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CORRELATION BETWEEN THE GROSS AND MICROSCOPIC APPEARANCE OF CO_2 LASER INDUCED PORCINE SKIN BURNS

(Interim Report)

by

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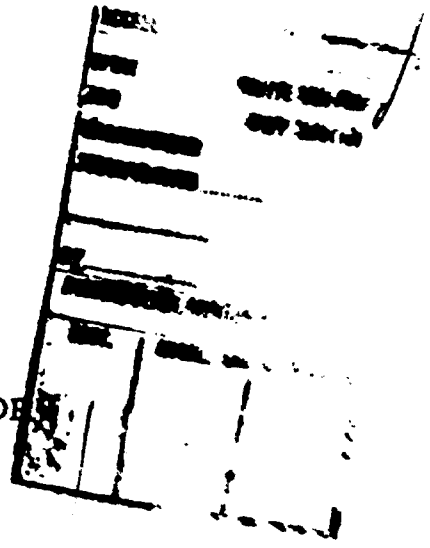

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Cutaneous Burns Induced by Laser Radiation
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ABSTRACT

CORRELATION BETWEEN THE GROSS AND MICROSCOPIC APPEARANCE OF CO_2 LASER INDUCED PORCINE SKIN BURNS

OBJECTIVE

To determine the correlation between the gross and microscopic changes in porcine skin exposed to varying times and power densities of CO_2 laser radiation.

METHODS

Routine H & E stained and histochemically prepared sections of porcine skin were examined microscopically for evidence of burn damage. The microscopic damage was initially compared to the macroscopic surface changes. The data was further categorized so that an indication of the relative power density (high, medium, low) used in producing the burns was included.

RESULTS AND CONCLUSIONS

As the burns increased in severity macroscopically, there was a commensurate increase in microscopic evidence of tissue damage. There was, however, some indication that burns which presented similar surface appearances but had been produced by high intensity CO_2 laser power densities, might have more actual tissue damage than those produced by a lower power density.

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INTRODUCTION

In an earlier report, threshold lesions produced in porcine skin by the CO_2 laser were studied (1). Evaluation of the burns in this study required only a visual determination of whether or not a particular power density and exposure interval combination produced a burn. This system was expanded for more severe burns so that an indication of the severity was included in the criteria for evaluation (2). The grading system devised was based on visible gradations in the surface appearance of cutaneous burns. However, the use of such a system in analyzing dose-response relationships presupposes that the correlation between surface appearance and severity remains constant regardless of exposure conditions. Hinshaw et al (3) found, for burns produced by radiation from a carbon arc, that this relationship was constant within the exposure times of .3 to 30 seconds and for burns ranging from erythema to patchy white burns. Because of the marked difference in the absorption by skin of carbon arc and CO_2 laser radiation, it was necessary to determine what relationship exists between surface appearance and tissue damage for lesions induced by CO_2 laser radiation. Therefore, this study was undertaken to determine if it is in fact valid to assume that the surface appearance of a CO_2 laser induced burn is an accurate indication of its severity. If so, an arbitrary, but meaningful, system for visual evaluation of cutaneous burns would then be established.

MATERIALS AND METHODS

The skin biopsies of cutaneous burns in this study were taken from the animals used to determine dose-response data for lesions induced by

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[1] Brownell, A. S., W. H. Parr, D. K. Hysell, and R. S. Dedrick. Threshold lesions induced in porcine skin by CO_2 laser radiation. USAMRL Report No. 732, 1967 (DDC AD No. 659347).

[2] Brownell, A. S., W. H. Parr, D. K. Hysell, and R. S. Dedrick. CO_2 laser induced skin lesions. USAMRL Report No. 769, 1968.

[3] Hinshaw, J. R., H. W. Bales, and H. E. Pearse. The relationship between surface grade and depth of damage of burns produced by radiant thermal energy. University of Rochester Atomic Energy Project Report UR-440, 1956.

CO_2 laser radiation. Equipment and procedures were identical up to the time of burn evaluation 24 hours post-lasing and have been previously reported (1).

The visual system for gross evaluation of the cutaneous burns was based upon subtle differences in the color, intensity and uniformity of the surface appearance of the lesion (2). Six categories were included: 0, no burning; 1-1, immediate erythema post-lasing which faded by 24 hours; 1-2, mild persistent erythema; 1-3, moderate to severe persistent erythema; 1-4, severe erythema with a bluish cast due to vesicle formation; 2-1, whitish coagulated burn.

Immediately after visual evaluation of the 24 hour burns, preselected pigs were terminated by an overdose of pentobarbital sodium injected intravenously via the anterior vena cava. Full thickness skin biopsies of individual burns were excised and bisected with one-half placed in 10% formalin fixative prior to processing for paraffin embedded histologic sections, while the other half was quick frozen and stored in liquid nitrogen (-193°C) for subsequent histochemical studies.

Histochemical evaluation of cutaneous enzymes in burned porcine skin was undertaken to facilitate microscopic determination of the depth of damage. As a result of pilot studies into the simplicity of technical preparation and ease of microscopic interpretation of a number of enzyme determinations (lactic dehydrogenase, isocitric dehydrogenase, succinic dehydrogenase, glucose 6 - phosphate dehydrogenase, nicotinamide adenine dinucleotide, and nicotinamide adenine dinucleotide phosphate diaphorase), only nicotinamide adenine dinucleotide-diaphorase (NAD-ase) determinations were used in this study. The histochemical procedure utilized frozen skin sections cut at 6-8 μ on an International cryostat at -20°C, affixed to clean glass slides and dried in a constant temperature oven at 37°C. They were then stained according to the method of Nachlas, Walker and Seligman (4). The sites of NAD-ase were demonstrated as deposits of blue formazan. The formalin fixed tissues were embedded in paraffin, sectioned, stained with Hematoxylin and Eosin (H & E) and examined for histologic evidence of damage.

