EFFECT OF ELECTRICAL STIMULATION ON FRACTURE HEALING IN BUFFALO CALVES-An Experimental Study.

A THESIS
SUBMITTED TO THE
GUJARAT AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE

OF

MASTER OF VETERINARY SCIENCE

(SURGERY)

BY

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ANAND CAMPUS, ANAND

1982.

A B S T R A C T

EFFECT OF ELECTRICAL STIMULATION ON FRACTURE HEALING IN BUFFALO CALVES - An experimental study.

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Effect of electrical stimulation was studied in 18 buffalo calves at 2 phases of healing i.e. at nineth and eighteenth day. The electrical stimulation was achieved by passing 20 microamperes of direct current at the fracture site. The fracture healing was evaluated by radiographic, arterio graphic, macroscopic and histo-morphological studies. Radiography failed to demonstrate any difference between the treated and control limbs. On arteriography significant increase in arterial supply was observed in treated limbs as compared to control, which probably may be responsible for acceleration of fracture healing. Histomorphological examination clearly revealed an acceleration in the healing process in treated limbs, when compared to the controls.

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C E R T I F I C A T E

This is to certify that the thesis entitled, "Effect of electrical stimulation on fracture healing in buffalo calves - An experimental study", submitted by Shri Sunil B. Thakur in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Surgery) of the Gujarat Agricultural University is a record of bonafide research work carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Major Advisor

Anand,
Date : 4-1-1983

A C K N O W L E D G E M E N T

With immense pleasure I, wish to place on record my deep sense of gratitude to Dr. M.N.Mannari, M.V.Sc.(Surgery), F.R.V.A.C. (Denmark), Professor and Head, Department of Surgery and Radiology, Gujarat College of Veterinary Science and Animal Husbandry, Anand, for guiding the research project, constant moral support in its execution and critical appraisal of the manuscript.

I am deeply indebted to members of advisory committee Dr. S.B.Kodagali, Professor of Gynaecology and Obstetrics, Dr. G.A.Prabhu, Professor of Physiology and Biochemistry for their invaluable criticism and concrete suggestions during the course of this investigation.

My sincere thanks are due to Shri K.V.Nageshwariah, M.E. (Electronics), Head of the Electrical Engineering Department, B. and B. Polytechnic, V.V.Nagar, for his help in devicing the electrical stimulator.

I am grateful to Dr. K.N.Vyas, Professor of Anatomy for providing generous help and advice during histo-morphological studies.

Words can hardly express, my gratitude to Dr. D.M. Tadkod, Associate Professor of Surgery and Radiology for generous and everwilling help at various stages of this study.

Sincere gratitude is extended to : Dr. K. Sukumaran, Hospital Superintendent, Dr. R.R.Parsania, Assistant Professor, Dr. B.M.Jani, Research Associate, Department of Surgery and Radiology for their valuable suggestions and help. Dr. S.T.Pandya, post-graduate student in Veterinary Surgery for his everwilling help during the course of the this study.

Shri R.A.Patel, Laboratory Technician, Department of Anatomy for his help during histomorphological studies.

All those staff members of Department of Surgery and Radiology who helped me directly or indirectly during this study.

Shri D.P.Padsumbia deserves appreciation for meticulous typing.

I am grateful to Dr. M.R.Patel, Principal, Gujarat College of Veterinary Science and Animal Husbandry for constant encouragement and moral support.

I thank Gujarat Agricultural University, for permitting inservice post-graduate studies.

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Anand,
Date : 27/8/1982

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INTRODUCTION

I. INTRODUCTION

The nature of bone and the manner in which it heals and remodels have evoked the curiosity of physicians and surgeons throughout the history of modern medicine. Animal experimentation over the past 30 years has yielded a wealth of information about osseous repair and remodelling. Modern medicine has made great strides in the successful treatment of fractures. However, the natural phenomenon of osseous regeneration has remained essentially unchanged. To achieve acceleration in this natural phenomenon, a great deal of work has been carried out. Many agents like collagen, cartilage extract, vesical mucosa and growth hormone and techniques like venous occlusion and electrical stimulation have been used for acceleration of fracture healing.

The idea to stimulate osteogenesis by delivering extremely low intensity current in bone and especially at the fracture site, is comparatively a new venture. The initial interest aroused about two decades ago when it was proved by a Japanese team led by J. Yasuda that bone also posses a peculiar property known as piezo-electricity. This sparked a chain of hectic activity in the field of research all over the world and many researchers tried to find out the utilization of this property of bone in the complex problem of fracture healing.

In the last two decades a lot of work has been done to acquire the knowledge about the electrical behaviour of normal bone and effect of electrical stimulation on fracture healing in laboratory animals, but as no such type of work has been done in bovines an attempt was made to study the same by keeping the following objectives in mind.

1. To study the feasibility of application of electric current to the fracture site in bovines.
2. To find out experimentally whether electrical stimulation accelerates the process of fracture healing or not? Which is to be evaluated by comparing the radiographic changes, macroscopic changes and histomorphology of normal callus and electrically stimulated callus.
3. To study the vascular pattern during normal healing of fracture and electrically stimulated fracture.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The earliest reports of the use of electrical energy to stimulate bone healing, seem to be from England in 1853 and from Boston in 1860. However more earnest efforts have been made in the last thirty years, due to a better knowledge of the fracture healing and the measures to hasten it.

Beginning with studies of the relationship between mechanical stress and the electrical potentials generated by stress, these investigations were later extended to include non-stress potentials and the response of bone to applied current.

Stress generated potentials were originally described by Yasuda et al. (1955) in Japan and by Bassett and Becker (1962); Shamos et al. (1963) in the United States.

I. STRESS GENERATED POTENTIALS IN BONE:

Yasuda et al. (1955) investigated stress generated potentials in bone. They found that compressed part of long bones showed a negative potential and part under tension showed positive potential.

Bassett and Becker (1962) also demonstrated generation of electric potentials by bone in response to mechanical stress. The potentials generated in these stressed bones, apparently, were not dependent upon cell viability since frozen and thawed or air-dried specimens

behaved like the fresh specimen. They further suggested that since bone is composed of crystals of hydroxy-apatite, piezoelelectric potentials may be responsible for the conversion of stress to electrical stimuli which may influence the activity of osseous cells directly.

Shamos et al. (1963) observed the same stress induced electric potential in a number of whole bones from different anatomical sites and species; both in bending and compression modes. Based on these findings they suggested that the surface charges which appear on the stressed bone may be the controlling factor in bone formation. The local electric fields resulting from such surface charges might be expected to influence the orientation and deposition of ions.

Steinberg et al. (1974) studied stress induced potentials in moist bones in vitro. The surface under compression was constantly electro-negative with respect to that of under tension.

The above studies show that the potentials which arise when the bone is mechanically stressed are not dependent on cellular viability, but arise mainly from organic component of bone and are electro-negative in areas of compression and electro-positive in areas of tension.

II. NON-STRESS GENERATED POTENTIALS:

Friedenberg and Brighton (1966) measured the resting potentials of fractured bone in rabbits. They found that the metaphysis had a negative potential in relation to diaphysis. Following fracture of shaft of tibia, the diaphysis became negative in relation to epiphysis and the metaphyseal potential also became more negative. The negativity of the diaphysis remained until the fracture united, after which the potential returned to normal. These resting potentials are also known as non-stressed potentials or bioelectric potentials and are electro-negative in areas of active growth and repair.

These potentials quite naturally led to experiments in which the current was applied to the bone.

III. EFFECT OF ELECTRICAL STIMULATION ON BONE:
(Experimental studies)

Yasuda et al. (1955), by using a battery of 1.5 volts, kept a current of one microampere passing through femur for three consecutive weeks. A ridge of callus was formed running through periosteum from pole to pole. The cathode side had more callus than the anode side. When current was between 1 - 100 microampere, bony callus was formed, but when the current was stronger than one milliampere bone destruction took place.

Bassett et al. (1964) studied effects of electric current on femora of eighteen adult dogs by implanting units consisting of two iridium platinum electrodes, a 1.4 volt mercury battery and a resister. After fourteen and twenty one days of operation, on microscopic studies, they found a greatly increased quantity of non-oriented young trabecular bone around the cathode.

Friedenberg and Kohanim (1968) studied the effect of electric stimulation on epiphysis and bone in rabbits. In this study they found that :

(i) Direct current stimulation of an epiphyseal line failed to stimulate the growth of rabbit tibias.

(ii) There was a tissue destruction around anode.

(iii) The area adjacent to the negative electrode, whether the epiphysis or metaphysis, frequently revealed minimal formation of new bone trabeculae or cartilage nodules.

O'Conner et al. (1969) carried out similar experiment as that of Bassett et al. (1964) in twelve dogs and found more bone around the cathode. Though their results agreed with Bassett et al., they cautioned its use in clinical application as they found considerable biological variation in the response of bone to electric current even within a single species.

Lavine et al. (1969) studied the effect of electric current on bone in rabbits. The test side consisted of a bony cortical defect of fixed size, drill holes were placed fifteen millimeter apart for the electrodes and the experimental defect was placed midway between them. The experimental power unit consisted of a 1.4 volt mercury cell, connected in a series with 174 K resister. The leads from the battery pack were copper wires fastened to platinum electrodes with silver epoxy cement. Alligator clips were placed in one end of the lead for monitoring. The rabbits were killed at the interval of one, two and three weeks. Gross and histological examination of bony defect revealed that after 2 -3 weeks direct current varying from 2 -3 microamperes markedly enhances healing in experimental as compared to control animals.

Friedenberg et al. (1970) studied bone reaction to varying amount of direct current. Right femur of the rabbit was used for the study. 1, 5, 10, 20, 50 and 100 microampere of current was passed. The rabbits were killed on the tenth post-operative day.

Roentgenograms of the specimen were not helpful in showing bone production or destruction except in higher amperages, bone destruction could be seen at the both electrode sites.

Microscopically bone formation occurred predominantly around the cathode. The optimum range of current for such formation was 5 - 10 microamperes with diminution of bone production above twenty microampere.

Friedenberg et al. (1971), studied the effect of direct current on rabbit fibula, in relation to the site of electrodes to the fracture site, and the fracture site of each animal was subjected to ten microampere galvanic current. Leads were placed in opposite (control) fibula but did not deliver any current. Each fibular fracture was studied by roentgenogram, stressed for rigidity and evaluated microscopically. The evidence strongly suggests that a cathodal current of this intensity placed within the fractured site stimulates fracture healing.

