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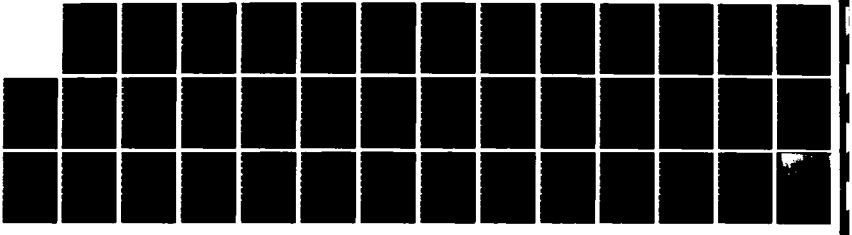

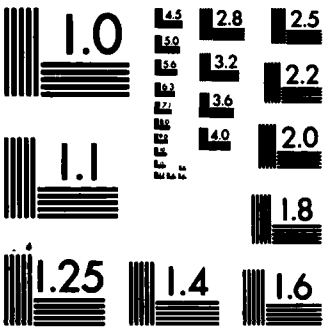


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Report on "Laser Assisted Microsurgical Anastomosis" (LAMA) of arteries to nerves in rats comparing histologic and mechanical results to conventionally sutured anatomosis.

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FINAL REPORT TO THE

OFFICE OF NAVAL RESEARCH

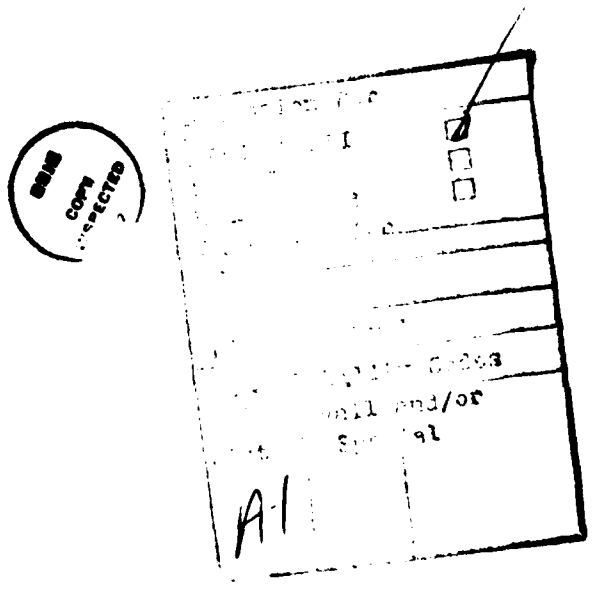
# LASER ASSISTED MICROSURGICAL ANASTOMOSIS

Department of Orthopaedics and Rehabilitation (D-27)
University of Miami School of Medicine
P.O. Box 016960
Miami, FL 33101

September 22, 1983

Principal Investigator
William E. Burkhalter, M.D.
Vice-Chairman and Chief, Hand Service
Department of Orthopaedics & Rehab. (D-27)
University of Miami School of Medicine
P.O. Box 016960
Miami, FL 33101
(305) 325-6416

Fiscal Responsible Officer



Dennis Cahill
Administrative Assistant
Dept of Ortho-Rehab
(305) 547-6996

---

CO- Principal Investigators

Douglas K. Dew, B.S.
Director, Laser Surgery Research Program
Dept of Ortho-Rehab
(305) 547-6202

Loren L. Latta, PH.D., P.E
Director of Research
Assistant Professor
Dept of Ortho-Rehab
(305) 324-3185

## Introduction

# "Application of Experimental $CO_2$ Laser Microsurgery"

D.K. Dew
University of Miami
School of Medicine

This Paper describes new experimental microsurgical procedures that utilize laser infrared energy emitted at 10.6 um to heat denature tissue components for use as a biological glue. Heating of tissue proceeds in four distinct steps: Heating, coagulation, evaporation, and carbonization. Heating past 100°C liberates cellular water followed by swelling, chariing and eventual disintegration leaving only residual ash. The speed of this process and the amount of tissue affected depends upon tissue absorption Characteristics, laser mode, spot size, laser wavelength, exposure time and power input. Laser surgical applications of the past twenty years have centered around evaporation and carbonization of tissue - its destruction. By keeping the tissue temperature in the range of heating and coagulation, a laser can be used for reconstructive purposes.

This carbon dioxide laser microsurgical technique takes advantage of the very high absorption of laser energy (at 10.6 um) by water in soft tissue to effect successful anastomoses of rat small arteries, veins, peripheral nerves, fallopian tubes, and vas deferens as well as closure of skin and corneal incisions. For this study, a SHARPLAN 733 Surgical $CO_2$ Laser System is used. A $TEM_{00}$ $CO_2$ laser beam is directed to the repair site by means of an articulating arm that attaches to a mircromanipulator mounted on a Zeiss operating microscope.

With the proper electro-optical parameters, the only tissue reaction the surgeon should observe is a mild blanching with slight constriction of the tissue at the site of laser repair; this subtle tissue reaction is best seen at very high magnification (40X). Accurate control of the power level, time exporsure, and spot size is critical; this has been achieved for each tissue type tested to date. Proper control of these electro-optical parameters avoids boiling and charring resulting in maintenance of the gross tissue architecture. By promoting only true coagulation, a controlled tissue denaturation can take place. To estimate the proper heat distribution within a hydrated tissue, laser coagulation of egg white was used as an experimental model. When proteins denature upon heating, hydrophobic regions are exposed and a gel-like matrix forms as the proteins coagulate.

