

# The Bioelectric Factors in Amphibian-Limb Regeneration\*

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The replacement of lost body parts by a specific growth process known as regeneration is an ability shared, to a varying extent, by all living things. In general, as one follows the evolutionary sequence, the ability becomes increasingly restricted. In man, the process is limited to the regeneration of certain tissues only, notably skin and bone. In a child the diaphysis of a long bone can be regenerated provided the periosteal tube is intact, whereas in an adult a fracture of a long bone can heal by replacement of the missing portion with functional osseous tissue. Although there is little doubt that factors such as nutrition, vascularity, and immobilization all play important roles in supporting this osseous healing process, the basic processes of cellular de-differentiation and subsequent re-differentiation which produce a replica of the missing portion represent true tissue regeneration. This process of bone healing is in marked contrast to the healing mechanisms in most other human tissues in which fibrous connective tissue is used to bridge the gap. It is evident, therefore, that the process of regenerative healing must entail specific factors not operating in other less adequate types of tissue healing. To control this type of tissue healing adequately, these specific factors must be identified and themselves controlled.

It is reasonable to expect that the same general controlling mechanisms are operative for all types of regeneration, and that knowledge of the regenerative process in another vertebrate may furnish a basis for a study of human regeneration. Fortunately, the peak of regenerative ability, as judged by the complexity of the structures regenerated, is present within the vertebrate phylum. The urodel, or salamanders, are present-day examples of the basic vertebrate type from which the higher vertebrates have been derived. Their fore and hind limbs are equivalent in anatomical structure and complexity to the human upper and lower extremities. Nevertheless, the larval-stage salamander is capable of regenerating a perfect replica of one of its limbs in a period of thirty to forty days. The adult salamander is similarly capable, although the required time is somewhat longer and the regenerated extremity may be slightly smaller than normal (Fig. 1). This process is so dramatically evident that it has stimulated study for literally centuries †. However, the basic controlling factors in the regenerative process and the factors operating to reduce the process in mammals have successfully resisted investigation until recently. Within the past few years, some significant observations have been made and the problem now can be approached from a new aspect.

In one line of inquiry Singer demonstrated the dependence of the regenerative process upon the presence of a critical amount of nerve tissue within the amputation stump<sup>22</sup>. He showed that this effect is not dependent on the type of nerve

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† Spallanzini (Prodromo, 1768): "But if the above mentioned animals recover their legs—how comes it to pass, that other land animals are not endowed with the same power? Is it to be hoped that they may acquire them by some useful disposition?"

tissue present. In the salamander, if the normal amount of peripheral nerve tissue is reduced by proximal nerve sectioning, then regeneration will not occur until either the missing nerves grow into the area or until neural tissue of any type, but sufficient in amount, is grafted into the area. Under certain specific circumstances, limb regeneration can occur in limbs devoid of nerves (parabiotic twinning<sup>27</sup>), indicating that under these circumstances the neural contribution may possibly be ancillary rather than primary. Although these experiments have, in general, left little doubt that some neural factor is necessary for the process of limb regeneration, the specific responsible factors associated with the nerve have yet to be identified.

Some recent investigations I reported furnished a possible clue to the identity of this neural factor<sup>2</sup>. The existence of direct-current electricity in living organisms was first observed by Galvani in 1792, and has received attention intermittently since then. This type of electrical activity, designated as the *bioelectric*



FIG. 1

An example of the regenerative ability of the salamander. The animal on the left had the right forelimb amputated one week prior to the photograph. The other specimen had the left forelimb removed at the same level fifteen weeks before. The regenerate is somewhat smaller than the normal limb, but the completeness of restoration is evident. These forelimbs are homologous to the human upper extremity, containing a humerus, radius, ulna, and carpal bones, in addition to the homologous soft structures. The regenerate restores almost completely the anatomical complexity and is a functional organ. It will be noted that the long bones, and, particularly, the spine of the specimen with the week-old amputation, are less distinct and appear somewhat less dense than the same structures of the other animal. This observation has been made repeatedly and is not due to any difference in technique. Its significance is unknown at present.

*field* or the *direct-current field*, must be carefully distinguished from the more familiar action-potential activity of the nerves and muscles. The bioelectric field is a steady-state potential, measured as direct-current potentials, on the surface of living organisms. The action potentials, on the other hand, consist of the transmission of pulses of membrane depolarization along the nerve or muscle fiber. Although the pulses are detected by electrical means, they do not involve the actual flow of an electrical current longitudinally in the fiber<sup>4</sup>. Since the techniques of measurement of action potentials are simpler, and since the action potentials apparently are a prime means of data transmission, this type of activity has been preferentially studied by most investigators.

