Spotsize Effects on Guinea Pig Skin Following Pulsed Irradiation

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Laser irradiation parameters such as wavelength, irradiance (W/cm^2), and pulse duration have been clearly shown to influence the extent to which tissue is damaged. The careful choice of these parameters can result in confining laser injury to specific targets in tissue. Spotsize, a parameter not commonly appreciated in the application of lasers to medicine and surgery, has been shown, in this study, to contribute to the ultimate outcome of laser effects in tissue. A series of histological events occurring in the skin are demonstrated to be directly related to the effects of spotsize on tissue at a fixed exposure time and wavelength. Many of these changes could contribute to unwanted adverse effects, such as scarring, which occur following certain laser therapies. *J Invest Dermatol 90:877–881, 1988*

The application of lasers to medicine and surgery over the last two decades has resulted in the use of many laser systems, each with different operating characteristics such as wavelength, power, and pulse duration. In addition, delivery systems have also varied from contact probes to focused and defocused beams, to spotsizes of different diameters ranging from 100 μm to 5 mm [1–10]. Attempts to review the literature for data to define "ideal" laser parameters for clinical applications have been hampered by difficulties in comparing the different combinations of wavelength, irradiance (power density), continuous versus pulsed durations, and the spotsize used in each study. For exposure durations shorter than the thermal relaxation time, the radiant energy per unit area, wavelength, and spotsize are sufficient to describe the laser irradiation. An additional problem has been the lack of a consistent and objective clinical end point reached by all therapists during laser treatment. While whitening of the tissue has been widely used as the visual marker of therapeutic effect, in practice, definitions of whitening imply a very wide range of tissue damage and, therefore, a great variability in the clinical end result. This has made evaluation of the published data, especially adverse effects, difficult.

Recently, great emphasis has been placed upon the use of lasers for selective targeting of chromophores within tissue to minimize some of the reported adverse effects resulting from nonspecific thermal injury such as scarring [1–3]. The appropriate combination of the three laser-irradiation parameters of wavelength, pulse duration, and radiant energy per unit area (J/cm^2) has been integral to the achievement of such selective destruction of chromophores in tissue [11–13]. It has also become apparent that other parameters, particularly spotsize (independent of irradiance), a parameter not commonly appreciated in the application of lasers to dermatology, also contribute to the ultimate outcome of laser effects on tissue [14]. The relative neglect of this parameter, except in ophthalmology [14–18], is clearly demonstrated by the wide variety of spotsizes which have arbitrarily been used in dermatology. For the treatment of portwine stains (PWS) alone, the following spotsizes have been used: a 5 mm diameter spotsize in combination with the 577-nm flashlamp pumped dye laser [9,10]; a 100-μm diameter spotsize has been used with either 577 nm or with combined 488, 514-nm wavelengths of the argon, to trace individual vessels in PWS and telangiectasia [4] and 1 mm diameter spotsize is commonly used with continuous-wave or pulsed CO_2, argon, and 577-nm lasers [1–3,5]. A change in spotsize in some cases has necessitated a change in irradiance (W/cm^2) in order to obtain the required clinical end point of whitening [4]. In general, a decrease in spotsize has resulted in an increase in the laser treatment irradiance and vice versa for a large spotsize [4,9,10]. Often such a change has been guided only by arbitrary clinical end points such as whitening. It is apparent, upon reviewing the literature, that the simplified rationales do not describe an understanding of the mechanisms responsible for the spotsize effects on skin. It is for this reason that this study was undertaken to establish and correlate the clinical, morphologic, and histologic effects of different diameter spotsizes on guinea pig skin following pulsed irradiation and in doing this, attempt to explain the laser-tissue interaction mechanisms responsible for these observed changes.

MATERIALS AND METHODS

Six albino Hartley guinea pigs were epilated 48 h prior to the study using a mixture of beeswax and rosin in a 4:1 ratio. Prior to epilation, laser irradiation, and each time before skin biopsies were taken, the guinea pig was anesthetized using intramuscular ketamine (12.5 mg/kg).

A Candela flashlamp pumped tunable dye laser LPDL-1 with pulse duration at 360 μsec was used at 577 nm. The laser beam was transmitted through a 1-mm diameter quartz fiber and focused to 5 and 3 mm diameter spotsizes using a planoconvex lens. A diameter spotsize slightly larger than 1 mm was achieved by placing the fiber 0.5 mm above the tissue. The energy (J) for each 360-μsec pulse at the end of the delivery system was measured using a Scientech Energy Meter calibrated to $\pm$ 10% accuracy. Radiant energy per unit area was approximated as the measured energy divided by the spot area. The basic term for radiant energy per unit area is fluence, but a more common term is energy density, which will be used in this paper.

