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April 5, 1993

Wound Healing after Laser Injury to Skin-

The Effect of Occlusion and Vitamin E

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ABSTRACT

The effects of argon and copper vapor laser irradiation of the skin of the Yorkshire pig were determined by gross and microscopic evaluation as a function of dose. For both lasers, the incident irradiance was between 3.5 and 4.5 watts/cm^2 for a 10-14 mm beam diameter with a nearly uniform intensity profile. The dose was varied by varying exposure duration. The minimal erythemic doses for the copper vapor and argon laser exposures were 35 $\pm$ 2 J/cm^2 (10 second exposure) and 22.4 $\pm$ 0.1 J/cm^2 (6 second exposure) respectively. Three dose levels ranging from a low dose causing light erythema, an intermediate dose, and a high dose causing dermal stasis were selected. The effect of topical and intramuscular vitamin E pretreatment and Op-Site wound dressing on wound healing was determined at each of these dose levels. Wound healing was defined by loss of primary eschar. Argon and copper vapor laser exposures to pig skin generally caused wounds with similar healing times, which were approximately 4 weeks after the intermediate dose in control pigs. Following intermediate exposures of the copper vapor laser, wound healing was significantly decreased by approximately one week for both vitamin E pretreatments and wound dressing. Following the corresponding argon laser exposures, wound dressing and only intramuscular vitamin E significantly decreased wound healing time by approximately one week. These treatments may be valuable for treatment of accidental laser skin injuries to man.

Key Words: Copper Vapor Laser, Argon Laser, Pig Skin, Histology, Wound Healing, Vitamin E, Wound Dressing.

INTRODUCTION

Laser systems are widely used in industry, medicine, and for military purposes. Lasers produce collimated electromagnetic radiation that is of high intensity. It is generally characterized by monochromaticity, a high degree of coherence, a small angle of divergence of the light beam and the possibility of optically focusing the radiation. Carbon dioxide laser radiation (10.6 um) is strongly absorbed by water molecules in skin. Absorption therefore takes place in the superficial layers of the skin (stratum corneum and upper epidermis) with heat conduction and subsequent damage to deeper tissues, depending on the dose. Injuries caused by CO_2 laser radiation of different power density and exposure time combinations have shown that the injuries to pig skin are similar to thermal burns[1]. The Argon (Ar) laser emits at several wavelengths including 488 and 514 nm (green light). The radiation can be absorbed by the chromophore melanin present in the epidermis and by hemoglobin present in the viable epidermis and dermis. The Copper vapor laser emits at 511 nm (green) and 578 nm (yellow). Hemoglobin absorbs the yellow emission four times more strongly than the green emission, while melanin absorbs the yellow emission 30 % less strongly than the green emission[2]. Radiation originating from the argon laser will cause more specific damage to the vascular tissue than is caused by the CO_2 laser, with the most specific vascular damage caused by the yellow emission from the copper vapor laser. This

selectivity has been used to advantage in the treatment of port wine stains by damaging ectatic blood vessels in the dermis[3].

The copper vapor laser is of particular interest for industrial applications because of its high power output. With rapid development of laser technology and expansion of applications, there is an increasing need for detailed information on the biologic effects and treatment of skin injury associated with this type of radiation.

This study determined the effects of argon and copper vapor lasers on lightly pigmented pig skin to correlate radiant exposure with subsequent injury to the skin. The effect of wound treatment on healing time was investigated.

MATERIALS AND METHODS

Animals

The pig was chosen as an experimental animal for this study due to its histologic and biochemical similarities to human skin[4],[5],[6],[7]. In recent years, a model has been developed using young domestic pigs for assessing epidermal regeneration in superficial wounds[8]. Female Yorkshire pigs (white skin color) weighing 15-30 kg were used in this study. They were quarantined for at least seven days after arrival and checked for possible disease or abnormal conditions. On the day of the experiment the

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pigs were premedicated intramuscularly with ketamine, rompun and atropine (2, 2 and 0.02 mg/kg, respectively), followed by anesthesia to effect with nembutal. Hair from both sides of the animal was removed with an electric clipper (Oster, Model A2, Milwaukee, WI) and the clipped area cleaned thoroughly with water.

Lasers

A continuous wave argon laser (Model I-100, Coherent Radiation, Palo Alto, CA) operating at 514 nm was used to make a series of exposures at independent sites on one side of the pig. A multiline copper vapor laser (Model MVL-2010-CU, C J Laser Corp, Dayton, OH) operating at wavelengths of 511 nm (67% of total output) and 578 nm (33% of total output) was used to make a second series of exposures at independent sites on the other side of the pig. The copper vapor laser operated at a pulse repetition frequency of 10 kHz with each individual pulse in the train having a duration of 50 ns (full width half maximum).

The lasers were configured as shown in Figure 1. The irradiance for all exposures was between 3.5 and 4.5 w/cm^2. Circular exposures 10 to 14 mm in diameter were produced. The intensity distribution for the lasers at the exposure plane was nearly uniform. Duration of exposure varied incrementally from 0 to 40,000 msec.

