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ELECTRICAL ENHANCEMENT OF HEALING IN COMBAT INJURIES TO HARD AN--ETC(U)
APR 79 R B TACKE, M H BARTLEY, R W FIELDS DADA17-72-C-2185 NL

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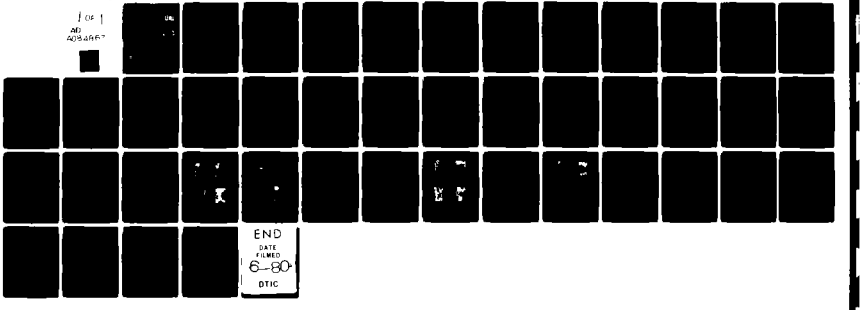

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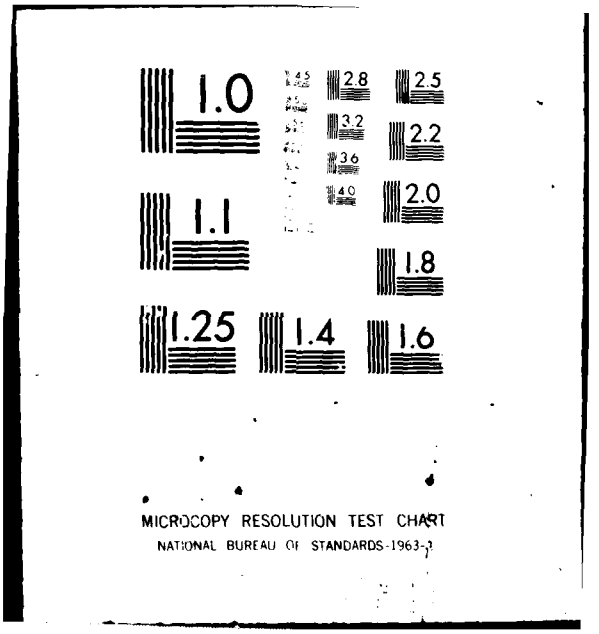
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Over the past decade a large body of experimental evidence has accumulated showing the positive influence of electric currents on bone metabolism. It is evident that modeling, remodeling and repair are regulated in part, through bone's piezoelectric properties as mechanical transducers in a closed loop system. In situations where enhancement of normal function of the transducer system is desirable, viz during bone repair, it would be advantageous to use an exogenous source of electric current.

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PROJECT SUMMARY
1972 - 1977

The research effort entitled "Electrical Enhancement of Healing in Combat Injuries to Hard and Soft Tissues" has resulted in the following:

Contract Year 1972-1973

1. Development of an experimental model capable of quantitative evaluation of the effects of the application of exogenous electric current to a healing circumscribed defect in the dog calvarium.

2. The establishment of all surgical, anesthetic and recovery techniques required for this model were developed.

3. Implanted electrodes were designed and produced.

4. Constant current power supplies were designed and produced.

5. Fourteen animals, ten experimental and four control, were operated and entered into the study.

6. A model for the evaluation of the application of electric current to healing wounds in soft tissue in vivo was developed.

7. A model for the evaluation of the application of electric current to cell cultures in vitro was designed and developed including unique cell culture chambers that are presently in use in several laboratories.

8. A tissue explant culture system was developed to further evaluate the effects of the controlled application of electric current to tissue culture.

9. Baseline values were collected on both animal models and on tissue culture media.

10. The following are offered as summary and conclusions for this contract year:

"Experimental programs are under development to study the feasibility of using electrical currents to routinely enhance the repair of hard and soft tissues. Rapid and efficient assessment depends on an integrated approach involving three distinct programs, listed below, which have been under development and are now mostly operational and productive:

a) Electrical currents in hard tissues in vivo

b) Electrical currents in soft tissues in vivo

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c) Electrical currents in cell and tissue cultures in vitro.

The application of direct currents has been found to influence wound repair in both positive and negative manners based upon the preliminary data to date.

a) Negative Influences. These influences are restricted to the area of the anode and appear to be coagulation phenomenon, probably of proteinacious material.

b) Positive Influences. In vivo studies of hard and soft tissue show more rapid organization of reparative elements and related vascular phenomena, enhanced production of ossified tissue, and increases in the magnitude of systemic indices of healing. The results of initial culture studies suggest electrically dependent enhancement of mitotic activity.

The positive influences of electrical currents on wound healing greatly outweigh the negative influences in efficacy of effect, in the relationship to specific elements of healing of deemed therapeutic importance, and because such negative influences could potentially be removed by placing anodal electrodes at remote locations and in electrical geometries of low current density."

Contract Year 1973-1974

1. The first quantitative evidence of the electrical enhancement of the reparative process in hard tissue was reported.

2. The feasibility of the use of electric current to accelerate the healing of hard tissue defects was established for the first time using quantitative methods that were worthy of statistical analysis.

3. The relative influence of anodal and cathodal current to the reparative process in hard tissue was quantitatively evaluated and measured.

4. The effective period of current application was determined and reported.

5. The effects of three currents densities were measured in the calvarium model and reported.

6. The quantitative data reported from the hard tissue studies was confirmed qualitatively in soft tissue in vivo studies and reported.

7. The relative effects of the anode and cathode were examined in monolayer cell culture and reported.

8. The following observations were stated in the summary of this contract year:

"The application of electric current does accelerate the reparative process in hard tissue."

"The optimal time for the application of this current is in the early stages of the reparative process."

Contract Year 1974-1975

The following was presented for publication as part of the annual summary report for contract year 1974-1975.

Work conducted by our laboratory in the study of the electrical enhancement of the reparative process of hard tissue has resulted in the following:

1. The development of a carefully controlled experimental model productive of quantitative data that can be subjected to rigid statistical analysis.

2. Statistically significant data demonstrating the positive effects of the application of an exogenous electric current to the healing wound in hard tissue.

3. Statistically significant data demonstrating the effect of electrode configuration when direct current is administered to a healing wound in hard tissue.

4. Statistically significant data demonstrating differences in the effects of varied current densities when applied to the healing wound in hard tissue.

5. Significant data demonstrating that administration of exogenous current early in the healing process is more efficacious than later administration.

6. Statistically sound data demonstrating no significant effect between the application of pulsed direct current and alternating current at the 1.0 microampere level.

7. Statistically sound data demonstrating no significant effect between the administration of direct and pulsed direct current at the 1.0 microampere level.

8. Valid observations yet to be confirmed by statistical analysis include a) reversal of the effects of polarity at higher current densities during the administration of direct current; b) the cytopathogenic effect of high-level direct current when applied to cell monolayers; c) the

production of periosteal hard tissue excrescences in fields of high current density; d) alternation in rates of bone apposition in sites remote from stimulated areas.

Contract Year 1975-1976 (This contract year due to delays in funding extends into 1977.)

Qualitative histological evaluation of those observations reported in previous years by this laboratory was pursued during this contract year. Observations included:

1. Enhanced bone formation stimulated by 1.0 microampere direct pulsed current for 21 days occurred in healing bone defects produced in the dog calvarium. This observation was confirmed histologically at 21 days, 42 days, 84 days and 168 days post wounding.

