ARTICLE

# Repair in the rabbit outflow system

IAN GRIERSON, WILLIAM UNGER, LYNNE WEBSTER, PENNY HOGG

**Abstract**

**An *in vivo* study was conducted to study repair processes in the injured rabbit outflow system. A uniform injury was produced by raising intraocular pressure (IOP) manometrically to 70 mmHg for 1 h. The recovery process, which was followed clinically for 8 weeks and morphologically for 6 weeks, led to the re-establishment of normal meshwork architecture within this period. The morphological studies included light microscopy, autoradiography and electron microscopy. The initial lesion consisted of large deficits in the meshwork with breakdown of cell-to-cell connections, loss of extracellular materials and disruption of the vessels of the aqueous plexus. There was a significant lowering of IOP in the first week of recovery, which thereafter climbed back to normal. Also in the first week the meshwork became infiltrated with inflammatory cells which cleared by 4 weeks. There was some meshwork cell death by either necrosis or apoptosis. The majority of meshwork cells became activated within the first few days and remained activated for at least the first 2 weeks. Tritiated proline incorporation was maximal between 1 and 2 weeks. Tritiated thymidine labelling was seen throughout, but only after the inflammation subsided was it clear that meshwork cells in all regions of the meshwork were proliferating. Our study provided no evidence that normal meshwork cells have a basal proliferative turnover level. Our injury model involved complete repair of the outflow tissues and that required meshwork cells to become activated, mobilise, undertake synthetic activity and proliferate. This is the first example, other than argon laser trabeculoplasty, where meshwork cells *in vivo* have been induced to divide. Possible therapeutic implications for glaucoma are discussed.**

*Key words* Apoptosis, Injury, Meshwork cells, Outflow system, Proliferation, Repair

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The healing and repair potential of the trabecular meshwork following various forms of trauma is still poorly understood and recent literature on the subject is lacking. Following trabeculectomy, quite obviously, the deficit is not filled in and repair activities seem to stop at the cut edge of the wound.[1,2] Similar events distinguish meshwork repair following trabeculotomy[3] and ciliary muscle disinsertion procedures.[4] After these major lesions, evidence of meshwork cell proliferation has not been reported[1–4] but meshwork cells do take on the morphological appearance of being 'activated'.[1,2,4] Activation is characterised by a nucleus rich in euchromatin, prominent rough endoplasmic reticulum, abundant Golgi vesicles and obvious lysosomes,[1,5] and has been described in various situations such as that following particulate insult to the meshwork[1,5–9] and during the early stages of organ culture.[10,11]

Perfusion of a range of substances into the meshwork, including cytochalasin B,[12] echothiophate,[13,14] EDTA,[15] alpha-chymotrypsin[16] and ethacrynic acid[17] among others, provokes meshwork cell loss, meshwork cell activation but limited repair and no clear evidence of cellular proliferation. Hamanaka and Bill[16] were surprised to find alpha-chymotrypsin effects several weeks to months after the insult with no evidence of regeneration of cell-denuded trabecular beams.

Major 'trabeculotomy-type' laser lesions also are associated with feeble meshwork repair activity.[18,19] It should be said that Van der Zypen *et al.*[19] did identify some trabecular beam repair, following Nd:YAG lasering, that was associated with meshwork cell activation and some cell proliferation, although the authors stated that 'degeneration appears, however, to eclipse proliferation'.

On the other hand argon laser trabeculoplasty (ALT), which delivers numerous discrete and superficial laser burns to the uveal portion of the meshwork, does seem to be associated with a more vigorous repair action which does not stop at meshwork cell activation but also involves a fair degree of meshwork cell proliferation. There is marked incorporation of tritiated thymidine into meshwork cells following ALT procedures[20–24] and the dividing cells repopulate the area of the burns. Enhanced thymidine incorporation persists for at least 2 weeks after laser application.[21]

It would seem that the repair mechanisms which take place in the trabecular meshwork are variable depending on the nature, and



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perhaps the extent, of the initial lesion. There are considerable gaps in our knowledge, particularly with respect to the excessive cell loss and tissue alteration that characterises the trabecular meshwork in primary open-angle glaucoma.[5,25,26] Might glaucomatous pathology be considered as a defective repair response,[5] especially as ALT is an effective, albeit temporary, means of stimulating the meshwork recovery and producing intraocular pressure (IOP) reduction?[27]

An earlier study from our group had shown that if the rabbit eye was maintained at 50 mmHg for 1 h with a manometer system, the architecture of the outflow system was compromised with loss of tissue cohesion and occlusion of the vessels of the aqueous plexus.[28] As the injury was uniform around the 360° of the outflow system and reproducible, we considered that a sterile pressure/flow lesion would give us valuable insight into meshwork repair. For the present study an even more severe injury was produced by exposure to 70 mmHg, and of particular interest during the recovery period were meshwork cell activation, evidence of matrix reconstruction, synthesis of extracellular components and whether or not proliferation could be demonstrated following non-laser-induced tissue damage.

## Materials and methods

### *Animals*

Adult New Zealand albino rabbits of either sex and weighing between 2.0 and 2.5 kg were anaesthetised with intravenous pentabarbitone (20 mg/kg, May and Baker), together with diazepam (20 mg/kg, Roche), supplemented with additional barbiturate to achieve a constant level of anaesthesia and respiration.

### *Elevation of IOP*

The anterior chamber of one eye of each of 30 rabbits was cannulated with a 23 gauge needle fitted to flexible tubing leading to an adjustable reservoir and, via a two-way tap, to a pressure transducer. The needle, line and tubing were filled with an isotonic saline solution. Initially the eye was isolated from the reservoir but open to the pressure transducer so that baseline IOP could be recorded. Thereafter the flow/pressure injury was achieved by adjusting the reservoir so that IOP climbed up to 70 mHg in a stepwise manner over a period of 3 min and remained at that pressure for 60 min.

After 60 minutes the reservoir was once again closed to the eye via the two-way tap and the IOP of the eye was allowed to fall to its new resting level. When the IOP was stable, the needle was removed from the anterior chamber. Topical antibiotic was applied to both eyes (1% chloramphenicol ophthalmic ointment) while the animals were recovering.

The rabbits were examined daily for the first 14 days for signs of inflammation and thereafter they were subjected to clinical examination at least once per week until the end of the experimental period; levels of tissue hyperaemia, vascular injection and aqueous flare were recorded in a standardised manner for experimental rabbits based on slit-lamp examination.[29] The pupil sizes were determined using fine dividers and IOP was measured with an applanation pneumotonometer (Alcon) calibrated for routine use on the rabbit eye. The maximum follow-up period for our study was 8 weeks.