Hambury et al. (1971) studied the effect of microampere electrical current on bone in vivo and its measurement using Strontium-85 uptake in eighteen white rabbits. They could not detect any difference between the radio isotopic uptakes at the site of anode and cathode of active implants. Therefore they concluded that no constant evidence of increase in bone growth at one electrode over that of other of an active implant can be shown by gross examination, histology, radiology and radio-isotopic assessment, although they suggested designing of experiments to achieve larger current.

Chamay et al. (1972) studied the influence of electric impulses on osteogenesis in twenty-six rabbits. In rabbits a generator produced fifty microamperes impulses of one second duration at nine seconds interval. Osteogenesis was recorded around the two active electrodes and a few necrotic foci were found about the cathode. It would appear therefore that there is osteogenic influence exerted by this dosage of electricity.

Pollis et al. (1973) studied the atraumatic osseous response to electric current. They inserted insulated wires with one to two cms of tip exposed into medullary canals of rabbit tibiae. A current of 2 to 10 micro-amperes for twentyone days caused bone to be formed, only at micro amperages of about twenty or below, particularly at the end of insulation. Maximum osteogenesis was at ten microamperes and only minimum effects were seen at 2 -5 microamperes.

Weigert et al. (1973) studied the effect of direct current on the rabbit tibia in relation to the site of electrode. The fracture site of each animal was subjected to 1 -10 microampere of DC current. The fracture healing was studied by radiology, histology, micro-angiography, Strontium-87 -m -scanography and by measuring the weight bearing capacity. The results of the experiments indicate that there was acceleration of

fracture healing due to electric stimulation which was more pronounced when the cathode was near osteotomy gap.

Connolly et al. (1974) investigated the effect of electrical stimulation on the biophysical properties of healing canine-fractures. They studied the torque strength, the mineral content and the structural organization. Increase in torque strength was found in stimulated fracture site. The authors have related the increased torque strength to better collagen or matrix.

Friedenberg et al. (1974) studied the response of non-traumatized bone to direct current by inserting the teflon insulated stainless steel cathode with a bare tip into the tibial medullary canal. In each group of rabbits 5, 10, 20, 40, 50 and 100 microampere of current was passed for 21 days. The direct constant current cathode caused bone to form at the site remote from any bone trauma in the medullary cavity. The osteogenic response was current dependent and the greatest bone response developed around cathode delivering 20 microampere of current. Excessive current i.e. 30 microampere and more caused osteonecrosis.

Lavine et al. (1974) carried out clinical and ultrastructural investigations of electrical enhancement of bone healing. This study showed that enhancement of bone formation by direct electrical current was reflected not only in clinical and X-ray film but also at microscopic and ultrastructural level.

Brighton et al. (1975) studied the cathodic oxygen consumption and electrically induced osteogenesis. The purpose of this experiment was to compare changes in oxygen and hydroxyl ion concentration that occur at the cathode at current levels known to be capable of inducing osteogenesis (10 to 20 microamperes) and with those changes that occur at the current levels known to be toxic to the bone (100 microamperes). It was concluded from this in vitro experiment that at 10 - 20 microamperes the oxygen tension in the vicinity of the cathode is lowered and the pH is moderately increased. At 100 microampere the oxygen tension is not lowered but the pH is increased dramatically. If these same changes occur in the vicinity of a cathode in vivo, then lowering the local tissue oxygen tension and raising the local pH may be mechanisms operative in electrically induced bone formation.

Martin and Gutman (1978) studied the effect of electric fields on osteoporosis of disuse in male rats. The right femurs of untreated rats were found to be atropic with respect to opposite limb. In the treated rats the immobilized femur was larger than the opposite bone.

Treharne et al. (1979) carried out in vitro study of electrical osteogenesis using direct and pulsating currents. The results indicated that electric current did cause osteogenesis in vitro and the direct current was more

effective in bone growth than pulsed current delivering the same total charge (coulombs).

Satyanand et al. (1980) ... studied the fracture healing by electrical stimulation in 24 rabbits. 10, 20 and 50 microamperes of current was passed in three groups of animals for 18 days. The extent of osteogenesis was studied by radiography, morphology and histology. From this study they concluded that a current of 10 to 20 microamperes is the safe range for osseous stimulation and yields more calcified callus. The maximum osteogenesis takes place at the cathodal end of electrical circuit.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

The present study was conducted on 18 clinically healthy male buffalo calves in the age group of six to eight months weighing 60 to 80 kg, which were selected randomly.

All the animals were observed for one week before commencement of the experiment. They were maintained under similar managemental and dietary conditions.

Before commencing actual experiment different types of fractures viz., tibial fractures, fracture of transverse process of lumbar vertebrae, metacarpal fractures were created and then electrically stimulated in pilot trials so as to determine the feasibility of contemplated procedures and to standardize the techniques involved. It was observed that creation of a rectangular defect in metacarpus was most feasible and it offered many advantages. Being a partial fracture complications like displacement of fragments and post-operative impairment of gait were not seen. For the same reason the need for immobilization of limb was not required.

The 18 animals were randomly divided into two groups (group I and group II) of nine animals each. A rectangular defect was created in both metacarpi and 20 microampere DC current was passed for 9 days in group I and for 18 days in group II.

The radiographic, arteriographic, macroscopic and histomorphological studies of all the animals of both the groups were recorded in two phases of healing i.e. on 9th and 18th post-fracture day.

SURGICAL TECHNIQUE:

A. Preparation of the animals

All the animals were fasted for 24 hours and deprived of water 12 hours prior to surgery. On the day of the experiment both the fore-limbs from knee to coronet were prepared for aseptic surgery.

B. Anaesthesia

The animals were restrained in lateral recumbency and deeply sedated with 10% chloral hydrate. Two per cent procaine hydrochloride (Novocain)* was infiltrated on the proposed line of incision and then tested for analgesic effects.

C. Creation of fracture

A crescent skin incision approximately three inches long was taken on the antero-medial surface of the midshaft of the right metacarpus. The bone was exposed and the periosteum was resected out. A rectangular defect of 17 x 10 mm was created with stainless steel chisels of same sizes. A stainless steel wire electrode (26 gauge) was anchored at the distal end of defect by passing it

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*Hoechst Pharmaceuticals Ltd., Bombay.

through a hole drilled distal to the fracture site. This electrode was ultimately connected to the negative pole of battery so that it served as cathode. Another stainless steel wire, anode, was anchored in the soft tissues proximal to the fracture site. Both the electrodes were insulated with polythene tube. The area was dusted with hostacycline* powder and the incision was closed by mattress sutures using silk. The electrodes were ultimately connected to the cell . The limb was lightly plastered to restrict the stretching of wires and subsequent breakage. Identical operation was carried out in the left forelimb, but the fracture site was not stimulated by electrically and hence it served as a control.

Electric stimulator:

A semi-invasive technique, as described by Brighton et al. (1975), was employed to fix the electric stimulator.

The circuit was made up of insulated copper wire, three volt dry cell, 0 - 100 K Ω variable resistance and a microampere meter with a scale of 0 - 100 microamperes.

The variable resistance and the microamperemeter was connected in series with the positive end of the cell, while the negative end was connected directly to the electrode using insulated copper wire. The cell and the

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*Hoechst Pharmaceuticals Ltd., Bombay.

variable resistance were kept in rexin case which was anchored over the back of the animal. The microampere-meter was used to check the strength of current flowing through the circuit. The current strength was kept constant by altering the resistance during the daily check-ups. The calves of first and second group were sacrificed on 9th and 18th day respectively.

Post-operative care:

After surgery the animals were shifted to the stalls, on recovery of anaesthesia they were made to sit and water was offered. Post-operative antibiotic Oxytetracycline hydrochloride (Oxystecline)* at the rate of 5 mg/kg was given for 5 days.

The current strength was checked twice a day by microamperemeter.

The limbs were examined for edema.

Following studies were made after sacrificing the animals on 9th and 18th day for 1st and 2nd group respectively.

Parameters Investigated:

I. Radiographic and Arteriographic Studies:

Arteriography was carried out in 6 animals of group I and in 7 animals of group II.

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*Sarabhai Chemicals, Baroda.

The limbs along with shoulder region were removed by cutting through the muscles of shoulder girdle. The brachial arteries were cannulated and perfused with normal saline until the clear fluid came out through the respective veins. About 15 to 20 ml of Barium sulphate (microbar)* suspension in water in proportion of 1.8 gms/ml was injected by glass syringe.

Arteries were ligated and radiographs were taken in medio-lateral and anterio-posterior positions from knee downwards with a constant exposure factors of 60 KV and 6 mas at 75 cms FFD. The arteriographs obtained were also employed for radiographic interpretation.

Quantitative analysis of the arterial supply was done in treated, control and normal arteriographs. Keeping the fracture site in the centre the site was divided into 40 squares with each of 1 cm^2. This almost covered the whole metacarpus. The arteriographs of the normal limbs were obtained from the special problem work of Parsania (1982).

The blood vessels in each square were counted, totalled and statistically analysed.

II. Macroscopic observations:

After the arteriography the fracture site was exposed by incising the skin. Macroscopic changes at the fracture

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*Eskay Fine Chemicals, Bombay.

site inclusive of the site of insertion of anode were observed and recorded.

III. Histomorphology:

Immediately after arteriography the fracture site as a whole was collected by careful sawing. The piece of bone was transferred to neutral buffer formalin for fixation. Then this fixed tissue was decalcified by a fluid containing

1. Formalin - 10 parts.
2. Concentrated nitric acid - 5 parts.
3. Distilled water - 85 parts.

Then ten microns thick cryostat sections were taken and were stained with hematoxylin - eosin for studying histomorphology.

Fig. 1. Instruments used for surgical procedure.

Fig. 2. Crescent shaped incision given over the metacarpus. Adequate exposure of metacarpus is evident.

