Eur Biophys J (1990) 18:267–276

European Biophysics Journal

Evidence from its temperature dependence that the β-dielectric dispersion of cell suspensions is not due solely to the charging of a static membrane capacitance

L. E. Ferris, C. L. Davey, and D. B. Kell

Department of Biological Sciences, University College of Wales, Aberystwyth, Dyfed SY23 3DA, United Kingdom

Received November 19, 1989/Accepted March 21, 1990

Abstract. *1.* A survey of the literature indicates that the apparent capacitance per unit area (C_m) of biological membranes is in general significantly greater than is that of 'artificial' phospholipid (black lipid) membranes (BLM). It is not possible, by quantitative arguments alone, to exclude that this simply reflects the idea that protein-containing biological membranes have a greater thickness than BLM. *2.* The *temperature-dependence* of the membrane capacitance for both solvent-containing and solvent-free BLM is negative. However, where appropriate data are available, it appears that the capacitance of biological membranes has a *positive* temperature-dependence, indicating a *qualitative* difference between natural and artificial membranes. *3.* Using a 4-terminal dielectric spectrometer, and the fitting program and electrode polarisation correction described in the accompanying paper, we have carried out a careful study of the temperature-dependence of the β-dielectric dispersion of a unicellular eukaryote (*Saccharomyces cerevisiae*) and a prokaryote (*Escherichia coli*). *4.* In the range 15–40 °C, the temperature-dependence of the β-dielectric dispersion (and thus in principle of C_m) in *S. cerevisiae* and *E. coli* is respectively +0.13 and +0.35% $(°C)^{-1}$. *5.* Flow cytometric measurements indicate that the cell size of *E. coli* is unchanged in the temperature range studied. *6.* These data strongly suggest that the β-dielectric dispersion in cell suspensions is not due solely to the charging of a 'static' membrane capacitance. It is proposed that the positive temperature coefficient of the β-dispersion reflects the contribution of temperature-dependent, partially restricted, lateral *motions* of the charged lipid and protein components of the cytoplasmic membrane.

Key words: Dielectric permittivity – Dielectric dispersion – Membrane capacitance – Protein mobility – Fluctuations

Offprint requests to: D. B. Kell

Introduction

The passive electrical or dielectric properties of tissues and of cell suspensions have beens studied since the last century (Stewart 1899; Hüber 1910). As reviewed by a number of authors (Schwan 1957; Geddes and Baker 1967; Grant et al. 1978; Schanne and Ceretti 1978; Pethig 1979, 1984; Stuchly and Stuchly 1980; Adey 1984; Chiabrera et al. 1985; Foster and Schwan 1986, 1989; Kell 1987; Pethig and Kell 1988), it is generally found that these properties are strongly frequency-dependent, exhibiting three major dispersions in the range DC-10^{11} Hz, known (according to a terminology of Schwan 1957) as the α-, β- and γ-dispersions. Our interest is focussed on the β-dispersion, which occurs typically in the radio-frequency portion of the electromagnetic spectrum.

As discussed in extenso, for instance by Schwan (1957); Cole (1972); Schanne and Ceretti (1978); Pethig (1979); Salter (1979); Schwan and Foster (1980); Stoy et al. (1982); Zimmermann (1982); Asami et al. (1984); Kell and Harris (1985); Foster and Schwan (1986, 1989); Pethig and Kell (1987); Davey et al. (1988); and Takashima (1989); the β-dispersion is normally considered to arise as a result of the fact that cell suspensions consist of more-or-less highly conducting interior and exterior phases separated by a poorly-conducting plasma of cytoplasmic membrane. The interfaces of this membrane are analogous to the plates of an electrical capacitor, so that the low-frequency-field-induced build-up of charge across the membrane is observable macroscopically as a large "static" capacitance between two extracellular electrodes. From the so-called suspension equations derived by Maxwell, and developed for this case by Fricke (1925), it became possible to calculate the membrane capacitance per unit area C_m, in μF cm^{-2}. The experiments of Fricke (1925) using erythrocyte suspensions led to a value of some 0.81 μF cm^{-2}, and since the membrane was known to be lipoidal in nature, with a dielectric permittivity in the range 3–10, it was argued from the equation for a slab dielectric that the membrane must have a thickness in the range 10–3 nm, a value consistent with those accepted

today. Numerous experiments on a wide variety of biological cells have led to the view that the biological membrane capacitance is a "constant", and of the order of some 1 μF cm^{-2} (Cole 1972).

Whilst is has proved difficult to measure C_m in whole cells using microelectrodes (although whole-cell clamp experiments are possible in large cells (Fernàndez et al. 1984; see also Sakmann and Neher 1983, 1984)), the bilayer black lipid membrane (Jain 1972; Tien 1974) has provided a convenient model system for the estimation of the capacitance of phospholipid bilayers. Such membranes are generally made either by painting a solution in a hydrocarbon solvent of the lipids of interest over a small hole in a Teflon® beaker (Muller and Rudin 1968) or by assembling them from monolayers adsorbed at the air-water interface (Montal and Mueller 1972) to form so-called solvent-free bilayers. The convenient geometry of BLM allows one to measure their capacitance directly using macroscopic electrodes in the 2 solutions on either side, and to estimate the permittivity of the different lipid regions. Since these are in series with each other, the capacitance is dominated by the lowest capacitance, due to the hydrocarbon core, and its permittivity ε_m may be calculated from the equation for a slab dielectric:

$$C_m = \varepsilon_m \varepsilon_0 \tag{1}$$

where C_m is the membrane capacitance per unit area, A the membrane area, d its thickness and ε_0 the permittivity of free space ($=8.854 \cdot 10^{-12}$ F/m).

The principal findings with BLM are as follows: *(a)* comparison of the thickness of the hydrophobic component of the membrane and the overall capacitance measured leads to a value for the permittivity of the hydrocarbon core of some 2.0–2.2, as expected (Hanai et al. 1964; Tien 1974; Benz et al. 1975; Laver et al. 1984; Dilger and Benz 1985); *(b)* BLM have an essentially "static" (frequency-independent) membrane capacitance at frequencies low with respect to their charging time (Hanai et al. 1964, 1965; Everitt and Haydon 1968), which at the usual conductivities employed is some tens of microseconds; *(c)* a very small dispersion due to interfacial layers may be observed at very low frequencies (<100 Hz; Fettiplace et al. 1971; Coster and Smith 1974; Perez and Wolfe 1989); *(d)* the presence of solvent increases the membrane thickness and hence decreases its capacitance from ca. 0.7 to some 0.4 μF cm^{-2} (Hanai et al. 1964; White and Thompson 1973; Tien 1974; Fettiplace et al. 1975; White 1978; Coster and Laver 1986; McIntosh et al. 1989); *(e)* as expected for hydrocarbon-like materials, and neglecting ranges in which phase transitions occur, it is found that increases in temperature increase the thickness of BLM, and also the percent incorporation of organic solvent, both effects thereby serving to *de*crease the membrane capacitance by some (0.3–2% $°C^{-1}$ overall (White 1970, 1975, 1978; Benz et al. 1975; Coster and Laver 1986).

Following Bangham's early work (see Bangham 1983), the liposome has also assumed popularity as a model system for the study of biological membranes. For a spherical shell suspension consisting of vesicles of radius r suspended to a volume fraction P, the dielectric increment ($\Delta\varepsilon = \varepsilon_{low} - \varepsilon_{high}$) for the β-dispersion is given by (e.g. Schwan 1957):

$$\Delta\varepsilon = 9 Pr\, C_m / 4 \varepsilon_0 \tag{2}$$

The β-dispersion in small liposomes has been studied by Schwan et al. (1970) and by Redwood et al. (1972). Because in these cases r is small, $\Delta\varepsilon$ was only ca. 1 unit in many cases, such that the data could be fitted to models in which ε_m lay anywhere between 3 and 12. However, using an elegant approach in which a *single, macroscopic* (solvent-containing) bilayer sphere could be formed and measured, a value for C_m of 0.54 μF cm^{-2} was obtained (Asami and Irimajiri 1984).

