THE EFFECTS OF HYALURONATE AND ITS INTERACTION WITH ASCORBATE ON AQUEOUS HUMOUR DYNAMICS. A CLINICAL AND LABORATORY STUDY

By

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"Where is the wisdom we have lost in knowledge?
Where is the knowledge we have lost in information?"

T.S.Elliot

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IOP	Intraocular pressure
NPE	Non-pigmented epithelium
PE	Pigmented epithelium
Na^+/K^+ATPase	Sodium-Potassium activated Adenosine Triphosphatase
Cyclic AMP	3', 5'-Cyclic Adenosine Monophosphate
BAB	Blood-aqueous barrier
TM	Trabecular meshwork
GAGs	Glycosaminoglycans
HA	Hyaluronic acid
NIF-SH	Non-inflammatory fraction of sodium hyaluronate
ICCE	Intracapsular cataract extraction
ECCE	Extracapsular cataract extracton
p/c IOL	Posterior chamber intraocular lens
IOL	Intraocular lens
BSS	Balanced salt solution
HPLC	High-performance liquid chromatography
DMEM	Dulbecco's modified Eagle's medium
DMEM + PSF	Dulbecco's modifed Eagle's medium with added antibiotics (penicillin, streptomycin, fungizone)

AA	L-Ascorbic acid
DHAA	Dehydro-L-ascorbic acid
µg	Microgrammes
mg	Milligrammes
g	Grammes
kg	Kilogrammes
µl	Microlitres
ml	Millilitres
mol	Moles
h	Hours
°C	Degrees Celcius
mmHg	Millimetres of Mercury
cSt	Centistokes
SEM	Standard error of the mean
SD	Standard deviation
iv	Intravenous
G	Gauge
vs	Versus
ns	No significant
ODS	Octadecyl silica gel
RSD	Relative standard deviation

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SUMMARY

Purpose. Healon (sodium hyaluronate 1%), a viscoelastic material widely used in ophthalmic surgery, may exacerbate the potentially harmful early intraocular pressure (IOP) rise observed postoperatively in cataract surgery. The results of *in vitro* studies demonstrate that such IOP elevations may be due to obstruction of the aqueous humour outflow pathways. Therefore Healon- induced IOP rises might be preventable if this biopolymer could be depolymerized into smaller molecules which could be easily cleared by the outflow pathways. Ascorbic acid (AA) depolymerizes hyaluronic acid *in vitro* in a concentration-dependent manner at concentrations of AA which are normally present in the aqueous humour. In the early postoperative period, aqueous AA levels are probably decreased due to damaging effects of surgical trauma on the blood-aqueous barrier (BAB). Furthermore, recent reports suggest that Healon exacerbates the postsurgical BAB breakdown. Collectively, these results suggest that the BAB breakdown occurring during surgery may result in a drop in the concentrations of aqueous humour ascorbate to levels too low to effectively depolymerize Healon. This suggests that if physiological levels of ascorbate could be maintained the postoperative Healon-induced IOP rise would be attenuated. The purpose of this study was to investigate if Healon-induced IOP rises can be attenuated by increasing aqueous AA levels in the aqueous humour in the immediate postoperative period. Clinical, animal and *in vitro* studies were conducted to investigate this hypothesis.

Methods and Results. In a pilot study the timecourse of plasma AA levels following a single 1g dose of ascorbate was investigated in five volunteers. Plasma AA peaked within 2-4h and remained high for 3-5h. An initial study

was conducted to determine the maximum increase in aqueous AA attainable after oral pretreatment with AA in 105 patients undergoing cataract surgery. Patients were divided into 5 groups based on the total dose of AA given preoperatively (0, 1, 2, 3 or 5g in two divided doses). The maximal aqueous AA levels ($\mu g.ml^{-1}$) were attained with the 2g dose (643±73; mean±SEM) which is approximately two-fold increase over non-pretreated patients. The effect of Healon on the postoperative IOP and the effect of AA on the Healon-induced IOP rise was then studied in a prospective randomized double-masked trial in 76 patients who underwent uncomplicated cataract surgery with and without the use of Healon. A total dose of 2g ascorbate or placebo was given before surgery and IOP was measured at 6 and 24h postoperatively. Healon-treated patients were further subdivided in three groups according to the volume of Healon used intraoperatively (<0.2ml, 0.2-0.35ml and >0.35ml). AA pretreatment did not influence the postoperative rise in IOP in any of the Healon groups. A large difference in IOP between operated and contralateral control eyes among no Healon and Healon-treated (in whatever volume) patients was observed at 6 and 24h. Significant increases in IOP in the operated eyes of all groups of patients who received various volumes of Healon were observed at 6h postoperatively, except for the group which received <0.2ml Healon. Significantly higher IOP rises were observed in the >0.35ml Healon than the <0.2 ml Healon, 0.2-0.35ml Healon or no Healon groups at 6h. A significantly higher percentage (77%) of patients who received >0.35ml Healon exhibited differences in IOP greater than 10mmHg between operated and control eyes at 6h as compared with that of no Healon, <0.2ml Healon or 0.2-0.35ml Healon groups. At 24h, there was still a high percentage (22%) in the >0.35ml Healon group with a difference in IOP greater than 10mmHg while in

all other groups, IOP returned to preoperative levels. In order to better understand the failure of AA pretreatment to prevent the Healon-induced IOP elevations, studies were conducted in 52 rabbits to determine whether the trauma of surgery may have been responsible for the absence of sufficient levels of AA in the aqueous humour postoperatively. In these studies, the effects of surgical procedures (corneal incision alone and corneal incision plus lens removal) and Healon on aqueous ascorbate concentration were investigated. An approximate 50% decrease in aqueous AA levels was observed 2-6h postoperatively (without the use of Healon), which persisted at 48h. However, an additional (18%) significant drop in AA levels was found in eyes which underwent lens removal with the use of Healon intraoperatively. Pretreatment with oral AA (50mg/kg body weight, 3h preoperatively) significantly increased AA levels, but did not attenuate the surgically- and Healon-induced drops in AA concentration at 6 and 48h postoperatively, although plasma AA levels remained elevated for about 10h. Aqueous humour and plasma ascorbate levels were determined by HPLC in both clinical and animal studies. Since the results of these studies suggested that AA levels decrease during surgery even with AA pretreatment, experiments were conducted in a human eye model in vitro (perfused ocular anterior segments) to determine whether the obstructive effect of Healon could be eliminated under conditions where a constant high concentration of AA could be obtained. Initial studies were designed to confirm that Healon decreases outflow facility. After establishment of a baseline outflow facility different concentrations of the commercial preparation of Healon (100%, 50% and 10%) in culture medium were perfused in a total of 22 eyes. The maximum amount of obstruction obtained was 55%, 30% and 12%, respectively. A significant decrease in outflow facility was observed with all

Healon solutions in a concentration-related fashion. The obstructive properties of Healon were consistent with histological findings. Similar to the Healon-induced rises in IOP observed in the clinical studies, Healon-induced reductions in outflow facility did not improve when the eyes were perfused in the presence of a constant high concentration of AA ($1000\mu g.ml^{-1}$) studied in 3 pairs of human eyes over a period of 6h.

Conclusions. A two-fold increase in aqueous AA levels can be achieved by oral administration of AA. Healon-induced IOP rises in the immediate postoperative period are dose-related. When more than 0.35ml of Healon is used, high IOP rises are observed for at least 24h. Surgery results in a 50% decrease in aqueous AA concentrations in the rabbit, and Healon has an exacerbating effect on this reduction. Healon reduces outflow facility in a dose-related manner and this reduction is not improved in the presence of a high concentration of AA. Postoperative Healon-induced IOP rises are not prevented by increased levels of aqueous humour ascorbate. Healon is not depolymerized by AA *in vivo*, or if it is, that depolymerization is not sufficient to break hyaluronate molecules down to a size compatible with easy egress from the anterior chamber.

PUBLICATIONS AND COMMUNICATIONS RESULTING FROM THIS STUDY

Karditsas SD, Wilson WS, Iqbal Z, Watson DG, Dutton GN, Midgley JM Effect of surgery on ascorbic acid levels in the rabbit aqueous humour. Proc IX Congr Soc Ophthalmol Europ, Brussels. 1992:189.

Karditsas SD, Dutton GN, Iqbal Z, Watson DG, Wilson WS, Midgley JM. Ascorbic acid in human aqueous is increased by preoperative oral administration. Proc IX Congr Soc Ophthalmol Europ, Brussels. 1992:189.

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Watson DG, Iqbal Z, Midgley JM, Pryce-Jones H, Morrison L, Dutton GN, Karditsas S, Wilson W. Measurement of ascorbic acid in human aqueous humour and plasma and bovine aqueous humour by high-performance liquid chromatography with electrochemical detection. J Pharm Biomed Anal. 1993;11:389-392.

Karditsas SD, Schroeder AM, Erickson KA. The effect of different dilutions of Healon (sodium hyaluronate) on outflow facility in the human eye in vitro. Invest Ophthalmol Vis Sci (ARVO Suppl). (Submitted for publication).

DECLARATION

I declare that this thesis embodies the results of my own research work, that it has been composed by myself in the Tennent Institute of Ophthalmology and in the Department of Pharmacology of the University of Glasgow, and in the Department of Ophthalmology, Massachusettes Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts, USA, between October 1990 and September 1993. This thesis does not include work forming part of a thesis presented by me for a degree in this or any other University.

PREFACE

The introductory material in the first chapter describes the fundamental anatomy and physiology of the anterior segment of the eye relevant to the basic hypotheses of this thesis. The literature on hyaluronic acid and the use of Healon^{R} (sodium hyaluronate 1%) in cataract surgery with particular emphasis on its exacerbating effect on postoperative intraocular pressure elevation is then reviewed. This is followed by a selective review on ascorbic acid pharmacology including its capacity to depolymerize hyaluronic acid. The central hypothesis along with the rationale, approach and specific aims underlying the body of work are then presented in subsequent chapters.

CHAPTER 1

INTRODUCTION

1.1 AQUEOUS HUMOUR DYNAMICS

1.1.1 Introduction

The term aqueous humour dynamics refers to the sum total of physiological events associated with the production and drainage of aqueous humour and the maintenance of normal intraocular pressure (Kaufman, 1985).

The human eye consists essentially of three layers enclosing the transparent refractive media (Figure 1). The outermost layer is made up of the sclera and cornea. The middle coat is mainly vascular, consisting of the choroid, ciliary body and iris. The innermost layer is the retina, containing the essential nervous elements responsible for vision - the rods and cones; it is continued forward over the ciliary body as the ciliary epithelium (Davson, 1990). The globe of the eye contains three chambers: the anterior chamber, the posterior chamber and the vitreous cavity. The anterior chamber is bounded anteriorly by the cornea, posteriorly by the front surface of the iris and the lens, and peripherally by the anterior chamber angle. The posterior chamber is bounded anteriorly by the iris, posteriorly by the anterior lens capsule and its zonule (suspensory ligament of the lens), and peripherally by the ciliary processes. The vitreous cavity is the largest cavity of the eye and is bounded anteriorly by the lens, zonule and ciliary body, and posteriorly by the retina and optic nerve.

The ciliary processes secrete aqueous humour into the posterior chamber of the eye. From here, the aqueous humour flows into the anterior chamber and leaves the eye by bulk flow via two pathways: (1) the conventional route

through the trabecular meshwork and (2) the unconventional or uveoscleral route. The chamber-angle tissues offer a certain amount of resistance to the outflow of aqueous humour. Intraocular pressure (IOP) builds up due to the inflow of aqueous, to a level sufficient to drive fluid through that resistance at the same rate at which it is produced. In the glaucomatous eye, this resistance is usually high causing elevated IOP, which is associated with damage to the optic nerve and subsequent loss in visual field.

1.1.2 The ciliary epithelia

The ciliary body forms a ring along the inner wall of the globe and extends from the iris anteriorly to the ora serrata posteriorly. The largest component of the ciliary body is smooth muscle that is arranged in three bundles: longitudinal, reticular and circular. The ciliary body is anatomically divided into two segments: the anterior pars plicata and the smooth posterior pars plana. The ciliary processes, approximately 70 in the human, project inward from the pars plicata region as radial ridges. They consist of a central core of highly vascularised connective tissue surrounded by a double layer of epithelium (Figure 2). The two layers face each other apex-to-apex, as a result of the invagination of the neuroepithelial layer during embryogenesis (Figure 3). The inner epithelial layer consists of non- pigmented epithelium (NPE), which lies adjacent to the posterior chamber and contains many mitochondria and rough and smooth endoplasmic reticulum. Tight junctions are present on the apical surfaces of these cells. The outer epithelial layer consists of the

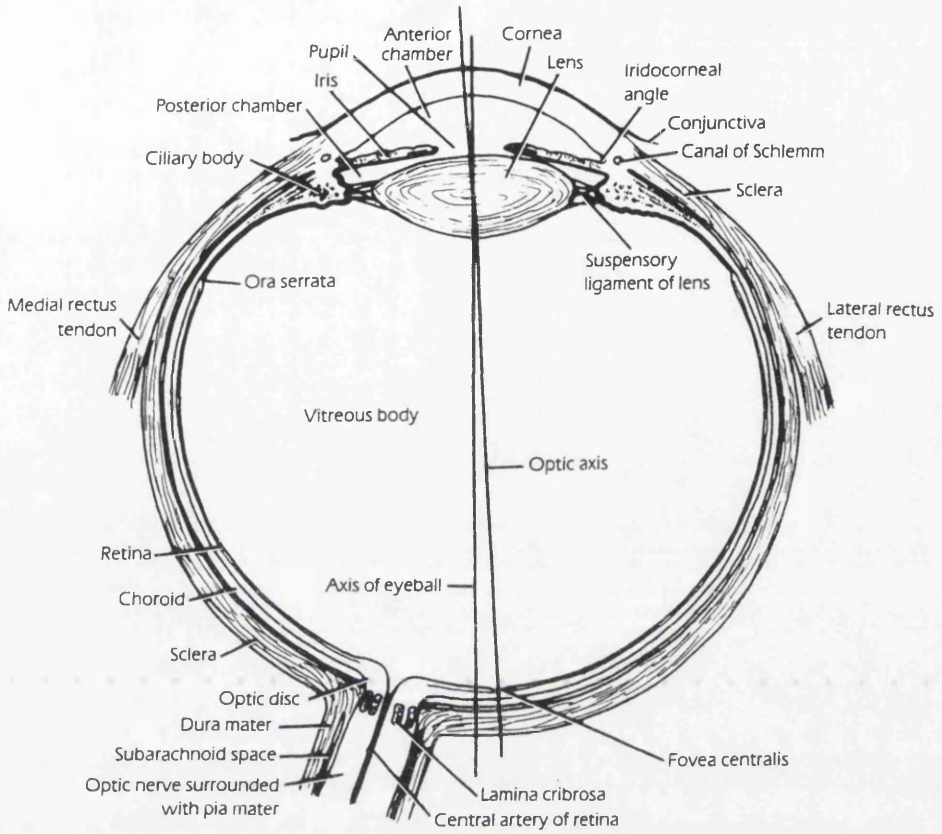

Figure 1 **Horizontal section through the human eyeball at the level of the optic nerve (Snell and Lemp, 1989).**

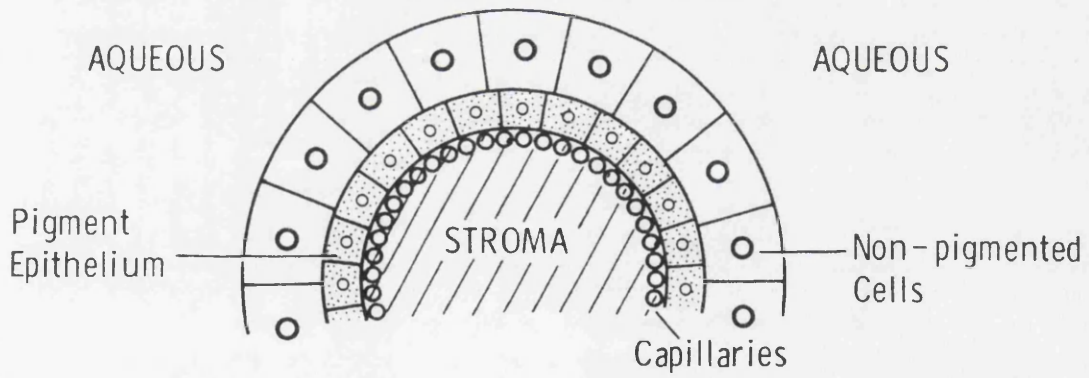

Figure 2 **Schematic cross-section of the ciliary epithelium of a human eye showing its relationship to the capillaries and aqueous humour (Fatt and Weissman, 1992).**

pigmented epithelium (PE). It rests on the stromal core. The pigmented epithelium contains melanosomes but is relatively poor in other intracellular organelles. The cells of this layer are coupled to each other and the NPE by gap junctions (Figure 4).

1.1.3 Production of aqueous humour

Aqueous humour is derived from the ocular blood supply and is essentially a modified filtrate of blood serum. Aqueous humour enters the posterior chamber via the ciliary processes. The production of aqueous humour occurs through a combination of diffusion, ultrafiltration and active transport/secretion (Caprioli, 1987, 1992). It is now well accepted that active transport of certain solutes by the ciliary epithelia is the most important process in the formation of aqueous humour. Specific transport systems of the NPE secrete potassium chloride, bicarbonate, some amino acids, glucose, ascorbic acid and some organic compounds into the posterior chamber. The active solute pump sets up a concentration gradient that forces an osmotic flow of water into the posterior chamber. The major transport system is the membrane-bound enzyme complex sodium-potassium adenosine triphosphatase (Na^+/K^+ATPase) which is located on the NPE membrane and is found in highest concentrations along the lateral cellular interdigitations. When this enzyme system is inhibited by ouabain, formation of aqueous humour is reduced by about 70% (Cole, 1977). Carbonic anhydrase is also involved in active transport in the ciliary body. Inhibition of carbonic anhydrase decreases the secretory activity of the ciliary epithelium (Garg and Oppelt, 1970; Maren, 1977).

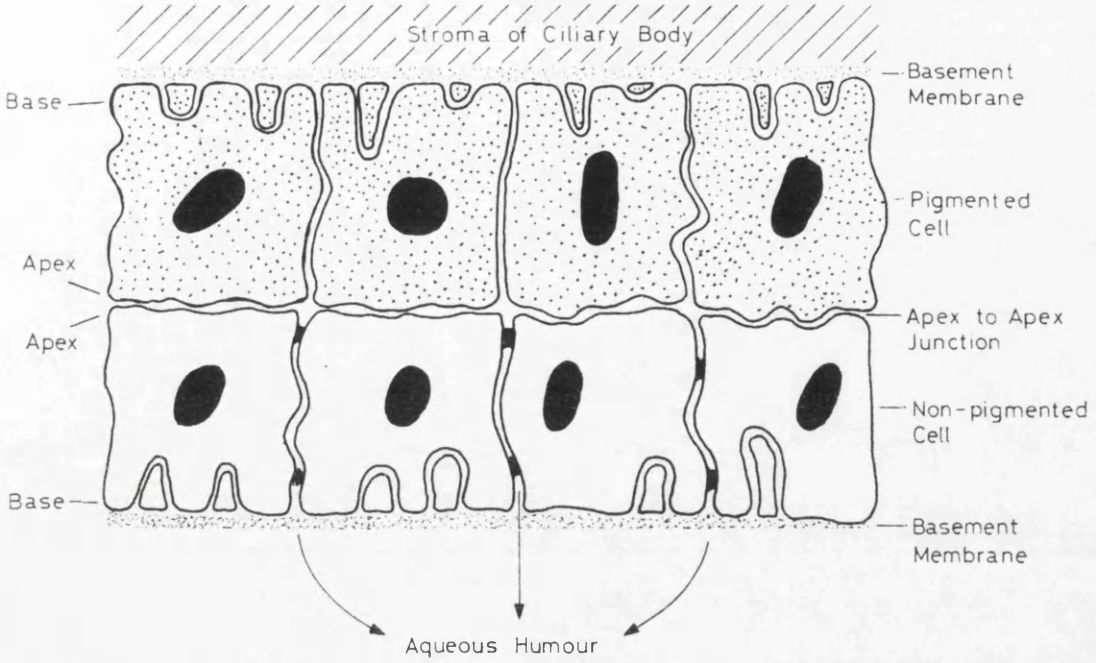

Figure 3 **The apex-to-apex relation of the two layers of cells of the human ciliary epithelium (Davson, 1990).**

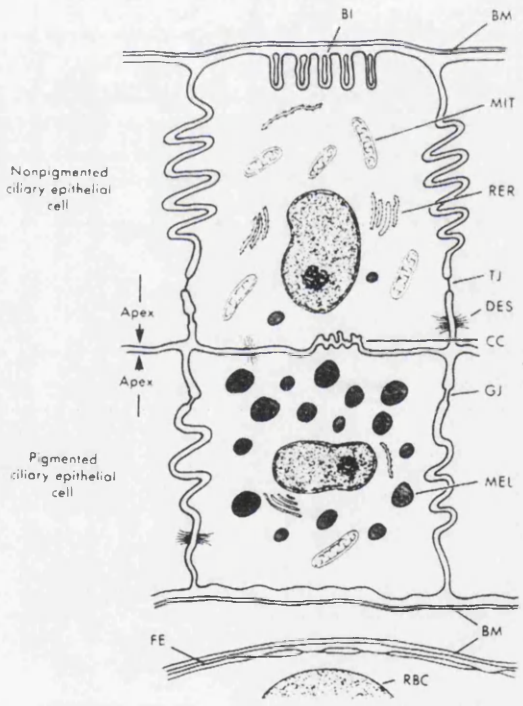

Figure 4

Schematic diagram of human non-pigmented and pigmented epithelial cells showing intracellular organelles and tight and gap junctions.