2. Maximum effects occurred in the initial time period of 21 days. Bone produced in the defects and on the periosteal surfaces in juxtaposition to the electrode (cathode) was essentially woven or fibrous bone.

3. Effects varied with current density and were associated with differences in the type of bone deposited. Highly active formation was characterized by trabecular bone while more slowly forming bone was composed of compact lamellar bone.

4. Prominent enhancement of bone apposition occurred at periosteal surfaces and showed two basic patterns; circumscribed outcroppings of bone resembling exostosis or diffuse increases in circumferential lamellae.

5. Complete perforation of the calvarium in localized areas of the defects produced wounds showing only limited tendency to heal. Healing was confined to regions of the defect in which bone was still present on the inferior surface.

6. Prominent inflammation was evident about the electrodes particularly the anode. This was also associated with black granular foreign material presumably produced at the electrode. The apparent breakdown of the electrode was progressive with foreign material seen even at the latest time period of observation (168 days).

7. Quantitative experiments to evaluate the effects of low density electric current on the reparative process in hard tissue in vivo resulted in data cells that did not differ significantly from the unstimulated controls.

Contract Year 1976-1977

A comparison of the effect of electric stimulation in different hard tissues was pursued under this annual contract.

The calvarium, the mandible and the humeri were utilized in each of ten animals. Half of the circumscribed defects were stimulated as test sites while the other half were used as unstimulated controls.
Observations included:

1. Electrical stimulation of circumscribed surgical defects in the calvarium, the mandible and the humerus resulted in significantly increased bone apposition when compared with unstimulated controls.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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ABSTRACT

Over the past decade a large body of experimental evidence has accumulated showing the positive influence of electric currents on bone metabolism. It is evident that modeling, remodeling and repair are regulated in part, through bone's piezoelectric properties as mechanical transducers in a closed loop system. In situations where enhancement of normal function of the transducer system is desirable, viz during bone repair, it would be advantageous to use an exogenous source of electric current.

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INTRODUCTION

Bioelectricity is found to be of great importance in the control of numerous processes among which are such diverse phenomena as: stimulation of the synthesis of macromolecules, limb regeneration, bone wound healing, modeling of bone, thrombosis formation, and cell membrane functions (1-9). This listing is by no means inclusive. Our concern here is with the influence which electrical currents have on healing bone wounds. A number of reports demonstrate electric potentials present in mammalian bone *in vivo*. Bones, when subjected to mechanical stresses, exhibit minute electric potentials in precise geometrical correlation to the mechanical forces of deformation. Another class of potentials, the static or standing potentials, also exists associated, it is thought, with life processes of the cellular populations of the bone tissue (6,10).

The above observations have provided impetus to many investigators involved in the study of both physiology and the healing of wounds to apply currents, both alternating and direct, using a wide range of current density to a variety of wounds (11-18). Varying degrees of success have been reported based upon classical biological and clinical endpoints which include "breaking strength", "bending strength", (tensile and compression strength), radiographic evaluation of the resultant callous and histological examination in hard tissue, the tensile strength of the so-called scar, gross inspection and microscopic examination of the scar in soft tissue. These biological endpoints are of undisputed value but yield qualitative or, at best, semi-quantitative results which do not furnish a direct answer to the question of whether or not the application of current does accelerate or enhance bone apposition. Further, it is reasonable to assume that without collection of quantitative data that can be subjected to statistical analysis the understanding of the mechanism by which acceleration or enhancement of the reparative process resulting from the application of electrical current occurs will be impossible.

Our focus of study is the quantitative demonstration of exogenous electric current enhancement of bone wound healing. After it was established that accelerated healing occurred, it became manifestly important to find out what conditions of employment would optimize the effects of exogenous currents. Time of application, wave forms and electrode configuration were evaluated quantitatively and qualitatively *in vivo* and *in vitro*.

ELECTRICAL CURRENTS IN HARD TISSUE REPAIR *IN VIVO*

GENERAL APPROACH

Over the past decade a large body of experimental evidence has accumulated showing the positive influence of electric currents on bone metabolism (19-26). It is evident that modeling, remodeling and repair are regulated through bone's piezoelectric properties as mechanical transducers in a closed loop system (1,2,7). In situations where enhancement of normal function of the transducer system is desirable, viz during bone repair, it would be advantageous to use an exogenous source of electric current.

One must keep in mind that there is a direct positive correlation in a given bone or portion thereof between the amount of calcified tissue (as determined by bone ash values) and the compression or "breaking strength" (27-28). It follows from this that the degree of bone healing is also directly related to the quantity of bone being formed in the wound. The rate of bone production can be directly determined by tetracycline labeling.

In previous annual reports we have shown that the application of exogenous currents accelerates the repair of wounds in bone. In addition these reports have yielded data suggesting what conditions might be more efficacious and establishes them quantitatively. Questions regarding the relative advantages or disadvantages of electrode configuration, wave forms and time of application have also been specified. The question posed in a previous annual report regarding possible mechanisms by which repair enhancement occurs was elucidated qualitatively in a serial sacrifice study of the histologic steps encountered in healing sites. The quantitative data, however, always supersede in biological importance the qualitative findings because the fundamental, practical questions to be answered remain: "What are the rates at which bone forms during healing process (normal and abnormal)?" and, further, "Does the electrical current supplied change these rates significantly?" A means of measuring bone accretion rates will always be necessary in order to validate any observed qualitative processes assumed to be instrumental in the acceleration of bone repair. Due caution must be exercised during the interpretation of qualitative studies not to confuse bone reparative processes with remodeling of bone.

In the past, we have used radiographic and histologic data to monitor the progress of the experiment. Paralleling the quantitative bone accretion studies (with tetracycline labeling), rat *in vivo* and primate fibroblast tissue and cell culture *in vitro* studies were conducted to inspect certain aspects of current level, wave form and electrode materials as well as to expedite feedback of information to the experimenter.

Below is a detailed account of our activities through June 1975.

Genetically-defined dogs, 10-12 months of age, weighing 25-35 lbs. total body weight were utilized in the in vivo quantitative measurement of calvarium defect repair. Under strict asceptic techniques with the animal in the prone position, general anesthetic was administered and the parietal bones were reached through a midline-incision extending from the occipital protuberance along the length of the sagittal crest.

