BIOELECTROCHEMISTRY - AN OUTLOOK

by R. O. Becker

Bioelectrochemistry, apparently a word coined specifically for this workshop, may be defined as that interdisciplinary field wherein electrochemistry and biology come together with the intent of applying the tools and techniques of the former to the problems of the latter. Implicit in this is the concept that this is something new and that this meeting represents the birth of a new science, holding great promise for the future of biomedical affairs. An "outlook" of where we are going requires a taking stock of where we are now and perhaps most importantly, how we got here. A simple review of our past history reveals the interesting fact that rather than being a new field of scientific endeavor, bioelectrochemistry actually antedates and lies at the foundation of many present-day sciences, including electrochemistry and biology. Our present concept of this field represents a final reunion of two antagonistic views which arose almost immediately following the birth of this science.

The foundation for bioelectrochemistry was laid by Sir William Gilbert, physician to Queen Elizabeth, when in 1600 he clearly separated electricity and magnetism in his volume "de Magnete." The following century saw the discovery of the electroscopy and the Leyden jar, methods of measuring and storing electricity, along with a procession of scientific giants such as Bacon, Harvey, Descartes and Newton; yet by its close biology was still concerned with such concepts as soul, animal spirits and aether. It remained for another 80 years to elapse before Galvani set the stage for the development of bioelectrochemistry. He was convinced that what he had discovered was electricity generated by the living organism that served a useful function for the organism. This view was quickly challenged by Volta who discovered bimetallic generation of electricity and ascribed all of Galvani's observations to that phenomenon. Controversy raged for the next several decades and despite the logical analysis of Von Humbolt that both were equally right, a breach developed which is only today being healed. Mateucci, who followed Galvani, discovered the electrical nature of the stimulus delivered by the marine ray fish, the rheoscopic nerve muscle preparation and most importantly, the current of injury. This latter is the generation of a voltage and current by all injured tissues, animal or vegetable. Little note was taken of Matteucci or his findings because duBois-Reymond very shortly discovered the action potential of nerve, a pulse-type phenomenon, easily measurable and of obvious biological significance. The theories and observations of Volta led to the gradual development of the science of electrochemistry, dealing primarily with the inorganic world and safely away from the problems of biology. Simultaneously, duBois-Reymond's finding laid the basis for modern electrophysiology dealing primarily with excitable tissues such as muscle and nerve and accepting only pulse-type phenomena as having significance. Bernstein developed his famous hypothesis of ionic flux through a semipermeable membrane to explain the action potential, a theory that has persisted almost intact until today. Bioelectricity, the concept that living organisms could generate steady state or slowly varying electrical phenomena by other mechanisms and that such phenomena could have major functional roles was relegated to the field of "vitalism," akin to old wives' tales and similar nonscientific mystical concepts as Mesmerism. Einthoven, the discoverer of the electrocardiogram, and Berger, the discoverer of the electroencephalogram, both measured underlying steady state or slowly varying potentials, along with their complicated pulse signals and considered them of equal importance. Nevertheless, the bulk of science rejected these observations, while simultaneously accepting the easily measured pulses. More recently, the work of many other investigators in the field of bioelectricity has been almost totally rejected. Notable among them was Burr, who, during the past several decades, measured DC potentials in many tissues and in a variety of organisms as diverse as oak trees and humans. Until the present decade the original concepts of Galvani and Matteucci were carried on by a small band of devoted fanatics scorned by the electrophysiologists and completely unknown to the electrochemists. Within the past decade observations have been reported using precise techniques that have required a reevaluation of this attitude.

This present meeting was the result of a chance encounter in an airliner between one of the present participants going to an orthopedic surgical meeting to present data on the stimulation of bone growth by small electrical currents and another of our colleagues, an electrochemist, concerned primarily with building better storage batteries. That meeting and this resultant workshop may well prove to have been events of considerable significance, since we appear to be rapidly approaching a situation that could mark the emergence of a completely new concept in biomedical disciplines of momentous import for good or evil.

Clinical medicine, despite its pretentions, is hardly a science; the majority of treatment modalities used are empirical and their effect on the living organism is in the nature of second-order phenomena. Today medicine has little more control over the basic physiological functions and properties of its patients than it had 50 years ago. The basic requirement in medicine is that the sick be made well and this art has, over its long history, never rejected a treatment that did this empirically. To the practicing physician, the result is what counts; how the treatment worked is of little concern. They appear to offer undreamed-of vistas of control over processes previously denied the physician, and we are now witnessing the beginning of a period of intense interest in and therapeutic application of low-magnitude electromagnetic forces in clincial medicine. Low intensity direct current via implanted electrodes has been used to stimulate healing of non-united fractures, a device with similar output is to be marketed by a prestigious manufacturer of operating room equipment for the purpose of producing rapid healing of indolent skin ulceration and burns, general anesthesia by the application of small direct (and modulated) currents to the head is in use in the Soviet Union and under clinical trials in this country, and finally, the ancient Chinese art of acupuncture is being expanded and enhanced by the application of low intensity direct current on the theory that bimetallic electricity may have been the actual therapeutic modality applied in the classical application. It is extremely interesting to note that what little scientific basis exists for these clinical applications is derived from the observations of Galvani, Volta and Matteucci and that present-day electrochemistry and electrophysiology have proven non-relevant, at least to these applications.

