MICROWAVE DIELECTRIC RELAXATION IN MUSCLE

A SECOND LOOK

KENNETH R. FOSTER, JONATHAN L. SCHEPPS, AND HERMAN P. SCHWAN,
Department of Bioengineering D2, University of Pennsylvania, Philadelphia, Pennsylvania 19104 U.S.A.

ABSTRACT The dielectric permittivity and conductivity of muscle fibers from the giant barnacle, *Balanus nubilus*, have been measured at 1, 25, and 37°C, between 10 MHz and 17 GHz. The dominant microwave dielectric relaxation process in these fibers is due to dipolar relaxation of the tissue water, which shows a characteristic relaxation frequency equal to that of pure water, ranging from 9 GHz (1°C) to 25 GHz (37°C). The total permittivity decrease, $\epsilon_0 - \epsilon_\infty$, due to this process accounts for ~ 95% of the water content of the tissue; thus, the major fraction of tissue water is dielectrically identical to the pure fluid on a picosecond time scale. A second dielectric process contributes significantly to the tissue dielectric properties between 0.1 and 1–5 GHz, and arises in part from Maxwell-Wagner effects due to the electrolyte content of the tissue, and in part from dielectric relaxation of the tissue proteins themselves.

INTRODUCTION

Because the macroscopic dielectric properties of tissue are determined by their large water content at microwave and ultrahigh frequencies (UHF), dielectric studies can probe the physical properties of the intracellular water on a picosecond time scale that is not accessible by other techniques. In particular, the dielectric relaxation frequency of pure water at room temperature is 20 GHz, due to the 8-ps rotational correlation time of the water molecules. Thus, microwave dielectric data from tissue can be used to compare the rotational correlation time of tissue water molecules with those of the pure liquid.

From earlier data on various excised mammalian tissues between 0.1 and 8.5 GHz at 37°C, we recently argued that the dielectric properties of most of the tissue water are the same as those of the pure liquid (1). However, these data did not extend past the relaxation frequency f_c of pure water, 25 GHz at that temperature, and other useful information (the tissue water content and orientation of muscle fibers in the electric field) was not available. These deficiencies resulted in some uncertainty in the interpretation of the data.

Our conclusions, moreover, disagreed with another recent study. Masszi showed (2), from the increase in conductivity of muscle tissue between 2 and 4 GHz, that the dielectric relaxation frequency of the intracellular water is apparently 20% lower than that of the pure liquid; similar results were found for gelatine suspensions (3). If correct, this would imply that even water molecules distant from the water protein interface are affected on a picosecond time scale by the water-protein interactions. However, this argument assumes that the dielectric properties of the tissue in this frequency range reflect only the dipolar relaxation of the tissue water. But other dielectric relaxation processes might also contribute at UHF and

low microwave frequencies in the tissue and confuse this interpretation. For example, protein solutions (4–6) and hydrated Sephadex gel (7) exhibit a dielectric dispersion between 0.1 and 4 GHz, thought to arise from dielectric relaxation of the "bound" water at the protein surface, or partial orientation of polar sidechains. If these or other relaxation mechanisms contribute significantly to the muscle conductivity data at low microwave frequencies, the apparent dielectric relaxation frequency of the tissue would be lower than that of its water fraction.

Using improved instrumentation, we have measured the dielectric properties of single muscle fibers from the giant barnacle, *Balanus nubilus*, from 0.01 to 17 GHz, at three temperatures between 1 and 37°C. At the lowest temperature, the dielectric relaxation frequency for pure water is 9.1 GHz, well within the frequency range of our measurements. The barnacle muscle fibers were chosen for these measurements because individual cells can be dissected from the animal immediately before each measurement, and are of a convenient size (~ 30 mg apiece) for the measurements; because the fibers can be precisely aligned in the sample cell so that the electric field is normal to the fiber axis, simplifying the interpretation of the data; and because the cells remain contractile for the short duration of the measurements, and are (in this sense) physiologically viable during the measurements. While there is no indication that tissue viability affects its properties at UHF and microwave frequencies, it seemed appropriate to choose a living preparation for these measurements.

Our data show that there are at least two dielectric relaxation processes that contribute to the dielectric properties of the tissue above 0.1 GHz. The relaxation frequency of one process is indistinguishable from that of pure water at the same temperature. At 25 and 37°C the lower frequency relaxation (called the UHF relaxation below) contributes significantly to the dielectric loss in the tissue at these microwave frequencies, and resembles the dispersion previously observed in protein solutions in this frequency range.

METHODS AND PROCEDURE

Dielectric measurements in the range 0.7–17 GHz were performed using the short-circuit transmission-line method introduced by Roberts and von Hippel (8). Our equipment and procedures are described in detail elsewhere (9) and will only be summarized here.

