6-1964

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Virgil V. Heinrich

Recommended Citation

Heinrich, Virgil V., "The Physiological Aspects of the Piezoelectric Phenomenon and its Application in Biologic Bone" (1964). *Loma Linda University Electronic Theses, Dissertations & Projects*. 1386.
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LOMA LINDA UNIVERSITY
School of Graduate Studies

THE PHYSIOLOGICAL ASPECTS OF THE PIEZOELECTRIC PHENOMENON AND ITS APPLICATION IN BIOLOGIC BONE

by

Virgil V. Heinrich

A Thesis in Partial Fulfillment of the
Requirements for the Degree
Master of Science in the Field of Orthodontics

June, 1964

I certify that I have read this thesis and that in my opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Thomas J Zwemer

Thomas J. Zwemer, D.D.S., M.S.,
Associate Professor and Chairman,
Department of Orthodontics

Robert L. Nutter

Robert L. Nutter, B.A., M.S., Ph.D.,
Associate Professor of Microbiology

Ralph R. Steinman

Ralph R. Steinman, D.D.S., M.S.,
Associate Professor of Oral Medicine

Robert W Woods,

Robert W. Woods, B.A., M.A., Ph.D.,
Associate Professor of Preventive
Medicine and Public Health

ACKNOWLEDGMENTS

With sincere thankfulness and appreciation, I give due acknowledgments to the following named persons:

Dr. Thomas J. Zwemer, Chairman, Department of Orthodontics, to whom I am indebted for guidance, inspiration and technical advice during the writing of this thesis.

Dr. Ralph R. Steinman, Associate Professor of Oral Medicine, for the hours of technical aid and research advice.

Dr. Robert W. Woods, Associate Professor of Preventive Medicine and Public Health for his invaluable contribution in the field of electronics and materials design.

Dr. Robert L. Nutter, Associate Professor of Microbiology, for his interest and consideration in this work.

To all those who helped in any way during the preparation of this thesis, I am deeply grateful.

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CHAPTER I

INTRODUCTION

Importance of the Research. The intent of this investigation is to correlate some of the physical principles of crystallites with the physiological aspects of bone in a biophysical approach. This thesis is to serve as a pilot study in the investigation and application of the indirect piezoelectric effect on crystallites on physiologic bone.

Objectives of the Research. 1. To determine the effects of microamperage current on viable bone. 2. To apply direct and direct pulsating current to viable bone. 3. To equate the effect with the indirect piezoelectric phenomenon. 4. To assess bone growth and provide a means for altering growth both in external configuration and ultrastructure. 5. To better understand the techniques involved in using dogs as research animals in bone studies.

CHAPTER II

REVIEW OF THE LITERATURE

Literature on Piezoelectricity. Piezoelectricity as a phenomenon was discovered in the year 1880 by the Curie brothers. They applied a weight on the surface of a crystal and produced a measurable voltage which was directly proportional to the weight applied. In the following year Lippman conversely predicted that a voltage applied to a crystal would produce a displacement. This hypothesis was then confirmed by the Curies. The word Piezo is derived from the Greek word meaning to press; therefore piezoelectricity can appropriately be called pressure electricity. It manifests itself only in insulating solids of which crystalline materials are the largest group exhibiting the effect. Hundreds of materials have been tested and a great proportion exhibit piezoelectric properties. Some of the most common in use today are quartz, rochelle salt, tourmaline and ammonium dihydrogen phosphate.[8] These crystals have many uses in the field of electronics, medicine and science. Commercially they are applied in microphones, earphones, hearing aids, audiometers, electronic stethoscopes, loud speakers, sonar devices and oscillators.[7]

Of special interest to this paper is the possible fact that the apatite crystals of the inorganic portion of bone and the collagen crystals of the organic portion of bone exhibit piezoelectric properties. Very little has been done in correlating piezoelectric principles of crystals to physiologic bone.

In 1955 Doctors Yasuda, Naguchi and Sata, in dealing with stimulation of callus formation in bones, applied compression and tension to bone femurs and produced callus formation in relation to strains applied. Two plastic rods were inserted through the femurs and connected by a spring to produce compression. Compressive forces directed parallel to the bone axis produced osseous callus in the form of a low ridge parallel with the long axis of the bone in the periosteum between the plastic rods. When compression was directed at right angles to the long axis of the bone, callus formation again resulted. This time it was in a crescent shape at right angles to the long axis of the bone in the periosteum between the plastic rods. The above callus formation was probably due in part to an accelerated metabolism in the part of the bone to which the dynamic energy was applied. These men observed and recorded positive electrical potentials at tension sites and negative electrical potentials at compression sites. They feel this shows that in the formation of a callus, dynamic energy is converted into electrical energy. They also suggested the possibility of forming callus merely by electrical energy, which would implicate the indirect piezoelectric effect as having an effect on the callus formation.

In continuing their research they applied one microampere of current through femurs for three consecutive weeks. The supply was 1.5 volts. Osseous callus formed between the two poles with the cathode pole exhibiting more callus formation than the anode side. They reported osseous callus formation when the current was between one and a hundred microamperes. When the current was increased to one milliampere, bone destruction took place. From this experiment the authors felt that dynamic energy, which was exerted upon the bones, was transferred to electrical energy and the latter played an important part in the callus formation.[20]

In 1963 Dr. Morris Shamos wrote on the "Piezoelectric Effect in Bone."[16] He reviewed the works of Drs. Fukada and Yasuda who measured piezoelectric constants of small specimens of compact bone. They concluded that the effect was truly piezoelectric and resulted in the collagen crystals of the bone slipping past each other.[5] Dr. Shamos also demonstrated the same stress induced electrical effect in various whole bones, both in compression and bending modes. The bones were prepared by removal of the periosteum and then placed in an ultrasonic cleaner with a detergent. After this the bones were thoroughly dried. Conductive paint was applied for electrodes. Leads were attached to these electrodes and connected to an electrometer which had an output chart recorder. The bones were placed in a shielded box to minimize electrical pickup. Compressive and bending forces were applied from the outside. Sudden application of a static force resulted in a potential difference between the electrodes proportional to the stress applied. Upon releasing the stress, the same voltage pulse appeared but with an opposite polarity. This voltage pulse was in the order of .3mV/kg. of applied force. It is evident that architecture of bone depends to some extent upon the forces acting upon them. Dr. Shamos feels that the surface charges which appear on stressed bone may be a controlling factor in bone formation. The electric fields which are produced from such surface charges might influence the orientation and deposition of ions or polarizable molecules.[16]

Dr. Bassett in his work on mechanically stressed bones has produced positive potentials in areas of tension and negative potentials in areas of compression. The magnitude of the direct current potential is in proportion to the amount of deformation. Stress potentials were not observed when the inorganic portion of the bone was removed. Dr. Bassett feels that this stress-induced potential affects osseous activity and may have an effect upon the ultrastructure of the bony matrix.[1]

Dr. Becker has shown bioelectric direct current potentials as being responsible or having an influence upon the regeneration of amphibian limbs.[2]

There has been very little work done in the demonstration of the piezoelectric effect in bone mineral other than these reviewed articles. It is possible that its importance in the orientation of bony trabeculations and its effect upon the ultrastructure of bone may have been underemphasized.