### *Morphology*

A total of 30 rabbits were killed at varied time periods by intravenous barbiturate overdose up to 42 days following the initial injury. The eyes were enucleated immediately and immersed in cacodylate-buffered glutaraldehyde; to facilitate fixative penetration, each globe was opened by deep postlimbal incision. Following not less than 4 h in primary fixative, the anterior segment of the eye was dissected under buffer and radial portions of the angle region were postfixed in buffered osmium tetroxide, dehydrated through graded alcohols and embedded in Araldite.

Semithin sections for light microscopy and thin sections for electron microscopy were cut on an Ultracut E ultramicrotome (Reichert-Jung). The semithin sections (1 μm) were stained with toluidine blue whereas the thin sections were mounted on copper grids and treated with uranyl acetate and lead citrate. The thin sections were screened and photographed in a Hitachi H600 transmission electron microscope.

Other segments of tissue from the same group of rabbits at the same time intervals up to 42 days after injury were examined and photographed by scanning electron microscopy. The appropriate tissue was dehydrated through graded alcohols or acetone and dried in a critical point drier (Polaron). The dried specimens were mounted on viewing stubs, sputter-coated with gold (Polaron) and examined in a Hitachi S520.

Separate studies were conducted for autoradiographic investigations. A total of 19 (tritiated thymidine) and 18 (tritiated proline) rabbits, at various times following initial IOP injury (2 days, 4 days, 1 week, 2 weeks, 4 weeks and 6 weeks, tritiated thymidine only at the 6 weeks time period) were injected intravitreally with the appropriate radiotracer either 24 or 48 (thymidine only) h before being killed (20 μl injection with a total activity of 20 μCi; Amersham International, UK). The enucleated eyes were fixed and specimens of angular tissue processed into Araldite in the manner described earlier.

Semithin sections of the test material (together with suitable positive and negative controls) were cut and placed on glass slides coated with chrome-alum and gelatine solution (BDH Ltd). After the sections had dried onto the slides they were coated with photographic emulsion (Ilford Ltd) and left in the dark at 4°C for 8 weeks. Then the slides were developed (Kodak D-19), washed, fixed and dried. Thereafter the sections were stained with toluidine blue. A further group of 11 rabbits, without IOP injury, had tritiated thymidine or proline (20 μl, 20 μCi) introduced into the vitreous of one eye either 24 (tritiated thymidine and proline) or 48 h

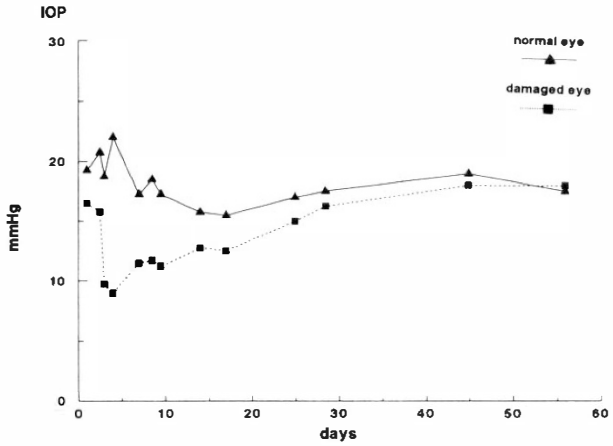


**Fig. 1.** *The mean IOP measurements (based on 30 rabbits) for the treated (squares) and contralateral (triangles) eyes over an 8 week time period. A 50% decrease in IOP is seen in the treated eyes over the first week.*

(tritiated thymidine only) prior to the animals being killed. The appropriate tissue samples were processed for autoradiography in an identical manner to that previously described.

## Results

### *Clinical features*

Following the IOP injury there were some mild signs of inflammation including conjunctival hyperaemia, limbal injection and flare in the anterior chamber. The features were at their most obvious between 3 and 6 days and were absent by 14 days. Applanation pneumotonometry showed a significant (paired *t*-test, $p < 0.001$) ipsilateral reduction in IOP which was maximal at 7 days. The pretreatment IOP measurements in the experimental eyes were 18.6 ± 2.2 mmHg (mean ± standard deviation), which went down to 8.7 ± 1.6 mmHg. Thereafter IOP recovered back to normal levels between 6 and 8 weeks. In the contralateral control eyes, there was a moderate (not statistically significant) rise in IOP over the first 4 days which then fell away (Fig. 1).

### *Normal morphology*

The rabbit's outflow system is located above the posterior extension of the chamber angle called the ciliary cleft. The meshwork is loosely organised with partial trabecular organisation.[28] A plexus of small aqueous-containing vessels, called the aqueous plexus,[30] serves a similar function to Schlemm's canal in primates. Between the vessels and the meshwork is a juxtacanalicular connective tissue (JCT) comparable in structure, if not extent, to that in the primate.

Following intravitreal injection of tritiated thymidine for either 24 or 48 h prior to enucleation there was no evidence of incorporation of thymidine into the nuclei of meshwork cells, endothelial cells of the aqueous plexus of vessels or into the cells of the pectinate ligaments, despite careful scrutiny of at least 12 levels of section around the 360° of tissue available for each of 6 eyes. Twenty-four hours after the intravitreal injection of tritiated proline, labelling in the normal meshwork was modest (Fig. 5a, column 0).

### *Morphology: Immediate damage*

The immediate effect of 70 mmHg IOP for 1 h was to produce large deficits in the meshwork tissues which could be seen clearly by scanning electron microscopy (Fig. 2a). Light microscopy and transmission electron microscopy revealed that cell-to-cell connections had been lost (Fig. 2b). Extracellular material (ECM) within the trabeculae was disorganised and disrupted. Some

