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An Electrochemical Consideration of Electromagnetic Bioeffects

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INTRODUCTION

Pulsed electromagnetically induced current (PEMIC) has been shown to stimulate the healing of delayed and non-union fractures (1-8). In addition, many cell and tissue systems have been affected by PEMIC having specific waveform parameters (9-18). It is important to consider the origins of the choice of these waveform parameters in order to relate them to a study of the mechanism of PEMIC bioeffects. This author was profoundly influenced by the early work of Becker (19) who proposed that electric fields play a substantial role in regeneration. Yasuda, Brighton, and Bassett (20-22) suggested that the pathway through which bone adaptively responds to mechanical input may be electrical. Pilla took the findings of these authors and used an electrochemical approach to predict a set of bioeffective electrical waveform parameters based on electrochemical kinetic interactions at the cell's surfaces (23-30). This approach ultimately led to the creation of PEMIC waveforms now in widespread clinical use for orthopaedic applications. It is the purpose of this chapter to review how electrochemistry has played a role in the electromagnetic modulation of cell and tissue behavior.

BASIC ELECTROCHEMICAL KINETICS APPLIED TO THE CELL SURFACE

Why consider electrochemical processes at the cell surface? The answer is that it provides a quantitative look at the cell's real-time responses to electromagnetic fields. Here it is important to note that the role of ions as transducers of information in the regulation of cell structure and function has gained widespread acceptance. Examples of ionic control mechanisms include: growth-factor activation of Na–K ATPase in fibroblasts (31,32), nerve growth-factor effects regulated by Na–K ATPase (33,34), Ca^{2+} regulation of the cell cycle via calmodulin (35,36), differential Ca^{2+} requirements of neoplastic vs. non-neoplastic cells (37,38), and Ca^{2+} dependent adenylate cyclase activation in macrophages (39). Ionic control mechanisms therefore represent a coupling mechanism for electromagnetic fields which can be quantitatively analyzed. The interaction of ions at the electrically charged interfaces of a cell is an example of a

potential or voltage dependent process. The following is a review of the basic electrochemical kinetic approach to quantitate these ionic and/or dipolar interactions.

The working concept of electrochemical information transfer *in vivo* (25) uses the analogy between the electrified interfaces at the electrode/electrolyte and the membrane/fluid junctions. A change in the electrochemical microenvironment of the cell can cause the structure of its electrified surface regions to be modified by, for example, changing the concentration of a specifically bound ion or dipole which may be accompanied by a modification in the conformation of molecular entities (such as enzymes) in the membrane structure. Basically, therefore, the regulatory interactions at a cell's surface are considered to have both potential and kinetic functions associated with the specific biochemical events to which these processes may be coupled.

If, as proposed, membrane structural changes that lead to a modification in membrane function involve or are caused by the basic electrochemical event of a specific adsorption (binding), it is easy to see how this process can be a key step in cellular response to the variety of external inputs which have been utilized. For skeletal tissue, the functional response to mechanic- al input can be envisaged to occur via at least two modalities involving electrochemical surface steps. If the cell membrane is not mechanically deformable (or does not experience significant force when in the collagen matrix), but is in direct membrane-collagen (matrix) contact via, for example, ligand bridging, then the known piezoelectric properties of the collagen bundles can modify the charge-charge interaction at the cell/collagen interface. On the other hand, certain membrane structures may be modified by mechanical input and result in new or increased specific adsorption and/or membrane transport. The latter may be more likely since it has been shown that the functional response of proliferative cartilage cells does not depend on whether they are bound to the matrix. Whatever structure responds directly to external force, it is clear that the cell membrane can exhibit new or modified charged-species interactions as required by the electrochemical information approach.

Under these conditions it would be expected that the cell membrane's electrical impedance would be modified, but in a different manner, dependent upon whether the cell exhibited direct or indirect response to mechanical input. In this context a variety of studies have shown that an electrical relaxation exists in bone tissue (alive or dead) when it is stressed (20,22). A portion of the slowly varying voltage function which is observable in bulk tissue at each stress input albeit very small, may be due to a transient change in the cell/collagen electrified interface structure. The time constant (kinetics) of this effect would be expected, as will be shown below, to be orders of magnitude smaller than the observed response function. Evidence that what the cell "sees" as a direct electrical input signal (in the absence of electrode effects) is indeed a much shorter-lived transient current, comes from attempts to mimic the stress-related relaxation signal. This has consistently failed to generate a biological response unless electrolysis and/or ion

migration effects were obviously present.

When current is injected via electrodes, cellular response can again involve electrochemical surface steps if the electrochemical microenvironment is modified. Without considering the gross effects of electrolysis at this point, the ionic distribution changes that occur over a period of time during direct current (DC) flow can indeed couple to the concentration dependence (isotherm) of a specifically bound entity. It has already been observed that ionic changes in the extracellular fluid can cause a modification in cell state function (11,23,25,26). In fact, simple changes in extracellular ionic microenvironment can influence the rate of cell differentiation and even redirect its developmental pathway (40-42). If electrochemical information transfer is operative in cellular control, then there should be a direct functional response to the (pure) injection of current, provided it reaches the relevant cell surface and that the wave- form parameters are chosen to modulate the kinetics of the directed electrochemical surface step. This chapter will consider the details of the type of charge interactions that could be involved in the cell's detection of, and reaction to, its immediate environment. In particular, the possible unifying nature of the electrochemical information transfer concept in cell regulation will be discussed in detail.

