

## SCIENTIFIC REPORT

# Histological and morphometric analysis of the effects of argon laser epilation

S Hanumanthu, L A Webb, W R Lee, J Williamson

*Br J Ophthalmol* 2003;87:984–987

**Aim:** To analyse the location and extent of tissue damage induced after argon laser epilation.

**Methods:** Laser burns were applied to the lid margins of four patients before excision for entropion ("live tissue") and the lid margin of one patient was lasered after an excision for ectropion ("dead tissue"). The laser burns were directed towards the lash follicle and between 10 and 50 burns were applied with an argon blue-green laser set at power 0.9–1.0 W, at 0.1–0.2 second duration and a 100 µm spot size. The tissues were processed for conventional histology. Serial sections were obtained and used for area measurements and three dimensional reconstructions of the burns to determine the volume and location of tissue destruction.

**Results:** The laser created a cone-shaped region of tissue ablation with surrounding coagulative necroses. Maximum burn depth was 1.2 mm in dead tissue and 0.8 mm in live tissue. Maximum necrosis depth was 1.4 mm in dead tissue and 0.9 mm in live tissue. Follicle depth ranged from 0.8 mm to 1.9 mm. Some of the burns had been misdirected in the dermis leaving target hair follicles intact, despite being of adequate depth.

**Conclusions:** The argon laser has some potential for ablation of lash follicles, but accurate placement of the burn is essential and energy levels greater than those currently recommended should be applied. The treatment is ineffective in patients unable to remain immobile.

epilation include inaccurate location of the burn and insufficient burn depth to destroy the follicle of the lash. To investigate the effects of conventional therapeutic levels, a histomorphometric study (including three dimensional reconstruction) was carried out on eyelid tissue lasered in vivo and in vitro.

## PATIENTS AND METHODS

### Patients

Ethics committee approval was obtained for this study. An information sheet was issued to patients explaining the purpose of the study, and informed consent was obtained.

### Source of specimens

Eyelid tissue was obtained from four patients (age range 67–86 years) who required block excision for trichiasis. Laser epilation to the target lashes was carried out immediately before surgery. A block of eyelid tissue including lid margin was also obtained from a patient after an entropion repair and this was used to study the effects of the laser on recently viable tissue.

### Details of laser application

An argon blue-green laser was used to apply the burns and details of the energy levels and the number of pulses is shown in Table 1. The total energy levels in joules was a product of watts × duration × number of pulses.

### Procedure of laser epilation

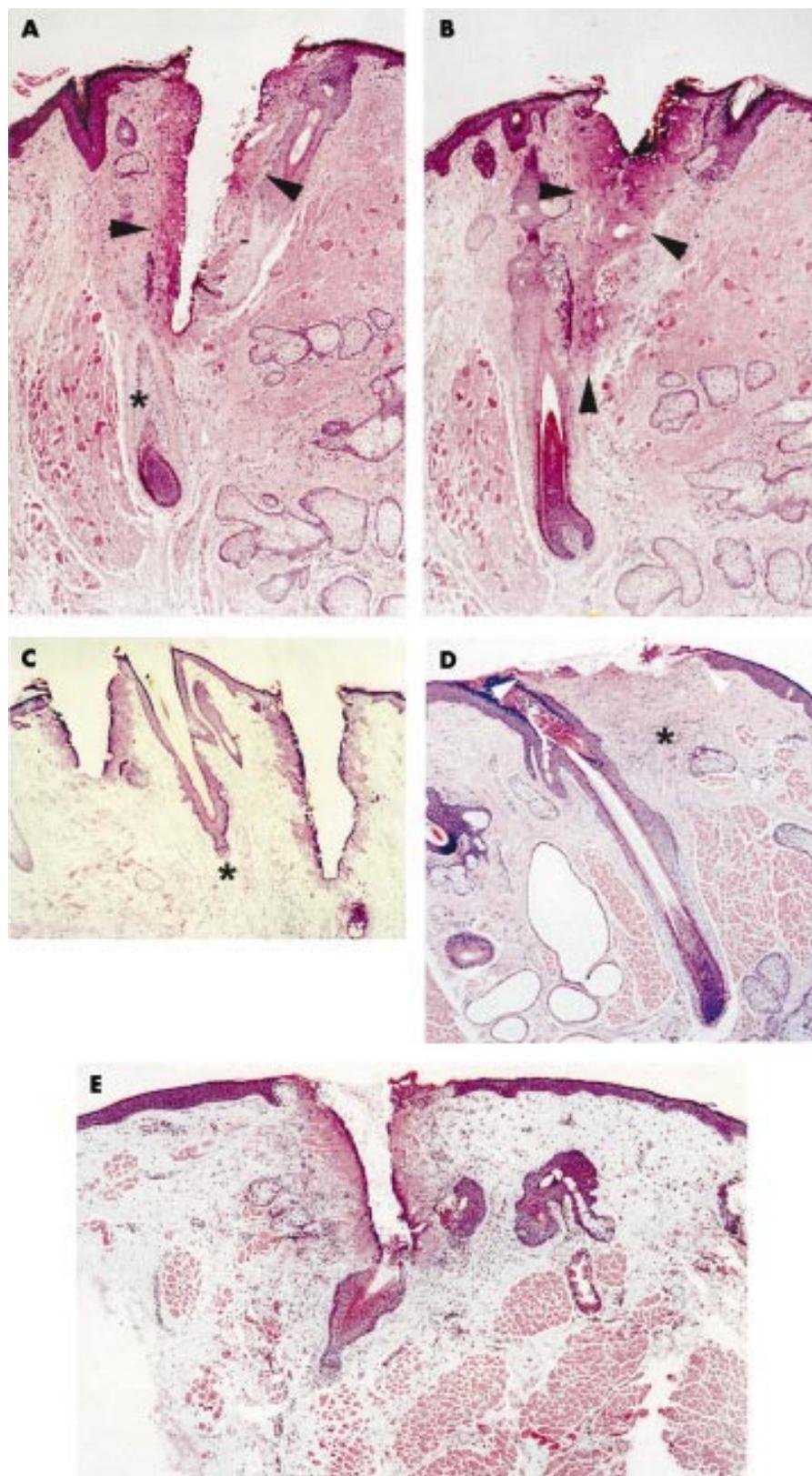
To study burns in non-viable tissue, a block of excised eyelid margin was held by forceps in front of the aiming beam of an argon laser (Coherent 900). The first one or two laser burns were directed at the base of the lash to divide it. The burns were applied along the presumed axis of the shaft of the lash.