Much of the work on the direct-current field potentials was done ten to twenty years ago. The concept arrived at then was that the bioelectric field consisted of a simple electrical dipole with no more structural complexity than a positive polarity of the cephalad portion, compared with the caudad portion of the animal. The source of the potential was assumed to be the total electrical activity of all of the cells of the organism. The usual measurements done at that time were between the nose and the tail (cephalad-caudad) or between two other arbitrary points only. Despite this amorphous concept, many observations were made, indicating some relationship between these potentials and various biological activities such as: fluid transport in plants<sup>9,10</sup>, anesthesia in animals<sup>5</sup>, and ovulation<sup>19</sup>, hypnosis<sup>20</sup>, and schizophrenia<sup>21</sup> in man. In particular, there appeared to be correlations with growth processes<sup>6,15,16</sup>. In recent years the relationship between direct currents and growth were investigated by artificially applying the currents to the organism. Acceleration of the rate of regenerative growth in tomato plants<sup>22</sup> and, conversely, complete regression of malignant tumors in mice<sup>12</sup> were reported to follow application of currents of appropriate polarity.

It therefore appeared worthwhile to re-evaluate the entire problem of the bioelectric field and its relationship to growth processes. The salamander was chosen as the experimental animal in view of its regenerative ability and its position in the vertebrate phylum. Furthermore, the moist skin of these animals permitted direct-current measurements to be done with less technical difficulty. Utilizing the techniques of equipotential line plotting (determining the imaginary lines on the surface of the animal which connect all points of equal potential), a complete field plot of the bioelectric field can be made. (For further concepts of the electrical field, the interested reader is referred to references 14 and 18.). If the bioelectric field is a controlling factor in growth processes, such control should be indicated by some change from the normal in the field plot. Therefore, the initial observations were concentrated on a determination of the field configuration in the intact animal. Rather than finding the expected simple dipole, a complex field was found with multiple positive areas, or electron sinks, and multiple negative areas, or electron sources (Fig. 2). This pattern was found uniformly in over fifty individuals of several different species. It became apparent that the spatial configuration of the bioelectric field coincided with the gross anatomical arrangement of the central nervous system. The positive areas (electron sinks) coincided with the three major cellular accumulations of the neuraxis (cranial, brachial, and lumbar enlargements). The negative areas (electron sources) correlated with the terminations of the major peripheral nerve outflows from these same areas. It was therefore postulated that the direct-current potentials were generated within the cellular aggregates and distributed by the peripheral nerves, with a completion of the circuit made through the soft tissues (or in the aquatic forms, through the environment). This hypothesis was tested in a number of ways. It was noted that complete section (adjacent to the cord) of the nerves to one ex-

tremity produced a prompt drop of the potential along the extremity to nearly zero. By applying the Hall effect (a physical measurement involving the interaction between a steady magnetic field and a moving direct current) it was shown that there was actual flow of electrical charges longitudinally in an extremity and that this flow ceased after nerve section. Finally, an analogue device, or model, of the central nervous system was constructed and was found to produce the same field plot and to show the same behavior as the living prototype. It must be emphasized, however, that although these experiments show a distinct relationship between the central nervous system and the bioelectric field, the specific tissue in the central nervous system responsible for the generation and transmission of the currents has not been identified. At this time we cannot say whether the

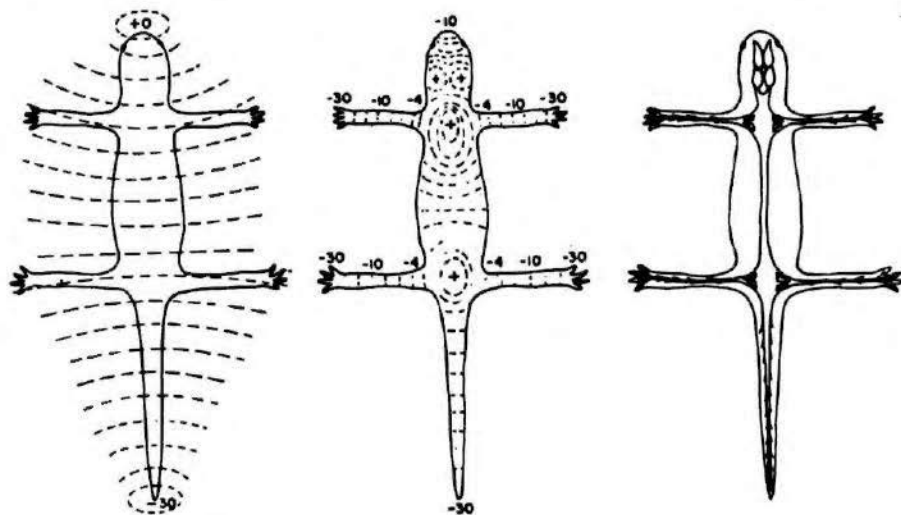


FIG. 2

The bioelectric field, previous dipole concept compared with organized field concept as related to the central nervous system.

The outline drawing on the left indicates the total body potential as measured by previous investigators (tip of nose reference electrode, tip of tail recording electrode, animal immersed in fluid medium). The equipotential lines as drawn represent the theoretical dipole field that was postulated. Such field determinations were never reported in the literature. This is the type of field plot I expected to find when the project was first begun.

