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MILLISECOND EXPOSURE OF PORCINE SKIN
TO SIMULATED CO_2 LASER RADIATION

(Final Report)

by

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13. ABSTRACT

To approximate the absorption of CO_2 laser radiant energy by skin and the subsequent distribution of thermal energy in this tissue, the skin was painted with a thin layer of India ink and exposed to ruby laser radiation. The resulting lesions were examined and graded both visually and histologically. The median effective dose for minimal erythema was determined to be 0.8 joules/cm^2 for an exposure time of 3.5 msec. Histologically measured depth of damage was determined for doses up to 2.8 joules/cm^2. It is concluded that the simulated experimental conditions are valid only for doses less than 1.4 joules/cm^2.

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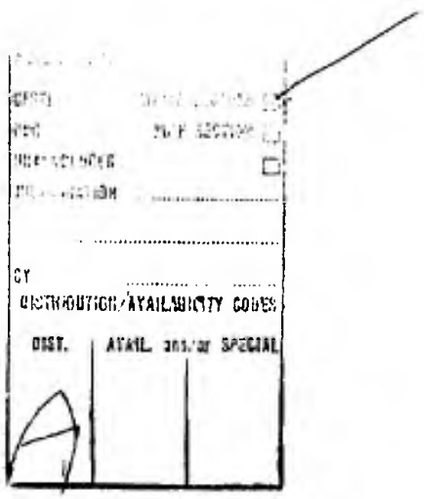

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ABSTRACT

MILLISECOND EXPOSURE OF PORCINE SKIN
TO SIMULATED CO_2 LASER RADIATION

OBJECTIVE

To provide interim threshold values for the induction of porcine cutaneous burns equivalent to a 3.5 msec exposure to high intensity CO_2 laser radiation.

METHODS

To approximate the absorption of CO_2 laser radiant energy by skin and the subsequent distribution of thermal energy in this tissue, the skin was painted with a thin layer of India ink and exposed to ruby laser radiation. The resulting lesions were examined and graded both visually and histologically.

RESULTS AND CONCLUSIONS

The median effective dose for minimal erythema was determined to be 0.8 joules/cm^2 for an exposure time of 3.5 msec. Histologically measured depth of damage was determined for doses up to 2.8 joules/cm^2. It is concluded that the simulated experimental conditions are valid only for doses less than 1.4 joules/cm^2.

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MILLISECOND EXPOSURE OF PORCINE SKIN TO SIMULATED CO_2 LASER RADIATION

INTRODUCTION

Published data by Brownell *et al* (1) give the dose-response relationship for varying degrees of cutaneous burns produced by CO_2 laser radiation within the power density range of 0.7 watts/cm^2 to 13.6 watts/cm^2 and exposure times of 0.2 to 40 sec. The very high power densities currently available with CO_2 lasers indicate a need to extend these studies to shorter exposure times to provide data for a more extensive evaluation of the hazards involved in the military use of these lasers.

In such burn studies, the laser beam requirements ideally are stable beam geometry, high power density, and a reasonably large cross sectional area with uniform power distribution. These characteristics eliminate the complexities introduced by radial heat flow when narrow beams or beams with a Gaussian power distribution are used. Because these requirements could not be met with the available CO_2 laser, an experimental design was devised to simulate the condition of CO_2 laser exposure.

The beam requirements can be easily met with the ruby laser by selecting a small uniform cross sectional area of the beam, expanding it and recollimating the apertured portion of the beam to the desired dimension.

The absorption coefficient of aqueous tissue for CO_2 laser radiation (10.6 μm) is approximately 700 cm^{-1} (2), giving a half value layer of tissue for this radiation of about 10 μm. Therefore, the absorption of CO_2 laser radiation by skin can be considered as essentially surface absorption. The absorption of ruby laser radiation will approximate that of CO_2 laser radiation if the ruby radiation is absorbed on the skin surface by a thin opaque layer of India ink. That is, the layer of ink will act to absorb the ruby laser radiation just as the skin surface absorbs the longer wavelength radiation of the CO_2 beam.

