

*Review article*

## **The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology**

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### **1. Introduction and scope**

There is much current interest in the electrical properties of biological materials, not least because of an increasing awareness of the possible physiological effects elicited by the absorption by tissues of non-ionising electromagnetic radiation (e.g. Presman 1970, Sheppard and Eisenbud 1977, Adey 1981, Illinger 1981, Konig *et al* 1981, Becker and Marino 1982, Fröhlich and Kremer 1983, Adey and Lawrence 1984, Chiabrera *et al* 1985, Marino and Ray 1986, Polk and Postow 1986). Studies of the ways in which electromagnetic energy interacts with tissues are also of importance to the continuing development of the use of RF and microwave hyperthermia (Hahn 1982, Storm 1983), in impedance plethysmography (Hill 1979, Mohapatra 1981, Wheeler and Penney 1982, Brown 1983, Anderson 1984, Penney 1986) and pneumography (Henderson and Webster 1978, Baker 1979), in electrical impedance tomography (Pryce 1979, Barber and Brown 1984), in the gentle thawing of cryogenically preserved tissue (Burdette *et al* 1980), in the use of pulsed electromagnetic fields to aid tissue and bone regeneration and healing (Barker and Lunt 1983, Pilla *et al* 1983) and in a variety of existing or projected biosensing devices (Kell 1986a). The use of dielectric measurements as a tool for studying molecular and cellular parameters is a well established technique of continuing importance (e.g. Grant *et al* 1978, Pethig 1979, 1984).

Historically, measurements of the electrical properties of biological materials hold a pre-eminent position in several areas of physiology and biophysics. Thus, Höbner (1910) measured the electrical impedance of suspensions of erythrocytes up to frequencies of 10 MHz and, finding that their impedance decreased with increasing frequency, concluded that the cells were composed of a poorly conducting membrane surrounding a cytoplasm of relatively low resistivity. Amongst the first indications of the ultra-thin, molecular thickness of this membrane were those provided by Fricke (1925), who obtained, from a theoretical and experimental analysis of the dielectric properties of red blood cell suspensions, a value of  $0.81 \mu\text{F cm}^{-2}$  for the erythrocyte membrane capacitance. By assuming a value of three for the relative permittivity of the membrane, Fricke derived a value for its thickness of 3.3 nm, a value within a factor of two of those currently accepted (see, e.g., Cole 1972, Schanne and Ceretti 1978, Kell and Harris 1985b and see later). Similarly, the observation of an inductance (negative capacitance) by Cole and Baker (1941) during measurements of the AC electrical properties of squid axons led directly to the concept of voltage-gated membrane pores, as embodied in the celebrated Hodgkin-Huxley (1952) treatment (Cole 1972, Jack *et al* 1975), as the crucial mechanism of neurotransmission.

Quantitative details of the molecular size, shape and extent of hydration of protein molecules were provided by the dielectric measurements of Oncley (1943), and these were extended in the laboratories of Hasted (Haggis *et al* 1951), Grant (1965) and Schwan (1965a) to provide important details of the physical nature and extent of protein hydration. As examples of how electrical and dielectric studies continue to provide new and unique knowledge in the biophysical and physiological sciences, we may mention measurements of the diffusional motions of proteins and lipids in cell membranes by Harris and Kell (Kell 1983, Kell and Harris 1985a, b, Harris and Kell 1985), studies of the mechanism of field-induced cell fusion and cell rotation in Zimmermann's laboratory (Zimmermann 1982, Arnold *et al* 1985) and by others (Fuhr *et al* 1985, 1986), studies of the influence of hydration on enzyme activity and intramolecular mobility (Bone and Pethig 1985, Finney and Poole 1984, Neumann 1986) and on protonic and ionic charge transport processes in proteins (Careri *et al* 1985, Morgan and Pethig 1986).

The major purpose of this review then is to document those factors which are believed most strongly to influence the way in which non-ionising electromagnetic (EM) radiation in the frequency range 1 Hz to 100 GHz interacts with biological materials. Now, since EM radiation is composed of both electrical and magnetic fields, we need in principle to consider not only the electrical properties such as permittivity and conductivity but also the magnetic permeability of the substances involved. However, apart from ferromagnetic materials such as magnetite, which is known to be associated with the orientational and navigational abilities of certain bacteria, insects and mammals (Gould 1984), most biological materials have a magnetic permeability equal to that of a vacuum. Equally, whilst magnetic fields are also able to interact with certain nuclear magnetic moments or with unpaired electrons associated with paramagnetic ions or free radicals, such interactions are highly specific and in general have little influence on the bulk electrical properties. Thus we shall be primarily concerned with the (complex) dielectric properties of biological materials, namely with the frequency-dependent magnitudes of the conductivity  $\sigma$  and relative permittivity  $\epsilon'$ .

We do not here attempt to give a full historical account of the development of this topic, an overview of which may be found elsewhere (Schwan 1957, Cole 1972, Grant

*et al* 1978, Schanne and Ceretti 1978, Pethig 1979). In addition, we shall be concerned mainly with the so-called linear passive electrical properties, i.e. those which are independent of the magnitude of the fields used to assess them; relevant aspects of the non-linear electrical properties of cells and tissues in the stated frequency range may be derived (mainly for neuromembranes) from Cole (1972), Jack *et al* (1975), Almers (1978) and Schanne and Ceretti (1978). Similarly, we shall not devote much space to methodological aspects, discussions of which may be found, for instance, in the review by Schwan (1963) and the books by Grant *et al* (1978) and Pethig (1979). Finally, tabulations of the dielectric properties of various tissues and biomaterials have been given by Geddes and Baker (1967), Schwan and Foster (1980), Stuchly and Stuchly (1980) and Pethig (1984). The present review is designed to extend these surveys and to provide an overview of the more persuasive theories which have been formulated in an effort to understand the observable electrical properties in terms of the underlying biophysical processes.

## 2. A summary of dielectric theory

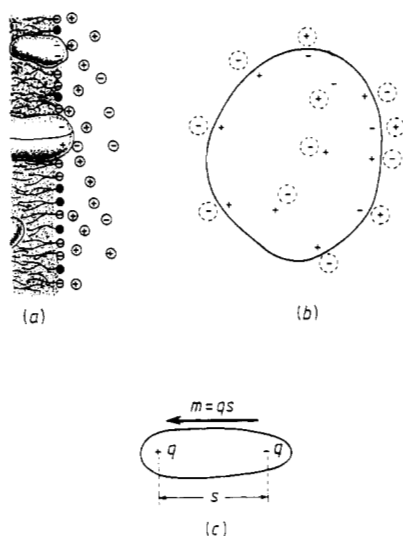
The passive electrical properties of a material held between two plane-parallel electrodes of area  $A$  separated by a distance  $d$  are completely characterised by the measured electrical capacitance  $C$  (units Farads) and conductance  $G$  (units  $\text{Ohm}^{-1}$  or Siemens), as defined in the following two equations:

$$G = A\sigma/d \quad C = A\epsilon\epsilon_0/d. \quad (1)$$

The conductivity  $\sigma$  is the proportionality factor between the electric current density and the electric field, and is a measure of the ease with which 'delocalised' charge carriers can move through the material under the influence of the field. For aqueous biological materials, the conductivity arises mainly from the mobility of hydrated ions and other processes to be described later. The factor  $\epsilon_0$  is the dielectric permittivity of free space, and has the value  $8.854 \times 10^{-12} \text{ F m}^{-1}$ , whilst  $\epsilon$  is the permittivity of the material relative to that of free space.  $\epsilon$  is (erroneously, but for historical reasons) sometimes referred to as the dielectric constant. The permittivity is proportional to the ratio of the charge to the electric fields, and reflects the extent to which 'localised' charge distributions can be distorted or polarised under the influence of the field. For biological materials, such charges are mainly associated with electrical double layers occurring at membrane surfaces or around solvated macromolecules, or with polar molecules which (by definition) possess a permanent electric dipole moment.

Figure 1 shows somewhat diagrammatic examples of electrical double layers at the surface of a membrane and around a globular protein, and the constitution of an electric dipole. The simplest molecular dipole consists of a pair of opposite, unit electric charges (of magnitude  $+q$  and  $-q$ ) separated by a vector distance  $s$ ; in this case, the molecular dipole moment  $m$  (which is an intensive property) is given as  $m = qs$  and has units of C m. For a complex protein molecule, such as that illustrated in figure 1, positive and negative charges arise from the presence of ionisable acidic and basic amino acid sidechains (see later) in the protein structure, and due to the large size of protein molecules these will give rise to a comparatively large permanent dipole moment whose value will vary with pH, molecular conformation and intramolecular mobility.

Each type of polar or polarisable entity will exhibit its own characteristic response to an imposed electric field; to describe this, we write the relative permittivity as a



**Figure 1.** An illustration of the electrical double layers formed at the surface of a charged biological membrane (a) and around a charged, aqueous globular protein (b). (c) A simple polar molecule, consisting in this case of a pair of opposite unit charges  $+q$  and  $-q$  separated by a distance  $s$  and possessing a dipole moment of  $m = qs$  C m.

complex function of the form (Debye 1929)

$$\epsilon^*(\omega) = \epsilon_\infty + (\epsilon_s - \epsilon_\infty)/(1 + i\omega\tau) \quad (2)$$

in which  $\epsilon_\infty$  is the permittivity measured at a frequency sufficiently high that the polar or polarisable entity is unable to respond to the electric field,  $\epsilon_s$  is the limiting low-frequency permittivity (sometimes called 'static' permittivity) where the polarisation is fully manifest,  $\omega$  is the angular frequency of the (sinusoidal) electrical field (in  $\text{rad s}^{-1}$ ),  $i$  is  $(-1)^{1/2}$  and  $\tau$  is the characteristic response time or relaxation time. The real and imaginary parts of the complex permittivity may also be expressed in the form

$$\epsilon^* = \epsilon' - i\epsilon'' \quad (3)$$

in which the real part  $\epsilon'$ , corresponding to the permittivity described in equation (1), is given by

$$\epsilon'(\omega) = \epsilon_\infty + (\epsilon_s - \epsilon_\infty)/(1 + \omega^2\tau^2). \quad (4)$$

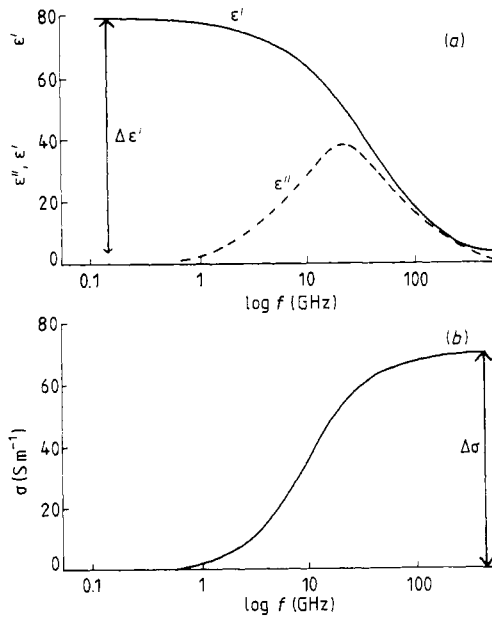
The imaginary component  $\epsilon''$ , known as the dielectric loss, corresponds to the dissipative loss associated with the movement of polarisable charges in phase with the electric field and is given by

$$\epsilon'' = (\epsilon_s - \epsilon_\infty)(\omega\tau)/(1 + \omega^2\tau^2). \quad (5)$$

It takes the form of a peak (loss peak) such as that shown for water in figure 2. The loss factor  $\epsilon''$  may also be defined in terms of a frequency-dependent conductivity as

$$\epsilon'' = \sigma(\omega)/\omega\epsilon_0 = (\sigma_0 + \sigma_d(\omega))/\omega\epsilon_0 \quad (6)$$

where  $\sigma_0$  is the steady state conductivity arising (predominantly) from mobile ions and  $\sigma_d(\omega)$  is the frequency-dependent conductivity arising from dielectric polarisation.



**Figure 2.** The dielectric dispersion exhibited by pure water at 20 °C, illustrated in terms of the change in (a) the real ( $\epsilon'$ ) and imaginary ( $\epsilon''$ ; dielectric loss) parts of the permittivity and (b) the frequency dependence of the conductivity. The low-frequency conductivity at neutral pH, due to the presence of  $H^+$  and  $OH^-$  ions, has a value of some  $5 \mu S m^{-1}$ .