Our technique required that the sample be confined at the end of a transmission line between a thin teflon plug and a short circuit. From measurements of the phase and magnitude of the wave reflected by the sample, the sample dielectric properties were calculated by solving the appropriate transmission-line equations (8). We used a section of precision 7-mm, 50-Ω coaxial transmission line to contain the sample, mounting the line vertically in a special jig that also held a precision dial gauge for accurate measurement of the sample thickness. The teflon plug was pushed an appropriate distance into the line, and the sample was placed above it to fill the sample chamber. A precision APC-7 short-circuit termination (A.P. Circuit Corp., New York, N.Y.) was then placed flush against the sample, with care being taken to exclude all air from the sample. By this arrangement electrical continuity in the transmission line was maintained, and the short circuit could be removed and replaced by other terminations of known impedance in the calibration procedure outlined below. The sample temperature was controlled by a water jacket coaxially surrounding the line, and monitored by a thermocouple embedded in the short circuit a few millimeters from the sample, in good thermal contact with, but electrically well-shielded from, the sample cell. The measurement of the sample temperature was accurate to within 1°C, as shown by control dielectric measurements on pure water, whose dielectric properties are well known.

The phase and magnitude of the voltage reflection coefficient from the sample-filled line was measured using a Hewlett-Packard (HP) microwave network analyzer system (Hewlett-Packard Co., Palo Alto, Calif.) consisting of a microwave oscillator (HP model 8620C), reflection test unit (HP model

8743A), network analyzer (HP model 8410B), and digital frequency counter (HP model 5342A). A computer routine (10) was used to calculate the dielectric properties of the sample from its measured reflection coefficient.

In microwave measurements of this sort, elaborate precautions are needed to obtain reasonably accurate data. Because of the transcendental equations that apply, the sample thickness should be such that about one-quarter of a wavelength of the radiation is contained in the sample (9). Therefore, at each measurement frequency, a different sample was used whose thickness was adjusted (by moving the teflon plug) to the proper value. Because the permittivity of the tissue (ϵ^*) is quite high, and the wavelength of the energy in the tissue is smaller than that in air by about the factor $1/\sqrt{\epsilon}$, this required sample thicknesses of < 1 mm at frequencies above 10 GHz. The small uncertainty in small thickness ($\sim$ 0.001 cm) resulted in a maximum theoretical error of 2% in the tissue dielectric permittivity and conductivity at frequencies above 10 GHz, and less at lower frequencies (9).

A potentially serious problem arises from unwanted reflections from the teflon plug and (to a smaller extent) from imperfections in the precision APC-7 coaxial connectors, as well as directivity errors in the network analyzer itself. The data were corrected using a vector calibration procedure, which assumes that all reflections and losses in the system can be modeled by a two-port error network in front of the circuit whose reflection coefficient is to be measured. This error network is characterized by a complex scattering matrix, whose elements are found by terminating the transmission line (without the tissue but with the teflon plug in place) by several known impedances, including two short circuits, located one-quarter of a wavelength apart, and a precision sliding 50-Ω load, located at three positions one-sixth of a wavelentgh apart (11). We repeated these calibration measurements each time the frequency or sample thickness was changed, or whenever a coaxial connector in the system was disturbed.

To test the system, we measured the permittivity and conductivity of distilled water over the range 0.7–17 GHz, with a maximum error of the order of 2% above 10 GHz, compared with data on water from the literature. The tissue results showed larger variability ($\sim$ 1 dielectric unit and $\sim$ 5% in the conductivity), presumbly because of variations in the water content of each sample. In addition to these microwave measurements, we measured the permittivity and conductivity of the tissue at frequencies between 1 and 100 MHz, using a Boonton R-X meter and capacitor sample cell (Boonton Electronics Corp., Parsippany, N.J.) described elsewhere (12).

Before each microwave measurement, one or several muscle fibers (depending on the frequency) were dissected free of the animal, blotted on filter paper, and gently wrapped around the center post of the

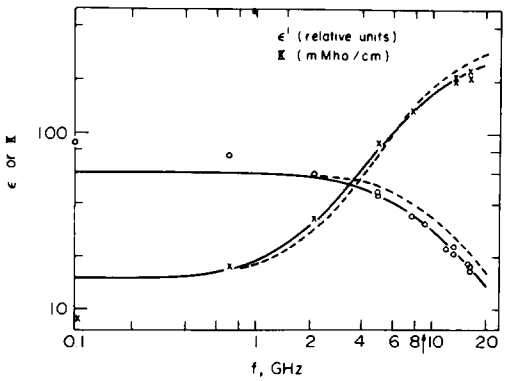

FIGURE 1 Dielectric permittivity and conductivity of barnacle muscle fibers at 1°C. The solid lines are fitted funtions assuming a Debye dispersion with f_c = 9.1 GHz (the same as for pure water at that temperature), ϵ_0 = 62, and ϵ_∞ = 4 dielectric units. The dotted lines are calculated values assuming f_c = 11 GHz, showing the sensitivity of the fit to small changes in the assumed relaxation frequency. The "static" conductivity κ_0^M was taken to be 15 mMho/cm, and is greater than the conductivity at 0.1 GHz (9.1 mMho/cm) because of the dielectric relaxation occurring above 0.1 GHz. An additional dielectric relaxation process contributes ~20 dielectric units to the tissue permittivity at 0.1 GHz.