Of natural membranes, only the squid giant axon has been much exploited for capacitance measurements using *transmembrane* electrodes. Here, the principal pertinent findings (Palti and Adelman 1969; Takashima 1976; Taylor et al. 1977; Almers 1978; Haydon et al. 1980; Fishman 1985) are *(a)* that the capacitance is strongly frequency-dependent, decreasing from some 1.1 μF cm^{-2} at low frequencies (say 1 kHz and below) to 0.5 μF cm^{-2} or less above 100 kHz; *(b)* that the frequency-dependence can be greatly decreased by the addition of channel-blockers such as tetraethylammonium, Cs^+, tetrodotoxin etc., so that C_m adopts the lower value more typical of BLM (suggesting strongly that transmembrane, voltage-induced ionic currents contribute to the larger values observed normally); *(c)* that the portion of the capacitance associated with ionic conduction is strongly temperature-dependent, *in*creasing with temperature, whilst that of the background capacitance is very small (Palti and Adelman 1969; Keynes and Rojas 1976; Keynes and Kimura 1983).

As reviewed above, the β-dielectric dispersion of tissues and of cell suspensions has been measured by numerous investigators since the time of Fricke, and analysed using (2), giving values for C_m that are generally in the range 1–1.5 μF cm^{-2}. When measurements are made with *extracellular* electrodes, the field-induced increases in membrane potential are so small as to make it implausible (in contrast to the case of nerve axons) that the higher value with respect to BLM is due to the induction of voltage-gated ion-channels (Pethig and Kell 1987). The question then arises as to what other types of mechanism can account for these 'high' values characteristic of biological membranes, and we have argued (Kell 1983, 1988; Kell and Harris 1985; Pethig and Kell 1987; Davey and Kell 1989) that the *motions* of proteins (and lipids) in biological membranes must contribute both to the apparent value of C_m and to the large *distributions* of dielectric properties commonly observed (and encapsulated in the Cole-Cole α (Cole and Cole 1941)). It is to be expected that protein motions, both intramolecular and in the plane of the membrane, have *positive* temperature coefficients, enabling in principle their contribution to the measured C_m to be assessed.

Whilst the temperature-dependence of the BLM capacitance is negative (see above), that of biological membranes has been found to be positive, both in the squid axon (see above) and in a variety of tissues and cell suspensions summarised in Table 1. In spite of the large

Table 1. A comparison of the temperature-dependence of the capacitance of artificial lipid membranes and of natural membranes reported in the literature. Calculations from tabulated data are obtained from $(C_h - C_l)/C_h$ where the subscripts l and h refer to the low and high ends of the temperature range studied, and are thus designed to *minimise* the absolute numbers. Citations are restricted to those in which the full relevant frequency range was measured, or in which measurements were extrapolated to a suitable "low-frequency" value using a Cole-Cole type of analysis. It may be observed that, despite quantitative differences, there is a clear *qualitative* difference in that artificial membranes (containing only lipids) invariably have a negative temperature coefficient whilst natural membranes (with one exception) have a positive one. It should be mentioned that in the one exception (Ballario et al. 1984), the capacitance was not measured but was calculated from a theoretical model. In the study of Adams (1987) measurements were performed using square pulses

Membrane	Solvent	dC/dT (%/°C)	Reference
Pure lipids			
Lecithin BLM	Hexadecane	−0.02	Fettiplace et al. 1971
Lecithin BLM			
(<30 deg C)	Hexadecane	ca. 0	Coster and Laver
(>30 deg C)	Hexadecane	−0.3	1986
	Tetradecane	−0.8	
+30 mM benzyl alcohol	Hexadecane	−1.4	
Oxidised cholesterol BLM	Decane	−0.79	White 1970
GMO BLM	Dodecane	−0.26	White 1975
	Tetradecane	−0.7	
GMO BLM	Squalene	−0.03	White 1978
Asolectin vesicles	None	< −1	Schwan et al. 1982
Natural			
Erythrocyte		+0.3	Schwan 1948
Erythrocyte (5–35 deg C)		−0.12	Ballario et al. 1984
(15–25 deg C)		0	
Chlorella sp.		+2	Hope 1956
Squid axon (3–20 deg C)		+1.36	Palti and Adelman
(3–40 deg C)		+0.65	1969
Brain (dog grey matter)		+0.75	Stoy et al. 1982
Brain (dog white matter)		+1.52	Stoy et al. 1982
Kidney (dog)		+1.39	Stoy et al. 1982
Liver (dog)		+0.82	Stoy et al. 1982
Liver (rabbit)		+2.17	Stoy et al. 1982
Muscle (dog)		+0.85	Stoy et al. 1982
Muscle (rat)		+0.89	Stoy et al. 1982
Pancreas (dog)		+1.60	Stoy et al. 1982
Spleen (dog)		+0.28	Stoy et al. 1982
T lymphocytes		+1.28	Surowiec et al. 1986
B lymphocytes		+1.24	
Lizard skeletal muscle			Adams 1987
Sceloporus white		+1.9	
Diposaurus white		+1.3	
Dipsosaurus red		+1.2	

number of instances of a positive temperature coefficient for the β-dispersion cited in Table 1, it would be naive to extrapolate these observations to the general case without seeking a more rigorous explanation for these findings. The volume fraction of "suspended" cells in tissues is very high, such that (2) does not apply, and although modifications for high volume fractions have been suggested (Hanai et al. 1979), there is no generally accepted analysis for cells of arbitrary shape (see e.g. Cole 1970; Foster and Schwan 1989). Thus one should limit one's analysis at this stage to cell suspensions with volume fractions $<0.2-0.3$. In addition, few studies of tissues have actually *displayed* the extent of polarisation, which can be very substantial in the media characteristic of tissues from higher organisms (Schwan 1963; Grant et al. 1978).

As discussed in more detail in the accompanying paper (Davey and Kell 1990b), many if not most biological systems possess overlapping dielectric dispersions, and it is necessary to be sure that one is considering *only* the β-dispersion when trying to analyse it. The relaxation time τ for the β-dispersion of a suspension of spherical shells depends upon the internal (σ_i) and external (σ_0) conductivities and the cell radius r, according to the relation:

$$\tau = r \cdot C_m [1/(\sigma_i) + 1/(2\sigma_0)] \quad (3)$$

Thus increases in temperature cause increases in conductivity which shift the characteristic frequency (and thus the capacitance at a fixed frequency) to higher values, although the magnitude of the dielectric dispersion is not affected by conductivity per se. In other words, it is the entire dielectric dispersion $\Delta\varepsilon$ which must be considered, and not merely the permittivity at a given frequency, since if $\Delta\varepsilon$ is not measured, this could lead to an *artefactual* assessment of a positive temperature coefficient for C_m because the temperature-dependent increases in conductivity would *necessarily* drive one towards the plateau region at the low-frequency end of the β-dispersion (or even into the α-dispersion). Further, measurements are invariably corrupted, to some degree, by the presence of electrode polarisation artefacts, for which correction must be made (see Kell 1987). Therefore in analysing dielectric data of the type of present interest a fitting procedure is necessary by which to obtain $\Delta\varepsilon$. In the present work we have made use of the fitting program described in the accompanying paper (Davey et al. 1990). It should be noted that this program makes fits only to the Cole-Cole equations, which are empirical, embodying a putative distribution of relaxation times in a single parameter, the Cole-Cole α (and many distribution may be fitted to the same data (Schwan 1957; Foster and Schwan 1989)). Whilst more physically based analyses are available (Dissado and Hill 1983; Hill and Jonscher 1983; Jonscher 1983), our interest here is only in obtaining the magnitude of $\Delta\varepsilon$, for which the Cole-Cole fitting procedure is quite suitable.