The middle outline drawing indicates the field plot that was actually found (using the two electrode equipotential line-plotting technique on an animal kept moist, but not immersed, in solution). The numerical values inserted along the extremities are typical ones found on an animal about to recover from anesthesia when the head sink areas are used as the reference electrode site and the recording electrode is located at the other appropriate sites.

The outline drawing at the right represents the gross arrangements of the central nervous system in the salamander. It will be noted that the neuraxis contains three major cellular accumulations, with major peripheral nerve trunks extending distally from each. The correspondence between the cellular accumulations and the positive sink areas, and between the nerve-trunk terminations and the negative sources, is apparent.

neurons themselves, the neuropil, or the supporting elements—the glia and Schwann cells—are the important structures.

These observations on the bioelectric field indicate that it is a highly organized activity, generated within and transmitted by elements of the central nervous system. It appeared that, as such, it could function as a very primitive data transmission and control system. This concept permits a restudy of many life processes, including growth, which are obviously under precise control but for which control systems have not yet been identified. In this paper I shall be concerned with limb regeneration, perhaps the highest expression of organized growth in the adult

animal. The demonstrated relationship between the central nervous system and the bioelectric field would appear to furnish a logical basis for Singer's observation relating any type of nerve tissue to regeneration. It appeared quite possible that the basic phenomenon that Singer observed was nerve-related direct-current electricity rather than specific neural function. A preliminary study of this relationship between the bioelectric field and growth-control processes has been completed and some encouraging results obtained.

#### METHODS

Several species of salamanders with regenerative ability (*T. viridescens*, *A. tigrinum*, *A. opacuum*, and *N. maculosus*) and a non-regenerating, but closely related amphibian, the grass frog (*R. pipiens*), were used as the experimental animals. The techniques of measurement used were the same as previously described<sup>2</sup> and will not be discussed here. The only change in this technique has been a refinement in the recording apparatus, permitting the simultaneous monitoring of the direct-current voltages from as many as eight different areas of the same animal. In the experiments in which direct current was administered to the animals, a precision direct-current voltage divider type of supply was used along with solid, silver-silver chloride electrodes. One electrode was large and encased in a pad soaked in saline solution to serve as the reference electrode. The other was small and glass mounted and was applied directly to the amputation stump. Animals were anesthetized with tricaine for most of the experiments, although the effects of other anesthetic agents on the field potentials were also studied. Deep anesthesia, regardless of the inducing agent, is accompanied by a complete field reversal; that is, the extremities become positive and the cellular areas become negative. Despite this polarity reversal, the equipotential lines remain the same and the field plot remains constant. Interestingly, the degree of reversal of field polarity correlates well with the depth of anesthesia, being fully reversed with the deepest anesthesia and slowly returning to normal as anesthesia diminishes. During the period (five to ten minutes) immediately preceding full recovery from anesthesia, the animals demonstrated a field plot of normal polarity and potential. These factors were taken into consideration in making the measurements. Determinations on wound and regenerate potentials were done just as the animals were fully recovered from anesthesia. In some instances, when restraint was used on non-anesthetized animals, similar results were obtained.

#### RESULTS

The presentation of experimental results will be divided into four sections: the electrical changes immediately associated with amputation; the subsequent changes during the healing or regenerative phase; the electrical changes during fracture healing; and the results of artificially augmenting the electrical changes in the course of regeneration.

##### *Section One*

Limb amputation in both regenerating and non-regenerating forms was followed immediately by a complete reversal of the electrical polarity at the amputation stump (Fig. 3). This change was not associated with any major change in the direct-current levels at other sites measured, except that the head voltages subsequently showed continuous fluctuation (wave forms lasting ten to fifteen seconds) during the period of recovery from anesthesia. These wave forms were found to be associated with any major damaging stimuli and, in this case, they probably represented the continuous stimulus from the amputation site. The

rapid reversal of electrical polarity at the site of amputation (with a shift from negative to positive voltage) may represent the cessation of direct-current electron flow in the associated nerve fibers. This cessation is a neural response to the trauma and does not necessitate sectioning of the nerve fibers themselves. It was noted that any stimulus producing cellular trauma—heat coagulation, radiation, freezing, fracture of the long bone of an extremity, or crush injury—produced a similar polarity shift, obviously without nerve section. The duration of the direct-

