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Electrochemical Mechanisms and the Control of Biological Growth Processes

Robert O. Becker

Veterans Administration Hospital
Syracuse, New York
and
Upstate Medical Center, Department of Orthopedic Surgery
Syracuse, New York

and

Arthur A. Pilla

Electrochemistry Laboratory, ESB Technology Center
Yardley, Pennsylvania
and
Bioelectrochemistry Laboratory, Orthopedic Research Laboratories
Departments of Orthopedic Surgery and Chemical and Applied Engineering
Columbia University, New York, New York

I. INTRODUCTION

One of the greatest challenges facing the life sciences today is the elucidation of the control mechanisms that regulate the cellular processes of growth, healing, response to injury, and the maintenance of the organizational pattern of the total individual. The determination of these mechanisms may well provide the cures for such diverse entities as cancer, arthritis, and heart disease, and, in addition, provide insight into the aging process as well. An immediate clinical dividend from such an investigation would be the control over healing processes in general, and possibly the stimulation of more effective modes of healing in man than provided by nature.

Since the advent of antibiotics, disease as a state of ill health caused by exogenous factors—bacteria, viruses, etc.—is largely

under control, using the tools provided by the biochemists. Therefore, we are left with a mounting burden of controlling diseases of endogenous or poorly defined etiology which is reflected in the gradual functional loss of portions of the body such as the joints, etc., due to a lack of effective healing or cellular replacement. The ability to control growth processes and possibly to enhance healing mechanisms would obviate all of the relatively ineffective measures currently in use, e.g., tissue or organ transplantation or replacement with artificial prostheses such as metallic joints or mechanical hearts. Thus, the study of growth control systems becomes a central point in present-day biomedical research.

The study of electrical activity associated with the majority of *in vivo* cellular processes is quite well known with respect to excitable-membrane (nerve, muscle, etc.,) events. Of increasing importance, however, are those electrical phenomena that are becoming to be associated with various basic processes including cell growth, total body organization, and fundamental neural activity. There is increasing evidence that these electrical factors are control signals that regulate these processes. This chapter will attempt to review the present status of knowledge in this area and indicate the basic electrochemical nature of these potentials.

There is a long history of the detection of standing (dc) electrical potentials on the body surface of many animals.[1] It remained as a vague concept not generally accepted by science until the development in the 1950's of instrumentation adequate to make precision measurements of such potentials. It is now generally accepted that all organisms possess a pattern of standing potentials on the body surface that reflects, in general fashion, the anatomical pattern of the underlying central nervous system.[2] These electrical patterns also demonstrate charges reflecting alterations in the function of the nervous system such as sleep, biological cycles, exposure to ionizing radiation, and anesthesia.[3] Trauma to a portion of the body produces in the total body potential pattern a local perturbation that has been called the "current of injury." This potential has some of the characteristics of a signal indicating to the organism that damage has been incurred.[4] The relationship of the current of injury to the central nervous system is complex and poorly understood, but it may play a role in the perception of pain. In a view of the evident relationships between the surface potentials and the central nervous system it was postulated that the surface dc potentials were produced by

similar electrical potentials associated with the nerves themselves. Considerable effort was, therefore, devoted to a search for the existence of standing or slowly varying potentials within the nerves themselves, and some success was obtained.[5] Attempts to determine the mechanism of production of these neural potentials encountered considerable technical problems due to the fragility of the tissue and its high electrolyte content; however, some indications were obtained for a solid-state electronic mechanism.[6] At present, our operational concept of the function of this system is that it is a primitive type of nerve mechanism that provides the overall control for the maintenance of the organizational pattern of the organism (i.e., relates new growth of a part to the total organism, etc.). This process requires a continuous monitoring of the receipt of injury and the state of repair. In addition, the evidence indicates that the dc neural potentials are also related to the ability of the nervous system to transmit data via the well-known action-potential system. The ability of a nerve to generate and transmit action potentials is dependent upon the dc system being in a certain state. At present, it is considered that this function of the dc system is the one that is influenced by the therapeutic techniques of acupuncture, hypnosis, and electroanesthesis.[7] Thus, there is growing evidence of the importance of this dc function of the central nervous system in the regulation of many basic life processes.

In addition to the organized electronic system contained in the nerves, at least one other tissue, bone, has many elements of a self-contained electronic mechanism approximating a self-organizing system from the cybernetic point of view. Based upon knowledge of this system, it has been possible to stimulate new bone growth, the growth of joint cartilage, and even growth of portions of amputated limbs, utilizing electrochemical stimulation techniques. This was achieved by studying and comparing the overall electrical events which occur naturally in various healing processes and subsequently attempting to reinforce or reproduce these events electrochemically. In each case, it is quite clear that the individual cell, which is the basic building block of all tissue, has been directly or indirectly influenced by electrochemical means. The manner in which this may be possible will be the major topic of this study.

Fundamental to these studies is the theory originally proposed by Szent–Gyorgyi[8] that biological materials might possess electronic properties akin to those of the solid state, being derived from

the precise organization of biological structures at molecular and submolecular dimensions. He postulated that these properties could be functionally important at such basic levels of cellular operations such as growth. If this premise is correct, then one could further postulate that changes in the electronic state of the cell's environment could produce alterations in basic cellular functions such as growth by producing perturbations in the cellular electronic systems. In order for these alterations to take place, it is necessary for electrical communications to and from, as well as between, cells to occur. Since all cells reside in an aqueous medium, the extracellular fluid, all such information transfers must involve an electrochemical link, even though some of the information may be processed in a solid-state fashion within the cell.

At present, the basic mechanisms governing the normal growth and replacement of cells are largely unknown. All cells of the body have finite lifetimes and, except for those of the central nervous system, are constantly being replaced. The cells of some tissues, for example, the gastrointestinal tract, have a high "turnover rate," while the rate of replacement of bone cells is quite low. Nevertheless, it is particularly important to note that this constant replacement is accomplished without error. New cells are being formed from old cells at exactly the rate required and of the type appropriate to the site. When an error occurs, then one encounters the situation of abnormal cellular growth; i.e., an excess of cells, inappropriate to the site and lacking cutoff controls. This we refer to as malignant growth, or cancer. It would appear obvious that before one can adequately understand the abnormal process, one must first understand the normal process.

Since the basis for all growth is cellular, the problem resolves itself into investigating the cellular mechanisms involved, in particular, the communication systems that call these mechanisms into action and guide and direct the subsequent growth. At present, it would appear that these are, at least in part, electrochemical in nature, and that the newly founded discipline of bioelectrochemistry occupies a key role in our access to these control systems.

While the majority of work and interest at the present time is directed toward bone and the stimulation of bone growth, it is of the utmost importance to emphasize that what are being manipulated by electronic means are the naturally occurring control

systems that pre-exist within the organism. These control systems are generalized in nature and not limited to bone growth control. Since all growth is the result of cellular mechanisms, regardless of the type of stimulation or the control system employed, it would appear appropriate to discuss at this point some of the basic concepts of cellular function necessary to a complete understanding of the control-system concept.

II. CELLS AND THEIR ELECTROCHEMICAL ENVIRONMENT

It is not within the scope of this study to review in detail the structure and function of cells. The interested reader is referred to excellent monographs on the subject.[9] A few details of cellular anatomy and function are important to the understanding of the

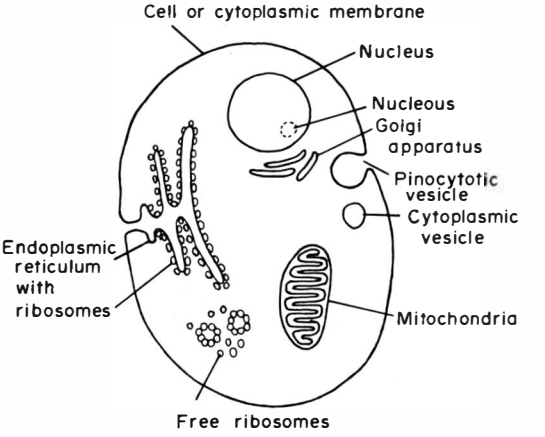


Figure 1. Simplified view of typical cell. The entire cell is a complex system of membranes separating various organelles, with the cytoplasmic membrane enclosing the entire unit. The nucleus contains the DNA genetic material; the nucleolus becomes particularly visible in the active nucleus. The Golgi apparatus is involved in cell-division processes. Cytoplasmic vesicles may accumulate waste or other products which are subsequently extruded by the cytoplasmic vesicle merging with the cytoplasmic membrane and subsequently becoming a pinocytotic vesicle, emptying to the outside. The mitochondria are the energy sources for the cell. Proteins that go to make up the cell itself are constructed by the free ribosomes, while proteins for "export" are manufactured by the ribosomes attached to the endoplasmic reticulum, which appears to communicate with the exterior.

concept of bioelectrochemical mechanisms, and these are summarized in Fig. 1. As can be seen, the cell consists of a number of membrane-bounded systems enclosed in a primary cytoplasmic membrane. It is at the latter that the cell interfaces with its environment and presumably receives the information that regulates its various functions. Of the internal organelles, the nucleus contains the genetic material, and the mitochondria are the primary energy-transferring structures. It is important to recognize that the nucleus of every cell contains *all* of the genetic codes for all of the many types of cells that make up the organism, and that what distinguishes one cell from another is merely the sequence of genes that are in operation. For each cell type there is a single such sequence of genes, referred to as a genetic operon, and in any given cell all other operons are repressed. It should, therefore, be possible to derepress other gene sequences and cause a conversion from one cell type to another. In the course of development of the organism from the original fertilized egg, there is not only growth, expressed by cell division and increase in numbers, but differentiation. This latter term refers to the gradual limiting of the genetic operons available to the cells until, at certain stages, cell and tissue types are fixed by repression of all operons except the one characterizing that specific cell type. In the course of this process, three general basic cell types are recognized: ecto-, meso-, and endodermal. The ectodermal cell type gives rise to skin and nerve; the mesodermal type to muscle, bone, and blood; and the endodermal type produces the gastroinestinal tract and its associated organs. Thus, in development, there is an orderly, organized process of great complexity which gradually converts an egg with total genetic potential (totipotent) to a multicellular organism in which each cell is operating under the instructions of one genetic operon only, with all the others being repressed (Fig. 2). After maturity is reached, the organism must have the capacity to repair injuries in an appropriate fashion. The lower animals demonstrate great reparative characteristics, being able to regenerate a complete organism from a single fragment. As one ascends the evolutionary scale, however, this process becomes limited in capability until in man the only tissue capable of true regeneration is bone. Apparently accompanying the decrease in healing ability is an increase in the incidence of abnormal growth such as cancer.[10] It would appear that in general, growth control

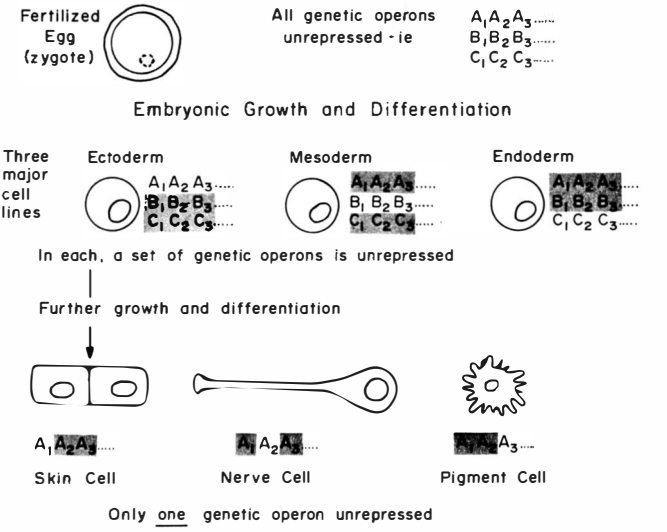


Figure 2. Simplified schematic of the genetic factors involved in cell differentiation. The fertilized egg contains a full complement of genetic operons to characterize any cell of the adult organism. A genetic operon is the set of genes that characterizes any single cell. In the fertilized egg, all operons are open and the growth process consists of the gradual delimiting (repression) of the genetic options available to the cells of the embryo. Early in development, three basic types of cells can be recognized that give rise to specific types of tissues. Theoretically, a *set* of operons would apply to each basic type, and finally in each tissue type there is only one operon functioning. The progression in schematic is from top down, and repressed operons are indicated by overshaded letters.

