidney (Goat) 30.5°C

MHZ	148 μs	138 μs	128 μs	118 μs	108 μs	98 μs	88 μs	78 μs
4.0	0.33	0.45	0.46	0.51	0.53	0.58	0.6	0.62
4.4	0.55	0.7	0.7	0.77	0.82	0.89	0.9	0.92
4.8	0.85	0.98	1.01	1.08	1.15	1.24	1.25	1.26
5.2	1.14	1.27	1.33	1.39	1.47	1.54	1.57	1.58
5.6	1.33	1.44	1.52	1.57	1.64	1.73	1.74	1.74
6.0	1.37	1.47	1.54	1.6	1.65	1.74	1.76	1.76
6.4	1.27	1.34	1.37	1.43	1.49	1.58	1.6	1.62
6.8	1.04	1.04	1.04	1.18	1.25	1.33	0.88	1.4
7.2	0.77	0.82	0.83	0.89	0.97	1.06	1.11	1.14
7.6	0.54	0.6	0.61	0.65	0.74	0.83	0.89	0.91
8.0	0.39	0.44	0.44	0.5	0.58	0.69	0.72	0.76
8.4	0.33	0.36	0.35	0.43	0.52	0.62	0.64	0.69
8.8	0.29	0.29	0.31	0.41	0.5	0.6	0.63	0.7
9.2	0.27	0.27	0.27	0.41	0.49	0.59	0.69	0.7
9.6	0.24	0.24	0.24	0.35	0.43	0.52	0.57	0.61

Table IV .8
Normalized Power Spectrum (Plotted)

MHZ	78 μs	88 μs	98 μs	108 μs	118 μs	128 μs	138 μs
4.0	5.48	5.48	4.9	4.12	3.78	2.89	2.69
4.4	4.47	4.28	4.18	3.47	2.92	2.09	2.09
4.8	3.42	6.35	3.28	2.63	2.08	1.5	1.24
5.2	2.84	2.78	2.72	2.21	1.72	1.34	0.94
5.6	2.33	2.33	2.28	1.82	1.44	1.16	0.69
6.0	2.18	2.18	2.08	1.62	1.35	1.02	0.61
6.4	2.11	2.0	1.9	1.39	1.03	0.66	0.46
6.8	2.58	1.45	2.14	1.6	1.09	0.00	0.00
7.2	3.41	3.18	2.78	2.0	1.26	0.65	0.55
7.6	4.53	4.34	3.73	2.74	1.61	1.06	0.92
8.0	5.8	5.33	4.96	3.45	2.16	1.05	1.05
8.4	6.4	5.75	5.48	3.95	2.3	0.51	0.76
8.8	7.65	6.67	6.32	4.73	3.0	0.58	
9.2	8.27	7.5	6.79	5.18	3.63	0.00	
9.6	8.1	7.5	6.72	5.1	3.28	0.00	
dB	4.64	4.27	4.02	3.07	2.18	1.21	1.09
N =	15	15	15	15	15	12	11
f(t)	7.02	6.97	6.95	6.86	6.63	5.54	5.39
σ^2(t)	3.25	3.31	3.26	3.38	3.56	2.02	1.95

$$\frac{df(t)}{dt} = -0.03$$

Attenuation Slope anmd Attenuation Coefficient

78 μs (7.16 MHZ) β	88 μs (7.13 MHZ) $\alpha = \beta f$	98 μs (7.14 MHZ) $\alpha = \beta f^{1.2}$
0.14	0.97	1.44
0.13	0.87	1.29
0.12	0.83	1.23

