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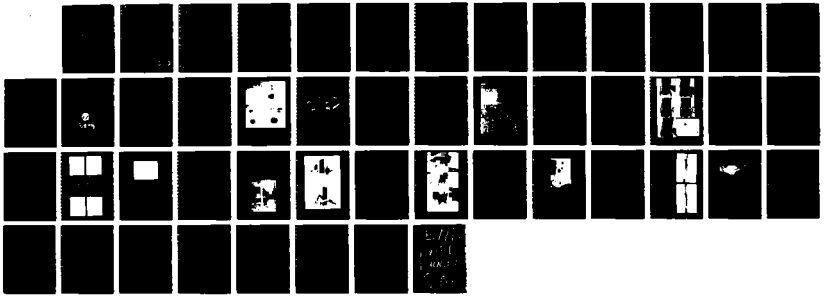

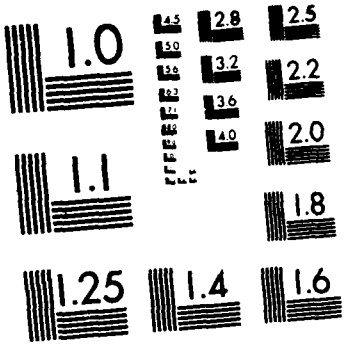

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ELECTRICALLY MEDIATED TRAUMA REPAIR

Annual Report

Richard B. Borgens
Associate Professor

DECEMBER 1987

Supported by

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Historically our group has been interested in the way that naturally produced currents of injury and their associated electrical fields are involved in controlling a cell or tissue's response to damage. Studies of these fields has led to novel applications of current to injury systems which have demonstrated that indeed a tissue (for example, bone) or a cell's (for example, a nerve fiber) regenerative capacity can be enhanced. In recent years we have focused on the nervous system and bone. We have demonstrated that severed nerves within the adult guinea pig spinal cord can be induced to regenerate and form functional connections. This electrically enhanced spinal cord regeneration is associated with a functional recovery of an otherwise permanent defect in a significant proportion of experimental animals in preliminary experiments. We have also learned that we can facilitate or enhance peripheral nerve regeneration in the adult guinea pig. This facilitated regeneration

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19. (Abstract continued)

has the important property of significantly reducing the time between nerve lesion and the onset of functional recovery. In bone we have developed a thorough approach to testing the possibility that the clinical use of applied fields may enhance the rate and biomechanical properties of normal fracture repair using a canine tibial fracture model. Our mission is to develop all of these techniques to the stage where they can be realistically considered for Human clinical trials. Key words: Mission Profiles.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

A. STATEMENT OF PROBLEM

Our laboratory has been interested in the role that natural and applied electrical currents may play in the regeneration of tissues in vertebrates (Borgens 1982, 1985, 1986, 1987, 1988a, 1988b). Our basic science investigations into the endogenous currents produced by injury has utilized the Ultrasensitive Vibrating Probe for the measurement of extracellular current (Jaffe and Nuccitelli, 1974). By characterizing the natural currents of injury - we then attempt to modify the tissue responses to injury by imposing electrical fields across injured tissues. This approach has been used in studies of bone fracture healing (Borgens, 1983), amphibian limb regeneration and development (Borgens, 1982, 1987), eye regeneration in Gastropod molluscs (Bever and Borgens, 1988), and the regeneration of reticulospinal neurons within the severed lamprey spinal cord (Borgens, 1979, 1981, reviewed 1987). In recent years we have investigated if such electrically mediated regeneration may hold promise for clinical treatment in the nervous system of man as well as a method to enhance the rate of fracture repair. It is the studies of CNS and PNS regeneration and fracture repair that has been supported by the current DOD (Dept. of Army) contract.

During the last year we have established that:

a. An applied electrical field will induce a robust regeneration of identified long tract axons within the hemisected spinal cord of the adult guinea pig (Borgens et al., 1986).

b. That this electrically facilitated regeneration is associated with a functional recovery of a specific and quantifiable long tract spinal reflex (The Cutaneous Trunchi Muscle Reflex - CTM). This defect is produced by severance of the ascending sensory afferents of the reflex loop and is permanent for the life of the animal. Applied fields induce a recovery rate in acutely injured guinea pigs of about 25%. (Borgens et al., 1987)

c. Applied fields induce a substantial increase in the rate of peripheral nerve regeneration in the adult guinea pig associated with substantial decrease in the latency between nerve trunk lesion and the onset of functional recovery (using a peroneal nerve lesion and toe spreading behavior as the experimental model) (McGinnis and Borgens, in preparation).

d. In collaboration with Dr. David Van Sickle, we are now applying fields in a canine tibial fracture model. Pilot experiments have been completed and will be discussed below.

The problems we wish to address in the upcoming contract year are these:

1. We need to develop a more complete understanding of the most optimal field strengths to influence CNS regeneration within the spinal cord. This may lead to increases in the percentage of animals that recover function in the CTM paradigm.

2. The CTM model addresses functional recovery in ascending sensory projections within the adult mammalian spinal cord - and we need to further develop behavioral models in the guinea pig that address descending motor projections.

3. Since we have now established that a functional recovery is possible after spinal cord lesion in the adult mammal, we need to develop a stimulation technique whereby both (ascending and descending) projections of nerves within the spinal cord can be induced to regenerate at the same time. This is the only intelligent approach to further development of a truly relevant clinical therapy.

4. We need to complete our preliminary studies of the effect of applied fields on peripheral nerve, and establish the optimum field strength to produce the greatest regenerative response.

5. In our fracture studies, we now possess a rigorous and reproducible fracture model using a canine tibial fracture as well as a thorough means to assess the biomechanical and anatomical characteristics of union between electrically treated and sham treated animals (explained below). We have further developed an implantable stimulator system designed specifically for the dog. We now need to complete these studies and determine if indeed applied currents can enhance the overall success or speed the rate of normal fracture repair using this clinically meaningful model system.

B. BACKGROUND

1. Applied Electric Fields and Nerve Regeneration

Since the early part of this century, it has been suggested that electric fields can influence the growth of nerve fibers. The merit of this notion was first demonstrated by S. Ingvar in 1920 on neurites in culture. Subsequently, Weiss (1934) and D. Ingvar (1947) found no effect of applied electric fields on neurites in vitro while Marsh and Beams (1946), Sisken and Smith (1975), and Jaffe and Poo (1979) all demonstrated a growth response toward the cathode. Finally, Hinkle, McCaig, and Robinson (1981) and Patel and Poo (1982) published what have come to be accepted as the definitive work in this series. They very clearly demonstrated, using isolated neurones from frog embryos, that: (1) neurites grow preferentially toward the cathode of a DC field, (2) greater numbers of neurones sprout neurites in the presence of a field, and (3) neurites facing the anode can be resorbed. These findings have recently been refined in a series of papers by McCaig (1986a, b, 1987). Patel and Poo (1984) extended these observations to focal currents applied to single growth cones. The accumulation of evidence from in vitro studies that electric fields can influence nerve growth has been paralleled by observation of electrically enhanced nerve growth in vivo. Borgens et al. (1977, 1979) noted that an element of electrically stimulated regeneration in frog limbs was a profusion of nervous tissue in the hypomorphic limbs that formed. In Xenopus, they found 40 times more nerve in the electrically treated limbs than in the sham treated control limbs.

The electrical enhancement of nerve regeneration in vivo has also been demonstrated in the central nervous system. Small DC currents passed across transected spinal cords of lamprey enhanced regeneration of the large reticulospinal axons (Borgens et al., 1981). Recent evidence has been presented that similar effects can be attained in the mammalian CNS (Borgens et al. 1986). That such electrically induced axonal regeneration can be associated with functional recovery of a specific spinal reflex has also been demonstrated (Borgens et al., 1987). Taken as a whole, both the in vitro and in vivo experiments indicate that electrically enhanced nerve growth and regeneration is a well established finding. It is important to note that all reports consistently demonstrate an enhanced growth toward the cathode and reduced growth or even degeneration toward the anode. Although several studies have been published on the electrical enhancement of mammalian peripheral nerve regeneration, none have shown convincing evidence of a response of PNS to applied fields (see Pomeranz 1984, 1986 and Kerns et al. 1986). Presently emerging from a variety of studies is the understanding that steady, biologically generated, electric fields play a significant role in both development and repair processes in a variety of tissues, including nerve (Borgens, 1982). The observation that applied electric fields can enhance nerve regeneration is consistent with this hypothesis.

a. The following is a summary of some of the well established observations of the effect of electric fields on nerve.

i) For embryonic nerves in culture (amphibian and chick) it is known that:

a) neurites grow preferentially toward the cathode (Jaffe and Poo, 1979; Hinkle et al., 1981); b) growth is inhibited or resorption initiated toward the anode (Hinkle et al., 1981; McCaig, 1986b); c) growing neurites can deviate to grow toward a cathode (Hinkle, et al., 1981; McCaig 1986b; Patel and Poo, 1984); d) fields can override contact guidance cues (McCaig, 1986a); e) both peripheral and central nerves respond (Jaffe and Poo, 1979; Hinkle et al., 1981); f) both regenerating and developing nerves respond (Jaffe and Poo, 1979; Hinkle et al., 1981); g) the percentage of neurones producing neurites is enhanced in a field (Hinkle et al., 1981); h) an apparent threshold for the effect exists at 10 mV/mm (Hinkle et al., 1981; Patel and Poo, 1982); i) nerves do not respond to alternating fields (0.1-1.0 Hz). Only DC fields are effective (Patel and Poo, 1982); j) There are different kinetics of the cathodal enhancement and the anodal inhibition of growth (McCaig, 1986b, 1987); k) alteration of growth cone morphology occurs (Patel and Poo, 1984; McCaig 1986b); l) focal fields applied through microelectrodes are also effective in orienting fibers (Patel and Poo, 1984).

ii) From in vivo studies in vertebrates it has been established that:

a) nerve growth is enhanced toward a cathode as compared to a sham or anode (Borgens et al., 1977, 1979); b) the extent of axonal dieback following lesion is inhibited toward a cathode and enhanced toward an anode (CNS) (Roederer et al., 1983); c) DC currents enhance

sprouting of intact peripheral nerves (Pomeranz et al., 1984); d) DC currents can induce regenerative growth in the mammalian spinal cord (Borgens et al., 1986b; e) electrically induced regeneration in the mammalian spinal cord can result in recovery of a specific reflex behavior (Borgens, et al., 1987). Since these last two observations were completed during the period covered by this annual report, they will be described here in detail.

We tested the possibility that an applied field could induce - or permit - a more luxurious growth of axons in the adult mammalian spinal cord as has been observed in non-mammalian vertebrates and in culture conditions. Before beginning this experiment we felt it was necessary to develop a proper mammalian model system and set of techniques to rigorously address this issue. Lastly we wished to be able to analyze these axons within thick sections of spinal cord (this bias was derived from our experience with the comparably unambiguous Lamprey cord preparation).

2. Applied Fields and the Mammalian CNS

We felt that to understand the nature of our application, we should develop a model that provided the following: (a) A means to analyze a subpopulation of long tract axons within a given spinal cord tract. (b) A means to discriminate these axon from fibers arising near the site of injury, sympathetic axons, or originally undamaged axons within the plane of the lesion (surviving fibers). (c) The highest degree of resolution at the light microscope level, providing a high degree of cytological detail but within relatively thick sections of tissue. This allows observations of fiber trajectories without having to reconstruct them from many individual sections. (d) An unequivocal ability to determine the precise plane of transection at any time post-injury. To accomplish this we adopted the use of a marker device similar to that previously used in brain lesions by A. Foerster (1985).