To study the effect of burns in viable tissue, the block of tissue due to be excised was identified on slit lamp examination. Topical or infiltrative anaesthetic was not used. The eyelid was rotated outwards and dried, and the patient asked to look in a

The recurrent nature of trichiasis has led to the development of numerous therapeutic techniques, including cryotherapy,<sup>1–3</sup> electrolysis and mechanical epilation,<sup>3</sup> and laser epilation.<sup>2–9</sup> None of these techniques is entirely successful in preventing recurrence of abnormal lash formation and only one report<sup>10</sup> includes histopathological verification of location and depth of the tissue destruction by an argon laser. The probable reasons for the unsatisfactory outcome in laser

**Table 1** Various energy levels used to create burns in the lid margin

Pathology reference no	Burns studied by histology	Number of pulses	Energy/pulse (W)	Duration (seconds)	Spot size (µm)	Energy (J)
Non-viable						
229LA	1	10	0.9	0.2	100	1.8
229RC	1	25	0.9	0.1	250	2.2
229RA1	1	10	0.9	0.1	100	0.9
229RA2	1	25	0.9	0.1	100	2.3
Viable tissue						
941003A	1	20	1.0	0.1	100	2.0
941003B	1	20	1.0	0.1	100	2.0
941002	1	34	1.0	0.1	100	3.4
941004	1	40	1.0	0.1	100	4.0



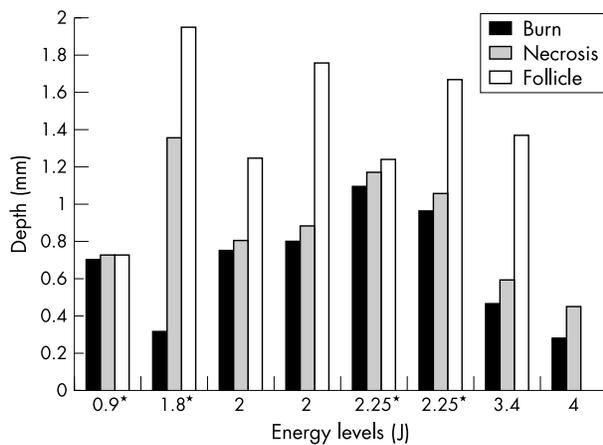
**Figure 1** (A) Section taken through the centre of a burn (229/LA: 1.8 J) in the non-viable tissue. A narrow burn path is clearly seen. Contiguous tissue damage is only evident in the narrow zone of surrounding tissue necrosis (arrowheads). The burn does not reach the hair follicle (\*) (haematoxylin and eosin (HE),  $\times 40$ ). (B) Section through the same burn as in (A), demonstrating the surrounding zone of coagulative necrosis (arrowheads) (HE  $\times 40$ ). (C) Two burns with broader profile than those shown in (A), lying each side of a hair follicle. The sections were taken from specimen 229RA in which two burns were applied (0.9 J left and 2.3 J right). The asterisk indicates the level of the hair bulb (HE  $\times 30$ ). (D) Section demonstrating a broad area of superficial tissue ablation and a triangular area of underlying necrosis (\*), are as a result of patient movement throughout the procedure (specimen 941003A: 2.0 J). A very deep lash follicle is seen within fibres of orbicularis (HE  $\times 35$ ). (E) Burn with a good profile, but which is misdirected and stops short of the presumed target follicle (HE  $\times 28$ ).

