Comparative Histological Studies of the Tunable Dye (at 577 nm) Laser and Argon Laser: The Specific Vascular Effects of the Dye Laser

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This study compares the histological changes occurring after argon laser and dye laser (operating at 577 nm) treatment of normal human skin. The initial effect of the argon laser is a diffuse nonspecific epidermal and upper dermal necrosis with subsequent cell death and a neutrophilic response at 48 hr. These changes occur at 15 joules/cm² and their extent closely correlates with the energy applied. In sharp contrast, the immediate effect of the dye laser is erythrocyte aggregation, vessel rupture, and hemorrhage. At 48 hr, there is a pattern of acute vasculitis in the upper dermis and a prominent perivascular neutrophilic response in the mid-dermis. Focal epidermal necrosis does occur but is relatively minimal, while skin appendages and collagen are preserved. The energy to produce these alterations is relatively small, approximately 3 J/cm². Thus, the dye laser at 577 nm can selectively damage the cutaneous vascular plexus and may provide a basis for treatment of cutaneous vascular lesions.

Lasers have been in clinical use in dermatology for over 15 yr for the treatment of a variety of cutaneous lesions. One of the initial studies reports the efficacy of the argon and ruby laser in the therapy of portwine stains [1]. Immediate histologic changes included diffuse coagulative necrosis of the epidermis and papillary dermis. Within 3 mo after treatment, the skin became markedly less violaceous, approximating normal skin color. The authors point out in this paper the importance of developing lasers which more specifically damage vascular structures rather than producing the nonspecific, diffuse coagulative necrosis caused by the argon and ruby lasers. Subsequently, other documentation of the value of argon laser therapy of portwine stains has been obtained in larger numbers of patients [2-6]. Argon laser therapy seems most effective in older patients with
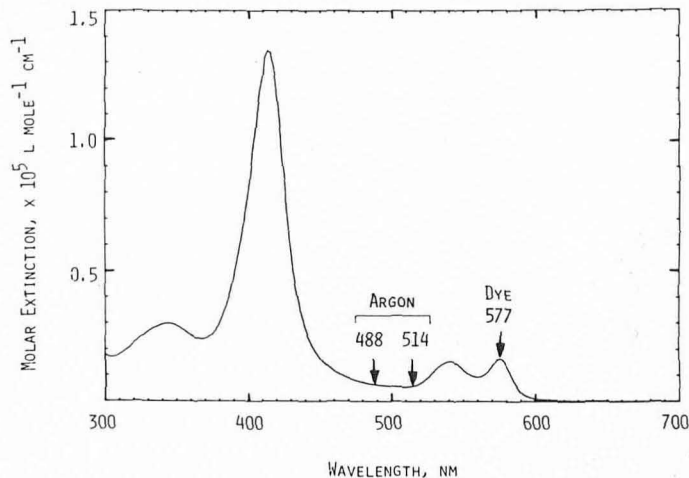