It is appropriate at this point to briefly consider the relation of membrane structure to function, and how interfacial electrochemical (non-faradaic) effects can be an integral part of this structure/function relationship. Among the various models of membrane structure (43,44) the dynamic fluid mosaic approach (45) appears to be the most consistent with observed behavior. The basic molecular components of cell membranes are lipids, proteins, and carbohydrates. Their molecular movements, conformations and interactions are without doubt influenced by the environment and can form part of a molecular feedback loop for cell regulation (46). Lipids are responsible for the structural integrity of the membrane. Membrane proteins are within this lipid fluid (integral proteins), or on the surface (peripheral proteins). They are highly mobile, and probably provide the structural modifications related to functional regulation.

Both the lipid and protein portions of the membrane contain hydrophobic and hydrophilic segments. The hydrophilic segments form the membrane side of the electrified interfaces at which specific adsorption (binding) can take place. Two distinct types of electrochemical interactions can occur at cell surfaces. The first involves all of the non-specific electrostatic interactions involving water dipoles and hydrated (or partially hydrated) ions. This structure is analogous to the electrode/electrolyte interface and can be contributory, along with lipid and protein asymmetry (47-49), to the observed dielectric response of the lipid and lipoprotein membrane structures (24,30). For small amounts of charge input, only minor modifications of this portion of a cell's surface structure would be expected. This is so for two reasons. The first relates to the fact that these non-specific electrostatic interactions are physically in series with the membrane

dielectric structure, primarily due to the lipid bilayer fluid. Under these conditions any charge perturbation that could satisfy the kinetic requirements of these interfacial structures will primarily be experienced by the lipid dielectric. In addition, these non-specific interactions are, to a good first approximation, governed by a Boltzmann distribution with respect to the aqueous layer. Thus, over this portion of the interface, water dipoles would be expected to provide the first layer of charge interaction with the membrane surface as opposed to the more specific ion interactions discussed below. Water dipoles followed by a rigid layer of partially hydrated ions form an equilibrium structure which would be perturbed only to a negligible degree by low-level charge injection.

A second type of charge interaction at a cell surface involves potential-dependent specific ad sorption (or binding). Here an ion or organic dipole can effectively compete with water dipoles and hydra ted ions for specific membrane sites. This type of interaction involves, for the aqueous phase, the steps of dehydration, displacement and binding (50,51). If this is to engender a membrane function change, then the structure of the molecular entity within the membrane at which the binding occurs can undergo modification. For example, the allosteric nature of certain enzyme surely allows this to occur (52). In addition to enzyme activity it is known that biochemical reactions on the cell surface involve charged reactants (53), and the surface potential (and therefore structure) is experienced by an ionic species involved in membrane transport (54,55).

The most straightforward method to quantitate the above approach, which also provides unambiguous parameters capable of being experimentally tested, is to generate the electrical impedance of each relevant electrochemical pathway. All variables in this study will be given in terms of the complex frequency plane using the Laplace transformation (56). This frequency variable, s, has a real part, σ, and an imaginary, $j\omega$ part which define the axes of the Laplace plane. Utilization of the Laplace transformation allows a time domain function (such as a pulsating current) to be expressed in terms of its frequency content. Utilization of this transformation along the imaginary ($j\omega$) axis results in the familiar Fourier transformation by which the frequency spectrum of time domain signals is often expressed. Determination of the impedance, $Z(s)$, of a cellular system will ultimately require, as will be shown in a later section, knowledge of the input pulsating current waveform and the pulsating voltage response of the membrane.

Here it will be considered that the physical passage of current into the membrane causes a change in its surface charge (transmembrane transport is neglected). The total current $i_T(s)$, can be considered, in light of the above discussion, to be the sum of a dielectric and double-layer charging portion $i_D(s)$ and a specific adsorption portion, $i_A(s)$. In other words, the total current can be written:

$$i_T(s) = i_D(s) + i_A(s) \tag{1}$$

Each of the above contributions to the total current can be related to the physical situation likely to exist at a living membrane.

Under linear conditions $i_D(s)$ represents the charging (discharging) of the surfaces of the lipid bilayer fluid which, because of structural asymmetry has different charge density on the intra- and extracellular surfaces, respectively. This structure behaves like a capacitor over the frequency range of interest for both observation and excitation (DC to 25 MHz). This is the well-known membrane dielectric capacitance, defined here as C_D. At each membrane/solution interface there exists, in addition, the electrostatic double-layer capacitance defined as C_i on the intracellular side and as C_e on the extracellular side. All charge separations corresponding to these membrane properties can be considered, to a first approximation, to lie on planes separated, for the dielectric capacitance, by 50–100 Å, and for the compact electrical double-layer by only a few Angstroms. Note that the latter is most certainly perturbed to some extent by the presence of carbohydrate on the extracellular surface of the membrane. However, this would be expected to be equivalent to surface roughness which would not change the basic capacitor analogy, but merely add a two-dimensional aspect. In view of all of the above, $i_D(s)$ may be related to the transient voltage response, $E(s)$, by:

$$Z_D(s) = E(s)/i_D\,(s) = [1/s][1/C_i + 1/C_D + 1/C_e] \tag{2}$$

which describes the frequency behavior of the non-specific electrostatic portion of the membrane structure. Examination of Equation (2) shows that $Z_D(s)$ represents pure capacitive behavior for three capacitors in series.

Experimentally it is often observed that the majority of new charge is associated with C_D because it is much smaller (approximately 0.5 μF/cm^2) than either C_e or C_i which, by analogy with the electrode/electrolyte interface (25), are in the range of 10 μF/cm^2 . This difference in interfacial and dielectric capacitance arises mainly because of the large difference in distance between the "planes" of charge separation associated with each capacitor (50–100 Å for C_D, and 1–6 Å for C_i and C_e). Because of this physical situation, it is important to realize that the majority of the voltage change, $E(s)$, in response to $i_D(s)$ will appear across C_D, indicating that, in the absence of specific adsorption, most of the membrane charge acceptance will be associated with its dielectric structure. In most cases, therefore, $Z_D(s)$ can be represented by a single capacitor, C_D, the charging of which in response to low-level pulsating current would not be expected to alter membrane structure in a functional (regulatory) pathway since its equilibrium (or resting) structure would remain unaltered.