The skin sections from both techniques (H & E and NAD-ase) were examined for evidence of burn damage by use of conventional light

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[4]Nachlas, M. M., D. G. Walker, and A. M. Seligman. A histochemical method for the demonstration of diphosphopyridine nucleotide diaphor... J. Biophys. Biochem. Cytol. 4: 29-38, 1958.

microscopy and placed into one of four categories: I, no detectable change; II, partial thickness epithelial damage; III, full thickness epithelial damage; IV, dermal damage.

Results from the macroscopic and microscopic observations were categorized, tabulated and examined for correlation between the two.

RESULTS AND DISCUSSION

Microscopic examination of the porcine skin biopsies showed lesions varying in severity from no detectable changes to dermal damage. There was also variation in the uniformity of the burn area. Generally, the partial thickness epithelial burns were quite uneven with areas of superficial epithelium remaining intact. Some full thickness epithelial burns were uneven and had scattered pockets of viable epithelium, while others were quite uniform. The dermal burns tended to be uniform in involvement.

Examples of the H & E stained skin sections are shown in Figures 1 - 4 (pages 6 - 9). Microscopically, the lateral margins of the epithelial burns were quite well delineated. The primary epidermal changes noted were those of nuclear pyknosis, eosinophilia of the cytoplasm and intracellular vacuolation. This vacuolation was particularly prominent in the more basally located epithelial cells. There frequently were vesicles filled with a fibrinous to fibrinopurulent exudate located at the base of the epithelial burns. The obvious dermal changes were extreme congestion of the underlying dermal capillaries and hypercellularity due to a leucocytic infiltrate of the dermis. In the more severe burns, there was morphologic evidence of erythrocytic aggregation and thrombus formation within the capillaries. There were, in some burns, scattered areas in the superficial dermis with coagulated collagen bundles which assumed a more basophilic staining appearance. With the more severe burns, examination of the root sheaths of the dermal hair follicles sometimes showed changes similar to those seen in the damaged epithelial cells. In many cases, the H & E stained skin sections did not demonstrate enough specific alterations in the appearance of the dermis to determine presence of and/or the extent of a dermal burn.

The histochemical preparation of frozen tissue sections permitted delineation of not only the lateral margins of the burns but also the depth of damage. In normal skin, the sites of NAD-diaphorase activity are concentrated in the epidermis; however, there are also scattered sites of activity within the dermis (Fig. 5, page 10). The lateral margins

and depth of burn damage were readily visualized by virtue of the destruction of enzymatic activity and therefore reduction or complete loss of staining intensity by the damaged tissue (Figs. 6 - 8, pages 11 - 13).

The preparation of frozen sections for histochemical evaluation was rendered more difficult, particularly in the more severe burns, because of a tendency for the damaged tissue to shear off from the underlying undamaged tissue. Cellular detail was also poorer in the histochemistry sections than in the formalin fixed H & E stained tissue. Since each technique tended to counter the other's deficiencies, it was possible to arrive at a more complete microscopic evaluation of individual burns by examining both the formalin fixed and the frozen skin sections.

The comparisons between the macroscopic and microscopic changes for the porcine skin burns are shown in Table 1 (page 16). There appears to be a definite correlation between the macroscopic appearance of a burn and the microscopic evidence of tissue damage. This was apparent particularly in those burns which microscopically had dermal damage (category IV). Of those burns classified as 2-1 macroscopically, 80% had dermal damage. In the other categories, 37% of the 1-4 burns, 18% of the 1-3, 6% of the 1-2, and none of the 1-1 burns had microscopic evidence of dermal damage.

The distribution of histologic changes for the various macroscopic categories is graphically presented in Figure 9 (page 14). In the category of microscopic damage which occurred most frequently for the 1-1 burns, there was no change; for the 1-2, 1-3, and 1-4 burns, it was full thickness epithelial; and for the 2-1 burns, it was dermal damage.

After examining the histologic appearance of the burns, it was apparent that the full thickness epithelial burns could be subdivided into two categories, based on the distribution of the lesion: spotty full thickness (III-A) and uniform full thickness (III-B). The category of microscopic damage, therefore, occurring most frequently for the 1-2 burns, is spotty full thickness, while the 1-3 and 1-4 burns are uniform full thickness (Fig. 10, page 15). In view of the multimoded beam pattern produced by the laser in these experiments (1), the spotty distribution of some burns may be related to the beam pattern rather than some intrinsic factor of the skin. This question can only be answered by examining skin exposed to a laser beam with a relatively uniform power density cross section.

In order to determine if there was a difference in tissue damage in macroscopically alike burns resulting from differing power densities, the burns were further categorized as having resulted from exposure to high, medium, or low intensity CO_2 lasing (Table 2, page 17). These categories were arbitrary and relative to the power densities used in this experiment. The high category included power densities from 13.6 to 7.6 watts/cm^2, medium from 4.7 to 2.5 watts/cm^2 and low from 1.7 to 0.67 watts/cm^2. The microscopic categories used were those first mentioned in that the full thickness epithelial burns (III) were not further broken into uniform (III-B) or spotty involvement (III-A). Examination of the results shows insufficient numbers of biopsies of 1-1 burns to draw any conclusions. Likewise, no trends can be observed in the microscopic classes I and II for the 1-2, 1-3, 1-4, and 2-1 burns. Discussion must, therefore, be limited to the microscopic categories III and IV for the 1-2, 1-3, 1-4, and 2-1 burns. Generally, it would seem that within these macroscopic categories, there may be a tendency for burns induced by the higher power densities to have more severe tissue damage. It should be noted that the differences in many cases are small and because of the low numbers of burns in each category, the significance of the differences is questionable. If the differences are real, then the visual appearance of a burn might not be sufficiently reliable criterion for categorization as to burn severity. Therefore, it would seem appropriate to expand this portion of the experiment so that sufficient data may be accumulated to permit realistic evaluation of the differences. Only then can any definite conclusions be made as to the efficacy of the macroscopic categorization in evaluating burn damage originating from the power densities and exposure intervals used in this experiment.

