Eye (1989) **3,** 719–726

Morphological Effects of Argon Laser Trabeculoplasty upon the Glaucomatous Human Meshwork

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Summary

Thirty four trabeculectomy specimens from open angle glaucoma patients who had under argon laser trabeculoplasty as part of their treatment, were studied by scanning electron microscopy. Three of these were also examined by transmission electron microscopy. The intervals between laser therapy and surgery ranged from one month to five years. A sub-group consisted of six patients who had received laser treatment on more than one occasion prior to surgery. Electron microscopy revealed distortion of trabecular beams, loss of trabecular endothelial cells and the development of a cellular sheet extending from the region of Schwalbe's line and covering the anterior surfaces of the anterior portion of the uveal meshwork. The sheet occluded the trabecular spaces of the region; thus, when extensive, contributing to laser trabeculoplasty failure. It is concluded that argon laser trabeculoplasty induces a repair process, in the form of repopulating a cellular deficient meshwork, but which can become detrimental as a consequence of its success.

The argon laser trabeculoplasty (ALT) method of Wise and Witter[1] has been shown to be successful in lowering the intraocular pressure of a high percentage of chronic open angle glaucoma patients.[2,3] ALT can defer surgery for the remaining lifespan of elderly patients and has controlled open angle glaucoma in others for over 10 years.[4] Unfortunately the beneficial intraocular pressure lowering effects of ALT may be superceded in the course of time to the extent that trabeculectomy is required.[3,4]

The means by which ALT produces the clinically observed decrease in pressure has been accounted for by both mechanical changes to the trabecular beams[1,5] and active cellular mechanisms.[6,7] Rodrigues *et al.*[6] described the ultrastructural changes induced by ALT in 22 trabeculectomy specimens obtained from six weeks to one year post treatment. Weber *et al.*[5] studied three similar specimens taken two to eight months post ALT but with conflicting results. Specifically, Weber *et al.*[5] did not observe the intratrabecular-space-occluding cellular sheet that was a feature of Rodrigues *et al.*[6] These specimens may be considered to represent failed ALT. It is the purpose of this communication to present our own cases (34) of ALT failure, in the hope that by elucidating the pathology of failure one may be able to more fully understand the cellular responses evoked by ALT and to reconcile the different findings of Rodrigues[6] and Weber[5] by virtue of being a third report.

Materials and methods

Thirty-four trabeculectomy specimens were available for study from eyes that had received

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Table I *The cases studied*

Case	*Time post ALT*	*Cell sheets (coronal×advance μm)*	*Speciment length* (coronal μm)*
1	4W	negligible	2050
2	1M	300×400	1900
3	1M	120×50	1400
4	1M	negligible	1200
5	1M	100×100	1700
6	2M	350×250	2050
7	2M	negligible	1500
8	4M	250×150	1700
9	7M	500×300	1700
10	8M	1000×200	1800
11	9M	300×300	1500
12	9M	375×60	1250
13	10M	200×200	1600
14	11M	700×350	1900
15	1Y 1M	150×150	1300
16	1Y 1M	250×200	2600
17	1Y 2M	350×200 & 200×200	1400
18	1+Y	150×150	2200
19	2Y 1M	300×250	2500
20	2Y 2M	1500×300	2250
21	2Y 6M	250×150	1900
22	3Y	150×200	3300
23	3Y 9M	350×300 & 300×300	3750
24	4Y	250×200	3750
25	5Y	250×250 & 400×400	2150
26	?	negligible	1800
27	?	150×150	1800
28	?	200×200	2150
29	2M and 1M	100×50 & 100×100	1650
30	1+Y and 1Y	2000×600	2050
31	2Y and 1Y7M	150×100 & 90×100	2700
32	3Y, 2Y and 4W	400×300 & 120×250	1600
33	1Y 10M, 5M and 3M	200×200 & 150×200	2900
34	1Y 9M, 1Y, 4M and 2M	500×300	2150

* Largest measurement of trabecular meshwork in the direction parallel to Schwalbe's line.

