BIOELECTROCHEMICAL PHENOMENA

Their role and exploitation in science and technology

Douglas B. Kell

Department of Botany & Microbiology, University College of Wales, Aberystwyth, Dyfed SY23 3DA, U.K.

A brief overview of some simple faradaic and non-faradaic electrochemical concepts for both DC and AC systems is given. The combination of living cells or biological molecules with electrochemical systems constitutes bioelectrochemistry. It is seen that (i) bioelectrochemical phenomena underlie a great many cellular processes, (ii) a great many recent technological innovations, in both analysis and synthesis, have developed directly from academic bioelectrochemical studies, and (iii) a plausible extension of modern bioelectrochemical ideas can lead directly to a variety of future scientific and technological advances.

Introduction and Scope

Following the realisation that the macroscopically observable chemical changes catalysed by living cells could be reproduced, to a greater or lesser extent, in cell-free extracts, biochemistry, and its cognate disciplines biophysics and molecular biology, might be said to have evolved through two major phases. Initially, emphasis was placed upon an analysis of the chemical intermediates in the metabolic (reaction) pathways used *in vivo*. However, with the realisation that such processes do not always proceed at the same rate and are therefore regulated, at genetic, structural and metabolic levels, it was necessary to proceed to a molecular analysis of the mechanisms underlying cellular regulation. Such studies have concentrated on the (biophysical) chemistry of living systems and of subsystems derived therefrom.

Yet, with the distinguished exceptions of ionmotive neurophysiological processes in higher organisms (Jack, Noble and Tsien 1975), and the membranous redox processes involved in free energy conservation (Nicholls 1982; Harold 1986), it is my opinion that the role of electrochemical or purely electrical phenomena in the control of the growth, development and metabolic activity of living cells has not gained either the prominence or acceptance which it deserves. The main thematic purposes of this review will therefore be to draw attention to, (a) the numerous existing and projected uses of bioelectrochemical phenomena *sensu lato* which may be exploited in the analysis, construction and improvement of a variety of scientific studies and processes of commercial importance, and (b) the various types of circumstantial evidence which lead one to the view that many novel mechanisms of (electrical) field/cell interactions remain to be uncovered. I take the view that this area of scientific and technological activity is particularly timely due to the spectacular advances in digital electronic instrumentation that have occurred over the past decade or so.

Fig. 1

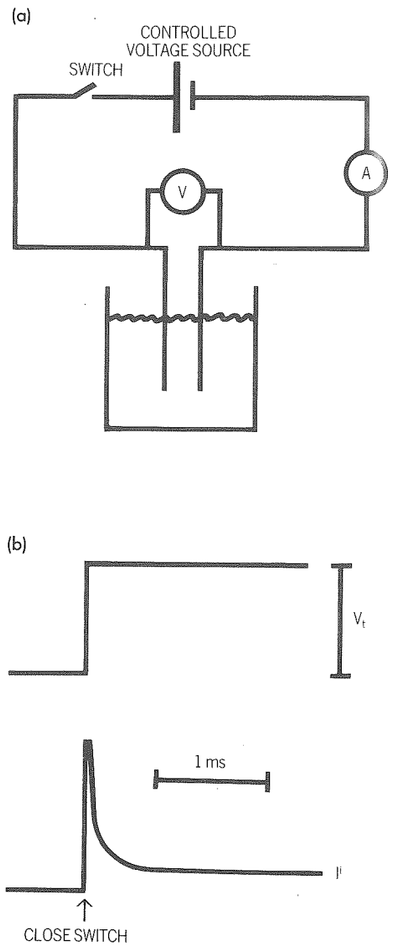
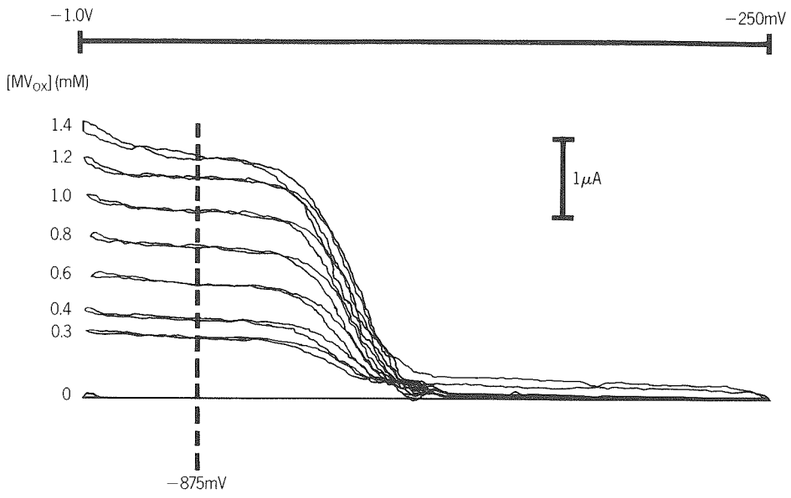


Fig. 1. (a) A simple DC electrochemical circuit consisting of 2 metallic electrodes connected to a controlled voltage source and immersed in an aqueous solution. The circuit is provided with a voltmeter (V) and Ammeter (A). (b) Approximate time-dependence of the voltage and current following the closing of the switch in (a) For further details, see text.

Fig. 2. (a) Electrochemical reduction of oxidised methyl viologen ('paraquat'), an electroactive compound. The supporting electrolyte consisted of 1M potassium phosphate pH 7, and methyl viologen was present at the mM concentrations indicated. The working electrode was a dropping mercury electrode and the reference electrode was of Ag/AgC1 (3M KC1). Voltage scans were from positive to negative at 10 mV/s and together with the measurements of current were performed with a PAR 174A polarograph. (b) For continuing faradaic current to flow under DC conditions an electron must pass across the interface to reduce the oxidised methyl viologen.

Fig. 2(a)



It is appropriate to begin by defining bioelectrochemistry, which I take to mean (Kell 1986a) "the study of the mutual interactions of electrical fields and biological materials, including living systems". To understand the type of subdisciplines which this encompasses, it is first necessary to clarify exactly what an applied electrical field can do to condensed matter and how one may measure and characterise this. We consider first the effect of a static (DC) field, with reference to Figure 1.