By defining the magnitude of a dielectric dispersion (figure 2(a)) as

$$\Delta\epsilon' = \epsilon'_s - \epsilon'_\infty \quad (7)$$

we obtain, by combination with equations (4) and (5),

$$\epsilon'(\omega) = \epsilon'_\infty + \Delta\epsilon' / [1 + (f/f_c)^2] \quad (8)$$

and

$$\sigma(\omega) = \sigma_s + (2\pi\epsilon_0 f^2 \Delta\epsilon') / f_c [1 + (f/f_c)^2] \quad (9)$$

in which  $f_c$  is the relaxation frequency or 'characteristic frequency' ( $f_c = 1/2\pi\tau$ ). The factor  $\sigma_s$  is the low-frequency limit of the conductivity that includes the steady state (DC) conductivity and dielectric losses associated with polarisation processes having relaxation frequencies significantly lower than that defined by  $f_c$  above. By putting  $f \gg f_c$ , a conductivity increment ( $\Delta\sigma$ ) is obtained (figure 2(b)) given by

$$\Delta\sigma = \sigma_\infty - \sigma_s = 2\pi f_c \epsilon_0 \Delta\epsilon'. \quad (10)$$

This shows that, as a dielectric dispersion (with a single relaxation time) is traversed by changing the frequency of measurement, the change in conductivity is directly proportional to the change in permittivity. This follows from the fact that the total energy in the field is constant (for a given voltage) and must either be stored (as reflected in  $\epsilon'(\omega)$ ) or dissipated (as reflected in  $\epsilon''(\omega)$ ) by the system with which it interacts. This allows an additional check upon the validity of the experimental data.

Equation (10) may therefore be written in the form

$$\tau = \Delta \epsilon' \epsilon_0 / \Delta \sigma. \quad (11)$$

Equations (10) and (11) hold strictly only for a process with a single relaxation time; however, they are obeyed reasonably well provided that any spread of relaxation times is not too large.

It is useful to note that by using frequency as the parameter a 'complex permittivity' plot of  $\epsilon''$  against  $\epsilon'$  may be obtained; this has the form of a semi-circle whose centre lies on the abscissa and which intersects the  $\epsilon'$  axis at the points  $\epsilon'_\infty$  and  $\epsilon'_s$ , and such a plot may be used to derive values for  $\epsilon'_\infty$  and  $\epsilon'_s$  when, for technical or other reasons, the frequency range over which one can measure is restricted in some way. This is known as a Cole-Cole circle and it is named after the brothers K S and R H Cole who first derived it (Cole and Cole 1941). A similar plot, the complex conductivity plot (Grant 1958), may be obtained by plotting  $\sigma'' (= \omega \epsilon_0 (\epsilon' - \epsilon'_\infty))$  against  $\sigma'$ , and used to derive the values of  $\sigma_s$  and  $\sigma_\infty$ . When the system exhibits only a single relaxation time, the frequency at which  $\epsilon''$  and  $\sigma''$  take their maximum values is equal to the characteristic frequency.

For real systems, and especially in biological work, it is commonly observed that the centre of the circular locus traced by the data in these complex plane diagrams lies *below* the abscissa. This is conventionally interpreted (but cf Jonscher 1983) in terms of the existence of an ensemble of processes possessing a significant distribution of relaxation times and contributing independently to the macroscopically observable dispersion of interest, although the appearance of these plots as arcs of a circle is only barely influenced by the exact type of relaxation time distribution function invoked (Schwan 1957). Nonetheless, a particular analysis of the foregoing (Cole and Cole 1941) is widely used in dielectric studies of biological materials since, though empirical, it allows an economy in the expression of the degree to which a particular relaxation exhibits heterogeneity in its relaxation times. We therefore briefly discuss the salient feature of the Cole-Cole analysis.

What Cole and Cole (1941) realised was that a simple empirical modification of the Debye equation could be derived which had the property that, whilst a plot of  $\epsilon''$  against  $\epsilon'$  would give a locus that traced the arc of a circle, the semi-circle so extrapolated would have a centre that both lay below the abscissa and which would make an angle  $\alpha\pi/2$  rad with the points ( $\epsilon'_s$ ) or ( $\epsilon'_\infty$ ), thus still permitting the estimation of  $\epsilon'_s$  and  $\epsilon'_\infty$ . This Cole-Cole  $\alpha$  appears in the modified Debye equation as follows:

$$\epsilon^*(\omega) = \epsilon_\infty + (\epsilon_s - \epsilon_\infty) / [1 + (i\omega\tau)^{1-\alpha}]. \quad (12)$$

Whilst this Cole-Cole  $\alpha$  is an entirely phenomenological variable (it lacks a proper molecular or physical basis), and Boyd (1980) surveys many other modifications of the Debye equation which may also be used to fit or to express dielectric data, the Cole-Cole formalism remains overwhelmingly the most common means of describing data from experiments on the passive electrical properties of biological systems. Furthermore, whilst the *actual* distribution of relaxation times embodied in the Cole-Cole formula is rather complex (e.g. Cole and Cole 1941, Hasted 1973), a variety of plausible relaxation time distributions (e.g. Gaussian, rectangular) gives behaviour that is for practical purposes indistinguishable from 'true' Cole-Cole behaviour (Schwan 1957). It is also true that the sum of two Debye processes can not be distinguished from a small distribution of relaxation times (Sheppard and Grant 1974). We may therefore characterise any dielectric dispersion observed in biological materials

in terms of three parameters only: the dielectric increment  $\Delta\epsilon'$ , the relaxation time ( $\tau$ ) or characteristic frequency  $f_c$ , and the Cole-Cole  $\alpha$ .

A simple physical model, which one may use to gain a mental picture of the processes occurring during the dielectric relaxation of a molecule with a permanent dipole moment, is one that considers the dipoles to be spheres whose rotation in response to the field is opposed by frictional interaction with the surrounding viscous medium. The relevant relaxation time for the orientation of such a sphere is given by

$$\tau = \chi/2kT \quad (13)$$

where  $\chi$  is a molecular friction coefficient which relates the torque exerted on the dipolar molecule by the applied electric field to the molecule's angular velocity,  $k$  is Boltzmann's constant and  $T$  is the absolute temperature. Assuming the dipole to be equivalent to a rigid sphere of radius  $a$  turning in a Newtonian hydrodynamic fluid of macroscopic viscosity  $\theta$ , the Stokes-Einstein relation gives  $\chi = 8\pi\theta a^3$  so that the relaxation time is

$$\tau = 4\pi\theta a^3/kT. \quad (14)$$

Considering that the model described is virtually as simple as one may conceive, equation (14) often gives remarkably good results, even for the case of water molecules rotating in bulk water. In bulk water, the distance between adjacent oxygen molecules is 0.28 nm, i.e.  $a = 0.14$  nm. At 293 K, the viscosity of water is  $10^{-3}$  kg m<sup>-1</sup> s<sup>-1</sup> (i.e. 1 mPa s), so that the value for the relaxation time of water at this temperature that one would derive from equation (14) is 8.5 ps, in excellent agreement with the experimentally observed value of 9.3 ps. However, it should be added that this relationship between viscosity and dielectric relaxation can also be accounted for using a model of water based on directed hydrogen bonds (Grant 1957). The relaxation time of 9.3 ps is equivalent to a characteristic frequency of some 17 GHz, and the frequency-dependent values of  $\epsilon'$  and  $\epsilon''$  for bulk water between 100 MHz and 100 GHz are given in figure 2(a). The corresponding increase in  $\sigma$  for 'pure' water as this frequency range is scanned is from about  $5 \times 10^{-6}$  S m<sup>-1</sup> to some 70 S m<sup>-1</sup>, and is displayed in figure 2(b). The low-frequency conductivity of the electrolyte solutions typical of higher eukaryotes (usually taken as equivalent to that of a 150 mM NaCl solution) is approximately 2 S m<sup>-1</sup>, so that above 2 GHz or so their conductivities are dominated by the water present (at a concentration approaching 55.5 M), and thus exhibit approximately the same frequency dependence as that shown in figure 2(b).

Following Kirkwood (1932, 1939), we may relate the dielectric increment (a macroscopic observable) to the dipole moment  $m$  and the molecular weight  $M$  (both intensive properties) of the polar molecule according to the relationship

$$\Delta\epsilon' = Ncgm^2/2\epsilon_0MkT \quad (15)$$

where  $N$  is Avogadro's number,  $c$  the concentration in kg m<sup>-3</sup> of the polar molecule in the solvent and  $g$  a parameter introduced by Kirkwood to account for molecular associations and correlation effects between the motions of solute and solvent molecules. Such effects are particularly noteworthy for hydrogen-bonded liquids, where the rotation of one molecule seriously disrupts the local hydrogen bonding and requires the correlated or cooperative reorientation of as many as four neighbouring water molecules to compensate for this (Hasted 1973, Hallenga *et al* 1980). For four-bonded water at room temperatures,  $g$  has a value of about 2.8, dropping to 2.5 at 100 °C. For zero-bonded water molecules the value of  $g$  is, of course, unity. Evidently, a value

for  $g$  of unity implies no intermolecular associations, or that the net value of such associations cancels out, as for a protein molecule in an aqueous environment where there will be numerous solute-solvent forces acting over a wide range of directions (South and Grant 1972). We shall see shortly that  $g$  values for  $\alpha$ -amino acids are of the order of 1.2.

### 3. Amino acids, peptides, proteins and DNA

#### 3.1. Amino acids

$\alpha$ -amino acids have the general formula  $R-C_\alpha(H)(NH_3^+)-COO^-$ , and thus exist in two forms of opposite optical activity due to the fact that, unless the sidechain  $R=H$  as in glycine,  $C_\alpha$  constitutes a chiral centre. These sidechains, of which twenty are particularly widespread in nature, determine in large measure the dielectric properties of proteins and polypeptides. Given the presence of both amino and carboxyl groups, the degree of ionisation of  $\alpha$ -amino acids is obviously strongly pH dependent; the doubly charged form shown above is known as a zwitterion and is predominant at neutral pH. The zwitterionic nature of amino acids has the consequence that their solvation by water is accompanied by a large negative change in volume, resulting from the strong electrostatic interaction between the polar water molecules and the two charged groups. Similarly, since the zwitterion represents a large dipole, neutral solutions of amino acids (which may have a negligible DC conductivity) exhibit a high permittivity and absorb infra-red radiation at  $1580\text{ cm}^{-1}$ , an absorption band (in wavenumbers) characteristic of the carboxylate ion, and not at  $1720\text{ cm}^{-1}$  as would be the case for an uncharged  $-COOH$  group.

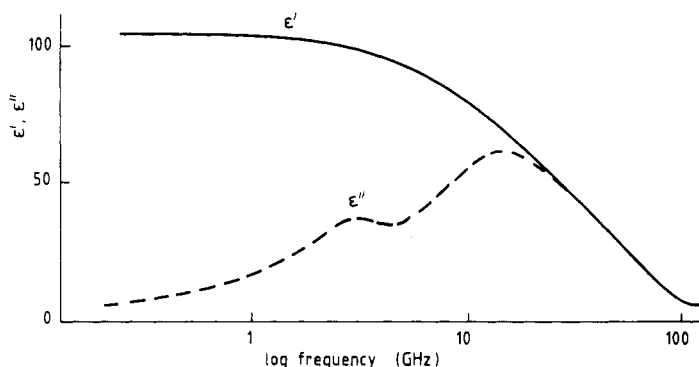
As mentioned above, the simplest  $\alpha$ -amino acid, and the only common one to lack a chiral centre, is glycine, in which  $R=H$ . The distance between the centres of the positive ammonium group and the negative carboxyl group should be about 0.32 nm, so that the effective dipole moment should have a value given by

$$m = qs = (1.6 \times 10^{-19}) \times (3.2 \times 10^{-10}) = 5.1 \times 10^{-29} \text{ C m} = 15.3 \text{ Debye units (D)} \quad (16)$$

( $1 \text{ D} = 3.33 \times 10^{-30} \text{ C m}$ ). (The displacement of one electronic charge through  $10^{-10} \text{ m}$  gives a dipole moment of 4.8 D.) This value of the dipole moment calculated for glycine solutions compares reasonably with that of 20 D obtained by Wyman (1934) from dielectric measurements. Dunning and Shutt (1938) showed further that the permittivity of glycine solutions is constant from pH 4.5 to pH 7.5 but falls sharply on either side of these pH values. The interpretation of this is that at the extremes of pH glycine possesses a single net charge only, so that the more dipolar, zwitterionic form disappears in acid or alkaline solutions.

The dipole moment per unit volume of a zwitterionic  $\alpha$ -amino acid is larger than that of water, so that an amino acid solution exhibits a greater low-frequency or static permittivity than does water, as is illustrated for glycine in figure 3. At room temperature, the characteristic frequency of the dielectric dispersion due to the rotation of glycine is 3.3 GHz (Grant *et al* 1978), so that the dispersion overlaps with that due to water (figure 3). Actually, because of the electrostatic interactions between the amino acid and the water molecules, the simple dipole model used in the derivation of equation (14) (and which would predict  $f_c = 12.56 \text{ GHz}$  for glycine) is invalid. Qualitatively, it is not surprising that the experimental finding is that the rotation of glycine is slower than that calculated on the basis that there are no molecular interactions between the solute and solvent save those frictional forces to be expected for a solid





**Figure 3.** The dielectric dispersion exhibited by a 1 M aqueous solution of glycine in its zwitterionic form. It may be observed that the low-frequency permittivity exceeds that of pure water (figure 2), whilst, as more evident in the loss peaks, the dispersion of the glycine overlaps that of the water molecules.

sphere rotating in an isotropic fluid. Quantitatively, no straightforward theoretical treatment of the dielectric behaviour of amino acid solutions exists. Thus, even in the case of the simplest amino acid, we lack a precise, quantitative molecular interpretation of the observable dielectric behaviour. We shall have further cause to ponder such difficulties when we consider the dielectric properties of proteins and more complex ensembles of biological molecules.