argon laser trabeculoplasty at some time in the course of treatment for chronic open angle glaucoma. The ALT procedure was performed by nine different surgeons, including: Mr. P. G. Watson (Cambridge, ten cases); Mr. E. D. Allen (Sunderland, nine cases); Mr. S. N. Rizk (Nottingham, five cases); Mr. R. A. Hitchings (London, three cases) and Mr. R. Bates (London, two cases). Technical information concerning the laser procedure and clinical details covering changes in IOP were not available in all instances. When described the ALT procedure closely followed that of Wise and Witter.[1] At the time of surgery the patients' ages ranged from 43 to 83 years. Table I outlines the number of cases studied with respect to the time ALT was performed prior to trabeculectomy.

Trabeculectomy specimens were fixed by immersion in buffered glutaraldehyde for at least 24 hours, followed by buffered osmium tetroxide for 1.5 hours. Each specimen was dehydrated through ascending concentrations of ethanol prior to critical point drying and coating with gold. The inner surfaces of the trabecular meshwork and peripheral cornea were studied using an Hitachi S520 scanning electron microscope. Transmission electron

microscopy of Araldite embedded material was performed on three cases andstudied using a Jeol 100C electron microscope.

Results

The most obvious abnormality of the inner surface of 30 out of the 34 trabeculectomy specimens was the presence of cellular sheets (Fig. 1) which encroached upon the anterior uveal meshwork, either 'advancing towards' or covering presumed laser impact sites. Smaller versions of the cellular sheets (wave fronts) were seen as significant irregularities in the outline of the corneal transitional zone endothelium. Both the larger cellular sheets and the smaller wave fronts were seen as continuations of theperipheral corneal endothelium. Table I relates the size of the trabeculectomy tissue and the dimensions of the cell sheets for each of the specimens studied.

One treatment with ALT

The one to five month post ALT group exhibited wave fronts or sheets which ranged in size from small 120 μm (wide, coronal) by 50 μm (advance, antero-posterior) to medium 300×400 μm.

All measurements are approximated as the cellular sheets are not regular rectangles and in some instances the exact termination of the peripheral cornea was unclear. The seven to 14 month post laser group displayed similar sheets but which were generally larger in one or more directions (Fig. 2). Examples ranged from 150×150 μm to 700×350 μm and 1000×200 μm. This larger response, which occluded a large amount of intratrabecular space, may correspond to an amalgamation of individual wave fronts or sheets. In the two to five year post ALT group there did not appear to be a correlation between size of sheet and time following laser treatment; since the largest sheet, measuring 1500×300 μm, was present in a two year two month post ALT specimen (Fig. 3). Whereas the case obtained five years after treatment exhibited a sheet only 250×250 μm in size. Transmission electron microscopy (Fig. 4) revealed that the occluding sheet and wave fronts were formed by a monolayer of cells which were continuous with those of the transitional zone endothelium.

In two cases, the cellular sheets were seen beneath the most superficial trabecular beams rather than over. In four instances, ranging from one to two months and an unknown time post ALT, no cellular sheet or wave front was conclusively identified.

