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REVIEW ARTICLE

ELECTRICAL CONTROL SYSTEMS AND REGENERATIVE GROWTH

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INTRODUCTION

One of the most pressing problems in biology today is the identification of the communication and control systems acting on living cells. More than a billion cells of hundreds of different types are exquisitly organized together into that unique entity, the human being. This incredibly complex unit begins with a single cell, the fertilized egg, which contains all of the genetic information for that specific individual. This code contains sub codes (genomes) coded for each specific cell type. Yet this identical code is contained in all of the different cells of the final organism, except

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for the germ cells, the eggs and sperm, which contain only half the code in preparation for the generation of a new individual. In embryonic growth, the total genetic code produces specific cell types by the expression of a single genome and the repression of all others. Muscle cells, for example, have only the "muscle" genome active. In the course of development, cells in areas where muscle is to appear must receive instructions from their environment causing them to repress all but the desired genome. Furthermore, muscle cells occur as organized complex entities in anatomically specific locations, attached to the proper bony structures and supplied with appropriate nerves and blood vessels. The factors that "instruct" certain cells to express the "muscle" genome and to organize in such specific fashion must be extracellular and must contain several levels of organization including a final one representing the total organism.

Viewed in this perspective, the DNA-RNA apparatus is not the "secret of life" but simply a tool that life uses to express an organized pattern in terms of living cells. The nature of this organized pattern has been largely a subject for philosophical discussion only. The original concept of the "morphogenetic field" developed by Weiss in 1939 (1) remains today the primary basis for discussion. If such an amorphous "field" does exist then it's action must be expressed by factors that communicate with cells in such a way as to alter their active genetic complement. Such factors however, must be capable of containing within themselves the capacity for "structure". Extensive study of embryonic development has led to

the identification of certain chemical inducers, that is,substances produced by one embryonic structure that induce neighboring cells to differentiate in a certain fashion. While such substances can work at the local level, they are themselves capable of only the simple structure of a diffusion gradient, and lack the organizational complexity to be the physical entities representing the morphogenetic field. In the 1800's there was considerable discussion concerning the role of electricity in this process. These concepts enjoyed considerable prominence as part of the general concept of "electrobiology" with broad applications in many areas of biology. In the early decades of the present century a sequence of events produced the total discreditation of the entire concept (2). However, within the past few decades this idea has again attained prominence, and today a respectable body of evidence exists indicating that electrical forces do indeed play a role in morphogenesis via their ability to communicate with living cells in such a fashion as to alter their genetic expression. The possibility that electrical forces are, at least in part, an expression of the morphogenetic field no longer seems so remote. This change has occured not as the result of the study of embryonic development but rather is the result of new investigations of the phenomenon of regenerative growth. Regeneration, the regrowth of portions of the adult body missing through trauma, represents a return to embryonal control systems and cellular activities within a localized area of the total organism. It therefore is a far simpler, more accesible expression of morphogenesis, much more amenable to scientific study.

REGENERATION

The study of regeneration began, of course, with the original scientific description by Spallanzani in 1768 (3). While the broad outlines of the process were sketched in early, new advances in technique and analysis are still providing new data and altering original concepts even at the descriptive level alone. As a general rule regeneration expresses its highest competence in the simple metazoans such as the flatworms and there is a gradual decline in this abiity as one ascends the phylogenetic scale. Combining organizational complexity and regenerative ability, the process seems to reach its highest competence in the tailed amphibians, the salamanders. These animals are capable of regenerating major portions of all their organized anatomical structures including, heart, brain, gut, eye, spinal cord and limbs. This latter has become the most studied system, not only because of the ease of experimentation it offered, but because of its competancy. It results in a multi tissue structure not only appropriately organized itself but also appropriately related to the remainder of the organism as a functional part of the total entity. Despite their small size and superficially primitive appearance, the salamanders are equally as complex as man. Regeneration of their forelimb involves the re-formation of a structure composed of bones, muscles, nerves and blood vessels quite analogous in organizaional complexity to the human arm.