DC electrochemistry

Figure 1(a) shows a simple 2-electrode arrangement in which a controlled voltage source (*potentiostat*) may be used, by closing a switch, to induce an electrical potential difference between two metal electrodes immersed in an aqueous solution or suspension of unspecified composition. Although, for reason which do not concern us here, it is usual to use three electrodes, and the 'battery' is constituted by an operational amplifier circuit incorporating feedback (Sawyer and Roberts 1974; Bard and Faulkner 1980; Bond 1980; Kissinger and Heineman 1984), the arrangement shown is suitable for heuristic (and many practical) purposes. The time-courses of the voltage and current are shown in Figure 1(b). Because we have specified that our voltage source is controlled, the set voltage is attained "instantaneously" and (since the resistance of the wires between the source and the terminals of the voltmeter is negligible compared with that of the aqueous solution) is maintained at its set value throughout the duration of the observation. The behaviour of the current, however, is entirely different, and until the steady state is attained is not uniquely dependent upon the voltage, in contrast to what one might expect from the well-known Ohm's Law relation that would suggest that the voltage = the current x the *resistance* (units: Ohms) of the aqueous phase between the electrodes. Why then does our simple electrochemical system not obey Ohm's Law?

The short answer is that this 'failure' of Ohm's Law is due to the presence of interfaces in the system (Bockris and Reddy 1970) and to the phenomenon of *electrode polarisation*. In the aqueous solution between the electrodes, the current is carried by positive and negative ions which move to the electrode of opposite sign in response to the electrical field (i.e. the voltage difference between the electrodes divided by the inter-electrode distance). However, whilst the current carriers in the external circuit are electrons in the metal wires, free electrons are not stable in aqueous solution. Therefore, for a continuing flow of current across the interface, an electron must react with a chemical species in the solution, i.e. some type of chemical reaction *(faradaic reaction)* associated with a change in valency of the aqueous current carriers is necessary. The negative electrode *(cathode)* will tend to effect the reduction of any suitable *(electroactive)* species in the solution whilst oxidation will occur at the positive electrode *(anode)*. If no special electroactive species are added, the faradaic reactions will involve the *electrolysis* of water to produce dihydrogen (by the reduction of protons) and dioxygen (by the oxidation of water). Such faradaic reactions are associated with an activation energy, and their rates are thus both finite and dependent upon the voltage on the electrode of interest.

Additionally, a charged electrode will attract ions of opposite sign to its surface, to form an "electrical double layer" in which there is a local and opposite charge imbalance (i) at the electrode "surface" and (ii) in the first two or three adjacent molecular layers of the aqueous phase. (In the bulk of the aqueous phase, electroneutrality is maintained). However, because the property of an electrical capacitor is that it stores charges on its plates, and possesses a *capacitance* (units: Farads) which is inversely proportional to the distance between the plates, there exists a large double-layer capacitance which must be charged up before a continuing (faradaic) current can flow. After the charging of the double-layer capacitance, the current becomes (most) limited, respectively, (a) by the rate of the faradaic electrochemical reactions at the electrode-solution interfaces, and (b) by the rate of diffusion of fresh electroactive material to the electrode surface(s).

Since the electrical resistance of the circuit at any instant is simply the ratio of the instantaneous voltage to the instantaneous current, it is fairly obvious that (after attaining the peak of the initial 'spike') the electrical resistance increases, and the electrical *conductance* (units: Siemens), which is the reciprocal of resistance, decreases, in mirror image to the behaviour of the electrical current shown in Figure 1(b). In the steady state, therefore, the (DC) electrical resistance, as determined from the ratio of the voltage to the current, will appear to be large, and will not simply reflect that of the bulk aqueous phase between the electrodes, whilst the apparent capacitance will be a complicated function of the electrochemical processes

Fig. 2(b)

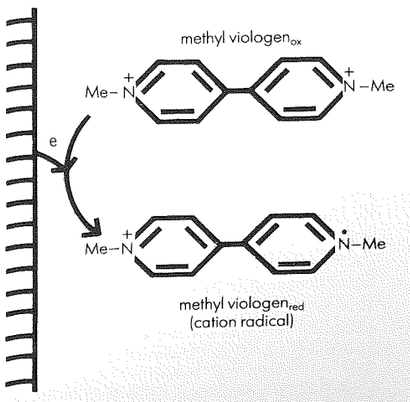
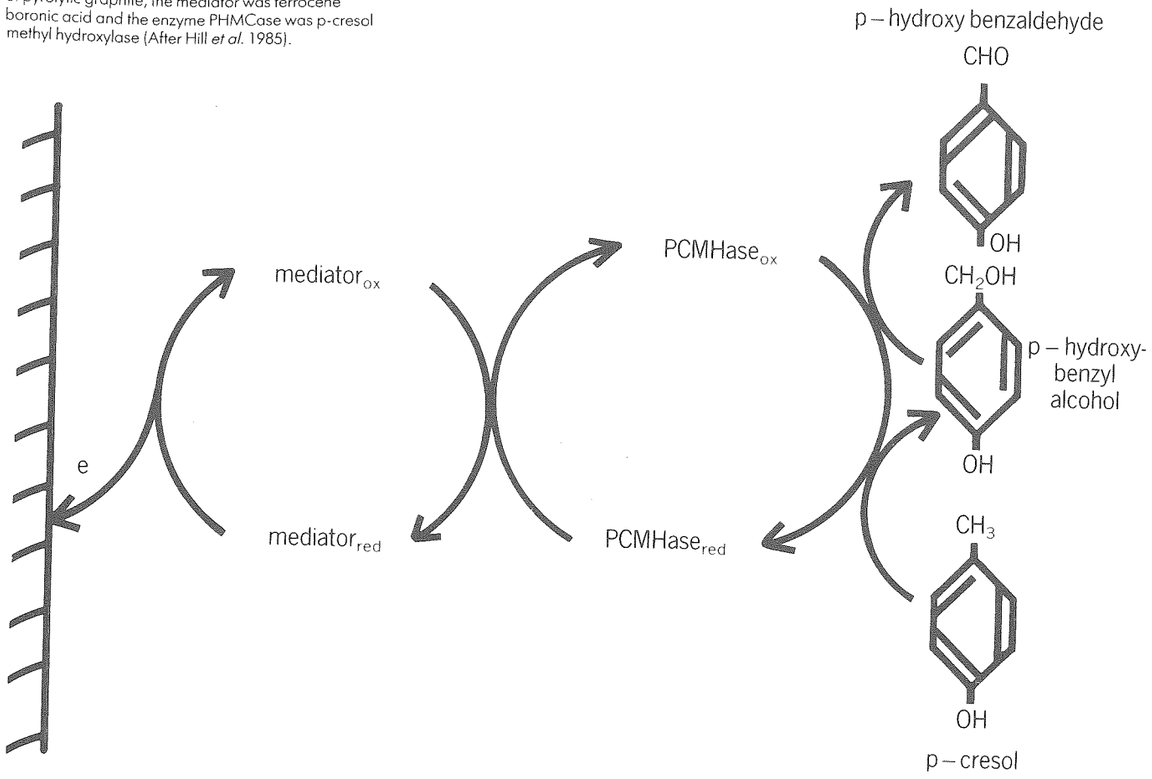


Fig. 3

Fig. 3. Bioelectrosynthesis. Use of an electrochemical system in concert with an appropriate electroactive (redox) mediator and an enzyme of the appropriate specificity to drive a chemical reaction. The anode was of pyrolytic graphite, the mediator was ferrocene boronic acid and the enzyme PHMCase was p-cresol methyl hydroxylase (After Hill *et al.* 1985).



taking place at the electrode-solution interface(s) and only weakly dependent upon the geometrical capacitance of the aqueous system.