Thus, tentative values for skin burns induced by CO_2 laser radiation can be obtained by the above technique. These values can provide interim guidance for safety standards.

METHODS

The radiation source used was a laser with a 14" x 3/4" ruby rod. The rod and dual flash lamps in a double elliptical cavity were water cooled. The capacitor banks were equipped with pulse forming networks providing a selection of pulse lengths rangin from 0.5 to 5 msec. An output up to 300 joules was available from th laser system.

The output beam of the laser was first directed through a 5/8" circular aperture. The beam then passed through neutral density filters and impinged upon a metal plate with a 1/4" circular aperture. This plate could be positioned so that the aperture selected a portion of the beam with a relatively uniform energy density distribution. The beam was then expanded and recollimated by means of two positive lenses. The selected beam was directed downward by a reflecting prism to impinge upon the target tissue. The beam from a 2 mW He-Ne laser directed axially through the ruby rod served as an aiming light to position the target tissue.

The pulse length was measured with a PIN-10 detector coupled to either a Tektronix 564 storage oscilloscope or a 545a scope with camera attached. The detector was shielded with a narrow band pass filter with maximum transmission at 6943 Å. Light scattered from the second aperture plate triggered the detector. In these experiments the pulse length varied between 3.4 and 3.7 msec.

The total energy in each pulse was determined by directing the final beam into a calibrated TRG 107 ballistic thermopile. The output of the thermopile was measured with an Electronic 19 recording potentiometer. Prior to each experiment the output of the laser was adjusted to the predetermined 100% level by adjusting the voltage on the capacitor banks. The beam was attenuated to the desired energy levels by introducing calibrated neutral density filters into the optical train. The output was calibrated prior to and following the termination of each experiment.

The energy distribution within the beam and the beam diameter in the plane of the target tissue were determined by two methods. First, an attenuated portion of the final beam was directed into a multiple beam splitter consisting of a high quality glass block 2" x 3" x 10" placed at a 45° angle to the incident beam. On either side of the block medium contrast projector slide plates were positioned so that the multiple beams exiting from the glass block were normally incident on the plates. The emergent beams were 2 cm apart. The glass block and film were enclosed in a light-tight box with the entrance aperture covered with a narrow band pass filter for transmitting ruby wavelength light only. The images on the negatives were scanned by a Densichron densitometer, Model 3853A, using a 1 mm aperture. By knowing the percent reflection at the glass-to-air interface, it was possible to calculate the relative intensities of the sequential exit beam and use the successive image densities to calibrate each film strip individually. The second method for analyzing the beam was to direct the beam onto black fogged and processed photographic paper. By subjecting the paper to a series of exposures with the beam intensity attenuated in a stepwise manner, the appearance of the paper could be related to incident intensity. In this series of experiments the maximum variation in energy density in the beam was measured as 20%, but in most cases was less. The beam diameter at the plane of the target tissue was 2.0 cm with a divergence of approximately 1/3 mm per 10 cm of path length. The energy density values given are the average over the target area.

Eight pigs with non-pigmented skin were used in these experiments. Their weights ranged from 25 to 50 pounds. The preparation of the animals for exposure was similar to that previously described (1). Acepromazine and Dial in urea-urethane were given as the preanesthetic and the anesthetic, respectively. The skin area on one side of each animal was divided into a grid of forty squares, with four rows and ten columns. Within each square a circle approximately 1" in diameter was painted with India ink containing a small amount of detergent. Three separate layers of ink were applied to each spot, each layer was allowed to dry before the next one was applied. Each site was carefully aligned with the laser beam so that the full laser beam impacted within the painted area. To minimize the effect of area to area variations in sensitivity, various energy densities were randomly assigned to each row. Each animal received approximately the same number of exposure combinations. Upon completion of the exposure pattern for each animal, the ink layer was gently sponged off.