In our studies (Borgens et al., 1986a) we performed transverse dorsal hemisections extending just ventral to the central canal. These lesions were made first with iridectomy scissors, followed by passage of a fine tungsten needle to verify the completeness of the lesion. This procedure completely severed the dorsal columns. Into this open incision (made with iridectomy scissors, followed by a tungsten needle), we placed the Foerster device (see figure in following section). At various times post transection, we analyzed the responses of axons to transection by intracellular filling with Horseradish peroxidase (HRP). Our observations (determined from studying cords that were between 12 hours post-transection and 90 days post-transection) were these: Dorsal column axons undergo retrograde degeneration, beginning immediately after transection, and continue to degenerate for about 1 week to 10 days afterward. The extent of dieback is on the order of 0.5 to 1 mm caudal to the lesion. At this point, these axons sprout and show a limited regenerative attempt. This endogenous regeneration was evidenced by bifurcated endings on large caliber myelinated axons, neurites sharply reversing direction, and terminal growth cones. Such regenerative responses did not result in linear elongation of more than a few hundred microns. Large caliber (4-10 um diameter) myelinated axons were rarely found within the glial scar.

This very limited growth of fibers is consistent with the observations of many previous studies (Ramon y Cajal, 1928; Gilson and Stensaas, 1974; Lampert and Cressman, 1969; Kiernan, 1979; and Berry, 1979).

a. Response to Applied Electric Fields.

We applied total currents of 1, 5, and 10 uA to dorsal hemisections in three experimental groups. Currents were imposed by means of a stimulator unit developed in this laboratory (for details refer to the following methods section). Briefly, the experiment was conducted in this manner: Active stimulators (or sham units for control groups) were implanted intraperitoneally in adult 0.5-1 kg guinea pigs. Stimulating electrodes were routed subdermally to the back of the animals and located within laminectomies 1.5-2 cm. rostral and caudal to the transection) (refer to diagram in the methods section). These electrodes were electrically continuous with the spinal cord via body fluids and cerebrospinal fluid filling this cavity. The electrodes did not, however, touch the cord itself. They were fastened within the walls of the vertebral column by suturing to the musculature of the back. All three incisions were closed.

One day prior to the termination of the experiment, animals were anesthetized and a fourth surgical approach to the cord was made. This was for making a shallow incision into which were placed crystals of HRP. This incision was closed and 12-18 hours later animals were sacrificed by anesthesia followed by perfusion - fixation. Spinal cords were dissected from the animals, the marking device removed, serial frozen sections were taken, and tissues were processed with diaminobenzidine for the HRP reaction and then dehydrated, cleared and mounted on slides.

b. Sham-Treated Animals

The appearance of axons within the control group at 50 days post-transection was unremarkable. In all ways these spinal cords appeared the same as untreated animals studied prior to beginning tests on the effects of applied fields. Briefly, the terminal ends of dorsal column axons were found well caudal to the plane of transection. In only two of eleven cases were there even axons visible within the caudal boundary of the glial scar- all other HRP filled axons in all other animals were caudal to this point by several hundred microns.

c. Current-Treated Animals

In animals whose stimulators produced 1 and 5 uA total current, HRP filled dorsal column axons were found to ramify throughout the glial scar caudal to the plane of transection. These axons displayed a variety of features indicative of active and vigorous regeneration including branched terminal endings, and fibers that diverted their vector of growth by 180-300 degrees to circumnavigate obstacles (such as capillaries within the newly formed glial scar). None of these axons, however, crossed over to the rostral segment of the spinal cord though they did grow near to the plane of transection. In the 10 uA series, all of the above features were observed, however in about 50 percent of these animals, axons traversed into the rostral portion of the spinal cord. They bridged the lesion not

by directly growing through the plane of transection, but by growing around it. The pathway taken by regenerating fibers was determined by their original position within the dorsal column. Laterally placed fibers grew into the fringe of the glial scar and followed its boundary to the more dense scar that encapsulated the lateral pin of the marker device. Fibers adhered to this closely, growing around it into the rostral portion of the cord. When on the rostral side of the original plane of transection, they then deviated their axis away from this area to return to their original position within the spinal cord, projecting toward the brain. Medial axons of the dorsal columns grew directly through the caudal portion of the glial scar to the plane of transection. At this point, they deviated their axis of growth sharply ventral and descended into the cord, sometimes growing caudally to regenerate around the swollen protuberance of the severed central canal. Axons also adhered tightly to the dense scar encapsulating the ventral pin of the maker device. After growing around this obstacle into the rostral portion of the spinal cord, fibers ascended the cord sharply (circumnavigating obstacles such as the marking device, or the rostral segment of the swollen central canal). When their original position within the cord was reached, axons turned abruptly and projected rostrally toward the brain.

It is important to note that these complicated pathways through foreign terrain (i.e. dorsal axons plunged well into the ventral cord in circumnavigating the lesion) underscore the striking nature of this axonal regeneration. Axons did not grow into grossly inappropriate regions of the spinal cord after crossing into the rostral segment. Since we had employed a marker for the exact level of transection, and since we had determined (in earlier studies) an average figure for expected retrograde degeneration after the acute severance - we can suggest that the total growth of these fibers at 50 days post transection was on the order of 1-2 cm.

In summary, untreated and sham-treated animals showed little power of axonal elongation after axotomy. In electrically-treated animals (especially the higher current series used) fibers regenerated around the scar into the rostral segment of the spinal cord. It should be remembered that our HRP filling procedure only marks a subpopulation of dorsal column fibers - it does not mark any intact fibers that enter the cord at more rostral vertebral segments. Thus, our sample is presently quite conservative and does not identify all possible regenerating fibers within even this one tract.

d. Recovery of function in the CTM reflex.

Our most recent studies have addressed the problem of functional effects of electric-field mediated regeneration in spinal cord sensory (CTM) pathways. Briefly: Using this novel propriospinal intersegmental reflex, we have shown that a weak electrical field applied across a lesion of the guinea pig spinal cord is associated with CTM functional recovery in a proportion of the current-treated animals. The functional defect is permanent in sham-treated controls.

e. The CTM Reflex

The CTM reflex is a behavioral function of cervical spinal cord motor units that depends on sensory input from lumbar and thoracic dorsal roots. The underlying circuitry of this reflex has been determined in some detail by Diamond and collaborators in the rat (Nixon et al. 1984; J. Diamond, personal communication). The behavior can be measured clearly and simply either visually or electromyographically to give quantitative information on both amplitude and spatial distribution (see figures in Methods section).

We can easily determine the spatial distribution of the reflex response by marking the back skin of the animal and observing skin movements in response to light tactile stimulation. There is a chronic unilateral loss of responsiveness to ipsilateral stimulation below a vertical hemisection of the lower-thoracic cord. We have found no evidence of recovery of this reflex on the lesioned side, at least during observation periods in excess of 1 year. There appears to be no capacity for contralateral pathways to contribute to functional restoration of the ipsilateral reflex. Therefore, chronic recovery of the reflex in conjunction with signs of regeneration of ipsilateral sensory pathways of the lateral tracts would be strong evidence indicative of functional recovery through reconnection of regenerated axons. We already have evidence of such functional recovery in a proportion of animals whose hemisected cords were exposed to 35 uA total current.

The reflex may also be quantified by digital analysis of videotape records and electromyography (see section C. 5. - Behavioral Techniques) This will allow relatively complete documentation of changes in the response subsequent to spinal lesions.

3. Applied Electric Fields and Fracture Repair

It is established that an applied direct electric current can enhance the healing of intractable bone lesions in humans (chronic non-unions) (Brighton, 1981). It is not clear whether this clinical procedure supplements naturally produced currents that are crucial for bone healing, or whether it promotes bone healing by some different mechanism. The existence of a steady current traversing bone could provide an experimental rationale for the mechanism of action of clinically applied direct currents however, prior to 1984 there were no reports of endogenous steady current in living bone. Using our ultrasensitive vibrating probe system, we explored the fields about intact as well as damaged bone of the laboratory mouse immersed in a natural medium. I will briefly recount the main points of this investigation (Borgens, 1984).

a) Intact living bone drives a substantial and steady electric current through itself. Current enters the primary cartilaginous and regions of bone, and enters and leaves the shaft.

b) A fracture to a bone produces an immediate and large leak of current into the lesion. These densities (on the order of 100 uA/cm^2)

decay to a stable level of about 5 ua/cm^2, which may persist indefinitely. The decay to this "plateau" level takes on the order of 5-15 minutes.

c) The initial large and declining currents are independent of cellular metabolism and are produced by a deformation of bone. This can be demonstrated since fixed bone or dry bone can produce these densities of current which eventually decay to zero. The steady plateau of about 5 uA/cm^2 is driven by a cellular battery, and is observed in only living bone. this "plateau current" is also responsive to changes in the ionic composition of the measurement media and is dependent on body temperature.

d) Finally, endogenous fracture currents are of the same polarity and of roughly the same magnitude as clinically applied currents that are successful in treating chronic fracture non-unions. This furthermore suggests that the defect in biological non-unions may ultimately be a defect in the electrophysiology of repair.

These measurements of endogenous current have suggested to us that natural currents and their associated voltages and fields may play a role on bone remodeling and repair. Support for this view could be gained from the clinical use of applied DC fields in healing fracture non-unions. However, as mentioned one cannot state unequivocally that such applied fields induce changes in hard tissue - apart from the complicated chemical changes that occur at the metallic electrodes. To this end we have begun studies of current mediated effects in rat long bones. We deliver current to the animals target tissues via long wick electrodes phased to salt bridges. These "aqueous wires" carry current from the voltage source to the tissues via a solution of electrolytes (mammalian ringers). In this way we can view any response to stimulations as being mediated by electrical effects.

In preliminary experiments we have driven total current of 1-3 uA between two wick electrodes surgically mounted next to undamaged adult rat femurs. The electrodes were about 1-2 cm apart. Current was delivered for 2 to 3 weeks at which time the animals were sacrificed, and femurs were removed (with a small portion of each electrode still attached), embedded in methracylate, sectioned longitudinally (50-70 um thick) and stained with villanova's metachromatic stain. At the present time we are now analyzing these sections quantitatively using a Zeiss Osteoplan Image Analysis System. However, it would be pertinent to describe several of the obvious histological distinctions between current-treated and sham-treated bones.

i) In all animals, new bone was deposited near the site of electrode attachment to the outside of the femur. Obviously, the physical irritation of the silastic tubes and suture against the periosteal surface induced osteogenesis. We estimate that 2-4 fold more bone was deposited around the electrodes in current-treated animals than in controls. At present, we are quantifying this as mentioned - however it is quite obvious that in the experimental group - their responses are exaggerated. In experimentals, bone was deposited on the external surface at both electrodes in such a fashion that the femurs were greatly distorted in shape.

ii) A particularly striking observation was that a large nodule of bone was deposited on the endosteal surface of the experimentally treated bone directly adjacent the electrode in contact with the outer surface of the diaphysis. In a few cases such nodules were observed adjacent both electrodes. This suggests that where current penetrated the diaphysis, bone was deposited on the inner surface (marrow cavity or endosteal surface). No such nodules were observed in sham-treated controls.

Altogether we can now suggest (with some experimental support), that: large, steady current are driven through long bones; these may be a component in the control of remodeling and repair; and that applied fields, free of electrode products, can indeed remodel the form and character of mammalian long bones. These observations and interpretations form the basis for our new studies in which we attempt to enhance the rate and strength the character of normal fracture repair. We use a clinical model - a canine tibial fracture (in collaboration with Dr. David Van Sickle's laboratory). We have developed a reproducible and rigorous fracture model as well as novel anatomical and biomechanical methods of analysis of union (described in next section).