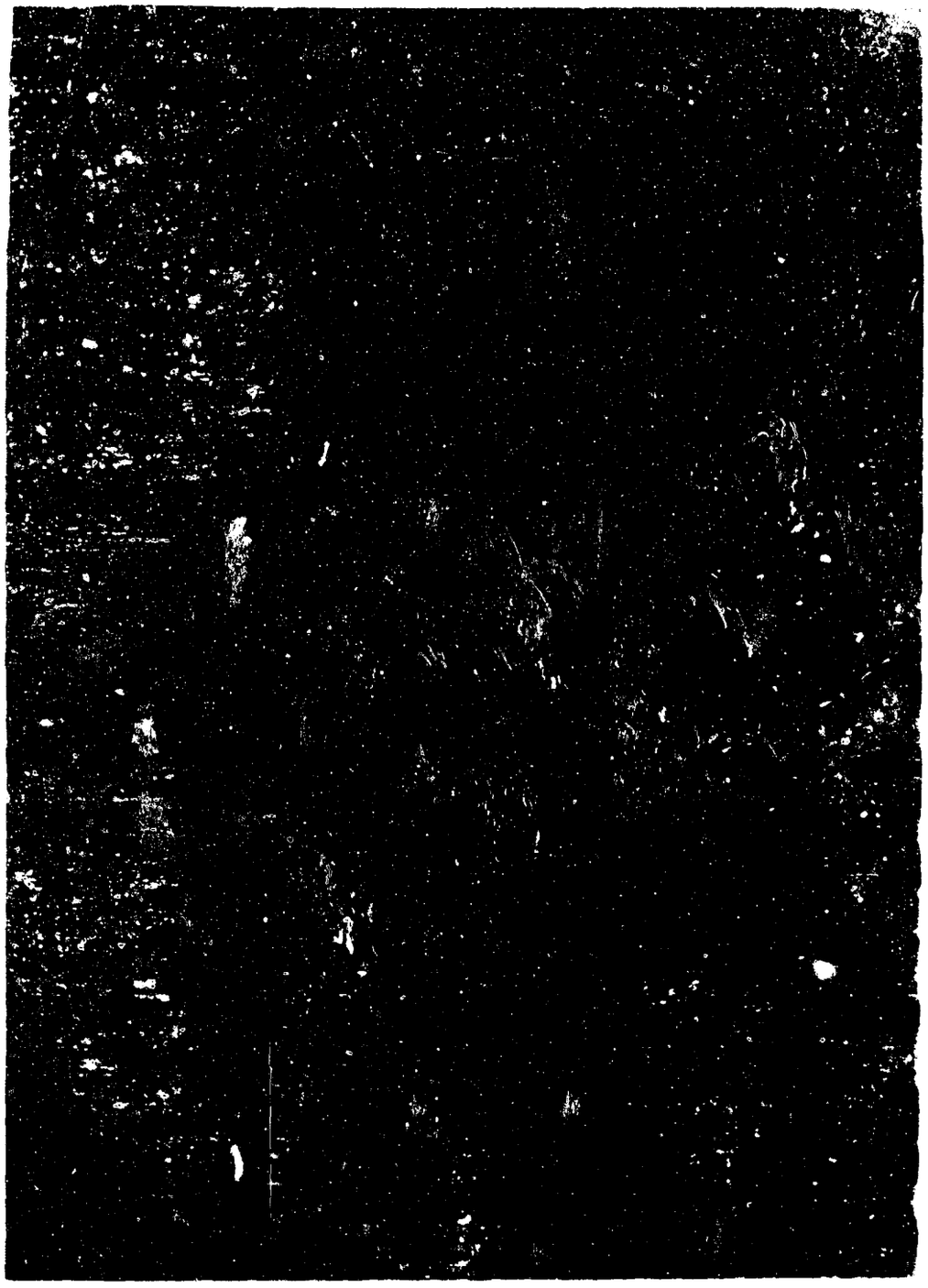

Fig. 1. Normal porcine skin. The separation between the cornified and non-cornified epithelium is artefactual. Hematoxylin & Eosin. X400.