Many of the cells forming the sheets, in

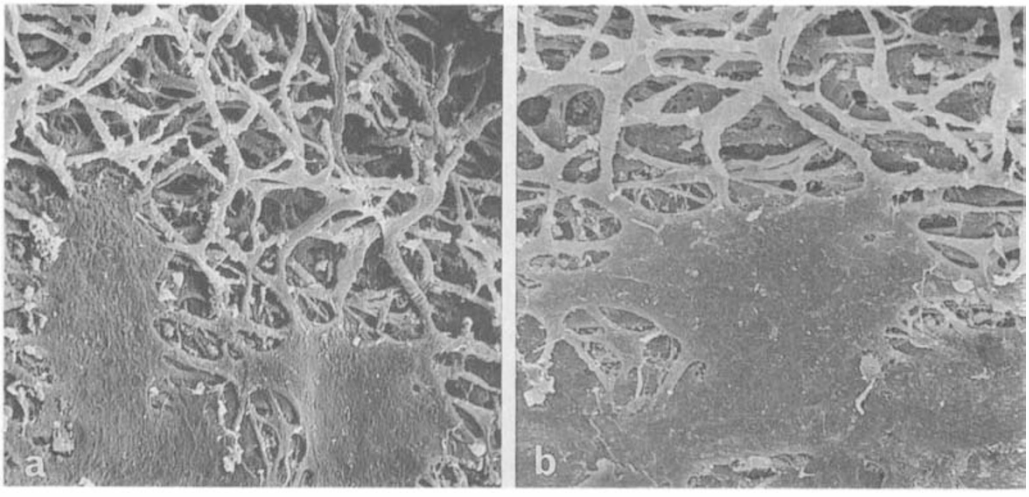

Fig. 1a. *Scanning electron micrograph showing two small endothelial sheets covering areas of the anterior uveal meshwork. One of the sheets is seen to overlie a shallow depression. (24 and 19 months post ALT, ×350).*

Fig. 1b. *The small, intratrabecular space occluding, sheet is 150μm wide×100 μm advance over the meshwork. It is seen to be continuous with the transitional zone endothelium. (24 and 19 months post ALT, ×400).*

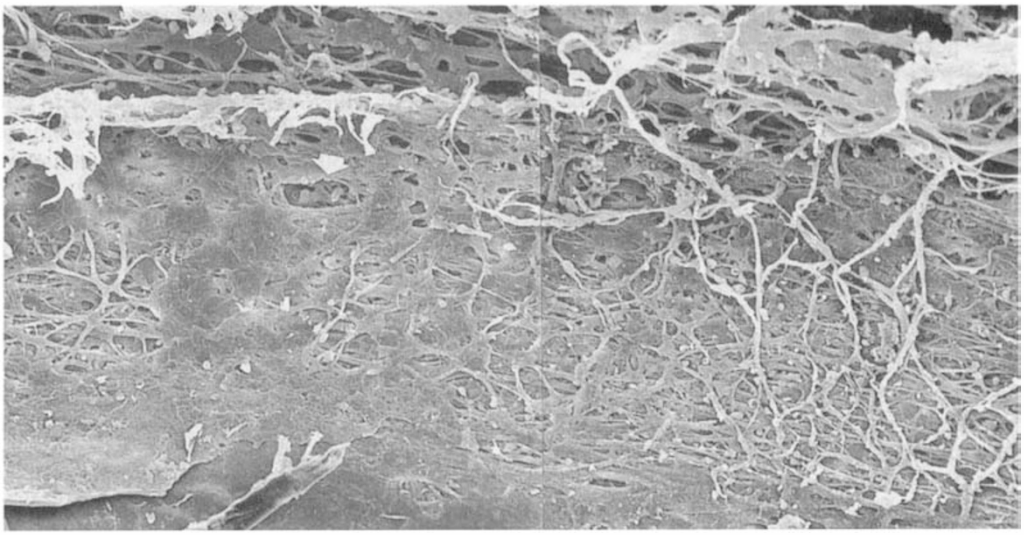

Fig. 2. *Low magnification montage showing a 350×200 μm cellular sheet encompassing on 50 μm hole (arrow). An inflammatory response is noted. (1y2m post ALT, ×210).*

common with a number of corneal transitional endothelial cells appeared activated, in that they exhibited numerous small microvilli-like surface processes (Figs. 4 and 5).

Some trabeculae at the margins of the occluding sheet (Fig. 6) or seen through deficiencies in the cellular cover were observed to be broken or distorted. Many were also seen to lack an enveloping endothelium (Fig. 7). A localised combination of distorted and denuded trabeculae were interpreted as being associated with the site of laser impact. The loss of trabecular cells resulted in connective tissue components becoming less compact, so causing the beams to appear thickened. A small number of surviving trabecular endo-