What other factors then affect the current flowing in an electrochemical circuit in which the DC voltage of one electrode (the *working electrode)* is clamped at a certain value relative to that of a reference electrode which maintains a "standard" potential? The most important factors are the concentrations of any electroactive species present and the voltage on the working electrode. Thus Figure 2 indicates the current measured in a circuit containing a solution consisting of a supporting buffer and and varying concentrations of the compound methyl viologen (MV: 1,1′-dimethyl -4, 4′ -bipyridylium). At the more positive voltages, little current flows, whilst as the potential on the working electrode is made increasingly negative the current increases until it reaches a plateau value which is limited by the rate of diffusion of the new material to the electrode surface (and thus increases in direct proportion to the concentration of MV). The explanation of this behaviour is that as the voltage becomes more negative, not only do the thermodynamics of the reaction by which MV is reduced become increasingly favourable but the reduction process itself increasingly rapid. The potential at which the current is half its maximum value is known (for reversible reactions) as the *mid-point potential* ($E_{½}$), and is characteristic for different electroactive (redox) compounds (e.g. Clark 1960; Fultz and Durst 1982). Thus the simple measurement of the current flowing when the working electrode has its potential clamped at a value significantly cathodic of $E_{½}$ (in this case some −875 mV relative to the reference electrode), permits one to derive the concentration of oxidised MV present in the system.

If one were to perform a reaction in which all MV were initially present in the reduced form, little current would flow. If one were then however to add a molecule which could itself be reduced by reduced MV, there would be an increase in current and the rate of the reaction would be proportional to the current. If the reaction is catalysed by an enzyme (such as a dehydrogenase) or by a suspension of living cells, the reaction in question is liable to be highly specific. Many such devices, so-called *biosensors*, have been built, and *amperometric* biosensors, in which the DC current is measured, provide a particularly popular means of constructing sensors and transducers (e.g. Albery and Bartlett 1985; Aston and Turner 1984; Davis 1985; Green and Hill 1986; Turner, Karube and Wilson 1986). The analytes to which such devices may be made responsive are really limited only by the specificity of the enzymes or cells which one may exploit, and we are currently surveying a number of obligately anaerobic microorganisms for novel enzymes suitable for this purpose.

Similarly, by running such devices "backwards", electroactive components may serve to produce electricity by means of biofuel cells (Aston and Turner 1984; Bennetto 1984), whilst if the configuration (though not the principle) is changed one may use such devices for the chiral or other synthesis of chemicals which are otherwise difficult to produce by non-biological routes (e.g. Simon *et al.* 1984, 1985). A typical arrangement, modified from the work of Hill *et al.* (1985) and in which the working electrode is here the anode, is shown diagrammatically in Figure 3. It is obvious that amperometric systems in which the voltage is held at a constant value provide a powerful and convenient means of both driving and monitoring any number of reactions of commercial significance. What happens when we choose to use a sinusoidally-modulated voltage (Kell 1986b and references therein)?

AC electrochemistry

Figure 4(a) shows a typical 2-terminal arrangement in which a sinusoidally-mounted voltage source is applied to a system under test. The system may be a purely electronic circuit or an electrochemical system of the type already discussed. The voltage now has a time-dependence of the form $V = V_m \sin \omega t$, where ω is the frequency in radian s^{-1}, and the current flowing in the circuit is of the form $i = i_m \sin(\omega t + \theta)$ where θ is known as the phase angle. The system is then characterised (figure 4(c)) by its *impedance* (units: ohms), which is a complex quantity, i.e. one which is a vector sum of "real" and "imaginary" parts. The real part R of the impedance Z is the resistance, whilst the imaginary part is the reactance $X = -1/\omega C$ where C is the capacitance. Thus $Z = R + jX$, where $j = (-1)^{½}$. The important points are that the impedance may be characterised solely in terms of (i) the ratio of the maximum voltage to the maximum current and (ii) the phase angle θ (figure 4(b)); the frequency, and sinusoidal nature, of the current is *unchanged* by the presence of the system under test. The *admittance* Y (units: Siemens) is the reciprocal of the impedance and is given by $Y = G + j\omega C$ where G is the *conductance*. It is assumed here that the measured impedance is independent of the magnitude of the applied voltage. The measured impedance is, however, generally strongly dependent upon the *frequency* of measurement.

Figure 5 shows a typical trace of the apparent conductance and capacitance of a simple ionic solution lacking added electroactive compounds and measured with a pair of Pt wire electrodes; obviously these (apparent) properties are highly dependent upon the frequency at which the measurement is taken. However, under the conditions of measurement, the electrical properties of the ionic solution *between* the electrodes are in fact quite independent of frequency; the measured frequency-dependence is due simply to electrochemical reactions at the electrodes and to the difficulties of forcing current through the interface, i.e. to the phenomena of electrode polarisation, as described above. The 'true' conductance and capacitance of the system between the electrodes are only observed at high frequencies, i.e. those at which the electrochemical reactions such as double-layer charging and interfacial charge transfer cannot take place since they are insufficiently fast.

Whilst such phenomena are of fundamental interest, and may be used to obtain important, rapid and accurate information on electrochemical kinetics (e.g. Bard and Faulkner 1980; Bond 1980; Sluyters-Rehbach and Sluyters 1986); or exploited in biosensing devices (Kell 1986b), they tend to obscure the intensive properties of the system between the electrodes. To observe the latter, it is necessary either to use electrodes with a large surface area (such as platinum black electrodes) or better (figure 6) a 4-terminal arrangement in which no current passes across the interface of the voltage-sensing electrodes so that they cannot undergo polarisation.