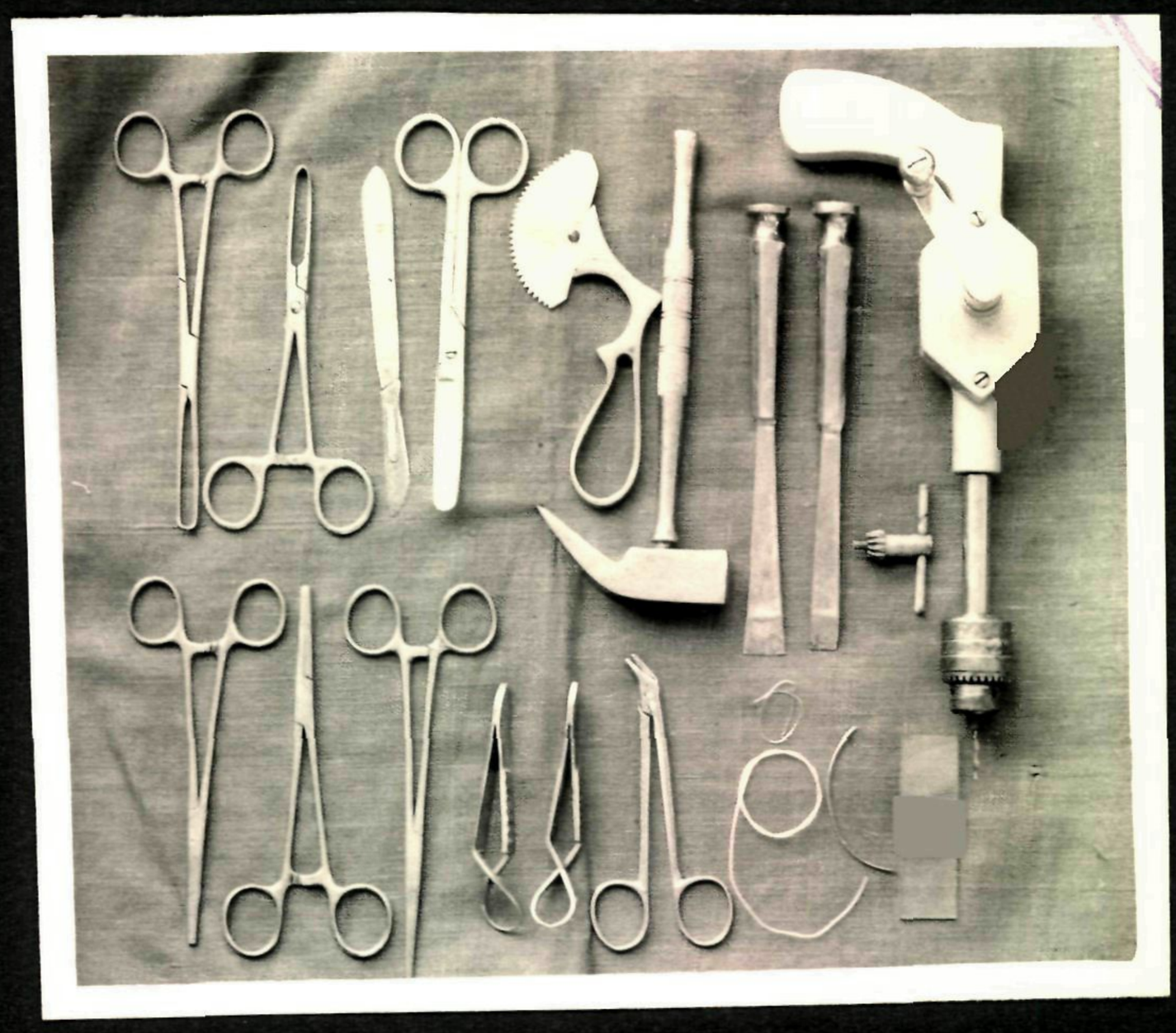
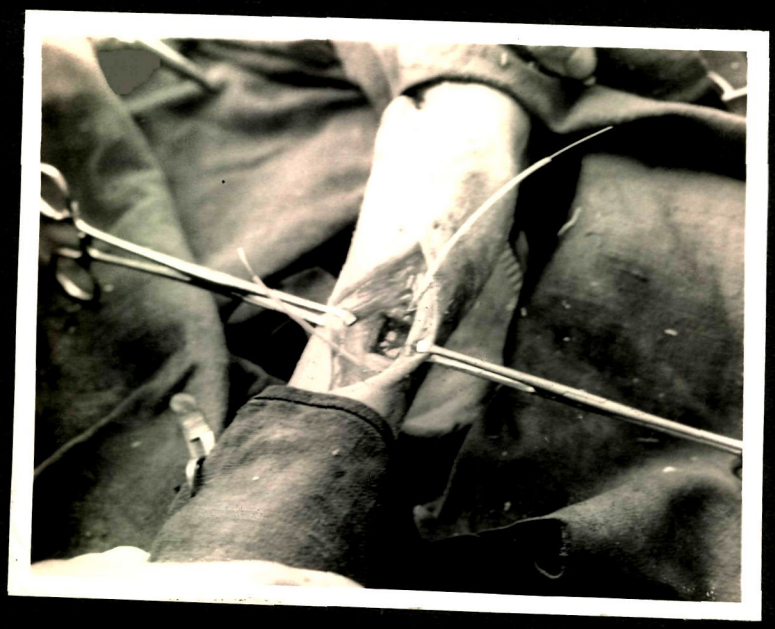


Fig. 3. Rectangular defect created in the metacarpus.

Fig. 4. Electrodes insulated with polythene tubing anchored.



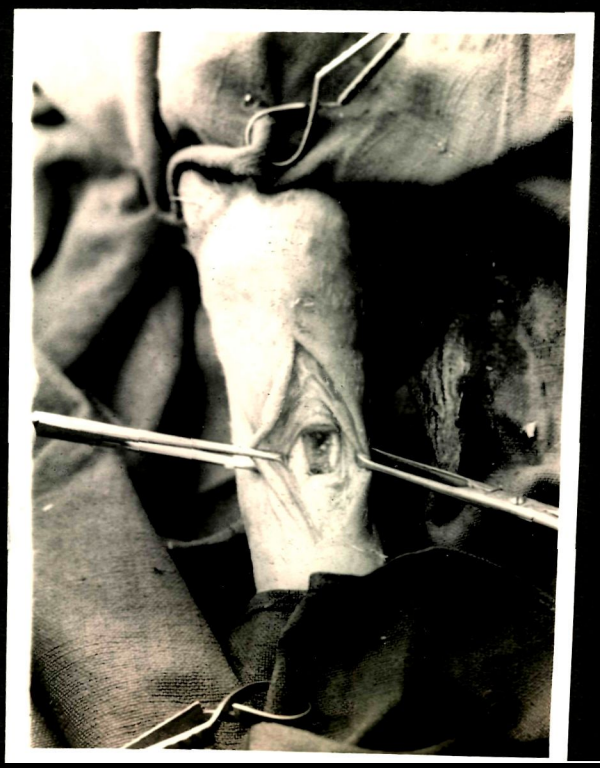
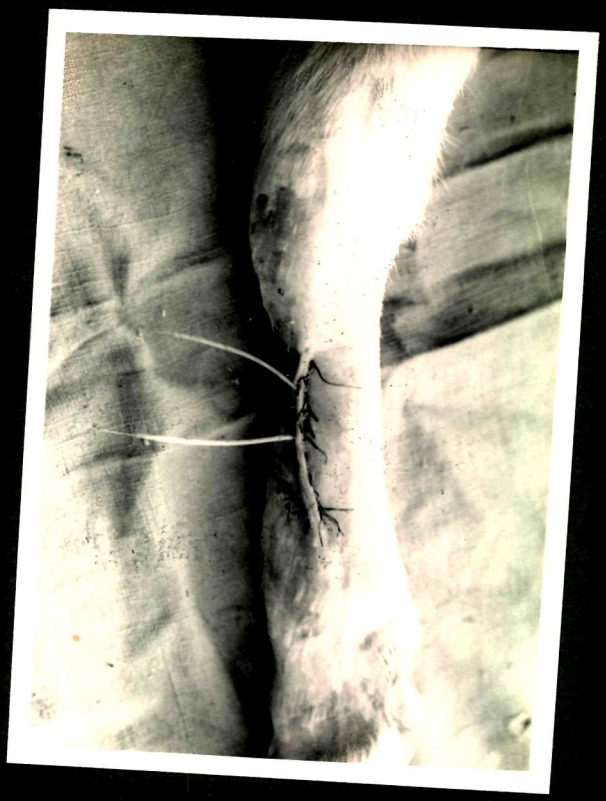


Fig. 5. Skin incision closed. The opposite ends of the electrodes are visible, which are to be connected to the electrical stimulator.

Fig. 6. Calf in a standing position with electric stimulator fixed over the back, the microamperemeter connected to check the current.