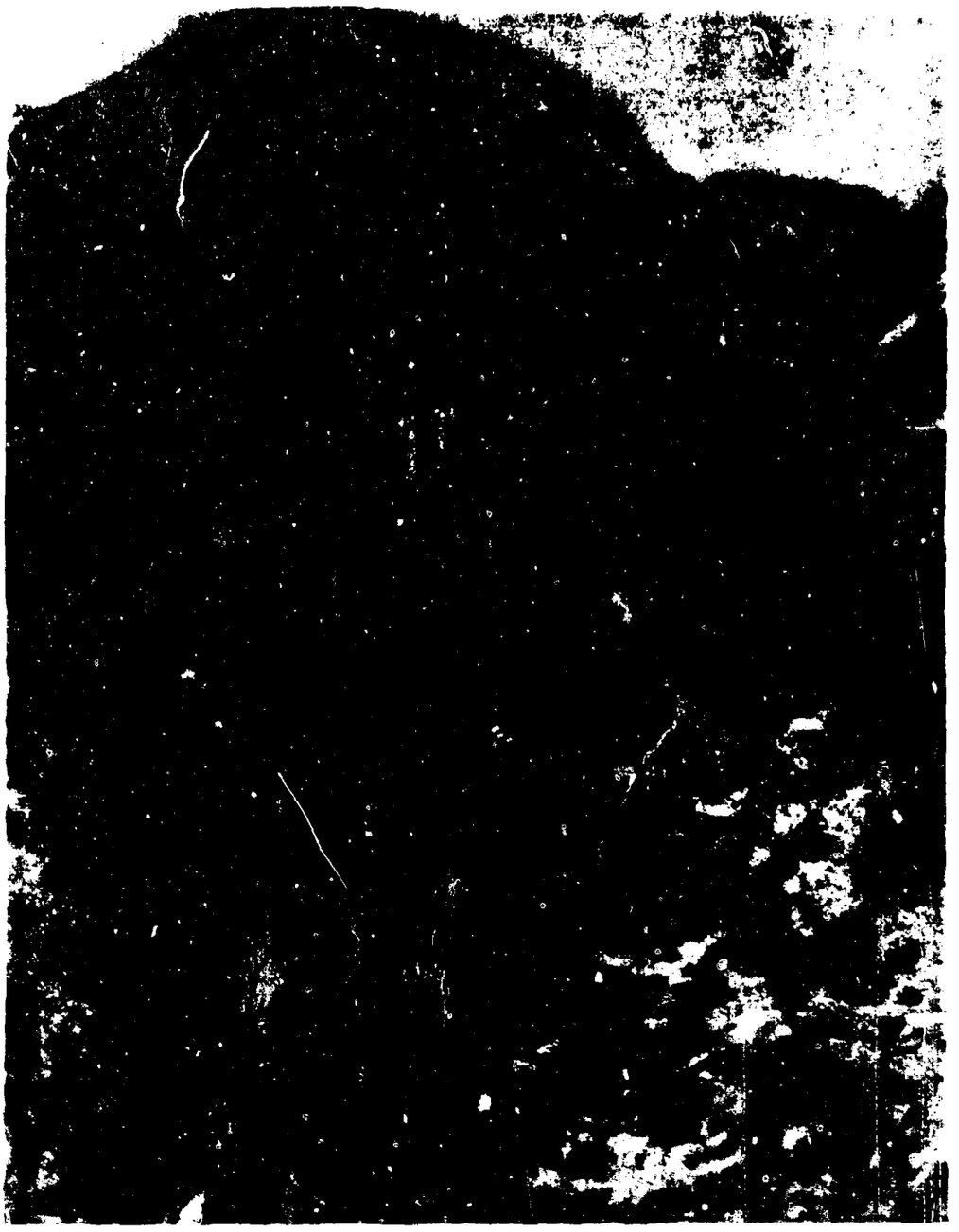

Fig. 2. Partial thickness epithelial burn 24 hours post-lasing. There is a zone of separation between the damaged and the undamaged epithelium. Even this early there has been a reparative response with increased mitosis and a somewhat thickened hyperplastic epithelium. The dermal tissues display a prominent inflammatory cell infiltrate. Hematoxylin & Eosin. X400.