Each animal was exposed to laser irradiance ranging from 1–13 J/cm^2, at approximately 1 J/cm^2 (0.2 J) increments for a 5-mm

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diameter spotsize, from 3–25 J/cm² for a spotsize of 3-mm diameter also at increments of 1 J/cm², and from 10–350 J/cm² at 5 J/cm² (0.04 J) increments, for a 1-mm diameter spotsize. Increments were achieved by varying laser output energy for each 360-μsec pulse. The energy density was increased until purpura threshold was achieved at each of the three spotsizes. These energy levels were used because doses below 1, 3, and 10 J/cm² for spotsizes of 5, 3, and 1-mm diameter, respectively, did not produce any clinically detectable effect. The criteria used for purpura threshold energy density was similar to those used in previous studies [5–13]; defined as the lowest laser energy density required to produce clinically detectable purpura filling the whole spotsize diameter within 10 min of laser irradiation.

Skin biopsies were taken prior to laser exposure, immediately after, and at three weeks following laser irradiation. The tissue was fixed in 10% formaldehyde and processed for light microscopic examination.

RESULTS

The average threshold energy densities for purpura for 360-μsec irradiation duration at 577 nm for each of the three spotsizes of 5, 3, and 1-mm diameter were 4.0, 6.0, and 27.0 J/cm², respectively. At purpura threshold energy density, the time to onset of morphologically detectable purpura filling the whole spotsize diameter as well as the time for which it persisted following laser irradiation, varied according to the diameter of the spotsize used. The onset of purpura, following exposure to a 5-mm diameter spotsize, occurred within 2–3 min and persisted longest, lasting up to 2 weeks. This was unlike the purpura produced following exposure to a 1-mm diameter spotsize, which only lasted between 3–5 days, having taken the longest time, up to 10 min to appear. The time taken for purpura to appear and clear, for a 3-mm diameter spotsize, fell between those times for 1 and 5-mm diameter spotsizes, taking between 3–5 min to appear and persisting for up to 10 days following laser exposure. The dependence of the onset and clearance upon spotsize was no longer clinically apparent when laser energy density increased to levels above purpura threshold energy density. Purpura, produced following exposure to suprathreshold doses at all three spotsizes, persisted for a longer time and appeared to be independent of spotsize.

Histology at Threshold Dose Histological examination of skin biopsies taken at 577-nm purpura threshold energy density at each spotsize revealed that the laser injury induced was confined only to blood vessels. These findings are very similar to changes previously described in other studies at this wavelength [11–13]. The overall change noted, at the three spotsizes, consisted of agglutinated red blood cell (RBC) masses filling laser damaged blood vessel lumens. However, a distinct difference was observed between the three spotsizes in the depth of blood vessels damaged within the dermis. The deepest vessels affected by threshold irradiation were found 0.6 mm from the dermal epidermal junction (DEJ) following exposure of skin to a 5-mm diameter spotsize. All blood vessels within the laser-exposed field, extending from the superficial vascular plexus to mid-reticular dermis (Fig 1*A*) were affected. By comparison, blood vessel injury following irradiation using a 3-mm diameter spotsize only extended to the upper reticular dermis, at approximately 0.4mm from the DEJ. In contrast to the 3 and 5-mm diameter spotsizes, there was a marked difference in the depth of blood vessel injury following irradiation using a 1-mm diameter spotsize. The damage in this instance was confined only to the uppermost part of the superficial vascular plexus lying approximately 0.2 mm from the DEJ (Fig 1*B*), and, unlike the other two spotsizes, none of the deeper blood vessels was affected.