Fig. 4(a)

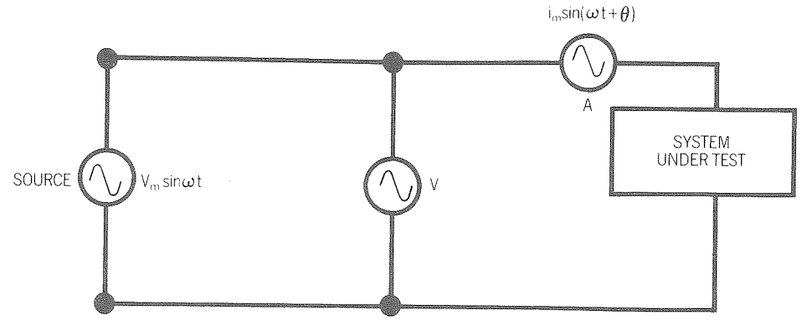


Fig. 4(b)

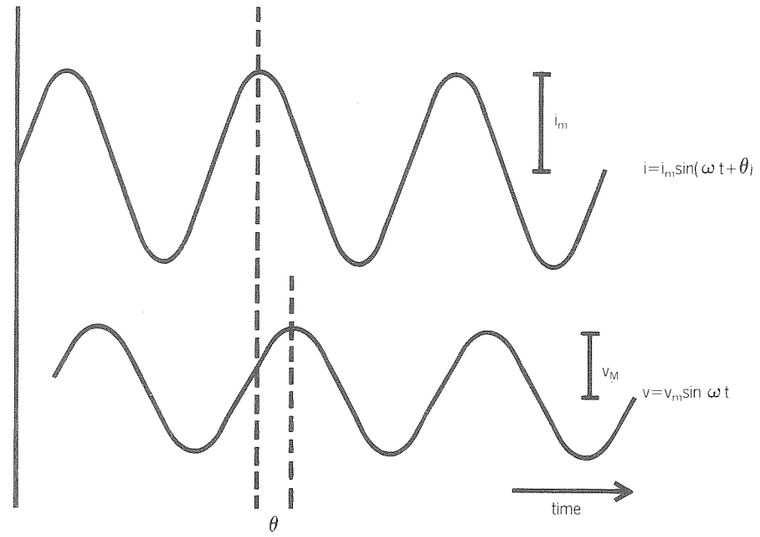


Fig. 4(c)

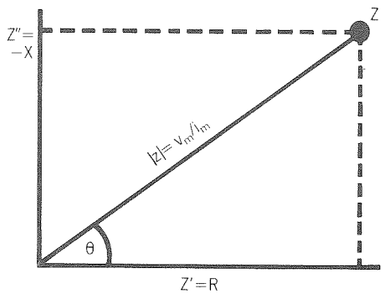


Fig. 4. (a) A simple AC electrochemical circuit, consisting of a controlled source of alternating voltage or current, an AC-sensitive voltmeter (V) and an AC-sensitive ammeter (A). (b) Typical time-dependent voltage and current flowing in the system as measured by the voltmeter and ammeter. The sinusoidal nature and frequency are the same, but there is a phase shift of θ radians. (c) The system under test may be characterised by its impedance, which is a vector quantity related to the modulus of the impedance $|Z|$ and the phase angle θ. Geometrical considerations indicate that the real part of the impedance is given by $|Z| \cos \theta$ whilst the imaginary part is $-|Z| \sin \theta$.

Fig. 5

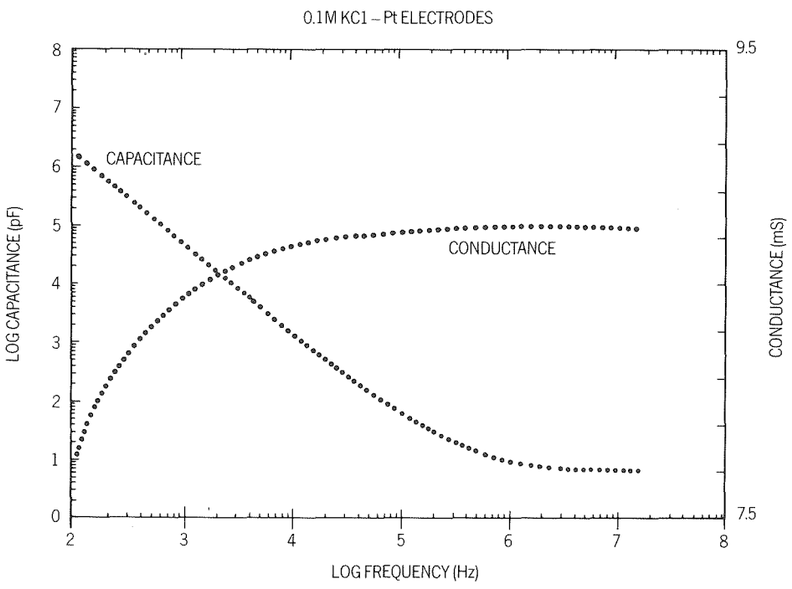


Fig. 5. Apparent conductance G and capacitance C of a 0.1M KC1 solution measured with two Pt wire electrodes (diameter 1 mm, length and separation 8 mm). The applied voltage was 50mV and the measuring system was an HP 4192A Impedance Analyser controlled by an HP85 microcomputer (Harris and Kell 1983). Only at high frequencies are the true geometric conductance and capacitance of the aqueous phase measured when a 2-electrode system is used.

Fig. 6

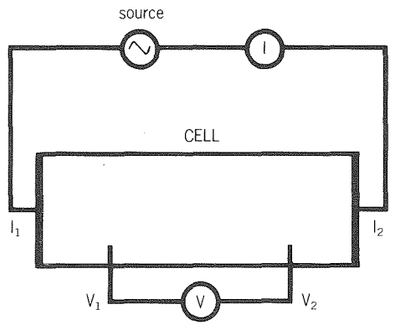
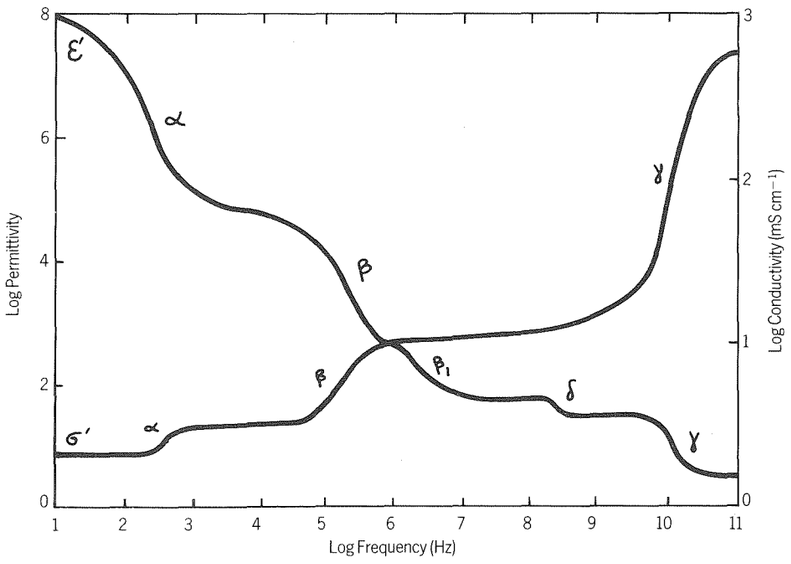


