

# 13

## *Bioelectrical Factors Controlling Bone Structure* \*

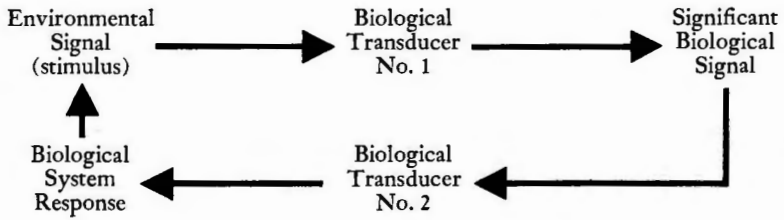
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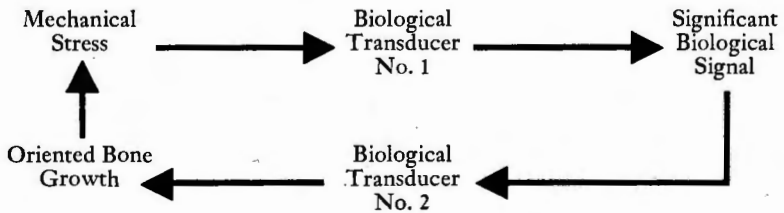
It has long been recognized that bone structure and mechanical stress are directly related. This relationship was first described in detail by Wolff,<sup>33</sup> in 1892, and now is commonly referred to as Wolff's law. The mechanisms responsible for this relatively unique phenomenon have been poorly understood. When bone responds to mechanical stress by differential growth so as to precisely resist the applied stress, it may be viewed as a stimulus-response growth system rather than a complex self-organizing system. Since this is the case, its behavior might be analyzed by modern control-system theory. The structure of bone is well known, almost down to a molecular level, and demonstrates an organization permitting it to be viewed as quasi-crystalline in nature. The water content of mature cortical bone is quite low,<sup>22</sup> and the major component of the structure is noncellular. The tissue can therefore be subjected to various physical and chemical manipulations and still retain significant elements of organization for study. These aspects of the physical nature of bone permit the application of solid state physical methods of experimentation to the problem of determining appropriate control-system mechanisms. This report sets forth data that links these two methodologies—control-system theory and solid-state theory—to the mechanism controlling bone architecture and growth.

In general, the simplest control system is a closed-loop feedback in which the response feeds back to the original signal and trends to cancel or null it out. An example is the control of blood calcium level as proposed by McLean.<sup>19</sup> A theoretical, more generalized biological closed-loop system may be diagrammed as follows:

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If such an analysis is applied to Wolff's law, it is possible to fill in two of the units in the system as follows:



The nature of the two biological transducers and the biological signal involved remains to be determined and is the subject of this report.

## METHODS

Bone samples were obtained from amphibian (bullfrog) and mammalian (human, dog, white rat) sources. Measurements of stress-generated electrical phenomena were made primarily on the amphibian tibio-fibula, because its size and shape were convenient as well as fairly uniform from individual to individual. The other types of bone were similarly tested and yielded results comparable to the amphibian bone. Tests using techniques designed to demonstrate solid-state characteristics (conductivity, photoconduction, fluorescence, etc.) were done primarily on normal mature human cortical bone; iliac crest, femur, and tibia were most frequently used. Amphibian and other mammalian bones were also studied by the same techniques and again demonstrated similar results. Sample amounts available from human material, however, were more suited to the preparation of identical size and configuration samples for these studies and consequently this source was primarily utilized.

### *Treatment of Bone Samples*

Specimens were surgically removed and were kept "dry," i.e., not exposed to normal saline solutions during dissection or storage. All soft tissue attachments, periosteum and endosteal cancellous trabeculae, were removed

mechanically, leaving only thin samples of outer cortical bone. In all cases, particular care was taken to remove residual blood. Samples requiring specific shapes or sizes (or both) were prepared by hand sawing at slow speed, followed by light shaping with fine Carborundum paper. Care was taken to prevent heating. Samples were used either immediately or stored under controlled conditions at 20°C. and 60 per cent relative humidity. Measurements of stress potentials were done on fresh samples. Various degrees of dehydration were produced by controlling the humidity of the equilibrating atmosphere, by heating in an oven at various temperatures and times, or by lyophilization. Demineralization was accomplished by constant agitation in 5 per cent formic acid until the samples were flexible.<sup>20</sup> The organic matrix was removed by refluxing with ethylenediamine for 30 to 40 cycles.<sup>22</sup> Both demineralized and anorganic samples were washed in running tap water for 24 hours, followed by distilled water for 24 hours. When various conductivity measurements were done on these samples, the original long axis of the bone was utilized as the current axis. In this fashion, the orientation of the collagen fibers and the crystal axes were maintained insofar as possible. No studies were done on powdered crystals.

### *Mechanical Stress*

The physical arrangement is diagramed in the upper portion of Figure 3 (see page 215). The currents generated by stress were detected by "painted on" silver electrodes with pressure contacts of fine platinum wire. A 10<sup>11</sup>-ohm resistor was inserted into the circuit, and the voltage generated across it by the current flow was measured with a Keithley 603 electrometer amplifier and recorded on a Brush Model BL 202 galvanometer recorder. Total system response was adequate to record a 100 c.p.s. sine wave without distortion or damping. Stress was applied by a series of calibrated weights suspended from a fine thread looped over the free end of the bone. In order to prevent displacement artifact, loading was gradual (1 second), and drop loading was avoided.

### *Conductivity Measurements*

The general physical arrangement is as shown in Figure 1. Teflon cabling and block polyethylene insulation were used throughout. Currents were measured with Keithley 600A or 603 electrometers. Temperature was measured with a standard glass mercury partial-immersion thermometer in contact with the sample. There was a measurement lag noted on the heating portion of a cycle, but cooling cycles proceeded slowly enough to permit equilibration. All records are cooling-cycle records except when otherwise specified. An initial period of equilibration was permitted until ionic currents were exhausted and steady current values reached.

