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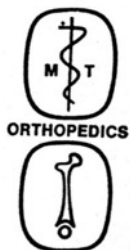
The Electrical Control of Growth Processes

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One of the salient characteristics of living organisms is their ability to heal themselves following physical injury. To the physician this is an absolutely essential part of the recovery from all pathological conditions characterized by tissue destruction. The primary facet of this important process is organized and directed growth, whether it be the simple regrowth of an epithelial surface in the human or the regeneration of an entire extremity in an amphibian. Such growth is directly proportional to the trauma and productive of a new structure that is harmonious with the total organism. If one applies the concepts of control system theory to this property of living organisms, the organism itself may be viewed as an automatic self-organizing system differing from the familiar automata of today only in complexity and the modality controlled. For example,

the modern home heating system functions as a simple, closed loop control system.

The controlled modality in this case is the air temperature, and a decrease in its value activates the input transducer, the thermostat. This produces an electrical signal which causes the heater to be turned on. The heater functions as the output transducer, changing the electrical signal into warm air. The output is constantly being sampled by the input transducer and when the appropriate temperature is reached, the transducer stops the control signal. This sampling of the output of the system by the input transducer is called the feedback. In certain systems the input transducer actually generates an error signal, a variable dependent upon the difference between the actual and the desired circumstance.

While this may appear to be an unnecessarily complicated way to view a commonplace device, the use of carefully defined terms and rigorous analysis enables us to predict exactly how such a system will function under a variety of cir-

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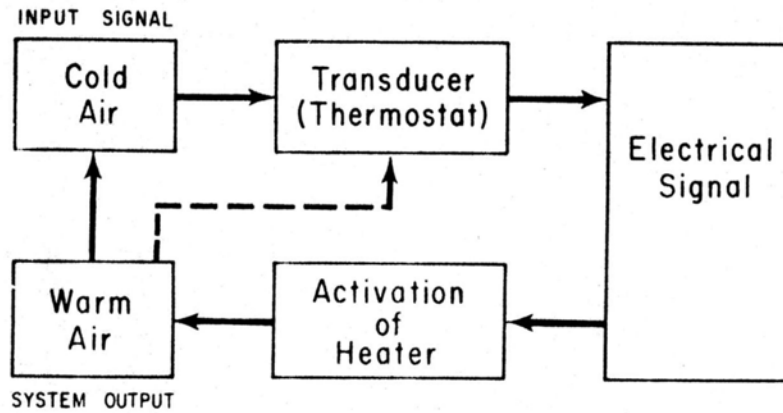


FIGURE 1 Simplified control schematic for home heating systems.

cumstances and using different devices. This technique has proven to be a very powerful analytical tool and when we apply it to the phenomenon of healing in living organisms, we derive a system surprisingly similar to our simple example.

In this case the controlled modality is the physical and functional integrity of the organism. Trauma, acting through some transducer mechanism, produces a signal proportional to

the tissue loss. This signal acting through a second transducer mechanism produces new growth, which eventually negates the original tissue loss.

While in this case, this may seem like an oversimplified and mechanistic approach to the problem of organismal growth, it offers the advantage of furnishing a basis for both theoretical and experimental analysis in a logical fashion. I believe that such an analysis may

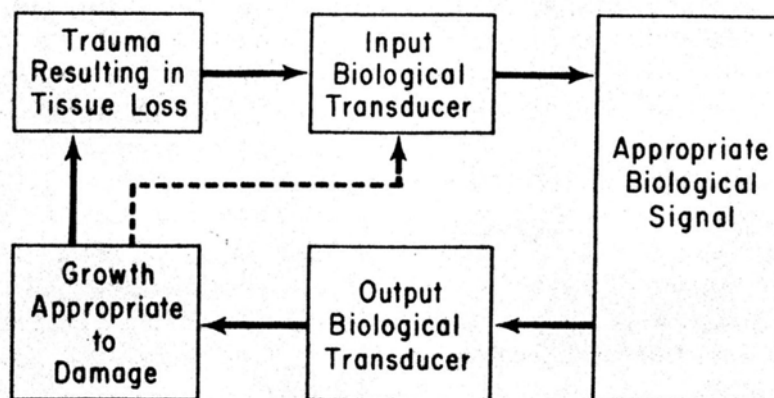


FIGURE 2 Theoretical control schematic for repair of tissue damage by growth in living organisms. Function would be quite similar to Figure 1 and again the feedback loop would gradually reduce the value of the input signal from the input transducer.