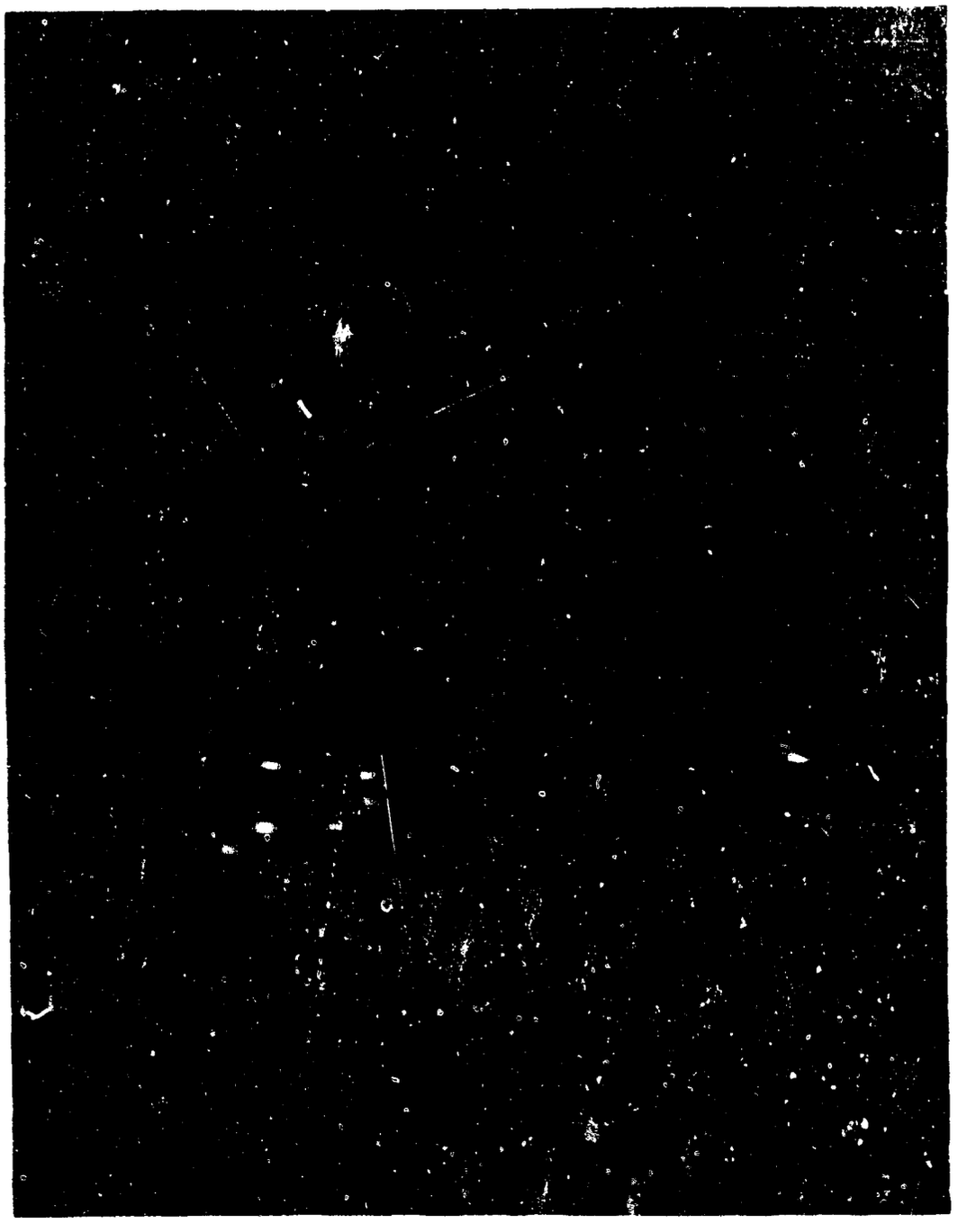

Fig. 3. Full thickness epithelial burn 24 hours post-lasing. The zone of separation between the damaged epithelium and undamaged dermis is filled with a fibrinopurulent exudate. There is extensive infiltration of inflammatory cells into the dermis and burned epithelium. The dermal capillaries are severely congested. Hematoxylin & Eosin. X400.

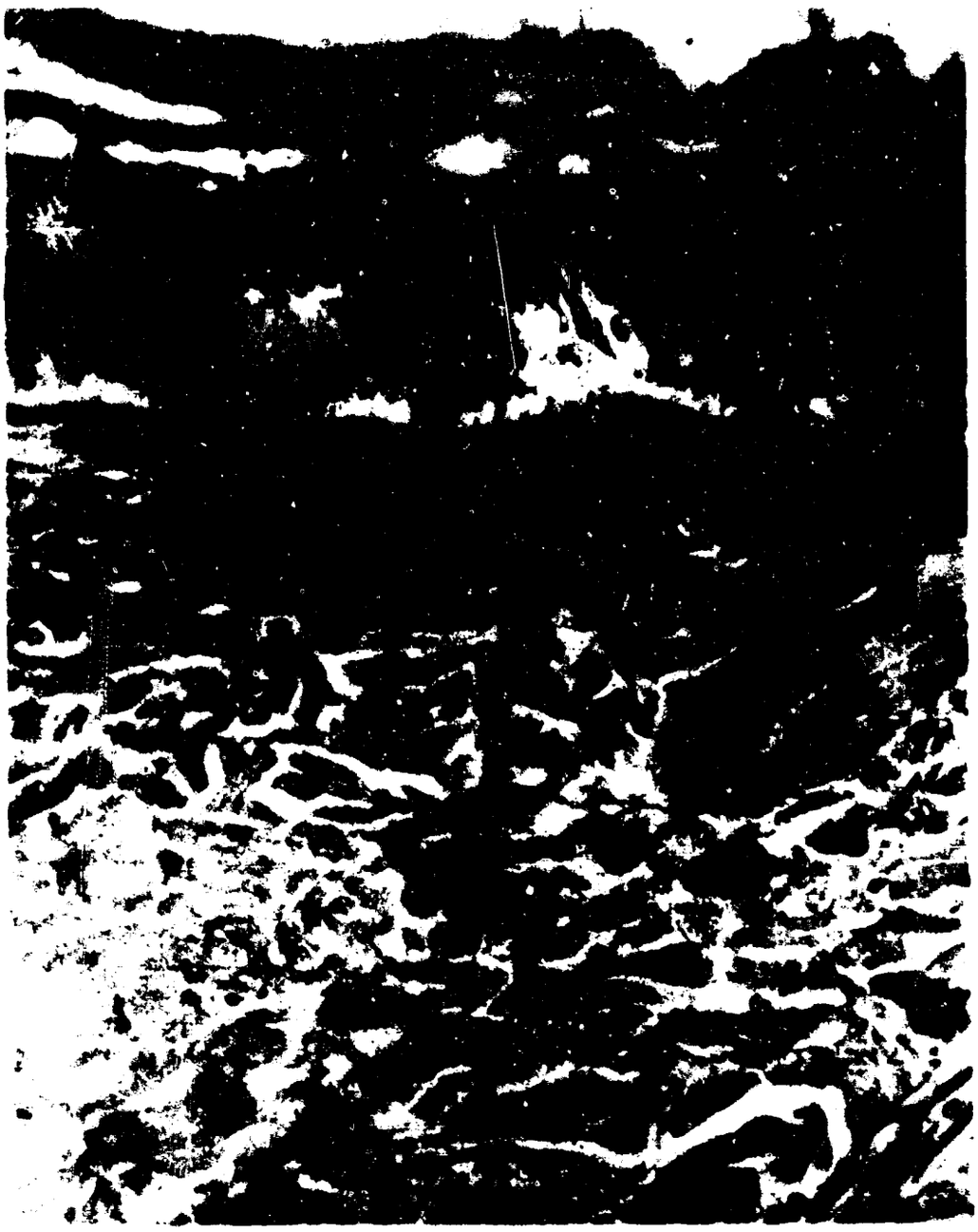

Fig. 4. Dermal burn 24 hours post-lasing. The cells of the deeper layers of epithelium have assumed an almost "picket fence" arrangement. The superficial dermal collagen fibers are denser and have a basophilic staining appearance. The dermal capillaries are congested and, in some cases, thrombi of aggregated erythrocytes are present. Hematoxylin & Eosin. X400.

