THE BIOLOGICAL EFFECTS OF ULTRASOUND AND ITS DETECTION AND MEASUREMENT

by

JOHN J. ALLEN

M.Sc. Dissertation, University of London, July, 1967.

ABSTRACT

The aim of the dissertation is to try and relate the various effects of high frequency sound waves on biological materials to the parameters of the wave and, also, to indicate how these parameters can be measured. A brief survey of the early work done in this field is followed by a discussion of the various absorption mechanisms which are responsible for the observed biological effects. This consists of two sections. The first deals with the absorption of ultrasound in cellular suspensions and intact tissue that causes purely thermal effects. These effects are characteristic of waves of small amplitude. The second section deals with higher amplitude waves which, although they cause heating effects, can also cause other more dramatic changes. The mechanisms considered are the mechanical stresses exerted on cells and the altered permeabilities of cell membranes due to the acoustic streaming effects of the direct beam and microstreaming effects due to the presence of bubbles or inclusions of resonant size. Also, the mechanical effects and production of free radicals associated with collapse cavitation are described. Then, follows a discussion of the possible absorption mechanisms in intact tissue. The various applications of ultrasound in medicine are mentioned. These are for diagnostic and diathermic procedures and in the treatment of Parkinson's and Ménière's diseases.

A discussion of the various parameters that should be measured in order to try and explain the observed biological effects is then made and this is followed by a description of a number of techniques that are available for this purpose. The parameters described are the frequency; transducer output; intensity distribution; temperature rise; physical characteristics of the media and the occurrence of cavitation and measurement of its intensity. The conclusion sums up the rather unsatisfactory position of this field at the present time.

CONTENTS

1.1 Introduction

Although the biological applications of ultrasound are now well-established in the medical field and for disrupting cells in order to study their contents, little is really known about the interaction of ultrasonic waves with cells. The position can be likened to the early days in the use of x-rays where, although used a great deal, little was known about the interaction of ionising radiations with cells. The main problem in both cases has been the difficulty of making the necessary measurements.

Many hundreds of papers have been published on the biological effects of ultrasound but the vast majority have been qualitative in nature and little or no information has been given about the parameters of the wave. Also, any figures quoted for values of the parameters are, in many cases, suspect. More recently, the position started to change and now many workers have conducted series of rigourously controlled experiments in order to elucidate the relevant mechanisms. A number of methods and devices have now been developed for measurement of the parameters in this field.

The dissertation aims to explain what mechanisms are responsible for the observed effects on cells. To be successful, it should be possible to relate these effects to one or more parameters of the wave. This has not really been achieved as detailed knowledge of the mechanisms is still incomplete.

1.2 History

The first person to observe any biological effects due to ultrasound was Langevin who, in 1917, during the course of his work on the propagation of ultrasound through water, found that small aquatic animals could be killed on prolonged exposure to the beam. The first detailed paper on this topic was due to Wood and Loomis (1927) who subjected a large number of different biological materials to the effects of ultrasound. Since then a vast number of papers have appeared on the biological effects of ultrasound, but it is to be regretted that in the majority of these, little consideration has been given to measurement of the various parameters of the wave. Thus, they are not very helpful in the elucidation of the physical mechanisms involved. It is only since the War that the fundamental importance of the need for accurate measurements has been sufficiently appreciated and the steps taken to remedy this.

It has been known for a long time that ultrasound causes destruction and death of simple organisms such as bacteria, viruses and cells (e.g. Harvey et al, 1928, Harvey, 1930 and Chambers and Gaines, 1932). The specimens were placed in a liquid medium, usually water, for irradiation purposes. The destruction of these was generally found to take place only in the presence of "cavitation" (this term generally meant that the intensity of the sound wave was high enough to cause growth and collapse of bubbles within the liquid. The exact meaning of this term will be discussed later). Variation of sensitivity to ultrasound was found among different strains of the same type of bacteria (Anderson et al, 1948).

A good summary of the early work done in this field has been given by Grabar (1953).

The effects of ultrasound on the complex molecules of very high molecular weight found in cells have been extensively studied. Changes in protein structure were first observed by Wu et al (1931) and changes in the structure of carbohydrates by Szent-Gyorgyii (1933). Knowledge of the structures of the various macromolecules found in cells has increased considerably since these first observations were carried out and it is now possible to relate changes in molecular weight to actual changes in the structure of the molecule.

Following the publication of the initial paper of Wood and Loomis in 1927, a large number of different living organisms were subjected to the effects of ultrasound. It was found that unicellular organisms, such as eggs and larvae, and small animals were easily killed. Resistance to the effects of ultrasound was found to vary among different species. The effects on living animals and humans have been extensively studied.

Lesions have been produced in most parts of a living body by different workers. Brain lesions have been of interest since Lynn et al (1942) first demonstrated how to obtain these in the brains of dogs. Irradiation of the central nervous system has produced both reversible and irreversible changes (Fry, 1958). As is to be expected, very high intensity ultrasound can completely destroy tissue and organisms.

The use of ultrasound for diathermy was the first medical application and was introduced in the thirties. The heating of body tissues due to the passage of ultrasonic waves has been of considerable use in the treatment of complaints that require some form of conventional heat treatment. Dussik, just after the War, was the first to try and visualise structures within the body. It is now a highly developed method of diagnosis especially in the soft tissue regions of the body (Gordon, 1963). Ultrasound has been used to treat a number of nervous diseases (the main one being Parkinson's disease) by implanting lesions deep within the brain. Another application is to Ménière's disease, an affliction of the inner ear causing vertigo and deafness. Newell (1963) has given a survey of the medical applications of ultrasound.

By treating cellular suspensions with ultrasound, it is possible to release many of their components into solution. This method has been used for a number of years (Stumpf et al, 1946) and is now a standard way of extracting components such as chloroplasts, enzymes, proteins and mitochondria in a relatively pure state.

2. Theory

2.1 Introduction

When dealing with the propagation of mechanical waves through biological media, it is possible to treat most of them as liquids (Fry and Dunn, 1962). This means that the only type of wave that can be successfully propagated is a longitudinal one, due to the inability of liquids to withstand shearing stresses. The densities and velocities of propagation for soft tissues have been found to be of the same order of magnitude as those for water. Densities vary between 1.02 and 1.07 $gm.cm.^{-3}$ and velocities are from 1450 to 1610 $m.sec.^{-1}$ (the only real exception is bone). However, their absorption coefficients are much larger than that for water.

Sound waves are produced at megacycle frequencies by means of piezoelectric crystals. Pond (1963) has described some of the transducers used in this field. Until recently, quartz was the only substance used, but now the relative fragility of the ceramic materials available (e.g. barium titanate and lead zirconate) has greatly improved and these are being used more and more. They possess the advantage that they can be shaped for focussing purposes. Magnetostrictive devices are also used and these usually operate in the lower ultrasonic range (up to about 50 kc/s). Cavitation effects are more easily produced at these frequencies than at the megacycle frequencies used with piezoelectric crystals.

In attempting to elucidate the mechanisms responsible for the biological effects of ultrasound, both progressive and stationary wave systems have been employed. For concentrating the power available, focussing is used.

2.2 Plane waves

Considering a plane wave vibrating into an unbounded and non-dissipative medium may oversimplify the actual experimental conditions but it gives an idea of the amplitudes of the various acoustic parameters. This is helpful as biological effects could depend on these values.

Assuming no attenuation and a plane wave, the following equations may be derived by means of simple wave theory:-

$$I = \frac{\rho c U^2}{2} \quad \text{. . . . . eqn. 2.1}$$

$$I = \frac{P^2}{2\rho c} \quad \text{. . . . . eqn. 2.2}$$

$$I = cE \quad \text{. . . . . eqn. 2.3}$$

Where I = acoustic intensity

ρ = mean density

c = velocity of sound

U = particle velocity amplitude

P = pressure amplitude

E = energy density

For such a wave the acoustic impedance is equal to the characteristic impedence (density x velocity). This is not true if attenuation occurs or the wave diverges. In practice, a plane wave will be approximated to if the dimensions of the transducer are very much larger than the wavelength of the generated sound. At 1 Mc/s the wavelength of the sound wave in water is 0.15 cm.

2.3 Reflection and Refraction

If a wave passes from one medium to another of different characteristic impedance, part of the energy is transmitted and the

rest reflected back into the first medium (assuming no absorption). With normal incidence, the reflection coefficient, α_r and the transmission coefficient, α_t are given by the following equations:-

$$\alpha_r = \left(\frac{\rho_2 c_2 - \rho_1 c_1}{\rho_1 c_1 + \rho_2 c_2}\right)^2 \quad . \quad . \quad . \quad . \quad . \quad \text{eqn. 2.4}$$

$$\alpha_t = \left(\frac{4\rho_1 c_1 \rho_2 c_2}{\rho_1 c_1 + \rho_2 c_2}\right)^2 \quad . \quad . \quad . \quad . \quad . \quad \text{eqn. 2.5}$$

Where $\rho_1 c_1$ denotes characteristic impedance of 1st medium

" $\rho_2 c_2$ " " " " " 2nd medium

If the angle of incidence is other than at 90° to the interface, then the possibility of mode conversion occurs (i.e. generation of shear waves). If the second medium cannot withstand shearing stresses, then the transverse wave produced is quickly damped with the consequent production of heat.

If $\rho_2 c_2 \gg \rho_1 c_1$ or $\rho_2 c_2 \ll \rho_1 c_1$ then most of the incident energy is reflected back into the first medium. Under favourable conditions this reflection gives rise to a stationery wave pattern which can be considered as the superposition of two progressive waves travelling in opposite directions.

2.4 Absorption

In all real media, a sound wave will be attenuated by various absorption mechanisms. If the absorption per unit path length is constant, then it is possible to define an absorption coefficient, α by means of the expression:-

$$A_x = A_o e^{-\alpha x} \quad . \quad . \quad . \quad . \quad \text{eqn. 2.6}$$

Where A_o = initial value at x = o of parameter (usually pressure)

A_x = amplitude of parameter at distance x away.

Since the intensity is proportional to the square of the amplitude of any of the acoustic parameters, the intensity absorption coefficient, μ, is given by:-

$$\mu = 2\alpha \quad . \quad . \quad . \quad . \quad \text{eqn. 2.7}$$

The amplitude absorption coefficient, α, is the one usually quoted but it can be described in a number of different ways. These are:-

(i) the absorption per unit path length i.e. α cm^{-1} or as the absorption per unit wavelength i.e. $\alpha\lambda$

(ii) the attenuation in the parameter from its initial value to $\frac{1}{e}$ of this value. The units are nepers. It is usually expressed in nepers $cm.^{-1}$ or per wavelength.

(iii) the usual decibel notation.

The relationships between these are as follows:-

1 neper $\equiv$ 8.7dB

1 dB $\equiv$ 4.35α

2.5 Diffraction

In practice, a transducer will not generate a plane wave and, especially at the megacycle frequencies encountered in this field, diffraction effects become quite important. The diffraction theory quoted below assumes a transducer vibrating as a solid piston in an infinite baffle into a non-absorbing medium (Kinsler and Frey, 1962). The acoustic field may be divided into two sections:-

(i) Fresnel region (or near field)

This is the space adjacent to the transducer and it is found that the axial acoustic intensity I_o goes through a number of maxima

and minima according to the equation:-

$$I_o = 2 \rho c U_o^2 \sin^2 \frac{k}{2}\left(\sqrt{r^2 + a^2} - r\right)$$

Where U_o = velocity amplitude at surface of transducer

r = distance along perpendicular to plane of transducer

$2a$ = diameter of transducer

$k = \frac{2\pi}{\lambda}$

and other symbols have their usual meaning.

The last minimum occurs at a distance given by $\frac{a^2}{2\lambda}$ While travelling this distance, theory indicates that the beam remains the same width as the transducer i.e. 2a. If the absorption coefficient is large, this picture is modified.

(ii) Fraunhoffer region (or far field)

The far field begins after the last axial minima. The intensity falls off according to the inverse square law and divergence of the wave occurs. The intensity at a polar angle θ to the line perpendicular to the axis of the crystal, I, is given by:-

$$I = \frac{\rho c k^2 V_o^2 a^4}{8 r^2} \left[\frac{2 J_1 (ka \sin \theta)}{ka \sin \theta}\right]$$

Where J_1 denotes a Bessel function of the first kind and first order

r = distance of point from centre of transducer and the rest of the symbols have usual meaning.

This differs from the equation for the intensity of a simple source by reason of the presence of the directivity factor, $\frac{2 J_1(ka \sin \theta)}{ka \sin \theta}$ which indicates the existance of side lobes.

The main lobe contains most of the energy but as the ratio $\frac{a}{\lambda}$ increases more energy is transferred to these lobes.

When $I = 0$, $\quad ka \sin \theta = 3.83$

$$\theta = \sin^{-1} 0.61 \frac{\lambda}{a}$$

This is called the half angle of spread and indicates the approximate angle of divergence (fig.1). The existence of side lobes outside this major lobe, however, further complicates the field.

2.6 Focussing of sound waves

Focussing is used in order to concentrate the power available from a given transducer thus increasing the acoustic intensity at a particular point. Another use is to minimise diffraction effects in the far field. The usual methods involve either converging plastic lenses or specially shaped ceramic transducers. A survey of the actual systems used has been given by Gordon (1964).

(i) Plastic lenses are generally made of polystyrene or perspex and are plano-concave; the back is coupled directly to the crystal. Different lenses with different focal lengths may easily be used, but to obtain a good focus a complex shape to the concave side of the lens is required. The main disadvantages are reflections due to impedance differences between the crystal, the lens and the medium and absorption losses in the lens itself. For a lens with a spherical concave surface and a small aperture, the focal length, f, is given by:-

$$f = \frac{nr}{n - 1}$$

Where r = radius of curvature of concave face

n = refractive index of material of lens

(ii) Shaped transducers are made of ceramic materials and are able to withstand high intensities for long periods. They have a fixed focus. Unfortunately, the resonant frequency of these materials varies with temperature and so the focal length will vary slightly. The field at the focus is similar to the Fraunhoffer region already considered; there being a main lobe and several side lobes of much smaller intensity. 84 % of the available energy passes through the main lobe. Assuming a spherical lens, the radius of the main lobe, r_F , is determined by the condition that the directivity factor $\frac{2J_1(ka \sin \theta)}{ka \sin \theta} = 0$.

From this $r_F = \cdot 61 \frac{R\lambda}{a}$

Where a = radius of transducer

$$R = \frac{a}{\text{aperture of lens}}$$

If the total output is W, then the average intensity in the main lobe in the focal plane is given by:-

$$I = 0.71 W \left(\frac{a}{\lambda R} \right)^2$$

Details of acoustic wave theory can be found in a number of text books on the subject e.g. Hueter and Bolt (1955), Blitz (1963), Kinsler and Frey (1962) and Stephens and Bate (1966). Fry and Dunn (1962) have given a detailed account of most of the theory required in this field.

3. Absorption of Ultrasound Leading to thermal effects

3.1 Introduction

When an ultrasonic wave is propagated through a medium it is attenuated and, assuming a wave of small amplitude, the energy absorbed appears mainly as heat. With large amplitude waves, other effects become important and these are discussed in section 4.1 Elucidation of the absorption mechanism is complicated by the inhomogeneous nature of biological materials with the consequence that reflection and refraction effects add to the dissipation of the available energy. Since also the majority of the attenuation now appears to occur at molecular level the complexity of biological materials makes a complete understanding of the absorption mechanisms impossible at the present time (Ackerman, 1962).

3.2 Absorption coefficients

A knowledge of the values of absorption coefficients and their variation with frequency and temperature of as many biological structures as possible is needed to elucidate absorption mechanisms. Not enough work has been done in this respect and the various values obtained by different workers tend to be inconsistent. The reasons for this are probably differences in the intensities used in the physical state of the media and in experimental and measuring procedure.

The only comprehensive list of values for absorption coefficients (and velocities of propagation) for mammalian tissues and organs based on the work of a number of workers in this field has been quoted by Goldman and Heuter (1956, 1957). Figs. 2 to 4 show the variation of absorption with frequency for a number of biological materials. It has been found that the absorption coefficient for most biological materials

is a direct power function of the frequency where the value of the power, in general, lies between 1.0 and 1.3 (Schwann, 1959).

The velocities of propagation of a compressional wave in soft tissues are mostly in the range 1450 to 1,610 metres $sec.^{-1}$ The velocity of sound in fatty tissues is less than that in water while most other tissues (e.g. muscle, liver, kidney and brain tissue) have velocities exceeding that of water. The only real exception is bone which has a propagation velocity of about 3,350 metres $sec.^{-1}$ For most purposes, the velocity can be assumed to be independent of frequency (Schwann, 1960).

The dependance of the absorption coefficient on the temperature of the medium has been studied for very few media. Carstensen and Schwann (1953) showed that blood exhibited a negative dependance. Kishimoto (1958), investigating ultrasonic absorption in bone, found an increase in absorption with temperature while Dunn (1962), irradiating the nerve tissue of young mice, found a positive temperature coefficient of absorption which was given by the empirical formula:-

$$\alpha = \frac{1}{10}\left[2 - e^{0.016(35-t)}\right]$$

where t = temp. of tissue in ^{o}C

3.3 Absorption mechanisms

Absorption of ultrasound by a medium occurs when there are time lags for the energy exchanged between the different degrees of freedom of the molecules and these time lags are not negligible compared with the period of the wave. This type of absorption is termed relaxational.

The classical absorption processes of viscosity and thermal conduction (the latter is not important in liquid-like structures) lead to a calculated value of the absorption coefficient which only

agrees with that observed in very simple media, and for all others is only a small fraction of the observed value. Classical theory indicates that, for a single relaxation mechanism operative, the absorption shows a maximum at a particular frequency known as the relaxation frequency. Also, the absorption exhibits a quadratic dependence on frequency (i.e. α/f^2 = constant) well below the relaxation frequency (Blitz, 1963). From fig. 5 it can be seen that as the number of operative relaxation mechanisms increases, the graph of absorption per wavelength against frequency tends to become more horizontal, and with an infinite number of mechanisms the graph would be horizontal. (A graph of absorption against frequency would be linear in this case). This would indicate the presence of high molecular weight compounds with many degrees of freedom. For many biological materials there is an approximately linear relationship between absorption and frequency over the frequency ranges as far investigated. To explain these results it is necessary to assume a large number of relaxation mechanisms uniformly distributed with frequency. With relaxation effects velocity dispersion must also occur. A hysteretic type of absorption will exhibit a linear dependence on frequency (Blitz, 1963) but no velocity dispersion. This type of absorption mechanism has been used to explain the absorption coefficients at high frequencies observed for bone (section 3.5).

To explain the observed absorption coefficients in biological materials, it is necessary to treat them as viscoelastic media possessing both shear and bulk (i.e. volume) viscosities (Ackerman, 1962). Fry and Dunn (1962) have said that cellular material can be treated as Newtonian systems (i.e. no viscosity changes with velocity gradient) and that above 100 kc/s only the bulk viscosity need be considered. The bulk viscosity

is composed of a number of different relaxation mechanisms about which only little is really known. These are thermal, structural and chemical relaxations. Thermal relaxations involve exchanges of energy between the external and the internal degrees of freedom of the molecule. Structural relaxations involve changes in the actual structure of the molecules (e.g. orientation of the molecules) due to the passage of the wave. Chemical relaxations involve changes in chemical equilibrium due to the sound wave.

Fry (1952) tried to explain the observed absorption by just a shear viscosity effect due to relative motion between particles and the suspending medium, and with a suitable choice of parameters obtained a linear relationship between absorption and frequency. Hueter et al. (1953), working on absorption of ultrasound in milk, found that this approach was not really justified and postulated a bulk viscosity with an appropriate distribution of relaxation times to explain the observed results. Since then, workers have used the bulk viscosity approach.

Knowledge of the temperature dependence of the absorption coefficient is also useful. A positive coefficient indicates the existence of unassociated molecules (Hueter and Bolt, 1955) and nerve tissue (Dunn, 1962) has such a coefficient. It rules out shear viscosity as an important mechanism as this decreases with increasing temperature. A negative coefficient is indicative of associated types of molecules. Blood shows a negative coefficient (section 3.4). The main difficulty in obtaining information about temperature coefficients is that living adult mammals are homeostatic.

3.4 Absorption of ultrasound by Blood

A large amount of work has been done by Carstensen and Schwann to try and work out the absorption processes that occur in mammalian blood. They chose blood initially as it is acoustically homogeneous and yet contains cells (however, these have no nucleus). They investigated a wide range of experimental conditions in the frequency range 800 kc/s to 10 Mc/s. The intensities used are not mentioned in the papers but El'Piner (1964) quotes them as being between 1 μW cm^{-2} and 1 mW cm^{-2} Their experimental technique is described in section 6.7.

Carstensen et al. (1953) first showed that proteins were responsible for most of the absorption. The cell structure itself and the water present contributed an almost negligible amount to it. The main protein in the blood cell is haemoglobin while in the surrounding plasma it is albumin (it contributes 60 % to the total plasma protein content, the rest is made up of globulins). It was found that the absorption was a direct function of the protein concentration and that it did not matter whether the haemoglobin was still in the cell or a suspension in water, the absorption figures were the same.

In two further papers, Carstensen and Schwann (1959, a, b) continued the study of the protein absorption in blood. They again used haemoglobin from various mammalian bloods. This was prepared by treating the red cells with toluene to cause haemolysis. Centrifuging removed the stromata and lighter elements leaving the high molecular weight protein, haemoglobin. Fig. 6 shows the absorption for various mammalian haemoglobins from 0.1 to 10 Mc/s. It can be seen that the absorption per wavelength shows small dependence on frequency i.e. a large number of relaxation mechanisms is operative. If relaxation

effects are the cause of the absorption, velocity dispersion must also occur. Although the actual dispersion was small it was definitely shown to be present (fig.7). They also showed the negative temperature coefficient of absorption for blood (fig.8).

However, it was found that the total absorption could not be completely explained by assuming an infinite number of relaxation mechanisms occuring within the complex protein molecules and this led to the idea of a non-protein process occurring (Carstensen and Schwann, 1957). This part of the absorption decreased with frequency and increased with dilution of the blood cells. It was explained by the relative motion between the cells and the suspending medium (the plasma) due to differences in their densities. The cells are not able to follow the oscillatory motion due to alternating forces, caused by the passage of the wave, as fast as the lower density plasma. Thus, there is a viscous effect taking place with consequent absorption of energy. An expression for the absorption due to this mechanism has been given by Fry and Dunn (1962). This was derived by considering elements stiff in shear compared with the surrounding medium. The formula indicates another type of relaxation process. An absorption curve taking into effect relative motion is shown in fig.9. It results in the levelling of a typical absorption curve containing a small number of relaxation times. Relative motion explains the observed facts since it is less effective the greater the percentage of red cells present (less motion possible) but increases with increasing frequency (less likely that the cells can follow the periodic motion due to the wave). Carstensen and Schwann (1957) derived a similar expression for the absorption due to relative motion. They

considered that a longitudinal wave on hitting a spherical object (e.g. a cell) was partially converted into a transverse wave and that this transverse wave was quickly damped because of the liquid medium, causing absorption of the ultrasonic energy.

It would now appear that the absorption of low amplitude ultrasound in blood is due to the large number of different relaxation processes associated with the protein molecules and, to a lesser extent, the relative motion between the cells and the plasma. Carstensen and Schwann (1959,b) developed a theory to confirm this idea of an infinite number of relaxation processes. By consideration of an infinite number of relaxation processes uniformly distributed with frequency, the derived equations fitted the experimental curves quite well. They are of the opinion that the wave disturbs the chemical and structural equilibrium within the protein molecules or in the protein-water complex. The range of the relaxation frequencies would be at least from 30 kc/s to 10 Mc/s. More recently Edmonds (1962) has extended these results up to 230 Mc/s. He used haemoglobin solutions which were prepared by the same methods as those used by Carstensen and Schwann and employed similar experimental conditions. He found that the same type of relaxation spectrum existed up to this frequency with a broad maximum in the region 10 to 100 Mc/s. Fry and Dunn (1962) say that there could be a large number of discrete relaxation frequencies (perhaps masked by the effects of relative motion). In fact, they showed that by the choice of two suitable relaxation frequencies it is possible to obtain an absorption curve that only increased 20 % over a ten-fold (0.66 to 6.6 Mc/s) range of frequencies. Fig.10 shows the

resultant curve, the two relaxation curves being indicated by dotted lines. One curve has a peak at about 1 Mc/s and the other at such a high frequency that only the quadratic part is present. Ackerman (1962) has favoured the idea of a large number of relaxation mechanisms with a uniform distribution of relaxation frequencies, but recognised that this is artificial as the origin of the relaxations is unknown.

