SESSION 1:1 ULTRASONIC ABSORPTION BY BIOMACROMOLECULES

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For the purpose of this workshop, it is important to treat the ultrasonic propagation properties of solutions of biological polymers because such investigations help provide explanations to the strange acoustical behavior in tissues. Detailed knowledge of the latter is essential for the intelligent employment of ultrasonic energy in medical practice and for assuring that the full potential of this form of energy will be realized in the health care field. It is the intent of this paper to review the macromolecular studies dealing with the physical and chemical mechanisms contributing to the absorption processes.

The following is a very brief treatment of one-dimensional acoustic wave theory in fluid media. As a plane, progressive acoustic wave propagates through a lossy medium, the pressure amplitude decays exponentially as defined by equation (1)

$$P(x) = P_o e^{-\alpha x} \qquad (1)$$

where,

P_o and $P(x)$ are the pressure amplitudes at the origin and at a distance x from the source, respectively, and α is the pressure amplitude absorption coefficient. The frequency-free classical absorption coefficient, as derived for fluids by Stokes (1845) and Kirchhoff (1868), is

$$\frac{\alpha}{f^2} = \frac{2\pi^2}{C_o^3}\left[\frac{4}{3}\eta_s + \eta_v + \frac{\gamma - 1}{C_p}\kappa\right] \qquad (2)$$

where,

ρ_o is the undisturbed density, c_o is the low-frequency speed of sound, η_s and η_v are the shear and volume viscosity coefficients, respectively, γ the ratio of specific heats, C_p is the heat capacity at constant pressure and κ is the thermal conductivity. The third term within the brackets is typically negligible compared with the viscous terms for aqueous solutions. Liquids in general, and biological media in particular, do not often exhibit such behavior and it is convenient to consider that relaxation processes are involved. Since the observed ultrasonic absorption in biological media is generally three to four orders of magnitude greater than that predicted by classical absorption mechanisms such relaxation processes are usually invoked to describe the "excess' absorption. A single relaxation process is one in which the propagation of an acoustic wave perturbs the system between only two energy states and the frequency-free absorption is represented by

$$\frac{\alpha}{f^2} = \sum_n \frac{A_n}{1 + \omega^2 \tau_n^2} + B \qquad (3)$$

where the first term represents the single relaxation process when n = 1 and B represents absorption due to classical mechanisms. However, since biological systems possess many energy states, it is necessary to consider many relaxation processes occurring and to sum over all n, as described in equation (3). Whether or not these processes are discrete or continuous is, at present, extremely difficult to determine.

the wave out of phase results in an absorption because of a structural relaxation process.

Figure 2, prepared by Hueter (1958), shows the absorption coefficient of several biological materials vs frequency on a log-log plot. The slope of the curve is the exponent of frequency upon which the absorption coefficient depends. This figure depicts materials of increasing biological complexity and illustrates correspondingly more complicated absorption behavior. For example, the 10-molar urea solution exhibits a slope of 2 indicative of classical viscous absorption. Homogenized milk, a suspension of fat molecules and casein complexes, exhibits a slope of near unity, a behavior which cannot be explained in terms of simple classical absorption behavior; other specimens show complex behavior, their slopes approaching 2 at the higher frequencies. Note also in figure 2 the differences between the ultrasonic absorption of whole liver and homogenized liver. The latter has the gross structure destroyed but leaves intact the molecular constituents.

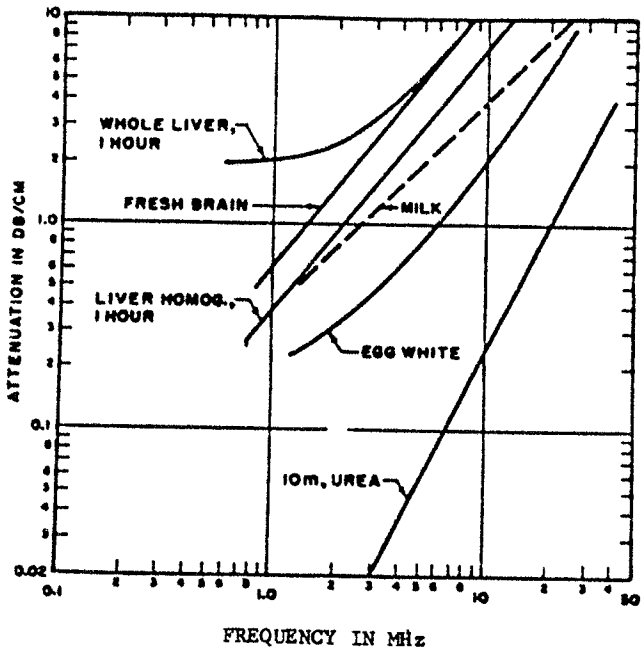

Figure 2. Acoustic amplitude absorption coefficient vs. frequency for materials of different biological complexity (Heuter, 1958).