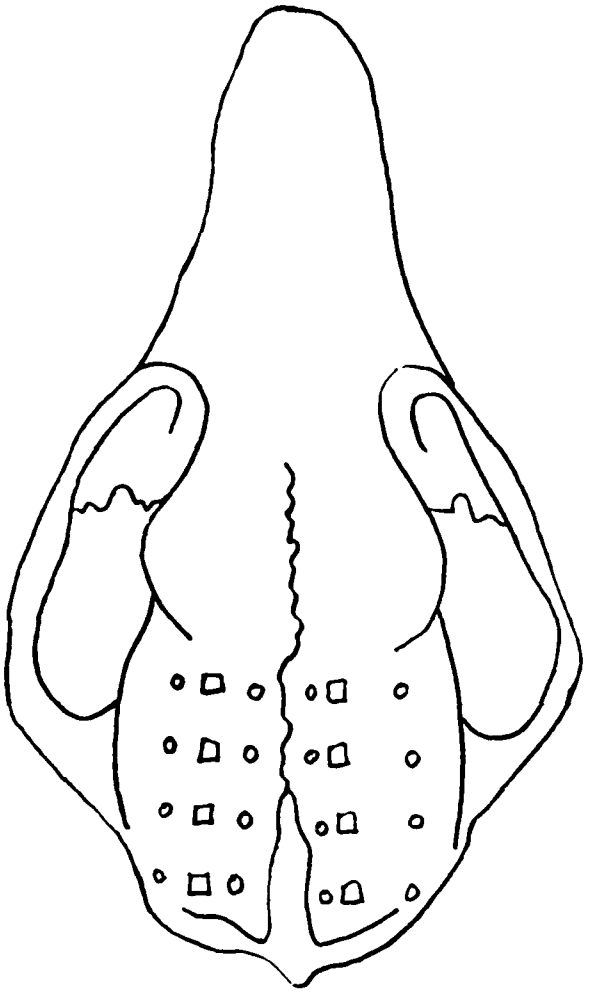
Fascia and muscles were separated by sharp dissection and retracted laterally on each side, exposing the parietal bones. Four circumscribed defects, 4 mm square, were cut through the cortical and spongy bone into the marrow of each bone using a dental handpiece with a concurrent flushing of the area with large volumes of physiological salt solution. Hemostasis was maintained in the defect using Gelfoam (29). Threaded stainless steel electrodes, 2 mm in diameter, with 34-gauge, Teflon-coated (8), stainless steel wire attached were (1) in the case of the balanced electrode system, implanted 8 mm from the center of each defect; and (2) in the case of the unbalanced electrode system, one electrode was implanted 4 mm from the center of the defect while the other was implanted 8 mm from the center of the defect (refer to Fig. 1). These wires were brought from the electrode site through the muscle and fascia and finally exposed through a small skin incision on the dorsal aspect of the neck of the animal at the fourth cervical vertabra. Hemostatic material was removed, and the entire area was again rinsed with large volumes of saline to remove debris. Muscle and fascia were approximated and closed using absorbable, silk, interrupted sutures. A small constant current, alternating or pulsed direct current power supply (refer to Fig. 2) was attached to the appropriate electrode pair, and the power unit with the additional electrode pairs was wrapped in a sterile dressing around the neck of the animal. This dressing was periodically removed and replaced to change electrode pairs and for routine inspection. Fully charged power sources were utilized for each healing interval. The current output of each power supply was measured prior to its use and upon its removal from the animal. In the rare instance of malfunction the data from the healing defect involved was considered unsuitable for analysis.

In the initial series of experiments, a constant direct current from a battery operated constant current power supply of 0.10 microampere was applied to a separate electrode pair for each of three consecutive 14-day healing intervals. This series was repeated using 1.0 and 10.0 microamperes. During the period of current administration, daily physical examination and weekly hematological and serum chemistry evaluations were reported on each animal to provide specific evidence of the time course of systemic indices of healing and to help identify individual abnormalities. Tetracycline hydrochloride (30), a label of bone apposition (31), was administered intravenously (10 mg/kg total body weight) at 1, 3 and 5 weeks postoperatively.

On completion of the experimental protocol, the 42nd postoperative day, the animal was sacrificed and a complete autopsy was performed, with special attention being given to all factors that are related to the reparative

FIGURE 1

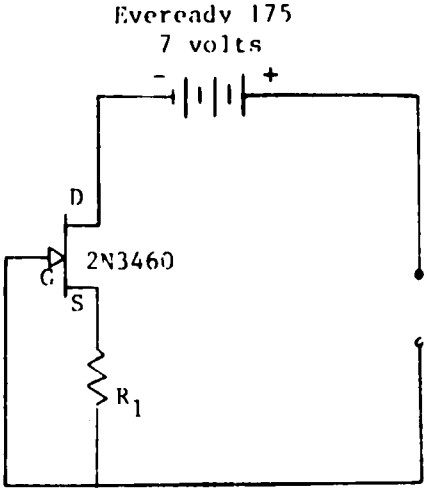
General Arrangement of Bone Defect - Electrode Pair Complexes



Top view of dog cranium indicating the general arrangement of the eight bone defect-electrode pair complexes. Each complex consists of a central defect 4 mm square straddled by two remote electrodes. The left depicts the balanced electrode system and the right the unbalanced electrode system. The electrical circuit of each complex is completely isolated from the circuit of other complexes and from ground, limiting the current flow between a particular electrode pair to a uniform density through the associated defect without spread to neighboring sites.

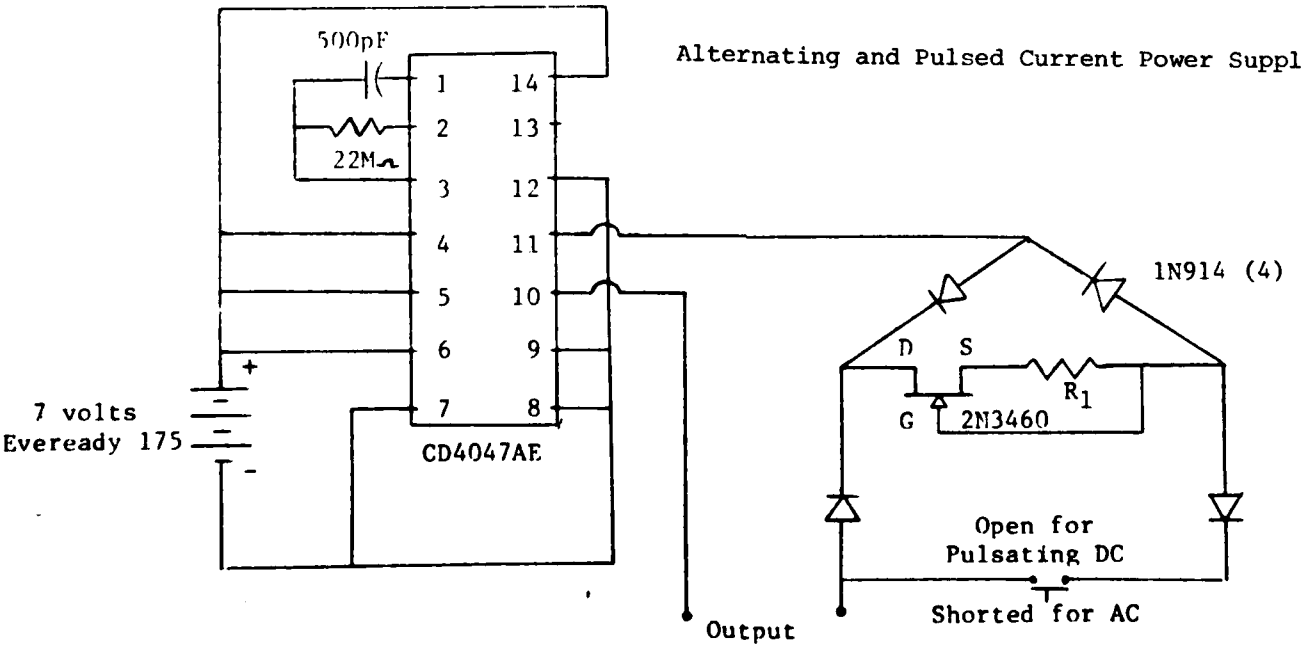
FIGURE 2

Power Supply - Schematic Diagrams



Direct Current Power Supply

R_1 - Selected to match individual 2N3460 to yield desired power output



Alternating and Pulsed Current Power Supply

R_1- Selected to match individual 2N3460 to yield desired power output

process. Parietal plates including the defects were removed, radiographed and fixed in acetone from which ground sections were prepared for tetracycline flourescent labeling analysis of the appositional bone growth. The experimental series were designed to provide both histological examination and flourescent analysis of two distinct specimens from each defect. Each animal affords a total of 8 defects as depicted in Fig. 1. Four of these were chosen at random as the test specimens, and the remaining 4 were controls, prepared in exactly the same manner as the other 4; but the subsequent administration of current was deleted. Additional controls included animals with defects but lacking implanted electrodes in order to determine the potential effect of the presence of stainless steel electrodes, and animals having both defects and electrodes but lacking current administration at any site in order to evaluate the effects of current spread from a test defect to the control sites.