well be productive of clinically important knowledge, particularly in two aspects. First, the healing process is present in its most spectacular form in the lower animals who have the capacity to regenerate major body parts. It is frequently stated that we have lost this ability as the price of complexity; this, however, is simply not true. The salamander, in regenerating a forelimb, produces a new structure fully as complex as the human arm in osseous, neuromuscular and vascular details. Since the human retains vestiges of single tissue regenerative ability in the healing of skin, bone and visceral organ defects, we may postulate that the basic control system is retained but inadequate in some detail to produce multitissue or organ regeneration. Might it not be too fanciful to postulate that knowledge of the workings of this system in the salamander may enable us to restore a greater measure of this "useful disposition" to the human? In the second case, assuming that our theoretical control system is reasonably correct and basic to most growth processes, one may postulate that at least some abnormal growths may be due to defective functioning of the system. One may further predict that regardless of the system component at fault, the control signal calling for increased growth would be present, albeit in an abnormal fashion. Identification of the signal in the normal system may enable us to negate the erroneous signal of tumor growth by appropriate means, possibly providing us with a much more effective measure of tumor control than presently available.

We have been engaged in such a study of normal growth reparative systems for several years, using limb regeneration in amphibia and post-embryonic bone growth in mammals as test systems. In both cases we have been successful in determining the basic outlines of the control systems involved. In each case the

control signal appears to be electrical in nature and this has enabled us to experimentally control these growth processes, in both accelerating and retarding fashions. It might be appropriate to point out that heretofore there has been no known means of increasing the rate of a healing process over its normal, or of causing new growths of normal tissue to occur without antecedent trauma.

Our method of approach involved first a logical analysis of the known facts using the principles of control systems theory. In the case of limb regeneration several aspects became obvious. First, the regenerative growth is appropriate to the area, i.e. a forelimb is produced to take the place of a missing forelimb; secondly, the growth is limited to a restitution of the missing part and does not continue thereafter in an uncontrolled fashion. From a control system point of view there must be constant information transfer taking place between the growing tissue and the remainder of the organism. Basically the growing tissue is informed of where it is with respect to the total organism, i.e. it is to become a forelimb or hindlimb, and it is furnished with complex and precise instructions regarding organization from the gross to the submicroscopic level. The organism in turn is apprised of the amount of reparative growth that has taken place compared with the amount necessary to replace the missing part. This second point lends itself well to consideration under the "error signal" concept. Biologically, the well-known phenomenon of the "current of injury" seemed to us to possibly represent such an error signal. Basically, little is known of this process and the explanation offered by Ostwald in 1890¹ is still generally accepted. His concept that the current was basically nothing more than the summation of the membrane potentials of all the damaged cells is

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obviously untenable since such currents are measurable days after the injury, long after the damaged cells have died. The major difficulty, however, was not this, but rather how did the current of injury convey information and what was the nature of the control system that accepted this as a valid signal? A possible clue was furnished by the work of Professor Marcus Singer, demonstrating a precise mathematical relationship between the total amount of nerve tissue in the amputation stump and the ability to regenerate a new extremity.²

He found that all types of nerve tissue were equally competent as long as present in more than a certain minimal amount and that this was not a function of the action potential-acetyl choline system but was related to some, as yet unidentified, property of the CNS.³ In this light, the failure to regenerate in higher animals may be due merely to decreasing ratios of nerve tissue to total cross-sectional area of amputated stump and Singer theorized that surgically increasing the nerve supply to the amputation site would permit normal regeneration, provided sufficient functioning neural tissue could be transplanted. He has been able to restore regenerative ability to two non-regenerating species of animals by this technique.⁴ Unfortunately, in the human the ratio of peripheral nerve tissue to extremity tissue is so low as to render it virtually impossible to procure sufficient nerve tissue to permit regeneration of one extremity.

We began our studies with this background but investigated primarily the electrical events associated with regenerative and non-regenerative healing of limb amputations. We found that the current of injury persisted in detectable but slowly decreasing amounts until healing was complete in non-regenerating forms, but that the electrical signal was quite different at an amputation site that was regenerating.⁵