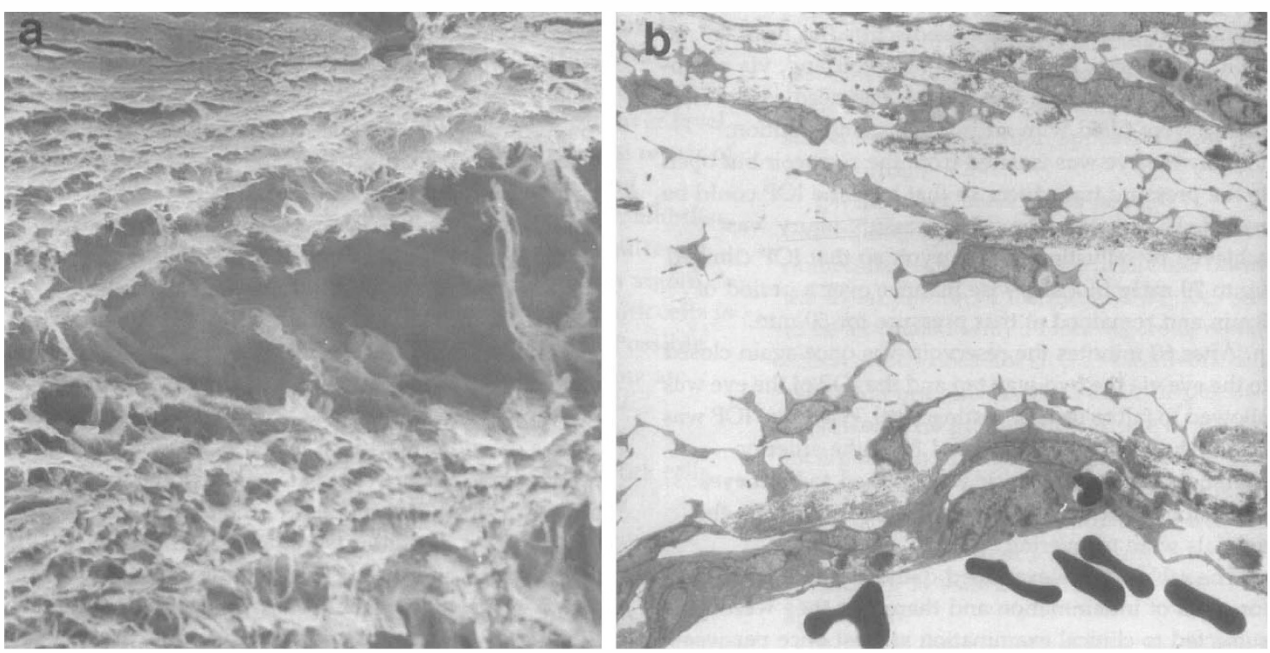


**Fig. 2.** *A scanning electron microscope (a) and a transmission electron microscope (b) view of the lesion immediately following IOP elevation (a, ×550; b, ×1750).*

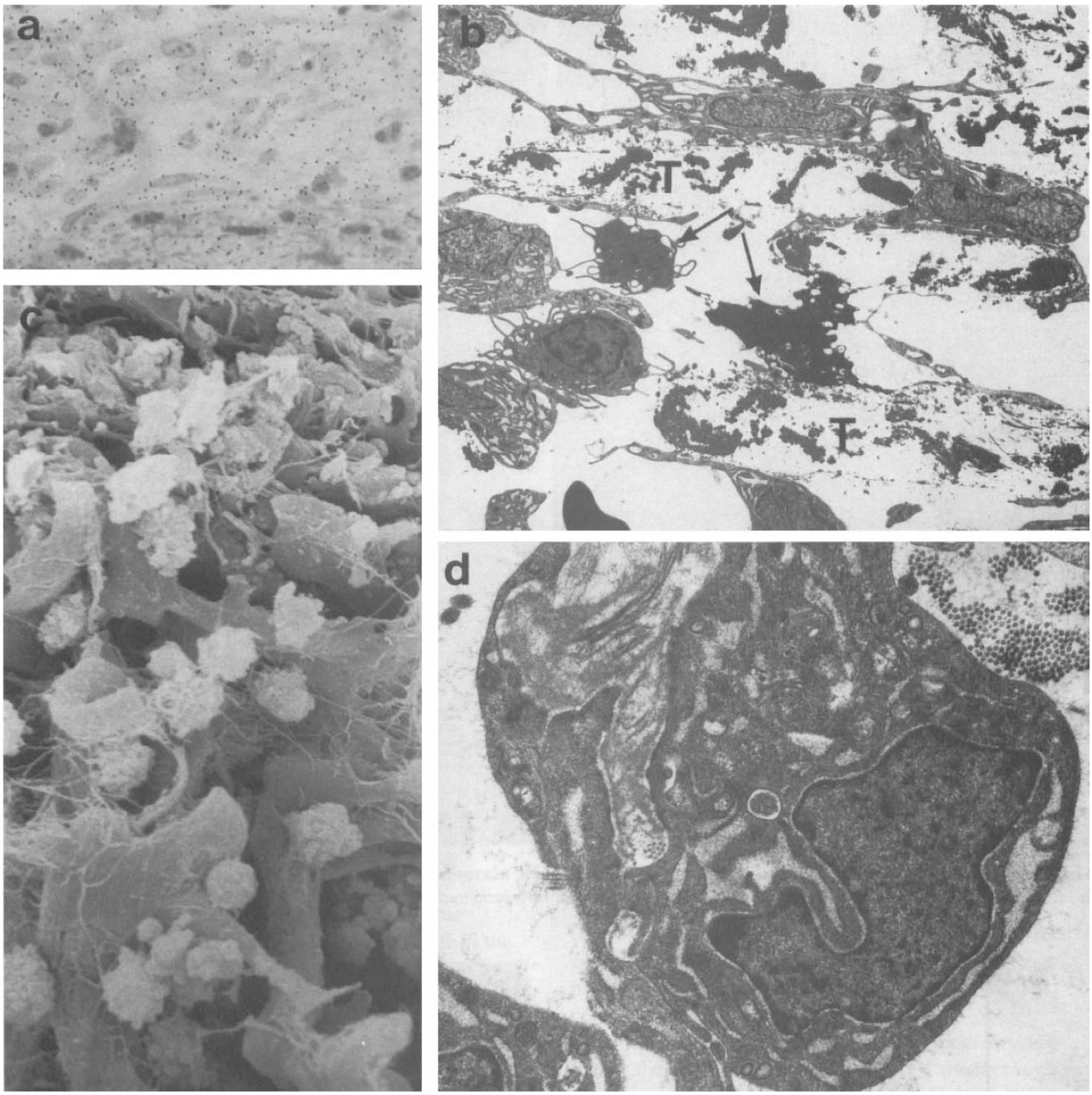


**Fig. 3.** *The outflow system 2 days after injury. (a) A light autoradiograph showing silver grains where there is tritiated proline incorporation. (b) A transmission electron micrograph showing damaged trabeculae (T) and dying cells (arrows) in the meshwork. (c) The inflammatory influx by scanning electron microscopy. (d) A rounded but activated meshwork cell with prominent rough endoplasmic reticulum by transmission electron microscopy (a, ×400; b, ×2500; c, ×1000; d, ×13 000).*

ECMs were scattered throughout the meshwork and deposited in the intertrabecular spaces. Many of the smaller vessels of the aqueous plexus were occluded and the larger ones had deficits in their endothelial lining. The JCT beneath the vessels was distinguished by dilated extracellular spaces containing little or no ECM.