## SUMMARY

Carbon dioxide laser microsurgical repair has thus been used successfully for the anastomosis of rat small arteries, veins, peripheral nerves, fallopian tubes, vas deferens as well as closure of skin and corneal incisions. The technique is faster, reduces surgical manipulation, is less technically demanding, and introduces less foreign material at the site of the lesion when compared to conventional suture techniques. Current laser microsurgical studies now underway include the use of the argon laser at 514 nm for microvascular repair.

# ARTERIAL REPAIR USING LASER

## Introduction

Lasers have been used to repair divided arteries, veins, fallopian tubes, uterine horns, vas deferens, nerves, and to close skin and cornea incisions, This study describes several variations of laser assisted small vessel anastomosis techniques in the rat.

Jain demonstrated the first successful in vivo end-to-end anastomosis of small arteries using a CW Nd:YAG laser. Morris, et al later reported on the end-to-end anastomosis of small arteries using a CW $CO_2$ laser. The following study describes several variations of laser assisted small vessel anastomosis techniques in the rat.

The following techniques were established in our study using either a $CO_2$ or Argon Laser:

1. End-to-end arterial anastomosis. (Anastomosis of rat abdominal aorta, carotid, temoral, and renal arteries.)

2. End-to-end venous anastomosis. (Common femoral vein)

3. End-to-side arterial anastomosis. (Femoral artery is used to bypass an obstruction in the common carotid artery as well as a procedure for carotid-carotid end-to-side.)

4. End-to-side venous anastomosis. (Porto-caval shunt.)

5. Vein graft. (Vein graft to common femoral artery.)

Rat vessels were dissected and after adequate exposure, they were clamped. The vessels were then transected and approximated with the help of two to five stay sutures depending upon whether the procedure was end-to-end or end-to-side.

For the $CO_2$ laser group, a TEM.. $CO_2$ laser beam from a SHARPLAN 733 laser was directed to the repair side by means of an articulating arm that attaches to a micromanipulator mounted on a Zeiss operating microscope. By using a low power (200-500 mw) $CO_2$ laser focused to a small spot size (approximately 0.5 mm), the vessels were fused together.

For the Argon Laser studies, the electromagnetic radiation at a wavelength of 514.5 nm was delivered to the tissue by a fiber optic device of approximately 600 um diameter. In order to develop the required power density of the output side, the beam of an Argon Laser was reduced in diameter by a factor of 11 with a simple optical system and directed into the fiber optic device. The ratio of laser power in to power out was approximately 8.

After laser repair was complete, the vascualr clamps were removed and the anastomotic site was observed for bleeding. In most cases, laser assisted microvascular repairs were without leaks. In those that did leak, the clamps were reapplied and the operating field was cleared of blood and thoroughly dried; the laser was then used to repair the bleeding site. Histological sections of the anastomotic site were later prepared for light microscopy and the tissue bond observed with H & E and Verhoeff's elastin stains.

Strain and bursting strength were measured using a mechanical system designed for in vivo testing.

The laser-assisted procedures were faster, involved reduced surgical manipulation and introduced less foreign material at the lesion site when compared to conventional suture techniques. It was fount that the fiber-optic delivery system of the argon laser was easier to manipulate than the articulating arm/micromanipulator system of the $CO_2$ laser.

# I. ARTERIAL REPAIR USING LAMA

## A. Objectives

1. Determine the histological process of healing and maintenance of patency time periods for conventional and laser small vessels.

2. Determine the properties of the bonding mechanism associated with the welding effect of the laser technique using pathological, biomechanical as well as clinical testing methods.

3. Obtain additional data on conventional microsurgical techniques to be used as the control model.

## B. The Animal Model

The femoral vessels of adult rats were used employing the standard dissection for routine exposure. One artery was anastomosed using standard 10-0 nylon (75mu needle) with microsurgical conventional technique. On a second animal, the other limb was anastomosed by the laser technique using a sharplan $CO_2$ 773 Surgical Laser System.

The respective anastomosis will then be studied as follows:

1. Patency Testing

Was don acutely and in stages as animals are sacrificed over time intervals up to 5-6 months. Patency tests were recorded on 35 mm and video tap photographed through the microscope. Follow-up in the initial phase of this study with follow-up intervals of 8-10 weeks were 88%. A second phase study revealed 92% patency rates (46/50).

2. Biomechanical Testing

(Please refer to appropriate section).

3. Pathological Analysis

Each anastomosis whether a control or from the laser technique were sectioned, stained and evaluated. (Please refer to appropriate section for detailed description).

4. Development of Laser/Optical System

All studies for FY 2 were carried out using existed laser surgery equipment, the $CO_2$ SHARPLAN 733 system. The department of physics has recently finialized design modification, on the system proposed to the ONR. Testing will be completed in the 2 month no-funds extension. Final reports on the $CO_2$ and Argon Laser systems will be presented at the end of the 2 month extension.

C. Rationale

The preliminary results presented and documented in this proposal for rat specimens strongly suggests that the laser technique is superior, faster, and far more clincially and scientifically applicable to today's demands of the microsurgeon and his/her expertise. Laser provides a more accurate, faster and far less technically demanding method to not only teach to surgeons but also to carry out in the operating room or, in fact, in a mass casualty occurrence. The technique to date, is in "it's infancy" but this should not deter or detract its tremendous application for the future. The only problems with using the laser in today's operating room theaters are:

1. The development of a more sophisticated laser/optical design.

2. The establishment of sound basic scientific data regarding the effects and description of wound healing using the laser (Pathological/ Histological Analysis).

3. The establishment of basic scientific data regarding the realtive strength, tensile properties and bursting analysis of the laser anastomosis (Biomechanical Properties).

4. The experience of a well qualified team of surgeons working to accumulate the data, documentation, and present the results to insure the credibility, safety and clinically numerous applications of the LAMA technique.