Following Singer's lead, we began investigating the peripheral nerves as the possible source of this electrical phenomena. It must be emphasized that the current of injury is a constant or slowly varying direct type current (DC) in contrast to the action potential which is, of course, propagated single pulses of electrical charge in the neural membrane. We readily detected DC potentials in the peripheral nerves and brain in non-traumatized animals, and discovered that they constituted an organized field pattern closely allied to the anatomical arrangements of the entire CNS.^{6,7,8} It became apparent that this DC field constituted a primitive data transmission and control system, apparently antedating the action potential system phylogenetically. This activity was found to be present in the CNS of all living organisms from flatworms to man and was, in each case, organized into a three dimensional field representative of the entire organism.^{9,10} Trauma produced measurable changes in all portions of the DC field in a manner suggesting a sensory function. Since the field was in effect a physical representation of the organism and electrical in nature, it could interact both with the current of injury and act as an information transfer mechanism indicating the relationship of the damaged part to the remainder of the organism.¹¹ The current of injury then is the input or error signal to the system and the difference between this electrical activity at the amputation site in regenerating forms may constitute the growth control signal derived from the neural DC system. Our methods of measurement indicated that the current of injury is normally positive in polarity and non-regenerating forms merely display a slow fall in its value back to the normal field potential at the amputation site during healing. Regenerating forms on the other hand displayed an initial positive polarity of similar magnitude

followed, however, by a rapid reversal to a high negative polarity, the peak negativity preceding the maximum cellular proliferation of the regeneration phase. In experiments on regenerating animals, we were able to accelerate the course of this process by artificially augmenting the negative polarity.⁵ It has been reported that tumors demonstrate high negative polarity¹² and Humphrey and Seal produced regression and disappearance of sarcoma 180 implanted in mice by administering very small direct currents orientated to reverse this negativity.¹³ While many of the details are lacking, we can identify some of the components of this growth control system at this time.

The current of injury produces a disturbance in the DC field pattern on both a purely electronic basis and also by producing an input signal directly to the DC organization of the brain. The neural DC field system integrates this information and generates an electrical output signal, via the nerve supply to the

extremity, which controls the regenerative process. It would appear that Singer's unknown neural factor is the neural DC system. While we have measured this output control signal, we do not know the nature of the output transducer, i.e. the mechanism involved in producing cellular proliferation in response to the output electrical signal. However, a direct effect of electrical current upon cellular activity has been reported in plants¹⁴ and we postulated a similar process in animals (experimental proof has been obtained in bone as will be detailed later in the present paper).

At this time, however, logical analysis indicated several other defects in our general theoretical construct. Primarily, the key component of the entire system is a new type of neural activity and while direct current potentials have been investigated to extense in the brain¹⁵ our proposal to extend this activity to the entire CNS and to invest it with control functions is hardly universally accepted. For

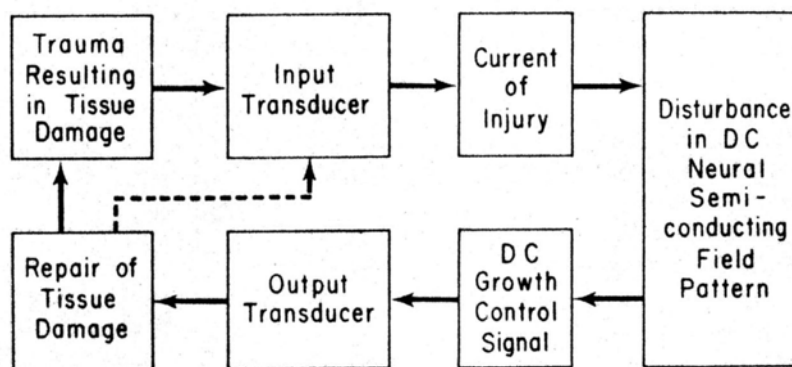


FIGURE 3 Present concept of the control system governing regenerative growth. Some increased complexity over Figure 2 is evident in that the input transducer produces an input signal (in this case some mechanism produces a current of injury proportional to trauma) which feeds into an active system (the neural DC potential system). This in turn produces an output or control signal which activates an output transducer (changing the output electrical signal into organized growth). Again a negative feedback loop is present between the final result and the system input which tends to reduce the input signal as the resultant growth approaches restoration of the normal state.