direction away from the region to be lasered. The laser application was the same as that described above. Tear secretions into the treatment area were dried before further applications. Treatment was continued until a visible pit (deemed to be of sufficient depth) was produced. Following the laser treatment,

patients were immediately transferred to a day theatre, where the block of tissue treated with the laser was excised.

**Histological and morphometric techniques.**

Following laser epilation and excision the tissue was fixed immediately in glutaraldehyde (2%) and cut into vertical



**Figure 2** A graph correlating energy versus depth shows an increase in tissue damage at levels >2 J. The depth of necrosis also increases with increase in the total energy applied. The relation between energy and depth of burn is non-linear. (The burns made in non-viable tissue are shown by an asterisk.)

blocks for paraffin histology. Serial sections (5  $\mu\text{m}$ ) were stained with haematoxylin and eosin. A Leitz Orthoplan microscope with a video camera was used to generate images on a computer monitor screen at a final magnification of  $\times 250$ : the image was calibrated with a micrometre slide. In each of the serial sections, the outline of the burn, and the associated region of necrosis were delineated using a manual cursor on a graphics tablet and the area of the defect with the maximum dimensions was used for cone and volume extrapolations.

The morphometric study was carried out by one observer (LAW): repeatability tests and interobserver tests were not performed. Area and volumetric analysis was provided by an image analysis programme (VIDS-V, Analytical Instruments, Pampisford, Cambridge, UK). Three dimensional reconstructions of burns and the adjacent structures were created from histological serial sections using a VIDS 3D reconstruction programme (Synoptics, Cambridge, UK).

## RESULTS

### Appearance of burns in non-viable tissue

In sections that contained the centre of a burn (Fig 1A), a triangular defect pointing towards the lash bulb was seen within the epidermis and dermis. Regions of coagulative necrosis surrounded each defect (Fig 1B). A narrow epidermal defect indicated accurate alignment of consecutive laser burns. Serial sections often contained two burns in different planes of section (Fig 1C).

### Viable tissue

In live tissue the burn profiles were more variable, generally being much broader than in dead tissue. The burn pits were filled with haemorrhage and tissue debris. In some sections a superficial defect was present (Fig 1D). In others, there were burns with a narrow profile, which stopped short of the target hair bulb (Fig 1E).

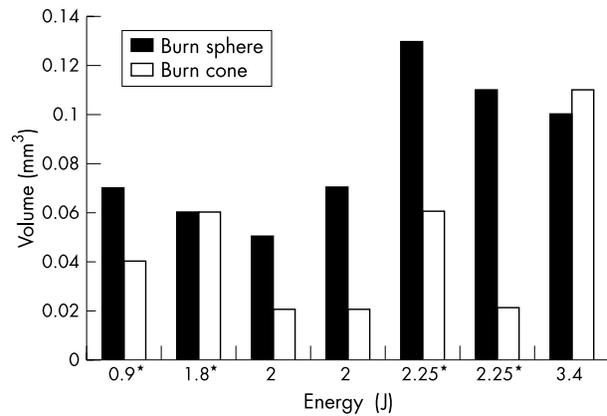
### Morphometry of the defects, areas of necrosis, and follicle depth

#### Non-viable tissue

The laser produced burn depths which varied between 0.7 and 1.1 mm and necrosis extended to a maximum depth of 1.3 mm (Fig 2). Burn widths varied between 0.3 and 0.5 mm and necrosis widths 0.6–0.7 mm.

#### Viable tissue

Burn depths varied between 0.5 mm and 0.8 mm, with depths of necrosis which extended to a maximum of 0.9 mm (Fig 2).



**Figure 3** A graph correlating energy and volume of burn. (The burns made in non-viable tissue are shown by an asterisk.)