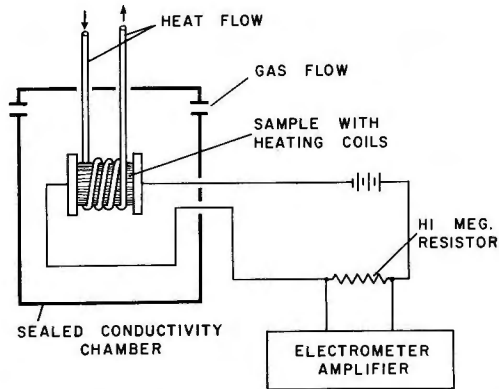


FIGURE 1. Diagram of apparatus used to measure conductivity variations with temperature. Samples were held in a screw-type clamp between silver plate electrodes. The ends of the sample were coated with silver paint before insertion into the clamp. Sample size averaged 5 by 3 by 2 mm., with the original long axis being the largest dimension. Value of the high megohm resistor was literally  $10^{11} \Omega$ , although  $10^9 \Omega$  was used on occasion with higher currents. The chamber was shielded both electrically and thermally.

### Photoconductivity

Small thin sections of whole cortical bone were affixed to a glass microscope slide with Silicone rubber RTV 120. Indium-mercury amalgam electrodes with fine platinum-wire contacts were employed. The light source was a direct-current-excited halogen lamp with a broad-band spectral output. The light beam was collimated to prevent direct light falling on the electrodes. Currents were measured and recorded with the same apparatus as in the stress measurements.

### Fluorescence

The same light source was used as in the photoconductivity measurements, except that the beam was filtered through a Corning-No. 9863 ultraviolet filter which absorbs most of the visible light and has an ultraviolet transmission range (10 to 90 per cent) between 2400 and 4000 Å. There is also a small transmission (10 to 40 per cent) in the near infrared region above 6800 Å.

Photometric absorption and transmission curves were run on thin sections, whole or treated, in a Beckman Model DU Spectrophotometer.

Determinations of majority carriers were done on purified samples of bone collagen and bone mineral by the hot-point method as modified by

Gould.<sup>11</sup> (For a discussion of the nature of majority carriers, see footnote on page 217.)

### Preparation of Tropocollagen Samples and Experimental Procedure

Tendons were procured from tails of three growing white Wistar rats (150 to 180 Gm.) and processed by a technique described by Ehrman and Gey.<sup>7</sup> This consists essentially of extracting the pooled, teased tendons in 1:1000 acetic acid for 24 hours at 4°C. The resultant material is centrifuged at 4°C. and the supernate dialyzed in Visking cellophane against running de-ionized water for 72 hours at 4° to 6°C. A clear, viscous fluid containing only trace amounts of sodium and calcium ions was obtained from this procedure. No fibers were detectable in the fluid by means of light microscopy.

One drop of this material was placed on a clean glass microscopic slide, and two 10 mil, pure-platinum electrodes introduced 1 mm. apart. These were connected to a 6-volt battery, with a 1-megohm variable resistor in series. A Hewlett-Packard meter was also placed in the circuit, in series, to monitor current. All experiments were conducted at 4°C. with 95 to 100 per cent relative humidity (Figure 2). Collagen bands were observed with

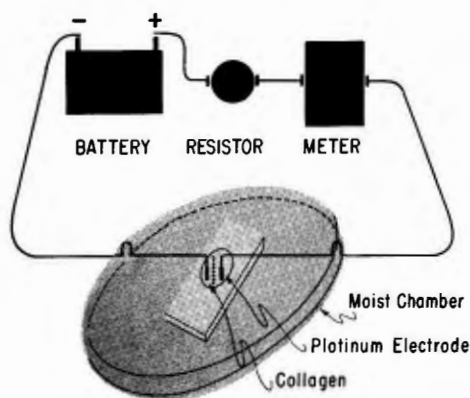


FIGURE 2. Scheme of experimental system used to orient collagen fibers. A 6-volt battery is connected in series with a 1-megohm variable resistor and a Hewlett-Packard 425-A meter to monitor current levels (0.2 to 2.0 microamperes).

the Leitz Ortholux microscope, fitted with a polarizer, after current ranging from 0.2 to 2.0 microamps had been passed through the drop for 5 to 30 minutes. In order to "fix" a band *in situ*, 1 drop of a 1 per cent sodium chloride solution was added to the drop of collagen solution. For staining, these preparations were washed gently with distilled water, passed through

ascending alcohol (from 50 per cent to absolute), and stained with eosin or aniline blue-picric acid. Enzymatic digestion studies were carried out by washing the salt-fixed collagen band in distilled water and immersing for 90 minutes in trypsin or crude clostridial collagenase at 37°C. (Additional details of technique and results will appear elsewhere.<sup>3</sup>)

## OBSERVATIONS

### *Electrical Response of Bone Subjected to Mechanical Stress*

It has long been suggested that bone, in view of its crystalline nature, had piezoelectric properties that might function as a stress transducer mechanism. In 1957, Fukada and Yasuda<sup>9</sup> reported such a phenomenon. They used frequencies of 2000 c.p.s. and noted not only direct but also indirect piezoelectric effects. Since both whole and demineralized bone showed the same effect, they related the phenomenon to the orientation of the collagen molecules into a quasi-crystalline matrix rather than to the content of bone mineral. In 1963 Shamos *et al.*<sup>27</sup> made similar observations but only on whole bone. Their samples were cleaned mechanically, treated ultrasonically in a detergent bath, and dried. Compressive and bending forces produced a potential difference appearing between ring electrodes around the shaft. The decay rate of the potentials was approximately 0.5 second, and release of stress was accompanied by another potential surge of equal magnitude but opposite polarity. The electrical output therefore approximated that of a classic piezoelectric device of multicrystalline nature rather than a single crystal.

Both homogeneous and nonhomogeneous piezoelectric crystals produce an alternating signal upon application and release of stress. The electrical sum in such a case is zero, and it is difficult to conceive of any continuous long-term process being directed in this fashion unless one interposes various other electrical circuitry to rectify the output. In addition, the classic concept of single-crystal piezoelectricity requires the absence of either a plane or axis of symmetry in the crystal structure. Most well-crystallized, nonbiological apatites have an axis and a plane of symmetry and therefore are not piezoelectric. While the apatite of bone is thought to be asymmetrical, its extremely small size might possibly alter its behavior in this regard.

In 1962 we reported the generation of electrical signals by freshly prepared bone subjected to bending stress rather than to compressive forces.<sup>2</sup> Considerable differences from the single-crystal piezoelectric effects were observed, including a tendency towards a nonsymmetrical output and a steady-state output during the period of steady-stress application.