Fig. 6. The principle of using 4 electrodes to determine the AC properties of a system without interference from electrode polarisation. Current from the AC source is measured with a vector ammeter and flows through the system via two current electrodes (I_1 and I_2). The voltage drop across the relevant part of the system is measured using two voltage electrodes (V_1 and V_2) connected to a vector voltmeter of high input impedance. Since a negligible current flows across the interface of the voltage electrode they are not subject to electrode polarisation.

Fig. 7. The frequency dependence of the permittivity ϵ' and conductivity σ' of a typical biological tissue, showing the major classical dielectric dispersions which may be discerned.

Fig. 7



Dielectric properties of biological systems: the effects of cells on electrical fields

The intensive, passive electrical (dielectric) properties of a system between two or more electrodes may be completely characterised by the frequency-dependent *conductivity* σ (units: S/m) and the dimensionless *permittivity* (dielectric constant) ϵ which are related to the measured conductance and capacitance by $\sigma = GK$ and $\epsilon = CK\epsilon_0$. K is a constant with units of reciprocal length known as the cell constant (for plane-parallel electrodes of area A separated by a distance d, K = d/A), whilst ϵ_0 is a constant known as the permittivity of free space and equal to the capacitance of a unit cell containing a vacuum, ϵ_0 has the value 8.854×10^{-12} F/m.

When the dielectric properties of a system are frequency-dependent, the system is said to exhibit *dielectric dispersion,* and a diagrammatic indication of the major dielectric dispersions exhibited by living cells is given in Figure 7. The frequency-dependence of the dielectric behaviour is due to the presence of mobile charged or dipolar species in the system, and the magnitude and frequency-dependence of these dispersions give information on the dynamic structural organisation of living systems. Briefly, the major mechanisms underlying the dielectric dispersions are thought to be: tangential relaxation of ions adjacent to charged cell surfaces (α-dispersion), charging of the capacitances represented by the cell membrane(s) (ß-dispersion), rotation of small dipoles, predominantly cytoplasmic water (γ-dispersion, protein rotation ($ß_1$-dispersion) and the rotation of water and side-chains bound to cellular macromolecules δ-dispersion (Grant *et al* 1978; Pethig 1979, 1984). Recently we noted a novel dielectric dispersion, the μ-dispersion, which may be largely ascribed to the lateral motions (*lateral electrophoresis*) of membrane-associated lipids, proteins and their counterions (Harris and Kell 1983; Kell and Harris 1985a, b; Harris and Kell 1985a). How may such behaviour, *which is independent of electrode properties,* and is therefore wholly non-faradaic, be exploited technologically?

Almost all types of biosensing devices which operate with a 'clamped' voltage might be designed such that their operating principle was not simply amperometric (as is usual) but relied upon the measurement of *impedance* (i.e. impedimetry) at one or more frequencies, including frequencies at which electrode properties *are* manifest (Kell 1986a), though little effort to exploit this idea seems to have been expended to date. However, impedimetric systems may be exploited in cases where there are *no* added electroactive substances present. For instance, we have been able to use impedimetry for the real-time estimation of the biomass content of laboratory and industrial fermentors, a long-standing and thorny problem (Harris and Kell 1985b) to which a solution is widely awaited.

The principle of our approach is that the magnitude of the ß-dispersion of a cellular suspension is proportional to the radius, membrane capacitance and volume fraction of the suspended phase. Since the former two are generally known, or may be assumed constant for a given type of cell, the capacitance or dielectric permittivity of a cellular suspension at frequencies between the α- and ß-dispersions is proportional to the cellular volume fraction or biomass. Particulate matter, which does not possess a cell membrane, does not contribute significantly to the dielectric properties in this frequency range. Thus a measurement of the RF permittivity of a culture provides an accurate and continuous measurement of its biomass content.

With such thoughts in mind, and in collaboration with Dr Bob Todd from Dulas Engineering, Machynlleth, and Dr Stephen Bungard, ICI Agricultural Division, we have developed a 4-terminal instrument, the Dulas Bug Meter (Harris *et al.* 1986; Kell *et al.* 1987; patents pending) which can measure the RF permittivity (and conductivity) of lossy (conducting) samples at frequencies between 0.2 and 10 MHz. Because the electrodes used are metallic, they may be autoclaved (an important consideration in fermentor instrumentation), and fouling of the electrodes is obviated by the periodic application of a high voltage pulse, which electrolyses the adjacent solution and effects electrochemical cleaning of the electrodes. This initiative was recently a recipient of one of 10 SMART awards ('Small firms Merit Awards in Research and Technology') for novel instrumentation sponsored by the Department of Trade and Industry. A photograph of the present device is given in Figure 8(a) whilst representative data obtained with it are given in Figures 8(b) and (c).

Whilst I have here mentioned only this particular application and this particular frequency range (which is suitable for the stated purposes), it should be evident that *measurements* using AC fields provide a route to the development of a large variety of novel instruments for all types of analytical and process control systems. Parenthetically, and in view of the current intellectual climate, it is perhaps worth mentioning that this development followed rather directly from a purely academic, Research Council-funded study of the organisation of bacterial membranes.