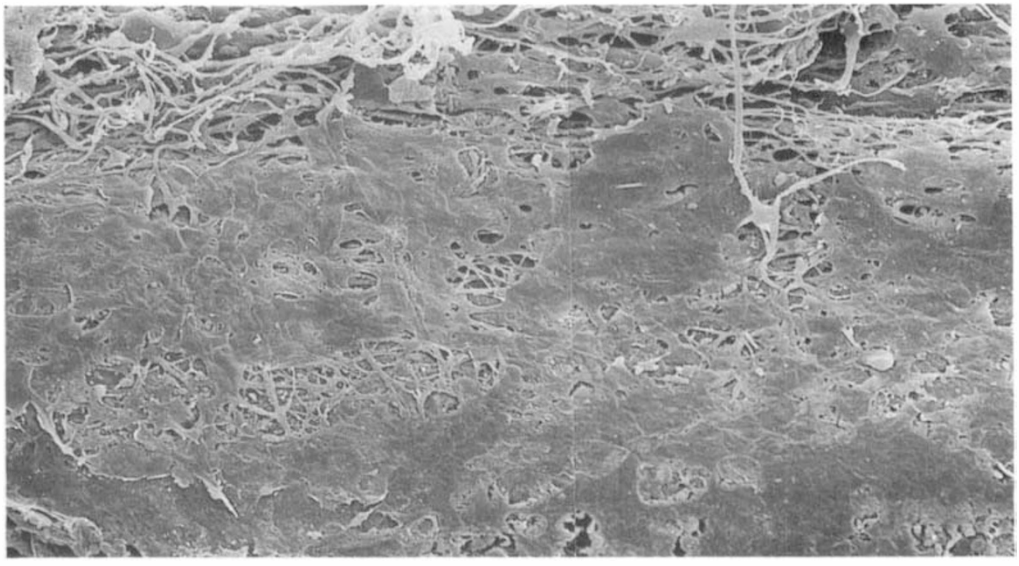

Fig. 3. *Low power montage showing extensive coverage of the uveal meshwork by connected cellular sheets. Deficiencies in the sheet reveal trabecular beams. (2y2m post ALT, ×210).*

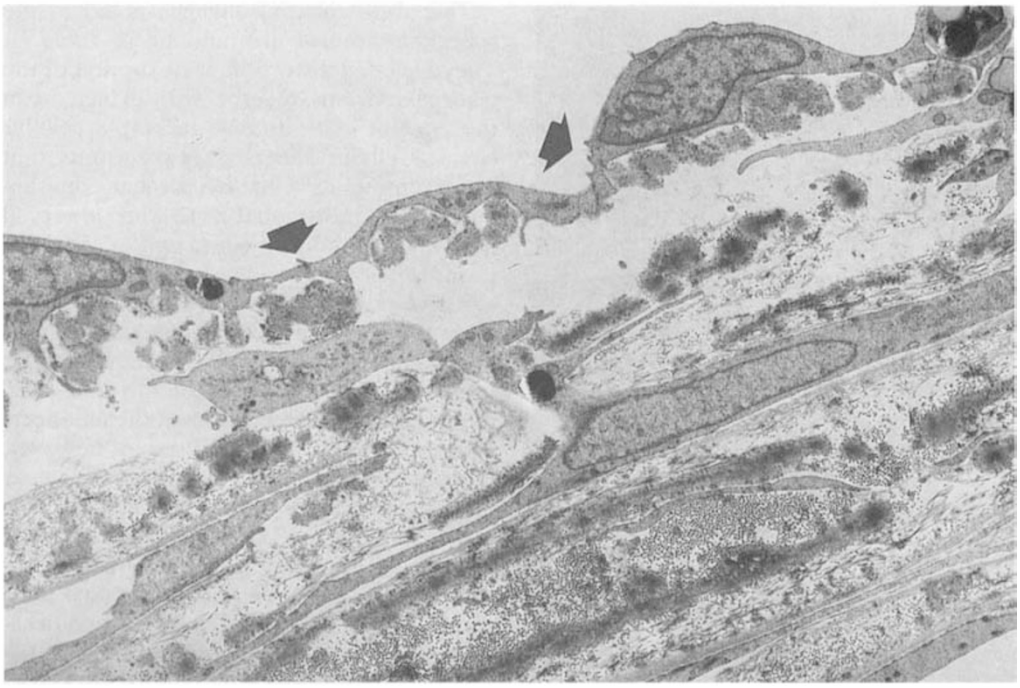

Fig. 4. *Transmission electron micrograph showing the intratrabecular space occluding sheet to be formed by a cellular monolayer. Small microprocesses (arrows) are present. The deeper trabecular beams appear normal. (14m post ALT, ×6,600).*

thelial cells around the laser impact site also exhibited signs of cellular activation. Six cases possessed a small number of long, thin migratory cells upon the corneal transitional en-