It is felt that by accomplishing the above four criteria, the LAMA technique may, if the preliminary observations continue to be valid, hold a tremendous contribution not only to the basic scientist but also the the surgeon and most importantly the patient in need of microsurgery.

## II. PATHOLOGY OF LASER ASSISTED MICROSURGERY ANASTOMOSIS

### A. Methods

The anatomy and progression the wound healing of the anastomotic sites of severed rat femoral arteries was studied using various histological and histochemical preparations for light microscopy. The wound healing of the laser assisted microsurgical anastomosis was compared with wound healing of vascular anastomosis using conventional microsurgical techniques with 10-0 nylon sutures.

The blood vessel segments were fixed immediately upon removal from the rats in 10% buffered formalin. The tissues were then dehydrated through a series of graded alcoholic solutions and embedded in paraffin.

Serial paraffin sections of the entire anastomotic site and adjacent tissues were prepared for histologic and histochemical studies, including some or all of the following techniques:

| | |
|---|---|
| Hematoxylin and Eosin | General anatomy of bonded tissues and healing wounds. |
| Verhoeff's Elastic Stain | Stains the elastic framework of the arterial wall. General connective tissue stain. |
| Masson's Trichrome Stain | Stains for muscle cells and fibrous connective tissue of blood vessel walls. |
| Phosphotungstic Acid Hematoxylin Stan | Stains fibrin to differentitate Fibrin from othe Fribillar, extra-cellular proteins. |

B. Background and Rationale

Preliminary morphologic studies have shown that the initial bond produced by the $CO_2$ laser is formed by an amorphous plug of protein. The biophysical and bio-chemical nature of this plug is unclear but may represent thermally denatured proteins from tissue and/or plasma fluids. Remnants of this plug remain in the healing wound for about four weeks; however, our few observations have not allowed any conclusions about how the plug affects the healing process.

The sutures used in microsurgery elicit an inflammatory reaction, including the formation of foreign body granulomas around the sutures, which can eventuate into obstructive dense fibrous scar or rupture of the vascular wall. The preliminary patholic observations have shown that the blood vessel wall anastomosis produced by the laser is not associated with such prominent inflammatory reactions. The progression of wound healing in the laser assisted microsurgical techniques were compared to that of the anastomosis produced by routine microsurgical techniques.

The relatively less traumatic laser anastomosis effected a healed wound which was less distorted by inflammation and less prone to be associated with thrombosis, vessel rupture and anerrysm formation when compared to the routine techniques.

The well structured external and internal elastic lamina of the rat femeral and carotid arteries are not reconstituted in the healed vascular anastomosis produced by laser. Irregularly arranged thin elastic fibrils and collagen are the extracellular components of the wound tissue at five weeks.

These observations are similar to those reported in light microscopic morphologic study of wound healing in microvascular surgery.

Light microscopic examinations of the ruptured vessels tested for tensile strength will identify the anatomic point(s) of anastomotic weakness at various times in the healing process. Comparison of the anatomy rupture in the laser anastomosis and the suture anastomosis will better define the relative strengths and weaknesses of the two techniques in producing an anastomosis which will maintain the functional integrity of the small blood vessels. The tensile strength measurements are nearly complete and the segments will be examined in the 2 month non funds extension.

To date, histologic specimens have veen prepared, photographed, and studied on 70 rats from animals sacrificed 5 minutes to 6 months post anastomosis.

The second phase of pathologic analysis will involve the re-endothenlialization of the arteries. Damage to the epithelial lining of small and large blood vessels (the endothelium) has been associated with thrombus formation. Scanning electron microscopic studies of luminal wound healing have been done in severed rabbit femoral arteries and veins (0.8-1.0 mm. in diameter) anastomosed by routine microsurgical techniques. (O"Brien, Bernard McC. Microvascular Reconstructive Surgery. Churchill livingstone, New York, 1977). Re-endothelialization of the wound begins within seven days and is complete at 28 days. However, few specimens were studied and no correlation with thrombus formation or mural wound healing was done. The evaluation of the role of the rat and pattern of re-endothelialization of damaged small blood vessels contributing to the success of microsurgical vascular anastomosis have not been done.

The surfaces of the sutures within the lumina of the blood vessels are the last to be covered by endothelial cells. The protein plug produced by laser may provide a more appropriate surface for re-endothelialization than the foreign material of the ultrafine sutures; therefore, it may enhance rapid healing of the luminal surface and decrease the change of thrombus formation.

Luminal wound healing comparing the rates and the patterns of re-endothelialization of severed rat femoral arteries joined together by laser and by routine microsurgical suture techniques will be studied with the scanning electron microscope up to 12 weeks post anastomosis in 7 groups of 12 animals each (6 laser and 6 sutured). The progression of the wound healing and the mophological changes seemingly associated with surgical failures, especially occlusive thrombus formation, will be evaluated. These vascular anastomosis are complete and the specimens will be examined in the 2 month no funds extension.

Transmission electron microscopy will also be used to study specific phenomena of the wound healing in laser. The ultrastructure of the protein plug relative to that of naturally formed fibrin thrombi and to the tissues surrounding the plug will give information concerning the biophysical and biochemical nature of the bond. The biochemical cellular events of the sequence of resolution of the plug as wound healing progresses as seen with the electron microscope will complement the study of those events by light microscopy. The production of the fibrillar extracellular components of the healing would and the relationship of their production fo the proliferation of the cells of the intima and the media will be studied by transmission electron microscopy. The effects, if any, of the plug on the progression of wound healing especially in the formation of the scar in the healed wound will be best analyzed by comparison of the light microscopic observations with those made by electron microscopy. These vascular anastomosis are complete and the specimens will be examined in the 2 month no fund extension.