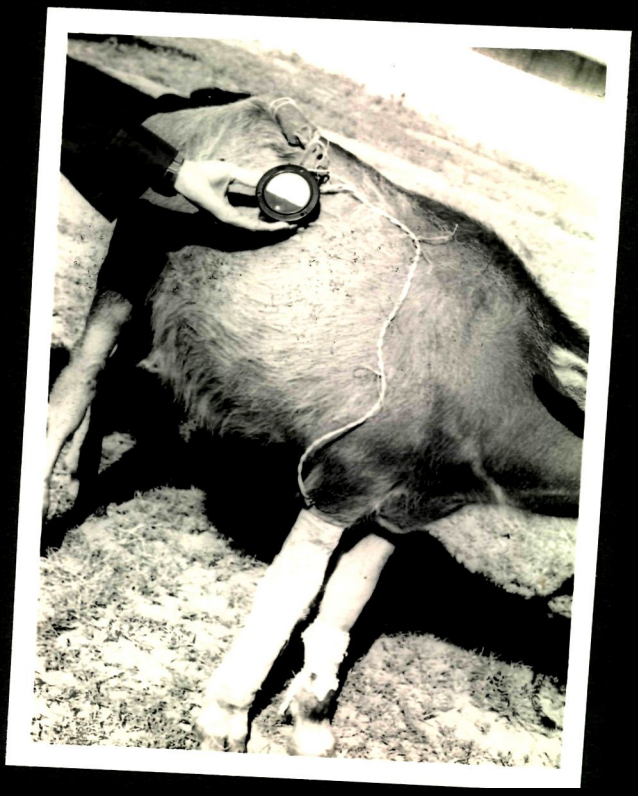
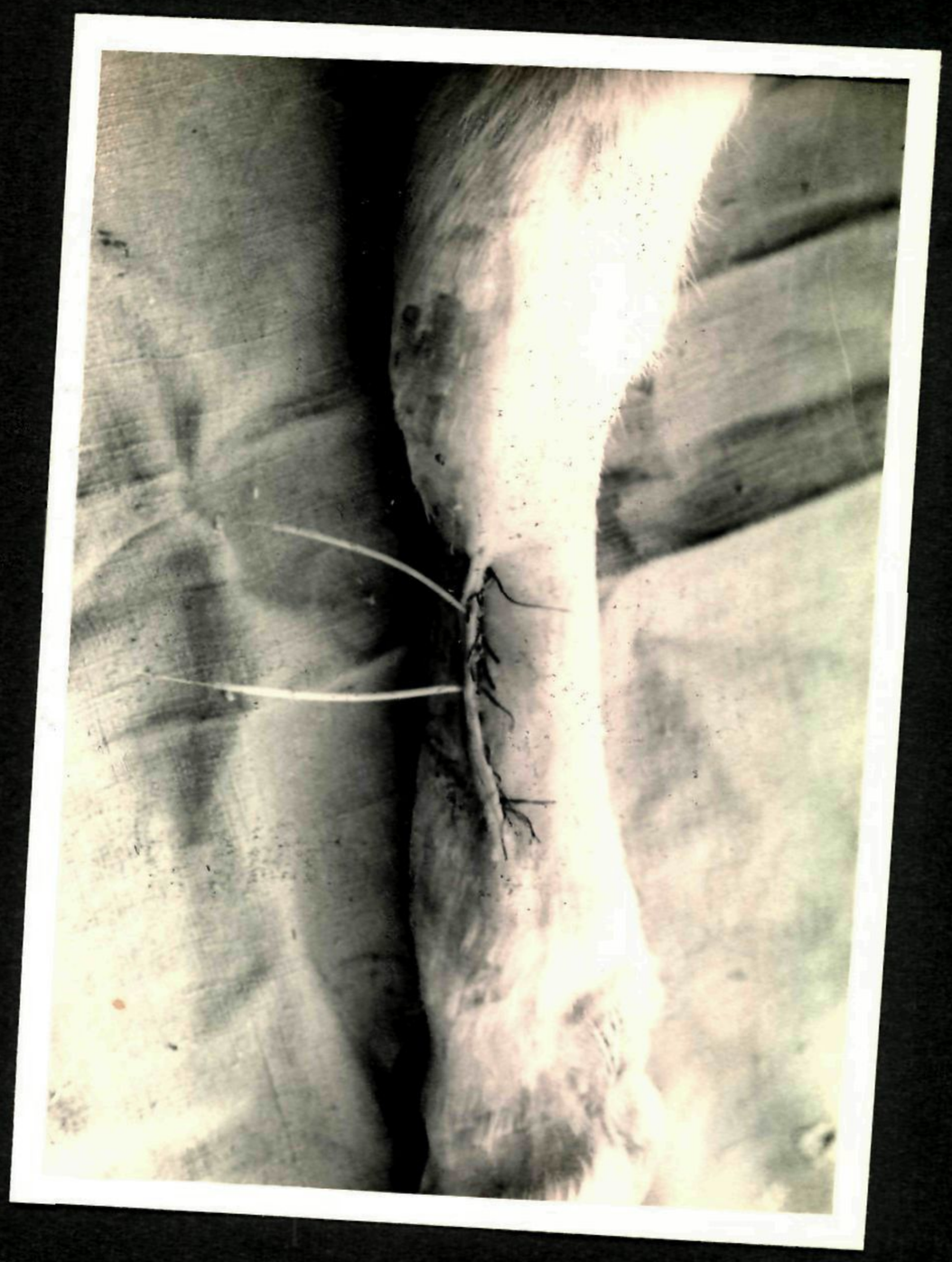


Fig. 7. Appliances used for electric stimulation.

Fig. 8. Diagram of the electrical circuit for electrical stimulation of bone.



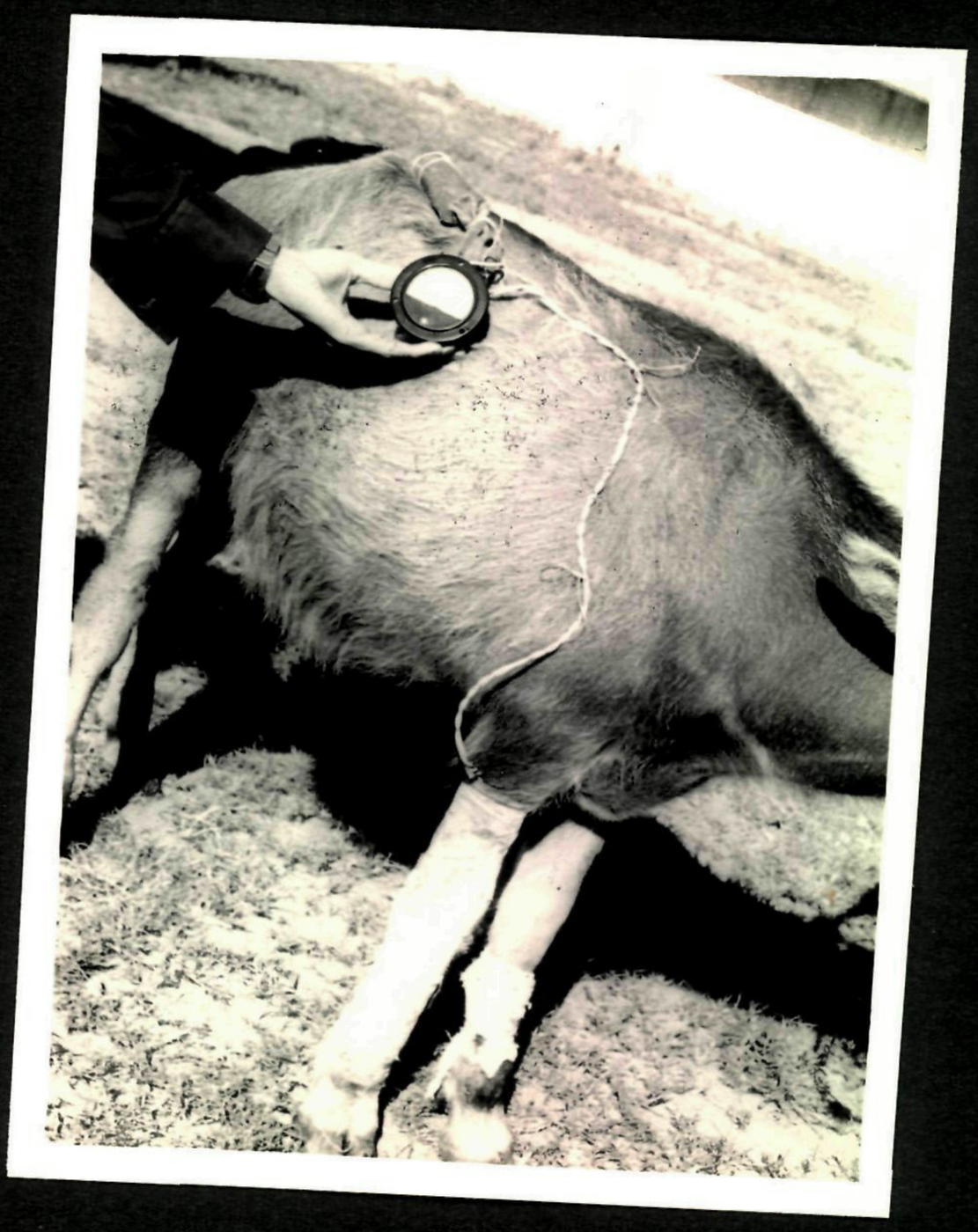
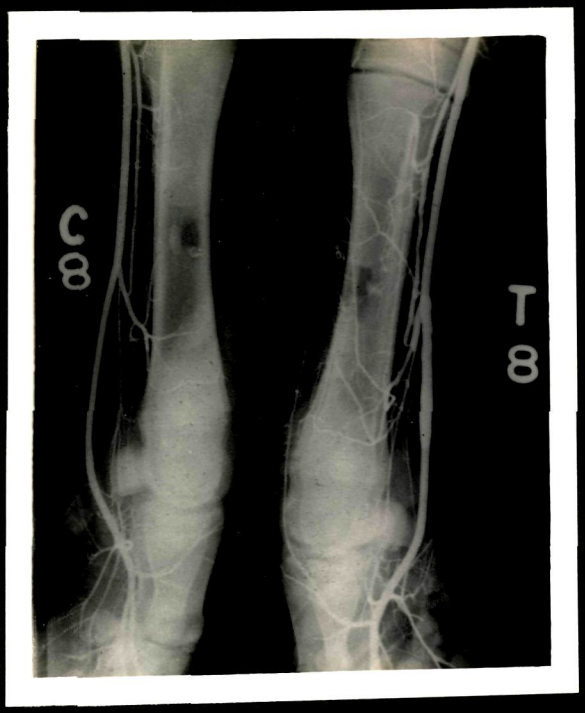


Fig. 9. Arteriograph,lateral view (9 days).