Literature on Physical Properties of Crystals. Classification of crystals has produced seven crystal systems and thirty-two crystal classes according to their shapes and dimensions. The symmetry which crystals possess determine whether the crystal will exhibit a piezoelectric effect or not. Crystals possessing a center of symmetry cannot be piezoelectric, because no stresses applied can produce a current or dipole moment, by the separation of the positive and negative charges. The greater the asymmetry of a crystal the greater the piezoelectric properties of the crystal.[8]

The crystallographic configuration of bone crystallites have not, as yet, been definitely determined. McLean describes the crystals of bones as rodlets or hexagonal prisms.[10] Robinson describes the crystals as minute hexagonal tablets.[15] Bourne reports cross sections of apatite particles as being arranged in hexagonal form.[3] It is possible that the comparison of a hexagonal form of apatite crystals to the known hexagonal form of piezoelectric quartz crystals can be significant in determining from a morphological basis that apatite of bone is piezoelectric.[17] While not conclusive evidence in itself, it does add to the possibility that apatite crystals possess piezoelectric properties, and therefore comply with the laws of the phenomenon.

Literature on the Composition and Physiology of Bone and Bone Mineral. Bone can be divided into two basic components. An organic portion of about thirty-five to fifty per cent by weight and sixty to seventy per cent by volume. It includes the cells, cementing substance and collagen fibers.[15] The collagen portion may be termed the crystalline component of the organic matrix and as described by Fukada and Yasuda may possess piezoelectric properties.[5] These investigators feel that the piezoelectric direct effect resulting from compressive strains on bone is due to the collagen "crystal" fibers slipping over each other and not the apatite crystal complex.

The other basic component is the inorganic portion of bone and it is also represented by a crystalline and noncrystalline portion. The noncrystalline portion includes such ions as sodium, magnesium, calcium and about twenty trace elements. The crystalline portion is basically an apatite matrix. It has the basic formula of $Ca_{10}P_6O_{26}H_2$.[3,9,10,12,15,18,19]

The apatite structure was simultaneously and independently determined in 1930 by Naray-Szabo and Mehmel. Its ultrastructure as its Greek name implies (= I decieve) ensures one of fertile fields for future research.[12] Crystallographically the apatite crystals are thin hexagonal plates having the basic dimensions, as described by Robinson with the use of the electron microscope, of 10 angstrom units high by 250 angstrom units wide by 500 angstrom units long.[15] The range as reported by many investigators is from 10-50 angstroms by 50-250 angstroms by 500-1200 angstroms.[3,10,12,15,19]

These crystals are aligned parallel to the collagen fibers in a close relationship with them. According to Engstrom three inorganic crystallites fill up a "period" of the collagen fibers.[19] In the formation of bone, it is felt that the deposition of collagen is first laid down. As the bone matures and calcifies, the apatite forms between these fibers and adds strength and hardness to the bone.[9]

The apatite lattice is manifested in several forms in bone. There is a carbonate apatite, a tricalcium phosphate hydrate, a floroapatite and a hydroxyapatite. It is felt, however, that the internal lattice structure of the basic apatite crystal remains stable and these various forms are then manifested only by surface exchange of ions.[10]

Literature on Methods of Producing or Altering Bone Growth. Various methods have been acclaimed for causation or alteration of osseous growth. Richards describes five methods of bone growth in theory. 1. By augmentation of blood flow. 2. By increasing the oxygen tension. 3. By increasing the temperature of bone within physiological limits. 4. By the application of distracting forces. 5. By the stimulation of the nerves supplying growing bone.

Miltner produced stimulation of bone growth by simple stripping of the periosteum.[11] The resulting trauma was considered the triggering mechanism for growth. Enlow describes bone growth as a result of remodeling due to both tension and compression.[4] Gelbke has demonstrated the influence of pressure and tension on growing bone.[6] Yasuda produced osseous callus growth by passing low microamperage current through bone.[20] Pease produced temporary stimulation of bone growth with the use of pins inserted within the bone.[13] Of significance is the theory of Wolff who proposes that changes in function of a bone are attended by definite alterations in the outer shape and in its internal structure.[18]

The need in the fields of medicine and orthodontics for controlled bone growth cannot be overemphasized. It is with this idea in mind that this thesis is being pursued.

CHAPTER III

MATERIALS AND METHODS

MATERIALS

<u>The materials and apparatus used in this research are as follows</u>:

1. Direct current outputs of two and twelve microamperes resulting from a one volt cell supply in combination with the appropriate resistors.
2. Direct current outputs of eight and sixty microamperes resulting from a nine volt supply in combination with the appropriate resistors.
3. Direct pulsating current outputs of eight and sixty microamperes resulting from a nine volt supply in combination with the appropriate transistors, capacitors and resistors. The multivibrator pulse rate, which is a factor of resistance times capacitance, is one second on and six seconds off.
4. Dead soft stainless steel wire for leads and three-quarter inch, #4 stainless steel bone screws. All conducting materials were coated with a nonreactive silicone base insulating plastic.
5. A regular armamentarian of surgical instruments including

anesthetic materials and plaster cast materials.

6. Four young dogs ranging in weight between twelve and twenty-five pounds.

TECHNIQUES

Three different techniques were used in the application of a current to the femurs of the test dogs. In the first technique the dog was anesthetized with a Sodium Butisol injection into the vein. The dosage being 35mg/kg. of body weight. The femur was surgically minimally exposed at about the middle of the shaft and two slots were drilled on opposite sides of the bone within the cortical plate. These slots were of approximate magnitude of one mm. by one mm. by four mm. and were undercut slightly with a #35 inverted cone dental burr. The leads were placed in the slots and held fast with dental amalgam. The current applied was two microamperes of direct current for three weeks. Two control slots were placed approximately ½ inch away. Amalgam was inserted in these slots but no current was applied. The entire unit of a one volt cell and resistor hooked in series was sealed in plastic and this miniature unit was buried between the fascial planes and was sutured to the underlying muscle planes to promote stability. The area was sutured and the dog was placed on antibiotics for the first week. X rays were taken of the area using high speed film at 65 KVP for two seconds. After three weeks the animal was sacrificed and the femur removed for observation.

In the second method of approach the dog was anesthetized and treated surgically similarily to the first, but instead of making slots in the cortical plate two small holes were drilled on each side of the bone and the soft stainless steel leads were looped through the two holes and fastened by twisting instead of using the amalgam. The leads and battery source were not embedded within the fascia, but were allowed to extend through the tissue and were embedded with their power source within the plaster cast material. The opposite leg in each case received the same surgical procedures including the lead wires, but with no power source. The area was sterilized with alcohol. Sterile gauze was wrapped around the leg before the cast material was applied. The current applied was 12 microamperes for three weeks at which time the animal was sacrificed and the two femurs removed, cleaned and examined. Techniques one and two, though necessary in the development of a workable technique, were abandoned for the third technique which was considered more reliable and accurate for our data collection.