Over the past few decades many of the observational details of the process have been extended and revised and we are now in a

position to describe it in full. Following traumatic amputation of a limb two events occur almost immediately. There is a proliferative response of the epidermal cells in the skin surrounding the wound and these begin to migrate over the wound surface. At the same time there is death and resorption of the terminal ends of the muscles, bone and nerves exposed in the stump. Within a few days the epithelial covering of the stump is complete and regrowth of the terminal nerve fibers occurs through all residual tissues until they penetrate the epithelial covering. The epithelium then becomes thickened, forming the apical cap, and the nerve fibers form a specific and unique structural association with the epithelial cells called the neuro epidermal junction (NEJ) (4). This structure is apparently essential for the remainder of the regenerative process; if the nerves are prevented from contacting the epithelial cells by any of a variety of techniques, all growth ceases and all residual tissues resorb back to the body wall and regeneration does not occur (5). In the 1950's Singer reported a number of observations indicating the dependence of regeneration upon a minimal amount of nerve tissue in the amputation stump (6). Apparently the requirement ultimately depends not on the nerve tissue alone but on the formation of the NEJ. If this event occurs, there is the rapid appearance of a mass of primitive appearing cells immediately beneath the apical cap. This structure is called the blastema and it has now been firmly established that it is formed by the de-differentiation of residual adult-type cells in the stump. These cells undergo a de-repression of their genetic apparatus, becoming primitive in

appearance and capable of mitotic activity as toti-potent cells capable of subsequently re-differentiating into other necessary adult cell types.* Through this mitotic activity the mass of the blastema is increased and it grows in an axial direction, similar to the limb bud in embryonic development. As this elongation occurs the more proximal cells of the blastema re-differentiate into the necessary mature cell types required to re-form the missing limb, apparently by the process of re-repressing the unwanted *geneomes*. As the distal portions of the blastema continue to undergo mitosis and increase the overall length of the regenerate, the organized structure continue to appear in the proximal portions with the development of organized muscles with appropriate nerve and blood supply, connected to appropriate bony structures with appropriate joints. This process is completed in a matter of weeks with the formation of the terminal digits and a total re-formation of the missing limb.

CONTROL SYSTEM FOR REGENERATIVE GROWTH

The majority of workers in this field view regeneration as a linear, continuous process with the same set of unknown factors controlling it throughout its course. This leads to the insurmountable obstacle of defining the factors containing the instructions for the total integrated extremity in order to understand any portion of the process. If one considers the "bits"

*The origin of the blastema was hotly contested for many years because of a predominant dogma that held that de-differentiation was impossible. In this view the process of differentiation was linear and non reversble. It has not been until the present decade that the weight of evidence has finally overthrown this dogma.

of information required to designate all of the details of the finished extremity, the impossibility of transmitting this type of information by any known mechanism becomes obvious. I would like to propose therefore that regeneration is a biphasic proess with each phase under different controls. In this view, the initial phase, that of the formation of the blastema, requires only a simple stimulus or signal capable of producing mesenchymal cell de-differentiation. The second phase, that of re-differentiating the primitive cells of the blastema into the organized tissues of the regenerate, contains the requirement for the transmission of the exceedingly complex instructions. Not only does this view offer an initial simplification of the problem but it also contains some clues as to the site from whence the instructions for each phase are issued. The blastema appears beneath the apical cap and its subsequent mitotic activity producing the longitudinal growth is limited to the same area. The stimulus for mesenchymal cell de-differentiation and the subsequent mitotic activity of the primitive cells thus formed would seem to come from the apical cap, specifically from the NEJ. As elongation of the blastema occurs re-differentiation instructions are given to the proximal cells not only to form an appropriate structure but to do so in the context of that structure being an appropriate part of the total organism. Therefore, whatever the nature of the instructions, they must be associated with the organism as a whole and must be coming from the remainder of the organism. This concept lends itself to additional analysis. For example, the growth of the blastema must be limited

both spatially and temporally. The regrowth of an extremity requires so much tissue and no more. Therefore, the control signals entering through the basal end of the regenerate producing the re-differentiation must contain a feedback function that gradually reduces the activity of the NEJ as completion of the structural re-formation nears. It would certainly appear reasonable to assign the overall control of the regenerative process to some function of the central nervous system.