Fig. 10. Arteriograph, anterio-posterior view (9 days).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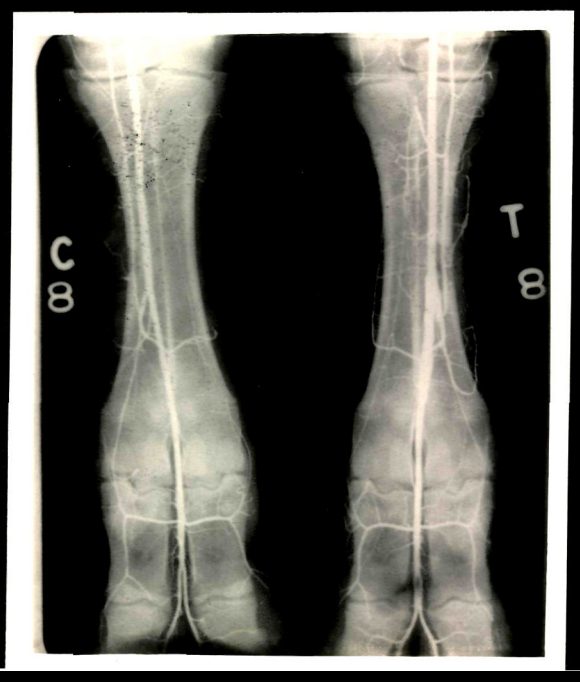
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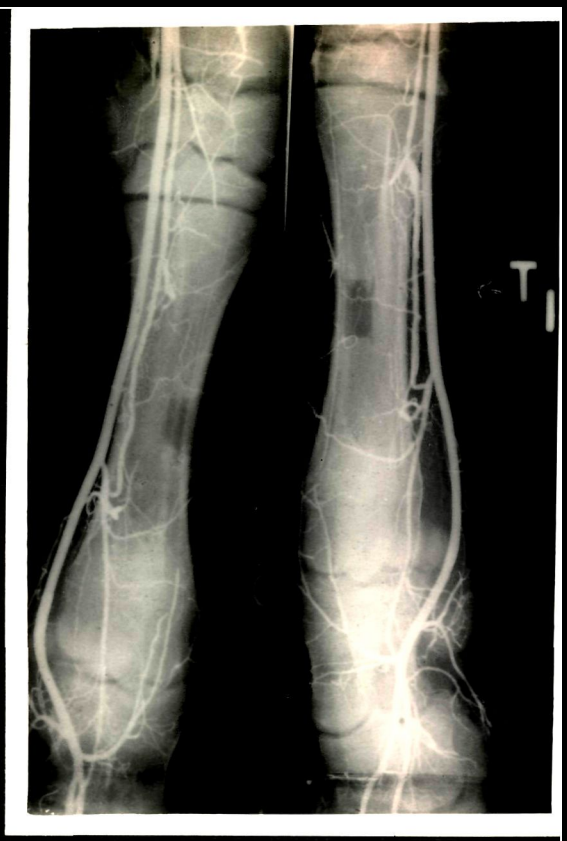
Fig. 11. Arteriograph, lateral view
(18 days).

Fig. 12. Arteriograph, anterio-posterior view
(18 days).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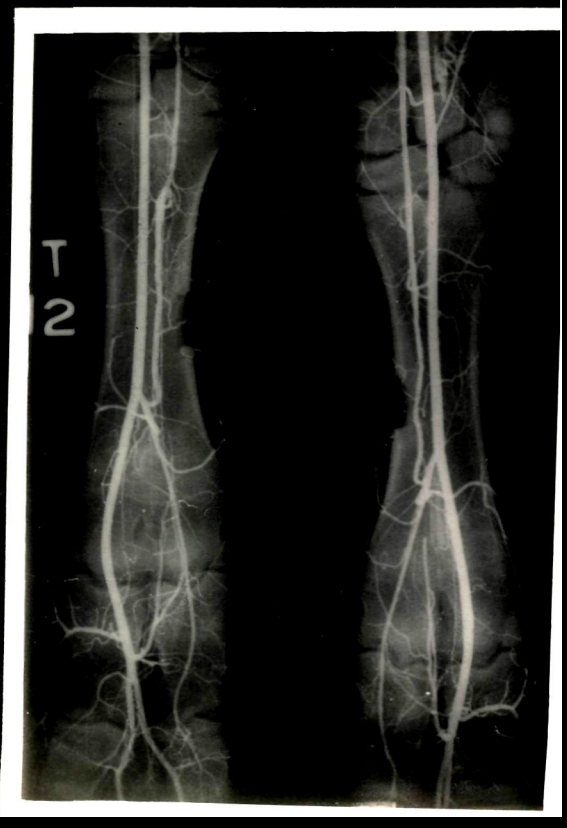
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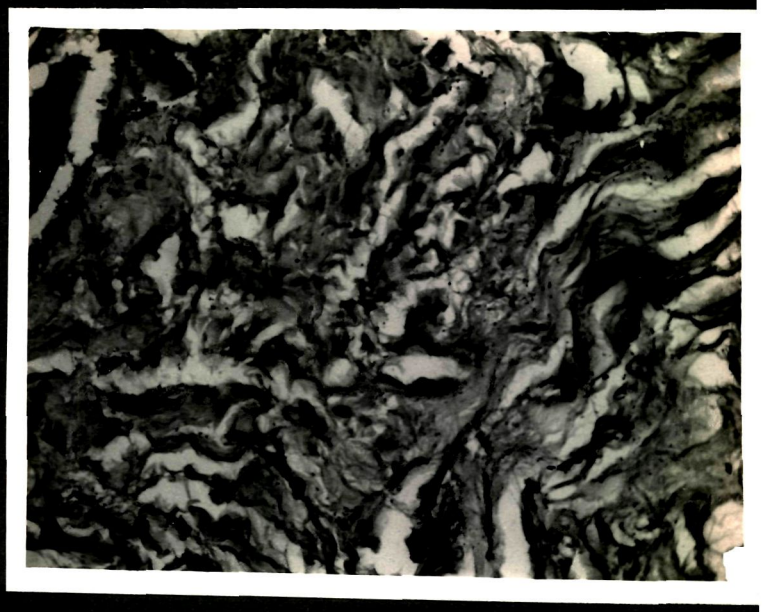
Fig. 13. Photomicrograph of 9 days treated callus. Note the fibrous tissue with infiltration of innumerable neutrophils. H and E stain X 170.

Fig. 14. Photomicrograph of 9 days control callus. Note the haemorrhagic areas with less fibrous tissue. H and E stain X 80.



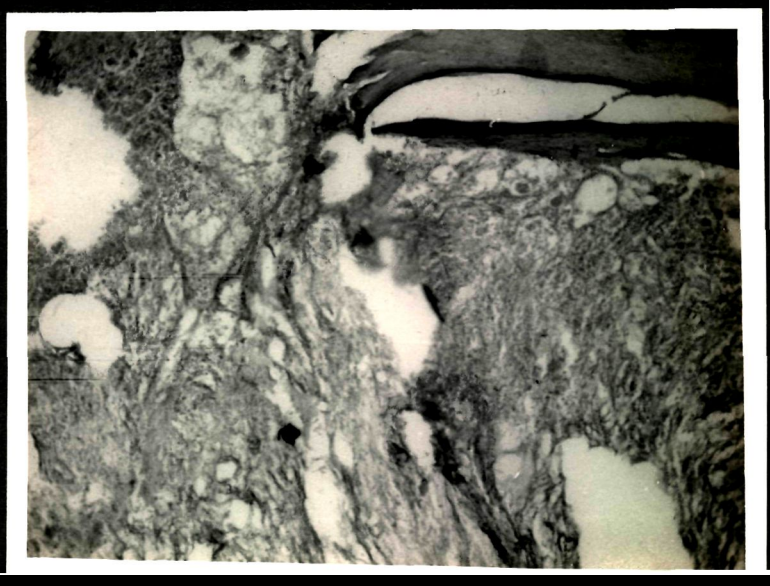
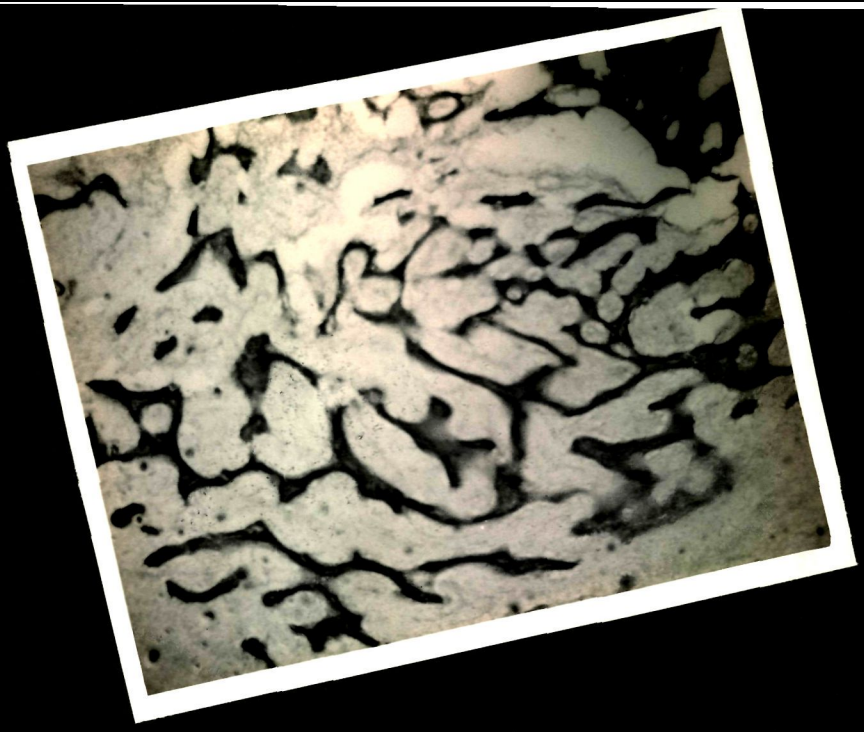
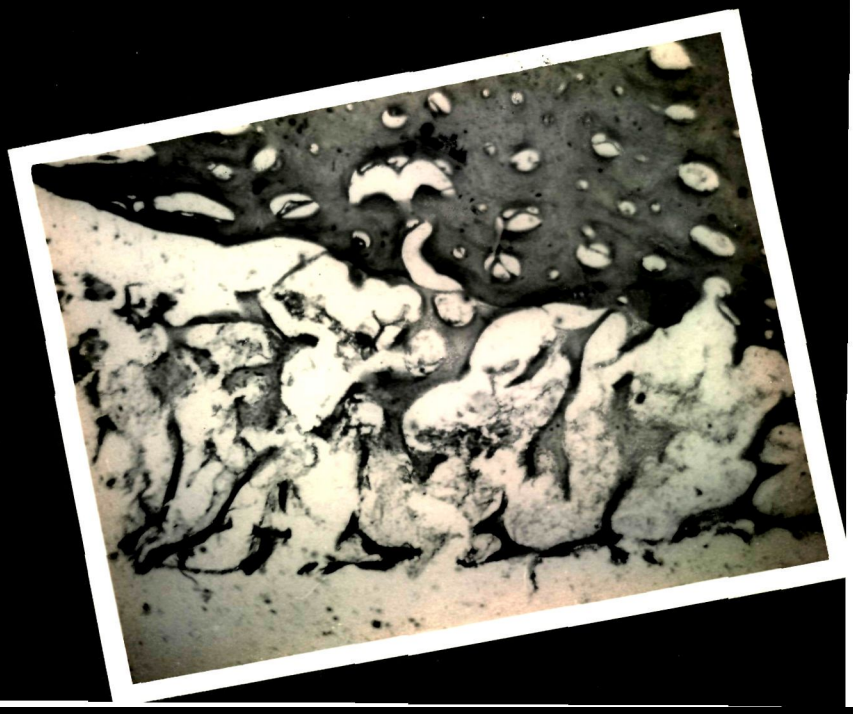


Fig. 15. Photomicrograph of 18 days treated callus showing dense trabecular formation with less intertrabecular space as compared to Fig. 16. H and E stain X 80.