The exposure sites were examined and visually graded 20 to 24 hours postexposure for the presence or absence of erythema. Following the gross evaluation of the exposure sites, biopsies were taken from a selected number of lesions to determine the depth of damage as a function of dose. The biopsy method and tissue preparation techniques were those previously described (3). Samples from each lesion were stained with Hematoxylin and Eosin (H&E) and evaluated histochemically for the enzyme nicotinamide adenine dinucleotide-diaphorase (NAD-ase) (4). The depth of the damage was measured microscopically by means of an eyepiece micrometer.

The absorption characteristics of the India ink layers were determined by measuring the reflectance of successive layers painted on human skin. The reflectance measurements were made with a Beckman DK spectrophotometer equipped with an integrating sphere reflectance attachment.

The erythema and histopathological data were graphically analyzed by the probit method of Litchfield and Wilcoxon (5) and the more exact mathematical method of Bliss (6, 7).

RESULTS AND DISCUSSION

The results of the measured reflectance of the India ink layers painted on human skin are given in the first five columns of Table 1. The second column gives the measured reflectance of the unpainted skin of each subject. The next three columns give the measured reflectance of each successive paint layer as applied to the same site on the skin. After the second layer of India ink the reflectance shows no further decrease. Essentially two layers of India ink constitute an infinitely thick optical layer for radiation of this wavelength.

The actual transmittance of the ink layers can be calculated. In the case of a translucent layer of material, by taking into account all

TABLE 1

Measured Reflectance at 6943 Å of Thin Layer of India Ink Painted on Human Skin

	Measured Reflectance				Calculated Transmittance	
Sample	Bare Skin	1st Ink Layer	2nd Ink Layer	3rd Ink Layer	Internal Trans. 1-Ink Layer	Total Trans. 3-Ink Layers
1	.670	.059	.043	.043	.167	.0015
2	.690	.048	.038	.038	.122	.0005
3	.630	.058	.048	.048	.132	.0008
4	.668	.055	.050	.050	.095	.0002
Average					.129	.0008

successive internal reflectances, the measured transmittance γ and reflectance ρ can be related to the internal transmittance T and reflectance R_0 (8). By modifying the general equations (8) to fit the present situation, the measured transmittance and reflectance can be described by the following equations:

$$\gamma = \frac{(1-r_1)(1-r_2)T}{1-r_1r_2T^2} \tag{1}$$

$$\rho = r_1 + (1-r_1)^2 \frac{r_2T^2}{1-r_1r_2T^2} \tag{2}$$

where r_1 is the reflection coefficient of the air-ink interface and r_2 is the reflection coefficient of the ink-skin interface. It is assumed that the internal reflectance of the ink is zero and that the reflectance values of the bare skin represent r_2, and the reflectance value of the three ink layers represent r_1. The original general equations were derived on the basis of both the incident and internal flux of light being completely diffuse. The use of normally incident light to make the measurements should introduce no serious error because of the high scattering and absorption characteristics of the India ink.

The internal transmittance for a single layer of ink can be solved by means of equation 2 and these values substituted in equation 1 to solve

the total transmittance of three layers of ink. The calculated values are shown in the last two columns of Table 1. The ruby laser beam is essentially completely absorbed in the three ink layers on the skin. Attempts to accurately measure the thickness of the ink were not successful; however, it is estimated their depth is no more than 10 μ.

The absorption of the radiation under these experimental conditions is essentially at the surface of the skin, and the subsequent distribution of the thermal energy should approximate closely enough the case of CO_2 laser radiation exposure to provide interim threshold values. Assuming that the dried ink layer is, for all practical purposes, carbon, its relatively high thermal conductivity should provide for nearly complete transfer of the generated thermal energy to the skin except for very high power densities and extremely short exposure times.

At the higher doses the absorbed radiation produced gross changes in the appearance of the ink layer. A slight glazing of the surface over the area of the impinging beam was apparent at exposure levels of about 1.4 joules/cm^2. At higher doses progressively more of the ink layer was removed. Obviously, when energy is expended in altering the ink layer and/or when its absorption characteristics are altered, there are departures from the proposed simulation model.