# ESTABLISHMENT OF MECHANICAL FAILURE CRITERIA FOR MICROSURGICAL ANASTOMOSIS OF SMALL ARTERIES WITH A LASER

# III. ESTABLISHMENT OF MECHANICAL FAILURE CRITERIA FOR MICROSURGICAL ANASTOMOSIS OF SMALL ARTERIES WITH A LASER

## A. Background

Once an artery has been anastomosed under the microscope, it is important to understand how fast tensile strength is gained in the artery to protect against the development of leaks and ruptures during post-operative rehabilitation. The patient generally begins some range of motion exercises within 72 hours post surgery and thus, the arteries must be able to withstand some tensile forces during active or passive range of motion of the adjacent joints. AT the present time, it is not known how fast strength develops in classically micro-sutured vessels utilizing standard 10-0 nylon sutures. It is important for this work to develop such "control" information for comparison to anastomosis by a laser "weld". Two failure mechanisms must be noted for these vessels at various periods of time post surgery: 1) development of leaks subsequent to pulsatile pressures which are applied from the fluid within during tensile stretching of the anastomosis site, 2) yield and actual rupture of the anastomosis site due to tensile forces. Neither of these two criteria have ever been reported in the literature or clinical experience of the surgeons performing the anastomosis.

## B. Methods

Adult rats had the femoral arteries exposed and anastomosed by two separate methods: 1) standard microsurgical 10-0 nylon (75 um) suture and, 2) laser welding of tissue as described in the earlier methods. At 0 day, 1 day, 3 days, 10 days, 21 days, and 12 weeks post anastomosis in both the conventional and laser suture vessles, mechanical tests were performed with follow-up histologic assessment of the failure site in the tissue.

In order to provide as realistic an environment as possible for failure testing, the following tests were carried out in vivo so that the normal pulsatile blood pressure of the rat will be maintained throughout the experiment. Each animal was anesthetized with Nembatol and the femoral artery which had previously been anastomosed was exposed and dissected free from the surrounding soft tissues over a distance of approximately 1 cm. The hip was then disarticulated and the remaining soft tissue connections of the limb with the trunk of the body was disconnected. All major vessels were ligated so that the only mechanical and circulatory connection between the limb and the animal was the intact artery at the anastomosis site. The main trunk of the body was then mounted on a table and the dissected limb mounted on an adjacednt sliding table for tensile testing. This table is designed as a relatively friction free system so that the force required to distract the table and the limb from the main portion of the body will primarily reflect the resistance force of the artery connecting the two systems.

This entire system was mounted under the dissecting microscope with a videotape camera attached. Behind the artery, a light color contrast background was established so that videotape recording of the image produced as sharp a contrast between the vessel and its background as possible. Contrast marks were placed on the artery on each side of the anastomosis site and at the proximal and distal ends of the regions immediately adjacent to the anastomosis site where scar tissue formation or thickening of the vessel was evident. Two marks also were placed possibly a millimeter apart, both proximal and distal to the anastomosis site in a region where the vessel appears to be relatively normal. These

marks were viewed through the operating microscope. In this manner, relatively position changes between these marks can be recorded as a function of time on each frame of the image so that strain measurements can be made optically from this test through the microscope.

Appropriate indications on the videotape image were made to indicate the onset of the initial leaking of the vessel and the final rupture of the vessel. The vessel was stressed with a constantly increasing force placed on the sliding table through a pulley system. The pulley system was attached to a receptacle which was counterbalanced so that the systems begin at an initial load of 0 and load was increased at a contrast rate with time by injection of mercury into the vessel at a constant rate controlled by a syringe and an infusion pump. A calibration run establishes the rate of load increase for this test and markers made on the videotape image indicate the onset of loading. In this manner, load level at any given time during their videotape recording was known by noting the frame number of the videotape image. Therefore, analysis of the data collected included a strain measurement for each region of the vessel surrounding the anastomosis site and at the anastomosis site as a function of force at a constant force rate with failure indicated for a given force and strain level.

This method of testing was chosen because of the fact that it proved to be extremely difficult to dissect whole specimens of artery free from the tissues and test them in a type of a clamping mechanism as was proposed originally in the first year of this project. Clamping artifacts created very artificial boundary conditions for this testing and because of the size of the vessels, it became impractical to attempt to produce a complete

pulsatile, flow system in vitro for the mechanical testing. The only artifacts created in this in vivo test are the change in boundary conditions around the vessels due to the dissection of soft tissues from the vessel within 1 cm. and any changes in blood pressure or pulsatile rate which would be associated with the anesthesia of the animal at the time of testing. Minor leaks created under these in vivo testing conditions might be easily repaired under normal in vivo conditions due to the tamponade effect of the surrounding soft tissues. Therefore, this test would represent the minimal bounds of behavior of the system expected to occur in vivo. Also, the dissection of the surrounding soft tissues might damage some of the superficial tissues of the arterial structure and weaken it compared to its normal in situ condition. Thus, this testing might be considered to be a lower bounds of behavior of the system in true in vivo, in situ conditions.

After this test was carried out on the experimental artery of each animal, separate series of animals were utilized for similar in vivo tests of intact arteries for control information.

During the 2 month no fund extension, results from the load and deflection data, stress and strain curves will be calculated for each zone of the tissue plotted simultaneously on a stress-strain graph. On the stress-strain graph a marker will indicate the onset of vessel leakage, yield in the tissue and the final tissue rupture with a note indicating wich zone of tissues failed for the given specimen. Strain calculations will be estimated by noting elongation of each of the zones and comparing it to the original length of each zone at each period of time during the tensile test.