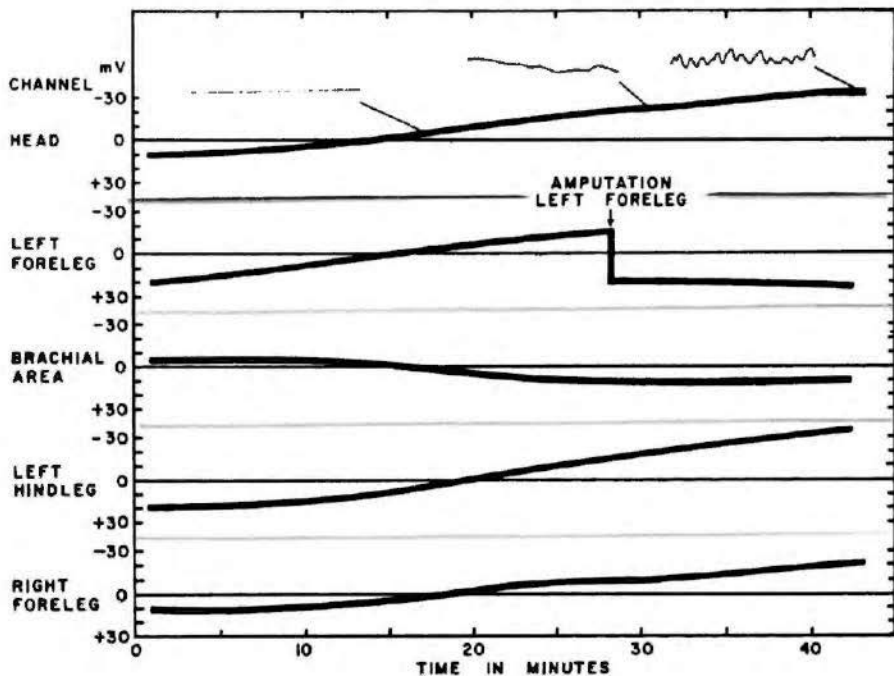


FIG. 3

Direct-current voltages monitored at five sites before and immediately after amputation of the left foreleg. During deep anesthesia (at left, on the time scale) a reversal of normal field pattern is present, with the extremities positive and the central areas negative to some degree. As anesthesia decreases, the voltages swing back through zero to normal polarities and potentials. Amputation of the left foreleg in this stage produces an immediate, lasting reversal of polarity back to the same levels present during deep anesthesia. This positive voltage not only persists to the point of full awakening, but actually increases slightly in magnitude. This positive voltage in the left forelimb is accompanied by a tendency to reversal of polarity, represented by a slower rate of change, in the contralateral right foreleg. The left hind limb, however, shows a steady return to normal value.

The three lines immediately above the heavy line representing the head-channel voltage (top) refer to the wave-form patterns seen in this channel with a faster speed of the recording paper (one millimeter per second) and a higher amplification than was used in the scale represented here. In deep anesthesia there is little or no wave-form pattern at this amplification; after amputation, however, continuous wave forms appear and increase in amplitude with time. These are the wave forms typical of the response to trauma; they actually represent variations in direct current since they have frequency periods ranging from five to twenty seconds. In this case, they apparently represent the response to the continuous trauma arising from the amputation.

current response appeared to be dependent upon the degree of trauma. At present, we can only speculate on the mechanism relating the cellular or tissue trauma to the changes in the bioelectric field itself. It is apparent that the trauma must be sensed in some way by the system to bring about a change in the direct-current activity of the appropriate cellular aggregate (brachial or lumbar). It has been known for some time that trauma involving cellular disruption causes a local "current of injury"<sup>8</sup>. Possibly, this is the local mechanism that influences the direct-current activity of the central nervous system. That there is some rela-

tionship between the peripheral nerve and the injury potential is evidenced by the decreased current of injury elicited from chronically denervated structures<sup>22</sup>.

### Section Two

The sequence of changes in the bioelectric field during the healing processes in regenerating forms was very different from the field-change sequence in non-regenerating animals. In the former group, a complex series of potential changes occurred that correlated well with the various stages of the regenerative process (Fig. 4). The initial positive polarity fell rapidly in value during the first two to three days, rose again during the fourth to sixth days to a value of about 50 per

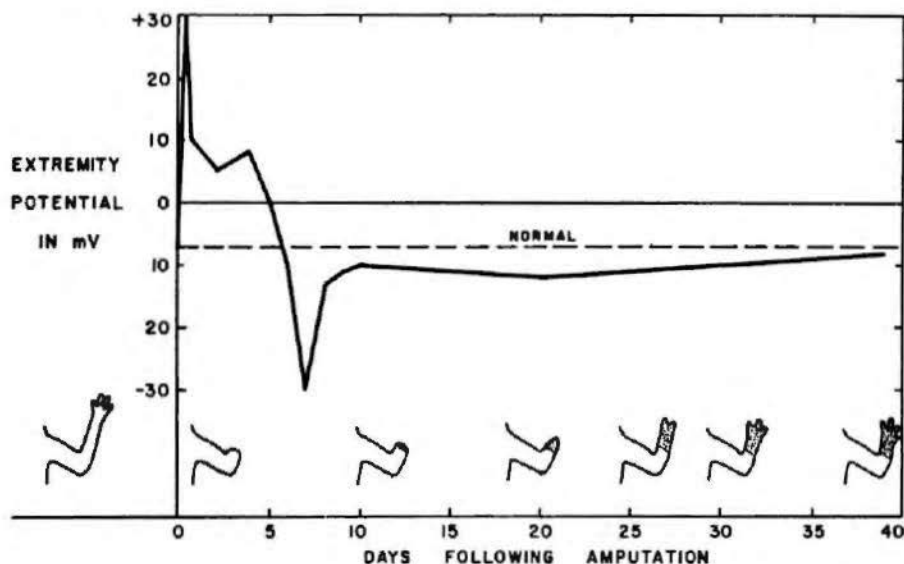


FIG. 4

The voltage changes in an extremity of a salamander during regeneration after amputation. The zero point on the horizontal scale immediately precedes amputation, which is concurrent with the very high positive peak in the voltage curve. The secondary positive peak in this case is on the fifth day and the negative maximum is on the eighth day. This negative bias of the regenerating limb is maintained until regeneration is complete. The normal limb voltage is about ten millivolts negative (interrupted line). The lower figures show schematically the stages of regeneration, which in this case was practically completed by the thirty-eighth to fortieth day.