3.5 Absorption of ultrasound by intact tissue and bone

It has been found that the absorption coefficients for intact tissues are between two and ten times greater than those for a cellular suspension such as blood, though the coefficients show the same type of frequency dependence. Fig.11 shows graphically the results assembled by Goldman and Hueter (1956, 1957). The spread of the results, shown by the shaded regions, indicates the different physical states of the specimens and experimental techniques used by the investigators from whom these results were obtained. In general, the more complicated structures (e.g. kidney and muscle) absorb ultrasound more strongly than the less complicated structures (e.g. fatty tissue).

Schwann (1959) mentioned that Pauly studied the effects on the absorption coefficient of grinding down liver tissue in a series of steps until the cellular structure was completely destroyed and then removed. Finally only a solution of the liver proteins was left. He found that 20% of the absorption coefficient disappeared with the initial step of grinding down the tissue, but the remaining 80 % did not change at all and must then have been of molecular origin. Changes in the hydrogen ion concentration (pH values) were found to cause

changes in the absorption values but did not alter the type of frequency dependence at all.

The actual macro-structure of the tissue is probably responsible for part of the absorption, as in the case of the liver tissue, but this will not completely explain the high observed values. The macro-structure contains inhomogeneities and, if these are much larger than the wavelength of the sound, will cause reflection and refraction effects due to the impedance differences (equations 2.4 and 2.5) Smith and Schwann (1957) have shown that the nuclei of liver cells absorb ultrasound more strongly than the proteins (the absorption coefficient was twice that for haemoglobin) and that if the proteins in intact tissue absorbed mechanical energy at the same rate as the nuclei then this would explain completely the observed values. Ackerman (1962) said that these high absorption coefficients could be due to protein differences or scattering at cell walls. Hawley et al (1965) determined the absorption coefficients of aqueous solutions of dextran of various molecular weights. They found that the absorption spectra were very similar to those obtained for beef haemoglobin solutions by Carstensen and Schwann (1957). From this they concluded that the absorption in tissue was not completely due to the proteins present but non-proteineous material could play a considerable part.

Variation in the absorption coefficient depending on the direction of orientation of the material to the wave has been found. This acoustic anisotropy is of two types. One is where the tissue has long connective fibres (e.g. nerves and muscles) and the second

where the tissue consists of a number of separate layers (e.g. skin, tongue). The absorption coefficient in the human sciatic nerve in the direction of the fibre axis was 0.35 cm^{-1} while in the direction perpendicular to the fibre axis it was 0.55 cm^{-1} at 3.4 Mc/s (Goldman and Hueter, 1956) For a wave travelling at right angles to fibres or tissue layers, impedance differences will cause reflections and, thus, higher values of the absorption coefficients will occur. It has been found that velocity anisotropy also occurs. (Goldman and Richards, 1954).

The age of the specimen appears to have considerable effect on the value of its absorption coefficient. In Fig.12, the effect of aging on liver is shown. About nine hours after death, the absorption coefficient starts to decrease. At about twenty hours, this ceases and the value for the coefficient levels off.

Bone has a much higher absorption coefficient than any of the soft tissues. Frederick (1965) has quoted values of 1.5 dB/cm for brain tissue at 1 Mc/s compared with 13 dB/cm.for skull bone at the same frequency. (The large variation found in values for absorption coefficients observed in bone is due to their inhomogeneous nature and variation in physical characteristics such as density). Absorption in bone shows a frequency squared dependance below 2 Mc/s but, above this frequency changes to a lower power frequency dependance, (Dunn 1965). Kishimoto (1958) had found this to be a linear relationship and put forward the idea of a hyteresis type of absorption. The velocity of sound in bone is about 3360 metres sec^{-1} (the actual value depends on the type and the state of the specimen) and this is about twice the value for any of the soft

tissues. Bone density is also much larger (average 1.85 gm.cm.$^{-3}$) and this means a large diffence in characteristic impedance between bone and a soft tissue. Consequently, there is a large change in acoustic impedance encountered by a sound wave in a tissue meeting a tissue-bone interface. Besides scattering and reflection, Herrick (1954) has suggested a mode conversion also occurs with shear waves being formed at the interface. These are rapidly attenuated with the consequent production of heat. Also, bone is a good absorber of ultrasound. These facts would explain the large temperature increases observed in bone (Herrick 1953).

In conclusion, it may be said that although the absorption coefficients vary from tissue to tissue, they all show the same type of frequency dependance. This appears to be due to the large number of relaxation mechanisms uniformly distributed with frequency. At present, details of these mechanisms are not available due to incomplete knowledge of the structure of the cell and the molecules within it, especially the proteins, which appear to play a considerable part in the absorption processes. It is to be regretted that there appears to be very little being done on the elucidation of these mechanisms at the present time.

4. Absorption of ultrasound leading to non-thermal effects

4.1 Introduction

With higher intensity sound waves, appreciable heating of the medium does occur and, in some cases, this heating alone is responsible for observed lethal effects in cells and small animals. However a further series of effects, quite distinct from those due to thermal action, are also found to occur. In many situations, their occurrence can be observed at intensities well below those resulting in appreciable heating, although in some cases it is necessary to employ a pulsed sound source in order to achieve a sufficiently high peak intensity for their production without consequent generation of heat.

The mechanisms that cause non-thermal effects appear to be acoustic streaming, the presence of bubbles and cavitation. It is not easy to separate the effects due to each of these mechanisms, but it is instructive to treat them separately at first.

4.2 Biological effects due to acoustic streaming.

It is a well known property of classical wave theory that second order effects give rise to a steady radiation pressure. If the medium is an absorbing one, then this pressure falls off with distance giving rise to a force that tends to accelerate the medium. Due to the effect of viscous forces, a uniform motion will exist and this is known as acoustic streaming (Stephens and Bate, 1966).

These streaming effects can cause important tangential motions along interphase boundaries giving rise to viscous stresses. The greater the velocity gradient in the region of the boundary the

the greater will be the stress (= viscosity x velocity gradient) acting, although its value will depend on a number of other factors and it is difficult to predict values in the neighbourhood of complicated cellular structures although a lot of work has been done in connection with simpler media (Nyborg, 1965). Although the stresses occuring are not very large (compared with those obtained due to cavitation) they can disrupt weak cellular structures and change permeability of cells as will be shown.

Nyborg and Dyer (1960) investigated the effects of a vibrating needle at 25 Kc/s on the walls of plant cells. They used a small section of elodea leaf which was mounted on a microscope slide. The effects on the cell were viewed microscopically. At low amplitudes of vibration, 0.1μ or less, there occurred an intracellular streaming which was orderly and occurred only within the cell in direct contact with the needle (Fig. 13). On increasing the vibration amplitude, (these values were calculated by measuring the vibration blur with a microscope eye-piece micrometer) chaotic motions resulted within the cell, cytoplasm being removed from the walls and the walls themselves ruptured. The tip of the needle did not have to be even in contact with the cell wall although this was its most effective position (these effects happened with vibration amplitudes between 5 and 10μ). They concluded that the boundary flow was great where the vibration amplitude varied rapidly with distance but it also depended for a complete explanation of the eddying motions on the location of the boundaries, the amplitude distribution and the

viscosity of the medium. In a further paper Dyer and Nyborg (1961) reported similar results obtained with other plant cells.

Wilson et al (1962) undertook similar experiments to determine the effects of ultrasound on marine eggs. They used a steel needle driven at 83 Kc/s on the unfertilised eggs of Asterias and Spisula. With the eggs, diameters were about 100 μ, held at the end of a micropipette under water and the needle applied directly to the surface of the eggs, two effects were noticed. The vibrations either caused the nucleolus to move around inside the nucleus, or the nucleolus remained still but rotated about itself, the rate of rotation increasing with increased ultrasonic intensity. In one Asterias egg, the nucleolus was observed to split into two halves. The effects of subjecting Asterias eggs in a suspension of salt water to similar vibrations was also studied. They found that the eggs tended to move towards the tip of the vibrating needle. It was observed that the part of the surface of the egg that approached closest to the tip was deformed and in several of the experiments large cone-like projections appeared on the egg surfaces. Wilson et al (1966) have reported similar results to these but have included full details of the experimental arrangements used and photographs illustrating the various motions observed.

Hughes and Nyborg (1962) undertook a series of experiments to try and differentiate between the various mechanisms that could be responsible for biological effects and one such experiment was to obtain cellular destruction without the presence of bubbles or any

cavitation effects. Suspensions of fresh erythrocytes (blood cells) were irradiated with a vibrating needle. The amount of cell breakage at different vibration amplitudes was determined by the amount of haemoglobin released into the solution. This was measured after centrifugation. The number of cells ruptured increased with increasing vibration amplitude as shown in Fig. 14. They concluded that the cell breakage was due to the stresses set up by the acoustic streaming (no bubbles were present). The streaming velocity was the order of 10 $m.sec^{-1}$ close to the actual tip of the needle. The streaming motions were circular as shown in Fig. 15.

Similar results were obtained using the protozoan, Tetrahymena pyriformis, a single-celled animal, with the same type of irradiation. However, using a low vibration amplitude and high speed photography (3000 frames per second), it was seen that not only were the cells distorted near the needle-tip (region of greatest streaming velocity) but their contents were seen to move in a circular fashion relative to the actual cell motion. They also studied the effects of using a vibrating needle on bacteria which need a much higher shear rate for disruption. Escherichia coli were used. By employing long periods of irradiation - up to one hour - only a small number of the cells were ruptured, the actual number being estimated by the amount of protein released into solution. The amount depended linearly on the amplitude of vibration. Subsequent light and electron microscopy confirmed the existence of broken cells.

Ackerman (1963) investigated the effects of ultrasound on canine erythrocytes both with cavitation present and with cavitation suppressed

by means of hydrostatic pressure. The same intensity was used in both cases. He found that rupture of the cells (this was measured spectrophotometrically) occurred without the presence of cavitation, though the number was much smaller than that obtained when cavitation effects were present. No mention is made of how cavitation was judged to occur and the intensity of the wave (frequency 495 Kc/s) was described as being intermediate between those used for physical therapy and surgery.

A series of experiments to study degradation of a suspension of the biomacromolecule, deoxyribonucleic acid (D.N.A.) was made by Hawley et al (1963). Although the measured intensities were high (25 and 31 $W.cm.^{-2}$) great care was taken to suppress cavitation by means of externally applied hydrostatic pressure. The frequency used was 981 Kc/s. Three devices were used to ascertain if cavitation did occur. A thermoelectric detector was inserted in the suspension (its response would become completely erratic in the presence of cavitation). An electrical meter was inserted in the feedback circuit of the transducer (cavitation would produce changes in the meter reading due to alteration of the load impedance presented to the transducer). With the onset of cavitation, the refractive index of the medium changes considerably due to bubble formation and ultra-violet light of wavelength 259 $m\mu$ was monitored in order to detect this. They found that there was a rapid initial decrease in molecular weight (as shown by ultracentrifuge and ultra-violet absorption studies) followed by a period in which little or no change in molecular weight occurred (Fig. 16). No change in optical density

of the suspension was observed (i.e. no denaturation occurred) implying that degradation was only due to breaks occurring along the backbone of the molecule. Similar results had been obtained with this molecule by Doty et al (1958). Hawley et al concluded that the mechanism of degradation was due to relative motion between the higher density D.N.A. molecules and the lower density solvent and the consequent stresses set up.

This behaviour is characteristic of a number of biomacromolecules but usually no changes are detected without "cavitation" being present (see section 4.6). The original molecule cannot be broken down into pieces of smaller and smaller molecular weight. There is a limit to this degradation (for D.N.A. the final molecular weight is $\sim 3 \times 10^5$ - its initial molecular weight being $\sim 10^6$ or 10^7) and it may indicate that only certain molecular bonds can be fractured. With D.N.A., the mechanism is purely mechanical and is thought to produce breaks in the phosphate - sugar backbone of the molecule. "Cavitation" produces different effects to these according to Hughes and Nyborg (1962).

It is well known that the presence of acoustic streaming increases the transfer of material through semi-permeable membranes (Nyborg, 1957) such as occur in cells. Hughes (1965) has pointed out that it is possible to completely empty red blood cells of their haemoglobin content without rupturing the cell wall, though this requires very delicate instrumentation. It would appear that using soluble proteins and enzymes as an indication of cell rupture is thus not a completely reliable method.

Acoustic streaming could help in accounting for the stimulation

of growth that has been reported. El'Piner (1964) studied the effects on maize seeds. They were immersed in water, and those irradiated for 5 mins. with a beam of intensity 5 $W.cm^{-2}$ (frequency 380 Kc/s) were found to germinate more quickly than ordinary seeds but those irradiated for 10 to 15 mins. at the same intensity tended to be killed. A possible explanation is that the acoustic streaming induced by the sound beam alters the permeability of the cell wall increasing the water taken up by the plant. Gordon (1963) has said that this could be increased by 10% to 35%. Earlier, Bronskaya and El'Piner (1959) reported that replacing the oxygen in the water with hydrogen produced no stimulating effects (oxygen plays a large part in the biochemical processes of plants). This is not the whole picture and El'Piner (1964) has suggested that also the ultrasound loosens the submicroscopic structures of the cells, causing swelling and assisting in the interaction with oxygen.

Changes in the permeability of the blood-brain barrier have been found by Bakay et al (1959). They noticed that, after irradiation of cat brains in order to cause lesions, there occurred greater transfer of tracer materials (trypan blue and radioactive phosphorus) from the blood plasma to the damaged tissue than to normal tissue. A possible explanation is that of altered permeability due to streaming.

Although it is tempting to assume that the prime mechanism in the quoted examples is that of acoustic streaming and its effects, the possibility of the presence of invisible bubbles or inclusions especially in the examples involving cellular structures has to be admitted.

4.3 Resonant bubble activity in a sound field

The propagation of sound waves in liquid-like media is complicated by the presence of bubbles containing gas. The presence of these will have a number of different consequences on the sound propagation, depending in particular on the size of the bubbles relative to the wavelength of the sound in the liquid.

The bubble is a very good source of microstreaming due to its volume oscillations (or other modes of oscillation) in the presence of a sound wave. This type of microstreaming can occur near any solid object in a sound field but a bubble, being compressible, is extremely effective. Nyborg (1959) has said that the streaming speeds can be greater by a factor of 10^6 compared with a sound field in which no bubbles are present. This bubble activity can occur at pressure amplitudes well below those required for collapse cavitation.

The microstreaming motions around bubbles in simple media such as water have been the subject of a great deal of study. The streaming patterns even in a medium like water are not simple. Elder (1959), investigating microstreaming around a bubble close to a rigid boundary, found four different types. The actual type of streaming that occurred depended on the intensity of the sound wave and the viscosity of the medium. The change from one type to another corresponded to a different mode of vibration of the bubble. He calculated that for a bubble in water and a sound wave of frequency 10 Kc/s, the velocity gradient in the neighbourhood of the bubble surface was of the order of 1.5×10^5 cm.sec.$^{-1}$ per centimetre. It

is not surprising, therefore, that bubbles are a source of greatly increased streaming. It was also found that the effects were greatest when the bubble was of resonant size i.e. when the natural frequency of vibration of the bubble coincides with the frequency of the sound wave. The formula for this natural frequency is:-

$$f = \frac{1}{2\pi r}\sqrt{\frac{3\gamma P_o}{\rho}}$$

Where f = frequency
r = radius of bubble
γ = ratio of principal specific heats
P_o = external pressure
ρ = density of liquid

For a bubble in water and with atmospheric pressure acting this formula reduces to:-

$$r = \frac{0.328}{f}$$

Where f is frequency in Kc/s and r is radius in cm.

For example at 1 Mc/s a resonant bubble will have a radius of about 3μ.

An example of the increased biological effects of ultrasound in the presence of bubbles has been described by Kolb and Nyborg (1956) and was due to Ackerman et al. They found that, using a single cell organism Paramecium caudatum, destruction of the cells occurred within a few minutes if bubble-induced streaming were present (the sound source was a small whistle and maximum pressure amplitude was 0.1 atmos.). In the absence of this streaming, the destruction took more than ten minutes.

Jackson and Nyborg (1958) put forward the idea that bubble-induced streaming could be responsible for certain biological effects. They had studied the streaming patterns above a pliable membrane

driven by a vibrating probe of small area underneath it and considered that the presence of a bubble close to a cell wall could similarly induce streaming in the cytoplasm. However, it was not possible to predict the streaming patterns due to the inhomogeneous nature of this material.

Hughes and Nyborg (1962) have used a brass probe drilled with a series of 50 holes, each of diameter just smaller than that of a resonant bubble at the frequency used, 20 Kc/s, to provide a source of bubbles. The amplitudes used were below those required for collapse cavitation effects. The results they obtained are discussed in more detail in section 4.6. They found that the bubbles were a good source of streaming and caused cell destruction.

4.4 Sound propagation in media containing gas bubbles

The size of the bubble is important in connection with the scattering of sound energy. If the bubble is the same order of size as or larger than the wavelength of the sound, it acts as a scatter of sound waves due to the large impedance differences between the gas in the bubble and the surrounding medium (see section 2.3). If the bubble size is less than the wavelength a number of processes become important (e.g. at 1 Mc/s, wavelength in water is 0.15 cm.). The bubble acts as a secondary source and a great deal of energy is scattered since an oscillating non-cavitating bubble can set the surrounding medium in motion due to its contractions and expansions. By considering the motion of a vibrating bubble as being similar to that of a simple damped oscillating system with one degree of freedom, Devin (1959) found

that energy was absorbed from the beam by a number of different mechanisms which he called the thermal, viscous and dissipation effects. The energy absorbed is due to heat conduction with the alternate compressions and expansions of the gas within the bubble, viscosity effects at the liquid-gas interface and the re-radiation of spherical sound waves from the actual bubble. The total absorption is due to the sum of these effects, but the proportion due to each depends on the frequency of the incident sound.

An interesting biological application of Devin's equations has been their application to explain the absorption figures for lung tissue by Dunn and Fry (1961). They used excised dog lung tissue, irradiated at a frequency of 980 Kc/s and a thermocouple as detector. For transmitted energy, the amplitude absorption coefficient was found to be 4.7 $cm.^{-1}$ To try and explain this high figure, the following model was used. The lungs were assumed to consist of a uniform distribution of spherical gas bubbles (radius .03 cm.) imbedded in a medium similar to water. Assuming that the sound wave causes the bubbles to vibrate and then to lose energy only by radiation, it was found by applying Devin's equations that the amplitude absorption coefficient should be 5.7 $cm.^{-1}$ at 1 Mc/s. The similarity between the two figures suggests that the absorption process in lung tissue is primarily one of radiation. It is extremely difficult to obtain ultrasonic pictures of the lung due to the enclosed air, but Dunn and Fry deduced the most favourable frequency at which to work, if any success was to be obtained. They assumed

that the absorption in actual lung tissue (no air present) was 0.1 $cm.^{-1}$ at this frequency and increased linearly with frequency. However, the absorption effects due to the presence of the bubbles decreased as the frequency increased. Since the total absorption was the sum of both, it was simple to deduce the frequency at which the minimum value for the absorption coefficient occurred. This was found to be 6 Mc/s (see fig.17). This frequency would then be the most appropriate at which to work.

4.5 Cavitation

Real liquids will normally be found to contain microbubbles (those having a radius of the order of 10^{-5}cm. are essentially stable) and impurities such as dust particles. Under the action of a sound wave these nuclei grow, mainly by rectified diffusion. At comparatively low amplitudes, stable oscillations of the bubbles are observed. At somewhat higher amplitudes, the bubbles become unstable and break up into a number of microbubbles, which form the basis for new bubble growth. At higher amplitudes, sudden and rapid growth and collapse of bubbles is found. These phenomena are referred to as "cavitation".

In many papers, no distinction is made between the collapse and stable forms of cavitation. Since these give rise to different effects, this is to be regretted. Stable cavitation occurs at lower intensities and gives rise to less violent effects than collapse cavitation. The collapse of bubbles can produce very high temperatures and pressures at the centre of the bubble (Noltingk and Neppiras, 1950

put these as high as 10,000°C and 10^6 atmospheres under suitable conditions) with the consequent transmission of a high intensity shock wave through the adjacent medium. Hueter and Bolt (1955) have said that for a bubble of initial radius 100 μ at 10 Kc/s and a sound pressure amplitude of 1 atmosphere, the subsequent collapse gave rise to a shock wave which had a measured pressure of between 200 and 500 atmospheres at a distance of 0.1 cm. from the bubble centre. Only collapse cavitation produces ions and free radicals in the solution. The nature of these depends on the medium itself and any gas dissolved in it.

The actual growth of the nuclei occurs in two different ways. The first takes place over a large number of cycles of the wave (probably, of the order of hundreds) and gives rise to the stable form of cavitation. The nuclei usually grow by a process known as rectified diffusion. During the rarefaction phase of the passage of a sound wave, gas diffuses into the cavity since the solution surrounding it is supersaturated with gas and during the compression phase the gas tends to leave the space as the solution surrounding it is now undersaturated. Since the surface area of the bubble space is greater during the rarefaction phase, there will be a net growth. Thus, the bubble grows with each cycle of the wave until it reaches its resonant size for the frequency used. Further increase in size makes the bubble unstable. It undergoes violent surface perturbations and oscillations and then collapses with the generation of a large number of microbubbles which stream away at high speed and form the nuclei for further bubbles. If the frequency is not high enough,

then the bubble will become buoyant before reaching its resonant size and will rise to the liquid surface. This is a familiar action known as 'de-gassing' of the liquid. The nuclei can also grow by a second method, that of coalescence. Nyborg (1957) has shown that a bubble of less than resonant size is attracted towards like bubbles.

The collapse form of cavitation appears to involve vapour-filled cavities or even voids in the liquid rather than the gas-filled bubbles encountered with stable cavitation. The growth and subsequent collapse of such cavities takes place over very few cycles. The negative pressure which occurs in the rarefaction phase must be large enough to overcome the cohesive forces in the liquid. Thus, violent expansion is succeeded by collapse with the production of very high peak pressures and other dramatic effects, such as sonoluminescence and the production of free radicals.

The occurrence and mechanisms of these different bubble phenomena are not completely understood at the moment. A good survey of the state of the work in this field is given by Flynn(1964). However, a number of different factors on which the onset of cavitation depends has been determined experimentally and, in some cases, theoretically. These are discussed by a number of authors (Noltingk and Neppiras, 1951, Webster, 1963 and Frederick, 1965 among others). These factors are as follows:-

1) The threshold intensity increases with frequency and an upper limit for the generation of cavitation is about 15 Mc/s.

2) The threshold intensity increases with increasing viscosity of the medium.

3) Increasing the hydrostatic pressure on the medium raises the threshold intensity.

4) The gas content of the liquid affects the threshold intensity. The more thoroughly a liquid is de-gassed, the more difficult it is to cause cavitation. Also, the distribution of impurities which act as nuclei for the initial cavities is also important.

5) It is found that the threshold intensity increases with increasing surface tension or decreasing vapour pressure of the liquid.

4.6 Biological effects due to cavitation

It is well known that certain types of cells can only be fractured in the presence of "cavitation". The actual type of cavitation is generally not specified but in the vast majority of reports, it appears to be the collapse form. The more fragile cells, as has been seen, can be ruptured purely by the mechanical stresses due to acoustic streaming or bubble-induced microstreaming. Generally speaking, it is the larger cells that are more fragile while the smaller ones are resistant to all but large amplitude sound waves. It has been found that the destruction rate of cells in the presence of collapse cavitation is frequency independant (Ackerman, 1962). Also, the number of cells remaining in a suspension decreases exponentially with time (Horton and Horwood, 1950, and Davies, 1959). This means that a graph of $\log_e N/N_o$ plotted against t, where N_o =

original number of cells and N = the number after time, t, will be a straight line (fig.18). This type of graph indicates that a single phenomenon may be responsible for the cell destruction. If cavitation is suppressed in a suspension of such cells, only a very small number are found to be ruptured even with long periods of irradiation (Neppiras and Hughes, 1964). The main problem concerning the mechanism responsible is whether this is due to additional chemical effects produced by collapse cavitation or the much greater mechanical stresses caused by the violent vibrations and collapse of bubbles. A third possibility is a combination of the two.

A set of experiments was carried out by Hughes and Nyborg (1962) to try and differentiate between the streaming and viscous effects due to vibrating bubbles (purely mechanical in nature) and those due to collapse cavitation (mechanical and chemical), and to see whether cells could be ruptured without the more violent effects of the latter. They used a brass bar driven at 20 Kc/s in a suspension containing E.coli (bacteria). The lower face of the bar was drilled with 50 holes whose size (200μ x 200μ) was slightly smaller than that of a resonant bubble at 20 Kc/s. The bubbles grew from air trapped within the holes. At low vibration amplitudes, the bubbles grew but remained on the face of the bar. As the amplitude was increased, the bubbles were seen to be vibrating until eventually a point was reached at which there was movement of the bubbles from the bar through the liquid with the subsequent growth (by coalescence) and collapse of bubbles with much consequent streamer activity and microbubble throw-off. This vibration amplitude (6.3μ) was

identified as that for the onset of collapse cavitation as at this point iodine was released from potassium iodide present in the solution (an indication of H_2O_2 production).