Stress calculations will be made by dividing the applied force measured during the test by the cross-sectional area measurements taken after the tensile testing procedure. Cross-sectional area measurements will be made with a mechanical device which has proven effective for measuring cross-sectional areas of tendon. This method represents a simple, repeatable means of estimating the cross-sectional area of tubular or cylindrical soft tissue specimens. The specimen is confined to a fixed space in a fisture and compressed by a plunger system within the contour of that fixture space using a micrometer with a spring ratchet mechanism to assure consistency of pressure on the tissue from one measurement to the next. The height of the plunger will then be measured by the micrometer in tenths of a thousand of an inch so that the cross-sectional area can be calculated as the remaining space in the fixture occupied by the tissue at the given pressure. In this manner, cross-sections were measured for each zone of the tissue in each specimen. From the stress-strain diagrams for each zone, a calculation can be made of the apparent modulus of elasticity, the apparent poissons ratio, the apparent nominal stress at the leakage, yield and rupture of tissue.

Each specimen after tensile testing was fixed in 10% formalin and mounted for longitudinal histologic sectioning to assess the tissue at the apparent failure site and to assess any ultrastructural or microstructural damage to the tissues in each of the zones under test.

C. Significance

The importance of the destructive, mechanical tests of these anastomosed arteries is to demonstrate the timing of the development of strength in the tissues in the various regions at and immediately surrounding the anastomosis site. A relative strength of the various regions can be also assessed as to continuity of the vessel for the prevention of hemorrhage as well as for the prevention of gross rupture of the anastomosis. These parameters are important for the timing of passive and active range of motion exercises of the patient post surgery and for the development of appropriate regimes for nursing and general rehabilitation of the patient. Proper handling of the limbs and the need for immobilization of the joints adjacent to the anastomosis site are not well understood at this time. If a particular anastomosis technique will not render the required strength to the vessel to allow for each range of motion of the adjacent joints within a few days post surgery, it will not be adequate for proper rehabilitation of the patient and prevention of joint stiffness and muscle atrophy post surgery. Final measurements and data analysis will be complete at the end of the no funds extension.

# LASER ANASTOMOSIS OF NERVES

# INTRODUCTION

## "CARBON DIOXIDE LASER REPAIR OF RAT SCIATIC NERVES"

Lasers have been used to reanastomose divided arteries veins, fallopian tubes, vas deferens, uterine horns, nerves, and to close skin and cornea.

This paper describes a new surgical technique that utilizes laser heat energy to repair transected rat sciatic nerves, and nerve grafts. The energy emitted at 10.6 um is used to heat-denature tissue components for use as a biological glue.

Present microsurgical technique allows the anastomosis of severed nerves using ultrafine sutures. The eventual functional success of such repairs is dependent upon accurate alignment of the ends and the use of impeccable surgical technique to minimize tissue trama. Although the sutures used in microsurgery are very small, the number of sutrues necessary to effect the anastomosis of small nerves still introduces a substantial amount of foreign material into the wound. The sutures used in microsurgery elicit an inflammatory reaction, including the formation of foreign body granulomas around the sutures. This can disrupt the regenerating axons; and thus, they cannot eventually reinnervate their proper target organs.

Rat sciatic nerves were dissected and after adequate exposure, they were transected. The severed ends were approximated with the help of two or three stay sutures. By using a low power carbon dioxide laser, defocused to a very large spot size (approximately 3 mm), epineurial repair of the severed ends was carried out. A TEM.. $CO_2$ laser beam from a SHARPLAN 733 Laser was

directed to the repair site by means of an articulating arm that attaches to a micromainpulator mounted on a Zeiss operating microscope. The 10.6 um wavelength has a very limited "coagulative" penetration that is well suited for epineurial closure. Immediately following laser repair the stay sutures were removed. It must be emphasized the selection of a long IR laser wavelength, large spot size, low power input, and short time exposure is critical for this technique to be effective.

The laser group was compared to three other groups of rats; sixty rats were studied. In one group, the transected nerves were anastomosed using conventional microsurgical technique; this involves using six 10-0 sututres placed around the circumference. In a second group of rats, the nerve was transected but not repaired. The third group of nerves was crushed twice by angulated jeweler's forceps for one minute. This crush injury resulted in separated axons but an intact epineurium. All surgical procedures were performed by one author.

Return of muscle function was recorded and nerve counts were observed; electrophysiology was assessed blindly. Longitudinal and proximal and distal cross sections were studied by light microscopy using H & E, LFB-PAS, and Bodian staining. Electron microscopy was also performed on the laser repaired nerves.

The progress of laser repaired nerve regeneration has been followed up to 9 months post-opratively. Nerve fiber integrity appears greater with laser as compared to microsurgical suture technique. The laser sealed epineurium appeared to channel the regenerating fibers into the distal stump well.

Bodian staining for axons shows relatively good axonal preservation distal to the site of laser union as early as 17 days.

CONCLUSION: This laser surgical procedure eliminates the presence of epineurial sutures, is faster than suture technique, minimizes surgical manipulation, and provides a circumferential coagulum weld of the epineurium preventing the escape of the regenerating axons at the site of lesion.

Further evaluation of recovery after laser epineurial repair will require human studies; human research protocols are now being outlined.

# I. LASER ANSTOMOSIS OF NERVES

## A. Introduction

Present microsurgical techniques allow the anastomosis of severed, small nerves using ultrafine sutures. The eventual functional success of such repaires is dependent upon the accurate alignment of the ends of the nerves and the use of impecable surgical techniques to minimize tissue trauma. Although the sutures used in microsurgery are very small, the numbers of sutures necessary to effect the anastomosis of small nerves relative to the size of the nerves still introduces a substantial bulk of foreign material into the wound. The granulomatous inflammatory response to these sutures can be responsible for considerable scarring. The regenerating axons sometimes cannot penetrate the scar tissue barrier; and thus, they cannot eventually reinnervate their target organs so the return of function can be severely compromised. Even with multiple suture, an epineurial seal can never be completely accomplished. Axons will wander thru the openings in the epineurium and into surrounding soft tissues.