FIG 1. Absorption spectrum of oxyhemoglobin showing the emission wavelengths of the lasers used. Note that the argon laser emission (488, 514 nm) is at wavelengths not as well absorbed by hemoglobin. This contributes to the nonselective damage caused by the argon laser. However, its longer pulse-width is the major factor which causes widespread necrosis beyond blood vessels. The maximum absorption of hemoglobin is near 415 nm but this radiation is not transmitted as deeply into the dermis as 577 nm radiation.
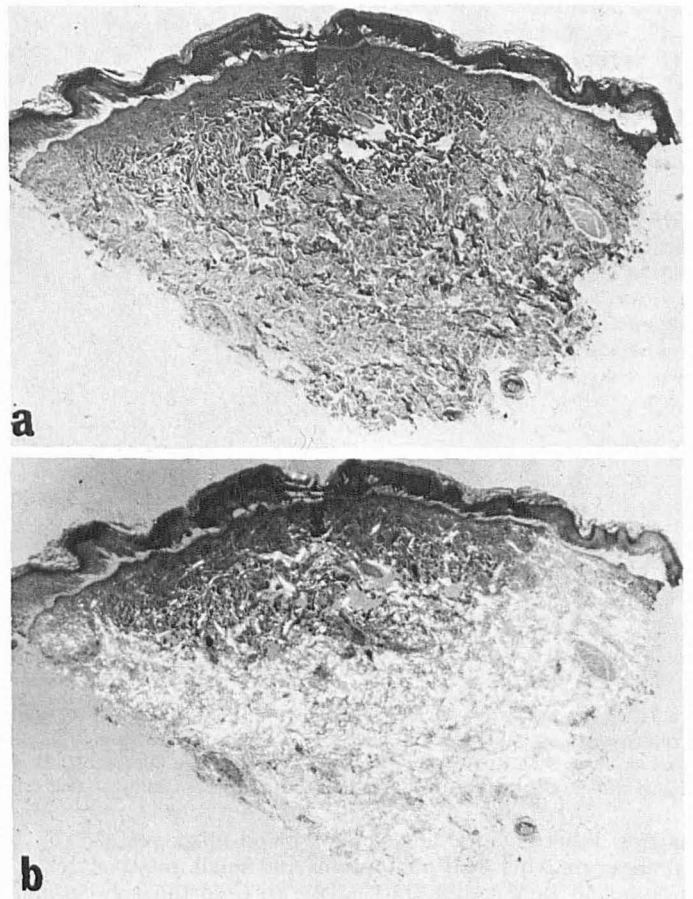
FIG 2. Argon laser—25 J/cm². Biopsy obtained immediately after laser treatment. a, Epidermal coagulative necrosis