Fig. 5. Normal porcine skin. Histochemical evaluation for the presence of NAD-diaphorase shows a heavy concentration of the enzyme in the germinal layers of epidermis; however, scattered sites of reactivity are present in the dermis. X400.

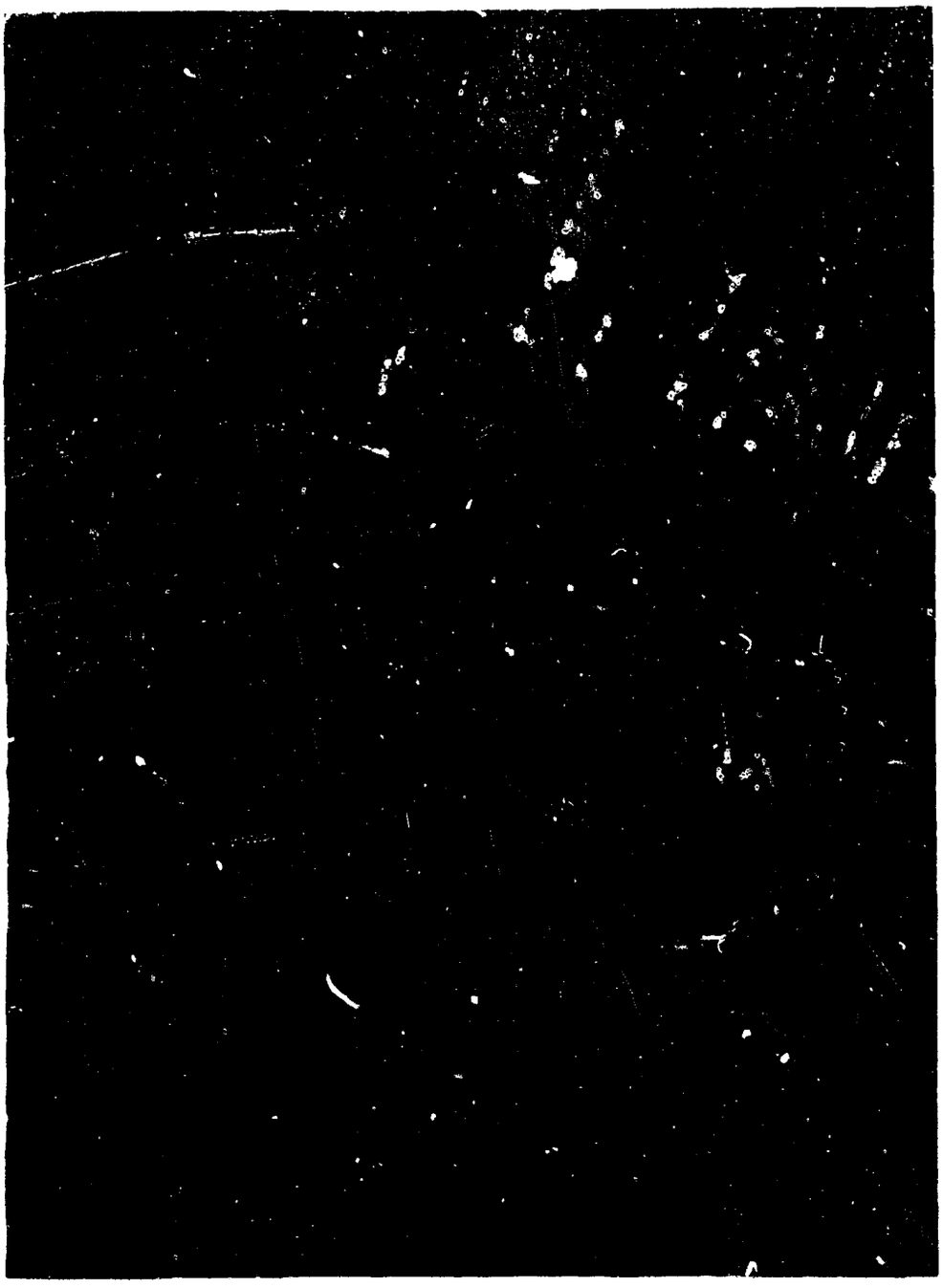

Fig. 6. Partial thickness epithelial burn 24 hours post-lasing. There has been degradation of NAD-diaphorase in the superficial layers of the epidermis as evidenced by the absence of staining. No alteration in enzymatic activity is apparent in the deeper epidermal layers or the dermis. X400.