### *Morphology: 1 to 4 days recovery*

By day 1, the disrupted and disorganised tissues of the meshwork contained polymorphonuclear leucocytes and occasional macrophages. On the second day, the polymorphs were beginning to degenerate and some were phagocytosed by native meshwork cells and also by incoming macrophages which were now entering the tissue in substantial numbers (Fig. 3b, c). The endoplasmic reticulum of the meshwork cells was prominent and dilated (Fig. 3d) and some meshwork cells exhibited rounding up. At this stage there was no tritiated thymidine incorporation into the nuclei of either meshwork cells or invading inflammatory cells. The incorporation of tritiated proline into the angular tissue was modest (Fig. 3a) but, on the basis of grain counts, was about twice the incorporation seen in injured controls (Fig. 5a).

By the 4 day recovery time period, polymorphs were rare but infiltrating macrophages were plentiful. The bulk of the meshwork cell population had activated nuclei with an abundance of euchromatin and an absence

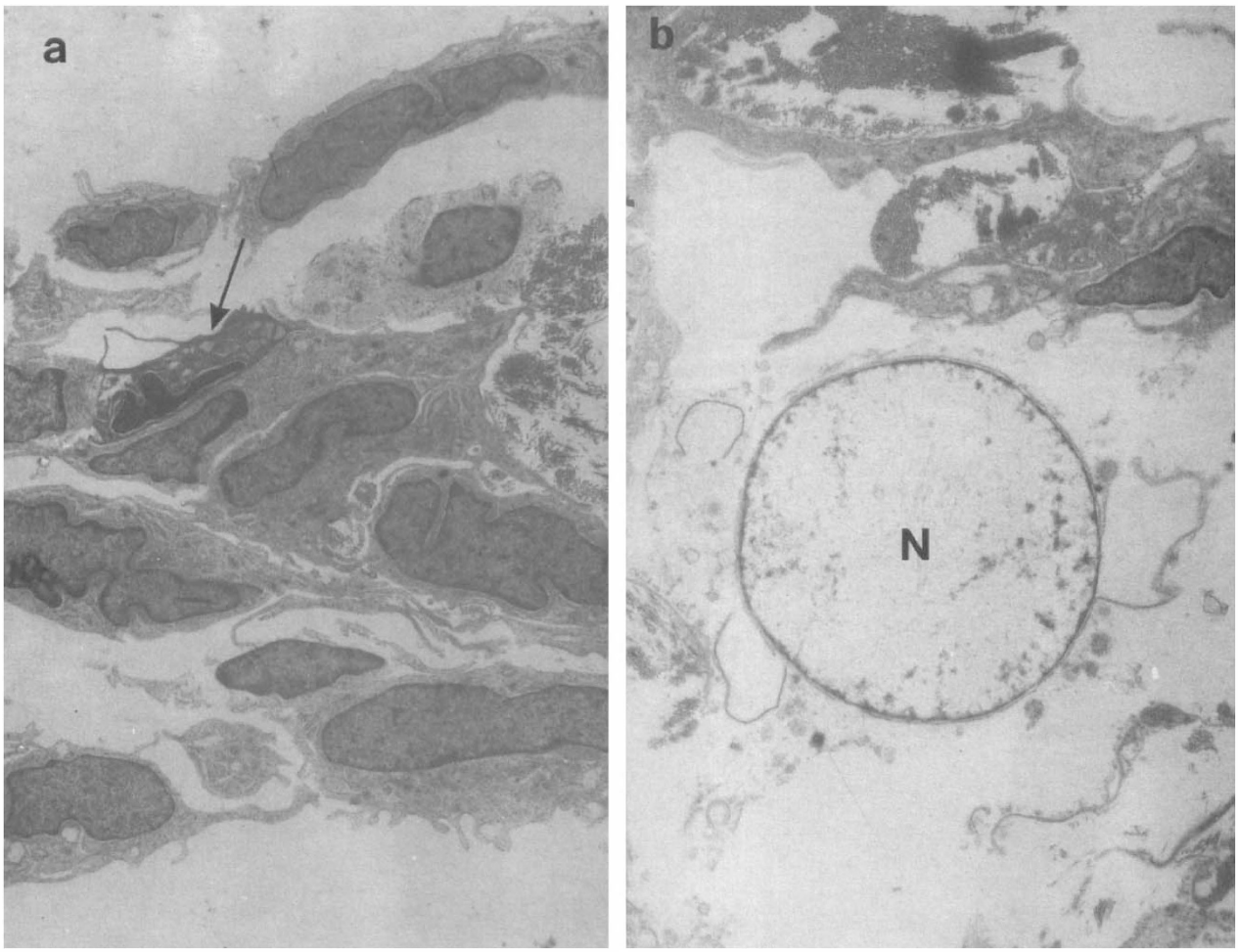


**Fig. 4.** *The meshwork at 4 days by transmission electron microscopy. (a) Some macrophages and activated meshwork cells. A cell dying by apoptosis is indicated (arrow). (b) A necrosed meshwork cell with a large swollen karyolytic nucleus (N) (a, ×250; b, ×6250).*