Histology

Following general anesthesia, elliptical biopsies were taken from the pigs at selected time points after laser exposure. The biopsies included the center and borders of the exposed area, and outlying normal tissue. Biopsies were fixed in 10% formaldehyde, imbedded in paraffin and separate sections stained using both hematoxylin-eosin and Masson's trichromat. Slides were prepared and the following scores established:

1) depth of injury (0,normal; 1, epidermis only; 2, epidermis and upper 1/3 of dermis; 3, epidermis and upper 2/3 of dermis; 4, epidermis and entire dermis; 5 subcutaneous tissue)

2) nuclei (0, normal; 1, pyknosis; 2, pyknosis and perinuclear vacuole; 3, pyknosis and elongation),

3) epidermal cytoplasm (0, normal; 1, loss of cytoplasmic basophilia; 2, basal layer edema/vesiculation; 3, necrosis; 4, slough)

4) connective tissue fibers (0, normal; 1, stained red with trichrome; 2, as in grade 1 with loss of fiber definition)

5) basement membrane zone (0, normal; 1, subepidermal cleft; 2, denuded; 3, ulcer)

6) <u>healing epidermis</u> (1, undermining; 2, flat basement membrane; 3, normal)

7) <u>fibers</u> (0, normal; 1, stained with red trichromat; 2, as in grade 1, with loss of fiber definition)

8) <u>scar</u> (1, slender fiber bundles, no dominant orientation. loose arrangement; 2, as in grade 1, with some horizontal orientation of fiber bundles; 3, mostly normal orientation, although fiber bundles remain slender and straighter than the normally undulating undamaged fiber bundles; 4, similar to grade 3, but fibroblasts are still more shrunken; 5, fibroblasts have attained their normal inactive appearance)

9) <u>vessels</u> (0, normal; 1, congestion; 2, hemolysis in upper 1/3 of dermis; 3, hemolysis in mid 1/3 of dermis; 4, hemolysis occurring in lower 1/3 of dermis; 5, hemolysis occurring in upper 1/2 of adipose tissue present on slide; 6, hemolysis occurring in deep adipose tissue)

10) <u>inflammatory cells</u>, (0, normal; 1, scattered polymorphonuclear neutrophils; 2, polymorphoneutrophils (PMNs) forming lateral "wall" at border of treated area; 3, a band of PMNs forming a complete base as well as wall of the ulcer).

11) <u>granulomas</u>, (0, absent; 1, present)

12) <u>appendages</u>, (0, normal, 1, damaged)

Photography

Individual exposure sites were photographed with a Nikon camera equipped with a Nikon flash attachment on Ektachrome-100 film.

Photomicrography

Photomicrographs were taken with an Olympus BH2 microscope equipped with an exposure control unit. Ektachrome T 64 film was used.

Effect of Exposure Time on Skin Damage.

Six pigs were prepared. Multiple circular sites of skin were exposed to the copper vapor laser radiation on one side of the pig and argon laser radiation on the other. Triplicates of 10 doses ranging from 0 to 50000 msec were applied. The animals were reanesthetized and each exposed site was observed visually and photographed at 2, 4, and 24 h post exposure. Biopsies were taken 48 hours after exposure.

Effect of Pretreatments or Wound Treatment on Healing

Twenty pigs were prepared and divided into four groups of 5 pigs each. A rectangular grid consisting of 3 rows of 1 inch by 1 inch exposure sites (36 squares), were drawn on both sides of the pig. On one side of the pig, twelve of the randomly selected squares received a low dose of radiation (10 sec) from the copper vapor-laser, twelve received a moderate dose (20 sec) and the remaining twelve a high dose (40 sec). A similar exposure

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pattern was followed on the other side of the pig for three doses
(6,15 and 35 seconds) from the argon laser. The four groups of
pigs consisted of a control group, a group treated with occlusive
dressing (Op-Site, Acme United Corp., Bridgeport, CT) immediately
following exposure, a group injected intramuscularly (shoulder)
with vitamin E (5 mg/kg total) in 3 doses given 24, 12 and 2
hours prior to exposure, and a group receiving topical vitamin E
(2 mg/cm^2 in 3 ul/cm^2 of 70% ethanol) 18 hours prior to
exposure. Levels of vitamin E were determined[9] in serum just
prior to laser exposure in control and treated groups.

Individual sites were photographed as often as 24 hours,
1,2,3,4,6,8, and 13 weeks following laser exposure. Randomly
selected exposure sites were chosen for biopsies at 2 days, 8
weeks and 13 weeks for copper vapor laser exposures and at 2
days, 5 weeks (low dose), 8 weeks and 13 weeks following argon
laser exposure. Photographic data was used to estimate wound
healing time by interpolation between the time the primary eschar
was last observed and the time it was no longer present.
Histological evaluations were done double-blinded and control
biopsies were compared with those from the treated groups at the
biopsy timepoints.

Statistical analysis: The measurement of healing time for each
laser injury with the three doses (low, moderate and high) was
subjected to an analysis of variance model to determine whether

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or not differences exist between the treatments (control, vitamin
E - systemic, vitamin E - topical and dressing) or between the
lasers. If significant differences occurred, the Dunnett's t-test
was applied to determine which treatments differ from the
control. All tests were done at the 0.05 level of significance.

Histological data were subjected to analysis of variance for
differences between the control and treatment groups. If
significant differences occurred, a nonparametric test was
applied to determine which treatments differed from the control.

The effect of laser dose on the histological parameters
"depth of injury", "scar", and "healing" was determined with
Kruskal-Wallis one-way analysis of variance using a Chi-square
distribution with two degrees of freedom. Criteria for
significance was $p < 0.05$.