Laser microsurgical repair of rat scratic nerves consists of a surgical procedure which eliminates the use of sutures, produces minimal neural damage, and allows precise alignment of the severed nerve ends necessary to maximize the efficiency of nerve regeneration. The relatively minimal tissue damage produced by the laser when compared to the routing microsurgical techniques and the confinement of the regenerating axons within the epineurium was found to decrease the incidence of a frequent complication of nerve anastomosis, the traumatic neuroma.

Nerve regeneration after conventional microsurgical anastomosis and laser assisted microsurgical anastomosis was compared using measurement of axonal transport, electrophysiologic and ultrastructure evaluations. Testing of the tensile strength of the "laser bond" at various stages of wound healing will provide an understanding of the critical factors in the rehabilitation of the patient with nerve repair and will be completed in the 2 month no funds extension. To date, wound dehiscence was found in none of the laser repaired nerves.

Previous work by Yahr and Strully (1964), demonstrated the feasibility of repair of arteries in vitro utilizing a laser. Further work by Jain and Gorisch (1979) demonstrated that not only repair but anastomosis of arteries and veins could be accomplished utilizing a laser in vivo. They were able to accomplish anastomosis to vessels down to .8 mm. in diameter and repair of vessles as small as .3 mm. in diameter with 80-90% patency rate. In 1981, Morris, Carter and Thomson reported on $CO_2$ laser anastomosis of arteries utilizing the laser to produce a "weld" of the peripheral soft tissue structures without damage or penetration into the interior structure. In 1982 Dew and LO (American Society for Laser Surgery)reported on experimental repair in vivo of nerves, vas deferens, skin, cornea, fallopian tubes, veins , arteries, in a feasibility study of $CO_2$ laser repair of various tissues. Also, in 1982, a specific preliminary report was given on the experimental work of this grant on the repair of nerves and the regeneration of those nerves after repair as described in this proposal. (American Academy of Neurology) subsequent papers were present at CLEO' 83, the American Fracture Association, the American Orthopedic Association, and an invited paper before the 5th International

Congress of Laser Medicine and Surgery.

B. Methods

Adult rats had the sciatic nerve dissected free of the surrounding soft tissue and the nerves experimentally treated as follows:

Group A (Sham nerves) - the sciatic nerve will be surgically exposed and then evaluated.

Group B (Cut Nerves - the sciatic nerve will be transected and left to regenerate without suturing the nerve ends.

Group C - (Crush nerves) - The sciatic nerve will be crushed for 45 seconds with jeweler's forceps.

Group D (Suture nerves) - An epineural repair will be performed using conventional 10-0 nylon microsurgical technique.

Group E (LAMA group) - the transected nerve will be anastomosed using the laser technique. Laser technique will consist of forming a coagulum bond of the epineurium - a completely circumferential weld of the epineurium.

C. REQUIREMENTS FOR LASER REPAIR:

There must be perfect, exact restoration of the original anatomic position of the nerves which can be accomplished by utilizing the small vessel patterns in the epineurium as alignment marks during the anastomosis. The laser energy must be controlled so as to avoid charring which occurs between 300 and 500 degrees centigrade. The temperature must also be kept below 100° centigrade, the boiling point of the tissue where bubbling becomes evident. These parameters can be controlled by the spot size, wave length of the laser and the time of the exposure. The laser being utilized is $CO_2$ with 10.6 micron wave length, the appropriate spotsize appears to be 1 to 3 mm. and exposure time 1/20 to 1 second for the present investigation. Temperature of the tissue must be maintained between 37° and 100° centigrade, the range of coagulation and denaturation of the protein. The laser "weld" can be likened to coagulation of egg white which has been measured to occur at

about 70° centigrade. This coagulation or denaturation of protein can be likened to a biological glue formation.

Sutures are initially placed only through the epineurium of the severed ends of the nerves to approximate the nerve ends for epineurial repair. Sutures must be placed with a very large "bite size" so that suture material does not pass close to the severed end of the epineurium. Two or three sutures seems adequate for approximation to an adequate degree for laser anastomosis. In this manner, after the anastomosis is completed the long loop of the suture can be pulled out without disrupting the laser anastomosis and the accompanying holes from the suture can be lasered shut. In areas where the epineurial alignment is not exact enough for laser anastomosis, the area can be patched with fat to obtain a complete seal of the epineurium around the transacted nerve bundle. Hemostatis must be absolutely perfect in the area and the field must be kept completely dried/ The slightest amount of water on the tissue could result in the tissue literally falling apart after the laser has been applied. The laser power levels are held to 100-1000 millowatts.

Since the initial studies have been relatively complete in these animal groups, and additional 30 animals will complete each of the groups described above.

D. Axonal Transport Studies

This part of the investigation studied the regeneration of motor axons in the rat sciatic nerve by labeling with axonally transported radioactive proteins. The early stages of regeneration as well as events that occur after axonal elongation were studied. We investigated the kinectics of the outgrowth of regenerating motor axons as well as

attempted to quantify the amount of neuroma formation by measuring activity above, at, and beyond the nerve lesion.

Sixty-five lewis rats will be divided into groups A through E. Each rat had an "experimental nerve" (right side) and "intact nerve" (left side) and thus serve as its own control.