Fig. 16. Photomicrograph of 18 days control callus. Note the sparce formation of trabeculae. H and E stain X 80.





RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

A. Anaesthesia:

Local infiltration anaesthesia with chloral hydrate narcosis was adequate for surgical procedure. Sharma et al. (1977) have also created fracture of metatarsus under chloral hydrate sedation and linear infiltration of site with local anaesthetic. Similarly Brighton et al. (1975) found local anaesthesia adequate for drilling the holes inside the bone and fixation of electrodes in 23 patients suffering from non-unions.

B. Surgical procedure:

The crescent shaped incision employed in the present study proved advantageous in exposing medial portion of metacarpus completely and creation of fracture was facilitated.

The steady and slow hammering of chiesel adopted to create rectangular defect proved satisfactory. However, splintering of the fragments occured in two animals. Bone defect of a fixed size was created so as to achieve uniform results. Sharma et al. (1977) employed oscillating bone saw in addition to chisel and hammer for creating fracture in midshaft region of metatarsus. Since uniform rectangular fractures could be created only with chisel and hammer the use of oscillating bone saw was not felt necessary. Similar bony cortical defects of a fixed size as a test

site for studying the effect of electrical current were employed, successfully, by Lavine et al. (1969).

It was observed that creation of a rectangular defect in metacarpus was most feasible and offered many advantages. Being a partial fracture complications like displacement of fragments and post-operative impairment of gait were not seen. For the same reason the need for immobilization of limbs was not required. Similarly, to avoid these complications Friedenberg et al. (1971) and Satyanand et al. (1980) created fracture of fibula in rabbits.

C. Electric stimulation:

The semi-invasive technique employed in the present study proved very practicable. Monitoring of a constant current was most feasible, as and when required. Since the rexin case was fixed on the back of the animal it did not interfere in the normal activities of the animal. Similar advantages were also recorded by Brighton et al. (1975) with the above method. The other technique available for electric stimulation viz., totally invasive technique as suggested by Bassett et al. (1964), Friedenberg et al. (1968), O'Connor et al. (1969) was not employed in the present study because of their inherent disadvantages.

Friedenberg et al. (1970) used 7.5 volt battery and passed 1, 5, 10, 20, 50 and 100 microamperes of current in different groups of rabbits and found maximum osteosynthesis at 20 microamperes.

Satyanand et al. (1980) used a 1.3 volt mercury cell and passed 10, 20 and 50 microamperes of current and concluded that a current of 10 to 20 microamperes as a safe range for osseous stimulation.

Based on their studies and findings, 20 microamperes of current was passed at the site of fracture by using a 3 volt cell in the present study.

A 1.5 volt cell as suggested by Srivastava and Saxena (1977) was employed for electric stimulation of the fracture site during the pilot trials. However, maintenance of current at a constant level of 20 microamperes was not possible with this voltage. So a 3.0 volt cell was used by which the constant current could be maintained throughout the experimental period. Cathode was placed at the fracture site, since it is at the cathode that maximum osteosynthesis occurs. Friedenberg et al. (1970) studied acceleration of fracture healing by placing the cathode at different places relative to the fracture site and recorded maximum osteosynthesis when the cathode was placed at the fracture site.

Evaluation of fracture healing:

I. Macroscopic observations

Macroscopically in the group I of 9 days, a whitish soft fibrous tissue was seen accupying the fracture gap in the treatment limb. However, the gap in the control limb was filled by a reddish tissue indicative of a blood clot.

In the group II of 18 days a whitish callus firm in consistancy bridging the fracture site was observed in the treated limbs. The callus was resistant to moderate external pressure, while in the control limbs whitish callus comparatively softer in consistancy covering the fracture site was noticed.

These findings of group II corroborated with the results of Satyanand et al. (1980). However Hambury et al. (1971) did not get any consistant evidence of increase in bone growth on gross as well as other examinations.

Lavine et al. (1969) concluded from gross examination that after 2 - 3 weeks direct current markedly enhanced healing in experimental as compared to control animals.

In the present study necrosis was always observed at the anode site, due to which dislodgement of anode was found in 3 cases which subsequently required refixation. This finding corroborates with Friedenberg et al. (1970, 1971 and 1974) as well as Chamay et al. (1972).

Infection of the fracture site was observed only in one animal of group II.

II. Radiography

Radiographs failed to demonstrate any difference between the fracture healing of control and treated limbs in both the groups. This observations is in agreement with Friedenberg et al. (1970) so far as group I of 9 days is concerned. They found that Roentgenograms of the specimen were not helpful in showing bone production on the tenth post-operative day in rabbits. However, Friedenberg et al. (1971) in another series of experiment of fibular fractures in rabbits observed radiographic evidence of accelerated fracture healing on eighteenth post-operative day by electric stimulation. Such evidence of accelerated fracture healing could not be noticed by eighteenth day in the present study, probably, this may be due to the difference in the fractures created. Similar radiographic evidence of dense callus formation on eighteenth post-operative day was observed by Satyanand et al. (1980) in treated fibulae of rabbits.

III. Arteriography

Arteriographic studies in both the groups showed apparent increase in vascularity at the treatment site as compared to control. In the treatment limbs the growth of

arterioles was more from dorsal metacarpal artery as well as from palmar metacarpal artery II and III. The branches of proximal and distal deep palmar arches showed extensive ramification when compared to control limbs. The maximum ramification and branching around the fracture site was from the distal deep palmar arch. There was an increase in vascular tributaries along the periosteum.

Table I

No. of arterioles surrounding the fracture site (Total 40 sq. cms area)

Sr. No.	Group I				Group II				Normal arteriographs	
	Lateral view		Anterio-posterior view		Lateral view		Anterio-posterior view		Lateral	Anterio-posterior
	C	T	C	T	C	T	C	T		
1.	71	100	70	105	31	47	47	52	24	51
2.	38	62	24	60	98	115	85	95	36	38
3.	53	62	50	75	90	114	86	85	38	45
4.	50	81	66	78	38	85	24	68	24	24
5.	61	45	56	30	39	42	36	38	40	65
6.	38	45	24	36	38	45	43	51	43	40
7.					73	114	75	115		

C = Control, T = Treatment.

The quantitative analysis of the increased number of arterioles is shown in Table I. When this data was subjected to paired 't' test significant increase in number of arterioles was found in treated limbs of groups I in both

lateral and anterior-posterior views. In group II significant increase was found in treated limbs, so far as the lateral view was concerned. However, no significant difference was found in anterio-posterior view in treated and control limbs. The fifth observation in group I was excluded from the analysis as the findings were in contrast to all other animals. This might be due to improper filling of arterioles with barium.

It is quite evident from the qualitative as well as quantitative results, that blood vessels assume a major role in the fracture healings. This increase is not limited only to the site of fracture but the whole bone becomes hyperemic. An increased vascularity was evident even in control limbs, when the arteriographs were compared with the normal limb arteriographs of buffaloes (Parsania et al., 1981). Teneff (1950) and Wray and Lynch (1959) observed similar increase in vascularity in early stages of fracture healing in dogs. Cavadias and Trueta (1965) while studying the vascular contribution to the fracture callus stated that "..... an outstanding fact appears, namely that the successful organization of the preliminary callus as well as the further development until reaching the final stage of repair, is in direct relation to the vascular activity elicited by the interrruption of bone continuity. The contribution of peripheral vessels, mainly periosteal, to the organisation

of the callus is much greater than that of endosteal vessels". Regional blood supply has been observed to be an important factor during fracture healing (Blevins, 1968). Rhinelander et al. (1968) observed that periosteal circulation was the chief source of blood to the healing area in case of displaced radial fracture in dogs. In view of the above facts increased vascularisation seems to be a natural phenomenon by the body mechanism towards healing of fractures. Since increased vascularisation was more in treated limbs when compared to the control limbs, it seems that electric stimulation probably brings about intense vascularisation resulting in accelerated healing response. This is in agreement with the findings of Weigert et al. (1973) in their study of electric callus in rabbits. Increased vascularisation has also been observed when other methods, other than electric stimulation, were employed for acceleration of fracture healing by different authors. Kumar (1977) recorded increased vascularisation in experimental femur and humerus fractures in buffalo calves when fresh autogenous conchal cartilage was used for acceleration of fracture healing. Khanna (1979) recorded increased vascularisation in the rib fracture of buffaloes with the use of cartilage extract. Tadkod (1980) observed intense soft tissue, periosteal and extra-periosteal vascularisation in the lower third tibial fractures on tenth and twentieth post operative days

through venous occlusion in buffalo calves. He opined that this was indicative of accelerated healing response.

It was interesting to note in the present study that the dorsal metacarpal artery was enlarged and filled up with barium in both the treated and the control limbs, more so in the treated limbs. Sapra and Dhingra (1974) and Parsania (1981) reported incomplete filling of this artery by barium in normal limb arteriographs of buffaloes. This was attributed to the fineness of this vessel by the authors. The complete filling up, enlargement and delineation of the dorsal metacarpal artery in the present study may probably due to increased vascularity of fracture site from this artery.

IV. Histomorphology:

Group I

Histomorphologically on the nineth post-operative day majority of electrically treated calluses showed extensive proliferation of fibrous tissue. Periosteally the fracture site showed innumerable fibroblasts with elongated nuclei, interspaced by a few neutrophils, Few budding capillaries were also evident. However, endosteally haemorrhagic areas still persisted at some places and these areas were invaded by many budding capillaries. Many neutrophils and few osteoclasts were

also observed in these areas. The histological picture in general was indicative of fibrovascular connective tissue.