In the third method, three-quarter inch #4 gauge stainless steel screws were inserted into opposite sides of the femurs. Both the experimental and control femurs received the bone screws. The technique allowed us to minimize surgical trauma to the tissue and periosteum in that only a short incision of approximately ¼ inch was made. The tissue was dissected to the bone and a small hole was drilled into the bone using a #4 round dental burr. The screw was then inserted and twisted until secure, yet avoiding extreme stress to the bone. The leads were priorly soldered to the stainless steel screws and as described in the second technique, leads and screw heads were outside the tissues and embedded in sterile gauze and plaster cast material. The currents applied to the animals were eight microampere and sixty microampere of direct constant current and eight microampere and sixty microampere of direct pulsating current with a pulse time of one second in every seven seconds. Again all legs had corresponding controls into which the screws were inserted, but no current applied. The power supply was nine volts and the current was continued for three weeks at which time the animals were sacrificed and their femurs removed. The femurs were thoroughly cleaned of tissue and then X rayed for 1½ seconds at 65 KVP. They were then photographed with Pan Fx film for complete records.

CHAPTER IV

RESULTS AND OBSERVATIONS

The observations and results of this experiment are based upon visual analysis using photographs, X rays and bone sections.

It is noted that in the development of the third technique, several dogs were used. Ribs, jaw bones, teeth and finally femurs were used using two to sixty microamperes of direct and pulsating current. Though all of this material is not included in the results, it gives us a good technical background upon which to base our results of the third and final technique.

Photographs were taken to show gross and cross-section structure. X rays were taken to show density and trabecular patterns. The results and observations appeared obvious and consistent throughout the cases.

Illustrations 11 and 12, which are the controls, give evidence of normal reactions of bone to trauma. The area around the leads and screws show slight osseous callus repair. Both sides of the bone show similar osseous growth and are indistinguishable in their appearance. No evidence of bony dissolution can be seen except in the immediate area of the screws. This is in accordance with the results of Miltner.[11] It is interestingly noted that in the controls of techniques 1 and 2, where the trauma is increased due to more extensive surgical procedures, the osseous callus growth is increased.

Illustrations 5, 6, 7, 8, 9, and 10 are the experimental femurs and present for our observation two different and distinct pictures. The negative poles appear to be very similar to the controls and show the osseous callus growth which follows the trauma. The positive poles on the other hand present a completely different picture. On the photographs and X rays these appear on the upper side of the bone.

From the two microampere current through the sixty microampere pulsating current, there is an increasing picture of increased bone dissolution at the positive pole. The photographs reveal cortical bone loss around the positive poles with the resulting necrosis. X rays show decreasing bone density in the field of the positive pole. It is noted that the wave of bony dissolution is radiated out in the form of a semicircle from the positive pole. The severity of this dissolution seems to be progressive and proportional to the amount of direct current applied. This can be observed in comparing Illustrations 5 and 6 with 9 and 10.

CHAPTER V

DISCUSSION AND INTERPRETATION OF FINDINGS

Six different direct currents were induced across the femurs of growing dogs. These included two, eight, twelve and sixty microamperes of direct continuous current for three weeks, and eight and sixty microamperes of direct pulsating current for three weeks.

Previous research has claimed osseous callus growth at sites of electrical stimulation with similar electric currents over the same period of time.[5,16,20] It is the feeling of this investigator that a direct electric field, while definitely having an effect, has an adverse effect upon the osseous structure at the anode. The results and observations of this experiment seem to substantiate this statement.

When one considers the bioelectric or physiologic currents of living things, he must understand these as part of a complex physiological system. The potentials that make up this system are derived from many factors. This system is vital to every living thing and in being an integral part of living things, becomes very difficult to isolate and mimic on a physiologic basis. It is because of this that certain principles or ideas which may be sound, are very difficult to demonstrate in a biophysical system.

This seems to be the case in using a direct current on physiologic bone. It is true that stress on bone produces a potential and this potential is a vital physiological result of the deformation of bony structure and ultrastructure.[1,16] The stress induces deformation, most likely in the crystalline structure of the bone, and this results in a dipole moment of the crystallites. If there is a constant current flow, however, it is no longer physiologic and bone destruction ensues. In the application of a direct current or pulsating current to bone of physiological magnitude, adverse effect can be explained by the fact that the electrical field that is set up depolarizes the ions in this field. Positive ions are then repelled from the positive pole and attracted to the negative pole. Negative ions are conversely repelled from the negative pole and attracted to the positive pole. If this is kept up for a continuous period of three weeks, physiologic chaos results. It is therefore possible that the area of bony dissolution as seen in the X rays at the positive pole is the result of the migration away of the many positive ions in bone. Such vital ions as Ca^{++}, Na^{+}, K^{+}, and Mg^{+} may be influenced by the electrical field. This would act to break down the inorganic or mineral portion of the bone in this area.

There is a possibility that alternating current can be applied to enhance or alter the growth of bone. Alternating current, however, may not be considered physiologic either, in that living tissues produce, by chemical means, bioelectric potentials which are continuous and do not alternate.

Further research, using direct and alternating current is indicated. There is a possibility that bone cultures grown on an elastic membrane would allow the researcher more control over his experiment. Currents could be applied to this culture as well as the stretching of the membrane in an effort to reproduce both the direct and indirect piezoelectric phenomena.

CHAPTER VI

SUMMARY AND CONCLUSIONS

As a result of the experiment on applying microamperages of current on bone, the following conclusions can be made:

1. Direct current, when applied to physiologic bone for a period of time, has a profound effect upon its morphological configuration and ultrastructure.
2. The mineral content at the positive pole is markedly reduced during the three-week period of applied current.
3. The amount of bony dissolution at the positive pole appears progressive to the amount of current applied.
4. The controls exhibited an increase in mineralization and callus growth as would be expected from the trauma during surgical procedures.
5. The negative pole does not characterize itself with the positive pole, but appears similar to the controls. When compared to the control, it appears the callus growth is the result of surgical trauma.
6. Further research, using bone cultures, to give the researcher more control of his experiment is indicated.