C. APPROACH AND METHODOLOGY

1. Surgical Implantation of the Stimulator

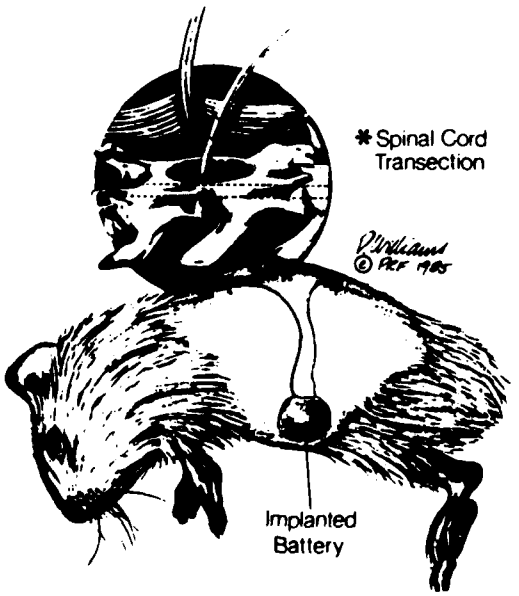

Figure 1.

This drawing shows the intraperitoneal battery implantation and the position of the subcutaneous electrodes. The inset shows the approximate end of the wick electrode adjacent the exposed spinal cord. Note that the electrode is not touching the cord and is fastened in this position by sutures to the musculature (a suitable length of suture is fastened to the tip of the electrode with silastic adhesive during the fabrication of the stimulator).

Animals are anesthetized with an i.m. injection of a mixture of 35 mg/kg ketamine HCL, 3.5 mg/kg acepromazine maleate, and 5 mg/kg Xylazine. The peritoneal cavity of the animal is opened by longitudinal incision. The stimulator is inserted into this cavity - the wick electrodes are left protruding after the cavity is sutured closed. A tunnel beneath the skin is made from this site with a blunt probe to exit a small hole in the mid-dorsal backskin. After the electrodes have been routed from the belly through this tunnel, the belly skin is then closed with surgical clips. The animal is then turned over, and three small laminectomies are performed. A central one for the lesioning of the spinal cord and the insertion of the marker device, and two others, one rostral and one caudal to this site for the implantation of the stimulating electrodes. These electrode sites are approximately 2 cm rostral and 2 cm caudal to the spinal cord lesion (for details of the lesioning technique see below). After the cord was exposed (at all three locations) the dura was opened with watch-markers forceps. Wick electrodes were gently secured within the vertebral column - but not touching or compressing the spinal cord.

2. Stimulator Design: See figure and legend on following pages.

Figure 2. (Next page)

A) These views of a unipolar battery stimulator show the: (a) coiled platinum iridium (PtIr) stimulating electrodes - positive and negative; (b) voltage source = two lithium dioxide manganese cells (Sanyo, CR-1220) in series providing 6 volts at 30 milliamp hours capacity; (c) constant current source - National Semiconductor LM-334; (d) resistor, which sets the magnitude of the total current delivered; (e) a current monitoring resistor (1K), to (f) monitoring leads from this resistor to exit animal to monitor stimulator operation); (g) complete unit coated in medical grade elastomer, and ready for sterilization and implantation.

B) This layout shows the components of an oscillating field stimulator that delivers a set current (i.e., 35 uA) with a field that reverses its polarity at a predetermined duty cycle (with a range of microseconds to hours). (s) Coiled PtIr stimulating electrode; (b) voltage source - two lithium dioxide manganese cells (Duracell, DL-2025) in series providing 6 volts at 120 milliamp hours capacity; (c) CMOS low power operational amplifier (Intensil ICL-7611); (d) CMOS 14-stage ripple-carry binary counter/divider with an internal oscillator (RCA-CD4060); (e) timing capacitor; (f) two resistors, with a timing capacitor set the frequency of the oscillator; other resistors are located beneath chip, out of field of view and set the magnitude of the total current delivered; (g) printed circuit board - produced at Purdue University; (h) The complete unit coated in medical grade elastomer and ready for sterilization and implantation.

The Unipolar Stimulator (A) has been designed to replace the older wick stimulator units. With proper implantation of the electrodes, a homogenous field, parallel with the long axis of the spinal cord, can be imposed across a lesion using the PtIr electrodes. One of these (the cathode) is situated near the spinal cord within a laminectomy, while the anode is placed about 2 cm distant on the other side of a lesion and situated on the axial musculature dorsal to the cord but beneath the skin. The voltage source is implanted subdermally and not within the peritoneal cavity (see manuscript in appendix). Contamination of the cord parenchyma by noxious electrode products liberated at the PtIr cathode has not been found to be problematic - while the anode can cause some tissue destruction. Hence it is removed to a distant location.

The Oscillating Field Stimulator (B) is designed to influence regeneration of ascending and descending projections of axons within the spinal cord while reducing electrically mediated dieback (Roederer et al., 1983). This unit can also be implanted subdermally, however both PtIr electrodes need to be positioned within laminectomies rostral and caudal to the spinal lesion) (see manuscript in appendix).

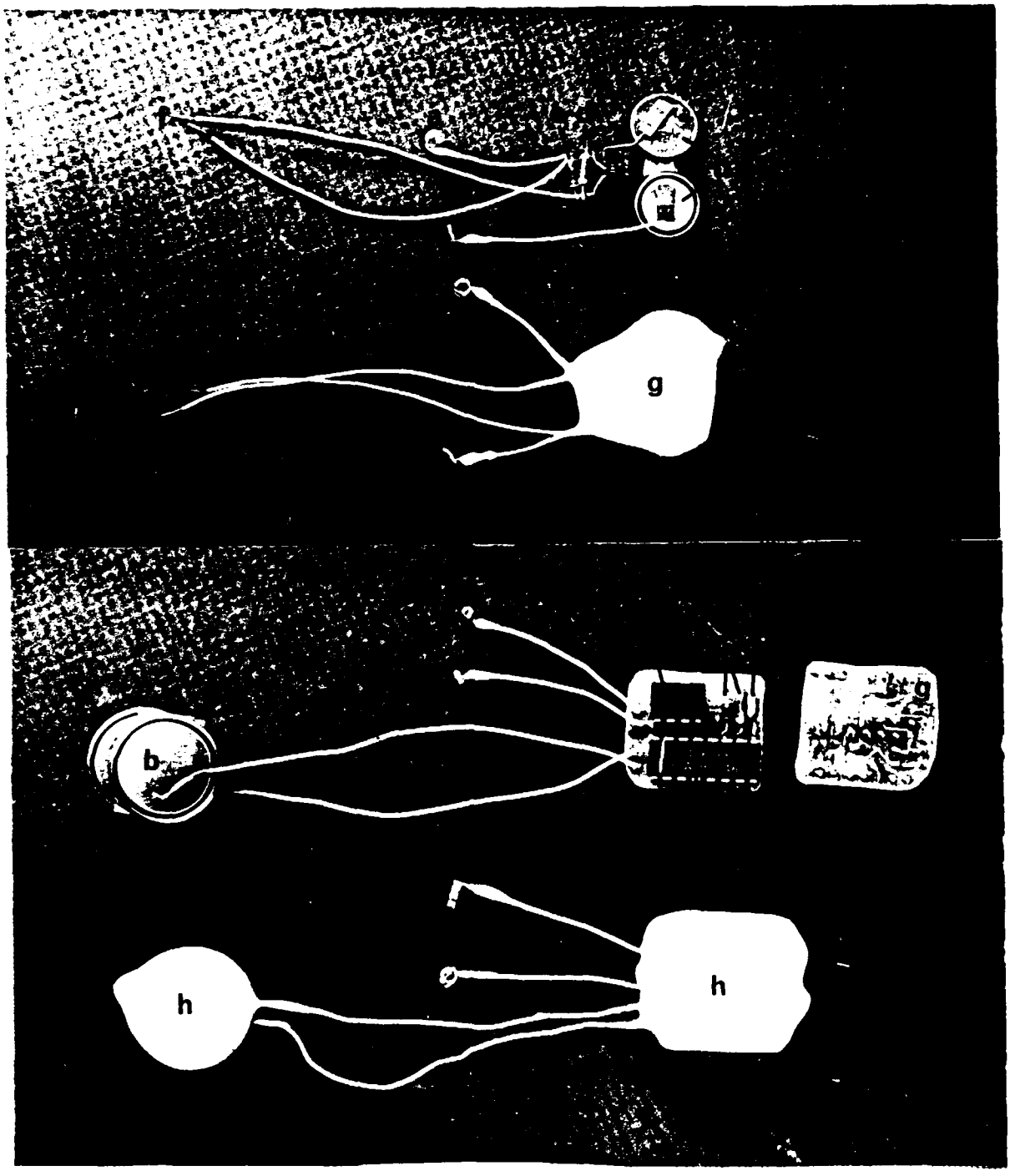

Figure 2.

3. Lesioning Technique and Use of the Marker Device

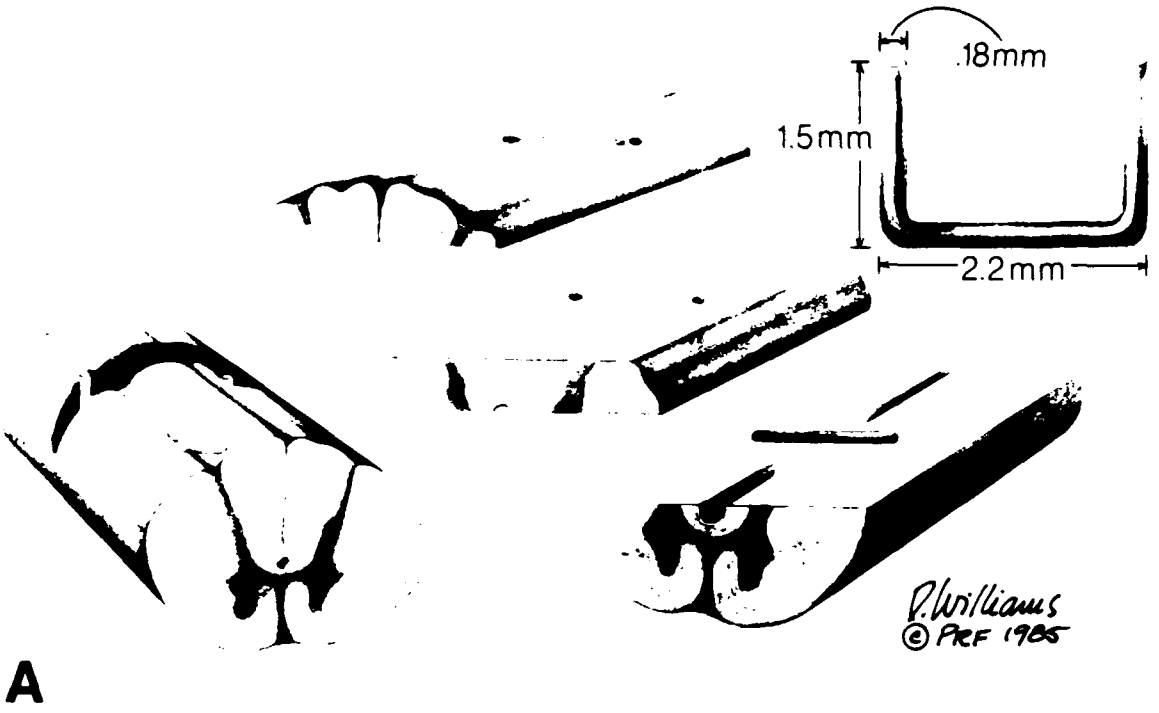

Figure 3.