The control calluses of these animals showed abundant haemorrhagic areas both periosteally and endosteally with slight fibroblastic activity at few places. The number of budding capillaries and neutrophils were comparatively less when compared to the treated calluses. However, many osteoclasts had invaded the haemorrhagic areas. At places organised blood clot could be observed especially at the endosteal side.

There was no difference in the histological picture of both the treated and control calluses in one experimental animal. Considerable haemorrhagic areas were still persisting at periosteal and endosteal region in both the calluses. These haemorrhagic areas were invaded by innumerable neutrophils. Few osteoclasts were also evident. The number of capillary buds observed in those calluses were very few. Fibroblastic activity was evident at very few places and was mostly restricted to the adjacent areas of bony fragments.

The electrically treated calluses of two experimental animals were observed to be well advanced in the healing process. Bony trabecular formation with wide intertrabecular spaces was evident at a few places, especially near the fractured fragments. However, surrounding these areas fibroblastic activity and well

organised clot was evident. Number of capillary buds was very less and so the invasion of neutrophils.

The control callus of one of these experimental animal did show sparce trabecular formation, however, haemorrhagic areas and fibroblastic activity was abundant. The neutrophils and budding capillaries were comparatively less. The control callus of the second experimental animal did not show any trabecular formation and the histological picture was almost similar to other control calluses.

Histomorphological studies could better demonstrate the acceleration of healing process in comparison to other parameters. The results observed are indicative of a better attempt at healing in the electrically stimulated limbs when compared to control limbs at nineth post-fracture day. Budding capillaries, an essential component of granulation tissue along with abundant fibroblasts and neutrophils in the calluses of electrically treated limbs could be demonstrated in majority of the animals in this group. Demonstration of areas of matrix in the form of trabeculae in two calluses was a further proof of acceleration of healing process. However, haemorrhagic areas with RBCs interspaced with neutrophils were still persisting in the calluses of control animals. Some fibroblasts and fibrinous meshwork though noticed in these calluses, were few. Importance of inflammatory

reaction during normal fracture healing has been shown by Cruess and Dumont (1975). The authors opined that invasion of capillaries and inflammatory cells was essential for absorption of the blood clot and necrosed bone at the fracture site. Laying of granulation tissue started after this absorption but there was some overlapping in this process as per the authors. Thus persistance of haemorrhagic areas at the fracture site of control limbs, in the present study indicated that these definitely lagged behind in the healing process when compared to the electrically treated limbs. Looking to the fibroplasia, budding capillaries and invaded neutrophils it can be very well presumed that the absorption stage had already passed in the electrically treated limbs and the healing process had advanced to the next phase of repairative process. Similar attempts at accelerated healing were observed in venous occlusion by Brookes and Helal (1968) in rabbits, Kruse and Kelly (1974) in dogs and Tadkod (1980) in buffalo calves. Further, Tadkod (1980) demonstrated differentiation of fibroblasts into osteogenic cells in the fibrous calluses of animals with venous occlusion. Friedenberg et al. (1970) while studying the bone reaction to varying amount of direct current on tenth post-fracture day in rabbits found that the bone formed to be predominantly osteoblastic type with some

areas of fibro-osseous metaplasia. The areas of bone formation were continuous with surrounding areas of cartilage.

Group II

By eighteenth day in almost all the electrically treated calluses the fibrovascular connective tissue was replaced by provisional cartilage, indicative of endochondral ossification. The osteoblasts invaded the chondroid tissue and had laid osteoid tissue at many places. The osteoid tissue was in the form of trabeculae and these trabeculae had coalesced to leave very less intertrabecular space. Varying degrees of compactness in the trabeculae of the osteoid tissue was evident. The compactness, especially at the periosteal and endosteal region of the fracture fragments, was almost comparable to the normal bone with establishment of Haversian system at places in a few of the specimen. Away from the fractured fragments few areas of fibroblastic activity with fibroblasts having elongated nuclei could be seen. The osteoblasts were observed lining the trabeculae.

The control calluses at this phase of healing were more cartilagenous. The trabecular formation was evident at places. However, these were sparce with wide intertrabecular spaces. The amount of fibrous tissue was more when compared to the treated calluses. In one of the specimen haemorrhagic areas invaded by many neutrophils

still persisted. No specimen showed compactness of bone and establishment of Haversian system.

Histomorphological evidence of acceleration of fracture healing could be demonstrated still more clearly in this group of animals where a constant 20 microampere current was passed at the fracture site continuously for 18 days. The type of ossification for the repair in both the treated and control calluses was predominantly endochondral in nature. However, the cartilage present was very less in the treated callus when compared to the controls. The presence of compact trabeculae with less intertrabecular spaces in the treated calluses was indicative of advanced healing process when compared to the controls. Further, the establishment of characteristic Haversian system in some of the treated calluses indicated a clear acceleration of fracture healing by electric stimulation. Formation of cartilage during fracture healing depends on the degree of rigidity of immobilization, chances of its formation depending on vascularization are more in unstable fractures (Braden and Brinker, 1976). Johnson et al. (1974) have stressed the importance of rigid fixation of long bone fractures through compression plating for detouring cartilage formation. However, in the present study cartilage was quite evident in both the treated and control animals, though only defects were created, where there was no

question of unstability. Cartilage can also occur whenever cellular proliferation outspaces the vascular regeneration (Ham and Leeson, 1961). Cartilage is also formed when oxygen tension is low and when oxygen is adequate bone is formed per primum (Cruess and Dumont, 1975). Cartilage formation observed in the present study could have been due to any of the above mentioned factors. Although arteriographic observations and the observed abundant capillaries at histomorphology of nineth day specimens almost exclude the possibility of lack of vascularization especially in the treated animals. It is possible that growth of the callus might have out-paced the vascularisation due to electric stimulation.

Histomorphological evidence of osteogenesis and acceleration of fracture healing through electric stimulation by direct current exists. Yasuda et al.(1955) obtained electric callus by stimulating normal bone by using a battery of 1.5 volts cells and passing a current of 1 microampere for three weeks. The authors postulated that since electricity always generated at parts of living organisms, which are active, greater potentials may also be expected in the callus. Similarly Bassett et al.(1964) found histological evidence of osteogenesis at the cathode on electric stimulation. He observed greatly increased quantity of non-oriented young trabecular bone about the

cathode on twentyfirst day. Lavine et al. (1969) also reported enhancement of bone healing, on histology, after application of 2 - 3 microamperes of current when compared to controls. Satyanand et al. (1980) observed osteoblastic activity, early calcification of cartilage tissue and some fibrous tissue on the eighteenth post-fracture day of the fibulae of rabbits when treated with 20 microamperes of direct current, while those of the controls showed organised highly vascular granulation tissue invaded with cartilage. Peripheral fibrous proliferation was also observed in the control calluses by the authors. Almost similar histological picture was evident in the present study in the treated limbs indicating enhanced fracture healing with 20 microamperes of constant current. However, the control calluses of the present study showed better healing process when compared to the control calluses of Satyanand et al. (1980). This probably may be due to the difference in the mode of creation of fracture and the species involved.

Many theories have been postulated by different workers on the mechanism by which constant direct current induces osteogenesis.

Becker et al. (1964) demonstrated orientation of collagen fibres in electrical field. In their opinion such orientation leads to spatial arrangement that

enhances calcification. Becker (1972) postulated that very small currents lead to cellular differentiation. Importance of oxygen tension at the fracture site were discussed by Brighton and Friedenberg (1974), Brighton et al. (1975) and Happenstall et al. (1975). These authors showed that the cathode consumed oxygen and produced hydroxyl radicals which helped in lowering the oxygen tension and raising tissue pH in the vicinity of the cathode. The authors were of the opinion that the low oxygen tension was favourable for bone formation and alkaline environment was favourable for laying of calcium. Brighton et al. (1981) while studying the treatment of non union with constant direct current opined that the microenvironmental changes occurring in the vicinity of the cathode lead indirectly to cellular changes which ultimately resulted in osteogenesis. They thought that, presumably, electricity acted directly on bone or cartilage, which probably activated cells cyclic AMP system. This resulted in the activation of various enzyme systems within the cell to bring about the cell's specific physiological response.

In view of the arteriographic, macroscopic and histomorphological findings in the present study, it can be emphatically said that constant electrical stimulation with 20 microamperes current does accelerate the healing process.

SUMMARY AND CONCLUSIONS

V. SUMMARY AND CONCLUSIONS

The experiment was conducted to study the effect of electrical stimulation on fracture healing in buffalo calves.

Eighteen animals were selected randomly and were divided in two groups (Group I and group II) of 9 animals each. A rectangular defect was created by chisel and hammer in both metacarpi under local infiltration anaesthesia and chloral hydrate narcosis. A constant 20 microamperes of DC current was passed continuously at the fracture site of right fore limb by a 3 volt cell and a potentiometer. The cathode was fixed at the fracture site and anode in the soft tissues proximal to fracture site, while the left limb served as a control. The animals of group I and group II were destroyed on nineth and eighteenth day, respectively.

The fracture healing was assessed by macroscopic, radiographic arteriographic and histomorphological studies.

Macroscopically in group I a whitish soft tissue was seen occupying the fracture gap in the treated limb. However, the gap in the control limb was filled by a reddish tissue indicative of blood clot.

In group II a whitish callus, firm in consistancy bridging the fracture site was observed in the treated limbs while in the control limbs whitish callus, comparatively softer in consistancy covering the fracture site was noticed.

In the present study necrosis was always observed at the anode site.

Radiographs failed to demonstrate any difference between the fracture healing of control and treated limbs in both the groups.

Arteriographic studies in both the groups showed apparent increase in vascularity at the treatment site as compared to control. Statistically, the number of arterioles showed a significant increase in the treated as compared to control limbs at 5% level of significance but no such difference was observed between the two groups. In the treatment limbs the growth of arterioles was more from dorsal metacarpal artery II and III. The branches of proximal and distal deep palmar arches showed extensive ramification.

It was interesting to note in the present study that the dorsal metacarpal artery was enlarged and clearly delinated in both the limbs, more so in treated limb as compared to control limb.

Histomorphologically, by 9th day an accelerated attempt towards healing was evident in the electrically treated callus through proliferation of fibroblasts and infiltration of cells as compared to control calluses. Trabecular formation was also seen in two treated calluses. By 18th day a definite acceleration of healing was evident

through marked osteoblastic activity and osteoid tissue. The trabeculae were compact with very little inter-trabecular space. Establishment of Haversian system was found in a few of the specimens. However, the control calluses were cartilagenous with sparce trabecular formation and wide intertrabecular space. Formation of Haversian system was not found in any of the control calluses.