Analysis of 100-150 micron-thick, ground, undecalcified, calvarium sections was accomplished utilizing a Leitz fluorescent microscope and an eyepiece micrometer (Bausch and Lomb). The mean of 12 micrometer measurements was recorded for each defect, test and control (31).

Daily physical examinations including body temperature, pulse rate and a description of the general state of health were maintained on each animal throughout the experiment.

All animals showed a minor temperature elevation (less than 1^{o} F.) for 1-2 days postoperatively with a return to normal by the third day. Pulse rate showed no significant change. All animals were described as appearing in either good or excellent health on the first postoperative day with the exception of three that showed mild to marked edema in the region of the head and neck. This was immediately alleviated by loosening the bandage around the neck of each animal to allow adequate venous return. These animals were then described as appearing in good or excellent health on the following morning.

At sacrifice, all animals were described as fully-developed, adult dogs in apparent good to excellent health, each showing a completely healed incision extending anterior from the occipital protuberance along the entire length of the sagittal crest.

The autopsies performed through a standard Y incision showed all organs of the thorax and abdominal cavities to appear within normal limits in the dogs examined. On gross examination, all the defects of the animals that had received current were in a more advanced state of repair than those animals that had not received current. Also by gross examination it appears that those defects having directly received current in the earlier stages of the reparative process show slightly more advanced repairs than both those receiving current in the later stages of repair and those defects in the same animal having received no current. This gross observation was confirmed by examination of the post-mortem radiographs.

Three direct current electrode configurations have been examined at 3 different current levels. The results of the aforementioned studies are summarized in Table I. We have extended the recent work to include comparisons of alternating, direct and pulsed direct current effectiveness in enhancing the rate of bone wound healing. These comparisons have been made at two current levels, 1.0 microampere and 0.1 microampere. These findings are presented in Table II. The effect of time of application has been refined and data compared from three time intervals (1-2 wk., 3-4 wk. and 5-6 wk.) utilizing direct, alternating and pulsed direct currents (Tables III and IV). Quantifications of data from all the studies ennumerated above is presented in units of bone accretion (microns) over the experimental period.

1. Effect of Electrode Configuration and Current Levels on the Rate of Bone Healing (Table I)

   Balanced electrode configurations at two current levels, 0.1 microampere and 1 microampere showtan approximately two-fold increase in the amount of bone accretion during the period of experimental stimulation. Both current levels, futhermore, under these conditions are not significantly different in their ability to effect rate changes. Inspection of the table will also show a remarkable reversal of effects at the proximate anode between the 0.1 microampere level (113 $\pm$ 79 μ) and the 10 microampere level (1162 $\pm$ 311 μ). The cathode placement proximate to the defect showed an increase uniformly in accretion rates at the three current levels employed.

2. Effect of Alternating, Direct and Pulsed Direct Current on the Rate of Bone Healing (Table II)

   Stated simply, all wave forms employed are capable of significant stimulation of bone defect healing. Individual comparisons of AC to DC, or AC to DC pulsed, or DC to DC pulsed failed to show significant differences between them.

3. Effect of Application Time and Wave Form on Bone Healing with Electric Currents (Tables III, IV and V)

   In a previous report (Table III) we noted a significant enhancement of healing when an early time period (1-3 wks.) was chosen for current application versus normal rates when the current was applied in a later time period (4-6 wks.) following wounding. Our recently-completed studies (Tables IV and V) have demonstrated that application at two early intervals, namely 1-2 wks. and 3-4 wks., show significant increases of bone accretion with direct (pulsed) current and alternating current. A fascinating additional finding is that when alternating current is employed in the 5-6 wk. interval, a significant enhancement of bone formation occurred. This finding is rather unexpected in view of

TABLE I

Bone Accretion in Circumscribed Surgical Defects in the Dog Calvarium Under Three Different Current Levels of Electric Stimulation and Three Electrode Positions.*

	Control	0.1 uA	Control	1.0 uA	Control**	10 uA
Balanced Electrode Configuration	461 ± 333 μ	807 ± 193.25 μ	475 ± 254 μ	865 ± 433 μ		
		t = 2.65 P 0.010 n = 20		t = 5.30 P 0.005 n = 82	Not Done	
Anode Proximate to Defect	461 ± 333 μ	113 ± 79 μ	475 ± 254 μ	518 ± 299 μ	481 ± 305 μ	1162 ± 311 μ
		t = 2.27 P 0.025 n = 17		t = .460 P Not Sig. n = 65		t = 5.09 P 0.005 n = 28
Cathode Proximate to Defect	461 ± 333 μ	785 ± 354 μ	475 ± 254 μ	1106 ± 682 μ	481 ± 305 μ	905 ± 370 μ
		t = 2.15 P 0.025 n = 21		t = 5.38 P 0.005 n = 67		t = 3.11 P 0.005 n = 29

*Measurements in μ ± standard deviation

**Control values in the 10 uA cell are derived from animals not having received current.

TABLE II

Comparison of Direct Current, Pulsed Direct Current and Alternating Current
At the 1.0 Microampere Level

1 Microampere Alternating Current vs. Control

$864 \pm 415\ \mu$ $470 \pm 218\ \mu$

$t = 7.410$
$P \leq 0.001$
$n = 47$

1 Microampere Direct Current (Pulsed) vs. Control

$877 \pm 495\ \mu$ $470 \pm 218\ \mu$

$t = 3.778$
$P \leq 0.001$
$n = 51$

1 Microampere Alternating Current vs. 1 Microampere Direct Current (Pulsed)

$864 \pm 415\ \mu$ $876 \pm 495\ \mu$

$t = 0.245$
$P \leq$ Not Sig.
$n = 48$

1 Microampere Direct Current vs. 1 Microampere Direct Current (Pulsed)

$1106 \pm 682\ \mu$ $877 \pm 495\ \mu$

$t = 1.155$
$P \leq$ Not Sig.
$n = 37$

1 Microampere Alternating Current vs. 1 Microampere Direct Current

$864 \pm 415\ \mu$ $1106 \pm 682\ \mu$

$t = 1.276$
$P \leq$ Not Sig.
$n = 33$

TABLE III

Early vs. Late Application of Direct Current (1.0 Microampere) to Circumscribed Surgical Defects in the Dog Calvarium

TREATMENT PERIOD:	BONE ACCRETION DURING THE EXPERIMENTAL PERIOD:
Control	488 $\pm$ 289 μ
*Early Application	890 $\pm$ 254 μ
**Late Application	371 $\pm$ 222 μ

t Test Control vs. Early Application
n = 48, t = 4.54, Significant at $P \leq 0.01$

t Test Control vs. Late Application
n = 48, t = 1.37, Not Significant

*Early application period = 1st 3-wk. period (Wks. 1-3)
**Late application period = 2nd 3-wk. period (Wks. 4-6)

TABLE IV

Comparison of Current Application at Varied Time Intervals During the Reparative Process

1 Microampere Direct Current (Pulsed)

Interval of Current Application Week (Post-Op)	1 - 2	3 - 4	5 - 6	Control
Bone Accretion in Microns	880 ± 350	924 ± 447	449 ± 296	410 ± 245