Finally, it follows from (2) that the dielectric increment of a cell suspension depends on the cell radius. Temperature-dependent changes in the cell radius would lead to positive or negative temperature coefficients of $\Delta\varepsilon$ as appropriate, simply due to changes in cell size (and its distribution), much as in the case of BLM. If the causes of the β-dispersion are to be clarified, it is clearly necessary to take cell size into account. For the same reason, the organism to be studied should be free of membrane invaginations, which can contribute greatly to the apparent value of C_m (Davey et al. 1988).

For these reasons, we have carried out a careful study of the temperature-dependent dielectric increment of the β-dispersion of a prokaryotic and a eukaryotic microorganism in suspension at volume fractions less than 0.2, fitting the data according to the procedure of Davey et al. (1990) so as to extract $\Delta\varepsilon$, and where possible measuring the temperature-dependent cell sizes by flow cytometry. In no case do we find evidence for a negative temperature dependence of $\Delta\varepsilon$ for the β-dispersion, but rather for a moderately significant positive temperature-dependence. We conclude from this that the β-dielectric dispersion is not due solely to the charging of a 'static' membrane capacitance but must also contain contributions from the (temperature-dependent) internal and lateral field-induced *motions* of membrane components.

Materials and methods

Source, maintenance, growth and preparation of organisms

Saccharomyces cerevisiae was obtained locally as a cell paste and stored at 4 °C until required. The paste was slurried in 40 m*M* KH_2PO_4 to a concentration of ca. 300 mg wet weight ml^{-1}. *Escherichia coli* K12 strain C600 from the laboratory collection was maintained on glucose nutrient agar, and resuscitated in R broth (10 g tryptone, 1 g yeast extract, 5 g NaCl, 1 g glucose per litre). A 0.5 ml sample of this culture was inoculated into 2–10 l of R broth or of modified medium M9 (containing per litre: Na_2HPO_4 6 g, KH_2PO_4 3 g, NaCl 0.5 g, NH_4Cl 1 g, glucose 2 g, $MgSO_4$ 1 mmol, $CaCl_2$ 0.1 mmol, threonine 20 mg, leucine 20 mg, thiamine 20 mg). Following overnight aerobic culture to late logarithmic phase, cells were harvested by centrifugation, washed and resuspended in 40 m*M* KH_2PO_4 pH 4 at a concentration in the range 50–150 mg dry weight ml^{-1}. All experiments were carried out within 5 h of the preparation of the washed cell suspensions.

Dielectric measurements were performed using a Bugmeter (Aber Instruments, Aberystwyth Science Park, Cefn Llan, Aberystwyth, Dyfed SY23 3AH, U.K.). This is a 4-terminal instrument (Harris et al. 1987) which provides sensitive and accurate measurements of capacitance and conductance in lossy media in the frequency range 0.1–10 MHz, with a minimum contribution from electrode polarisation phenomena. The electrodes consisted of 4 coplanar stainless steel or gold-plated pins. The temperature of the contents of the reaction cell was controlled via a water jacket or by circulating hot air, and in some cases was monitored using an AD590KH temperature sensor (RS components, Uxbridge, Middlesex). The measurement of electrode polarisation was carried out in the same media in which the cells were suspended, adjusted so as to give the same conductivity at 100 kHz as that of the relevant cell suspension. Dielectric data, corrected for electrode polarisation, were analysed as described (Davey and Kell 1990 b).

Flow cytometry was carried out using a Skatron Argus instrument (Skatron Ltd, P.O. Box 34, Newmarket, Suffolk, U.K.). This instrument, based on the design of Steen (1983, 1986; see also Skarstad et al. 1985) allows one to measure the cell size and its distribution by forward light scatter. The instrument was calibrated using latex beads (Sigma, Poole, Dorset U.K. and Dyno Particles A/S, Lillestrøm, Norway) of known diameter (C.V. <2%). Cells were removed from the dielectric chamber and added at a final concentration of 25 mg ml^{-1} to 25% glutaraldehyde. Under these conditions their flow cytometric properties remained stable for many days. They were analysed in the Skatron Argus at a concentration of approx. $5 \cdot 10^6$ ml^{-1} using a PMT voltage of 470 V.

Results

Figure 1 shows a typical dielectric spectrum at 30 °C of a suspension of *S. cerevisiae* (300 mg wet wt = 100 mg dry weight ml^{-1}) as used in the present work, corrected for the small amount of electrode polarisation found, and the best fit of the data to a single (β-) dispersion carried out using the spreadsheet program described in the accompanying paper (Davey et al. 1990). The overall fit in this case is to better than 0.6%, and in every case is to better than 1%, the dielectric parameters being: $\Delta\varepsilon = 790$, characteristic frequency (f_c) = 3.0 MHz, Cole-Cole $\alpha = 0.075$. Changing the estimated dielectric increment by as little as 5 permittivity units, the characteristic frequency by 0.1 MHz, or the Cole α by 0.025 led to nearly a doubling in the percent error of the fit, indicating the accuracy achievable with the present method. It is also clear from Fig. 1 that whilst the frequency range available to us permitted us to reach the low-frequency plateau of the β-dispersion, the high-frequency plateau was not reached. Since it is the dielectric *increment* in which we are interested, the importance of the fitting procedure is evident. The dielectric increment of ca. 8 permittivity units (mg dry wt/ml)$^{-1}$ is in line with those observed

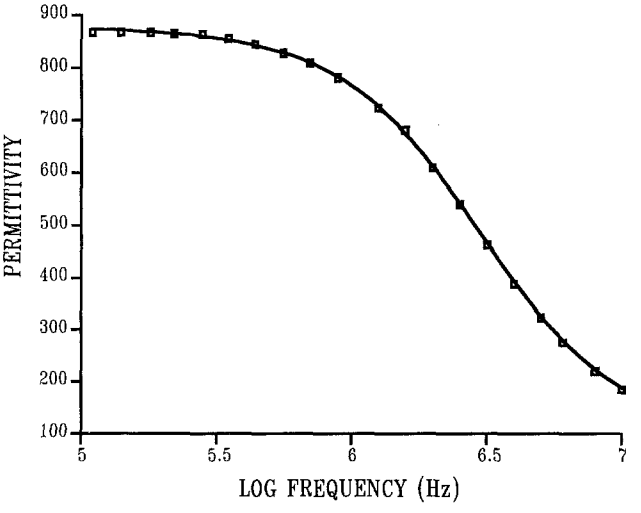


Fig. 1. Dielectric properties of *S. cerevisiae*. A suspension of *S. cerevisiae* (100 mg dry wt · ml^{-1}) was prepared, and its dielectric behaviour assessed, corrected for electrode polarisation and fitted to the Cole-Cole equation, as described in the Materials and Methods section. The temperature was 30 °C. □: data. ——: fit

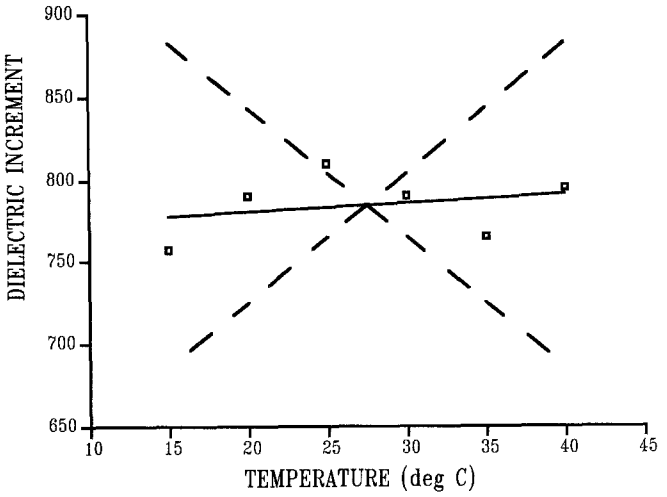


Fig. 2. Effect of temperature on the dielectric increment of suspensions of *S. cerevisiae*. Dielectric measurements were performed as described in the legend to Fig. 1, except that the temperature was varied as indicated. The full line indicates the best fit by linear regression to the data obtained, whilst the dotted lines indicate the changes to be expected if the dielectric increment changed by $\pm 1\%$ $(°C)^{-1}$, taken around the mid-point of the range of observations