coaxial sample cell with its longitudinal axis normal to the direction of the electric field. The short-circuit termination was affixed with all air carefully excluded from the sample cell; then the sample was allowed to equilibrate at the desired temperature. Measurements were repeated at least three times at each temperature and frequency, using a different tissue sample each time. Several samples of tissue were dried to constant weight at 105°C to determine their average water contents, which were found to be 80.4 ± 1.3 wt % water (from eight separate determinations). Assuming the density of protein to be 1.3, this is equivalent to a volume fraction 0.16 of solid in the tissue. The measured water content of the fibers was in good agreement with results from a previous study (13).

RESULTS AND DISCUSSION

The dielectric permittivity and conductivity of the fibers at 1°C above 0.1 GHz are shown in Fig. 1; the data at the higher temperatures appear quite similar and are not shown. Below 0.1 GHz, the permittivity increases with decreasing frequency to reach high values at low radiofrequencies (e.g., 10^4 dielectric units at 1 MHz), typical of all soft tissues (12).

In this study, we are interested primarily in the dielectric relaxation processes occuring above 1 GHz, where the dipolar relaxation of tissue water occurs. We will examine the relaxation properties of the bulk tissue water and look for evidence for additional dielectric relaxation processes contributing to its dielectric properties at microwave frequencies.

Dielectric Relaxation Frequency of Tissue Water

The dielectric relaxation properties of pure water and dilute electrolyte solutions are well known at microwave frequencies (14, 15). The complex dielectric constant ϵ^* of dilute electrolyte solution at frequency f is accurately described by the Debye equation

$$\epsilon^* = \epsilon_\infty - \frac{j\kappa_0}{2\pi f \epsilon_r} + \frac{(\epsilon_0 - \epsilon_\infty)}{1 + jf/f_c} \equiv \epsilon' - j\epsilon'', \tag{1}$$

where ϵ_∞ is the permittivity at frequencies far above the relaxation frequency f_c, ϵ_r is the permittivity of free space (a constant), ϵ_0 is the permittivity at frequencies much lower than f_c, and κ_0 is the (frequency-independent) conductivity from the ions. Since most dielectric relaxation processes in tissue are relatively slow on the picosecond relaxation time scale of the tissue water (16), Eq. 1 should be a reasonable approximation at sufficiently high frequencies. However, great care must be taken in fitting tissue dielectric data to this equation: if additional relaxation processes also occur on a sufficiently short time scale, the fitted values of the parameters in Eq. 1 will not reflect the dielectric properties of the tissue water alone, but will include a contribution from other relaxation processes as well. Since there is no plateau in the permittivity of tissue below 1 GHz (where the permittivity of pure water is essentially constant), we expect this interference from other relaxation processes.

Applying this equation to tissue, κ_0 and ϵ_0 now become the conductivity and permittivity due to all other dielectric relaxation processes but tissue water relaxation, and are denoted by κ_0^M and ϵ_0^M. The ionic contribution to the conductivity is denoted by κ_0^{ion}, which is close to the tissue conductivity near 10–100 MHz (16). We will numerically fit the data at 1°C to Eq. 1. The data at higher temperatures do not extend past f_c for pure water (which is 20 GHz at 25°C), and, as shown below, the "UHF dispersion" affects the microwave data more at these higher temperatures. Thus, a direct fit of the data from tissue at 25 or 37°C is impractical.

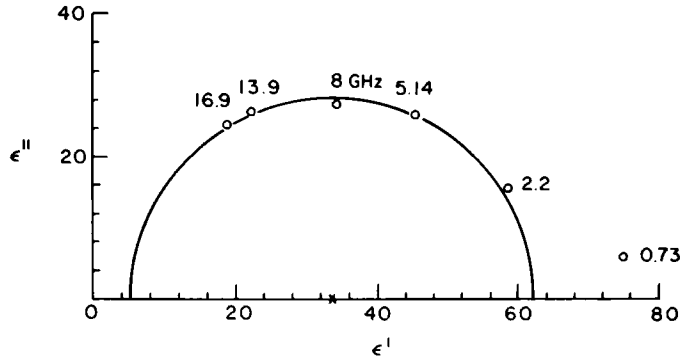

FIGURE 2 Dielectric data from Fig. 1, replotted showing the semicircular behavior characteristic of a simple Debye dispersion for the intracellular water.