mechanisms diminish in competency as one ascends the evolutionary ladder (Fig. 3). In man, all tissues other than bone are healed either by modest enhancement of the normal rate of replacement of cells or by scar formation. Only certain tissues are capable of the former process; these are primarily characterized by a high cellular turnover rate; e.g., skin and digestive tract. Other tissues having slow turnover rates heal primarily by the formation of a fibrous tissue scar. It was one of the aims of our study to determine the operational characteristics of the control system regulating regenerative healing in lower organisms and, after determining which ones were missing or inadequate in the human, to replace them by external means.[11]

In those animals capable of extensive regeneration, the cellular process utilized is one of dedifferentiation. This can be viewed

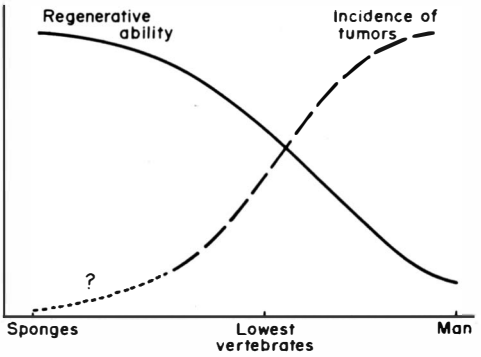


Figure 3. Evolutionary relationship to growth processes. This figure presents probably an oversimplified view of the general concept that the more primitive organisms have greater regenerative and reparative abilities and less abnormal growth occurrences than the more advanced organisms. The lowest vertebrates, where the curves cross, are represented by the salamander. This animal is a vertebrate with anatomical structuration basically analogous to the human. The incidence of malignant growth is quite rare in this form (but not unknown), and it has the ability to regrow, by a process termed regeneration, a complete extremity following amputation. The regrown extremity is entirely analogous to the human, having the same bone, muscle, blood vessel, and nerve structuration. It is interesting to note that the process of encephalization follows roughly the same curve as the incidence of tumor. This refers primarily to the percentage of total nerve mass that is sequestered in the brain and may possibly furnish an explanation for the lessening of growth control with increasing organismal complexity. The relationship between innervation and growth is subtle and only a few observations have been made, primarily those of Singer.[17]

basically as a return of a cell to a more primitive cell type characteristic of an earlier stage of development. The mechanism employed is obviously the derepression of the genetic operon characterizing a more basic cell type. For example, in certain regenerative processes in the salamander, the red blood cells (RBC's) that constitute the blood clot at the site of injury have been found to be able to dedifferentiate into primitive mesodermal cells and subsequently redifferentiate as either cardiac or skeletal muscle, bone, or cartilage. A simpler, but equally adequate, reparative process for single tissues is a partial dedifferentiation within surviving cells of the injured tissue to a more primitive cell of the same tissue type, followed by subsequent rapid mitosis, with the formation of a mass of cells adequate to heal the defect, and, finally, with maturation back into the original cell type.

It is obvious that these processes must be the result of a control system which has provisions for sensing the original injury, its position and extent, and subsequently relating the reparative process to the remainder of the organism (i.e., after amputation of the right foreleg in a salamander, a right foreleg is regenerated, oriented appropriately to the site, and functionally connected to the total organism via the central nervous system). Simultaneously, the control system must be able to continuously sample the extent of the repair process and bring it to a close when it is adequate to correct the defect. Since all of the growth is accomplished through the agency of cellular activity, the cells must be capable of both receiving instructions from the control system and in some fashion indicating their own operational status to the system. Biologically speaking, the signals received by the cells may be divided into two general types. The first being the notification that a traumatic event has occurred and that some growth process is necessary. The cellular reponse to this signal would be that of dedifferentiation. This signal is obviously of a simple nature and its information content is minimal. The second type of signal indicates the type of cells that are to be produced to repair the injury. The cellular response in this case would be redifferentiation into the cell type necessary, and, therefore, the signal must be capable of transmitting more information content than the initial signal (i.e., what types of cells are to be produced, in what quantity, and with what spatial relationships to each other to produce an organized structure). Over both of these information systems must be an additional one which relates the growth produced to the remainder of the organism and is responsible for the production in certain animals, of a complex multitissue extremity which is anatomically and functionally connected to the organism. Considerable work has gone into one such specific growth system, limb regeneration in the salamander, and, while it has not been particularly viewed from the control-system or information-transfer point of view, much useful information can be gathered from a review of the literature in this field.[12]

All cells in a living organism are suspended in an aqueous medium containing a variety of inorganic ions (primarily, sodium, potassium, chlorine, and calcium) and an extremely complex variety of organic molecules, including peptides, proteins, and lipids, many of which are dipolar and potentially electroactive. In the course of

development of the organism from the fertilized egg, slight changes in the cation content of this extracellular fluid have been reported to produce major defects in developmental pattern.[13] In the situation where the cells of the adult organism are stimulated to grow in response to injury, the two classes of signals derived from the growth control systems must be transmitted to the cells via this extracellular fluid. One can then postulate that these signals may be a combination

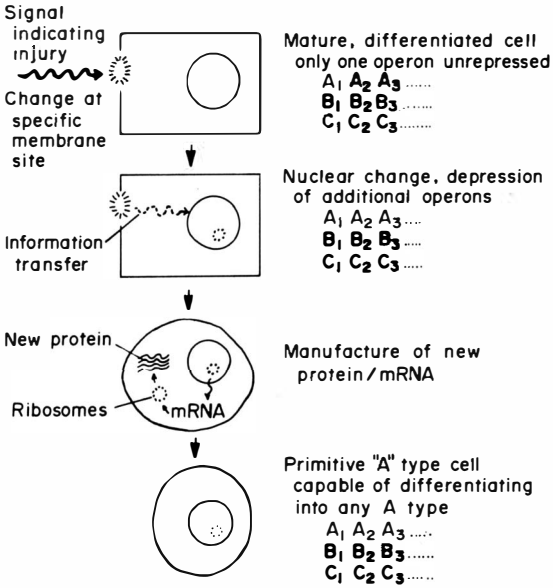


Figure 4. Theoretical schematic of events occurring in one type of cell response to injury. It is postulated that injury produced local changes in electrical environment and chemical species. These could produce a specific change on the membrane of specialized cells in the area, the membrane alteration being meaningful for the cell. It is obvious that this initial step could well be electrochemical in nature. Once the membrane event has occurred, we must postulate an information transfer to the nucleus which activates the nucleus. A nucleus becomes visible and an alternate set of genetic operons becomes derepressed. This depression brings about the formation of new messenger RNA (mRNA) which furnishes the template for a new set of cellular proteins. At the completion of the process of destruction of the prior set of cell proteins and the construction of the new set, the cell is a new type of cell. If the change has been in the direction of a more primitive type cell with several genetic operons available (as pictured), the process is termed dedifferentiation. This results in a stock of cells capable of transforming into a variety of tissue types adequate to repair the injury by regrowing the missing portion.

of purely electrical factors, such as field configuration, current and power densities, etc., and chemical factors such as specific ion concentrations resulting from the electrical fields or the release of specific charged molecules, either locally or systematically as the result of trauma. Whatever the nature of the signal, it is impressed upon the cell at the cytoplasmic membrane–extracellular fluid interface. It is obvious that the membrane must be able to sense a variety of signals; from the simple one that indicates trauma and produces dedifferentiation to those more complex signals that indicate which, of several, cell types are to be produced. Since the nucleus contains the genetic operons and is not in contact with the cytoplasmic membrane and obviously cannot "see" the external electrochemical environment, one must postulate an additional signal-processing link between the cell membrane and the nucleus which then calls forth the appropriate nuclear response. In this light, the cell membrane becomes the structure by which the cell senses its environment and responds appropriately. The membrane, therefore, must be capable of discriminating between a variety of signals, and its levels of organization and function must be correspondingly sophisticated. It is perhaps well to indicate at this point some brief facts about the mechanism employed after a new genetic operon is derepressed and begins to exert its influence. The primary characteristic that distinguishes one cell from all others is the protein composition of the cell. The new genetic operon, therefore, must bring about simultaneously breakdown of existing proteins and their reconstitution into new proteins by recombination of the basic amino acid sequences. Details of this process, which involve primarily RNA, as distinguished from DNA, which is the genetic material itself, may be obtained from several reviews of the subject.[14] The entire sequence of signal production, transmittal to the cell, and subsequent cellular mechanism is summarized in Fig. 4.

At the present time, some data are available on the general characteristics of the growth control systems, the signals employed, and the cellular responses in several specific growth systems. These are: the limb regeneration system in which a complex, multitissue structure is formed; the fracture healing and response to mechanical-stress growth systems in bone; and the growth-response system of joint cartilage. The remainder of this chapter will discuss these systems in general, and, in particular, from the electrochemical and information-transfer point of view.

III. REGENERATIVE GROWTH AND ELECTROCHEMICAL MECHANISMS

Regenerative capacity (see Fig. 5 for an outline of the cellular events in regenerative growth), as previously noted, diminishes as one ascends the evolutionary scale. Much significance has been attached to this and has given rise to the common concept that

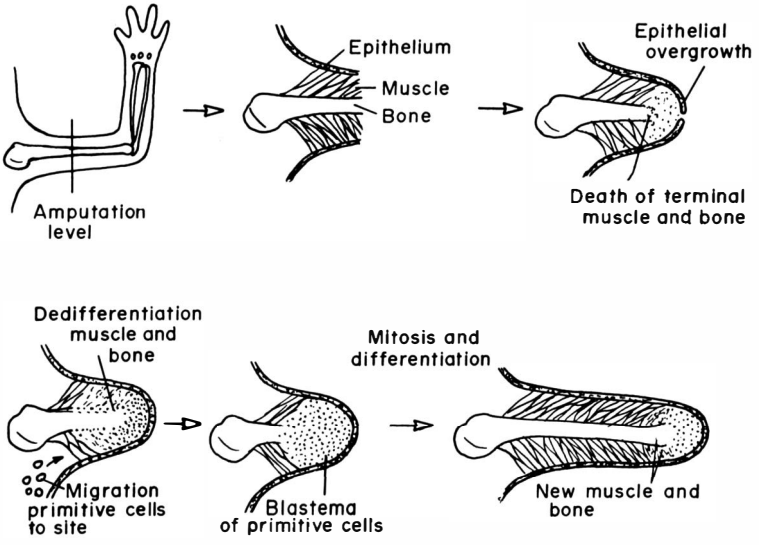


Figure 5. Schematic representation of the events occurring in limb regeneration in the salamander (figures read from left to right). (Upper left) Illustration of the level of amputation, note that the bone structure is equivalent to the human. (Upper middle) Immediately following amputation. (Upper right) Status at 5–7 days: the epithelium overgrows to cover the wound and some death and degeneration of residual muscle and bone occurs. (Lower left) 7–15 days. Early cellular events include some migration of primitive cells to the area and some dedifferentiation of the surviving bone and muscle cells in the area. The final cell type accumulating in the area is primitive mesenchymal. (Lower middle) 14–21 days. The cells from the preceeding step organize into a mass of primitive, highly active cells called the blastema. (Lower right) 21 + days. The blastema enters a phase of active mitosis and grows longitudinally. The most proximal portions redifferentiate back into the mature cell types necessary to reconstruct the missing portions. The most distal portion remains blastemal in nature and continues to proliferate. As it elongates, the redifferentiated portions form the missing structures in fully appropriate fashion: the organization is normal, including joints, muscle attachments, blood vessels, and nerve. As the blastema reaches the portion destined to be the hand, proliferation ceases and this final blastemal remnant redifferentiates into the appropriate structures, bringing the process to a close.

mammals cannot regenerate because the complexity of the structure to be formed is too great for whatever the communication system is that is responsible. That this is erroneous is evidenced by the simplest review of comparative anatomy. The salamander, the primitive-type vertebrate, has changed little since its first appearance in the fossil record, and its anatomical structure is basically quite similar to ours. When a salamander regenerates a foreleg, the structure formed has bones, joints, nerves, blood vessels, and muscles extremely similar to man's. The kinds of information transmitted in producing this multitissue organized structure from the formless mass of primitive cells known as the blastema cannot be much less than that which would be required for a mammalian extremity. From the time of the first scientific description of limb regeneration in salamanders by Spallanzini in 1768 to the present date, the question of why these animals have this capacity and we lack it has intrigued scientists.