It can be seen from fig. 19 that destruction of the E. coli cells occurs readily without the presence of collapse cavitation. Also, on the same graph, are the results obtained using a smooth polished probe without holes (cavitation was not observed here until much higher amplitudes of vibration were used). Similar results were obtained using a suspension of D.N.A. This important macromolecule can be degraded in two ways. It can be broken purely by the mechanical stresses set up in the medium due to streaming as discussed in section 4.2. However, free radical attack due only to the collapse form of cavitation produces the breaking of the hydrogen bonding between the bases and a reduction in hypercromicity (very probably mechanical attack is present as well). Below the 6.3 μ vibration amplitude, there was a breakdown of the molecule as indicated by a viscosity decrease (fig. 20). There was no reduction in hypercromicity. After the 6.3 μ amplitude was reached, the breakdown occurred more rapidly and an increase in the optical density was found due to denaturation. In this case, the mechanical and chemical effects due to the passage of the sound wave have been elegantly separated.

Hughes (1961) had studied the effects of collapse cavitation on yeast cells, which are extremely resistant to ultrasonic waves. Two magnetostrictive transducers were used with stated electrical inputs of 50 W. and 500 W. at a frequency of 19-20 Kc/s (the transducer

output was not measured). The number of cells ruptured was estimated by determining the amount of nitrogenous material released from the cells into the solution or, where this method was inconvenient, the amount of protein released. Added nuclei in the form of particles whose diameters were 10 μ - 15 μ increased cavitation effects and thus the numbers of cells fractured (increased cavitation effects were probably due to the fact that the particles contained trapped air which served as nuclei for bubble growth). Hughes also showed that increasing the viscosity of the suspending liquid or lowering the surface tension lessened the rate of destruction (also, decreasing cavitation effects). It was also found, (Hughes and Rodgers, 1961) that there was no connection between the rate of radical formation (this was estimated by determining the amount of iodine released from potassium iodide present in the solution) and the rate of cell destruction. However, the inactivation of certain enzymes was found to depend on the free radicals present. Comparison of the rate of inactivation of the enzyme alcohol dehydrogenase by a probe that caused little free radical formation with that by one that caused a great deal of it showed that the latter was much more successful. Also, Hughes found that certain components leaked from the cells before the walls were ruptured. When the yeast cells were irradiated for two to three minutes with the 50 W. probe, it was observed that although very few cells were empty or even damaged (shown by phase-contrast and electron microscopy), protein, alcohol dehydrogenase and 260 mμ absorbing material were present in the

solution. It was found that small nucleotides were released early while the large nucleic acid molecules were released at a later stage.

The question of the part that free chemical radicals play in the biological mechanisms is fairly complex and not yet completely understood. Weissler (1960) investigated this aspect using a suspension of haemoglobin. He used a focused concave transducer operating at 400 Kc/s with an output of 20W. (measured by substitution calorimetry). The estimated focal intensity was of the order of a few hundred $W.cm^{-2}$. The absorption spectrum of the suspension was observed by means of a Beckman spectrophotometer. The haemoglobin molecule consists of the protein globin which is attached to the haeme, a complex iron-containing substance. After irradiation for about three minutes, the absorption peak changed from 4150 Å to 4050 Å indicating the formation of methehaemoglobin (due to the sonochemical oxidation of the ferrous part of the molecule to ferric). There was then a decrease in the absorption peak until after about thirty minutes it was completely flattened. By means of an ultracentrifuge it was found that the haeme and globin parts of the molecule were no longer attached. There was also found to be some aggregation or degradation of the protein though which of these happened depended on the gases present in the solution (and, thus, the free radicals formed). In the presence of dissolved air, Weissler showed that the sonochemically formed nitrous and nitric acids were responsible respectively for the formation and destruction of the methehaemoglobin. The free radicals formed from the water

such as H_2O_2, OH and HO_2 were much less important and did not affect the reaction. El'Piner (1961) has investigated the effects of various gases dissolved in the medium on different biomacromolecules and found that oxygen caused degradation with consequent repression of their functional characteristics (or even complete inactivation) while hydrogen caused aggregation with consequent preservation of these functional charactertistics. Fig.21 shows the effects on the molecular weight of two enzymes, pepsin and trypsin, in the presence of various dissolved gases and with collapse cavitation present.

Ackerman (1962) investigated the effects of high intensity ultrasound on red blood cells. By means of electron microscope studies, he showed that the cells were torn mechanically, concluding that the cell walls were torn by the shearing stresses set up by the collapsing bubble cavities. Although various free radicals were formed from the water, he considered that the concentration of radicals was several magnitudes too small to be responsible for breaking down the cell wall by chemical attack.

Definitely cavitation plays a major part in the mechanisms. If cavitation is suppressed, the effects on cells are severely limited. Other mechanisms, however, must play a part. Streaming effects can cause certain changes as has been shown but only on the weaker cells. The chemical effects due to collapse cavitation have been shown to cause changes only to the molecules released after destruction of the cell walls. A mechanical mechanism would appear to be the main one and is connected with the bubble collapse

phase of the cavitation. But whether it is due to radical resonance just before the bubble collapses, the production of the shock waves associated with the collapse of the bubble or the resultant microstreaming and viscous stresses set up in the region of the collapsed bubble is a matter for conjecture at the moment.

4.7 High amplitude effects in intact tissue

It has been found that other effects, quite distinct from the purely thermal effects discussed in section 3.5, occur when high amplitude waves are used or focussing is employed in intact tissue. The elucidation of mechanisms then becomes more complex. It has not been possible to make satisfactory measurements at the site of irradiation even in experimental animals and any damage caused by the wave can only be investigated after the animal is killed. Generally, focused ultrasound is used to increase the available intensity and most of the observed damage is found within the main focal lobe (section 2.6).

Fry et al (1950) irradiated the spinal cords of frogs and paralysis of their hind legs was taken as the end-point. The frequency used was 0.98 Mc/s and intensities were of the order of 35 $Wcm.^{-2}$ By using pulsed waves, they showed that paralysis was not dependant on the temperature rise. In a further paper (1951), they found that paralysis of the frogs still occurred even if an external hydrostatic pressure, which they considered was sufficient to inhibit any chance of cavitation at the frequency used, was applied. The effects were, however, reduced at the higher external pressure (fig.22). From the graphs, it can be seen that there is a minimum

pressure amplitude below which no effects occurred. Lehmann (1953) put forward the theory that selective heating was important together with a mechanical effect such as diffusion. Welkowitz (1955) suggested a mechanical mechanism based on the unidirectional forces produced by the wave itself. Later work (Dunn, 1957) tended to contradict this theory. Hueter et al (1956), on the basis of their work on irradiating the spinal cords of mice and using paralysis of the hind legs as an end-point, postulated a temperature dependant mechanical effect originating at weak points within the tissue.

Although it is agreed that cavitation is not a main mechanism, the part played by the heating of the medium is not easy to determine. If the biological effects were solely due to heating then the dosage for a given effect (e.g. paralysis of the hind legs) can be expressed as I x t where I is intensity and t the time of irradiation (assuming no heat losses). On the other hand, if the amplitude of one of the parameters (e.g. pressure) is responsible then a relationship of the type A x t (or $I^{\frac{1}{2}}$ x t), where A is amplitude of parameter, should apply. Some authors have found the first type of relationship occurring (e.g. Curtis, 1965, who investigated the effects of ultrasound on the intact mouse liver and used hepatic lesions as the end-point) and other authors the second (e.g. Fry, 1958). These results need not contradict each other. Hueter et al (1956) have pointed out that the reaction kinetics of the molecules in cellular materials are extremely temperature dependent, making it very difficult to separate thermal and non-thermal effects. A great deal depends on the temperature of the specimen and the rate at

which heat is removed from the irradiated area. Various authors (e.g. Lehmann et al, 1957 and Ackerman, 1962) have shown that the effects on cellular materials due solely to heating are different from those observed with ultrasound.

On the bases of a large amount of work on the irradiation of the spinal cords of mice, the following empirical formula relates the intensity with the time for paralysis of 50% of the sample using results such as shown in fig. 22 (Fry, 1958):

$$t^{-1} = 0.209\ I^{\frac{1}{2}} - 1.42$$

Where I = intensity ($W.cm^{-2}$), t = time of irradiation (secs.)

It has been found that using intensities on the C.N.S. less than those required for irreversible effects, can result in temporary blockings of nerves (Herrick, 1953, Young and Henneman, 1961). The intensity range for these effects is small. Fry (1958) has suggested that this could be a good method of obtaining information about neural pathways. By interrupting these with ultrasound, it is possible to relate them to identifiable changes in behaviour.

At the present time, the whole subject of high amplitude effects in tissue is a matter of controversy and the literature contains many contradictions. The main mechanism appears to be mechanical and very temperature dependent. The occurrence of cavitation is very difficult to prove one way or the other. Evidence appears to suggest that it does not occur (Fry, 1958) but vacuolization of the protoplasm has been observed in the subsequent histological examination of onion roots irradiated at intensities of 110 $W.cm^{-2}$ by Lehmann et al (1957).

Bell (1957) observed this, also, in liver tissue, the diameter of the holes being about 1μ.

The technique of using pulsed waveforms has not been used very much. It would appear that this could be a good way of separating effects that are due to heating from those that are purely mechanical in nature.

4.8 Discussion of other effects of ultrasound

In the previous sections an attempt has been made to explain the interaction of ultrasound with biological material. The examples were chosen as they helped to elucidate some of the mechanisms responsible. This is probably not the complete picture and various other mechanisms could play a smaller part. There are a large number of variable quantities present in a sound wave and it is extremely difficult to separate the effects due solely to one of them.

Little consideration has been given to the effects of the direct sound beam on an object placed in its path. Simple calculations (equations 2.1 to 2.3) indicate values for the different parameters. For instance with a $1 W.cm^{-2}$ intensity beam at 1 Mc/s in water, the pressure amplitude oscillates between + 1.73 and - 1.73 atmospheres. This occurs 1,000,000 times each second. The maximum acceleration of the medium under these conditions is 73,000 x g. Though these values are fairly large, they act for only short periods of time. Fatigue could occur to an object in the medium due to repeated oscillations. The direct sound beam could have a number of indirect effects such as the breakage of weak molecular bonds.

It has been shown that the destruction rates of cells in a suspension is largely frequency-independant. Ackerman (1962), however has shown that at certain frequencies the sensitivity of cells to ultrasound was greatly increased. It is known that mechanical systems have certain resonant frequencies. His interpretation for cells was that it is a mechanical resonance involving the cell walls and that knowing the values for the resonant frequencies leads to some knowledge of the physical properties of the cells. He proposed two types of cell model to explain the resonances and, with a suitable choice of physical constants, these both agree with experimental results. What part this resonant effect can play in the operative mechanisms is not very clear at the moment.

Another interesting effect is the combination of sound waves and X-rays. Conger (1948) found that the number of chromosome aberrations on Tradescantia paludosawas increased by a factor of 1.3 when using sound waves (the frequency used was 91 Kc/s) and X-rays together than with X-rays alone. His explanation was that the sound waves stirred up the broken chromosomes preventing any reunions (this could occur with X-rays alone). Woeber (1954) has used ultrasound in conjunction with X-rays in the treatment of various types of skin cancer. The first subjects were 120 rats. It was found that the addition of ultrasound enabled a reduction of the X-ray dose by up to 40%. Woeber (1965) has reported that the results obtained with humans have been very encouraging.

Chromosomal aberrations, which lead to permanent changes in succeeding generations, have been obtained using ultrasonic radiation. These mutations have mainly been observed with plant cells (Wallace et al, 1948 and Newcomer, 1954). Most mutations observed have been of the point type. Selman and Counce (1953) have observed that, in eggs subjected to the effects of ultrasound, the nucleus and cytoplasm were left in unusual positions and abnormal development followed in some cases. The mechnism operative in causing mutations is not clear but Gordon (1963) has suggested that a depolymerising action occurs similar to that observed with some biomacromolecules.

5. Applications of ultrasound

5.1 Diathermy

This application of ultrasound is a direct consequence of the fact that when ultrasound passes through a medium, energy is absorbed and appears ultimately as heat. The greater the value of the absorption coefficient, the greater the amount of heat produced. In addition to heat, some authors consider that "stirring" of the cells and their contents occurs, especially in the soft tissues of the body, although the evidence for this is very difficult to prove one way or the other. Diathermy is used to treat certain rheumatic and arthritic conditions. The use of ultrasound is usually a better method of heating body tissues than other conventional heat treatments such as microwave therapy due to the more effective distribution of the heat generated within the tissue. The actual heat produced depends on the absorption coefficient of the tissue at the frequency used. At low frequencies, absorption is small but increases linearly with increasing frequency. Since the impedance of the tissues does not change, the actual penetration of the sound beam will decrease with increasing frequency. Schwann (1960) defines the depth of penetration as the distance covered by the wave in order to reduce its amplitude to $\frac{1}{e}$ $(= 0.37)$ of its original value. Some values for these figures at different frequencies are given in Fig. 24. From these figures it can be seen that ultrasound is very effective for heating muscle under a layer of fat due to the small absorption coefficient of the latter. As mentioned before, the absorption coefficients of tissues with a high water content are small and,

hence, depth of penetration is large. The reverse is true for those tissues with a high degree of structural order. Bone is a good absorber of ultrasound and, in addition, causes mode conversion (section 3.5). This means that bone will be strongly heated compared with surrounding tissue.

Ultrasound is applied to the tissue by means of a conventional transducer (Frederick, 1965) with water or oil as the coupling medium. The intensities used vary with different workers and complaints but the maximum appears to be about 3 $W.cm.^{-2}$ Above this, pain is experienced by the patient. The frequency employed depends on the depth of the tissue to be irradiated. If the tissue is near the skin a higher frequency can be used (more absorption, greater heating) than for a tissue deep in the body. The range of frequencies used is between 1 and 3 M c/s.

Aldes (1957) has stressed that a daily check of intensities, duration of irradiation and frequency used should be undertaken. He normally used intensities between 0.2 $W.cm.^{-2}$ and 1.5 $W.cm.^{-2}$ at frequencies between 0.8 and 1 Mc/s on a large number of arthritic patients. During a five year period and the treating of about 4,000 cases, it was found that 78% of these obtained overall relief after one series of irradiations (12 doses, the periods varying from 3 to 20 minutes at intervals of 48 hours). The remaining 22% were not completely free of symptoms after the one series and had to have another one or two series before complete relief was obtained. To prevent permanent damage to the tissue of the patient, if acute discomfort was registered by the patient due to excessive

temperature rise, the intensity was cut down. This appears to be the only way in current practice of preventing too large an intensity being applied. Noltingk and Terry (1958)have quoted other results obtained using ultrasound for diathermic applications.

Patrick (1966) has mentioned more recent applications of this form of diathermy for treating such ailments as painful scars, fractures and nerve root pains. Details of intensities and dosages are given. She is of the opinion that pulsed ultrasound is more effective than the unpulsed type as the latter can lead to the production of too much heat if adequate precautions are not taken. This would tend to imply a non-thermal mode of action.

5.2 Diagnosis

The physical basis of this application depends on the fact that the body consists of many materials of different acoustic impedances. When a travelling wave crosses a boundary between two media of different impedance then part of the incident energy is reflected and the rest transmitted. According to equation 2.4 the greater the difference in impedance of the two media, the greater the fraction of the energy that is reflected. For two given media, the reflected fraction is greatest when the incident wave-front is at right angles to the boundary. The reflected energy is detected by the same transducer (a pulsed waveform is usually employed) and the pulse displayed on a suitable oscilloscope. This method is especially useful in the diagnostic investigation of soft tissues where X-ray techniques are not of much value. The main difficulty is the similarity of the values for all soft tissues. The density of most mammalian soft tissues varies between

1.02 and 1.07 $gm.cm.^{-3}$ and this leads to values of the characteristic impedance between 1.5 and 1.7 $gm.cm.^{-2}sec.^{-1}$ Thus, the reflected signals are small. Added to this, absorption reduces the amplitude of the wave further. Strictly speaking, the complex acoustic impedance should be considered if a diverging beam is employed or absorption occurs.

The equipment and techniques have developed considerably since Dussik and his co-workers tried, just after the war, to get ultrasonic pictures of the ventricles of the brain by transmitting sound through one side of the brain and using a second transducer on the opposite side as a receiver. The results were not very successful, owing to the short time intervals between transmitted and received pulses. It was only with the advent of high speed oscilloscopes that satisfactory progress could be made.

The general idea is to send out a triggered pulse, lasting a few millionths of a second, from the transducer into that part of the body under investigation, using a suitable coupling medium, and then to display the received pulse together with the initial pulse on an oscilloscope. If the speed of the sound in the tissue is known, then its thickness can be determined. By means of complicated scanning equipment, two dimensional pictures can be built up (Howry, 1957). Although the peak intensity of the pulse is high, the average sound intensity is very low - thus, no appreciable heating of the medium occurs.

The choice of frequency is generally a compromise. To receive discernable echoes from two objects close together is only possible theoretically when their distance apart is equal to or greater than

the wavelength of the sound. In practice, it is generally at least two or three times the wavelength. Consequently, it would be best to employ as high a frequency as possible. However, since absorption increases with frequency, the deeper the site to be investigated, the lower the frequency that must be used to receive a discernable echo. The frequencies in use vary from 1 Mc/s (wavelength = 0.15 mm. in water) when large distances such as those in the brain are encountered, up to 15 Mc/s (wavelength = 0.01 mm.) when investigating such structures as the eye. Due to absorption the signal amplitude will be reduced to $e^{-2\alpha x}$ of its original value where x is the depth of the interface and α the absorption coefficient.

Information about the intensities used is not plentiful. Elder (1963) has discussed the intensities he used in his investigations of the heart. 200 pulses per second were used with a maximum duration of 5μ secs. each. Thus, the actual period during which the ultrasound is propagated is 1/1000th. of each second. Peak intensities of 80, 40 and 20 $W.cm.^{-2}$ were employed at frequencies of 1, 2.5 and 5 Mc/s. respectively and these give mean intensities of 0.08, 0.04 and 0.02 $W.cm.^{-2}$ These values, according to Elder, are one tenth of the intensity required to produce any sensation of warmth in the body. Gordon (1959) irradiated a cat's skull at twice the normal operating voltage and three times the repetition rate for five minutes. Killing of the animal and subsequent histological examination revealed no apparent damage to the brain.

Ultrasound has been used to visualise a number of different parts of the body. The detection of tumours is sometimes possible owing to

the fact that malignant tissue reflects more sound than normal tissue (Wild and Reid, 1957). Various eye complaints such as detachment of the retina can be rapidly diagnosed, and the measurement of ocular distances determined with a high degree of accuracy ($\pm$ 0.2 mm.) according to Baum (1964). Although initial work on the brain was disappointing due to interference of the skull and the actual complexity of the brain itself, it is now possible to visualise the structure (de Vlieger, 1963) and to detect the presence of tumours by displacement of the mid-line structure (Pell, 1964). Much work has been done on the abdomen and associated organs (Howry and Gordon, 1964). Kossof et al (1965) have visualised the pregnant uterus. For safety reasons, they irradiated approximately 100 pregnant mice for two minutes with continuous 1 Mc/s sound at full output (about 15 W). The mice were apparently unharmed if the transducer was properly coupled to their skin. The average power of the pulsed wave employed in practice was about 1 mW.

Though now a widely used and successful method of diagnosis, little is really known about the mode of propagation of a sound wave through a structure as complicated as the human body. Schwann (1963) has stressed this fact, pointing out it is not a very satisfactory position in which to be.

5.3 Neurosurgery

It has been known for many years that lesions can be obtained in practically all parts of the body using ultrasound of sufficient intensity but the only area in which practical use of this has been made is the brain and the central nervous system. By using focused beams of

sound, very high intensities can be obtained within the focal volume and discrete lesions can be formed deep within the brain without disturbing the intervening medium. Lesions as small as 2 $mm.^3$ at 1 Mc/s have been obtained by Fry (1958) and 0.2 $mm.^3$ at 2.5 Mc/s by Ballantine et al (1956) in this way. The lesions that occur consist of a central region of killed tissue surrounded by a peripheral region of disturbed and damaged tissue. Although clinical effects are immediately apparent (i.e. within a few seconds), lesions do not show up in subsequent histological examination until at least ten minutes after irradiation. The white matter of the brain is more easily damaged than the grey. The resistance of components in the white matter to the effects of ultrasound increases in the following order: myelin sheaths, axis cylinders, cells glia and then blood vessels (Fry, 1958). Ultrasound breaks down the blood-brain barrier though the blood vessels themselves are not impaired, as has been mentioned before (Bakay et al, 1959).

By destroying specific small areas of the brain, it is possible to eliminate or minimise certain diseases that occur due to malfunction of the central nervous system such as Parkinson's disease, a number of types of involuntary movement and tractible pain. The majority of cases treated have involved Parkinson's disease, (Meyers et al, 1960). This disease causes unsteady movements and rigidity of limbs. The frequencies used vary between 1 and 3 Mc/s depending on the depth to be irradiated (the absorption coefficient for brain matter at 1 Mc/s is about 0.1 $cm.^{-1}$). The intensity within the focal volume can be as high as 900 $W.cm.^{-2}$ and the total time of irradiation up to three seconds.

In using ultrasound for neurological applications, it is essential that the lesions are accurately placed and that effects are reproducible. Because of this, very expensive equipment and expert handling are required (Fry and Dunn, 1962). This method has a number of limitations, (Hughes, 1964). A good description of the physical factors involved has been given by Lele (1962a) and a statistical evaluation of their results has been given by Basauri and Lele (1962). Meyers et al (1960) have given clinical results of their work. Since the work done in this field has been fairly successful, it is probable that it will be extended to other sites such as the spine or malignant tumours.

The mechanisms which appear to be responsible for biological effects in intact tissue have been discussed in section 4.7.

5.4 Ménière's Disease

This is an affliction of the inner ear and causes dizziness and vertigo. It is due to a fault in the fluid-filled cavities of the semicircular canals. Conventional surgery is needed for about 10% of the cases but usually results in loss of hearing. By using ultrasound the vestibular apparatus can be destroyed but there is the possibility of causing facial paralysis due to proximity of the facial nerves. However, it does preserve the hearing. The method was initially developed by Arslan who has used it with much success. The canal is surgically exposed from behind the ear and the bony section is flattened to receive the probe (Arslan, 1964). The ultrasound destroys the neuroceptor cells in the semicircular canals. The beam must be narrow because the facial nerves are very close and a small rise in

temperature will destroy them. No histological change is visible immediately after irradiation, it being days before any is apparent. The end-point is determined as follows. If warm or cold water is added to the ear, the eyes turn to one side or the other. This happens due to stimulation of the nerve endings. If the vestibular nerve endings are destroyed then this does not happen. The irradiation is generally kept up for a few more minutes to ensure that the end-point or nystagmus has been reached. The same type of surgery has been used to cure certain types of vertigo by Wolfson (1965).

It would appear that the mechanism here is primarily a thermal one, but there is histological evidence that the answer is more complex than this (Arslan, 1965).

6. Measurements

6.1 Introduction

If certain assumptions are made about a medium, namely that a plane wave is considered and no attenuation of it occurs, then a set of simple relationships exist relating the various parameters (equations 2.1 to 2.3). While such assumptions are approximately valid for many media, considerable departures from them are to be found in the case of media containing macromolecules and membrane structures characteristic of biological materials. Because of this, only measurement of all the various parameters will give a detailed picture of the behaviour of the wave. Also, any device used for measuring a parameter must necessarily alter the characteristics of the medium due to differences in acoustic impedance, absorption, thermal capacity and conductivity.

It is necessary to know how the available energy is distributed and what biological changes it causes during its passage through tissue or biological suspension. It is not possible to describe the effects satisfactorily due to incomplete knowledge of absorption mechanisms and the complexity of biological structures. Also, the difficulty of making measurements in intact tissue creates difficulties.

In order to try and elucidate biological mechanisms and to obtain reproducible results, the following parameters at least should be known: the frequency and method of operation of the transducer; the total output of the transducer; the intensity

distribution and geometry of the beam in the medium; the rise in temperature produced by the wave; the physical chacteristics of the medium and the occurrence and intensity of any cavitation effects. The first part of the section will consist of a brief discussion about the various necessary measurements and the second part an account of the available methods with special reference to those designed specifically for work in this field.

6.2 Parameters to be measured

(a) Frequency and method of operation

A transducer is usually driven at its resonant frequency (especially for power applications) or one of the higher harmonics of it. The determination of the frequency of operation is important both in explaining the behaviour of the sound wave and in elucidation of any frequency-dependent effects.

A complete description of the experimental arrangements are essential for repeatable results to be obtained. Very little description is found in many papers which helps to lead to lack of reproducibility of results in this field.

(b) Total output of the transducer

The total output of a transducer is an important parameter to know. Only with the advent of the medical applications of ultrasound has the accurate measurement of output been the subject of much study, and a number of devices developed for this purpose. Obviously, the dosage applied to a patient is critical. In some papers, the output or the intensity per sq.cm. of the emitting

surface is quoted but little or no idea is given of how the value was obtained.