DES=desmosomes; BI=basal infoldings; BM=basement membrane; CC=ciliary channels; FE=fenestrated capillary endothelium; GJ=gap junction; MEL=melanosome; MIT=mitochondrion; RBC=red blood cell; RER=rough endoplasmic reticulum,;TJ=tight junction (Caprioli, 1992).

The NPE probably plays the dominant role in aqueous humour formation, but the exact nature of its interaction with the PE remains unclear.

1.1.4 Composition of aqueous humour

The volume of aqueous humour in the anterior and posterior chambers of the human eye is about 200-250µl and 60µl, respectively and turns over approximately once every 100min (Caprioli, 1992).

The aqueous humour contains no cellular components, but has most of the small molecular weight electrolyte and nutrient constituents of serum. In addition, some constituents (eg, ascorbate) are in much higher concentration than in serum, suggesting that they are actively transported into the aqueous humour. The presence of the blood-aqueous barrier excludes large molecules from the aqueous humour. Small molecules such as urea and some amino acids enter the aqueous humour by diffusion from plasma. Serum proteins are present in the aqueous but at very low levels. The concentration of proteins in the aqueous humour varies with molecular size. They are mainly restricted to the smaller molecular weight proteins such as albumin and the beta-globulins. Glucose is found in the aqueous in slightly lower levels than in serum. Lactate, in contrast, is present at higher levels than in serum. Oxygen is present at a partial pressure of 55mmHg. The cation balance in aqueous humour is affected by the surrounding tissues. The major anions are chloride and bicarbonate. Organic constituents show a higher degree of variation from plasma concentrations. Steroid hormones and insulin are also present and enter into the aqueous by simple diffusion. However, prostaglandins, appear to be actively released into the aqueous by intraocular tissues such as the iris and

possibly the trabecular meshwork. Cyclic AMP has been detected at a concentration about the same as in the serum. Other substances, such as hyaluronic acid, sialic acid, trivalent chromium ions, vitamin B_{12} and monoamine metabolites have been found in the normal aqueous humour of various species.

Diffusion, aqueous flow, metabolism and interaction with surrounding tissues, make the composition of anterior chamber aqueous humour different from that of the posterior chamber aqueous humour. Most studies concerning the composition of the aqueous humour describe that which is obtained from the anterior chamber (Newell, 1986). Human studies often relate to fluid removed at the time of cataract extraction (Newell, 1986).

1.1.5 Function of aqueous humour

The secretion of aqueous humour generates the IOP required for an optically efficient globe. The flow of aqueous provides nutrition for the avascular ocular tissues that it bathes: the posterior cornea, trabecular meshwork, crystalline lens and anterior vitreous. The constant flow of aqueous humour replenishes nutrients that have been taken up by the avascular tissues and carries away their metabolic waste products (Caprioli, 1992).

1.1.6 The blood-aqueous barrier (BAB)

The tight junctions (zonulae occludentes) between the NPE cells and between the non-leaky endothelial cells of the iris capillaries exclude large molecules from the aqueous. Together, they form the blood-aqueous barrier

(anterior blood-ocular barrier), meaning that substances encounter difficulty in passing from the one fluid to the other.

A breakdown of the BAB may be brought about by a large variety of experimental and pathological conditions (Table 1), so that substances such as proteins, that are normally almost completely excluded from penetration, now appear in the aqueous humour in large amounts. By contrast, the concentrations of constituents that are secreted into the aqueous humour through an active transport mechanism and are found in much higher concentrations than in plasma (eg, ascorbate) will fall. This inflow of plasma-like or 'plasmoid' aqueous or 'secondary aqueous' is responsible at least in part for the increased IOP that is found after paracentesis in rabbits (Al-Ghadyan, 1979). Local synthesis and release of prostaglandins has been implicated in the irritative response after mechanical trauma to the eye which results in miosis, vasodilation and penetration of plasma protein into the aqueous humour which is usually, but not always, accompanied by a rise in IOP. The phase of acute rise in IOP usually gives way, after a short time, to a prolonged phase of lowered IOP (reactive hypotonia). Breakdown of the BAB occurs after intraocular surgery and may play an important role in the postoperative events that determine surgical outcome. The breakdown of the BAB is evidenced clinically by the presence of the so-called "flare". Disruption of the BAB has been reported in the contralateral eyes of patients who have had cataract extraction and lens implantation surgery (Miyake et al, 1984a, 1984b) and in the contralateral eyes of rabbits following anterior chamber paracentesis (Kottow and Seligman, 1978). Rabbit and clinical studies showed that residual viscoelastic materials can contribute to and/or augment the breakdown of BAB and/or interfere with barrier recovery following the trauma of surgery (Miyake

and Mizuno, 1986; Machi et al, 1989; Tsurimaki and Shimizu, 1991a, 1991b).

TABLE 1: PROCEDURES PRODUCING A BREAKDOWN OF THE BLOOD-AQUEOUS BARRIER (Eakins, 1977)

Trauma	Endogenous mediators
Mechanical injury to iris, lens	Histamine
Contusions	Bradykinin
Paracentesis	Prostglandins
	Serotonin
Chemical irritants	Acetylocholine
Nitrogen mustard	
Formaldehyde	Miscellaneous
Acid burns	Bacterial endotoxins
Alkali burns	X-ray irradiation
	Laser irradiation
Nervous activity	
Stimulation of trigeminal nerve	
Immunogenic mechanisms	
Bovine serum albumin	

1.1.7 Aqueous humour outflow tisue anatomy

The aqueous humour flows from the posterior chamber through the pupil into the anterior chamber and leaves the primate eye by bulk flow via two pathways (conventional and unconventional) at the anterior chamber angle (Figure 5).

Total outflow of aqueous humour is the sum of the conventional flow through the trabecular meshwork (F_{trab}) and flow via the unconventional or uveoscleral route (U).

1. Conventional aqueous outflow: In this pathway, aqueous humour leaves the eye through the trabecular meshwork, across the juxtacanalicular tissue into Schlemm's canal and from there into the collector channels and the aqueous veins and finally, to the episcleral veins and general venous circulation. The trabecular meshwork is sieve-like with many progressively smaller pores until Schlemm's canal is reached. Outflow is IOP-dependent through this pathway. Outflow can be summarized as follows:

$$\mathbf{F_{trab} = (P_i - P_e)C}$$

Where: F_{trab} = trabecular outflow

P_i = intraocular pressure

P_e = episcleral venous pressure

C = facility of outflow

The P_e in a normal eye is about 8mmHg, making the pressure drop across the outflow tissues about 7mmHg assuming an average P_i of 15mmHg. Outflow facility in normal human eyes is approximately $0.3\mu l.min.^{-1}mmHg^{-1}$ (Hart, 1992).

2. Unconventional aqueous outflow: In this pathway, aqueous humour leaves the eye by traversing between ciliary muscle bundles and out to the suprachoroidal space where it is absorbed into the uveal circulation. Additionally, it is thought to leak through the sclera into the orbital tissue. This system is thought to be IOP-independent. In the monkey eye in vivo, it may account for as much as 70% of total outflow (Bill, 1971; 1977). In human

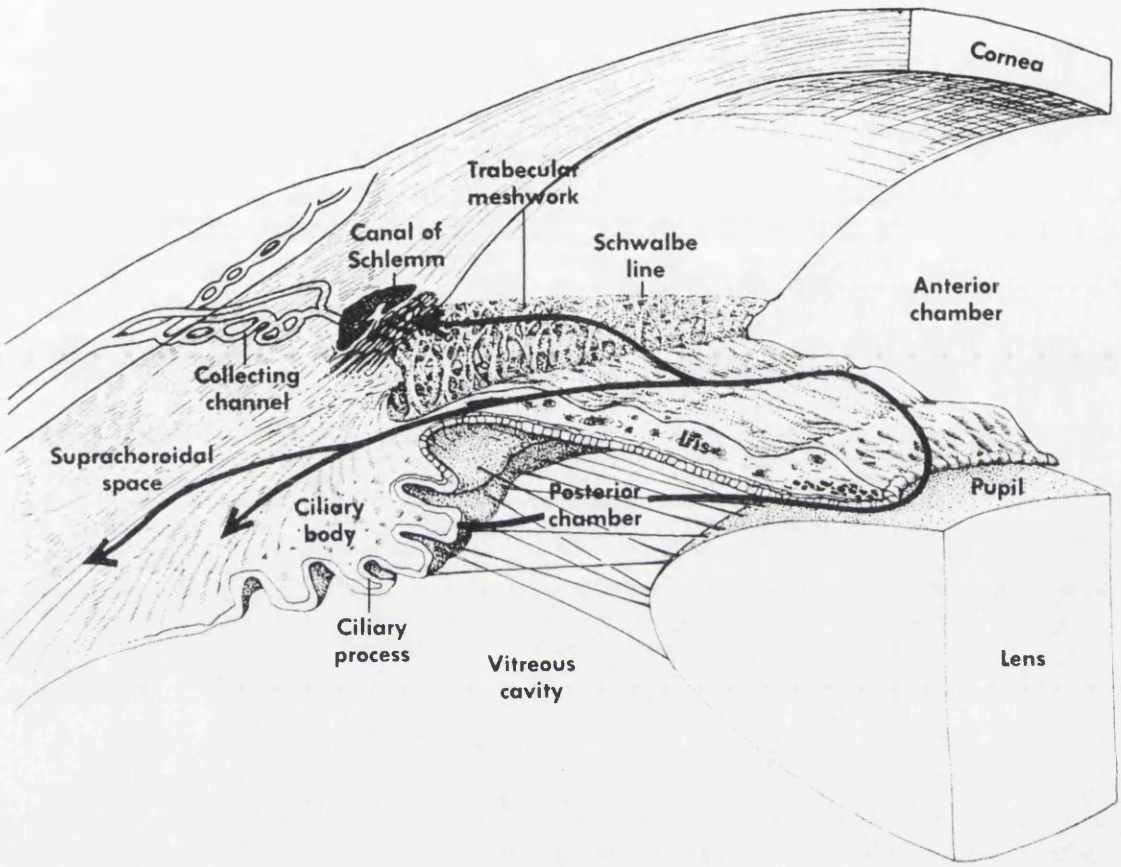

Figure 5 **The aqueous humour outflow pathways of the human eye.**
Aqueous humour is secreted by the non-pigmented ciliary epithelium into the posterior chamber. It flows through the pupil into the anterior chamber and leaves the anterior chamber through the trabecular meshwork, which opens into the canal of Schlemm. In humans, about 20% of the aqueous humour leaves the eye through the suprachoroidal space and ciliary muscle spaces (the uveoscleral or unconventional route) (Newell, 1986).

eyes, estimates have ranged between 10% and 30% of total outflow depending on the age of the eye and the method of measurement (Bill and Phillips, 1971).

1.1.8 Intraocular pressure

Intraocular pressure (IOP) is the tissue pressure of the structures in the anterior chamber of the eye. In normal individuals it varies from a low of 10mmHg to a high of about 22mmHg with a mean of about 15mmHg (Hollows and Graham, 1966). The IOP is a dynamic parameter which oscillates with respiration and ocular pulse pressure (Newell, 1986). IOP follows a circadian rhythm being lowest at night and varies seasonally, being highest in winter (Vareilles et al, 1977; Bar-Elan, 1984), but there is little agreement amongst authors as to the times of peak or low pressure. IOP is increased in response to increased arterial and/or venous pressure in the head, such as that which occurs when the head is below the heart or with a Valsalva manoeuvre. It also rises on response to contraction of the extraocular muscles and to pressure on the eyelid. The basis for the average steady-state IOP is the inflow of aqueous humour from the ciliary processes and the resistance to outflow of aqueous humour through the drainage pathways. As derived mathematically, pressure is force per unit area. In Ophthalmic practice, the units are expressed as mmHg. IOP can be measured indirectly by tonometry (indentation or applanation) or directly by a pressure transducer connected to an intracameral cannula. In clinical practice, measurement of IOP is performed by applanation tonometry which measures IOP by subjecting the eye to a force that flattens the cornea. Two methods have been devised. In the first, the area of the cornea being applanated is held constant and a variable force is applied. In the second, the

force applied to the cornea is held constant and the area applanated varies. Goldmann applanation tonometry is the prime example of variable force applanation tonometry. It is based on the Imbert-Fick law (Goldmann and Schmidt, 1957), which states that the pressure within a sphere (P) is roughly equal to the external force (f) needed to flatten a portion of the sphere divided by the area (A) of the sphere which is flattened: $P = f/A$ or $PA = f$.

1.1.9 Mathematical modelling of aqueous humour dynamics

In a physical system, flow (F) is defined as the volume (V) of fluid moving from one place to another in a unit of time (T): $F = V/T$ (Moses, 1987).

In the human eye the average rate of flow is $2.5\mu l.min^{-1}$ (Brubaker, 1991). Under steady state conditions, the rate of aqueous flow into the eye (F_{in}) is equal to the rate of aqueous flow out of the eye (F_{out}):

$$\mathbf{F_{in} = F_{out}}$$

Fluid flow always requires energy. In aqueous humour dynamics, the energy is supplied by hydraulic force, osmotic force and membrane pumps.

1. Hydraulic flow: Hydraulic flow is driven by a difference in tissue pressure across a barrier:

$$F_{hydraulic} = C (P_1 - P_2)$$

Where: $F =$ flow

$C =$ coefficient of facility of flow through the barrier (as defined by Poiseuille's law)

$P_1 - P_2 =$ the difference in tissue pressure of the two sides of the barrier

According to Poisseuille's law for streamline flow through a cylindrical tube:

$$C = \pi r^4/nL$$

Where: r = radius of the tube

n = fluid viscosity

L = length of the tube

C = coefficient of facility

In terms of fluid dynamics, the reciprocal of tissue coefficient of facility of flow is resistance, or R. Therefore: $R = 1/C$ and $P_1-P_2 = FR$

The outflow of aqueous humour through the conventional route is driven primarily by hydraulic force. The resistance to aqueous outflow offered by the outflow pathway tissues results in a damming back of aqueous humour into the eye which stretches the cornea and sclera, building sufficient pressure to drive the aqueous humour through the outflow pathways. In terms of aqueous humour outflow, the smaller the pores in the aqueous outflow pathways, the higher the outflow resistance (or the lower the outflow facility). Further, because facility is proportional to the fourth power of the radius, a small decrease in the dimensions of the outflow pathways would result in a large decrease in outflow facility.

2. Osmotic flow: Osmotic flow is driven by hydrostatic pressure (generated by the solvent) and osmotic pressure (generated by dissolved materials) resulting from an unequal distribution of material across a membrane permeable to the solvent only. Where there is an osmotic pressure difference, solvent flow will continue from the low solute side to the high solute side until the pressure head across that barrier is zero. The pressure head is the

difference in total pressure (osmotic + hydrostatic) on the two sides of the barrier.

3. Flow generated by metabolic pumps: Pumping of fluid across a concentration gradient occurs across many of the body's epithelial barriers. In the ciliary processes, Na^+/K^+ ATPase releases the energy for many of the molecules transported into the aqueous humour.

1.1.10 Aqueous outflow physiology

1. Functional morphology of outflow tissues: The trabecular meshwork is thought to be the tissue offering the greatest amount of resistance to aqueous outflow, and is thought to be the site of the pathogenesis underlying the increased IOP in glaucoma. Much of what is known about the site of abnormal outflow resistance in glaucomatous and normal human eyes comes from early classic studies of Grant (1963). He measured outflow facility in enucleated eyes before and after an ab interno trabeculotomy. He found that approximately 75% of the resistance to aqueous outflow was proximal to Schlemm's canal in normal eyes. Further, he found that enucleated eyes from individuals with glaucoma had a low outflow facility in vitro, and that the abnormal resistance to outflow was eliminated by removal of the trabecular meshwork.

The trabecular meshwork consists of three layers (Figures 6 and 7). The outermost layer is the cribriform layer (also referred to as the juxtacanalicular layer or the endothelial meshwork), which consists of several layers of

endothelial cells embedded in extracellular matrix. The corneoscleral layer consists of a sieve-like matrix of equatorial plates covered by endothelial cells. The innermost layer, the uveal meshwork, consists of strands of loose connective tissue, each strand being lined by endothelial cells. Within the limbus, the drainage pathways consist of Schlemm's canal, the internal collector channels and the aqueous veins, which typically carry only aqueous humour.

The ciliary muscle is involved in both conventional and unconventional aqueous outflow pathways. The longitudinal fibres, which are outermost, probably subserve changes in outflow facility when the ciliary muscle contracts. The reticular portion of the muscle consists of a large amount of connective tissue as well as muscle fibres. Its physiological function is not clear. The circular portion of the muscle subserves accommodation.

2. Aqueous outflow physiology: Much of our knowledge of drug effects on the outflow pathways has been derived from experimental anterior chamber perfusion of monkey eyes in vivo.

Outflow facility is typically measured by two-level constant pressure perfusion of the anterior chamber as originally described by Barany (1964). This involves placement of two needles into the anterior chamber; one connected to a perfusion reservoir and the other to a pressure transducer. The pressure in the eye is set by the height of the reservoir's meniscus at about 2.5mmHg above the resting IOP in order to have a net flow of perfusion solution into the eye. After several minutes, the meniscus of the reservoir is raised again to effect an IOP of about 10mmHg higher. The pressure is alternated between these two levels throughout the measurement period.

Outflow facility is calculated as:

$$C_{total} = (F_2-F_1)/(P_2-P_1)$$

Where: C_{total} = total outflow facility

F_1 = measured flow from reservoir at P_1

F_2 = measured flow from reservoir at P_2

P_1 = 2.5mmHg above spontaneous IOP

P_2 = 12.5mmHg above spontaneous IOP

Recently, an organ culture human eye preparation, suitable for outflow physiology studies has been introduced (Erickson-Lamy et al, 1991; Erickson-Lamy, 1992). In enucleated intact eyes or organ cultured anterior segments, outflow facility is measured at constant pressure after the method originally described by Grant (1963). Again, the anterior chamber is connected to a perfusion reservoir set at a given height above the limbus of the eye (typically equivalent to 10-15mmHg) and facility is measured as: **C = F/P**

3. The nature of aqueous outflow resistance: It is generally agreed that most of the resistance to aqueous outflow via the conventional route is located proximal to Schlemm's canal. However, there is controversy as to the exact site and nature of that resistance. Further, it is not completely understood exactly how aqueous humour crosses the endothelial lining of Schlemm's canal. A pressure/flow sensitive invagination-balooning of the inner wall cells has been observed; the so-called giant vacuoles (Johnstone and Grant, 1973; Kayes, 1975; Grierson and Lee, 1975b; Tripathi, 1977; Johnstone, 1979; Ainsworth and Lee, 1990). It is thought that aqueous humour leaves the anterior chamber through these vacuoles (Figure 8), however, a paracellular route may also exist.