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certain technical reasons, this DC system must be associated with a constant or slowly varying electrical current longitudinally in the nerves. Membrane depolarization mechanisms are not at all suitable to accomplish this and we proposed that this current was produced by semi-conduction, or similar solid state mechanism, in the neurone itself. The nature of nerve tissue does not lend itself well to solid state techniques and while reasonably good indirect evidence for semiconduction in nerve tissue was obtained,¹⁶ convincing proof for such a marked departure from current thinking was lacking. A second deficiency in the system concept was also apparent; all vertebrates, whether capable of limb regeneration or not, have retained the capacity for regeneration of certain tissues including bone. In fact, fracture healing in the human demonstrates a high level of competence in tissue regeneration yet bone has no nerves.¹⁷

This logical analysis, appearing at first to negate our entire theoretical construct, set us on a course of investigation that has resulted in further substantiation to our original thesis. Initially, in keeping with the unity of nature,

we postulated that bone should have a similar electronic control system governing its growth but that the entire system should reside in the bone itself and be independent of the central nervous system. Rather than working with fractures and their subsequent regenerative healing, bone offered us a much simpler growth system more amenable to control system analysis. Wolff stated in 1892 that bone growth occurs in response to mechanical stress in such a fashion as to produce an anatomical relationship best able to resist the applied stress.¹⁸ This can easily be expressed as a function of a simple control system.

In our initial experiments, aimed at detecting the biological signal, we found that bone did produce detectable electrical currents when subjected to bending stresses. The amplitude of the current was proportional to the amount of stress and the polarity indicated the direction — i.e. areas of compressional stress were negative and areas of tensional stress were positive.¹⁹

This electrical response therefore was directly related to the important parameters of the applied stress and was considered likely to be the actual control signal. In view of the aneural

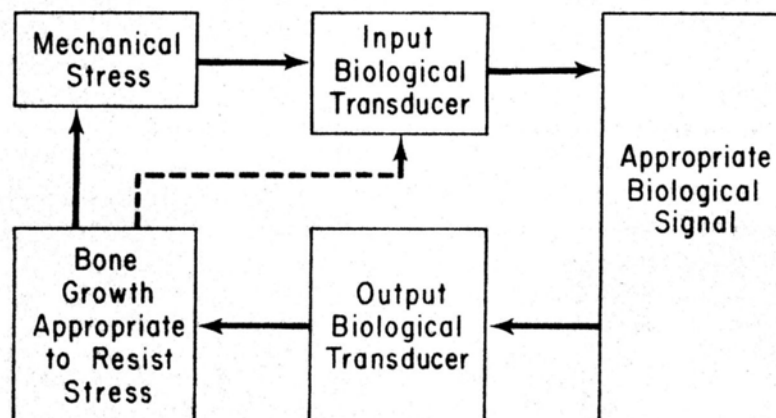


FIGURE 4 Theoretical control system regulating bone growth in response to mechanical stress.

nature of the bone, the signal must be the result of some specific property of the bone matrix itself, and again a semiconducting system was considered. It soon became apparent that bone offered several unique advantages in this regard over nerve tissue. The structure of bone from the gross to the molecular level has been determined with a high degree of precision as a result of the work of the electron microscopists over the past decade.

This has shown rather conclusively that an exceedingly precise organization exists between the crystals of the bone mineral, apatite, and the collagen fibers at the molecular level. Since these two structures are the major constituents of the bone matrix and are so uniquely related, any theory concerning the electrogenic properties of bone must take this fact into consideration.

It must be emphasized that neither the

collagen fiber nor the apatite crystals are cellular or living in nature. Collagen fibers are the product of cellular activity in the fibroblasts and are organized into the spatial relationships specific for bone by some growth control system. The apatite crystals are deposited on the collagen fibers apparently by simple physiochemical means. The water and cellular content of mature cortical bone are small and, therefore, spatial organization at the molecular level is dimensionally stable and the tissue can be subject to the manipulations necessary for analysis by solid state physical techniques (i.e. drying, cutting, shaping, depositing metallic electrodes, etc.).

Obviously neural tissue, being entirely cellular in nature, with a high water content is not suitable for such direct experimentation. Furthermore, the organization within a single neurone is much less well understood. It would