The study of regeneration, however, was limited to observation only until 1945, when Rose published the first report of the production of partial limb regeneration in the adult stage of an animal not normally capable of this function.[15] He showed that adult frogs could be induced to partially regrow a foreleg if exposed to hypertonic saline several times a day, and he attributed his results to the saline preventing epithelial overgrowth. However, the following year Polezhaev obtained the same result by simply needling the amputation site daily.[16] Both of those observations can be interpreted as indicating that, at least in part, the extent of the trauma is related to the regeneration response. In the next decade, Singer reported a direct relationship between the mass of nerve in an extremity and the ability of the extremity to be regenerated.[17] A quasi-mathematical relationship was established, and he again succeeded in inducing the same extent of limb regeneration in the adult frog as Rose and Polezhaev by transplanting excess nerve into the extremity. Singer postulated the existence of a chemical factor produced by the nerve as the responsible agent, but has not yet satisfactorily demonstrated its existence.[18] In 1958 Zhirmunskii published a report that the current of injury (CI) in muscle was directly related to the extent of innervation of the muscle.[19] The CI is a phenomenon that extends back to the foundations of electrochemistry since it was, in part, responsible for the phenomena that Galvani first observed. The CI was extensively studied by Matteuci in 1838, who found that it was

related to the extent of the trauma and could, in fact, be multiplied by series stacking of injured tissues.[20] At present, little attention is directed toward the CI despite the fact that the textbook explanation for its occurrence is obviously inadequate.[21]

All of the foregoing observations lent themselves to the following integration.

Since innervation enhancement can produce regeneration without increasing the extent of trauma, and, conversely, increasing the extent of trauma without increasing innervation can produce regeneration, the inference may be drawn that a factor common to both innervation and trauma might be the responsible agent: Trauma is directly related to both CI and regeneration; nerves are directly related to both CI and regeneration; possibly CI is related to regeneration. Therefore, it may be postulated that the CI is quantitatively related to regeneration.

One of the present authors reported in 1960 on a simplistic experiment designed to test this hypothesis. The CI was measured from the time of amputation to the completion of healing in a group of adult salamanders and a similar group of adult frogs.[22] The initial CI was identical in both groups, but shortly a major difference was revealed which appeared to be related to the appearance of the blastema in the salamander. An attempt to duplicate the salamander's sequence of potential change in the frog by injection of externally generated current failed due to technical problems. In 1965 however, Smith succeeded in inducing limb regeneration in adult frogs by insertion of an appropriately oriented bimetallic electrogenic device.[23] The extent of the regenerative growth produced was equivalent to that obtained by Rose, Polezhav, and Singer. In the interim, our laboratory had been working on the electrical characteristics of bone and their relationship to bone growth systems, including fracture healing, a single-tissue regenerative system. This work will be discussed in detail later in this chapter; however, it did lead to a general consideration of regenerative-growth control.[11] It was hypothesized that all regenerative growth, including multitissue limb regeneration, was a two-step process directed by the two types of control signals previously discussed. The initial step is the formation of an adequate blastema by cellular dedifferentiation in response to an appropriate level of CI at the injury site. Once an adequate blastemal mass is present, then growth

and redifferentiation can occur under a second set of much more sophisticated controls which specify tissue types etc. It was further theorized that the failure of regeneration growth in the Mammalia is due to failure to generate an adequate initial signal (i.e., inadequate CI resulting from the decreased peripheral nerve-to-tissue ratio in these animals) rather than a failure in the more sophisticated second data-transmission system. Our work on the fracture healing system enabled us to define the appropriate level of current that should be effective as the initial signal at the cellular level. Working *in vivo* directly with the cells responsible for fracture healing, it was found that the initial cellular responses to appropriate electrical factors occur only within a narrow range of current and voltage, with both upper and lower limits.[24] The simple silver–platinum bimetallic junction devised by Smith[23] was studied and found to be producing currents 15 times greater than the optimal, in fact well beyond the upper effective limit. Currents closer to the theoretically optimal range could, however, be produced by interposing a high-value resistor between the two metals. While the devices were found to be unpredictable due to a variety of uncontrolled factors, they could be made to fall within a broad range of current values, and a preliminary study of the effect of producing an adequate initial signal (CI) at the site of injury in mammals was undertaken. The 21-day-old white rat was chosen as the experimental subject and amputations were done through one foreleg at a level mid-way between shoulder and elbow joints. A surprisingly high percentage of animals in whom a bimetallic device with interposed 10-megohm resistor was implanted demonstrated organized growth of bone, joint cartilage, muscle, nerve, and blood vessels;[25] most frequently, the major structure formed was the missing portion of the humerus down to and including the proximal portion of the elbow joint (Fig. 6). The organizational pattern of the multitissue structures formed was within normal limits, and it is presently our interpretation that the device inserted serves as the initial simple signal necessary to produce the required cellular response and that the subsequent organized growth was derived from the naturally occurring second data-transmission systems. Obviously, the mammal is capable of transmitting the necessary information to produce a structure of such complexity. The key to the process quite apparently is in simulating something close to the initial signal which then brings

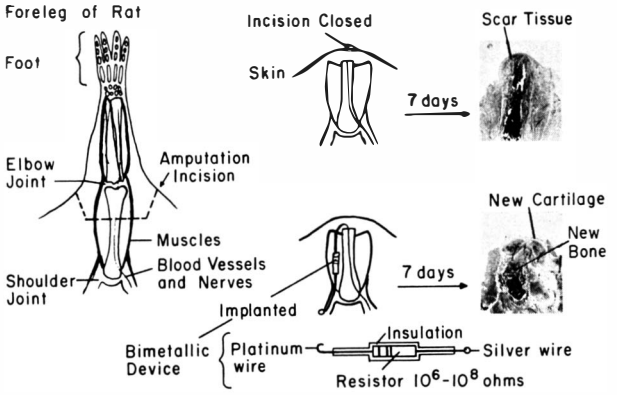


Figure 6. Representation of the experiment designed to restore a regenerative-type electrical environment in the amputation stump of the rat foreleg.

The figure on the left indicates the basic anatomy and the level of amputation. The skin is sutured closed over the stump in this case. The upper row demonstrates the control animals and at the upper right is a low-power photomicrograph of a longitudinal section through the amputation site, 7 days post operative. The skin is removed prior to sectioning and all that is visible is the rounding off of the stump and closure with scar tissue.

The lower row demonstrates the experimental animals. The diagram shows the placement of the bimetallic electrogenic device. At the lower right is a similar low-power photomicrograph at 7 days postoperative. In this instance, the bone and muscle have regrown and, in fact, a normal elbow joint has been formed.

about the formation of a blastema adequate to support organized growth.

One of the primary problems at present is the cell or tissue of origin of this blastema. The placement of the distal electrode into medullary cavity and the histological appearance of the amputation site in the first few hours after device insertion both appeared to indicate that some cell or cells of the bone marrow were stimulated to dedifferentiate into primitive mesenchymal cells. (It is possible that some of the white blood cells of the peripheral circulation are involved, but the extent of their contribution cannot be gauged, as yet.) The cellular appearance of the blastema was essentially similar to that noted in the lower vertebrates (Fig. 7). One interesting aspect, however, was the presence of a noticeable number of very dark-staining, densely compacted, cells. These resembled the melanocytes which have been noted to be present at the site of cardiac regeneration in

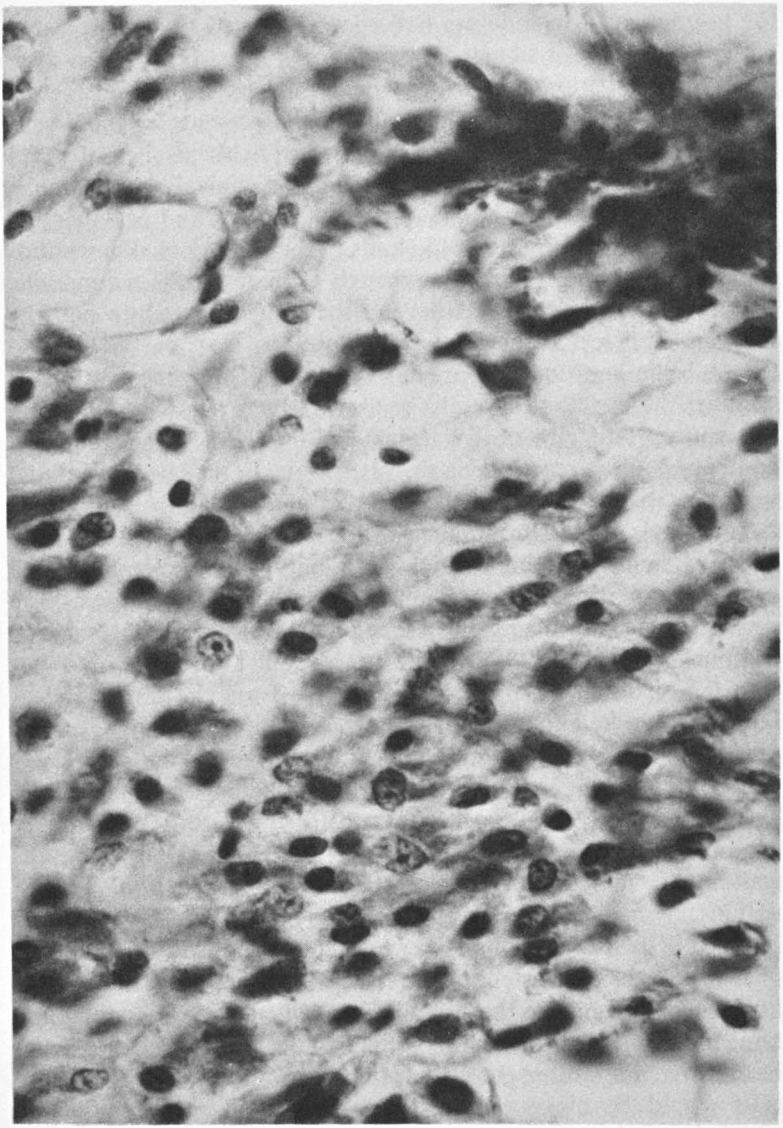


Figure 7. Photomicrograph of the blastema-like cells mass formed at five days in the experimental series of rats (540 ×). The cells are quite active with prominent, open-type nuclei and clearly visible nucleoli. Some larger cells are present which include dark-staining pigment which may be melanin.

the amphibian. Melanocytes have been reported to be associated with the process of tissue differentiation,[26] but the modern view limits their function primarily to epidermal pigmentation. Nevertheless, it is interesting to speculate on the presence of these cells containing large amounts of stable free radicals at sites of rapid cellular differentiation and growth. A related clinical observation is that heavily pigmented humans such as Negroes and Melanesians are prone to the formation of keloids in the healing of skin wounds (the keloid is a noticeable overgrowth of tissue at the wound site, perhaps interpretable as a failure of the control system to halt the reparative process at the appropriate level).

A basic question that arises as a result of our observations is whether the metallic electrodes serve merely as passive conductors for appropriate electrical parameters or whether they actually contribute to the reaction via dissolved ions. In the latter event, the question arises as to what the effect would be of substituting different metals for the terminal electrode. In an attempt to assay the effect of single (not bimetallic), presumably inert, metals on the cellular population of the bone marrow, a series of experiments was performed, again utilizing the 21-day-old white rat as the test subject. A 4-mm length of 99.99 % fine wire was inserted approximately 3 mm into the medullary cavity of the humerus from the same foreleg-amputation site. The terminal 1 mm was crimped over the exposed cortex of the humerus and the wound closed at the skin level. Metals used were silver, platinum, gold, palladium, and tantalum. The animals were sacrificed at 7 days postoperative and the extremity examined histologically. A surprisingly varied spectrum of results was noted, with the silver wire producing marked bone growth within the medullary cavity (presumably by changing the bone marrow cells into osteoblasts), while gold produced an extensive growth of the periosteum (the tissue covering the bone). We attempted to duplicate these changes in cell cultures of mammalian bone marrow, because with this technique the cellular changes could be followed much more closely on a time base and changes within the cells would be more visible. Using the standard technique, in which the original marrow sample is diluted with the culture medium until a sparsely populated monolayer is produced, absolutely no cellular changes were yielded with any type of wire placed in the culture directly at the cell-monolayer level. However, cell cultures

diluted much less, so that in the monolayer each cell had contact with several other cells (close neighbors), produced very interesting cellular behavior patterns. These have not been fully analyzed, but they consisted of, in general, preferential migration of certain cell types to "zones" several cell diameters from the wire itself as well as changes in the morpholgy of the cells and in some cases rapid dedifferentiation of certain cells. The differences in the responses of the two cell dilutions, even though the same types of cells were present in each case, is very instructive. Cells are extremely sensitive to their environment and can sense very rapidly such changes as whether or not they are still in the proximity of other marrow cells or whether they appear to have no close neighbors. We have noted that under the latter circumstances that dedifferentiation to a standard, primitive, simple type occurs much sooner than in the former situation. Presumably then, when close neighbors are sensed, the tissue type is much more stable. The differential response to the immersed metal wires indicates that the stable tissue type is necessary for the specific reactions to occur. One is forced to conclude that the responses to the metallic wire particles represent active responses rather than toxic changes. These active responses must be the result of the minute electrical charges, and the minute concentrations of ions introduced by the "inert" wires being sensed by the cells. Thus, the simplistic experiments at the organismal level aimed at the regenerative control system have led us to a sophisticated technique enabling us to begin studying the effects of extremely small electrochemical changes in the environment upon cells in various functional states.