Histology at Suprathreshold Doses A range of histologic changes was observed in skin exposed to 577-nm suprathreshold laser energy density and are summarized in Table I. Unlike the vascular specific injury observed at purpura threshold energy density, the damage inflicted upon skin at suprathreshold doses involved not only blood vessels but also other dermal structures, such as collagen. The most striking feature seen upon examination of skin exposed to these energy densities was the presence of a concentric pattern in the histologic changes observed within the laser-exposed field. Following exposure of skin to energy densities of between 0.5–1.5 J/cm² above purpura threshold energy density (Table I), a change occurred at the center of the irradiated site which consisted, initially, of edema between collagen bundles. Also scattered throughout this central area of collagen change were widely dilated, empty lumina lined by what seemed to be endothelial cells (Fig 2*A*). These structures were most likely to be empty blood vessels. A band of laser-affected blood vessels filled with individually identifiable RBC, formed a semicircular collarette around this central area of collagen change (Fig 2*B*). This ring of injured blood vessels extended in a semicircle from the dermal epidermal interface to as deep as the mid-reticular dermis, in some cases (Fig 3*A*). Although these blood vessel lumen appeared packed with RBCs, the cell membranes around individual cells were discernable and appeared well preserved (Fig 3*B*). These RBC changes were in sharp contrast to those found in a second, outer zone, where the damage to blood vessels appeared more severe, even though they were anatomically a greater distance from the center of the irradiated site than the

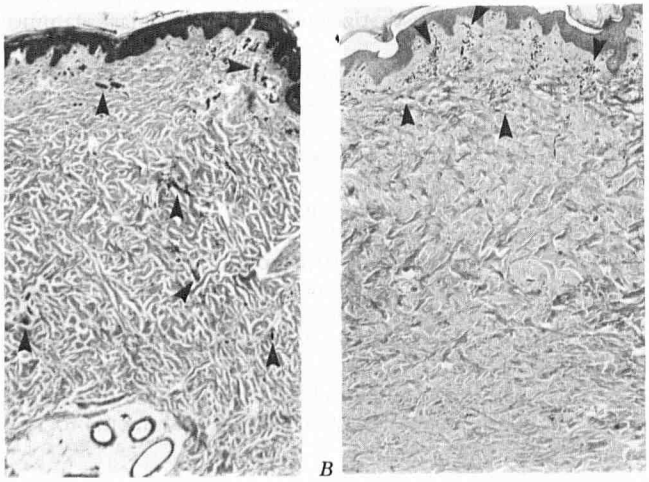
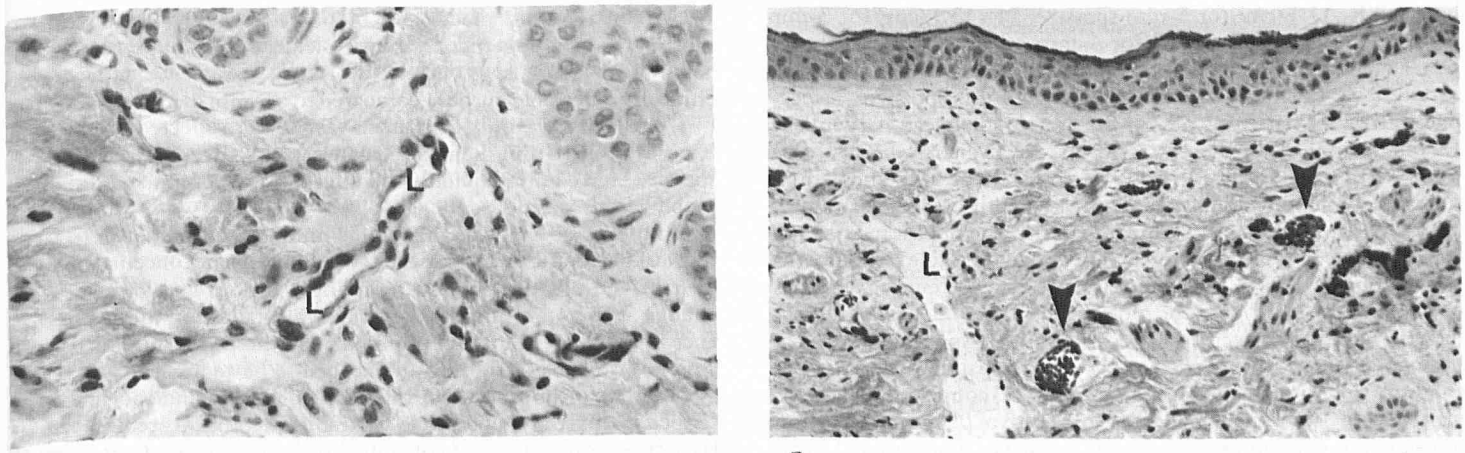


Figure 1. *(A)* Blood vessel injury *(arrowheads)* extending to mid-reticular dermis following exposure to threshold energy density, of 577-nm irradiation using a 5-mm diameter spotsize. 4×. *(B)* Superficial vascular injury confined to vessels in upper papillary dermis *(arrowheads)* following irradiation using a 1-mm diameter spotsize at threshold energy density. 4×.