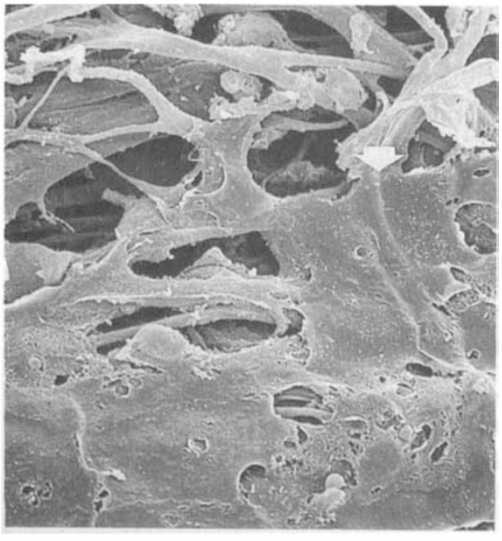

Fig. 5. *The flat plate-like cells are shown to be occluding the intratrabecular spaces. Microprocesses are seen as mottling of the cell surface (arrowed cell). (2y2m post ALT, ×1,000).*

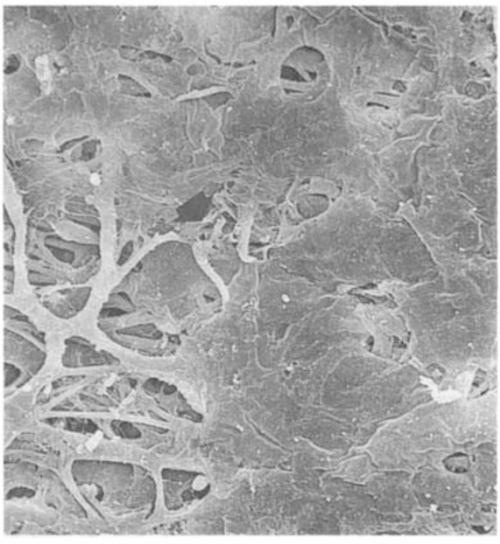

Fig. 6. *Showing distorted trabeculae (arrow) at the lateral margin of a cellular sheet. (1y2m post ALT, ×600).*

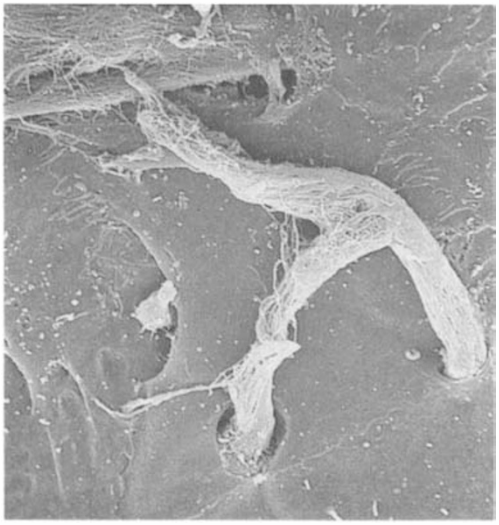

Fig. 7. *Higher magnification of a broken trabeculum, protruding from an occluding sheet. The beam lacks an overlying endothelium. (2y1m post ALT, ×3,000).*

dothelium. It was not possible to identify the direction of travel.

Multiple treatment with ALT

Six of the cases studied were of patients who had received ALT on two or more occasions prior to trabeculectomy. All of the specimens exhibited one or more intra trabecular space-occluding cellular sheets (Fig. 8) which were seen to be continuous with the endothelial cells of the transitional zone. The smallest individual sheet measured 100 μm (wide) by 50 um (advance), whilst the largest was 2000 by 600 um and covered most of the excised meshwork. As with the single treatment cases individual specimens displayed distorted and denuded trabeculae, cellular activation and cell migration. It was not possible to distinguish between older and more recent laser lesions.