(c) Distribution of the intensity

Even when considering a transducer vibrating as a simple piston source into an unbounded homogeneous medium, the resultant intensity distribution does not follow the simple pattern of the plane wave. The theoretical equations for this are given in section 2.5. Adjacent to the transducer lies the region known as the Fresnel region in which the axial intensity passes through a number of maxima and minima, the width of the wave pattern being the same as the transducer diameter. Due to the large absorption coefficients of biological materials, this region is important especially in diathermic applications. After the last minimum, the beam enters the Fraunhoffer region in which divergence occurs and the intensity falls off with the square of the distance. Most of the acoustic energy for a given transducer is concentrated in the main lobe and the energy carried by the side lobes decreases with increasing frequency. The distribution obtained with mathematical techniques does help to explain wave patterns in simple media. In practical media, the sides of the container give rise to reflection and standing wave effects, and bubbles further complicate the intensity distribution. In intact tissue, no real picture is, at present, possible.

Location of the focal region and the intensity distribution within it are important if focussing of the sound wave is employed.

This is especially true in the neurosurgical applications of ultrasound where focal intensities are extremely high. It was mentioned in section 2.6 that the focal area consists of a main lobe surrounded by a number of much smaller lobes. Up to 84% of the acoustic energy flows through the main lobe. Knowledge of the focal position can be worked out by theory but is not of too much value when considering complex biological media.

(d) Temperature

Since most of the energy absorbed by a medium is ultimately degraded to heat, a knowledge of the temperature rise is required especially when dealing with the propagation of ultrasound through living structures. The temperature rise produced will depend on the absorption coefficient and thermal capacity of the medium. This rise will also depend on the rate at which heat is removed from the irradiated region (this is especially important in tissue irradiation).

(e) Physical characteristics of the medium

Elucidation of the mechanisms requires some knowledge of the physical chacteristics of the medium. Examples of these are the density, viscosity and presence of any dissolved gases. To describe the progression of a sound wave through a biological structure, the nature of the materials that compose it and the locations of the interfaces are required. Also, the absorption coefficients and velocities of propagation are essential. The absorption coefficients quoted generally mean the gross coefficient since they take into

account all losses from the main beam due to reflection, refraction and diffraction effects. The different values of absorption coefficients of tissues obtained are probably due to differences in age, preparation and tension of the specimen. The propagation velocity is needed to calculate the characteristic impedance of the medium in order that reflection coefficients at the different interfaces can be determined (section 2.3).

(f) Occurrence of cavitation and measurement of its intensity

Detection of the occurrence of cavitation and the ability to distinguish between the stable and collapse forms are essential in the elucidation of mechanisms. Although many of the dramatic effects of ultrasound occur in the presence of cavitation, in other cases, steps must be taken to ensure that it does not occur. Some indication of the actual intensity of the cavitation would also be of interest for obvious reasons.

6.3 Measurement of frequency

The measurement of the frequency of operation is done in the usual way. If a high impedance oscilloscope is connected across the input to the transducer, then the frequency can be determined if the speed of the trace is known.. Resonance of the crystal will be indicated by a maximum of the received signal. Alternatively, the frequency can be compared with that of a variable frequency generator by means of Lissajou's figures (Stephens and Bate, 1966). The frequency stability of piezoelectric crystals appears to be quite good (Hughes,1963) but a meter in the input circuit (e.g. oscillator

plate voltage) can be used to monitor any electrical change due to frequency drift.

In connection with magnetostrictive transducers, Hughes (1961) has mentioned that it was possible to tune his transducer (resonant frequency was 19 Kc/s) by listening to changes in the noise emitted. This noise was not of a frequency of 19 Kc/s but a mixed noise produced by the cavitation. Neppiras and Hughes (1964) used an accelerometer device to detect resonance. The transducer was megnetostrictive and a small prepolarized crystal of lead zirconate titanate was attached to a threaded base which was screwed into the transducer face. The output from the crystal was monitored on a valve voltmeter and any drift from resonance was shown on this.

6.4 Measurement of total output

One method, which is to be regretted, is of assuming that the transducer is perfect so that the acoustic output is equal to the electrical input. This would mean an energy conversion efficiency of 100% whereas in practice this efficiency may only be of the order of 40% or even less. An accurate but time consuming method of obtaining the output is to work out the efficiency by means of an Argand diagram (Frederick, 1965). For the loaded condition, the transducer must be vibrating into the medium for which the output is required. Neppiras (1965) using this method at fairly high intensities (4 - 10 $W.cm.^{-2}$ at 20 Kc/s) obtained reliable figures for efficiency and power that agreed with calorimetric methods.

Radiation pressure devices are reliable and simple to operate. They work on the principle that a second order effect in sound waves produces a steady component of force on an interface of different acoustic impedance placed in the beam's path. Measurement of this radiation pressure force, F, (usually by means of a beam balance arrangement) leads to a value for the power, P, given by the equation:-

$$P = F \times c$$ Where c = velocity of sound in the medium

For this simple formula to apply, the interface must be either a perfect absorber or reflector of sound or be mounted at an angle of 45° to the incident beam. The force on the interface corresponding to a power of 1 watt is 0.069 gm. for the absorbing surface and 0.138 gm. for the reflecting surface (water being the medium). The main problems with these devices is the elimination of standing waves which give rise to resonance effects. Also, results at high intensities are not accurate due to streaming and cavitation. A number of radiation pressure devices have been designed to measure the output of transducers used in medical applications.

The radiometer due to Wells et al (1963) was of classical design (fig. 25), consisting of a balance arm which pivoted about its midpoint. On one side was an absorbing (or a reflecting) surface mounted at 45° to the incident beam while on the other side of the fulcrum was an adjustable counterweight. The beam was directed vertically downwards onto the angled plate. The instrument was immersed in water. With the beam incident on the plate, the

adjustable counterweight was moved until the balance arm became horizontal again. By consideration of the principle of moments, the force on the plate was determined and hence the power of the beam evaluated. A perfect absorber was approximated to by lining the plate surface with neoprene. A perfect reflector was obtained by using two thin metal sheets cemented together with an air gap between them. The reflected beam was eventually directed into a multiple absorber lined with neoprene. Using a transducer working at its resonant frequency, they found that using the reflecting surface the intensity was found to be 1.33 $\pm$ 0.03 watts but the absorbing surface led to a value of 1.31 $\pm$ 0.02 watts. The second result was considered to be more accurate as the relecting surface introduced angulation errors due to beam divergence.

A similar radiation pressure device has been designed by Newell (1963) for the measurement of the output of medical transducers but possesses the advantage that it can measure powers from watts down to the order of milliwatts by changing the wires that compose the framework of the balance. With the apparatus at its most sensitive (using thinnest wires), Newell obtained a deflection of 1mm. for a weight of 0.004 gm. This corresponds to the force due to a 60 mW. beam. If it is calibrated for fixed wires (deflection per centimetre is known), then the output of a transducer may be readily determined.

Wells et al (1964) designed another radiometer to work at the very low power outputs of pulsed transducers or of the tiny transducers

used in some medical applications. This method depends on the principle that a small sphere or interface suspended by a wire will be deflected from its rest position due to the radiation pressure of the beam. In Well's instrument (fig. 26), a hollow metal vane, 5.5 cm. in diameter, was mounted at 45° to the on-coming beam. Two heavy members were cemented either side of the vane to keep it in this position (the vertical force is always much greater than the horizontal force). It was suspended by two phosphor bronze wires, 83.7 cm. in length, from the top of the apparatus. The beam was deflected vertically down by the vane into a multiple absorber lined with neoprene. Assuming a wire of zero weight and cross-section, the following equations are derived by resolving the forces in two directions (fig. 26):-

$$F = T \sin \theta$$

$$W = T \cos \theta$$

Where F = horizontal force (radiation force)

W = vertical force (weight)

T = tension in wire

θ = angle of deflection

From these two equations, $\frac{F}{W} = \tan \theta$

Since $\frac{d}{l} = \tan \theta$, where l = length of the wire, d = horizontal displacement of vane, the following relationship follows:-

$$F = \frac{dW}{l}$$

Thus, by measuring d and knowing W and l, a value for F and hence P, can be found. The actual deflection was measured by a

travelling microscope and it was worked out theoretically that a 1 mW. beam would cause a deflection of about 0.02 cm. However, several errors arise with this type of instrument. Investigation showed that any error due to the weight of the wires, their resilience and any changes in length was negligible; as was that due to surface tension effects. In practice, the main error ($\pm$ 2 %) was found to be due to dust particles that occur on the surface of the water. However, this could be avoided by totally immersing the suspension wires. A lighter vane would increase the sensitivity but its weight becomes more temperature and pressure dependent. The apparatus needs a very substantial base if vibration effects are to be eliminated. They claimed that the instrument is accurate to within $\pm$ 3% for a power of 2 mW. This value for the error was obtained from the mean of a series of ten measurements from the source of 2 mW. The minimum deflection that could be detected corresponded to a beam of 0.01 mW, but it is very difficult at these powers to separate any deflection due to draughts and vibrations from that due to a sound beam.

Other methods have been developed by workers to monitor power outputs. Gordon (1963) developed an instrument known as the Friston ultrasound intensitymeter at the Royal Ear Hospital. Again the basic arrangement is similar to Well's original pressure balance. However, it is very sensitive and can record forces as small as 0.00017 gm.wt. with the aid of sophisticated additional apparatus. In normal use, it can record powers down to 1.7 mW.

Kossoff (1962) described a method in which the radiation pressure due to a sound beam was used to depress a float partially immersed in carbon tetrachloride (fig. 27). The stem of the float was usually calibrated directly in watts.and intensities of up to 25 watts could be measured with an accuracy of ± 5%. More recently, Kossof (1965) has greatly improved the sensitivity by using a Mettler balance. Using a reflecting target a sensitivity of 0.04 mW has been obtained.

Calorimetric methods are of two types; the constant flow method (Brown and Goodman, 1965) and the thermal isolation method (Mikhailov, 1964) but neither appears to have proved popular for biological applications. The calorimeters work on the principle that all the sound energy is used to heat the liquid and from the temperature rise it is possible to calculate the transducer power. The best results with thes instruments are obtained at high intensities even with cavitation present (Neppiras, 1965). The main difficulty appears to be to stop any heat generated within the transducer being transfered to the absorbing medium. At low intensities, this heat swamps any heat due to the absorption of ultrasound by the liquid due to imperfect thermal insulation between the source and the calorimeter.

The only application of a calorimeter measurement in this field appears to be that of Wells et al (1963). Their calorimeter is shown in fig. 28. It was filled with carbon tetrachloride which has a much larger absorption coefficient than the sphere

containing it (this means that the sphere will absorb little power from the beam). The entry tube was off-set to prevent the beam being re-radiated out of the calorimeter. The entry tube was filled with constantly flowing water to remove any heat generated within the transducer. There is only a small loss in power due to any reflections at the water/carbon tetrachloride interface as their characteristic impedances differ by only 0.67%. Original trials with the instrument had used water instead of carbon tetrachloride but, due to very slow heat transfer, non-linear responses were obtained. The temperature rise in the calorimeter was found by means of a number of thermocouples suspended inside the sphere. The acoustic power can be calculated knowing the rate of temperature rise and the mass of the absorbing liquid. Using a transducer whose output was 1 watt (as determined by a radiometer), a value of 1.06 ± 0.07 watt was obtained with the calorimeter. The discrepancy was probably due to possible errors in the volume estimation of the calorimeter and divergence of the beam. For clinical work, it was maintained that such results were of sufficient accuracy.

6.5 Measurement of the intensity distribution

Measurement of the intensity distribution is important because of the complex nature of the acoustic field radiated by a transducer. The main drawback again to all the methods described is that observations can only be made in a simplified medium, usually water. In a biological medium, especially tissue, this picture will naturally be modified but at the present time there is no method of obtaining

any picture of this distribution. It has be to assumed that the intensity distribution both in a simple medium and a complex biological material is similar. A summary of the various methods used to visualise acoustic fields in order to study intensity distribution has been given by Rozenberg (1955). The main methods of interest are those of ultrasonically sensitive chemicals and Schlieren photography. The acoustic field can also be investigated with the aid of a small piezoelectric probe or thermocouple.

Chemical methods are a simple and cheap way of visualising an acoustic field. An interesting method developed by Kossof (1962) after an idea by Bennett (1952) gives fairly accurate results ($\pm$ 15%) of the intensity distribution. The transducer under test was allowed to vibrate into an iodine solution. Arranged at intervals and mounted at right angles to the path of the beam, were a number of thin polyethylene sheets (these are transparent to ultrasound) on which was deposited a film of starch. The effect of ultrasound is to accelerate the well known starch/iodine reaction which turns the starch blue. When the beam is propagated it takes about 5 to 10 seconds for the patterns to develop. Where the intensity is greatest, the colour change will be greatest (Kossof showed that the density of the colour was directly proportional to the ultrasonic intensity at that point in the range 0.2 to 2 $W.cm.^{-2}$ Above this value, cavitation effects begin to occur and these complicate the results). Due to the rapid deterioration of the sheets, it is advisable to film them as soon

as possible in order to obtain a permanent record of the intensity distribution.

Another method described by Arklangel'skii (1967) could be used in a similar manner. It concerns the accelerated developing rates of photographic paper in the presence of ultrasound. There is the usual darkening effect which increases with increasing sound intensity. By using an arrangement similar to Kossof's, except that the iodine is replaced by developing solution and the sheets with photographic paper, which must be transparent to ultrasound, similar measurements can be made. Arklangel'skii found that silver iodide gave the best results. He also found that, using undeveloped photographic paper, cavitation produced development of the paper if there were no developing agents present in the solution and it occurred after a few minutes irradiation. This was probably due to sonoluminescence. If cavitation effects do not occur, then a developing agent is necessary and complete development of the paper requires a time of the order of tens of minutes.

Schlieren photography has proved to be of considerable use in visualising the side lobes and, thus, the divergence of ultrasonic beams emitted from transducers. This system depends on the fact that a mechanical wave propagated through a medium causes density changes. Light is shone through the medium and any density gradients are manifested as intensity changes in the image. Two interesting applications of Schlieren photography have been those due to Bullen (1963) and Kossof (1964). Both were investigating

the design for a focused transducer for treating Ménière's disease. This necessitated a transducer of very small emitting surface but high intensity. Knowledge of the divergence of the beam was essential due to the proximity of the facial nerve during irradiation.

The intensity distribution can be scanned mechanically by means of a small piezoelectric probe. However, its presence will tend to distort the fields even when its physical size is extremely small. It can also give rise to standing wave systems which will cause resonant effects. At megacycle frequencies the receiver can cause cavitation effects because of imperfect wetting of the crystal face due to surface discontinuities which encourage bubble formation, (Lele, 1962). This can be avoided by using a crystal covered with silicone rubber. The voltages produced by the pressure charges at the crystal are generally displayed on an oscilloscope. The intensity of the wave is proportional to the square of the pressure and hence to the square of the voltage. The main advantage of using such probes is the wide range of intensities to which they will respond. Intensities from microwatts to the order of watts per sq.cm. can be satisfactorily measured.

A thermocouple designed specifically for this field has been developed by Fry and Fry (1954, a and b). The instrument, shown in fig.29, consists of two separated polyethylene diaphragms containing an imbedding medium with the tiny thermocouple, 0.0005 in. in diameter, fixed at the centre. The imbedding medium, which

should be chosen to ensure a good acoustic impedance match, is usually castor oil when working with water or similar liquids. Also in fig. 29, there is shown the thermoelectric e.m.f. generated due to the passage of a one second pulse of ultrasound. There is an initial sharp rise due to viscous forces between the wire of the thermocouple and the imbedding medium. Then follows the effects due to true absorption and this section is approximately linear. When the pulse has passed, there is an initial rapid decrease of the e.m.f. due to removal of the viscous forces. The subsequent slow decrease is due to the cooling of the medium. Fry and Fry used 0.1 sec. pulses for intensity distribution studies as thermal recovery was quicker. They have shown (1954,a) that considering the linear portion of the graph:-

$$I = \frac{\rho C}{\mu} \frac{dT}{dt}$$

Where I = acoustic intensity

ρC = heat capacity/unit volume of imbedding medium

μ = intensity absorption coefficient of imbedding medium

$\frac{dT}{dt}$ = temperature gradient

If certain criteria are satisfied, then the intensity absorption coefficient, μ , can be found if the acoustic intensity, I, is known. If μ is known then I can be found. The intensity absorption coefficient is equal to twice the pressure amplitude coefficient.

The main advantages of this thermocouple method are that it is

very small and stable; it does not distort the acoustic field to any great extent and can determine an absolute value for the acoustic intensity. It has been used by Dunn and Bryer (1962) for frequencies as high as 2000 Mc/s. The main disadvantages are that an intensity of 1 W/cm^2 is needed for a suitable output and the instrument cannot be used to investigate the temporal waveform of a wave. For measuring purposes, the thermocouple is connected to an oscilloscope via a low noise amplifier. The results are then seen visually or photographed. A magnetic oscillograph can also be used.

The focal length of a concave transducer and the distribution of the intensity within the focal region are of critical importance especially in irradiation of brain material in order to produce lesions. Hughes (1964), discussing dosimetry in neurophysical uses of ultrasound, said discrepancies of 0.6 mm. had occurred between computed and observed values of the focal length in work on animals. Due to the temperature dependence of the focal distance and the inhomogeneity of brain tissue, the focal length must necessarily alter from that calculated in simple media.

A simple method of determining the focal point of a concave transducer has been mentioned by Gordon (1964). The transducer vibrated into water and was coupled to a commercial flaw detector, thus acting as transmitter and receiver. A small metal sphere, 3 mm. in diameter, was moved in two directions, one along the axis of the crystal and the other at right angles to this axis until the maximum echo was received by the transducer. This meant that a maximum of

the transmitted energy was reflected back and denoted the focal point. This method is claimed to measure focal lengths to within small fractions of a millimetre.

The focal intensity can be calculated from theory (section 2.6). The main lobe, which carries up to 84% of the incident energy, is the most important. It can be investigated by means of a piezo-electric probe or thermocouple. Due to the fact that these instruments can cause cavitation effects in the medium, because of the high intensities within the focal region, it is sometimes necessary to take measurements using lower transducer outputs and then extrapolate the graph of focal intensity against some parameter of the electrical input, such as the transducer driving voltage. Lele (1962a)has given an excellent survey of the physical problems associated with focal measurements and the production of lesions with ultrasound. The actual shape of lesions formed in living tissue has been found using subsequent histological examination to be ellipsoidal. This method means the sacrifice of a number of animals and an elegant method developed by Lele (1962b)has been used to study the effects of the various acoustic parameters on the size and shape of the lesions formed. The irradiation of strain-free methyl-methacrylate with focused ultrasound caused 'lesions' (due to structural changes) to be formed below the surface. This material becomes double refracting when strained and the strained areas are visualised as interference patterns if viewed between a polariser and analyser. This method was initially developed to study

reproducibility of 'lesions' and the effects on their size and shape due to change of the various acoustic parameters. A linear relationship was obtained between the log. of the volume and the log. of the pulse duration and, also, between the log. of the volume and the log. of the intensity. These results were obtained using three different frequencies. It is thus possible to get the desired size of lesion by a suitably chosen intensity and pulse duration. Also, the actual shape of the 'lesion' was investigated. The effect of pulse interval on the 'lesion' was of interest. It was found that if the pulses were separated by a time interval of less than 300 secs. the effects were cumulative. Above 300 secs. transient 'lesions' were obtained which disappeared before the next pulse was propagated. These transient 'lesions' were also obtained using threshold intensities. Lele concluded from this that the mechanism responsible for the 'lesions' was of a thermal origin. Reproducible and consistent results were obtained with this method.

6.6 Measurement of temperature

The usual method of measuring temperature changes involves using a calibrated thermocouple such as the developed by Fry and Fry. A thermocouple, because of its small size, possesses the advantage that it can be directly imbedded in the medium of interest. Fry and Fry (1953) have investigated the temperature rise in the spinal cords of rats with this method and Herrick (1953) the temperature rise in the femurs of dogs. This method is not practical in humans, and in diathermic applications the only indication of temperature

rise is provided by the patient himself.

Chemical methods relying on the use of thermosensitive substances (Ernst and Hoffman, 1953) could be employed with biological suspensions but do not appear to be of any importance.

6.7 Measurements of physical characteristics of the media

A number of the physical characteristics (e.g. density, viscosity etc.) can be measured by well-known methods and will not be described here. The only two which will be discussed in detail are the measurement of absorption coefficients and the velocity of sound.

a) Absorption coefficients

Fry and Dunn (1962) have described the principles behind the usual methods of determining the absorption coefficients of biological materials. This is to send the sound beam through different thicknesses of the specimen mounted at normal incidence to the beam. These are immersed in a simple medium, such as water, between a transmitter and receiver. A probe (e.g. piezo-electric) can be used to measure the amplitude of the received sound wave. By plotting the received voltage against the thickness of the specimen, a value for the amplitude absorption coefficient, α, can be found. Using a calibrated thermocouple (such as that developed by Fry and Fry) as detector leads to a value for the intensity amplitude coefficient, μ. Continuous wave methods can be used but pulse methods are more convenient and result in little heating of the medium. By using the reflected waves, it is possible to find the absorption coefficient. By using two thicknesses of

the specimen, both reflection and absorption coefficients can be found. If continuous wave or pulse methods are not possible at normal incidence, the beam can be made to intercept the material at an oblique angle. This method avoids any reaction on the transducer due to direct reflected energy and eliminates any energy in the transmitted beam due to multiple reflections in the tissue.

Schwann and Carstensen (1952) have used the arrangement shown in fig. 30 for the measurement of absorption coefficients in blood and this method employs the usual pulse method. The separation between the two transducers was kept constant and they were moved along a fixed axis. The blood was separated from the water by a thin membrane permeable to ultrasound. In fact, any reflections due to impedance differences were very small. The received and initial pulses were displayed on the oscilloscope. By means of an accurate decade attenuator, the loss in amplitude was determined. Knowing the value for the absorption in water to a high degree of accuracy, the absorption coefficient for blood was determined. This method was also used for measurements with solid tissue. The vessel was completely filled with water and the tissue was supported between two plastic windows. The transmission loss was found by comparing the output amplitude with and without the tissue being present.

The measurement of absorption coefficients in tissue presents more difficulties since the tissue has first to be excised from

the animal and then suitably supported in a simple medium for irradiation. This leads to changes in the physical properties of the tissue. A typical arrangement was that used by Dunn and Fry (1961) and shown in fig. 31. A special absorption chamber containing castor oil was used to eliminate reflections and the coupling medium was degassed saline solution. The detector was a calibrated thermocouple. By using two thicknesses of excised lung tissue, a value for the intensity amplitude coefficient, μ, was determined. Placing the thermocouple between the lung tissue and the source and then altering the path length yielded a value for the reflection coefficient by analysis of the standing wave system formed. For bone, the method used is similar, slices of the bone being placed between the transducer source and a suitable receiver (Kishimoto, 1958 and Bullen et al, 1963). Bullen et al (1963) used their data on transmission to calculate a value for the velocity of sound in bone.

b) Velocity of propagation

The first measurements on the velocity of sound in biological media to appear in the literature seem to be those of Ludwig (1950). His experimental arrangement, shown in fig. 32, was based on the original pulse techniques developed by Pellam and Galt (1946). Ludwig, however, used separate crystals as transmitter and receiver. X-cut quartz crystals were used. Triggered pulses were used to drive a radio frequency oscillator at the resonant frequency of the crystal. The pulse was propagated through the tissue, mounted

between the two crystals. The received pulse was then amplified and displayed on an oscilloscope. Since the speed of the oscilloscope was known, the time for the pulse to pass through the specimen was determined and hence the velocity was found. Anisotropy of velocity with fibre direction in samples of beef muscle was also demonstrated. Frequencies of 1.25 Mc/s and 2.5 Mc/s were used for the measurements.

Schwann and Carstensen (1952) also used their experimental arrangement (fig. 30) to measure the velocities of propagation in blood. The separation of the crystals in this case could be varied by means of a micrometer screw control. The wavelength of the sound was found by comparing the phase of the received signal with that obtained directly from the generator while the distance between the two crystals was altered. Knowing the wavelength and the frequency of the sound, the velocity was calculated. During the course of their work, knowledge of velocity dispersion, if present, was needed. The same form of apparatus was used (Carstensen, 1954). A barium titanate crystal driven at frequencies between 0.3 Mc/s and 10 Mc/s was used as transducer. The separation between the crystals was arranged for a minimum response between the input and output signals. The separation was then altered until the initial and transmitted signal were again out of phase. Knowing the wavelength, the velocity was found. An accuracy of one part in ten thousand is said to be possible with this method.

Goldman and Richards (1954) used an interferometer technique to measure velocities in biological liquids and tissues (fig. 33).