Variation of [illegible] Centroid with Depth (in Micro Second) kidney 30.5 (Deg.C.)
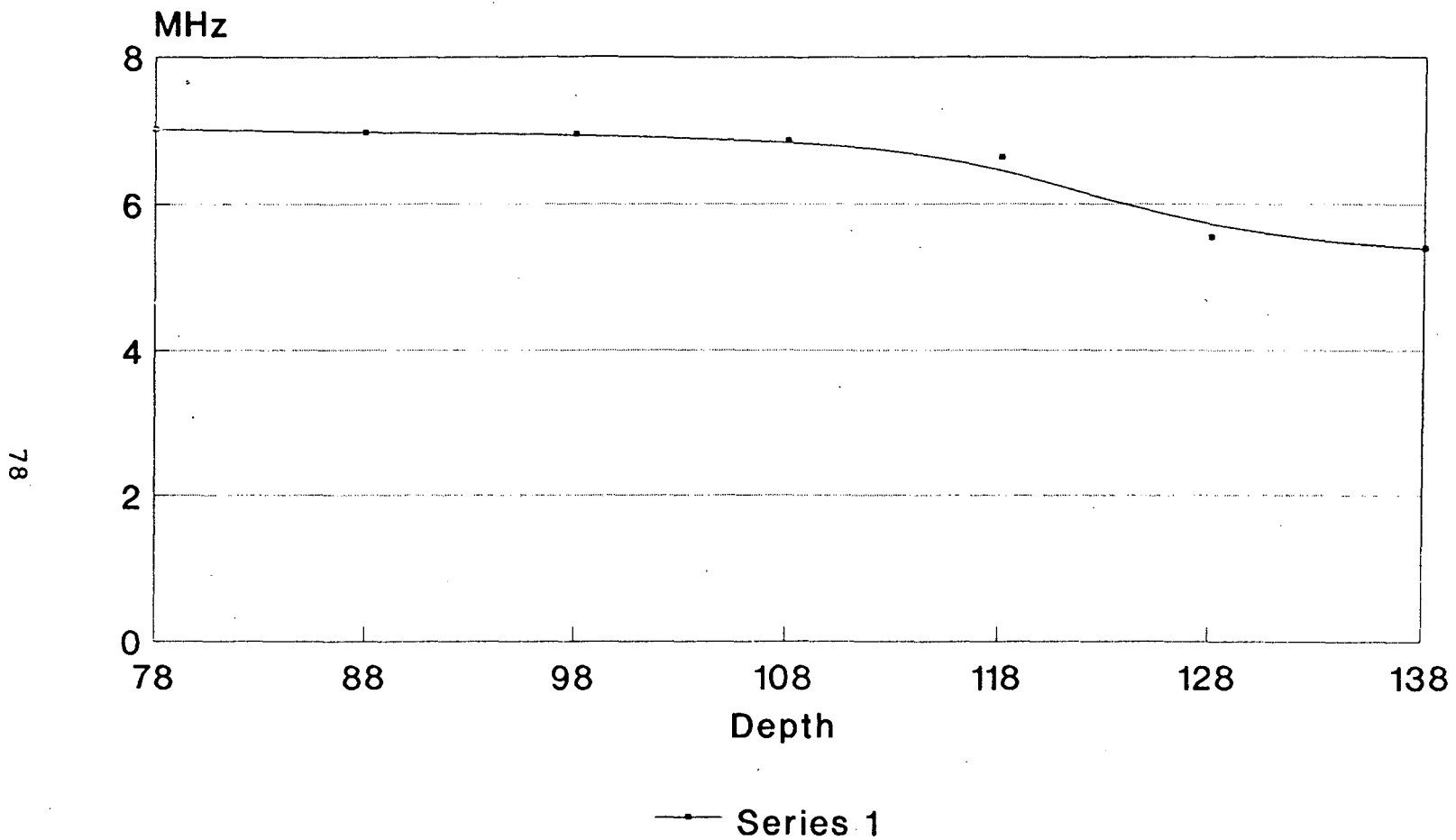
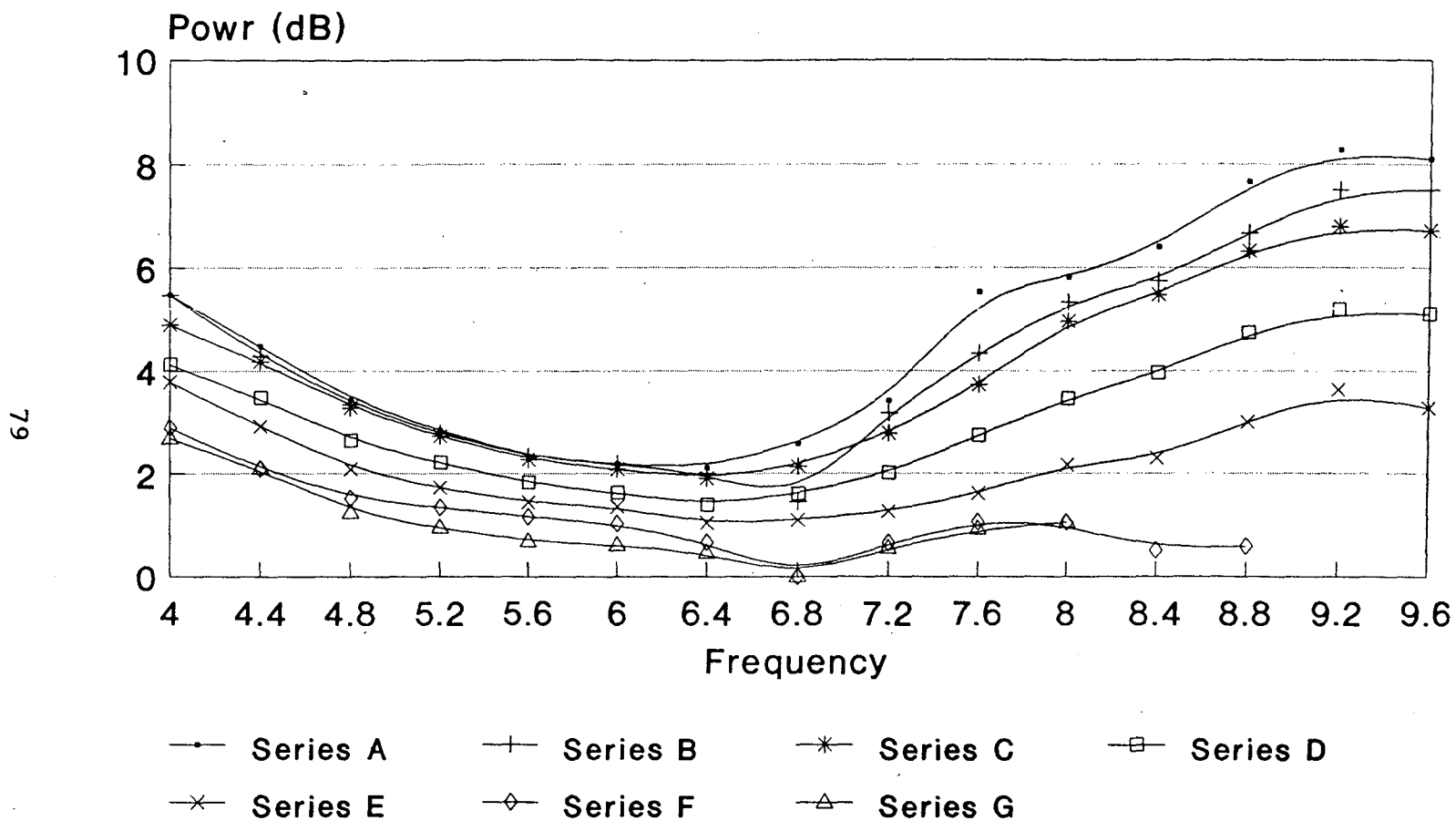# NORMALIZED POWER SPECTRUM (Kidney Fixed) 30.5 (Deg. Centigrade)

Table IV .9

Kidney (Goat) 33.5°C

Smooth voltages at different frequencies at particular depth.