Discussion

It has become apparent that the beneficial effect of ALT as a means of lowering the intraocular pressure is only temporary in some glaucoma patients.[3] In the present study, trabeculectomy specimens were examined from eyes that had had their intraocular pressure controlled, following ALT, for up to five years before requiring surgery. A second group had received ALT on more than one occasion.

The ultrastructural changes noted in the specimens studied are outlined in Table II. They included distortion or disruption of the trabecular beams, together with an increase in the amount of trabeculae lacking a cellular cover. Cellular sheets or wave fronts that were continuous with the corneal endothelium of the transitional zone were observed partially or totally to cover and/or 'advance towards' the presumed laser impact site. The size and extent of the intratrabecular space-occluding sheet was not unequivocally related to the time interval between ALT and surgery. In general the one to five month post ALT group had less extensive endothelial sheets than the seven to 14 month group; however, exceptions were noted. The two to five year group was clearly not time related. The largest single cellular sheet observed, 2000 um wide by 600 um advance over the uveal meshwork, was present in a specimen taken from an eye that had received ALT on two occasions—one year and more than one year—prior to surgery. This is considered to represent an amalgamation of individual lesion wave fronts or sheets. Seven specimens possessed two completely separate cell sheets. The production of large or multiple cell sheets was not surgeon related.

The changes induced by ALT in human glaucomatous angular structures have been documented by Rodrigues' *et al.*[6] (22 cases) and Weber *et al.*[5] (three cases). Weber confirmed Rodrigues' observations of trabecular disruption and tissue debris formation but did not observe Rodrigues' finding of sheets of abnormal trabecular and/or corneal endothelial cells which covered the laser burns. We can confirm both Rodrigues' and Weber's observations since four or our cases did not exhibit a cellular sheet. In two of our cases the endothelial sheet was present beneath the most superficial beams of the uveal meshwork. The lack of an observed cellular sheet could also be a trabeculectomy sampling problem or that the laser impact site was in the posterior meshwork. Van der Zypen and Fankhauser[8] studied the effect of ALT upon the monkey trabecular meshwork and concluded that it is advantageous to irradiate the posterior trabeculae so as to minimise corneal endothelial involvement.