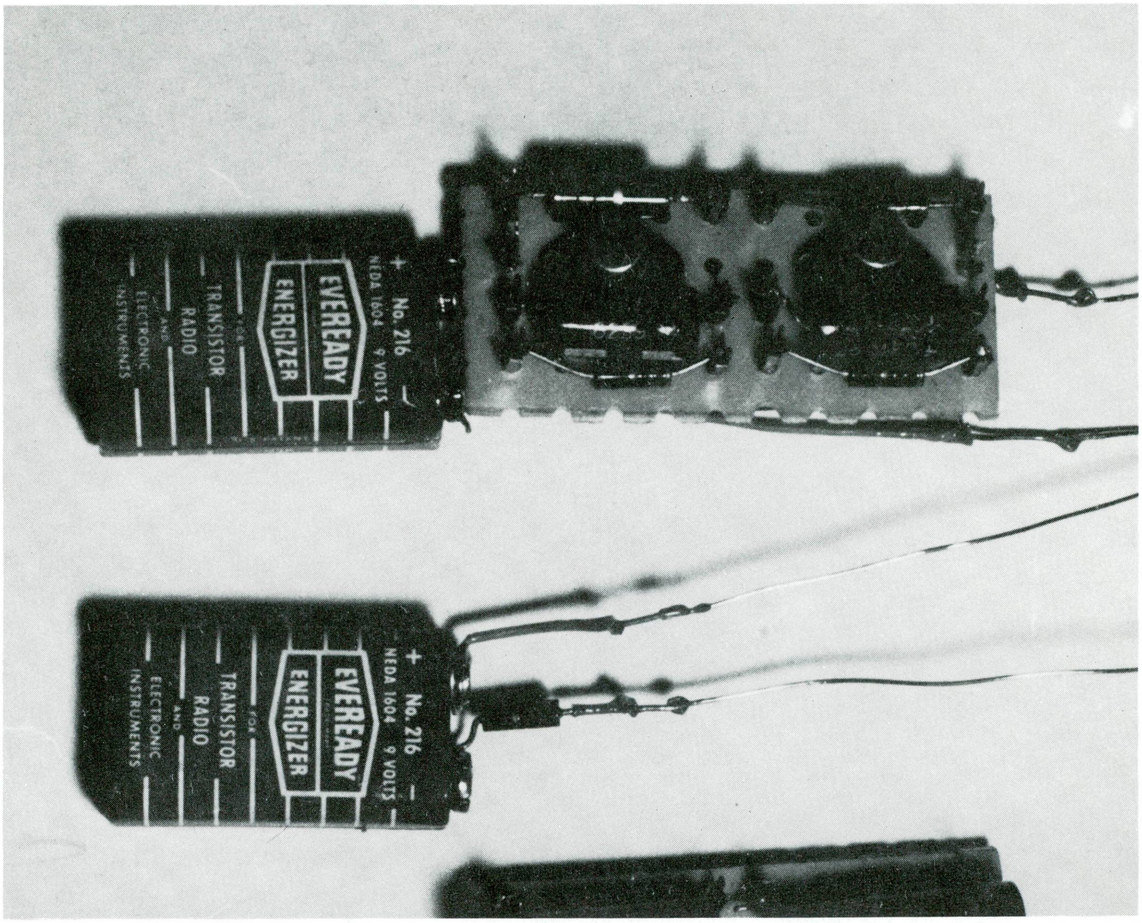

ILLUSTRATION 1

Direct Current and Direct Pulsating Current Supply Systems.

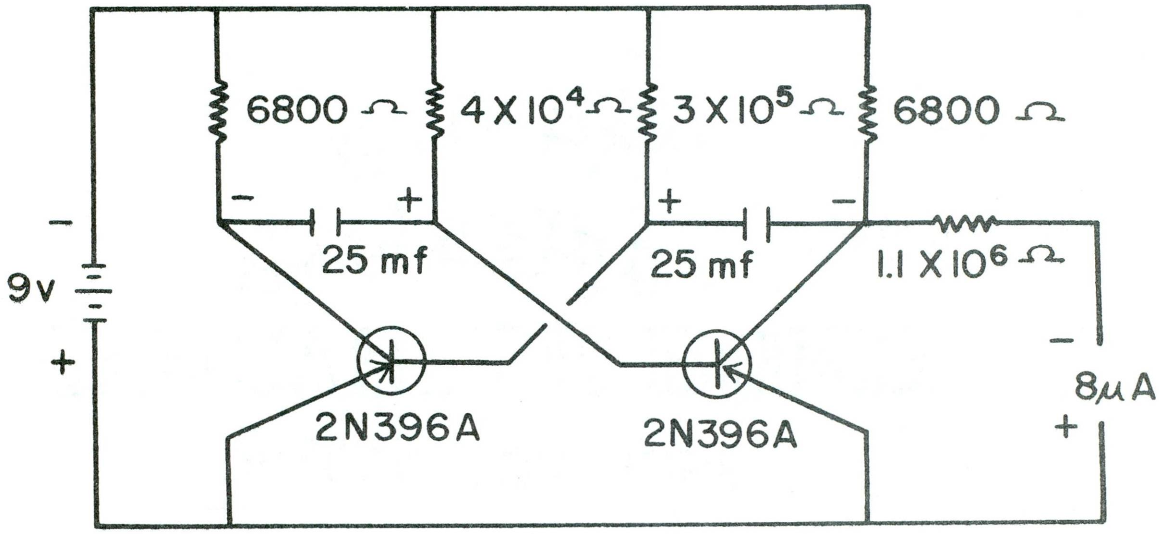

ILLUSTRATION 2

Multivibrator Circuit.

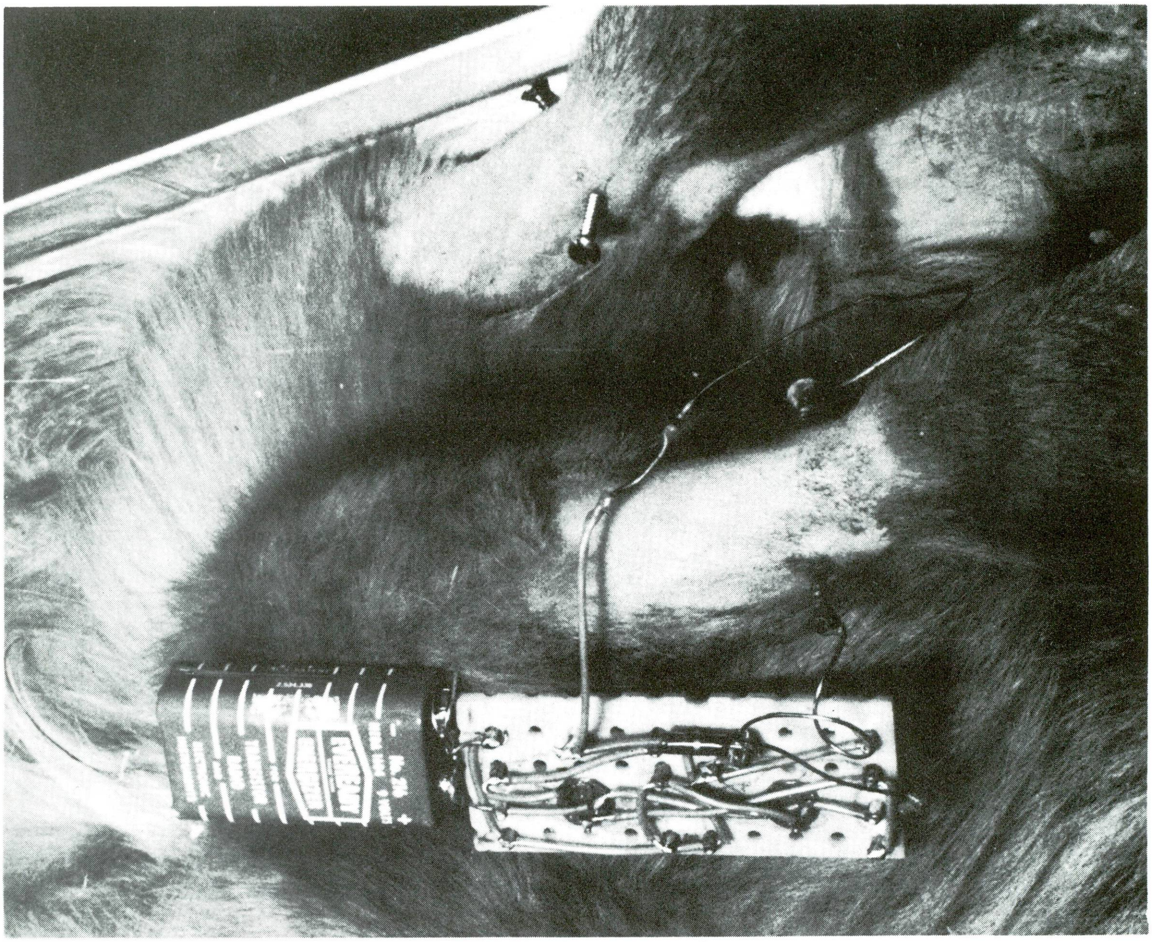

ILLUSTRATION 3

Multivibrator and Control Placed Using Stainless Steel Screw Technique.