Table I. Correlation of Spotsize with Laser Energy Density Associated with 360-μsec Exposure at 577 nm Required to Produce Different Histologic Endpoints at the Center of the Laser-Irradiated Site

Histologic Endpoint	Diameter Spotsize (mm)	Radiant Energy Per Unit Area (J/cm²)	Radiant Energy Per Unit Area Above Threshold Energy Density (J/cm²)
Dermal edema and empty, dilated blood vessels at center of spotsize	5	5	+1
	3	7.5	+1.5
	1	27.5	+0.5
Loss of identifiable collagen fiber detail.	5	8.0	+4
	3	11.0	+4
	1	70.0	+43
Denatured mass of collagen bundles. Epidermal vacuolization and streaming.	5	10.0	+6
	3	13.00	+7
	1	150.0	+123
Epidermal and dermal necrosis.	5	13	+7
	3	20	+14
	1	200	+173

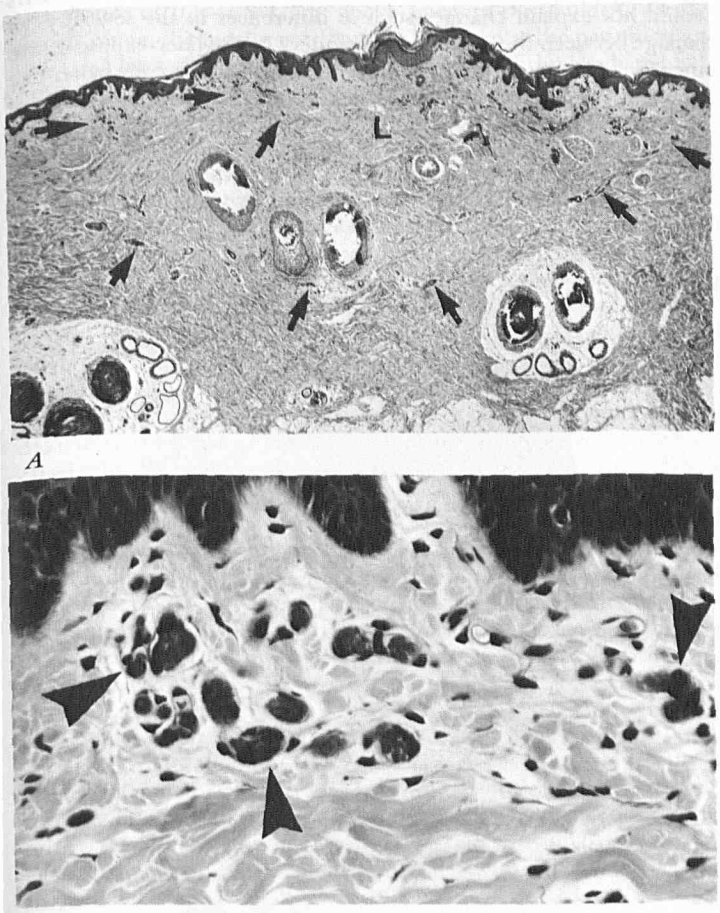


A B

Figure 2. *(A)* Empty, dilated lumina (L) lined by cells most likely to be endothelial cells seen at the center of the laser exposed site at between 0.5 – 1.5 J/cm^2 above threshold. (40×). *(B)* The inner ring blood vessels *(arrowheads)* immediately adjacent to the central area of collagen change. Note dilated lumen (L) (10×).

inner zone of less-damaged blood vessels. The RBCs within the vessels in this outer ring formed large intravascular agglutinated masses and, unlike the RBCs in the inner ring, the membrane structures around individual cells were not discernable (Fig 3C). In addition, there was also a striking change in the tinctorial quality of these cells. The endothelial cell nuclei of blood vessels forming the outer ring appeared pyknotic and in some cases, blood vessel wall structures themselves could no longer be identified (Fig 3C).