IV. ELECTROCHEMICAL MECHANISMS AND BONE GROWTH

The vertebrates (fish, amphibians, reptiles, birds, and mammals) are distinguished from other animals by the presence of a hard, supporting endoskeleton. This furnishes a base for muscle action by means of hinges or joints in the endoskeleton and has permitted the evolutionary development of remarkable varieties of life forms, culminating in man, who uses his muscles and skeleton to manipulate his environment. Except in the case of man, who usually provides for his disabled fellows, the existence of an intact endoskeleton is

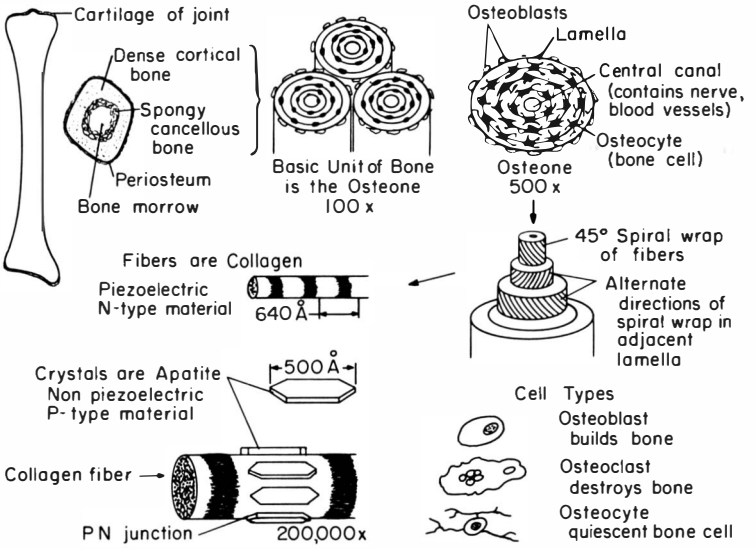


Figure 8. Schematic representation of bone organization. The figure reads clockwise from the top left, and each separate figure is at increasingly higher magnification. The precise organizational pattern extends from the gross appearance (top left) to the electron microscope level (lower left). At each step the organizational pattern is striking, and this tissue is quite evidently nonrandom and constructed in a sophisticated fashion. Important points are: the reverse spiral pattern of the collagen fibers in each lamella (middle right), the three types of bone cells (lower right), and the precise arrangement of the inorganic crystals on the organic collagen fiber (lower left). One can easily predict that the properties of such a structure, at all levels of organization, would be greater than those attributable to the sum of the components.

essential for survival. The demands placed upon the endoskeleton as it developed in the course of vertebrate evolution have been met by a unique material, bone, which has no analogue in the nonliving world of materials science.

In order to provide an appropriate reference for our discussion, it is necessary to provide the reader with some basic information on the structure of bone and the types of growth it exhibits. Bone is basically a nonliving, biphasic material, having two major components, a fibrous protein, collagen, and a mineral crystal, apatite, which have an extremely precise relationship with each other (Fig. 8). While the majority of the structure is nonliving, the collagen is the result of cellular activity and must be present in order for the deposi-

tion of mineral to occur. Furthermore, in the intact structure there is a precise relationship between the small number of living cells incorporated into the matrix and the organization of the matrix itself. Death of the cells results in ultimate dissolution of the nonliving remainder, so it may be assumed that the presence of the living cells is necessary in some way for the integrity of the nonliving matrix. Mature bone has a low water content, approximately 7% by weight, and this plus the dimensional stability of the material and its well-characterized structure make it the one biological material most suited to application of solid-state physical methods of experimentation. We have determined that the basic elements of the bone matrix have some semiconduction properties, with collagen being an *n*-type semiconductor and apatite a *p* type.[27] The association between the apatite and collagen has been shown to be epitaxial,[28] and some evidence has been developed for a *p*–*n* junction-type structure being formed by these two units.[29] It has also been well established that collagen has piezoelectric properties[30] and that the piezoelectric effect determined in bone is the result solely of the collagen.[31] Collagen, in addition, demonstrates a well-characterized electron paramagnetic resonance which varies with temperature,[32] and it also is capable of generating stable free radicals

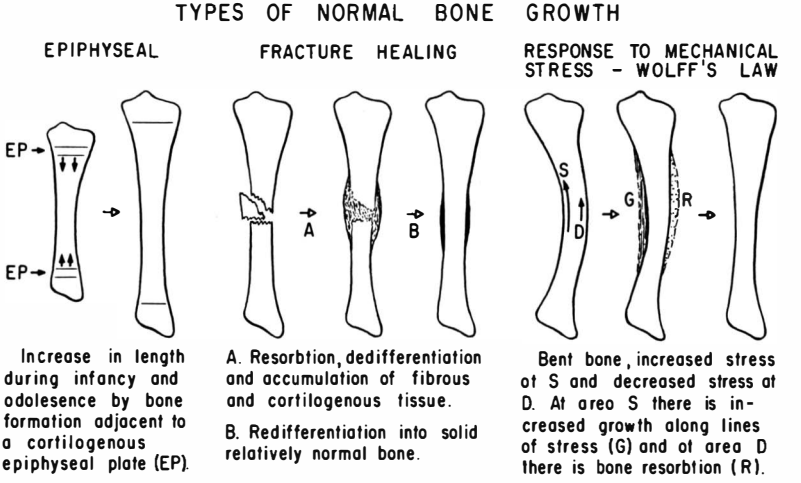


Figure 9. Representation of the three different types of normal bone growth.

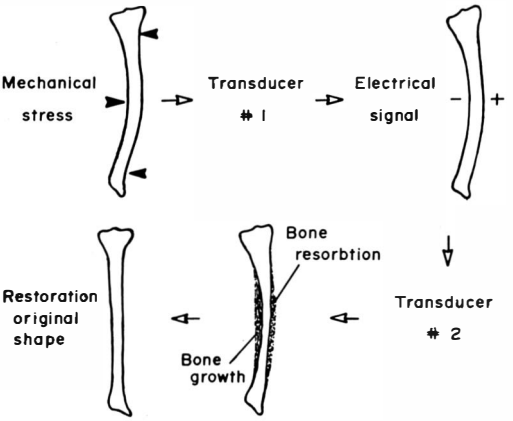


Figure 10. The growth response of bone to mechanical stress, and deformation viewed as a feedback control system. The figure reads from the top left clockwise and is self-explanatory. The two transducer mechanisms are theoretically predicted, while the remainder of the depicted events are directly observable.

at sites of fiber disruption.[33] It is our belief that several of these properties are of functional significance in the growth control system evidenced by bone.

Of the demands placed upon bone by the organism, the most important are that: it must grow as the organism grows in size, it must respond to applied mechanical stresses by growth as needed to increase its load-carrying capacity, and it must heal, when physically stressed to failure, with tissue of the same mechanical properties. All of these requirements are met by the specialized growth control systems illustrated in Fig. 9.

The growth response to mechanical stress appears to be amenable to control-systems analysis, and a simple closed-loop negative-feedback control system could be theorized for it (Fig. 10). It was postulated that the signal generated by mechanical stress is electrical and further that it should have a polarity imbalance in order to indicate directivity of stress. Bone was found to be stress electrogenic in accord with this concept, the electrical signal having the required polarity imbalance.[34] Areas of compressional stress where bone growth would subsequently occur were negative and

areas of tensional stress, bone resorption, were positive. It was then theorized that if the stress-generated electrical potentials were meaningful signals, the injection of a dc voltage of appropriate values into normal bone should produce bone growth at the negative electrode and bone resorbtion at the positive electrode. This was confirmed by observation of the results from the injection of approximately 3 μA at 1.3 V.[35] While the initial report indicated only growth response at the cathode, subsequent evaluation of the data has shown resorbtion to be occurring at the anode. The growth and resorbtion were both the result of differential cellular activity, dependent upon the polarity, and based upon unknown mechanisms. A contributory mechanism might be the differential migrations of newly formed precursors of collagen which produce collagen fibers oriented at 90 degrees to the direction of current flow. This phenomena however, has been found to be based upon *p*H gradients and requires voltage sufficiently high to produce the pH shifts.[36] This property, therefore, might be operable at close ranges from the cell wall, where such voltages may be present, but could not be a factor at greater distances. It is, therefore, quite possible to construct a control-system schematic for bone growth in response to mechanical stress with all of the portions reasonably well identified (Fig. 11).

The validity of this concept has been subsequently confirmed by other investigators,[37] and this represents, to our best knowledge,

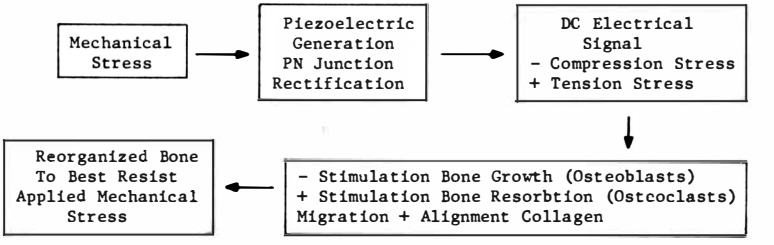


Figure 11. Final form of the control system responsible for bone growth in response to mechanical stress. The two transducers have been identified as shown. Based upon this data, the concept was developed that electrical forces applied to cell systems could produce effects (other than Joule heating, etc.) by furnishing meaningful signals to activate pre-existing control systems. If this concept proves to be correct, one would predict a rather wide variety of control systems that could be effected. Since the resultant effects cannot be predicted in advance, some caution should be exercised in clinical applications.

the first identification of such an electrically driven growth control system in this detail.

The prime question remaining in the system, however, is the mechanism involved in the cellular responses to the electrical parameters. A partial answer to this has developed from our investigation of the fracture-healing phenomenon. We theorized that the stress to failure might produce electrical voltage lasting for some time following the fracture as a result of the residual stresses or resulting from the generation of free radicals of the fractured surfaces. A nonmammalian species was chosen for this study, the bull frog, whose tibia happens to be a straight, well-developed, bone of approximately 2 cm in length. It is easily fractured in a controlled fashion, yielding comparable anatomical situations for analysis from a large experimental population. The literature on fracture healing in nonmammals is sparse, but the few papers that existed indicated that the cellular mechanisms were comparable to the mammalian: proliferation of the periosteum, migration of the new cells into the fracture hematoma, and subsequent development of a blastema between the bone ends, with maturation into bone via intermediate stages of fibrous tissue and cartilage.[35] Electrical determinations pre- and postfracture gave results as expected in regard to duration and indicated the existence of both a complex field pattern between the bone ends and a polarity difference between the bone matrix itself and the periosteum (Fig. 12). Histological evaluations were done to correlate the cell changes with the

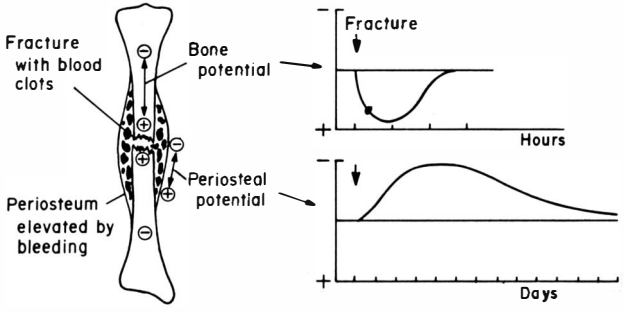


Figure 12. The electrical events associated with fracture healing. The figure is self-explanatory and should be compared with Fig. 8 for anatomical and structural orientation.

electrical variations noted, and the expected periosteal proliferation was not observed. What was noted, however, was a process of dedifferentiation in the red blood cells constituting the hematoma between the bone ends. It should be noted here, that all vertebrates except the mammals have red blood cells containing intact nuclei, the mammals having developed red cells which in the final stages of maturation extrude their nuclei and assume a binconcave shape which facilitates gas exchange. The amphibian red cells, with their intact nuclei containing the complete genetic material for the individual, are capable of dedifferentiating into more primitive cell types. Depending upon the circumstances and the cell type required for healing, the dedifferentiated cell then undergoes redifferentiation wherein the genetic code for the cell type required is placed into action and the primitive cell becomes a mature cell of the necessary type. The histological evidence indicated that what was happening in the amphibian fracture was a dedifferentiation of the red blood cells of the hematoma add their subsequent redifferentiation as cartilage cells which then transformed into bone. It was postulated that this mechanism is triggered by the electrical conditions at the site of the fracture. This hypothesis was tested by exposing normal frog red blood cells, suspended in saline solutions, to various voltages applied through immersed electrodes. At voltages and currents approximating those calculable on the fracture itself, the red cells underwent morphological transformation identical to those observed in the fracture hematoma.[39] Subsequent evaluation by cytochemical and autoradiographic techniques has established that true dedifferentiation was occurring in these cells.[40] This test system has proven to be of great utility in evaluating the basic mechanisms involved in the reaction of cells to minute electrical potentials, and the concepts evolved will be discussed in more detail in the next section of this chapter.