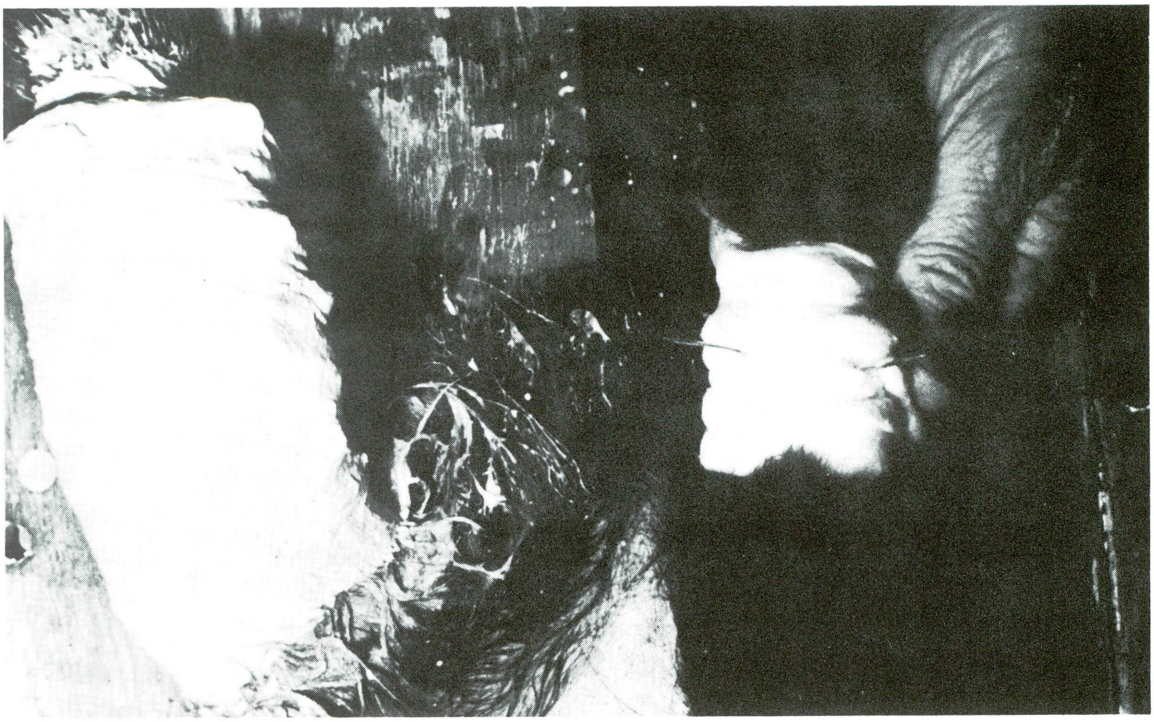

ILLUSTRATION 4

Leads and Cast Material in Place.

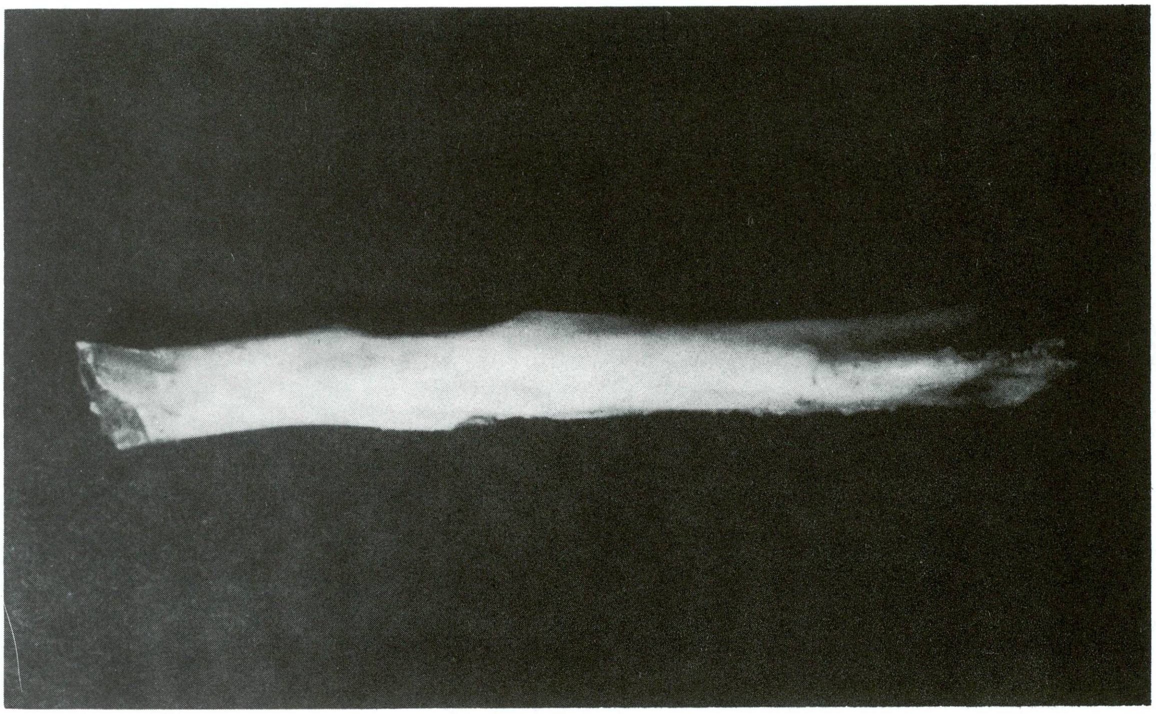

ILLUSTRATION 5

Photograph of Eight Microampere Direct Pulsating Current.