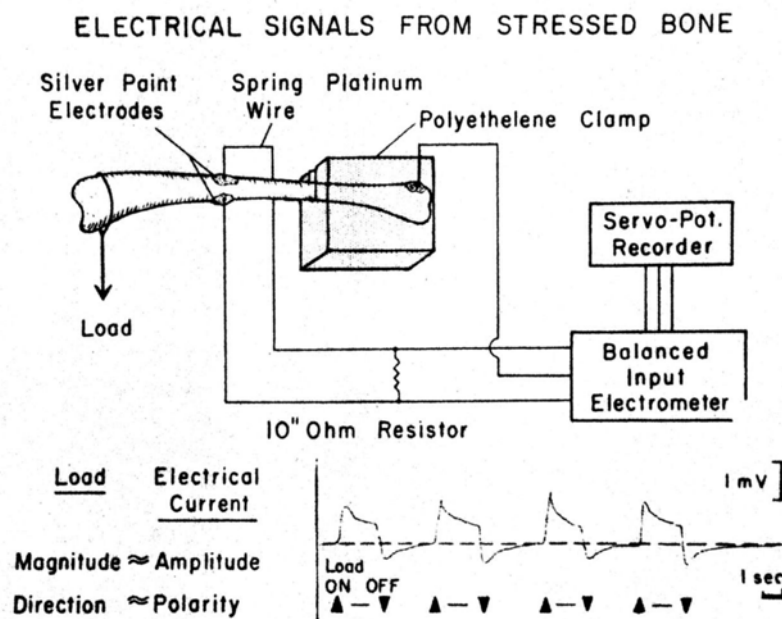


FIGURE 5 The nature of the electrical phenomena produced by long bones under bending stress.

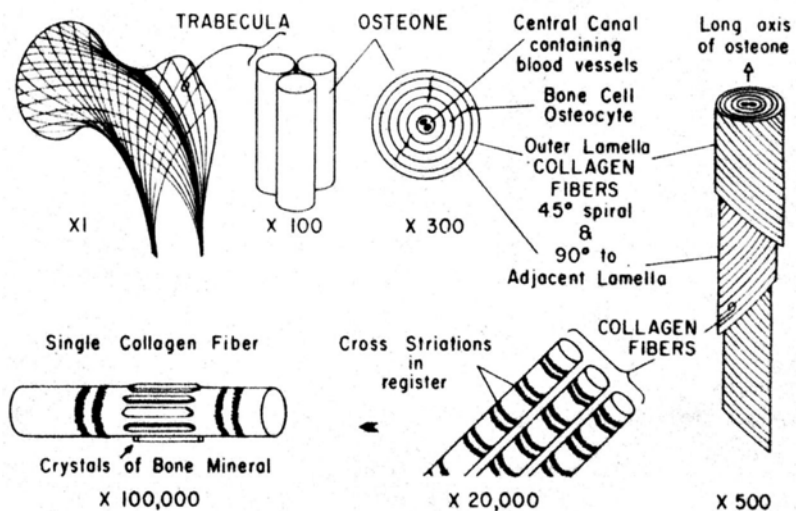
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be out of place in a paper such as this to discuss all of the experimental data resulting from our work on the solid state characteristics of bone; details have been published and the interested reader is referred to them.²⁰⁻²⁴ The data indicated that not only are collagen and apatite semiconductors separately, but that their association in bone produces a specific semiconducting device known as a PN junction diode.

Manufactured inorganic PN diodes are employed extensively in electronic equipment and their properties and characteristics have been extensively studied. They are well known

transducing devices, capable of transforming a variety of forces, including mechanical stress, into electrical currents. We have been able to demonstrate appropriate diode properties in bone such as rectification, photoconductive—photovoltaic effects and electronic action spectra. In addition the technique of electron paramagnetic resonance (EPR) has demonstrated free electron resonances in both collagen and apatite and indicated that the resonance spectrum of whole bone is something more than the simple additive spectra of the two components.^{23,24} It appears quite justifiable therefore to con-

FIGURE 6 The organization of bone. Except for cells which constitute about 5%, mature bone is non-living and composed primarily of collagen fibers with apatite crystals deposited upon them. At the gross levels this matrix is organized as trabeculae which are concentrated in areas of minimum stress and which follow the lines of the applied stress. At the microscopic level, the trabeculae are composed of osteones which lie longitudinally within the trabeculae. Each osteone is composed of "onion peel" lamellae of collagen fibers. Within each lamella the fibers are parallel to each other and spiral at 45° to the long axis of the osteone. The direction of the spiral is reversed in each adjacent lamella. At the electron microscopic level the collagen fibers are not only in parallel but their cross striations are in register. The apatite crystals are deposited upon the collagen fibers between the 640A cross striations and the long axis of the crystals are parallel to the long axis of the fiber. We stand and move about, therefore, on a non-living material that draws its strength from its organizational pattern at the molecular level.