Although this concentric profile and progression of histological changes were seen to occur at all three spotsizes (Table I), the depth to which collagen and vascular alterations occurred also appeared to be affected by spotsize (Fig 4). Blood vessel injury, at purpura threshold energy density, following 5-mm spotsize irradiation, affected vessels to a depth of 0.6 mm from the DEJ. This contrasted markedly to 1-mm spotsize where the blood vessel damage only extended 0.2 mm from the DEJ. Although initially the depth of blood vessel injury increased proportionately with increasing energy density, the depth ceased to increase at around 0.9 mm ± 0.1 mm from the DEJ, for all three spotsizes, in spite of increasing laser energy density. This plateau was reached at energy densities of



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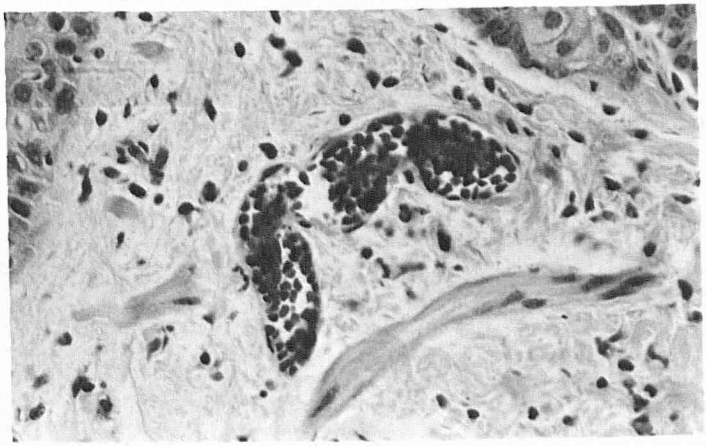

B

C

Figure 3. *(A)* Low power view showing two semicircular rings of blood vessels *(arrows)* around dilated lumen *(L)*, which forms the center of the spotsize (4×). *(B)* High power view of RBCs within vessels forming the inner ring of blood vessel damage where individual cells are discernable and membranes are well preserved (40×) .*(C)* RBCs at the outer ring forming intravascular agglutinated masses where the cell membranes are not discernable. Endothelial cells around these vessels cannot be identified (20×).

between 8–11 J/cm² for 5-mm spotsize, 11–13 J/cm² for 3-mm spotsize, and 70–150 J/cm² for 1-mm spotsize. Following this plateau, a second increase in the depth of blood vessel damage occurred at 10, 13, and 150 J/cm for 5, 3, and 1-mm spotsizes, respectively (Table I), flattening off, forming a second plateau at a depth of 1.2 mm from the DEJ. The latter occurred at an energy density of 13 J/cm², 20 J/cm², and 200 J/cm² for spotsizes of 5, 3, and 1 mm, respectively. The second increase in the depth of vascular injury, following the first plateau, coincided with the appearance of necrosis at the center of the laser-exposed field seen to extend downwards from the DEJ (Fig 4). The latter occurred at energy densities of 13, 20, and 200 J/cm² for spotsizes of 5, 3, and 1 mm, respectively. The depth of necrosis varied according to spotsize, starting as an isolated area of collagen denaturation in the papillary dermis after exposure to a 5-mm diameter spotsize (Fig 4*A*) compared to necrosis involving the epidermis and dermis to a depth of 0.7 mm for a 1-mm spotsize (Fig 4*B*) following exposure to energy densities of 13 and 200 J/cm² for 5 and 1-mm diameter spotsize, respectively.

In addition, a progression of changes also occurred at the center of the laser-exposed field as the laser dose increased to above-threshold energy density. Collagen bundles changed from being edematous to basophilic masses with loss of identifiable collagen fiber detail. These changes were accompanied by epidermal injury (Table I), which ultimately resulted in necrosis of both the epidermis and dermis at the highest energy density tested. In spite of a change in the spectrum of collagen injury, the concentric pattern of histologic change remained a constant feature. The two inner and outer rings of injured blood vessels consistently surrounded this central area of collagen change in all the specimens examined and the damage to the inner ring of blood vessels always remained less severe compared to the outer, peripheral ring.

When laser injury was so severe that dermal and epidermal necrosis occurred, such extensive dermal injury inevitably healed by scar formation. Scattered throughout this scar tissue was an increased number of small blood vessels with lumina filled with RBCs.

DISCUSSION

The clinical observation of an increase in the clearance rate of purpura following exposure of skin to threshold energy density of 1-mm diameter spotsize was explained by the histologic findings described in this study. Injury resulting from 1-mm diameter spotsize irradiation only affected the blood vessels lying in the uppermost part of the superficial vascular plexus even though considerably higher laser energy density (27 J/cm²) was needed to produce clinically detectable purpura compared to a 5-mm diameter spotsize (4 J/cm²). Because only superficial vessels were affected following exposure to a 1-mm diameter spotsize, the injury induced at this spotsize cleared more quickly than did those where the damage to blood vessels was deeper.