These preliminary observations have been extended in the present investigation. Further study of the stress-generated electrical phenomena demon-

stated an initial current surge that decayed in a few seconds to a steady current output. This steady current then persisted as long as the stress was applied (Figure 3). Experimental difficulties with direct-current record-

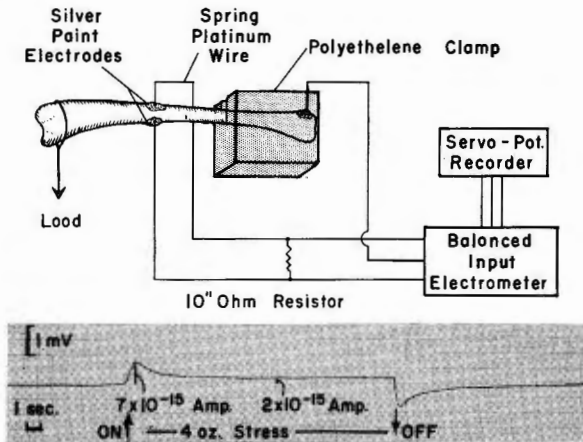


FIGURE 3. Diagram of experimental setup for stress current and potential detection (upper portion) and recording of standard stress response in amphibian tibiofibula (lower portion). Note the initial high current surge followed by the steady output during the application of the steady stress. The reversed current flow on stress release does not equal that passed during stress application.

ing have limited the period of observation to a maximum of 60 seconds; there was no measurable decay in this current during this period (Figure 4B, C, D). On release of stress, some current flow in the opposite direction was evident, but the total amount is considerably less than that observed during the stress application. Cyclic stress application at physiological frequencies resulted in considerable "pumping" effect which seemed to take advantage of the initial high deflection (Figure 4A). Under these conditions, considerable unidirectional current flow was obtained. The polarity of the current in all instances was the same as that previously reported (i.e., compression side negative with respect to tension side). The amplitude of potentials generated as measured by the current output was roughly proportional to the degree of deflection under load. The initial high output is apparently the result of the initial deflection, while the prolonged, low level output results from the deformation creep occurring under constant load conditions.<sup>30</sup>

The oriented nature of the potential and current pattern established throughout the stressed region provides a suitable biological control signal

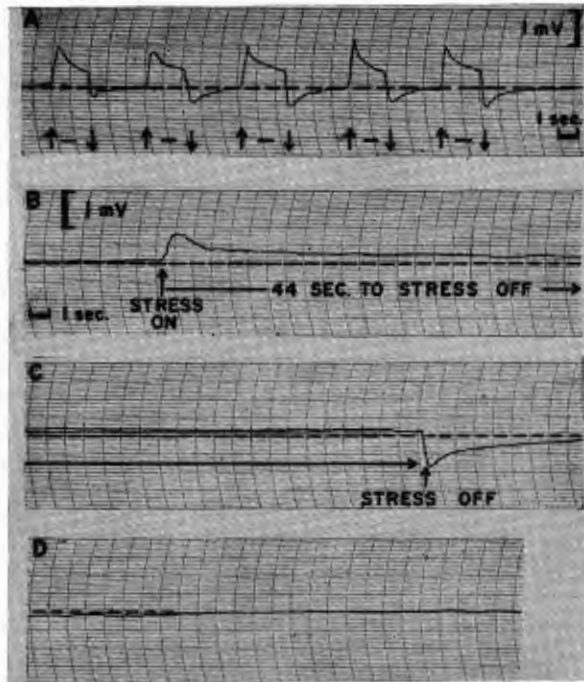


FIGURE 4. A: Pumping effect of repeated 2-oz. stress applied to amphibian tibio-fibula at cyclic rate as shown. The dotted line is the zero, isopotential line. The current flow ( $10 \times 10^{-15}$  amperes) during stress application periods in one direction is much greater than the reserve flow ( $5 \times 10^{-15}$  amperes) during stress release cycles.

B, C, D: Prolonged recording of stress current showing time decay. Stress was applied constantly for a period of 44 seconds. During the constant stress, a constant current of approximately  $2 \times 10^{-15}$  amperes was recorded with no discernible tendency to decay. Stress release is again accompanied by a current surge in the opposite direction but with a constant decay rate, reading zero in 16 seconds.

in the hypothetical control system. Previous reports have indicated that unidirectional direct currents can exert a significant biological effect. (<sup>4,14,18,23</sup>) In all of these reports increased growth rate was related to relative negative potentials. The compressed side of a stressed bone displays such a relative negativity, and it is in this region that osteoblastic activity characteristically is observed.

The differences between these stress-generated electrical currents and potentials, and those produced by classic piezoelectric crystals may indicate a different method of production. It has long been known that single crys-



tals of semiconducting substances have altered electrical properties when subjected to stress.<sup>6</sup> In addition, semiconducting devices having positive-negative (PN) junctions \* have nonclassic piezoelectric properties. Most recently, Rindner and Nelson<sup>21</sup> have shown that the PN-junction zone itself is exceedingly sensitive to minute amounts of stress. Bone matrix is basically a two-component system in which crystals of apatite and collagen frequently are precisely oriented. Therefore, the possibility was considered that these units may form multiple PN junctions capable of responding to mechanical stress.

Considerable work has been done on semiconduction properties of various biological materials, notably proteins.<sup>24, 25</sup> These properties have further been shown to have great functional significance in such processes as photosynthesis.<sup>5</sup> In all this work so far, purified crystalline samples have been used. Few efforts have been made to apply these techniques to organized tissues because of the complexities of the living system as well as technical difficulties. However, as previously noted, the physical characteristics of bone appear to be ideally suited for such an attack.

#### *Studies of the Semiconduction Properties of Bone*

*Electrical Conductivity of Bone.* One of the major characteristics of a semiconductor is its ability to conduct varying amounts of current under a constant potential in response to variations in temperature. Without going into great detail, this property may be explained by stating that the charge carriers in such materials may be freed from their ground state by thermal energy and thus be made available for current conduction. Increasing amounts of thermal energy make increasing numbers of charge carriers available, increasing the sample conductivity. This phenomenon is noted over the range of physiologically significant environmental temperatures, and is in contradistinction to metallic conductors where large numbers of conduction electrons are always available. Electrical insulators, on the other hand, have their electrons so tightly bound that great amounts of energy are required to free them sufficiently to carry a current. In practice, direct-

\* Semiconducting solids conduct electrical currents by means of charge carriers moving through the crystal lattice. These carriers may be negative in sign (electrons) and the solid is then designated as "N" type. They may also be positive in sign ("holes" being absences of electrons within the lattice, or on occasion, protons). The solid is then "P" type. Semiconductors may carry current by means of more than only charge carriers; however, if a carrier of one sign is more prevalent, it is designated as the "major" carrier, and the other charge carriers are then "minority" carriers. Bonding the crystal lattice of two such solids (one with positive, the other with negative majority carriers) produces a PN junction that has rectification properties (a diode) as well as stress sensitivity. The classic definition of piezoelectricity has been expanded to include devices of this sort, and at this time any solid producing electrical phenomena on application of a mechanical stress is considered to be piezoelectric regardless of the presence or absence of planes of symmetry. The interested reader is referred elsewhere<sup>6, 12</sup> for more extensive discussions of these principles.

current potentials are applied to the samples under study, and the current passed is measured as the temperature is varied between 20° and 50°C. (Figure 1). When the log of the current is plotted against the reciprocal of the Kelvin temperature, an intrinsic semiconductor will show a straight line with a slope proportional to the activation energy (energy required to raise an electron from the ground to excited state—usually expressed in electron volts, eV). The addition of various impurities in minute amounts (“doping”) changes the thermal sensitivity and various deviations from the straight-line plot are produced.