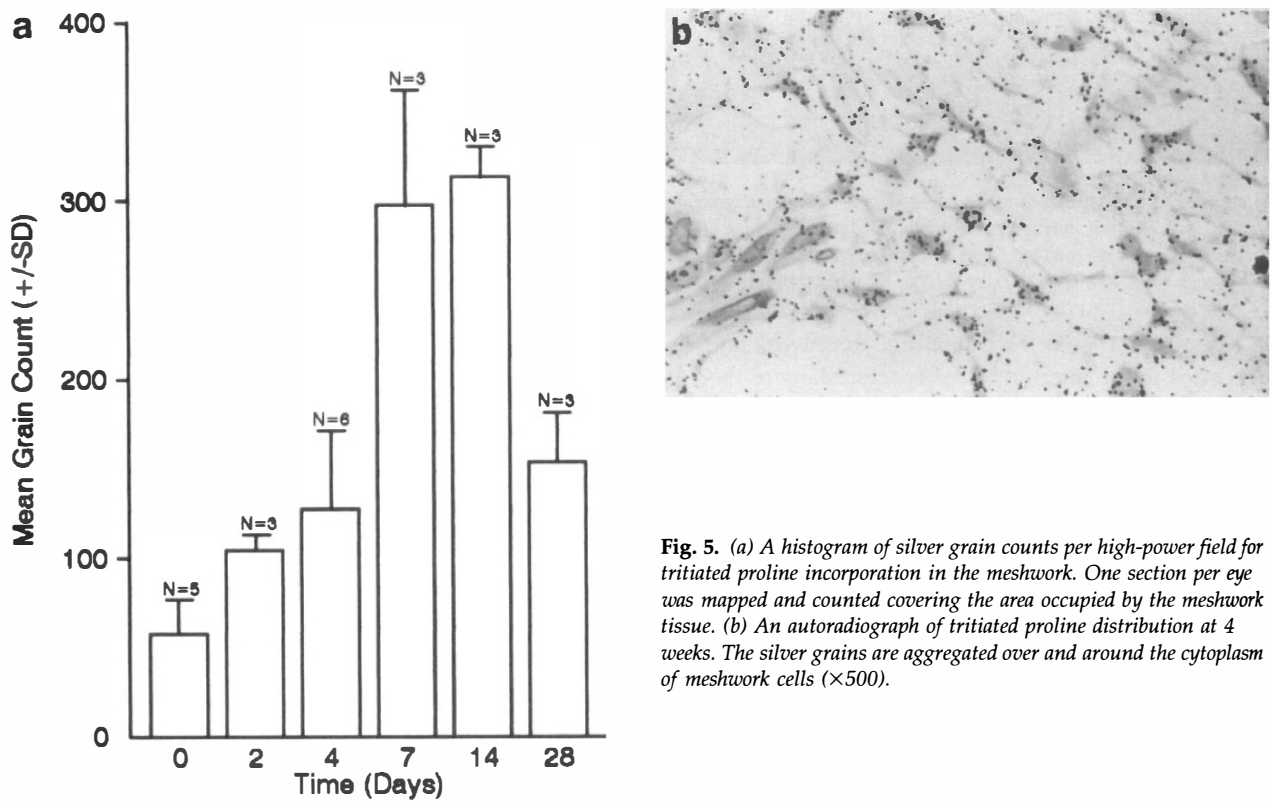


**Fig. 5.** *(a) A histogram of silver grain counts per high-power field for tritiated proline incorporation in the meshwork. One section per eye was mapped and counted covering the area occupied by the meshwork tissue. (b) An autoradiograph of tritiated proline distribution at 4 weeks. The silver grains are aggregated over and around the cytoplasm of meshwork cells (×500).*

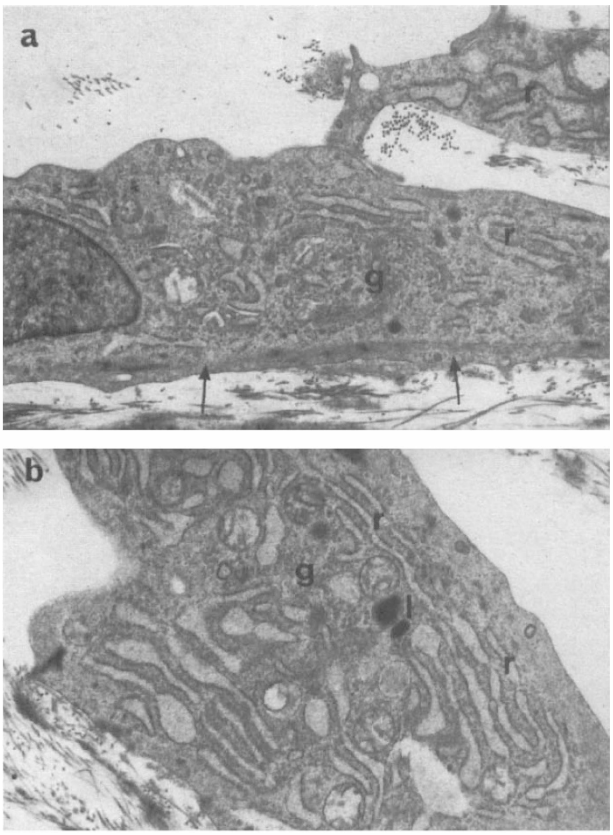


**Fig. 6.** *Activated meshwork cells at 14 days by transmission electron microscopy; they are particularly rich in rough endoplasmic reticulum (r), Golgi vesicles (g) and lysosomal inclusions. (a) Spindle-shaped cells dominate and they often have basally located stress fibres (arrows). (b) Partially rounded but activated cells like this one are now rare and do not have stress fibres (a, ×11 000; b, ×16 000).*

of heterochromatin (Fig. 4a). Rough endoplasmic reticulum was well developed and obvious within the cytoplasm of most meshwork cells.

A number of meshwork cells were seen to be dead or dying. The cells exhibited the two well-known forms of cell death: necrosis and apoptosis. Some cells had grossly swollen karyolytic nuclei, swollen organelles and exhibited cytoplasmic rarefaction (Fig. 4b), the classical features of necrotic death, whereas others had shrunken pyknotic nuclei (Fig. 4a) with limited organelle changes and other features more in line with apoptosis.

By 4 days tritiated thymidine labelled the nuclei of some cells within the meshwork area but it was difficult in this tissue, infiltrated with inflammatory cells, to determine whether or not some of the labelled cells were native meshwork cells. The tritiated proline incorporation at 4 days was similar to that seen at 2 days (Fig. 5a).

### *Morphology: 7–14 days recovery*

During the 7–14 day period of recovery, the meshwork cells still had well-developed rough endoplasmic reticulum and nuclei with little heterochromatin (Fig. 6). The Golgi apparatus was pronounced, mitochondria were distinctive, coated vesicles were common and lysosomal inclusions were scattered in the cytoplasm. The activated cells often had distinctive aggregated bundles of 4–6 nm actin microfilaments situated towards the cell base and running with the long axis of the cell (Fig. 6a). The activated meshwork cells were sometimes elongated and spindle-shaped and there was less evidence of rounding up, which had been a common feature at earlier stages of recovery (Fig. 6b). The meshwork was still disorganised and occasional lysed cells were still noted up to 14 days.

Throughout the angular region there was a high incidence of cells which had incorporated tritiated thymidine into their nuclei. Up to 8–10% of cell nuclei in the central region of the meshwork were positively labelled (Fig. 7, insert b). Careful examination of the positive cells indicated it was likely that the majority were invading macrophages, although their numbers were much in decline. Some labelled nuclei were possibly meshwork cells, based on their elongated morphology and association with trabecular ECM, but such identification was not entirely sound and a level of tissue disruption still remained which made it hard to distinguish between labelled cells with any high degree of confidence. It was at the 7 and 14 day time periods that incorporation of tritiated proline was at its highest (Fig. 5a). The grain counts at 7 and 14 days were much the same but they were 5 times the level found in the control, uninjured meshwork specimens.