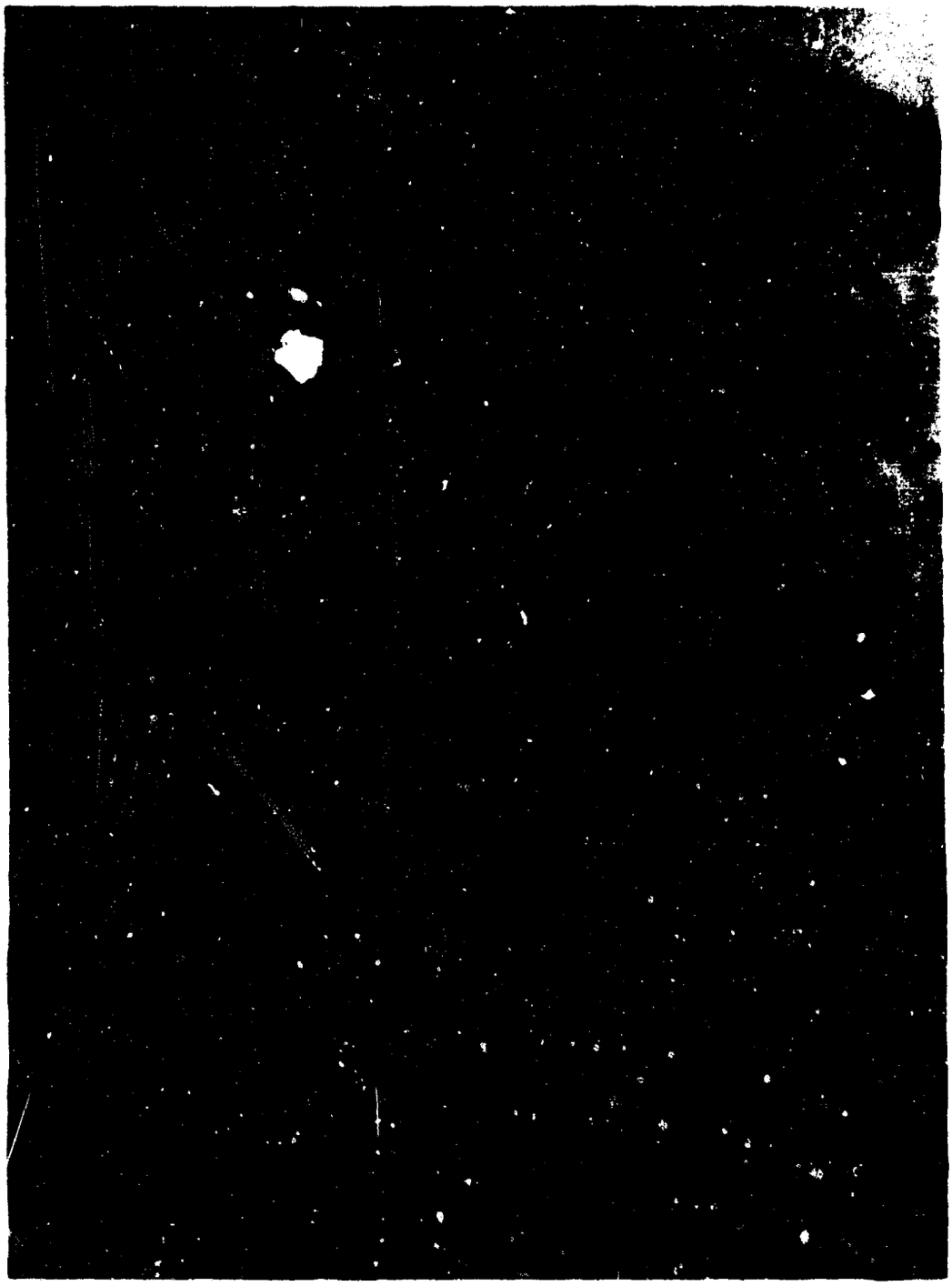

Fig. 7. Full thickness epithelial burn 24 hours post-lasing. Enzymatic activity in the epidermis has been destroyed by the burn. No alteration of enzyme seems apparent in the dermis. X400.

Fig. 8. Dermal burn 24 hours post-lasing. NAD-diaphorase activity is absent in the epidermis and superficial dermis. Burn depth is readily apparent due to the sharp demarcation between damaged and normal dermal enzymatic activity. X400.