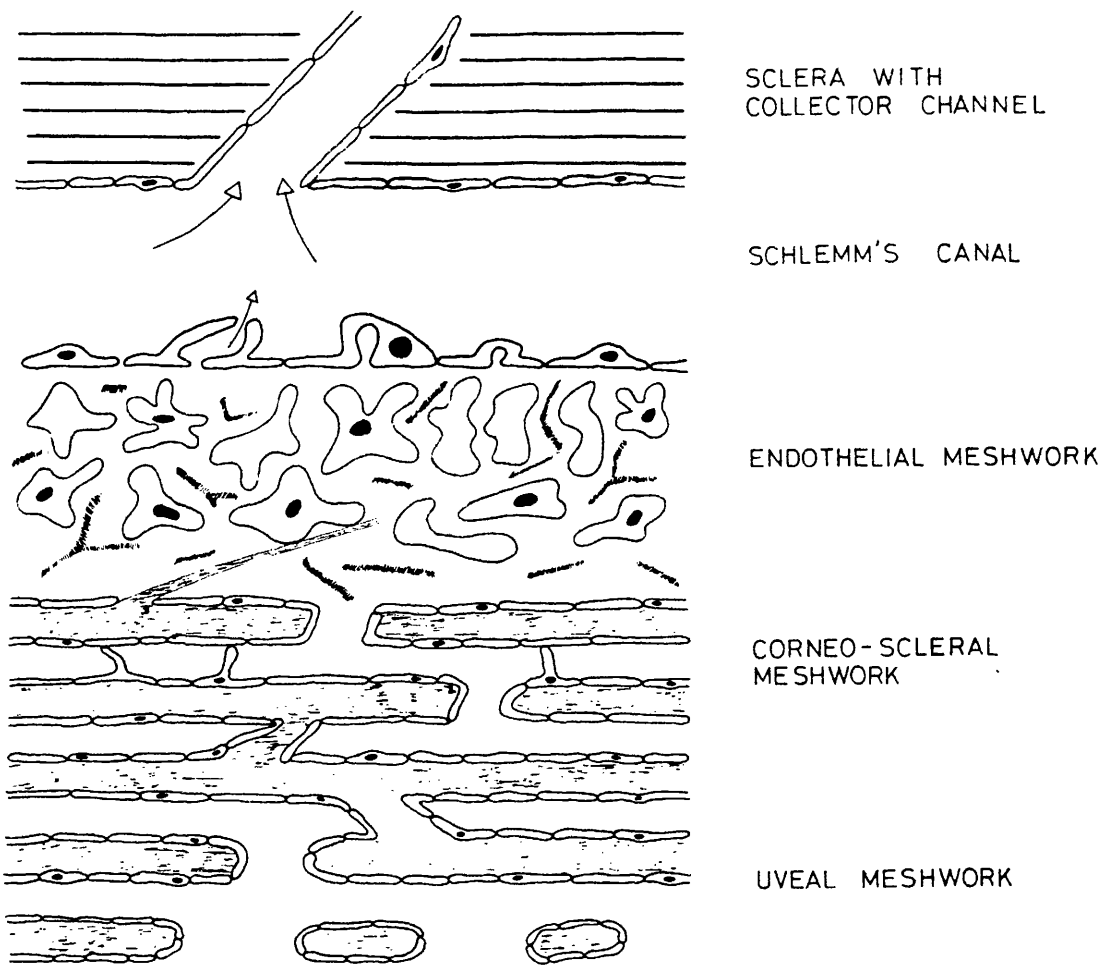

Figure 6 **Schematic representation of a section of the trabecular meshwork through chamber-angle tissue (Bill, 1975).**

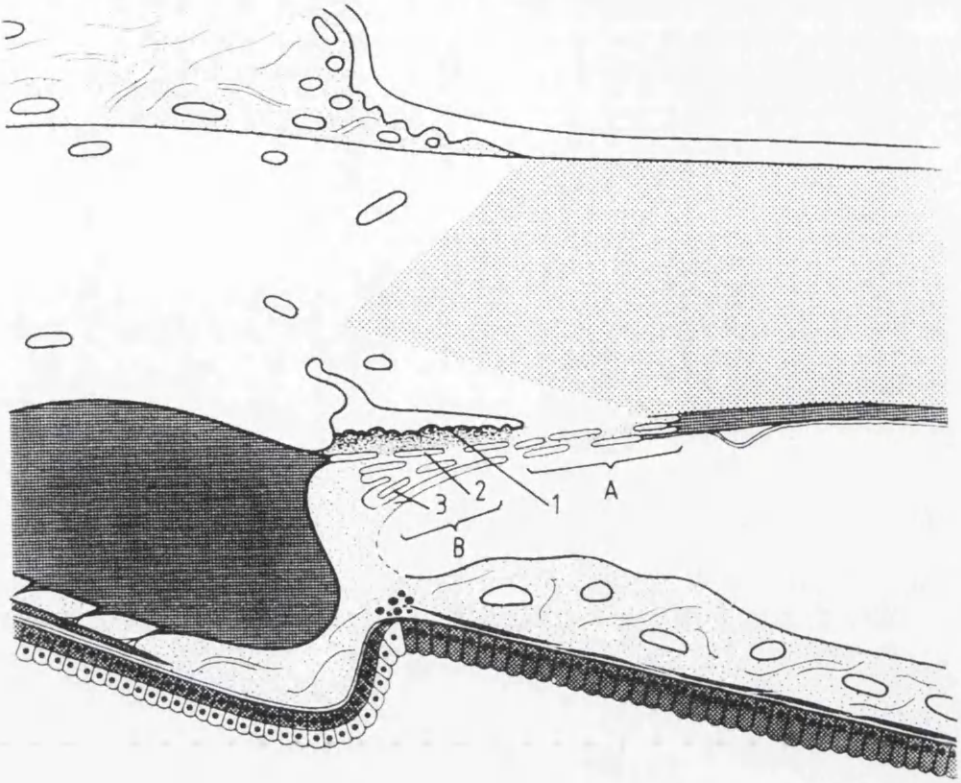

Figure 7 **Sagittal section through the chamber-angle and anterior portion of the human ciliary body (schematic drawing). Localisation of Schlemm's canal and trabecular meshwork. A: non-filtering part of the meshwork; B: filtering part of the meshwork; 1: cribriform layer; 2: corneoscleral layer; 3: uveal layer (Rohen, 1986).**

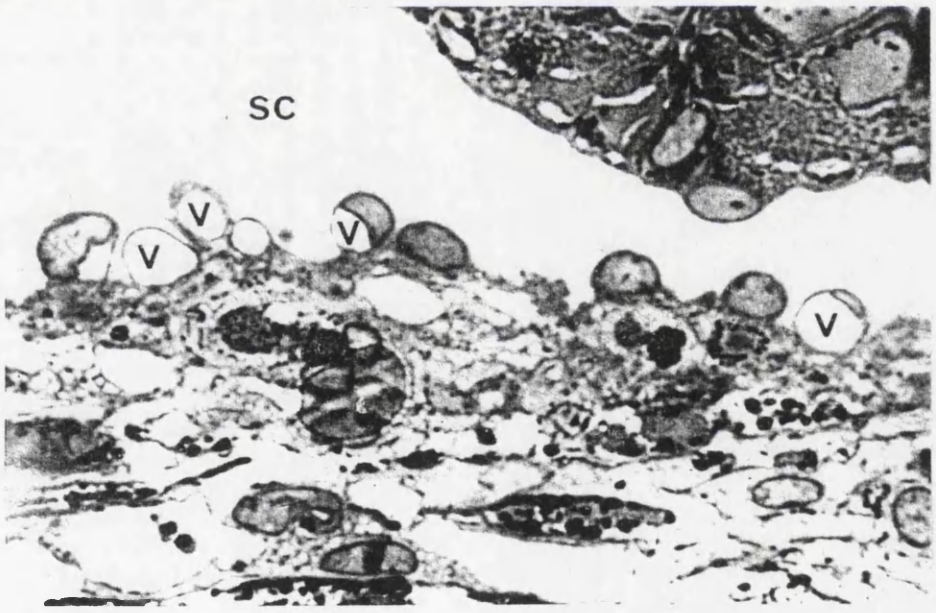

Figure 8 **Light micrograph of the human trabecular wall of Schlemm's canal (SC) showing giant vacuoles (V) (Tripathi, 1977).**
The lining cells are characterized by prominent bulging nuclei and vacuolar structures (v). Araldite section, x1306.

With regard to the nature of outflow resistance, some investigators have hypothesised that the resistance is mediated by tight junctions between cells while other investigators have implicated the extracellular material in the juxtacanalicular meshwork as the site of resistance. There are experimental studies that support both views.

4. Influence of the ciliary muscle on trabecular outflow physiology: Contraction of the ciliary muscle mediates increased outflow facility as well as changes in accommodation. The ciliary muscle is highly innervated with cholinergic fibres and contracts in response to stimulation by muscarinic agonists. Although there was some thought that pilocarpine's effect on outflow facility might be directly on the outflow pathway cells, experiments by Kaufman and Barany (1976) showed that the effect was mediated primarily by ciliary muscle contraction. Ciliary muscle contraction results in changes in the architecture of the conventional aqueous outflow pathway. However, whether and how these changes are involved in the effect on outflow facility is poorly understood.

5. Influence of the ciliary muscle on uveoscleral outflow physiology: The absence of a barrier between the trabecular beams and the ciliary muscle bundles results in a bulk fluid movement of aqueous humour into the spaces between ciliary muscle bundles and out into the suprachoroidal space. It has been shown that contraction of the ciliary muscle with cholinergic agents results in decreased uveoscleral outflow, due to an obliteration of the tissue spaces between ciliary muscle bundles (Bill and Walinder, 1966; Grierson et al, 1978). In contrast, chronic treatment with PGF_{2alpha}, which lowers IOP,

results in an increase in uveoscleral flow, which may be due to degradation of extracellular material in the spaces between the muscle bundles (Kaufman and Crawford 1989).

1.2 HYALURONIC ACID AND HEALON

1.2.1 Introduction

Hyaluronic acid (HA), along with chondroitin sulphate, keratan sulphate, heparan sulphate and heparin are the major glycosaminoglycans (also known as mucopolysaccharides) which constitute the polysaccharide chains in proteoglycans. Proteoglycans consist of units made of polysaccharide (about 95%) and protein (5%). These large polyanions bind water and cations and thereby form the extracellular medium or ground substance of connective tissue. Proteoglycans are important in determining the viscoelastic properties of joints and of other structures that are subject to mechanical deformation, acting as shock absorbers and structure stabilisers.

HA is synthesized in the cell membranes (Prehm, 1984) and is present in nearly all connective tissue matrices of vertebrate organisms. The highest concentrations of HA occur in synovial fluid, skin, vitreous body and certain specialised tissues such as umbilical cord and rooster comb. Levels of HA are also high during foetal development and in tissue repair and regeneration. Hyaluronate in the vitreous was discovered by Meyer and Palmer (1934). HA in the blood is present in a very low concentration (in human 0.015 - 0.055$\mu g.ml^{-1}$) originating mainly from the lymph, which in turn collects it from the connective tissue matrices (Engström-Laurent et al, 1985; Delpech et al, 1985). From the blood, HA is picked up by the liver and here metabolised by lysosomal enzymes. The turnover of HA in the bloodstream is normally in the range of 0.3-1.0$\mu g.min^{-1}$/Kg body weight (Laurent and Laurent, 1981; Fraser et al, 1983).

Sodium hyaluronate is a very long molecule and its repeating disaccharide units consist of sodium D- glucuronate linked to an N-acetyl-D-glucosamine molecule by a b 1→4 glucoside bond. The two disaccharide units are linked together with b 1→3 glucoside bonds forming the tetrasaccharide segment of a long, unbranched chain (Figure 9).

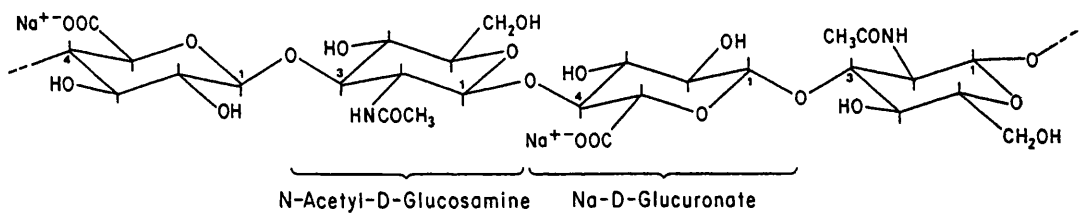

Figure 9 **The tetrasaccharide segment of a sodium hyaluronate chain.**

A 4 million molecular weight hyaluronate molecule contains approximately 10000 disaccharide units and has a length of 10m (Bothner and Wik, 1987). This long polysaccharide chain has not been shown to contain any covalently bound proteins, peptides, amino acids, other sugar molecules or nucleic acids. Nor is there any evidence of branching or of permanent crosslinks between the individual chains. Most important, the long polysaccharide chain is stabilised by hydrogen bonds and water bridges formed between adjacent individual sugar molecules and between neighbouring chains. X-ray diffraction studies of hydrated sodium hyaluronate membranes and sheets indicate ordered structures associating two molecular coils into a soluble

helical structure. The long molecular chain forming a random, kinked coil configuration constitute the basis of the specific viscoelastic properties of the molecules in a physiological ionic environment (Dea et al, 1973; Arnott et al, 1983). HA molecules in water or in physiological salt solution occupy a large molecular domain. This sponge-like molecule contains much water. The hydrated specific volume of a sodium hyaluronate molecule in physiological salt solution is 2000-6000ml per gram. This means that when a solution contains 0.16-0.5mg sodium hyaluronate per ml solvent (0.016-0.05% solution), all the solvent space is occupied (Balazs, 1986). The unique rheological properties of the hyaluronate molecule stem from its large molecular volume and from the extensive interaction and entanglement of the molecular coils (Gibbs et al, 1968; Comper and Laurent, 1978).

The property of viscoelasticity is the essence of usefulness of the viscoelastic materials. Viscosity is a measure of a solution's resistance to flow. It is dependent on the solution's degree of movement, also known as the shear rate. To facilitate optimal intraocular manipulations, a high viscosity at low shear rates (for space maintenance and tissue protection), a moderate viscosity at medium shear rates (allowing instrumentation and implantation) and a low viscosity at high shear rates (allowing injection via a cannula) is desired. The decrease of viscosity with increasing shear rate is also referred to as pseudoplasticity (shear thinning behaviour). The viscosity of a solution can be increased by increasing either the concentration or the molecular weight of the solute (Bothner and Wik, 1987, 1989). Viscosity is complemented by the property of elasticity, a measure of the capacity of a solution to resist deformation. When energy is transmitted to a viscoelastic solution at a low frequency or slow impact, the solution reacts primarily as a viscous compound.

However, when energy is transmitted at a high frequency or fast impact, the solution reacts as an elastic compound or gel. This allows a viscoelastic to be introduced into the eye via a 27 or 30 gauge cannula and yet maintain the intraocular space in which it is placed even in the face of an open incision. On the molecular level, this means that under low frequencies, the polysaccharide coils slip by each other and conformation and configuration rearrangement results in viscous flow. At high strain frequencies, this rearrangement cannot occur. Under these conditions, the molecular chains deform and mechanical energy is stored as elasticity. Thus, it acts as a reversible mechanical energy-storing and energy-dissipating system (Pruett et al, 1979). The degree of elasticity increases with increasing molecular weight of an elastic compound (Larson et al, 1989).

1.2.2 Hyaluronic acid in the anterior chamber of the eye

In the eye, HA is found not only in the vitreous, but also at a much lower concentration in the aqueous humour and in the connective tissue of the anterior chamber angle. HA was detected in the aqueous humour over 50 years ago (Meyer and Smyth, 1938). The concentration of HA in the aqueous humour is about 1-3$\mu g.ml^{-1}$; about 1% of that in the vitreous (Laurent, 1981; 1983).

The source of HA found in the aqueous humour is not entirely clear. Some authors state that the majority of it derives from the vitreous cavity and only a small amount from the corneal endothelium, which is covered by a thin layer of HA, and where hyaluronate-binding sites have been found (Madsen et al, 1989; Härfstrand et al, 1992). However, other studies suggest that the

majority of hyaluronate derives from local sources (ie, ciliary body). Detailed studies in rabbit and cattle have shown that in both species, HA is polydispersed; ie, it shows a broad range of molecular weight values (Laurent and Granath, 1983). HA found in the rabbit aqueous humour has an average molecular weight higher than that of vitreous; moreover, the turnover of HA in the vitreous is only 15% of that in the aqueous, the latter being estimated as 3μg/day (Laurent and Fraser, 1983). These findings imply that in the rabbit aqueous humour HA is not a simple degradation product of vitreous HA; some or possibly most of it probably originates from the anterior segment of the eye. In adult cattle as in rabbit a large proportion of the HA in the aqueous humour is of higher molecular weight than that of the vitreous (Laurent and Granath, 1983). However, unlike rabbit, cattle aqueous contains HA of lower molecular weight that could have originated from the vitreous by diffusion. Studies using explants of human and monkey ciliary body as well as carefully dissected tips of the ciliary processes support the view that these tissues may secrete HA into the aqueous humour (Rohen et al 1984, Schachtschabel et al 1984).

1.2.3 Hyaluronic acid and the trabecular meshwork

The major chemical components of the human trabecular meshwork (TM) are type I collagen and elastin fibres. In addition, basement membrane-like structures consisting in part of type IV collagen have been identified in human TM (Rodrigues et al, 1980). The TM is rich in glycosaminoglycans (GAGs) and also contains fibronectin and laminin (Mizokami, 1977; Rodrigues et al, 1980; Polansky et al, 1984; Sramek et al, 1987). GAGs are localised on the surface of the TM cells and Schlemm's canal and are in close association with

the connective tissue elements and extracellular spaces of the TM. Histochemical studies, mainly using alcian blue or colloidal iron, combined with electron microscopy, have demonstrated a broad spectrum of GAGs in the TM in a variety of animal species and in humans, with HA appearing as the most abundant species in human TM (Zimmerman, 1957; Armaly and Wang, 1975; Grierson and Lee, 1975a; Mizokami, 1977; Knepper et al 1981; Acott et al, 1985).

Many years ago, Barany (Barany 1953/54, 1956; Barany and Woodin, 1955) showed that perfusion of the aqueous outflow system with hyaluronidase resulted in an increase in outflow facility. Studies on hyaluronidase-infused rabbit eyes suggest that HA is an important component of the aqueous outflow resistance (Knepper et al, 1984). This was shown by comparing the effect of testicular hyaluronidase (which partially degrades not only hyaluronate but also chondroitin sulphate and possibly dermatan sulphate) with HA lyase (which degrades only HA specifically and completely). The latter was found to be more effective than testicular hyaluronidase in degrading HA of the TM and in reducing aqueous outflow resistance. HA lyase has a wider pH range than testicular hyaluronidase and at physiological pH completely removes all of the HA without affecting other GAGs present in the TM. These findings suggest that HA, more than other GAGs, may play a role in controlling outflow facility.

Hyaluronidase has not been detected in native aqueous humour. However, there is some indirect evidence for hyaluronate-degrading activity in surrounding ocular tissues. HA was injected into the anterior chamber of rabbits and the HA content of the iris-ciliary body reached a high level after 2h but returned to normal level within 24h (Miyauchi and Iwata, 1984). This

rapid elimination was attributed to enzymatic breakdown after diffusion into the iris-ciliary body and the angular aqueous plexus/sinus.

The concentrations of HA in glaucomatous individuals were found to be normal, (Laurent, 1983) observations that cast some doubt on the commonly held view that a hyaluronidase sensitive material in the aqueous may be one of the causes of impaired outflow facility in glaucoma. This would not, however, exclude a possible accumulation of HA or other GAGs in the TM, not necessarily originating from the aqueous, as a factor in impaired outflow facility.

1.2.4 The non-inflammatory fraction of sodium hyaluronate and the development of Healon

In 1972, Balazs et al suggested the use of sodium hyaluronate, which is a natural viscous material, as a vitreous substitute for posterior segment ocular surgery, but unexpected problems soon emerged. It turned out that although highly purified, sodium hyaluronate contained an inflammatory component which caused a characteristic invasion of mononuclear cells (mainly macrophages) when injected into tissue compartments (Balazs, 1986, 1989). The infiltration of mononuclear cells was accompanied by an increased protein concentration that reached a maximum 48h after injection.

The vitreous cavity of the primate eye proved to be especially sensitive to this inflammatory reaction. It took several years to develop the necessary reproducible and reliable techniques to prepare the so-called non-inflammatory fraction of sodium hyaluronate (NIF-SH).

In 1976, the NIF-SH extracted from such tissues as umbilical cords and

rooster combs was isolated and assayed in the primate eye. This preparation neither caused ocular inflammation even after multiple injections, nor did it potentiate the inflammatory reaction induced by other known inflammatory agents. This NIF-SH fraction was first manufactured by Biotrics, Inc. (Arlington, MA) and later by Pharmacia AB (Uppsala, Sweden), and is currently marketed by Kabi Pharmacia Ophthalmics AB (Uppsala, Sweden) under the trade name Healon in disposable syringes containing 0.5ml of 1% sodium hyaluroante in aqueous buffer. Healon is registered in some countries as Healonid. Healon contains $10\pm1 mg.ml^{-1}$ NIF-SH dissolved in a sterile, preservative-free phosphate buffer (0.146M NaCl, 0.34mM NaH_2PO_4, 1.5mM Na_2HPO_4; pH 7.2 ± 0.2). The molecular weight of Healon is 2-4 x 10^6 , its viscosity 100-300 x 10^3 centistokes and its colloid-osmotic pressure 9mmHg. The concentration of impurities such as proteins (as amino acids), nucleic acids and sulphated GAGs in the solution is very small; less than $0.005 mg.ml^{-1}$, $0.03 mg.ml^{-1}$ and $0.03 mg.ml^{-1}$, respectively.

The introduction of Healon has given birth to the concept of viscosurgery. The term viscosurgery was coined by Balazs to describe surgical procedures which employ viscoelastic solutions to produce certain surgical effects. In ocular surgery, viscosurgery is used to protect eye tissues from mechanical trauma, to create and maintain tissue space for surgery and to permit the manipulation of tissues without mechanical damage.

1.2.5 Turnover of hyaluronate and the non-inflammatory fraction of sodium hyaluronate in the eye

The half-life time of NIF-SH in the anterior chamber depends on the viscosity of the solution and the amount injected (Denlinger and Balazs, 1988). The more NIF-SH injected and the greater the viscosity of the injected solution, the more time is required for the NIF-SH molecules to clear the anterior chamber. In one study (Schubert et al, 1981), 6 samples of 1% NIF-SH of differing viscosities (ranging from 10 to 940 x 10^3 centistokes) were injected into the owl-monkey eye in two volumes replacing approximately 50% and 75% of the volume of the anterior chamber (0.22 and 0.35ml, respectively). With the smaller volume, the rate of disappearance depended entirely on the viscosity, with the highest viscosity solution cleared from the anterior chamber with a rate of 50µl/h and the lowest viscosity cleared the eye with a rate of 120µl/h. When the larger volume of the aqueous humour was replaced, the initial rates of disappearance were much slower; the lowest viscosity and highest viscosity samples cleared the anterior chamber at rates of 30 and 70µl/h, respectively. These experiments showed that 24h after the injection only 27% of the high viscosity-low volume solution remained compared with 50% of the high viscosity-high volume solution. By 48h all solutions except for the high viscosity-high volume solution had cleared from the eye. By 72h 15% of the high viscosity-high volume solution still remained in the anterior chamber. In these experiments, the molecular size of the hyaluronate within the anterior chamber did not change. Therefore, there appears to be no endogenous capacity for degradation. It is interesting to note that the maximal increase in IOP in all cases occurred during the first 7h and returned to normal

24h after injection, a time at which as much as 370 times the normal concentration of hyaluronate still remained in the anterior chamber.