To translate the results on the fracture-healing system in amphibians to mammals, we have utilized the rabbit as the experimental animal. Similar voltages have been noted by us and other workers,[41] and in this case, it is important to distinguish the voltages that appear on the periosteum from those that appear on the bone itself. The high negative spike appearing on the periosteum has been shown (by experiments in which negative potentials were applied to the intact periosteum in the absence of a fracture) to stimulate

mitotic activity in the periosteum itself, with the subsequent production of bone-forming cells, osteoblasts. We have determined that the mammalian bone marrow (a tissue containing a variety of cells, including the immature stages of the red blood cells which contain nuclei) is involved in the fracture repair by a process of dedifferentiation with subsequent redifferentiation into fibrocartilage and bone. Thus, the mammal has two cell systems involved in fracture healing, one quite analogous to the primitive amphibian system, and the other, periosteal proliferation, etc., apparently specifically developed in the mammal. While the latter has not definitely been shown to be electrically driven, all preliminary data tend to support this concept.

We have, thereby, been able to demonstrate that two of the three growth control systems of bone are electrically driven by the minute stress-generated potentials derived from the bone matrix itself. It is apparent, however, from both clinical[42] and experimental data[43] that factors contributed by the nervous system play a role in both of these growth processes. Presumably these factors are electrical in nature and interact with the locally generated potentials. The third growth control system, epiphyseal growth, by which growth in length and consequently increase in body size are produced, is something totally different. The appearance and position of the epiphyseal growth plates are genetically determined, and extirpated embryonic limb rudiments explanted into tissue culture medium will demonstrate growth and development of a normal bone in the absence of mechanical stress. While it is known from clinical observation that mechanical stress will increase the growth of an epiphyseal plate, this is evidently superimposed on a basic rate. The question of what factor or factors drive this activity is unanswered at the present time. It is our opinion, however, that the role played by the central nervous system (CNS) in producing unified growth and in relating the various parts of the organism to the total organism is related to the steady-state or slowly varying electrical potentials measurable in the CNS itself. These potentials have been observed, described, and correlated with many basic biological activities, but their generating sources, patterns, and functions are little understood at present. From a philosophical viewpoint, it would appear unrealistic to expect that nature, having developed two sophisticated electrically driven growth-control

systems, would develop other systems for other growth phenomena, at the cellular level. One might, on this basis, reasonably expect to find widespread growth-controlling properties of electrical parameters, including an effect upon the epiphyseal-plate growth rate.

A tissue intimately related to bone is cartilage; it evidences itself in many forms, from the specifically oriented epiphyseal plate to the cartilage "callus" that forms at the fracture site and is the precursor of the soft, unorganized, bony callus. Perhaps its most clinically important form is the hyaline cartilage that provides the bearing surface of joints. While the joint is anatomically and mechanically a complex structure, the ultimate tissue upon which load bearing occurs is the joint cartilage, and it appears to be particularly well organized and designed to withstand those stresses. Unfortunately, in the mammal, this tissue has limited reparative potential, and injuries to the joint cartilage are repaired not by the well-organized hyaline cartilage, but by fibrocartilage, a tissue type much less capable of withstanding applied mechanical loading. This unfortunate fact leads to the prevalence of arthritis within the human population today.

Considerable work has been done on the problem of the healing of joint cartilage in the mammal; some of which indicated that the surviving cartilage cells at the borders of an injured area attempt to repair the defect by mitotic activity.[44] Nevertheless, this process is slow and ineffectual and is overwhelmed by a much more rapid outgrowth of fibrous tissue from the bone marrow in the depths of the cartilage defect.

It was theorized that the current of injury from the damaged site is insufficient to produce an adequate response from the residual cartilage cells, and that increasing the CI might selectively increase the proliferation rate of the cartilage cells without concomitantly increasing the rate of fibrous-tissue proliferation. A series of experiments was performed on rabbits in which a standard defect was produced on the femoral condyle (the rabbit's knee joint) and a resistor-limited bimetallic device surgically implanted so as to terminate the active electrode in the center of the defect. It was found that the rate of cartilage production could be materially enhanced by this technique and that coverage of 50% of the defect with anatomically normal (both grossly and microscopically) hyaline cartilage could be accomplished in two to three weeks.[45] It was

recognized previously that the bimetallic devices are not the optimal tools to utilize for growth stimulation, being difficult to construct with a reliable range of voltage and current, and, also, being quite limited in the values obtainable. One, therefore, can conclude from this preliminary series of experiments that mammalian joint cartilage is capable of regrowth and that this process can be markedly enhanced by electrical stimulation at very low voltages and currents. It seems reasonable to postulate that with devices producing optimal ranges of current and voltage, clinically useful cartilage regrowth may be entirely possible.

In summary, studies on bone and cartilage, from the theoretical viewpoint of electrically driven growth control systems, have been successful in demonstrating the existence of such systems and hold promise for theraputically useful tools in the near future.

V. BIOELECTROCHEMICAL MODELING*

The previous sections of this study, as well as many others,[22,25,30,34,35,39,46–56] have shown that fundamental cellular activity can, indeed, be influenced by the electric and/or magnetic environment. Studies on the amphibian erythrocyte (red blood cell) *in vitro* show conclusively that the cell responds directly to local variations in the electric field. (Magnetic field reactions have not yet been quantized, but there is some indication that this also plays a role, possibly via voltage induction.) In all of these cases, it is most probable that the living cell senses changes in its microenvironment via its cytoplasmic membrane–extracellular fluid interface, i.e., it is electrified, and as such, will have very analogous properties to the electrode–solution interface. This means that the membrane may respond to changes in local electric field in much the same manner as an electrode via potential-dependent interfacial processes such as electrostatic and specific-adsorption (binding) charge acceptance and charge transfer. Any or all of these processes will modify the structure of the interfacial region, resulting in a different interaction of the cell with its environment, thereby eliciting a possible trigger for an appropriate modification in cell function.

In order to construct a membrane model which takes into account interfacial processes, it is necessary to understand the nature

*The treatment here depends for validity upon the attaining of linear conditions.

of the electrified interface. By definition, an interface represents a discontinuity in the nature of species which are in close proximity to each other. In order to accommodate this, component species from each bulk phase take on different distributions and orientations than those which they have in the bulk. When this happens, electroneutrality conditions break down across the interface and a potential develops. This potential is a measure of the manner in which the ions and dipoles which make up each side of the interface distribute themselves for minimum free-energy requirements. This distribution consists of a region in which the ions, dipoles, and other charge carriers make up a relatively rigid (Helmholtz or inner) layer on each side of the interface and a less rigid (diffuse layer) region extending from, e.g., the solution side of the interface into bulk. The manner in which the ions and dipoles distribute themselves in the inner layer can be governed solely by electrostatic requirements, or by both electrostatic and specific-adsorption requirements. In the former case, these requirements are governed solely by such quantities as charge, charge density, etc. However, in the case of specific adsorption, the interactions are governed by the physical and chemical properties of the species involved. Thus, whereas the concentrations of components in the interfacial region would be expected to be proportional to their bulk concentration (all other things, e.g., charge density, being equal), in the case of electrostatic interactions, the concentration of a specifically adsorbed species may, of course, be grossly out of proportion with respect to its bulk contribution. Diffuse-layer interactions are only electrostatic in nature and, as such, are nonspecific. Since the cells of most living (higher) organisms bathe in an electrolyte of relatively high total concentration (approximately 0.2 *M*), the potential drop across the diffuse layer is relatively small compared to that which exists across the inner layer.[57] Thus, in addition to the preferred rigid and perhaps specific interactions in the layers immediately bounding the interfacial region, there are preferred (nonrandom), less rigid, electrostatic interactions in the electrolytic planes just outside the Helmholtz layer. The diffuse region is responsible for the electrokinetic effects such as electrophoresis and streaming potential. It must be remembered, however, that while these phenomena allow the potential across the diffuse double layer (zeta potential) to be measured, this potential represents nonspecific interactions of the

cell with its environment, and contributes less and less to the total potential drop as the extracellular-fluid ionic (or charge carrier) concentration increases.[57] This means that even though the zeta potential allows partial charge calculations to be made, these quantities certainly do not reflect the total organization of ions and dipoles in the whole interfacial region.

The above discussion illustrates that the structure of the membrane–extracellular fluid interface can, indeed, be dependent upon changes in the electric and/or magnetic environment. Thus, it can be envisaged that any change in potential across or along the interfacial region will be sufficient to modify the distribution of ions and dipoles, *even if current does not pass across the interface.* However, in order for as complete a model to be developed as possible, it is reasonable to expect in some cases that charge transfer may also occur at the interface. This means that either electron conduction may occur from one end of a molecule to the other in the membrane structure or that enzymes may be present in this structure to mediate the transfer. In this case, it is, of course, possible for current to pass across the membrane, and if a redox mechanism is necessary for the cell to respond, then it would be expected that the direction of current flow across the interface could play an important role in cellular response.

It is, therefore, evident that there are three fundamentally important potential-dependent phenomena, the response of which to artificial electrical changes can indeed be relevant to a change in a living cell's function. It is to be noted that mass transport *across* the membrane has not been envisaged as a *primary* response to the change in electric environment. Thus, a "change in the permeability" of a membrane, while certainly basically important, is not essential to the interfacial model presented here. Rather, the processes envisaged, particularly that of specific adsorption, may explain the manner in which the membrane changes permeability. The model to be discussed here then considers that a change in potential across the membrane–extracellular fluid interface can result in both electrostatic and specific-adsorption charge acceptance (or ion–dipole population change), along with the possibility of charge transfer. If a species undergoing specific adsorption and/or charge transfer at an interface is in relatively low bulk concentration, it may, in addition, be necessary to consider mass transport of that species *up to* (or away from) the interfacial region. Mass transport

within the membrane phase is not considered to contribute substantially, if it occurs, to the overall response.

In essence, therefore, the model to be presented in this study considers that a membrane may be the site of processes other than transport. Since it is evident that most of a cell's interactions with its environment occur before, or at least nearly simultaneously with, any change of permeability, it then appears appropriate that interfacial phenomena play a role.[58–61] An example of this would be cell-cell and cell–tissue interactions which occur, for example, in thrombosis.[62–64] It is thus the specific aim of the theoretical approach given below to emphasize particularly the potential-dependent interfacial phenomena discussed earlier.