MHZ	148 μs	138 μs	128 μs	118 μs	108 μs	98 μs	88 μs	78 μs
4.0	0.3	0.37	0.39	0.42	0.45	0.48	0.51	0.54
4.4	0.52	0.58	0.69	0.67	0.72	0.77	0.81	0.84
4.8	0.82	0.91	1.0	0.99	1.08	1.13	1.18	1.2
5.2	1.13	1.22	1.27	1.31	1.39	1.49	1.55	1.55
5.6	1.35	1.42	1.45	1.5	1.61	1.71	1.77	1.77
6.0	1.42	1.42	1.48	1.55	1.67	1.77	1.81	1.82
6.4	1.34	1.34	1.34	1.45	1.54	1.64	1.7	1.71
6.8	1.12	1.12	1.12	1.22	1.31	1.41	1.47	1.5
7.2	0.85	0.85	0.87	0.93	1.02	1.12	1.2	1.25
7.6	0.61	0.61	0.64	0.68	0.76	0.87	0.95	1.25
8.0	0.45	0.45	0.45	0.5	0.57	0.71	0.77	0.85
8.4	0.35	0.35	0.35	0.43	0.47	0.63	0.7	0.77
8.8	0.28	0.31	0.38	0.4	0.45	0.62	0.69	0.78
9.2	0.26	0.3	0.35	0.38	0.44	0.61	0.7	0.8
9.6	0.21	0.25	0.29	0.31	0.34	0.5	0.61	0.64

Table IV.10

Normalized Power Spectrum

dB	78 μs dB	88 μs dB	98 μs dB	108 μs dB	118 μs dB	128 μs dB	138 μs dB
4.0	5.1	4.61	4.08	3.52	2.92	2.28	1.82
4.4	4.17	3.85	3.41	2.28	2.2	2.46	0.95
4.8	3.3	3.16	2.79	2.39	1.64	1.72	0.9
5.2	2.75	2.75	2.4	1.8	1.28	1.01	0.67
5.6	2.35	2.35	2.05	1.53	0.92	0.62	0.44
6.0	2.16	2.1	1.91	1.4	0.76	0.36	—
6.4	2.12	2.1	1.75	1.2	0.69	—	—
6.8	2.54	2.36	2.0	1.36	0.74	—	—
7.2	3.35	2.99	2.32	1.58	0.78	0.2	—
7.6	6.23	3.85	3.08	1.91	0.94	0.42	—
8.0	5.52	4.67	3.96	2.05	0.92	—	—
8.4	6.85	6.02	5.1	2.56	1.78	—	—
8.8	8.9	7.83	6.9	5.46	3.1	2.65	8.8
9.2	9.76	8.48	7.55	4.57	3.3	2.58	1.24
9.6	10.94	9.54	8.52	5.8	3.38	2.8	1.51
dB	5.07	4.44	3.86	2.63	1.69	1.55	1.51
N	15	15	15	15	15	11	8

Centroid and spectral variance

	78 μs	88 μs	98 μs	100 μs	118 μs	128 μs	138 μs
	7.18	7.1	7.09	6.92	6.73	6.35	5.92
	3.1	3.2	3.27	3.48	3.88	4.48	4.5

$$\frac{df(\bar{t})}{dt} = -0.02$$

Velocity of ultrasound in Kidney (Fixed) at 33.5°C 1552 M/S

78 μs (7.16 MHZ) β	88 μs (7.13 MHZ) $\alpha = \beta f$	98 μs (7.14 MHZ) $\alpha = \beta f^{1.2}$
0.105	0.757	1.22
0.089	0.635	0.939
0.076	0.539	0.798