### *Morphology: 4–6 weeks recovery*

Macrophages were virtually gone from the tissue by 4 weeks and were absent by 6 weeks. Well-organised trabecular structures were re-established by 4 weeks (Fig. 8) and generally throughout the meshwork effective cell-to-cell associations were in place. By 6 weeks, gap junctions became evident between many of the cell contacts (Fig. 9).

Throughout this period the meshwork cells were losing their activated appearance. At 4 weeks rough endoplasmic reticulum, mitochondria, Golgi vesicles and lysosomal structures were less in evidence. On the other hand, aggregates of actin microfilaments were still in evidence and many cell nuclei were still electron-lucent due to paucity of osmiophilic heterochromatin. However, by 6 weeks, aggregated cytoplasmic microfilaments were rare and heterochromatin was more prevalent in the nuclei. By 4 weeks, the endothelium of the vessels of the aqueous plexus was without obvious deficits and the JCT tissue had re-established the ECM content of its extracellular spaces (Fig. 10).

Also at 4 weeks it was possible, with the absence of infiltrating inflammatory cells, to show convincingly that some native meshwork cells were incorporating tritiated thymidine into their nuclei, and in excess of 24 silver grains per nucleus was considered beyond just being DNA repair (Fig. 7 and inserts a and c). Such cells were identified in the JCT (Fig. 7 insert a) and part of the interlinking network of cells that form the loose trabeculae (Fig. 7 insert c). The 2 animals labelled for 24 h and the 1 labelled for 48 h with intravitreal tritiated

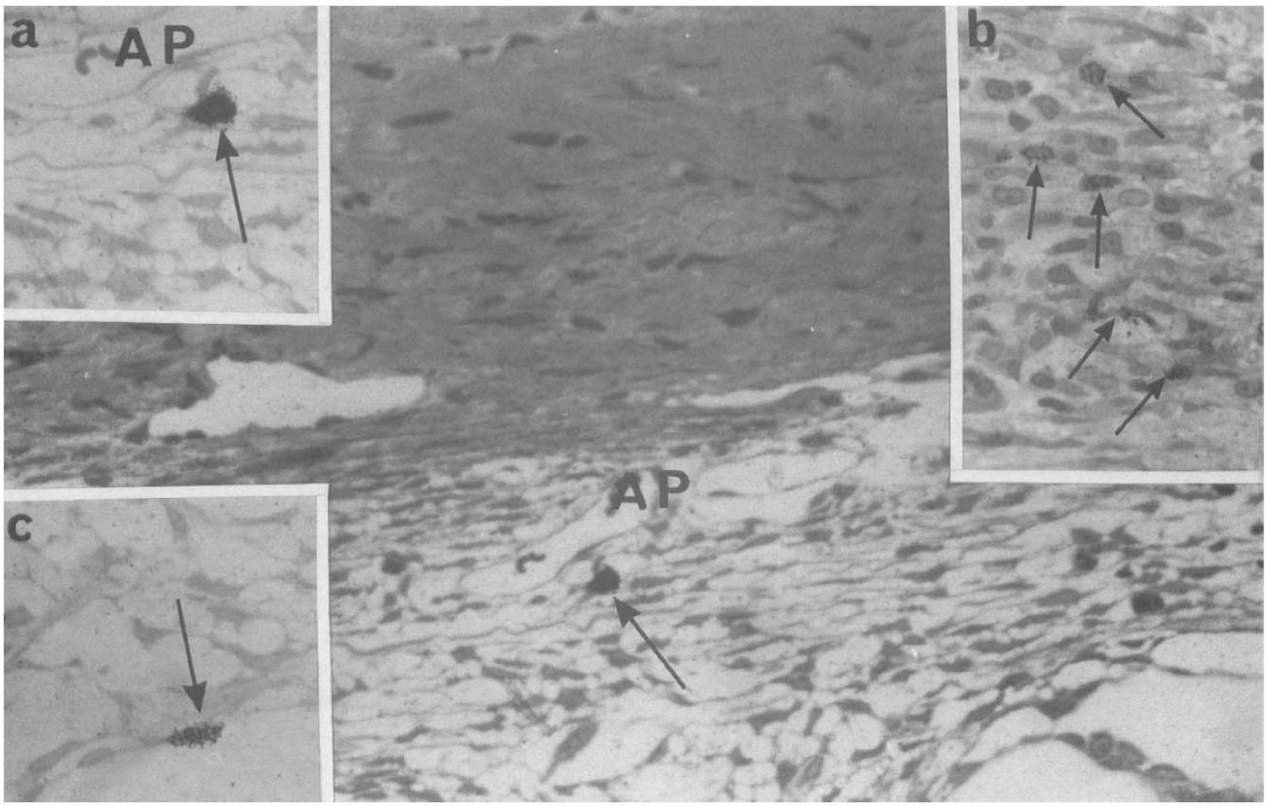


**Fig. 7.** *An autoradiograph of tritiated thymidine incorporation into the meshwork at 4 weeks. One cell in the juxtacanalicular connective tissue region, adjacent to vessels of the aqueous plexus (AP), is labelled (arrow) and is seen to be a meshwork cell at higher power (insert a). The insert c shows a labelled meshwork cell (arrow) in the chamber angle region at high power, also at 4 weeks. By way of contrast, at 1 week (insert b) there are still too many inflammatory cells present to be sure whether meshwork cells are labelled; arrows indicate some labelled cells. (×350; inserts a and c, ×600: insert b, ×400).*

thymidine at the 4 week time period all had much the same incidence of labelled nuclei. There were between 2 and 5 labelled nuclei per meridional section of meshwork which gave a labelling index of around 2.5–3.5%. Nuclear labelling at 6 weeks could be identified but, by then, it was extremely hard to find. Tritiated proline incorporation at 4 weeks had dropped to half that seen during the 7 and 14 day periods (Fig. 5a). However, at 4 weeks, tritiated proline was clearly seen to be associated with the cytoplasm of meshwork cells (Fig. 5b).