The importance of these applications cannot be denied, but their enthusiastic acceptance by clinical medicine before the basic knowledg of how the electrical parameters actually interact with the living organism is not without hazard. One must take note of the fact that we are not dealing with a chemical agent which has a specific second-order biological effect, but with forces that make up the very fabric of the control systems that nature uses in the organism itself. When we apply direct current to stimulate growth, we are enhancing the naturally occurring current of injury and the cellular response is one of either a marked proliferation or a return to a more primitive level of cellular organization, both processes commonly associated with malignant growth such as cancer. At this writing one cannot be sure that such a side effect as malignant transformation may not be associated with electrical treatment of this type, with its appearance delayed until several years after application. One may well question how many persons would have been treated in the interim. A similar possible hazard of long-term behavioral alteration is present with applications of such forces to the central nervous system. In additio as soon as low-level electrical forces are accepted as a therapeutic modality, one may with confidence expect the appearance of large numbers of frauds and quacks with extravagant claims for their treatments which will be extremely difficult to disprove. The increase of interest in electronically assisted acupuncture in France has already led to this situation. Perhaps most importantly, lack of basic knowledge regarding mechanisms of action could result in overlooking applications of even greater therapeutic importance. Finally, acquisition of such control over basic life processes could lead medicine into areas in which a system of medical and social ethics has not yet been established.

It should be obvious that we are in a time of abrupt transition in bioelectrochemistry from esoteric considerations of synthetic membranes and electrode processes to such clinically relevant areas as growth acceleration and production of anesthesia. That the transition is not without considerable danger is likewise evident. Appropriate measures are urgently required to fill in the gap between application and knowledge; such measures can only come from interdisciplinary basic research aimed at the central question: How do low-level electrical (and magnetic) phenomena interact with the living organism? From the viewpoint of one who has been in the intellectually satisfying, but scientifically unrewarding field of bioelectricity for the past 15 years, I should like to present one approach to this problem which hopefully will provide a stimulus for the interdisciplinary cooperation which is so necessary.

Certain theoretical concepts have been evolved in our laboratory which have proven to be of value. First, the site of interaction must involve portions of cells which have electronic properties permitting interaction to occur with very low magnitude electrical parameters. Solid state electronic properties such as semiconduction, junctional states, etc. would appear to have the necessary characteristics. Over the last two decades, the electron microscope has demonstrated many stable, highly organized sub-cellular structures that conceivably could have such functions, and, in fact, some evidences for such properties have been presented.

Secondly, these properties must not be fortuitous, but should constitute portions of control systems regulating basic biological functions, so that interactions would produce effects representing perturbations in these functional systems.

Thirdly, all interactions should not involve properties or principles not presently known to either physics, electrochemistry or biology; it must not be necessary to invoke "magic" or divine intervention.

My initial venture into this field was a study of the possible relationship between Matteucci's current of injury and the subsequent healing of the injured part. A rather striking difference was noted in the time course of the injury potential in animals capable of regenerating a limb and closely related species that lacked this ability. Since a definite relationship between the capacity to regenerate such structures and the nervous system had been established by Singer, we spent considerable time subsequently studying the DC activity of the central nervous system. It was possible to establish that this property was capable of transmitting data and exercised certain basic control functions; in addition, some evidence for a Hall effect interaction with external magnetic fields was demonstrated indicating the possibility of a semi-conduction mechanism as the source of the potentials. Nerve tissue proved to be recalcitrant to application of more direct solid state techniques and in the early 1960's we turned to a study of bone as a tissue more suited to this technique.

Bone in the living organism exists with many of the qualities of a solid state material, with only about 75% by weight living cells and a like amount of water. The matrix, composing the remainder, is a highly organized (accurate to the Angstrom level) two-phase material that is non-living and extra cellular. The one phase, the protein collagen, is produced by the cells in an extremely accurate spatial pattern; after its production, the mineral phase, hydroxy-apatite, is deposited in an epitaxial fashion on it. Collagen is piezoelectric and has several unique electronic properties, one of which is that it is an N-type material. The apatite mineral is a P-type material and the combination of the two has some characteristics of a PN junction diode.

One of the growth processes exhibited by bone is a differential growth response to mechanical stress with growth and deposition of new bone in areas of compression and resorption of bone in areas of tension. The resultant anatomical structure is that which is best able to resist the applied stress. This is a biological mechanism of great value, imparting a plasticity and resistance to varied stresses obviously lacking in any inorganic material. An analysis of this growth system indicated that it was a closed loop negative feed-back type control system based upon the stress electrogenic properties of the bone matrix and a differential response of the bone cells responsible for the growth to the minute electrical currents and potentials produced. Subsequent experiments have shown that implanted battery operated devices are capable of simulating the same system and result in controlled growth of new bone. (This observation is the primary basis of the present clinical application.)