Fraser et al (1981), Laurent and Fraser (1983) and Laurent et al (1988) studied the disappearance of hyaluronate from the rabbit eye with labelled polysaccharides. They found that when hyaluronate is injected into the vitreous it leaves the tissue by diffusion and that the disappearance rate is molecular weight dependent. They also found that endogenous hyaluronate in the vitreous has a half-life of about 70 days similar to that in humans. Trace amounts of hyaluronate injected into the anterior chamber are transported by aqueous flow into the general circulation and have a half-life of 60-80 min in the eye. When concentrated sodium hyaluronate (1%) was injected into the anterior chamber, the disappearance rate was slower. When 0.05 or 0.2ml was injected, initial disappearance rates corresponding to half-lives of 9 and 14h, respectively, were recorded. The rate accelerated, however, as the substance disappeared from the eye.

1.3 CATARACT SURGERY AND HEALON

1.3.1 Lens and cataract

The crystalline lens is a transparent biconvex structure, located directly behind the iris and pupil and anterior to a shallow depression in the vitreous face, the lenticular fossa. It is approximately 10mm in diameter and 4mm thick (Newell, 1986). It is held in position by its suspensory ligament (zonule) which connects the lens to the ciliary body. The zonule is composed of fine fibrils of modified collagenous tissue on the outer surface of the lens capsule. The zonules attach to the lamellar portion of the lens capsule on either side of the equator and to the basement membrane of the ciliary epithelium in the valleys between the ciliary processes. The ciliary attachment is long and fibres may extend to the pars plana of the ciliary body. Other fibres attach to the anterior vitreous face. The insertions extend about 2mm in front and 1mm behind the lens equator. The lens is composed of (1) a lens capsule that envelopes the entire lens, (2) an anterior lens epithelium located immediately beneath the anterior lens capsule and (3) a lens substance consisting of the cortex (newly formed lens fibres with nuclei) and the nucleus (a dense central area of fibres that have lost their nuclei).

The lens capsule is a smooth, homogenous, acellular structure. The anterior capsule is the basement membrane of the anterior lens epithelium and is the thickest basement membrane in the body. The posterior capsule is the basement membrane of lens cells (fibres). The lens substance consists of elongated lens fibres. Young lens fibres form at the equator and migrate towards the anterior of the lens. They contain nuclei and are attached to their

basement membrane, the posterior capsule. Mature lens fibres have lost their nuclei and are no longer in contact with the posterior capsule. These form most of the substance of the lens. They are packed into an increasingly dense, central nucleus. The lens fibre proteins are composed of a water-soluble fraction, the crystallins, and a water-insoluble group.

The term cataract is used to describe the condition of opacification of the crystalline lens of the eye. Age- related (senile) cataract is the commonest form of cataract and appears to be an accentuation of normal ageing changes which occur in the lens though, occasionally, it is seen as early as 40 years of age. It is divided into two main types: a senile opacification of the nucleus of the lens and a senile opacification of the cortex. A large number of senile cataracts possess both nuclear and cortical opacities. Age-related cataract is the main cause of blindness and visual impairment throughout the world (Leske and Sperduto, 1983; Kupfer, 1984; Foster and Johnson, 1990). With the ageing of the population, the overall prevalence of visual loss due to lens opacities continues to increase every year. More than 17 million persons worldwide are estimated to be blind from cataract (Foster and Johnston, 1990). Surgical removal is the only treatment for cataract and cataract extraction is the single most common intraocular surgical procedure.

Extraction of age-related cataract may be performed in two ways: by removal of the entire lens (by cryoextraction), including its capsule (intracapsular cataract extraction; ICCE) or by excision of a portion of the anterior capsule followed by expression of the nucleus and cortical clean-up with retention of the posterior capsule (extracapsular cataract extraction; ECCE). Phacoemulsification is a type of ECCE in which the lens matter is broken up by ultrasonic vibration into an emulsion which can then be aspirated

from within the capsular bag and replaced with infusion fluid. The ICCE was the method of choice until early 1980s. Since then, ECCE has become increasingly more popular because of the use of posterior chamber intraocular lenses (p/c IOL) that require an intact equatorial lens capsule for support. In ECCE, the IOL, with its haptics, is inserted into the ciliary sulcus (a groove between the root of the iris and the ciliary body) or into the capsular bag. The latter is known as endocapsular or envelope-like technique.

1.3.2 Cataract surgery and corneal endothelial cell damage

Corneal clarity is dependent on the endothelium which serves as a barrier and physiological pump in maintaining stromal dehydration. Injury to corneal endothelial cells is a risk in cataract surgery, particularly when cataract removal is combined with IOL implantation. Endothelial damage may occur at any stage in even routine procedures in the course of any type of cataract surgery from the opening of the anterior chamber with manipulation of the cornea to insertion of an IOL following cataract extraction.

In most instances of cataract surgery-induced damage, a transient insufficiency of endothelial function results causing striate keratopathy (corneal decompensation) which in some instances may cause permanent visual dysfunction. Additional trauma to the endothelium due to IOL implantation is likely to compromise the endothelium further. Corneal endothelial cells do not regenerate through mitosis. Therefore, damage to cells during anterior segment surgery, resulting in a loss of endothelial cell density, is thought to be a major factor in postoperative corneal oedema. Bourne and Kaufman (1976) reported a 62% decrease in endothelial cell density following IOL implantation. This

damage is thought to have resulted from contact between the cells and the surface of the implant and varies with the style of the implant used (Kaufman and Katz, 1976). When cells are lost, the denuded area is covered by enlargement and sliding of remaining cells resulting in a net decrease in cell density (Khodadoust and Green, 1976). It is thought the function of the endothelium as a whole is compromised by the decreased cell density. Early attempts to avoid surgically-induced endothelial cell damage involved the filling of the anterior chamber with air, balanced salt solution, or Hartmann's solution to buffer the endothelium against trauma. These attempts resulted in limited success, as the air and solutions were lost as the cornea was retracted.

1.3.3 Viscoelastic materials and cataract surgery

Endothelial dystrophy following cataract surgery has almost become a preventable complication since the advent of endothelial specular microscopy in the late 1970s. This technological advance contributed greatly to the knowledge of age and disease-related changes in the density of corneal endothelial cells. Careful studies have shown which procedures and techniques result in the least traumatic loss of endothelial cells. Methods of cataract removal and styles of IOL have changed because of these findings (Hoffer, 1979, 1982). Since the recognition by Bourne and Kaufman (1976) and Forstot et al (1977) that corneal endothelial cell loss is greater after cataract extraction and IOL implantation than after cataract extraction alone and that contact between the IOL and the endothelium at the time of surgery is a major factor in endothelial damage (Kaufman and Katz, 1976), several substances have been investigated for their protective effect. The rationale

behind the use of these substances has been to prevent contact between the IOL and the corneal endothelium by maintaining the anterior chamber shape and to prevent endothelial damage by coating the IOL or the endothelium or both, should contact occur. The literature describes the average endothelial cell loss in the hands of experienced surgeons in the 1970s to be about 50% (Forstot et al, 1977; Binkhorst et al, 1978; Sugar et al, 1978; Binkhorst, 1980). The substances that have been investigated to various extents in animal and human eyes include balanced salt solution (BSS), air, plasma, serum, albumin, gamma globulin, TC199, polyvinylpyrrolidone, methylcellulose, chondroitin sulphate and sodium hyaluronate (Kirk et al, 1977; Bourne et al, 1979; Soll et al, 1980; Fechner and Fechner, 1983; MacRae et al, 1983; Aron-Rosa et al, 1983; Fechner, 1985; Rosen et al, 1986). Of these substances, sodium hyaluronate was the first one introduced commercially and is the most widely used.

Healon was first used in anterior segment surgery animal lens implant experiments by Miller et al (1977) and was later successfully used in human cataract surgery (Graue et al, 1980; Miller and Stegmann, 1980; Pape and Balazs, 1980). Healon reduced postoperative corneal endothelial swelling and cell loss when it was used during cataract surgery. Its clinical use has resulted in reported endothelial cell loss of only 9.7% to 18% (Miller and Stegmann, 1980, 1981, 1982; Pape, 1980; Lazenby and Broocker, 1981; Percival, 1983).

However, not all reports have shown a significant protective effect of Healon. Hoffer (1982) and Bourne et al (1984) were unable to demonstrate significant decreases in postoperative endothelial cell loss with the use of Healon during ECCE with p/c IOL. These authors suggested that the protective effects of Healon might be more demonstrable in operations normally accompanied by greater cell loss or unusually traumatic surgery.

Some of the many uses of viscoelastic substances during cataract surgery as reviewed by Liesegang (1990) are shown in Table 2 and have been described in detail by many authors (Miller et al, 1977; Pape, 1980; Pape and Balazs, 1980; Percival, 1981; Hoopes, 1982; Stegmann and Miller, 1982; Holmberg and Philipson 1984a, 1984b; Polack, 1986, Larson et al, 1989). A number of viscoelastic substances are currently available for use in intraocular surgery (Table 3) and several new preparations are in various stages of development. Healon was the first viscoelastic substance introduced commercially and is the standard against which newer viscoelastic substances are compared. Apart from cataract surgery and vitreoretinal surgery, viscoelastics are used in penetrating keratoplasty (Polack, 1981; Steele, 1983), glaucoma surgery (Hung, 1985; Raitta and Setälä, 1986), anterior segment reconstruction (Roper-Hall, 1983; Drews, 1986), plastic surgery (Lerner and Boynton, 1985), eye muscle surgery (Clorfeine and Parker, 1987) and tear dysfunction states (Limberg et al, 1987). It is estimated that viscoelastics are used in about 60% of all ophthalmic procedures and about 75% of all cataract extraction procedures.

TABLE 2: USES OF VISCOELASTIC MATERIALS IN CATARACT SURGERY

In extracapsular cataract extraction

- Cushion anterior chamber
- Fill, maintain and restore the depth of the anterior chamber
- Coat and protect corneal endothelial cells
- Secure anterior chamber with vitreous pressure
- Push back choroidal haemorrhage
- Prevent scrolling of the anterior capsule of the lens
- Break of posterior or anterior synechia
- Hydraulic separation of nucleus and cortex
- Maintain mydriasis
- Tamponade bleeding vessels on iris or in wound
- Restore globe integrity when vitreous is lost
- Ease evacuation of nucleus by injection under capsule
- Help better wound adaptation with fully constituted globe
- Help reposition of detached Descemet's membrane
- Protect cornea by acting as epithelial wetting agent
- Ease passage of limbal sutures

In IOL insertion

- Expand capsular bag or ciliary sulcus for insertion of the implant
- Coat intraocular lens
- Tamponade break in posterior capsule and push back vitreous for placement of implant

In phacoemulsificaton

- Coat corneal endothelium to protect from eddy currents of irrigating fluids or whirling lens particles
- Cushion introduction of various large instruments through small opening
- Push iris - vitreous or iris - capsule diaphragm back and maintain deep anterior chamber
- Help rotate nucleus within capsular bag

In intracapsular cataract extraction

- Protect the corneal endothelium from heat and exposure drying, as well as from the effects of cryoextraction
- Coat and protect corneal endothelium from potentially sliding contact between the lens and the cornea
- Help for manipulating the iris diaphragm in postextraction vitreous/iris bulge
- Restore and maintain the anterior chamber depth during wound closure
- Help for localising bleeding from a peripheral iridectomy and haemostasis
- Prevent fluid vitreous from having access to the wound area and thereby its incarceration
- Reform the posterior chamber and therefore create the space into which a retropupillary intraocular lens will be inserted with consequential protection of the anterior hyaloid membrane
- Deepen the anterior chamber angle to facilitate placement of the loops of an anterior chamber intraocular lens.

TABLE 3: COMMERCIAL VISCOELASTIC PREPARATIONS

Preparation	Viscoelastic material	Concentration ($mg.ml^{-1}$)	Manufacturer
Healon	Sodium hyaluronate	10	Pharmacia
AmVisc	Sodium hyaluronate	10	IOLAB
AmVisc Plus	Sodium hyaluronate	16	IOLAB
Viscoat	Sodium hyaluronate Chondroitin sulfate	38 30	Alcon
Occucoat	Hydroxyl-methylcellulose	20	Storz

1.3.4 Complications of Healon

Although Healon is a useful surgical tool, besides its high cost, there are disadvantages and complications resulting from its use. Healon is a highly viscous clear solution at rest very similar to the vitreous and is therefore difficult to identify when both are present in the anterior chamber. If Healon is left inside the eye, inflammatory cells, red blood cells and lens material may remain suspended in the anterior chamber, giving the appearance of a 'plastic' anterior uveitis (Larson et al, 1989). This may delay the reabsorption of these materials and cause persistent inflammation. Sodium hyaluronate contained in Healon is extracted from avian tissues and despite vigorous purification

procedures, minute amounts of protein are present. Although immunogenicity studies in both animals and humans showed no evidence of immunological reactions (Richter, 1979; Richter et al, 1979), the possibility of idiosyncratic reaction following its use remains (Hultsch, 1980).

Potential endothelial cellular toxicity is another disadvantage of Healon. Although Healon does not decrease corneal wound strength (Arzeno and Miller, 1982), some reports suggest that it may have toxic effects on the corneal endothelium (Meyer and McCulley, 1989; Slack and Hyndiuk, 1994).

The most serious complication of the use of Healon is the induction of early (<24h) postoperative intraocular hypertension over and above that reported following cataract surgery before its advent (Rich et al, 1974; Haimann and Phelps, 1981; Tuberville et al, 1983). This augmentation and prolongation of the IOP elevation may occur especially if the Healon is left within the eye postoperatively. This has been referred to as Healon-block glaucoma (Hoffer, 1982).

The role of Healon as a cause of postoperative intraocular hypertension has been controversial. The original reports on the use of Healon did not mention any postoperative IOP rise. In several studies no statistically significant differences in IOP were observed after use of Healon compared with controls (Pape and Balazs, 1980; Miller and Stegmann, 1981; Stegmann and Miller, 1982; Holmberg and Philipson, 1984a, 1984b). However, other studies showed significant rises in IOP and advocated aspiration or dilution of Healon at the end of surgery (Pape, 1980; Lazenby and Broocker, 1981; Olivius and Thorburn, 1985) . The discrepancies in results may be due to differences in surgical technique (ECCE or ICCE, and whether or not the viscoelastic was removed at the end of surgery) and in the postoperative times that the pressures

were monitored. Most clinicians record the first postoperative IOP measurement after about 24h (1st postoperative day) and the early postoperative IOP rise passes, in most cases, unnoticed. In a prospective randomized investigation of postoperative IOP in patients undergoing ICCE with or without Healon, Passo et al (1985) found that the maximal difference in IOP between the Healon group and the control group occurred approximately 16h postoperatively. Cherfan et al (1983) emphasized the importance of early postoperative measurements when evaluating the effect of Healon on postoperative IOP and stated that IOP measurements made one day postoperatively were insufficient. Collectively, the IOP readings in studies which did not show increased IOP were taken 24h or more after surgery. The presence of increased IOP more than 24h after cataract surgery may be a consequence of excessive surgical trauma or a compromised trabecular meshwork (Liesegang, 1990).

1.3.5 Methods to reduce Healon-induced postoperative intraocular pressure elevation

A number of investigators have advocated methods to attenuate the Healon-induced IOP increases. Some authors have reported successful attenuation of the rise in IOP by prophylactic treatment with beta blockers, acetazolamide and/or miotics (Miller and Stegmann, 1981, 1982; Percival, 1982; Lewen and Insler, 1985; Anmarkrud et al, 1992). However, the use of pre- or postoperative anti- glaucoma remedies is often ineffective and/or slow to take effect. There is no consensus that pre- or postoperative therapy with acetazolamide, miotics or beta-adrenergic antagonists prevent the postoperative

IOP pressure elevation induced by viscoelastics (Krupin et al, 1989).

The practice of diluting or removing Healon from the anterior chamber to prevent postoperative increases in IOP has been recommended by many authors (Pape, 1980; Lazenby and Broocker, 1981; Miller and Stegmenn, 1981, 1982; Hoffer, 1982; Stegmann and Miller, 1982; Cherfan et al, 1983; Glasser et al, 1986). Pape (1980) reported higher mean IOP values one day postoperatively in non- irrigated eyes than in irrigated eyes (38.3 and 19.4mmHg, respectively). Cherfan et al (1983) found that using a minimal quantity of Healon and diluting it with BSS at the end of surgery lessened but did not prevent postoperative increases in IOP. Olivius and Thorburn (1985) measured IOP following ECCE and IOL implantation, with and without irrigation of Healon at the end of the surgery. When Healon was left in the eye, 35% of the patients had an IOP increase of greater than or equal to 20mmHg during the initial ten hours, compared with 11% of the group in which Healon was washed out. Twenty hours after surgery, the irrigated eyes had a lower mean IOP than the non-irrigated eyes (15.6mmHg vs 23.8mmHg). Glasser et al (1986) reported that the IOP elevations peaked within 4h after instillation of Healon and were significantly reduced by anterior chamber washout. However, although irrigation appears to attenuate the IOP increase, substantial increases in IOP can still occur postoperatively. Bourne et al (1984) aspirated Healon at the end of surgery but still observed a significant rise in IOP in the 1st postoperative day compared with the preoperative value. Similarly, Naeser et al (1986) found that in spite of aspiration of Healon, a significant rise in IOP was observed after 6h both in comparison with the preoperative values and with controls. Anmarkrud et al (1992) studied the effect of Healon on the acute IOP rise after ECCE with p/c IOL and found a significant rise in IOP at 3-6h postoperatively, whether or

not Healon was aspirated.

It is clear that the success of the present attempts to attenuate Healon-induced IOP rises either by anti-glaucoma drugs or by irrigating/aspirating Healon at the end of surgery is at best limited.

1.3.6 Mechanism of Healon-induced intraocular pressure elevation

The mechanism underlying Healon-induced increases in IOP probably involves an obstruction of aqueous outflow through the conventional outflow routes. Support for this hypothesis comes from clinical as well as laboratory data. The relative lack of success of acetazolamide in significantly preventing the Healon-induced rise in IOP supports the hypothesis that Healon causes a transient but massive obstruction of the trabecular meshwork rather than increased production of aqueous humour. The observation that the Healon-induced increase in IOP occurs quickly and is short-lived (i.e. <24h) further supports the hypothesis that the trabecular meshwork is obstructed with fragments of hyaluronate or with small accumulations of undiluted hyaluronate. These clinical observations are supported by laboratory findings. Berson et al (1983) found a 65% decrease in outflow facility one hour after the instillation of sodium hyaluronate into the anterior chamber of enucleated human eyes compared with a 32% decrease when the hyaluronate was immediately irrigated from the anterior chamber. However, after three hours outflow facility showed a further overall decrease to 60%. They concluded that irrigating the anterior chamber after using sodium hyaluronate does not eliminate the possibility of severe postoperative glaucoma (i.e. anterior chamber washout delayed but did not prevent the reduction in outflow facility).

It is not clear whether obstruction of the outflow pathways occurs because of the high viscosity of the Healon solution or because of the high molecular weight (and size) of the polymers. If the egress of Healon from the anterior chamber is viscosity-dependent as is thought by Pape (1980), substances with higher viscosity would theoretically have greater difficulty leaving the anterior chamber and consequently cause greater increase in IOP. However, Schubert et al (1984) found that low viscosity solutions of NIF-SH caused a greater and more prolonged increase in IOP in the owl monkey than high viscosity solutions of NIF-SH. Levy and Boone (1989) suggested that the viscosity of the hyaluronate solution can be increased while the likelihood of IOP elevation can be reduced by using lower molecular weight hyaluronate molecules. In the rabbit eye, instillation of sodium hyaluronate decreased outflow facility, but a mixture of hyaluronate and hyaluronidase produced no significant change in outflow facility (Hein et al, 1986). Other rabbit studies found that IOP increased with sodium hyaluronate and that the recovery time to normal pressure was not dependent on molecular weight or concentration of sodium hyaluronate (Iwata et al, 1984). However, the recovery times of protein and ascorbate concentration to normal levels were longer than those obtained after injection of lower molecular weight and lower concentrations of sodium hyaluronate. A rapid recovery of the aqueous humour components to normal levels is presumably desirable after anterior segment surgery because the constituents of aqueous humour are necessary to maintain good conditions for the metabolism of the anterior segment tissues.

1.3.7 The use of Healon in compromised eyes

Routinely performed cataract surgery often causes ocular hypertension in the first hours after surgery (Rich et al, 1974; Haimann and Phelps, 1981; Tuberville et al, 1983). The elevations of IOP after cataract surgery are miltifactorial and may be due to debris, residual lens material, products of inflammation, prostaglandin release, inflammatory cells, red blood cells, breakdown of the blood-aqueous barrier, suture-induced changes in the trabecular meshwork or watertight wound closure. The immediate effect of cataract extraction on IOP continues to generate conflicting reports from a reduction or little change to universal elevation. However, there is reasonable agreement that the pressure effects are not permanent in the normal eye, but there is no consensus as to the long-term effects in the glaucomatous eye (Galin et al, 1978).

Elevation of IOP following uncomplicated cataract extraction is a common though certainly not innocuous phenomenon. Pain, corneal oedema, optic nerve damage - glaucomatous or ischaemic - and possible inhibition of wound healing are all serious consequnces of increased postoperative IOP (Kolker, 1977; Hayreh, 1980; Bartov et al, 1984).

The normal eye ordinarily tolerates transient elevations in IOP without a deleterious effect on the ultimate visual outcome. However, in the eye with advanced glaucomatous cupping and severe visual field loss, even a transiently elevated IOP can have devastating effects on the remaining visual field. In a large series of patients with severe glaucomatous damage who underwent intraocular surgery, Kolker (1977) found that 2 of 23 eyes (8.7%) lost central vision after cataract extraction. The loss was attributed to elevation of IOP

postoperatively.

The early cataract IOP rise in glaucoma patients is greater both in frequency and magnitude than that reported in similar cataract surgery in non-glaucomatous eyes. (Savage et al, 1985; McGuigan et al, 1986; Krupin et al, 1989; Calissendorff and Hamberg-Nyström, 1993).