This analysis of the regenerative process as a biphasic control system also contains the possibility of some practical application, even if all of the details involved are not known. For example, the paucity of the regenerative process in the human has been assigned to a variety of causes from, "the human is just too complex an organism", a view that does not stand close scrutiny, to, "mammalian cells have lost the ability to form a blastema", not supported by the occurance of fracture healing and the occurance of finger tip regeneration in humans below the age of 10 years (7). There is, however, a basic difference between the human and the salamander that may have a bearing on this problem. In both forms, the total percentile of nerve tissue compared to total body mass is thought to be about the same, but in man the majority of nerve tissue is concentrated in the brain, while in the salamander it is dispersed throughout the body with major portions in the extremities. If the formation of the NEJ is the one most essential element required for the formation of a blastema, it can be postulated that man's inability to form this structure because of prior committment to

development of cerebral structures is the cause of the scarcity of regenerative growth in the human. If the nature of the relatively simple signal produced by the NEJ can be determined, its replication in the human might lead to the development of a blastema. In that case it is possible that sufficient competency is retained by the mammallian nerve tissues to produce some measure of appropriate re-differentiation.

EXPERIMENTAL FRAMEWORK

The foregoing background and theoretical analysis provides a framework for reviewing the more recent studies of regeneration. In a paper of this type it is not possible to cover all aspects of the subject, and the interested reader is referred to more complete recent reviews (2,8) for more information.

Modern research on regeneration began with Rose's observation of the restoration of partial regeneration of adult frog forelimbs by the repeated application of hypertonic saline (9). This was the first instance of the restoration of even a small measure of regenerative growth to a species normally lacking this capacity. Rose attributed it to the saline preventing the epithelial overgrowth, however, the following year the observations of Polezhaev (10) provided an alternate explanation. He reported the same degree of regeneration in the same animal by simply repeated pricking of the amputation stump with a needle. Both observations could be interpreted as the result of increasing the extent of the injury beyond that of the original amputation. A decade later, Singer,

following up on his observations of the importance of nerve suply for regeneration, produced the same extent of limb regeneration in the same animal by the transplantation of additional functioning nerve into the amputation stump (11). It would appear that there is a common factor of some sort between increased trauma and increased innervation. Shortly after Singer's report Simukhin observed the acceleration of regenerative growth in tomato plants when the current of injury was artificially increased as well as the converse effect when it was similarly reduced (12). The original observation of the current of injury was made by Galvani who mistakenly attributed it to "animal electricity" a rather amorphous, vitalistic concept (13). The true nature of the phenomenon was determined by Matteucci in 1847. He described it as a steady direct current measurable between a site of injury and uninjured portions of the same animal (14). In a long series of experiments he defined many parameters including a direct relationship between the magnitude of the current and the extent of the injury. Following the demise of electrogiology in the early 1900's the current of injury was postulated to be a second order phenomenon produced by the leakage of the transmembrane potential from injured cells, and without physiological significance. However, in 1958, Zhurmunskii reported that the current of injury was also directly related to the extent of innervation (15). Thus, it appeared to me at the time that the current of injury might be the factor common to both increased trauma and increased innervation in stimulating regeneration. It seemed to me also that the best experiment would be to simply compare the

currents of injury following amputation in animals capable of regeneration with that measured in animals lacking that ability. I chose the foreleg amputation in the frog to compare with the same injury in the salamander, expecting that the magnitude and duration of the current in the salamander would be significantly greater than in the frog. My initial results were quite disappointing, both animals demonstrated an immediate positive potential of the same magnitude and that in the salamander fell back to the base line in about 3 days while the frog's current of injury was maintained at the highly positive level. The experiment was almost terminated at that point, however, continued measurements disclosed the startling fact that the polarity of the salamander's current completely reversed, continuing across the base line to become highly negative. During this rise in negativity the blastema appeared and throughout the remainder of the regenerative process the current in the salamander remained negative while that in the frog remained positive during scarification and epithelialization. While direct current measurements are notoriously difficult and fraught with artifact, the polarity reversal of such magnitude mesured with identical techniques would seem to be a genuine observation (16). At the time I formed the concept that the time sequence of changes in the current demonstrated by the salamander could be considered a "regenerative type current of injury" while that demonstrated by the frog was of the "non-regenerating" type. This concept appears to have stood the test of time fairly well. It is to be regretted that even today the bias among some workers against electrical factors is so great that in otherwise