Effects of electrical fields on cells

We noted that DC fields could be used to deliver faradaic currents that might be used either in the *measurement* of reactions or analytes or to *drive* chemical reactions. Similarly, we may wonder whether, if AC electrical fields may be used to *measure* cellular properties, can they be used to *affect* them? The answer is of course in the affirmative. However, for a variety of reasons, including the complexity of the ideas and systems involved, and the diversity of the literature on this topic, the idea that electrical fields can affect cellular processes has had something of a chequered history, and still lacks general acceptance. Therefore, and since I regard the fact that electrical fields of relatively low intensity *can* affect cellular processes as amply proven, I simply cite several reviews which list the enormous number of papers in which low-intensity electrical fields *have* been shown to affect one or more metabolic reactions (Presman 1970; Sheppard and Eisenbud 1977; Adey 1981; Konig *et al.* 1981; Becker and Marino 1982; Zimmermann and Vienken 1982; Barker and Lunt 1983; Fröhlich and Kremer 1983; Pilla *et al.* 1983; Adey and Lawrence 1984; Goodman and Henderson 1986). The use of such electrical fields in the stimulation of bone healing (Becker and Marino 1982;

Fig. 8(a)

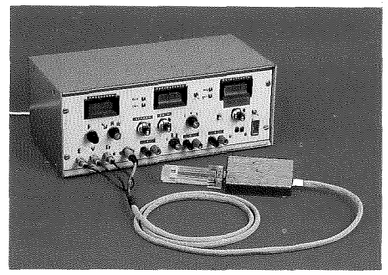


Fig. 8(b)

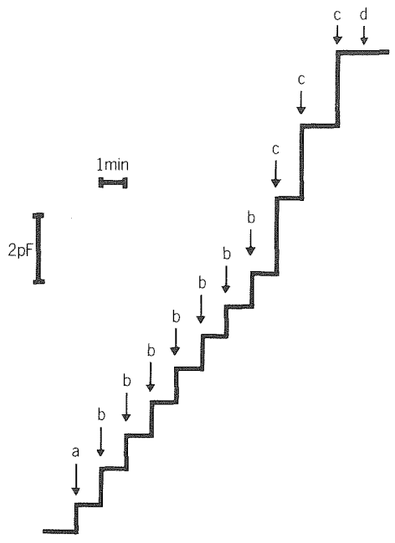


Fig. 8(c)

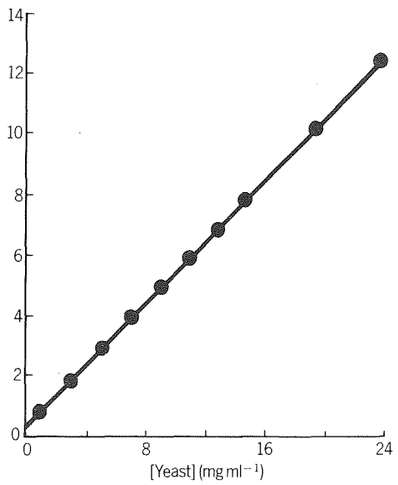


Fig. 8. (a) The Dulas Bug Meter, an instrument for the real-time estimation of cellular biomass and developed as a collaborative project between the University College of Wales, ICI Biological Products Business and Dulas Engineering. (b) and (c) The radio-frequency capacitance of suspensions of yeast *(Saccharomyces cerevisiae)* measured with the Dulas Bug Meter. The reaction medium consisted of 0.2M sorbitol plus 5mM Tris chloride pH 7. (b) Time-dependent behaviour. The Meter output was backed off to give an initial reading of approximately 0 pF. At the point marked a, cells were added from a concentrated suspension to a final concentration of 1 mg dry wt/ml, whilst at the points marked b, aliquots corresponding to a change in biomass concentration of 2 mg/ml were added. At the point marked c, cells were added so as to increase the biomass content by 5 mg/ml, whilst at the point marked d, powdered calcium carbonate was added to a final concentration of 30 mg/ml. The Bug Meter is effectively insensitive to non-cellular, particulate material. (c) Relationship between the capacitance change and the dry weight of cells, using the data in (b).

Fig. 9

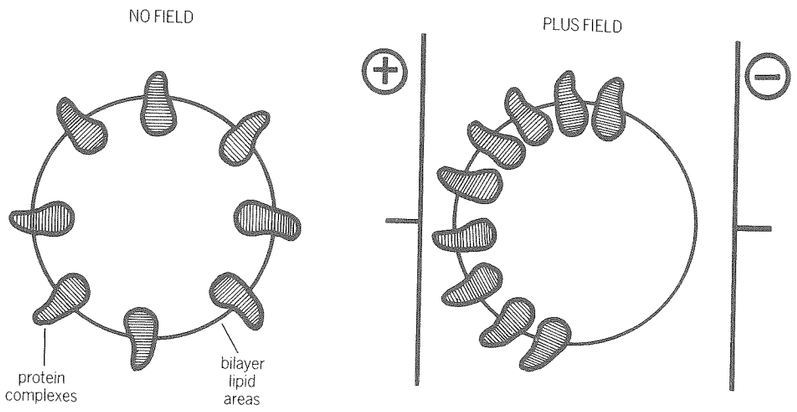


Fig. 9. The principle of lateral electrophoresis of cell membrane components. In the absence of an electrical field, the disposition of proteins in the membrane is liable to be more-or-less random. In a low-frequency or DC field, however, there will be a tendency for proteins (and lipids) that are mobile in the cytoplasmic membrane to move to one or another cell pole. This may affect the kinetics of such enzymes or the polarity of growth. For further details see Kell and Harris (1985a, b; Kell and Westerhoff 1985).

Barker and Lunt 1983) is particularly well known. What then are the mechanisms of such field effects on biological processes?

Unfortunately we have to say that whilst there are many theories, there is at present not a single well-documented case in which a satisfying *mechanistic* explanation of the effect of an electrical field on a living cell has been given. Since any such effects must be mediated via the cell's enzymes, we should begin by realising of course that any electrical field of the appropriate frequency can be absorbed by an enzyme and thereby change its kinetic behaviour (see above). The percentage of enzymes in the system that are actually affected by the field is related to the electric field strength and to the effective dipole moment of the absorbing species (see e.g. Kell and Harris 1985a). Similarly, the phenomenon of 'lateral electrophoresis' (figure 9; Poo 1981) provides a ready explanation of how a steady DC field can polarise the growth and hence development (Rathore and Goldsworthy 1985; McGillivray and Gow 1986) of an organism.

There remains, however, the apparent paradox of how an AC field of an intensity which is low relative to the background thermal energy, and whose 'average' potential is in any event zero, can significantly affect the kinetics of an enzyme. One solution (e.g. Fröhlich and Kremer 1983; Adey and Lawrence 1984; Kell and Westerhoff 1985) resides in the fact that living systems may transduce free energy by non-thermal means, and there is certainly an abundance of circumstantial evidence in favour of this view (Welch and Kell 1986). Further, even if proteins act as purely isothermal, passive devices, calculations have recently shown that the biophysical properties required to permit free energy transduction from an oscillating external electrical field are in fact common to most proteins, especially membranous ones (Westerhoff *et al.* 1986). Whilst this is not the place to discuss the quantitive energetic considerations underlying this statement, it is obvious from the foregoing that we really are now beginning to obtain a proper understanding of some of the means by which an electrical field may affect an enzyme.