Therefore, the strong possibility that Healon exacerbates IOP increases due to surgery potentially places glaucomatous eyes at even greater risk. An anterior chamber filled with Healon may stress the ability of the outflow mechanism to eliminate it in a timely fashion, especially if there is a history of a compromised outflow. This condition could be extremely dangerous if the pressure rises high enough to cause closure of the central retinal artery (Hoffer, 1982). The glaucomatous eye already has a compromised outflow system that would be expected to tolerate stress poorly. A number of other obstructing substances are present in the perioperative period to lead to such a decrease in outflow facility. In glaucomatous eyes because their pupils are often insufficiently dilated due to long-term miotic therapy or previous laser and surgical therapy, sphincterotomy is often required. Despite sphincterotomy, the width of the opening during capsulotomy and irrigation-aspiration is often considerably smaller than in normal eyes with cataract. Hence, there is more iris trauma and more inflammatory mediators are released.

High ocular pressure can decrease blood flow in the optic nerve head (Hayreh, 1980) since blood flow is dependent on perfusion pressure which is equal to mean blood pressure in the nutrient vessels minus IOP. It is therefore possible that individuals with atherosclerotic vessels and poor optic nerve blood flow may be very susceptible to even moderate elevation in IOP. Indirect evidence for this can be found in the series of cases reported by Hayreh, who

described 11 patients who developed anterior ischaemic optic neuropathy following cataract surgery. There is no firm evidence that severe IOP for several hours after cataract extraction in healthy eyes is harmful. Perioperative anti-glaucoma drugs are therefore not routinely used in all patients undergoing cataract surgery. However, in view of Hayreh's report, certain patients may benefit from the use of perioperative drugs to prevent pressure elevation. Any patient who has had anterior ischaemic optic neuropathy soon after cataract extraction in one eye is probably at great risk to develop it in his second eye after cataract surgery. Hayreh recommends that such a patient should receive perioperative acetazolamide and timolol. The use of these drugs should also be considered in any patient who has had atherosclerotic anterior ischaemic optic neuropathy, not related to cataract surgery or who has well documented diffuse atherosclerosis (i.e. coronary heart disease, cerebral infarction and/or severe claudication). Furthermore, such patients should also have their IOP measured 4-8h after surgery so that additional pressure-lowering treatment can be given if prophylactic medications fail to prevent severe ocular hypertension.

The possibility that the use of Healon can, in itself, cause increased pressure means that it must be used with care. If any method of obviating that risk can be found, it would be an invaluable contribution to safe ophthalmic surgery, since it undoubtedly offers many benefits.

1.4 ASCORBIC ACID

1.4.1 Introduction

Ascorbic acid (AA), also referred to as L-ascorbic acid or vitamin C, is a water-soluble vitamin widely distributed in the animal and plant kingdoms. It is of major importance in nutrition, maintenance of good health and the food industry. Scurvy is the deficiency disease caused by lack of AA and has been known since the time of crusades especially among Northern European population, who subsisted on diets lacking fresh fruits and vegetables. In 1932, the anti-scorbutic factor from lemon juice was isolated (Waugh and King, 1932).

1.4.2 Chemistry of ascorbic acid

AA is a six-carbon ketolactone (Figure 11) with molecular weight of 176.1 ($C_6H_6O_8$) structurally related to glucose from which is synthesized both chemically and biologically.

AA is a potent reducing compound which is reversibly oxidized in the body to dehydro-L-ascorbic acid (DHAA), which is also biologically active (Figure 11). DHAA can be converted by further oxidation to diketogulonic acid which is biologically inactive. AA has an optically active carbon atom and antiscorbutic activity resides almost totally in the L-isomer. Another isomer, erythorbic acid (D-isoascorbic acid or D-araboascorbic acid or D-erythorbic acid or D-ascorbic acid) has very weak antiscorbutic action but has a similar redox potential. The reason for the lack of stronger antiscorbutic action of

erythorbic acid is probably the incapacity of the tissues to retain it in the quantities in which AA is stored.

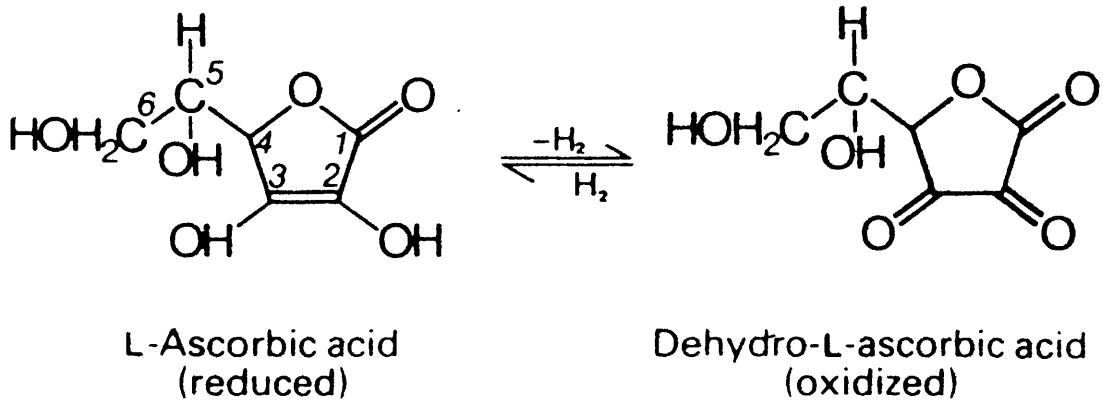

Figure 10. Structural formulae of L-ascorbic acid and dehydro-L-ascorbic acid.

Although it is called an acid, AA is actually not a carboxylic acid but a lactone and an enediol, and it is the enediol group (-C(OH) = C(OH)-) which is mainly responsible for the molecule's acidic and reducing properties. The reversible oxidation-reduction with DHAA is AA's most important chemical property and the basis of its known physiological activities. AA is a monovalent anion at physiological pH. Solid ascorbic acid is stable when dry and gradually darkens on exposure to light. Degradation reactions of AA in aqueous solution depend on several factors such as pH, temperature, the presence of absence of oxygen and the presence or absence of metals. In aqueous solution it is more sensitive to alkali than to acids. It is most stable at pH 4-6. Ascorbic acid is sensitive to heat. In the presence of oxygen and heat, it is oxidized at a rate proportional to the temperature rise. DHAA undergoes

irreversible hydrolysis to diketogulonic acid. Most metals, especially copper, catalyse the oxidation of AA. Metaphosphoric acid inhibits the catalytic oxidation of ascorbic acid by copper by chelating the copper in a non-ionic form that prohibits the initial formation of a complex with AA. Another catalytic reaction which accounts for loss of the vitamin occurs with enzymes such as AA-oxidase (ascorbate oxidase). Metaphosphoric acid inactivates AA-oxidase (Sauberlich et al, 1982).

1.4.3 Biosynthesis of ascorbic acid

The human organism cannot make its own AA because through evolution and genetic mutation it has lost a particular enzyme system (L-gulono-gamma-lactone oxidase) which can transform a sugar like glucose or galactose into AA. Thus, man (as well as guinea pig and monkey) must rely upon outside sources of AA to cover their needs. However, rabbits,like most other animals, possess the enzyme system and can produce AA. The liver is the site of AA synthesis (Chatterjee, 1973).

1.4.4 Physiological functions of ascorbic acid

AA functions as a cofactor in a number of hydroxylation and oxidation reactions by transferring electrons to enzymes that provide reducing equivalents (Levine, 1986). Thus, it is required for or facilitates the conversion of certain proline and lysine residues in procollagen to hydroxyproline and hydroxylysine in the course of collagen synthesis, the oxidation of lysine side-chains in proteins to provide hydroxylysine for carnitine synthesis, the conversion of folic

acid to folinic acid, microsomal drug metabolism and the hydroxylation of dopamine to form noradrenaline (Marcus and Coulston, 1991). AA promotes the activity of an amidating enzyme thought to be involved in the processing of certain peptide hormones, such as oxytocin, antidiuretic hormone and cholecystokinin. By reducing non-haem ferric iron to the ferrous state in the stomach AA also promotes intestinal absorption of iron. In addition, AA plays a role, albeit a poorly defined one, in adrenal steroidogenesis. At the tissue level, a major function of AA is related to the synthesis of collagen, proteoglycans and other organic constituents of the extracellular matrix in such diverse tissues as tooth, bone and capillary endothelium.

AA is an antioxidant and free radical scavenging nutrient protecting cells from damage by oxidants (Padh, 1991). Free radicals can be damaging to biological systems. Some evidence exists that AA has a number of physiological effects, the mechanisms of which are not understood. A few of these effects are: detoxification of histamine, phagocytic functions of leukocytes, infections, metabolism of drugs (induction of microsomal enzymes), formation of nitrosamine, expression of acetylcholine receptor, leukotriene biosynthesis and lipid metabolism.

A number of enzyme systems are stimulated by AA. AA keeps the metal ions in these enzymes in their reduced forms and it may have no obligatory role in catalysis (Padh, 1991).

1.4.5 Absorption and excretion of ascorbic acid - Assessment of nutritional status

AA is readily absorbed in the gastrointestinal tract by an energy Na^+-dependent active transport mechanism and absorption of dietary AA is nearly complete (Kallner et al, 1977). When exogenous AA is given in a single oral dose, absorption decreases from 75% at 1g to 20% at 5g. Both gastrointestinal absorption and renal tubular reabsorption of AA appear to be saturable processes (Melethil et al, 1986). AA is present in the plasma and is ubiquitously distributed in the cells of the body.

Although no data have been found to clearly show that any one tissue or organ acts as a storage reservoir of AA, leukocytes must be regarded as likely candidates.

Loh and Wilson (1971) who worked with subjects, who had been receiving a daily supplement of the vitamin for several weeks, showed that there was good correlation between leukocyte and plasma concentrations only until the leukocytes were saturated with the vitamin. Thereafter, there was no further change in the leukocyte AA concentration, although the plasma level continued to rise reflecting increased intake. These correlations suggest that leukocyte levels reflect the amount of AA available for storage, while plasma AA levels reflect the metabolic turnover rate. The above report is in good agreement with a more recent study (Omaye et al, 1986).

During vitamin C repletion leukocyte AA responded to dietary intake of the vitamin but not as rapidly as plasma AA (Omaye et al, 1986).
In addition, leukocyte AA levels dropped slowly during depletion, only reaching zero just before the onset of clinical symptoms of scurvy. For these

reasons, leukocyte AA levels are considered as a better indicator of vitamin C status than plasma. Adequate ingestion of vitamin C is associated with AA concentrations in plasma over $5\mu g.ml^{-1}$ ($28\mu M$) whereas concentrations of $1.5\mu g.ml^{-1}$ ($8.5\mu M$) are seen in individuals with frank scurvy (Marcus and Coulston, 1991). When the latter value ($8.5\mu m$) is reached the total body store of the vitamin at this time approximates 300mg. When the intake of ascorbate is raised, the concentration in plasma also increases at first linearly. Relatively few studies have examined the disposition kinetics of AA in humans after administration of exogenous high doses. The elimination half-life of the ascorbate pool in humans is approximately 16 days (Tietz, 1986). In contrast to this relatively slow turnover time, the exogenous high dosage of ascorbic acid, in excess of the body store, appears to be eliminated rapidly. In a human study, healthy volunteers' body stores have been saturated with the vitamin by receiving 1g of AA daily for several weeks. After this dosing period, saturation was assumed when urinary recoveries of AA (during 24h) on two occasions, separated by 3-7 days were within ± 10% of each other. The elimination half-life following an iv injection of 1g of ascorbate was found to be 3h (Yung et al, 1978). Following a single oral administration of a range of AA megadoses (0.5-20g), Melethil et al (1987) similarly reported that plasma AA levels peaked at 3h post-dosing and returned to pretreatment values at 10-12h. However, Zetler et al (1976) estimated the elimination half-life as 13-29h, values considerably greater than the previous study.

The majority of dietary AA is eliminated by the kidney. One route of metabolism of AA in man involves its conversion to oxalate. In addition to the presence of AA, DHAA and oxalate in urine, lesser amounts of a number of catabolites are also present.

1.4.6 Human requirements for ascorbic acid

The recommended dietary allowances (RDA) for AA is 30mg/day in Britain and 60mg/day in USA. Under special circumstances more AA may be required to achieve normal concentrations in the plasma. For example, South African miners have been observed to require 200 to 300mg of vitamin C daily to maintain a plasma concentration of 7.5$\mu g.ml^{-1}$ (Visagie et al, 1975). Requirements may increase in certain diseases, particularly infectious disease and also following surgery (Irvin et al, 1978). In its latest recommendations (1989) the National Research Council of the United States has suggested higher intake (100 mg/day) for smokers and an increment of 10 mg/day for pregnant women. Determination of the optimum requirement for AA has unfortunately remained a controversial topic; amounts of up to several grams per day have been advocated in the public press as well as in professional journals (e.g. Pauling, 1970).

1.4.7 Food sources of ascorbic acid and scurvy

AA is widely distributed in both plants and animals probably in equilibrium with DHAA. It occurs in significant quantities in fruits (especially citrus fruits) and fresh vegetables.

Today, incidents of scurvy surface occasionally in infants fed exclusively on cow's milk which is deficient in vitamin C, in aged persons on limited diets, and in adults where there is associated poverty, alcoholism, and nutritional ignorance. Evidence exists showing that even with undefined scorbutic

symptoms, vitamin C levels can be low, causing marked diminution in resistance to infections and slower healing of wounds.

1.4.8 Ingestion of megadoses of ascorbic acid

Reports suggesting that daily intake of megadoses (gram doses) of AA are beneficial for the prevention and treatment of several disorders, including cancer, have led to widespread consumption of vitamin C supplements. Despite contradictory reports, the consensus from an extensive literature review by Rivers (1987) is that these adverse health conditions are not improved by ingesting large doses of ascorbic acid.

Several reviews of the safety of megadoses of vitamin C concur that AA is safe at very high levels of intake for prolonged periods of time (Rivers, 1987; Gerster and Moser, 1988; Hathcock, 1991). In addition, adverse affects have not been reported in recently published placebo-controlled, double-masked studies with dosages of vitamin C up to 10g/day taken for several years (Moertel et al, 1985; Bordia and Verma, 1985; Bucca et al, 1990; Taylor et al, 1991).

1.4.9 Factors that affect ascorbic acid nutritional status

Low dietary intake of AA leads to low plasma and leukocyte concentration of ascorbate, low urinary excretion of the vitamin and eventually to the appearance of clear deficiency signs and symptoms. However, a number of other factors can also affect the apparent vitamin C nutritional status regardless of the intake of the vitamin.

a) Age: A number of surveys have shown that old people have low plasma and leukocyte AA levels. This can be attributed to a great extent, to a low intake of vitamin C where there is associated poverty, alcoholism and nutritional ignorance. However, there is also some evidence that ageing may affect vitamin C reserves in the body, regardless of intake. A number of studies in man and guinea pigs have shown that, allowing for variations in intake, there is an age-related fall in plasma and leukocyte AA concentration (Brook and Grimshaw, 1968; Burr and Rajan, 1972; Davies et al, 1976; Bates et al, 1979; Newton et al, 1985).

b) Sex: Most of the surveys that have shown age-related changes in vitamin C status have also shown a marked sex difference. Women tend to have a higher concentration of AA in plasma and leukocytes than do men; recent studies have shown that plasma AA levels are higher for females than for males having the same intake of AA (Jacob et al, 1988; Taylor et al, 1991)

c) Oral contraceptive agents: Rivers (1975) reviewed a number of studies of interaction between oral contraceptives and vitamin C status and concluded that a fall in plasma and leukocytes AA concentrations occur in women taking oral contraceptive agents.

d) Smoking: Cigarette smoking may adversely affect ascorbic acid metabolism in humans and a number of studies have suggested that plasma and leukocyte AA levels are reduced in smokers as compared with non- smokers (Pelletier, 1970; Hoefel, 1977; Schectman et al, 1991)

1.4.10 Determination of ascorbic acid

Various procedures based on the reduction-oxidation properties of AA are used for determination of AA and DHAA in foods and biological samples. Titrimetric, fluorometric or spectrophotometric principles are used for the analysis (Roe and Keuther, 1943; Denson and Bowers, 1961). Depending upon the method selected, either the reduced form or the oxidized form of AA (DHAA) or total AA levels can me measured. Although often quite accurate, the techniques can be laborious and time consuming (Johnsen et al, 1985). In addition, interfering substances present in the samples may affect the specificity and accuracy of the measurement. Recently, due to advances in high-performance liquid chromatography (HPLC), quantitative measurement of ascorbic acid and its metabolites have become possible. Advantages of HPLC techniques include: minimum sample preparation, fast analysis time, high specificity and sensitivity (Pachla and Kissinger, 1976; Tsao and Salimi, 1981). In addition, they offer the feasibility of quantitation in extremely small volumes and in specimens with very low concentrations; this is a major advantage in biochemical and particularly in ocular research due to the small volume of sample which can usually be obtained.

1.5 ASCORBIC ACID AND THE EYE

1.5.1 Introduction

Harris (1933) and Birch and Dann (1933) first discovered a much higher concentration of AA in aqueous humour than in plasma; this has since been confirmed by several investigators (Table 4). High levels of AA have also been found in other ocular tissues, notably lens and vitreous (Table 4), but there are wide differences in the distribution of AA within the eye in different species. DHAA is also present in the ocular tissues but at lower extent (Taylor et al, 1991).

1.5.2 Role of ascorbate in the eye

The exact role of AA in the eye is still not clear and it has been suggested that its most important role may not yet be known (Heath, 1962; Garland, 1991; Rose and Bode, 1991). Protection against oxidative damage, by scavenging reactive free radicals generated by sunlight, appears to be one of the most notable functions of this compound.

Some of the other putative functions of ascorbic acid in the eye include:

1. Promotion of corneal wound healing, especially of deep ulcers following alkali burns (Levinson et al, 1976; Pfister and Paterson, 1980; Nirankari et al, 1981).
2. Modulation of metabolism of arachidonic acid in the iris, ciliary body and cornea (Hurst et al, 1989).
3. Modulation of production of fibronectin and laminin and synthesis of

glycosaminoglycans in trabecular meshwork cell culture (Higginbotham et al, 1988; Schachtschabel et al, 1989; Yue et al, 1990).

4. Operation as a redox system (ascorbic acid/dehydroascorbic acid) linked to the maintenance of reduced pyridine nucleotide levels (Reddy, 1971; Varma et al, 1987).
5. Development of the lens and maintenance of its transparency during development (Garland, 1991).
6. Elimination of oxygen (thus decreasing the probability of oxidative insult) from the lens (Pirie, 1965a 1965b; Spector and Garner, 1981; Lohman et al, 1986; Garner et al, 1983; Linklate et al, 1990; Eaton, 1990) and cataract prevention (Jacques et al, 1988; Robertson et al, 1991).
7. Protection against UV irradiation (Ringvold, 1980; Chandra et al, 1986; Devamanoharan et al, 1991). Higher aqueous ascorbic acid levels are found in diurnal than in nocturnal animals; it may be an adaptation that protects the eye against solar irradiation. (Reiss et al, 1986).
8. Prevention of riboflavin-mediated light-induced damage to the cation pump and decrease of the photoperoxidation of the membranes in the lens. In both cases damage is thought to be the result of superoxide generation (Varma et al, 1979; Varma and Richards, 1988).
9. Protection against light-induced loss of retinal pigment epithelial cells and photoreceptor cells (Khatami et al, 1986; Organisciak et al, 1990).
10. Inhibition of the activity of polymorphonuclear leukocytes in inflammed ocular tissues acting as endogenous anti-inflammatory factor (Williams et al, 1984; Williams and Patterson, 1986).
11. Contribution in delaying age-related macular degeneration (Tso, 1985; Eye Diseae Case-Control Study Group, 1993).

1.5.3 Ascorbate, free radicals and the eye

The term free radicals represents reactive molecules with an unpaired electron. Free radicals are of varying degrees of toxicity to the body. Many of them are derived from atmospheric oxygen. When a single electron is added to the most stable state or ground state O_2 molecule, the product is the superoxide anion radical:

$$O_2 \rightarrow O\cdot^-_2$$

The body is endowed with a family of enzymes referred to as superoxide dismutases which metabolise $O\cdot^-_2$ to H_2O_2:

$$2O\cdot^-_2 + 2H+ \rightarrow H_2O_2 + O_2$$

Direct two-electron reduction of O_2 also leads to H_2O_2. Hydroxyl free radicals may also be generated by the reaction of superoxide radical with peroxide (Haber-Weiss reaction):

$$O\cdot^-_2 + H_2O_2 \rightarrow O_2 + OH^- + OH\cdot$$

as well as via H_20_2 in the presence of Fe^{2+} or Cu^+(Fenton reaction):

$$Fe^{2+} + H_20_2 \rightarrow Fe^{3+} + OH^- + OH\cdot$$

$$Cu^+ H_20_2 \rightarrow Cu^{2+} + OH^- + OH\cdot$$

Hydroxyl radicals are highly reactive and can directly damage cell components. AA and glutathione are part of the biological system that protects against the damaging oxidative effects of free radicals and peroxides that are continuously produced as minor by-products of tissue metabolism. Since such reactions are enhanced by the presence of oxygen and by light, the ocular tissues and fluids are at considerable risk.

In the aqueous, the problem is exacerbated by the absence of the major

detoxifying enzymes (superoxide dismutase, catalase, glutathione peroxidase) and by the presence of riboflavin which catalyses the formation of hydrogen peroxide.