excellent reviews (17) this initial work is only partially reported and this key observation omitted. Such a significant electrical phenomena appearing at the site of injury seemed very unlikely to arise de novo unrelated to any other similar factor. In a long series of experiments I was able to define, with some precision, a direct-current generating and transmitting function associated with the central nervous system (18,19). Finally, I proposed that this direct current was organized as a data transmission and control system of a primitive type with one of its control functions being the regulation of growth and healing (20). Lending some support to this concept was Bodemer's observation, reported in 1964, of partial restoration of limb regeneration in the adult frog by electrical stimulation of the nerve supply to the amputation site (21). Shortly thereafter, Smith reported the same result with the implantation of bimetallic, electrogenic devices directly into the amputation site (22). He observed regenerative responses with both anode-distal and cathode-distal orientations, although the latter was more successful in this regard. Later, using a simple refinement in his bimetallic devices that permitted control over the levels of current and voltage produced, we were able to stimulate partial forelimb regeneration in the laboratory rat (23). This was the first successful restoration of such ability in the mammal by any technique. Our results were much more skewed in the direction of the cathode distal orientation, a fact attributable to the more appropriate current levels from our devices. Similar restoration of limb regeneration in the same animal has recently been reported by

others (24,25). We had been able to predict the most appropriate level of current density to stimulate rat limb regeneration as a result of our observations on the electrical system regulating fracture healing in the frog (26). We observed that the electrical changes following fracture were quite different from those following limb amputation in this animal. In keeping with the concept that fracture healing is a regenerative process, they paralled those of limb amputation in the salamander. More significantly, we observed that the fracture blastema was produced by the de-differentiation of the nucleated red blood cells in the initial fracture hematoma. In the frog, as in all vertebrates except the mammal, the normal circulating red cells retain their nucleus. This observation enabled us to expose normal red cells in vitro to various levels of electrical current and voltage in an attempt to produce the same de-differentiation. We found a rather narrow range of electrical parameters that produced the morphological changes of de-differentiation. This range approximated that measured in the hematoma and was considerably lower than tht produced by Smith's bimetallic devices. In our mammalian limb regeneration experiments we interposed various values of resistors between the two wires to arrive at the effective level of current. Most importantly, Harrington established that the electrically produced changes in the frog red blood cell were in fact de-differentiation by measuring the changes in DNA, RNA and protein composition (27). From this work we have inferred that the functional role of the specific "regeneration type" electrical phenomena at the site of limb amputation and

fracture healing is to produce de-differentiation of susceptible cell populations to produce the initial blastema.

ORIGIN OF THE REGENERATION SIGNAL

The concept of a neural origin for the electrical phenomena associated with regeneration has not been universally accepted. Jaffe and his co-workers have preferred to attribute their origin to the epidermal cells (28,29). Nevertheless, they have similarly stimulated frog forelimb regeneration with implanted direct current devices and have emphasized the necessity for a distal negative orientation (30), and have proposed similar general growth controlling functions for the electrical phenomena (31). In their view, wounding the skin simply permits a flow of direct current setting up a circuit between the site of injury and the uninjured tissues. While there is no doubt that the skin is an electrically active tissue, attributing the major share of the stimulation and control of regeneration to it seems to be an over simplification. Far too much data exists attesting to the importance of the nerves in this process. Obviously, the electrical currents, whatever their source, are major factors in the regenerative process, but they must be working in conjunction with some other system reflecting the total organization of the individual, and the skin does not appear to be an adequate candidate for this function. That the situation is far from being fully understood is best indicated by the recent report of Rose (32) in which he obtained normal regeneration in denervated limbs by simulating the "regenerative type" current. Amputation of a

denervated limb usually leads to regression of the residual tissues back to the body wall, and this observation would seem to firmly establish a neural origin for the currents as well as demonstrate the fact that they are the single most important factor in the regenerative process. At the same time, however, it is difficult to understand how the regenerate could be appropriate in type and orientation when it was essentially disconnected from the rest of the organism. It is not clear from the data whether nerve fibers entered the regenerating tissues at their proximal end.