To quantitate the specific adsorption process, it is necessary to consider both its potential and concentration dependencies. The surface concentration, Γ, of the specifically adsorbed species can be equal to the number of ions (or dipoles) that penetrate the oriented water dipole layer. The specific adsorption current, $i_A(s)$ of

Equation (1) will be utilized by the membrane to create a net change in the surface concentration, $\Delta\Gamma(s)$, of the bound species. In addition, the rate of specific adsorption can be considered, under linear conditions, to be adequately represented by first-order kinetics. Specific adsorption current i_A(s) may be represented by:

$$i_A(s) = q_e s \Gamma_e \Delta\Gamma_e(s) \tag{3}$$

wherein only binding of a single species at the extracellular interface (subscript *e*) is considered (realizing that the adsorption of several species can occur, and at each interface), and q_e is a coefficient representing the dependence of interfacial charge upon the surface concentration of the bound species.

Equation (3) can lead to an expression for the impedance of specific adsorption, $Z_A(s)$, if the quantity $\Delta\Gamma(s)$ can be related to experimentally accessible parameters. This can be done, for the linearized conditions of this study, if a specific kinetic expression relating binding to potential changes is used. For this it is convenient to write:

$$\Gamma_e(s) = [v_e/\Gamma_e s][\Delta\Gamma_e(s) + aE(s)] \tag{4}$$

which states that the rate of change of surface concentration of the binding species is a function of the change in potential, $E(s)$, via its potential dependence, a, and the exchange rate constant, v_e, taking into account that two species may not occupy the same site.

The adsorption impedance $Z_A(s)$ can now be written using Equations (3) and (4), as:

$$Z_A(s) = 1/q_e a[(1 + \Gamma_e s/v_e)/\Gamma_e s] \tag{5}$$

Inspection of Equation (5) shows that the specific adsorption process is functionally equivalent to a series $R_A - C_A$ equivalent electric circuit. The heterogeneous adsorption process thus behaves as a lumped parameter system wherein the kinetic term is given by:

$$R_A = 1/q_e a v_e \tag{6}$$

As expected, R_A is inversely proportional to the exchange rate constant. C_A, which represents the accumulation of charged species at the kinetic site in question, is given by:

$$C_A = q_e a \Gamma_e \tag{7}$$

C_A is directly proportional to the resting concentration of adsorbed species about which its perturbation exists. It is now possible to construct the equivalent electrical model of a cell membrane at which the two considered processes exist. Inspection of Equations (1), (2) and (5) shows that the two current pathways consist of C_D, in parallel with R_A and C_A, which are themselves in series.

The above discussion illustrates (for a very simple case) the manner by which membrane charging can be utilized by the cell as a real-time event in its regulatory

process. The relative rates of these non-specific and specific interfacial electrochemical steps is expected, from physical considerations alone, to be significantly different. In the context of electrochemical information transfer, this kinetic separation allows the concept of rate modulation selectivity to be considered. For example, if the specific adsorption process involves a regulatory enzyme, its average activity could be increased by affecting a net change in the surface concentration of bound ions. The first requirement for this is to satisfy the kinetics of, for example, the process described above during each current perturbation. As expected and observed (10,12,16-18), waveform duration is one of the most important parameters to achieve this result. The second requirement is to achieve sufficient charge injection to satisfy the potential dependence of the adsorption process. This would be relatively easy if there were no other adsorption processes with possible overlapping kinetics and potential dependences. This is certainly not the case, although for a given tissue in a given developmental, repair or maintenance phase it is possible that the overriding regulatory process involves a single family of membrane-bound entities. In view of this, it is reasonable to assume that a relationship will exist between waveform amplitude and width over the available selectivity range.

The real situation is not, however, as simple as just described. Because low-level perturbations are employed, linear or very near linear conditions can be expected in terms of the real-time direct response to the pulsating current input. It is therefore necessary to add the important variable of pulse repetition rate to the selectivity requirements. Of significance to cell function is the degree of kinetic coupling between biochemical follow-up reactions and the triggered electrochemical surface events. It thus becomes a question of the kinetics of the molecular control loop within the cell. These considerations mean that the new boundary conditions for the control loop must be maintained each time the surface process is expected to be involved in the loop kinetics. The number of times the new loop conditions must be present for a functional consequence is, of course, unknown. In view of the physical nature of the surface processes involved in electrochemical information transfer, it is not expected that the repetition rate window will be as narrow as that in, for example, the alpha rhythm brain activity.

The above discussions provide a simplified quantitative picture of electrochemical information transfer in terms of cell surface regulation. It is obvious that the myriad surfaces and junctions which a cell exhibits may play a key role in its response to functional modification. However, recent evidence (57,58) tends to suggest that the cell surface is one of the major factors in cell regulation, and it may be the target area for current injection. It is obvious that the two membrane charging pathways given above allow an approach to selectivity in the choice of input current waveform by virtue of the different relaxation times associated with each process. Thus, for a given amplitude, pulse duration could be employed to achieve a selective response. Selectivity on the basis of pulse duration alone is, of course, not sufficient since, at the very least, the

microenvironment of the cell, its state of function, position in cycle, etc., will contribute to the ultimate functional response.