This artist's drawing shows the placement and the dimensions of the marking device (adopted from A. Foerster, J. Comp. Neurology., 210:355-356, 1982). Transections of the dorsal column are first made with a sharpened pair of iridectomy scissors, followed by a pass with an electrolytically sharpened tungsten needle. This ensures that the transverse hemisection is complete down to the level of the central canal. Into this lesion is placed the marking device, its upright "poles" pointing dorsally. This device is left in the lesion during the experiment and removed after sacrifice and fixation. After fixation procedures, longitudinal - horizontal sections are taken after the removal of the device. We use sections taken on a "Vibratome", as well as frozen sections obtained on a cryostat. The position of holes left within the spinal cord tissues serve to mark the lateral and ventral boundaries of the lesion. The cord is about 3.5 - 3.8 mm wide at the level of the central canal. To prevent the loss of the device, a small square of surgical silastic (Dow Corning 500 - 1) is placed above the laminectomy, and held in place when the musculature is surgically closed.

4. Histological Procedures

a. HRP Application:

One day prior to the termination of an experiment (about 50 days post-transection in our published experiments; times vary in our new experiments), animals are anesthetized and another laminectomy performed, 2-3 vertebral segments caudal to the original lesion. Crystals of horseradish peroxidase (HRP) (Sigma type VI) are placed into this fresh, shallow, transverse incision of the cord. We have found that excellent results can be obtained if prior to the insertion of the crystals, 3-4 drops of 2% dimethyl sulfoxide (DMSO) are placed into the cut. A small piece of gelfoam is placed over the incision after the insertion of HRP crystals to limit diffusion of HRP away from the lesion. 12-18 hours later animals are anesthetized and fixed by perfusion with 2.5% glutaraldehyde in phosphate buffer.

This use of a second spinal cord lesion for the application of HRP has allowed us to concentrate our attention on axons clearly projecting through the area and to avoid confusion from those fibers which may enter from the periphery in the immediate area of the initial lesion. We have already performed experiments to confirm that HRP is not taken up by intact fibers in the surroundings (Borgens et al., 1986a). The axons which transport the HRP are a mixed population, however, of first and second order sensory neurons and it would be interesting to determine the precise origin of some of the regenerating fibers that are seen in the presence of applied fields. In some experiments, therefore, we will apply HRP to cut dorsal roots 2-3 segments caudal of the lesion and apply HRP in a pool restricted to the area of the dorsal root, in order to visualize the central projections of identified primary afferent fibers around the lesion. The chief advantage of this, in addition to identifying cells of origin, will be the possibility of staining more repeatable samples of the axon population.

b. HRP Development:

The fixed spinal cord is dissected free and the marking device is removed gently. This is accomplished by first clipping the device with fine-tipped cutters at the base of only one vertical wire. Each segment of the device is gently pulled out of the fixed cord. The device can be removed in this way without any damage to the cord except at the lower margin of the spinal cord where the small incision was made by the wire cutter itself. There is no damage to the upper portion of the cord, that containing the dorsal columns. Vibratome or cryostat sections are placed in individual small containers to maintain sequence and are processed for HRP staining as follows: overnight in 0.1 M cacodylate buffer (pH 7.4) at 4°C; sections warmed to room temperature; 3-4 minutes in 0.5% hydrogen peroxide in absolute methanol; 1 minute in absolute methanol; three rinses of 5-10 minutes each in 0.1 M phosphate buffer; 0.4% sodium borhydride in 0.1 M Tris buffer for 30 minutes (Wood and Cohen, 1979); 15 minutes in 0.1 M cacodylate buffer (pH 5.1) (Malmgren and Olsson, 1977); 30 minutes in 0.3% diaminobenzidine (DAB) in the same cacodylate buffer; 1 hour in 0.1% hydrogen peroxide in DAB-cacodylate buffer and a final rinse in cacodylate buffer (pH 7.4). Sections are mounted on chrome-alum slides and

counterstained with 1% neutral red at pH 3.3. Mounted sections are dehydrated, cleared in xylene, and mounted with permount. In some cases, alternate sections are silver impregnated (Goshgarian 1977; Guth et al., 1980). A Leitz Orthoplan Universal microscope is used for photomicrography, using both brightfield and darkfield optics.

c. Morphometric Analysis:

A simple but effective technique of quantifying axon numbers and the extent of dieback and regeneration is described in Borgens et al.(1987). This consists of using the HRP stained fibers as a population sample. Individual horizontal sections through the dorsal spinal cord were viewed on the light microscope at a standardized magnification of 200x. An eyepiece hairline reticle was then used to define a sample line at selected distances from and parallel with the plane of transection. All HRP stained axons crossing the sample line were counted. The relative numbers found at different distances from the lesion plane, totaled over a number of consecutive sections could then be used as an indication of the relative extent of dieback and regeneration, particularly between experimental groups.

This technique is a useful approach to rapid estimation of axon numbers, though in our further studies we shall be quantifying axonal morphometry in more detail. There are a number of potential problems in using the HRP stained axons alone, mainly associated with the variability of uptake and transport of the marker. Also, in the initial studies no measurement of the axon caliber was included, and it is possible that electric field effects include some size- related properties, perhaps associated with the caliber-dependent factor in secondary loss of axons noted in contusion injury model (Blight 1983, Blight and DeCrescito 1986). We shall therefore be employing more detailed morphometric analyses based on transverse semi-thin sections of the cord stained with toluidine blue, which we have used extensively in the cat spinal cord contusion injury. We

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Legend for Figure 4. (Next page)

Responses of dorsal column axons to electric fields in a chronically injured spinal cord.

This spinal cord was 2 months post-transection when a field was placed across the lesion. Two months later regeneration was assayed with anterograde HRP filling (as in Borgens et al., 1986b).

A) Columns of axons reach within 100 um of the exact plane of transection (hatched line) having penetrated the glial scar. Axon terminals were observed in many of these fibers. Growth cones (arrows) are apparent in a) and in higher magnification in b). Many of the fibers in this column were traced through a ventral trajectory to circumnavigate the scar into the rostral segment.

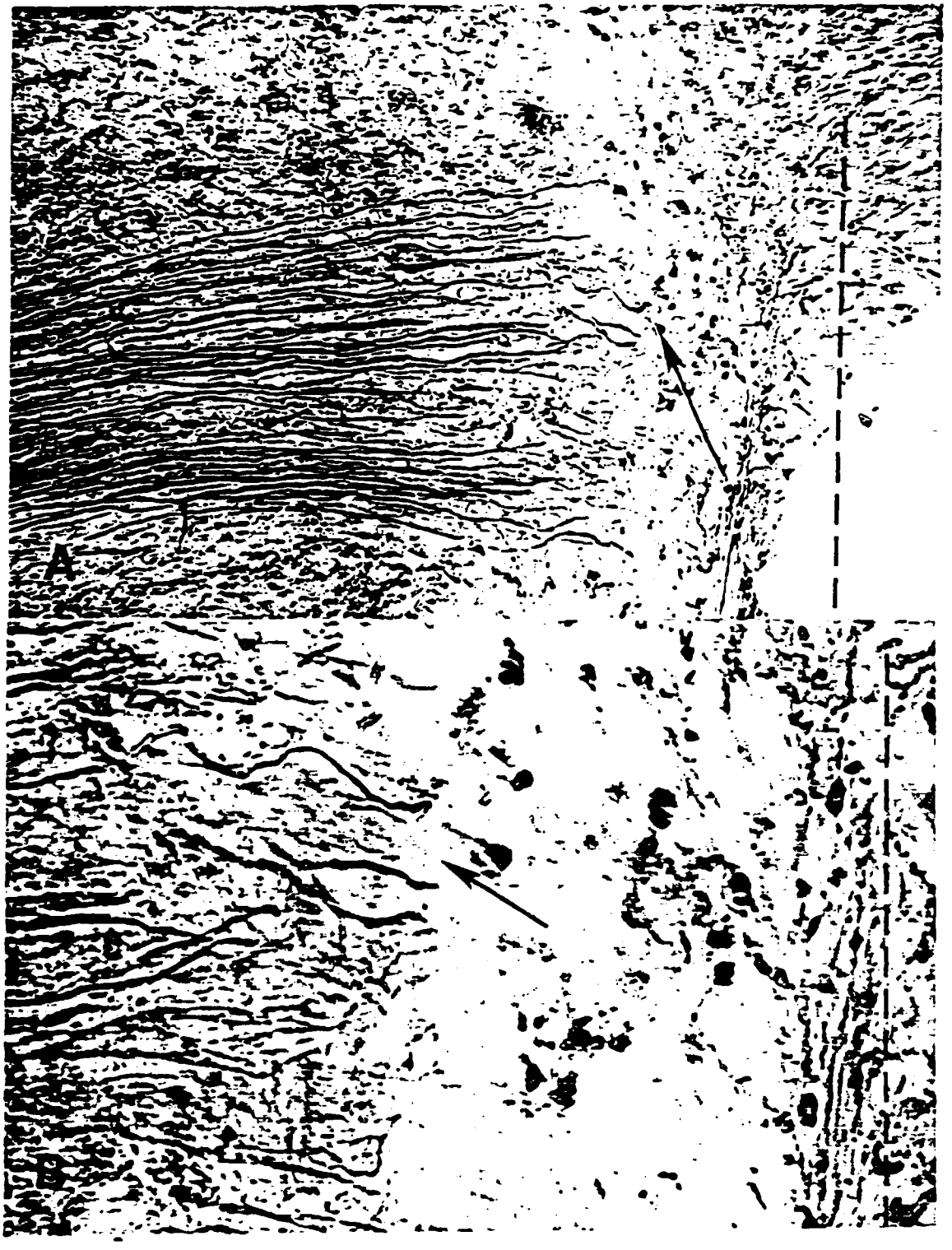


Figure 4.

have recently established by correlative electron microscopic studies that such light microscopic techniques are capable of accurate sampling of myelinated axons throughout their caliber range when using a 100X oil-immersion objective. These more accurate methods of analysis however, cannot be used by themselves to analyze regeneration unequivocally and these will have to be related to parallel measurements of HRP stained axons in thick horizontal or vertical sections of the lesion site, such as we have already employed in the dorsal column lesion experiments, which allow the course of individual axons to be traced through or around the lesion.

5. Behavioural Techniques

a. The CTM Reflex:

We have concentrated on three behavioral repertoires that require the integrity of known spinal cord long tract ascending or descending projections: the Cutaneous Trunchi Muscle (CTM) reflex, which requires ascending sensory spinothalamic tract integrity; the Vestibulo Spinal Free Fall Response which requires the integrity of the descending (motor) components of the Vestibulospinal tract and other lateral tract components; and the Hindlimb Placing Response which requires the integrity of the descending corticospinal tract. Severance of these tracts within the cord pr a permanent behavioral deficit that is clearly analyzable - functionally, quantitatively, and physiologically. Below is a description of our three approaches to a quantitative evaluation of the integrity of these tracts at the behavioral level - the most important of the clinical responses to spinal cord repair based on axonal regeneration.