While many workers have emphasized the importance of the epidermal cap, they have tended to view it as a collection of epidermal cells alone. In 1960, Hay first described the complex structure formed by the association of the epidermal cells and the penetrating nerve fibers (33). This structure, already referred to as the neuro-epidermal junction (NEJ), is not only a highly developed entity formed by an exceedingly close association between these two cell types, but it is also unique, found only in the epidermal cap of regenerating extremities. Despite its obvious importance, this structure is not even referred to in a recent general review of the regenerative process (17). Regeneration cannot occur in the absence of either the nerves or the apical cap or even if the conjunction between the two is prevented. Obviously, the structure formed by the association of these two tissues is a key element in the regeneraive process. We believe that we have recently identified the NEJ as the structure responsible for producing the specific "regenerative-type" electrical activity. In a series of experiments designed to

determine the effect of transplanting functional nerve tissue into the mammalian bone marrow cavity we encountered difficulties with keeping the nerve in position. A surgical procedure was finally worked out in which the sciatic nerve was introduced into the marrow cavity of the rat femur at the junction of its upper and mid thirds. In order to maintain it in position, the hind limb was amputated through the femur at the junction of the mid and lower thirds. The sciatic nerve, which exited the marrow cavity of the femur at that site, was then introduced through a hole in the skin flap covering the amputation site so that approximately 1 cm was left on the outside. This portion of the nerve quickly dried and became firmly attached to the skin retaining the remainder within the marrow cavity even though the animals quickly became active and used the amputation stump. To our surprise, a few weeks after beginning the experiment a number of the animals displayed a considerable measure of regeneration distal to the original amputation line. In these animals tissue examination with standard techniques suggested the presence of nerve fibers in the epidermis. This was later confirmed using special stains for nerve tissue. Very clearly there was lateral growth of fibers into the epidermis with the formation of neuro-epidermal junctions identical to those described by Hay (33). In a follow up series of experiments several additional observtions were made (34). Control animals subjected to the same procedure except the nerve was not introduced through the skin failed to demonstrate either NEJ formation of any measure of regeneration. In other control animals the same procedure was used except in their

case the nerve was not passed through the bone marrow but was deviated through the soft tissues to a hole in the skinflap on the lateral aspect of the amputation stump, passed through the hole and exited on the exterior. While typical NEJ's were formed at this site, these animals demonstrated the same lack of regenerative growth as the other controls. We concluded that while the NEJ was necessary for regenerative growth it must have a source of competant cells to act on, in this case the bone marrow population. The most significant observations resulted from the measurement of the electrical potentials. In those rats in which a neuro-epidermal junction was formed, regardless of position, the time sequence of voltage changes was identical to the "regenerative type" demonstrated by the salamander. Where no NEJ was formed, the voltages followed the "non-regenerating type" sequence demonstrated by the frog. Clearly, the electrical factors associated with regenerative growth are produced by the NEJ. Similarly it would seem that they produce their effect by causing the de-differentiation of residual mesenchymal cells sensitive to this voltage sequence. Interestingly, Chang and Snellen have reported the same "regeneration type" electrical changes accompanying the normal, spontaneous regeneration of punched out lesions in rabbit ears (35). They made no observations of nerve tissue contribution although according to Grimes and Goss (36) nerve is not essential to this activity.