It is possible to express the dielectric behaviour of amino acids in aqueous solution in the form

$$\epsilon' = \epsilon'_1 + \delta c \quad (17)$$

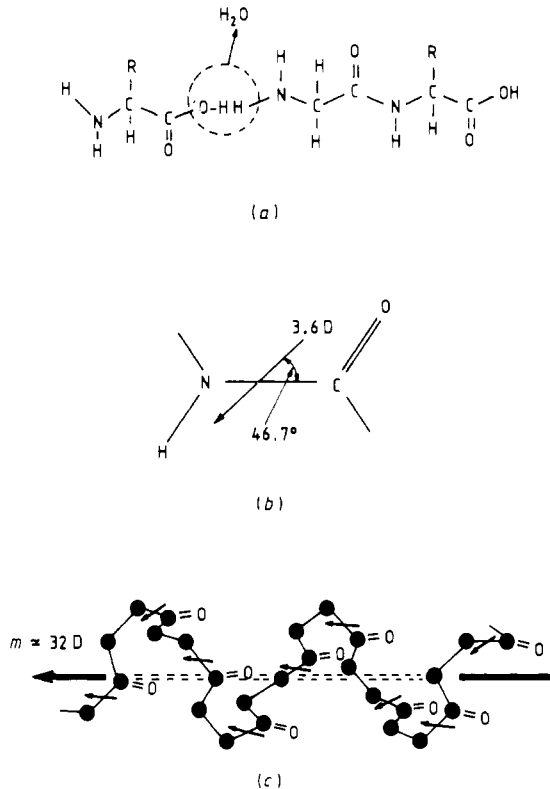
where  $\epsilon'$  and  $\epsilon'_1$  are the permittivities of the solution and pure solvent, respectively.  $c$  is the molar concentration of the solute and  $\delta$  is a constant which is related to the total dielectric increment  $\Delta\epsilon'$  by the expression

$$c\delta = \Delta\epsilon' - \Delta\epsilon_\infty. \quad (18)$$

In this equation,  $\Delta\epsilon_\infty$  is the high-frequency decrement and is equal to the amount that the static permittivity of pure water is lowered by the presence of the solute (Grant *et al* 1978, p 175, Young *et al* 1982).  $\delta$  therefore quantifies the increase in polarisation due to the presence of the amino acid of interest. Bearing in mind that pure water has a molarity of 55.5, it is perhaps not surprising that the measured permittivity of amino acid solutions varies more or less linearly with the concentration of solute (i.e. that  $\delta$  is constant), even for what are, from a biological standpoint, rather high concentrations of amino acid. For aqueous solutions of  $\alpha$ -amino acids at 25 °C,  $\delta$  has a value of some 26–28 permittivity units  $M^{-1}$  for frequencies up to 1 GHz and concentrations up to 2.5 M. If we take the value for the dipole moment of glycine (15.3 D) as being typical, a value for the Kirkwood  $g$  of approximately 1.2 may be obtained from equation (15) for amino-acid solutions.

### 3.2. Polypeptides and proteins

The way in which polypeptide chains are built up is shown in figure 4(a). These peptide bonds take the form of an amide linkage resulting from the condensation of the  $\alpha$ -amino and  $\alpha$ -carboxyl groups of adjacent amino acids. As the polypeptide increases in length, the distance between the terminal amino and carboxyl charges also



**Figure 4.** The construction of an  $\alpha$ -helical structure in a protein. (a) The formation of a peptide bond, via the condensation of the  $\alpha$ -amino and  $\alpha$ -carboxyl groups of  $\alpha$ -amino acids. (b) The magnitude and direction of the dipole moment possessed by an unmodified peptide bond. (c) The arrangement of the dipole moments of individual peptide bonds in the classical  $\alpha$ -helix structure of a protein.

tends to increase, and thus the effective dipole moment and observed dielectric increment. For polyglycine chains up to the heptapeptide, the value of  $\delta$  increases according to the relation

$$\delta = 14.51n - 5.87 \quad (19)$$

where  $n$  is the number of chemical bonds between the terminal amino and carboxyl groups (Pethig 1979, p 83).

Protein molecules are composed of one or more polypeptide chains folded in a complex, fractal geometry (Creighton 1983). The static and dynamic three-dimensional structure of proteins is controlled at different levels of organisation by non-covalent interactions between the peptide bonds, amino acid sidechains and solvent molecules, to form regions of  $\alpha$ -helix or  $\beta$ -pleated sheet (Salemme 1983), for example (Kabsch and Sander 1983). The N—C bond in the peptide units has a partial double-bond character, so that the six atoms  $C_\alpha NHCOC_\alpha$  are coplanar. In addition, the C=O bond is itself polar, so that the peptide bond possesses a permanent dipole moment. A relatively straightforward quantum mechanical calculation (Pethig 1979, pp 44-9) gives the magnitude of this dipole moment as approximately 3.6 D, directed at an angle of  $46.7^\circ$  to the C—N bond axis, as illustrated in figure 4(b). Since each peptide unit possesses a permanent dipole moment, polypeptide chains take the form of strings of connected dipoles.

The contribution that an individual dipole of moment  $m$  makes to the polarisability of a medium is proportional to  $m^2$ . For a completely rigid linear polymer chain of  $n$  regularly spaced dipoles of unit moment  $m$ , fixed and directed normally to the chain, the overall contribution to the polarisability will lie between zero and approximately  $nm^2$ , depending upon whether the dipoles are additive or cancel vectorially. If the dipoles are perfectly free to rotate, the contribution will be  $nm^2$ . For most proteins,  $n$  is a large number, typically 100–1000, so that the contribution of the dipoles of the peptide units to the overall dipole moment of the protein molecule, and hence to the permittivity of an aqueous protein solution, is extremely sensitive even to the 'static' polypeptide configuration.

A good example (Wada 1976) in which the peptide group moments are purely additive is the extended  $\alpha$ -helix configuration depicted in figure 4(c). The axis of a rigid polypeptide  $\alpha$ -helix is directed at approximately  $56^\circ$  to the C—N bond of the constituent peptide units and, since the peptide dipole moment is itself directed at an angle of  $46.7^\circ$  to the C—N bond, the peptide contribution to the total moment parallel to the axis of the helix will be

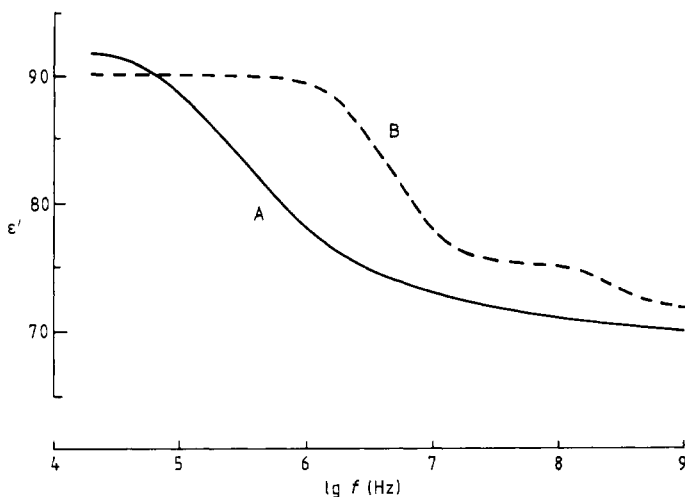
$$m = 3.6n \cos(9.3^\circ) = 3.6n \text{ D.} \quad (20)$$

The 'classical'  $\alpha$ -helix configuration (Pauling *et al* 1951) contains 3.65 peptide units per helix turn, so that even the relatively small helix of not quite three turns shown in figure 4(c) will have a dipole moment of some 34 D. Such a helix dipole moment is equivalent to there being half a positive electronic charge at the N terminus and half a negative electronic charge at the C terminus. However, dipole-dipole interactions between neighbouring  $\alpha$ -helical and other segments of the main polypeptide backbone influence the overall 'static' or 'average' polypeptide conformation (Sheridan *et al* 1982) and vice versa (Blundell *et al* 1983). Indeed, the subtle interplay between short- and long-range functional linkages between different parts of a protein molecule represents a primary focus of many studies of the effects of site-directed mutagenesis on protein structure and function (Ackers and Smith 1985), and opens up a number of interesting possibilities for the improvement of our interpretation of the dielectric behaviour of protein molecules.

We saw above that, even in the case of glycine, uncertain degrees of interaction between the solute and the solvent gainsaid our ability to properly interpret the dielectric behaviour observed. This problem is compounded in the case of proteins for two particular reasons: (i) whilst x-ray and other measurements can give us a good idea of the *static* structure of proteins, we still know rather little about how mutual interactions between different groups and domains of the protein serve to control the *dynamics* of the constituent groups (Welch 1986), and (ii) the number of atoms in typical proteins is so large that we cannot expect, *even in principle*, to gain a full picture of the dynamic organisation of a protein molecule and how this affects its functional behaviour (Welch *et al* 1982, Somogyi *et al* 1984, Kell 1986b). In the latter case, the argument runs as follows (e.g. Jaenicke 1984, Kell 1986b). A protein of molecular weight 20 kDalton might contain 200 amino acids and possess, or be able to explore, some  $10^{80}$  conformational states. Since the universe itself is 'only' some  $10^{17}$  s old (Barrow and Silk 1983), even if we allowed our protein to explore these states at a rate of  $10^{15} \text{ s}^{-1}$ , it cannot conceivably explore all of those available on any relevant timescale. If we therefore consider, for instance, the transition from the unfolded to the folded state, the above reasoning alone means that we cannot state whether or not a protein in a particular conformation is residing in a local or a global free energy

minimum. These considerations themselves raise difficulties of a philosophical nature, some of which are explored elsewhere (Kell 1986b, Welch and Kell 1986).

The value of the effective dipole moment for small globular proteins, as obtained from measurements of the dielectric increment and the use of equation (15), is typically of the order of several hundred D or 5–20 kDalton<sup>-1</sup> (e.g. Takashima 1969, Gerber *et al* 1972, Grant *et al* 1978, Pethig 1979). This corresponds to a measured dielectric increment in the range of 1–15 relative permittivity units for a concentration of 1 g (100 ml)<sup>-1</sup>. Examples of the dielectric dispersions exhibited by solutions of non-enzymic proteins are given in figure 5 for bovine serum albumin (BSA) and myoglobin (MB). The fall in  $\epsilon'$  from a value of about 90 to about 70 is due mainly, if not entirely, to the rotational relaxation of the protein molecules, and is referred to as the  $\beta$  dispersion. Consistent with this interpretation, but not conclusively so (since changes in the bulk solvent viscosity can be transmitted to motions of mobile groups on the protein surface and/or within the protein and vice versa (Beece *et al* 1980)), is the finding that the relaxation time of the  $\beta$  dispersion is proportional to the medium viscosity (Takashima 1962, Laogun *et al* 1984). However, BSA is a larger molecule than MB, so that its relaxation time (cf equation (14)) is greater. Further, given the size of protein molecules (diameter 1–10 nm), the dipole moments observed correspond to those expected if the dipole is constituted by a few electronic charges only (say less than five) separated by a molecular diameter, suggesting that the organisation of proteins is such that the majority of the vector dipoles of which they are constituted cancel to a high degree.



**Figure 5.** The dielectric properties of aqueous globular proteins. (A) Bovine serum albumin (BSA) at a concentration 100.8 mg ml<sup>-1</sup> (data after Essex *et al* 1977). (B) Myoglobin at a concentration of 10% w/v (data after Grant *et al* 1978).

For frequencies below 10 kHz (for BSA) and below 1 MHz (for MB), the rotational motion of the protein molecules can contribute fully to the polarisability of the solutions and, as the polarisability (per unit volume) of a protein is greater than that of a water molecule, the resultant (static) permittivity is greater than that of pure water. In contrast, as the frequency is raised above the rate at which the protein molecules can

reorientate by rotation, the protein molecules contribute through their atomic and electronic polarisation only, and thus the permittivity drops below the value of around 80 expected for bulk water at these frequencies. As mentioned above, this dielectric decrement should be added to the  $\delta$  defined in equation (17) in order to obtain the dielectric increment to be used in equation (15).