We can graphically fit the data at 1°C to the Debye equation by plotting the permittivity ϵ' vs. its imaginary part, $\epsilon'' = (\kappa - \kappa_0^M)/(2\pi f \epsilon_r)$ on the complex plane, where κ_0^M is estimated from the conductivity near 1 GHz (15 mMho/cm; from Fig. 1). Eq. 1 plots as a semicircle whose center is on the real axis at $(\epsilon_0 - \epsilon_\infty)/2$, with the point at $f = f_c$ at the top of the semicircle. This behavior is shown by the data at 1°C (Fig. 2). The data point at 0.73 GHz never falls on the semicircle, irrespective of the exact value of κ_0^M chosen. The point at 2.2 GHz is somewhat sensitive, and the remaining points in Fig. 2 relatively insensitive, to the choice of κ_0^M, because at the higher frequencies the tissue conductivity is much larger than κ_0. Thus the tissue data at 1°C are well described by the Debye equation at frequencies above 5 GHz, with the data point at 2.2 GHz falling on this locus or not, depending on the assumed value of κ_0^M. The apparent value of f_c for the tissue is evidently close to 9 GHz, as found for pure water at 1°C.

A numerical fit of the data at 2.2 GHz and above at 1°C to Eq. 1 yields[1] $\epsilon_0 = 62.0 \pm 0.4$, $\epsilon_\infty = 7.9 \pm 0.4$, $\kappa_0^M = 17.6 \pm 0.3$ mMho/cm, and $f_c = 8.3 \pm 0.4$ GHz, in good agreement with the values indicated in Fig. 2 for these respective parameters. The approximate standard errors for the fit indicated above were calculated from a numerical evaluation of the partial derivatives of Eq. 1 with respect to the fitted parameters; but the uncertainty in the fitted parameters must also reflect any systematic error in the data (<5% error from this source) and would be somewhat larger. The fitted value of f_c is 7% below the respective value for water (9.1 GHz at 1°C). However, this parameter is sensitive to the data point at 2.2 GHz; deleting that point yields a value of f_c of 10 GHz; evidently the permittivity at this frequency is still affected by the lower frequency relaxation evident below 1 GHz in Fig. 1. Considering only the dielectric data between 2 and 4 GHz, Masszi (3) noted a 20% reduction in f_c below that of water. Since this difference diminishes when higher frequency data are included in our fit, it apparently results from remnants of relaxation processes occurring primarily at lower frequencies. As shown in Fig. 1, our data are well-fitted by the Debye equation, with f_c chosen

[1]We wish to acknowledge the use in this work of subroutine STEPIT, written by J. P. Chandler of Oklahoma State University and distributed by the Quantum Chemistry Program Exchange of Indiana University. Note that the data points shown in Fig. 2 each represent averages of 4–10 separate measurements; we used 26 data points to determine four fitted parameters.

equal to that of pure water. This is also the case with the data at 25 and 37°C. The dielectric relaxation frequency of tissue water is close, if not precisely equal, to that of pure water. We now estimate what fraction of tissue water contributes to this microwave relaxation in tissue.

Dielectric Permittivity of Tissue Water

This fraction of tissue water that contributes to its microwave dielectric properties can be found from ϵ_0^M in Eq. 1 by using an appropriate mixture theory to be discussed below. Because of the interference from the lower frequency dielectric relaxation in the tissue, evident in Fig. 1, this parameter cannot be directly read from Fig. 1. Nevertheless, the dominant character of the water dispersion at microwave frequencies allows us to calculate ϵ_0^M by the following different methods.

(*i*) From the microwave conductivity data from Eq. 1:

$$\kappa = \kappa_0^M + \frac{2\pi\epsilon_r(\epsilon_0^M - \epsilon_\infty) f^2/f_c}{1 + (f/f_c)^2}, \tag{2}$$

which is a linear function of $f^2/\,[1 + (f/f_c)^2]$, whose slope is proportional to $(\epsilon_0^M - \epsilon_\infty)/f_c$. Since variations in the assumed value of f_c will merely shift the abscissa, the slope $(\epsilon_0^M - \epsilon_\infty)/f_c$ is relatively independent of f_c. The conductivity plots for muscle at two temperatures are shown in Fig. 3, which were calculated assuming f_c equal to the respective values for pure water. These plots clearly show the interference due to the lower frequency UHF dispersion. The limiting values of κ_0^M and ϵ_0^M obtained from the slopes are summarized in Table I.

(*ii*) From the equation

$$\epsilon_r(\epsilon_0^M - \epsilon_\infty) = \frac{\kappa_\infty - \kappa_0^M}{2\pi f_c}, \tag{3}$$

which can be derived from Eq. 2, where κ_∞ is the limiting value of the conductivity at high frequencies. This approach requires knowledge of the difference $\kappa_\infty - \kappa_0^M$ which may be estimated from Fig. 1 or, better, from a circular plot of the data $\omega\epsilon'\epsilon_r$ vs. κ in the complex admittance plane analogous to the dielectric plot shown in Fig. 2. At 1°C, a value of $\kappa_\infty = 280$ mMho/cm is obtained and, hence, a value of $\epsilon_0^M - \epsilon_\infty = 56$, in good agreement with Table I.

(*iii*) From the circular plot shown in Fig. 2, or equivalently, from a numerical fit of the data to Eq. 1. The fitted values for ϵ_0^M and κ_0^M agree well with the extrapolated values at 1°C listed in Table I. Methods (*ii*) and (*iii*) work best when the data extend above f_c.