with subjacent collagen necrosis and disorganization is present (H&E, ×32). b, Loss of collagen's capacity to polarize is apparent in the zone of necrosis (H&E, polarized light, ×32).
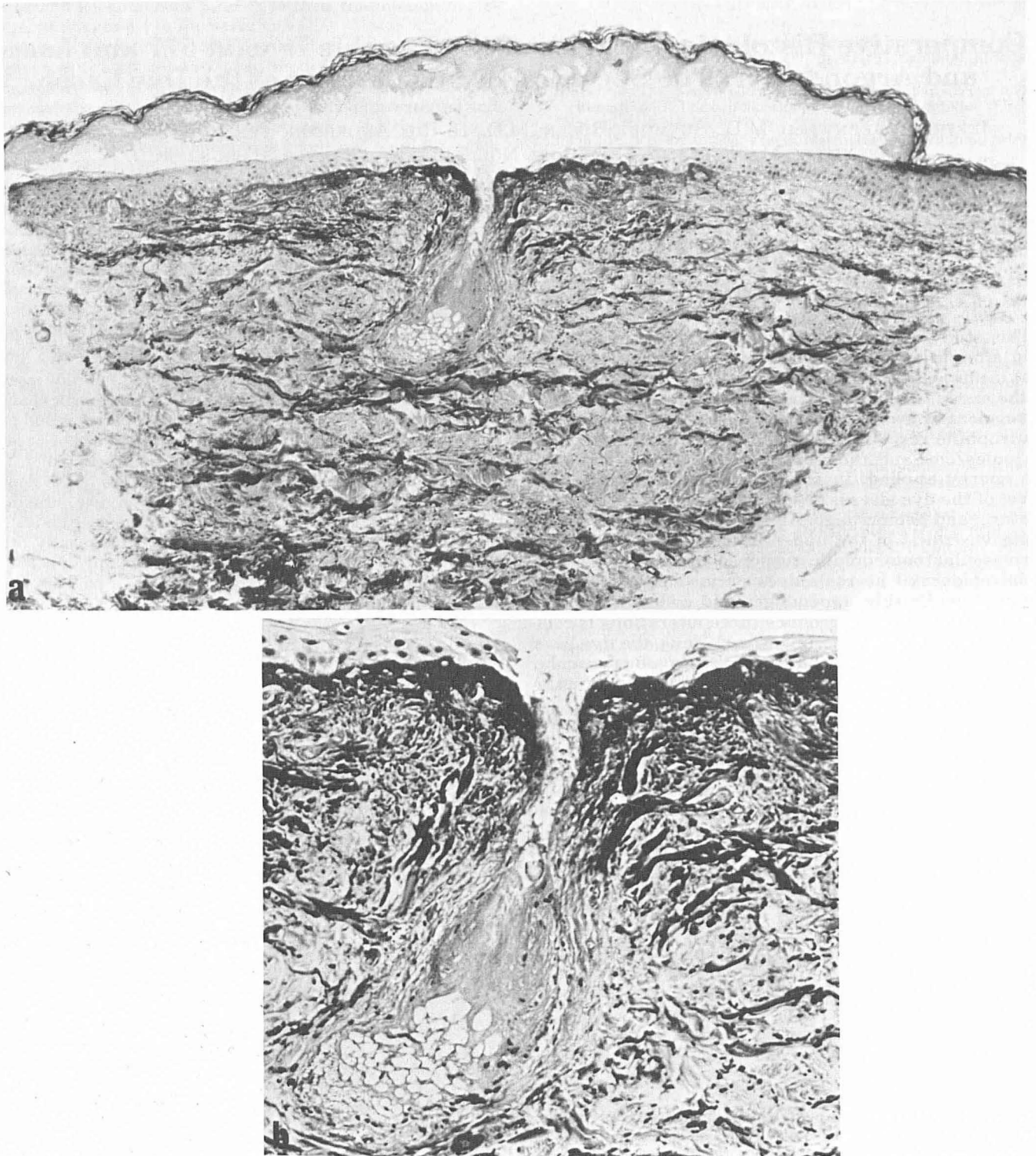
FIG 3. Argon laser—30 J/cm². Biopsy obtained 48 hr after laser treatment. *a*, A subepidermal blister has a roof of necrotic epithelium and a base that is re-epithelialized (Verhoeff-van Gieson, ×62). *b*, Hyperchromatic collagen bundles reveal the extent of collagen necrosis. Although there is no collagen alteration about the lowermost portion of the sebaceous gland, the entire pilosebaceous unit is necrotic. (Verhoeff-van Gieson, ×140).