In view of the above, the total current $i_T(t)$, which could flow in response to an input perturbation is the sum of the charging current, $i_D(t)$, which satisfies both electrostatic and specific-adsorption requirements, and the faradaic current, $i_f(t)$, which may satisfy the latter and the redox process. Thus,

$$i_T(t) = i_D(t) + i_f(t) \tag{1}$$

In this particular model it will be assumed that the electrostatic interactions may be satisfied by all of the ions and dipoles which may be present in the interface. In addition, the specific adsorption and charge transfer influences a single (minority) species j, which is as expected if it can be considered that any given interface does not act in a nonselective manner to external electrical stimulus. For this case, $i_D(t)$ is given by

$$i_D(t) = \frac{dq}{dt} = \left(\frac{\partial q}{\partial E}\right)_{\Gamma_j} \frac{d\eta}{dt} + \left(\frac{\partial q}{\partial \Gamma_j}\right)_E \Gamma_j \frac{d\Delta\Gamma_j}{dt} \tag{2}$$

where q is the total change in charge, E the potential across the interface before the applied perturbation, η the change in potential caused by the perturbation, Γ_j the initial surface concentration of the specifically adsorbed species j, and $\Delta\Gamma_j$ the relative change in Γ_j given by $[\Gamma_j(t) - \Gamma_j]/\Gamma_j$.

Equation (2) shows that the change in interfacial charge as the result of an artificial electric perturbation is given by normal capacitive charging determined by the coefficient $q_E = (\partial q/\partial E)_{\Gamma_j}$.* In addition, a portion (often, the major portion) of the charging

*This may be via the dielectric capacitance of the membrane or due to surface-charge behavior.

current goes toward changing the interfacial concentration of j governed by the coefficient $q_{\Gamma_j} = (\partial q/\partial \Gamma_j)_E$. The second portion of the total current, $i_f(t)$, is given by

$$i_f(t) = (\partial i_f/\partial E)_{C_j^b}\eta + (\partial i_f/\partial C_j)_E C_j^b \Delta C_j(0, t) \tag{3}$$

where C_j^b is the bulk concentration of species j and $\Delta C_j(0, t)$ represents the relative change in concentration of j at a plane $x = 0$, which is defined as that plane just adjacent to the specifically adsorbed layer. This equation shows that the faradaic current depends upon the change in potential across the interface, governed by the coefficient $I_E = (\partial i_f/\partial E)_{C_j^b}$ as well as on the concentration of the charge-transferring and specifically adsorbing species, governed by the coefficient $I_{C_j^b} = (\partial i_f/\partial C_j)_E$. Note that both faradaic and charging processes are functionally coupled since $\Delta\Gamma_j$ and ΔC_j are, of course, dependent upon each other. The relation can be established in a manner similar to that employed in electrode kinetics by establishing an exchange adsorption–desorption rate constant, v_j,[65,66] which relates to adsorption kinetics; the following can then be written

$$d\Delta\Gamma_j/dt = (v_j/\Gamma_j)[\Delta C_j(0, t) - \Delta\Gamma_j(t) + a\eta(t)] \tag{4}$$

where a represents the potential dependence of adsorption $\partial\Gamma_j/\partial E$.

In order to be able to employ equations (2) and (3) to describe the behavior of the membrane–extracellular fluid interface when subjected to an artificial electric perturbation, it is necessary to obtain an explicit expression for $\Delta C_j(0, t)$. This can be performed using Fick's second law written for linear diffusion as*

$$\frac{\partial \Delta C_j(x, t)}{\partial t} = D_j \frac{\partial^2 \Delta C_j(x, t)}{\partial x^2} \tag{5}$$

where D_j is the diffusion coefficient of species j. To solve equation (5), the boundary conditions at the interface ($x = 0$) are given by equations (2) and (3) and

$$d\Delta\Gamma_j/dt = D_j[\partial \Delta C_j(x, t)/\partial x]_{x=0} + i_f/\eta F \tag{6}$$

(where F is the faraday) which states that $\Delta\Gamma_j$ may occur by mass transport or charge transfer, or both. The remaining boundary

*Note that the variation in concentration considered in this model refers only to the aqueous phase. This is valid when mass transport is not limiting in the membrane phase.

condition is that for $x \to \infty$, at which $\Delta C_j(\infty, t) = 0$, meaning that there is no variation in concentration at distances sufficiently far from the interface at any time.

For this study, it is considered that the most relevant property of the interface is its impedance. This is so since it is a characteristic function of the system (independent of the perturbing waveform). To do this, it is useful to employ the Laplace transformation by which a time-domain function, $f(t)$, or operation, may be transformed to a complex-frequency domain via:

$$F(s) = \int_0^\infty f(t) \exp(-st)\, dt \tag{7}$$

where s is a complex frequency variable given by $s = \sigma + j\omega$, in which ω is the real and $j\omega$ the imaginary part. The variable s defines a complex plane known as the Laplace plane[67,68] allowing both real, $z(\sigma) = \eta(\sigma)/i_T(\sigma)$, and complex, $Z(j\omega) = \eta(j\omega)/i_T(j\omega)$, impedance functions to be obtained. This approach has allowed both theoretical and experimental development in interfacial studies. Of particular importance is the fact that impedance can be studied artifact-free at frequencies up to 100 MHz.[67,69] This is of relevance in the study of adsorption phenomena, where time constants relevant to the structure of the interface could go undetected if a wide frequency range were not examined.[70]

Using all of the above, we find the following expression for the faradaic portion of the impedance $Z_F(s)$

$$Z_F(s) = \frac{(RT/nFI_E)(A_j + Y_j) + I_{C_j^b} C_j^b / gI_E}{A_j(I_{C_j^b} C_j^b anF/gI_E + 1) + Y_j} \tag{8}$$

and that for the charge-acceptance portion, $Z_D(s)$, by

$$Y_D(s) = \frac{1}{Z_D(s)} = q_{\Gamma_j} A_j \left[\frac{I_E + a(nFY_j + I_{C_j^b} C_j^b)}{nF(A_j + Y_j) + I_{C_j^b} C_j^b} \right] + q_E s \tag{9}$$

where

$$A_j = nF\Gamma_j s/(1 + \Gamma_j s/v_j) \tag{10}$$

and

$$Y_j = nFC_j^b (D_j)^{1/2} (s)^{1/2} \coth[\delta (D_j)^{1/2} (s)^{1/2}] \tag{11}$$

In addition, $g = n^2F^2/RT$ and the other terms have their usual significance.

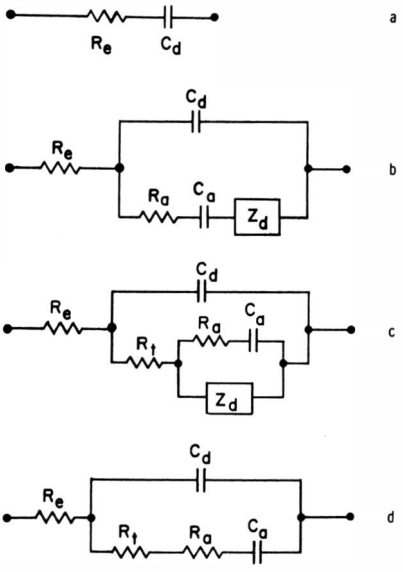


Figure 13. Aperiodic equivalent-circuit models by which a membrane may respond to electrochemical information input: (a) Electrostatic charge acceptance, (b) electrostatic and specific-adsorption charge acceptance, (c) coupled faradaic and interfacial processes, and (d) as (c) except negligible, mass transport.

Equations (8) and (9) describe the manner in which a membrane–extracellular fluid interface responds to an electric perturbation which changes the potential across the interfacial region. They appear complex, but the number of parameters is not too large to enable numerical analysis to be carried out. In general, Z_F and Z_D are in parallel, but the manner in which these quantities can be analyzed does not readily allow aperiodic (frequency-independent) equivalent electric circuits to be proposed. In order to illustrate the build-up of an interface from elements which ultimately lead to equations (8) and (9), it is useful to make the following general observations:

1. If charge acceptance due to electrostatic requirements is the only mode by which an interface responds to a change in potential, then it is clear that $Z_F \to \infty$ and all terms in equation (9) relating to specific adsorption and charge transfer disappear (e.g., $q_{\Gamma_j} \to 0$). In this case, the total impedance, $Z_T(s)$, is given by

$$Z_T(s) = 1/q_E s + R_e \tag{12}$$

where R_e is the electrolyte resistance between any perturbation input point and the interface (i.e., bulk current path), and it is possible in this case to express the system unambiguously in terms of the equivalent circuit shown in Fig. 13a, where $C_d = 1/q_E$. This is the simplest of the interface models and represents nonselective response.

2. In the case of response by the interface using both electrostatic and specific-adsorption charge acceptance, $Z_F \to \infty$ and all terms in equation (9) relating to charge transfer disappear. Thus,

$$Y_D(s) = q_{\Gamma_j} a A_j Y_j/(A_j + Y_j) + q_E s \tag{13}$$

which is represented by the aperiodic equivalent circuit shown in Fig. 13b. Here, selective and nonselective charge acceptance are seen as two parallel current paths. The latter is given by C_d, defined above, and the former by

$$R_a = 1/q_{\Gamma_j} a v_j \tag{14}$$

$$C_a = \Gamma_j/q_{\Gamma_j} a \tag{15}$$

and

$$Z_d = 1/Y_j \tag{16}$$

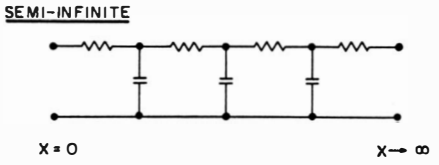
where R_a, the equivalent adsorption resistance, is a function of the exchange adsorption rate, v_j, as expected; C_a, the equivalent adsorption capacitance, is a function of the surface concentration, Γ_j; and the diffusion impedance, Z_d, exhibits the well-known square-root frequency dependence in the case of semi-infinite linear response and bulk time-constant response for finite conditions (see Fig. 14).

3. In order to couple all processes, it is convenient to assume that the potential dependence of adsorption, a, is negligible. This effectively negates the functional coupling between i_f and i_D and allows the following to be written:

$$Z_F(s) = \frac{RT}{nF}\frac{1}{I_E} + \frac{I_{C_b^j} C_b^j}{g I_E}\frac{1}{A_j + Y_j} \tag{17}$$

and

$$Z_D(s) = 1/q_E s \tag{18}$$



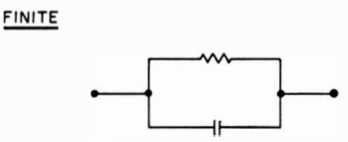


Figure 14. Aperiodic equivalent electric circuits for linear diffusion. Semi-infinite behavior denoted by RC transmission line and finite behavior denoted by lumped RC parallel circuit.

which state that the effect of specific adsorption is, in fact, reflected only in Z_F. This is illustrated in Fig. 13c, where C_d is as above, and

$$R_t = RT/nFI_E \tag{19}$$

$$C_a = g\Gamma j I_E / I_{C_j^b} C_j^b \tag{20}$$

and

$$R_a = gI_E / I_{C_j^b} C_j^b v_j \tag{21}$$

$$Z_d = I_{C_j^b} C_j^b / gI_E Y_j \tag{22}$$

It can be seen that not only are the faradaic and specific adsorption processes functionally coupled, but there is, in addition, a parameter coupling due to the general formalism employed for i_f [equation (3)]. Thus, if R_t reflects a negative i_f vs. E slope, the result would cause R_a, C_a, and Z_d to become negative. The rationale, of course, is that if R_t were negative, then the whole impedance spectrum would necessarily have to be shifted in order to maintain system stability.

4. A final case of interest is obtained if mass transport is negligible. This occurs if the bulk concentration of species j is sufficiently high. In this manner, all interfacial processes in which j plays a role do not result in a significant concentration gradient for

it. In this case, $Y_j \to 0$ and, if $a \to 0$ then,

$$Z_F(s) = \frac{RT}{nF}\frac{1}{I_E} + \frac{I_{C_j^b} C_j^b g I_E}{A_j} \tag{23}$$

and

$$Y_D(s) = \frac{q_{\Gamma_j} I_E}{(nF + I_{C_j^b} C_j^b / A_j) + q_E s} \tag{24}$$

which show remarkably simple behavior as illustrated in Fig. 13d, wherein all the parameters are as for the previous case.

The above four illustrations demonstrate how a relatively simple interface can be described by progressively adding the processes that may play a role in its response to an electrical perturbation. In general, there are two or three predominant time constants which are sufficient to describe the electrochemical events which may take place. It is to be noted that this is for single-species behavior only, which is entirely reasonable if a membrane responds in a very specific manner. However, extension of this treatment to any number of species is entirely straightforward. Only the algebra gets more complex, not the concepts. It has thus been shown that, even for an insulator (at dc) membrane, an entirely reasonable physical picture can be created in terms of simple electrochemical interfacial processes by which a living cell may recognize specific environment changes.