AA present in the aqueous humour at high concentrations can react with oxygen or hydroxyl radicals to yield hydrogen peroxide, becoming oxidized to DHAA. The hydrogen peroxide which is itself toxic to the cornea at high concentrations (Riley and Giblin, 1983), can then be reduced to water by non-enzymatic reaction with glutathione, with formation of the disulfide. Thus, the continuous supply of ascorbate and glutathione may effectively detoxify any injurious free radicals produced in the anterior segment.

Ionising radiation often induces cataract, perhaps, by increased formation of hydroxyl and superoxide radicals. AA is present at high concentrations in the aqueous humour, lens and cornea of man and many animals. Its ability to scavenge $O\cdot^{-}_2$, $OH\cdot$ and singlet oxygen (reactive form of oxygen) may be of importance, although on the other hand, it has been suggested that light-induced degradation of AA in the aqueous humour may be a source of hydrogen peroxide that has to be removed by glutathione, peroxidase and catalase. Ascorbate has been observed to protect the lens ion-transporting ATPase enzyme against damage by an $O\cdot^{-}_2$ generating system in vitro (Halliwell and Gutteridge, 1985).

1.5.4 Ascorbate in the aqueous humour

AA is present at high levels in the aqueous humour of many species. In the human aqueous humour the concentration of AA is some 10-30 times that in the plasma (Table 4). Koskela et al (1989) suggested that the high

concentration of AA in the aqueous is a long- term adaptation to solar radiation. This gives support to the original hypothesis of Reiss et al (1986) that AA is present when needed to protect against sunlight. McGahan (1985) evaluated the levels of reduced and oxidized AA in plasma and in aqueous and vitreous of the rabbit. Although no DHAA was detected in the ocular fluids, it was present in plasma, sometimes at a concentration exceeding that of the reduced molecule.

1.5.5 Aqueous humour ascorbate saturation

Active transport sytems usually have a limit beyond which an increase in substrate concentration will produce no further increase in transport. When this limit is reached, the system is said to be 'saturated'. The ascorbate transport system into the aqueous humour is saturable.

At high serum concentrations of AA, there is a maximum rate of transfer of this substance into the aqueous humour in different species including man and rabbit. As the blood level of AA rises, the aqueous AA level reaches saturation. Kinsey (1947) has demonstrated that in the rabbit, the AA concentration in the aqueous humour increased rapidly within 1h with increasing serum concentrations until the level into the aqueous reached $500\mu g.ml^{-1}$ (ie, twice the normal values). This occurred when the concentration in the serum was $30\mu g.ml^{-1}$. With higher blood levels, no further increase in the quantity of AA in the aqueous humour was observed, even though the concentration in the serum was elevated to $1300\mu g.ml^{-1}$. The saturation level of AA ($500\mu g.ml^{-1}$) in the aqueous humour was maintained as long as the blood level remained constant. Linner (1952) has shown that the maximum rate of transfer of AA across the BAB in guinea pigs is reached at a

TABLE 4: CONCENTRATIONS OF ASCORBIC ACID IN PLASMA, AQUEOUS HUMOUR, VITREOUS BODY AND LENS

Plasma ($\mu g.ml^{-1}$)	Aqueous humour ($\mu g.ml^{-1}$)	Vitreous body ($\mu g.ml^{-1}$)	Lens ($\mu g/g$ wet tissue)	Species	Reference
3.5	167			Rabbit	Kinsey, 1953
7	195	81		Rabbit	Reddy and Kinsey, 1960
	128			Rabbit	Heath et al, 1961
8	153			Guinea pig	Heath et al, 1961
7	186	81		Human	DeBerardinis et al, 1965
3.5	208			Monkey	Gaastarland et al,1979
5.6	176	77		Rabbit	McGahan, 1985
0.8	200		206	Human	Ringvold et al, 1985
	186			Human	Reiss et al, 1986
10	160	360	190	Human	Varma, 1987
4.1	346	70	130	Rabbit	Varma, 1987
	132			Guinea pig	Varma, 1987
5.3	135		209	Human	Taylor et al, 1991

plasma AA level of about 20μg.ml^{-1} and that the saturation concentration of AA in the aqueous humour is about 300μg.ml^{-1}. The same investigator subsequently found (Linner, 1954) that in man, the maximum rate of transfer of AA across the BAB is reached at a plasma concentration of about 10μg.ml^{-1}, while the concentration of AA in the aqueous humour rises to about 220μg.ml^{-1}. By raising the plasma AA level of guinea pigs, saturation kinetics can be demonstrated in the aqueous humour (Becker, 1967; Berger et al, 1988). The concentration of AA in the aqueous is proportional to plasma AA levels over a fairly wide range of values and falls precipitously in scorbutic guinea pigs (Linner, 1952; Hughes et al, 1971; Giblin et al, 1984). The dependence of aqueous AA on dietary intake was confirmed by Bates and Cowen (1988). It is well documented that a decline in vitamin C status occurs in the elderly (Bates et al, 1979; Newton et al, 1985) and it was clearly shown that the aqueous humour of guinea pigs exhibited an age- related decline in AA levels (Bates and Cowen, 1988). In the rabbit, a single either subcutaneous or iv injection of AA was able to raise the aqueous ascorbate level up to two to three times the original level for at least 8-12h and with a higher dosage of AA this saturation level was reached faster than with lower ones (Reim et al, 1978). Other rabbit studies also showed that intraperitoneal injection of AA increased the level four-fold in the plasma and two-fold in the aqueous humour (Giblin et al, 1984).

In humans, Ringvold et al (1985) have found that there was a significant elevation of aqueous AA in cataractous patients after 1000mg oral intake (500mg x 2, 2 and 12h preoperatively), but not after 500mg administration as a single dose 2h before surgery. Taylor et al (1991) described the ability to increase AA concentration in plasma and aqueous humour following ingestion

of a 2g supplement (500mg qid) of ascorbate (165% and 57% increase vs unloaded patients in plasma and aqueous humour, respectively).

1.5.6 Ascorbate in the vitreous body

In some species, AA is found in higher concentration in the vitreous body than in the plasma; the gradient in rabbits being posterior chamber greater than vitreous greater than plasma (Reddy et al, 1961). In cattle, on the contrary, the level in the vitreous is half of that in plasma (Balazs, 1960). It is unclear whether the ascorbic acid within the vitreous results from diffusion from the aqueous humour or from synthesis within the vitreous body (Jacobson, 1967). The gel characteristic of the vitreous body does not seem to correlate with the AA content; the latter correlated most directly with the dietary intake of AA. It has been suggested that AA fulfils a function of HA degradation, breaking the linkages responsible for the organisation of the HA molecules (Swann, 1969). Nevertheless, the large variation found among animals did not correlate well with the viscosity of the gel, nor did it correlate well with the degree of syneresis, although the concentration was lower in rabbit embryos than in the mother animal (Balazs, 1954, 1968). While its role in liquefaction of the vitreous gel is unclear, AA has been found to participate in the absorption of ultraviolet rays in the vitreous body (as it does in the aqueous humour).

1.5.7 Ascorbate and the lens

Varma and Richards (1988) measured AA levels in lens and aqueous in eight animal species. The values of lens/aqueous concentrations of ascorbate

ranged from 0.44 in the rabbit to 2.6 in the horse. Values around 1.0 - 2.0 are often presented for man. It has not been determined which form of AA is preferred for transport in the lens. It is well documented that there is a rapid loss of AA from the tissues of guinea pigs deprived of dietary AA. Hughes et al (1971) noted that the brain and the lens are unique in retaining considerable AA after other organs have been depleted. Animals were maintained on a scorbutic diet for up to 90 days and then killed for procurement of tissues. The concentration of AA fell much less rapidly in the lens than in the aqueous and vitreous humours. This suggests either that the lens has a remarkable capacity to extract AA from surrounding fluids or that AA taken into the lens is more tightly sequestered than in most other tissues.

1.6 DEPOLYMERIZATION OF HYALURONIC ACID BY ASCORBIC ACID

Numerous studies have demonstrated that AA depolymerizes HA in vitro (Niedermeier et al, 1967; Wong et al, 1981; Miyauchi and Iwata, 1984; Motohashi and Mori, 1985). In these studies the rate of AA-induced depolymerization is measured either as the loss of viscosity or as the lowering of molecular weight of the HA. This depolymerization appears to occur in a concentration-related manner (Wong et al, 1981; Motohashi and Mori, 1985; Fink and Lengfelder, 1987) at least for AA levels up to 1000μM (176μg.ml^{-1}), which is approximately the physiological aqueous humour AA concentration (Table 4). The mechanism of AA-induced depolymerization of HA is unclear. There is strong evidence that oxygen-derived free radicals, especially the highly reactive hydroxyl radical, may play an important role in the depolymerization process (Wong et al, 1981; McNeil et al, 1985). The presence of traces of heavy metals such as iron or copper, which act as catalysts, seems to be critical in the reaction of formation of hydroxyl radicals and the following mechanism has been suggested (Harris et al, 1972; Wong et al, 1981; Scarpa et al, 1983; Motohashi and Mori, 1985). Ascorbic acid acts to maintain the iron ions in the ferrous form by reducing ferric ions:

Fe^{3+} + ascorbate → Fe^{2+} + dehydroascorbate

then, $Fe^{2+} + 0_2 \rightarrow Fe^{3+}\ 0\cdot^{-}_2$

and then H_20_2 reacts with Fe^{2+} to produce hydroxyl radicals:

$$H_20_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH\cdot$$

Hydroxyl radicals are probably the ultimate species that depolymerize HA. This reaction scheme can explain the inhibition of depolymerization by scavengers of hydroxyl radicals (such as formate, mannitol, thiourea, ethanol) and by catalase (Wong et al, 1981). The reports that iron (II) salts enhance the AA-induced depolymerization of HA and that the rate of depolymerization is reduced by the specific iron chelator desferrioxamine, strongly supports the theory that the above iron-catalyzed Haber-Weiss reaction regenerating OH· is the most probable depolymerizing mechanism for hyaluronate (Harris et al, 1972; Wong et al, 1981; Motohashi and More, 1985).

CHAPTER 2

RATIONALE AND SPECIFIC AIMS OF THE STUDY

RATIONALE AND SPECIFIC AIMS OF THE STUDY

Clinical studies investigating the effect of Healon on postoperative IOP in patients who have undergone cataract extraction with or without IOL implantation have suggested that within the first 24h potentially dangerous increases in IOP can occur, even if an attempt is made to remove the Healon. Further, an in vitro study has shown that such IOP rises may be due to obstruction of the aqueous humour outflow pathways. This suggests that Healon-induced IOP rises might be ameliorated if it was depolymerized into smaller molecules which could be more easily cleared by the outflow pathways.

Ascorbate, at levels known to be present physiologically in the aqueous humour, is known to depolymerize hyaluronate in vitro. In the immediate postoperative period, ascorbate levels probably are greatly decreased due to the damaging effect of surgical trauma on the blood-aqueous barrier. Some reports have also shown that the use of Healon intraoperatively may exacerbate the postsurgical breakdown of the barrier and/or interfere with barrier recovery.

Collectively, these studies suggest the general hypothesis that postoperative IOP spikes due to the use of Healon in cataract surgery might be attenuated by increasing the concentration of ascorbate in the aqueous humour in the immediate postoperative period. The following lines of investigation were followed in order to address this general hypothesis:

A. Documentation and study of Healon-induced rise in IOP. More specifically:

Specific aim A.1: Study of the magnitude and timecourse of IOP rise in the early postoperative period with and without the use of Healon in patients who undergo planned ECCE with p/c IOL implantation.

Specific aim A.2: Documentation of the magnitude and timecourse of obstructive properties of Healon in the human eye in vitro (perfused human ocular anterior segment).

B. Test and study of the hypothesis that postoperative IOP rises due to Healon might be attenuated by increasing aqueous humour ascorbate to a level which might depolymerize hyaluronic acid. More specifically:

Specific aim B.1: Determination of the extent and duration of any change in postoperative aqueous humour ascorbate concentration following the trauma of surgery in rabbits (in absence or presence of Healon).

Specific aim B.2: Study of the maximum attainable level of ascorbate in the aqueous humour by raising plasma ascorbate levels by administration of systemic ascorbate.

Specific aim B.3: Study of the effect of ascorbate on any Healon-induced change in outflow facility in the human eye in vitro (perfused human ocular anterior segment).

Specific aim B.4: Investigation of whether a high concentration of ascorbate in the aqueous prevents or attenuates postoperative IOP rises following cataract surgery with intraoperative use of Healon.

CHAPTER 3

MATERIALS AND METHODS

3.1 CLINICAL STUDIES

Patients admitted to the Department of Ophthalmology, Western Infirmary, Glasgow for planned ECCE with p/c IOL between October 1990 and February 1993 were invited to participate in these studies. Procedures were reviewed and approved by the Hospital Ethical Committee and informed consent was obtained from all patients meeting the enrolment criteria.

3.1.1 The effect of systemic ascorbate pretreatment on aqueous humour ascorbate concentration

An initial investigation was conducted to determine the maximum increase in aqueous humour ascorbate attainable after systemic pretreatment with oral ascorbate in patients undergoing planned ECCE.

Patients: A total of 105 patients agreed to participate in this study. The patients included 44 males and 61 females ranging in age from 50 to 93 (72.6 ± 10.1; mean ± SD).

Pretreatment with oral ascorbate: Patients were divided into 5 groups based on the amount of ascorbate given preoperatively: those receiving no ascorbate (n=56) or a total dose of 1g (n=11), 2g (n=17), 3g (n=18), or 5g (n=3). Demographic data are pesented in Table 5. Oral ascorbate was administered as Ascorbic Acid tablets BP 500mg BP (APS Ltd, West Yorkshire, UK) in two equal doses administered the evening before surgery (10 pm) and the morning of surgery (8 am, one to six hours before surgery). Aqueous humour and plasma samples were obtained between 9.00 am and 2.00 pm on the day of surgery.

TABLE 5: PATIENT DEMOGRAPHICS OF ASCORBATE PRETREATMENT STUDY

				Sex			
		Age		Male		Female	
Ascorbate(g)	No	Mean±SD	Range	No	%	No	%
0	56	71.2±10.9	50-93	25	45	31	55
1	11	74.8± 8.6	63-86	5	45	6	55
2	17	72.9±10.9	56-92	6	35	11	65
3	18	72.6± 9.7	56-92	7	39	11	61
5	3	71.0±12.5	61-85	1	33	2	66

Collection of aqueous humour samples for analysis of aqueous humour ascorbate levels: the patients' pupils were dilated one hour before surgery with two drops of phenylephrine hydrochloride 10% (Minims, Smith & Nephew plc, Romford, Essex, UK) and two drops of cyclopentolate hydrochloride 1% (Minims). During the operation, an aqueous humour sample (30-200µl) was aspirated with a microsyringe through a 25 gauge (G) needle prior to entry of the anterior chamber after a partial thickness corneoscleral incision had been made. Each aqueous humour sample was immediately added to a preweighed sample tube containing 200µl of metaphosphoric acid (Aldrich, Milwaukee, WI, USA)solution (2.5% in H_20).

Collection of plasma samples for analysis of plasma ascorbate levels: a blood sample was withdrawn by antecubital venipuncture using an evacuated heparinised tube approximately at the same time as the aqueous humour collection. The blood was centrifuged for 3 min at 5,000g and a 0.5ml aliquot

of the plasma was mixed with 0.5ml of metaphosphoric acid solution (2.5% in H_20).

Both aqueous humour and plasma samples were immediately stored at -20°C for up to six weeks until analysis.

3.1.2 Pilot study of plasma ascorbate levels following ascorbate ingestion

Five healthy adult volunteers (four males and one female, 28-50 years of age), after giving informed written consent, participated in a study designed to determine the timecourse of plasma ascorbate levels following ingestion of a single bolus dose.

Each subject ingested 1g of ascorbic acid and blood samples were obtained at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15 and 24h. Plasma was immediateely separated and stored until analysis as described previously. One day prior to and during the experimental day the subjects avoided foods known to be high in ascorbic acid content. On the experimental day, the subjects ingested the assigned dosage on an empty stomach after overnight fast. Food was withheld for the next four hours. All subjects were non-smokers.

3.1.3 The effect of Healon upon the immediate postoperative IOP and the effect of ascorbate upon the immediate IOP rise as a sequel to the use of intraoperative Healon

A prospective, randomized, placebo-controlled double- masked trial was conducted to study the magnitude and timecourse of early postoperative rises in IOP after cataract surgery with and without the use of Healon. A further

aim was to investigate whether pretreatment with ascorbate would prevent any Healon-induced IOP rise.

Patients: patients undergoing routine, planned, uncomplicated ECCE with p/c IOL were enrolled in this study. Exclusion criteria for participation in the study included the following:

1. history of intraocular surgery or disease (apart from age-related macular degeneration) in either eye
2. preoperative IOP > 21mmHg in either eye
3. family history of glaucoma, ocular hypertension or exfoliation syndrome
4. diabetes mellitus or patients taking beta-blockers or corticosteroids
5. state of health precluding proper examination and assessment

Patient data were also excluded from data analysis in the case of any intraoperative or postoperative complications. Only one eye per patient could be included in the study. The patients were randomized using randomization tables to one of the following four treatment groups:

1. no ascorbate (placebo)/no Healon
2. no ascorbate (placebo)/intraoperative Healon (0.1- 0.5ml)
3. ascorbate (2g)/no Healon
4. ascorbate (2g)/intraoperative Healon (0.1-0.5ml)

A total dose of 2g of ascorbate or placebo (as the results of the previous study showed that a maximal attainable level of ascorbic acid into the aqueous humour could be achieved with this dosage) was given preoperatively in two doses as noted above and aqueous ascorbate levels were determined.

A total of 94 patients were entered into the study and 76 completed the study. Eighteen patients did not complete the study for the following reasons: 7 required sphincterotomy due to insufficiently dilated pupils; 5 had rupture of

the posterior capsule with loss of vitreous requiring insertion of an anterior chamber IOL; 2 had immediate postoperative macroscopic hyphaema; 1 developed postoperative endophthalmitis; 1 required a secondary surgical procedure due to wound leaking; 2 did not receive the morning dose of ascorbate (or placebo). The patients included 37 males and 39 females ranging in age from 39 to 92 (71.9±11.5; mean ± SD). Demographic data are presented in Table 6 for all patients who completed the study.

TABLE 6: PATIENT DEMOGRAPHICS OF ASCORBATE-HEALON STUDY

		Age		Sex			
				Male		Female	
	No	Mean±SD	Range	No	%	No	%
No ascorbate/ No Healon	10	71.0± 9.9	51-86	8	80	2	20
No ascorbate/ Healon	28	72.2±11.4	39-92	13	46	15	54
Ascorbate/ No Healon	10	76.0±10.9	50-90	4	40	6	60
Ascorbate/ Healon	28	70.2±12.2	46-91	12	43	16	57

IOP measurements and surgical procedure: A complete anterior segment examination and bilateral IOP measurements with a calibrated Goldmann applanation tonometer using a Haag-Streit slit-lamp (model 900) were taken the evening before surgery between 4 pm and 8 pm. One drop of lignocaine hydrochloride 4% and fluorescein sodium 0.25% (Minims) was applied to both eyes before measurement. The mean of two IOP values measured 5 min apart was calculated. All pupils of the experimental eyes were dilated 1h preoperatively as described above. Each operation was performed under general anaesthesia or retrobulbar anaesthesia. In the latter case an additional topical administration of 2-3 drops of oxybuprocaine hydrochloride ($4mg.ml^{-1}$) (Minims) was applied to the eye before surgery. One drop of chloramphenicol eye drops BP (Minims) was instilled to the eye before the beginning of surgery.

ECCE with p/c IOL was performed in all cases in a similar fashion by one of five experienced ophthalmic surgeons as described below.

A partial thickness superior limbal or clear corneal incision was made through 120° with a disposable razor blade knife. A sample of aqueous humour was then removed with a microsyringe through a 25G needle and treated as described previously, after which a perforating stab incision was made at the 12 o'clock position. Healon (0.1-0.25ml) was then injected through a 27G cannula to deepen the anterior chamber. An anterior capsulotomy was performed using a cystotome by an envelope-like (endocapsular) technique in a horizontal plane 1/4 - 1/3 superiorly. A gentle hydrodissection and mobilization of the nucleus of the lens was carried out with a needle and complete opening of the remaining incision (120°) was made with scissors. The nucleus was expressed with an irrigating Vectis. The cortical remains

were aspirated with a syringe during gravity-fed irrigation through a coaxial cannula. Hartmann's solution (Baxter Healthcare, Norfolk, England)with adrenaline BP ($1:10^6$) was used as the irrigant. The posterior capsule was manually vacuum- cleaned or polished and additional Healon (0.1-0.25ml) was injected in some cases to separate the anterior and posterior capsules and to reform the capsular bag before IOL implantation. A C-loop or tripod posterior chamber intraocular lens was inserted and every effort was made to place the haptics in the capsular bag. The central part of the anterior capsule was then removed. Immediately after lens implantation and centration, an irrigating/aspirating procedure was undertaken in an attempt to remove as much Healon as possible. The incision was closed with continuous or interrupted 10-O monofilament Polyamide (nylon) sutures or interrupted 8-O coated Vicryl sutures. The fornix-based conjunctival flap was reapproximated with one or two coated Vicryl 8-O sutures. One drop of chloramphenicol eye-drops BP (Minims) was applied to the eye at the end of surgery. No miotics, antibiotics or steroids were given and the eyes were not bandaged.

Although the Healon syringe has no calibrations the volume used for each patient was estimated by measuring the length of the column of residual Healon in the syringe.