1-2 Wks. vs. Control — $t = 3.349$, $P \leq 0.005$, $n = 18$

3-4 Wks. vs. Control — $t = 3.360$, $P \leq 0.005$, $n = 22$

5-6 Wks. vs. Control — $t = 0.294$, $P \leq$ Not Sig., $n = 13$

1-2 Wks. vs. 3-4 Wks. — $t = 0.223$, $P \leq$ Not Sig., $n = 18$

1-2 Wks. vs. 5-6 Wks. — $t = 2.376$, $P \leq 0.05$, $n = 14$

3-4 Wks. vs. 5-6 Wks. — $t = 2.331$, $P \leq 0.05$, $n = 18$

TABLE V

Comparison of Current Application at Varied Time Intervals During the Reparative Process

1 Microampere Alternating Current

Interval of Current Application Week (Post-Op)	1 - 2	3 - 4	5 - 6	Control
Bone Accretion in Microns	840 ± 482	1262 ± 605	803 ± 371	473 ± 201

1-2 Wks. vs. Control	t = 2.149 P ≦ 0.05 n = 16
3-4 Wks. vs. Control	t = 3.801 P ≦ 0.005 n = 17
5-6 Wks. vs. Control	t = 2.437 P ≦ 0.025 n = 19
1-2 Wks. vs. 3-4 Wks.	t = 1.327 P ≦ Not Sig. n = 13
1-2 Wks. vs. 5-6 Wks.	t = 0.168 P ≦ Not Sig. n = 15
3-4 Wks. vs. 5-6 Wks.	t = 2.055 P ≦ 0.05 n = 16

previous work in this laboratory which showed only early periods of applications effective.

When examining the time periods (1-2, 3-4 and 5-6 wks.) compared to each other at 1 microampere direct (pulsed) current, it is evident that the two earlier time periods (1-2 and 3-4 wks.) do not show a particular advantage one over the other (880 $\pm$ 350 μ vs. 924 $\pm$ 497 μ), but they both yield a significant advantage over currents applied in the latest period (5-6 wks., 449 $\pm$ 296 μ).

In consideration of the data from the 1 microampere AC study, it is equally evident that enhanced healing patterns exist with all accretion rates in the three intervals elevated over controls. It is further evident that at the middle time period (3-4 wks.) healing rates are slightly more elevated though not to a significant degree over the first interval (1-2 wks.) but are significantly elevated over the third time period (5-6 wks.).

In five animals we created eight surgical defects but no electrodes nor current were utilized. In an additional five animals eight defects and their associated electrodes were placed but current was not applied. The bone appositon rate from these defects did not differ significantly from comparable control sites in animals which did receive current application in test sites.

All data reported in the tables were examined using a student t test with the knowledge that they failed to meet all of the assumptions required for parametric analysis. This method was utilized for convenience and it was assumed that the reported significance levels would be lower with its use than with the use of nonparametric analysis. Portions of the data were subsequently analyzed using nonparametric analysis and in all cases the level of significance proved to be more significant than when examined by parametric analysis.

Experiments conducted from October 1976 through April 1977 utilized the same fundamental approach as those reported above and were designed to expand the calvarium model of wound healing to include an in depth histological examination of the effect of the application of exogenous electric current over an extended temporal period and to investigate the effects of lower current densities on the reparative process in hard tissue.

The histological evaluation utilized the experimental model described above (page 3, paragraphs 1 and 2) the following were examined histologically.

1. Circumscribed surgical defects receiving 1.0 microampere pulsed direct current constantly for 21 days post-wounding utilizing the unbalanced

electrode system (cathode proximate to defect) were compared to similar defects that had received no stimulation in a sequential series at 3, 6, 12, and 24 weeks post-wounding.

2. Sections of calvarium (no surgical defect) receiving 1.0 microampere pulsed direct current in an electrode system of identical geometric dimension used above for a period of 21 days post-surgical electrode implant were compared to analogous sections in the same sequential series described in Condition 1.

3. Circumscribed surgical defects from specimens having no electrodes were evaluated at the end of the 6 month period.

4. Circumscribed surgical defects from specimens having electrodes implanted but having received no electrical stimulation were evaluated at the end of the 6 month period.

5. Sections of calvarium from specimens having received electrode implants but no surgical defects and no stimulation were examined at the end of the 6 month period.

Conditions 1 and 2

A total of sixteen animals were operated and stimulated for twenty-one consecutive days post-surgery with 1.0 microampere pulsed direct current utilizing the unbalanced electrode system (cathode proximate to defect).

Four animals were sacrificed at the end of the twenty-one day period, four were sacrificed at the end of a six week period post-surgery, four at the end of twelve weeks and the remaining four at the end of twenty-four weeks. This provided a total of eight sites per experimental condition and period of healing for histological examination.

The test and control sites from all animals having surgical defects consist of a rectangular area of bone extending 1.0 millimeter beyond the lateral borders of the surgical defect and 2.0 millimeters beyond each electrode. The test and control sites of specimens having no surgical defects consist of a rectangular area of bone extending 2.0 millimeters beyond each electrode pair and 3.0 millimeters laterally from an imaginary line connecting the two electrodes (this area represents the same area that is examined in those sites having surgical defects.

Following decalcification and paraffin embedment, the sites were sectioned at 6 microns paralleling the plane connecting the two electrodes. All sections were retained, however, selected sections were stained with hematoxylin and eosin and mounted for microscopic examination.

One section per each one-half millimeter of the site were examined (approximately 12 sections per site).

Conditions 3-5

Using the same calvarium healing model, three additional dogs were subjected to surgical intervention at the beginning of the experiment. Of 24 available sites, eight were randomly selected for each of the following conditions: surgical defect with no electrodes, electrodes with no surgical defects and surgical defects with electrodes. All animals were sacrificed at the end of a 24 week period. Specimens were processed in the same manner described for Conditions 1 and 2.

Histological examination of those circumscribed defects having received 1.0 microampere pulsed direct for a period of 21 days post wounding that were harvested on the 21st post wounding showed more active bone formation than in the unstimulated control sites. This is evidenced by a greater amount of woven bone in the electrically stimulated regions as well as increased numbers of bone forming sites together with increased size or length of surface involved in new bone appositon. The cells involved are also larger, more cuboidal with prominent cytoplasmic basophilia in the stimulated sites in contrast to control sites where frequently the cells are flatter assuming a less active histological appearance with smaller amounts of cytoplasm, Figure 3A and 3B. The differences between positive and negative poles were primarily in the degree of inflammatory infiltration and bone formation about the respective poles. Consistently there was greater inflammation about the anode than around the cathode. Also evident was fine black granular material strongly suggestive of metal particles in the region of the electrode but primarily in the region of the anode. The inflammatory infiltration entered into nearby marrow spaces as well as along the superior periosteum laterally for some considerable distance in the 21 day series of animals. Also noted was extensive periosteal bone formation in the juxta-electrode regions primarily about the cathode. Much of this bone was in the form of lamellae but in those animals with more activity stimulated osteogenisis there is the formation of trabeculae in the configuration of a "T" extending perpendicularly from the periosteal surface. These have been described by Lacroix (32) as indicating exceedingly active bone formation. The excrescences of bone thus formed by electric stimulation strongly resemble exostosis. As one examined the lateral sections (those cut from the lateral aspect of each defect parallel to an imaginary line connecting the anode and cathode) the following changes were noted: there was bone formation on the walls and completely across the floor of the defect, of course greater in amount in the stimulated sites. In some stimulated sites bone formation in the center of the defect actually was in excess of that on the periphery but in most instances the central areas showed bone formation which lagged slightly behind that on the lateral walls.