The above discussion presents a working model of electrochemical information transfer by which the injection of low level current (in the $\mu A/cm^2$ range) can provide functional selectivity in the kinetic modulation of cell regulation. This method of predicting current waveform parameters is valid only when current is applied without concomitant electrolysis effects. When the latter are present the chemical microenvironment is under continual modification usually in an uncontrolled manner (with one notable exception (59)). Under these conditions the basic step of electrochemical information transfer may be present in the regulatory events occurring as the cell adapts to the modified environment. Interestingly, it is then possible to kinetically modulate the response to this new environment if the cell's surfaces and junctions are involved, by the superimposed injection of pure current. Indeed, if the kinetics of cell response to a modification in its chemical environment can be modulated, it then becomes possible to speak of synergistically enhancing the action of pharmacological agents, either by allowing a significantly reduced concentration to be employed, or through a basic enhancement of effectiveness. Preliminary experiments involving the lectin activation of peripheral human lymphocytes has shown this to be possible (60). A notable increase in the ability of concanavalin A to activate DNA synthesis was observed in the presence of injected current configured for specific adsorption selectivity. The implication of this in the possible enhancement of pharmacological agents is evident. In the context of bone repair, it is conceivable that the implanted electrode (used under controlled electrolysis conditions) and induced current techniques may well be synergistically employed for certain clinical situations.

GENERATION OF PEMIC WAVEFORMS — RELATION TO CELL IMPEDANCE STUDIES

To generate a voltage and current in tissue it was first decided that the induced waveform should have basically rectangular characteristics. This resulted from the kinetic analyses given above, which showed that excitation of real-time charge interactions can be more selectively accomplished if the driving voltage is relatively constant during the perturbation. In addition, the fact that inductive coupling results in a bipolar waveform has to be taken in to account. In other words, if the model given in the previous section is valid, then the potential-dependent specific adsorption process can be more selectively perturbed if the driving waveform at the cellular level looks potentiostatic to the cell surface. This does not imply that waveforms other than rectangular will not provide a bioeffect. However, it does imply that rectangular waveforms may provide a greater degree of selectivity.

PEMIC waveform parameters in tissue are directly related to the electrical

characteristics of the coil. For any coil the induced electromotive force (EMF) is proportional to the rate of change of current in the coil (dI_c/dt). The evaluation of this quantity for a given coil perturbation results in a description of the shape of the induced waveform in vacuum, air, and all nonmagnetic homogeneous conducting media in which the resulting current flow is not high enough to produce a sufficient back EMF for phase relationships to cause a waveform modification. The electrical characteristics of the coil relevant to PEMIC generation are its resistance, R_c, and inductance, L. The coil impedance is:

$$Z_c = R_c + Ls \tag{8}$$

from which dI_c/dt may be evaluated if the coil driving function is known. The coil-driving function $V(t)$ may be arbitrary and constructed such that the coil current $I_c(t)$ rises from and returns to zero in an arbitrary manner. From the electrochemical information transfer concept it was decided that cell surface electrochemistry should be modulated using voltage control. This means that the electric field at the cell interface should have a rectangular shape. To generate this field, the coil-driving function, $V(t)$, should be such that coil current, $I_c(t)$, rises and falls linearly from zero. The relative slopes may be equal or very asymmetrical, giving rectangles of differing amplitude and widths for dI_c/dt during coil charge and discharge. The Electro-Biology, Inc. coils are driven with a voltage step which is shut off in a time much shorter than the coil time-constant ($\tau_c = L/R_c$).

The coil current for an air-core inductor for a voltage step V_0 is:

$$I_c(t) = V_0/R_c[1 - exp(tR_c/L)] \tag{9}$$

Equation (9) shows that $I_c(t)$ rises exponentially to the short-circuit value (V_0/R_c) at a rate determined by the coil time constant, $\tau_c = L/\mathrm{R_c}$. The waveform of the induced voltage is a direct function of dI_c/dt, which is

$$dI_c/dt = V_0/L\,[exp(-tR_c/L)]. \tag{10}$$

Equation (10) clearly shows that, to achieve a rectangular-type induced waveform when V_0 is applied, should be greater (by 10 times) than the desired pulse width. This can be achieved by proper choice of L and R_c. One modality is to keep L relatively small so that safe driving voltages (<25 V) can be employed. Note that, as given by Equation (8), the maximum induced voltage (as t → 0) is inversely proportional to coil inductance for a given V_0. Effective coil resistance can be kept small by utilizing heavy magnetic wire and connecting cable (14 to 16 B&S gauge). With the above taken into account, it is easy to see that, for a given T_c, V_0 can be applied to the coil for as long a time as the following relation is approximately valid:

$$dI_c/dt = V_0/L\,(1 - tR_c/L) \tag{11}$$

Over the time during which Equation (11) holds, an induced voltage waveform in the form of a step having some negative slope will be achieved. To maintain the relative rectangular nature of the induced voltage, pulse-shaping circuitry allows $I_c(t)$ to be kept linear during magnetic-field collapse.

The waveform described in Figure 1 has been utilized with pulse widths varying from 20 to 400 μsec in the main polarity *T1*, and from 2 μsec to 6 msec *T2* in the opposite polarity. The pulses are repetitive, usually between 1–100 Hz and are sometimes set up as pulse bursts . The signal in primary use for recalcitrant bone-fracture repair is a 5-msec burst of pulses having 200 μsec main and 20 μsec opposite polarity. The repetition rate within the burst is approximately 4.4 KHz and the signal repetition rate is 15 Hz. To understand the rationale for the creation of a burst-type waveform it is useful to consider real-time cell-waveform interactions. For this it is useful to recall that the geometric dependence of the dosage of inductively-coupled current is predictable (61). Further, it has been shown that both Laplace and Fourier transforms are useful to describe various frequency characterizations of induced waveforms (29,62,63). Here this analysis is reviewed in the context of optimal coupling to cell surface electrochemical kinetics.