The second and entirely separate process of growth in bone is fracture healing, which, incidentally, is almost the sole residium in the human of regenerative healing (regenerative healing in contradistinction to scarification healing, is a growth process in which a missing structure is completely replaced by a cellular process characterized by the early appearance of a mass of primitive cells known as the blastema). A study of this process disclosed that fracture (or stress to failure) results in the production of small but relatively long duration electrical phenomena. The fracture blastema in humans and other mammals is apparently the result of mitotic proliferation of cells of the periosteium; in all other vertebrates, it appears to be the result of dedifferentiation of the nucleated erythrocytes (red blood cells) in the blood clot that forms between the ends of the bone (mammals have red blood cells that are lacking nuclei, that structure being discarded in the final stages of maturation of these cells. Presumably the resultant "cell" is more efficient as a gas exchanger, but is completely lacking in any other normal cellular function including the ability to dedifferentiate into a more primitive cell and participate in healing processes). The dedifferentiated red blood cell of these other vertebrates has the characteristics of a primitive cell and is capable of redifferentiating into a mature cell of a different type; in this case, cartilage, which then is converted into bone.

It was found that this important cellular process could be produced by exposing normal nucleated red cells in lucite chambers to electrical parameters simulating those found at the fracture site. This preparation enabled us to make some observations concerning the importance of certain electrical parameters in this process. Similarly it was possible to make some observations on the biological events that occurred in these cells and the importance of other factors such as certain chemical substances, notably hormones. It should perhaps be emphasized at this time that while all of the determinations were carried out on non-mammalian red blood cells, we have observed that human white blood cells, particularly lymphocytes and monocytes and many of the cells in mammalian bone marrow (the tissue in which both white and red blood cells are formed) respond to similar electrical factors *in vitro*, by similar morphological dedifferentiation.

The nature of the exact interaction of the electrical factors with the cells is presently unknown. The situation *in vitro* is complicated; cells of a high dielectric constant, suspended in an aqueous solution of weak electrolytes with two metallic electrodes passing a small current. The *in vivo* situation is several orders more complicated with the aqueous solution containing many more electrolytes plus a variety of larger molecules and the electrical source being the bone matrix with solution interfaces that cannot even be guessed at at this time. However, one theoretical factor would seem to be probable: The effect of the electrical factors (current, voltage or field) must be primarily at the cell surface or cell membrane. While this structure by electron microscopy appears deceptively simple, we know from functional studies that it must be exceedingly complex. At the present time it is difficult to integrate the physical and biological views of the cell membrane; nevertheless, it is here that the cell interfaces with its environment, exchanges information, and passes coded instructions that determine the cell's behavior. It is obvious that a major factor in the cell's environment is electrochemical and the possibility exists that this is the nature of many cellular communication mechanisms.

Whatever the nature of the events at the membrane, it has been determined that the electrical factors provide something akin to a trigger action and that the subsequent cellular changes that occur utilize rather well-known cellular chemical processes, involving the interaction of the DNA-RNA mechanism, protein breakdown and reformation, etc. It is perhaps worth noting at this time that all cells of the body have the complete genetic complement for the total; and that all that is necessary to change one cell to another is to repress that section of genetic coding (genome) that characterizes the original cell type and derepress the genome for the desired cell type. The new genome produces the new cell type by destroying the proteins (the matrix of the cell) appropriate to the former type and synthesizing a complement of proteins appropriate for the new cell type.

This process, while of obvious complexity, is likewise of obvious tremendous importance. The initiating factor for the process is undoubtedly electrochemical in nature and may be simulated by simple devices and means. It has most recently been demonstrated that mammalian cells are responsive to low-level electrical forces and that in certain types dedifferentiation occurs. This phenomenon has already been used to produce partial regeneration of amputated limbs in laboratory rats. From a clinical point of view, the immediate implications are revolutionary for medical practice.

Since we have largely overcome the infectious diseases, the primary concerns of clinical medicine are with the degenerative conditions in which destruction of important structures occurs, unaccompanied by an effective healing process (arthritis, heart disease, etc.) or in which normal growth processes go awry (cancer). Placing in the hands of the clinician the tool whereby effective repair processes can be stimulated would undoubtedly be a major step in enhancing medical care. Nonetheless, we must not lose sight of the fact that we are coming closer to "playing God" than medicine has ever come before. These possibilities are being placed in the hands of the biomedical profession through the application of poorly understood electrochemical mechanisms. The message is obvious; the need is urgent that we learn all that we can about the basic mechanisms of action, and learn it quickly. In the interim we must accept our ethical and moral responsibilities as physicians and scientists and insist upon cautious, well-planned and controlled human applications of this extremely powerful tool until such knowledge is at hand.