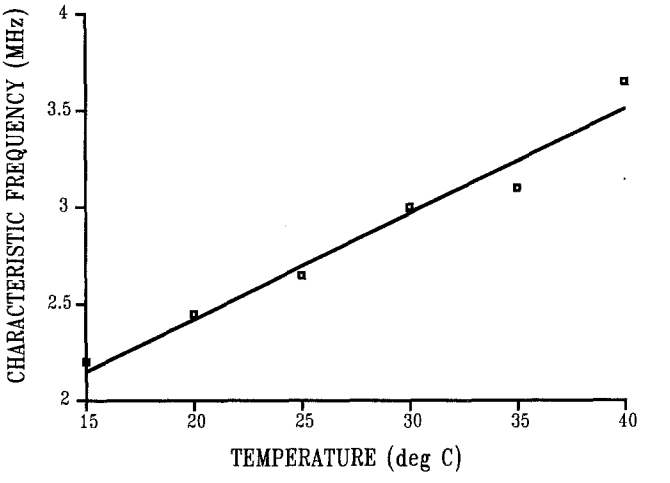
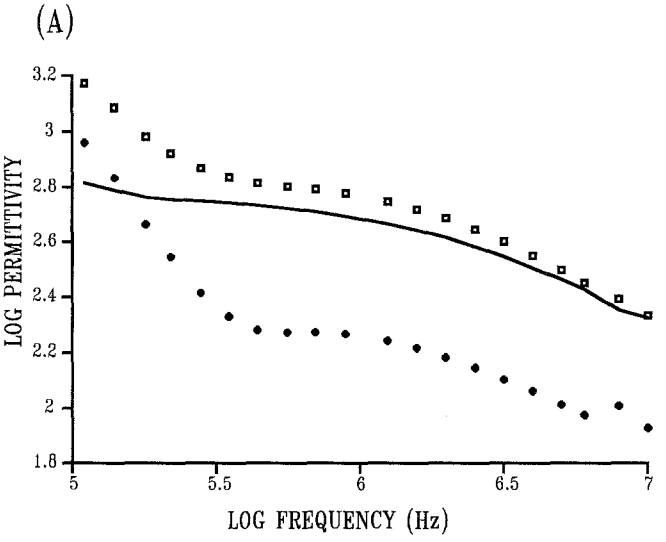


Fig. 3. Effect of temperature on the characteristic frequency of the β-dielectric dispersion of *S. cerevisiae*. Data were obtained exactly as described in the legend to Figs. 1 and 2



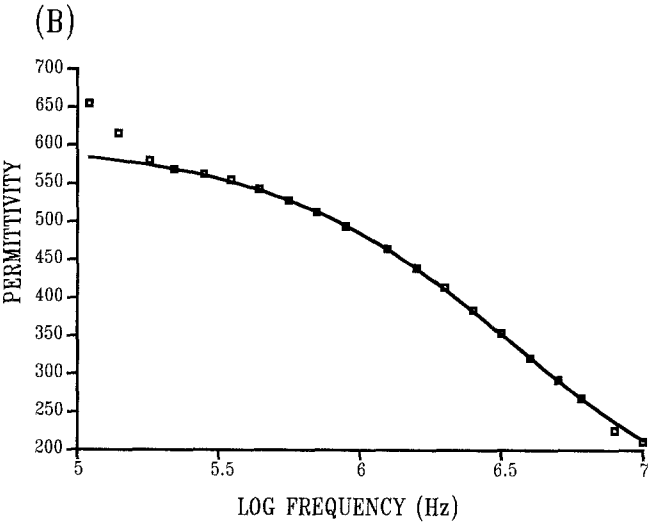


Fig. 4 A, B. Dielectric properties of *E. coli*. A suspension of *E. coli* (106 mg dry wt $\cdot$ ml^{-1}) was prepared, and its dielectric behaviour assessed, as described in the Materials and Methods section. **A** Raw data. This shows the data obtained with the suspension (□), a "polarisation control" consisting of a solution of the same low-frequency conductivity (◇), and their difference (——). **B** Fitted data. This shows the dielectric dispersion due to the cells alone (from **A**) (□) and the fit used to obtain the dielectric increment (——)

previously by us (Harris and Kell 1983; Harris et al. 1987) and others (Asami et al. 1980).

When the experiment of Fig. 1 was repeated using batches of the same suspension of cells at different temperatures, the dielectric increments observed varied as shown in Fig. 2. Figure 2 also gives the line of best fit of these data to a straight line together with a plot of the lines corresponding to values (taken through the mid-point of the range of observation) of $d(\Delta\varepsilon)/dT$ of $\pm 1\%$ $(°C)^{-1}$. It is evident that there is a small positive correlation between the dielectric increment and the temperature for the β-dispersion of *S. cerevisiae*, of in this case $+0.07\%$ $(°C)^{-1}$. Over 4 separate experiments, we obtained an overall value of $d(\Delta\varepsilon)/dT$ of $+0.13 \pm 0.08\%$ $(°C)^{-1}$ (mean $\pm$ SD).

The value of obtaining the overall dielectric *increment* is further substantiated when we plot the characteristic frequencies observed as a function of temperature (Fig. 3), where it may be observed that f_c is essentially linear with temperature, as may be expected from the temperature-dependent increase in conductivity of ionic solutions. Thus measuring $d(\Delta\varepsilon)/dT$ at but a single frequency would lead to the appearance of an *artefactually* positive value, simply because of the temperature-dependent change in f_c.

Because the temperature coefficient of the dielectric increment of *S. cerevisiae* was rather modest, we could not exclude that in this case it might be due to a small temperature-dependent increase in cell radius (see 2). If this were the *sole* cause of the observations, it would

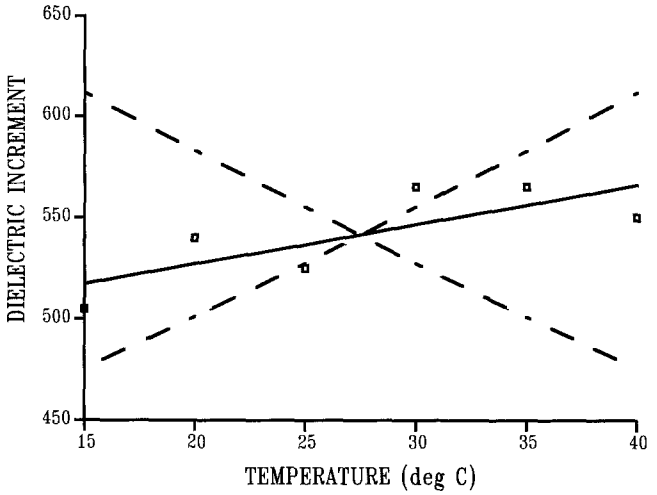


Fig. 5. Effect of temperature on the dielectric increment of suspensions of *E. coli*. Dielectric measurements were performed as described in the legend to Fig. 4, except that the temperature was varied as indicated. The full line indicates the best fit by linear regression to the data obtained, whilst the dotted lines indicate the changes to be expected if the dielectric increment changed by $\pm 1\%$ $(°C)^{-1}$, taken around the mid-point of the range of observations