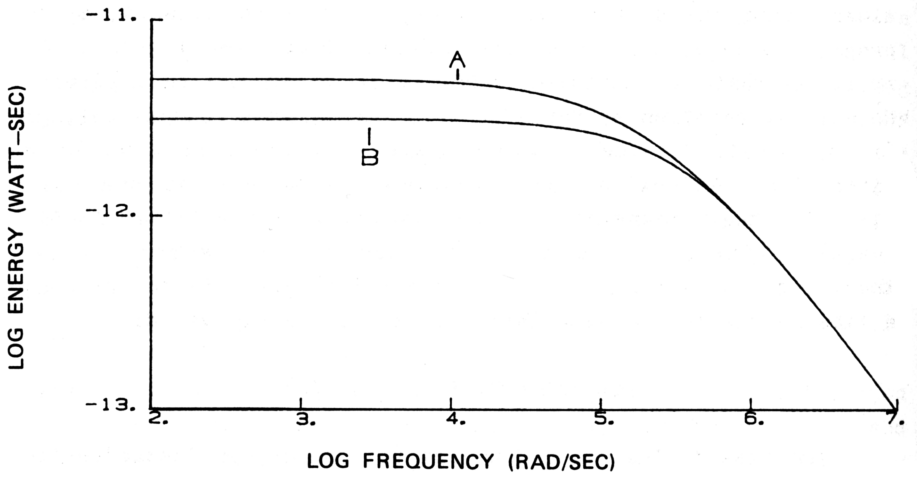

Figure 1. Schematic of the basic waveform of the (PEMIC) induced electric field vector considered in this study. The amplitudes of the main polarity (*A*) and opposite polarity (*B*) portions are controlled by the coil driving voltage. Note that $A \times T1 = B \times T2$ (*T1* and *T2* are the respective polarity pulse durations) so that for a larger *T2*, the opposite polarity amplitude (*B*) is lower when *A* is constant. The waveform exists in tissue and represents that voltage which can drive current into the specific adsorption-like pathway at the cell membrane. In a typical application the induced electric field is generated using two identical (10 × 10 cm) air-gap coils wound with 50 turns of No. 14 copper magnet wire set for approximately Helmholtz (magnetically-aiding) behavior (i.e., the intercoil distance is 6.5 cm). The effective amplitude reported for many *in vitro* and *in vivo* studies appeared to be that for which *dB*/*dt* ranged from 1–3.5 Gauss/μsec.

To quantitate waveform-cell interactions it must be pointed out that PEMIC appears to act as a trigger (29,63). Even the most powerful signal reported does not input sufficient energy to account for changes in processes as simple as ion fluxes (63). Also , it is clear that the overall biological response is not related in a simple linear manner to the average total power in the signal. For example, several systems exhibit similar responses to a single pulse and a pulse burst containing 21 of these pulses, both at identical amplitude and repetition rate (12,18,64). These two waveforms differ by a factor of 21 in total average power. Furthermore, there is overwhelming evidence that, for bipolar PEMIC type waveforms, the parameters of the narrow high-amplitude portion of the signal are most correlatable with the observed bioeffect (12,18,63,64). Finally it has recently been reported (64) that nearly interchangeable repetition rate and amplitude dose curves can be obtained for repetitive single pulses having constant pulse width. Of most interest is the observation that the shape of the dose-response curve is greatly dependent upon pulse duration, being generally narrower as pulse width decrease (64). The above suggests that the dosage correlation for waveform-cell interactions should take into account response kinetics.

Impedance measurements at living cell membranes provide data for a time-constant due to the specific adsorption-like pathways at the cell membrane. The values obtained strongly correlate with the frequency range within which the single pulse and pulse burst waveforms have similar power levels. It is therefore of importance to examine the real-axis transformation of the power in the adsorption pathway, $P_A(\sigma)$. This is evaluated starting with $P_A = VI_A$ where I_A is obtained via the model for which Equation (4) is written. Thus, for a bipolar waveform having a main polarity duration, *T1*, and amplitude, *A*, and an opposite polarity duration, *T2*:

$$P_A(\sigma) = [A^2/(\sigma + 1/TA)]\left[1 - e^{-(\sigma+1/TA)T1} + (T1/T2)^2 e^{-\sigma T1}\left(1 - e^{-(\sigma+1/TA)T2}\right)\right] \quad (12)$$

where *TA* is the specific adsorption (ion binding) time constant [see Equation (4)]. For $T2 << T1$, that portion of the waveform having duration *T2* will have predominant power levels over the higher-frequency regions of this spectrum of the power. The main advantage of Equation (12) is that it clearly shows the effect of pulse duration on the power levels present over the frequency ranges of most effective coupling to *TA*. For example, Figure 2 shows the effect of a unipolar pulse having *T1* equal to (B, Figure 2) and longer than (A, Figure 2). Clearly, pulses significantly longer than *TA* do not provide more usable power in the specific adsorption pathway over the relevant frequency regions. Use of the model-dependent spectrum of the power [Equation (12)] for various signal configurations allows the effect of differing ratios of *T1*/*T2* to be predicted. This is performed by adjusting amplitude, *A*, at constant repetition rate to obtain levels over the frequency range shown in Figure 2 that fall within the effective dose range observed for the system under study. This has been successfully tested on the cell adhesion assay

system as described elsewhere (64).