cent of the initial, and then fell, crossing the zero line sometime between the fifth and tenth day. This was followed by a prompt negative shift of high amplitude, with maximum negativity occurring somewhere between the eighth and the fifteenth day. This negative polarization was very high, exceeding in millivoltage both the normal limb negativity and the post-traumatic positivity. This negative polarity remained elevated during the phase of axial growth and differentiation, and as late as the fortieth day after amputation the now almost completely regenerated extremity was still more negative than all the others in more than 50 per cent of the animals. This experiment involved a group of fourteen animals and, except for minor differences in the amplitude of the voltages, they all followed the same sequence of changes.

The anatomical and histological changes during the regenerative process are divided arbitrarily into two phases. Initially, there is the accumulation, at the site of amputation, of a mass of undifferentiated cells. The origin of these cells seems to be somewhat in doubt, although certain quantitative measurements have

recently indicated that they are derived from remaining tissue of the limb and that there is a movement of cells from the proximal tissues into the stump<sup>7</sup>. This undifferentiated cell mass is known as the blastema. It increases in size without evidence of differentiation until sometime about twenty to twenty-five days after amputation (in the adult), when it begins to show axial growth and differentiation and enters the second phase. The observed time course of the voltage-change sequence suggests that the initial positive polarity may be associated with relatively non-specific healing processes or with the blastema formation, whereas the negative polarity appears to be definitely associated with the differentiation growth phase. It was noted that those species and individuals which naturally have a slower rate of regeneration showed a correspondingly slower rate of electrical potential change; that is, the two processes were time related.

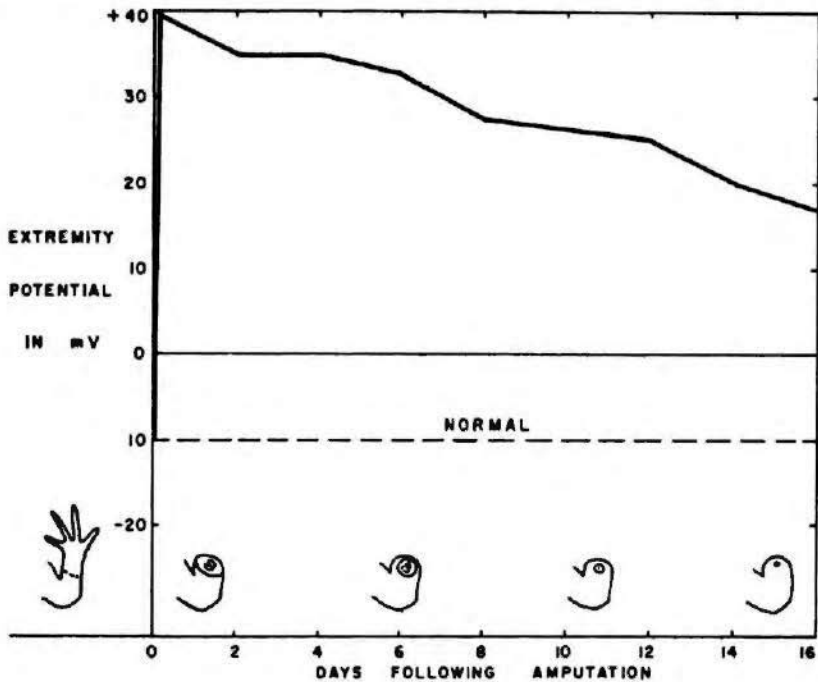


FIG. 5

Voltage changes during the healing process following amputation of the forelimb of a frog (a non-regenerating form). The zero point on the horizontal scale immediately precedes amputation, which is again concurrent with the very high positive polarity. Unlike the salamander (Fig. 4) this high positive polarity is maintained for a long period, dropping slowly to nearly normal levels as healing progresses almost to completion. The normal field pattern in this animal is similar to that of the salamander, and the extremities are invariably negative during the last stages of recovery from anesthesia (interrupted line).

Although the non-regenerating forms demonstrated the same immediate electrical-polarity reversal following amputation, they did not show the same subsequent sequence of electrical-potential changes as the regenerating forms (Fig. 5). The highly positive potentials remained elevated and fell only slowly toward zero potential as healing occurred. Only when healing was complete did the extremity begin to show negative potential and then it was generally lower than normal in value for some days. It must be emphasized that not one of twenty-four specimens so examined showed even a transient shift to negative voltages until healing was completed.