VI. ELECTROCHEMICAL INFORMATION TRANSFER

In order to test the above model in a general fashion, it is necessary to choose a cell system in which some biological correlation to an external electrical stimulus exists. This is so since it will otherwise be virtually impossible to obtain a meaningful electrical response. Thus, independent of the fact that penetration of the cell with a microelectrode could be physically destructive, it is important to realize that a transmembrane potential measurement is only very ambiguously relatable to that of the membrane–extracellular fluid interface because of the various contributions to the total potential observed (from the membrane, and the *two* interfaces).

Studies of bone-fracture repair[39] in the frog (*Rana pipiens*) have illustrated (as seen above) that a specific cellular response

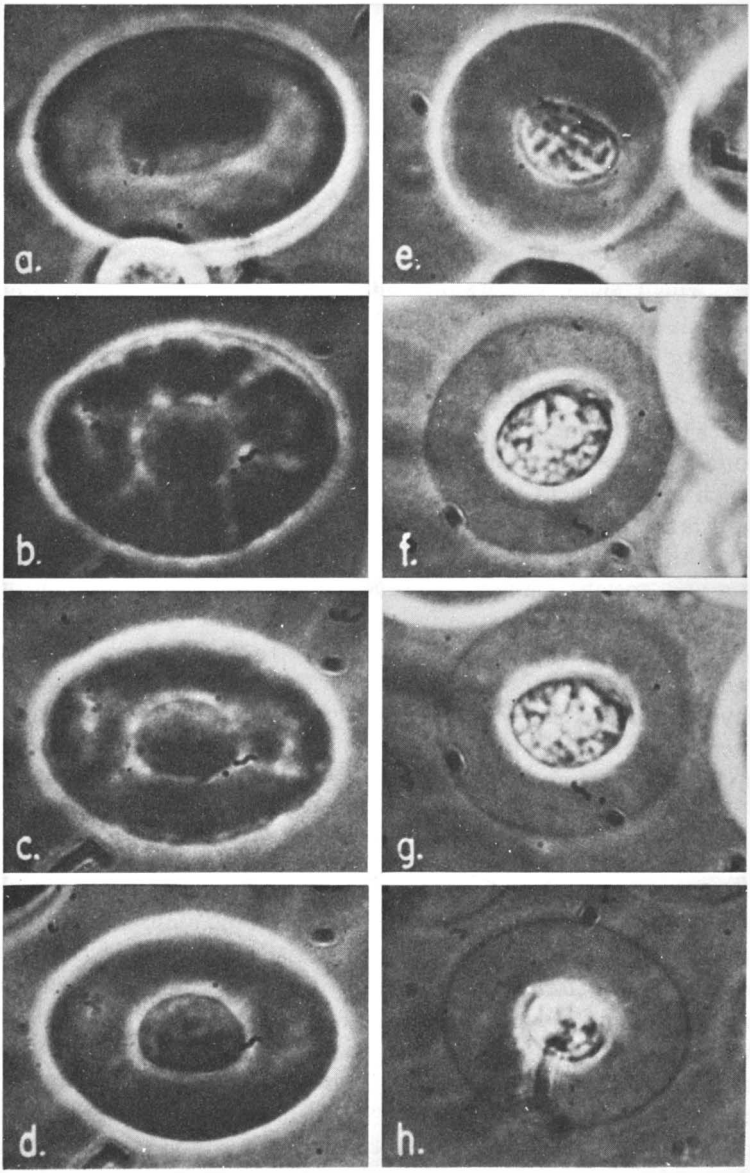


Figure 15. Amphibian erythrocyte (RBC) morphology changes during dedifferentiation. Here *a* is the normal RBC and *h* is the most embryonic or mesenchymal-like one. From R. O. Becker, *Clin. Orthop.* **73**, 169 (1970). Light microscope 1000 ×.

occurs in which the cell first becomes more embryonic-like (less specialized), after which it responds to information allowing it to redevelop along pathways necessary for complete tissue regeneration. The initial cellular process appears necessary before further repair can take place. In the specific case of amphibian bone-fracture repair, the nucleated red blood cell (erythrocyte) dedifferentiates in order to enable it to respecialize as a bone-producing cell (osteoblast). The dedifferentiation of this red blood cell (RBC) is observable under the light microscope as a series of morphology changes. These are illustrated in Fig. 15. Here, the normal erythrocyte may be seen in the top left-hand corner (a). The sequence of cellular changes proceeds through the lettered pictures, with (h) being a picture of the most embryonic-like cell.

If the trigger for dedifferentiation in the above case is electrochemically mediated, it should be possible to artificially change the electrochemical environment and cause the cells to undergo the required morphology changes. This was previously attempted[39] by placing platinum electrodes in a suspension of RBC's diluted 500:1, and passing minute amounts of current (10^{-9} A). After waiting a certain period of time, the cells were observed to dedifferentiate, always starting near the electrode and slowly proceeding into bulk electrolyte. These dc experiments have recently been performed[60] using standard electrochemical potentiostatic (voltage clamp) techniques wherein the possibility of deleterious faradaic processes (e.g., *p*H changes, Cl_2 evolution, etc.) was kept to a minimum. This approach also assured constant background electrolysis conditions for systematic studies by maintaining the platinum electrode potential constant.

When using platinum as the electrode material for stimulation, it is important to realize that there is a limited potential range over which the electrodes may be potentiostatted without causing a major modification in any given cell's electrochemical environment. It has previously been determined[71] that the optimum potential range between which the cells may be stimulated is situated from 0.1–1.0 V anodic to the potential at which hydrogen evolution occurs in physiological electrolyte, about -0.65 V with respect to the saturated calomel reference electrode (SCE). In this potential range, it has been established that the dc current varies (almost linearly) between 0.1 μA cm^{-2} at $+0.1$ V to 1 μA cm^{-2} at $+1.0$ V,

thereby varying the electric perturbation experienced by the cells in bulk suspension by one order of magnitude.

The reference electrode employed in these studies was a saturated calomel electrode isolated from the suspension vessel by an elaborate salt bridge. This was found necessary because of the toxic effect of ions foreign to the compatible saline mixture.

For this work, a suspension of frog RBC's were placed in a 5-cc plankton counting chamber, made specifically for a Zeiss inverted microscope stage, and kept at 25°C (normal for amphibian studies). The platinum-wire working (0.5-cm^2) and counter (5-cm^2) electrodes were placed at the bottom of the chamber so that a portion of them were on the same plane as the cells which lie on the bottom cover plate. The reference electrode input was via a small (1-mm i.d.) Teflon tube which places the salt bridge in electrochemical contact with the chamber. The tube was placed about 1 mm away

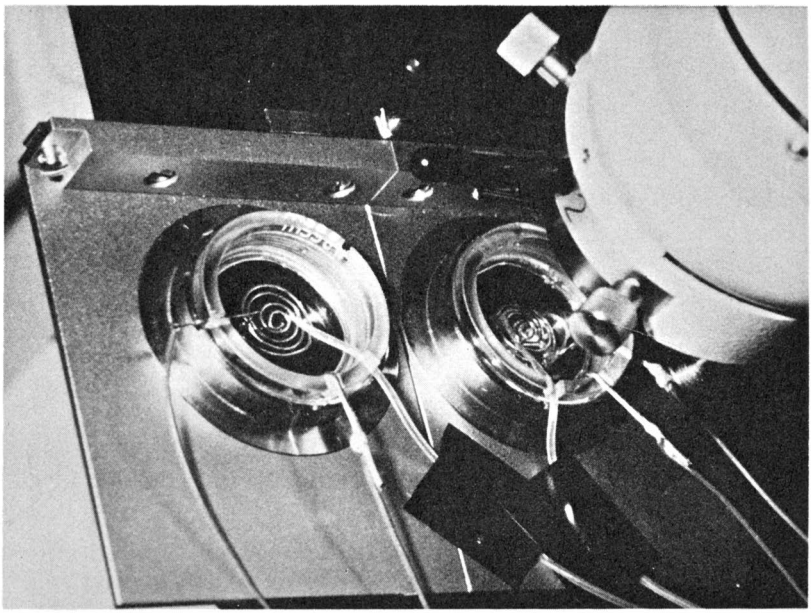


Figure 16. View of the plankton counting chambers with platinum electrodes and Teflon tube leading to SCE reference electrode for electro-chemically mediated cell dedifferentiation. Double chambers were employed to compare dc and transient response of cells taken from a frog at identical times.

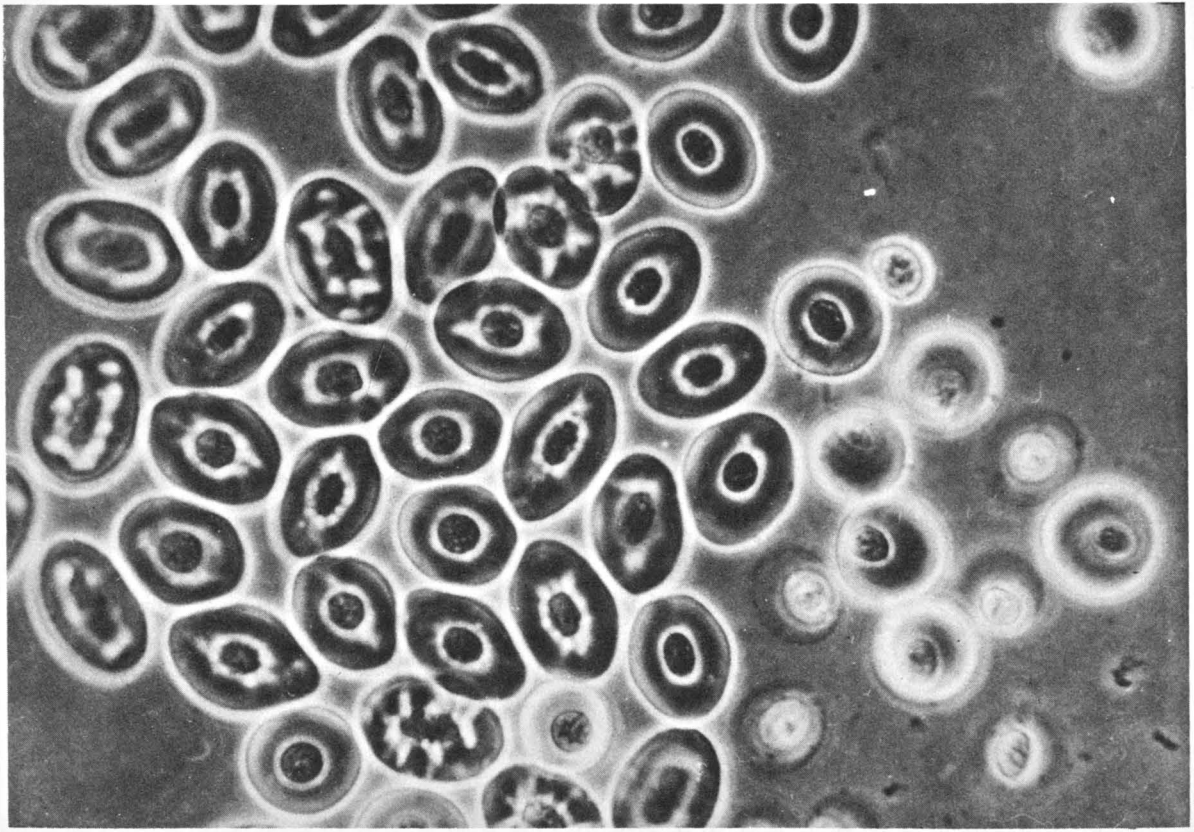


Figure 17. Light microscope (400×, reduced 40%) view of electrochemically mediated amphibian RBC dedifferentiation using dc stimulation. The platinum electrode is at the lower right-hand corner at +1. V vs. RHE. Note that dedifferentiation progresses from electrode area and that all morphological forms are present.

from the working electrode. A photograph of the chamber is shown in Fig. 16.

Electrochemical stimulation was applied to the cells via a Tacussel PIT 20-2A potentiostat driven by a Tacussel GSTP-2 function generator supplying square and triangular driving functions as well as dc potential control. In the initial experiments utilizing dc perturbation, cellular dedifferentiation under these conditions commences from the electrode and progresses into bulk solution. A typical example of this is shown in Fig. 17, in which the working electrode is closest to the right-hand corner, at which the most advanced stages of dedifferentiation can be seen. Examination of Fig. 17 shows that all of the cellular morphology changes seen in Fig. 15 are present.