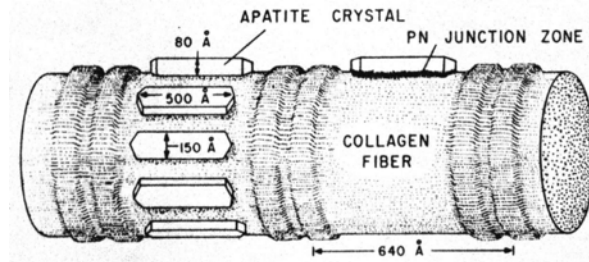


FIGURE 7 Schematic representation of the collagen fiber apatite crystal relationship and the PN junction zone formed.

clude that the generation of electrical currents by bone under stress is the result of the transducer action of the uncountable numbers of apatite-collagen junction diodes. It remained, therefore, to demonstrate the nature of the second transducer—the mechanism whereby the stress generated electrical signals is converted into directed new growth. Two mechanisms were considered; first, the electrical currents may determine the position of deposition of newly formed collagen fibers and secondly, cellular activity may vary in the electrical field. Both of these have been investigated and confirmatory evidence obtained for each.

The precursor of the collagen fiber is the tropocollagen molecule, a very long thin structure approximately 10\AA by 2400\AA . This material can be extracted from formed collagen and prepared as a solution. Typical collagen fibers can then be precipitated from this solutions by slight alteration in the pH; however, in this instance a randomized, felt-work of fibers is formed, not at all resembling the side by side linear arrays seen in bone. We found that the passage of a very weak direct electrical current through such a solution will produce a migration of the tropocollagen molecules towards the negative pole where parallel, linear

arrays will be formed. These parallelly arranged collagen fibers are at right angles to the current direction and near to, but not in contact with, the negative pole.

The significance of this finding lies in the fact that the position and alignment of newly formed bone in a long bone under bending stress is on the concave side with collagen fiber orientation parallel to the original shaft. In such a bone the concave side is negative, the convex side is positive and there is an effective current flow between the two. The migration of tropocollagen in such a field would be towards the concave side with alignment parallel to the original shaft, precisely the same position that the newly formed collagen fibers naturally assume. While this seems to support the theory in a most satisfactory fashion, other evidence indicates that migration of tropocollagen over long distances from the parent fibroblast is most unlikely. Therefore, we still require some direct cellular effect of the electrical field to produce an osteoblastic response locally in the vicinity of the negative pole. This cellular response would perforce be associated with an increase in tropocollagen production and the field effect would then be to produce orientation of the newly formed collagen fibers, with

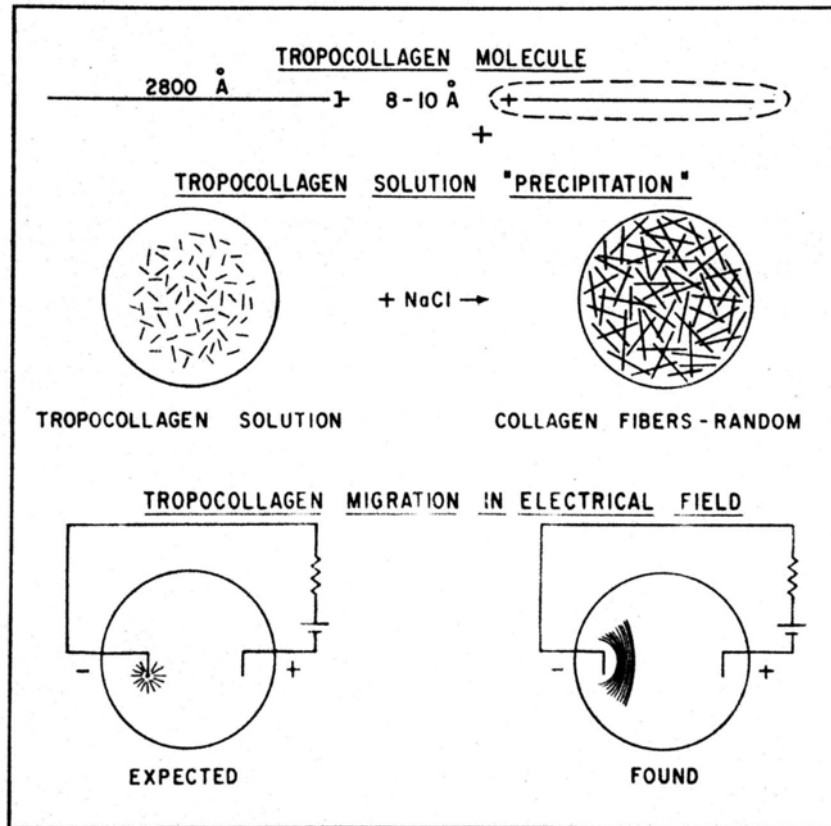


FIGURE 8 Schematic representation of tropocollagen behavior in solutions with and without applied electrical current.