As shown by the histological sections, all damage was confined to the epidermal layers of the skin. The depth of damage in any specific lesion was quite uniform. In the mild burns the cellular changes consisted of nuclear pyknosis and intracytoplasmic vacuolation (Fig. 1).

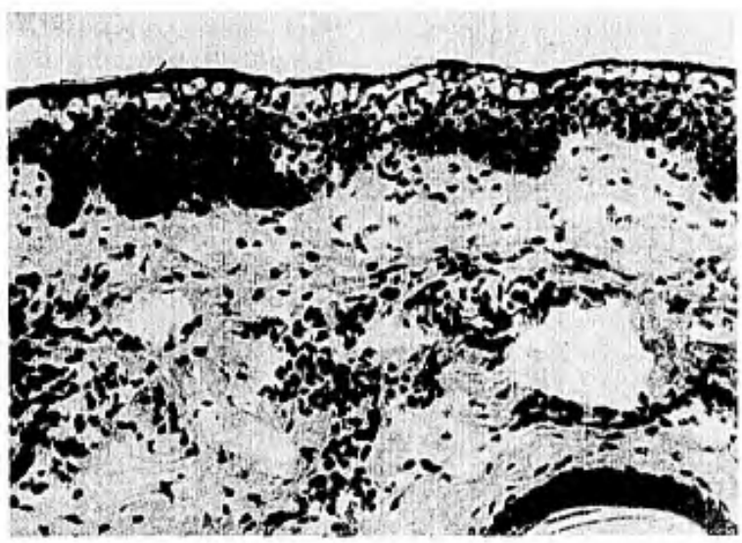

Fig. 1. H&E stained section of a mild burn. Nuclear pyknosis and cytoplasmic vacuolation of the superficial epithelium. Damaged tissue is pinker than normal epithelium.

In the more severe burns there were seen, in addition, prominent vesicle formation (Fig. 2) and focal dermal leucocytic infiltrates. In the H&E

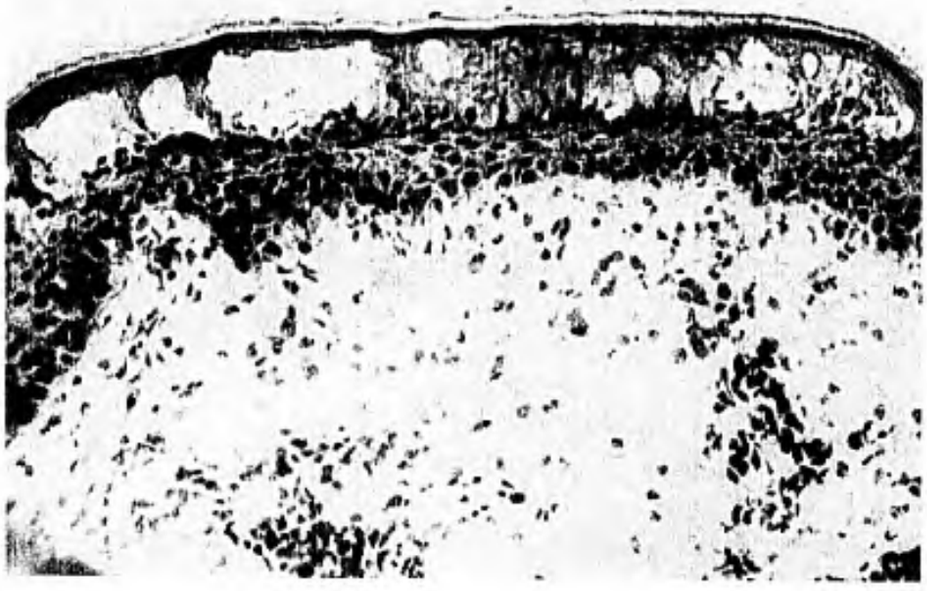

Fig. 2. H&E stained section of a severe burn. Deep involvement of epithelium with tendency to form vesicles. Superficial layers of dermis are more cellular because of inflammatory leucocytic infiltrate.