The healing process in these frogs consisted in granulation tissue and scar formation with gradual epithelialization of the raw surface from the skin edges (essentially the same healing process as in man). These animals are related to the salamanders, and in the larval stages are themselves capable of complete limb regeneration. They lose this ability in the adult form. However, the adult frog can be made to regenerate a nearly complete limb by grafting extra, functioning neural tissue into the amputation stump<sup>25</sup>. This is interpreted in the light of Singer's other findings as indicating that the normal density of nerve tissue in the adult frog limb is below the level necessary to sustain regeneration. Electrical

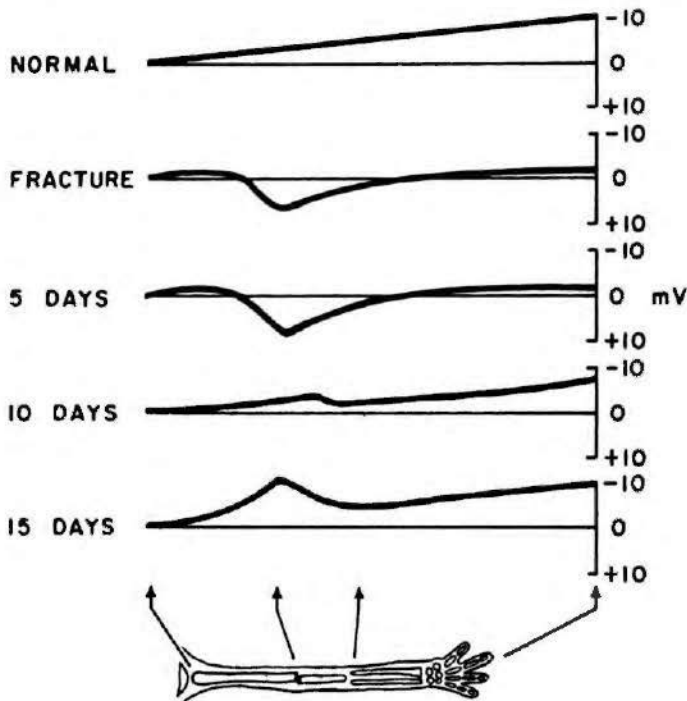


FIG. 6

Voltage changes observed during fracture healing. The simple fracture was produced at the junction of the lower and middle thirds of the humerus. The normal voltage gradient progressing peripherally along the limb is shown by the upper-line graph. This gradient is measured by placing the reference electrode over the brachial area and sweeping the recording electrode distally. Normally, there is a smooth curve of increasing negativity obtained by this method.

The lower four graphs show the disturbances in the limb-voltage gradient induced by the fracture at the times indicated. The sequence of local voltage changes is similar to that shown by the total extremity during regeneration (Fig. 4).

field measurements on intact frogs demonstrated a direct-current field pattern similar to that of the salamander, having the same spatial relationship to the anatomical arrangement of the central nervous system. Similarly, trauma caused the same polarity reversal in the potentials. However, the highly specific sequence of potential changes accompanying regeneration in the salamander did not occur in the non-regenerating frog. I have not had the opportunity to measure the potentials in a frog that was regenerating a limb secondary to nerve-grafting procedures, and attempts to drive the electrical potentials of the stump artificially in a regenerating sequence failed because of technical problems related to repeated anesthesia.

## Section Three

After fracture of a long bone in the salamander, the same polarity reversal immediately appeared with a skin zone of positive potential around the area of the break. The subsequent voltage-change sequence during the healing of the fracture was similar to that appearing during limb regeneration, although somewhat lower in magnitude (Fig. 6). The difference in magnitude of the potential would appear to be related to the fact that the injured part is within the soft tissues and that it is situated along the normal limb voltage gradient. Thus far, no correlations have been made between various stages of the fracture-healing process and the voltage-change sequence, primarily because of the difficulty in obtaining good roentgenograms of these small extremities.