Conclusions

Following main conclusions can be drawn from the present study:

1. Local infiltration anaesthesia with chloral hydrate narcosis was found to be satisfactory for creation of fracture in the middle of the metacarpus.
2. Crescent shaped incision adequately exposed medial surface of metacarpal bone and fascilitated creation of a rectangular defect.
3. Use of two sizes of chisel for creating a clear cut rectangular defect of uniform size proved to be satisfactory.
4. Stainless steel wire electrodes insulated with polythene tubings were found advantageous for electrical stimulation since they could be anchored very easily.

5. A 3 volt cell was found to be sufficient for maintainance of 20 microamperes of constant electric current. The device could be conveniently fixed on the back of the animal in a rexin case.

6. Electrical stimulation of the fracture site resulted in increased vascularity and probably, this helped in accelerating the healing process.

7. Radiographs failed to delineate the difference in the healing process between the treated and control limbs.

8. The repair of fracture defects in buffalo calves is mainly through endochondral ossification irrespective of immobilization.

9. Macroscopic, arteriographic and histomorphological evidence exists to show that electric stimulation results in accelerating the healing process of bony defects.

10. Histomorphological studies provide a better picture of the bone healing process than radiography, since changes in structural components of the bone are visualized better.

R E F E R E N C E S

R E F E R E N C E S

Bassett, C.A.L. and Becker, R.O. (1962). Generation of electric potentials by bone in response to mechanical stress. Science. 137:1063-1064.

Bassett, C.A.L., Pawluk, R.J. and Becker, R.O. (1964). Effects of electric current on bone in vivo. Nature. 204:652-654.

Becker, R.O. (1972). Augmentation of regenerative healing in man. A possible alternative to prosthetic implantation. Clin. Orthop. 83:255-262.

Becker, R.O., Bassett, C.A.L. and Bachman, C.H. (1964). Bioelectric factors controlling bone structure. In Bone Biodynamics. Edited by H.M. Front. Little Brown and Company. Boston. Pp. 209-232. Cited by Friedenberg et al. (1974). J. Bone and Joint Surg. 56A:1023-1030.

Blowins, W.E. (1968). Bone vascularization and its effect on fracture healing. Iowa State Univ. Vet. 3:69-74.

Braden, T.D. and Brinker, W.O. (1976). Radiographic and gross evaluation of bone healing in dog. J. Am. vet. med. Ass. 169:1318-1323.

Brighton, C.T., Adler, S., Black, J., Itada, N. and Friedenberg, Z.B. (1975). Cathodic oxygen consumption and electrically induced osteogenesis. Clin. Orthop 107:277-282.

Brighton, C.T., Black, J., Friedenberg, Z.B., Esterhai, J.L., Lorraine, J.D. and Connoly, J.F. (1981). A multi-center study of the treatment of non-union with constant direct current. J. Bone and Joint Surg. 62A:2-13.

Brighton, C.T. and Friedenberg, Z.B. (1974). Electrical stimulation and oxygen tension. Ann. New York Acad. Science. 238:314-320.

Brighton, C.T., Friedenberg, Z.B., Zemesky, L.M. and Pollis, R.P. (1975). Direct current stimulation of non-union and congenital pseudoarthrosis. J. Bone and Joint Surg. 57A:368-377.

Brookes, M. and Helal, B. (1968). Primary Osteoarthritis, venous engorgement and osteosynthesis. J. Bone and Joint Surg. 50B:493-504.

Cavadias, A.K. and Trueta, J. (1965). An experimental study of the vascular contribution of the fracture of callus. Surg. Gynaec. Obstet. 120:731-747.

Chamay, A., Richez, J. and Bieler, L. (1972). The influence of electric impulses in osteogenesis. Rev. Chir Orthop. 58:389 (Original not seen. Abstr. Surg. Gynaec. Obstet. (1974) 138:502).

Cruess, R.L. and Dumont, J. (1975). Current concepts: Fracture healing. Canad. J. Surg. 18:403-413.

Friedenberg, Z.B., Andrews, E.T., Smolenski, B.I., Pearl,B.W. and Brighton, C.T. (1970). Bone reaction to varying amounts of direct electric current. Surg. Gynaec. Obstet. 131:894-899.

Friedenberg, Z.B. and Brighton, C.T. (1966). Bioelectric potentials in bone. J. Bone and Joint Surg. 48A:915-923.

Friedenberg, Z.B. and Kohanim, M. (1968). The effect of direct current on bone. Surg. Gynaec. and Obstet. 127:97-102.

Friedenberg, Z.B., Roberts, P.G., Didizian, N.H. and Brighton, C.T. (1971). Stimulation of fracture healing by direct current in the rabbit fibula. J. Bone and Joint Surg. 53A:1400-1408.

Friedenberg, Z.B., Zemesky, L.M., Pollis, R.P. and Brighton, C.T. (1974). The response of non-traumatized bone to direct current. 56A:1023-1030.

Ham, A.W. and Leeson, T.S. (1961). Histology, Fourth Ed. J.B. Lippincott Company, Philadelphia Pq. U.S.A. p.331.

Hambury, H.J., Watsons, Sivyer, A. and Ashley, D.J.B. (1971). Effect of microampere electrical current on bone in vivo and its measurement using strontium-85 uptake. Nature. 231:190-191.

Heppenstall, R.B., Grisilis, G. and Hunt, T.K. (1975). Tissue gas tensions and oxygen consumption in healing bone defects. Clin. Orthop. 106:357-365.

Johnson, J.H., Lowe, J.E., Fessler, J.F. and Amstutz, H.E. (1974). The musculoskeletal system. Text Book of large animal surgery. Edited by Oeheme, F.W. and Prier, J.E. The Williams and Wilkins, Company, Baltimore. Pp. 232-339.

Khanna, A.K. (1979). An investigation into the effects of some osteogenic agents in fracture healing in buffalo calves. M.V.Sc. thesis. Punjab Agricultural University, Ludhiana, India. Cited by Tadkod (1980), Ph.D. dissertation.

Kruse, R.L. and Kelly, P.J. (1974). Acceleration of fracture healing distal to venous tourniquet. J. Bone and Joint Surg. 56A:730-739.

Kumar, R.V. (1977). Study of some immobilization techniques in the repair of long bone fracture in bovines with special references to the effect of some osteogenic agents. Ph.D. dissertation. Punjab Agricultural University, Punjab, India. Cited by Tadkod (1980). Ph.D. dissertation.

Lavine,L.S., Lustrin, I., Rinaldi, R.A., Shamos, M.H. (1974). Clinical and Ultrastructural investigations of electrical enhancement of bone healing. Ann. N.Y. Acad. Sci. 238:552-563.

Lavine,L.S., Lustrin, I. and Shamos, M.H. (1969). Experimental model for studying the effect of electric current on bone in vivo. Nature. 224:1112-1113.

Lavine,L.S., Lustrin, I., Shamos, M.H., Rinaldi, R.A. and Liboff, A.R. (1972). Electric enhancement of bone healing. Science. 175:1118-1120.

Martin, R.B. and Gutman, W. (1978). The effect of electric fields on osteoporosis of disuse. Calcif. Tissue. Res. 25:23-27.

O'Connor, B.T., Charlton, H.M., Curry, J.D., Kirby, D.R.S., and Woods, C. (1969). Effects of electric current on bone in vivo. Nature. 222:162-163.

Parsania, R.R. (1981). Comparative arteriography of the distal parts of limbs in the Buffalo, Ox and Sheep. Special problem submitted to Gujarat Agricultural University, Gujarat, India.

Pollis, R.P., Zachary, B., Friedenberg, Z.B. and Brighton, C.T. (1973). The atraumatic osseous response to electric current. J. Bone and Joint Surg. 55A:653.

Rhinelander, F.W., Phillips, R.S., Steel, W.M. and Beer, J.C. (1968). Microangiography in bone healing. J. Bone and Joint Surg. 50A:643-662.

Sapra, R.P. and Dhingra, L.D. (1974). The blood vessels of the thoracic limb of buffalo (Bubalus bubalis). The arteries. Anat. Anz. 135:116-139.

Satyanand, Debral, P.K., Basu, P.K. (1980). Fracture healing by electrical stimulation. Indian Journal of Surgery. 42:338-3 .

Shamos, M.H., Lavine, L.S., Shamos, M.I. (1963). Piezo-electric effect in Bone. Nature 197:81.

Sharma, A., Singh, H., Singh, A.P., Pandiya, S.C. and Amreshkumar (1977). Angiographic studies in metatarsal fractures in buffaloes. Indian J. Anim. Sci. 47:626-629.

Srivastava, K.P. and Saxena, A.K. (1977). Electrical stimulation in delayed union of long bones. Acta Orthop. Scand. 48:561-565.

Steinberg, M.E., Busenkell, G.L., Black, J. and Edward Koresteff, (1974). Stress induced potentials in moist bone in vitro. J. Bone and Joint Surg. 56A:704-713.

Tadkod, D.M. (1980). Clinical, Radiological and Histochemical studies on healing pattern of tibial fractures in buffaloes with special reference to venous occlusion. Ph.D. dissertation. Punjab Agricultural University, Ludhiana, India.

Teneff, S. (1950). Experimental studies on vascularization of bony calluses. J. Int. Coll. Surgeons. 13:186-191.

Treharne, R.W., Brighton, C.T., Koresteff, E. and Pollack, S.R. (1979). An in vitro study of electrical [illegible] osteogenesis using direct and pulsating currents. Clin. Orthop. 145:300-306.

Weigert, M., Wernahan, C., Mellerowicz, H. and Bemden, R. (1973). Stimulation of longitudinal growth and fracture healing by electric current. Orthopaedic Surgery and Traumatology. Edited by Delchef, J., Demarneffe, R. and Vander, E.V. Experta Medica. Amsterdam, Netherlands. Pp. 546-547.

Wray, J.B. and Lynch, C.J. (1959). The vascular response to fracture of the tibia in the rat. J. Bone and Joint Surg. 37A:1292-1293.

Yasuda, I., Naguchi, K. and Sata, T. (1955). Dynamic callus and electric callus. J. Bone and Joint Surg. 37A:1292-1293.