To understand how an electrical field may affect a complex metabolic process, we must take cognisance of the fact that the turnover number of an enzyme and the rate of flux through a metabolic pathway of which it is a part may be only loosely related. This realisation, due in particular to the work of Kacser, Burns, Heinrich and Rapoport (see Westerhoff *et al.* 1984), now opens up the possibility of giving a proper, quantitative description of the way in which cellular processes are controlled by *any* external parameter (including an electrical field), and of providing a *rational* approach to the improvement of biotechnological proc esses generally (Kell and Westerhoff 1986a, b). Armed with these theoretical considerations, a logical corollary is that just as drugs may be used to activate or inhibit specific (target) enzymatic processes, so might we expect in the future that electrical fields of appropriate waveforms will be found to exhibit specificity. The idea of *designing* an appropriate waveform for a particular purpose, whilst as yet speculative, requires only the same logical processes as does that of designing a chemical compound for such a purpose, and it seems at least plausible that the former, if not the latter, might be done in a manner which minimises side-effects. The commercial possibilities in this area, if reasonably distant, are obviously unlimited.

The earlier part of this section considered the effects on cells of electrical fields of an unspecified (but generally low) intensity. In the remainder of the article I shall turn to some of the very exciting possibilities, which are now being realised, of exploiting electrical fields of *high* intensity. The main procedures involved here are known as electroporation, electrotransformation and electrofusion.

Fig. 10

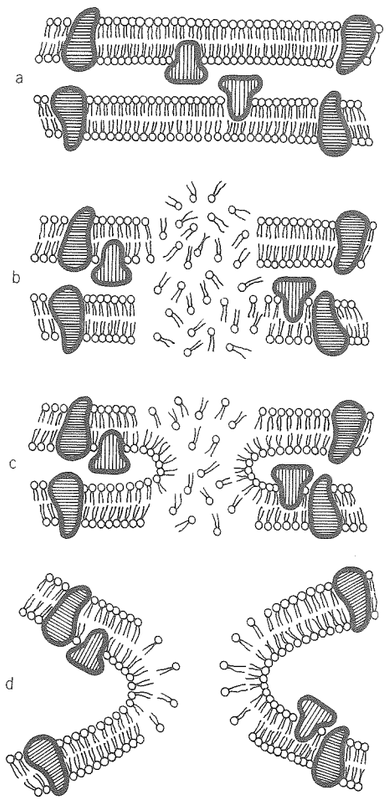


Fig. 10. Some of the molecular processes possibly occurring during the electrofusion of cells. Two adjacent lipid membranes (a) are exposed to an electronic pulse of high field strength, causing an electrical breakdown via disorganisation of the bilayer structure (b). This may lead to the formation of bridge structures between the bilayers of the two membranes during the subsequent resealing process (c) and to spherical two-cell aggregates (d). (After Zimmermann and Vienken 1982).

Electroporation, electrotransformation and electrofusion

A Coulter counter is a well-known device for the sizing of particles (see Harris and Kell 1985b); its principle is that a particle passing through a small orifice through which current is forced to flow, causes a fluctuation in the potential difference between the ends of the orifice which may be measured and which is proportional to the particle size. In 1973, Zimmermann *et al.* noted that the apparent size of *Escherichia coli* cells in a Coulter counter was not, in contrast to the theory, independent of the voltage difference between the plates which the orifice separated, and reasoned correctly that the high electrical fields were causing a transient (reversible) dielectric breakdown of the cytoplasmic membrane surrounding the cells, an effect which became more substantial the higher the electrical field and which was first noted in different circumstances by Sale and Hamilton (1967).

Following these observations, Zimmermann's group and others have studied in detail the electric field-dependence of the integrity of cellular membranes (e.g. Zimmermann 1982; Dimitrov and Jain 1984; Zimmermann *et al.* 1984, 1985) and have found that transient dielectric breakdown (electroporation or electric field-induced pore formation) may be exploited in two ways in particular: (a) to facilitate the entry of material to which the cell is normally impermeant, and (b) actually to fuse cells of different types. The former is of especial biotechnological interest in cases in which one wishes to transfer a gene into a cell, whilst the latter, which is of benefit for the creation or breeding of hybrid cells, such as those capable of producing monoclonal antibodies, is facilitated by devising conditions in which the cells are adjacent to each other just prior to the fusing pulse.

Whilst the mechanisms of electroporation and electrofusion are probably not identical, they share certain similarities, the crucial one being that the electrical field can induce a transmembrane potential which, by electromechanical forces, causes the disruption of the normally ion-impermeable membrane organisation. Provided that the electric field pulse does not last long (say $<10-50\mu s$), for otherwise *irreversible* membranes, permeabilisation occurs, the pores formed in the cell membrane can reseal, and if two cells are adjacent at this time they may fuse (figure 10).

Concluding remarks

What I hope to have been able to convey in this short and selective review is (i) that biolectromechanical phenomena underlie a great many cellular processes, (ii) that a great many recent technological innovations, in both analysis and synthesis, have developed directly from academic bioelectrochemical studies, and (iii) that, even without reference to biochips and the like (van Brunt 1985), a plausible extension of the more insightful ideas current in bioelectrochemistry can provide a springboard to numerous important areas of the science and technology of the not-too-distant future.

Acknowledgements

I thank the SERC and ICI Agricultural Division for financial support and Dr John Hedger and Professor Gareth Morris for reading the manuscript.