In an important collaborative study of the dielectric properties of BSA, Essex *et al* (1977) noted two components of the relaxation process underlying the  $\beta$  dispersion, arising from the fact that the BSA molecule is not spherical but possesses an axial ratio of some 3:1, so that it is capable of rotating about each of the axes of the equivalent ellipsoid of revolution. Additionally, these workers established that the small decrease in permittivity (the  $\delta$  dispersion) in the frequency range from about 10 to 200 MHz could also be resolved into two subprocesses; the process with a shorter relaxation time was deemed to arise from the relaxation(s) of water molecules bound to the protein. Figure 5 also shows a  $\delta$  dispersion centred at around 100 MHz for MB, which has recently been discussed in full by Grant *et al* (1986). The effects of fluctuations in the position of protons on the protein structure were considered to be the two most probable contributions to the other component of the  $\delta$  dispersion observed in BSA. As discussed elsewhere (Kell and Harris 1985b), the use of intramolecular cross-linking reagents represents a favourable experimental opportunity for assessing the contribution of amino-acid sidechain motions. That the motions of amino-acid sidechains, and entire domains of the protein, are both asymmetric (Bialek and Goldstein 1985, Glover *et al* 1985) and collective (i.e. non-linear) (see e.g. Somogyi *et al* 1984, Welch 1986, Kell 1986b) not only contributes to the difficulty in refining such interpretations (Kell and Hitchens 1983) but provides some of the more interesting and advanced possibilities for the direct detection of specific protein molecules (Kell 1986a). In this latter regard, it is worth stating that we know of no study in which the dielectric behaviour of an enzyme has been studied under turnover and non-turnover conditions; in view of the possibility that ligand binding or enzymatic activity might (transiently) be accompanied by very significant (resonant) changes in the dipole moment of the enzyme (Fröhlich 1975), it is to be hoped that such studies, aided by technical advances capable of the rapid registration of dielectric behaviour, will soon be forthcoming. Finally, we note that the addition of proteins to glycerol-water mixtures actually leads to the preferential exclusion of glycerol from proteins (Gekko and Timasheff 1981). Thus the addition of glycerol to a protein solution should not significantly affect that part of the  $\delta$  dispersion which is due to the rotation of bound water (since such bound water will not experience the macroviscosity of the glycerol relative to the forces exerted on it by the protein). In contrast, the ability of glycerol to effect viscous damping on at least some of the intramolecular motions of the protein should thus lead to a shift in frequency of that part of the  $\delta$  dispersion due to the motions of protein sidechains. Obviously the relaxation time of the protein's  $\beta$  dispersion will be increased in proportion to the microviscosity. Such an approach would seem to offer interesting and novel possibilities for the dissection of the mechanistic bases of the  $\delta$  dispersion of proteins.

Other processes that have been considered to influence the dielectric behaviour of protein solutions include relaxation of the diffuse double layer surrounding the protein molecule and 'surface' conduction processes associated with the movements of protein-bound ions. Nowadays, however, such effects are considered to contribute little to the dielectric behaviour of protein solutions (Grant *et al* 1978, Pethig 1979). Finally, the dispersion centred around 20 GHz for both the BSA and MB solutions, and indeed for

all aqueous biological material, is due to the relaxation of 'bulk' water molecules.

It is generally found that the relaxation times for proteins calculated on the basis of equation (14) lead to molecular radii that are larger than the values obtained from x-ray diffraction experiments. The interpretation of this is that there is a layer or two of water molecules (the hydration sheath) so strongly associated with the protein surface (and indeed visible as such in high-resolution (<0.15 nm) x-ray work) that they remain bound to the protein molecule as it rotates. Obviously, these water molecules experience a strong (electrostatic or dipolar) interaction with the protein and will tend qualitatively to relax at a frequency much lower than that of bulk water. This is discussed in more detail in a later section.

### 3.3. Deoxyribonucleic acid (DNA)

In its native configuration, DNA comprises two anti-parallel helical chains of nucleotides. It may take the form of linear rods or closed circles with varying degrees of supercoiling, and may have a molecular weight in the range  $10^6$ – $10^9$  kDalton. Since the two helical chains point in different directions, the dipole moments of each backbone cancel one another. Although the DNA molecule has no net permanent dipole moment due to the presence of the helices, early dielectric measurements (e.g. Takashima 1963, 1966, Hanss 1966) showed conclusively that DNA molecules possess a large dipole moment directed *along* the axis of the double helix. This dipole moment arises from the fact that the DNA molecule bears a net negative charge when in neutral aqueous solution, so that it will be surrounded by an atmosphere of counter-cations. These counter-cations will tend to be displaced along the surface of the macromolecule under the influence of an applied electric field and thereby give rise to a large induced dipole moment. The relaxation time of the resulting dielectric dispersion will depend upon the effective mobility of the ions along the macromolecule's 'surface', and for rod-shaped macromolecules will be given by

$$\tau = \pi \epsilon_1 L^2 / 2uzq^2 \quad (21)$$

where  $\epsilon_1$  is the effective permittivity of the surrounding ionic atmosphere of  $z$  ions per unit length,  $u$  is the (two-dimensional surface) counter-ion mobility,  $q$  is the charge on the ions and  $L$  is the length of the DNA molecule. The expectation that  $\tau$  is proportional to  $L^2$  is indeed borne out in practice (Takashima 1967). Typical dielectric relaxation times for DNA molecules as received are of the order of 1 ms, whilst values of the dielectric increment are large and of the order of 1000.

Apart from a dielectric dispersion ( $\alpha$  dispersion) arising from the induced dipole moment associated with the electrical double layer around the macromolecule, DNA solutions (colloids) exhibit one or more smaller dispersions in the frequency range 1–50 MHz (e.g. Mandel 1977, Takashima *et al* 1984). The relative permittivity of a 1% solution of DNA is shown for the frequency range 0.2 MHz–10 GHz in figure 6. The high-frequency tail of the  $\alpha$  dispersion is clearly evident, as well as the more rapidly relaxing dispersion centred in this case around 20 MHz; the latter is considered to arise from motions of polar groups within the DNA molecule (Takashima *et al* 1984). As with the protein solutions, dielectric dispersion above 1 GHz is dominated by the relaxation of bulk water, and does not differ appreciably from that observed in pure water.

The finding of Takashima *et al* (1984) that the high-frequency (1–10 GHz) dielectric behaviour of an aqueous solution of DNA does not differ appreciably from that of pure

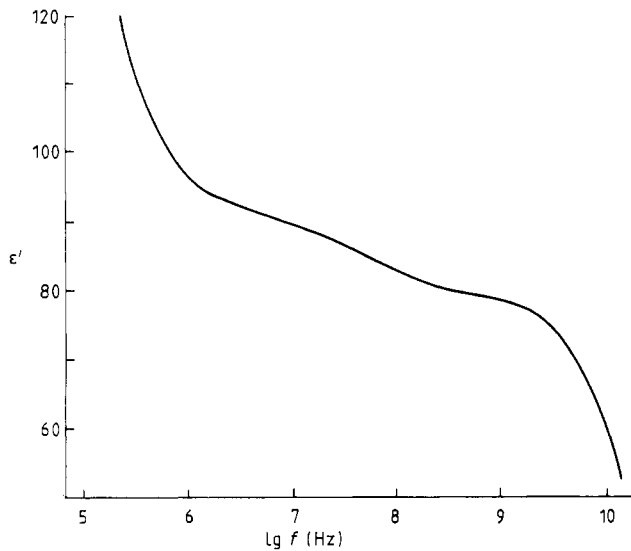


Figure 6. The dielectric properties of a 1% solution of calf thymus DNA (data after Takashima *et al* 1984).

water is in contrast to recent reports that large, *resonant* absorptions can take place in DNA solutions at these frequencies (Swicord and Davis 1982, 1983, Edwards *et al* 1984, 1985). These latter workers have provided detailed evidence for the possibility that aqueous solutions of monodisperse, plasmid DNA at room temperature can resonantly absorb microwave energy, and that the mechanism for this absorption is related to the coupling of the electric field to acoustic modes (standing waves) in the macromolecule. If such resonance effects, which are really only visible against the 'background' dielectric properties of the solvent when the DNA is very homogeneous, can be confirmed (and an extensive effort by Grant and colleagues has failed to do this (see Gabriel *et al* 1987)), they will have significant implications. These implications include the possibility that biochemical processes might be influenced by highly selective microwave frequencies of low intensity (Fröhlich 1980, Fröhlich and Kremer 1983) and that acoustic modes (phonons, solitons, etc) could provide a dissipation less mechanism for transporting free energy over large molecular distances (see Kell and Hitchens 1983, Kell and Westerhoff 1985, Kell 1986b and references therein). Obviously, confirmation of such findings would similarly stimulate the search, using dielectric methods, for resonant modes in proteins as well.

Since they may form a variety of secondary structures, nucleic acid molecules might be expected to exhibit a dielectric dispersion at very low frequencies ( $f_c < 10$  Hz). Whilst this is not likely to be easily observed directly by dielectric means (for technical reasons), the hysteretic behaviour of such structural transitions has led to the development of an extremely useful orthogonal pulsed-field gel-electrophoretic technique for the separation of intact chromosomal DNA (Schwartz and Cantor 1984, Townson 1986). Such developments provide an excellent illustration of the use of dielectrically-based methods in analytical and preparative biochemistry and biophysics.

#### 4. Bound water in biological systems

Although most (but not all) of the hydrophilic amino-acid residues of a protein molecule will be situated on the outside 'surface' (Connolly 1983) of the protein in contact with

the aqueous environment, significant areas of the protein surface are likely to be made up of hydrophobic regions. In lysozyme, for instance, approximately half of the exposed protein sidechains are non-polar (Lee and Richards 1971), although *functionally*, as judged by hydrogen-exchange kinetics, 'full' hydration is attained when less than half the surface residues have actually *been* hydrated (Schinkel *et al* 1985). The water molecules surrounding such hydrophobic groups will be forced to form networks of hydrogen bonds with each other in a way that differs from those characteristic of normal, bulk water; computer simulations indicate (Lee *et al* 1984) that such modifications in water structure can extend at least 1 nm into the bulk liquid from the hydrophobic surface. Indeed, dielectric studies of the effect of hydrophobic solutes on water have shown (Hallenga *et al* 1980) that the dielectric relaxation times are increased, suggesting that there is an increased degree of interaction (presumably hydrogen bonding) in the immediate neighbourhood of the hydrophobic solutes.

In a protein solution, one may expect that some water molecules will form hydrogen bonds with the protein structure and others will experience strong electrostatic interactions with charged groups such as those in glutamate and lysine residues. Water molecules near charged membrane surfaces will be similarly affected. All of these factors result in the formation of a 'layer' or two of water molecules near biomacromolecular surfaces, characterised by the fact that they have physical (including dielectric) properties which differ from those of normal bulk water. Such water molecules are usually referred to as 'bound' water, the dielectric behaviour of which has recently been reviewed for several types of biological molecule (Grant *et al* 1986).

The existence of protein-associated or bound water, which as a result of its hindered rotational mobility exhibits dielectric properties which differ from bulk water, was first indicated by studies of the dielectric behaviour of protein solutions at radiowave frequencies (Oncley 1943) and later confirmed by measurements at microwave frequencies (Buchanan *et al* 1952). On extrapolating the data observed to frequencies below that where the relaxation of bulk water occurs, Buchanan *et al* found that the value of the relative permittivity was lower than expected. This was ascribed to the existence of water 'irrotationally' bound to the protein molecules, in an amount corresponding to 0.2–0.4 g g<sup>-1</sup> of the protein. The identification of the  $\delta$  dispersion in terms of the relaxation of such bound water was made by Schwan (1965a, b) and Grant (1965) during studies of haemoglobin and albumin solutions.

By making measurements of the dielectric properties of protein powders of various degrees of hydration, effects associated with the rotation of the protein molecules themselves and with the relaxation of ions in electrical double layers are largely avoided, so that the dielectric properties of the protein-bound water may be investigated directly. Measurements by Bone *et al* (1977, 1981) of the microwave dielectric properties of protein powders as a function of the degree of hydration indicate that some 7–8% of the water molecules associated with a fully hydrated protein are so bound as to be unable to contribute to the dielectric polarisation at 10 GHz, a frequency at which normal bulk water exhibits a marked dielectric absorption. Subsequent investigations (Bone and Pethig 1982) of the  $\delta$  dispersion for hydrated lysozyme powders indicated that approximately 36 water molecules are tightly incorporated into each lysozyme molecule and as such form an integral part of the overall protein structure. It was also concluded that vibrational motions within the protein structure contribute to the  $\delta$  dispersion, especially at the higher levels of hydration, where the water appears to act as a molecular plasticiser or lubricant for the protein (Bone and Pethig 1985), a point of substantial significance to our understanding of the relationship between



protein flexibility and enzyme kinetic properties (Rupley *et al* 1983). Such considerations may also lead to a re-evaluation of the relative significance of the motions of bound water and protein sidechains in contributing to the  $\delta$  dispersion in aqueous solutions of globular proteins.