The eyes that did not receive intraoperative Healon were operated on with an identical technique with the exception that Hartmann's solution with adrenaline was used instead of Healon. The decision to use Healon was made before the start of surgery.

Postoperative IOP was measured approximately 6h (5-7h) and 24h (22-25h) after surgery by the same investigator (SDK) with the same applanation tonometer with patients in a sitting position as preoperatively.

Analysis of aqueous humour and plasma ascorbate

High-performance liquid chromatography (HPLC) on ODS silica with cetyltrimethylammonium bromide as an ion-pairing reagent and electrochemical detection (ECD) was used to determine ascorbic acid in human and rabbit aqueous humour and plasma as described in detail by Watson et al, 1993. Hydroquinone was used as the internal standard.

Chemicals: Ascorbic acid, hydroquinone, cetyltrimethylammonium bromide, metaphosphoric acid, sodium acetate and glacial acetic acid were obtained from Aldrich Chemical Co. (Gillingham, Dorset, UK). HPLC grade acetonitrile and water were obtained from Rathburn Chemical Co. (Walkerburn, Peebleshire, UK).

Preparation of samples: Stock solutions of hydroquinone and ascorbic acid (each 1mg.ml^{-1} in water) were freshly prepared each time before analysis.

200µl of trichloroacetic acid (30% solution) was added to a 50µl sample of aqueous humour which had been preserved with metaphosphoric acid. The sample was then centrifuged (9000g.min); 1µg of hydroquinone was added to 100µl of the supernatant which was then diluted to 1ml with mobile phase. A 50µl volume of the sample was then injected into the chromatograph using an autosampler.

200µl of trichloroacetic acid (30% solution) was added to the plasma samples which had been preserved with metaphosphoric acid. The sample was then centrifuged (15000g.min) to remove precipitated protein; 1µg of hydroquinone was added to 500µl of the supernatant which was then diluted to 1ml with the mobile phase. A 50µl volume of the sample was then injected

into the chromatograph using an autosampler.

Instrumentation: A Hewlett-Packard 1082 HPLC system with an autosampler was used in the analyses. The instrument was fitted with a 5μm spherisorb ODS-1 reversed phase column which was protected with a SGE ODS-1 guard cartridge system (Burke Analytical, Glasgow, UK). The HPLC system was linked to an electrochemical detector (LC-4A amperometric detector; Bioanalytical Systems). The potential of the detector was set at O.6V versus an Ag-AgCl reference electrode. Oxygen-free nitrogen was used for the operation of the pneumatically driven autosampler. The mobile phase was prepared by dissolving sodium acetate (0.08M) and cetyltrimethylammonium bromide (0.001M) in acetonitrile-water (5:95, v/v); the pH of the solution was adjusted to pH 4.2 with glacial acetic acid. The flow rate was adjusted to $1ml.min^{-1}$.

Calibration curve: Solutions containing 1μg of hydroquinone and 0.5-5μg of ascorbic acid in 1ml of the mobile phase were prepared. The solutions were then injected into the chromatograph. A standard curve was constructed using a fixed amount of hydroquinone as the internal standard and varying the amount of ascorbic acid over the range $0.5\text{-}5\mu g.ml^{-1}$. The curve was linear ($r=0.998$). The precision was determined by injecting five replicates of the same sample of diluted aqueous humour containing $246.4\mu g.ml^{-1}$ of ascorbic acid; the RSD was 1.49%. The reproducibility was determined on five separate analyses of the same sample of aqueous humour containing $311.8\mu g.ml^{-1}$ of ascorbic acid; the RSD was 1.63%. The retention times of ascorbic acid and hydroquinone were approximately 4 and 6min, respectively. Standard solutions of ascorbic acid and hydroquinone were prepared each time before analysis.

3.2 ANIMAL STUDIES

A total of 52 adult New Zealand white rabbits (2.5-4.6 kg, either sex) were used in these studies. The rabbits were housed in the Physiology Animal House, University of Glasgow, subjected to natural daylight hours and given free access to water and food. The rabbits were handled in accordance with Home Office regulations. Prior to experimental manipulation, the eyes were examined with the surgical microscope and determined to be normal and free of inflammation.

When pretreatment with ascorbate was required, rabbits were subjected to oral administration of Sodium Ascorbate (Sigma Chemical Co, St Louis, MO, USA) solution (100mg.ml^{-1} in H_2O) using a 10ml syringe fitted with a silicone rubber tube (5cm in length). The dose given was 50mg/kg body weight, 3h before surgery.

Blood samples were withdrawn by puncture of the marginal ear vein and then treated as described in clinical studies.

Heparin (Monoparin, CP Pharmaceutical, Wrexham, UK) was administered by iv injection (2000U.Kg^{-1}) in the marginal ear vein to all rabbits 1h before surgery.

Anaesthesia was induced and maintained with nitrous oxide-oxygen (75:25%) containing halothane BP (3%) in an open system with evacuation of exhaled gases.

Both surgery and subsequent paracentesis were carried out under general anaesthesia with supplementary topical anaesthesia using amethocaine eyedrops BP (Minims). A partial thickness corneal incision (120°) was made with a disposable razor blade knife. A sample of aqueous humour (60-100µl)

was removed with a microsyringe through a 27G needle and immediately mixed with an equal volume of metaphosphoric acid solution (2.5% in H_20), after which a perforating stab incision was made at the 12 o'clock position, followed by a complete (120°) opening with scissors. Hartmann's solution containing heparin ($1OU.ml^{-1}$) was infused into the anterior chamber and the incision closed after 15 min with interrupted nylon sutures (10-0).

Paracentesis was performed with a microsyringe through a 27G needle to penetrate the cornea 1-2mm from the unoperated limbus.

Chloramphenicol eye drops BP (Schering, Suffolk, England) were instilled before and after surgery.

In all cases, an aqueous humour sample from the fellow untouched eye at 48h served as a contralateral control. At the end of each experiment, euthanasia was carried out using nitrous oxide and halothane. Both aqueous humour and plasma samples were stored at -20°C for up to six weeks until analysis of ascorbate by HPLC as described in clinical studies.

3.2.1 Timecourse of the effect of surgery on aqueous humour ascorbate levels

The effect of surgery (corneal incision) on aqueous humour ascorbate levels at different postoperative timepoints was determined unilaterally in two series of experiments. In the first series (n=7), corneal incision and withdrawal of aqueous humour was performed unilaterally and further samples were obtained at 6, 12, 24 and 48h postoperatively. In the second series (n=7), further samples were obtained at 2, 5, 8, 24 and 48h.

3.2.2 Plasma ascorbate levels following oral administration of ascorbate

A total of 7 rabbits was used in this study designed to investigate the timecourse of plasma ascorbate levels following a single oral administration of ascorbate. Each rabbit received 50mg/Kg body weight of ascorbate and blood samples were obtained at 0, 45, 90, 135, 180, 225, 280, 325, 615 and 795 min.

Plasma was immediately separated and later assayed for ascorbic acid by HPLC as described in clinical studies.

3.2.3 The effect of surgery and Healon on aqueous humour ascorbate concentration

A study designed to investigate the effects of surgical procedures and Healon on aqueous humour concentration of ascorbate included 4 groups as follows:

1. corneal incision alone/no Healon (n=5)
2. corneal incision alone/Healon (0.25ml) (n=5)
3. corneal incision and lens removal/no Healon (n=6)
4. corneal incision and lens removal/Healon (0.5ml) (n=6)

Withdrawal of aqueous humour was performed at the time of surgery and a further sample was obtained at the end of surgery from both the experimental and contralateral control eyes.

Corneal incision, lens removal and all surgical manipulations were conducted as previously described.

Intraoperative Healon was used to deepen the anterior chamber (0.25ml) and to reform the capsular bag (0.25ml). No IOL was inserted and no attempt

was made to remove the Healon at the end of surgery.

The eyes that did not receive Healon were operated on with an identical technique with the exception that Hartmann's solution with heparin was used instead of Healon.

Analysis of aqueous humour ascorbate levels was performed by HPLC as described in the clinical studies.

3.2.4 The effect of systemic ascorbate pretreatment on postoperative aqueous humour levels of ascorbate following the intraoperative use of Healon

A total of 9 rabbits divided into two groups was used in this study designed to investigate the effect of systemic ascorbate pretreatment on the postoperative aqueous humour concentration of ascorbate following the use of Healon:

1. Corneal incision and lens removal/Healon (0.5ml) + ascorbate pretreatment (n=5)
2. Corneal incision and lens removal/Healon (0.5ml) (n=4)

The rabbits of group 1 received, orally, 50mg/kg body weight of ascorbate 3h preoperatively. Corneal incision, lens removal, intraoperative use of Healon and all surgical manipulations were performed as previously described. Withdrawal of aqueous humour was performed at the time of surgery and further samples were obtained at 6 and 48h.

The rabbits of group 2 (control) did not receive ascorbate pretreatment and followed an identical procedure.

3.3 IN VITRO STUDIES

Studies were conducted in perfused ocular anterior segments of human eyes to determine the effect of Healon on facility of outflow and the effect of ascorbate on the Healon-induced reduction in outflow facility.

Human eyes: A total of 28 normal human eyes, from donors having no history of intraocular surgery or intraocular disease ranging in age from 60 to 92 were used in these studies. Eyes were obtained from the New England Eye Bank (Boston, MA, USA) or from the National Disease Research Interchange (NDRI; Philadelphia, PA, USA).

Preparation of eyes for perfusion: Tissue was dissected and placed into culture medium (Optisol, Chiron Inc, Irvine, CA, USA) within 12h of death and shipped chilled to Boston via overnight mail. Techniques previously described were used for the preparation and perfusion of tissue (Erickson-Lamy et al, 1991; Erickson-Lamy, 1992).

Anterior segments were dissected (usually by the eye banks) under sterile conditions as follows. The eye was bissected at the equator, and the anterior segment was dissected. The lens zonule was cut and the lens removed, after which the iris was grasped at the pupillary margin and gently pulled away. Finally, the choroid and ciliary body were removed by grasping the choroid close to the ora serrata and gently pulling it along with the ciliary body away from the underlying sclera. The anterior segment was then placed in culture medium and stored chilled until the time of perfusion.

Perfusion technique: The corneoscleral shell with attached outflow tissue was mounted onto a specialised perfusion apparatus (Figure 11) and perfused at 15mmHg with culture medium (Dulbecco's Modified Eagle's Medium; Sigma

Chemical Co, St Louis, MO, USA) with added antibiotics (penicillin; 50 $U.ml^{-1}$, streptomycin 50$\mu g.ml^{-1}$; fungizone [amphotericin] 5$\mu g.ml^{-1}$; all from Sigma Chemical Co) (DMEM + PSF) at 37°C in a 5% CO_2 environment. Under these conditions, the tissue remains viable for up to one week. Outflow facility was calculated as the ratio of the measured flow of culture medium into the chamber to the perfusion pressure: $C = F/P$ ($\mu l.min^{-1}. mmHg^{-1}$). All determinations of outflow facility consisted of an average of 4-5 consecutive 5-min facility determinations, commencing when the rate of fluid flow into the perfusion chamber had reached steady state (usually 20 - 30 min after onset of perfusion).

3.3.1 Assessment of the effect of Healon vs a sham injection on outflow facility (pilot study)

In a pilot study the effect of Healon vs a sham injection on outflow facility was assessed in a pair of human eyes (74-year old female). In this experiment after establishment of a baseline outflow facility in both eyes, the perfusion chambers were emptied. In the experimental eye the perfusion chamber was filled with 300µl of Healon via an injection with a 27G needle at the limbus and outflow facility measurements continued for 3h. In the contralateral eye the perfusion chamber was filled with 300µl of culture medium (DMEM + PSF) and outflow facility measurements resumed for the same period of time.

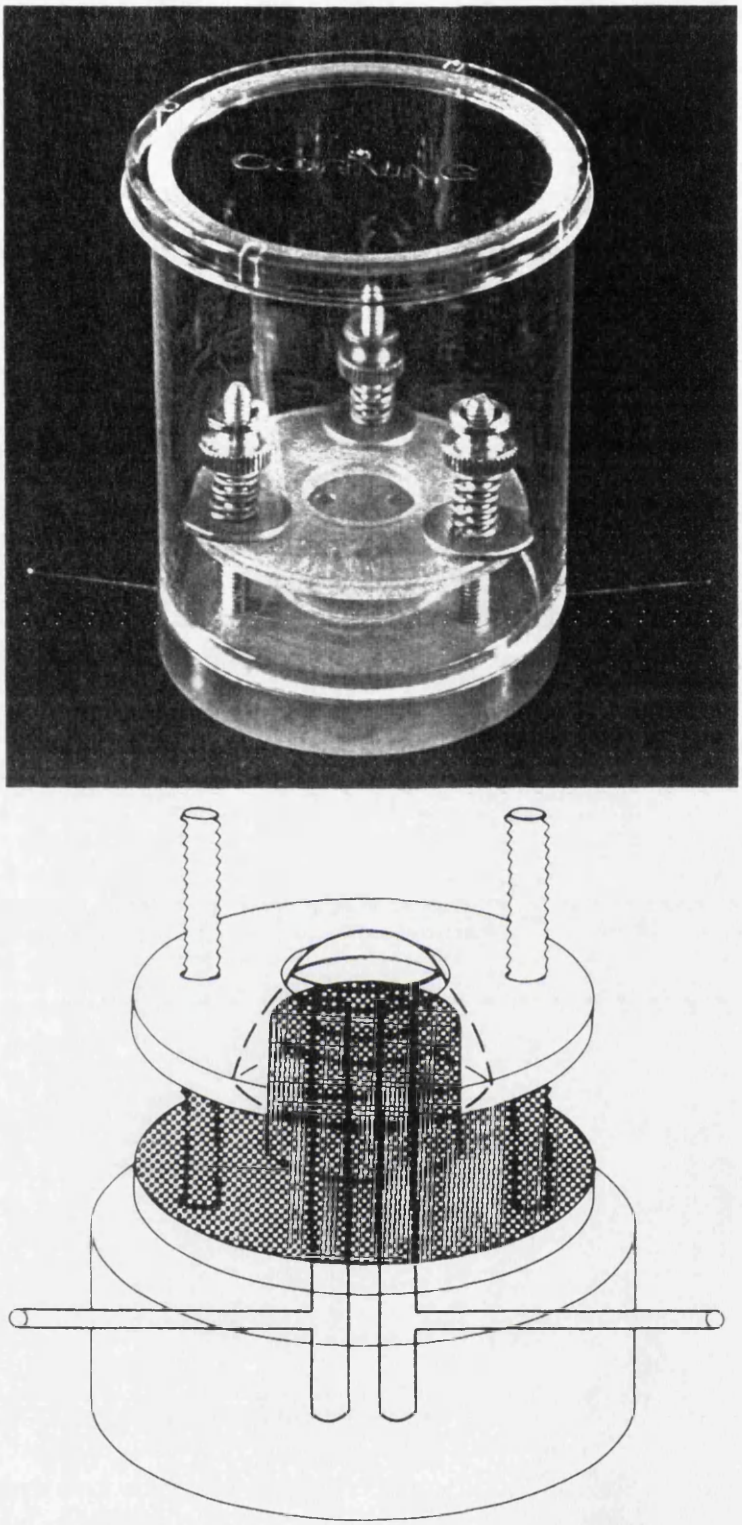

Figure 11 A: The perfusion apparatus. B: Diagrammatic representation off the perfused human ocular anterior segment model. For the sake of clarity,, one of three clamping posts was omitted from the diagram (Erickson-Lamy et al, 1991).

3.3.2 Assessment of the effect of different dilutions of Healon on outflow facility

After establishment of a baseline outflow facility, the chamber contents of 20 eyes were emptied and the chamber was refilled with 300µl of 3 different concentrations of Healon (100%, 50%, 10%). The 50% and 10% concentrations of Healon were prepared from the commercial product containing sodium hyaluronate (1%) by thorough vortex mixing with culture medium (DMEM + PSF) immediately prior to the injection. In some experiments, Healon was injected at the limbus using a 27G needle. In other experiments, Healon was injected through the outflow port of the perfusion apparatus. After Healon injection, outflow facility measurements resumed for 3-24h. A paired comparison was made in each eye between pre-Healon baseline outflow facility and post-Healon outflow facility.

3.3.3 Assessment of the effect of ascorbate on the Healon-induced reduction in outflow facility

In these studies, the effect of a high constant concentration of ascorbate ($1000\mu g.ml^{-1}$) was assessed on the Healon- induced outflow facility reduction.

After establishment of a baseline outflow facility in 3 pairs of eyes (69, 81 and 86-year old male donors) the perfusion chambers were emptied and 300µl of Healon (50% dilution in culture medium) was injected through the outflow port of the perfusion apparatus in both eyes. In the experimental eye the perfusion was then carried out with added ascorbate ($1000\mu g.ml^{-1}$ in culture medium) and outflow facility was measured for 6h. In the

contralateral control eye the perfusion was continued with culture medium without added ascorbate and outflow facility was measured for the same period of time.

Morphological examination of perfused outflow tissue: After perfusion with Healon with or without added ascorbate, anterior segments were fixed at perfusion pressure (15mmHg) with freshly prepared Karnovsky's fixative (4% glutaraldehyde, 5% paraformaldehyde, 0.1 M Na cacodylate, 0.5mg.ml^{-1} $CaCl_2$) for 30 min followed by immersion in 1% alcian blue in 3% acetic acid (pH 2.5). In the pair of human eyes of the pilot study, alcian blue was not used. Tissue was processed for light microscopy according to standard protocols and morphological examination was carried out.

CHAPTER 4

RESULTS

4.1 THE EFFECT OF HEALON ON POSTOPERATIVE IOP FOLLOWING CATARACT SURGERY (Specific aim A.1)

The results of postoperative IOP, measured 6 and 24h after ECCE with p/c IOL in patients who received or did not receive Healon are shown in Table 7 (top half). In this study, Healon-treated eyes were further subdivided into different groups based on the volume of Healon used intraoperatively: <0.2ml Healon, 0.2-0.35ml Healon and >0.35ml Healon (Table 7, bottom half). There was no statistically significant difference between age and sex among groups. These data show that the initial IOP was not different between the operated and contralateral control eye preoperatively in all groups. Six hours after surgery there was a significant increase in IOP in the operated eyes of all groups except for the group receiving <0.2ml Healon, with the 0.2 - 0.35ml group having a mean difference of about 7mmHg, similar to the no Healon group, and the > 0.35ml group having an average difference of about 17mmHg. At 24h there were no large differences in IOP between operated and control eyes in any of the groups. However, there was a statistically significant average elevation of 3mmHg in the operated eye relative to the control eye in the >0.35ml Healon group.

Although on the average IOP was not greater 6h postoperatively in the <0.2ml Healon group, the pressure differences between operated and control eyes ranged from the operated eye having a pressure of 10mmHg less than to 12mmHg greater than the fellow control eye. Pressure differences at 6h ranged from -1 to 17mmHg, -5 to 36mHg and -4 to 37mmHg in the no Healon, 0.2-0.35ml Healon and >0.35ml Healon groups, respectively (Table 7).

Table 8 shows the significance of difference in IOP between operated and control eyes among no Healon- and Healon-treated (in whatever volume) eyes at 6 and 24h postoperatively.

Comparisons among the different treatment groups showed that the IOP difference between operated and contralateral control eyes at 6h postoperatively was significantly higher in the >0.35ml Healon group than in all other groups. Interestingly, the IOP difference was significantly less in the <0.2ml group than in either of the groups treated with larger volumes of Healon, but although lower not from eyes receiving no Healon (Table 9 and Figures 12 and 13).

Table 10 shows the incidence of difference in IOP greater than 10mmHg between operated and contralateral control eyes among patients who received various volumes of Healon during cataract surgery. The significance of these incidences of differences at 6h postoperatively are presented in Table 11. These tables show that a significantly higher percentage of patients (77%) who received >0.35ml Healon exhibited difference in IOP greater than 10mmHg between operated and control eyes at 6h postoperatively as compared with that of 35%, 10% and 42% in the no Healon, <0.2ml Healon and 0.2-0.35ml Healon groups, respectively. At 24h, there was still a high percentage of patients (22%) in the >0.35ml Healon group with a difference in IOP greater than 10mmHg. However, in all other groups IOP returned to preoperative levels.

TABLE 7: IOP IN HUMAN EYES FOLLOWING THE USE OF INTRAOPERATIVE HEALON

			IOP (mmHg) *mean ± SEM*			*Range*
	Time (h)	n	Operated Eye	Control Eye	O-C	O-C
No Healon	baseline	20	13.5±0.7	13.3±0.6	0.2±0.3	-4 to 3
	6	20	19.4±1.3	12.1±0.6	7.3±1.4***	-1 to 17
	24	20	11.3±1.0	12.7±0.7	-1.4±1.0	-12 to 6
Healon	baseline	56	13.7±0.4	14.0±0.4	-0.3±0.3	-5 to 5
	6	56	23.7±1.4	12.7±0.4	11.0±1.5***	-10 to 37
	24	56	14.2±0.8	13.3±0.4	0.9±0.8	-12 to 17
Vol. of Healon (ml) **0**	baseline	20	13.5±0.7	13.3±0.6	0.2±0.3	-4 to 3
	6	20	19.4±1.3	12.1±0.6	7.3±1.4***	-1 to 17
	24	20	11.3±1.0	12.7±0.7	-1.4±1.0	-12 to 6
<0.2	baseline	10	14.1±0.8	14.7±1.0	-0.6±0.4	-3 to 1
	6	10	15.6±1.9	14.8±1.1	0.7±2.3	-10 to 12
	24	10	11.4±1.1	14.2±1.4	-2.8±1.5	-12 to 3
0.2-0.35	baseline	19	12.8±0.7	12.8±0.8	0.1±0.5	-5 to 5
	6	19	18.8±1.8	11.6±0.8	7.2±2.1**	-5 to 36
	24	19	11.9±1.0	12.0±0.7	-0.1±0.9	-7 to 8
>0.35	baseline	27	14.1±0.6	14.8±0.5	-0.5±0.5	-5 to 3
	6	27	29.9±2.0	12.8±0.4	17.2±2.0***	-4 to 37
	24	27	17.0±1.3	14.0±0.5	3.1±1.3*	-7 to 17

The top half of the table shows data gathered from all patients in the study. In the bottom half, the Healon-treated patients are subdivided according to the volume of Healon used, intraoperatively. Patient numbers in each group are indicated (n). IOP *measurements were taken in operated (O) and contralateral control (C) eyes prior to surgery (baseline) and 6h and 24h* postoperatively. The differences in IOP between operated and control eyes (O-C) are significantly different from zero by the two-tailed paired t-test: (*$p<0.05$; **$p<0.01$;***$p<0.001$). All patients received pretreatment with ascorbate (2g) or placebo, but this distinction is ignored for the present purpose since no significant effect of ascorbate was evident (see Table 17).