RESULTS

Macroscopic Observations of Copper Vapor and Argon Laser Skin
Damage

Exposures causing minimal erythema are given in Table 1 for
the two lasers. Longer exposures resulted in immediate vascular
stasis in the exposure area, and gave the skin a pale appearance
compared to the surrounding unexposed skin. The longest
exposures did not cause any immediate degradation of the stratum
corneum or hair; these structures appeared transparent to the
effects of the lasers. Five to seven days following intermediate
exposures (Table 1), damage to the viable layers of the skin

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resulted in degradation of the entire epidermis.

Microscopic Aspects of Copper Vapor and Argon Laser Skin Damage

General

The area of damage is rather sharply demarcated laterally. The epidermis invariably showed damage to varying degrees. Depth of involvement of the dermis and subcutaneous fat roughly correlated with intensity and time of exposure. Seldom were significant changes seen below the mid level of the adipose layer. Microscopic examination of laser exposed skin at the selected time points did not distinguish the effects of the copper vapor vs argon lasers.

Photomicrographs were taken of control (non-irradiated) pig skin (Figure 2) and pig skin exposed to 20 second irradiation from the argon laser (Figure 3) and copper vapor laser (Figure 4) 24 hours after exposure. In both the argon and copper vapor exposures, streaming of nuclei in the lower layers of the epidermis and loss of collagen bundle structure in the dermis are evident. Clefts or separations between the epidermis and dermis and congestion of blood vessels in the dermis are also evident.

Acute morphologic changes:

Epidermis:

In biopsies taken shortly after the laser exposure (48 hr) the general structure of the epidermis was disturbed to a degree

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roughly correlated with duration of exposure. These histologic changes were considered to be edema of the basal layer, subepidermal cleft formation, and slough (separation at the basement membrane). Specimens taken at a later time sometimes showed epidermal necrosis and ulceration.

Cellular changes included loss of cytoplasmic basophilia (perhaps due to decrease of cytoplasmic RNA), pyknosis & loss of nuclei. "Pyknosis & elongation" refers to a curious parallel arrangement of elongated nuclei resembling a field of wind-blown wheat "blowing" away from the center of the lesion. This pattern is seen in lesions resulting from copper vapor and argon lasers alike. This may be a heat effect, since it is also seen in dermatologic biopsies following use of the common tool of the dermatologist, the hyfrecator. Brownell et al[1] reported a similar arrangement of elongated nuclei after CO_2 laser exposure and attributed the effect to heat.

The most severely damaged epidermis may take on a kind of "cooked" appearance microscopically, probably corresponding to the "white burn" visual pattern. Here the epidermis had lost its cytoplasmic basophilia and the nuclei were pyknotic and generally elongated, but the epidermis remained attached to the basement membrane without clefts or edema. Perhaps this effect was the result of denaturation of all cellular enzymes.

Appendages:

The damages to appendages consists of sloughing of the

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epithelial cells in the sweat glands, and pyknosis of the nuclei in the sheaths and bulbs of the hair follicles. These cells often exhibited the curious pyknosis and parallel elongation of nuclei that was also seen in the epidermis. This was seen even in hair bulbs located in the subcutaneous fat, in spite of the fact that the depth of dermal damage may only be apparent in the upper 1/3 of the dermis. The hair shaft may serve to conduct energy more efficiently into the tissue than does the surrounding dermal connective tissue.

Dermis:

The most reliable evidence of laser damage to collagen fibers was a kind of homogenization. The collagen bundle was a paler blue when the Masson stain was used and the individual fibrils were indistinct. Color changes to a red appearance fibers stained with Masson stain were not a reliable indicator.

Fat:

Adipocytes were never involved in the early stages, although vessels in the subcutaneous fat were often damaged. It is possible that some of the curious granulomas seen in the healing stage may be composed partially of modified lipid.

Vascular changes:

Altered vessels outline the zone of laser damage, and were separated from the damaged area by a narrow "grenz" zone of apparently normal fibers. Vessels (capillaries and small venules)

were distended and congested. Fibrin was often found in larger, deeper venules in the dermis & underlying fat. Focal necrosis was occasionally seen in vessels with muscular walls. This change appeared at random and could not be correlated with the type of laser used.

Abnormalities of arterioles were not common. Pyknosis of nuclei in the muscular coat may occasionally be seen in specimens exhibiting significant laser damage.

Erythrocytes:

Simple congestion of capillaries and venules did occur, but was not a precondition for the more significant changes observed within the damaged vessels. Frequently the vessels contained amorphous, brightly eosinophilic material which may be hemoglobin released from lysed cells. Erythrocyte membranes were, to varying degrees, still evident. Extravasation of erythrocytes was not common.

Inflammation:

In the acute phase polymorphonuclear neutrophils appeared to play a rather minor role, considering the degree of damage, although they may be seen in small numbers. Lymphoid cell proliferation was not significant. Eosinophils were normally present in pig skin.

<u>Late changes</u> (5,8 and 13 week biopsies)

Ulcers:

Ulcers exhibited a ragged exposed base of condensed and eosinophilic collagen in contrast with denuded dermis (epidermis separated from the dermis at the basement membrane). There was usually an accumulation of polymorphonuclear neutrophils at the border of the ulcer forming a "wall". A band of PMNs at the base of an ulcer is uncommon; perhaps they cannot migrate into the area. Another curious feature of the ulcers is that they were always at the lateral extremity of the damaged area and rarely made up more than about 1/4 of the total surface of the treated area. Furthermore, they were never deep.