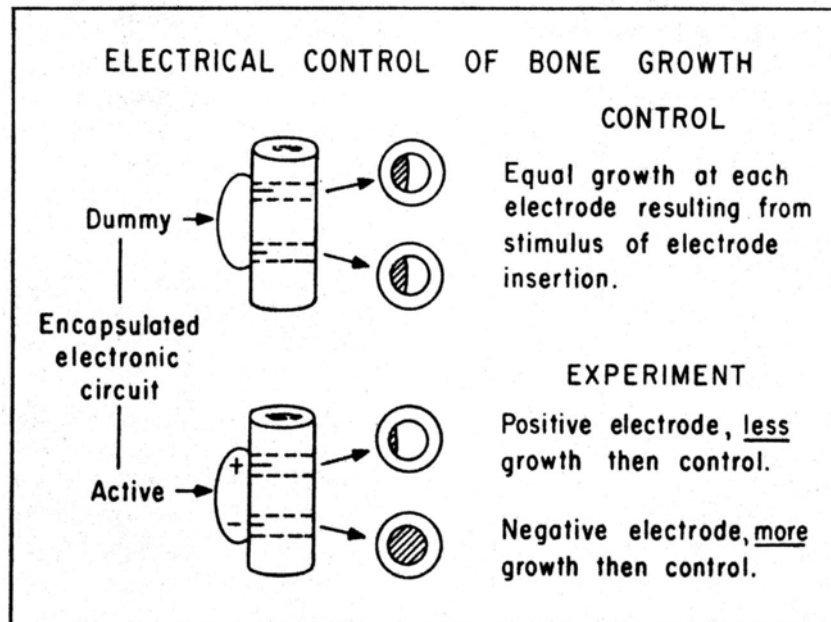


FIGURE 9 The growth response of bone in vivo to small direct electrical current. The growth resulting from the surgical stimulus in the controls is diagrammed at the top. The differential growth response (to 3μA) is diagrammed in the lower portion.

the required alignment, adjacent to the cells in the area of active osteogenesis.

To determine if such a local cellular response could be produced by small direct currents, *in vivo* experiments were done at the orthopedic research laboratories of Columbia University.²⁵ Small battery powered devices were constructed in such a fashion as to be implantable adjacent to the femoral shaft in the dog. Direct current was delivered to two electrodes spaced one cm. apart, projecting a few mm. into the medullary cavity. Inactive devices were implanted as controls into the opposite femur, where the stimulus of the surgery alone produced a small osteogenic response at both electrodes. In the active devices, with current levels between 2.3 and 3 μ A, massive osteogenesis was observed at 14 days in the vicinity of the negative electrode, becoming normally appearing mature bone at 21 days.

Consideration of all of the data indicated a

direct effect upon mitotic rate in the region of the negative electrode. Current levels considerably in excess of what we used have been tried by other experimentors with resultant bone destruction.²⁶ This is entirely in accord with the control system concept in that normal functioning would occur only within a certain physiological range of control signal values. The nature of the second transducer therefore appears to be a combination of two effects and we can now complete our hypothetical control system (Figures 10 and 11) with a fair degree of certainty.

The system applies only to bone growth in response to mechanical stress,²⁶ and does not pertain to the healing of fractures; however, our most recent experiments²⁷ have indicated that similar phenomena exist in the fracture healing sequence and that a not too dissimilar closed loop electronic control system is again the regulating factor.

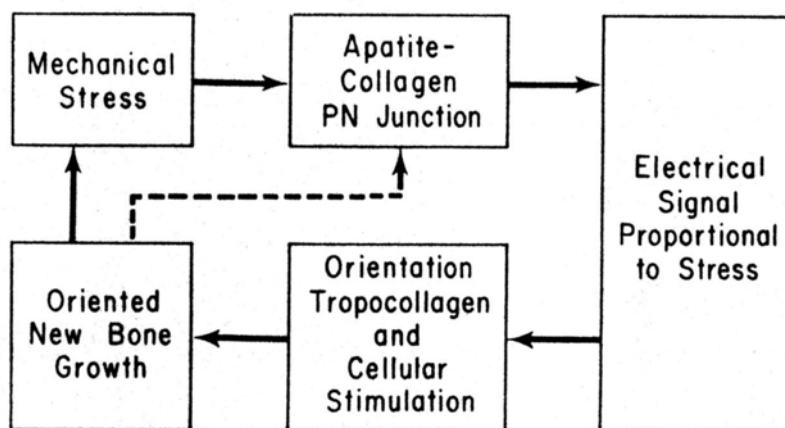


FIGURE 10 Final control system regulating bone growth in response to mechanical stress. This system is simpler than the neural regulated electronic system controlling organ regeneration in that there are no active components. The bone is a completely self-contained, self-organizing system that once set in motion requires only a continuous supply of tropocollagen to maintain itself. The negative feedback works back into the PN junctions by the new growth gradually reducing the stress applied.