In these studies, the role of the free and bound water in bone was evaluated first. Figure 5 shows the current versus time plots for samples of bone

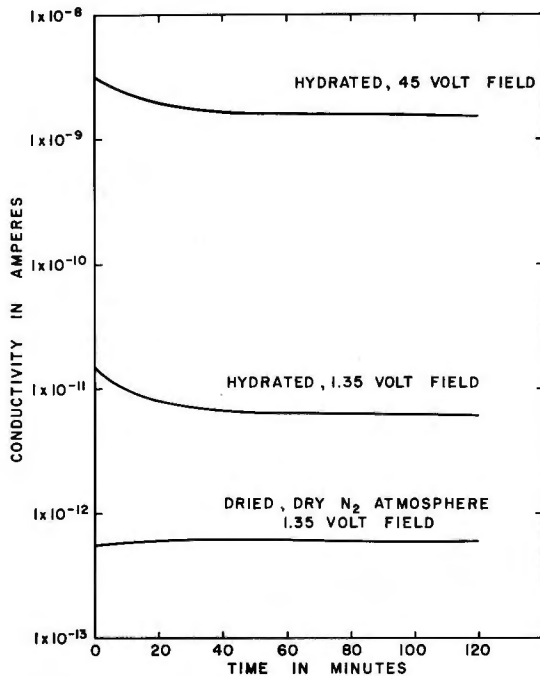


FIGURE 5. Conductivity changes with time for whole bone with various degrees of hydration, subjected to a constant electrical potential. The hydrated samples were human cortical bone, removed and prepared within 1 hour of the start of the experiment.

at various degrees of hydration and electrical potential. Initially there was a decrease in the current in the fresh as well as the partially dehydrated samples, indicating an ionic current. This current, produced by the migration of ions within the free-water compartment, decreases as the supply of free ions is exhausted, and the total current reaches a steady value which is main-

tained by the applied voltage for indefinite periods. The oven-dried samples (water free) demonstrated a steady current throughout the observational period. Removal of the bound water decreased the steady current at any potential by about one order of magnitude. These results are similar to those noted by Rosenberg<sup>24</sup> on the electronic conduction in hydrated versus dried crystalline hemoglobin. In both instances the conduction mechanism is electronic rather than ionic in nature, but the presence of the bound water of hydration markedly facilitates the mechanism.

Conductivity variations with temperature for whole bone samples in various states of hydration are shown in Figure 6. All samples demonstrated in-

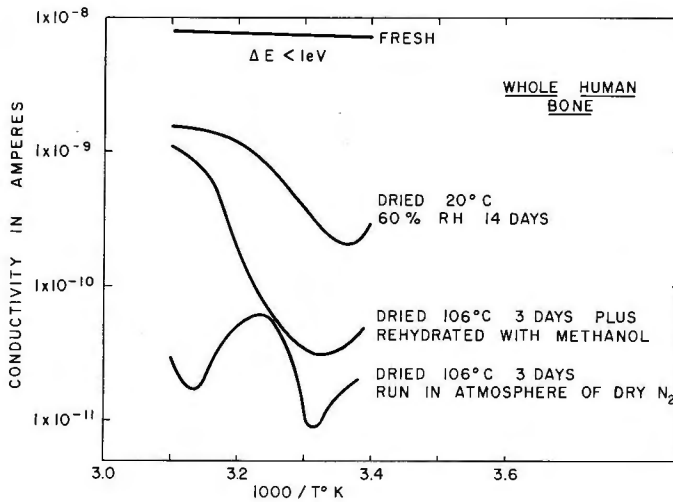


FIGURE 6. Conductivity changes with temperature variations in whole human cortical bone. The current-carrying capacity at any given temperature decreases with decreasing water content. All samples were subjected to 1.35-volt fields and only cooling curves are shown.

creasing conductivity with increasing temperature. The removal of various amounts of water decreased the conductivity and altered the slope of the curve. The oven-dried sample was particularly interesting in that it showed a conduction peak at approximately 37°C. Similar curves were noted for lyophilized samples, although the peak was relatively less intense. "Rehydration" of the dried samples by exposure to methanol vapor at 20°C. returns the conductivity to near that noted for the bone with bound-water content only. Rosenberg<sup>25</sup> obtained similar results by similarly hydrating crystalline hemoglobin with methanol vapor. Activation energies for whole, fully hydrated bone averaged about 1 eV. For partially dried (20°C., 60 per cent relative humidity, R.H.) bone (presumably with bound water intact) the

value was about 2.5 eV. Values for the completely dried sample were difficult to assess because of the complex curve obtained.

Bone collagen alone (obtained by demineralization) showed a temperature-dependent conductivity curve very similar to that described for other proteins,<sup>24</sup> with an activation energy of approximately 5 eV. (Figure 7). Hydration of the fibers is again of some significance as shown in the difference between the dry and the hydrated curves. The measurements charted were taken with current flow parallel to the normal long axis of the fibers. Conductivity measured transverse to the long axis was slightly lower at any given temperature.