purple lesions containing ectatic blood-filled vessels [7]. In younger patients with pink lesions and small, relatively bloodless vessels, poor results and scarring are unfortunately common [7]. The results of argon laser therapy of tattoos has also been discouraging due to the degree of scarring [5,8].

Lasers are capable of very high power density, extremely short pulses and virtually absolute monochromaticity. Because of their high power density, absorption of laser radiation can lead to extremely high focal temperatures and result in coagulation necrosis or boiling of tissue, features which permit the laser to be used for cutting and destruction of tumors. After therapy of portwine stains the argon, ruby or CO_2 lasers, diffuse coagulation necrosis occurs throughout the immediately exposed field. We believe it is possible to utilize other properties of the laser to achieve photon-tissue interactions which are quantitatively and qualitatively different from those seen after

exposure to conventional sources [9] and which also permit selective destruction of specific structures within skin [10]. In this report, the tunable dye laser, an instrument which allows high power density, short pulse duration and wavelength selection, is operated at 577 nm. Based on the optical properties of human skin and blood [10], vessel size and thermal diffusion theory, radiation at this wavelength and of pulse durations less than approximately 1 millisecond should destroy blood vessels while sparing other dermal structures and the overlying epidermis [10]. The absorption spectrum of oxyhemoglobin and the wavelengths of the argon and dye lasers used in this study are given in Fig. 1. In this report, we compare the clinical responses and histologic effects of a dye (at 577 nm), and an argon laser, confirm the nonselective changes produced by the argon laser, and demonstrate the relatively specific vascular destructive capability of the dye laser.

MATERIALS AND METHODS

Different sites of normal volar forearm skin of 10 healthy young Caucasian subjects were exposed to 10 pulses each from a dye laser operating at 577 nm. The laser used was a coaxial flashlamp-pumped tunable organic dye laser with output up to $\frac{1}{2}$ joule per pulse, in a pulse-width of 350 ns (full-width at half-maximum). The laser, supplied by Phase-R Corporation, was focused into a 1-mm diameter solid core step-index quartz optical fiber (Math Associates, QSF 1000) to produce spot diameters from 1 to 4 mm. The dye used was Yellow II (Rhodamine 575) which was prism tuned to 577 nm. Energy density was varied by varying the voltage on the laser's flashlamp supply. The exposure fields were 1 mm in diameter and the exposure doses were varied so that each subject received at least 2 exposures to 1, 2, 3, and 5 J/cm². No local anesthesia was used.

Single sites of normal volar forearm skin were subjected to ten 1-mm diameter pulses from an argon laser (Coherent Radiation Model No. 1000) in 7 healthy young Caucasians in doses ranging from 12.5 J/cm² to 40 J/cm². The argon laser emits radiation at both 488 and 514 nm simultaneously. The exposure durations were 200 milliseconds, provided by a shutter.