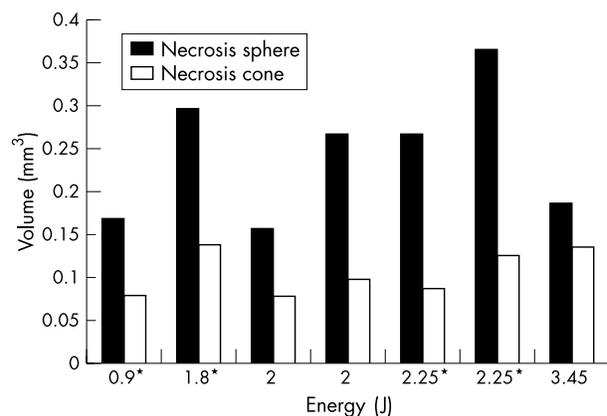
Burn widths in live tissue were up to twice that in dead tissue and varied from 0.3 mm up to 1.0 mm, despite a similar spot size of 100  $\mu\text{m}$  (0.1 mm). Necrosis widths ranged from 0.6 to 1.0 mm. The high energy burn (4 J) did not produce a deep burn because of patient reactions.

Hair follicle depths obtained from both live and dead tissue samples ranged from 0.8 mm to 1.9 mm.

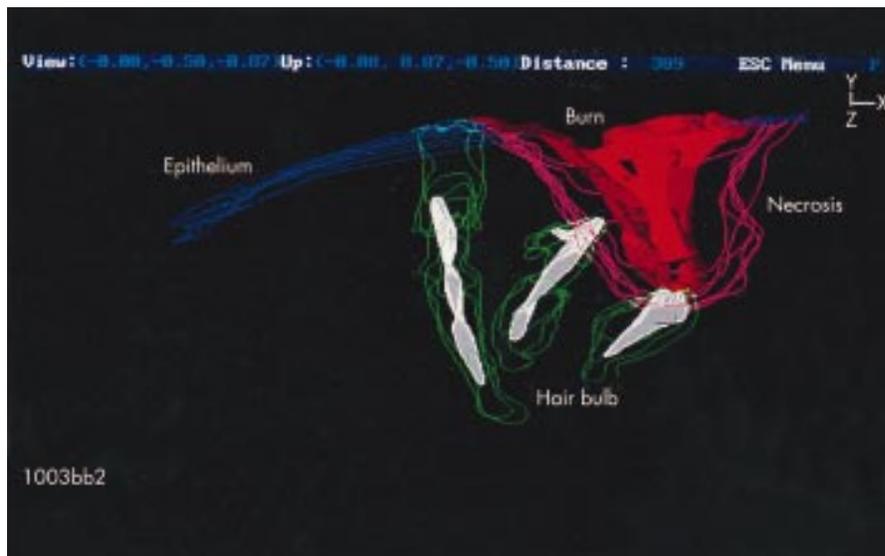
### Correlation between energy delivered and volume of tissue destroyed

Plotting burn energy against depth revealed a tendency to increase tissue damage at levels greater than 2 J (Fig 2). The depth of necrosis also increased with the total energy applied. The relation between energy and depth of burn was non-linear. An initial depth of 0.7 mm was achieved using 1.0 J, but the depth increased only by approximately 0.2 mm per additional joule input thereafter. Total energy levels of >3 J achieved superficial burns as a result of the patient's inability to remain immobile.

From the two dimensional images in serial sections, the image analysis programme was used to reconstruct the volume of tissue destroyed in terms of spheres and cones (Figs 3 and 4). The calculated values were similar for energy levels of the order of 1–2 J, but the burn cone and necrosis cone values were higher with 3.4 J. Calculation of sphere volumes introduced an inconsistent pattern. This trend was evident with 1.0 J of laser energy (delivered at 100  $\mu\text{m}$  spot size at 0.1 second duration) ablating less than  $2.0 \times 10^{-2}$  mm cube of tissue.



**Figure 4** A graph correlating energy and volume of necrosis. (The burns made in non-viable tissue are shown by an asterisk.)



**Figure 5** Computer reconstruction of burn. The burn and surrounding necrosis transects two cilia, but is directed away from the follicles and is of insufficient depth.

### Results of three dimensional reconstruction

By reconstructing in three dimensions the block of tissue undergoing laser ablation, the spatial relation between the burn and the hair follicles could be visualised. In one example, reconstruction demonstrated a burn that had cut across two hair shafts, but was insufficiently deep to reach either follicle, and was directed away rather than towards a hair follicle (Fig 5).

### DISCUSSION

The energy levels used in the present investigation were very similar to those described recently by Basar *et al*,<sup>11</sup> who claimed a success rate of 75% and a burn depth of 2–3 mm. Efficient energy delivery of the laser requires accurate focusing of the base of the burn, allowing smoke to clear and the maintenance of a tear free lid margin. The absence of these complications provided greater burn depths with better profiles as was noted in the results obtained in dead tissue.