Centroid VS Depth
Kidney 33.5 (Deg. Centigrade)
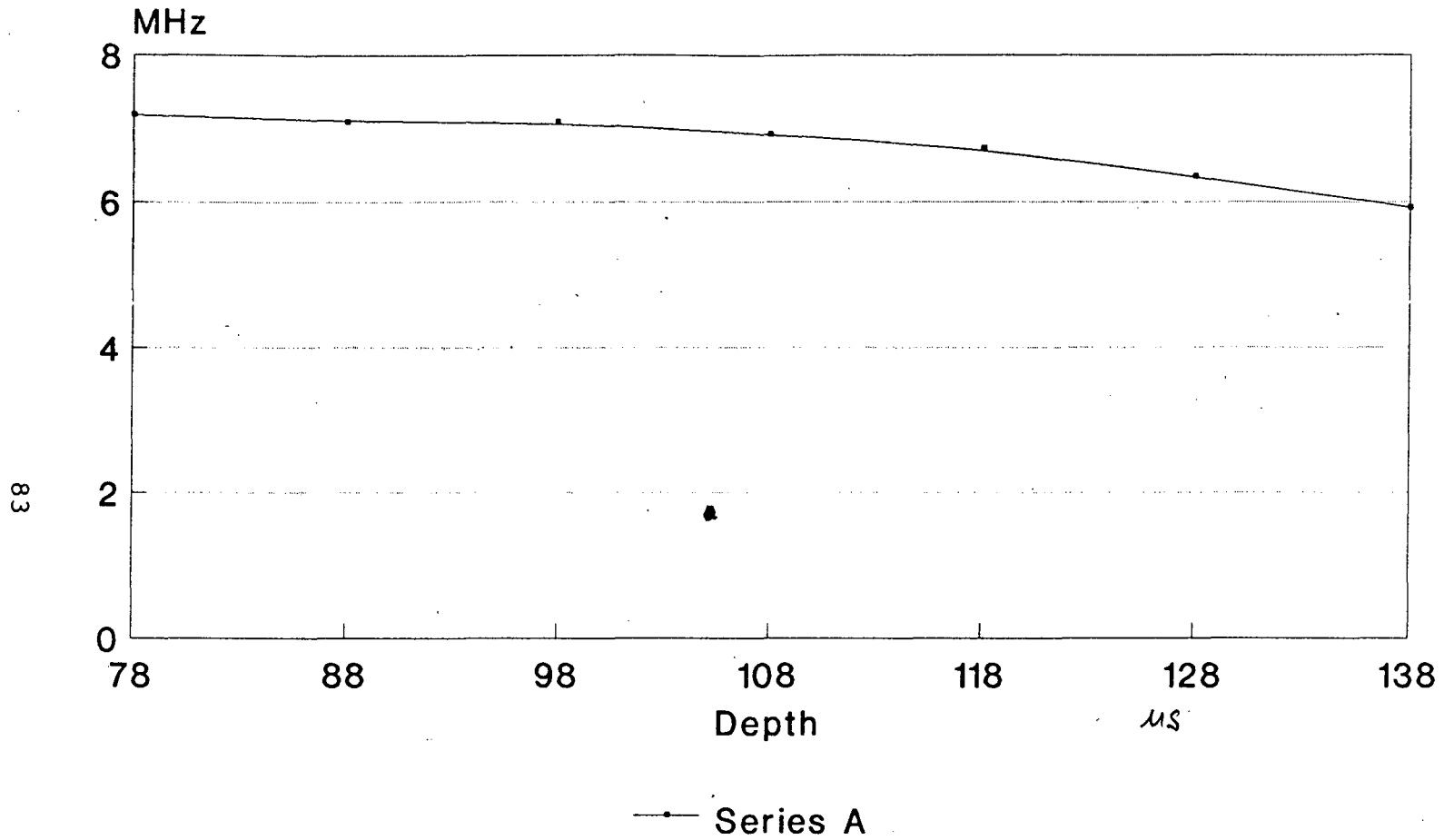
NORMALIZED POWER SPECTRUM (Kidney Fixed)
33.5 (Deg. Centigrade)

Power (dB)

Frequency

—•— Series A   —+— Series B   —✳— Series C   —▫— Series D

—✕— Series E   —◇— Series F   —△— Series G
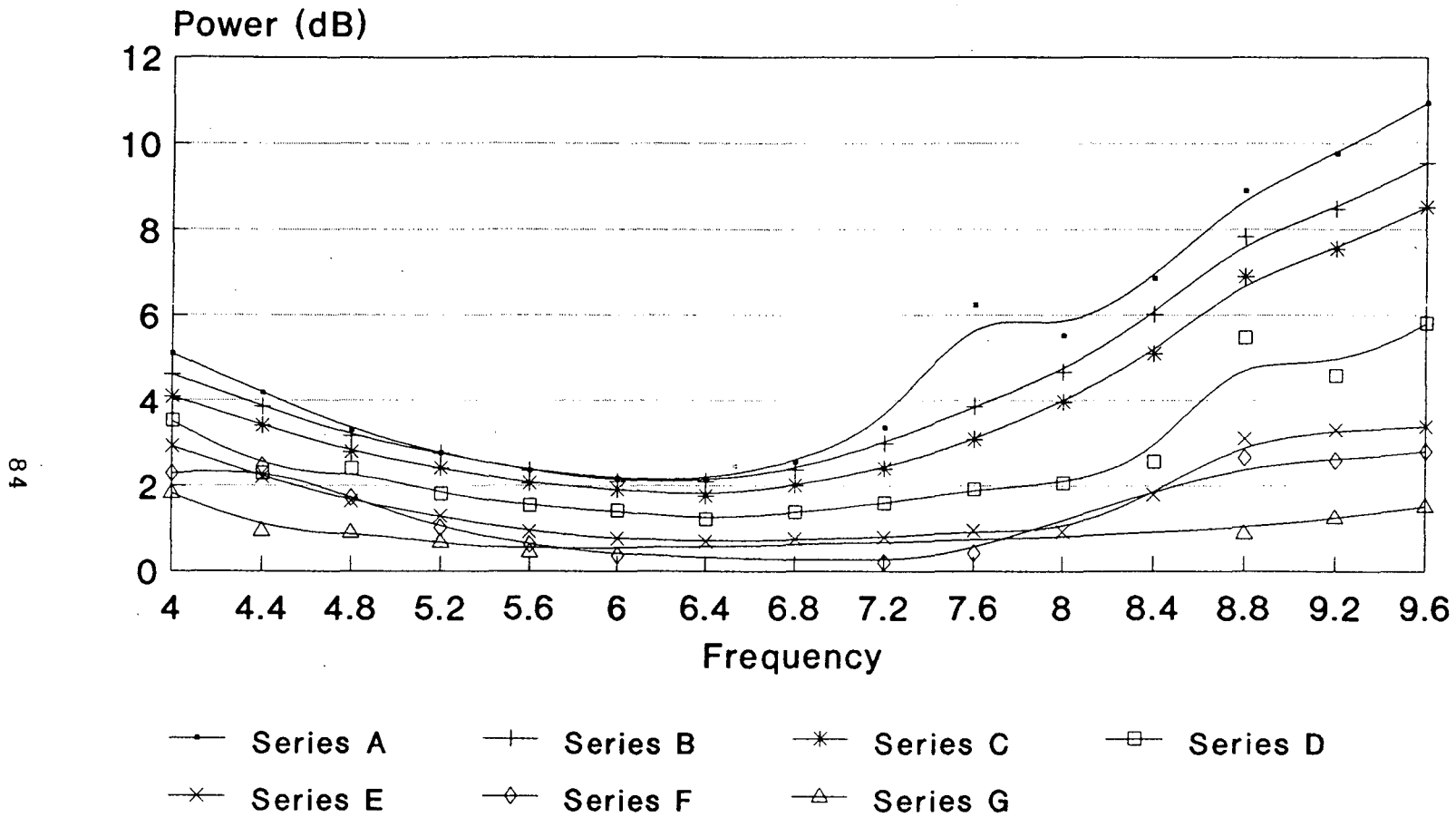
Table Iv .11