Gross clinical changes were recorded immediately, at 24 hr, and at 48 hr. Trephine punch biopsies of exposed sites were performed: immediately in 12 instances, twice at 24 hr, and 16 at 48 hr (dye laser); immediately in 7 instances and 5 at 48 hr (argon laser). Xylocaine (2%) without epinephrine was used for anesthesia. Biopsy specimens were fixed in formalin, dehydrated, embedded in paraffin, and cut at 4 μm thickness; hematoxylin and eosin, Masson-trichrome, and Verhoeff-van Gieson stains were done. Two subjects were also treated with the dye laser tuned to 590 nm; biopsies were not obtained at this wavelength.

RESULTS

Argon Laser

Energies less than 15 J/cm² (per pulse) produced no gross alterations. At 20 J/cm², the skin immediately turned white and at 25 J/cm² and 30 J/cm² a crust developed within 24 hr after exposure. Discomfort was first experienced at 20 J/cm².

Histologically (Fig 2, 3), immediate changes at lower dose levels were those of keratinocyte vacuolization; with higher doses, epidermal coagulation necrosis was present. Adjacent to the cauterized epidermis, a zone of keratinocyte vacuolization change was noted. Dermal collagen necrosis (cauterization) was regularly observed as distortion and basophilia in hematoxylin and eosin stains, iron hematoxylin affinity in Verhoeff-van Gieson preparations, loss of ability to polarize and irregular staining with Masson-trichrome. The depth of histologically identifiable necrosis varied from .125 to .3 mm of dermis; all cellular elements were destroyed within this zone. At 48 hr, the epidermis was necrotic and completely detached; 1.0 mm of epidermal regeneration was observed. The collagen necrosis was evident and varied from 0.2 to 0.8 mm dermal thickness. The pilosebaceous apparatus was extensively necrotic both within and beneath the region of collagen necrosis. In the zone subadjacent to the collagen necrosis, necrotic vessels and sweat glands were also observed; a slight neutrophilic infiltrate was present.

Thus, the initial response to argon laser treatment is a

necrosis of epidermis and superficial dermis (Fig 6). After 48 hr, cell death becomes evident in structures (vessels, sweat glands, and pilosebaceous apparatus) of the deeper dermal zone. There is an excellent overall correlation between depth of epidermal and collagen necrosis and exposure dose (Fig 4). Only a mild acute inflammatory response was observed at the 48-hr period. No anatomic components were selectively destroyed.

Dye Laser

In contrast to the argon laser, gross effects on normal skin were seen at lower doses. At 3–5 J/cm² purpuric macules and papules were noted but the epidermis appeared intact. When wavelength was changed from 577 to 590 nm, no changes in the skin could be observed at energies varying from 1–5 J/cm². Histologically, the primary alterations were virtually exclusively vascular in nature and basically involved the superficial vascular plexus (SVP) (Fig 5). The arterial and venular arcades ascending to the superficial plexi were generally spared. Clusters of tightly aggregated erythrocytes which had an orange hue were observed in the SVP. Endothelial and transmural necrosis associated with rupture and hemorrhage was manifest in the SVP as well as occasionally in the upper rami of the ascending vessels. Endothelial nuclear pyknosis, cytoplasmic vacuolization, and condensation were noted as well. In a few biopsies, mature neutrophils were marginating and emigrating through the arteriolar and venous walls in the upper reticular dermis; no karyorrhexis was present. These vessels had little endothelial alterations or vessel necrosis.

At 48 hr, venular walls were necrotic, replaced by fibrin; polymorphonuclear leukocytes and karyorrhexis were evident, a pattern of "acute vasculitis." However, some superficial vessels demonstrated apparent reconstitution of endothelial cells. Hemorrhage was less striking than in the initial biopsies. The "vasculitic" change occurred in approximately the upper 0.5 mm of dermis. In deeper dermis (0.5–1.1 mm) a more diffuse predominately perivascular PMN leukocytic infiltrate was noted occasionally associated with lymphocytes.