In those cases where there was perforation of both inner and outer tables little or no bone was formed in the area of perforation even when electrically stimulated though bone was formed on adjacent areas where the floor of the defect was intact. Also noted was the tendency for excess bone formation on the inner table when the electrode was placed rather closer to the inner table surface.

Sporadically, diploic spaces and vascular channels showed apposition of lamellar bone on the bony walls of these spaces which produced the appearance of osteosclerosis. In some instances the formation was situated on the walls of these spaces and in contact with electrodes in a manner suggesting that current flow may have followed vascular pathways.

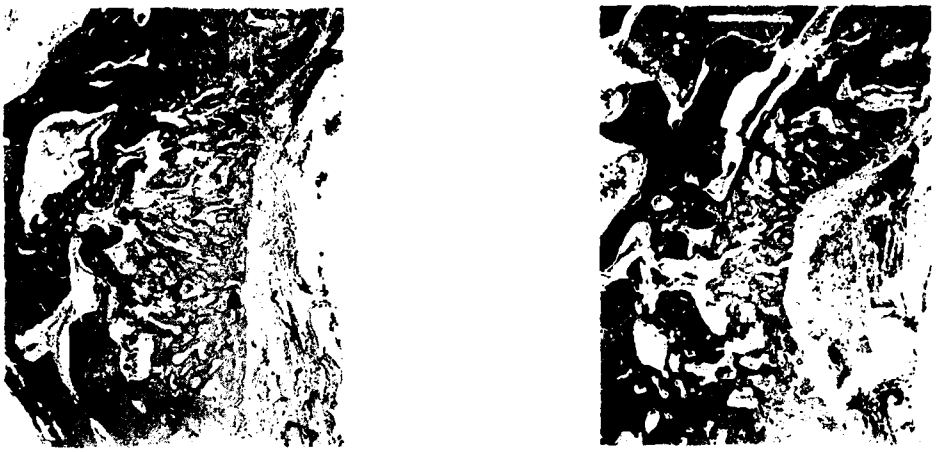
Defects having received current for 21 days that were sacrificed at 42 days post wounding were more advanced or mature in their healing than those at 21 days. The trabeculae are broader and many are composed of lamellar type bone instead of the woven bone prominent in the earlier periods of healing. Periosteal bone is still being laid down particularly about the electrodes. Osteoblastic activity, however, is leveling off. There are fewer and smaller areas of bone formation with the presence of less active appearing osteoblasts. The inflammatory reaction around some of the electrodes has caused extensive bone destruction in these sites. The infiltrate is polymorphous containing admixtures of neutrophils, macrophages, lymphocytes and plasma cells. The areas of complete calvarium perforation as in the 21 day animals showed little tendency to close, Figure 4A and 4B.

Defects having received 1.0 microampere pulsed direct current for a period of 21 days post wounding that were harvested on the 84th day post wounding showed bony defects nearly filled with mature lamellar bone. This was true of treated and non-treated sites. Almost all the defects showed a slight central depression which was deeper, in some instances significantly so, in the control sites. The control defects also were more porous and still composed of thick trabs of bone while stimulated sites were denser having smaller vascular spaces and showed conversion to osteonal bone. Considerable inflammation was present about many of the anodes in which current had been applied, Figure 5A and 5B.

At 168 days sacrifice the defects treated during the first 21 days with 1.0 μ amperes pulsed direct current showed conversion of the trabecular bone in the defect to well ordered lamellar bone much of which was in the form of concentric lamellae or osteonal bone. Treated and controls showed slight depression over the central portion of the defect sites as was mentioned above for the 84 day period.

Areas of bone formation on the periosteal bone surfaces showed attempts at remodeling and were somewhat reduced in size from those observed at earlier time periods. These areas were often difficult to discern because the external bone contours had been smoothed out presumably by osteoclastic activity. Osteoclasts themselves were not numerous on these surfaces but adequate time was available for these cells to have made their presence, performed their clastic activity and disappeared before observation could be made. Ordinarily osteoclastic activity during remodeling is a much more rapid process than bone apposition. There may be at least an order of magnitude difference in their rates.

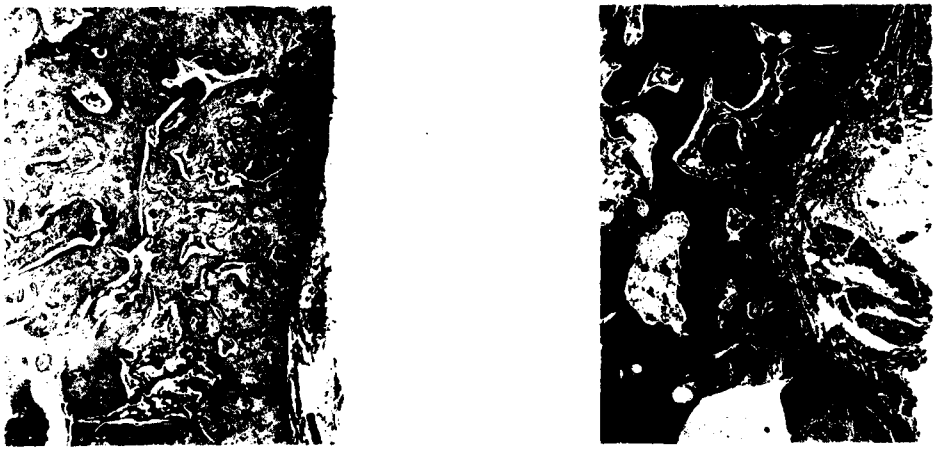
FIGURE 3



A B

Comparison of circumscribed surgical defects harvested 21 days post wounding. Figure 3A received 1.0 microampere D.C. for 21 consecutive days post wounding. Figure 3B was not electrically stimulated.

FIGURE 4



A B

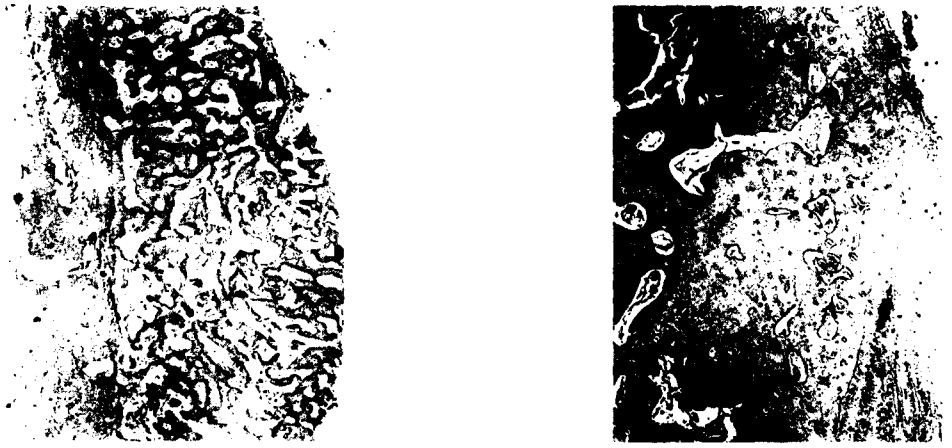
Comparison of circumscribed surgical defects harvested 42 days post wounding. Figure 4A received 1.0 microampere D.C. for 21 consecutive days post wounding. Figure 4B was not electrically stimulated.

FIGURE 5

A B

Comparison of circumscribed surgical defects harvested 84 days post wounding. Figure 5A received 1.0 microampere D.C. for 21 consecutive days post wounding. Figure 5B was not electrically stimulated.

FIGURE 6



A B

Sections of calvarium harvested 21 days post electrode implant. Figure 6A received 1.0 microampere D.C. for 21 consecutive days post electrode implant. Figure 6B was not stimulated.