These experiments were performed with the working platinum electrode held at different anodic potentials which, as indicated above, allowed an increase in current to be obtained. In addition, at a given potential, the electrode surface area was increased, thus allowing increased bulk current. In every case, the rate at which dedifferentiation occurred was directly proportional to the current, Fig. 18. This was observed by actual cell count, usually at the end

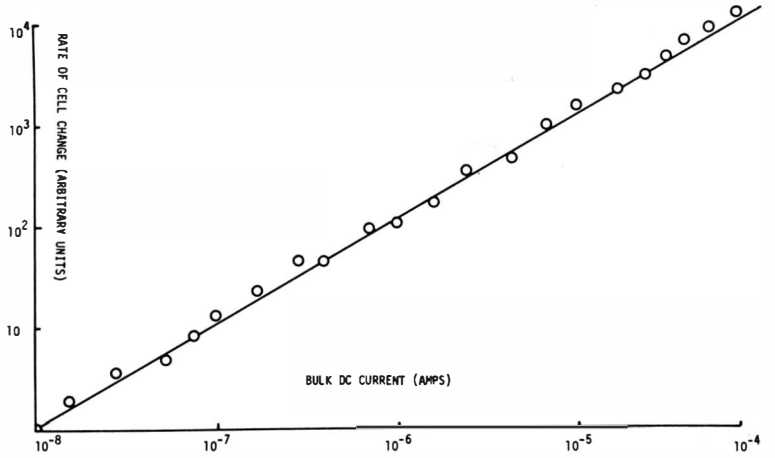


Figure 18. Cellular morphology change rate as a function of applied dc current using platinum electrodes at both different potentials and with increasing surface area at the same potential.

of the first 15–30 minutes of stimulation. In every case, the cell changes started with those in closest proximity to the electrode. In view of the maximum total currents which were passed in this and the previous study (approximately 1 μA cm^{-2}), the maximum potential difference which any of the RBC's could experience is 10^{-5} V. If these membranes are insulators, resulting therefore in a change in potential along the interface of 10^{-5} V μ^{-1}, the resulting change in interfacial structure is negligible. It remained, therefore, to explain the actual driving force for the dedifferentiation process in this case.

Since the results appeared to depend upon the maximum current rather than the electrode polarization, it was decided to investigate a possible change in tonicity caused by the different transference numbers of the current carrying ions involved. As is well known, the majority cations in this particular saline are Na^+, K^+, and Ca^{2+}, which have different transference numbers and different concentrations. It is the latter which plays the most significant role as far as the time required for the dedifferentiation process to initiate. Standard transference-number calculations[72] show that while the Na^+ ion carries 96% of the cationic current, K^+ carries 3%, and Ca^{2+} merely 1%. Therefore, as current is being generated at the electrode, there would tend to be accumulation of Ca^{2+} ions near the positive electrode as it is left behind by the K^+, which carries 3 times more cationic current. Thus, the concentration ratio $[Ca^{2+}]/[K^+]$ in a given plane with respect to that occupied by the electrode would tend to increase at a rate which is directly dependent upon the total current passing through the system. (Na^+ can be neglected in this case since its relative concentration with respect to K^+ and Ca^{2+} remains essentially unchanged.) The physical picture is then that a change in the concentration ratio $[Ca^{2+}]/[K^+]$ occurs, initiating at the electrode surface and progressing into bulk solution. Convective mixing is negligible since the cells are all on the bottom plate of the chamber, at which a static boundary layer exists over the first few microns. In addition, bulk electroneutrality requirements are satisfied due to the high Na^+ and Cl^- concentrations which overwhelmingly satisfy this. The time required for the initiation of differentiation is very roughly related to that required to establish a 5–10% change in the $[Ca^{2+}]/[K^+]$ ratio.

It would thus appear that the effect of dc electrochemical stimulation is, in fact, related to a local change in tonicity as a result of the background current flowing in the perturbation system. Dedifferentiation in this case always commences with those cells nearest the electrode surface since this is where the change in $[Ca^{2+}]/[K^+]$ ratio starts. It is to be noted that higher currents do not change this phenomenon, they merely accelerate its commencement and progression into bulk solution, provided that no deleterious faradaic processes occur. These experiments have, in fact, provided a rather unique possibility to vary the immediate bulk concentration of two components of the ionic environment, the K^+ and Ca^{2+} ions. This is one manner by which the structure of the membrane–extracellular fluid interface can be modified. While it is clear that these results give approximately the variation in surface concentration of K^+ and/or Ca^{2+} which must be caused in order to trigger dedifferentiation, it has not yet been demonstrated that a change in potential across the interface can have the same result.

If a comparison may be made of adsorption processes at electrodes with those which may occur at membranes,[73] it is reasonable to estimate that a 0.1-μC change in interfacial charge per cm^2 of *active sites* is all that is required to cause a 5% change in surface concentration. Again drawing from electrode behavior, specific adsorption capacitance is often approximately 100 $\mu F\ cm^{-2}$, which means that a change in potential across or along the interface of 10^{-3}–10^{-2} V should be sufficient to cause dedifferentiation provided it can be applied in accordance with the adsorption time constant.

It is clear from the above that dc cannot be employed because of both the change in tonicity effects and the practical limitation of an upper limit on the current value, exceeding which would require excessive electrode polarization.* However, it is entirely possible to provide larger currents using the large electrode–electrolyte double-layer capacitance of the stimulating electrodes (10–50 $\mu F\ cm^{-2}$). Thus, by applying ac or pulses to drive the electrode within the potential ranges mentioned above (0.1–1.0 V vs. RHE), it is possible to achieve up to 10-mA-cm^{-2} peak current. This is sufficient, in

*Note that the use of salt bridge electrodes to pass large dc currents in the absence of electrolysis effects did not elicit cell charges, in accordance with the specific adsorption model given earlier which prohibits dc current passage.

view of the specific resistivity of the saline suspension medium (50 Ω cm), to cause the interface to experience up to a 10^{-1}-V change in potential.

The above approach was employed by exposing the cells to controlled potential pulses repeating at frequencies from 10 Hz up to 10^6 Hz. These were applied in such a way as to maintain the same peak current and thus expose the RBC's to the same potential change. These were performed while simultaneously exposing identical cells from the same frog to dc polarization (see Fig. 16) set for maximum dedifferentiation rate (+1.0 V vs. RHE). In this manner, spatial patterns and dedifferentiation initiation rates could be compared.

The experiments were performed such that the field pattern set up in the plankton counting chamber exposed the cells to a relatively constant voltage gradient from one electrode to the other. In this way, an approximate voltage threshold could be established as a function of the frequency of the applied signal. At points 1 mm from each electrode, the voltage drop was able to be varied from 10^{-4} to 10^{-1} V, thereby exposing the cells at each frequency to several voltage gradients.

Preliminary results from these experiments show that the dedifferentiation process is no longer dependent upon background current being passed at the electrodes to establish local tonicity changes. Rather, at each applied frequency, the spatial pattern of dedifferentiation, reflecting the total rate of dedifferentiation, shows that this quantity depends upon the electric field values in the extracellular fluid. The largest distance away from the electrode over which dedifferentiation occurs in a uniform manner was for applied frequencies between 10^2 and 10^4 Hz. In addition, at this frequency, it appears that all cells exposed to a gradient less than 0.5 mV did not undergo function changes, in surprisingly good agreement with the crude calculation given above. These results are summarized in Fig. 19, which was established at a total gradient in bulk of 10^{-2} V. The peak at 10^3–10^4 Hz is a reflection of dedifferentiation occurring at a uniform rate at the greatest distance from the electrode at a given bulk-voltage gradient.

The dedifferentiation rate shown in Fig. 19 can be employed as though it were the response to an applied voltage-controlled ac waveform. It this case, the reciprocal of the function in Fig. 19

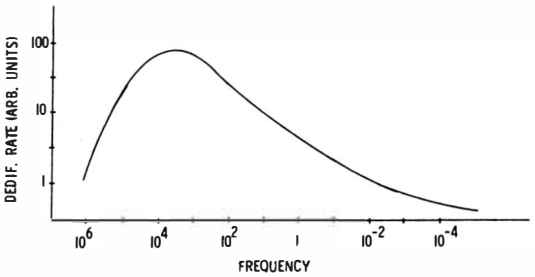


Figure 19. Dedifferentiation rate as a function of the repetition rate of applied pulses.

is directly proportional to the impedance of the system. Examination of the results in this manner shows that from the peak (10^3–10^4 Hz) to lower frequencies, a variation in $1/(\omega)^{1/2}$ changes to $1/\omega$ as the frequency becomes lower. This behavior is very indicative of the process represented by the aperiodic circuit represented in Fig. 13b. Thus, the process is one in which specific adsorption coupled with semi-infinite linear diffusion is the observable quantity correlateable with the cellular-morphology changes. Above the peak frequency, no straightforward functional dependence is observable. This is most probably due to the fact that electrostatic rearrangement of the ions and dipoles at the interface predominates, as shown by the quantity C_d in Fig. 13b. Since this is a nonspecific process, the cell would not be expected to employ this as a useful piece of information and the rate of dedifferentiation would be expected to decrease, as is observed.

In order to illustrate that indeed the cells were responding to a potential variation, it was established that the vector orientation of the bulk field was not important. For any given field strength, the behavior reported in Fig. 19 was observed with the spatial orientation of the dedifferentiated cells following the field distribution independent of the way it was distributed. This appears to lend support to the mosaic membrane model discussed by Lenard and Singer[74,75] in which globular proteins are nonuniformly distributed throughout the membrane. In this way both the RBC's and the specific membrane site which receives the electrochemical information may be randomly distributed with respect to the geometry of the electric field.

The above indicates in a very preliminary manner that an interfacial process could be a step in cellular dedifferentiation. In this particular case, both Ca^{2+} and K^{+} are implicated through the comparison of dc and pulse experiments. This cell, which has a nonexcitable membrane, appears able to understand electrochemical information input via a specific adsorption process. It is tempting at this point to anticipate that the initial step in the triggering of an excitable membrane may involve a very similar process, particularly since it is independent of the vector orientation of the input perturbation. In any case the model developed in the previous section appears able to be employed in a semiquantitative manner to correlate cellular dedifferentiation with electrochemical information input.

VII. THE FUTURE OF BIOELECTROCHEMISTRY

The future of bioelectrochemistry would appear to lie in interdisciplinary cooperation between scientists attacking problems at multiple levels. We would like to propose that one of the most important problems that could be explored is the area of cellular communication. This would include the mechanisms involved in the transmissional messages to cells, the mechanisms of receipt of the messages, and the mechanisms involved in communication between cells. As indicated earlier in this chapter, application of the concepts of electrochemistry to these problems appears to be most promising. Such projects must perforce include studies of the transmission of electrochemical information through aqueous media, the chemical species involved and their electronic characteristics, and, finally (perhaps crucially), the characteristics of the cell membrane from the electrochemical point of view. In addition to electrochemists, such projects must include solid-state physicists and many varieties of biologists. We feel that satisfactory pursuit of such objects will add a new dimension to biology and establish the study of bioelectrochemistry as a fully recognized scientific discipline central to what seems to be the last great frontier in life science. While full application of bioelectrochemistry to medical therapeutics must await the outcome of studies such as these, the bioelectrochemist has much to contribute to the present experimental studies ongoing in this area at the present time. As indicated earlier in this chapter, medicine

is a particularly empirical science and the presentation of data indicating that useful therapeutic results may be obtained by the application of electrical currents and voltages to living systems has led to the appearance of several techniques now being clinically applied. Medical science evidently does not require ultimate knowledge of mechanisms involved in useful techniques prior to the application of these techniques to the patient population. At present, techniques being explored in this fashion include electrical growth control systems, electronarcosis and electrosleep, and most recently, electroacupuncture. It is regrettable but true that most of these applications have not considered such basic aspects of electrochemistry as the electrode reactions involved, the distinction between purely electrical effects and those mediated by evolved metallic cations, or logical evaluation of the material best suited for implanted electrodes. These are obviously areas of competency of electrochemists, who should be involved directly in such ongoing studies.

In summary, the application of the principles of electrochemistry to the basic problems of the biological sciences would appear to offer a new and unique way of looking at these problems and the potential for markedly advancing our knowledge of how things biological function.

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