From these data we find that the limiting permittivity ϵ_0^M of the tissue is ~ 30 units below that of water, which is consistent with other protein suspensions of similar solid content at UHF and microwave frequencies (16). Using the mixture equation to be discussed below, we can calculate the apparent volume of the tissue ϕ, which does not contribute to the tissue polarizability above 1 GHz. These results are summarized in Table I. We find that the apparent value of ϕ is ~ 0.20, which is ~ 25% higher than the known solid fraction in the tissue (0.16). A similar discrepancy found in protein solutions (4–6) is thought to arise from bound water adjacent to protein surfaces which cannot reorient quickly enough with the applied microwave field. At most, the noncontributing water fraction in this tissue amounts to 5% of the tissue water, with the remaining water having the same permittivity and dielectric

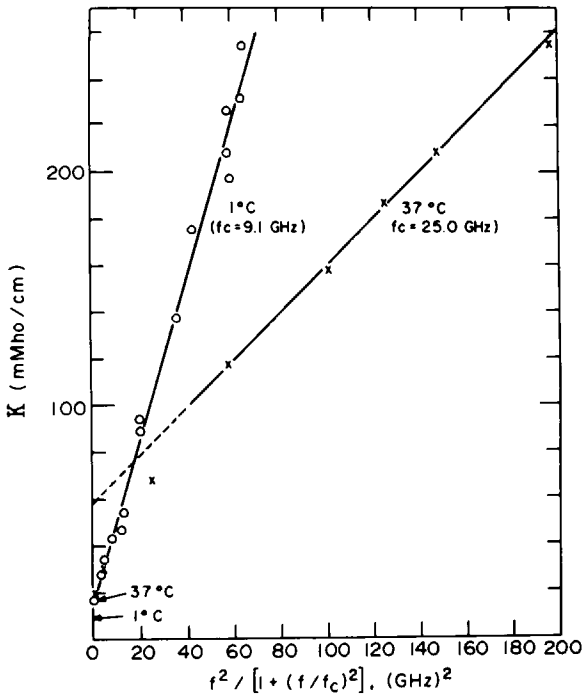

FIGURE 3 Tissue conductivity at 1 and 37°C, plotted vs. $f^2/[1 + (f/f_c)^2]$, with f_c assumed equal to the relaxation frequency of pure water at the respective temperature. The conductivity of the tissues at 100 MHz at these two temperatures is indicated also. The data at 37°C show that a second dielectric relaxation process adds significantly to the tissue conductivity above 1 GHz. This UHF relaxation occurs at lower frequencies at 1°C, and is evident in this plot by the 6-mMho/cm difference between the intercept of these data (κ_0^M = 15 mMho/cm) and the conductivity at 100 MHz (κ_0 = 9.2 mMho/cm). The permittivity of the barnacle tissue at 37°C is shown in the next figure.

TABLE I
SUMMARY OF RESULTS

Measured properties (100 MHz)			Derived properties from microwave data		
Temperature	ϵ	κ*	ϵ_0^M‡	κ_0^M§	(Volume-fraction solid) ϕ‖
°C	*(relative units)*	*(mMho/cm)*			
1	88	9.2	62	17	0.20
25	88	14.2	57	33	0.17
37	88.6	17.2	49	58	0.23

*These conductivity values agree well with the conductivity of the cytoplasm, measured at low frequencies using an entirely different technique (see reference 17). The conductivity of the tissue was essentially constant from ~10 MHz to over 100 MHz.

‡Calculated from the data in Fig. 3, assuming ϵ_∞ = 5. This is the permittivity contribution to the tissue from the intracellular water, which has the same dielectric relaxation frequency as the pure liquid.

§Calculated by plotting the microwave conductivity vs. $f^2/[1 + (f/f_c]^2)$ and extrapolating the water contribution to the conductivity to zero frequency. This is the ionic conductivity plus the conductivity added by the UHF relaxation.

‖Calculated using the Rayleigh mixture formula (Eq. 6), assuming ϵ_p (protein) = 5 and $\epsilon_\infty = \epsilon_0^M$. ϵ_w was the permittivity of pure water at the respective temperature.

relaxation time as bulk water. It has been suggested (4–6) that rotational reorientation of bound water partly contributes to the small dielectric dispersion in protein solutions observed at UHF and low microwave frequencies. We now discuss the much larger dielectric dispersion found in the muscle fibers at UHF and low microwave frequencies.

UHF Relaxation in Barnacle Muscle

By fitting the microwave dielectric data to Eq. 1, we obtain a limiting conductivity κ_0^M which is due to all mechanisms of dielectric loss in the tissue other than rotational relaxation of the "bulk" tissue water. Since we have shown elsewhere that the bulk conductivity of barnacle muscle at 0.1 GHz is close to that of the cytoplasm itself (17), and (from Fig. 1) this conductivity is only half of κ_0^M, there must be additional dielectric relaxation mechanisms effective at frequencies above 0.1 GHz. This UHF relaxation is evident also from the permittivity data (Fig. 1) and conductivity plots (Fig. 3).