Healing epidermis:

Evidence of healing of the epidermis was provided by the appearance of "tongues" of epidermal cells slipping under the damaged epidermis at the borders of the treated area. Although a few of the leading cells in the tongue were often somewhat vacuolated, they otherwise appeared normal. Mitoses were not seen at these late times.

Healed epidermis:

In the early stages the epidermis lacked well formed rete ridges.

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Scar formation:

No unusual patterns or scar formation were discerned. The effects of copper vapor versus argon lasers could not be distinguished.

Granulomas:

Some of the sections showed curious granulomas at the interface between dermis and subcutaneous fat. They were usually accompanied by fibrosis which extended in strands out into the adipose tissue. The granulomas were made up of lymphoid cells & giant cells, often multinucleated. They surround spaces which may represent liquefied fat. It is probable that these granulomas were late manifestations of laser damage to subcutaneous tissue.

Appendages:

In older lesions (8 and 13 week biopsies), no appendages were evident in the scars.

Effect of Exposure Time on Skin Damage

Based on microscopic observations, the threshold for argon laser damage began abruptly in the epidermis at approximately 5 seconds (about 20 J/cm^2). With longer exposure times, dermal and vascular changes gradually became apparent. Microscopic changes in the copper vapor laser experiments followed a similar pattern but with a threshold time between 5 & 10 seconds. The histologic findings agreed rather well with the visual inspection of the

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skin (Table 1).

Effect of treatments on wound healing

Blood levels of vitamin E after topical and intramuscular treatments are given in Table 2. Blood levels were not significantly different after topical application or intramuscular injections.

A statistical comparison of histological parameters (see Methods Section) between control and treatment sites did not reveal any significant differences at the various time points. Histological values (see Methods Section) for "nuclei", "epidermal cytoplasm", "connective tissue fibers", "basement membrane zone", "fibers", "vessels", and "inflammatory cells" returned quickly to normal after 2 days, preventing discrimination of treatments. Values for "depth of injury", "healing epidermis", and "scar" were too variable to detect an influence of treatments.

Table 3 provides mean values of wound healing time, as defined by loss of primary eschar, for the three exposure levels in control and treatment groups. Healing time was nearly the same for the three dose levels and does not reflect the degree of damage. The increase in damage with dose can be seen in the histological parameters "depth of injury" (Figs 5-6).

The low dose exposures did not produce a sufficient injury to discriminate treatment effects (Table 3), and it was difficult

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to distinguish between eschar and discoloration of the skin. Only dressing after copper vapor laser exposure produced a significant improvement in wound healing time. At the medium dose (Table 1) for both lasers, dressing and intramuscular vitamin E caused a significant decrease in wound healing time. Topical vitamin E also resulted in a decrease in wound healing time after laser exposure, but the decrease was significant only for the copper vapor laser. At the high laser doses, the extent of skin damage may have overwhelmed treatment effects, with only the dressing showing an effect after copper vapor laser exposure.

Effect of laser type of wound healing time

The differences in energy at the low dose (Table 1) preclude an exact comparison between healing times after copper vapor and argon laser exposures. Additionally, the exposure areas for the argon laser were of necessity smaller than those for the Cu vapor laser so that the radiant exposures (Table 1) could be kept nearly the same for the medium and high dose groups. Since wounds heal from the perimeter, the argon exposure sites might be expected to heal faster. Only the wounds following the low dose control and vitamin E (im) argon exposures healed faster than their copper vapor counterparts (Table 3).

DISCUSSION

The copper vapor laser has recently been introduced for

industrial applications requiring high power output. Few laser studies with the pulsed copper vapor laser or argon laser have been performed with a relevant animal model such as the pig. Because skin is the largest organ of the human body directly accessible to laser radiation, the risk of accidental or potentially deliberate exposure is significant, even though damage to the eye may occur at much lower radiation exposure levels.

In this study, wound dressing (Op-site) or intramuscular vitamin E decreased mean wound healing time by approximately 1 week after medium laser exposures. It has been shown that wound dressing can contribute to accelerated healing of burn wounds in general[10] and of CO_2 laser burns in rats[11]. The main advantages of an impermeable or semi-permeable cover to a wound are protection of the injured site from excessive fluid loss and protection of the wound from exogenous pathogens. Vitamin E has been shown to protect cells against injury[12]. Specifically, it was proposed that this vitamin acts as a scavenger of free radicals[13,14]. Following laser irradiation, free radicals have been demonstrated in some biological materials[15,16].

Vitamin E was shown to penetrate the skin and to be absorbed into the systemic circulation[17]. Twenty-four hours following topical application, the skin contained the highest level. In this study, blood levels of vitamin E after a single topical application of vitamin E were comparable to those obtained after

multiple intramuscular injections. Although topical vitamin E generally reduced mean wound healing time relative to controls, the effect was significant only after medium copper vapor laser exposure.