Two quartz crystals were used, one of them movable. The frequencies used were 1, 2, 4, 12 and 36 Mc/s. The input and received signals were mixed separately with the frequency generated by a common oscillator to produce two audio signals. These were then displayed as Lissajou's figures on an oscilloscope. The movable crystal was mounted on a micrometer screw and the wavelength was determined from the distances between the positions of phase coincidence observed on the oscilloscope. Knowing the frequency (to an accuracy of one part in twenty thousand) the velocity was found. The temperature was controlled within 0.1° C and the velocity was determined to within one part in a thousand. Tissue could be used with this apparatus but must necessarily be compressed as the path length was altered.

El'Piner (1964) has described a more recent method using an interferometer to measure velocities in aequeous solutions of molecules. It is based on the fact that when a sound wave is passed through a layer of material, maxima and minima of transmission occur depending on the thickness of the layer. A maximum occurs if the thickness is a whole number of half wavelengths and a minimum if it is an odd number of quarter wavelengths. In the arrangement (fig. 34), the layer of liquid was varied in thickness by adjusting the distance between two glass rods, 10.cm. long, to which the transmitting and receiving crystals were attached. The separation could be measured to within $\pm$ 5 mμ. By measurement of the number of maxima, n, for a particular change in thickness, d, it was

possible to determine the wavelength ($\lambda = \frac{2d}{n}$) of the sound. Knowing the frequency (in this case it was 1.4 Mc/s), the velocity of sound was found. This method has been used to study the variation of velocity in aequeous solutions of biopolymers which had been irradiated in the presence of dissolved oxygen or hydrogen, in order to determine degrees of hydration.

Hawley et al (1965) used standard pulse-echo techniques in their investigations with aequeous solutions of dextran. An absorption chamber was used in which the path length was varied by moving the quartz transducer relative to an acoustic reflector. The echo traces were displayed on an oscilloscope. From the oscillographs obtained of the exponential time-amplitude relations of the trace of the initial echo as the path length was varied, values for the velocity of sound (estimated accuracy $\pm$ 3%) and the absorption coefficient ($\pm$ 5 %) were calculated in the normal way.

Knowing the velocity, it is possible to calculate the real part of the acoustic impedance of the medium i.e. the characteristic impedance, ρc. The contribution due to the complex part involves consideration of absorption data. Lugwig (1950) found that the complex component of the impedance in tissues was negligible.

Most of the methods described for measurement of absorption coefficients and velocity apply only to liquids. Tissues, particularly those with irregular structures such as bone, present many difficulties and the published data on such measurements is very meagre.

6.8 Occurrence of cavitation and its measurement

The methods of determining the onset of cavitation are really only applicable to liquids or suspensions. At the present time, the occurrence of cavitation in intact tissue appears to be unlikely because of the high viscosity.

The visual method is sometimes employed. By passing light (or other electromagnetic radiation) through the solution, the onset of cavitation is determined by increased opacity of the medium due to the generation of bubbles and streamers. A change in volume of the solution also occurs and this, too, can be observed.

By analysis of the noise spectrum, it is fairly easy to determine the onset of cavitation and to differentiate between the stable and collapse forms. This method has been referred to by Hueter and Bolt (1955). A wide-band hydrophone is used to examine the spectrum. Although bubbles may be formed by degassing of the medium, no noise spectrum is obtained. With stable cavitation present, a line spectrum is observed containing the generated frequency and various harmonics of it. If collapse cavitation occurs, a spectra is obtained formed by harmonics and sub-harmonics of the generated frequency and these are superimposed on a white noise backgraound. Neppiras (1965) said that the sub-harmonics are due to non-linear radial motions of bubbles driven above their natural frequency and that they will have a strong sub-harmonic component if the generated frequency is an integral multiple of the natural frequency of vibration.

The impedance of a medium decreases drastically at the onset of cavitation due to bubble formation. By electrical measurements of the

input to the transducer, it is possible to determine the point at which the cavitation 'unloading' occurs since the power supplied to the transducer will alter considerably.

The other methods of interest are based on the sonochemical effects of collapse cavitation (they will not occur if stable cavitation only is present). The traditional method is the liberation of iodine from potassium iodide dissolved in the liquid. However, it is only qualitative. A recent method has been described by Neppiras (1965) using this chemical to give an idea of the intensity of cavitation. Potassium iodide with carbon tetrachloride and starch are dissolved in the solution. A measured quantity of sodium bi-sulphite is added. This delays the iodine reacting with the starch to give the well-known blue colour until the following reaction has been completed:-

$$2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6$$

When this is finished, the iodine will react with the starch and the blue colour will appear first where the cavitation effects are greatest. Knowing the quantity of sodium bisulphite added and the time before the blue colour appears will give an indication of the cavitation intensity.

Weissler and Hine (1962) have suggested another method to determine cavitation intensity. They used the liberation of chlorine from carbon tetrachloride. The chlorine formed reacted with an orthotolidine reagent to give a yellow colour, whose intensity was measured by means of a spectrophotometer. An accuracy of $\pm$ 10% has

been claimed for this method. The optical density of the solution is a direct function of the amount of chlorine present and it would appear that this is also linearly related to the cavitation intensity.

6.9 Other measurements

The field is not completely specified by measurement of the previous parameters. Until the mechanisms are completely explained, it is not possible to say which measurements are the essential ones. At present, as many as possible are needed in order to try and relate the observed effects to one or more parameters of the wave. The importance of such factors as the ambient pressure and temperature, pressure of dissolved gases, non-uniformity of the field, the intensity and the intensity gradient is not completely understood.

There are other measurements which would be useful, but at present there are no satisfactory techniques for making them. These are:-

i) the measurement of microstreaming effects and the calculation of the stresses that they exert on cell walls and biomacromolecules,

ii) more detailed knowledge of the physical properties of cells e.g. the elastic properties of the walls and viscosities of the liquids inside the cells,

and iii) the ability to make measurements in intact tissue and especially to detect cavitation (if it occurs).

Conclusion

Ultrasound acts on living materials by means of a number of different mechanisms. Heating of the medium occurs due to absorption of the ultrasonic energy. The viscous stresses due to acoustic streaming effects of the direct sound beam or, more important, microstreaming due to presence of resonant bubbles or inclusions cause stirring of cellular contents, alter cell wall permeabilities and even cause disruption of weak cells. Finally, collapse cavitation produces gross disruptive effects which are associated with the collapse phase of the bubbles. In intact tissue, although there is considerable evidence that, in addition to heating, mechanisms that are non-thermal in nature may operate under certain conditions, an adequate account of the basis for the observed effects has not yet been given.

Each of these mechanisms will depend for its mode of action on a different set of parameters describing the sound wave and the medium. At the moment, it is not certain which are the important parameters. The acoustic intensity distribution of the wave in the medium is of primary importance for any discussion of the mechanisms. Considering the heating effect, the frequency must be known since the absorption coefficient is a function of the frequency. For calculating the magnitude of viscous stresses, the velocity gradient needs to be known. For collapse cavitation the pressure amplitude is of great importance, determining the onset of the growth and subsequent collapse of bubbles but this also depends on the amount

and nature of any dissolved gases, the ambient pressure and the viscosity of the medium. In intact tissue, which parameter is the most useful is a matter for conjecture but it is useful to know (if possible) the value of the pressure amplitude.

In specifying conditions for any irradiation, it is desirable to know the following: the frequency of the wave, the output of the transducer and the intensity distribution of the acoustic energy within the medium. Also, measurable physical characteristics of the medium and any other parameters of the wave (e.g. pressure and velocity amplitudes) that can be determined should be known. Indication of any occurrence of cavitation is most important.

Measuring techniques for the following are available: the frequency, the output of the transducer, the intensity distribution (only in a simple medium), the rise in temperature and the occurrence of cavitation (the methods are restricted to consideration of solutions.)

At the moment, there are no satisfactory techniques for visualising the intensity distribution within intact tissue nor of detecting the onset of cavitation if, indeed, it takes place at all. Calculation of the stresses set up by microstreaming effects would be very helpful. Information about the physical properties of cells such as viscosity of cytoplasm and nucleus and elastic properties of the cell walls would be of the utmost importance.

In helping to explain the biological effects of ultrasound, the following lines of research would appear to be very useful. Ackerman's work on cellular resonances could lead to increased knowledge of the physical properties of cells. He has developed a suitable mathematical treatment to try and explain the observed maxima in cell destruction rates. Two simple cell models were proposed and, with a suitable choice of the physical constants, both led to values for the resonant frequencies that agreed with observed values. Development of more sophisticated cell models could be tested in this way.

Nyborg has tried to explain the streaming motions observed in the cytoplasm and nucleus of suitably suspended cells irradiated by ultrasound in terms of the classical acoustic theory developed to explain non-linear effects in simple Newtonian liquids. Although the compexity of cellular structures makes any sort of explanation extremely difficult, this method could prove very useful in explaining the effects of acoustic waves on cells.

In general, there would appear to be a need for a much more critical investigation of the basis for the mechanisms that appear to be non-thermal and non-cavitational in nature and the studying of these effects in situations where neither heating nor cavitation are present.

ACKNOWLEDGEMENTS

I would like to thank Dr. R.W.B. Stephens of Imperial College for his keen interest and advice in the preparation of this dissertation. I would like, also, to express my thanks to Dr. C.R. Hill of the Institute of Cancer Research who has provided me with much help and many suggestions in the course of the writing of this dissertation.

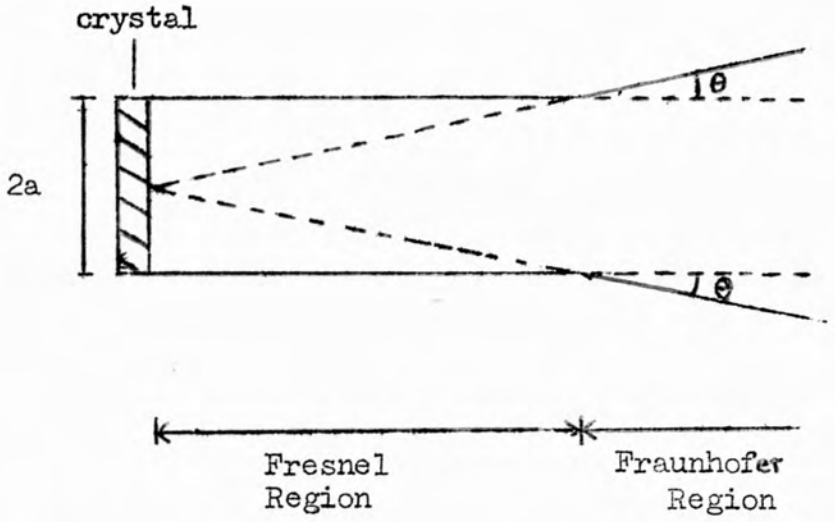

Fig. 1 Acoustic field pattern radiated by a simple source

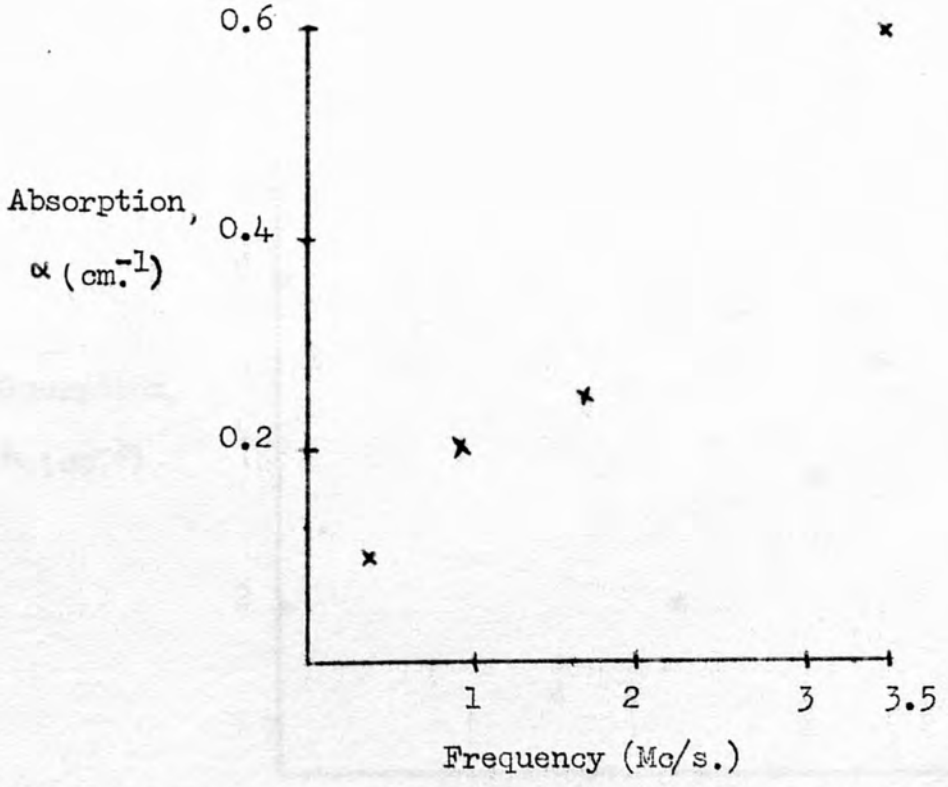

Fig.2 Absorption of ultrasound in cow muscle (Goldman and Hueter, 1956)

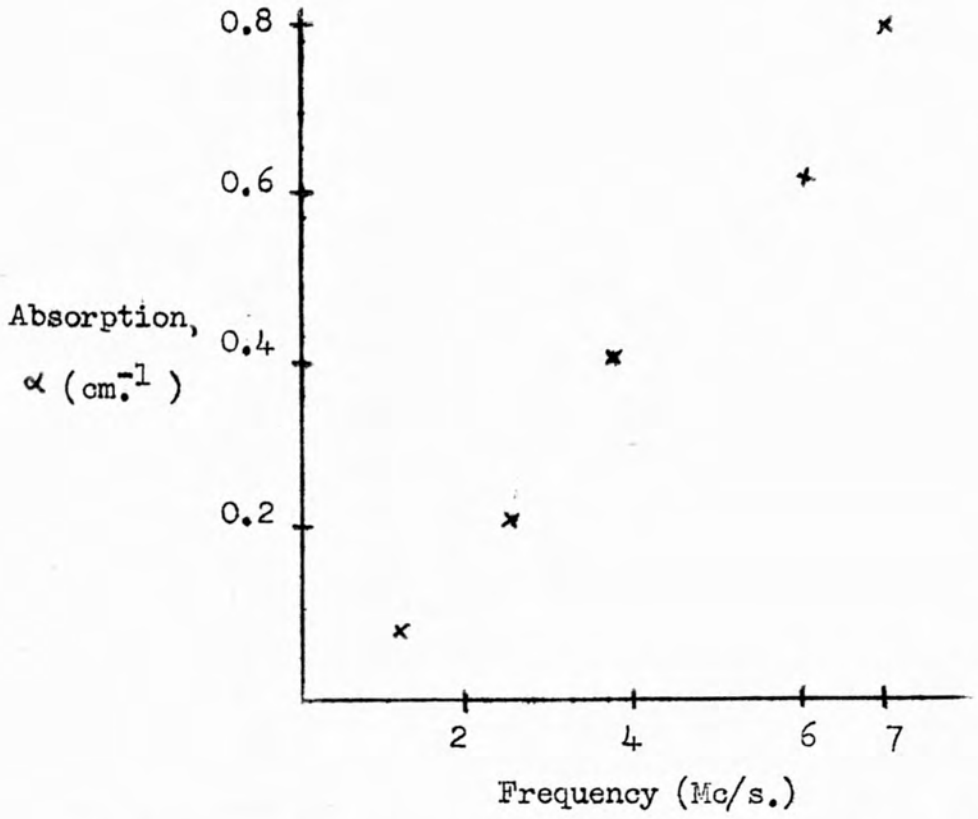

Fig.3 Absorption of ultrasound in pig fat (Goldman and Hueter, 1956)

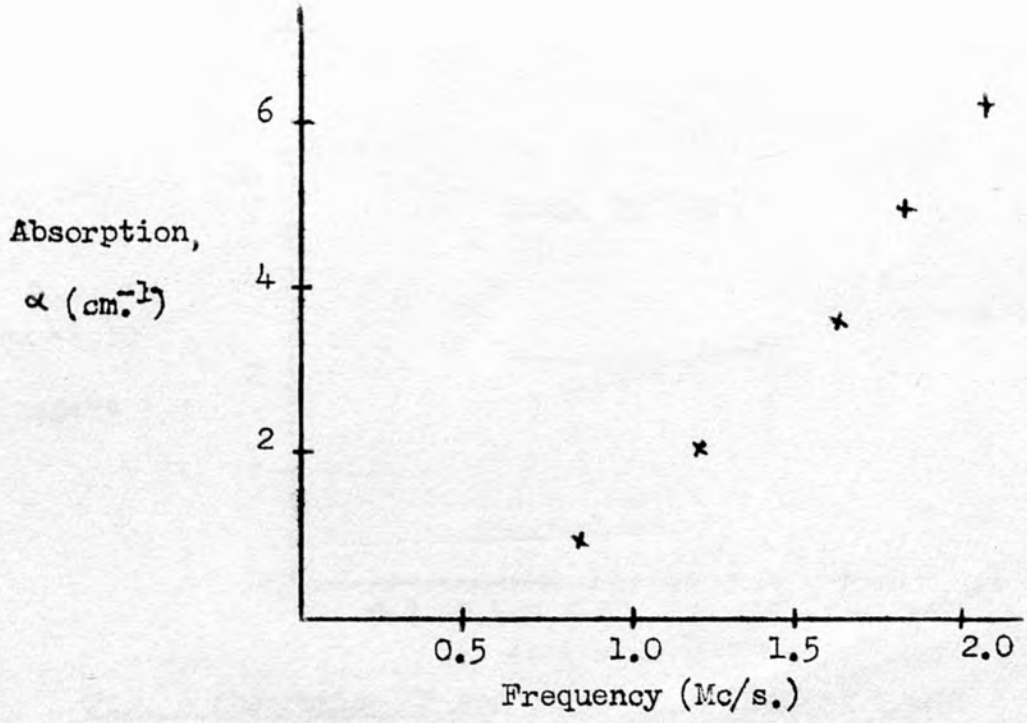

Fig. 4 Absorption of ultrasound in human skull bone (Goldman and Hueter, 1956)

(a) One relaxation mechanism

(b) Moderate number of mechanisms

(c) Very large number of mechanisms

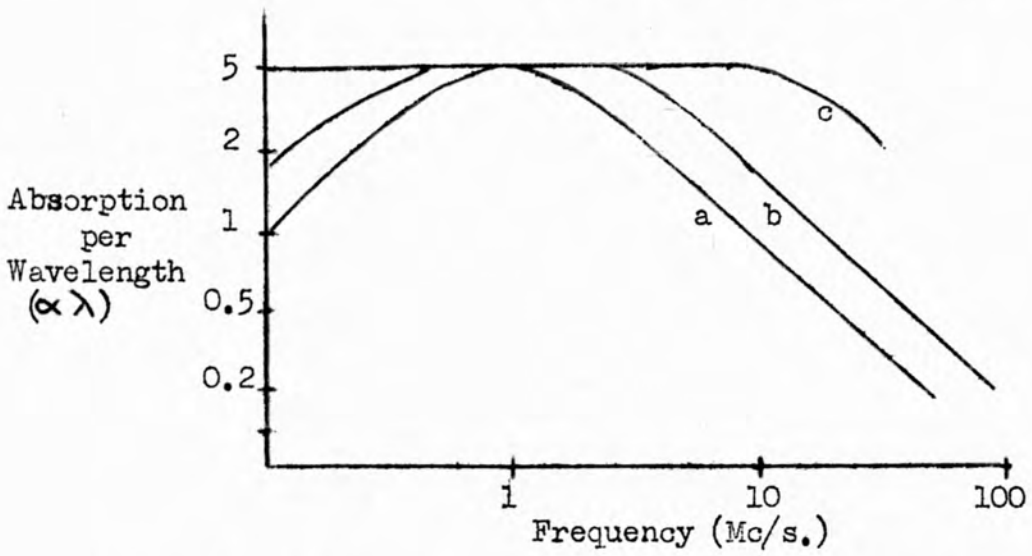

Fig. 5 Variation of absorption per wavelength with frequency when relaxation mechanisms operative (Schwann, 1959)

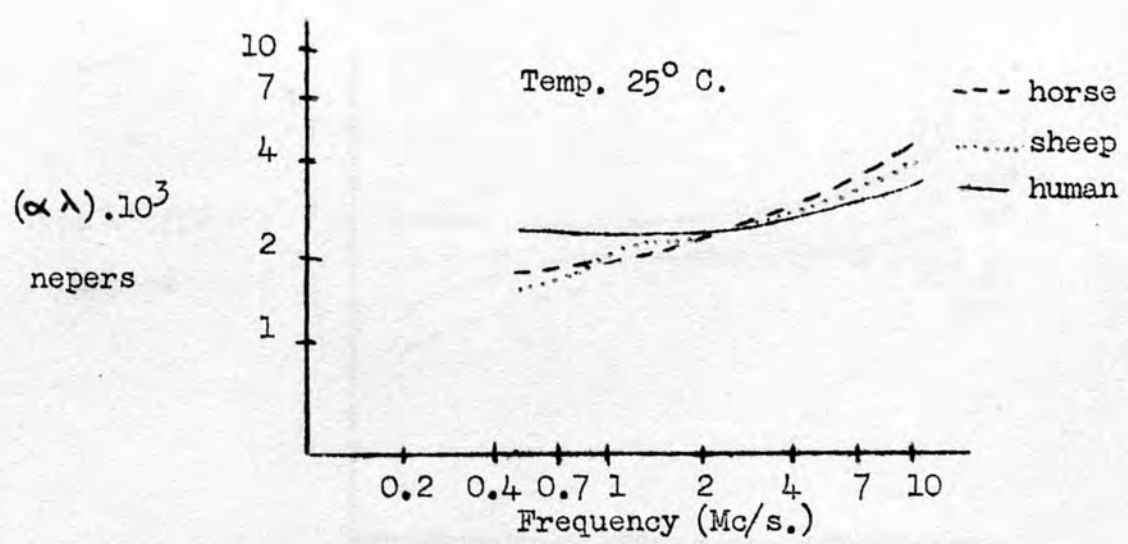

Fig. 6 Absorption of sound in solutions of various mammalian haemoglobins (Carstensen and Schwann, 1957)

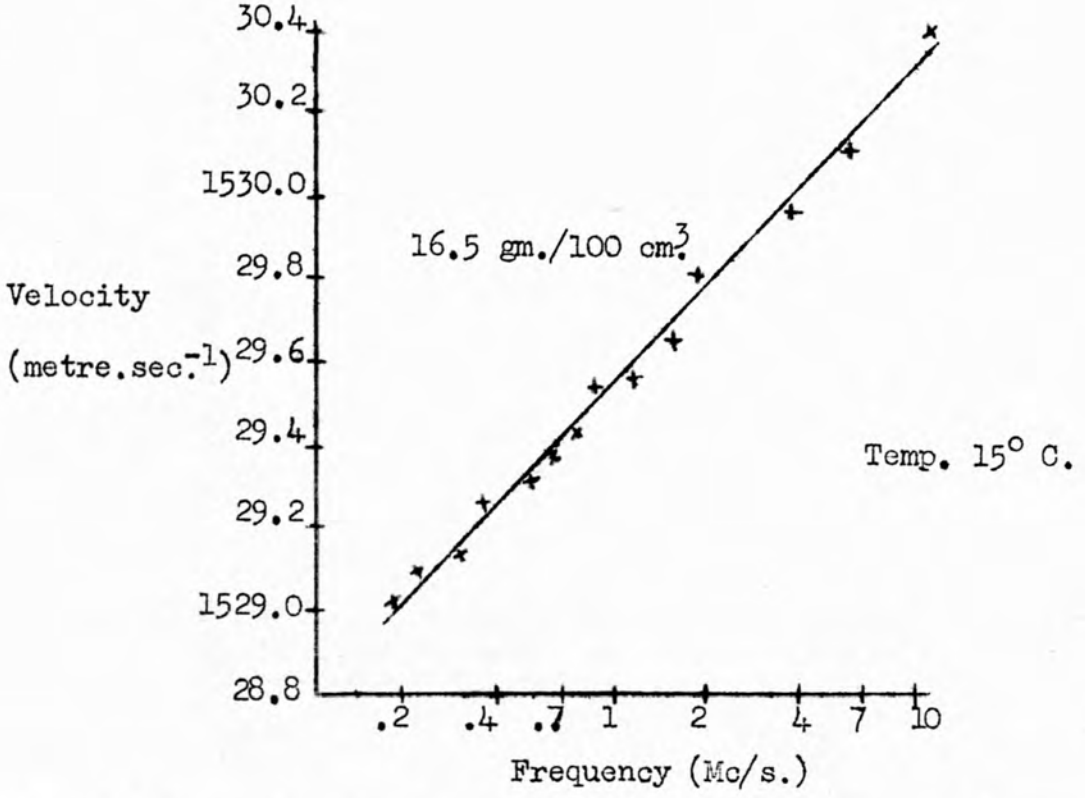

Fig. 7 Dispersion of velocity of sound in solutions of human haemoglobin (Carstensen and Schwann, 1957)