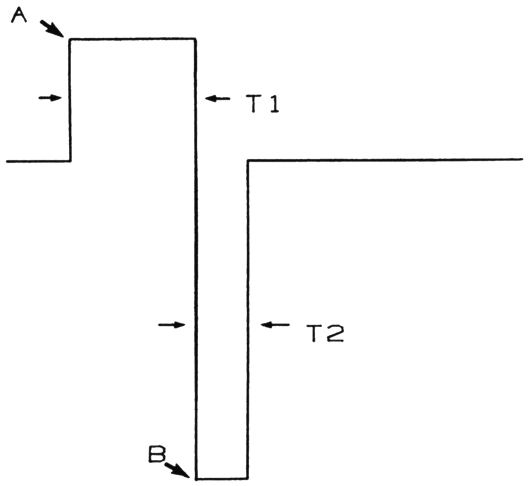

Figure 2. Spectra of the power via Equation (12) in an ionic membrane pathway functionally equivalent to specific adsorption for repetitive unipolar pulses having *T1* = 5 (B) and 200 (A) μsec. For both pulses *TA* = 5 μsec. Shows that the power in the target pathway is governed by the kinetic response [*TA*, Equation (2)].

The above shows that there is a real feasibility of using a model-dependent spectral analysis of the power in an ion binding pathway as a means to correlate the bioefficacy of electrical waveforms having widely different time parameters. The implication is that waveforms exhibiting power levels within given amplitude and frequency bands can effectively modulate cell and tissue function. Use of this analysis on results from a variety of different systems shows that Equation (12) offers a remarkable degree of correlation for the configuration of bioeffective waveforms. Of greatest mechanistic importance is the predictable effect of pulse width. As reported elsewhere, the cell adhesion assay allowed an effective assessment of the combined effect of *TA* and pulse width. These studies appear to s how that the most effective predictions and correlations exist for *TA* of 1–5 μsec. Referring to the cell impedance data (30), it is clear the specific adsorption pathway appears the most likely to contain the ionic transduction process through which PEMIC couples to modulate cell function.

It may be noted that repetition rate was not specifically included in Equation (12). This was intentional since systematic results from several systems (64) appear to show a relative independence of effect on repetition rate over the range 2–60 Hz. This finding reflects the non-linear portions of the cellular response to PEMIC. The cause for this behavior may be due to the refractory time of the trigger site (63).

Notwithstanding the above analysis, the creation of the pulse-burst waveform in present clinical use was based on a consideration that the electrochemical processes to which PEMIC should couple had relaxation times in the millisecond range (25,30). This

was prior to much of the *in vitro* evidence now available. Given the desire to create a safe portable PEMIC delivery unit for clinical use it was reasoned that a burst waveform would enable an effectively wider pulse to be seen by the cell. The parameters were chosen such that the opposite polarity width was significantly shorter than that of the main polarity. In this way it was hoped that the narrow portion of the waveform would be too fast for the proposed slow kinetics. The cell process would respond to the envelope of the burst portion of the waveform. As subsequent studies have shown, this reasoning was erroneous because the cell/waveform interaction appears most effective when kinetics in the microsecond range are considered. Thus a single repetitive pulse having the same characteristics as the 21 pulses in the clinical pulse burst is essentially as effective as the burst waveform when applied with the same amplitude and repetition rate. This has been known since 1981 and it is surprising to this author that this waveform as well as others having much narrower main and opposite polarity pulse widths, which can offer significantly more efficient coupling to cell surface electrochemistry, have not yet seen clinical use.

References

1. Bassett, C.A.L., Caulo, N. and Kort, J. Congenital pseudarthrosis of the tibia: treatment with pulsing electromagnetic fields. Clin. Orthop. 154:136-148, 1981.

2. Bassett, C.A.L., Mitchell, S., Norton, L. and Pilla, A.A. A repair of non-unions by pulsing electromagnetic fields. Acta Orthop. Belg. 40:706-724, 1978.

3. Bassett, C.A.L., Mitchell, S.N. and Gaston, S.R. Treatment of ununited tibial diapyseal fracturs with pulsing electromagnetic fields. J. Bone Joint Surg. 63A:511-523, 1981.

4. Bassett, C.A.L., Mitchell, S.N. and Gaston, S.R. Pulsing electromagnetic field treatment in ununited fractures and failed arthrodeses. J. Am. Med. Assoc. 247:623-628, 1982.

5. Bassett, C.A.L., Mitchell, S.N., Norton, L., Caulo, N. and Gaston, S.R. Electromagnetic repairs of nonunions, in *Electrical Properties of Bone and Cartilage*, C.T. Brighton, J. Black, and S.R. Pollack, Editors. Grune and Stratton: New York. p. 605-630, 1979.

6. Bassett, C.A.L., Mitchell, S.N. and Schink, M.M. Treatment of therapeutically resistant non-unions with bone grafts and pulsing electromagnetic fields. J. Bone Joint Surg. 64:1214-1220, 1982.

7. Bassett, C.A.L., Pilla, A.A. and Pawluk, R.J. A non-operative salvage of surgically-resistant pseudarthrosis and non-unions by pulsing electromagnetic fields. Clin. Orthop. 124:128-143, 1977.

8. Kort, J.S., Schink, M.M., Mitchell, S.N. and Bassett, C.A.L. Congenital pseudarthrosis of the tibia: treatment with pulsing electromagnetic fields. Clin. Orthop. 165:124-137, 1982.

9. Bassett, C.A.L., Valdes, M.G. and Hernandez, E. Modification of fracture repair with selected pulsing electromagnetic fields. J. Bone Joint Surg. 64A:888-895, 1982.