## Discussion

### *Injury to the outflow system*

The present study investigated a 'repair model' in the rabbit which produced IOP/flow-induced damage to the outflow system. The lesion was uniform around the 360° of the chamber angle and was distinguished by extensive loss of cohesion between meshwork cells, dispersion of ECM materials and damage to the vessels of the aqueous

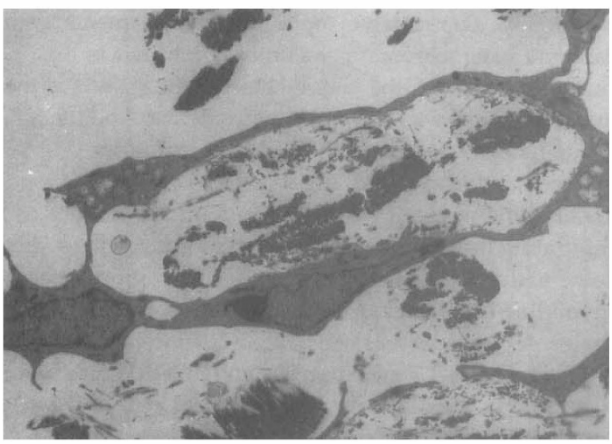

**Fig. 8.** *A transmission electron micrograph of a normal trabeculum at 4 weeks (×5000).*

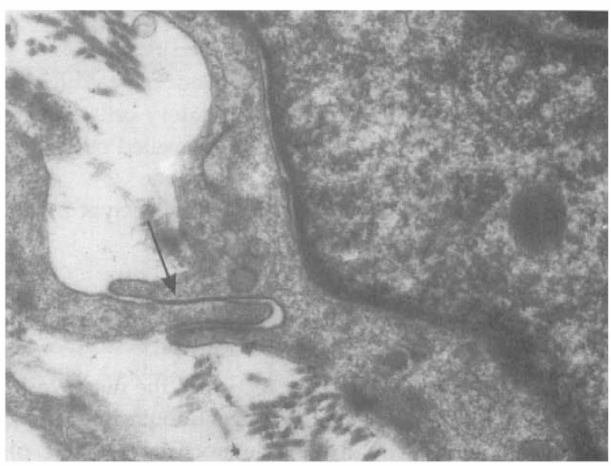

**Fig. 9.** *Transmission electron micrograph of a tongue-in-groove association formed between adjacent meshwork cells at 6 weeks. A gap junction (arrow) elaborates the intercellular space (×40 000).*

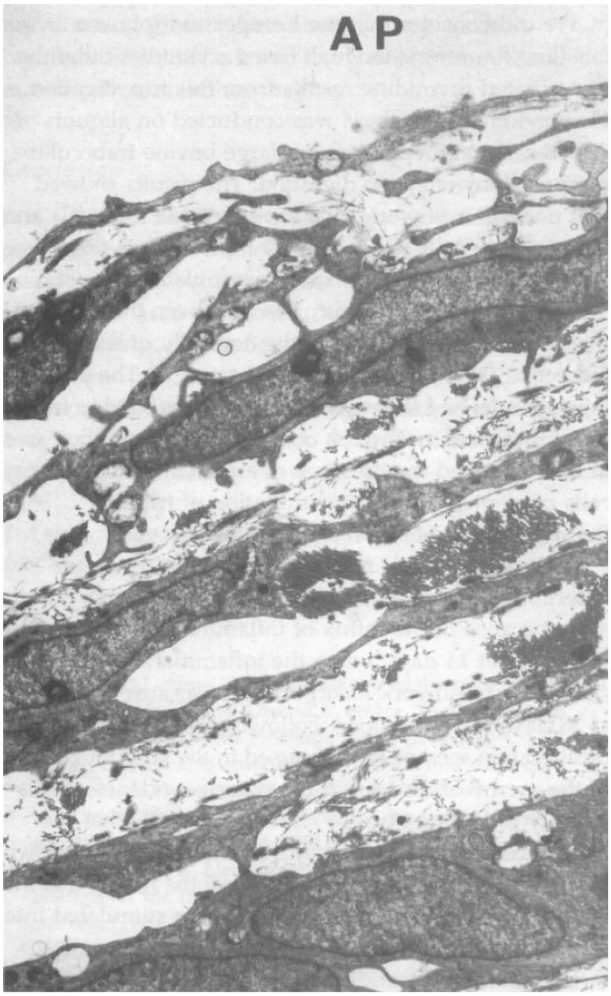


**Fig. 10.** *A transmission electron micrograph of the juxtacanalicular connective tissue area beneath a vessel of the aqueous plexus (AP) at 4 weeks when it is, by this time, indistinguishable from normal (×7000).*

plexus. A trabeculitis developed over the next few days in which polymorphs were prominent initially and then macrophages dominated.

The IOP in the eyes with the injured outflow system dropped by over 50% in the first week of recovery but then gradually climbed to normal over the next few weeks. Separate experiments (not reported here) showed that the gross facility of outflow, elevated while IOP was low, was back to normal by the sixth week of recovery. Functional normality coincided reasonably well with the re-establishment of normal tissue architecture and anatomy, which was between 4 and 6 weeks.

We have reported an injury model where the repair activity of the invading cells and native meshwork cells is sufficiently robust to establish a functionally and structurally normal outflow system. During the repair process we were able to show that meshwork cells became activated, probably synthesised at least some of the replacement ECM and proliferated to replace losses. As such our experimental system differs from findings from other trauma situations like those caused by trabeculectomy, trabeculotomy and ciliary muscle disinsertion[1–4] where repair in general and repair activity by meshwork cells specifically is far more limited. The crucial difference might be the lack of an effective migratory scaffold following severely destructive injury.[1] Without a scaffold or effective substrate, the biological range of activities that meshwork cells could undertake would be severely curtailed; so, by this means, repair activity becomes self-limiting.

That both necrotic and apoptotic cell death was seen in the meshwork following the IOP/flow lesion was surprising. A trauma/inflammation experimental system of the type used in this study usually is considered to be associated with necrotic death.[31] It is likely, however, that the two processes of cell death occur within the same lesion together more often than we suspect. Recently, for example, within the eye literature it was reported that necrosis and apoptosis were both in evidence after experimental lens extraction.[32] In our model, we visualised necrotic events far more often than apoptotic ones, but given the speed with which apoptotic debris is removed from its surroundings[31] it is unsafe to assume that our lesion is dominated by necrosis.

### *Activation of meshwork cells*

Activation of meshwork cells seems to be a universal response of these cells to injury or irritation stimuli ranging through surgery,[1–4] laser,[19] phagocytic particle insult,[1,5–9,24] noxious agents[12–14,33] to some *in vitro* culture conditions.[10,34] It has been associated particularly with a heightened metabolic state needed for the phagocytosis of debris entering the meshwork.[1,6–8] Indeed meshwork cell activation was first reported by Rohen and Van der Zypen[6] after they had induced meshwork cells to phagocytose non-organic materials. There was some evidence of meshwork cell phagocytosis in this study but not a lot.