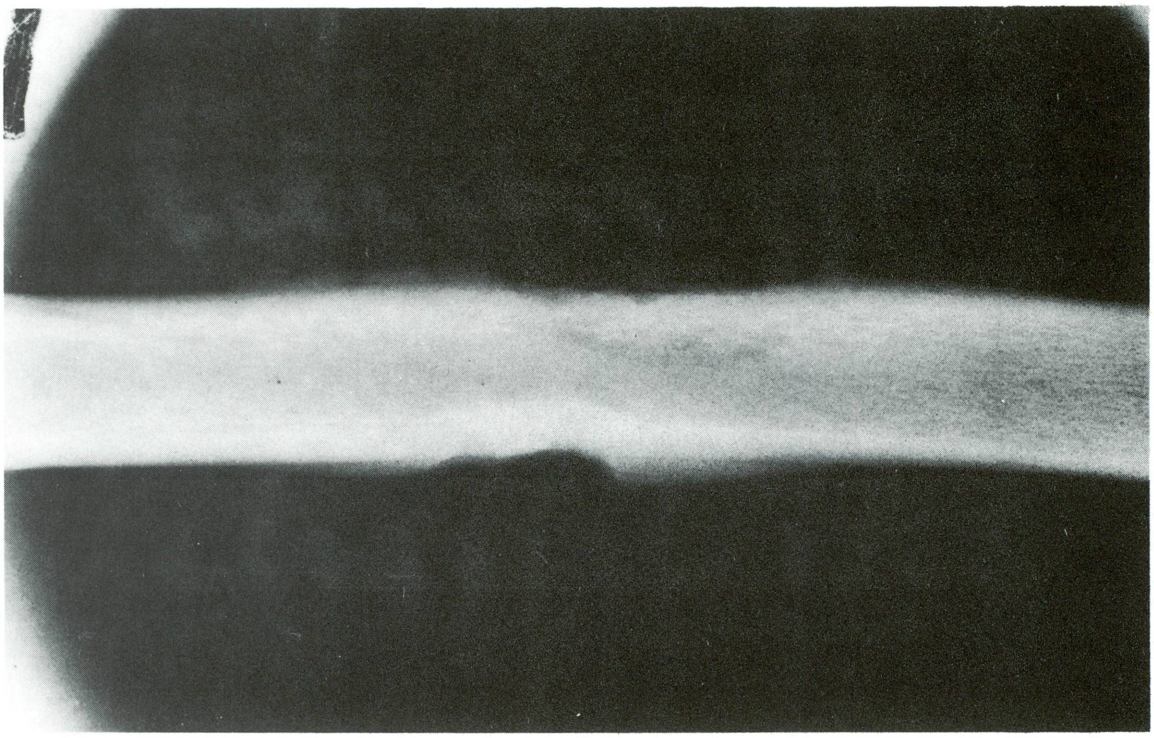

ILLUSTRATION 6

X ray of Eight Microampere Direct Pulsating Current

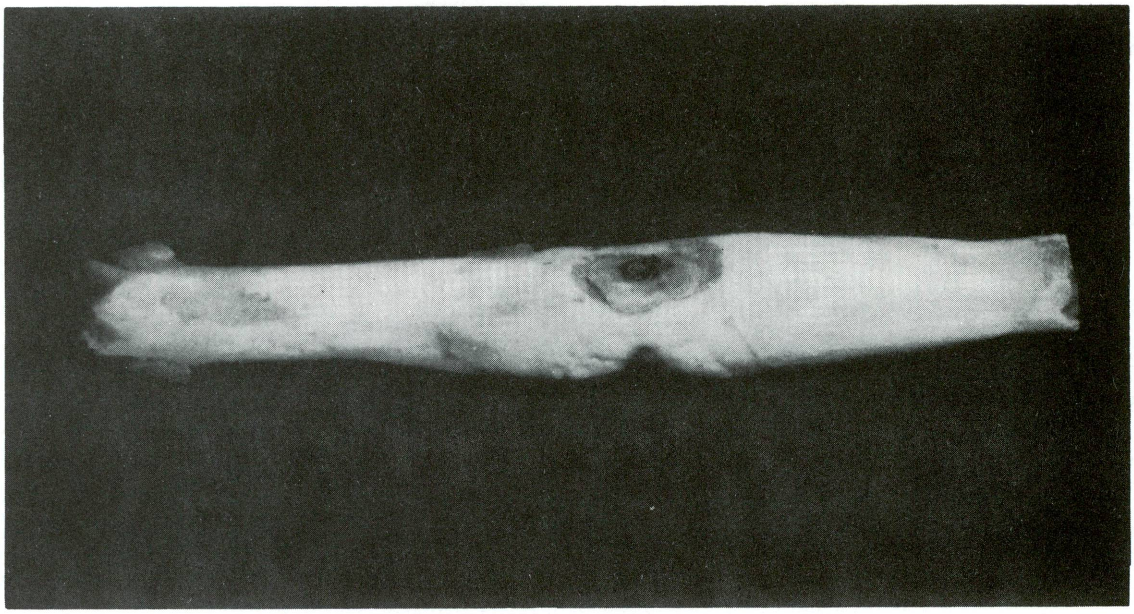

ILLUSTRATION 7

Photograph of Sixty Microampere Direct Current.

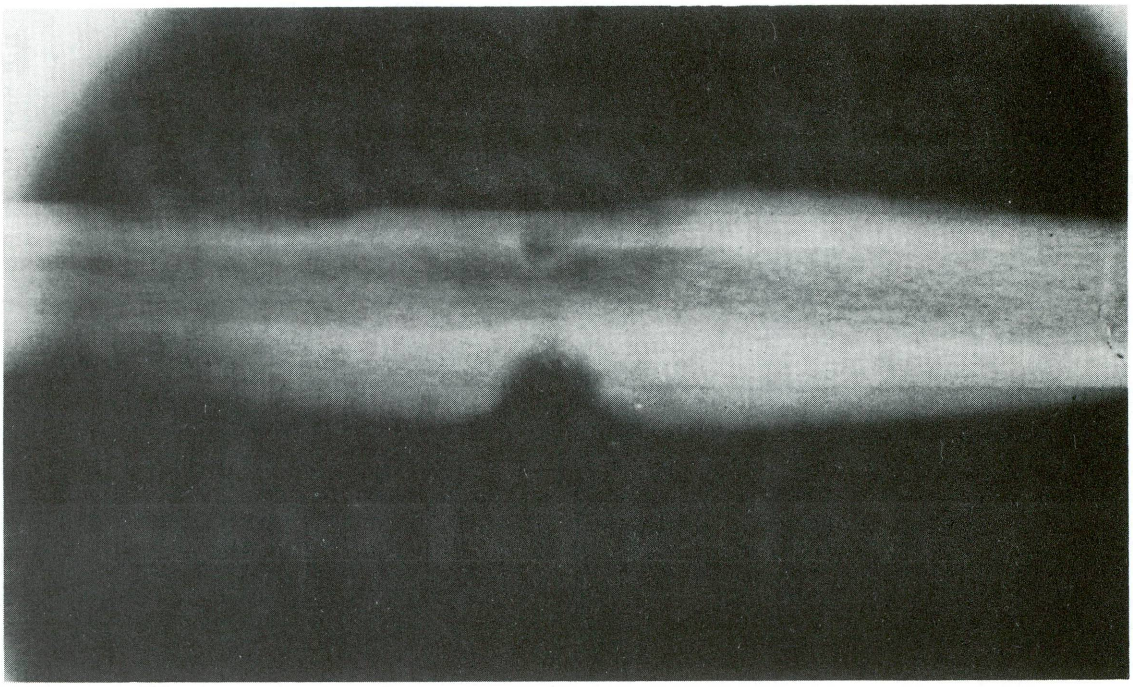

ILLUSTRATION 8

X ray of Sixty Microampere Direct Current.