stained sections, the damaged epidermis assumed a pinker than normal appearance. The cellular changes noted were essentially the same as those seen in the CO_2 laser skin study (1), with the exception that there was no indication of physical damage to capillaries. In the histochemical sections, the damaged tissue showed a loss of enzymatic activity as manifested by the absence of staining (Figs. 3 and 4). In all cases there was a sharp demarcation between enzymatic active and inactive tissue. The microscopic appearance of the lesions did not suggest any steam bleb formation or ablative effects. If one compares Figure 1 with Figure 3 (both from the same lesion) and Figure 2 and Figure 4 (both from the same lesion), it is apparent that there is good correlation between the tissue damage demonstrated by histochemical and routine H&E techniques. The absence of a marked gradation in the intensity of the erythema in this study probably relates to the fact that all microscopic evidence of tissue damage was confined to the epidermal layers of the skin. The erythema may be a response only to chemical products released by the damaged epithelial tissue.

Fig. 3. Loss of NAD-diaphorase activity in superficial epithelium of lesion shown in Figure 1. Depth of damage is uniform.

Fig. 4. Loss of NAD-diaphorase activity in major portion of epithelium of lesion shown in Figure 2. Some vesicle formation at the junctional area.

The visual evaluations of the erythemic response from 304 exposures are listed in Table 2. Figure 5 describes the percent of positive responses as a function of the logarithm of the dose. If the two negative responses at the high doses of 1.4 and 1.8 joules/cm^2 are not included in the analysis, the graphical probit method yields the following threshold values:

median effective dose (ED_{50}) = 0.77 joules/cm^2

slope function (S) = 1.13

The 95% confidence limits are .74 to .80 joules/cm^2. The slope function reflects the rate of change of the percent response with respect to the dose (5).

TABLE 2

Visual Erythemic Responses to Laser Radiation

Energy Density (joules/cm^2)	No. of Exposures	No. of Responses -	No. of Responses +	% Positive Responses
.28	16	16	0	0
.35	16	16	0	0
.46	12	12	0	0
.58	34	34	0	0
.71	33	24	9	27
.89	34	4	30	88
1.12	34	0	34	100
1.42	38	1	37	97
1.80	38	1	37	97
2.25	27	0	27	100
2.83	22	0	22	100
	304			

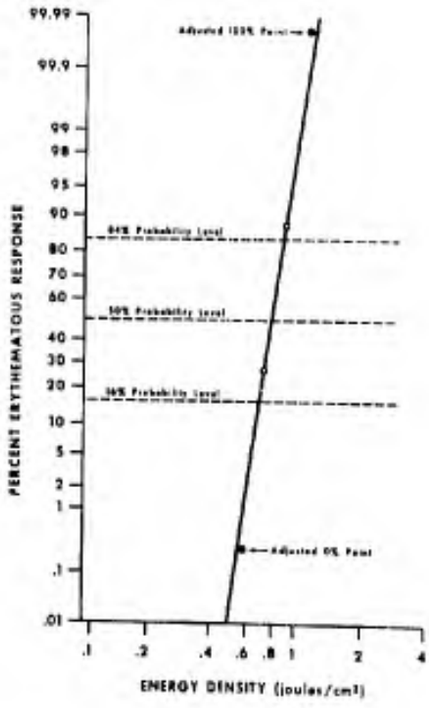

Fig. 5. Probit regression plot: percent erythemic responses versus dose of ruby laser radiation incident on blackened porcine skin.

The two negative values cause the data to be heterogenous and widen the confidence limits. Analysis of the data by the more exact mathematical method of Bliss (6, 7), including the two negative responses, gives the following threshold values:

$$ED_{50} = 0.78$$

$$S = 1.18$$

The 95% confidence limits for the median effective dose are 0.61 to 1.0 joules/cm^2. The slope function for the erythemic response obtained in these experiments is the same as that found in the studies of the response to CO_2 laser radiation for exposure times of 0.2 to 20 seconds (1). In both cases as the dose is increased by a given factor the change in percent effect is the same.