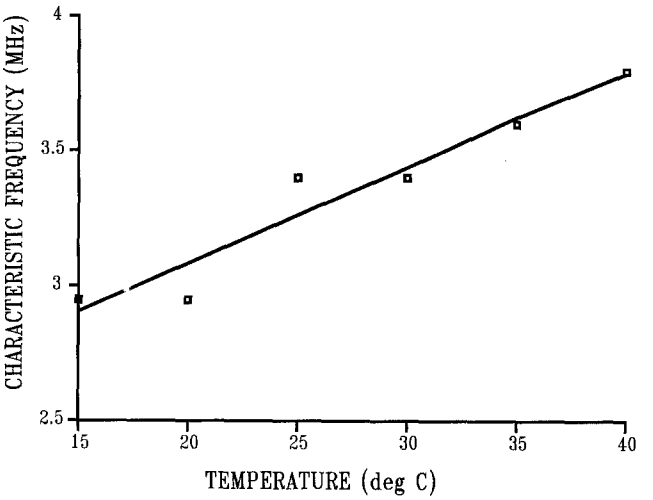


Fig. 6. Effect of temperature on the characteristic frequency of the β-dielectric dispersion of *E. coli*. Data were obtained exactly as described in the legend to Figs. 5 and 6

escape detection by our flow cytometer, since percentage changes of this magnitude are at the limit of detection of the instrument, corresponding to $\pm 1-2$ channels over the temperature range studied. We therefore turned our attention to *Escherichia coli*.

Figure 4 A shows a dielectric spectrum of a suspension of *E. coli* cells (106 mg dry weight ml^{-1}) at 25 °C, together with the polarisation control. Due to cell leakage and endogenous metabolism (which in contrast to *S. cerevisiae* causes an increase in the low-frequency conductivity (Firstenberg-Eden and Eden 1984)), the low-frequency conductivity is in this case some 8 mS cm^{-1}, greatly increasing the extent of electrode polarisation, which is also displayed in Fig. 4 A. However, as may be observed in Fig. 4 B, the fitting routine allows an accurate correction for electrode polarisation except at the 2 lowest frequencies of measurement. The dielectric parameters for this experiment are: $\Delta\varepsilon = 525$, $f_c = 3.4$ MHz, Cole-Cole $\alpha = 0.28$. Relative to *S. cerevisiae*, and much as observed by Asami et al. (1980), the lower dielectric increment of ca. 5 permittivity units (mg dry wt)$^{-1}$ · ml follows from (2), whilst the significantly higher Cole-Cole α seems to be a feature of all bacteria studied (Harris and Kell 1985 b).

When the experiment of Fig. 4 was repeated on batches of the same cell suspension, the data displayed in Fig. 5 were obtained. The best fit to the data gives a slope of $+0.4\%$ $(°C)^{-1}$ for the temperature-dependence of the dielectric increment of the β-dispersion of *E. coli*. From 4 separate experiments we obtained a value of $0.35 \pm 0.19\%$ $(°C)^{-1}$ (mean $\pm$ SD). The characteristic frequency of the β-dispersion for *E. coli* is greater than that for *S. cerevisiae*, both because of the greater conductivities and of the smaller cell radius. The temperature-dependence of the characteristic frequency of the β-dispersion of *E. coli* is shown in Fig. 6. As with *S. cerevisiae*, f_c increases linearly with temperature over the range studied.

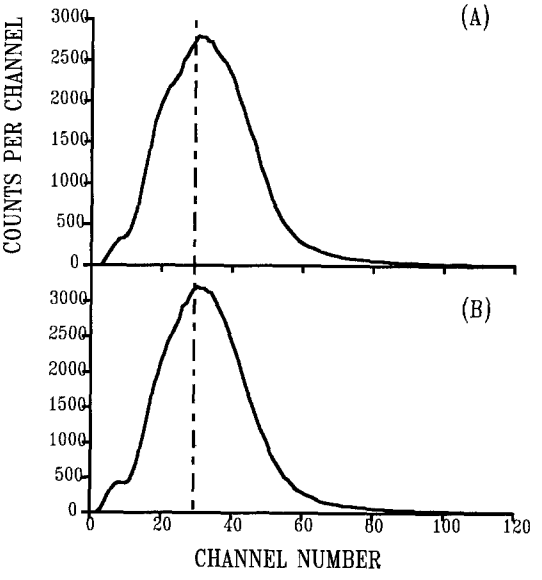


Fig. 7 A, B. Effect of temperature on the cell size distribution of *E. coli*. Samples were taken from the dielectric cell, treated with glutaraldehyde and analysed by flow cytometry as described in the Materials and Methods section. The shoulder between channel numbers 1 and 10 is an artefact due to the presence of very small impurities in the sheath fluid of the flow cytometer. **A** Sample taken at 15 °C. **B** Sample taken at 40 °C

To clarify whether the positive temperature coefficient of the dielectric increment for the β-dispersion of *E. coli* is due to changes in cell size, we studied the temperature-dependence of the cell size of our suspensions by flow cytometry. Figure 7 shows the flow cytometric forward-light-scattering data obtained with cells at the extremes of temperature of the range studied, viz. 15 °C (Fig. 7 A) and 40 °C (Fig. 7 B). It may be observed that the change is cell size is negligible over the 25 °C range encompassed. Thus changes in cell size are not the cause of the positive temperature coefficient for the β-dispersion of *E. coli*.

Discussion

It is now well recognised that the β-dielectric dispersion of biological tissues and cell suspensions is dominated by the electrical charging of the molecularly thin membranes surrounding biological cells. Since the work of Fricke (1925), it has been possible to estimate the capacitance of this plasma or cytoplasmic membrane, and a value of ca. 1 $\mu F\ cm^{-2}$ has become widely accepted (Cole 1972). By contrast, studies with black lipid membranes have consistently given a value that may be less than one half of this (when organic solvents are present) and at all events certainly lower than this (when they are not). It is possible to argue on a quantitative basis that this difference simply reflects the *existence* of proteins in biomembranes (where they may take up volume fractions of 75% and area fractions as great as 50% (Hackenbrock 1981; Kell and Westerhoff 1985)). However, where measurements have been made, it is found that neither the existence of water-filled pores (Weaver et al. 1984; Pethig and Kell 1987), nor the (hydrophobic core of) integral, polytopic membrane proteins (Casadio et al. 1988) could account for the greater value of C_m obtained with biological membranes.

Whilst *quantitative* differences in C_m might perhaps be ascribable to a greater *thickness* of biological than artificial membranes (Fettiplace et al. 1971), we have noticed (Table 1), stimulated by the article of Muller (1985), that there exists a *qualitative* difference that can not be so explained: namely, that artificial (pure lipid) bilayer membranes have a negative temperature coefficient but that that of natural biological membranes is positive. The former case is to be explained as follows: most substances (including water at temperatures above 4 °C) expand on heating, and thus contain fewer dipoles per unit volume which can contribute to the dielectric increment for a dispersion of the Debye (1929) type (Hasted 1973). Similarly, BLM become thicker, both naturally and by the incorporation of more organic solvent if present, and thus have a decreased value of C_m, as the temperature is increased. Independent measurements of the thickness of plasma membranes in cell suspensions and tissues are essentially impossible. However, especially given that they do not change their membrane area by incorporating preformed vesicles, as can some higher eukaryotes (Glaser and Donath 1988), prokaryotes cannot increase their cell radius (and membrane area) without decreasing their membrane thickness. Whilst temperature-dependent *decreases* in membrane thickness in prokaryotes would cause a positive temperature-dependence of C_m, this type of mechanism would invariably lead to increases in cell *radius*, which are measurable.

Thus, the purpose of the present work was to enquire more closely than has previously been possible into the causes of the 'high' value of the C_m of biological membranes, via a careful assessment of the temperature dependence of the magnitude of the β-dispersion of two microorganisms. Although these effects are relatively modest. This was possible because we have developed a convenient fitting procedure for the deconvolution of dielectric data, and its correction for electrode polarisation phenomena (Davey et al. 1990).