10. Chiabrera, A., Grattarola, M., Viviani, R. and Braccini, C. Modeling of the perturbation induced by low-frequency electromagnetic fields on the membrane receptors of stimulated human lymphocytes. Studia Biophysica 91:125-131, 1982.

11. Chiabrera, A., Hinsenkamp, M., Pilla, A.A., Ryaby, J., Ponta, D., Belmont, A., Beltrame, F., Grattarola, M. and Nicolini, C. Cytofluometry of electromagnetically controllec cell dedifferentiation. J. Histochem. Cytochem. 27:375-384, 1979.

12. Christel, P., Cerf, G. and Pilla, A.A. Modulation of rat radial osteotomy repair using electromagnetic current induction, in *Mechanisms of Growth Control*, R.O. Becker, Editor. Charles C. Thomas: Springfield. p. 237-250, 1981.

13. Dixey, R. and Rein, G. ^{3}H-noradrenaline release potentiated in a clonal nerve cell line by low-intensity pulsed magnetic fields. Nature 296:253-256, 1982.

14. Goodman, R., Bassett, C.A.L. and Henderson, A.S. Pulsing electromagnetic fields induce cellular transcription. Science 220:1283-1285, 1983.

15. Liboff, A.R., William Jr., T., Strong, D.M. and Wistar Jr., R. Time-varying magnetic fields: effect on DNA synthesis. Science 223:818-820, 1984.

16. Luben, R.A., Cain, C.D., Chen, M.C-Y., Rosen, D.M. and Adey, W.R. Effects of electromagnetic stimuli on bone and bone cells *in vitro*: inhibition of responses to parathyroid hormone by low-energy low-frequency fields. Proc. Natl. Acad. Sci. USA 79:4180-4184, 1982.

17. Norton, L. Effects of a pulsed electromagnetic field on a mixed chondroblastic tissue culture. Clin. Orthop. Relat. Res. 167:280-290, 1982.

18. Smith, S.D. and Pilla, A.A. Modulation of newt limb regeneration by electromagnetically induced low level pulsating current, in *Mechanisms of Growth Control*, R.O. Becker, Editor. Charles C. Thomas: Springfield. p. 137-152, 1981.

19. Becker, R.O. The bioelectric factors in amphibian limb regeneration. J. Bone Joint Surg. 43A:643-656, 1961.

20. Bassett, C.A.L. and Becker, R.O. Generation of electric potentials by bone in response to mechanical stress. Science 137:1063-1064, 1962.

21. Brighton, C.T., Friedenberg, Z.D., Zemsky, L.M. and Polis, R.R. Direct current stimulation of non-union and congenital pseudarthrosis. J. Bone Joint Surg. 58A:368-377, 1975.

22. Fukada, E. and Yasuda, I. On the piezoelectric effect of bone. J. Phys. Soc. Japan 12:1158-1162, 1957.

23. Becker, R.O. and Pilla, A.A. Electrochemical mechanisms and the control of biological growth processes, in *Modern Aspects of Electrochemistry*, J.O.M. Bockris, Editor. Plenum Press: New York. p. 289-338, 1975.

24. Margules, G.S., Doty, S.B. and Pilla, A.A. Impedance of living cell membranes in the presence of chemical tissue fixative. Adv. Chem. 188:461-484, 1980.

25. Pilla, A.A. Electrochemical information transfer at living cell membranes. Ann. N.Y. Acad. Sci. 238:149-169, 1974.

26. Pilla, A.A. Mechanisms of electrochemical phenomena in tissue growth and repair. Bioelectrochem. Bioenerg. 1:227, 1974.

27. Pilla, A.A. Electrochemical information transfer and its possible role in the control of cell function, in *Electrical Properties of Bone and Cartilage*, C.T. Brighton, J. Black, and S.R. Pollack, Editors. Grune and Stratton: New York. p. 455-490, 1979.

28. Pilla, A.A. Membrane impedance as a probe for interfacial electrochemical control of living cell function. Adv. Chem. 188:339-359, 1980.

29. Pilla, A.A. Electrochemical information transfer at cell surfaces and junctions—application to the study and manipulation of cell regulation, in *Bioelectrochemistry*, H. Keyzer and F. Gutman, Editors. Plenum: New York. p. 353-396, 1980.

30. Pilla, A.A. and Margules, G.S. Dynamic interfacial electrochemical phenomena at living cell membranes: application to the toad urinary bladder membrane system. J. Electrochem. Soc. 124:1697-1706, 1977.

31. Lopez-Rivas, A., Adelburg, E. and Rozengurt, E. Intracellular K^+ and the mitogenic resonse of 3T3 cells to peptide factors in serum-free medium. Proc. Natl. Acad. Sci. USA 79:6275-6279, 1982.

32. Mendoza, J., Wigglesworth, N.M., Pohjanpelto, P. and Rozengurt, E. Na entry and Na-K pump activity in murine, hamster, and human cells—effect of monensin, serum, platelet extract, and viral transfomration. J. Cell Physiol. 103:17-27, 1980.

33. Boonstra, J., Skaper, S.D. and Varon, S.J. Regulation of Na^+,K^+ pump activity by nerve growth factor in chick embryo dorsal root ganglion cells. J. Cell Physiol. 113:28-34, 1982.

34. Boonstra, J., Van der Sagg, P.T., Moolenarr, W.H. and DeLaat, S.W. Rapid effects of nerve growth factor on the Na^+,K^+-pump in rat pheochromocytoma cells. Exp. Cell Res. 131:452-455, 1981.

35. Chafoules, J.G., Bolton, W.E., Hidaka, H., Boyd, A.E. and Means, A.R. Calmodulin and the cell cycle: involvement in regulation of cell-cycle progression. Cell 28:41-50, 1982.