Kidney (Goat) 36.5°C

Smooth voltages at different frequencies at particular depth.

MHZ	148 μs	78 μs	88 μs	98 μs	108 μs	118 μs	128 μs	138 μs
4.0	0.29	0.54	0.51	0.48	0.45	0.42	0.39	0.35
4.4	0.51	0.84	0.81	0.77	0.7	0.67	0.65	0.58
4.8	0.81	1.2	1.18	1.13	1.08	0.99	0.93	0.91
5.2	1.13	1.55	1.55	1.49	1.40	1.31	1.29	1.22
5.6	1.34	1.77	1.77	1.71	1.61	1.5	1.45	1.42
6.0	1.41	1.82	1.81	1.77	1.67	1.55	1.48	1.43
6.4	1.33	1.71	1.7	1.64	1.54	1.45	1.34	1.29
6.8	1.11	1.5	1.47	1.41	1.31	1.22	1.13	1.06
7.2	0.84	1.25	1.2	1.12	1.02	0.93	0.87	0.84
7.6	0.6	1.0	0.95	0.87	0.76	0.68	0.64	0.6
8.0	0.44	0.85	0.77	0.71	0.57	0.5	0.46	0.44
8.4	0.34	0.77	0.7	0.63	0.47	0.43	0.37	0.35
8.8	0.27	0.78	0.69	0.62	0.45	0.4	0.34	0.31
9.2	0.25	0.8	0.7	0.62	0.44	0.36	0.32	0.29
9.6	0.2	0.74	0.6	0.56	0.41	0.31	0.29	0.24

Table IV.12

Normalized Power Spectrum Kidney 36.5°C

MHZ	78 μs dB	88 μs dB	98 μs dB	108 μs dB	118 μs dB	128 μs dB	138 μs dB
4.0	6.02	4.9	4.38	3.82	3.22	2.57	1.63
4.4	4.33	4.02	3.58	2.75	2.37	2.11	1.12
4.8	3.41	3.27	2.89	2.5	1.74	1.2	1.01
5.2	2.75	2.75	2.4	1.86	1.28	1.15	0.67
5.6	2.42	2.42	2.12	1.59	0.98	0.69	0.5
6.0	2.22	2.17	1.98	1.47	0.82	0.42	0.12
6.4	2.18	2.13	1.82	1.27	0.75	0.0	0.00
6.8	2.62	2.44	2.08	1.44	0.82	0.16	0.00
7.2	3.45	3.1	1.5	1.69	0.88	0.3	0.00
7.6	4.44	3.99	3.23	2.05	1.09	0.56	0.00
8.0	5.72	4.86	4.16	2.25	1.11	0.39	0.00
8.4	7.1	6.27	5.36	2.81	1.84	0.73	0.25
8.8	9.21	8.15	7.22	4.44	3.41	2.0	1.2
9.2	10.1	8.94	7.89	4.91	1.71	2.14	1.29
9.63	11.36	9.97	8.94	6.24	3.81	3.23	1.58
dB	5.16	4.63	4.04	3.14	1.82	1.18	.94
N	15	15	15	15	15	14	10
f(t)	7.13	7.11	7.09	6.84	7.1	6.37	6.14
	3.27	3.22	3.28	3.5	4.31	4.28	4.53