The threshold energy for induction of significant vascular damage is approximately 2–3 J/cm². However, even at 1 J/cm² slight alterations consisting of minimal hemorrhage and erythrocyte agglutination were noted. Edema, hemorrhage, and slight neutrophil infiltrate were the only dermal changes noted with these energy levels. Dermal fibroblasts appeared normal cytologically and in number. No alteration of collagen or elastic tissue was observed using Masson-trichrome and Verhoeff-van Giesen staining.
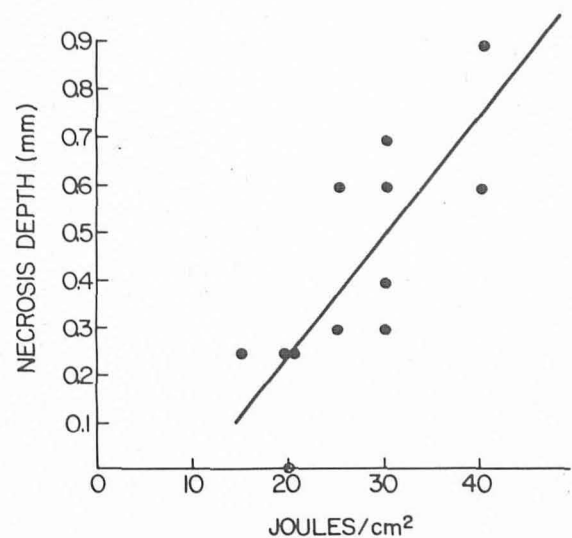

FIG 4. Relationship of depth of skin necrosis (epidermis and dermis) to energy applied with the argon laser. A good correlation exists between the amount of energy (J/cm²) and depth of necrosis.
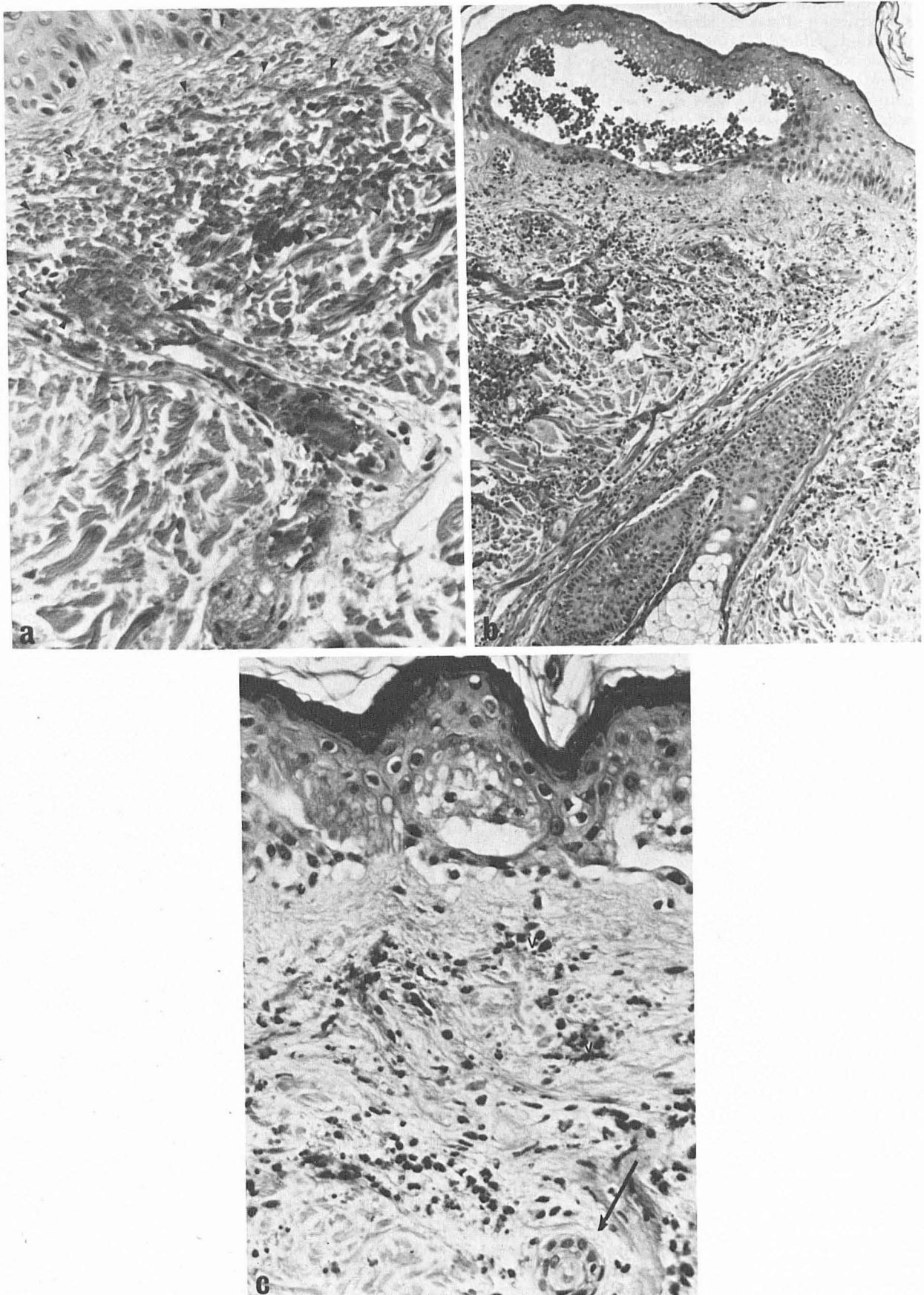
FIG 5. Tunable dye laser—577 nm, 3–5 J/cm². *a*, Immediate effects include erythrocyte aggregation, vessel rupture (*large arrowhead*) and hemorrhage (*small arrowheads*) (H&E, ×365). *b*, At 48 hr, changes of neutrophil-mediated necrotizing vasculitis were apparent. Small foci of epidermal necrosis with blister formation were present in a few specimens (H&E, ×145). *c*, Karyorrhexis of neutrophils and necrosis of vessels (*V*) were noted in 48 hr specimens. Note the sparing of the eccrine duct (*arrow*) (H&E, ×320).