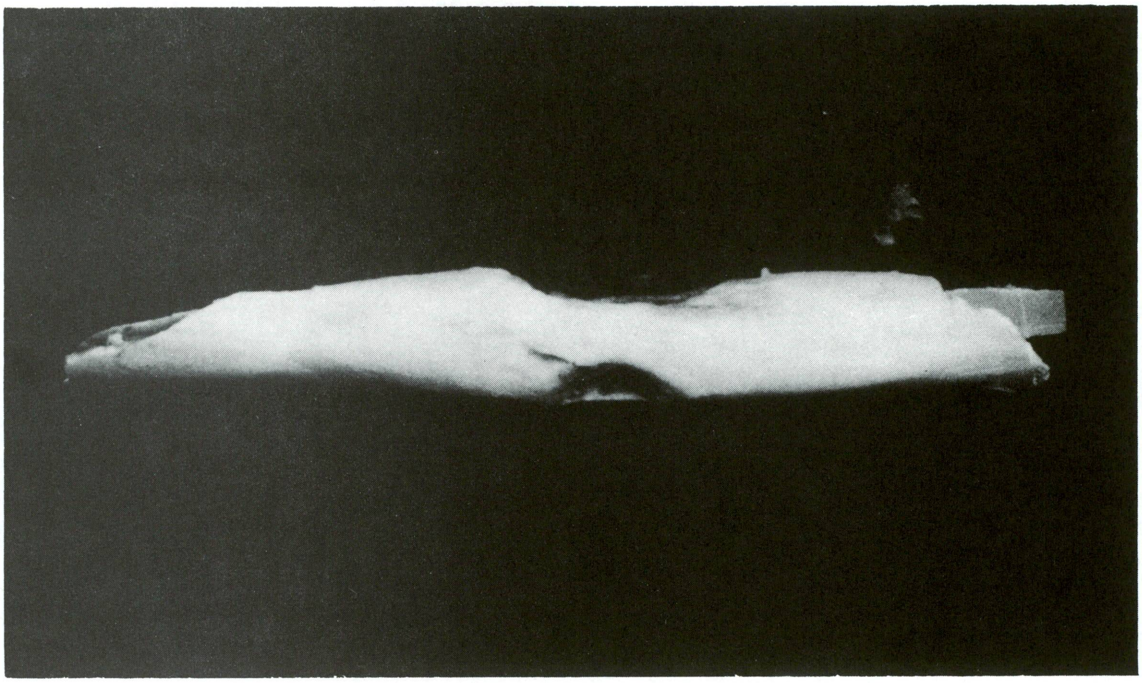

ILLUSTRATION 9

Photograph of Sixty Microampere Direct Pulsating Current.

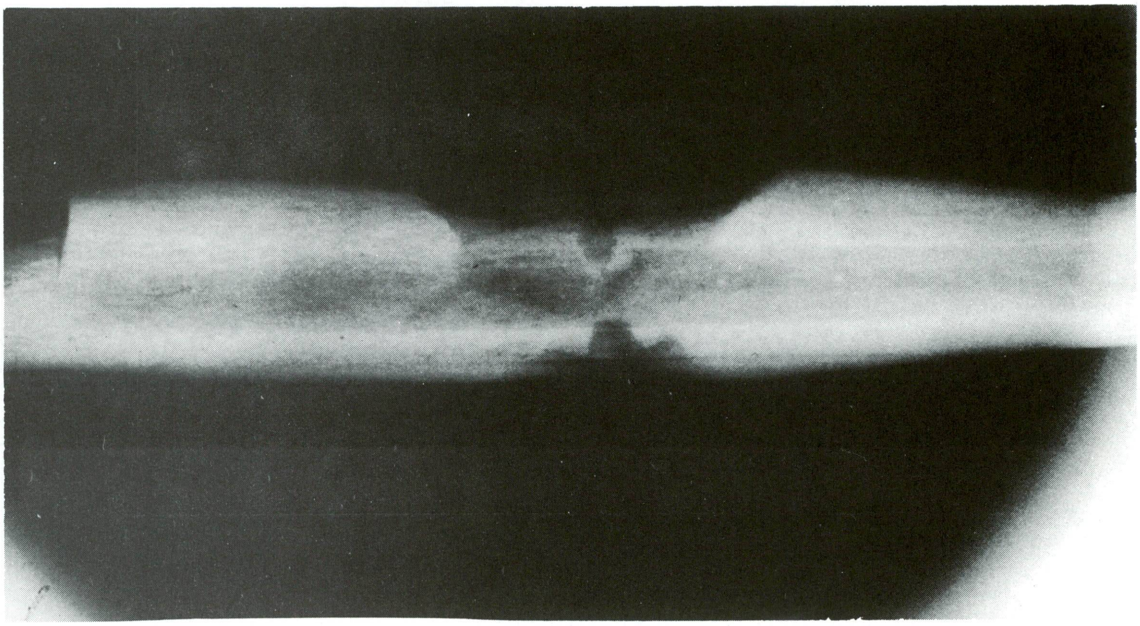

ILLUSTRATION 10

X ray of Sixty Microampere Direct Pulsating Current.

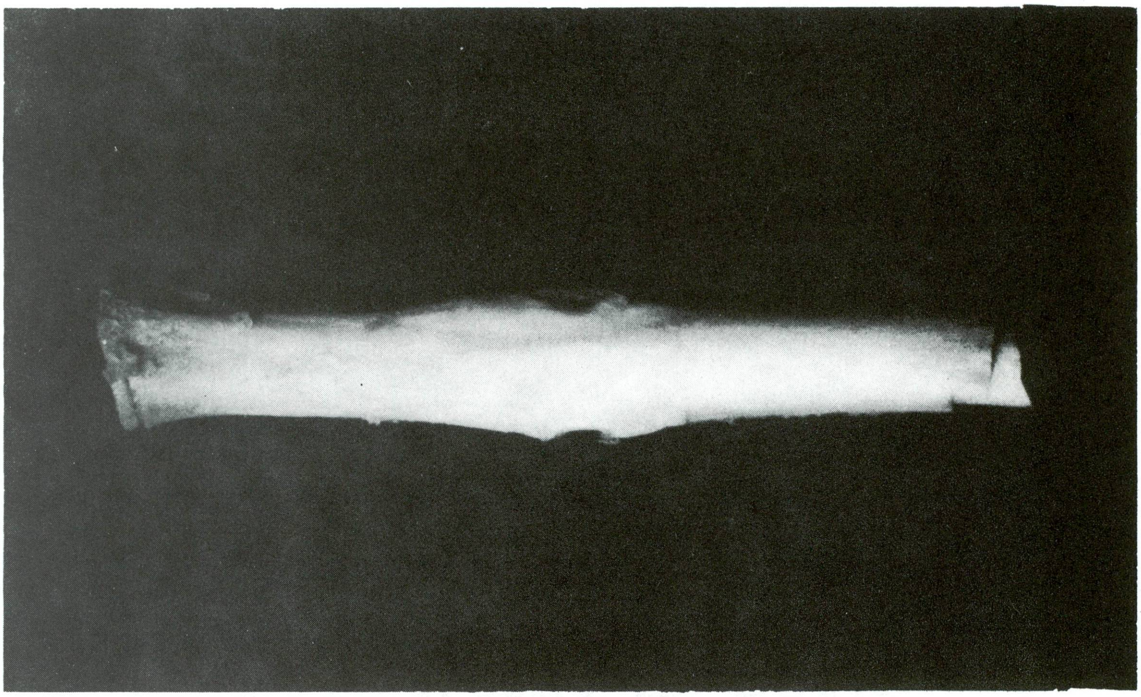

ILLUSTRATION 11

Photograph of Control.