Slope of Centriod

$$\frac{df_{(t)}}{dt} = -0.02$$

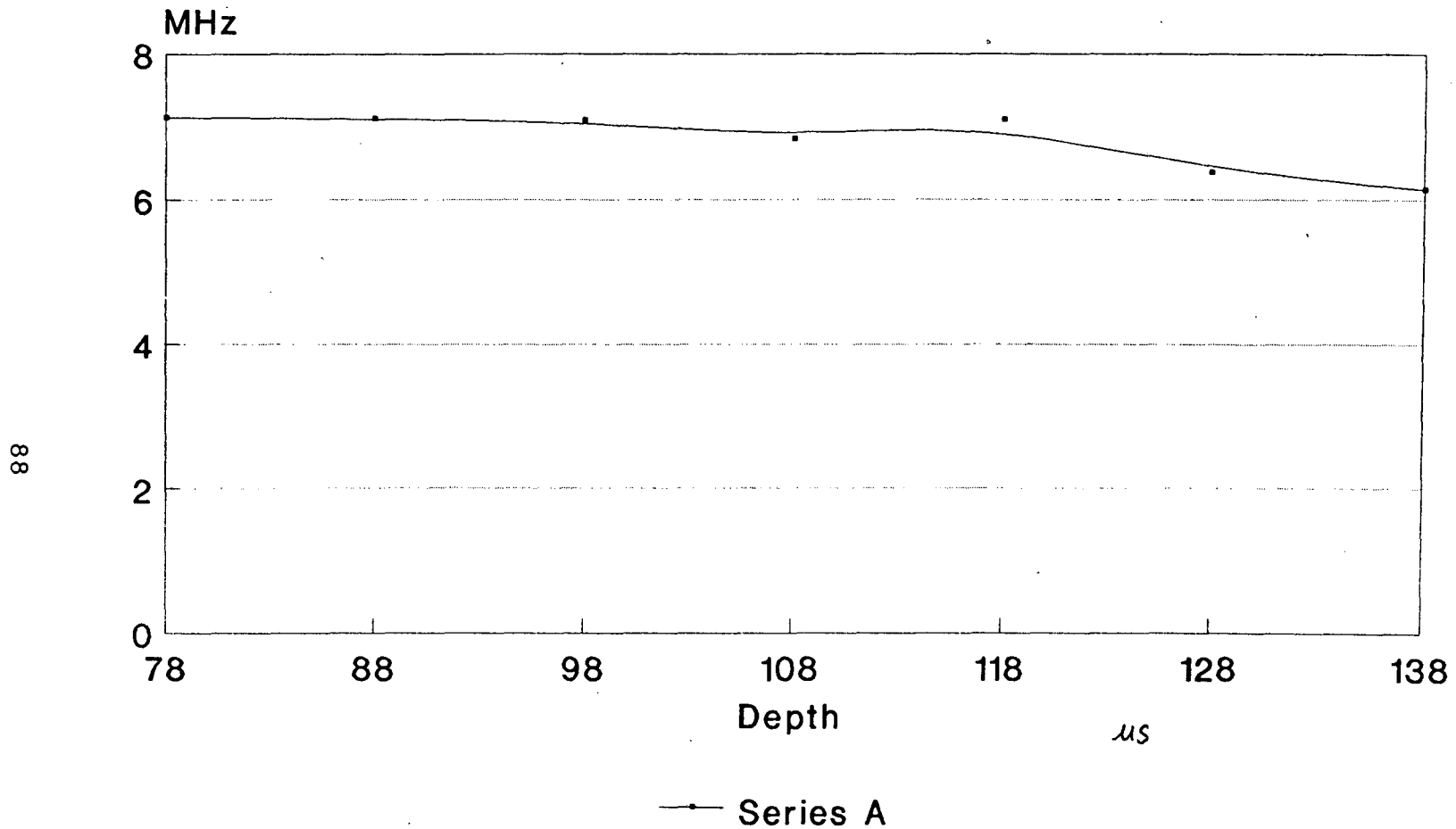Velocity of ultrasound at 36.5°C is kidney (fixed) =
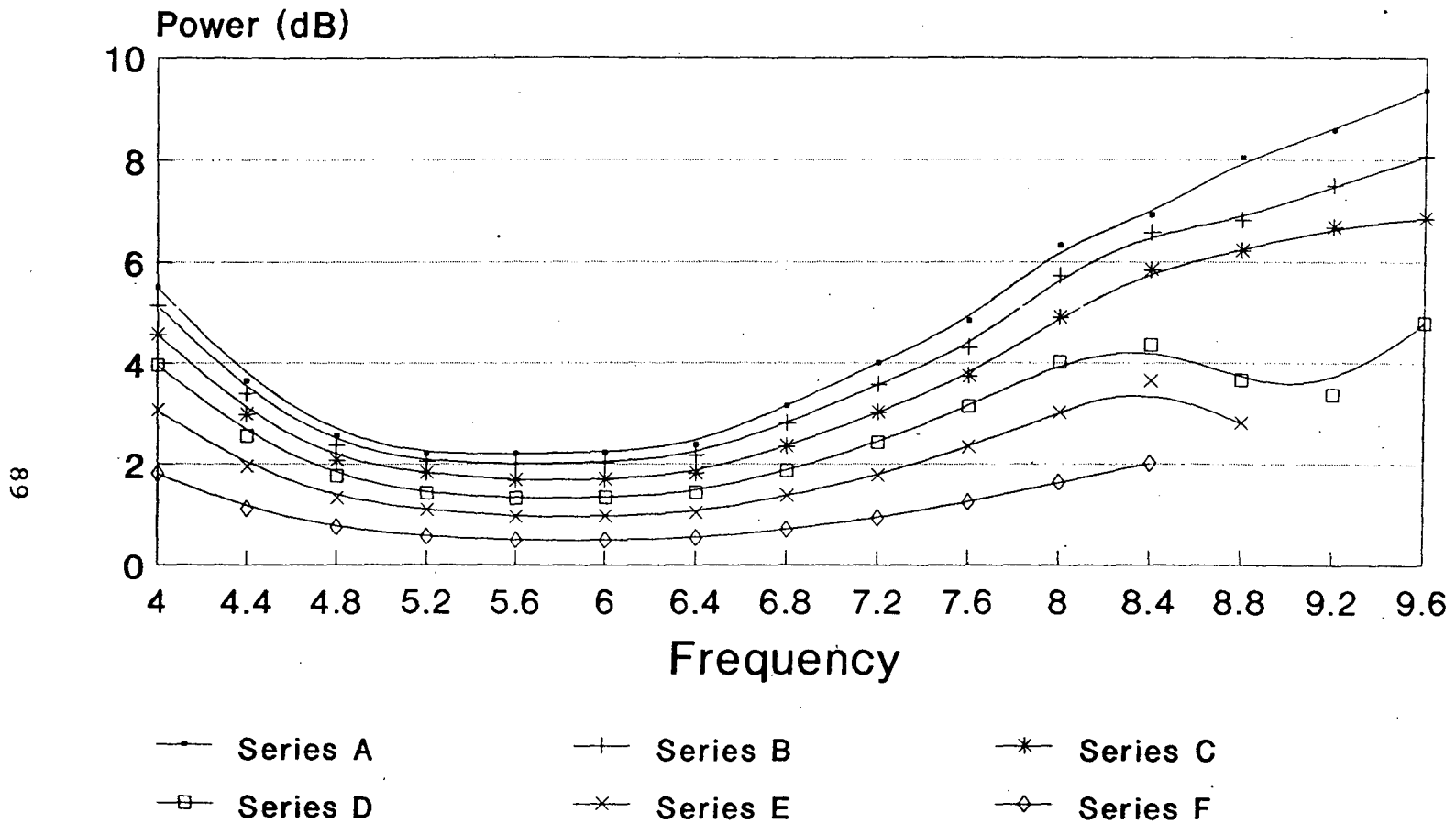
155100 Cm/sec