The cutaneous trunci muscle reflex can be documented a) visually; b) electromyographically; c) electrophysiologically.

i) Photographic documentation of skin contraction is presented in Fig. 5. This technique is simple but clear. The back of the animal is shaved and marked with grid lines which can be aligned with index lines drawn on a card positioned adjacent to them. During stimulation, skin contractions cause a relative shift in the positions of skin and card index lines. We have found that a more accurate quantitative analysis of skin movement can be performed in the following way: The back of the animal is shaved under light sodium pentobarbital anesthesia (c 30 mg/kg, i.p.) and marked with a pattern of india ink dots. The skin can then be stimulated by light pinching with forceps, or light touch with a brush to produce reflex movements of the skin that can be recorded with a video camera positioned directly above the animal. A stopwatch placed in the field of the camera assists in the frame-by-frame reconstruction of the movement.

To analyze the movements we have used a commercially available (Magic) video-image digitizer attached to a Macintosh Plus computer. This allows succeeding frames of the video recordings to be digitized in a dot matrix pattern. Each digitized image is then transferred to a graphics program (Superpaint) which allows superimposition of a succession of images. Finally, the image from the most extreme point of skin contraction can be superimposed on the image preceding the onset of contraction to give a

complete vectorial representation of the movement of the skin. To make this image still clearer, we have found it useful to replace the simple digitized image with a pattern of standardized dots obtained by superimposing "graphics tool" patterns over the center of each digitized ink mark before the stage of superimposition of frames. A selection of recordings with this procedure in the normal animal is shown in Fig. 6. This newly developed system allows us to analyze the movements in great detail and should reveal important features of the process of functional recovery in the CTM system, both with regard to spatial distribution and timing. We are currently in the process of obtaining a full description of the normal reflex activity in the unlesioned guinea pig.

ii) Electromyographic recording of the CTM reflex is now performed routinely in this laboratory (Fig. 7). The elements of the technique are described in detail in (Borgens et al. 1987). One of the important contributions of the electromyogram is in providing information about the duration and pattern of muscle activation in the reflex, which may show significant differences in the motor organization of normal and lesioned animals.

iii) Using straightforward bipolar stainless steel stimulating electrodes, exposed dorsal cutaneous nerves may be stimulated by brief (0.1 ms) electrical pulses in the usual way. The motor output of the reflex can be recorded either electromyographically or at the level of the brachial plexus output in the lateral thoracic nerve of the brachial plexus - the main source of motor innervation to the CTM (Cooper and Schiller 1975). A compound action potential recorded with a bipolar electrode in such an arrangement is shown in Fig. 8.

Figure 5. (Next page)

Photographs illustrating the CTM reflex in a guinea pig 6 months after thoracic right lateral hemisection of the spinal cord (arrow). A) The back skin of the guinea pig shaved and marked with ink grid lines. B) Forceps lightly pinching flank skin produce contraction of the CTM muscle (note drawing together of grid lines rostral to stimulus on the right side below lesion to show the grid lines. D) Stimulation by pinching lightly below the lesion produces no CTM reflex. E) Stimulation above the level of the lesion on the right successfully elicits CTM contraction. F) Oscilloscope trace of electromyogram recorded from subcutaneous stainless steel wire electrodes. The lower trace indicates application of tactile stimulation. Scale: sweep duration = 1 sec. Full scale = 5 mV.

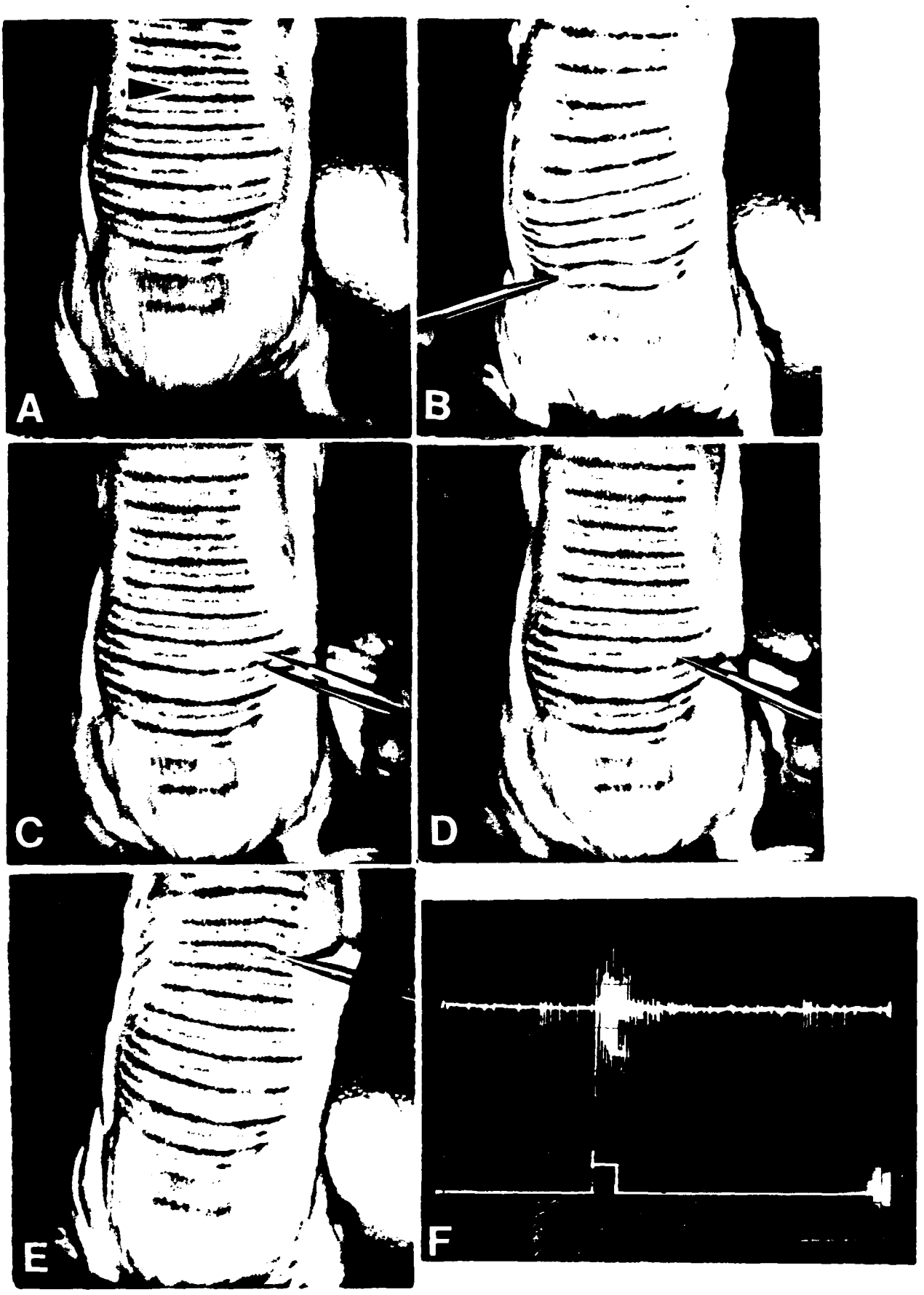
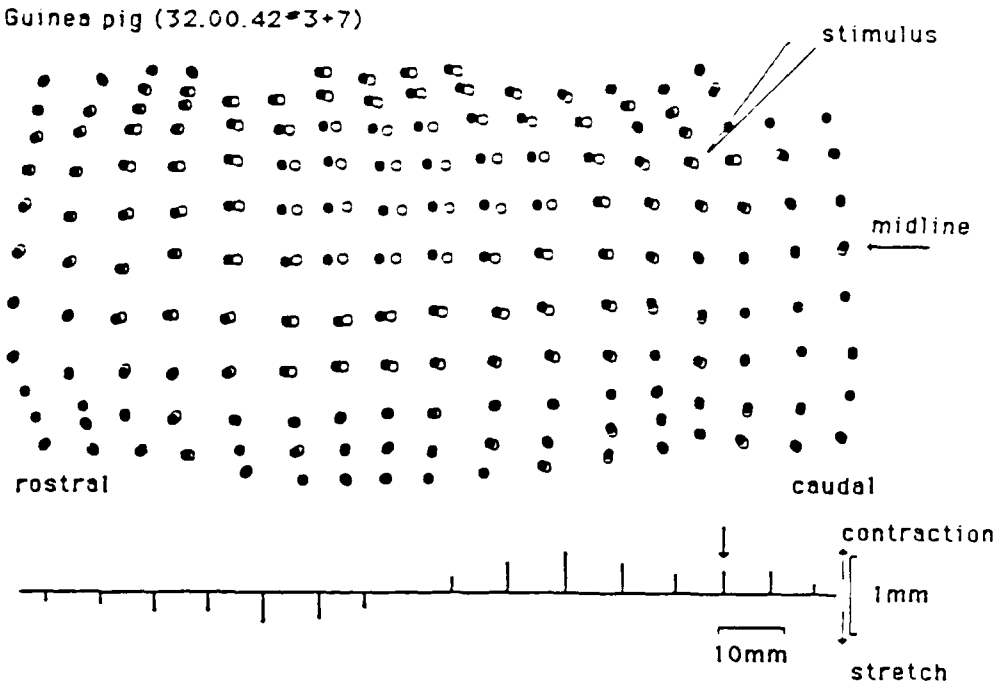


Figure 5.

Figure 6. (Next page)

The CTM reflex of guinea pig and rat compared by digitized analysis of videotape recordings. Animals were lightly anesthetized with sodium pentobarbital (30 mg/kg), the back shaved and a pattern of india ink dots applied to the skin. The animal was placed on a graduated background, together with a stop-watch, and recorded from above with a video camera. The guinea pig was stimulated with light touch, the rat with maintained pinch of the skin, using watchmaker's forceps. Individual frames of videotape were digitized with a Magic interface and stored on a Macintosh Plus computer. A graphics program (Superpaint) was used to superimpose uniform dots on the ink marks. Two frames were selected for superimposition, one just before the response (filled symbols) and one at the peak of skin contraction (open symbols), for each animal. A more accessible measure of the pattern of skin contraction was obtained by measuring the distance between pairs of dots along the rostro-caudal axis, in line with the stimulus point. The movement over 3 rows and 3 columns of dot pairs was averaged to give the histogram display shown below the overall representation of the back skin. The distribution of skin contraction in the two species was found to be quite similar, though smaller in amplitude in the guinea pig, and not maintained for the duration of the stimulus, as it was in the rat. The site of contraction of the skin relates directly to the stimulus point, though most of the observed movement in this instance is rostral to the stimulus.


Guinea pig (32.00.42*3+7)
stimulus
midline
rostral
caudal
contraction
1mm
10mm
stretch

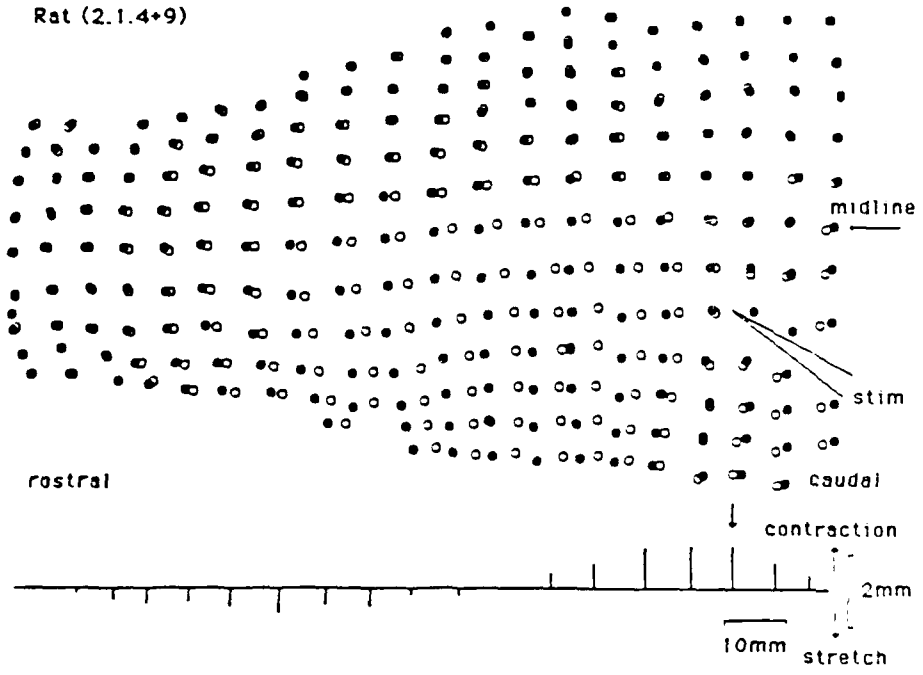

Rat (2.1.4+9)
midline
stim
rostral
caudal
contraction
2mm
10mm
stretch

Figure 6.