Relative Frequency of Microscopic Damage for the Macroscopic Burn Categories

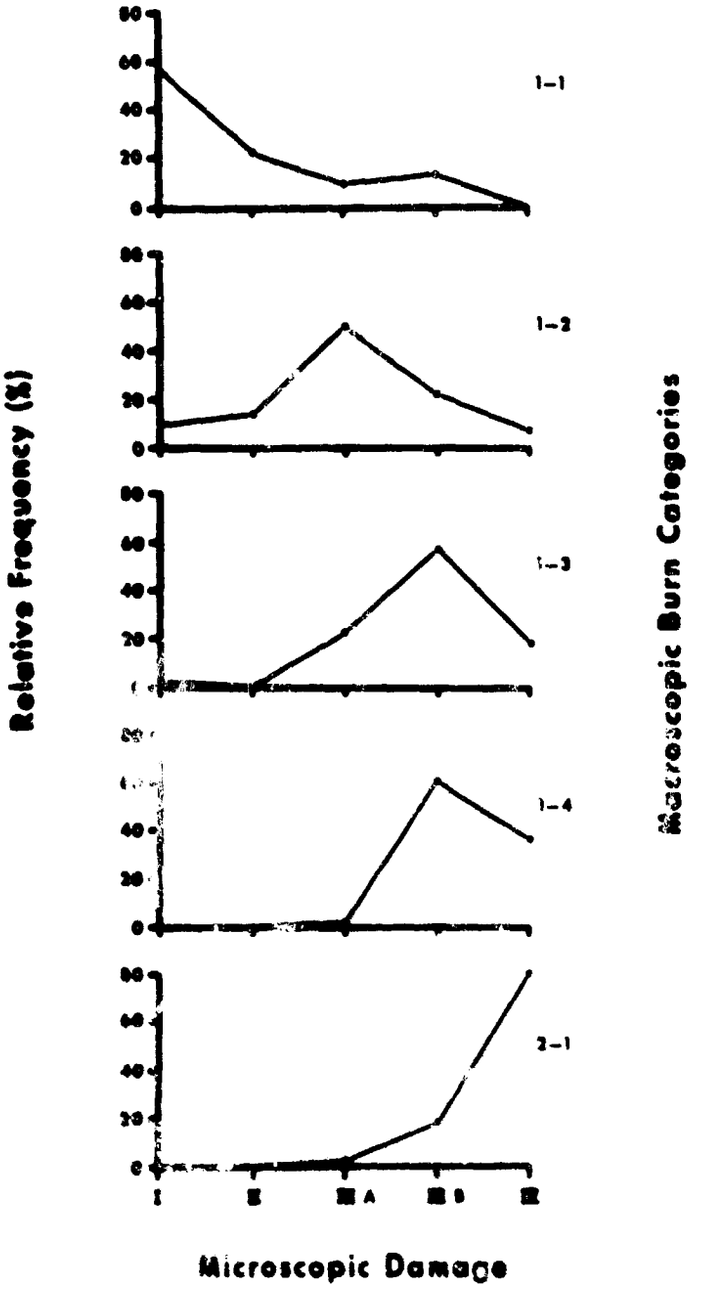

Figure 3

Relative Frequency of Microscopic Damage for the Macroscopic Burn Categories

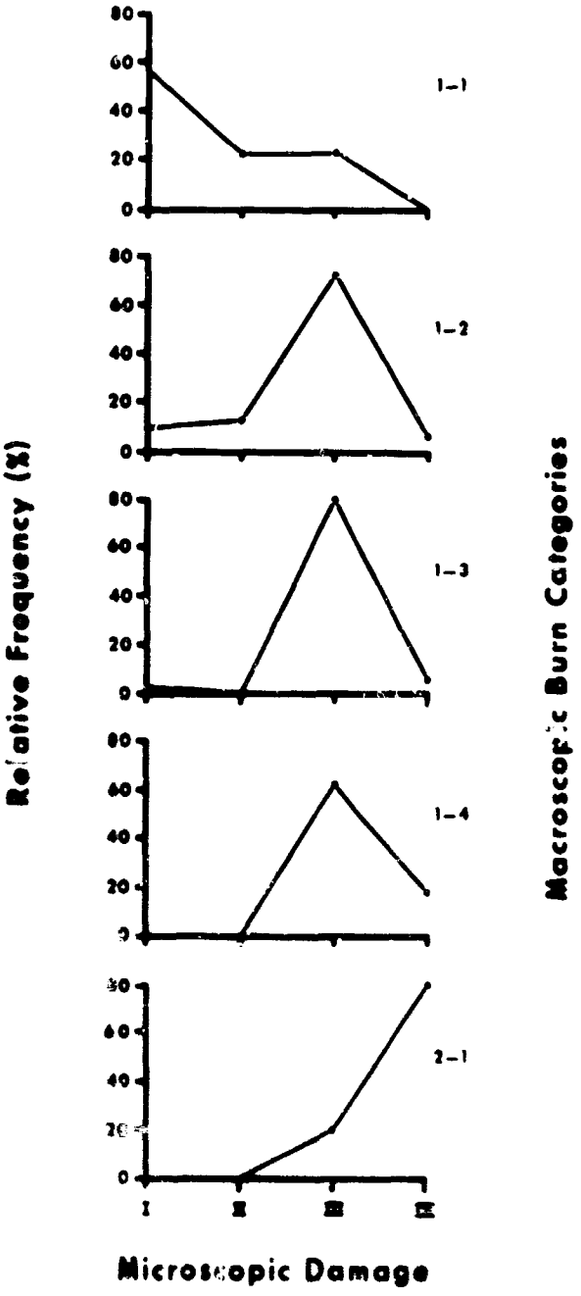

Figure 10

TABLE 1

Distribution of Microscopic Lesions for Macroscopic Categories of Burn Damage

Macroscopic Observations		Microscopic Findings			
Grade	Number	No Change I	Partial Epithelial II	Full Thickness Epithelial III	Dermal IV
1-1	23	56% (13)	22% (5)	22 % (5)	
1-2	77	9% (7)	13% (10)	72% (55)	6% (5)
1-3	44	2% (1)		80% (35)	18% (8)
1-4	41			63% (26)	37% (15)
2-1	50			20% (10)	80% (40)

( ) = Absolute number of lesions.

TABLE

Relationship of Power Density to the Distribution of Microscopic Lesions for the Macroscopic Categories of Burn Damage

Grade	Number	Power Density	No Change I	Partial Epithelial II	Uniform Full Thickness Epithelial III	Dermal IV
1-1	4	Low	75% (3)		25% (1)	
	18	Medium	56% (10)	22% (4)	22% (4)	
	1	High		100% (1)		
1-2	42	Low	10% (4)	10% (4)	75% (32)	5% (2)
	17	Medium	12% (2)	12% (2)	70% (12)	6% (1)
	18	High	6% (1)	22% (4)	61% (11)	11% (2)
1-3	11	Low			82% (9)	18% (2)
	14	Medium	7% (1)		93% (13)	
	19	High			68.1 % (13)	32% (6)
1-4	19	Low			79% (15)	21% (4)
	12	Medium			58% (7)	42% (5)
	10	High			40% (4)	60% (6)
2-1	14	Low			29% (4)	71% (10)
	14	Medium			21% (3)	79% (11)
	22	High			14% (3)	86% (19)

( ) = Absolute number of lesions.

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13. ABSTRACT

The skin of white pigs exposed to CO_2 laser radiation was biopsied and examined for histologic evidence of damage. As the burns increased in severity macroscopically, there was a commensurate increase in microscopic evidence of tissue damage. Limited data suggests that even though burns present similar surface appearances, those produced by high intensity CO_2 laser power densities might have more actual tissue damage than those produced by a lower power density. Insufficient data was available to determine if the differences were significant. (U)

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