On the other hand, the presence of numerous activated meshwork cells did fit reasonably well with the rise in the incorporation of tritiated proline. The highest grain counts for tritiated proline were at 7 and 14 days, the time period when activated meshwork cells were most notable. Incorporation into inflammatory cells also took place but at 14 days their numbers were very much on the wane and at this point incorporation appeared to be at its highest.

It seemed to us that in the IOP/flow injury model, meshwork activation was more to do with an upregulation of ECM synthetic activity than with the clearing up activity of phagocytosis. Tritiated proline is a constituent of collagens and other ECM materials and its more avid incorporation into the cells of the outflow system following injury might be indicative of ECM repair, especially as much of the ECM in the meshwork and JCT was re-established 6 weeks after injury. We know that meshwork cells do synthesise collagens and previously we have investigated the process of incorporation of tritiated proline into different collagen types by bovine meshwork cells *in vitro*.[34] Tritiated glucosamine and $^{35}$S-sulphate have been used as tracers to provide autoradiographic evidence that sulphated glycosaminoglycans are produced by meshwork cells in

the normal hamster outflow system[35] and $^{14}$C-glucosamine has been used in much the same manner in strips of organ-cultured human meshwork.[11]

Some activated meshwork cells had distinctive aggregated bundles of actin microfilaments in their cytoplasm. These cells had an elongated spindle-shaped profile and the aggregates were not found in more rounded meshwork cells seen in the earlier stages of repair. The aggregates have the characteristic features of actin stress fibres which have been seen previously in meshwork cells, but they have been appreciated more often *in vitro*[36–41] than *in vivo*.[42,43]

Actin-rich stress fibres are often associated with the contractile form of fibroblasts in skin wounds called myofibroblasts[44] and myofibroblasts are associated with wound repair. The myofibroblast phenotype is not the exclusive property of true fibroblasts and has been shown to be adopted by diverse ocular cells in injury, a well-known example being retinal pigment epithelium.[45] Myofibroblasts are thought by ourselves and others to exert isometric force and act as biological staples to hold the injured tissue together while ECM formation is completed.[45] It may be that the spindle-shaped, activated meshwork cells with stress fibres, seen in abundance in the later stages of our experiments, have such a role in the recovering meshwork. On the other hand, the cells we saw may just belong to the normal spindle-shaped meshwork cell subtype recognised by Coroneo *et al*.[39] but seen more readily in injury. It is worth mentioning that the spindle subtype from the meshwork is thought to be a contractile cell.[39]

### *Replication of meshwork cells*

It remains unknown whether or not there is any turnover of cells within the normal trabecular meshwork. If there is any replacement activity it is well below that of normal cell loss from the ageing outflow system.[5,25,46,47] However, the highest determination of tritiated thymidine labelling in the normal meshwork was that of Kempel and Johnson,[24] which ranged between 2.17% and 0.46% in the cat with an overall average of 0.95%. Other estimates have been far lower, ranging from 0.3% to 0.4% in human organ culture,[20,21] where culture conditions favour growth and incorporation of label, to 0.1% in cynomolgus monkeys[22] and 0 in the present investigation.

The disparity between our observations and those of Kempel and Johnson[24] may be rationalised by differences in our radiolabelling procedures, ' oth of which are invasive. We introduced a smaller volume of radiolabel (20 μl) into the vitreous whereas Kempel and Johnson introduced 100 μl on two occasions into the anterior chamber following paracentesis. We, at a more remote site from the target tissue, risked under-labelling (which might account for our zero index) whereas Kempel and Johnson risked a local tissue injury response and subsequent DNA repair in the outflow pathway (which might explain their high labelling index).

We did consider that the Kempel and Johnson labelling figure was too high based on studies other than our tritiated thymidine results from this investigation. Flow cytometric analysis was conducted on aliquots of 70 000 cells removed from the large bovine trabecular meshwork by enzymic digestion. The results showed that normal meshwork cells were arrested in $G_0/G_1$ and if there were cells in either M or S phase then they were far fewer than 1% of our sample populations.[5]

The ALT procedure has up to now been the only local injury model which induces the normally quiescent trabecular meshwork cells to replicate.[20–24] The present injury model had the weakness that it was hard to follow the activities of meshwork cells while inflammation was at its height and the presence of abundant inflammatory cells prevented effective quantitation of tritiated thymidine incorporation into meshwork cells.

Incorporation into meshwork cells could not be determined before and including the 7 day recovery stage because of the influx of inflammatory cells. However, at 14 days, when the inflammation was subsiding, meshwork incorporation was suspected and at 28 days it was obvious. Following ALT the proliferation seemed to be focused in the anterior portion of the meshwork[21,24] but there was no evidence of this polarity following our IOP/flow lesion. Whether the difference is species- or insult-related remains unknown but our finding does show, for at least the rabbit, that the whole meshwork has the potential to be stimulated into cellular replication given the right circumstances.

In both the ALT and the IOP/flow systems the injury is not massive and leaves adequate scaffolds for meshwork cell migration and reorganisation; whether or not that is relevant to the induction of meshwork proliferation remains to be determined. Acott *et al*.[21] were convinced 'the regulatory signal for cell replication is medium-borne', particularly because meshwork cell proliferation also took place well away from the site of lasering and therefore was likely to be initiated by a soluble mediator or mediators. At least one group has put forward vasoactive intestinal peptide (VIP) as a possible ALT-induced stimulant of meshwork cell growth.[48]

Both ALT and the present IOP/flow injury produce a localised inflammation and it is reasonable to speculate that cytokines and growth factors released during the inflammatory episode may be crucial for meshwork cell proliferation. On the other hand a wide range of pathology associated with inflammatory insults to the ouflow system has been reported but no local proliferation was mentioned in these circumstances.[49] Also puzzling was the finding, in an experimental study, that inflammation provoked by zymosan did not promote tritiated thymidine incorporation into meshwork cells.[24] *In vitro* it has been shown that phagocytosis can inhibit meshwork cell division.[50] Perhaps excessive phagocytic action *in vivo* has a confounding effect on replication? There are many confounding effects, including the potential inhibitory action of aqueous humour,[51] and one of its constituents,

transforming growth factor beta.[52] They serve to highlight how little we still know about the control of meshwork cell numbers *in vivo*.

### *Conclusion*

It is accepted that the present injury model is far removed from the pathobiological events which are associated with primary open-angle glaucoma.[25,26] However if, as is suspected, increase in or maintenance of meshwork cell numbers is a future potential therapeutic direction in the management of IOP, then the model, and others of a similar type, may give important insights into the proliferation control mechanisms and repair capabilities of meshwork cells.

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