Figure 7. (Next page)

(From Science article: Behavioral Recovery Induced by Applied Electric Fields After Spinal Cord Hemisection in Guinea Pig, Volume 238, pp. 366-369, October 16, 1987).

Electromyograph (EMG) recordings of CTM responses in a control animal at 61 days after hemisection; EMGs were recorded from subdermal wire electrodes, amplified with a Grass P15D preamplifier, and displayed on a Tektronix 5113 oscilloscope. Electrodes (c) were placed in the brachial region on either side of the midline (dashed line). Stimulation was performed by lightly touching the shaved skin with a pair of watchmaker's forceps (without pinching). Timing of stimulus contact (upward deflection in each of the four lower traces) was obtained by connecting the forceps in a circuit, which included a battery, and was completed through the animal to a moist ground electrode beneath the foot. Current was limited to < 1 uA. The surface of the skin was dampened at the stimulus site for electrical contact. Traces A, B, C, and D show examples of EMG with stimuli at sites a, b, c, and d. Trace D shows lack of response to stimulation below the hemisection (h). The unresponsive are (stippled area) was a persistent deficit after hemisection. The small repetitive signal on all traces represent the electrocardiogram.

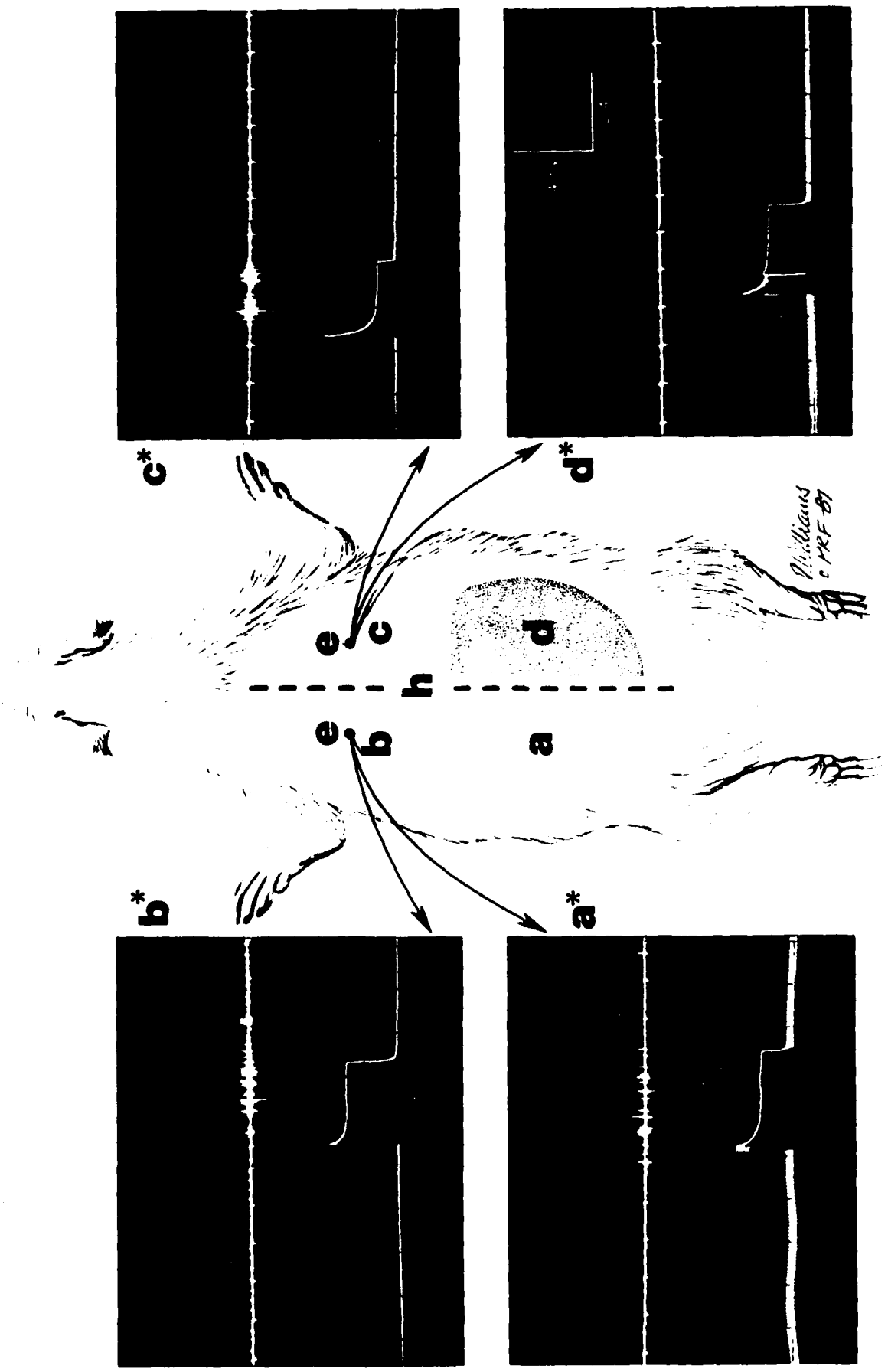


Figure 7.

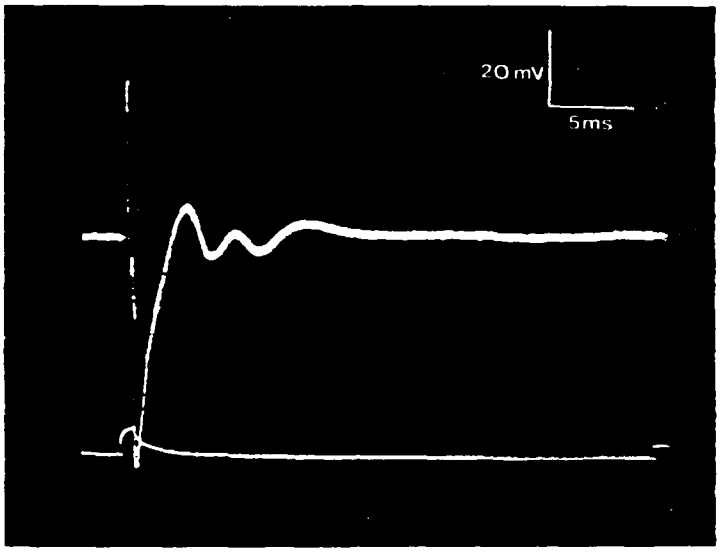


Figure 8.

Using bipolar stimulating and recording electrodes, this compound AP was recorded at the brachial plexus (lateral thoracic nerve) after stimulation of a lower thoracic (T11) dorsal cutaneous afferent. Stimulus intensity = 7 volts, 1 msec. duration.

The importance of this multimodal analysis of the CTM reflex will eventually lie in the information it will provide on the nature of reflex recovery in animals exposed to electric fields. The precise timing of the reflex conduction and contraction, together with the distribution of the receptive fields and the motor activity will provide the basis for a more invasive electrophysiological and morphological investigation, which will be required eventually to relate the recovery of function to effects of electric fields on regeneration. Visual and electromyographic techniques can be used at intervals throughout recovery.

b. Vestibulospinal Free-fall Responses

Free-fall responses (FFR) will be documented both photographically and electromyographically with the apparatus illustrated in Fig. 9. This allows the guinea pig to be supported in a harness and dropped into free fall for a short distance repeatedly. The characteristic toe-spreading

movement in the hindlimbs is recorded photographically by a camera mounted below the frame, its shutter release triggered by an array of photodiodes as the animal drops through the path of a light beam directed at them. Electromyographic responses in the hindlimb muscles can also be recorded during the drop response with intramuscular wire electrodes, similar to the technique used by Gruner in the cat and rat (Gruner et al. 1984, 1987). The photographic technique has the advantage of little discomfort to the animal. It can be carried out routinely without anesthesia and by a trained technician since the data collection is essentially automatic. The electromyographic recordings will by used in the event of clear visual signs of recovery in order to measure the temporal characteristics of the recovered response for comparison with the normal reflex.

c. The Tactile Placing Response

The tactile placing response is a hindlimb reflex that is uniquely dependent on information descending from the motor cortex (together with some cerebellar interactions). This long-loop reflex has been studied in detail, particularly in cats (Amassian 1979; Amassian et al 1972; Bard 1933; Bregman and Goldberger 1982), but also with some basic observations in rodents (Brooks 1933; Brooks and Peck 1940) that confirm the general similarity of corticospinal control in these animals. The key importance of this pathway, in addition to its distinct motor function is the compact anatomical distribution of corticospinal pathways in the rodent spinal cord (Revely, 1915; Brown, 1971; Schreyer and Jones, 1982). The great majority of corticospinal fibers in the rodent descend in the deepest region of the dorsal column. This should allow selective transection of the motor arm of the reflex loop with effective sparing of relevant sensory fibers in the lateral and dorsolateral funiculi.

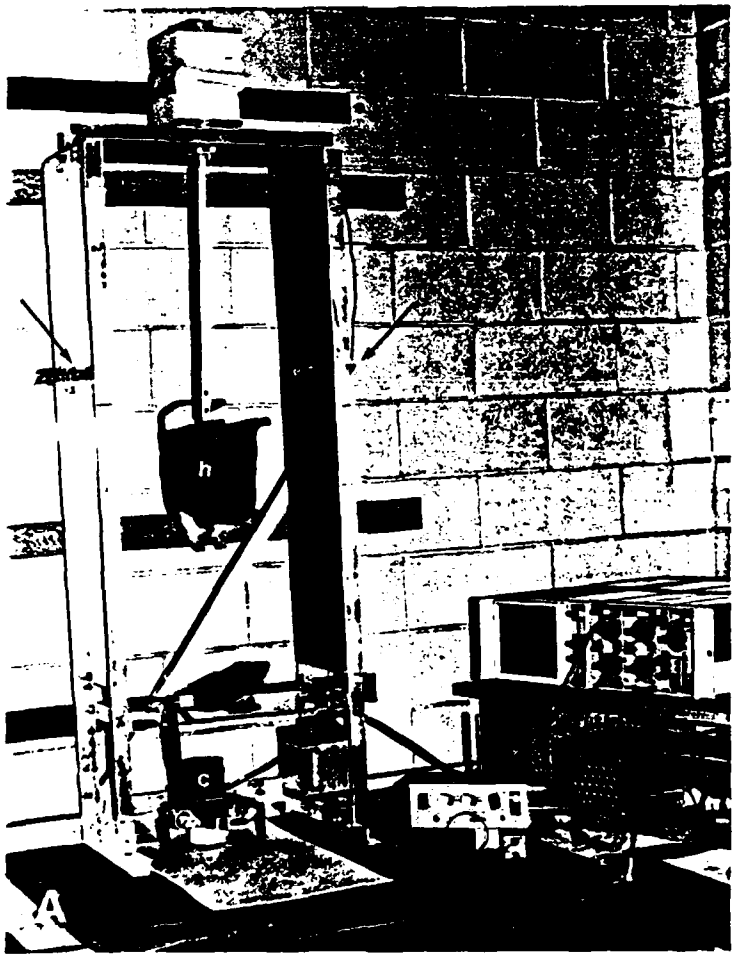
With the animal suspended in a supporting harness, tactile stimulation of the dorsal surface of the hindlimb elicits a placing movement, bringing the plantar surface of the limb up to the level of the stimulus. Figure 10 shows the use of a ledge to elicit bilateral responses in normal and lesioned animals. Moving the suspended hindlimbs of a normal alert animal (A) slowly up to the edge of a board produces a rapid reflex placement of the feet onto the board. An animal with a complete lesion of the dorsal column, including the corticospinal tracts (B), shows no placing response in either hindlimb in the presence of light tactile stimulation. Placing only occurs in such an animal when the hindlimbs are strongly abducted, initiating a proprioceptive placing response (Bregman and Goldberger 1982) probably involving local flexion reflexes, or when the animal is startled by generalized (auditory, tactile, visual) stimuli, probably through excitation of bulbospinal pathways that mediate a general postural resetting. An animal with a chronic right lateral hemisection of the spinal cord (C) shows a clear low threshold placing response only on the intact left side and not on the side ipsilateral to the lower thoracic section.