When these measurements were carried out on suspensions of *S. cerevisiae*, we found a small positive temperature coefficient for the β-dispersion of some $0.13 \pm 0.08\%\ (°C)^{-1}$ (mean $\pm$ SD), although we were unable in this case absolutely to exclude a contribution to this from changes in cell size (and/or its distribution) since the overall change under our conditions would have equated to 1 light-scattering channel on our flow cytometer, the system noise.

When similar measurements were carried out on suspensions of *E. coli*, however, the temperature coefficient of the β-dispersion was much greater, viz. $0.35 \pm 0.19\%\ (°C)^{-1}$ (mean $\pm$ SD). Measurements of the cell size distribution at different temperatures showed that changes in this were negligibly small, and certainly far too small to account for a change in dielectric increment of nearly 9% over the temperature range studied. It is also worth pointing out that the cell size distribution per se was far too small to account for the large values of the Cole-Cole α observed both herein and in other prokaryotes (see also Pauly 1963, Kell and Harris 1985a, b).

How then can one account for the positive temperature coefficient of the β-dispersion of prokaryotes? It is well known that temperature can increase the polarisability (Gascoyne et al. 1981; Careri et al. 1986) and flexibility (Welch et al. 1982; Finney and Poole 1984; Somogyi et al. 1982) of proteins, as well as the mobility of proteins in biological membranes (e.g. Webb et al. 1981; McCloskey and Poo 1984). Certainly electric fields can cause the lateral motions of membrane-located proteins (Jaffe 1977; Sowers and Hackenbrock 1981; Poo 1981; Chiabrera and Rodan 1984), and thus permit one to measure them (Kell 1983; Kell and Harris 1985a, b; Pethig and Kell 1987). Thus a temperature-dependent *increase* in C_m is best viewed as a temperature-dependent change in the extent to which lipids and proteins can diffuse in the plane of the cytoplasmic membrane. This explanation would not be tenable were lipids and proteins free to diffuse rapidly and randomly throughout the plane of the membrane over the range of temperatures encountered, at their viscosity-constrained rates. However, from what is known of this matter, it indeed turns out that they are not (Kaprelyants 1982, 1988; Kell 1984, 1986) although the molecular mechanisms underlying this restriction on motion, especially in prokaryotes, have yet to be clarified.

Based in part upon the effects of cross-linking reagents, we have argued elsewhere (Kell 1983, 1988; Harris and Kell 1985; Kell and Harris 1985a, b; Symons et al. 1986; Pethig and Kell 1987) that natural and field-induced *motions* of membrane components must contribute to the β-dispersion of cell suspensions. Because of the relative distances involved, the dielectric observability of lateral motions is expected to be much greater than that of rotational ones (Kell and Harris 1985a). Similarly, Uhlendorf (1984) has indicated that the contamination of liposomes by fatty acids, and the lateral diffusion of these molecules, can contribute to dielectric relaxations. (In our earlier work, changes in the aqueous viscosity had surprisingly large effects on the dielectric behaviour. However, we were not aware of the papers of Hughes et al. (1981, 1982), who had shown that the relative sensitivity

of the lateral mobility of membrane proteins to the 'aqueous' "viscosity" is greatly increased if they protrude from the membrane 'surface', and it has since been shown that the viscosity of the bacterial periplasm is very much greater than had been suspected, ca. 1 Pa · s (Brass 1986; Brass et al. 1986). Given that (in contrast to liposomes made of single lipids) natural membranes exhibit no sharp gel-to-liquid phase transition (e.g. Casal and Mantsch 1984; Devaux and Seigneuret 1985), but a broad distribution of temperature-dependent heat capacity changes (e.g. Nagle 1980; McElhaney 1982), we suggest that the simplest explanation of the present data is that they reflect a contribution to the β-dispersion of a temperature-dependent, restricted lateral mobility of the components of the microbial cytoplasmic membrane. This would serve simply to explain the high values of the Cole-Cole α observed in the β-dispersion of the prokaryotic cytoplasmic membranes (with their high protein : lipid ratio). Further experiments using purified, reconstituted systems, should help fully to clarify this mechanism.

Acknowledgements. We thank the Science and Engineering Research Council, U.K. and Aber Instruments, Aberystwyth, for financial support, and Robert Hill, Gerard Markx, Ton Muller, Alun Price and Gary Salter for many useful and stimulating discussions.

References

Adey WR (1981) Tissue interactions with non-ionising electromagnetic fields. Physiol Rev 61:435–514

Almers W (1978) Gating currents and charge movements in excitable membranes. Rev Physiol Biochem Pharmacol 82:96–190

Asami K, Hanai T, Koizumi N (1976) Dielectric properties of yeast cells. J Membr Biol 28:169–180

Asami K, Hanai T, Koizumi N (1980) Dielectric analysis of *Escherichia coli* in the light of the theory of interfacial polarisation. Biophys J 31:215–225

Asami K, Irimajiri A (1984) Dielectric dispersion of a single spherical bilayer membrane in suspension. Biochim Biophys Acta 769:370–376

Asami K, Irimajiri A, Hanai T, Shiraishi N, Utsumi K (1984) Dielectric analysis of mitochondria isolated from rat liver. I. Swollen mitoplasts as simulated by a single-shell model. Biochim Biophys Acta 778:559–569

Ballario C, Bonincontro A, Cametti C, Rosi A, Sportelli L (1984) Conductivity of normal and pathological human erythrocytes (homozygous – thalassemia) at radiowave frequencies. Z Naturforsch 39C:160–166

Bangham AD (1983) Liposome letters. Academic Press, London

Benz R, Fröhlich O, Laüger P, Montal M (1975) Electrical capacity of black lipid films and of lipid bilayers made from monolayers. Biochim Biophys Acta 394:323–334

Brass JM (1986) The cell envelope of Gram-negative bacteria: new aspects of its function in transport and chemotaxis. Curr Top Microbiol Immunol 129:1–92

Brass JM, Higgins CF, Foley M, Rugman PA, Birmingham J, Garland PB (1986) Lateral diffusion of proteins in the periplasm of *Escherichia coli*. J Bacteriol 165:787–794

Careri G, Giansanti A, Rupley JA (1986) Proton percolation on hydrated lysozyme powders. Proc Natl Acad Sci 83:6810–6814

Casal HL, Mantsch HH (1984) Polymorphic phase behaviour of phospholipid membranes studied by infrared spectroscopy. Biochim Biophys Acta 779:381–401

Casadio R, Venturoli G, Melandri BA (1988) Evaluation of the electrical capacitance in biological membranes at different phospholipid to protein ratios. A study in photosynthetic bacterial chromatophores based on electrochromic effects. Eur Biophys J 16:243–253

Chiabrera A, Rodan GA (1984) The effect of electromagnetic fields on receptor-ligand interaction: a theoretical analysis. J Bioelectr 3:509–521

Chiabrera A, Nicolini C, Schwan HP (1985) (eds) Interactions between electromagnetic fields and cells. Plenum Press New York

Cole KS (1970) Dielectric properties of living membranes. In: Snell F, Wolken J, Iverson G, Lam J (eds) Physical principles of biological membranes. Gordon and Breach, New York, pp 1–15

Cole KS (1972) Membranes ions and impulses. University of California Press, Berkeley

Cole KS, Cole RH (1941) Dispersion and absorption in dielectrics. 1. Alternating current characteristics. J Chem Phys 9:341–351

Coster HGL, Laver DR (1986) The effect of benzyl alcohol and cholesterol on the acyl chain order and alkane solubility of bimolecular phosphatidylcholine membranes. Biochim Biophys Acta 861:406–412