Whatever the total controls over the regenerative process are, it seems apparent that the concept proposed earlier of a two-phase system has considerable support. The simple direct current signal

generated by the NEJ produces de-differentiation of populations of susceptible cells and leads to the initial development of the blastema. This signal is, of course, quite inadequate of itself to control the total amount of blastemal growth or to provide the instructions necessary for appropriate re-differentiation. However, the involvement of the nervous system as an essential part of the structure producing the initial signal would seem to support the contention that this same system is involved in the feedback mechanism that limits the total growth, and quite possibly in the mechanism producing the re-differentiation as well. If this concept is to have any credance whatever, it must be extended to include at least some suggestive evidence on the elements of the central nervous system that may be involved and the mechanism or mechanisms employed in this information transfer. Surprisingly, there is evidence existing in both of these areas.

ROLE OF THE CENTRAL NERVOUS SYSTEM

There has been a persistent idea that the morphogenetic field is somehow connected with or expressed by electrical factors, even though the originator of the field concept, Weiss, has strenuously denied any role for electrical factors in any life process. The usual mechanism by which the nervous system operates, the action potential, is a prime example of a biological digital signal, and the entire action potential system is, except for the neuro-hormones, a good example of a digital data handling system. To express a morphogenetic field in such a systems' terms, while not impossible,

would be cumbersome indeed. A much better system would be one communicating information in an analog fashion; the morphogenetic field, after all, is nothing more than an analog expression of anatomical information. Furthermore, the morphogenetic field, if it does exist, must be a primitive function, having appeared early in biogenesis. The evidence for the central nervous system having such a system, expressed as direct current activity with potentital gradients etc. is actually considerable. Reference has already been made to some of my own investigations of this activity (18,19) but this must be considered in terms of a much larger bulk of data previously reported by other workers (37,38). At a time when it was certainly not fashionable in science to work on such maters, these scientists reported evidences for actual current flow in neural tissues that was extra-neuronal, outside of the neurones themselves. While we generally conceive of the central nervous system as being composed mainly of neurones, the fact is that the vast majority of this tissue is made up of various types of peri-neural cells -- glia and ependymal cells in the brain and spinal cord and Schwann cells accompanying all of the peripheral nerves. These cells are in contact with each other, forming a contiguous system which so pervades the central nervous system that no nerve exists without a complete envelopment by one type or another of these cells. With the neurones being essentially imbedded in a continuum of perineural cells and with the direct currents in the system being extra neural, the postulate that the peri-neural cells are the source of the electrical phenomena seems inescapable. There is evidence to support

this view. Kuffler and Potter (39,40) have demonstrated direct current potentials in the glial cells of the leech with current transmission between individual glial cells for considerable distances. Electrical coupling between glial cells themselves and between glial cells and neurones in the mammal has been reported by Walker and Hild (41). In addition, evidence has been accumulating that the role of the nervous system in healing and growth processes is actually the work of the peri-neural cells and not the neurones themselves. In patients with diabetic neuropathy, disturbances in growth and healing are commonly obsered. Fractures in these patients not only do not heal but frequently go on to resorption of the involved bones, reminiscent of the resorption of the denervated salamander extremity following amputation. Greenbaum, et al (42) and Thomas and Lascelles (43) have reported that the lesions of the peripheral nerves in such cases are in the Schwann cells rather than in the axons themselves. Finally, Maden, in a beautiful series of experiments (44) has shown that the single, vital element necessary for paradoxical regeneration in the axalotl is the Schwann cell. Such regeneration is paradoxical only in the sense that cellular elements producing the regrowth come from distal tissues. While his experiment cannot be reviewed in full detail in this paper, he combined selective radiation and amputation in such a manner that he was able to produce limb stumps with nerves completely devoid of Schwann cells or stumps otherwise similarly treated with nerves having a full complement of Schwann cells accompanying them. In both instances the necessary requirements for regeneration were present-