The physical state of water associated with DNA molecules has also been investigated by dielectric means. Microwave studies (Cross and Pethig 1983) on herring-sperm DNA in the temperature range 90–300 K have shown that a considerable proportion of the bound water, corresponding to some 280 molecules per DNA helix turn, exhibits properties significantly different from those of bulk water, especially in terms of their dielectric relaxation below 273 K. Indeed, an effective depression of the freezing point of this water of no less than 138 K was observed in this study (Cross and Pethig 1983), indicating that the loss of rotational mobility with decreasing temperature is a much more gradual process than occurs in the normal (and cooperative) water-ice transition. Computer simulations by Clementi (1983) suggest that, for levels of hydration up to some 270 water molecules per helix turn, the ensemble of water molecules rather accurately mirrors the electrostatic field generated by the DNA backbone.

The strength of ion pairs and hydrogen bonds between amino and carboxylate groups in proteins will obviously be a strong function of the degree of hydration of the protein. As the hydration is increased, the ability of protons to move between such groups will similarly be increased. The presence of fluctuating populations of protons will be of great relevance to the dielectric properties of structural and membrane-bound proteins, and also of significance in the analysis of proton-conducting pathways in general (see Westerhoff *et al* 1984). In an important series of measurements, Careri *et al* (1980) obtained significant details of the protein-water interaction for lysozyme (see Finney and Poole 1984). By measuring the infrared absorbance at  $1580\text{ cm}^{-1}$  (an absorbance characteristic for the carboxylate anion) and the specific heat as a function of hydration, these workers found that the first water molecules to bind to lysozyme interact with the ionisable carboxylate and ammonium groups. At around 5 wt% hydration ( $0.05\text{ g water g}^{-1}\text{ lysozyme}$ ), a redistribution of the proton population occurred, together with transitions in the protein-water and water-water hydrogen-bonding networks. Rates of proton transport as a function of the degree of hydration of protein powders have been described by Gascoyne *et al* (1981), Behi *et al* (1982), Careri *et al* (1985) and Morgan and Pethig (1986), and these measurements indicate that protons are able to migrate relatively freely in protein structures. In particular, the dependence of the time constant for proton motions on the extent of hydration, and the effect of a substrate analogue, strongly suggested that such motions are collective in nature and directed towards the enzyme's active site (Careri *et al* 1985). They may appropriately be treated by percolation theory (Careri *et al* 1986). Cyclodextrins have found some use as model enzymes, and studies by Bone and Pethig (1983) have shown that the flip-flop hydrogen bond networks that occur in the cyclodextrin hydrates also provide a useful model system for the study of proton conductivity.

## 5. Biological electrolytes

Mammals have a total water content amounting to some 65–70% of their body mass, and apart from the effects of dissolved macromolecules and membrane surfaces (Clegg 1984), the dielectric properties of this water are influenced by the presence of dissolved ionic salts. The physical effects of such dissolved ions on the dielectric permittivity

of biological fluids arise from more than just the volume effect of replacing polar water molecules by charged but non-polar ionic particles. In particular, the strong electric field around each ion has the effect of orienting the water molecules, reducing the way in which they can rotate in response to an applied electrical field. As for the case of amino-acid solutions, an equation of the form of equation (17) may be used to describe the permittivity  $\epsilon'$  of dilute electrolytes and that ( $\epsilon'_1$ ) of the pure aqueous solvent in terms of a dielectric decrement, as follows:

$$\epsilon' = \epsilon'_1 - \delta c \quad (22)$$

where  $\delta$  is the sum of the decrements arising from the cation and from the anion and is given by

$$\delta = \delta^+ + \delta^- \quad (23)$$

Values for  $\delta^+$  and  $\delta^-$  for various ions in water are given in table 1 for concentrations ( $c$ ) up to 1 molar, a value sufficient to cover all biologically relevant cases except that of halophilic micro-organisms. Thus, to estimate the extent to which, say, KCl will reduce the permittivity of water, we note that the total decrement value for  $K^+$  and  $Cl^-$  is  $\delta = 11$  (table 1). Thus, since the relative permittivity of pure water  $\epsilon'_1 = 79$  at 23 °C, the relative permittivity of a 1 M aqueous KCl solution at this temperature is 68.

**Table 1.** Dielectric decrement values for some ions in aqueous solution.

Cation	$\delta^+ (\pm 1)$	Anion	$\delta^- (\pm 1)$
Na <sup>+</sup>	8	Cl <sup>-</sup>	3
K <sup>+</sup>	8	F <sup>-</sup>	5
Li <sup>+</sup>	11	I <sup>-</sup>	7
H <sup>+</sup>	17	SO <sub>4</sub> <sup>2-</sup>	7
Mg <sup>2+</sup>	24	OH <sup>-</sup>	13

As well as the effect of lowering the relative permittivity of the aqueous solvent, dissolved ions will generally decrease its relaxation time. (An ion for which this is not the case is the proton.) To a first approximation, this may be considered to result from the disruption by the solvated ions of the normal hydrogen-bond structure of pure water. For concentrations of dissolved ions less than 1 molar, this may be expressed in terms of a relaxation frequency increment by the equation

$$f = f_1 + c\delta f \quad (24)$$

where

$$\delta f = \delta f^+ + \delta f^- \quad (25)$$

represents the effect of the cation and the anion. Values for  $\delta f^+$  and  $\delta f^-$  are given in table 2, and may be used to estimate how far the relaxation frequencies for various electrolytes differ from the value of 17.1 GHz accepted for pure water at 20 °C.

The overall AC conductivity of a biological (or other) material is given by

$$\sigma(\omega) = \sigma_1 + \omega\epsilon_0\epsilon'' \quad (26)$$

where  $\sigma_1$  is the conductivity arising from the electric-field induced motions of the various ions in the electrolyte. It has the approximate value:

$$\sigma_1 = q \sum z_i n_i u_i \quad (27)$$

**Table 2.** Relaxation frequency increment values for some ions in aqueous solution.

Cation	$\Delta f^+$ ( $\pm 0.2$ ) (GHz)	Anion	$\Delta f^-$ ( $\pm 0.2$ ) (GHz)
H <sup>+</sup>	-0.34	OH	0.24
Li <sup>+</sup>	0.34	Cl	0.44
Na <sup>+</sup>	0.44	F <sup>-</sup>	0.44
K <sup>+</sup>	0.44	SO <sub>4</sub> <sup>2-</sup>	1.20
Mg <sup>2+</sup>	0.44	I <sup>-</sup>	1.65

where  $i$  denotes an ionic species with valency  $z$ , concentration  $n$  and electrical mobility  $u$ . The mobility values (extrapolated to infinite dilution) for some biologically relevant ions are listed in table 3.

Since a pH value of 7 corresponds to there being  $10^{-7}$  mol of H<sup>+</sup> and OH<sup>-</sup> ions in a litre of water, i.e. to a concentration of  $6.03 \times 10^{19}$  H<sup>+</sup> and OH<sup>-</sup> ions l<sup>-1</sup>, the conductivity of pure water at, say, 24 °C may then be calculated from equation (27), using the mobility values given in table 3, as  $5.4 \mu\text{S m}^{-1}$ . In fact, H<sup>+</sup> ions rarely exist as such in water, since they are rapidly hydrated to the H<sub>3</sub>O<sup>+</sup> ion and hydrated versions thereof. The mobility of the H<sub>3</sub>O<sup>+</sup> ion is given in table 3, where it may be observed that it is the same as that of the proton. The explanation for this is that this apparent mobility of the hydronium ion is dominated by, and hence equal to, the rate at which protons transfer between neighbouring hydrogen-bonded water molecules.

It is usually more convenient to deal with the effective molar conductivities of ions; these are given in table 4 for dilute solutions, so that the conductivity of a dilute electrolyte can then be obtained from the equation

$$\sigma = \sum m_i \sigma_{mi} \quad (28)$$

**Table 3.** The electrical mobility of some ions in dilute aqueous solution at 25 °C.

Cation	Mobility ( $10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ )	Anion	Mobility ( $10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ )
H <sup>+</sup> , H <sub>3</sub> O <sup>+</sup>	36.3	OH <sup>-</sup>	20
K <sup>+</sup>	7.6	CH <sub>3</sub> <sup>-</sup> , I <sup>-</sup>	7.7
Na <sup>+</sup>	5.0	F <sup>-</sup>	5.4
NH <sub>4</sub> <sup>+</sup>	7.6	Br <sup>-</sup>	7.8
Ca <sup>2+</sup>	6.2	SO <sub>4</sub> <sup>2-</sup>	8.3

**Table 4.** Molar conductivity of some ions in dilute aqueous solution at 25 °C.

Cation	Conductivity ( $10^{-4} \text{ S m}^2 \text{ mol}^{-1}$ )	Anion	Conductivity ( $10^{-4} \text{ S m}^2 \text{ mol}^{-1}$ )
H <sup>+</sup> , H <sub>3</sub> O <sup>+</sup>	350	OH <sup>-</sup>	193
K <sup>+</sup>	73	Cl <sup>-</sup> , I <sup>-</sup>	74
Na <sup>+</sup>	48	F <sup>-</sup>	52
NH <sub>4</sub> <sup>+</sup>	73	Br <sup>-</sup>	75
Ca <sup>2+</sup>	119	SO <sub>4</sub> <sup>2-</sup>	160

**Table 5.** Water content values for various tissues and organs. Values derived from Altman and Dittmar (1972) except those for fat (unpublished data of first author) and those for ocular tissue (Gabriel *et al* 1983).

Tissue	Wt % water content	Tissue	Wt % water content
Bone	44-55	Muscle	73-78
Bone marrow	8-16	Ocular tissues	
Bowel	60-82	choroid	78
Brain		cornea	75
white matter	68-73	iris	77
grey matter	82-85	lens	65
Fat	5-20	retina	89
Kidney	78-79	Skin	60-76
Liver	73-77	Spleen	76-81
Lung	80-83		

where  $m_i$  is the molar concentration of ions of type  $i$  having a molar conductivity  $\sigma_{mi}$ . The dominant ions in the extracellular fluids of human tissue are those of sodium and chlorine, each at a concentration of some 150 mM. From tables 4 and 5 and equation (28), the conductivity of tissues such as liver and muscle should be approximately  $1.4 \text{ S m}^{-1}$ , and indeed, as described later (see table 7), this accurately predicts the conductivity at frequencies around 1 GHz where the electrical properties of tissues are determined not by the tissue structures and organisation themselves but by the electrolyte content. However, it should be stressed that the electrical conductivity at lower frequencies (and hence over a longer distance of ion movement) might be significantly lower, possibly reflecting frictional interactions between ions and dissolved or cytoskeletal materials. Thus, for those studying this problem of the organisation of the cellular cytoplasm, it may be quite misleading to state that the conductivity of a tissue or cell suspension at 1 GHz is the same as that to be expected on the basis of its ionic content and hence that there is no special organisation of the aqueous cytoplasm. This is because, as discussed in this context by Clegg (1984) for water molecules, the relevant conductivities are those which are observed over a longer range, and hence a longer timescale, than that equivalent to 1 GHz. At least in erythrocytes (Pauly and Schwan 1966) and prokaryotes (Marquis and Carstensen 1973) it is found that the electrical conductivities estimated *in situ* at frequencies lower than 1 GHz are smaller by a factor of approximately three than those to be expected on the basis of the number and nature of the ions present and indeed observed upon disruption of the cell membrane and dilution of the cellular contents. The extent to which this 'conductivity decrement' is a function of the physiological state of a given organism, and varies *between* organisms, is not known, but there are reasons to suppose (Kell and Westerhoff 1985) that the greater degree of cytoplasmic organisation to be expected in thermophilic micro-organisms might be reflected in the conductivity decrement of such organisms.

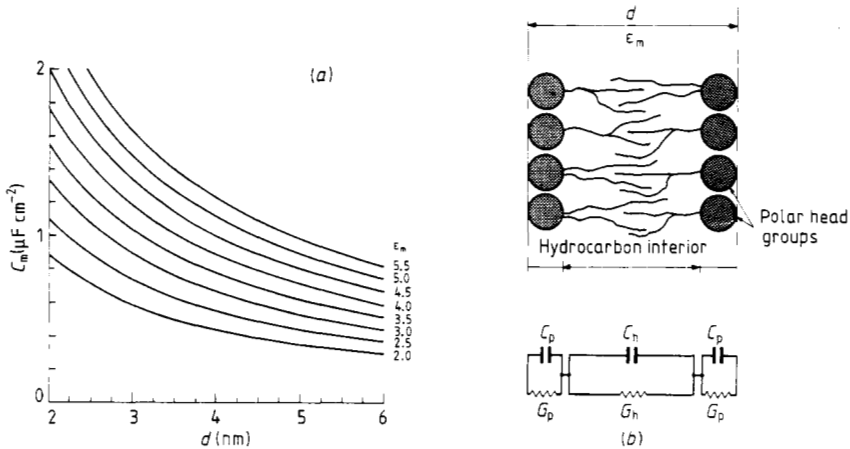
## 6. Membranes and cells

Cell membranes are generally structures with a thickness of about 5 nm, composed mainly of a bilayer of long-chain lipid molecules in, on and through which are dispersed proteins. An approximate value for the effective capacitance per unit area ( $C_m$ ) of

such a membrane can be found using the equation for a slab dielectric:

$$C_m = \epsilon_0 \epsilon_m / d \quad (29)$$

where  $d$  is the thickness of the membrane and  $\epsilon_m$  the permittivity of the membrane-forming material. Values of  $C_m$  as a function of  $d$  are plotted, for various values of  $\epsilon_m$ , in figure 7(a), where it may be seen that  $C_m$  does not exceed  $0.5 \mu\text{F cm}^{-2}$  if  $\epsilon_m = 2.5$  and  $d = 5 \text{ nm}$ . Whilst correct within a factor of two or so, this is in disagreement with the widely held textbook view (Cole 1972) that biological membranes have a static capacitance of  $1 \mu\text{F cm}^{-2}$ , and it is of interest to enquire as to why this may be so.