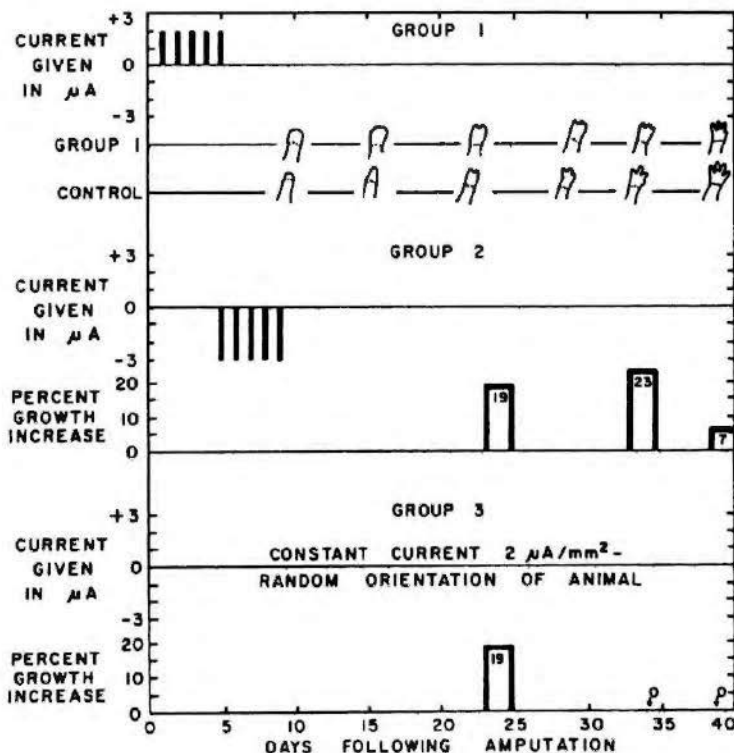


FIG. 7

The results of administration of current of various polarities to the regenerating forelimb of the salamander. The upper three lines (Group 1) refer to the series of animals given positive current during the first five days after amputation. The increase in blastema formation and the slower rate of re-differentiation in the experimental animals are schematically shown. The center two lines (Group 2) refer to the animals given negative current from the fifth to the ninth day inclusive (upper line). The percentage increase in axial growth of the experimental limbs over controls is shown on the lower line. A period of growth acceleration appeared to take place from the twentieth to the fortieth day, with a maximum acceleration about the thirty-fifth day (23 per cent). The lower portion (Group 3) refers to the animals exposed to constant current, with random orientation of the animal permitted. The solitary spurt in axial growth on the twenty-fourth day is thought to have been the result of changes in ionic strength in the tank. In Groups 2 and 3, all measurements were made with an ocular micrometer at five to ten-day intervals.

It is an apparently well accepted fact that fracture healing is a regenerative process<sup>17</sup>. This being the case, the observed regenerative type of voltage-change sequence is entirely appropriate. The relationship between the density of the peripheral nerves and the regenerative process was determined only in the case

of limb regeneration. What the relationship is in the case of fracture healing is a matter of speculation. As previously stated, neither injury to the nerve itself nor proliferation of the nerve fibers need be considered as prerequisites for the sequence of electrical-potential changes. Certainly, the fracture is productive of sufficient local currents of injury to inform the bioelectric field that such an event has transpired. The electrical-potential changes can be produced functionally by the appropriate cellular aggregate (brachial or lumbar) without such anatomical alterations.

#### *Section Four*

Three separate experiments were performed in which external current was administered to regenerating forms after amputation. In the first group, six larval salamanders were given two microamperes of current directly to the amputation stump for five to ten minutes per day for the first five postoperative days. This current was oriented so as to enhance the normally occurring positive polarity at that time. On the tenth to twelfth day, all six of these animals demonstrated a blastema almost twice as large as that noted in a simultaneous control group. It was difficult to quantitate these differences since the blastema is three-dimensional and so small that it does not lend itself to precise measurement. An observation of equal importance to the increased size of the blastema was the subsequent retardation of regeneration in the phase of axial growth and differentiation. In all of the control group there developed initial digitation of the regenerate on the sixteenth day, whereas the experimental group did not reach this stage until four to six days later. These results are summarized in Figure 7, upper section.

In the second experiment, six adult animals were given three microamperes of current directly to the amputation stump for five to ten minutes per day from the fifth to the tenth day. This current was oriented to enhance the normally occurring negative polarization at that time. There was no discernible difference in blastema formation compared with a simultaneous control group; however, subsequent axial growth, as measured by ocular micrometer at intervals of five to six days, exceeded that of the control group by significant amounts (Fig. 7, center section). The comparisons of growth rate were made by measuring by ocular micrometer the length of the regenerated limb in the six experimental and six control animals. The average results in each group were determined, and the average percentage increase in axial growth of the experimental animals over the controls was calculated at different time intervals. It was noted that all of the experimental animals showed a greater length of regenerate compared with the most rapidly growing control animal. The total size of the regenerates, however, was small; a 25 per cent difference refers to less than one millimeter in actual length.

Digitation, as an index of differentiation, also appeared to be accelerated in the experimental group, but this observation was difficult to quantitate. The technical difficulties encountered in this experiment necessitated that small numbers of animals be utilized. However, despite the lack of a statistically significant sample, the consistency of the results observed suggest that some growth-accelerating effect was obtained. In the animals receiving positive polarity current from the first to the fifth day after amputation, an increase in tissue resistance was noted on the third day, necessitating twice the previous voltage to deliver the same amount of current. The same phenomenon was noted on the eighth day in the series receiving negative polarity current from the fifth to the tenth day. In each case, current of the opposite polarity was easily passed, which would appear to indicate that there was some rectifier type of mechanism in the tissues. The significance of these observations is not known.