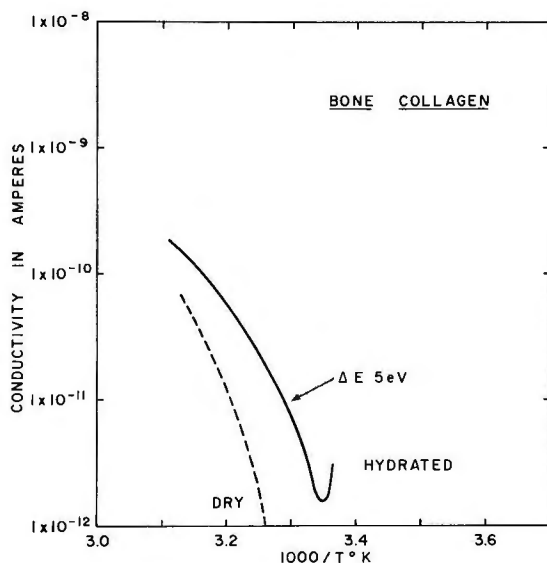


FIGURE 7. Conductivity changes with temperature variations in bone collagen obtained from human cortical bone. A field of 1.35 volts was used, and the "dry" curve was obtained in an atmosphere of dry nitrogen.

The conductivity versus temperature relationships for bone mineral along the original long axis of the bone are shown in Figure 8. The "hysteresis loop" for the hydrated sample is best explained by desorption of water on the heating cycle at 30° to 35°C. and corresponding absorption at the same range on the cooling cycle. The flat portion of the cooling curve from 45° to 35°C. indicates an activation energy of approximately 1 eV. As previously noted, the cooling curve was considered to be more accurate for this purpose than the heating cycle.

Oven-dried bone mineral measured in an atmosphere of dry N<sub>2</sub> demonstrated a curve similar to that of oven-dried whole bone with a conduction

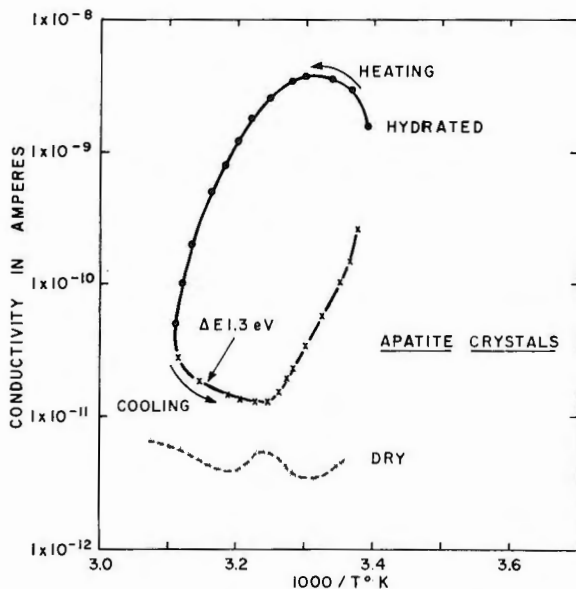


FIGURE 8. Conductivity changes with temperature variation for bone mineral obtained from human cortical bone. Both heating and cooling curves for one cycle are shown for the hydrated sample. Heating is begun at the right-hand end of the solid-line curve. Decreasing condition is noted between 30° and 35°C., dropping very sharply with increasing heat up to 50°C. Cooling from 50° to 35°C. is accompanied by a fairly linear decrease in conductivity from which the activation energy is estimated. Cooling below 35°C. results in increasing conduction at a rate parallel to the previous decrease with heating.

The cooling curve is shown for the dry sample run in dry nitrogen, although the heating curve was quite similar to it. Permitting this sample to rehydrate by exposure to room air, at 60 per cent R.H., produced a curve similar to that of the hydrated sample.

peak at 37°C. Whether this is actually related to the mineral or to residual organic material is presently not known. The "hysteresis loop" observed with hydrated samples suggests that certain water components of the mineral phase are very loosely coupled to the crystals. However, it is evident that this water is responsible for major conductivity variations of an electronic nature, possibly related to protonic conduction.

The foregoing data indicated that bone has a definite semiconduction property. Before discussing additional evidence for this property, let us re-examine the phenomenon of stress potentials in bone. If these potentials and currents are generated by a semiconduction mechanism, they should

demonstrate a temperature dependence. Figure 9 illustrates the effect on the stress currents of increasing the temperature of the bone by 5°C. All other conditions being equal, a temperature elevation from 20°C to 25°C. nearly doubles the electrical output. The process is reversible, and cooling to the original temperature results in a return to the original output. It is concluded, therefore, that the stress-generated currents show a temperature dependence similar to that of the semiconduction properties of the matrix.

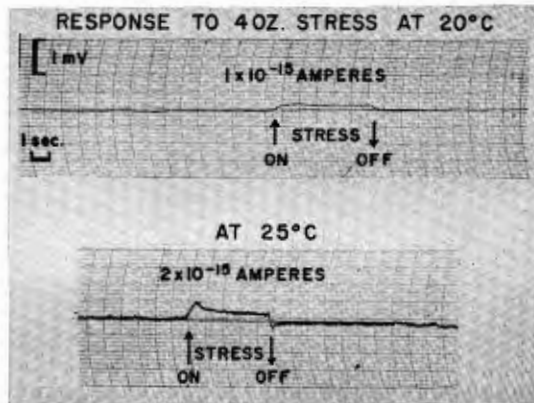


FIGURE 9. The effect of a 5°C. temperature change on the stress response of an amphibian tibio-fibula. Approximately twice as much current is obtained at 25°C. as at 20°C. from the same sample under the same load conditions.

*Photoconductivity.* Light of a proper wavelength is capable of producing excited charge carriers in semiconductors, the light energy being transferred to electrical energy. The energy of light photons is inversely proportional to the wavelength, the shorter the wavelength, the higher the energy per photon. In general, such mechanisms are studied by applying a direct-current electrical field to the sample while illuminating it with light of various wavelengths. The production of excited charge carriers during the illumination permits an increase in the current flow (over the "dark" current). In preliminary experiments we have utilized broad-band light sources (with a wide range of frequencies from ultraviolet to infrared) and produced measurable photo currents in whole bone samples with applied potentials of as little as 1.35 volts. Illumination in the vicinity of the negative electrode appeared to be more effective (Figure 10). The rise time of the photo current was found to be approximately 100 milliseconds. Isolated collagen and bone mineral are both photoconductors, with the former being about five times as efficient as the latter. The stress-generated potentials may be substituted for the external field in photo-

conductivity and again illumination on the concave (negative) side produces a larger current flux. From these preliminary experiments it appears that the whole bone matrix has photoconduction properties similar to those of other organic semiconductors.<sup>15</sup>