Since major molecular constituents of biological cells, save water, are globular proteins, it is pertinent to discuss briefly their conformation since one unsolved problem is concerned with the importance of the role of molecular conformation in determining the ultrasonic absorption spectra (Kessler and Dunn, 1969; Lang et al., 1971; O'Brien and Dunn, 1971). Proteins are polyelectrolytes which possess both positive and negative charges. They consist of one to several polypeptide chains, each chain being composed from the approximately 20 more common amino acids. The sequential ordering of these amino acids along the chain determines the primary structure. The secondary structure is due to the intrapolypeptide chain hydrogen bond formation, which produces the helical and sheet formations of the chain. A tertiary structure results from the twisting of the chain into layers, crystals or fibers, viz., a rigid compact globule in the case of globular proteins. If the protein contains only a single polypeptide chain, as in serum albumin, the tertiary structure is the highest form of structural ordering possible. For proteins which contain more than one polypeptide chain, for example, hemoglobin consists of 4, the quaternary structure defines the relationships between these noncovalently linked chains (save for disulfide links). The spatial configuration which a macromolecule assumes in aqueous solution is determined by its structuring. For example, macromolecules possessing tertiary or quaternary ordering are compact and rigid, whereas those with only secondary structuring assume a helical or rigid rod configuration. With negligible forces between any components of the chain, the macromolecule assumes a random coil configuration within the solution.

Figure 3, prepared by Carstensen (1960), exhibits the influence of molecular conformation on the ultrasonic absorption spectra of aqueous solutions of globular proteins. It is seen that the absorption spectra of solutions of hemoglobin, a globular protein composed of four polypeptide chain and having quaternary structure, and plasma, a solution primarily of the globular protein albumin, a single polypeptide chain having tertiary structure, are distinctly different from that of gelatin, a protein which assumes a random coil configuration in aqueous solution.

Figure 4 shows the excess frequency-free absorption per unit concentration for several biomacromolecules and supports the view that structuring contributes demonstrably to the ultrasonic absorption spectra. Both dextran (Hawley et al., 1965), a carbohydrate, and polyethylene glycol (O'Brien, 1968; Kessler et al., 1970), a synthetic

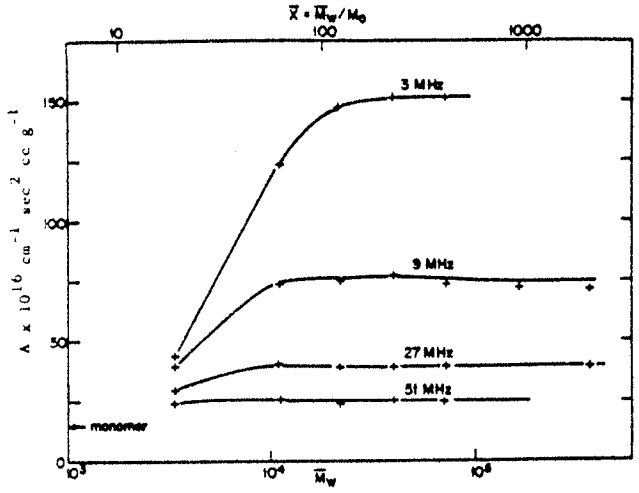

Figure 5. Frequency-free absorption magnitude as a function of weight average molecular weight at four frequencies, 20.0°C. The average degree of polymerization $\bar{X}$ is denoted on the superior abscissa (Hawley & Dunn, 1969).