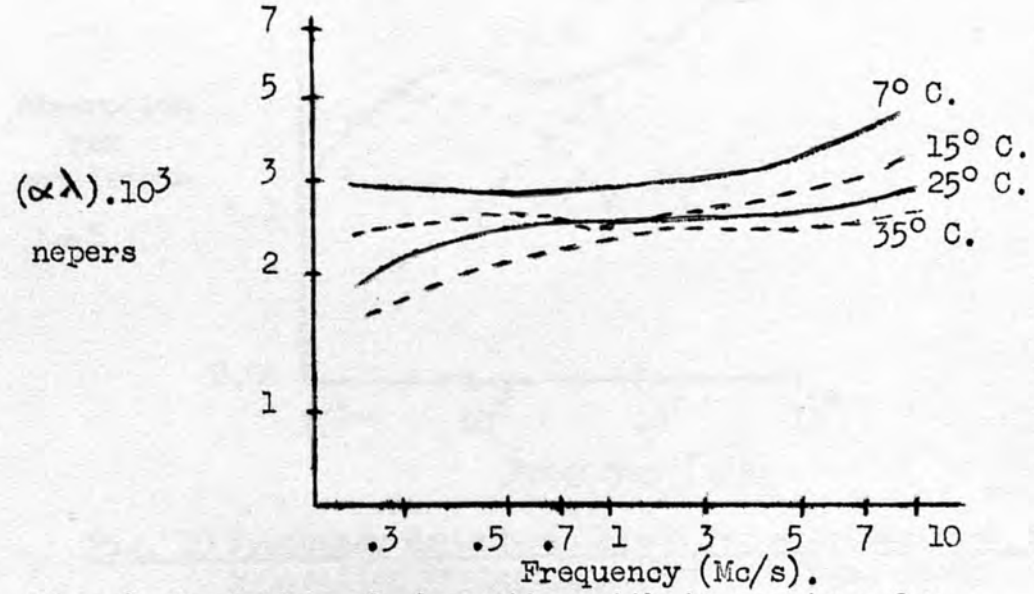

Fig. 8 Variation of absorption with temperature for human haemoglobin (Carstensen and Schwann, 1957)

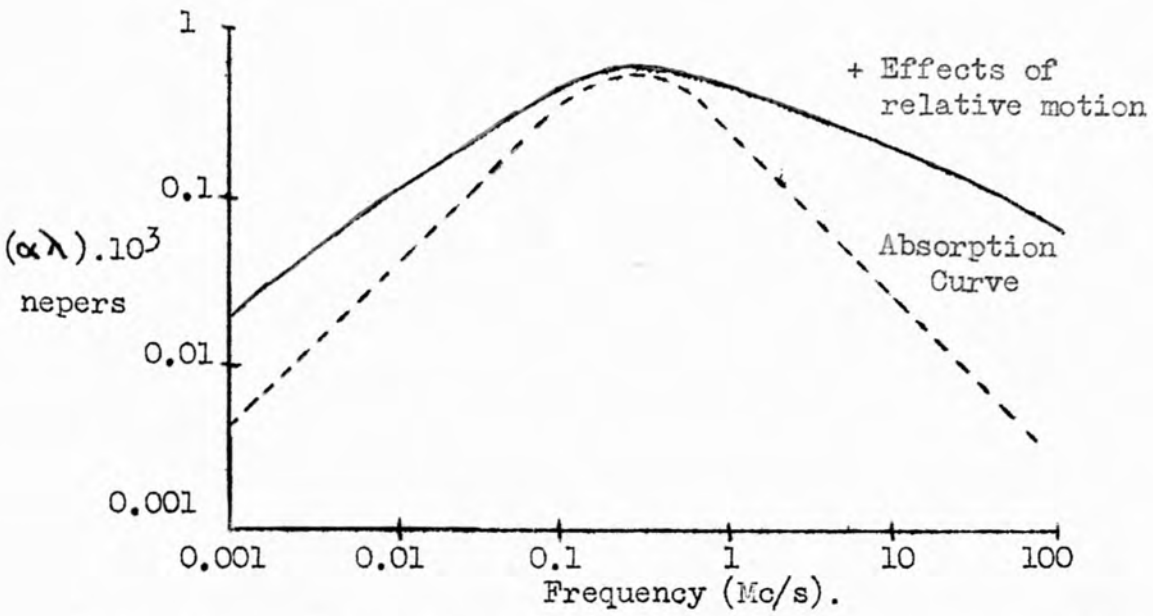

Fig. 9 Comparison of the frequency dependance of $\alpha\lambda$ due to relative motion and relaxation absorption (Carstensen and Schwann, 1957)

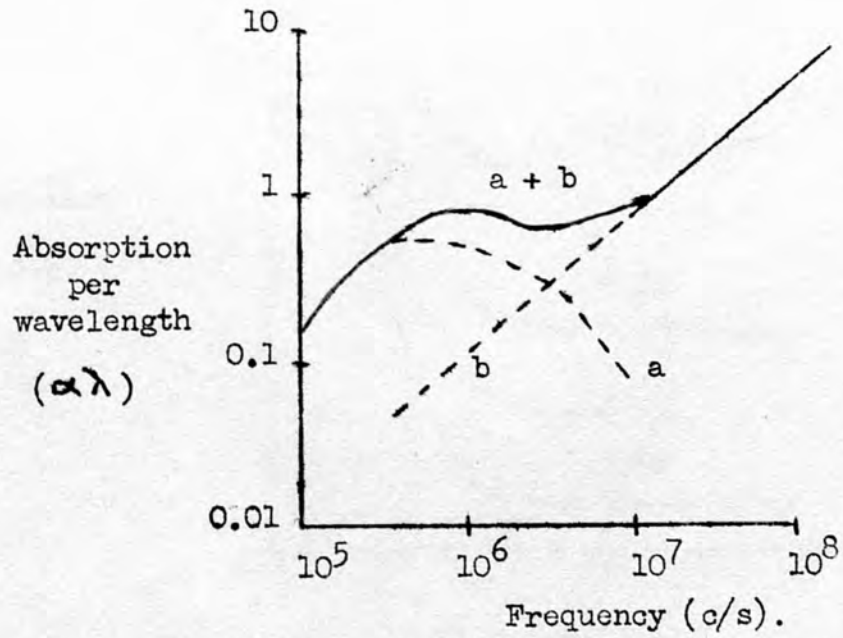

Fig. 10 Frequency dependance of $\alpha\lambda$ for a system with two relaxation frequencies (Fry and Dunn, 1962)

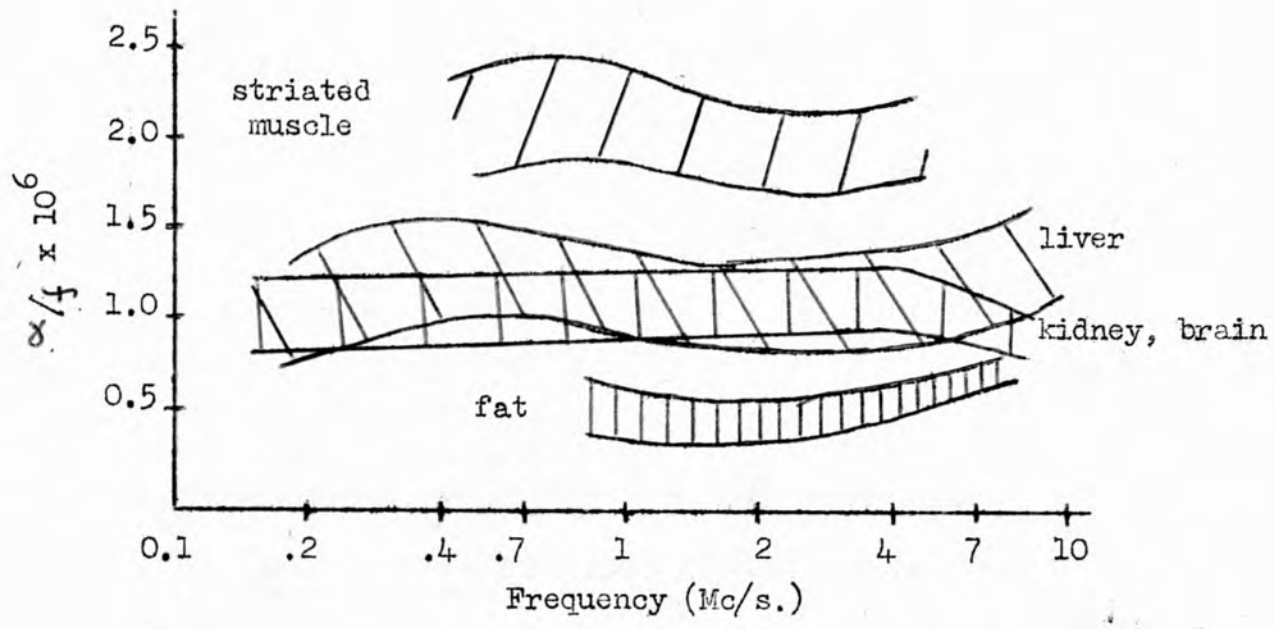

Fig. 11 Amplitude absorption coefficient (in db/cm.) per wavelength v. frequency for several mammalian tissues (Dunn, 1965)

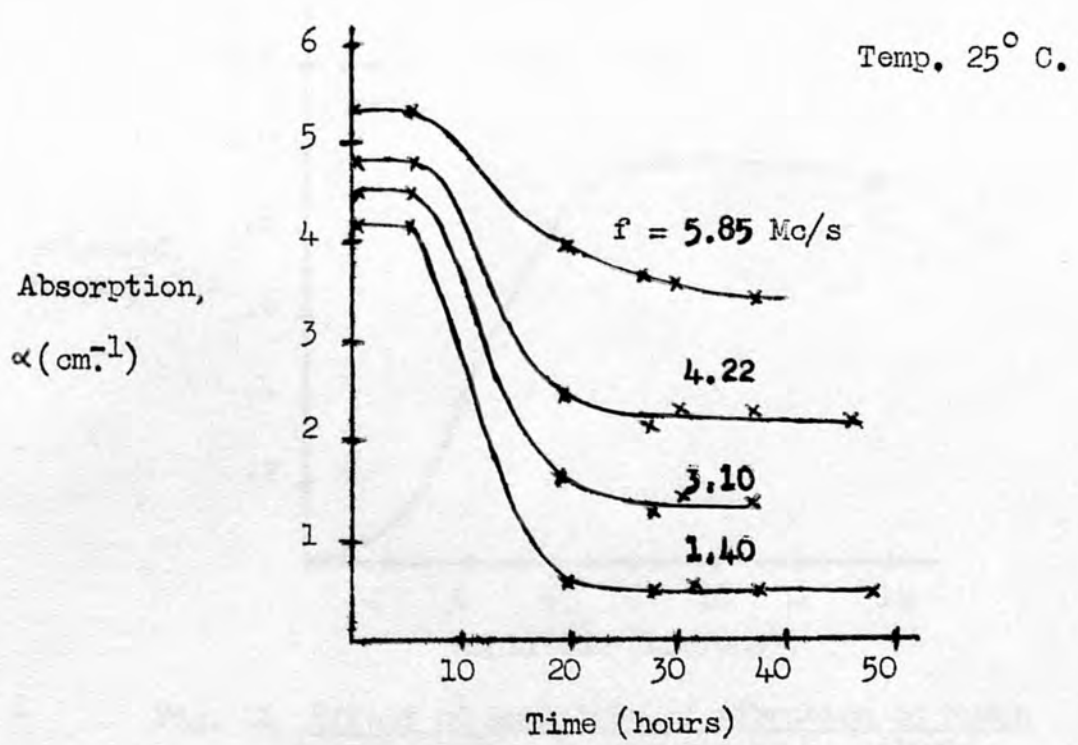

Fig. 12 Ultrasonic absorption coefficient in liver as a function of aging time (Dunn, 1965)

Arrows show path of motion of particles

Fig. 13 Ultrasonically induced motion in cells. (Dyer and Nyborg, 1960).

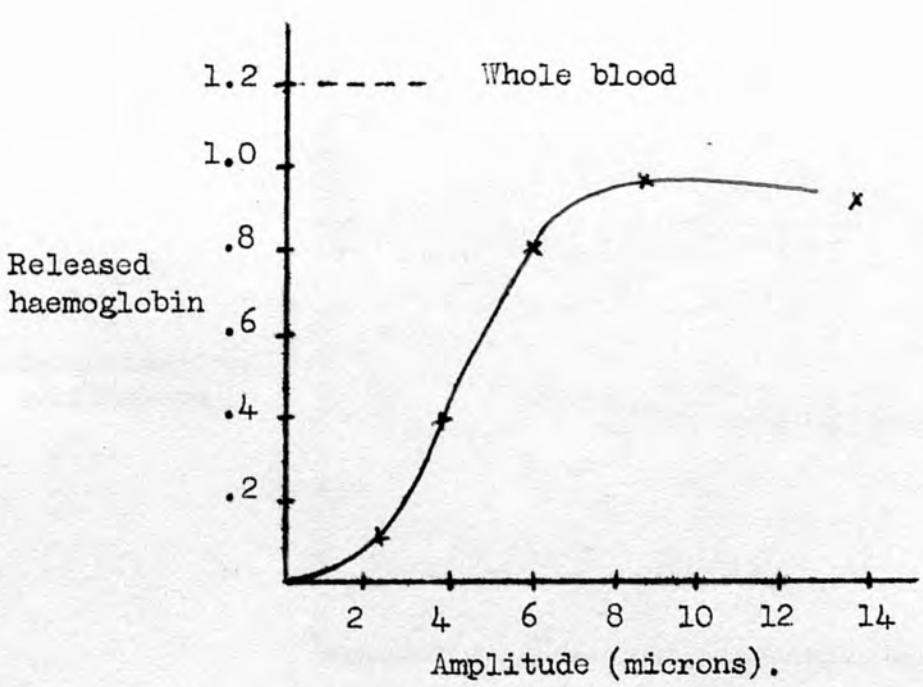

Fig. 14 Effect of amplitude of vibration on human erythrocytes as measured by haemolysis (Hughes and Nyborg, 1962).

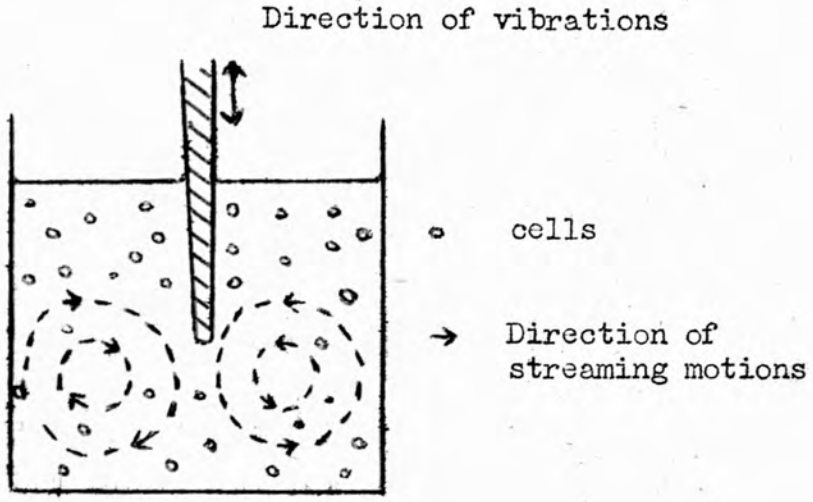

Fig. 15 Diagram of streaming motions due to the vibrating needle (Hughes and Nyborg, 1962).

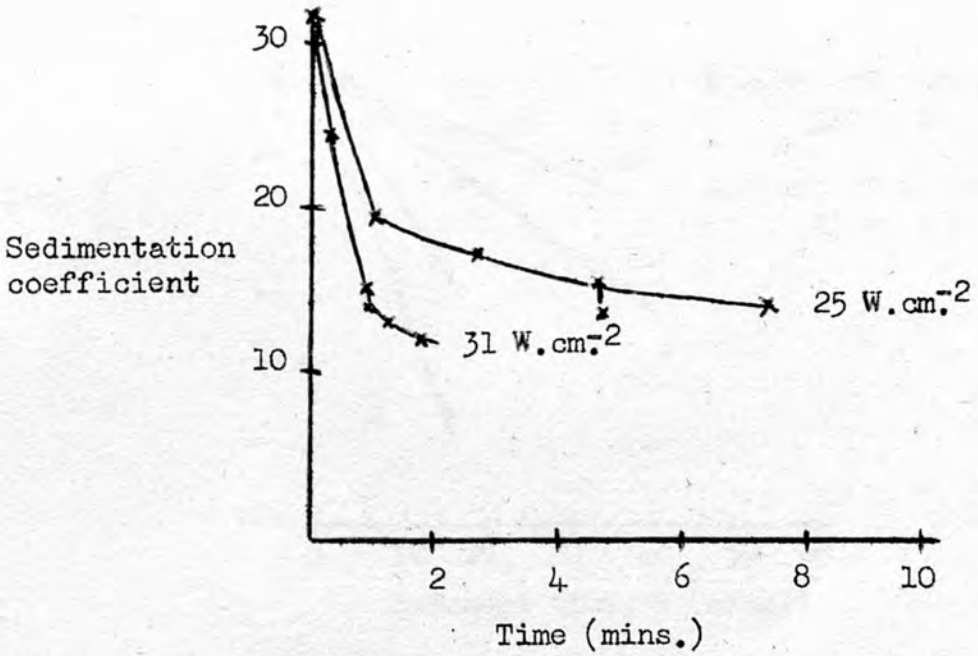

Fig. 16 Sedimentation coefficient of D.N.A. v. irradiation time (Hawley et al, 1963).

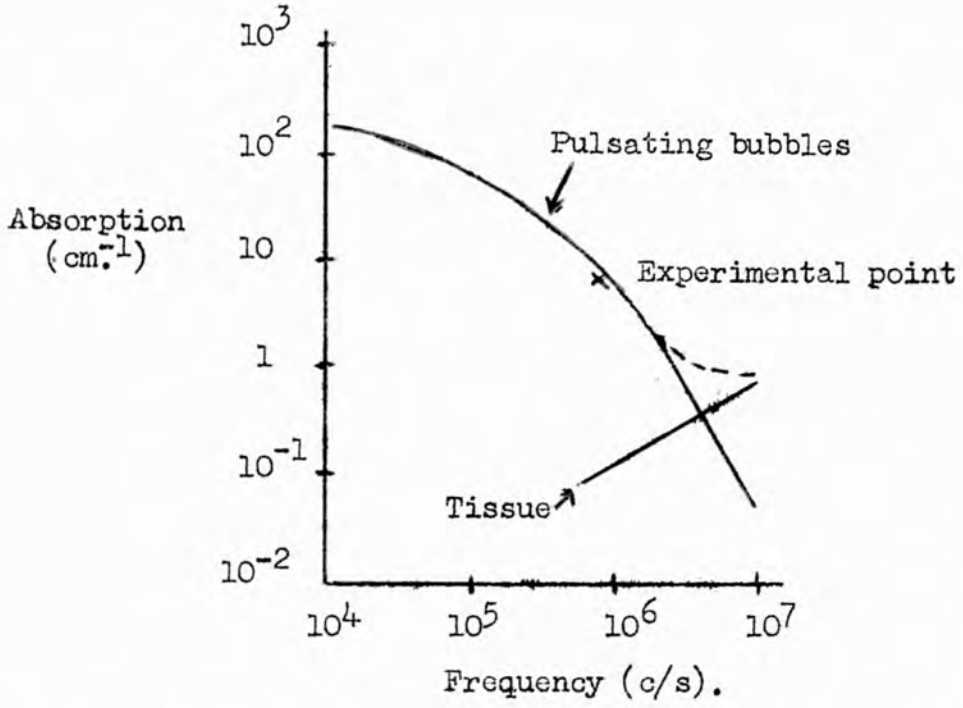

Fig. 17 Amplitude absorption coefficient per unit path length in lung v. frequency for bubble radius of 0.3 mm. (Dunn and Fry, 1961).

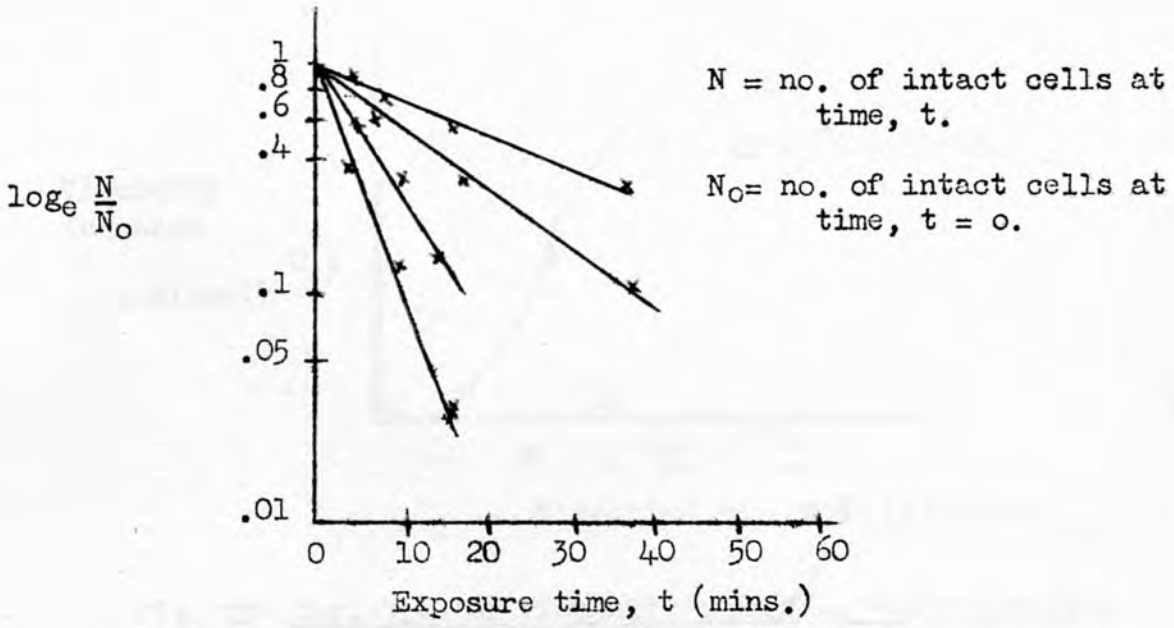

Fig. 18 Logarithmic curves of cell suspensions exposed to cavitation (Ackerman, 1962).