There are several processes that could contribute to this UHF relaxation behavior, in addition to rotational relaxation of bound water. (*i*) The huge dielectric permittivity of tissue at lower radiofrequencies arises from a Maxwell-Wagner effect due to the cell membranes (16, 18). Since the relaxation frequency for this dispersion is inversely proportional to the diameter of the particle, intracellular organelles or the sarcoplasmic reticulum might contribute somewhat to the dielectric permittivity in the higher radiofrequency range. For example, mitochondria exhibit a strong Maxwell-Wagner effect centered near 3 MHz, whose tail extends well above 20 MHz (19). (*ii*) The protein suspended in electrolyte should exhibit a Maxwell-Wagner dispersion at several hundred megahertz, which results from free ions in the suspending medium of the cytoplasm collecting against the low permittivity, low conductivity protein. (*iii*) The suspended proteins may well exhibit a relaxation of their own, since it has been demonstrated that the effective dielectric constant of the hydrated hemoglobin and albumin molecule changes drastically with frequency between 0.1 and 1 GHz (4–5).

Both possibilities (*ii*) and (*iii*) are analyzed by using dielectric mixture theory. Because the myofibrils were oriented perpendicular to the electric field, the Rayleigh formula is used to relate the complex permittivity of the mixture (tissue) ϵ_t^* to that of the protein ϵ_p^* and suspending fluid ϵ_t^* (18):

$$\frac{\epsilon_t^*}{\epsilon_w^*} = \frac{\epsilon_p^*(1+\phi) + \epsilon_w^*(1-\phi)}{\epsilon_w^*(1+\phi) + \epsilon_p^*(1-\phi)}, \tag{4}$$

where ϕ is the volume fraction of protein.

By straightforward but tedious algebraic manipulation, ϵ_t^* given above can be written in the form of Eq. 1, with the following parameters:

$$\epsilon_0 = \epsilon_\infty + \frac{4\phi(1-\phi)(\kappa_p\epsilon_w - \kappa_w\epsilon_p)^2}{[(1+\phi)\epsilon_w + (1-\phi)\epsilon_p][(1+\phi)\kappa_w + (1-\phi)\kappa_p]^2} \tag{5}$$

$$\epsilon_\infty = \epsilon_w \frac{\epsilon_p(1+\phi) + \epsilon_w(1-\phi)}{\epsilon_w(1+\phi) + \epsilon_p(1-\phi)} \tag{6}$$

$$\kappa_0 = \kappa_w \frac{\kappa_p(1+\phi) + \kappa_w(1-\phi)}{\kappa_w(1+\phi) + \kappa_p(1-\phi)} \tag{7}$$

$$\kappa_\infty = \frac{4\phi(1-\phi)(\kappa_p\epsilon_w - \kappa_w\epsilon_p)^2}{[(1+\phi)\epsilon_w + (1-\phi)\epsilon_p]^2[(1+\phi)\kappa_w + (1-\phi)\kappa_p]} + \kappa_0 \tag{8}$$

$$f_c = \frac{1}{2\pi\epsilon_r}\frac{\kappa_p + \kappa_w + \phi(\kappa_w - \kappa_p)}{\epsilon_p + \epsilon_w + \phi(\epsilon_w - \epsilon_p)},$$

where

$$\epsilon_\alpha^* = \epsilon_\alpha - \frac{j\kappa_\alpha}{\omega\epsilon_r} \quad (\alpha = p, w, \text{or } t).$$

Here, the subscripts 0 and ∞ refer to the limiting values of the parameters at frequencies respectively far below and far above f_c given by Eq. 8; they are not to be confused with the limiting values for the microwave dispersion due to rotational relaxation of tissue water discussed above. It is safe to assume that the protein conductivity κ_p is much smaller than that of the suspending medium κ_w, so these equations simplify considerably. Since it turns out that $f_c \ll 1$ GHz for the Maxwell-Wagner dispersion, ϵ_∞ in Eq. 6 can be identified with ϵ_0^M extrapolated from the microwave data at 1–17 GHz, and this equation can be used to calculate the apparent volume fraction of the water in the tissue $(1 - \phi)$. Assuming that $\epsilon_p \ll \epsilon_w \simeq 80$ in this microwave frequency range, we get the results shown in Table I.

If the effective permittivity of the hydrated protein is smaller than, or at least comparable to, that of water (as is the case with globular proteins [4–6]), Eq. 8 predicts the relaxation frequency f_c for this Maxwell-Wagner dispersion to be near 250 MHz at 1°C for a medium conductivity κ_w of $\sim$ 12 mMho/cm.[2] Therefore, at least part of the change in dielectric properties of the tissue with frequency above 0.1 GHz probably arises from an interfacial polarization process, which is a common feature of suspensions of materials with different dielectric properties (20).