**Figure 7.** (a) The relationship between the static capacitance of a biological membrane of thickness  $d$  and the permittivity values given, based upon equation (29). (b) Organisation and electrical equivalent of a phospholipid bilayer. Because the capacitance  $C_h$  of the hydrocarbon region is the lowest of those given, and is in series with them, it dominates the observable electrical properties.

In the case of a simple bilayer membrane, composed of a single type of phospholipid, an estimate of  $d$  may be obtained from a measurement of  $C_m$  and vice versa. In most cases, the membrane capacitance is largely determined, to within 1 or 2%, by the hydrophobic phospholipid core (see figure 7(b)) (Hanai *et al* 1965, Everitt and Haydon 1968, Coster and Smith 1974, Almers 1978, Laver *et al* 1984) and the value of  $d$  is reasonably well established from molecular models and from x-ray, electron and neutron diffraction measurements (the latter also gives estimates of the hydration of the hydrocarbon interior (Knott and Schoenborn 1986)). A value of 2.0–2.2 has therefore become accepted for the relative permittivity of black lipid membranes (BLM) (e.g., see Hanai *et al* 1964, Tien 1974, Tien and Diana 1968, Benz *et al* 1975, Laver *et al* 1984, Dilger and Benz 1985, Kell and Harris 1985b), depending upon the phospholipid composition and the solvent used in their preparation (Fettiplace *et al* 1975).

It is reasonable to assume that the surface area of an energy-coupling biological membrane is composed of approximately 30% protein (Hackenbrock 1981, Capaldi 1982), that these proteins are arranged in complexes of about 4 nm diameter such that each complex contains one 'permeant' aqueous pore of diameter 1 nm (Edmonds 1981), and that the permittivity of the hydrophobic core is 2.2. For a spherical microbial cell of radius  $0.55 \mu\text{m}$  there will then be approximately 14 500 aqueous pores with a total surface area of  $\frac{1}{83}$  of the geometrical surface area of the cell. If each of these pores is

assumed to consist of water with a permittivity equal to that of bound water (which we may take, from what is known of the capacitance of electrode/electrolyte interfaces, to have a value of 10 (Bockris and Reddy 1970)), then since the pores are in parallel with the hydrophobic core the total membrane permittivity will be raised to a value of about 2.32 ( $=2.2 + \frac{10}{83}$ ). This value is hardly different from that of a pure phospholipid membrane given above, even when, as here, the assumptions that give rise to it are rather extreme. Thus the presence of aqueous pores, even transient ones (Weaver *et al* 1984a, b), cannot underly the paradox that the permittivity of biological membranes appears to be somewhat greater than that of BLM. Since the interior of transmembranous, integral proteins is likely to be no less hydrophobic than that of the hydrocarbon core of the membrane, it would also seem implausible to invoke a significant contribution of the proteins *per se* to the bulk permittivity and thus to the macroscopic, static electrical capacitance of biological membranes, unless one assumes that the intramembranal protein/lipid interfaces are extensively hydrated, a possibility for which no present evidence exists.

The early measurements of biological membrane capacitances which led to the widely accepted value for  $C_m$  of  $1 \mu\text{F cm}^{-2}$  (see Cole 1972, Schanne and Ceretti 1978) were carried out on cell *suspensions*, using *extravesicular* electrodes. In the case of spherical cells with a negligible transmembrane conductance at the frequencies of interest, the following equations are used to describe the electrical properties of the  $\beta$  dispersion arising from the presence of the cell membrane (see, e.g., Schwan 1957, Kell and Harris 1985b):

$$\varepsilon'_1 = \varepsilon'_x + 9PrC_m/4\varepsilon_0 \quad (30)$$

$$\sigma'_1 = \sigma'_0(1 - P)/[1 + (P/x)] \quad (31)$$

$$\sigma'_x = \sigma'_0[1 + 3P(\sigma'_i + \sigma'_o)/(\sigma'_i + 2\sigma'_o)] \quad (32)$$

$$\tau = rC_m[(1/\sigma'_i) + (1/2\sigma'_o)]. \quad (33)$$

In these equations, the subscripts 1 and  $\infty$  indicate that the measurements are made at (or extrapolated to) frequencies that are very low and very high relative to the characteristic frequency  $f_c (=1/2\pi\tau)$ ,  $P$  is the volume fraction of the suspended phase,  $r$  the cell radius, the subscripts i and o refer to the (isotropic) inner and outer phases which the cell membrane separates,  $x$  is a shape-dependent 'form factor' ( $x=2$  for a sphere) and the other symbols are as above. A nomograph has been published to aid the rapid calculation of  $f_c$  and hence  $\tau$  from equation (33) (Kell and Burns 1986).

Equation (30) shows that (as did Fricke (1925)) one may estimate the value of  $C_m$  from measurements of the dielectric increment of the  $\beta$  dispersion, provided that the charging of a 'static' membrane capacitance is the only mechanism contributing to dielectric relaxation in the relevant (radio-) frequency range, and translate this into a value for the membrane permittivity via equation (29). However, an examination of the available literature suggests that the majority of membrane capacitances found using extracellular electrodes to be greater than  $0.9 \mu\text{F cm}^{-2}$  appear to correlate with an overestimation of the membrane permittivity (e.g. Asami *et al* 1976, 1980, 1984), although in one of these studies (Asami *et al* 1980) a plot of  $\Delta\varepsilon'$  against  $P$  does not extrapolate to the origin as required by equation (30) (Harris 1985). However, it is worth drawing attention to the fact that a judicious use of measurements of the RF permittivity of cellular suspensions has allowed us to develop a novel and convenient method for the real-time estimation of (microbial or other) biomass in laboratory and

industrial fermentations, a particularly thorny and long-standing problem (Harris *et al* 1987, Kell *et al* 1987).

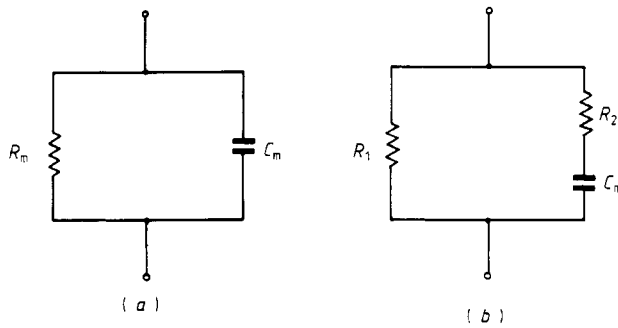
In some neurophysiological studies, measurements of  $C_m$  have been made with *transmembrane* electrodes (Takashima 1976, Haydon *et al* 1980). In this case, whilst the transmembrane capacitance may exceed  $1 \mu\text{F cm}^{-2}$  at low frequencies, the sensitivity of this value to inhibitors of the opening of ion channels, and the fact that the value drops below  $0.5 \mu\text{F cm}^{-2}$  at higher frequencies (which are still low compared with the value of  $f_c$  to be expected for a classical  $\beta$  dispersion caused by a Maxwell-Wagner type of mechanism (see later)), suggests again that the true value for  $C_m$  in biological membranes is probably not greater than perhaps  $0.6 \mu\text{F cm}^{-2}$ . In the case of neuromembranes monitored with transmembrane electrodes, it is evident that the larger values are associated with (the highly non-linear) gating currents and the consequent transmembrane movement of ions in response to the large transmembrane electrical fields which are set up under the conditions of this type of measurement.

In the case of measurements with *extracellular* electrodes, the transmembrane electrical potentials ( $V_m$ ) which are set up in response to the applied field are miniscule and given (e.g., Zimmermann 1982) by the equation

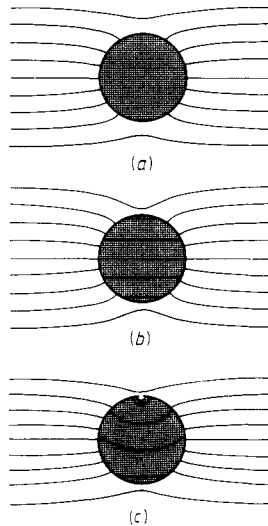
$$V_m = 1.5rE_0 \cos \phi / [1 + (\omega\tau)^2]^{1/2} \quad (34)$$

where  $E_0$  is the macroscopic electrical field between the electrodes and  $\phi$  is the angle between the plane of the membrane and the direction of the electric field. Thus gating currents and field-induced transmembrane current flow as occurring in neuromembranes are not likely to underlie the high values for  $C_m$  invoked in some studies. One possibility at least is the (partially) restricted lateral electrophoresis of double layer ions and membrane lipids and proteins, as has been discussed in detail elsewhere (Kell 1983, Harris and Kell 1985, Kell and Harris 1985a, b, Kell and Westerhoff 1985). The lateral electrophoresis of membrane components has also been observed under the microscope (Poo 1981, Sowers and Hackenbrock 1981) and is likely to contribute to field-induced modulation of biochemical activities (Chiabrera and Rodan 1984, Grattarola *et al* 1985, Kell and Harris 1985b).

Electrically, we may represent the resistive and capacitive properties of a cell membrane as an equivalent electrical circuit of the form of figure 8(a), where the membrane capacitance  $C_m$  is shown in parallel with the membrane resistance  $R_m$ . A feature of such a circuit is that with increasing frequency the membrane resistance is increasingly short-circuited by the reactance ( $1/\omega C_m$ ) of  $C_m$ . The consequence of this in terms of the electrical properties of the cell is shown in figure 9(a) and 9(b). At low frequencies (figure 9(a)) the resistance of the cell membrane insulates the cell interior (cytoplasm) from an external electrical field and no current is induced within the cell interior. Thus the cell appears as an insulating spheroid and decreases the effective conductivity of the aqueous suspension, as embodied in equation (31). Indeed, measurements of the *low-frequency* conductivity of a suspension and the fluid in which it is suspended have been exploited to provide a rapid estimation of the biomass content of immobilised cell suspensions, an otherwise extremely difficult task (Lovitt *et al* 1986). At higher frequencies (figure 9(b)), the short-circuiting effect of the membrane capacitance allows the electric field to penetrate into the cell until at a sufficiently high frequency the effective membrane resistance becomes vanishingly small and the cell appears dielectrically as a spheroid composed of the cytoplasm dispersed in the suspending electrolyte. Thus the effective permittivity and conductivity of a cell suspension will respectively fall and rise with increasing frequency, leading



**Figure 8.** (a) The equivalent electrical circuit for a cellular membrane, as assessed using transmembrane electrodes.  $R_m$  and  $C_m$  are the membrane resistance and capacitance respectively. (b) An equivalent electrical circuit for a cell or vesicle (suspension) as observed using external electrodes.  $R_1$  represents the resistance of the extracellular fluid whilst  $R_2$  represents the access admittance by which the membrane capacitance  $C_m$  may be charged. The resistors may additionally be drawn in series with another capacitor to represent the geometric (high-frequency) capacitance of the space between the electrodes. The circuit as drawn possesses a single relaxation time (i.e. dispersion) corresponding to the  $\beta$  dispersion observed in vesicular, cellular or tissue material.

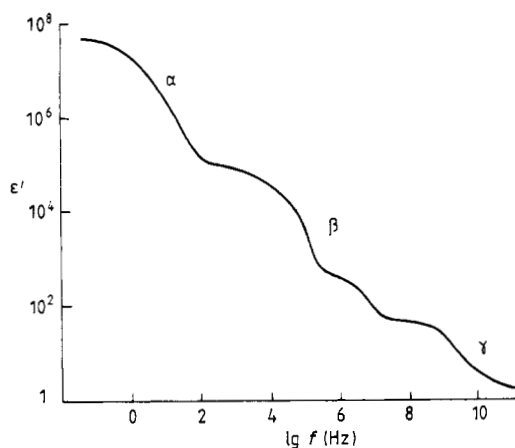


**Figure 9.** The flow of current at different frequencies relative to the characteristic frequency of the  $\beta$  dispersion of a cellular system. (a) At low frequencies, the membrane resistance shields the cell interior from the applied electrical field. (b) At higher frequencies, the membrane resistance is progressively shorted out by the membrane capacitance, such that the field enters the cell, current flows through the cell interior and the measured conductivity is increased. (For further details, see e.g. Zimmermann 1982, Kell and Harris 1985b.) (c) The effect of a small conducting pore in the cell membrane is to change the voltage drop across the membrane so that it is inhomogeneous and less than that in the situation described in (a).

to the existence of a dielectric dispersion, the  $\beta$  dispersion, of roughly the same form as the  $\beta$  dispersion exhibited by tissues (figure 10). The existence of a large, frequency-dependent dielectric increment in heterogeneous materials is generally known as the Maxwell-Wagner effect.