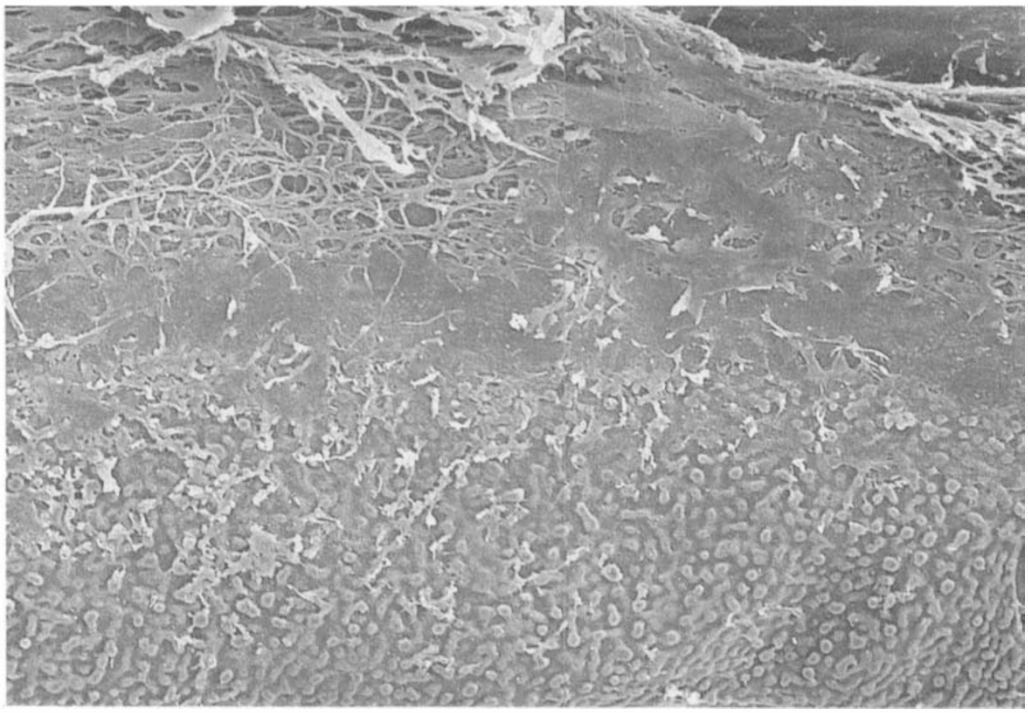

Fig. 8. *Low power montage showing a 500×300 μm cell sheet. Note that the Hassal-Henle excrescences of the peripheral cornea lack a covering endothelium. (ALT×4, ×120).*

The means by which ALT causes the clinically observed decrease in intraocular pressure in human glaucomatous eyes has not been established, other than that a direct communication between the uveal meshwork and Schlemm's canal is not required.[1] Mechanical, heat induced shrinkage of trabecular collagen has been suggested to cause the circumference of the meshwork to become smaller, move inwards and thus reopen the interbrabecular spaces.[1,5] It is of interest that the temperature required to cause collagen to contract is increased when it is under tension.[9] Bylsma *et al.*[10] argue against a purely mechanical reaction by commenting that the time course of ALT response is rather slow, whilst heat-induced shrinkage would be relatively quick.

The cellularity of the trabecular meshwork is known to be reduced as a consequence of normal ageing[11,12] and with chronic open angle glaucoma.[13,14] It has also been shown that trabecular endothelial cells are lost from the normal human trabecular meshwork when ALT is performed both *in vivo* before enucleation[15] and *in vitro* upon donor eyes.[7,16] We suspect that ALT may cause a further loss of trabecular cells in glaucomatous eyes at and near the laser impact site as evidenced by the amount of denuded trabeculae observed. A significant increase in trabecular endothelial cell number has been noted within two days of *in vitro* ALT in human organ cultures.[7,10] The proliferating cells[7,10] are considered to be a specialised population that are located anterior to the filtering portion of the meshwork,[16,17] at the corneal transitional zone.[18] It has been suggested that these proliferating cells migrate to the laser impact site[16] and perform the functions of normal trabecular endo-

Table II *The effects of ALT*

1. Trabecular endothelial cell death.
2. Distortion of trabecular beams.
3. Activation of surviving trabecular cells.
4. Activation/proliferation of transitional zone endothelial cells.
5. Wave front/cell sheet production.
6. Occlusion of laser impact site.
7. ? occlusion of meshwork.

thelial cells such as phagocytosis and glycosaminoglycan production; both factors that are known to affect the rate of aqueous drainage.[7] We did not observe evidence of cell division within the occluding sheet cells or at the transitional zone. However, we did observe cellular activation of surviving trabecular cells, sheet cells and transitional zone cells. Activation was seen as an increase in the amount of cellular surface processes. Trabecular endothelial cells are known to adopt an active form as a consequence of a number of insults, including tissue injury.[18] The production of surface microprocesses by cultured meshwork cells has been shown to be a sign of phagocytic activity.[19] As it has been suggested that the proliferating endothelial cells observed after ALT treatment might be phagocytic,[7,10] then such stimulated cleaning function, if long lasting, could be involved with the success of ALT. However, when these cells are present in such numbers as to form the large confluent sheets seen in some of our cases, it could form a physical barrier to aqueous outflow. If this appearance is representative of the complete 360°of the trabecular meshwork it must contribute to ALT failure. Thus we suggest that ALT can be considered to induce a repair mechanism, in the form of repopulating a cellular deficient meshwork but which ultimately becomes non-beneficial as a result of its success.

The authors would like to thank Mrs. P. Goodwin for secretarial assistance and Mr. Allen (Sunderland), Mr. Rizk (Nottingham) and Mr. Watson (Cambridge) for providing most of the specimens. Support has been provided by the International Glaucoma Association (IGA) and the Wellcome Trust.

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