The threshold level for minimal reaction produced on human skin by the argon laser ranged from 4.0 to 8.2 J/cm^2 for Caucasian skin and 4.5 to 6.0 J/cm^2 for Black skin[18]. The fifty percent probability for producing a minimal reaction on the skin was found to decrease with estimated or measured absorption of the skin at the wavelength of 500 nm. Tan and coworkers[19] reported that yellow (577 nm) wavelength laser irradiation of port-wine stains resulted in less damage to the epidermis as compared to argon (514 nm) or CO_2 (10,600 nm). The 577 nm wavelength was chosen to maximize absorption by hemoglobin and minimize absorption by epidermal melanin. The unpigmented epidermis of the Yorkshire pig skin should not be immediately damaged by exposure to either the argon or copper vapor laser. In vitro and in vivo pig skin permeability studies could not detect a difference in the percutaneous absorption of N,N-diethyl-m-toluamide between argon laser exposed and control skin samples[20].

The copper vapor laser used in this study is a pulsed laser with a frequency of 10k hz, while the argon laser is continuous wave. Approximately one third of the copper vapor laser's energy came from the 578 nm wavelength, while the remainder (511 nm) was close to the argon laser output at 514 nm. The wavelength

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differences should not affect the biological response of unpigmented skin. The waveform differences had little effect on wound healing times (Table 3). At the low dose copper vapor laser exposures, a trend for greater depth of injury was observed as compared to corresponding results for the Argon laser (Figs 5,6). However, the higher radiant exposure of the copper vapor laser (Table 1) probably accounted for this observation.

This study was designed to collect basic data on the effects of argon and copper vapor lasers on pig skin, and to follow the healing process of the injury caused by these lasers. Other factors which may influence the injury such as hyperemic (flushed) skin and skin color etc. will require further investigation.

CONCLUSIONS

Argon and copper vapor laser exposures to pig skin resulted in wounds with similar healing times. Wound dressing and intramuscular vitamin E treatment significantly decreased wound healing time by approximately 1 week after medium laser exposures.

Table 1. Exposure time and radiant exposure (J/cm^2) for the induction of skin damage to a selected degree.

Laser	Minimal erythema[a]	Vascular stasis[b]
Cu Vapor[c]	10 sec (35 $\pm$ 2 J/cm^2)	40 sec (138 $\pm$ 9 J/cm^2)
Argon[c]	6 sec (22.4 $\pm$ 0.1 J/cm^2)	35 sec (129 $\pm$ 1 J/cm^2)

[a]Exposure that caused light erythema for at least 24 hours

[b]Exposure that caused a white burn with stasis, without charring the epidermis

[c]The intermediate copper vapor exposure (20 sec) resulted in a radiant exposure of approximately 70 J/cm^2 to the skin; for the intermediate argon laser exposures (15 sec), the radiant exposure was approximately 55 J/cm^2

Table 2. Plasma levels of Vitamin E following topical and
intramuscular injection.

Administration	Vitamin E Concentration[a] (mg/100 ml plasma)	N
Topical	0.30 $\pm$ 0.11	5
Intramuscular	0.24 $\pm$ 0.16	4

[a]Mean $\pm$ 1 SD

Table 3. The influence of vitamin E pretreatment or wound dressing on healing time following laser injury to pig skin.

Laser	Treatment	Mean Healing Time in Weeks[a]		
		Dose[b]:low	medium	high
Cu Vapor	Control	3.5 ± 1.0	4.1 ± 1.5	3.9 ± 1.0
Cu Vapor	Dressing	2.7 ± 1.0[c]	2.8 ± 1.3[c]	3.0 ± 1.0[c]
Cu Vapor	Vit E (im)	3.2 ± 1.4	3.2 ± 1.0[c]	3.8 ± 1.5
Cu Vapor	Vit E (top)	3.0 ± 0.9	3.3 ± 1.1[c]	3.2 ± 0.9
Argon[d]	Control	2.8 ± 0.9	3.9 ± 1.0	3.7 ± 1.1
Argon	Dressing	2.5 ± 1.0	2.9 ± 1.2[c]	3.1 ± 1.3
Argon	Vit E (im)	2.3 ± 0.9	3.0 ± 1.1[c]	3.5 ± 1.2
Argon	Vit E (top)	2.9 ± 1.1	3.4 ± 1.4	3.0 ± 1.4

[a] Mean healing time ± 1 SD as measured by loss of primary eschar after low, medium, and high dose laser exposures

[b] Low dose (10 second copper vapor and 6 second argon laser exposures) produced minimal erythema; medium dose (20 second copper vapor and 15 second argon laser exposures) produced intermediate effects between low and high dose; high dose (40 second copper vapor and 35 second argon laser exposures) resulted in vascular stasis. See Table 1 for the corresponding radiant exposures.

[c] Significantly different ($p < 0.05$) from respective controls

[d] Copper vapor vs argon laser healing times were found significantly different ($p < 0.05$) only for the low dose control and low dose vitamin E (im) exposures

ACKNOWLEDGEMENT

FIGURE LEGENDS

Figure 1. Diagram of laser configuration for conducting cutaneous exposures. The helium-neon lasers were used to maintain the exposure plane.

Figure 2. Photomicrograph of non-irradiated pig skin.

Figure 3. Photomicrograph of pig skin following 20 second irradiation from the argon laser.

Figure 4. Photomicrograph of pig skin following 20 second irradiation from the copper vapor laser.