36. Whitfield, J.F., Boynton, A.L., MacManus, J.P., Rixon, R.H., Sikorska, M., Tsong, B., Walker, P.R. and Swierenga, S.H. The roles of calcium and cyclic AMP in cell proliferation. Ann. N.Y. Acad. Sci. 339:216-240, 1981.

37. Boynton, A.L., Whitfield, J.F., Isaacs, R.J. and Trembley, R.G. Different extracellular calcium requirements for proliferation of nonneoplastic, preneoplastic, and neoplastic mouse cells. Cancer Res. 37:2657-2661, 1977.

38. Hazleton, B. and Tupper, J. Calcium transport and exchange in mouse 3T3 and SV40-3T3 cells. J. Cell. Biol. 81:538-542, 1979.

39. Gearsa, D., Seitz, M., Kramer, W., Grimm, W., Till, G. and Resch, K. Ionophore A23187 raises cyclic AMP levels in macrophages by stimulating prostaglandin E formation. Exp. Cell Res. 118:55-62, 1979.

40. Barth, L.G. and Barth, L.J. The sodium dependence of embryonic induction. Develop. Biol. 20:236-262, 1969.

41. Barth, L.G. and Barth, L.J. ^{22}Sodium and ^{45}calcium uptake during embryonic induction in *Rana pipiens.* Develop. Biol. 28:18-34, 1972.

42. Barth, L.G. and Barth, L.J. Effect of the potassium ion on induction of notochord from gastrula ectoderm of *Rana pipiens.* Biol. Bull. 146:313-325, 1974.

43. Davson, H. and Danielli, J.F. *The Permeability of Natural Membranes, 2nd Ed.* London: Cambridge University Press. 1952.

44. Robertson, J.D. Unit membranes: a review with recent new studies of experimental alterations and a new subunit structure in synaptic membranes, in *Cellular Membranes in Development*, M. Locke, Editor. Academic Press: New York, 1964.

45. Singer, S.J. and Nicholson, G.L. The fluid mosaic model of the structure of cell membranes. Science 175:720-731, 1972.

46. Mueller, G.C. Biochemical perspectives of the G1 and S intervals in the replication cycle of animal cells: a study in the control of cell growth, in *The Cell Cycle and Cancer*, R. Baserga, Editor. Marcel Dekker: New York, 1971.

47. Bretscher, M.S. Membrane structure: some general principles. Science 181:622-629, 1972.

48. Tsai, K. and Lenard, J. Asymmetry of influenza virus membrane bilayer demonstrated with phospholipase C. Nature 253:554-555, 1975.

49. Zwaal, R.F.A., Roelofsen, B. and Colley, C.M. Localization of red cell membrane constituents. Biochim. Biophys. Acta 300:159-182, 1973.

50. Parsegian, V.A. Ion-membrane interactions as structural forces. Ann. N.Y. Acad. Sci. 264:161-174, 1975.

51. Urry, D.W. Basic aspects of calcium chemistry and membrane interaciton: on the messenger role of calcium. Ann. N.Y. Acad. Sci. 307:3-27, 1978.

52. Singer, S.J. The molecular organization of biological membranes, in *Structure and Function of Biological Membranes*, L.I. Rothfeld, Editor. Academic: New York, 1971.

53. Goldstein, L., Levine, Y. and Katchalsky, E. A water-insoluble polyanionic derivative of trypsin. II. Effect of the polyelectrolyte carrier on the kinetic behavior of the bound trypsin. Biochemistry 3:1913-1919, 1964.

54. McLaughlin, S. The mechanism of action of DNP on phospholipid bilayer membranes. J. Membr. Biol. 9:361-372, 1972.

55. McLaughlin, S., Szabo, G., Eisenman, G. and Ciani, S.M. Surface charge and the conductance of phospholipid membranes. Proc. Natl. Acad. Sci. USA 67:1268-1275, 1970.

56. Cheng, D.K. *Analysis of Linear Systems*. London: Addison Wesley. 1959.

57. Edelman, G.M. Surface modulation in cell recognition and cell growth. Science 192:218-226, 1976.

58. Harrison, R. and Lunt, G.G. *Biological Membranes: Their Structure and Function*. New York: Wiley. 1975.

59. Becker, R.O. and Spadaro, J.A. Treatment of orthopedic infections with electrically generated silver ions. J. Bone Joint Surg. 60A:871-881, 1978.

60. Conti, P., Gigante, G.E., Alesse, E., Cifone, M.G., Fieschi, C., Reale, M. and Angelettie, P.U. A role for Ca^{2+} in the effect of very low frequency electromagnetic field on the blastogenesis of human lymphocytes. FEBS Lett. 181:28-32, 1985.

61. McLeod, B. and Parker, R.A. Calculated induced currents in a model non-union fracture stimulated by Helmholtz coils or resistively coupled parallel plates. J. Bioelectricity 3:41-56, 1984.

62. Pilla, A.A. and Kaufman, J.J. Electromagnetic modulation of cell function: frequency characterizations of input waveforms. J. Bioelectricity 3:3-18, 1983.

63. Pilla, A.A., Schmukler, R.E., Kaufman, J.J. and Rein, G. Electromagnetic modulation of biological processes: consideration of cell-waveform interaction, in *Interactions between Electromagnetic Fields and Cells*, A. Chiabrera, C. Nicolini, and H.P. Schwan, Editors. Plenum: New York, 1985.

64. Fox, K., Pilla, A.A., Hellman, K. and Ryaby, J. Pulsating electromagnetic fields and rosette formation at macrophage membranes. in 4th Annual Bioelectrical Repair and Growth Society, Kyoto, Japan. 1984.