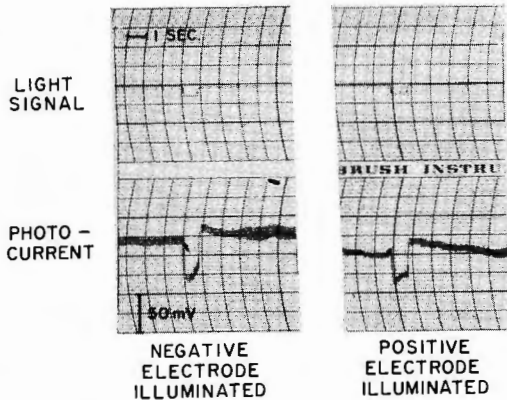


FIGURE 10. Photoconductivity in whole human cortical bone. A "surface cell" was utilized, i.e., electrodes were painted on at either end of the same flat surface of a sample 6 by 3 by 0.5 mm. A 1.5-mm. spot of white light was used to illuminate the bone in the vicinity of either electrode, avoiding direct illumination of the electrode itself. Light pulses of less than 1 second were obtained by a hand-operated shutter mechanism and are shown on the upper tracing as recorded from a photo diode behind the sample. A field of 1.35 volts was applied across the electrodes. High-speed recordings (not shown) have indicated a photo current rise time of less than 80 milliseconds.

*Light Absorption and Transmission.* In semiconductors a knowledge of the light absorption properties may yield information regarding the band structure since there may be a correlation between the wavelength (and the energy) of the absorbed photons and the energy necessary to provide a conducting charge carrier or to excite fluorescence.

Absorption curves were obtained from thin (approximately 1 mm.) sections of whole cortical bone as well as bone mineral and collagen prepared from the original sample. The curves are shown in Figure 11. The absorption minimums at 5000 and 11,500 Å in the whole bone also appear in the bone mineral and collagen. A similar absorption spectrum was obtained for three samples of natural mineral apatite (Figure 12).

It may be noted that the collagen exhibits a rather broad absorption minimum extending over a range of wavelengths from above to below those of apatite. Kommandeur<sup>16</sup> has indicated the extremely complex na-

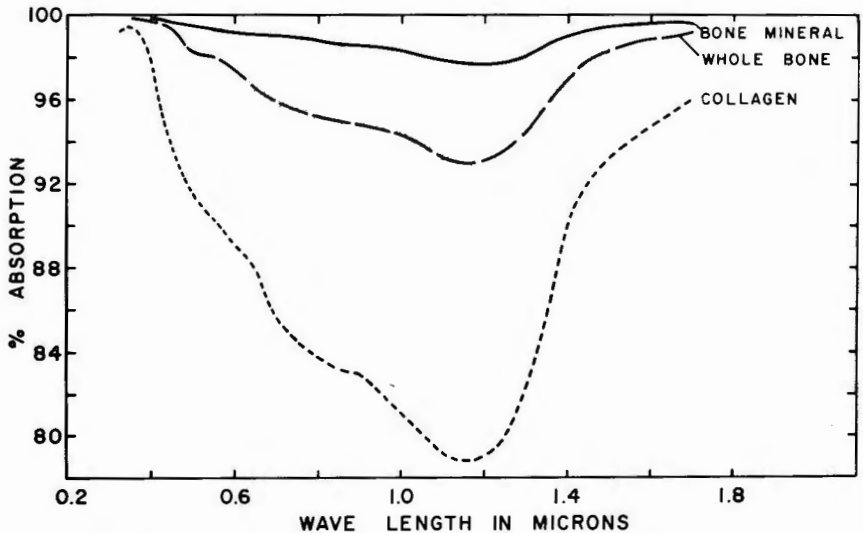


FIGURE 11. Spectral absorption curves of whole bone, bone collagen, and bone mineral. All samples were derived from a single specimen approximately 1 mm. thick. After deproteinization, the mineral sample still retained the original size, shape, and orientation. The demineralized sample, however, showed considerable shrinkage and some warping.

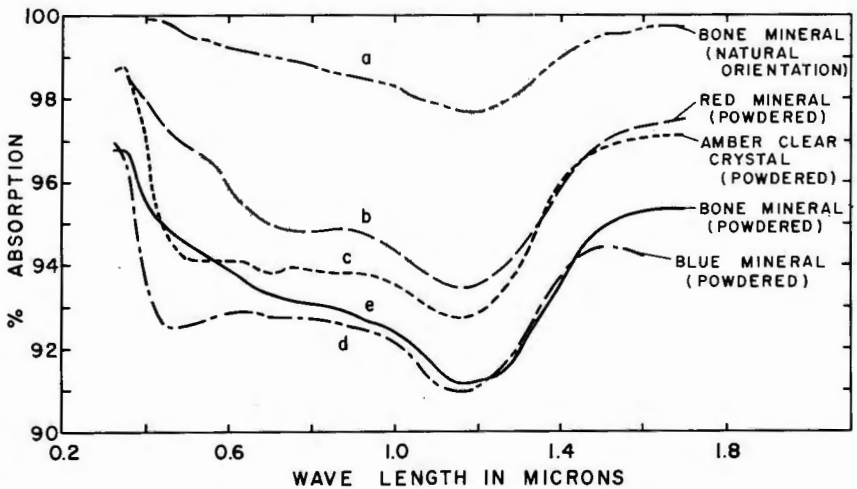


FIGURE 12. Spectral absorption curves for bone mineral and several samples of natural mineral apatite. Curve a was obtained from a sample of anorganic bone, 1 mm. thick, with its original orientation. Curves b, c, and d are from powdered mineral apatites, while curve e was obtained from powdered bone mineral. Since ordinate values depend upon sample thickness, the curves should be used to compare distribution shapes only.



ture of the crystalline semiconductor. Until experimentation is completed it can only be stated that wavelengths absorbed by both collagen and bone mineral are in the energy range including the activation energies determined by thermal conductivity measurements.

*Fluorescence.* In fluorescence, a substance bombarded by photons selectively radiates some of the energy received. Not all substances fluoresce. Of those which do, there are differences in the distribution of the emitted radiation, in the efficiencies of the mechanism, and in the types of radiation to which they are susceptible. The emitted photons have less energy than those of the incident radiation. Thus, visible fluorescence requires irradiation by ultraviolet.

Some observations have been made on whole bone as well as on bone collagen and mineral. When bombarded *in vacuo* with cathode rays, the fluorescence of these substances is just barely visible. Under ultraviolet light the fluorescence of all these materials is much greater. Visual observations were made of fluorescence excited by light of different wavelengths from a Beckman DU spectrophotometer. Collagen fluoresces with incident light of wavelength 2250 Å or greater, apatite with wavelength of 2950 Å or greater. Although fluorescence seemed to increase markedly as the irradiation approached 4000 Å, the power output of the light increased in the same fashion, and at present it is not possible to determine what wavelengths are most efficient in exciting fluorescence.