Figure 5. Depth of injury (see "Materials and Methods" for description) for biopsies at the various time points after argon laser exposure. Clear columns represent the low dose, hatched columns represent the intermediate dose, and cross-hatched columns represent the high dose. Asterisks indicate significant differences ($p < 0.05$) between the designated columns and the corresponding low dose exposures.

Figure 6. Depth of injury for biopsies at various time points after copper vapor laser exposure. Clear columns represent the low dose, hatched columns represent the intermediate dose, and cross-hatched columns represent the high dose.
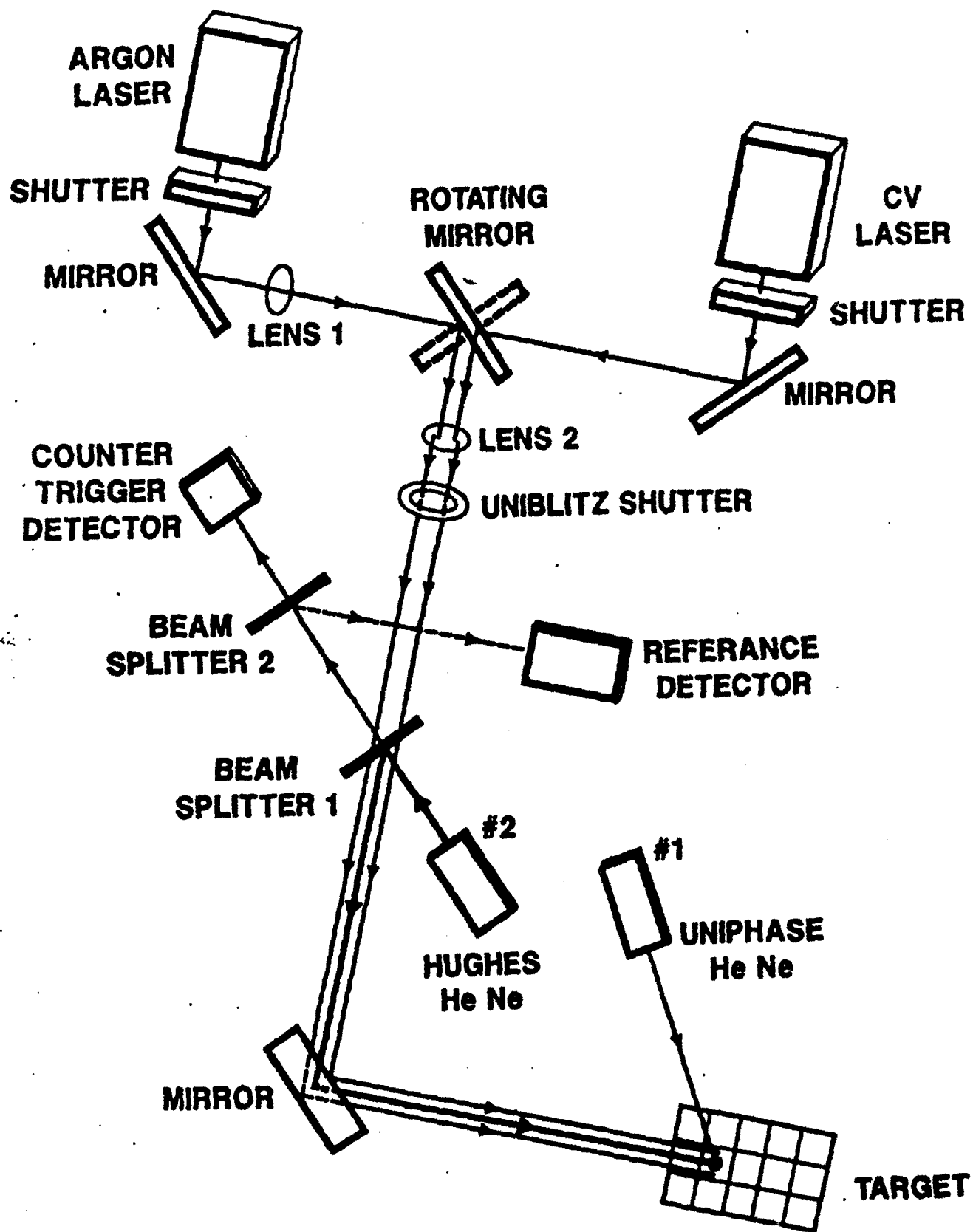
ARGON LASER