Coster HGL, Smith JR (1974) The molecular organisation of bimolecular lipid membranes. A study of the low-frequency Maxwell-Wagner impedance dispersion. Biochim Biophys Acta 373:151–164

Davey CL, Kell DB (1989) Low-frequency dielectric properties of cell suspensions. In: Paris R (ed) Electric field phenomena in biological systems. IOP Short Meetings Series vol 21. IOP, London, pp 51–62

Davey CL, Kell DB (1990) The dielectric properties of cells. What can they tell us about cellular physiology and the mechanisms of field/cell interactions? In: O'Connor M, Bentall R, Monahan J (eds) Emerging electromagnetic medicine. Springer, Berlin Heidelberg New York

Davey CL, Kell DB, Kemp RB, Meredith RWJ (1988) On the audio- and radio-frequency dielectric behaviour of anchorage-independent, mouse L929-derived LS fibroblasts. Bioelectrochem Bioenerg 20:83–98

Davey CL, Markx GH, Kell DB (1990) Substitution and spreadsheet methods for fitting dielectric spectra of biological systems. Eur Biophys J 18:255–265

Debye P (1929) Polar molecules. Dover, New York

Devaux P, Seigneuret M (1985) Specificity of lipid-protein interactions as determined by spectroscopic techniques. Biochim Biophys Acta 822:63–125

Dilger JP, Benz R (1985) Optical and electrical properties of thin monoolein lipid bilayers. J Membr Biol 85:181–189

Dissado L, Hill RM (1983) A cluster approach to the structure of imperfect materials and their relaxation spectroscopy. Proc R Soc A390:131–180

Everitt CT, Haydon DA (1968) Electrical capacitance of a lipid membrane separating two aqueous phases. J Theor Biol 18:371–379

Fernàndez JM, Neher E, Gomperts BD (1984) Capacitance measurements reveal stepwise fusion events in degranulating mast cells. Nature 312:453–455

Fettiplace R, Andrews DM, Haydon DA (1971) The thickness, composition and structure of some lipid bilayers and natural membranes. J Membr Biol 5:277–296

Fettiplace R, Gordon LGM, Hladky SB, Requena J, Zingsheim HP, Haydon DA (1975) Techniques in the formation and examination of "black" lipid bilayer membranes. In: Korn ED (ed) Methods in membrane biology, vol 4. Plenum Press New York, pp 1–75

Finney JL, Poole PL (1984) Protein hydration and enzyme activity: the role of hydration-induced conformation and dynamic changes in the activity of lysozyme. Comments Mol Cell Biophys 2:129–151

Fishman HM (1985) Relaxations, fluctuations and ion transfer across membranes. Progr Biophys Mol Biol 46:127–162

Foster KR, Schwan HP (1986) Dielectric properties of tissues. In: Polk C, Postow E (eds) CRC handbook of biological effects of electromagnetic fields. CRC Press, Boca Raton, Florida, pp 27–96

Foster KR, Schwan HP (1989) Dielectric properties of tissues and biological materials: a critical review. CRC Crit Rev Biomed Eng 17:25–102

Fricke H (1925) The electric capacity of suspensions with special reference to blood. J Gen Physiol 9:137–152

Gascoyne PRC, Pethig R, Szent-Györgyi A (1981) Water structure-dependent charge transport in proteins. Proc Natl Acad Sci 78:261–265

Geddes LA, Baker LE (1967) The specific resistance of biological material: a compedium for the biomedical engineer and physiologist. Med Biol Eng 5:271–293

Glaser A, Donath E (1988) Osmotically-induced vesicle-membrane fusion in plant protoplasts. Studia Biophys 127:129–138

Grant EH, Sheppard RJ, South GP (1978) Dielectric behaviour of biological molecules in solution. Clarendon Press, Oxford

Hackenbrock CR (1981) Lateral diffusion and electron transfer in the mitochondrial inner membrane. Trends Biochem Sci 6: 151–154

Hanai T, Haydon DA, Taylor J (1964) An investigation by electrical methods of lecithin-in-hydrocarbon films in aqueous solutions. Proc R Soc Ser A281:377–391

Hanai T, Haydon DA, Taylor J (1965) Polar group orientation and the electrical properties of lecithin bimolecular leaflets. J Theor Biol 9:278–296

Hanai T, Asami K, Koizumi N (1979) Dielectric theory of concentrated suspensions of shell-spheres in particular reference to the analysis of biological cell suspensions. Bull Inst Chem Res Kyoto 57:297–305

Harris CM, Kell DB (1983) The radio-frequency dielectric properties of yeast cells measured with a rapid, automated, frequency-domain dielectric spectrometer. Bioelectrochem Bioenerg 11: 15–28

Harris CM, Kell DB (1985) On the dielectrically observable consequences of the diffusional motions of lipids and proteins in membranes. 2. Experiments with microbial cells protoplasts and membrane vesicles. Eur Biophys J 13:11–24

Harris CM, Todd RW, Bungard SJ, Lovitt RW, Morris JG, Kell DB (1987) The dielectric permittivity of microbial suspensions at radio frequencies: a novel method for the real-time estimation of microbial biomass. Enzyme Microbial Technol 9:181–186

Hasted JB (1973) Aqueous dielectrics. Chapman and Hall, London

Haydon DA, Requena J, Urban BW (1980) Some effects of aliphatic hydrocarbons on the electrical capacity and ionic currents of the squid giant axon. J Physiol 309:229–245

Hill RM, Jonscher AK (1983) The dielectric behaviour of condensed matter and its many-body interpretation. Contemp Phys 24: 75–110

Hope AB (1956) The electric properties of plant cell membranes. I. The electric capacitance of suspensions of mitochondria, chloroplasts, and *Chlorella* sp. Aust J Biol Sci 9:53–66

Hübner R (1910) Eine Methode die elektrische Leitfähigkeit im Innern von Zellen zu messen. Arch Gesamt Physiol Mensch 133:237–259

Hughes BD, Pailthorpe BA, White LR (1981) The translational and rotational drag on a cylinder moving through a membrane. J Fluid Mech 110:349–356

Hughes BD, Pailthorpe BA, White LR, Sawyer WH (1982) Extraction of membrane microviscosity from translational and rotational diffusion coefficients. Biophys J 37:673–676

Jaffe L (1977) Electrophoresis along cell membranes. Nature 265: 600–602

Jain MK (1972) The bimolecular lipid membrane: a system. Van Nostrand, New York

Jonscher AK (1983) Dielectric relaxation in solids. Chelsea Dielectrics Press, London

Kaprelyants AS (1982) Dynamic protein ensembles in biological membranes. Biochem USSR 47:739–748

Kaprelyants AS (1988) Dynamic spatial distribution of proteins in the cell. Trends Biochem Sci 13:43–46

Kell DB (1983) Dielectric properties of bacterial chromatophores. Bioelectrochem Bioenerg 11:405–415

Kell DB (1984) Diffusion of proteins in prokaryotic membranes: fast, free, random or directed? Trends Biochem Sci 9:86–88

Kell DB (1986) On the lateral mobility of proteins in prokaryotic membranes. Biochem Soc Trans 14:825–826

Kell DB (1987) The principles and potential of electrical admittance spectroscopy: an introduction. In: Turner APF, Karube IS, Wilson GS (eds) Biosensors: fundamentals and applications, Oxford University Press, Oxford, pp 427–468

Kell DB (1988) Dielectric spectroscopy of biological systems. ISI Atlas Sci Biochem 1:25–29

Kell DB, Harris CM (1985a) On the dielectrically observable consequences of the diffusional motions of lipids and proteins in membranes. 1. Theory and overview. Eur Biophys J 12:181–197

Kell DB, Harris CM (1985b) Dielectric spectroscopy and membrane organisation. J Bioelectr 4:317–348