On the other hand, the histologic findings and the mechanisms responsible for the presence of the two semicircular concentric rings of damaged blood vessels surrounding a central area of collagen change at suprathreshold energy density, in addition to the difference in the degree of injury imposed upon these two rings of blood vessels, remained intriguing and needed to be addressed.

The relationship between the depth of injury and spotsize described in this study appeared to contradict theoretical predictions of laser effects on tissue. If it is assumed that 577-nm laser-irradiation affects tissue solely by thermal transfer, the energy from a small, 1-mm diameter beam at high energy densities should penetrate more deeply into the tissue than the lower energy density associated with the larger spotsizes. However, this did not appear to be the case in our study and the reverse effect was observed. Because the spotsize diameters of 5, 3, and 1 mm differed so markedly, it seemed more appropriate to compare differences in laser spotsize effects on tissue following exposure of the tissue to the same laser power (J) rather than doses (J/cm²).

Dermal tissue is highly scattering [17], the effect being a rapid attenuation of the collimated laser beam and diffusion of the scattered light. Portions of the scattered light that are absorbed produce heating beyond the original beam [19]. Because the histologic changes being described in this study differ markedly from expected changes based on energy density, it was reasonable to hypothesize that some of these observed changes might result from scattering and an alteration in the optical properties of dermal tissue following exposure to the laser beam at different spotsizes. However, even this would not explain changes such as differences in the severity of damage between the center and periphery of the laser-exposed site since the intensity of the light is predicted to decrease away from the center of the beam. Therefore, we propose that a combination of thermal and optical effects might be responsible for the histologic changes being described here. We postulate that during the 360-μsec pulse duration, the laser-exposed tissue was being ther-