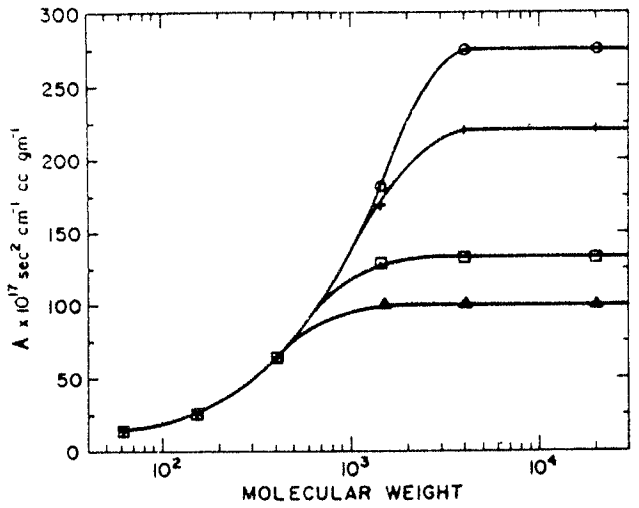

Figure 6. Dependence of absorption of polyethylene glycol on molecular weight: ⊞, all frequencies; ⊙, 6 MHz; +, 10 MHz; ⊡, 30 MHz; △, 60 MHz (Kessler et al., 1970).

while at 3 MHz the absorption is attributed to side chain dissociation. Parker et al. (1968) concluded that the observed ultrasonic absorption behavior in aqueous poly-L-lysine solution can be attributed to the helix-coil transition.

The pH of the solutions is another parameter that has been varied to study the ultrasonic absorption processes and a number of aqueous solutions of amino acids have been investigated, viz., serine and threonine (White et al., 1971), glycine (Applegate et al., 1968; Hussey and Edmonds, 1971a), glutamic acid, aspartic acid and alanine (Applegate et al., 1968) and arginine and lysine (Hussey and Edmonds, 1971b). Absorption maxima have been observed within the pH ranges 2 to 4 and 11 to 13, with such peaking being described quantitatively by assuming that the proton transfer reaction dominates the absorption process. When the pH of aqueous biopolymer solutions is varied alteration of the molecular charge distribution occurs thus affecting the conformation of the biopolymer. Here, buried amino acid side chains of globular proteins become exposed to the environment of the solvent and thereby affect the acoustical properties of the solution. Figure 7 shows the excess frequency-free absorption per unit concentration versus the solution pH for bovine serum albumin at four frequencies (Kessler and Dunn, 1969). Note

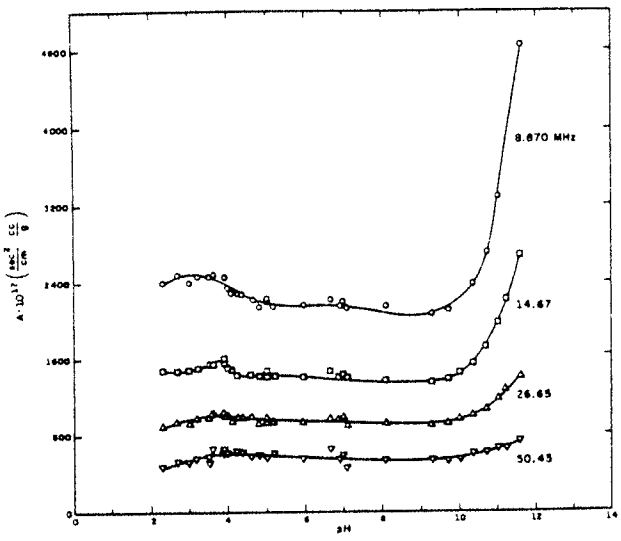

Figure 7. Ultrasonic absorption titration curve for bovine serum albumin (T = 20.0°C) (Kessler, 1968; Kessler & Dunn, 1969).

the peaking around pH 2 to 4 and the rapid increase in absorption beyond pH 10. Figure 8 shows the same parameters for hemoglobin, exhibiting in addition to the peak in the acid region one in the alkaline region around pH 11-13 (O'Brien and Dunn, 1972). These peaks have been correlated with the proton transfer reaction in which it is postulated that the pressure variations of the propagating acoustic wave perturbs the proton from the solvent (water) to the solute, an amino acid side chain, and vice versa. The energy required to drive the proton between the differing energy states is derived from the acoustic wave process and represents increased absorption. The pH val-

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