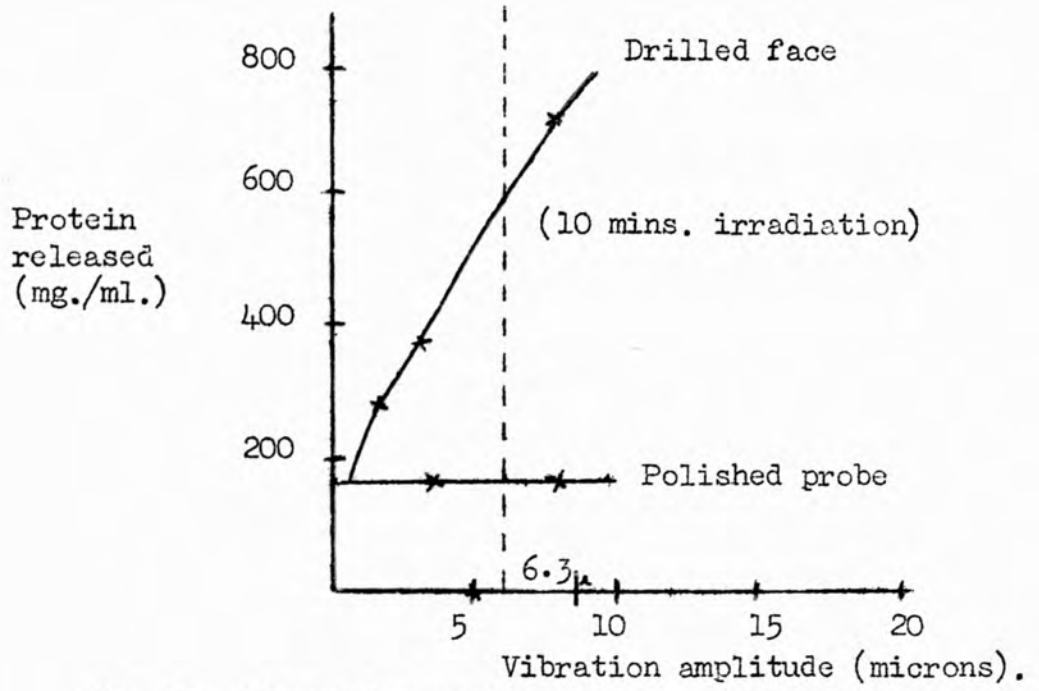

Fig. 19 Disruption of Escherichia coli by drilled and polished probe (Hughes and Nyborg, 1962)

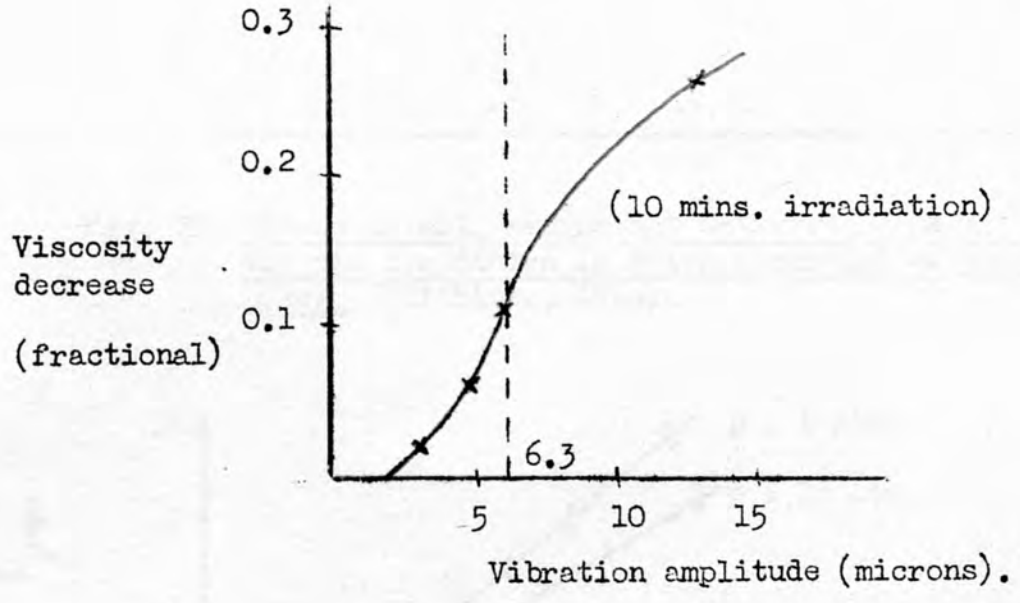

Fig. 20 Variation of viscosity of D.N.A. with vibration amplitude (Hughes and Nyborg, 1962)

Enzyme	Duration of irrad.(mins)	Gas saturating enzyme soln.	Mol.wt. of enzyme before/after irrad.	Change in mol.wt.(%)	Change in enzymatic activity(%)
Pepsin	0	Air	37,700	0	0
"	15	Air	32,700	-14	-38
"	0	Hydrogen	37,800	0	0
"	15	Hydrogen	41,000	8	0
"	30	Hydrogen	41,000	8	0
"	30	Argon	32,100	-13	30
"	30	Helium	37,000	0	0
Trypsin	0	Air	37,700	0	0
"	15	Air	32,700	-13	-75
"	30	Air	28,000	-24	-85
"	0	Hydrogen	37,000	0	0
"	30	Hydrogen	49,200	30	0
"	60	Hydrogen	49,200	30	0

Fig. 21 Change in mol. weight and activity of pepsin and trypsin irradiated in water saturated with various gases. (El'Piner, 1964).

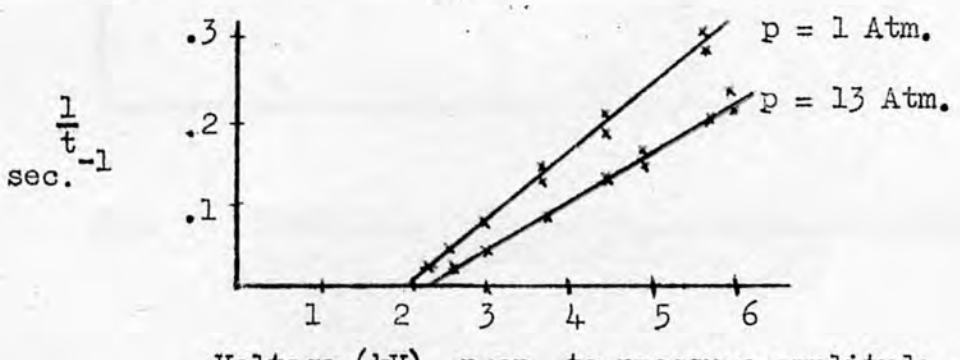

Fig. 22 Relation between crystal driving voltage and min. time for paralysis. (Fry et al, 1951).

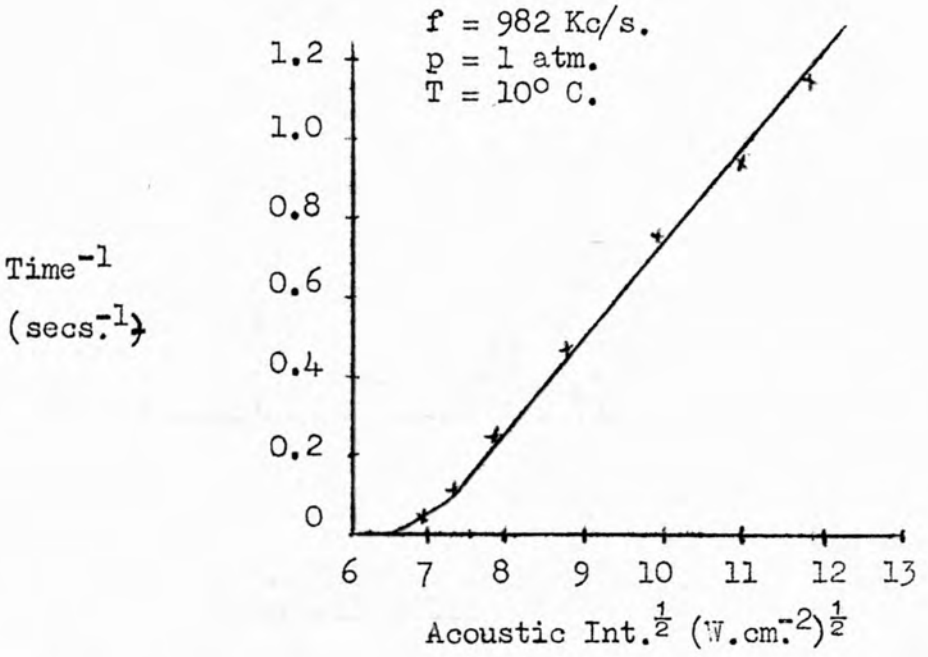

Fig. 23 Intensity$^{\frac{1}{2}}$ v. reciprocal min. time for paralysis (Fry, 1958).

Frequency (Mc/s.)	Relative Depth of penetration (cm.).		
	Bone	Muscle	Fat
0.5	0.15	5	40
1	0.04	2.5	15
2	0.01	1	4

Fig. 24 Depth of penetration in a number of different tissues (Schwann, 1960)

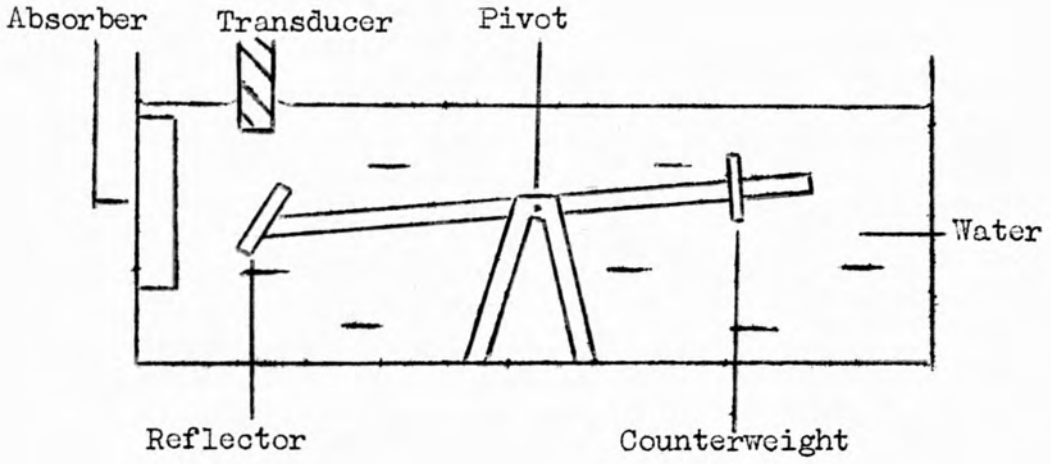

Fig. 25 Schematic diagram of a radiation-type sound intensity meter. (Frederick, 1965).

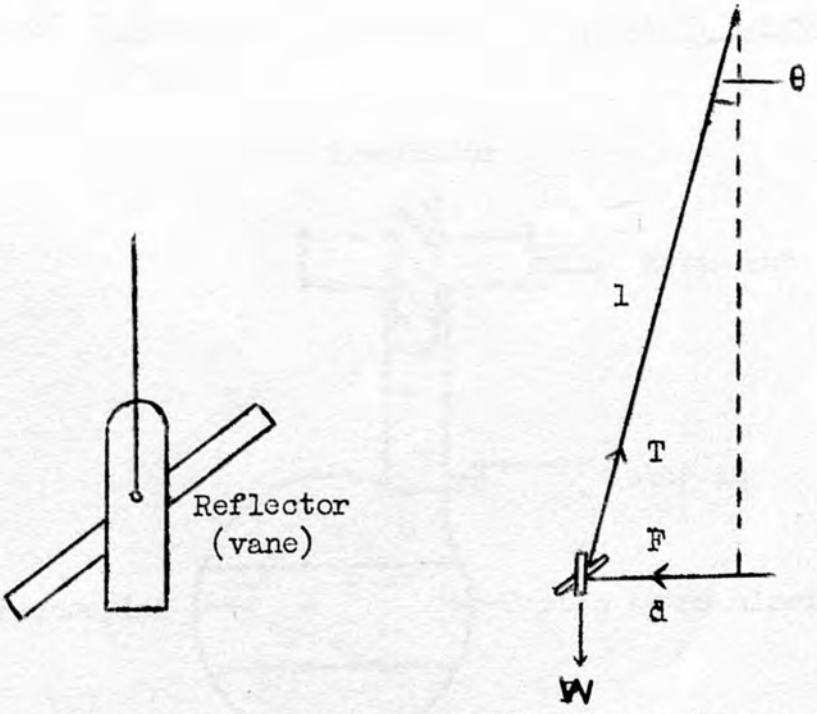

Fig. 26 Radiometer designed to measure small intensities (Wells et al, 1964).

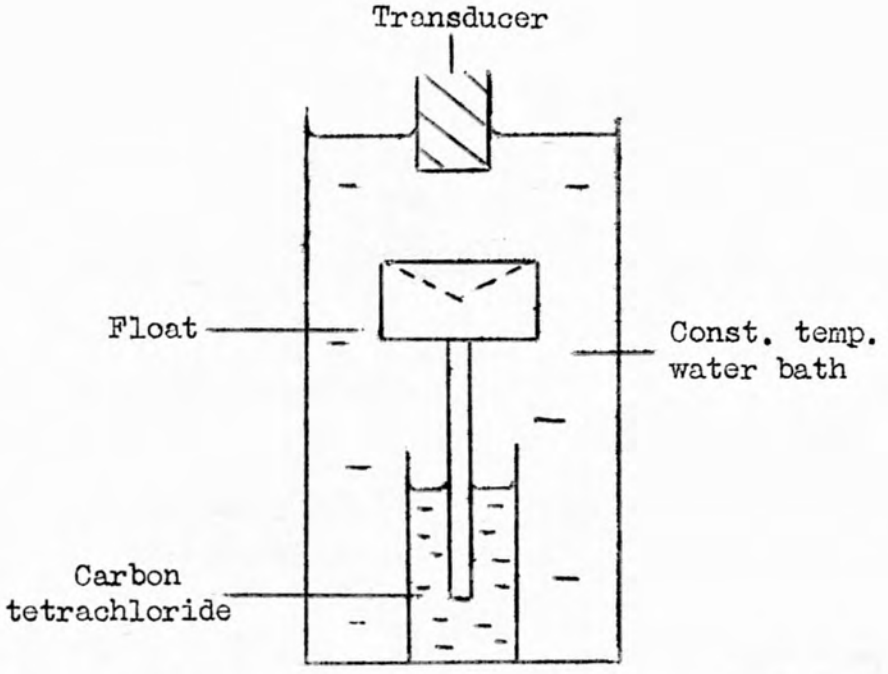

Fig. 27 Measurement of transducer output by depression of float (Kossof, 1962).

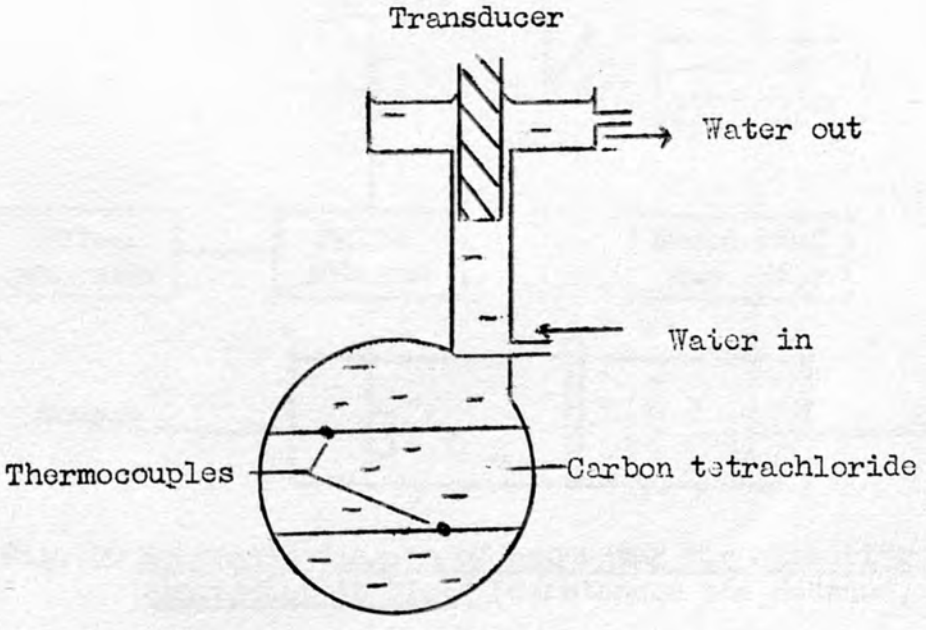

Fig. 28 Calorimeter (Wells et al, 1963)

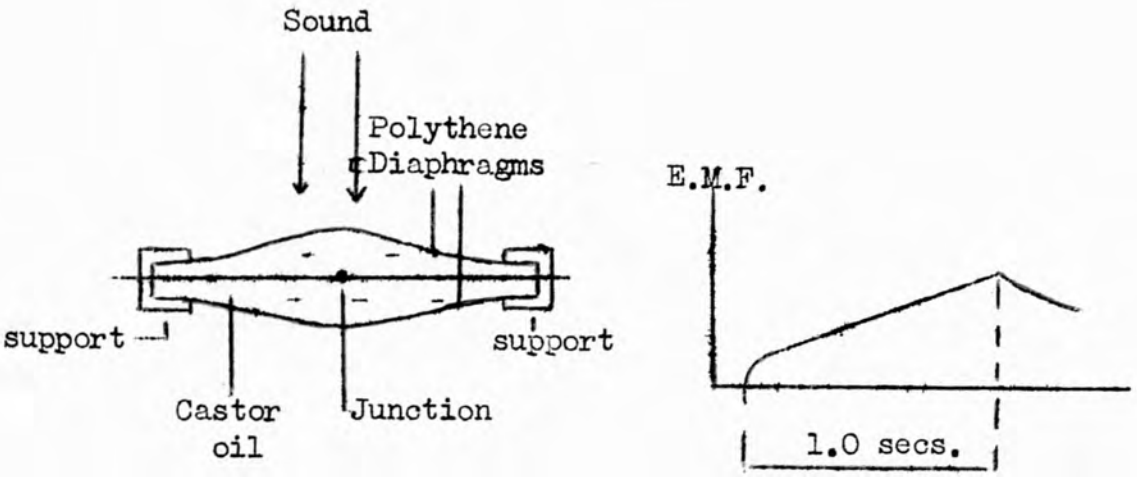

Fig. 29 Thermocouple and thermoelectric e.m.f. produced in response to a 1 sec. pulse. (Fry and Fry, 1954).

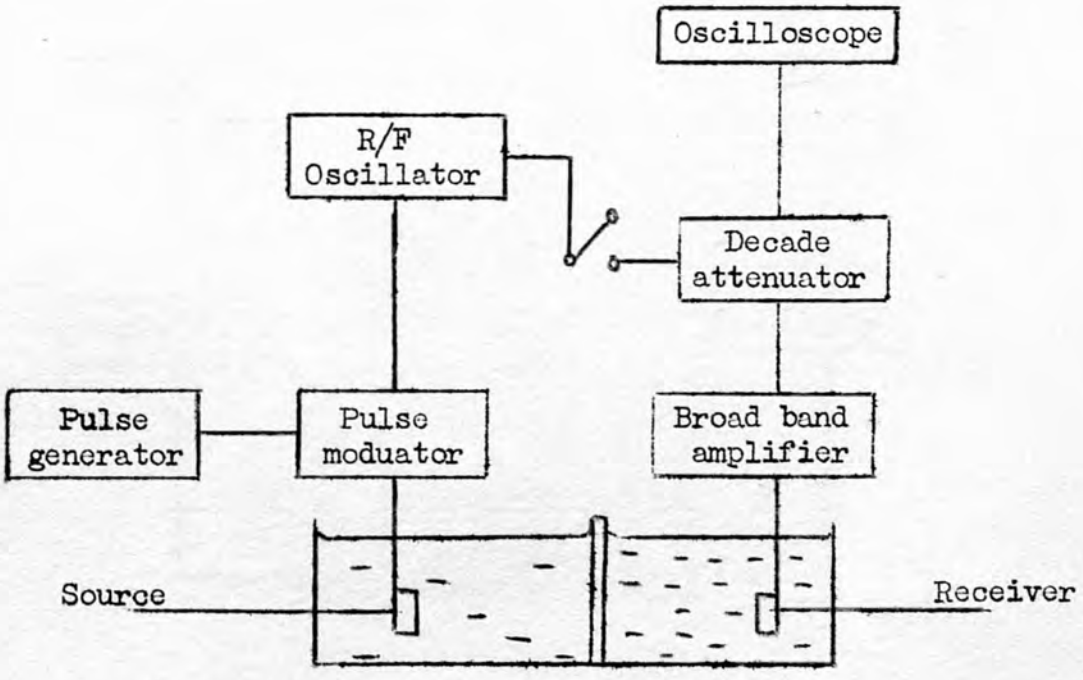

Fig. 30 Schematic diagram of apparatus for measuring absorption in blood (Carstensen and Schwann, 1952)

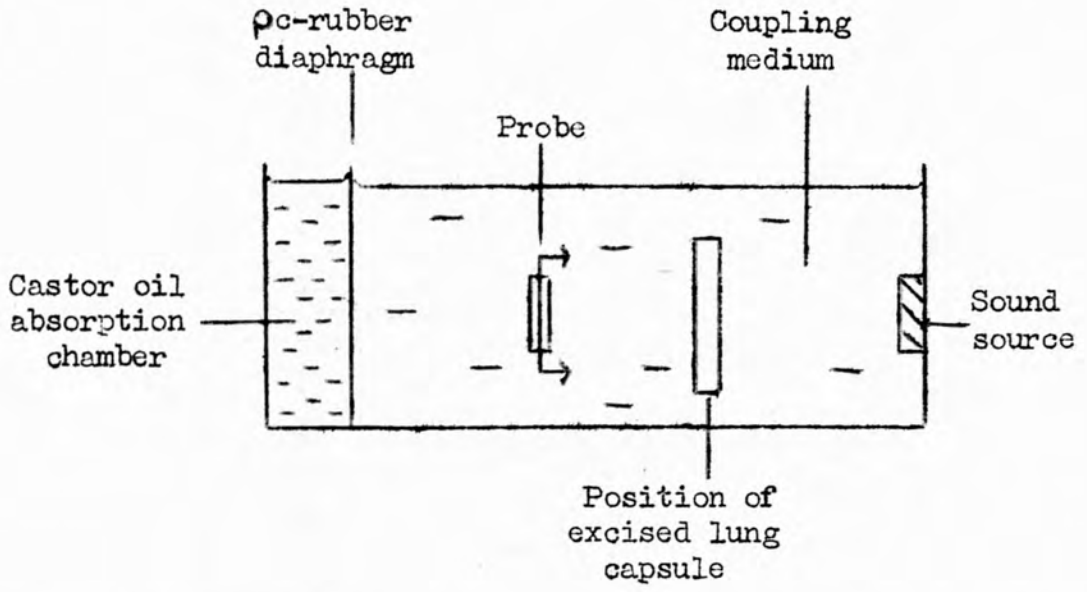

Fig. 31 Measurement of absorption in lung tissue (Fry and Dunn, 1961).

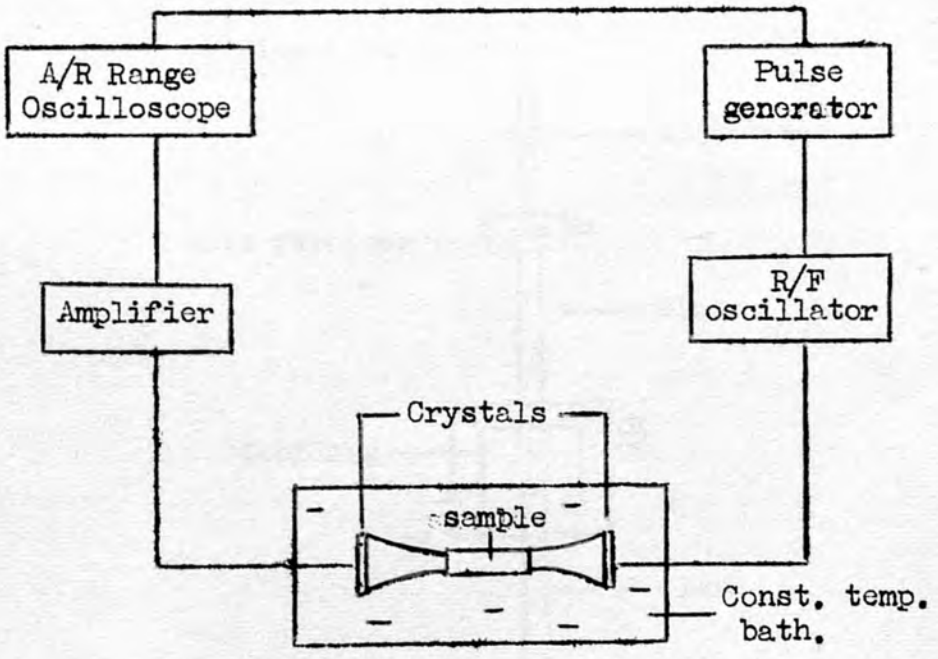

Fig. 32 Schematic diagram of apparatus for measuring velocity of sound through tissues (Ludwig, 1950)

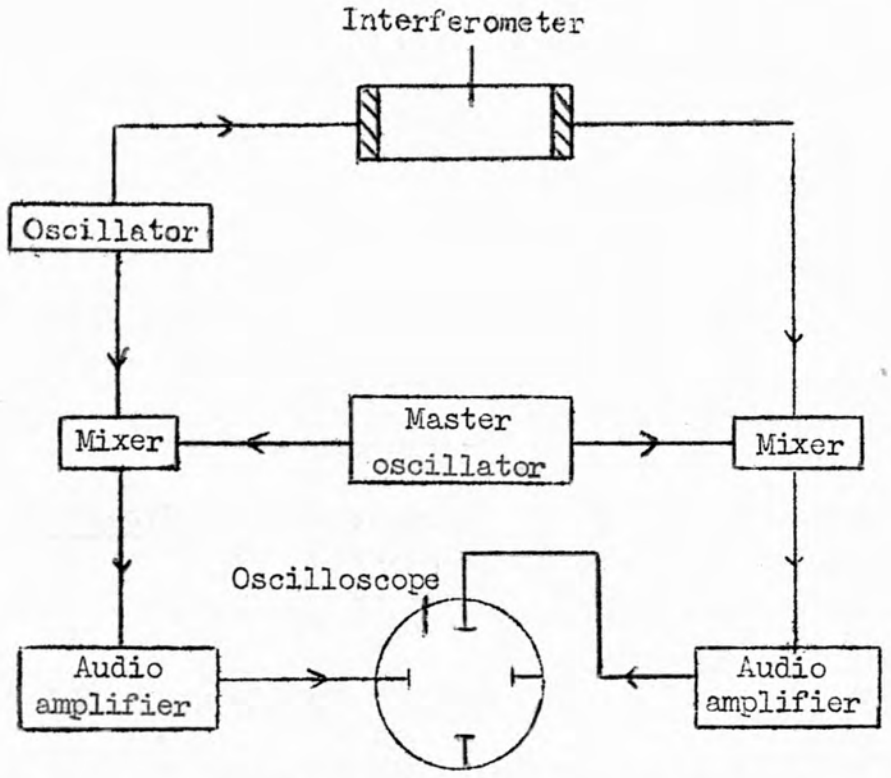

Fig. 33 Block diagram of interferometer measuring system (Goldman and Richards, 1954)

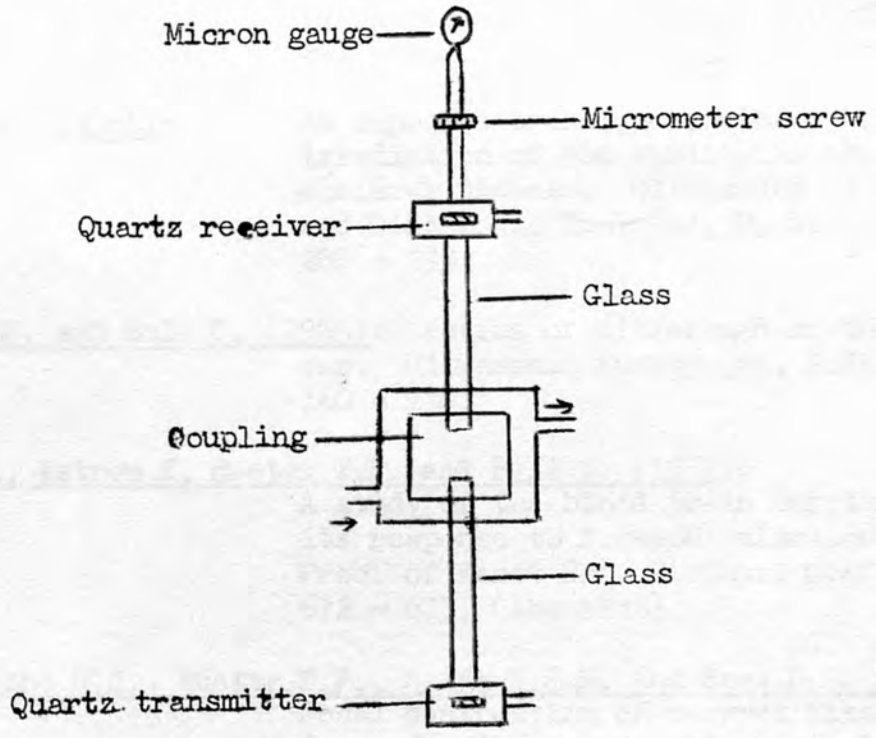

Fig. 34 Interferometer (El'Piner, 1964)

REFERENCES

Ackerman E. (1962): Biophysical Science (Prentice Hall), 204 - 245

Ackerman E. (1963): Rupture of canine erythrocytes in ultrasonic fields in which cavitation has been suppressed. J.A.S.A. 35, 809. (Abstract)

Aldes J.H. (1957): Indications and contraindications for ultrasonic therapy in medicine, Ultrasound in Biology and Medicine (ed. E. Kelly), 66 - 98.