The representation of a completely insulating membrane, as depicted in figures 9(a) and 9(b) is obviously only an approximation. Voltage-dependent, ion-conducting





**Figure 10.** An idealised representation of the way in which the permittivity of a biological tissue such as skeletal muscle might vary with frequency, showing the three major dispersions ( $\alpha$ ,  $\beta$  and  $\gamma$ ) generally observed.

channels are known to exist in cell membranes and these could act as localised areas of low membrane resistance. Whilst the magnitude of the  $\beta$  dispersion is rather insensitive to the macroscopic conductivity of the membrane (Schwan 1957, Harris and Kell 1985), Klee and Plonsey (1974) have shown that such areas can alter the field pattern across a cell membrane, as indicated for example in figure 9(c). The exact role of such behaviour in modifying the quantitative dielectric properties of cell suspensions (Casaleggio *et al* 1984, 1985, Marconi *et al* 1985) is as yet uncertain, but is of obvious relevance to studies of electroporation and electric-field-induced cell-to-cell fusion (Zimmermann 1982) and of the ionic currents implicated in developmental processes by the aficionados of the vibrating probe approach (Nuccitelli 1986). The facts that (i) the characteristic frequency of the  $\beta$  dispersion is dependent upon the cell radius and (ii) the existence of conducting ion pores is both voltage and frequency dependent means that no hard rules can be drawn up to assess the contribution of such behaviour to the  $\beta$  dispersion in the absence of reasonably detailed electrophysiological knowledge of the system of interest. One might well imagine that patch clamp studies (Sakmann and Neher 1983, 1984, Owyer 1985) could be of benefit here, although they are not without their pitfalls (Kell 1986c).

A simple equivalent electrical circuit which is often used to represent a cell or vesicle suspension is shown in figure 8(b), where  $R_1$  represents the resistance of the extracellular medium,  $C_m$  the membrane capacitance and  $R_2$ , the so-called access impedance, is a composite function of the membrane and cytoplasmic resistances.

As with aqueous globular proteins, cell membranes contain ionisable acidic and basic groups; additionally, because of the predominance of acidic phospholipids, most cell membranes bear a net negative charge at physiological pH values. As depicted in figure 1(a), therefore, there will be an electrical double layer at the membrane-solution interface on either side of the membrane. We may therefore expect, as with DNA colloids, a dielectric dispersion due to relaxation effects within at least the extracellular electrical double layer. Such a dispersion is known as the  $\alpha$  dispersion and is similar to that shown for a 'typical' muscle tissue in figure 10. In micro-organisms it is much more evident in Gram-positive than Gram-negative cells, a consequence of

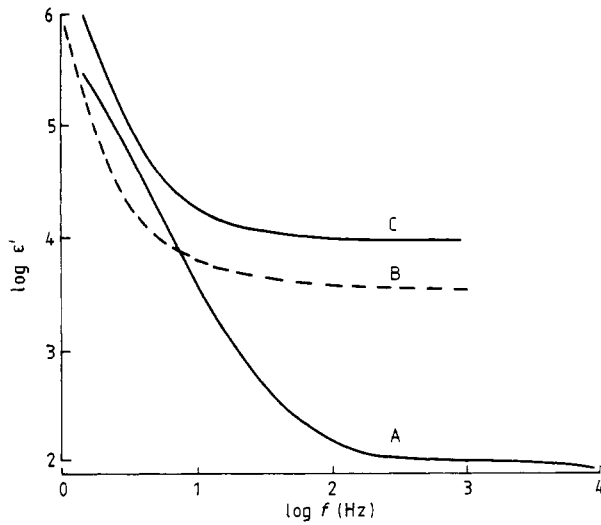
the very different organisation of the cell envelope in the two types of prokaryote (Harris and Kell 1985), although no exact theory exists due to the molecular roughness of the cell surface.

## 7. Tissues

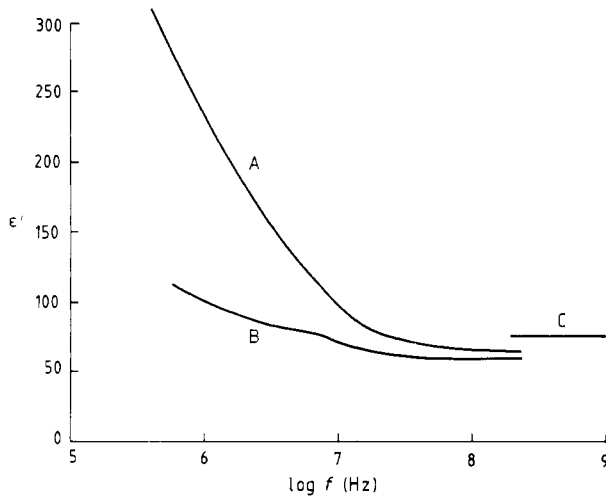
The dielectric permittivity of biological tissues typically decreases with increasing frequency in three major steps (i.e. dispersions) which are designated the  $\alpha$ ,  $\beta$  and  $\gamma$  dispersions, an idealised and widely cited representation of which (Schwan 1957) is given in figure 10. It should be mentioned, however, that in some cellular systems, the  $\alpha$  and  $\beta$  dispersions are by no means as well separated as suggested in figure 10. The  $\alpha$  dispersion is generally considered to be associated with the relaxation of ions tangential to charged membrane surfaces, while the  $\beta$  dispersion is shown as a doublet in figure 10 so as to illustrate that it is composed of both a Maxwell-Wagner effect at cell membranes and the rotation of proteinaceous material (and probably bound water). The  $\gamma$  dispersion arises mainly from the relaxation of free water within the tissues. Not the least of the problems of interpreting the dielectric behaviour of tissues arises from the fact that the volume fraction of the suspended phase approaches one, so that existing exact biophysical or mechanistic explanations, which hold only for cell suspensions of well defined geometries present at a volume fraction less than 0.2, are inapplicable.

For technical reasons connected with the measurement of very small phase angles in the presence of potentially large artefacts due to electrode polarisation (even in four-terminal measurements; see, e.g., Schwan 1963, Grant *et al* 1978, Kell 1986a), rather little work has been reported concerning the dielectric properties of tissues in the frequency range up to a few kHz. The early work of Schwan (1954) showed that skeletal muscle possessed a significant  $\alpha$  dispersion which decreased with time following the excision of the tissue, a finding qualitatively consistent with the view that the  $\alpha$  dispersion depends upon the physical integrity of cell membranes. Singh *et al* (1979) described a study of the low-frequency dielectric properties of freshly excised kidney and *in vivo* measurements, using external electrodes, of normal and malignant breast tissue. A representative selection of these data is shown in figure 11, where it may be seen that malignancy appears to influence the observed dielectric behaviour rather markedly. Another clear example of the  $\alpha$  dispersion in tissues may be found in the work of Kosterich *et al* (1983) on excised rat femurs.

The dependence of the  $\beta$  dispersion upon the integrity of cellular membranes was indicated by the work of Pauly and Schwan (1964), in which the effect of the detergent digitonin in lysing the fibre membrane of bovine eye tissue was studied (figure 12). Nonetheless, these and related experiments on erythrocytes (Pauly and Schwan 1966) used rather high concentrations of detergent, and it is also possible that the structural basis for any other type of mechanism underlying the  $\beta$  dispersion (e.g. the diffusion of membrane components) was also destroyed by the addition of the detergent. In view of current controversies regarding the interpretation of the effects of much lower concentrations of detergent on the organisation of the cytoskeleton and microtrabecular lattice (Schliwa *et al* 1981), one might be prudent to regard these early studies as less definitive than they might at first sight appear, although it must be added that disruption of the cell structure is certain to have a major effect on the  $\beta$  dispersion. Thus far we have considered the classical mechanism of the  $\beta$  dispersion in terms of the short-circuiting of the membrane resistance by the membrane capacitance and, whilst this



**Figure 11.** Dielectric permittivity of human kidney (A), normal breast tissue (B) and breast tissue containing a malignant tumour (C), as measured using a four-electrode technique (data after Singh *et al* 1979).

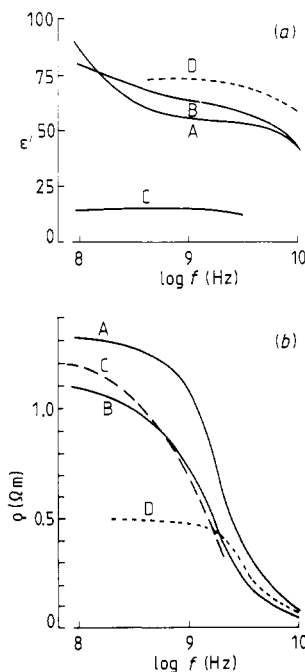


**Figure 12.** Dielectric permittivity of a suspension of bovine eye lens fibres (A), the same, after lysis of the cell membrane using digitonin (B), and a 0.9% saline solution at 27 °C (C).

is now the accepted interpretation, it does not altogether emphasise that the underlying physical mechanism resides in the interfacial polarisations associated with the fact that the membrane-solution boundary marks the interface between two heterogeneous structures. As indicated above, there is a build-up of charge at the interface between two dissimilar dielectrics, so that this gives rise to an interfacial or Maxwell-Wagner-Sillars type of polarisation. The equations for the magnitude and relaxation time of the dispersion in the case of a spherical shell membrane vesicle have been given above; other cases such as solid particles and oil-in-water and water-in-oil emulsions are discussed, for instance, in Pethig (1979, ch 4).

Figure 12 also shows the frequency dependence of the permittivity of an aqueous 0.9% w/v KCl solution, a concentration corresponding to the ionic strength of extracellular fluid. It may be observed that the dielectric properties of the tissue are rather similar to that of the ionic electrolyte above a frequency of some 50–100 MHz, although, due to the presence of non-polar membranous and other materials, the permittivity of the tissue between 0.1 and 1 GHz is slightly lower.

At these high frequencies, let us say above 100 MHz, it is to be expected that the dielectric properties of the tissues will largely reflect the properties of the intra- and extracellular electrolytes, and in particular will exhibit a dielectric dispersion corresponding to the relaxation of water dipoles, as in figure 2. To assess this, we give data for the high-frequency permittivity and resistivity of several tissues and a 0.9% saline solution in figure 13. Resistivity values are used the better to indicate differences between the tissues and the solution. It is clear that the dielectric behaviour of tissues in this frequency region is greatly influenced by the tissue water content, so that muscle, with a typical water content of 75% w/w, exhibits a much higher permittivity and conductivity than does fat (which typically has a water content of some 5–20% w/w). Since the tissue dielectric properties are rather independent of the structure and organisation of membranes at these frequencies, it is also to be expected that they will be little changed as a function of time post mortem. This expectation is in contrast to the lower-frequency behaviour, and is borne out in practice (Kraszewski *et al* 1982, Surowiec *et al* 1985, 1986). Taking these facts together, then, it is rather obvious that dielectric methods might be suitable for the rapid non-invasive determination of the water and/or fat contents of all sorts of tissues and biological (and other) materials,



**Figure 13.** The high-frequency permittivity (a) and resistivity (b) of rat brain (A), rat muscle (B) and rat adipose tissue (C) (at one fifth scale) obtained using an *in vivo* probe (data after Burdette *et al* 1980). (D) a 0.9% saline solution (data from Schwan and Li 1953 and Schwan *et al* 1976).

and indeed microwave dielectric methods have long been used to assess the moisture content of stored grain (e.g., see Pande 1975, Pyper 1985), a variable of great economic importance. The water content of various tissues is tabulated in table 5.