1. Group A ("Sham nerves") - The sciatic nerve was surgically exposed and then evaluated.
2. Group B ("Cut nerves") - The sciatic nerve was transected and left to regenerate without suturing the nerve ends.
3. Group C ("Crush nerves") - The sciatic nerve was crushed for 45 seconds with jeweler's forceps.
4. Group D ("Suture nerves") - An epineural repair was performed using conventional six 10-0 nylon with conventional microsurgical technique.
5. Group E ("LAMA group") - The transected nerve was anastomosed using the LAMA technique. The laser technique consists of forming a coagulum bond of the epineurium - a completely circumferential weld of the epineurium.

Axonal transport studies utilized a modification fo the techniques described by Forman and Berenberg* as outlined below.

I. Operative Procedure and Microinjections

A. Conventional microsurgical anastomosis.

B. Laser assisted microsurgical anastomosis.

C. Bilateral laminectomy utilizing electric drill. ($L_4$, $L_5$ region)

D. Microinjection of radioactive amino acids utilizing a glass micropipette/micromanipulator/Hamilton syringe/hydraulic micrpdrive system.

II. Construction of radioactivity profile

A. Sacrifice animal (24 hours)

B. Nerve preparation

C. Scintillation counting (Packard 2425)

D. Data analysis

*Forman, D.S. and Berenber, R.A., "Regeneration of Motor Axons in the Rat Sciatic Nerve Studied by Labelling with Axonally Transported Radioactive Proteins". Brain Research 156 (1978) 213-225.

E. Electrophysiological Studies

The presence, amplitude, latency and waveform of compound action potentials were used to monitor the regeneration of the sciatic nerve of the rat. We used muscle compound action potentiali as a measure of conduction velocity, number of active fibers, and dispersion of the action potentials. Electrophysiological findings were correlated with caonal transport studies to further evaluate laser peripheral nerve repair.

In light of the fact that regeneration is an extremely complex process involving a bundle of many nerve axons, it is impossible to study accurately the regeneration of any given axon. Therefore, the presence or absence of a compound action potential was used to indicate regeneration of the bundle of nerve fibers to the muscle most distal to the sight of sciatic nerve severed.

A hewlett-Packard HP150B myograph wired with shielded cable via the output to an electrical switchbox was used to stimulate the electrode. The electrode consists of 4 paris of platinum wire electrodes spaced 2 mm. from each other with a 1 mm. space between each of the two wires in each pair. The electrical switch box allows for stimulation to occur at any of the four stimulation pairs. The complete electrode assembly consist of a semi-circular channel 10 mm. long of approximate width to hold the sciatic nerve of an adult rat. Within the semi-circular canal are placed the platinum wires which are bent into a U-shape at distances described. The electrode is constructed of non-conducting material to minimize the possibility of spreading the stimulation of the nerve. Two Grass Instruments sub-dermal recording electrodes are positioned, on sub-cutaneously and the other at the muscle group being tested. This is connected to the input

channel of the Hewlett-Packard electromyograph where the resulting compound muscle action potential is recorded with an oscilloscope and photographed with a polaroid camera. Electrical pulse signals of 1,000 microseconds in duration generated by the Hewlett Packard are used to stimulate the nerves in this study. The recording electrode is placed subcutaneously and serves as a reference electrode against which the electrodes placed in the muscle can record changes in the ionic current as induced by the electrical signal. These extremely small voltage changes are amplified by the Hewlett Packard myograph and recorded as a wave form.

The amplitude of the wave gives a general assessment as to the strength of the voltage. The amplitude of this signal is quite variable and depends greatly on the degree of stretch of the muscle, the nerve and any debree on the nerve itself. Therefore, measure of amplitude is not taken to be a valid finding in this study and will not be used to assess the quality of nerve regeneration. The most valuable findings with this type of stimulation are the presence or absence of a wave which indicates whether the nerve could or could not transmit an impulse and the conduction velocity of that nerve as determined by measuring from the 0 point on the time axis, that is the immediate moment of stimulation, the amount of time it took for the electrical signal to cause stimulation in the muscle shown by a rise in the amplitude of that wave. By using a stimulation point along the nerve at two different distances through the switch box, one can calculate the amount of time it took from each of those points to the moment that the muscle was stimulated and thus, the conduction velocity can be calculated along the nerve.

In all instances, the recordings were made on coded nerves and the data analyzed by the authors without knowledge of the type of repair, initial trauma, ets.. Final decoding of the data took place for statistical comparisons.

These compount action potentials have been recorded on approximately 70 nerves to date in the study and only a few more measurements are required to complete this phase of the work. However, many hours of assessment of the data and quantitation of information collected to date is still required. This will be completed in the 2 month non funds extension. Initial findings indicate laser to be superior to suture repair of rat Sciatic nerves.

F. Histological Studies

I. Light Microscopy of samples of each of the nerves after anastomosis at various time periods post surgery were prepared to assess the constituents in the repair, the cytologic health of the tissue, the orientation of regenerating axons and the presence or absence of inflammatory response. Constituent tissues were assessed utilizing a silver impregnation with a trichrome counter stain to differentiate axons from fibrous tissue and other constituents of the repair. Orientation of these tissues can be well identified with these silver impregnation techniques. Standard hemotoxin and eosin staining were utilized for cytologic assessment of the response of the tissues to the repair process. Histochemical analysis was made using esterase stains to analyze the inflammatory response at the anastomosis site. Histopathology revealed neural integrity to be greater with the laser as compared to microsurgical suture techniques. Longitudinal and proximal and distal cross section, showed excellent axonal growth and preservation distal

to the site of laser repair. In all cases, the laser sealed epineurium appeared to channel the vegenerating fibers across the lesiun and into the distal stump nell. This was not the case with suture repair which showed frequent neuroma formation post operatively.