Argon Laser

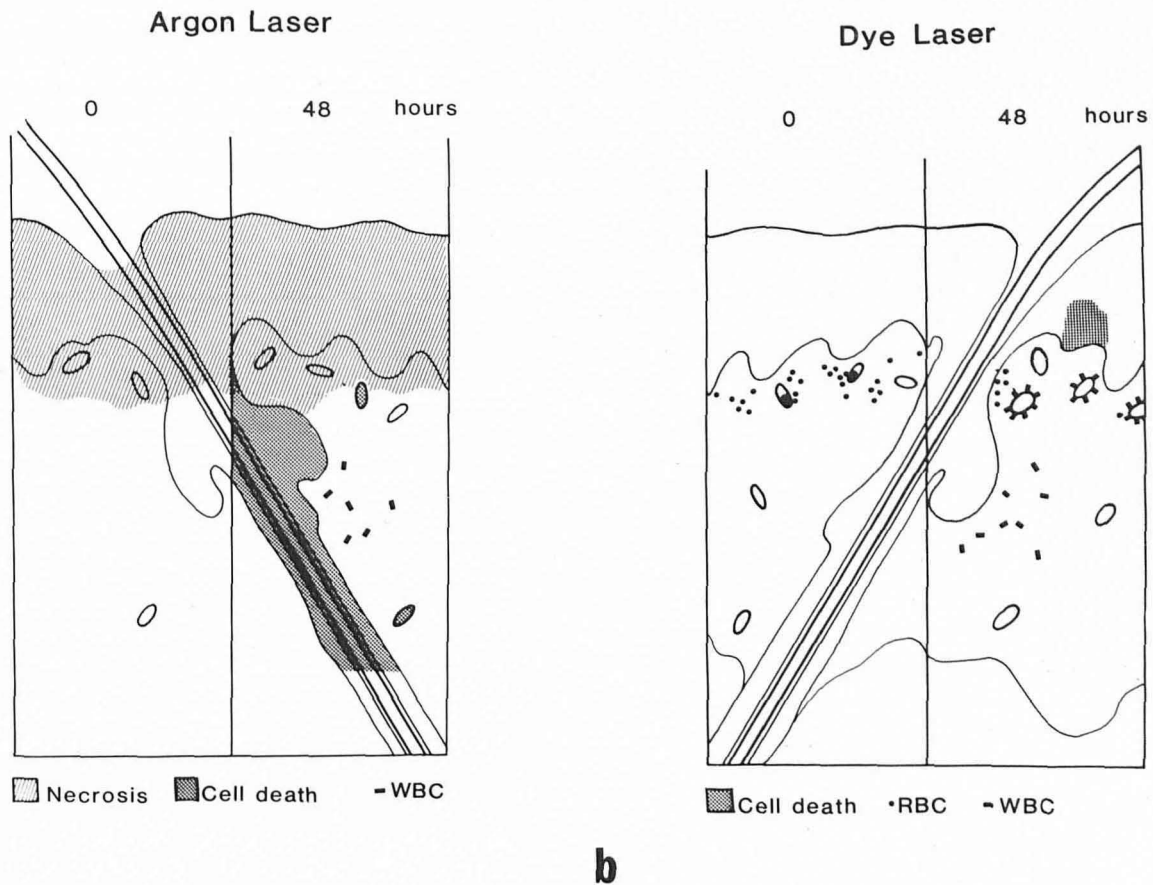## Dye Laser

a   b

FIG 6. Comparative features of argon laser and tunable dye laser (577 nm) at 0 and 48 hr. *a*, The argon laser nonspecifically causes necrosis in a superficial zone. *b*, The tunable dye laser causes relatively specific vascular damage.

The threshold for significant epidermal alterations appeared to be approximately 3 J/cm^2, since energy levels of 1–2 J/cm^2 caused no epidermal changes. In biopsies in which 3 J/cm^2 had been administered, only small foci of epithelial cell necrosis and basal cell vacuolization were noted. However, at 5 J/cm^2, full thickness epidermal necrosis and subepithelial blister formation were apparent. At this energy level, the most severe vascular damage was present. However, necrosis of collagen, pilosebaceous apparatus, and sweat glands was not seen.

Thus, the initial response to dye laser treatment is erythrocyte aggregation, vessel rupture, and hemorrhage (Fig 6). At 48 hr, a pattern similar to that observed in acute vasculitis is present in the upper dermis (0.5 mm thickness), but a primarily perivascular neutrophilic infiltration is observed fully 1.0 mm beneath the epidermis. There is, in addition, a lymphocytic response. Epidermal necrosis was present only in some of the biopsies, despite conspicuous vascular damage. Necrosis of the pilosebaceous apparatus and sweat glands was not observed.

DISCUSSION

It is possible to use light to severely damage blood vessels while sparing other connective tissue structures and overlying epithelium. By using laser radiation of appropriate wavelength, pulse duration and energy density, dermal blood vessels can be severely damaged at exposure doses which do not alter the epidermis, appendages, fibroblasts, or other cells. These effects depend on several of the properties of dye laser radiation. 577 nm was chosen so that hemoglobin in vessels would absorb the radiation. Although hemoglobin absorbs maximally near 420 nm, radiation at this wavelength is not transmitted as deeply into dermis and has greater absorption by melanin. Despite the weaker absorption by HbO$_2$ at 577 nm, a large fraction of the 577 nm radiation incident upon vessels 20 μm in diameter or larger is absorbed, and other considerations related to dermal optical scattering favor choice of this band [11]. When the dye laser was tuned to 590 nm, no vessel damage was seen at doses which severely affected vessels at 577 nm, confirming the necessity of absorption by HbO$_2$ for the production of vessel damage.

Most of the optical energy absorbed in tissues is rapidly converted to heat by nonradiative processes. When very high peak powers are used, absorbing cells can be vaporized. The pulse-width of the dye laser is 350 ns. This is a short time compared to the calculated thermal relaxation times (on the order of milliseconds) for structures the size of small vessels [10]. In sharp contrast, the argon laser is not at a wavelength absorbed as well by hemoglobin and this particular argon laser's 200 ms allows time for extensive diffusion of heat generated in blood vessels to the entire exposed field, thereby resulting in nonselective thermal destruction.

It is well established that nonspecific thermal necrosis by argon laser radiation is often therapeutic for portwine stains. Whether widespread tissue denaturation is necessary for this treatment is not known. Selective effect on the enlarged blood-filled vessels might improve the therapeutic results. Selective destruction of blood vessels may have *important* uses in the treatment of other vascular lesions in skin or eye or in internal *organs approachable by fiber-optical instruments*. Further selective alteration of specific tissue components may be achieved by varying pulsewidth, wavelength and energy of lasers and other radiant energy delivery systems.

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Announcement

The Fourth Annual Westwood Carolina Conference on Clinical Dermatology will take place October 22-25, 1981 at the Hyatt Hotel at Palmetto Dunes, Hilton Head Island, South Carolina. For information and registration, contact Dermatology Educational Services, Post Office Box 4207, Kenmore, New York 14217, (716) 884-1758.