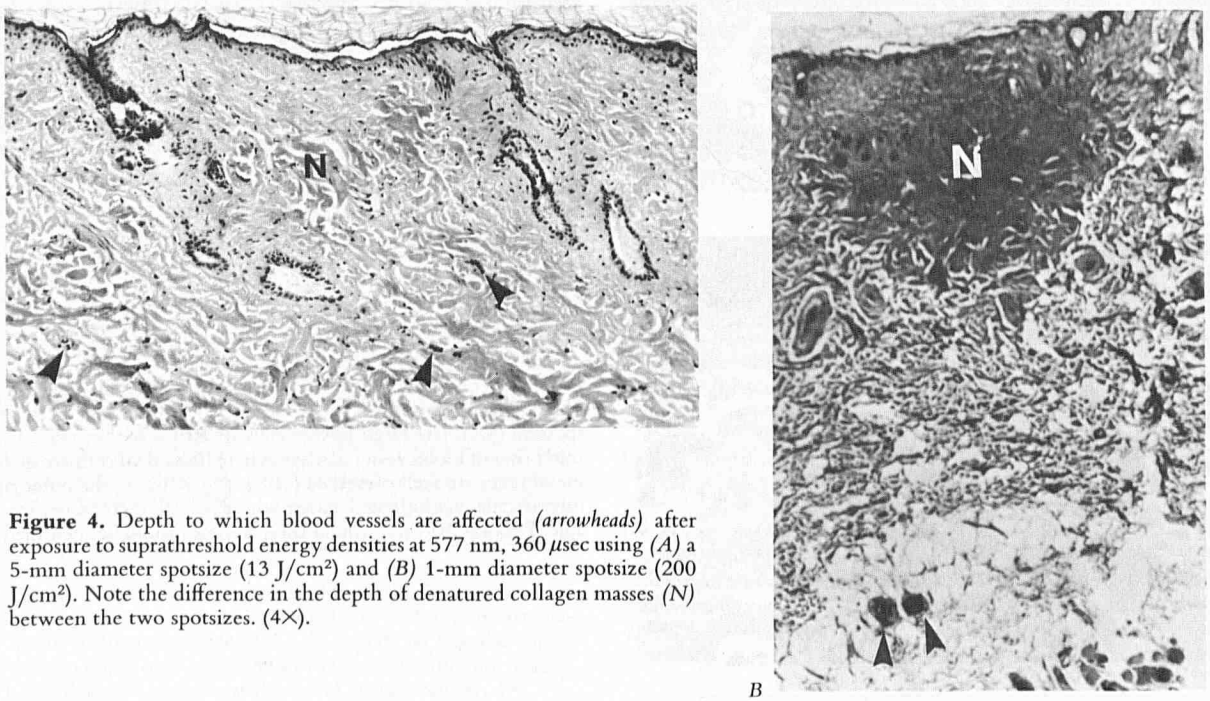

Figure 4. Depth to which blood vessels are affected *(arrowheads)* after exposure to suprathreshold energy densities at 577 nm, 360 μsec using *(A)* a 5-mm diameter spotsize (13 J/cm²) and *(B)* 1-mm diameter spotsize (200 J/cm²). Note the difference in the depth of denatured collagen masses *(N)* between the two spotsizes. (4×).

mally altered in such a way that a change occurred which altered the index of refraction of the tissue [20]. This in turn affected the way in which the laser beam was being distributed and scattered within this tissue.

It was also evident from the histologic changes described here that the tissues within the laser-exposed field were being heated to different temperatures. Based on histology, the temperature profile across the beam must have consisted of three peaks; one at the center, a second at the inner ring of blood vessel injury, and the third at the outer ring. The unique ability to use the dermal blood vessels as temperature probes in our in vivo model system provided a means for monitoring tissue injury [21]. In the absence of such probes, early detection of subtle histologic changes would otherwise have been missed. Not only was it possible to detect that areas of 577 nm induced tissue damage using these probes but also that they also provided a means for monitoring subtle morphologic changes in RBCs within the laser-exposed vessels. In this way, clues, such as the path taken by the beam, including the depth of penetration, could easily be detected during exposure to the 360-μsec pulse.

Although it could be argued that some dermal structures such as collagen were devoid of a 577-nm absorbing chromophore, and were therefore transparent to this wavelength, this argument would certainly not explain the presence of the widely dilated blood-vessel-like structures seen at the center of the laser-exposed field, which were absent in control specimens. Under normal physiologic conditions, nonirradiated blood vessels usually contain RBCs within them. Following laser irradiation, the structures being described here appeared empty, were widely dilated, and were seen scattered between edematous collagen bundles. Such evidence supports the occurrence of a thermal event; similar histologic changes having previously been described following application of direct heat to skin at 50°C [22]. Typically, the center is most severely damaged following a direct burn injury, with the damage becoming less severe towards the periphery. In our study, the reverse effect was observed when skin was exposed to 577-nm, 360-μsec duration irradiance of 0.5–1.5 J/cm^2 above purpura threshold. Over this small range, the most severe damage was seen at the periphery and the area least affected, occurred at the center of the irradiated field (Table I). It could be argued that such a change might have resulted from exposure of skin to a nonuniform laser beam. However, this would not explain the presence of the three consistent temperature peaks across the irradiated site. Furthermore, careful analysis of the laser beam profile itself did not substantiate this hypothesis. In addition, Motemedi et al [23] have recently described the occurrence of similar concentric patterns of temperature profile differences measured with a thermal camera when Agar gel, used as a phantom medium, was exposed to argon and Nd:YAG lasers.

The therapeutic implication of less dermal damage associated with a 5-mm spotsize compared to a 1-mm spotsize at the same power for comparable vascular insult is clearly demonstrated in this study. This is particularly relevant in the case of the 1-mm spotsize diameter because higher energy density was required to produce clinically detectable purpura at threshold: 27 J/cm^2 for a 1-mm spotsize versus 4 J/cm^2 for a 5-mm spotsize. In addition, the vascular specific damage following a 1-mm spotsize at threshold was confined to vessels extending only 0.2 mm from the DEJ, whereas vessel injury after a 5-mm spotsize extended as deep as the mid-reticular dermis. Furthermore, at all these energy densities, a 1-mm spotsize produced more dermal damage compared to 3 and 5-mm diameter spotsizes (Fig 4*A,B*). Although it was possible ultimately to injure deeper vessels using the 1-mm spotsize, this was done at the expense of severe dermal damage (Fig 4*B*). Therefore, when attempts are made to ablate blood vessels in the dermis using a 1-mm spotsize, the problem of damage to nonvascular structures such as collagen must be considered. In summary, if a 1-mm spotsize is required therapeutically, a "successful" result can only be achieved when a careful balance is struck between the desired depth of blood vessel damage and the degree of dermal damage induced; the latter should be carefully chosen to minimize scar formation.

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