II. Electron Microscopy: Model proteins, examinations and laser epineurial repair.

Method Model control protein and lasered coagulated protein were fixed with 2.5% glutaraldehyde (in 0.05 M cocadylate buffer, PH 7.4) post fixed with 17. $O_5$ $O_4$ (in same buffer) dehydrated in an ethanol series and embedded in british Araldite. Sections were cut with a diamond knife on an LKB ultramicrotome, collected on 300 mesh grids, and examined in a phillip 300 EM after "staining" in saturated 50% ethanolic uranyl acctafe and lead citrale.

Electron microscopy of normal and laser coagulated model egg white revealed distinct differences. Normal model protein showed homogenous ultra structural appearance with occasionally small areas of dark stained proteins. Laser coagulated model protein showed coagulation of protein with different stainability. Occasionally, homogenous finer and lightly stained material were observed in the background. At high magnification of laser coagulated egg albumim detailed gel-like matrix was evident. This proper controlled laser irradiation has demonstrated morphological alterations in a model protein system.

Cross sectional electron microscopy of laser repaired peripheral nerves confirmed the results of light microscopy obscruative concernia neural integrity. Laser nerves were examined at 30 days and 9 months post-operatively. Axons were well nylinated, normal in appearance, and metabolically active. Few if any degenerated fibers were seen distal to the anastomosis at these time periods. Axons were well organized

## CONCLUSION

The literature contains numerous attempts to document nerve regeneration both in clincial and laboratory setting. (see bibliography). There still exists a tremendous controversy over the most effective type of suture repair (epineural vs. perineural) and the most effective method of neasuring nerve regeneration. Although the laser assisted microsurgical method is still experimental and under investigation in or laboratory; our axoral transport platophysiological and ultravascular findings indicat to be inferior to laser epineurial repair, conventional microsurgical repair.

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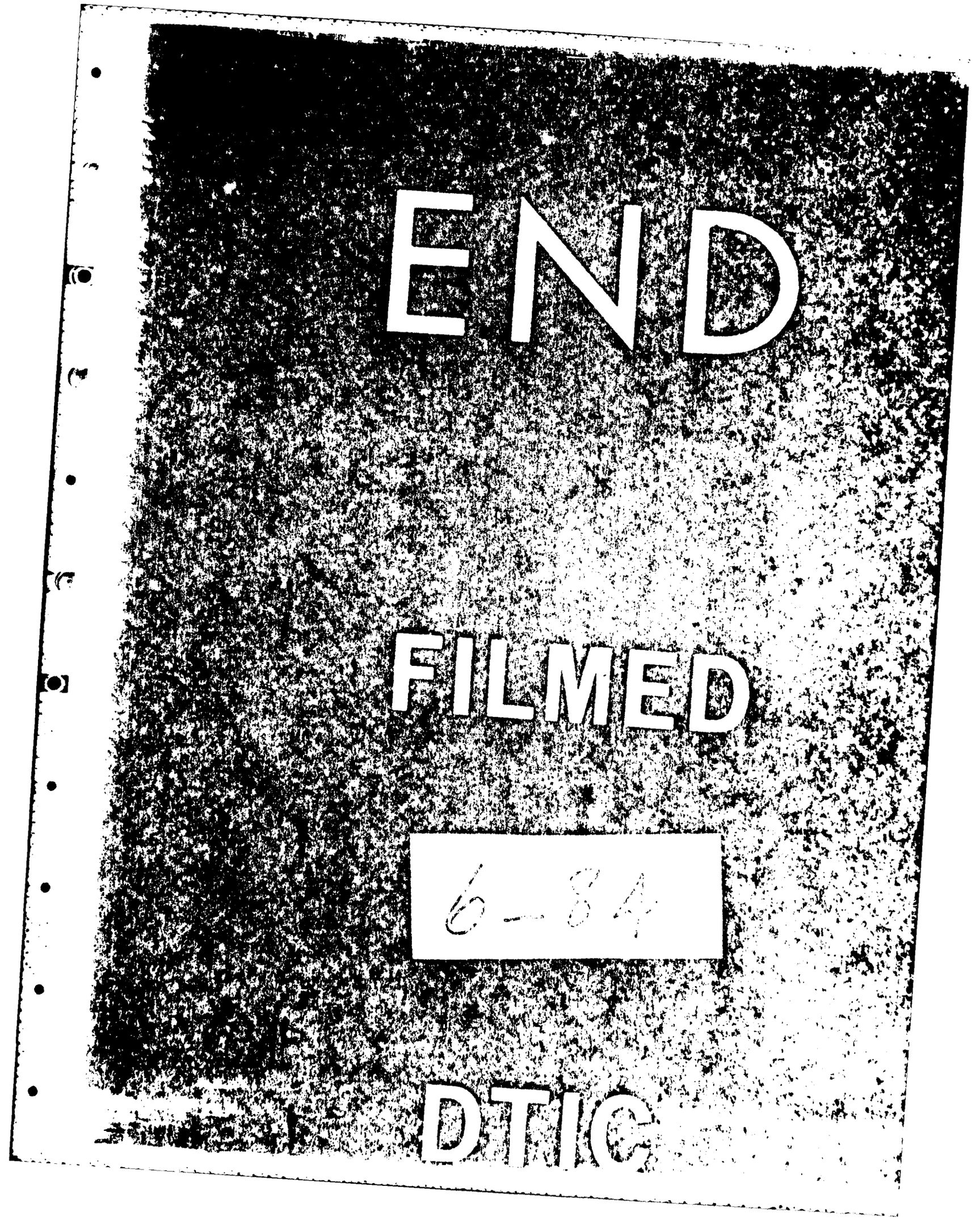
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