Anderson T.F., Boggs S. and Winters B.C. (1948): The relative sensitivities of bacterial viruses to intense sonic vibrations. Science 108, 48.

Arkangel'skii M.E. (1967): Action of ultrasound on the processes of photographic development and fixing (Review). Soviet Physics, Acoustics, 12, 241 - 248.

Arslan M. (1964): An improved technique of the ultrasonic irradiation of the vestibular apparatus by Ménière's disease. Ultrasound As a Surgical and Diagnostic Tool (ed. D. Gordon), 288 - 293.

Arslan M. and Sala O. (1965): Action of ultrasound on the internal ear. Ultrasonic Energy (ed. E.Kelly), 160 - 178.

Bakay L, Astrom K, Hueter T.F. and Bell E. (1959): A study of the blood brain barrier through its response to focused ultrasound. Proc. of First Nat. Biophys. Conf. 672 - 673. (Abstract).

Ballantine H.J., Hueter T.F., Nauta W.J.H. and Sosa D.M. (1956): Focal destruction of nervous tissue by focused ultrasound: biophysical factors influencing its application. J. of Exp. Med. 104, 337 - 350.

Basauri L. and Lele P.P. (1962): A simple method for production of trackless focal lesions with focused ultrasound: statistical evaluation of effects of irradiation on the C.N.S. of the cat. J. Physiology, 160, 513-534.

Baum G. (1964): Opthalmic ultrasonography: Ultrasound As a Surgical and Diagnostic Tool (ed. D. Gordon), 176 - 184.

Bell E. (1957: Some changes in liver tissue which survives irradiation with ultrasound. Ultrasound in Biology and Medicine (ed. E. Kelly), 203 - 225.

Bennet G.S. (1952): A new method for the visualization and measurement of ultrasonic fields. J.A.S.A. 24, 470 - 474.

Blitz J. (1963): Fundamentals of Ultrasonics (Butterworth)

Bronskaya L.M. and El'Piner I.E. (1959): The stimulating action of ultrasonic waves on the germination of corn seeds. Soviet Physics, Acoustics, 5, 507 - 508.

Brown B. and Goodman J.E. (1965): High Intensity Ultrasonics: Industrial Applications (Iliffe)

Bullen M.A., Wells P.N.T., Freundlich H.A. and Angell James G. (1963): Physical survey of the ultrasonic treatment of Ménières disease. Ultrasonics, 1, 2-8.

Carstensen E.L., (1954): Measurement of dispersion of velocity of sound in liquids. J.A.S.A. 26, 858 - 861.

Carstensen E.L., Kam Li and Schwann H.P. (1953): Determination of the acoustic properties of blood and its components. J.A.S.A., 25, 286 - 289.

Carstensen E.L. and Schwann H.P. (1957): Acoustic properties of blood and its components. Ultrasound in Biology and Medicine (ed. E.Kelly), 1 - 14.

Carstensen E.L. and Schwann H.P. (1959a): Absorption of sound arising from the presence of intact cells in blood. J.A.S.A., 31, 185-189.

Carstensen E.L. and Schwann H.P. (1959b): Acoustic properties of haemoglobin solutions. J.A.S.A., 31, 305 - 311.

Chambers L.A. and Gaines N. (1932): Some effects of intense audible sound on living organisms and cells.J.Cellular and Comp. Physiology 1, 451 - 471.

Conger A.D. (1948): The cytogenic effect of sonic energy applied simultaneously with x-rays. Nat.Acad. of Sciences, 34, 470 - 474.

Curtis J.C. (1965): Action of ultrasound on the intact mouse liver. Ultrasonic Energy (ed. E.Kelly) 85 - 116.

Davies R. (1959): Observations on the use of ultrasound waves for the disruption of micro-organisms. Biochemica et Biophysica Acta, 33, 481 - 493.

Devin C. (1959): Survey of thermal, radiation and viscous damping of pulsating air bubbles in water. J.A.S.A., 31, 1654 - 1667.

Doty P., McGill B.B. and Rice S.A. (1958): The properties of sonic fragments of deoxyribonucleic acid. Proc.Nat.Acad. of Sciences. 44, 432 - 438.

Dunn F. (1957): Comments on mechanical mechanism of destructive effects of sound on tissue. J.A.S.A. 29, 395 - 396.

Dunn F. (1962): Temperature and amplitude dependance of acoustic absorption in tissue. J.A.S.A., 34, 1545 - 1547.

Dunn F. (1965): Ultrasonic absorption in biological materials. Ultrasonic Energy (ed. E.Kelly), 51 - 65.

Dunn F. and Bryer J.E. (1962): Generation and detection of ultra-high-frequency sound in liquids. J.A.S.A., 34, 775 - 778.

Dunn F and Fry W.J. (1961): Ultrasonic absorption and reflection by lung tissue. Phys. in Med. and Biol. 5, 401 - 410

Dyer H.J. and Nyborg W.L. (1960): Ultrasonically induced motion in cells and cell models. I.R.E. trans. on Med. Electronics ME-7, 163 - 165.

Dyer H.J. and Nyborg W.L. (1961): Characteristics of intracellular motion induced by ultrasound. Proc. Third Internat. Conf. on Med. Electronics, 445-449.

Edmonds P.D. (1962): Ultrasonic absorption of haemoglobin solutions. Biochemica et Biophysica Acta, 63, 216 - 219.

Elder I. (1963): Ultrasoundcardiography: Ultrasound as a surgical and diagnostic tool (ed. D. Gordon) 124 - 144.

Elder S.A. (1959): Cavitation microstreaming. J.A.S.A., 31, 54-64.

El'Piner I.E. (1961): Ultrasonic waves acting on biomacromolecules Soviet Physics, Acoustics 6, 399 - 406.

El'Piner I.E. (1964): Ultrasound: Physical, Chemical and Biological Effects. (Consultants Bureau).

Ernst P.J. and Hoffman C.W. (1952): New methods of ultrasonoscopy and ultrasonography. J.A.S.A., 24, 207-211.

Flynn H.G. (1964): Physics of Acoustic Cavitation in Liquids. Physical Acoustics (ed. W.P.Mason), 1B, 57-172.

Frederick J.R. (1965): Ultrasonic Engineering.(Wiley), 317-358.

Fry W.J. (1952): Mechanism of ultrasonic absorption in tissue. J.A.S.A., 24, 412 - 415.

Fry W.J. (1958): Intense Ultrasound in Investigations of the C.N.S. Advances in Biol. and Med. Physics (ed. by C.A. Tobias and J.H. Lawrence) VI, 282 - 348.

Fry W.J. and Dunn F (1962): Ultrasound: analysis and experimental methods in biological research. Physical Techniques in Biological Research (ed. W.L. Nastuk), IV, 261 - 394.

Fry W.J. and Fry R.B. (1953): Temperature changes produced in tissue during ultrasonic irradiation. J.A.S.A., 25, 6 - 11.

Fry W.J. and Fry R.B. (1954a): Determination of absolute sound levels and acoustic absorption coefficients by thermocouple probes - theory. J.A.S.A., 26, 294-310.

Fry W.J. and Fry R.B. (1954b): Determination of absolute sound levels and acoustic absorption coefficients by thermocouple probes - experiment. J.A.S.A., 26, 311-317.

Fry W.J., Wulff V.J., Tucker D.and Fry F.J. (1950): Physical factors involved in ultrasonically induced changes in living systems: I. Identification of non-temperature effects. J.A.S.A., 22, 267-876.

Fry W.J., Wulff V.J., Tucker D. and Fry F.J. (1951): Physical factors involved in ultrasonically induced changes in living systems: II. Amplitude duration relations and the effect of hydrostatic pressure for nerve tissue. J.A.S.A., 23, 364-368.

Goldman D.E. and Hueter T.F. (1956): Tabular data of the velocity and absorption of high-frequency sound in mammalian tissues. J.A.S.A., 28. 35-37.

Goldman D.E. and Hueter T.F. (1957): Errata: Tabular data of the velocity and absorption of high-frequency sound in mammalian tissues. J.A.S.A., 29, 655.

Goldman D.E. and Richards J.R. (1954): Measurement of high frequency sound in mammalian soft tissues. J.A.S.A., 26, 981-983.

Gordon A.G. (1963): The use of ultrasound in agriculture. Ultrasonics, 1, 70-77.

Gordon D. (1959): Echo-encephalography: ultrasonic rays in diagnostic radiology. Brit.Med. J. 1, 1500-1504.

Gordon D. (1963): Ultrasonic rays in medical diagnosis - a survey. Med. Electronics and Biol. Eng. 1, 51-59.

Gordon D. (1964): Ultrasound As a Surgical and Diagnostic Tool (Livingstone).

Graber P. (1953): Biological Actions of Ultrasonic Waves. Advances in Biological and Medical Physics (ed. by J.H. Lawrence and C.A. Tobias), III, 191-246.

Harvey E.N. (1930): Biological aspects of ultrasonic waves, a general survey. Biol. Bull. 59, 306-325.

Harvey E.N., Harvey E.B. and Loomis (1928): Further observations on the effects of high frequency sound waves on biological material. Biol. Bull. 55, 459-469.

Hawley S.A., Macleod R.M. and Dunn F. (1963): Degradation of D.N.A. by intense, non-cavitating ultrasound. J.A.S.A., 35, 1285-1287.

Hawley S.A., Kessler L.W. and Dunn F. (1965): Ultrasonic absorption in aequeous solutions of high-molecular-weight polysaccharides. J.A.S.A. 38, 521-523.

Herrick J.F. (1953): Temperatures produced in tissues by ultrasound: experimental studies using various techniques. J.A.S.A., 25, 12-16.

Horton J.P. and Horwood M.P. (1950): The relationship of acoustical energy to the lethal effects of ultrasonic vibrations. Science, 113, 693-695.

Howry D.H. (1957): Techniques used in ultrasonic visualization of soft tissues. Ultrasound in Biology and Medicine (ed. E. Kelly), 49-65.

Howry D.H. and Gordon D. (1964): Ultrasonic tomography. Ultrasound As a Surgical and Diagnostic Tool (ed. D. Gordon), 103-123.

Hueter T.F., Ballantine H.T. and Cotter W.C. (1956): Production of lesions in the C.N.S. with focused ultrasound: a study of dosage factors. J.A.S.A., 28, 192-201.

Hueter T.F. and Bolt R.M. (1955): Sonics (Wiley).

Hueter T.F., Morgan H. and Cohen M.S. (1953): Ultrasonic attenuation in biological suspensions. J.A.S.A., 25, 1200-1201.

Hughes B. (1964): Use of ultrasound in neurosurgery. Ultrasound As a Surgical and Diagnostic Tool (ed. D. Gordon), 252-271.

Hughes D.E. (1961): The disintegration of bacteria and other micro-organisms by the M.S.E.-Mullard ultrasonic disintegrator. J. of Biochem. and Microbiol. Tech. and Eng. 3, 405-433.

Hughes D.E. (1965): Biological effects of ultrasound. Science Journal 1, 7, 39-45.

Hughes D.E. and Nyborg W.L. (1962): Cell disruption by ultrasound. Science 138, 108-114.

Hughes D.E. and Rodgers A. (1960): Some experiments on the disintegration of bacteria by ultrasonics. Proc. Second Internat. Conf. on Med. Electronics. 397-400.

Jackson F.J. and Nyborg W.L. (1958): Small scale acoustic streaming near a locally excited membrane. J.A.S.A. 30 614-619.

Kinsler and Frey (1962): Fundamentals of Acoustics (Wiley).

Kishimoto T. (1958): Ultrasonic absorption in bones. Acustica 8, 179-180.

Kolb J. and Nyborg W.L. (1956): Small scale acoustic streaming in liquids. J.A.S.A. 28, 1237-1242.

Kossof G. (1962): Calibration of ultrasonic therapeutic equipment. Acustica 12, 84-90.

Kossof G. (1964): Design of the C.A.L. ultrasonic generator for the treatment of Ménières disease. I.E.E.E. Trans. on Ultrasonic Eng. SU-11, 2, 95-101.

Kossof G. (1965): Balance technique for the measurement of very low ultrasonic power outputs. J.A.S.A. 38, 880-881.

Kossof G., Garrett W.J. and Robinson D.E. (1965): An ultrasonic echoscope for visualising the pregnant uterus. Ultrasonic Energy (ed. E. Kelly), 365-376.

Lehmann J.F. (1953): The biophysical mode of action of biological and therapeutic ultrasonic reactions. J.A.S.A. 25, 17-25.

Lehmann J.F., Baldes E.J. and Krusen F.H. (1957): Destructive effects of high intensity ultrasound on plant tissues. Ultrasound in Biology and Medicine (ed. E.Kelly), 177-190.

Lele P.P. (1962a): A simple method for production of trackless focal lesions with focused ultrasound: physical factors. J.Physiology, 160, 494-512.

Lele P.P. (1962b): Irradiation of plastics with focused ultrasound: a simple method for evaluation of dosage factors for neurological applications. J.A.S.A. 34, 412-420.

Ludwig G.D. (1950): The velocity of sound through tissues and acoustic impedance of tissues. J.A.S.A. 22, 862-866.

Lynn J.G. Zwemer R.L. and Cwick A.J. (1942): The biological applications of focused ultrasonic waves. Science, 96, 119-120.

Meyers R., Fry F.J., Fry, W.J., Eggleton R.C. and Schultz D.F. (1960): Determination of topological human brain representations and modification of signs and symptoms of some neurologic disorders by the use of high level ultrasound. Neurology, 10, 271-277.

Mikhailov I.G. (1964): Methods of visualising absolute intensities of ultrasonic waves in liquids and solids. Ultrasonics, 2, 129-133.

Neppiras E.A. (1965): Measurements in liquids at medium and high ultrasonic intensities. Ultrasonics, 3, 9-17.

Neppiras E.A. and Hughes D.E. (1964): Some experiments on the disintegration of yeast by high intensity ultrasound. Biotech. and Bioeng. 6, 247-270.

Newell J.A. (1963a): A radiation pressure balance for the absolute measurement of ultrasonic power. Phys. in Med. and Biol. 8, 215-221.

Newell J.A. (1963b): Ultrasonics in medicine. Phys. in Med. and Biol. 8, 241-264.

Noltingk B.E. and Neppiras E.A. (1950): Cavitation produced by ultrasonics. Proc. Phys. Soc. 63B, 674-685.

Noltingk B.E. and Neppiras E.A. (1951): Cavitation produced by ultrasonics: theoretical conditions for the onset of cavitation. Proc. Phys. Soc. 64B, 1032-1038.

Noltingk B.E. and Terry N.B. (1958): Ultrasonic applications. Technical Aspects of Sound (ed. E.G.Richardson), II, 123-198.

Nyborg W.L. (1957): Physical aspects of high amplitude sound phenomena. Ultrasound in Biology and Medicine (ed. E.Kelly), 15-29.

Nyborg W.L. (1965): Acoustic Streaming. Physical Acoustics (ed. W.P. Mason), IIB, 265-331.

Nyborg W.L. and Dyer H.J. (1960): Ultrasonically induced motions in single plant cells. Proc. Second Internat. Conf. on Med. Electsonics, 391-396.

Nyborg W.L., Gould R.K. and Jackson F.J. (1959): Sonically induced microstreaming applied to a surface reaction. J.A.S.A. **31**, 706-711.

Patrick M.K. (1966): Ultrasound in physiotherapy. Ultrasonics, 4, 10-14.

Pell R.L. (1964): Ultrasound for routine clinical investigations. Ultrasonics, 2, 87-89.

Pellam J.R. and Galt J.K. (1946): Ultrasonic propagation in liquids: I Application of pulse technique to velocity and absorption measurements. J.Chem. Phys. 14. 608-614.

Pond, J.B. (1963): The use of High Intensity Mechanical Waves in Biological Research. M.Sc. dissertation, London.

Rozenberg L.D. (1955): Survey of methods used for the visualization of ultrasonic fields. Soviet Physics , Acoustics, 1, 105-116.

Schwann H.P. (1959): Absorption of ultrasound by tissues and biological matter. Proc. I.R.E. 47, 1959-1962.

Schwann H.P. (1960): Absorption and energy transfer of microwaves and ultrasound in tissues: Characteristics. Med. Phys. (ed. O.Glasser), 3, 1-7.

Schwann H.P. (1963): Ultrasonic rays in medical diagnosis - a survey (letter). Med. Electronics and Biol. Eng. 1, 563.

Schwann H.P. and Carstensen E.L. (1952): Ultrasonics aids diathermy experiments. Electronics, 25, 7, 216-220.

Selmann G.G. and Counce S.J. (1953): Abnormal embryonic development in drosophila induced by ultrasonic treatment. Nature 177, 503-504.

Smith A.and Schwann H.P. (1957): Ultrasonic absorption and velocity of sound in cell nuclei. Proc. 1st. Nat. Biophys. Conf. 671-672 (Abstract).

Stephens R.W.B. and Bate A.E. (1966): Acoustics and Vibrational Physics (Arnold).

Stumpf P.K. Green D.E. and Smith F.W. (1946): Ultrasonic disintegration as a method of extracting bacterial enzymes. J. Bacteriology, 51, 487-493.

Szent-Györgyi A. (1933): Chemical and biological effects of ultrasonic radiation. Nature 131, 278.

Takahashi W.N. and Christensen R.J. (1934): The virucidal action of high frequency sound radiation. Science 79, 415.

de Vlieger M., de Starke A., Molin C.E. and van der Ven C.(1963): Ultrasound for two-dimensional echo-encephalography. Ultrasonics 1, 148-151.

Wallace R.H., Bushnell R.J. and Newcomer E.H. (1948): The induction of cytogenic variations by ultrasonic waves. Science, 107, 577-578.

Webster E. (1963): Cavitation. Ultrasonics 1, 39-48.

Weissler A. (1960): Effects of ultrasonic irradiation on haemoglobin. J.A.S.A. 32, 1208-1212.

Weissler A. and Hine E.J. (1962): Variations of cavitation intensity in an ultrasonic generator. J.A.S.A., 34, 130-131.

Welkowicz W. (1955): Mechanical mechanism of destructive effects of sound on tissue. J.A.S.A. 27, 1142-1144.

Wells P.N.T., Bullen M.A., Follett D.M., Freundlich H.F. and James J.A. (1963): The dosimetry of small ultrasonic beams. Ultrasonics, 1, 106-110.

Wells P.N.T., Bullen M.A. and Freundlich H.F. (1964): Milliwatt ultrasonic dosimetry. Ultrasonics, 2, 124-128.

Wild J.J. and Reid J.M. (1957): Progress in the techniques of soft tissue examination by 15 Mc/s pulsed ultrasound. Ultrasound in Biology and Medicine, (ed. E. Kelly). 30-48.

Wilson W.L., Wiercinski F.J., Nyborg W.L. and Sichel F.J. (1962): Observations on marine eggs subjected to ultrasonic vibration. Biol. Bull. 123, 518.

Wilson W.L., Wiercinski F.J., Nyborg W.L., Schitzler R.M. and Sichel F.J. (1966): Deformation and motion produced in isolated living cells by localized ultrasonic vibration. J.A.S.A. 40, 1363-1370.

Woeber K. (1959): Combination of ultrasound and x-ray radiation in the treatment of cancer. Internat. J. Phys. Med. 4, 1, 10-19.

Woeber K. (1965): The effect of ultrasound in the treatment of cancer. Ultrasonic Energy (ed. E. Kelly), 137-149.

Wolfson R.J. (1965): Ultrasonic surgery for various types of vertigo. Ultrasonics, 3, 147-148.

Wood R.W. and Loomis A.L. (1927): The physical and biological effects of high frequency sound waves of great intensity. Phil.Mag. and J.Science, 4, 417-436.

Wu H and Liu S. (1931): Coagulation of egg albumin by supersonic waves. Proc.Soc.Exptl.Biol.Med., 28, 782.

Young R.R. and Henneman E. (1961): Functional effects of focused ultrasound on mammalian nerves. Science, 134, 1521-1522.

GLOSSARY

Asterias: a member of the starfish family.

Bacteria: unicellular organisms having no nucleus.

Chloroplasts: minute bodies found in plant cells. They contain chlorophylls.

Chromosomes: thread-like structures found in the nucleus; they carry the genes.

Cytoplasm: substance within the cell exclusive of the nucleus.

Deoxyribonucleic acid (abbrev. D.N.A.): a nucleic acid. Found only in the nucleus of the cell.

Elodea: an underwater plant.

Enzymes: catalysts produced in living organisms. All known enzymes are proteins.

Erythrocytes: red blood cells.

Escherichia coli (abbrev. E. coli): bacterium found in human intestine.

Haemoglobin: red, respiratory pigment in blood cells. Mol. wt. ~68,000.

Haemolysis: the lysis or solution of red blood corpuscles.

Mitochondria: bodies found in cytoplasm that are responsible for nearly all energy production.

Mutations: the spontaneous production of new genetic traits in organisms.

Nucleolus: dense rounded mass in the cell nucleus.

Nucleotides: basic units from which the nucleic acids are built.

Nucleus: complex spheroidal mass essential to life of most cells.

Paramecium caudatum: a unicellular animal found in fresh water. Length up to 3 mm.

Pepsin: enzyme found in human stomach.

Plasma: the fluid portion of blood. It contains soluble proteins and inorganic salts.

Protein: high molecular weight compounds found in cells consisting of aggregates of amino acids.

Spisula: a bivalve mollusc.

Stromata: transparent filmy framework of red blood corpuscles.

Tetrahymena pyriformis: a unicellular animal found in water, similar to Paramecium caudatum.

Tradescantia paludosa: a tropical and sub-tropical herb.

Trypsin: enzyme found in the pancreatic juice.

Tumour: a growth formed by abnormally dividing cells. Can be malignant or non-malignant.

Virus: composed of protein and nucleic acid. Recognised by its toxic effect on cells.