The problems associated with the argon laser photocoagulation technique are widely reported in the literature<sup>1–10</sup> and those experienced in the present study were similar. Most importantly, all but one of the burns was of insufficient depth to reach the deeper lash follicles. This problem was highlighted by Huneke<sup>7</sup> who felt that a high clinical recurrence rate was, in part, because of a failure of penetration and suggested a burn depth of 2–3 mm. Gossman *et al* measured the depth of tissue vaporisation both in rabbit models<sup>2</sup> and humans<sup>9</sup> using a calibrated gauge and identified the required depth in cadavers which identified lash follicles 1.5–2.0 mm from the eyelid margins. Bartley and Lowry<sup>8</sup> suggested that rotating the lid away from the globe allowed the laser to be directed parallel to the lash shaft, and Gossman *et al*<sup>9</sup> stated that by using high magnification, a small stump of a lash could often be visualised at the base of the burn allowing better beam orientation. Despite achieving some burns in vivo with an excellent profile (narrow neck, deep burn), these were found on three dimensional image reconstruction to have missed the target follicles. This was because, once through the epithelial surface, it was not possible to ensure that the burn was in coaxial with the hair shaft and follicle.

Three out of four patients experienced little or no discomfort and remained still during the procedure so that some uniformity was obtained in the energy versus depth results. None the less, the fact that burn widths in live tissue were up to twice that in dead tissue simply reflects the effect of minor eyelid movements. In the patient who experienced

pain, histology revealed shallow burns extending to the superficial fibres of the orbicularis muscle, so that any movement of the head or lid results in broad based shallow burns that are completely ineffective.

Increasing the duration of the burn to 0.2 seconds was effective in producing a deep burn with a good profile in 10 laser bursts in dead tissue (Fig 1A), and energy at this level has been reported as producing minimal discomfort.<sup>6</sup> More prolonged burns require infiltrative anaesthesia in addition to topical anaesthesia.<sup>9</sup> It may therefore be reasonable to give infiltrative anaesthesia to allow more accurate burn placement, but this would not overcome the problem of misdirection once below the epithelium.

### Authors' affiliations

**S Hanumanthu, L A Webb**, Department of Ophthalmology, Royal Alexandra Hospital, Paisley, UK

**W R Lee**, Tennent Institute of Ophthalmology, Gartnavel General Hospital, Glasgow, UK

**J Williamson**, Southern General Hospital, Glasgow, UK

Correspondence to: Professor William R Lee, Department of Ophthalmology, Gartnavel General Hospital, Great Western Road, Glasgow G12 0YN, UK; wr1v@clinmed.gla.ac.uk

Accepted for publication 9 September 2002

### REFERENCES

- Sullivan JH**, Beard C, Bullock JD. Cryosurgery for treatment of trichiasis. *Trans Am Ophthalmol Soc* 1977;**74**:189–202.
- Gossman DM**, Brightwell JR, Huntington AC, *et al*. Experimental comparison of laser and cryosurgical cilia destruction. *Ophthalmic Surg* 1992;**23**:179–82.
- Bartley GB**, Bullock JD, Olsen TG, *et al*. An experimental study to compare methods of eyelash ablation. *Ophthalmology* 1987;**94**:1286–9.
- Berry J**. Recurrent trichiasis: treatment with laser photocoagulation. *Ophthalmic Surg* 1979;**10**:36–8.
- Campbell DC**. Thermoablation treatment for trichiasis using the argon laser. *Aust N Z J Ophthalmol* 1990;**18**:427–30.
- Sharif KW**, Arafat AF, Wykes WC. The treatment of recurrent trichiasis with argon laser photocoagulation. *Eye* 1991;**5**:591–5.
- Huneke JW**. Argon laser treatment for trichiasis. *Ophthal Plast Reconstr Surg*. 1992;**8**:50–5.
- Bartley GB**, Lowry JC. Argon laser treatment of trichiasis. *Am J Ophthalmol* 1992;**113**:71–4.
- Gossman MD**, Yung R, Berlin AJ, *et al*. Prospective evaluation of the argon laser in the treatment of trichiasis. *Ophthalmic Surg* 1992;**23**:183–7.
- Yung CW**, Massicotte SJ, Kuwabara T. Argon laser treatment of trichiasis: A clinical and histopathologic evaluation. *Ophthal Plast Reconstr Surg* 1994;**10**:130–6.
- Basar E**, Ozdemir H, Ozkan S, *et al*. Treatment of trichiasis with argon laser. *Eur J Ophthalmol* 2000;**10**:273–5.