A third group of six salamanders were taken on the first day after amputation and placed in a standard aquarium which had a steady direct electrical current flowing through the water in one direction. The current density was regulated to produce two microamperes per square millimeter, and the animals were permitted to assume random orientation in respect to the direction of current flow. There were no changes in the rate of regeneration in this group except for a solitary, but striking, increase of about 19 per cent on the twenty-fourth to twenty-fifth day of measurement (Fig. 7, lower section). Subsequent measurements showed no significant changes in rate, and the group completed regeneration at about the same time as the control group. In reviewing the experimental records, it was noted that two days prior to the twenty-fourth day the water in the tank was supplemented. Since Kappers observed some time ago that the trophic responses of aquatic organisms to direct-current fields depended upon ionic concentration, similar factors are presumed to have been in action here. To evaluate properly this random-orientation-steady-current study, strict control over ionic parameters would be necessary.

Since completing these experiments, it has come to my attention that an experiment, similar to the last one described, was done with large numbers of animals by Frazee in 1909. He reported a statistically significant increase in the rate of regeneration when direct current was passed through tanks housing larval salamanders with amputated limbs. The techniques available at that time were insufficient to indicate the current density, and knowledge of the bioelectric field was fragmentary. These results therefore could not then be interpreted in any satisfactory frame of reference. It is of interest to note that Frazee had traced similar experimental attempts to influence the rate of regeneration by electrical means as far back as 1840<sup>22</sup>.

#### DISCUSSION

Growth processes are not only complex in nature but cover a continuous spectrum of abilities and potentials. Whether one is considering the embryonic growth of an entire organism from a single fertilized ovum or the simple closure of a skin defect by epithelial proliferation, the process is quite evidently under precise control. Tumor and keloid formation are usually cited as evidence of loss or disturbance of the control mechanisms. Whether or not the same mechanisms are operative in all of these conditions can only be speculated upon at this time in view of the extreme paucity of information.

Philosophically, the phenomenon of growth is such a basic process that one would expect that exceedingly primitive control mechanisms are operative and that all of these processes bear some interrelationship. The aforementioned work of Singer, establishing the relationship between the peripheral nerves and limb regeneration, is an important beginning. However, this neural effect should not be interpreted as involving any of the known functions of the nervous system. Since any type of nerve tissue is equally potent, such known parameters as the action potential cannot be involved. It appears more likely that some very basic property of the nervous system, or its associated tissues, is the controlling mechanism. From the evidence presented in this paper, the thesis is proposed that the direct-current activity of some portion of the central nervous system constitutes a true data transmission and control system and that one of the output parameters of the system is the control of the growth processes involved in regeneration and repair. Since the electrical-field activity is not related to the system that produced action potentials and may even be a function of such tissues as the Schwann-cell syncytium, the observation that any type of nerve tissue will bring

about regeneration becomes more logical. There must exist certain linkage mechanisms between the cells of the organism and the bioelectric field itself, both in an input or sensory fashion and as an output or control mechanism. Considering system inputs, the relationships demonstrated between trauma and the changes in the field potentials appear to be mediated by the current of injury generated at the cellular level. Regarding outputs of the system, the influence of a steady-state direct-current potential on cells has been previously demonstrated in the central nervous system<sup>24</sup>. In this case the rhythmic activity (as action potentials) of cortical neurons can be modulated by changing the direct-current level existing in the environment of the neurons. More recently, it was demonstrated that cells of a regenerating tissue have electrical charges that are different from cells of the same tissue in its normal state<sup>3</sup>. An environmental parameter for all cells of the organism is the direct-current or bioelectric field potential generated in their area. This field could influence the site of an injury so as to cause accumulation of cell types bearing specific electrical charges at points having specific electrical potentials. In this fashion, the output portion of the direct-current field system can be linked to cells, and a true closed loop, data transmission and control system, is established for the growth process.

The preliminary nature of these experiments must be stressed. In all probability, the electrical field changes accompanying growth and healing processes are much more complicated (both temporally and spatially) than are reported here. Nevertheless, it would appear that certain specific alterations in the bioelectric field accompany the process of regeneration and that these alterations do not appear in the case of less adequate healing processes. It is surprising that the application of intermittent electrical currents produced an acceleration in the regenerative process. If the field potentials are the control mechanism, they must be considered to be operative in a continuous fashion. In these experiments, it must be assumed that the observed alterations were produced in an animal which normally has the capacity to regenerate an extremity, and that the added potentials in this case reinforced those which were naturally occurring. Although it would appear that continuous currents would have to be applied to reverse natural potentials, the successful attempt to inhibit malignant-tumor growth by intermittent field reversals must be noted<sup>12</sup>.

The application of similar techniques to the study and control of growth processes in man requires much more investigation both in animals and in human beings. Only recently has a technique been evolved permitting the precise measurement of the direct-current field in the human being<sup>1</sup>. Initial measurements indicated the presence of a human field system similar to that of the salamander, bearing the same spatial relationships to the central nervous system. Anesthesia was found to produce a similar tendency toward reversal of the field potentials. However, the cranial portions of the bioelectric field in man show a much greater complexity than in animals, apparently being influenced by the emotions and sensory inputs. Despite this, the general relationship between the human and the amphibian bioelectric field are remarkably similar.

In summary, bioelectric direct-current potentials have been shown to constitute an organized data transmission and control system. Relationships indicating some control function have been established between the direct-current field and the process of limb regeneration.

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