Attenuation slope ß and

Attenuiation coeficient ßf and $\beta f^{-1.2}$ is given below at different depth

78 μs	88 μs	98 μs
(7.16 MHZ)	(7.13 MHZ)	(7.14 MHZ)
β	$\alpha = \beta f$	$\alpha = \beta f^{1.2}$
0.102	0.725	1.074
0.093	0.659	0.976
0.079	0.563	0.833

Centroid VS Depth
Kidney 36.5 (Deg. Centigrade)

NORMALIZED POWER SPECTRUM (Liver Fixed) 36.5 (Deg. Centigrade)

Results and Discussion

Diffraction effects are cause of error when estimating the frequency dependent attenuation of ultrasound in biological tissues in reflection mode. The unbiased value of the slope of attenuation can be obtained once Time Frequency representation is corrected. We have obtained attenuation slope and attenuation coefficient at different depth.

The change in the spectral properties of signals reflected from far limits in the range of a medium can be used to characterise the medium.

Spectral differnces will indicate the incremental changes that the spectra undergoes due to propagation through the media and due to diffraction. Since the media are known to have approximately linear with frequncy attenuation characteristics. We would expect the spectral difference to exhibit a linear with frequency trend. Any additional functional from in the spectral differnce can be then attributed to diffraction effects.

The resulting collection of averaged spectra are given in power spectrum curve . From the power spectum we observe that the spectral band width reduces with range. Since the resolution capability of a pulse is determined by its band

width, the resolution also decreases with range, a result commonly observed in diagnostic ultrasound images.

The slope attenuation can be estimated from a down shift in the frequency of the reflected spectra with range (Kue, 1984). O'Donnel (1983) suggests using reflection from a plane to compensate for the location of centre-frequency estimate determined from signals reflected from liver we have used reflection from the same samples at different depth for the location of cetre frequency estimate determined from signal reflected from the tissues.

Attenuation at different depth are given below kidney (Fixed in 10% formaline)

Temp.	Depth	β (dB cm^{-1} MHZ^{-1})	βf (dB cm^{-1})	$\beta f^{1.2}$ (dB cm^{-1})
30.5°C	78 µs	.14	.97	1.44
	88 µs	.13	.87	1.29
	98 µs	.12	.83	1.23
33.5°C	78 µs	.105	.757	1.22
	88 µs	.089	.635	.939
	98 µs	.076	.539	.798
36.5°C	78 µs	.102	.725	1.074
	88 µs	.093	.659	.976
	98 µs	.079	.563	.833

Liver (Fixed in 10% Formaline)

Temp	Depth	β (dB cm^{-1} MHZ^{-1})	βf (dB cm^{-1})	$\beta f^{1.2}$ (dB cm^{-1})
30.5°C	78 µs	0.13	0.912	1.346
	88 µs	0.111	0.77	1.135
	98 µs	0.091	0.628	0.924
33.5°C	78 µs	0.11	0.787	1.67
	88 µs	0.094	.667	0.988
	98 µs	0.079	0.563	0.833
36.5°C	78 µs	.101	.721	1.068
	88 µs	.089	.0638	0.945
	98 µs	.077	.55	0.815

According to Kue the average value for the normal liver approaches 0.55% dB cm^{-1} MHZ^{-1} at 12 cm. The result due to present work are given above. The difference in result may be due to the following factors :

a.   Specular echo noise : Besides the diffraction, time varying, frequency filter there is a scattering, time varying frequnecy function related to the degree of inhomogeneity in real tissue. Such an effect, due to the presence in tissue of any large scatterer, may

introduce a bias into attenuation slope estimation. We call this frequency dependent effect "specular noise".

b. We used non focussed transducer of 10 MHZ.

c. The experimental variance may be the same of this variation.

Conclusion

When an ultrasound pulse is transmitted into a medium, the power spectra of the reflected signal vary as a function of range from this transducer. These spectral changes are caused by diffraction effects, due to finite sized aperture, and the properties of the medium. When the properties of the medium are to be determined from the back scattered signals, the diffraction effects tend to mask these properties especially in near field. He recorded the amplitude of the back scattered signals at different frequencies in the far field.