With a broad-band ultraviolet source (Halide lamp with Corning No. 9863 filter) whole bone fluoresces ivory to blue-white in color, collagen is intensely blue, and bone mineral emits a dull brick-red color. Three samples of natural mineral apatite were also noted to fluoresce a similar brick red. Spectral energy distributions have not yet been made for any of these substances.

*Majority Carrier Determinations.* The data thus far presented indicate that both major components of bone, collagen, and apatite are semiconductors and that the stress-generated electrical phenomena are related to this property. As previously noted, the PN junction is an exceedingly stress-sensitive structure. The postulate was made that the collagen-apatite relationship produced multiple PN junctions throughout the osseous structure. It has recently been possible to measure the electrical characteristics of the apatite-collagen PN junction. The experimental details and results are illustrated in Figures 15 and 16 in the Addendum. Measurements were made of the majority carriers (i.e., the sign of the major population of charge carriers) in anorganic bone and demineralized bone samples. All samples of apatite have been found to have predominantly P type carriers, while all bone collagen samples were predominantly N type. Electrical resistivities of the two substances are quite different, and the technique does not permit estimation of the relative numbers of carriers available in unit

samples. In addition, the presence of minority carriers cannot be detected by the method employed. However, the uniformity of observations indicates clearly that the two substances are opposite in sign. The stress-generated electrical phenomena may then be directly related to the multiple PN junctions formed by the precise relationship between the apatite crystals and the collagen fibrils.

A valid biological signal (the stress-generated electrical activity) and an appropriate transducer mechanism for its production (PN semiconductor junction) have been identified. The characteristics of the second transducer mechanism, relating the signal to directed bone reorganization, remain to be determined. While several possible mechanisms have been considered, the following data will be limited to one which has been evaluated in some detail.

#### *Orientation of Collagen Fibers in Solution by Weak Electrical Currents*

During osteogenesis the organic matrix is elaborated by the cells before mineralization occurs. In fact, collagen fibrils in this matrix nucleate and orient calcium hydroxyapatite crystals.<sup>10, 31</sup> It has previously been reported<sup>26</sup> that soluble collagen will migrate in an electrophoretic apparatus with currents of 25 milliamperes, forming a membrane with random fiber orientation on the cathode plate. Therefore, it seemed plausible to study the effects of weaker electric fields on solutions of acid-soluble collagen. These investigations demonstrated that tropocollagen units or collagen

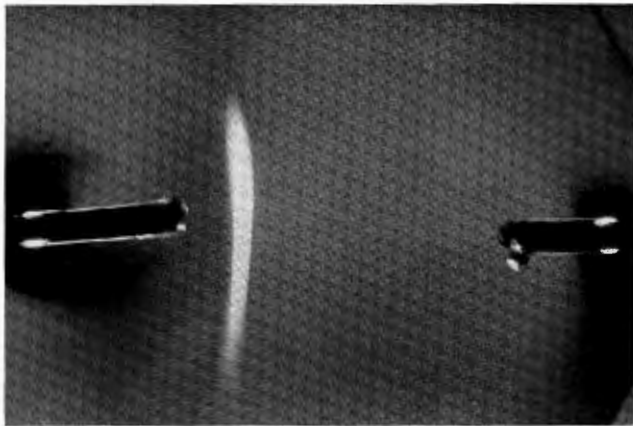


FIGURE 13. Photomicrograph of "unfixed" preparation of collagen after 5 minutes, at 0.5-microampere current. Salt had not been added yet. Cathode on left, anode on right. Note band closer to cathode than anode, with slight convexity toward anode. This preparation contained no polarizable or microscopically visible material prior to introduction of current. Polarized,  $\times 30$ , before 50% reduction.

molecules could be oriented into parallel structures by currents ranging from 0.2 to 2 microamperes. If the solution had been dialyzed free of salt before the current was turned on, a long curvilinear, polarizable band appeared (in 5 to 50 minutes) perpendicular to the field, and at varying distances from the cathode, depending upon the current density (Figure 13). The weaker the current, the closer the band approached the cathode. When the current was turned off, the band disappeared (diffused away) within several minutes or was drawn to the opposite pole if the polarity was reversed. When salt was added to the system after a band was formed, it neither diffused away when the current was turned off nor could it be moved significantly by current reversal. Such bands were digested by collagenase, but not by trypsin. They demonstrated parallel-oriented fibers that could be stained with eosin or picric acid-aniline blue and that polar-



FIGURE 14. Photomicrograph of band similar to the one shown in Figure 13 30 minutes after adding 1% NaCl. Cathode was several fields away, just to the left of this photograph. Note fine, parallel fibers, oriented perpendicular to the electrical field, along the left-hand portion of the band which faces the cathode. Eosin; phase contrast,  $\times 1000$ , before 45% reduction.

ized. These fibers, presumably collagen, were found running parallel to the long axis of the band on its concave face next to the point-like cathode (Figure 14). Observation with polarized light before salt precipitation suggested that the fibers were parallel to the long axis of the band on its concave side but that some perpendicular orientation was present on the concave side. This arrangement was not seen in the stained preparations. Electron micrographs of the fibers are currently being obtained to determine periodicity and fibril orientation.

These studies suggest that collagen in solution is affected by very weak currents, of the levels obtained in bone under stress. The magnitude of the current and the rapidity of its action suggest that this method might prove valuable in studying other macromolecules and their aggregation or polymerization patterns. Since fibrils can be oriented *in vitro* by weak currents and fields, it is conceivable that they might also be oriented *in vivo* by a similar mechanism.<sup>31</sup> This mechanism, however, probably is not the only way in which collagen is oriented in living systems. For example, orderly bundles of collagen were observed circumferentially about silicone rods used to produce tension in tissue cultures of bone cells.<sup>1</sup> In this latter system, mechanical factors seemed to be directly responsible for orienting collagen fibers.

These observations—that both electrical and mechanical factors seem to affect collagen alignment directly—may help to explain the highly ordered structure of lamellae and osteones in bone. They probably do not account for the synchronous phases of osteogenesis and osteolysis that establish the ultimate architectural pattern of a bone. Therefore, it is important to study also the action of electrical currents on osseous cells.