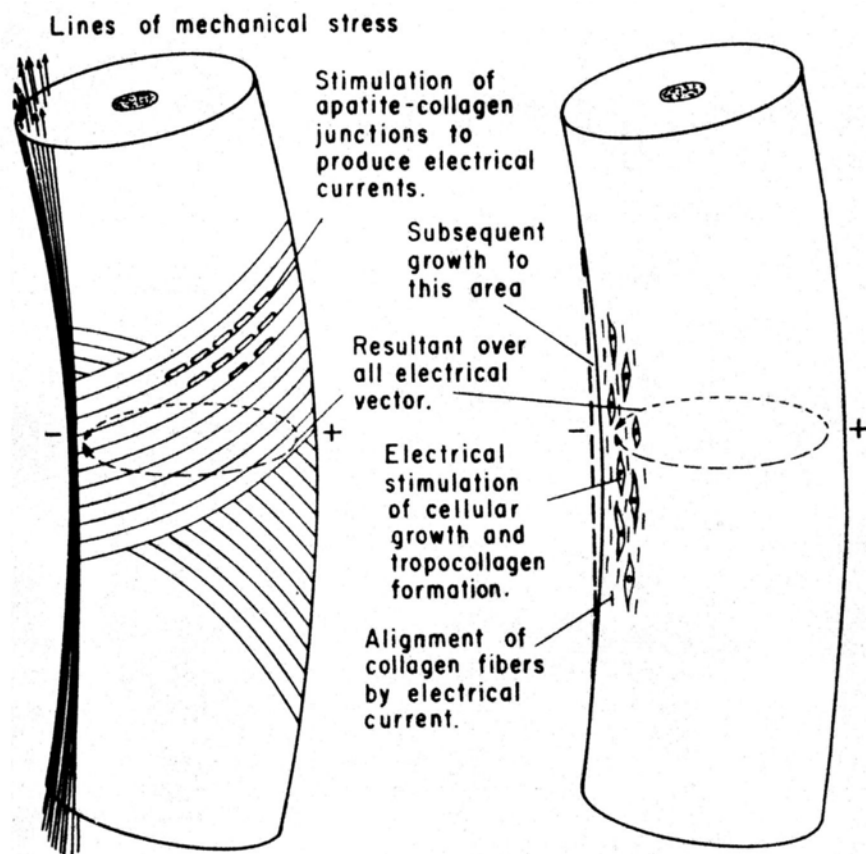
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The superiority of bone over implantable metallic devices (of initially higher mechanical strength) lies in its ability to remodel itself in response to the changing mechanical stresses placed upon it. This ability is simply the resultant of the innate organization of bone and its properties as a self-organizing system. The cells are responsible only for the production of new collagen fibers and remodeling theoretically could take place *in vitro* provided one started with a mature bone under stress and furnished a sufficient supply of tropocollagen to the solution. It should be pointed out that the original size and shape of bone is the resultant of genetic factors and that the process we are

describing is limited to the post embryonic period.

We have now studied two entirely dissimilar growth systems of living organisms, limb regeneration and bone growth in response to mechanical stress. Both are of vital import to the organism displaying them and both are represented, at least in part, in the human. Each has been found amenable to analysis by the techniques of control system theory and in each case actual experimentation has produced verification of the hypothetical control system. These systems are based upon the electronic properties residing in the molecular architecture of the tissues themselves with

FIGURE 11 Schematic representation of both halves of self-contained control system. On the left the major stress lines are activating the apatite-collagen PN junctions producing multiple small local currents, all orientated in the same direction, which produce an overall electrical vector between the negative compression side and the positive tension side. On the right these local vectors are producing the deposition of newly formed collagen along lines of stress, and the overall electrical vector is producing osteoblastic stimulation in the negative area. The resultant is new bone formation in the con-cavity, orientated with the collagen fiber longitudinally and parallel to the force vectors.



information transfer occurring by means of small electrical currents. Some measure of control over growth processes has already been obtained by injecting appropriate electrical signals into in vivo systems.

This line of investigation appears to be particularly fruitful and promises to yield in the near future much more effective measures of control over growth and possibly other vital functions as well.

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