The microscopic evaluation of depths of damage in 148 representative exposure sites is given in Table 3. The cornified epithelium in these animals was approximately 20 μm in thickness. Since this layer is normally devoid of enzymatic activity and cellular detail, the minimal lesion which

TABLE 3

Depth of Tissue Damage by Laser Radiation

Energy Density (joules/cm^2)	No. of Lesions	No. of Lesions with Depth of Damage (μm)					
		<20	20-30	30-40	40-50	50-60	60-70
.71	8	8*					
.89	20	18	2(10)				
1.12	22	3	16(86)	3(14)			
1.42	26		16(100)	9(38)	1(4)		
1.80	25		3(100)	12(88)	10(40)		
2.25	25			1(100)	14(96)	8(40)	2(8)
2.83	22			1(100)	5(95)	11(73)	5(23)
	148						

*Includes one sample with a definite microscopic lesion.

Values in parentheses are the percent showing damage at that depth or greater.

consistently could be detected microscopically had to be at least severe enough to extend deeper than 20 μm. Of the eight samples from sites exposed to .71 joules/cm^2, only one had a definite microscopic lesion. Of these samples, 63% were positive in the visual evaluation for erythemic response. The depth of damage data is presented graphically in Figure 6 as a probability function of the logarithm of the dose.

The results of the probit analysis of these data are given in Table 4. Although there is no statistically significant difference in the two values, the calculated value for microscopically detectable damage is somewhat higher than the erythema threshold. Thus, the evaluation of damage by observable erythema is as sensitive, if not more sensitive, than the detection by histological techniques.

The slope functions for the greater depths of damage appear to be somewhat higher than for the 20 to 30 μm depth. It can be noted from Table 3 that essentially all the values for depths of damage beyond 30 μm were the result of exposures to energy densities of 1.4 joules/cm^2 and greater. This was the range of exposures in which the appearance of the

TABLE 4

Summation of Values from Probit Analysis for Depth of Damage

Depth of Damage (μm)	ED_{50}	95% Confidence Limits ED_{50}	Slope Function S	95% Confidence Limits S
20-30	1.0	.95 - 1.05	1.12	1.10 - 1.15
30-40	1.5	1.34 - 1.57	1.23	1.16 - 1.31
40-50	1.9	1.76 - 2.05	1.21	1.13 - 1.30
50-60	2.4	2.24 - 2.64	1.23	1.14 - 1.32
60-70	3.4	2.81 - 4.09	1.30	1.19 - 1.42

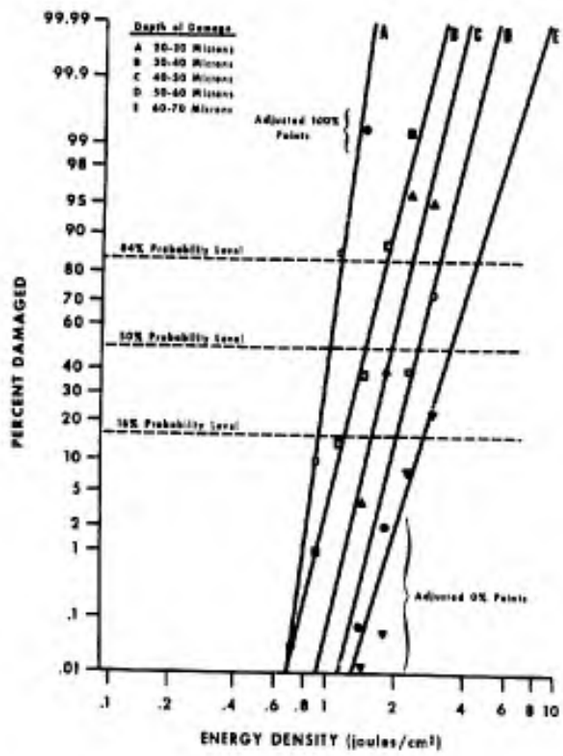

Fig. 6. Probit regression plot: percent damage extending to indicated depths versus dose of ruby laser radiation incident on blackened porcine skin.

ink layer was changed by the incident beam. As power densities increased, energy dissipated in the ink layer or lost by reradiation or scattering by the plume evidently decreased the effectiveness of the beam.