However, part of the dielectric dispersion in this frequency range also is due to the tissue solids themselves. Fig. 1 indicates an increase of $\sim$ 26 dielectric units above the permittivity $\epsilon_0^M = 62$ indicated in Fig. 2, which is the contribution of the tissue water. Thus, with $\phi = 0.16$, a value of $\epsilon_p \simeq 90$ is found from Eq. 5 for the effective permittivity of the protein at 0.1 GHz. This value is three to four times larger than that calculated for hydrated hemoglobin at 100 MHz using a similar procedure. Presumably, this difference is due in part to the geometric differences between the fibrous contractile protein and the globular hemoglobin. Fig. 4 compares the dielectric properties of the muscle, and quantitatively similar suspensions of gelatine and hemoglobin. The agreement between the gelatine and muscle data is striking; their divergence below 0.1 GHz is evidently due to the effect of cell membranes. The dielectric permittivity of gelatine follows a negative power function of frequency at least to kilohertz frequencies (21–23), indicating that there is a broad range of dielectric relaxation processes occurring in the gelatine, and presumably in the muscle protein as well. These effects arise in

[2]This κ_w is the conductivity of the aqueous phase of the cytoplasm excluding the suspended protein. It was calculated from Eq. 7 from the measured tissue conductivity at 0.1 GHz (9 mMho/cm) and assuming a volume fraction ϕ of protein to be 0.16. As we show below, $\epsilon_p \approx \epsilon_w$ at 0.1 GHz, so the predicted f_c for this Maxwell-Wagner dispersion would actually be closer to 100 MHz. The point here is that this relaxation mechanism probably affects the dielectric data above 0.1 GHz to a large but uncertain extent.

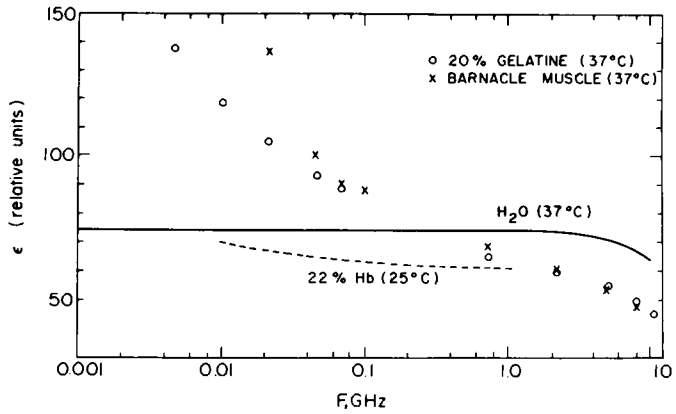

FIGURE 4 Dielectric permittivity of gelatine at 37°C, showing a large increase in the permittivity below 1 GHz. Also shown is the measured permittivity of a 22% hemoglobin solution (4) and barnacle muscle (this study) for comparison. The conductivity of the 20% suspension of gelatine and muscle, when plotted as in Fig. 3, shows the same evidence of two dielectric relaxations above 1 GHz as muscle. Since the dielectric permittivity of the barnacle and gelatine suspensions are similar above 0.1 GHz, the same dielectric processes may occur in both samples at these high frequencies. The divergence between the gelatine and barnacle properties below 0.1 GHz is probably due to the contribution of cell membranes to the dielectric properties of the tissue, which becomes more pronounced at lower frequencies.

part from dielectric relaxation in the protein itself, from rotational relaxation of its bound water, and also from counter ion polarization mechanisms at the protein-electrolyte interface, which are a dominant cause of dielectric relaxation in polymeric solutions and which also show a wide range of effective correlation times (24–27).

The molecular mechanisms for this UHF dispersion in the barnacle muscle and gelatine are probably too complex to completely sort out. But clearly, this dispersion extends somewhat above 1 GHz, and overlaps with the water relaxation effects. Since Masszi (2, 3) fitted dielectric data over only 2–4 GHz to the Debye equations, these effects probably led to his conclusions that most of the water molecules in the tissue and gelatine have longer dielectric relaxation times than in the pure liquid. In contrast, we find that above a few GHz, the same Debye equation correctly fits the dielectric data from the tissue and pure water, provided that proper allowance is made for the volume fraction of protein which has, at microwave frequencies, a much lower permittivity than that of pure water.

This work was partially supported by the Office of Naval Research (contract N00014-77-C-0392) and grant 1R01 CA26046-01, awarded by the National Cancer Institute, Department of Health, Education and Welfare.

We thank Mr. Richard Stoy for the dielectric measurements using the Boonton RF bridge, and Mr. Benjamin Epstein for assistance with the data analysis. We also thank Mr. Albert W. Friend, Jr. and the Naval Medical Research Institute for the loan of the microwave network and analyzer.

Received for publication 13 August 1979.