Histological examination of sections of calvarium that received 1.0 microampere pulsed direct current but did not have circumscribed defects for a period of 21 consecutive days post surgical intervention (see methods section page 7 paragraph 9) that were harvested upon cessation of the current showed mound-like excrescences of bone formed about the cathode on the periosteal surfaces. These resembled exostosis occasionally found on the periosteal surfaces of bone in otherwise normal persons untreated by electrical current. It is assumed in these latter instances that genetic influences are at work. In addition to these rather circumscribed areas there is a more diffuse increase in the rate of periosteal bone formation, greater near the cathode and gradually diminishing in intensity as the anode is approached. About the anode there often appears actual diminution of bone growth and/or frank destruction.

Bone being laid down on the outer table periosteal surface varies in character from rapidly forming trabecular bone to slowly forming circumferential lamellae. In the most active sites perpendicular trabeculae of bone growth from the periosteal surfaces and gradually assume a "T" shape as cross members sprout laterally from their superior aspect. These then fuse with those of nearby trabeculae and a new periosteal surface is created. The marrow spaces formed below the surface then gradually fill with lamellar bone or osteons.

Also observed was the tendency for lamellae of bone to form on the lateral walls of diploic or marrow spaces thus producing a minor degree of osteosclerosis. The tendency for bone filling of these spaces diminished as one left the region of the cathode.

Considerable inflammation of bone is present about the electrode particularly the anode with extension into the nearby diploic or marrow spaces and for some distance along the periosteum. In these earlier time periods the exudate is predominantly neutrophilic.

When stimulated areas were compared to sections of calvarium that went unstimulated until harvest at the 21 day interval the following differences were manifest. No exostosis or bony excrescences were to be seen in untreated sites, and concomittantly, there was little or no inflammation about the electrode. In almost every instance the serations of the screw electrode could be discerned. Occasional inflammatory cells could be seen in some cases present in the periosteal connective tissue. This was an adventitious finding and was far from consistent.

Examination of the sections in the region of the negative electrode showed that the effect was carried to the margins of resection of the specimen site, i.e. bone formation was diffuse over the experimental site being evaluated but concentrated within the space of 2 mm. or so from the electrode; while

the regions more proximate to the positive electrode showed diminished bone formation or actual destruction in the peri-electrode region associated with a robust inflammatory response and apparent disintegration of the electrode. Foreign granular material, however, was not as evident in these sections as in those from later time periods.

Examination of the sections of calvarium that were cut lateral to the imaginary line connecting the two electrodes demonstrated that maximum bone formation was occurring in the region directly between the electrodes in the stimulated sections and revealed no inordinate degree of periosteal bone formation in the unstimulated controls. Figure 6A (stimulated) and Figure 6B (control) illustrate the above observations.

Those sections of calvarium receiving current for 21 consecutive days post electrode implant and harvested 42 days post implant showed a conversion of woven trabecular bone (callus) seen at 21 days to a more mature lamellar bone with thicker trabs and the conversion of trabecular periosteal bone to circumferential lamellae on the periosteal surface when compared to the control sections harvested at the 42 day interval. Histological differences between the positive and negative electrodes included as usual a greater degree of bone destruction and inflammation about the electrodes with more extreme loss of bone about the anodes.

Lateral aspects of the stimulated sections showed extension of the effects to the borders of the experimental site. Effects gradually diminish towards the edges, however, and are most prominent for 2 mms. or so from the anode when compared to the same area in the control sections. Figure 7A (stimulated) and 7B (control) illustrate the above observations.

Sections of specimens that were stimulated for 21 days following the electrode implant and harvested on the 84th day showed total conversion of bone formed as a consequence of electric stimulation to lamellar type bone. There is some tendency for recontouring of the periosteal surface with slight diminution of the periosteal bone excrescences while their respective controls were entirely devoid of discontinuities and bony exostoses.

Examination of the negative electrode showed as in the other series inflammation about the cathode but less inflammation than around the anode.

Sections from the lateral aspects of defects showed newly formed bone while little or none was evident in control sections. Figure 8A (midline section) and 8B (lateral section) illustrated these observed differences.

Sections from the lateral aspects of defects showed newly formed bone while little or none was evident in control sections. Figure 8A (midline section) and 8B (lateral section) illustrated these observed differences.

FIGURE 7

A B

Sections of calvarium harvested 42 days post electrode implant. Figure 7A received 1.0 microampere D.C. for 21 consecutive days post electrode implant. Figure 7B was not stimulated.

FIGURE 8

A B

Sections of calvarium harvested 84 days post electrode implant. Figure 8A received 1.0 microampere D.C. for 21 consecutive days post electrode implant and was taken from the center of the specimen. Figure 8B, from the same specimen, is taken from its lateral edge.

Examination of sections of calvarium stimulated for 21 days following electrode implant and harvested on the 168th day post implant showed slight irregularities of the periosteal surface due to new bone formation about the cathode with extension toward the anode. This bone is lamellar but there is evidence of recontouring and associated with the presence of occasional giant cells. Their respective controls showed no changes from previous time periods. The surfaces are smooth.

Examination of the negative electrode site showed more bone apposition near the anode. There is somewhat less inflammation than in previous time periods but there is still some persistence of an inflammation reaction about the electrodes and evidence of black granular foreign material in these regions.

Comparison of these sites with electrodes that had not been stimulated showed little or no inflammatory reactions about the electrodes and little or no new lamellar bone found on periosteal surfaces. Figure 9A (stimulated) and 9B (unstimulated) compare these respective electrodes both positive and negative. Examination of sections taken from the lateral borders of stimulated specimens showed extension of effects nearly to the border of resection when compared with their respective controls.

Histological examination of surgical defects from dogs that received no electrical stimulation harvested on the 168th day post wounding showed no marked increase in the amount of new periosteal, circumferential lamellar bone nor surface irregularities due to exostosis formation. There was formation of bone in the defects which was not at this stage markedly different in quality and quantity from stimulated sites.

When compared to the control defects from the 168 day test dogs, no histological differences were noted.

The above defects from unstimulated animals, when compared to the stimulated defects harvested at 168 days post wounding showed little difference histologically either as to quality or quantity of bone formed except in those areas noted previously in which there was complete perforation.

Unstimulated surgical defects having electrodes implanted when compared to defects having no electrodes implanted showed no essential differences from one another.

Unstimulated specimens of calvarium from between implanted electrodes when compared to unstimulated surgical defects from animals receiving no current that were sacrificed on the 168th day post surgery were also similar in their essential details to series in the preceding two paragraphs.

FIGURE 9

A B

Comparison of sections of calvarium harvested 168 days post electrode implant. Section 9A received 1.0 microampere D.C. for 21 consecutive days post implant. Section 9B was not stimulated.

Pulsed direct current at the 1.0 microampere level again demonstrated its ability to stimulate bone formation. The qualitative, histological investigation confirmed previous quantitative studies of bone defect healing using precise tetracycline labelling techniques. Bone produced under the influence of electric current varied in amount and composition as a function of time following application. Early in the course of wound healing the new bone showed rapid growing "fibrous" or "woven" bone composed of thin trabeculae possessing a matrix of interwoven collagen fibers. Most of these trabeculae were initially arranged at right angles to the defect surface. As impressive as new bone formation was in the defect the growth of trabeculae from the periosteal region about the cathode was even more remarkable. In these sites the formation of "T" shaped trabeculae indicated an extremely rapid growth rate in the initial 21 day observation period. Two millimeters or so from the electrode this morphologic sign of rapid growth showed diminution and gradually trabecular bone was converted into bone characteristic of slow growth, i.e. circumferential lamellae extending toward the margins of surgical resection in the experimental site. Periosteal bone formation stimulated by electric current materially exceeded the regional acceleration phenomena (R.A.P.) effect of merely raising the periosteum. A logical deduction accounting for these differences in growth pattern is that the rate and type of bone formed is dependent to a considerable degree on current density.