Another unknown factor in the simulated experimental design is the extent to which the ink layer will distort the thermal input to the skin relative to that of an equivalent CO_2 laser radiation exposure of the bare skin. Because of its finite thermal coefficients, the ink layer will change the shape of the thermal pulse to the skin. The maximum temperature will be reduced and the thermal episode extended over a longer period of time. When the power density is low enough and the exposure time long enough so that the thermal energy is transmitted to the skin before the temperature of the ink layer reaches the point when it is no longer acting as a passive thermal transducer, the effect should be minor. Considering the thinness of the ink layer and its relatively high thermal conductivity and low heat capacity relative to that of the skin, the lower dose levels used here in the simulated experimental design should be reasonably valid. Comparative results from the calculations based on thermal models--one an opaque two-layer system using the thermal constants of carbon for the ink layer, the other a single layer diathermous model using the absorption coefficient for CO_2 laser radiation by aqueous tissue--suggest that the resultant thermal effects in the skin will differ very little. However, under the circumstances it is suggested that the value of 0.8 joules/cm^2 be considered only as an interim value and as an upper limit for equivalent CO_2 laser radiation exposure.

CONCLUSIONS

The thermal input to porcine skin subsequent to the absorption of ruby laser radiation in a thin, opaque layer of India ink on the surface of the skin will approximate, under certain conditions, that resulting from exposure of non-blackened skin to equivalent doses of CO_2 laser radiation. Doses of less than 1.4 joules/cm^2 given in 3.5 msec appear to satisfy these conditions. Above 1.4 joules/cm^2 the appearance of the ink layer indicates that the experimental design is not a valid approximation of CO_2 laser radiation exposure.

The median effective dose to produce minimally detectable erythema 24 hours postexposure is 0.77 joules/cm^2 with an exposure time of 3.5 msec. The median effective dose to produce histopathological damage to a depth of 20 to 30 μm is 1.0 joules/cm^2 for the same exposure time.

These values may be considered as interim, approximate threshold damage levels until verified by equivalent exposures to CO_2 laser radiation. Experiments using higher power densities of CO_2 laser radiation should be carried out to determine dose-response relationship for more severe damage levels.

LITERATURE CITED

1. Brownell, A. S., W. H. Parr, D. K. Hysell, and R. S. Dedrick. CO_2 laser induced skin lesions. USAMRL Report No. 769, 1968 (DDC AD No. 669610).

2. Hardy, J. D. and C. Muschenheim. Radiation of heat from the human body. V. The transmission of infra-red radiation through skin. J. Clin. Invest. 15: 1-9, 1936.

3. Hysell, D. K. and A. S. Brownell. Correlation between the gross and microscopic appearance of CO_2 laser induced porcine skin burns. USAMRL Report No. 778, 1968 (DDC AD No. 676578).

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

5. Litchfield, J. T., Jr. and F. Wilcoxon. A simplified method of evaluating dose effect experiments. J. Pharm. Exp. Therap. 96: 99, 1949.

6. Bliss, C. I. The calculation of the dosage-mortality curve. Ann. Appl. Biol. 22: 134, 1935.

7. Finney, D. J. Probit Analysis. Cambridge, Mass.: University Press, 1952.

8. Brownell, A. S., H. F. Kuppenheim, E. D. Evans, and P. Prager. Optical properties of excised porcine skin. I. Surface reflection factors. USAMRL Report No. 373, 1958 (DDC AD No. 209090).