If the propre accounts are taken for diffraction effect, tissue depth and specular reflections then short time Fourier analysis can be applied to measure frequency dependent attenuation for in-vivo condition (generally focussed transducer are suitable for this purpose).

ABSTRACT

Temperature dependence of velocity at 4 MHZ for different biological substances (liver and kidney and eggs) have been measured. We have also investigated the effect of fixation on velocity. Measurements of attenuation coefficient and estimation of attenuation slope was also accomplished.

Sound speed measurement in the biological medium (tissues) is one of the most important parameter for ultrasound tissue characterisation. We have rep.orted the effect of fixation on velocity and its temperature dependence. Fixing the tissue was accomplished by placing it in 10% formaline. Except durig measurement tissue was stored in refrigerator at 4°C. Unfixed tissues were stored in 0.9% sl.ine at 4°C. We observed that formaline fixing reduces propagation speed. In liver and kidney (goat) the average percentage decrease was 1.35 at 4 MHZ. Kremakau et (1981) observed that mean values for all tissues types at 1 MHZ are 1562 and 15523 for fresh and fixed human brain tissue. Bamber (1979) observed 1.5% decrease in velocity due to fixation at 4 MHZ. Other workers have also reported similar result in samples of glioma and astrocytoma. However it is difficult to explain decrease of propagation speed because

upon fixation molecules become more rigid and the bulk modules (for fixed tissues) would lead one to expect an increase in propagation speed. Most probably an increase in density for fixed tissues off sets the bulk modules change. Therefore the increase in density seems to be the cause of decrease of velocity.

The effect of temperature on propagation speed are also discussed. We find that the temperature coefficient is positive for (liver and kidney) within the temeprature range 30.5 to 39.5°C but the rate of increase is very slow. In the case of egg yolk different result have been obtained. No temperature coefficient could be established because the speed sometime increases or decreases with temperature.

We have also measured attenuation of ultrasound in egg and egg yolk at 20°C, 22°C and 24°C by Kremakau method (1981). Higher temperature may induce certain kind of irreversible changes in egg characteristics. (like egg white may become opaque).

Attenuation appears to be one of the promising quantitative parameters for tissue characterization. The acoustic attenuation coefficient measured in dB/cm is known to increase linearly with frequency of liver tissue. The slope of this linear function, has been shown to be an

indicator of tissue state. Because the attenuation coefficient in soft tissue is monotonically increasing fucntion of frequency in low (MHZ) range, spectral analysis has been used to quantify this parameter. In particular, the slope of a linear fit to the attenuation coefficient over a limited band width has been shown to be a reliable index of tissue state in a number of vitro studies.

Short time Fourier analysis is well suited for processing tissue ecographic signals which are non stationary. We have investigated the use of short - Time Fourier analysis to provide an estimation of the ecographic spectral composition as a function of tissue. It permits tracking of the depth dependence of the local round the trip transfer function. It has been shown that the depth dependence of the spectral centre of mass or centriod allows simple determination of the frequency dependent alttenuation. Moreover, the non invarient transfer function due to diffraction, which increases the low frequency compnent of transducer has also been corrected. It has been shown that the spectral running moments of short time Fourier analysis is correlated to the attenuation slope. That is, the time dependence of the spectral centroid allows one to deduce easily the frequency dependent

attenuation. We used liver and kidney (goat) fixed in 10%
formaline for forty eight hours as experimental sample. We
recorded attenuation slop and attenuation coeffcient at
$30.5^\circ C$, $33.5^\circ C$ and $36.5^\circ C$.

Diffraction effects are cause of error when estimating
the frequency dependent attenuation of ultrasound in
biological tissues in reflection mode. Due to diffraction
effects associated with finite aperture, the beam patterns
for each frequency within the bandwidth of the incident
pulse are different. In the far field of any aperture, the
beam pattern is simply related to the spatial Fourier
transform of the aperture, when the spatial frequency
variable is proportional to the ultrasonic frequency.
Consequently, for this reason, the beam pattern at each
ultrasonic frequency is of the same form. We have recordded
the echo-amplitudes at different frequency and differnt
depth and the corresponding power spectrum.

Panly and Schrvan (1971) observed that the acoustic
attenuation was a linear function of the frequency and
postulated that this behaviour was primarily the result of
macromolecular relaxation process. The ß denotes attenuation
slope and $= ßf$ or $= ßf^{1.2}$ denotes atlenuation coeficient
in dB cm^{-1}. Diffraction correction was made and experiment

accomplished for in vitrio measurement. We used non focussed transducer (10 MHZ).

The change in the spectral properties of signals reflected from the far limits in the range of a medium can be used to characterise the medium. From the power spectrum curve we observed that the spectral bandwidth reduces with range. Since the resolution capability of a pulse is determined by its bandwidth, the resolution also decreases with range a result commonly observed in ultrasound images.

The spectral content of the echo signals scattered by an identified slice of soft tissue (located at a mean distanced from the probe) may be found by calculating the power spectrum within a time window central around time $z = 2d/C$ where C is the mean speed of sound. The short-time Fourier analysis performs the calculation of the power spectrum through a sliding time window.

The spectral running moments of the short terms Fourier analysis are simply correlated to attenuation slope. The knowlede of the local spectial centroid shift and of the local spectral variance allows simple estimation of attenuation slope β(dB cm^{-1} MHZ^{-1}) without any assumption of pulse shape.

In present study we have fixed the tissue under investigation (liver and kidney) in 10% formaline and the sample was preserved at 4°C, in both experiments (velocity and attenuation slope estimation).

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