References

Adey, W. R. 1981 *Physiol Rev. 61,* 435.
Adey, W. R. and Lawrence, A. W. (Eds) 1984 *Nonlinear electrodynamics of biological systems,* Plenum Press, New York.
Albery, W. J. and Bartlett, P. N. 1985 *J. Electroanal. Chem. 194,* 211.
Aston W. J. and Turner, A. P. F. 1984 *Biotechnol. Genet. Eng. Rev. 1,* 89.
Bard A. J. and Faulkner, L. R. 1980 *Electrochemical Methods,* Wiley, Chichester, U.K.
Barker A. T. and Lunt, M. J. 1983 *Clin. Phys. Physiol. Meas. 4,* 1.
Becker R. O. and Marino, A. A. 1982 *Electromagnetism and Life,* University of New York Press, Albany, N.Y.
Bennetto, H. P. 1984 *Life Chem. Rep. 2,* 363.
Bockris J. O'M. and Reddy, A. K. N. 1970 *Modern electrochemistry, Vols 1 and 2,* Plenum Press, New York.
Bond, A. M. 1980 *Modern polarographic methods in analytical chemistry,* Marcel Dekker, New York.
Clark, W. M. 1960 *Oxidation-reduction potentials of organic systems,* Williams and Wilkins, Baltimore, Maryland.
Davis, G. 1985 *Biosensors J. 1,* 161.
Dimitrov D. S. and Jain, R. K. 1984 *Biochim. Biophys. Acta 779,* 437.
Frohlich H. and Kremer, F. (Eds) 1983 *Coherent excitations in biological systems,* Springer, Heidelberg.
Fultz M. L. and Durst, R. A. 1982 *Anal. Chim. Acta 140,* 1.
Goodman R. and Henderson, A. S. 1986 *Bioelectrochem. Bioenerg. 15,* 39.
Grant E. H., Sheppard R. J. and South, G. P. 1978 *Dielectric behaviour of biological molecules in solution,* Oxford University Press, Oxford.
Green M. J. and Hill, H. A. O. 1986 *J. Chem. Soc. Faraday Trans.* I, *82,* 1237.
Harold, F. M. 1986 *The vital force; a study of bioenergetics,* Freeman, Oxford.
Harris C. M. and Kell, D. B. 1983 *Bioelectrochem. Bioenerg. 11,* 15.
Harris C. M. and Kell, D. B. 1985a *Eur. Biophys. J. 13,* 11.
Harris C. M. and Kell, D. B. 1985b *Biosensors J. 1,* 17.
Harris C. M., Todd R. W., Bungard S. J., Lovitt R. W., Morris J. G. and Kell, D. B. *Enz. Micr. Technol.,* in press.
Hill H. A. O., Oliver B. N., Page D. J. and Hopper, D. J. 1985 *J. Chem. Soc. Chem. Comm.* 1469.
Jack J. J. B., Noble D. and Tsien, R. W. 1975 *Electric current flow in excitable cells,* Oxford University Press, Oxford.
Kell, D. B. 1986a *Modern Bioelectrochemistry* (F. Gutmann and H. Keyzer, Eds), p xi, Plenum Press, New York.
Kell, D. B. 1986b *Biosensors; fundamentals and applications* (Turner, A. P. F., Karube I. and Wilson, G. S., (Eds), p429; Oxford University Press, Oxford.
Kell D. B. and Harris 1985a *Eur. Biophys. J. 12,* 181.
Kell D. B. and Harris, C. M. 1985b *J. Bioelectricity 4,* 317.
Kell D. B., Samworth C. M., Todd R. W., Bungard S. J. and Morris, J. G. 1987 *Studia Biophys.,* in press.
Kell D. B. and Westerhoff, H. V. 1985 *Organised multienzyme systems: catalytic properties* (G. R. Welch, Ed.) p.63 Academic Press, New York.
Kell D. B. and Westerhoff, H. V. 1986a *Trends in Biotechnol. 4,* 137.
Kell D. B. and Westerhoff, H. V. 1986b *FEMS Microbiology Revs. 39,* 305.
Kissinger P. T. and Heineman, W. R. (Eds) 1984 *Laboratory techniques in electroanalytical chemistry,* Marcel Dekker, New York.
Konig H. L., Krueger A. P., Lane S. and Sonnig, W. 1981 *Biologic effects of environmental electromagnetism,* Springer, Heidelberg.
McGillivray A. M. and Gow, N. A. R. 1986 *J. Gen. Microbiol. 132,* 2515.
Nicholls, D. G. 1982 *Bioenergetics,* Academic Press, London.
Pethig, R. 1979 *Dielectric and electronic properties of biological materials* Wiley, Chichester, U.K.
Pethig, R. 1984 *IEEE Trans. Electr. insul. EI-19,* 453.
Pilla A. A., Sechaud P. and McLeod, B. R. 1983 *J. Biol. Phys. 11,* 51.
Poo M-m 1981 *Ann. Rev. Biophys. Bioeng. 10,* 245.
Pressman, A. S. 1970 *Electromagnetic fields and life,* Plenum Press, New York.
Rathore K. S. and Goldsworthy, A. 1985 *Bio/Technology 3,* 1107.
Sale A. J. H. and Hamilton, W. A. 1967 *Biochim. Biophys. Acta 148,* 781.
Sawyer D. T. and Roberts, J. L. 1974 *Experimental electrochemistry for chemists,* Wiley-Interscience, New York.
Simon H., Bader J., Gunther H., Neumann S. and Thanos, J. 1984 *Ann. N. Y. Acad. Sci. 434,* 171.
Simon H., Gunther H., Bader J. and Neumann, S. 1985 *Ciba Found. Symp. 111,* 97.
Sluyters-Rehbach M. and Sluyters, J. H. 1986 *Comprehensive Chemical Kinetics 26* (C. H. Bamford and R. G. Compton, Eds.) p.203, Elsevier, Amsterdam.
Turner A. P. F., Karube I. and Wilson, G. S. (Eds) 1986 *Biosensors: fundamentals and applications,* Oxford University Press, Oxford.
Van Brunt, J. 1985 *Bio/Technology 3,* 209.
Welch G. R. and Kell, D. B. 1986 *The fluctuating enzyme* (G. R. Welch, Ed.) p.451, John Wiley, Chichester, U.K.
Westerhoff H. V., Groen A. K. and Wanders, R. J. A. 1984 *Biosci. Rep. 4,* 1.
Westerhoff H. V., Tsong T. Y., Chock P. B., Chen Y. and Astumian, R. D. 1986 *Proc. Natl. Acad. Sci. 83,* 4734.
Zimmermann, U. 1982 *Biochim. Biophys. Acta 694,* 227.
Zimmermann U., Schultz J. and Pilwat G. 1973 *Biophys. J. 13,* 1005.
Zimmerman U. and Vienken, J. 1982 *J. Membr. Biol. 67,* 165.
Zimmerman U., Vienken J. and Pilwat, G. 1984 *Investigative Microtechniques in Medicine and Biology* (J. Chaven and L. Bitensky, Eds) p.89, Marcel Dekker, New York.
Zimmermann U., Vienken J., Halfmann J. and Emeis, C. C. 1985 *Adv. Biotechnol. Proc. 4,* 79.