Figure 9.

A) This device measures visual and electromyographic responses to vestibulospinal free fall. The guinea pig is secured in a canvas harness (h) attached to a free fall lever with a travel of about 2 feet. After the lever is released, the animal passes a photodiode light beam (arrows), a circuit is broken and triggers the shutter release of a camera (c) focused on the hind limbs (recording the spreading reflex or the lack of it). This circuit also triggers the sweep of an oscilloscope which can record 4 channel electromyograms from indwelling electrodes situated in forelimb muscles (after Gruner et al., 1984).

B) (Next page) photograph of the bilateral toe spreading reflex in response to free fall, a routine procedure using the device in A).

C) (Next page) Same animal as in B), showing a typical loss of toe spreading reflex; a characteristic and permanent defect caused by a spinal lesion of the ventral white matter.



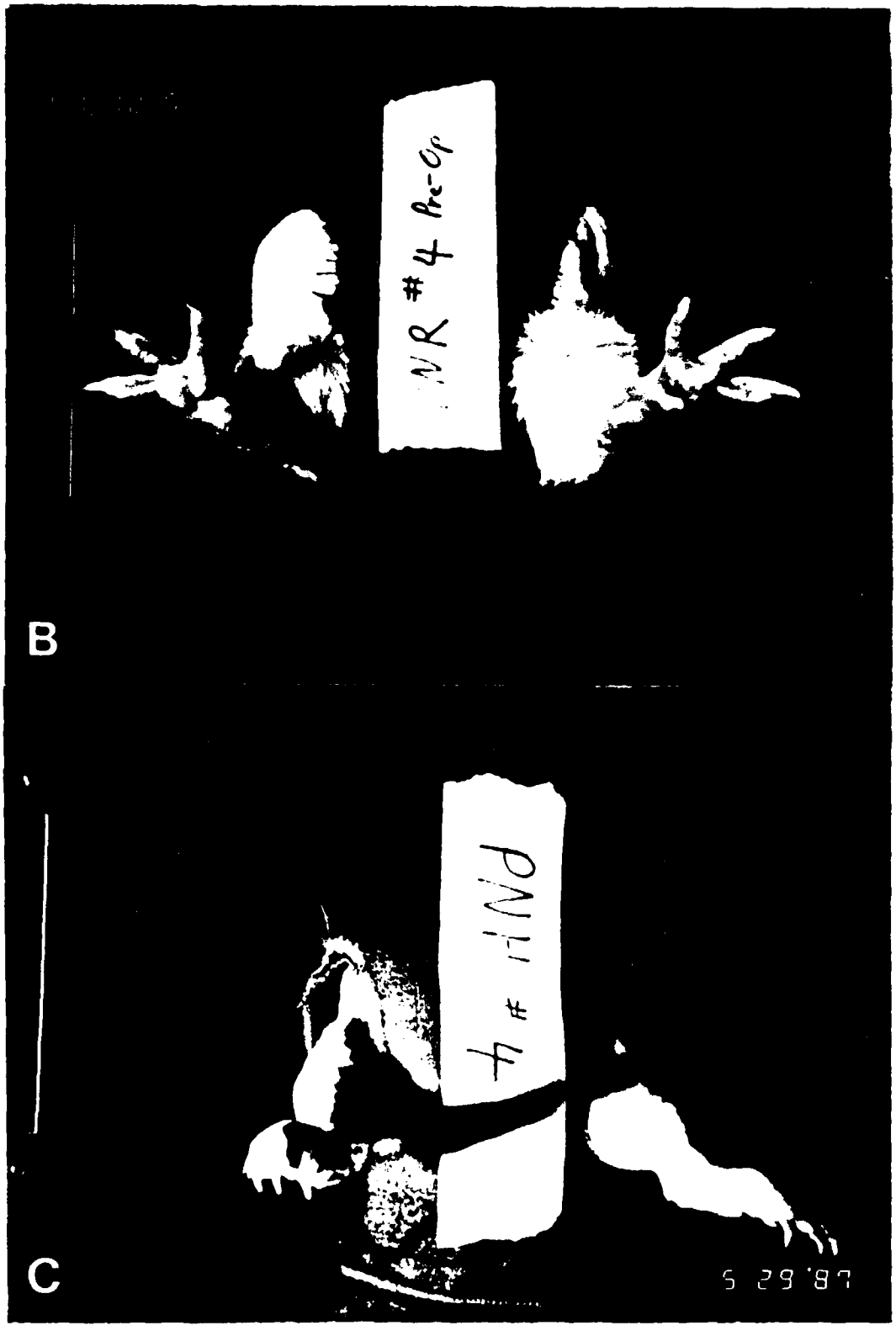


Figure 9.

6. Procedures for Randomizing and Blinding

Our initial studies involved anatomical analysis of regenerative responses included the direct examination of the chronic lesion at the time of analysis of outcome. Behavioral studies will be somewhat more vulnerable to potential bias in performance and analysis. It will therefore be necessary to randomize the lesioned animals and blind those investigators performing the scoring of observed functional recovery. Since the stimulators are normally implanted before the lesion of the spinal cord is made (except in the chronic treatment experiment), the randomization of lesioned animals to experimental and sham control groups will be performed by coding stimulators so that active and sham units are not known to the surgeon at the time of lesioning and implantation. The experiments on field polarity will also require the electrodes to be coded for polarity by the electronic technician responsible for their fabrication.

7. Statistical Analysis

The statistical evaluation of differences between treated and control populations is an important aspect of any treatment study. The final means of statistical analysis can only be chosen when the data are available, but certain considerations can be taken into account in planning such a study. The morphological data to be gathered will allow a number of comparisons between treated and untreated groups. From longitudinal sections of anterograde HRP stained material we shall be able to count the number of stained axons entering the scar, reaching the level of the lesion and passing beyond it. From the transverse 1 um plastic sections, we will be able to sample total axon numbers, at different levels in relation to the plane of the original lesion, as a measure of the extent of axonal dieback and elongation. From these sections we will also derive sample measurements of axon caliber and myelination index, and their spatial distribution with respect to the lesion. Simultaneous measurements of tissue area will allow accurate estimation of the total axon population characteristics, based on sampling probability (Blight, 1983a; Blight and DeCrescito, 1986).

Figure 10. (Next page)

Photographs illustrating placing responses in guinea pigs. The animals were suspended in a canvas sling, with the limbs normally extended. The dorsal surfaces of the hind feet were brought into light contact with the edge of a wooden step. In normal animals (A) this resulted in a rapid bilateral placing response. In animals with complete bilateral transections of the corticospinal tracts (B) this tactile placing response was absent in both legs. Right lateral hemisection of the lower thoracic cord (C) resulted in loss of tactile placing ipsilateral to the lesion. These deficits appear to be permanent. The animal shown in (C) was 6 months post lesion at the time of the test.

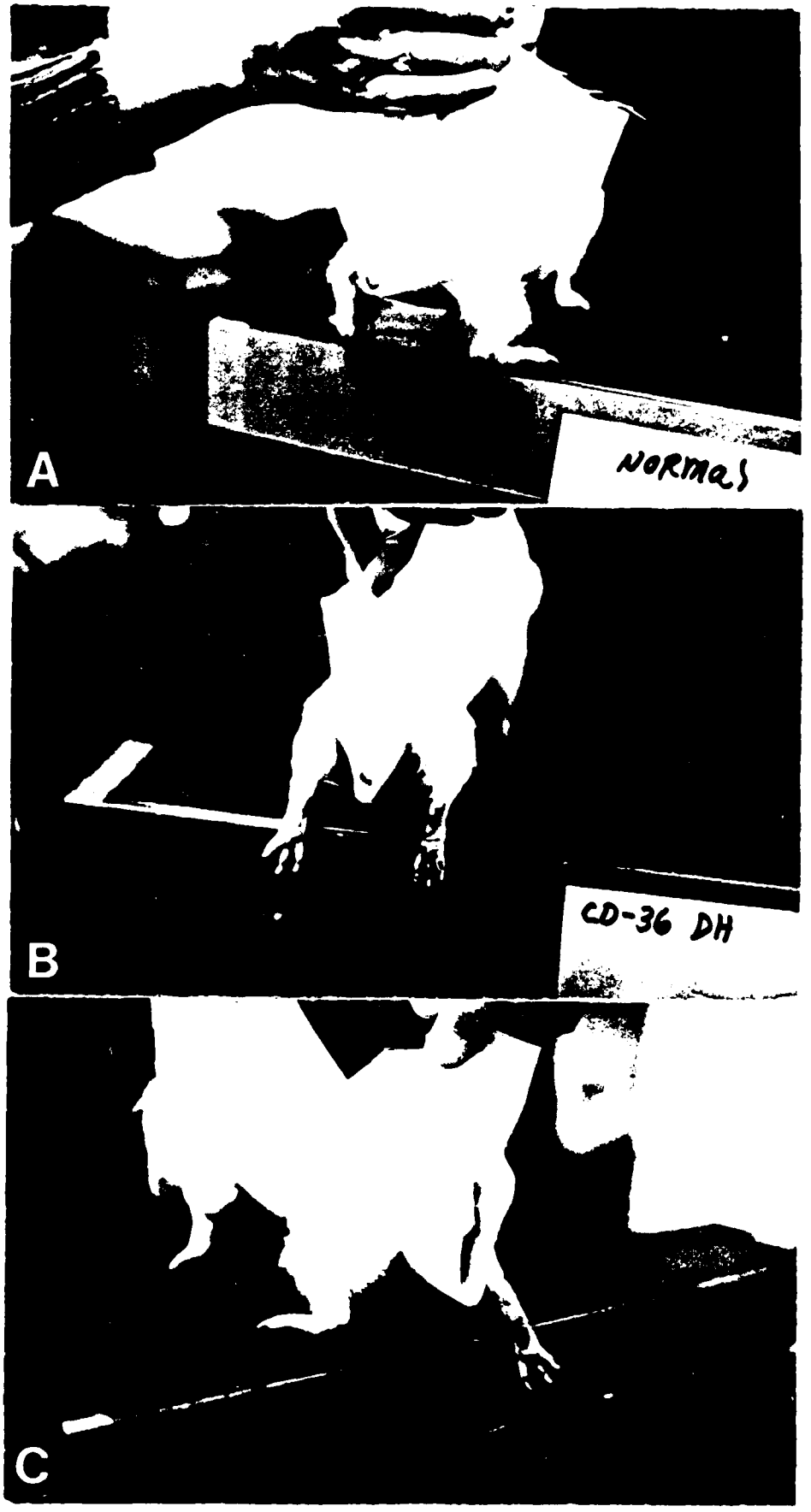


Figure 10.