#### DISCUSSION AND CONCLUSIONS

In 1941 Szent-Györgyi<sup>28</sup> first suggested that semiconduction mechanisms may be of functional significance in biological systems. He has subsequently made a number of observations and has extended his original thesis in a recent monograph on the subject.<sup>29</sup> Such solid-state mechanisms have been shown to be basic reactions in photosynthetic processes,<sup>5</sup> where light photons provide sufficient energy to produce excited molecular states. Dark reactions (not light activated) have been much more difficult to study because the energies required to raise electrons of proteins to conduction-band levels have been considered unavailable in biological systems. Recently, Huggins and Yang<sup>13</sup> arrived at the conclusion that strong electron donors or acceptors can produce mammary cancer provided that the steric configuration of the molecule permitted it to attach to a receptor site in the tissue, resulting in charge transfer. As previously noted,<sup>4, 14, 18, 23</sup> the

injection of large numbers of free charge carriers into living systems has been shown to influence growth patterns. In the simple system under study in this report, the energy requirement is fulfilled by conversion of mechanical to electrical energy in the macromolecular lattice of the bone. Konev and Katibnikov<sup>17</sup> have made observations on the luminescence of mechanically stretched collagen fibers that indicate a similar conversion. Obviously, this concept still permits other methods of altering the lattice properties. It is suggested that the regenerative growth of fracture healing may involve a similar mechanism, with currents of injury furnishing the "error signal." Substances such as vitamin D and certain of the hormones which are active in minute amounts may function by being incorporated into the lattice as impurities or by providing for charge transfer reactions. It also appears feasible to evaluate certain bone diseases from this point of view. Within a living system, the semiconduction mechanism may be altered by injection of large numbers of free carriers, by the addition of new lattice impurities, or by administration of molecules having proper steric and electronic properties to react with the lattice. Should the semiconduction properties of bone be changed by such factors, the electrical signals generated by stress would likewise be altered, and a structural change in the bone would be expected. It seems possible, therefore, that manipulations of the control system may eventually result in a clinically useful control of bone growth and architecture.

#### SUMMARY

1. Bone has many characteristics of a semiconductor. These characteristics are related to the semiconduction properties of each of the two major components of the organized bone matrix, plus structured water. The functional unit formed between the collagen fibrils and apatite crystals is a PN junction.

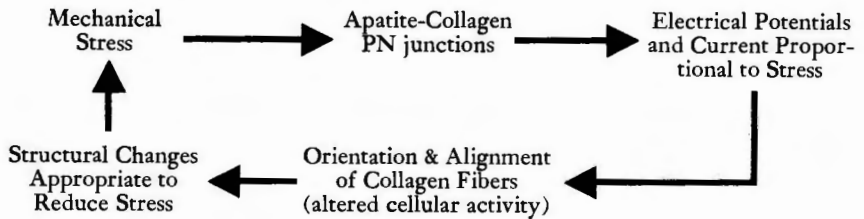
2. Stress applied to bone produces electrical currents and potentials proportional to the magnitude of the stress and with a polarity determined by the direction of the stress. These electrical phenomena are believed to be due to the stress sensitivity of the multiple PN junctions.

3. Tropocollagen units in solution may be oriented into linear parallel structures by the application of electrical currents and fields equivalent to those produced by stress in whole bone.

These observations may be inserted into their appropriate positions in the theoretical closed-loop growth control system.

This scheme is not yet complete since the action of electrical currents on cell function in bone has not yet been defined clearly. Furthermore, additional mechanisms may be operative at each step. Despite these consider-

ations, however, the present concept may furnish a logical framework for further investigation.



Finally, it should be emphasized that stress-generated electrical current is only one of many factors controlling the behavior of bone. For example, isolated limb rudiments have an inherent capacity to develop characteristic shapes in tissue culture.<sup>8</sup> This behavior indicates that genetic factors play a dominant role in establishing the initial pattern of bone growth. Once a bone is formed, however, the stress potential may provide a dynamic, finite, control mechanism which permits adjustment in structure to meet new functional demands.

#### ADDENDUM

It has been possible to measure the electrical characteristics of the apatite-collagen PN junction. The experimental details and results are illustrated in Figures 15 and 16.

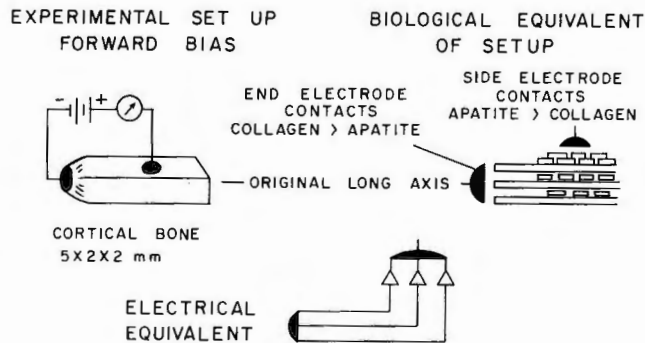


FIGURE 15. Details of experimental setup to measure the apatite-collagen PN junction characteristics. Whole human cortical bone was used in its normally hydrated state. There is undoubtedly some resistance and capacitance in the circuit as well as some PN diodes in the reverse direction; however, the overall effect of this electrode placement is as illustrated in the electrical equivalent—a mass of diodes in parallel.

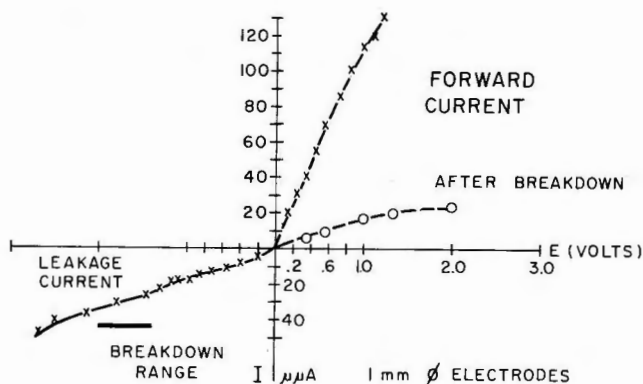


FIGURE 16. Electrical (diode) characteristics of the apatite-collagen PN junction. A typical voltage-current curve for a diode is obtained. Considerably more current is passed for a given voltage applied in the forward direction (side or apatite electrode positive, etc.) than for the same current in the reverse direction. If the breakdown range (reverse current) is not exceeded, one can repeat the forward voltage-current curve many times over. However, if breakdown is exceeded, forward-current characteristics become equal to reverse current and the rectifying properties are lost. This drop in forward current rather than a rise in reverse current after breakdown indicated that breakdown phenomena are associated with an increase in forward resistance—possibly a physical disruption of the apatite-collagen junction.

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