From our observations of the sites of accidental perforations of the calvarium and their relative lack of healing it became obvious that the periosteum of the outer table far exceeds the ability for bone formation of the osteoblastic cells lining the surface of the inner table. This may be due to the number of osteoprogenitor cells available for recruitment to functional osteoblasts or may be a combination of this and genetic characteristics of cells on these surfaces (intrinsic ability to form bone), or variations in other exogenous stimuli of bone formation (piezoelectric, hormonal, nutritional, blood supply, etc.). We do not yet know the exact differences in how populations of osteoblastic cells perform their functions in various specific anatomic locations, viz., on the outer periosteal, medullary, osteonal or endosteal surfaces of long bones. Normally these sites are load bearing and stimulated by relatively higher levels of piezoelectric activity. These anatomic differences in rates of healing under the influence of electric current stimulation need further elucidation experimentally.

The course of healing in the later time periods beyond 42 days following surgery showed a lessened formation rate and even some regression of periosteal bone associated with remodeling of the sites of exuberant bone formation in the "exostoses". One can only speculate at this time about the permanent effects of a short-term current application at the periosteal surface and the possibility of maintaining the bony outgrowths by periodic current applications to the periosteum in order to maintain the level of bone newly formed by the initial current application. If this newly formed bone could be maintained for more than the 168 day period this technique might be useful for bone augmentation in a number of clinical stiuations including: bone augmentation over edentulous ridges, in periodontal infrabony pockets, restoration

of facial bone contours in traumatically induced defects, i.e. combat wounds and containment of bone loss in osteoporosis.

Another important observation deserving comment was the presence of inflammation about the electrodes particularly about the anode occurring during and for extended periods (up to 147 days) following the application of current. The passage of current was apparently associated with breakdown of the metallic electrodes as evidenced by the accumulation of black granular foreign material about electrodes. The breakdown continued even after the current application ceased. Increasing amounts of foreign material built up in the electrode region over the post-current application period (up to 147 days) and was associated with inflammation and bone loss. This change could be ascribed to iontophoresis and could be mitigated by the use of more biologically compatible electrode systems.

A total of ten animals for each current level (0.01 and 0.001 microamperes) were examined in the second study of 1977 using the hard tissue in vivo system (see page 3 paragraphs 1 & 2). Each animal provided a total of eight experimental sites used at random in the following manner. Direct current and pulsating direct current at the same current level were applied to two additional sites simultaneously during week two of the reparative process and discontinued. This was repeated using two new sites during the third week of the reparative process. The two remaining sites served as controls. The above protocol was followed on five animals at the current level of 0.01 microampere and five animals at the current level of 0.001 microampere.

In addition, a protocol designed to compare alternating current with direct current was followed. The eight experimental sites on each animal were used in the following manner: direct current ("unbalanced" electrode system, cathode proximal to defect) and alternating current ("balanced" electrode system) see Figure 1 at the same current level was applied simultaneously to two of the eight electrode sites selected at random for the first week post-surgery of the reparative process, and then discontinued. Current of the same waveform and level was applied to a second pair of randomly selected sites during the second week of the reparative process and discontinued. This was repeated at two additional sites during the third week. The remaining electrode sites served as controls. The above protocol was followed on five animals each at the current levels of 0.01 and 0.001 microamperes.

On completion of the experimental protocol, the 22nd post-operative day, the animal was sacrificed and a complete autopsy was performed, with special attention being given to all factors that were related to the reparative process. Parietal plates containing the defects were removed, processed and analyzed according to procedures described earlier (page 6 paragraphs 2 and 3).

Examination of the data demonstrated that the current supplied at the 0.01 and 0.001 microampere level had no significant effect on the repair of circumscribed surgical defects in the dog calvarium when evaluated by our quantitative methods which do not describe qualitative differences.

EXPERIMENT (CONTRACT YEAR 1976 - 1977)

MATERIALS AND METHODS

The last series of experiments dealing with the efficacy of electrical enhancement of bone wound healing was completed in 1978. In this study of the effects of exogenous current, we compared the differences in healing rates of similarly created bone defects in three anatomical sites, the calvarium (parietal bone), mandible and humerus. This afforded us the opportunity of assaying bone healing rates under the influence of electrical currents in weight bearing versus non-weight bearing bones. It was posited that healing rates in the weight bearing, stressed humerus would be augmented by greater piezoelectrical effects in comparison to the relatively quiescent parietal bone and the intermittently stressed mandible.

Following procedures employed previously for the unbalanced electrode configuration (page 3 paragraph 2) 8 test bone defect sites (4 treated, 4 control/animal) and their corresponding electrodes were placed in the calvaria. The wires were brought from the electrode site through the muscle and fascia and finally exposed through a small skin incision on the dorsal aspect of the animal's neck at the fourth cervical vertebra. Hemostatic material placed in the defects according to the established technique was removed and the area rinsed with a large volume of saline to remove debris. Muscle and fascia were approximated using absorbable, uninterrupted suture and the skin approximated and closed with interrupted sutures. A small constant current power supply was attached to the appropriate parietal plates, humeri and mandibles, containing the defects were removed, radiographed and fixed in acetone. Later ground sections (around 100 μ) were prepared for analysis of fluorescent labeling to determine the appositional rate.

The flourescent analysis was accomplished as on previous occasions with a Leitz flourescent microscope and an eye piece micrometer (Bausch & Lomb). The mean of 12 micrometer measurements was recorded for each defect, test and control. Bone accretion was also recorded in numerous sites remote from surgical defects and from areas of current application in an attempt to normalize the large variance anticipated between animals.

RESULTS AND DISCUSSIONS

The results of the final year experiments extend the observations from previous years. From the data gathered (Table VI), it is evident that exogenous electric current is capable of increasing the amount of bone accreted during healing of experimental bone defects compared to unstimulated controls. Comparable augmentation of healing was observed in the three skeletal sites studied. Healing in the parietal bone was enhanced 1.85 times the normal rate while the mandible and humerus experienced increases of 2.43 and 1.46 the normal rates respectively. Absolute amounts of parietal and mandibular bone accretion were similar 440 μ vs. 471 μ in the 42 day period of observation. The amount of bone produced in the humerus (mean = 2769 μ) was greatly increased even under normal conditions (mean = 1892 μ). However, of the three skeletal sites the degree of electrical enhancement was smallest in the humerus (1.46). Parietal bone accretion was decreased absolutely from that of previous experiments. One possibility for this observation may be the great number of experimental sites prepared and the severity of the surgical procedures involved. The stress of these procedures might well interfere generally with healing.

TABLE VI

COMPARISON OF THE EFFECT OF APPLICATION OF 1 MICROAMPERE DIRECT CURRENT TO CIRCUMSCRIBED DEFECTS IN THE DOG CALVARIUM, MANDIBLE AND HUMERUS

Calvarium		Mandible		Humerus	
Test	Control	Test	Control	Test	Control
440 ± 342*	238 ± 170	471 ± 240*	194 ± 164	2769 ± 686*	1892 ± 491
N = 76		N = 60		N = 54	
t = 2.812		t = 4.117		t = 3.468	
$p < 0.01$		$p < 0.01$		$p < 0.01$	

* Measurements in $\mu \pm$ Standard Deviation

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