The behavioral tests will provide quantitative data on the recovery of function between groups. The recovery of the CTM response can be measured most effectively in terms of shifts in the receptive field area from acute to chronic stages. The free-fall response can be measured photographically in terms of the frequency of toe-spreading reflexes and electromyographically in terms of latency of extensor activation. (The average amplitude of responses can also be taken as some indication of the relative strength of the reflex between groups, through it is not a reliable indicator between individual animals because of variations in the characteristics and position of electrodes). Tactile placing will be measured in terms of frequency of response to a repeated stimulus as well as from features of the motor performance.

The ability to detect significant differences between groups will depend a) on the variance of the characteristic examined, b) on the magnitude of the differences, and c) on the size of the samples used. Significance testing will also depend on the nature of the data examined. Data on frequency of occurrence (e.g. of recovery of a reflex, of axons passing the lesion) can be examined with a chi-square test. More graded measurements from experimental groups (such as axon number or receptive field size) can be examined with parametric statistical tests, such as Student's t-test (unpaired), if the data indicated a normal distribution of the parent population. Otherwise, the more flexible non-parametric tests such as Wilcoxon or Mann-Whitney will be used to examine the significance of apparent differences in group values. We have used such standard statistical techniques for analysis of our previous experiments on the effects of fields, using similar numbers of animals in experimental groups.

8. Electrical Enhancement of Fracture Repair

a. <u>The Fracture Device</u>

A device (the Fracturator) at Purdue under the direction of Dr. David Van Sickle (Department of Anatomy) and Dr. Ben Hillbery (Department of Mechanical Engineering) was developed to produce a reliable and consistent fracture to living bone as well as aid in the analysis of the biomechanical properties of callus formation and union.

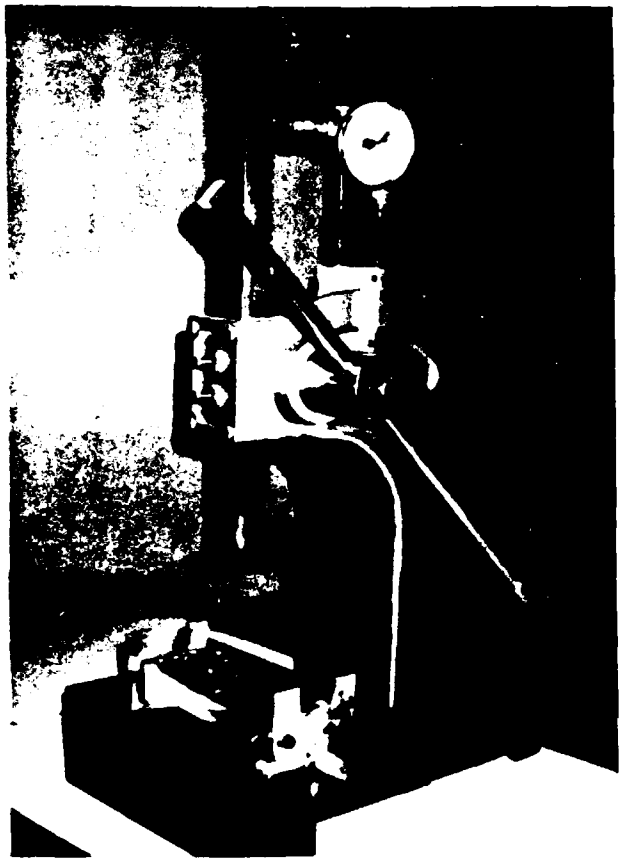


Figure 11.

The Fracturator.

Since the last annual report, considerable modification and testing of the device has been carried out. The three most notable modifications have been: a) a force dial with three times the capacity of the original was purchased and installed; b) a metric scale was added to the platform; c) an impactor with a wider head was made and installed. The reason for item b) was, at the time of refracture, to allow for placement of of the healed leg in as exact a position in the jig of the Fracturator as when originally fractured, thus providing biomechanical data. Following the fracture studies on cadaveric limbs, living bones were fractured in vivo and the vasculature of the experimental limb infused with latex to determine the extent of bruising by direct observation. With the larger impactor, the force was distributed over a larger area and the damage to the blood vessels was kept to an absolute minimum.

b. Experimental Protocol: Pilot Study

Four male random source dogs (37-50 lbs.) were acquired from a licensed dealer and were vaccinated and identified. Each dog was anesthetized, the right tibia radiographed for normalcy, fractured in the Fracturator, the electrical stimulators implanted, the leg dynamically casted and reradiographed. At two and four weeks, the dogs were anesthetized, cast removed, the fracture site radiographed and examined with ultrasound. The battery packs were checked twice a week. Tetracycline was administered on a "2-6-2" basis initially and five days prior to euthanasia at six weeks past fracture. From the serial radiographs one could observe the typical progression of callus elaboration and mineralization with electrical enhancement; the soft callus formation is more evident in ultrasonographs; the degree of in vivo vascularization of the callus as demonstrated by transosseous venography (evident on the angiogram in Figure 14). Following necropsy, one half of the area of fracture callus was decalcified for paraffin histology and histochemistry while the other half was infiltrated with plastic, and sectioned undecalcified to preserve the tetracycline labeling and mineral content, stained and studied.

The purposes of this pilot study (to "trouble-shoot" these coordinated procedures) have been achieved, some new analytical techniques (e.g. ultrasonography) with outstanding success. The investigative team has isolated and solved a variety of problems from cast adjustment; methods of refracturing the callus; to improved methods of monitoring the battery packs. Controls were omitted in this pilot trial since we wished to "flesh out" the entire protocol. An unexpected dividend was that it was determined from clinical experience that the amount of radiological fracture callus produced was larger and appeared sooner than that normally expected. The entire system is now operative and will be an excellent system with which to study the biology of fracture callus and its modification.

Figure 12. Post fracture radiograph of right foreleg of Dog #23355 prior to cast application and electrical pack implant.

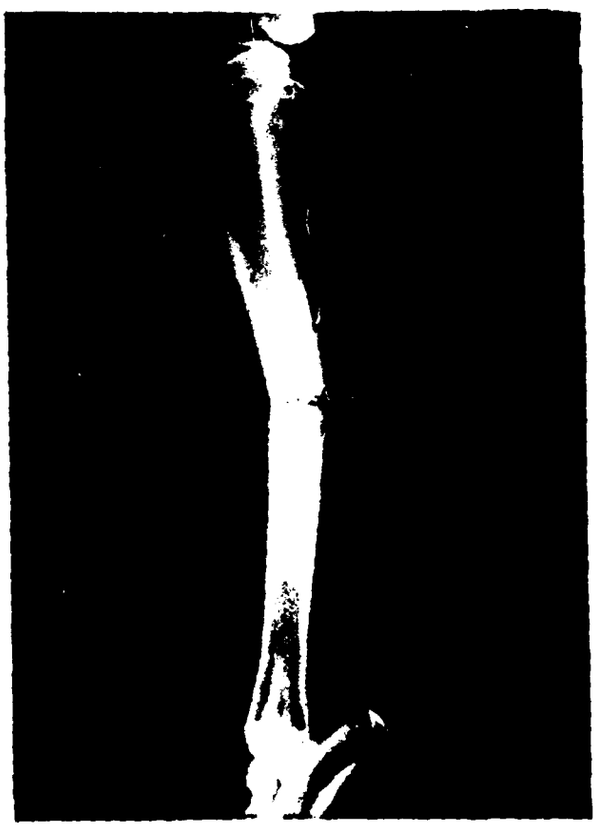


Figure 13. Radiograph of right foreleg of Dog #23355 two weeks post fracture. Note stimulator electrodes in situ.

Figure 14. In vivo angiogram following transosseous venography at six weeks post fracture. (Dog #23459) demonstrating the vascular network of the callus in the living animals. The functional vascular bed of the callus can be well visualized by this technique giving further data on the ongoing repair process in control (sham-treated) and experimental (current treated animals).

D. CONCLUSIONS

1. Applied electrical fields can indeed affect the regeneration of acutely severed single nerve fibers within the adult mammalian CNS and PNS.

2. A means to study discreet behaviors (functional changes after spinal cord injury) in both ascending (sensory) and descending (motor) has been developed.

3. A functional recovery of a discreet sensory modality (the CTM reflex) has been demonstrated in the adult guinea pig.

4. A rigorous means of producing a reproducible fracture to dog tibia (in situ) has been developed.

5. A rigorous means to biomechanically and anatomically analyze such union in normal dog fracture has been developed. Our first pilot studies of an electrical enhancement of tibial repair using all of the above methods has been successful.

E. RECOMMENDATIONS

It is clear that acutely injured CNS in the mammal can respond to early intervention with the use of exogenous applied electrical fields. With respect to the spinal cord, this application is almost noninvasive (requiring no surgery itself) and has been demonstrated to affect a robust regeneration of both myelinated and unmyelinated fibers within the spinal cord (Borgens et al., 1986a, 1986b). We now have demonstrated that such regeneration can be association with a functional recovery in a specific behavioral deficit (induced by spinal cord lesions). We recommend that these studies be pursued to find the most optimum and effective means of electrical stimulation of cord to produce the greatest degree of functional recovery (both on a population level, i.e., higher responding percentage of animals); and at the individual level, i.e., a more appropriate return of function after therapy. We view these studies at present to be near the stage for clinical testing in cases of natural occurring spinal injury in dogs with eventual trials in human spinal cord injuries as a long-term goal.

Our pilot studies on the peripheral nervous system have demonstrated that elements of the PNS will respond as well. The clinically relevant aspect of this study is an enhanced rate of regeneration. Clinically, this would produce a decrease in the time that one might expect a functional return in injured (denervated) tissues after nerve lesion; as well as increase the distance from nerve lesion to target tissue that may be effectively dealt with by conventional microsurgical techniques in conjunction with electrical field applications. Our recommendation is to pursue such studies toward a clinically relevant means of enhancing peripheral nerve repair.

We recommend that our studies on the dog tibia fracture model move from the pilot just completed to a full study in 1988. We suggest the possibility based on our new investigations that even normal repair processes in fractured long bone may be accelerated with relevant applications of electrical fields.

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GLOSSARY

Dorsal Columns These large spinal cord tracts are bundles of neurons that project into the spinal cord from segmental ganglia lying just outside the cord itself. Sensory information (largely) is carried to the brain by these tracts that ascend the cord.

Laminectomy Surgical exposure of the spinal cord within the vertebral column.

Neurite A general and non-specific term for a neuronal process.

Wick electrode An aqueous "wire". Stimulating electrodes fashioned from a silastic tube, filled with mammalian ringers and a cotton string (the "wick"). Thus, current is carried to the tissues by a conductive solution similar to body fluids and not by metallic wires (which contaminate the tissues with electrolysis products).

Orthodromic and Antidromic stimulation and recording. Experimentally evoked Action Potentials whose conduction pathway is in the same direction as natural conduction are orthodromically stimulated. For example: orthodromic stimulation of a motor neuron would involve stimulating near the soma (or ganglion) and recording at the periphery. Antidromic stimulation and recording would be the reverse of this regimen.

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