SHUTTER
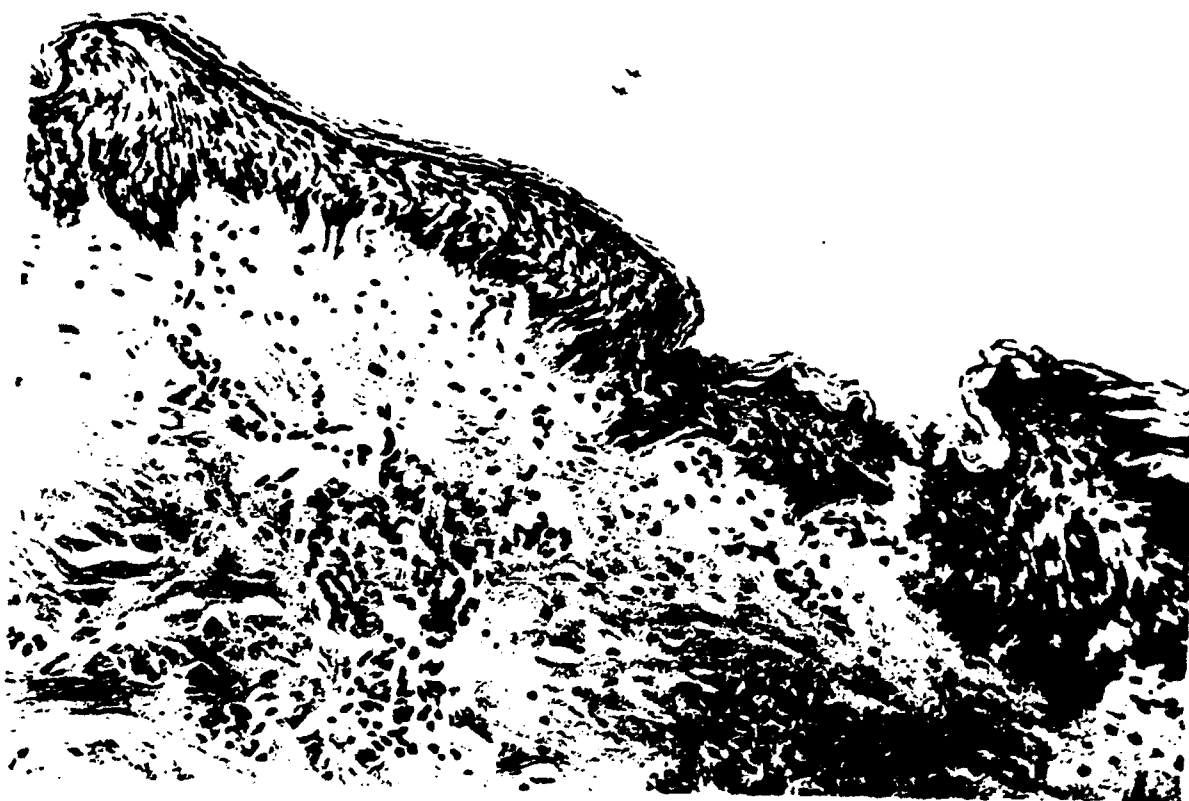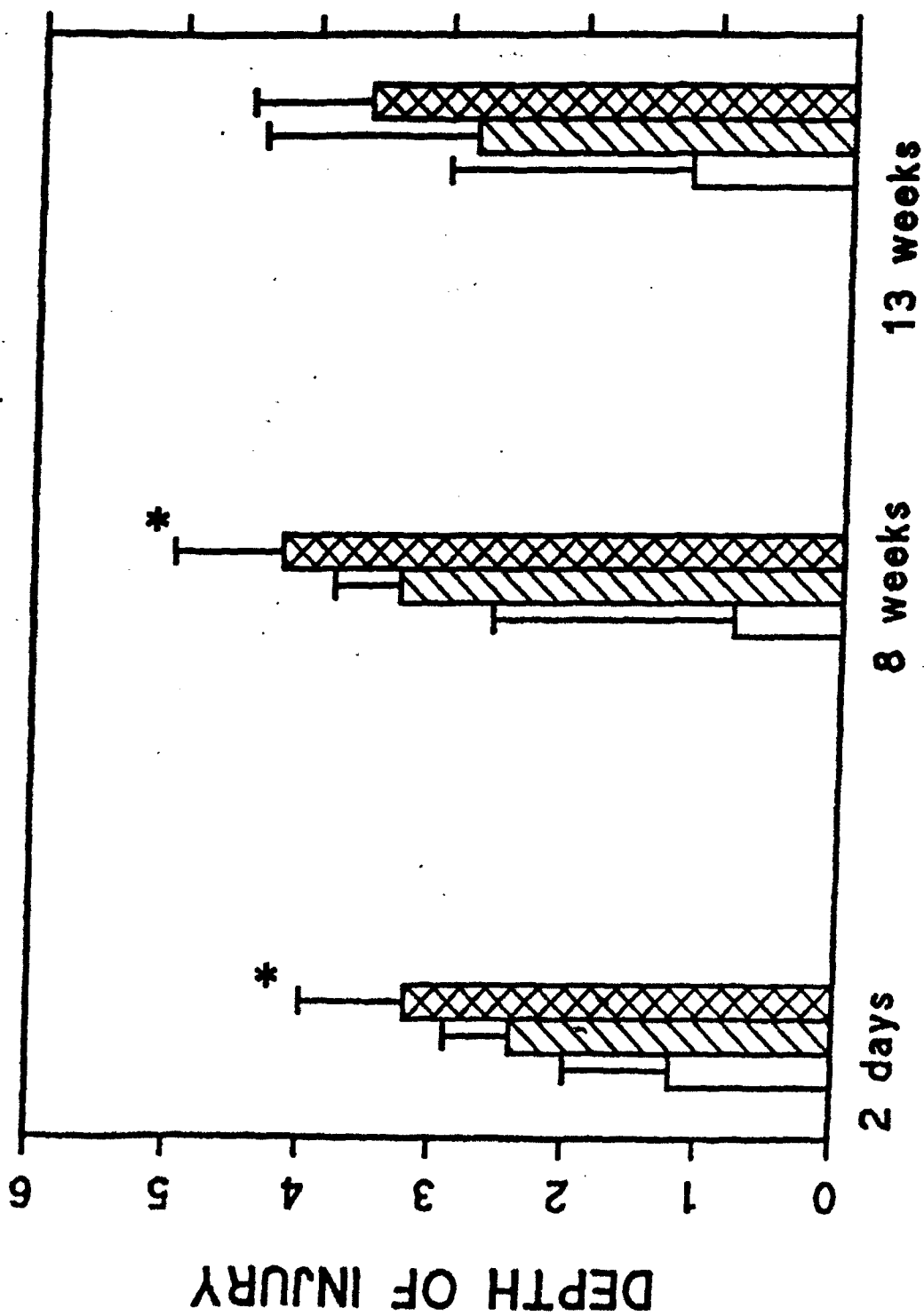
MIRROR