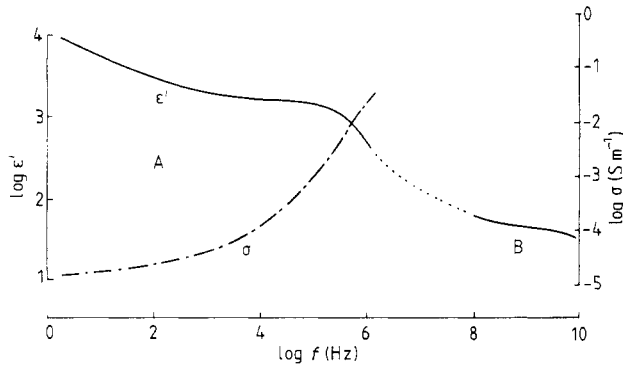
Schwan and Foster (1977), studying the microwave dielectric properties of liver, muscle and skin, concluded that these properties could be ascribed to the bulk water content with an appropriate correction being made for the presence of bound water and solid material. The same group came to a similar conclusion concerning the microwave dielectric properties of canine brain tissue (Foster *et al* 1979) and a variety of normal and malignant tissues (Foster *et al* 1980, Schepps and Foster 1980). More recent studies have confirmed (Foster *et al* 1982b, Stuchly *et al* 1982, Gabriel *et al* 1983, Smith and Foster 1985) that high-water-content tissues in particular, and also ionic and non-ionic microemulsions (Foster *et al* 1982a, Epstein *et al* 1983) and polymer solutions (Foster *et al* 1984), exhibit dielectric properties that can be accounted for by a simple mixture theory provided that a contribution from bound water is included. A similar conclusion has been arrived at by Clegg *et al* (1982), who also point out (i) that the estimation by physical methods of the degree to which tissue water is normal bulk water is extremely sensitive to the model used in the interpretation of the data, and (ii) that the degree of motional freedom ascribed to tissue water is highly dependent upon the time- (and hence distance) scale considered. As discussed above, this last point is of some significance to the estimation of intracellular conductivity values.

### 7.1. Skin

Many therapeutic and diagnostic techniques rely upon the application of electrical fields or the measurement of electrical properties. Since skin tissue often constitutes the interface between the biological and electronic parts of the system, its dielectric properties are of some interest and importance (Salter 1979, Gabriel *et al* 1986). The dielectric properties of skin are largely determined by the *stratum corneum*, which has a thickness (in non-calloused areas) of some 15  $\mu\text{m}$  and consists largely of dead cells. These dead cells are formed mainly of keratin and membranous matter and tend to wear off, to be replaced by underlying epidermal cells.

The dielectric properties of skin show considerable variability over different parts of the body, the macroscopic electrical admittance being greatest in those parts, such as the palms, which are most freely supplied with sweat ducts. Rosendal (1945) measured the dielectric properties of pieces of wet, freshly excised skin approximately 1 mm thick, and obtained values for the effective capacitance and resistance at 1 and 10 kHz of 4.6 nF  $\text{cm}^{-2}$  and 34.9 k $\Omega$   $\text{cm}^2$ , while a value of 6.2 k $\Omega$   $\text{cm}^2$  was obtained for the effective series resistance of the skin plus the underlying tissue. If the relative permittivity of dry, keratinous and membranous material is taken for the sake of argument to have a value of ten, these results may be interpreted to show that the capacitive, resistive element of skin, which provides the uppermost layer of the protective barrier between the body tissues and the environment, has a thickness of the order of 2  $\mu\text{m}$ .

The average *in vivo* electrical properties of skin in the range 1 Hz-1 MHz, determined by Schwan (1965b) and by Yamamoto and Yamamoto (1976), are shown in figure 14. An interpretation of these properties was approached via a consideration of the inhomogeneous structure and composition of skin and of the way in which this varies from the skin surface to the underlying dermis and subcutaneous tissues. It



**Figure 14.** The dielectric permittivity and conductivity of skin at 37 °C. Data based on those of Yamamoto and Yamamoto (1976) and Schwan (1965b).

may be noted that skin possesses a relatively weak  $\alpha$  dispersion (at least down to 1 Hz), and this relative lack of a significant dispersion in the frequency range 1 Hz–10 kHz is plausibly ascribed to the dead nature and low conductivity of the *stratum corneum*. Clar *et al* (1982) have found that the dispersion exhibited by normal skin in the frequency range 0.5 Hz–10 kHz can be described in terms of two separate relaxations centred at frequencies of approximately 80 Hz and 2 kHz. It was tentatively suggested that the origin of these dispersions lay in the *stratum corneum* and was associated with the relaxation of ions surrounding the corneal cells. The dielectric response of psoriatic skin was found, as might be expected, to differ significantly from that of normal skin. Finally, it is worth drawing attention to the fact that the electric impedance of those parts of the skin at which the points and meridians of acupuncture are located is significantly less than that of the surrounding tissue (see Becker and Marino 1982, Jakoubek and Rohlicek 1982), a fact which may be presumed to be of diagnostic value and may plausibly underlie the mechanism by which signals are transmitted around the body by means of the meridians of acupuncture. Whilst the relevant current carriers are not known, the fact that the locations of the meridians do not correlate with any known anatomical features might be interpreted to mean that the current carriers are protons.

### 7.2. Other tissues, including tumours

The relative permittivity and conductivity of various tissues at 37 °C are summarised in tables 6 and 7, respectively. These data, whilst considered reliable, are derived from the references cited, and might most suitably be taken to represent ‘average’ or ‘typical’ values (Lin 1975), since the physiological conditions and the history of the organisms from whence the tissues were derived are very rarely given. Indeed, it does not seem out of place to remark that the advent of rapid, digital impedimetric instrumentation means that one can now contemplate many more systematic studies than have hitherto been possible of the effect of various treatments and environments on the dielectric properties of biological systems, with a view to answering biologically relevant questions. The ranges of tissue water contents given in table 5 may be used to estimate the likely spread of electrical properties to be expected for the various tissues.

The results shown in figure 11 for breast tumours indicate that the permittivity of cancerous tissue may be significantly greater than that of normal tissues, over a wide

**Table 6.** The relative permittivity of biological tissues at 37 °C for various frequencies commonly used for therapeutic purposes.

Material	13.56 MHz	27.12 MHz	433 MHz	915 MHz	2.45 GHz	Reference
Artery	—	—	—	—	43	Brady <i>et al</i> (1981)
Blood	155	110	66	62	60	Schwan (1965b)
Bone						
(with marrow)	11	9	5.2	4.9	4.8	Pethig (1979)
(in Hank's solution)	28	24	—	—	—	Schwan (1954)
Bowel						
(plus contents)	73	49	—	—	—	Hahn <i>et al</i> (1980)
Brain						
(white matter)	182	123	48	41	35.5	Foster <i>et al</i> (1979)
(grey matter)	310	186	57	50	43	Foster <i>et al</i> (1979)
Fat	38	22	15	15	12	Burdette <i>et al</i> (1980)
Kidney	402	229	60	55	50	Burdette <i>et al</i> (1980), Stoy <i>et al</i> (1982)
Liver	288	182	47	46	44	Burdette <i>et al</i> (1980), Stoy <i>et al</i> (1982)
Lung						
(inflated)	42	29	—	—	—	Schwan (1985b), Hahn <i>et al</i> (1980)
(deflated)	94	57	35	33	—	Schwan (1965b), Hahn <i>et al</i> (1980)
Muscle	152	112	57	55.4	49.6	Schepps and Foster (1980)
Ocular tissues						
(choroid)	240	144	60	55	52	Gabriel <i>et al</i> (1983)
(cornea)	132	100	55	51.5	49	Gabriel <i>et al</i> (1983)
(iris)	240	150	59	55	52	Gabriel <i>et al</i> (1983)
(lens cortex)	175	107	55	52	48	Gabriel <i>et al</i> (1983)
(lens nucleus)	50.5	48.5	31.5	30.8	26	Gabriel <i>et al</i> (1983)
(retina)	464	250	61	57	56	Gabriel <i>et al</i> (1983)
Skin	120	98	47	45	44	Schwan (1965b), figure 13
Spleen	269	170	—	—	—	Stoy <i>et al</i> (1982)

frequency range, a trend first noted by Fricke and Morse in 1926. This feature is also indicated by the data presented in table 8 and is particularly clearly drawn in the work of Bottomley and Andrew (1978) on rat liver and that of Rogers *et al* (1983) on mouse muscle. The conductivity of tumours also seems to be greater than that of the normal tissue from which they are derived, and this property (Foster and Schepps 1981) might be of use both in the further development of the RF and microwave hyperthermic treatment of tumours and in impedance imaging systems, much as NMR imaging systems rely upon the fact that the water in tumours exhibits markedly different transverse and

Table 7. The conductivity ( $S\ m^{-1}$ ) of biological tissues at 37 °C.

Material	13.56 MHz	27.12 MHz	433 MHz	915 MHz	2.45 GHz	Reference
Artery	—	—	—	—	1.85	Brady <i>et al</i> (1981)
Blood	1.16	1.19	1.27	1.41	2.04	Schwan (1965b)
Bone						
(with marrow)	0.03	0.04	0.11	0.15	0.21	Stoy <i>et al</i> (1982)
(in Hank's solution)	0.021	0.024	—	—	—	Stoy <i>et al</i> (1982)
Brain						
(white matter)	0.27	0.33	0.63	0.77	1.04	Foster <i>et al</i> (1979)
(grey matter)	0.40	0.45	0.83	1.0	1.43	Foster <i>et al</i> (1979)
Fat	0.21	0.21	0.26	0.35	0.82	Burdette <i>et al</i> (1980)
Kidney	0.72	0.83	1.22	1.41	2.63	Burdette <i>et al</i> (1980), Stoy <i>et al</i> (1982)
Liver	0.49	0.58	0.89	1.06	1.79	Burdette <i>et al</i> (1980), Stoy <i>et al</i> (1982)
Lung						
(inflated)	0.11	0.13	—	—	—	Schwan (1965b), Hahn <i>et al</i> (1980)
(deflated)	0.29	0.32	0.71	0.78	—	Schwan (1965b), Hahn <i>et al</i> (1980)
Muscle	0.74	0.76	1.12	1.45	2.56	Schepps and Foster (1980)
Ocular tissues						
(choroid)	0.97	1.0	1.32	1.40	2.30	Gabriel <i>et al</i> (1983)
(cornea)	1.55	1.57	1.73	1.90	2.50	Gabriel <i>et al</i> (1983)
(iris)	0.90	0.95	1.18	1.18	2.10	Gabriel <i>et al</i> (1983)
(lens cortex)	0.53	0.58	0.80	0.97	1.75	Gabriel <i>et al</i> (1983)
(lens nucleus)	0.13	0.15	0.29	0.50	1.40	Gabriel <i>et al</i> (1983)
(retina)	0.90	1.0	1.50	1.55	2.50	Gabriel <i>et al</i> (1983)
Skin	0.25	0.40	0.84	0.97	—	Schwan (1965b), figure 13
Spleen	0.86	0.93	—	—	—	Stoy <i>et al</i> (1982)

spin-lattice relaxation times from that of the surrounding tissues (Hazlewood *et al* 1974). NMR results have also shown up the fact that the water content and sodium concentration of tumour tissues are higher than in normal cells (Damadian and Cope 1974, Pool *et al* 1981). The low-frequency dielectric results may also be related to the fact that cancerous cells have a reduced (less negative) membrane potential (Cone 1969, Cone and Tongier 1971) and an altered ability to take up positive ions (Ambrose *et al* 1956, Purdom *et al* 1958). By incorporating these known differences in the water content and ionic compositions of the two types of tissue (normal against tumorous), Grant and Spyrou (1985) have recently and successfully been able to model the interfacial polarisations so as to reproduce the dielectric differences that were observed by Bottomley and Andrew (1978).



Table 8. Relative permittivity ( $\epsilon$ ) and resistivity ( $\rho$   $\Omega$ m) values for some tumour tissues at 37 °C.

Material	13.56 MHz		27.12 MHz		433 MHz		915 MHz		2450 MHz		Reference	
	$\epsilon$	$\rho$	$\epsilon$	$\rho$	$\epsilon$	$\rho$	$\epsilon$	$\rho$	$\epsilon$	$\rho$		
Hemangiopericytoma	136	0.91	106	0.88	57	0.73	55.4	0.62	50	0.35	Schepps and Foster (1980) Bottomley and Andrew (1978) Hahn <i>et al</i> (1980) Rogers <i>et al</i> (1983)	
Intestinal leiomyosarcoma	309	1.2	183	1.1	62	0.81	60	0.67	54	0.38		
Splenic hematoma	297	1.56	243	1.35	54	1.08	52	0.94	49	0.53		
Rat hepatoma D23	305	1.35	178	1.15	—	—	—	—	—	—		
Normal rat liver	167	2.05	110	1.90	—	—	—	—	—	—		
Canine fibrosarcoma	48	5.6	29	5.3	—	—	—	—	—	—		
Mouse KHT tumour	30	1.55	19	1.51	—	—	(Before hyperthermia)	—	—	—		
normal muscle	—	—	~135*	—	61	0.89	(After 43 °C hyperthermia)	60	0.62	54		0.39
	—	—	~90*	—	56	1.08	56	0.66	48	0.38		

\* extrapolated data.

It is worth considering the fact that tumour cells are more electronegative than normal cells (Ambrose *et al* 1956) and that tumour tissues are similarly more electronegative (i.e. have a more negative surface potential) than normal tissues (Schaubel and Habal 1969, 1970). Such results would suggest that structurally less differentiated tissues are more electronegative than normal tissues, as observed also for regenerating tissue by Becker (1961). In conclusion, it is evident that dielectric methods can usefully complement more traditional approaches, and, particularly in concert with modern molecular biological techniques, might provide new insights into the structural and electrical differences between normal and transformed cells.

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