Kell DB, Westerhoff HV (1985) Catalytic facilitation and membrane bioenergetics. In: Welch GR (ed) Organized multienzyme systems: catalytic properties. Academic Press, New York, pp 63–139

Keynes RD, Kimura JE (1983) Kinetics of sodium activation in the squid giant axon. J Physiol 336:621–634

Keynes RD, Rojas E (1976) The temporal and steady-state relationships between activation of the sodium conductance and movement of the gating particles in the squid giant axon. J Physiol 255:157–189

Laver DR, Smith JR, Coster HGL (1984) The thickness of the hydrophobic and polar regions of glycerol monooleate bilayers determined from the frequency-dependence of the bilayer capacitance Biochim Biophys Acta 772:1–9

McCloskey M, Poo M-m (1984) Protein diffusion in cell membranes: some biological implications. Int Rev Cytol 87:19–81

McElhaney RN (1982) The use of differential scanning calorimetry and differential thermal analysis in studies of model and biological membranes. Chem Phys Lipids 30:229–259

McIntosh TJ, Simon SA, Dilger JP (1989) Location of the water-hydrocarbon interface in lipid bilayers. In: Benga G (ed) Water transport in biological membranes, vol I; from model membranes to isolated cells. CRC Press, Boca Raton, Florida, pp 1–15

Montal M, Mueller P (1972) Formation of bimolecular membranes from lipid monolayers, and a study of their electrical properties. Proc Natl Acad Sci 69:3561–3566

Mueller P, Rudin DO (1968) Bimolecular lipid membranes: techniques of formation, study of electrical properties, and induction of ionic gating phenomena. In: Passow H, Stampfli R (eds) Laboratory techniques in membrane biophysics. Springer, Berlin Heidelberg New York, pp 141–156

Muller AWJ (1985) Thermosynthesis by biomembranes: energy gain from cyclic temperature change. J Theor Biol 115:429–453

Nagle JF (1980) Theory of the main lipid bilayer phase transition. Ann Rev Phys Chem 31:157–195

Palti Y, Adelman WJ (1969) Measurement of axonal membrane conductances and capacity by means of a varying potential control voltage clamp. J Membr Biol 1:431–458

Pauly H (1963) Über die elektrische Kapazität der Zellmembran und die Leitfähigkeit des Zytoplasmas von Ehrlich-Aszites-tumorzellen. Biophysik 1:143–153

Perez E, Wolfe J (1989) Oestradiol changes the dielectric structure of bilayer membranes. Eur Biophys J 16:23–29

Pethig R (1979) Dielectric and electronic properties of biological materials. Wiley, Chichester

Pethig R (1984) Dielectric properties of biological materials: biophysical and medical applications. IEEE Trans Electr Insul EI 19:453–474

Pethig R, Kell DB (1987) The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology. Phys Med Biol 32:933–970

Poo M-m (1981) *In situ* electrophoresis of membrane components. Annu Rev Biophys Bioeng 10:245–276

Redwood WR, Takashima S, Schwan HP, Thompson TE (1972) Dielectric studies on homogeneous phosphatidylcholine vesicles. Biochim Biophys Acta 255:557–566

Sakmann B, Neher E (1983) Single-channel recording. Plenum Press, New York

Sakmann B, Neher E (1984) Patch clamp techniques for studying ionic channels in excitable membranes. Annu Rev Physiol 46:455–472

Salter DC (1979) Quantifying skin disease and healing *in vivo* using electrical impedance measurements. In: Rolfe P (ed) Non-invasive physiological measurements, vol 1. Academic Press, London, pp 21–68

Schanne OF, Ceretti ERP (1978) Impedance measurements in biological cells. Wiley, New York

Schwan HP (1948) Die Temperaturabhängigkeit der Dielektrizitätskonstante von Blut bei Niederfrequenz. Z Naturforsch 3b: 361–367

Schwan HP (1957) Electrical properties of tissue and cell suspensions. Adv Biol Med Phys 5:147–209

Schwan HP (1963) Determination of biological impedances. In: Nastuk WL (ed), Physical techniques in biological research. vol VIB. Academic Press, New York, pp 323–407

Schwan HP, Foster KR (1980) RF-field interactions with biological systems: electrical properties and biophysical mechanisms. Proc IEEE 68:104–113

Schwan HP, Takashima S, Miyamoto VK, Stoeckenius W (1970) Electrical properties of phospholipid vesicles. Biophys J 10: 1102–1119

Skarstad K, Steen HB, Boye E (1983) Cell cycle parameters of slowly growing *Escherichia coli* B/r studied by flow cytometry. J Bacteriol 154:656–662

Somogyi B, Damjanovich B, Welch GR (1984) The dynamic basis of energy transduction in enzymes. Biochim Biophys Acta 768:81–112

Sowers AE, Hackenbrock CR (1981) Rate of lateral diffusion of intramembrane particles: measurement by electrophoretic displacement and rerandomisation. Proc Natl Acad Sci 78:6246–6250

Steen HB (1983) A microscope-based flow cytophotometer. Histochem J 15:147–160

Steen HB (1986) Simultaneous separate detection of low angle and large angle light scattering in an arc lamp-based flow cytometer. Cytometry 7:445–449

Stewart GN (1899) The relative volume or weight of corpuscles and plasma in blood. J Physiol 24:356–373

Stoy RD, Foster KR, Schwan HP (1982) Dielectric properties of mammalian tissue from 0.1 to 100 MHz: a summary of recent data. Phys Med Biol 27:501–513

Stuchly MA, Stuchly SS (1980) Dielectric properties of biological substances – tabulated. J Microwave Power 15:19–26

Surowiec A, Stuchly S, Izaguirre C (1986) Dielectric properties of human B and T lymphocytes at frequencies from 20 kHz to 100 MHz. Phys Med Biol 31:43–53

Symons M, Korenstein R, Harris CM, Kell DB (1986) Dielectric spectroscopy of energy coupling membranes. Chloroplast thylakoids. Bioelectrochem Bioenerg 16:45–54

Takashima S (1976) Membrane capacity of squid giant axon during hyper- and depolarisation. J Membr Biol 27:21–39

Takashima S (1989) Electrical properties of biopolymers and membranes. Hilger, Bristol

Taylor RE, Fernàndez JM, Bezanilla F (1977) Squid axon membrane low-frequency dielectric properties. In: Adelman WJ, Goldman DE (eds) The biophysical approach to excitable membranes. Plenum Press, New York, pp 97–106

Tien HT (1974) Bilayer lipid membranes (BLM): Theory and practice. Dekker, New York

Uhlendorf V (1984) Fatty acid contamination and dielectric relaxation in phospholipid vesicle suspensions. Biophys Chem 20: 261–273

Weaver JC, Powell KT, Mintzer RA, Ling H, Sloan SR (1984) The electrical capacitance of bilayer membranes. The contribution of transient aqueous pores. Bioelectrochem Bioenerg 12:393–404

Webb WW, Barak LS, Tank DW, Wu E-s (1981) Molecular mobility on the cell surface. Biochem Soc Symp 46:191–205

Welch GR, Somogyi B, Damjanovich S (1982) The role of protein fluctuations in enzyme action: a review. Progr Biophys Mol Biol 39:109–146

White SH (1970) A study of lipid bilayer membrane stability using precise measurements of specific capacitance. Biophys J 10: 1127–1148

White SH (1974) Temperature-dependent structural changes in planar bilayer membranes: solvent "freeze-out". Biochim Biophys Acta 356:8–16

White SH (1975) Phase transitions in planar bilayer membranes. Biophys J 15:95–117

White SH (1978) Formation of "solvent-free" black lipid bilayer membranes from glyceryl monooleate dispersed in squalene. Biophys J 23:337–347

Zimmermann U (1982) Electric field-mediated cell fusion and related electrical phenomena. Biochim Biophys Acta 694:227–277