REFERENCES

1. SCHWAN, H.P., and K. R. FOSTER. 1977. Microwave dielectric properties of tissue. Some comments on the rotational mobility of tissue water. *Biophys. J.* **17**:193–197.
2. MASSZI, G., A. SZUARTO, and P. GROF. 1976. Investigations on the ion- and water-binding of muscle by microwave measurements. *Acta Biochim. Biophys. Acad. Sci. Hung.* **11**:129–131.

3. MASSZI, G. 1972. Dielectric relaxation and water structure in gelatine solutions. *Acta Biochim. Biophys. Acad. Sci. Hung.* **7**:349–357.
4. PENNOCK, B. E., and H. P. SCHWAN. 1969. Further observations on the electrical properties of hemoglobin—bound water. *J. Phys. Chem.* **73**:2600–2610.
5. GRANT, E. H., S. E. KEEFE, and S. TAKASHIMA. 1968. The dielectric behavior of aqueous solutions of bovine serum albumin from radiowave to microwave frequencies. *J. Phys. Chem.* **72**:4373–4380.
6. ESSEX, C. G., E. H. GRANT, R. J. SHEPPARD, G. P. SOUTH, M. S. SYMONDS, G. L. MILLS, and J. SLACK. 1977. Dielectric properties of normal and abnormal lipoproteins in aqueous solution. *Ann. N.Y. Acad. Sci.* **303**:142–155.
7. KOIDE, G. T., and E. L. CARSTENSEN. 1976. Dielectric properties of Sephadex and its water of hydration. *J. Am. Chem. Soc.* **80**:55–59.
8. ROBERTS, S. and A. VON HIPPEL. 1946. A new method for measuring dielectric constant and loss in the range of centimeter waves. *J. Appl. Phys.* **17**:610–616.
9. FOSTER, K. R., J. L. SHEEPS, R. D. STOY, and H. P. SCHWAN. 1979. Dielectric properties of brain tissue between 0.01 and 10 GHz. *Phys. Med. Biol.* In press.
10. NELSON, S. O., C. W. SCHLAPHOFF, and L. E. STETSON. 1974. Computation of dielectric properties from short-circuited waveguide measurements on high or low loss materials. *IEEE Trans. Microwave Theory Tech.* **MTT-22**:342–343.
11. DA SILVA, E. F., and M. K. MCPHUN. 1978. Calibration of an automatic network analyzer using transmission lines of unknown characteristic impedance, loss, and dispersion. *Radio Electron. Eng.* **48**:227–234.
12. SCHWAN, H. P. 1963. Determination of biological impedances. *Phys. Tech. Biol. Res.* **6**:323–407.
13. REISIN, I. L., and G. N. LING. 1973. Exchange of ^{3}HHO in intact isolated muscle fiber of the giant barnacle. *Physiol. Chem. Phys.* **5**:183–208.
14. GRANT, E. H., R. J. SHEPPARD, and G. P. SOUTH. 1978. Dielectric Behavior of Biological Molecules in Solution. Oxford University Press, Oxford. 145–184.
15. HASTED, J. B. 1973. Aqueous Dielectrics. Chapman and Hall, London. 136–174.
16. SCHWAN, H. P. 1957. Electrical properties of tissue and cell suspensions. *Adv. Biol. Med. Phys.* **5**:147–209.
17. FOSTER, K. R., J. M. BIDINGER, and D. O. CARPENTER. 1976. The electrical resistivity of cytoplasm. *Biophys. J.* **16**:991–1002.
18. COLE, K. S. 1972. Membranes, Ions and Impulses. University of California Press, Berkeley. 12–38.
19. PAULY, H., L. PACKER, and H. P. SCHWAN. 1960. Electrical properties of mitochondrial membranes. *J. Biophys. Biochem. Cytol.* **7**:589–601.
20. DUKHIN, S. S. 1971. Dielectric properties of disperse systems. *Surf. Colloid Sci.* **3**:83–165.
21. FRICKE, H., and L. E. JACOBSON. 1939. A dielectric study of the gelatine-water system: anomalous dispersion in bound (oriented) water. *J. Phys. Chem.* **43**:781–796.
22. FRICKE, H., and E. PARKER. 1940. The dielectric properties of the system gelatine-water. II. *J. Phys. Chem.* **44**:716–726.
23. MASUZAWA, M., and C. STERLING. 1968. Gel-water relationships: dielectric dispersion. *Biopolymers.* **6**:1453–1459.
24. COLE, R. H. 1977. Dielectric theory and properties of DNA in solution. *Ann. N.Y. Acad. Sci.* **303**:59–73.
25. MANDEL, M. 1977. Dielectric properties of charged linear macromolecules with particular reference to DNA. *Ann. N.Y. Acad. Sci.* **303**:74–87.
26. SCHWARZ, G. 1972. Dielectric relaxation of biopolymers in solution. *Adv. Mol. Relaxation Processes.* **3**:281–295.
27. MINAKATA, A. 1977. Dielectric dispersion of polyelectrolytes due to ion fluctuation. *Ann N.Y. Acad. Sci.* **303**:107–120.