nerve-fiber regrowth and epithelial cell regrowth-yet absolutely no regeneration occured in those animals with nerve fibers unaccompanied by Schwann cells. One is forced to conclude that the nerve alone is not the responsible agent, nor is the nerve in combination with the epithelial cap, but that the Schwann cells must also be present. As with most good experiments, Maden's work has led to further questions, for example, "could regeneration be sustained by the Schwann cells alone?".Maden's technique does not permit this experiment and to the best of my knowledge the literature contains no reports dealing with this. However, in an unpublished study of fracture healing in denervated limbs of the rat done in my laboratory we observed that fracture healing was delayed until restoration of the Schwann cell sheaths had occured, after which it followed a normal course as to duration and morphology. During this period, the nerve fibers themselves had just begun to regrow and would not cross the gap for a month or more. Therefore in this instance, the growth process seemed to be dependent upon the Schwann sheath rather than the neurones. Taken all together, the foregoing data seem to indicate that the peri-neural cell system might be the generating and transmitting mechanism for the direct current potentials and currents that play a role in the growth process. Obviously, this concept is not conclusive at this time and some discrepancies and uncertainties exist. For example, Hay's data on the neuro-epidermal junction (33) shows quite clearly tht while the regrown nerve fibers are accompanied by a normal Schwann sheath, the Schwann cells stop at the level of the basement membrane of the epidermal cap and only naked

nerve fibers enter the epithelial cell mass and are involved in the NEJ proper. Maden's data, on the other hand clearly demonstrate tht the Schwann sheath is the vital element in the regenerative process. He postulated that they were the source of the blastema, however, Hay's paper shows readily identifiable Schwann cells at the level of the basement membrane with underlying blastemal cells. Unfortunately at this time we do not know whether normal NEJ's were formed in Maden's animals or if the Schwann cells in Hay's experiments revealed any specific organization at the point where they stopped accompanying the nerve fibers.

Despite these uncertainties, which are ammenable to experimentation, I believe it is possible to sketch in, in some detail, the sequence of events and the mechanisms that seem to be involved in the process of limb regeneration (Figure 1). There would appear to be several advantages offered by this analysis. First, it simplifies the problem of control factors by dividing the process into two phases, one requiring controls of far less sophistication than the other. Secondly, it provides a theoretical framework, based on present information, for further experimentation in an organized fashion. For example, the role played by the neuro-epidermal junction is crucial for Phase I events with its influence being expressed through the generation of a specific type of direct electrical current. Evaluation of the mechanism of production of the current by electrochemical or electrical techniques might prove to be both interesting and productive of insignts into the entire process of regeneration. Thirdly, it provides a rationale for the

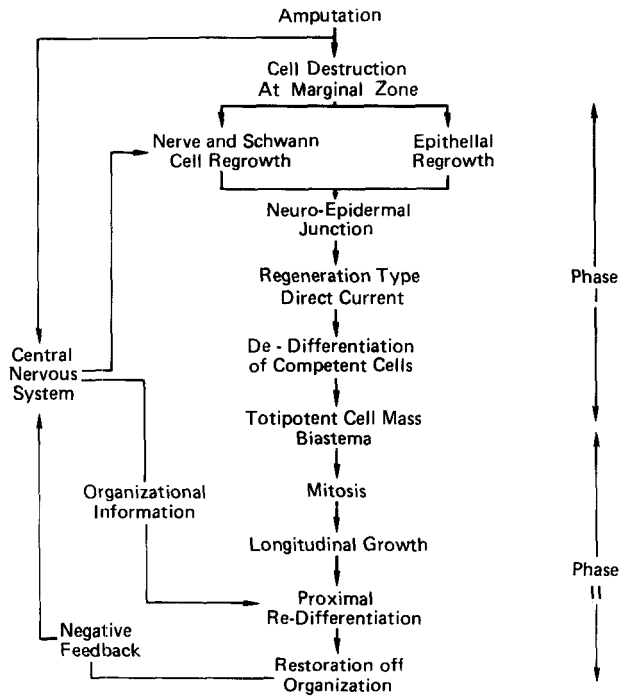

Figure 1

Sequence of Events in the Process of Limb Regeneration

involvement of the central nervous system in a regulatory role over a morphogenetic process, a situation preferable to both the rather vitalistic concept of morphogenetic fields and the ultra reductionist concept of genetic predeterminism.

Despite the uncertainties remaining in our understanding of the mechanisms controlling limb regeneration, the studies of the past few decades have clearly shown that naturally occuring electrical factors regulate genetic expression at the cellular level. This is a major

advance in our understanding of one of the basic problems in biology and could well led to insights into other life processes.

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