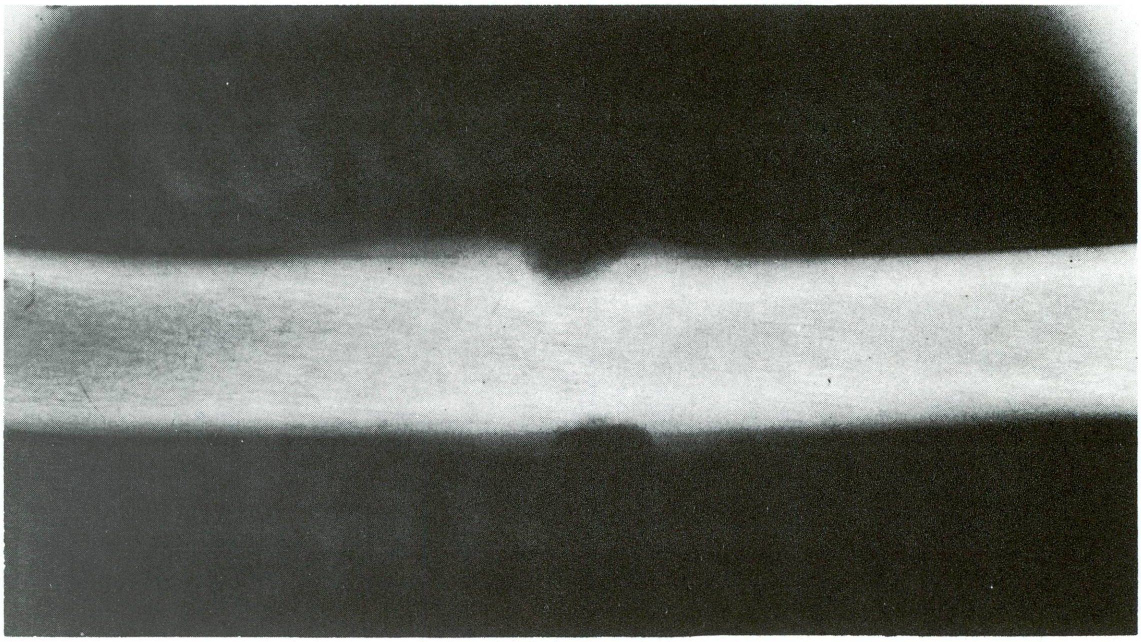

ILLUSTRATION 12

X ray of Control.

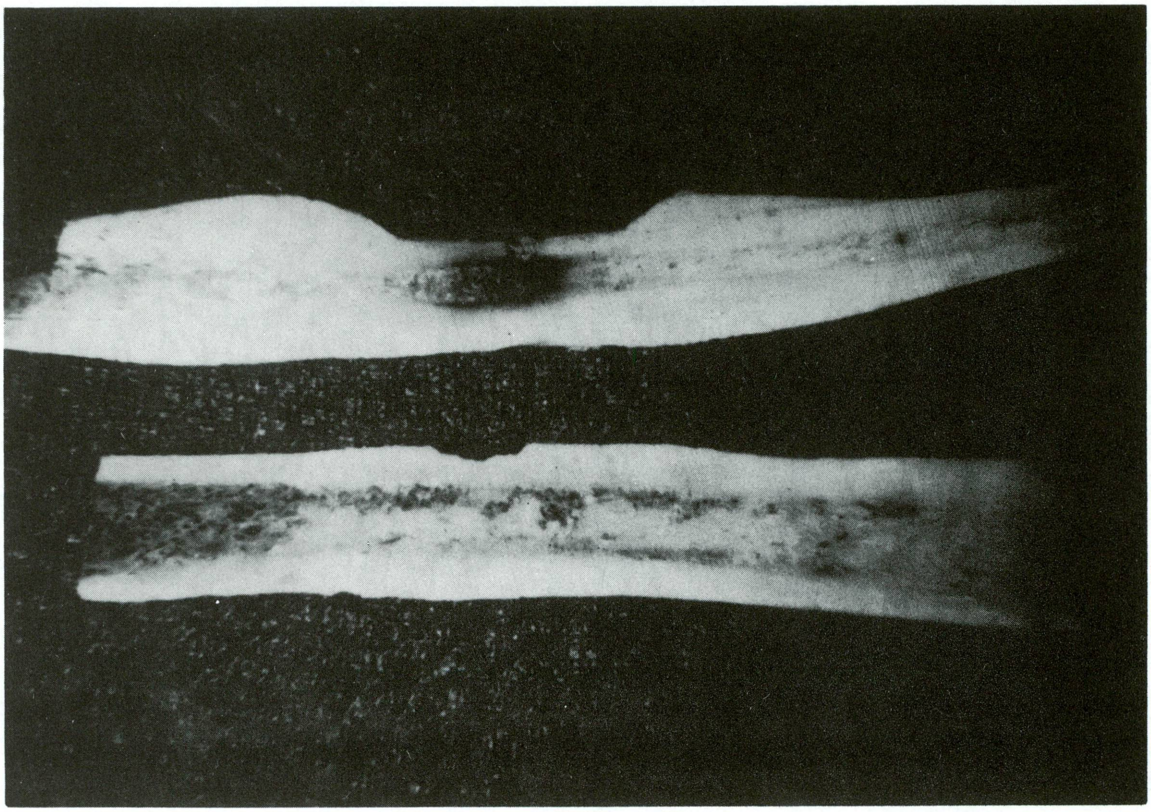

ILLUSTRATION 13

Photograph of Cross-section Comparing Sixty Microampere Direct Pulsating Current Above, with Control Below.

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ABSTRACT

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An Abstract of a Thesis in Partial
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June, 1964

ABSTRACT

The relationship between stress and bony architecture has long been recognized, but the actual cellular machanism which causes the alteration is still somewhat vague. Piezoelectric properties and bioelectric potentials have been proposed as partial answers.

It is the purpose of this investigation to better understand and correlate the relationship of electric potentials or bioelectric potentials to the formation and transformation of physiologic bone.

It is very likely that the crystallites of bone possess the physical properties that would allow them to exhibit the piezoelectric phenomenon.

In the technique of this work a direct current and a pulsating current across the femur shafts of growing dogs were applied for three weeks. The currents ranged from two to sixty microamperes.

The results obtained were in discord with previous literature in that adverse effects to direct current were observed in all cases at the positive poles. Previous literature has claimed osseous growth at both poles, especially the negative pole.

These results are explained in the nature of the electrical field that is produced from a direct current.

The field acts to depolarize the ions in the area. This results in the migration of positive ions away from the positive pole. Such ions of bone as Ca++, K+, Na++ and Mg+ are driven away from this area and result in a breakdown in the inorganic or mineral ultrastructure of the bone.

The conclusions of this thesis are:

1. Direct current adversely effects bone growth.
2. Demineralization occurs at the positive poles.
3. Bony dissolution and necrosis ensues following demineralization.
4. Trauma results in Osseous callus growth with increased mineralization of the area affected.