LENS 1

ROTATING MIRROR

CV LASER

SHUTTER

MIRROR

LENS 2

UNIBLITZ SHUTTER

COUNTER TRIGGER DETECTOR

BEAM SPLITTER 2

REFERANCE DETECTOR

BEAM SPLITTER 1

#2

#1

HUGHES He Ne

UNIPHASE He Ne

MIRROR

TARGET

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

DEPTH OF INJURY

TIME AFTER AR LASER EXPOSURE (CONTROLS)

2 days   8 weeks   13 weeks

TIME AFTER CU LASER EXPOSURE (CONTROLS)
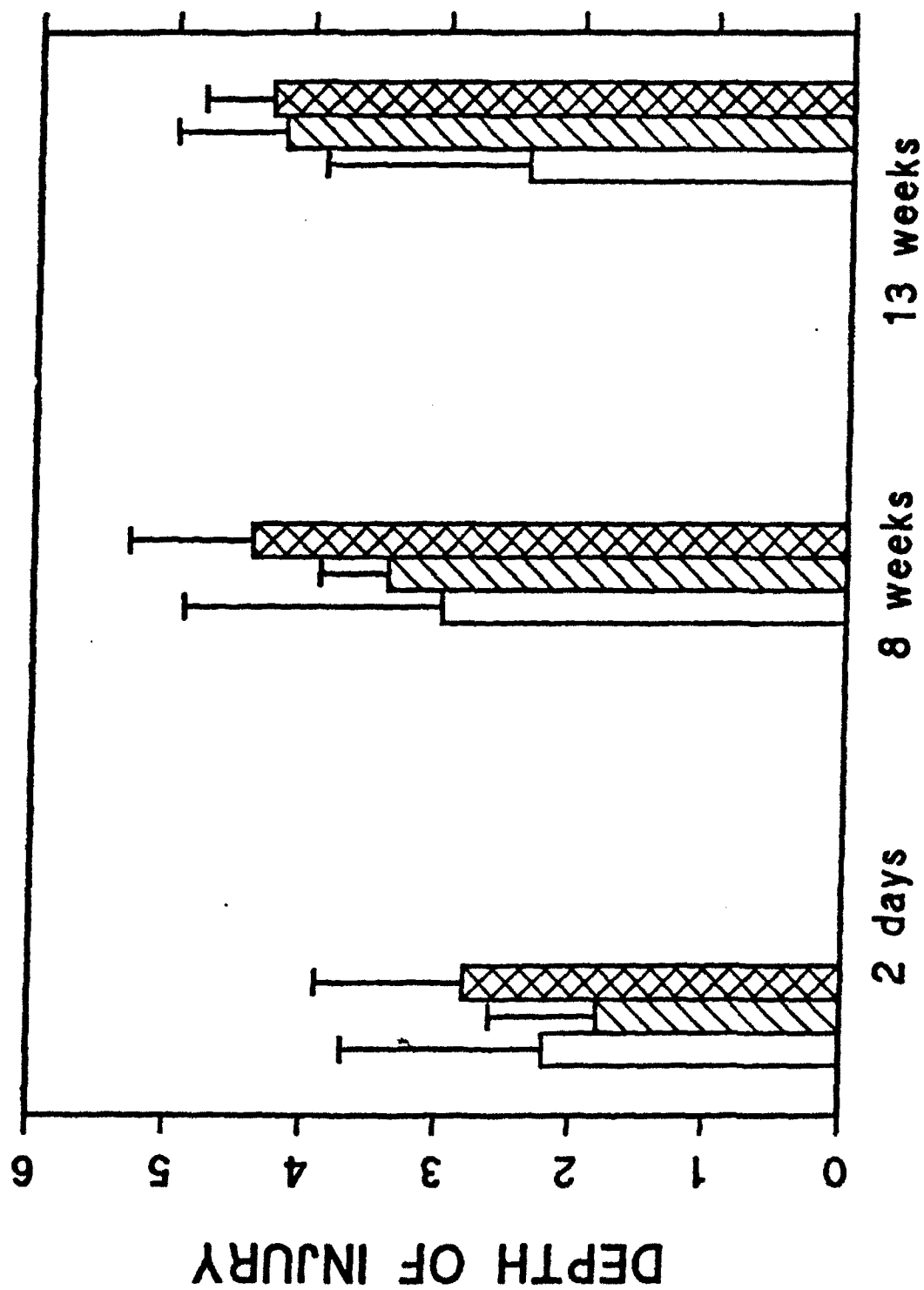
DEPTH OF INJURY

2 days   8 weeks   13 weeks

FIGURE 6

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