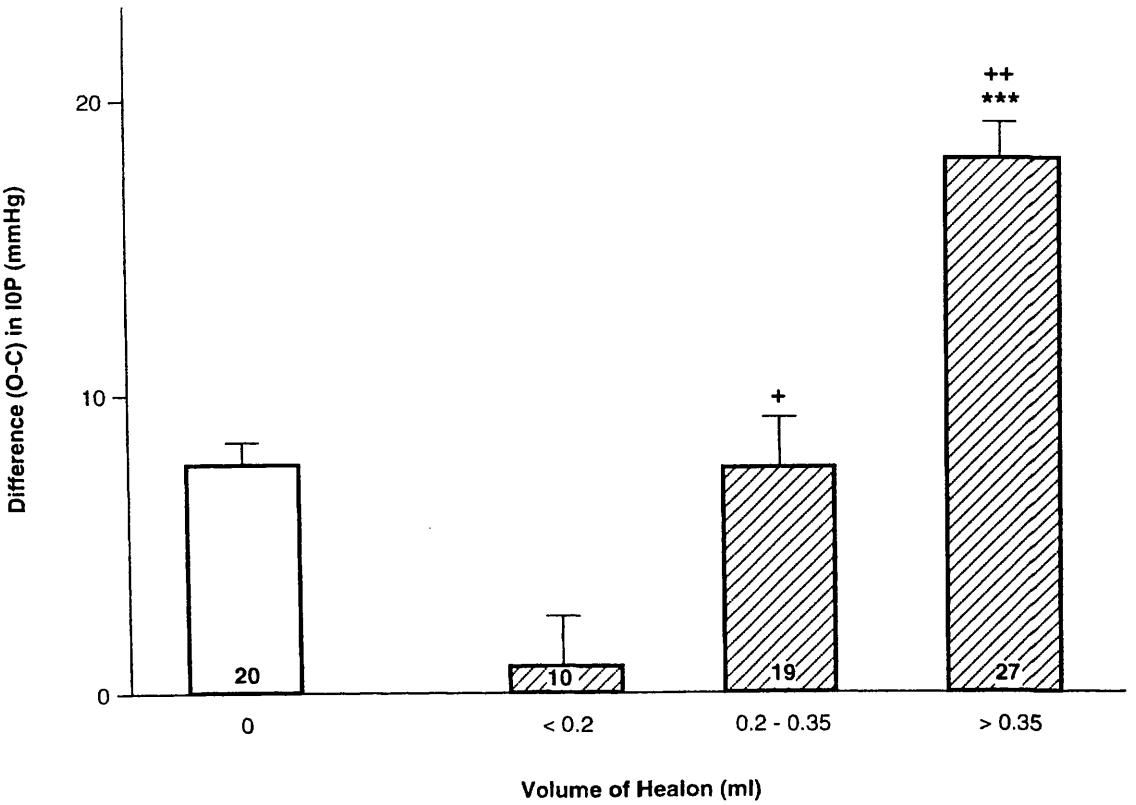

Figure 12 Differences in IOP between operated and control eyes following the use of various volumes of Healon during cataract surgery 6h postoperatively.

Graphical representation of data in Table 7, column 6 showing the difference (O-C) in IOP between operated (O) and control (C) eyes at 6h after cataract removal. The differences are shown in relation to the volume of Healon used during the operation. Patient numbers in each group are indicated within the blocks. Statistical significance of the difference (determined by the two- tailed unpaired t-test) between eyes receiving >0.35ml Healon and those which did not: ***$p<0.001$; between >0.35ml and <0.2ml Healon treatment: ***$p<0.001$; between >0.35ml and 0.2-0.35ml Healon treatment: ++$p<0.01$; between 0.2-0.35ml and <0.2ml Healon treatment: +$p<0.05$. Similar statistical significance was also revealed by the non-parametric (distribution-free) Mann-Whitney U test.

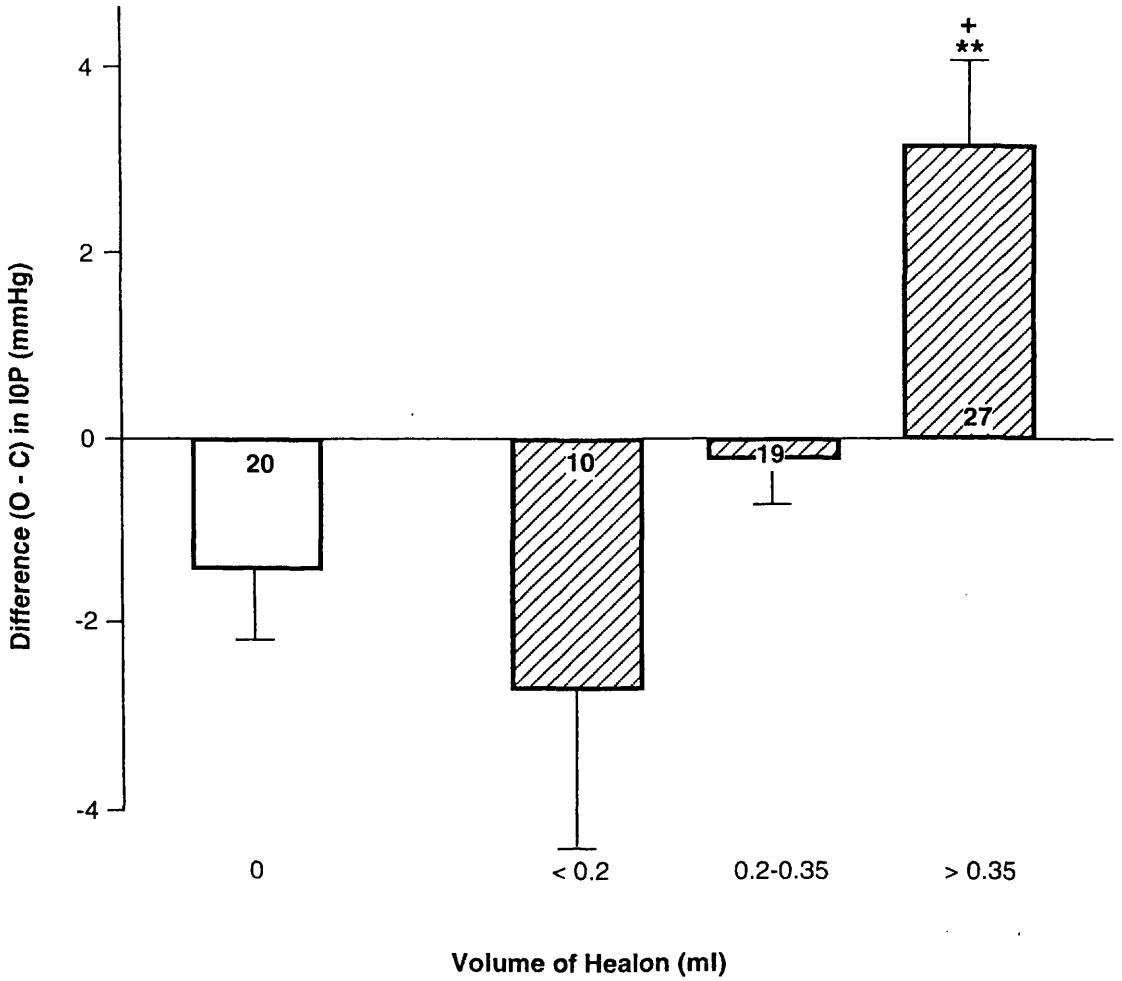

Figure 13 Differences in IOP between operated and control eyes following the use of various volumes of Healon during cataract surgery 24h postoperatively.

Graphical representation of data in Table 7, column 6 showing the difference (O-C) in IOP between operated (O) and control (C) eyes at 24h after cataract removal. The differences are shown in relation to the volume of Healon used during the operation. Patient numbers in each group are indicated within the blocks. Statistical significance of the difference (determined by the two- tailed unpaired t-test) between eyes receiving >0.35ml Healon and those which did not: **$p<0.01$; between >0.35ml and <0.2ml Healon treatment: **$p<0.01$; between >0.35ml and 0.2-0.35ml Healon treatment: +$p<0.05$. Similar statistical significance was also revealed by the non-parametric (distribution-free) Mann-Whitney U test.

TABLE 8: SIGNIFICANCE OF DIFFERENCES IN IOP BETWEEN OPERATED AND CONTROL EYES AMONG NO HEALON- AND HEALON-TREATED PATIENTS

Group Compared	p
A. At 6h after surgery	
Healon vs No Healon	0.08
B. At 24h after surgery	
Healon vs No Healon	0.07

The difference in IOP between operated and fellow control eyes 6h and 24h postoperatively was compared among patients who did not receive Healon and all those who received Healon (in whatever volume) intraoperatively. Statistical significance (p) of these differences was determined by the two-tailed unpaired t-test. Similar statistical significance was also revealed by the non- parametric (distribution-free) Mann-Whitney U test.

TABLE 9: SIGNIFICANCE OF DIFFERENCES IN IOP BETWEEN OPERATED AND CONTROL EYES AMONG PATIENTS RECEIVING VARIOUS VOLUMES OF HEALON INTRAOPERATIVELY

Groups Compared			p
A. <u>At 6h after surgery</u>			
Healon >0.35ml	vs	No Healon	<0.001
Healon >0.35ml	vs	Healon <0.2ml	<0.001
Healon >0.35ml	vs	Healon 0.2 - 0.35ml	<0.01
Healon 0.2-0.35ml	vs	No Healon	ns
Healon 0.2-0.35ml	vs	Healon <0.2ml	<0.05
Healon <0.2ml	vs	No Healon	ns
B. <u>At 24h after surgery</u>			
Healon >0.35ml	vs	No Healon	<0.01
Healon >0.35ml	vs	Healon <0.2ml	<0.01
Healon >0.35ml	vs	Healon 0.2-0.35ml	<0.05
Healon 0.2-0.35ml	vs	No Healon	ns
Healon 0.2-0.35ml	vs	Healon <0.2ml	ns
Healon <0.2ml	vs	No Healon	ns

The difference in IOP between operated and fellow control eyes 6h and 24h postoperatively was compared among groups of patients receiving stated volumes of Healon intraoperatively. Statistical significance (p) of these differences was determined by the two-tailed unpaired t- test. Similar statistical significance was also revealed by the non-parametric (distribution-free) Mann-Whitney U test.

TABLE 10: INCIDENCE OF DIFERENCES IN IOP GREATER THAN 10mmHg BETWEEN OPERATED AND CONTROL EYES AMONG PATIENTS RECEIVING VARIOUS VOLUMES OF HEALON INTRAOPERATIVELY

Time (h)	Volume of Healon (ml)	No of Patients	No of patients with ΔIOP >10mmHg	Percentage
6	0	20	7	35
	<0.2	10	1	10
	0.2-0.35	19	8	42
	>0.35	27	21	77
24	0	20	0	0
	<0.2	10	0	0
	0.2-0.35	19	0	0
	>0.35	27	6	22

IOP measurements were taken at 6h and 24h after cataract removal in human eyes which received the stated volume of Healon intraoperatively. Column 3 shows the total number of patients in each treatment group and column 4 shows the number of patients with differences in IOP greater than 10mmHg between operated and contralateral control eyes.

TABLE 11: SIGNIFICANCE OF INCIDENCE OF IOP DIFFERENCES GREATER THAN 10mmHg BETWEEN OPERATED AND CONTROL EYES 6h POSTOPERATIVELY AMONG PATIENTS RECEIVING VARIOUS VOLUMES OF HEALON

Groups Compared			p
Healon >0.35ml	vs	No Healon	<0.01
Healon >0.35ml	vs	Healon <0.2ml	<0.01
Healon >0.35ml	vs	Healon 0.2-0.35ml	<0.05
Healon 0.2-0.35ml	vs	No Healon	ns
Healon 0.2-0.35ml	vs	Healon <0.2ml	0.07
Healon <0.2ml	vs	No Healon	ns

Statistical significance (p) of these incidences was determined by the chi-squared test.

4.2 THE EFFECT OF HEALON ON OUTFLOW FACILITY IN THE HUMAN EYE IN VITRO (Specific aim A.2)

A pilot study designed to assess the effect of Healon vs a sham injection on outflow facility was conducted in one pair of human eyes and the results are presented in Figure 14. This Figure shows that the injection of Healon resulted in an average reduction in outflow facility of 64% over a period of 3h when compared with baseline. The contralateral eye which underwent a sham injection of culture medium showed a modest increase in outflow facility over the same period of time.

The result of this study is consistent with the histological findings reported by an independent ocular pathologist (Professor WR Lee, Western Infirmary, Glasgow): "In the eye which was not exposed to Healon, there is some outward movement of the uveal and corneoscleral layers and giant vacuoles are present in Schlemm's canal where the canal remains open. In the Healon perfused tissue, there is more displacement of the meshwork and the canal of Schlemm is much narrower. Giant vacuoles are not seen in significant numbers in the Healon perfused system. It would appear from this material that the Healon is lying on the inner surface of the meshwork and the pressure is forcing the various layers of the meshwork out against the scleral sulcus" (Figures 15A and 15B).

Table 12 shows the results of a study designed to assess the effect of different dilutions of Healon on outflow facility. This table shows that Healon substantially decreases outflow facility in the human eye in a concentration-related fashion at levels lower (ie, 50% and 10%) than the commercial preparation used in intraocular surgery.

The maximum obstruction studied over a period of up to 24h was 55%, 30% and 12% for the 100% (undiluted), 50% and 10% Healon dilution, respectively. This maximum obstruction typically lasted for at least 1h and in cases of higher concentrations of Healon (ie, 50% and undiluted) up to 6h or more.

In some instances, the obstruction was apparent immediately after the instillation of Healon or diluted Healon into the perfusion chamber. In other cases, there was a delay of up to 6h before the maximum obstruction became apparent. All eyes which received Healon and most eyes at the lower concentrations exhibited at least some degree of obstruction. However, in four eyes (two receiving 10% and two receiving 50% dilution) no obstruction was noted and, in fact, the outflow facility modestly increased.

A typical graphical representation of the reduction in outflow facility following the injection of Healon (50% dilution) is presented in Figure 16. The histological findings of this eye are shown in Figure 17.

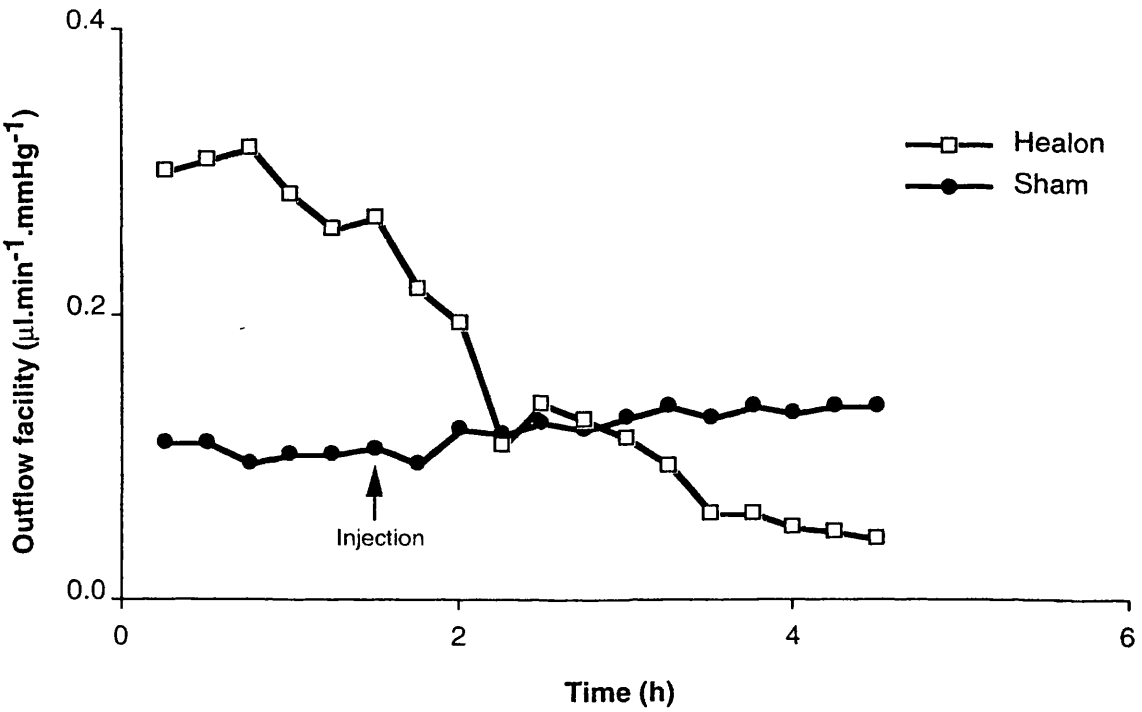

Figure 14 The effect of Healon vs a sham injection on outflow facility in the human eye in vitro.

Anterior segments from a pair of human eyes (74-year old female) were perfused with culture medium at 15mmHg at 37°C in a 5% CO_2 environment as described in Materials and Methods. After ninety minutes of perfusion (arrow) the perfusion chamber was emptied and refilled with 300µl of Healon or culture medium via an injection with a 27G needle at the limbus. Data show that Healon progressively and substantially reduces outflow facility over a period of 3h, while the sham injection results in a modest increase in outflow facility.

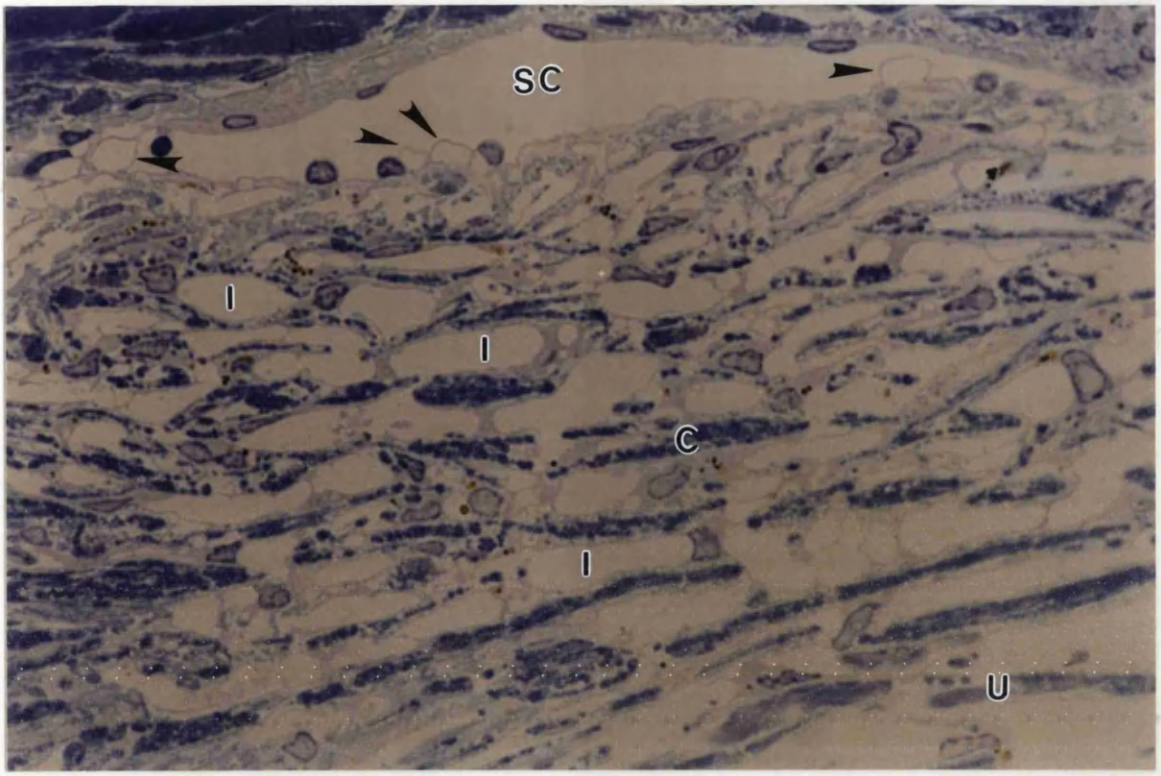

Figure 15A Light micrograph of a human eye perfused with culture medium. Semithin section of the trabecular meshwork of the eye (74-year old female) perfused with culture medium. Both the uveal (U) and corneoscleral (C) layers are open with large intertrabecular spaces (I). Schlemm's canal (SC) is open and a number of giant vacuoles (arrowheads) can be seen lining the cribriform layer. Toluidine blue, x630.

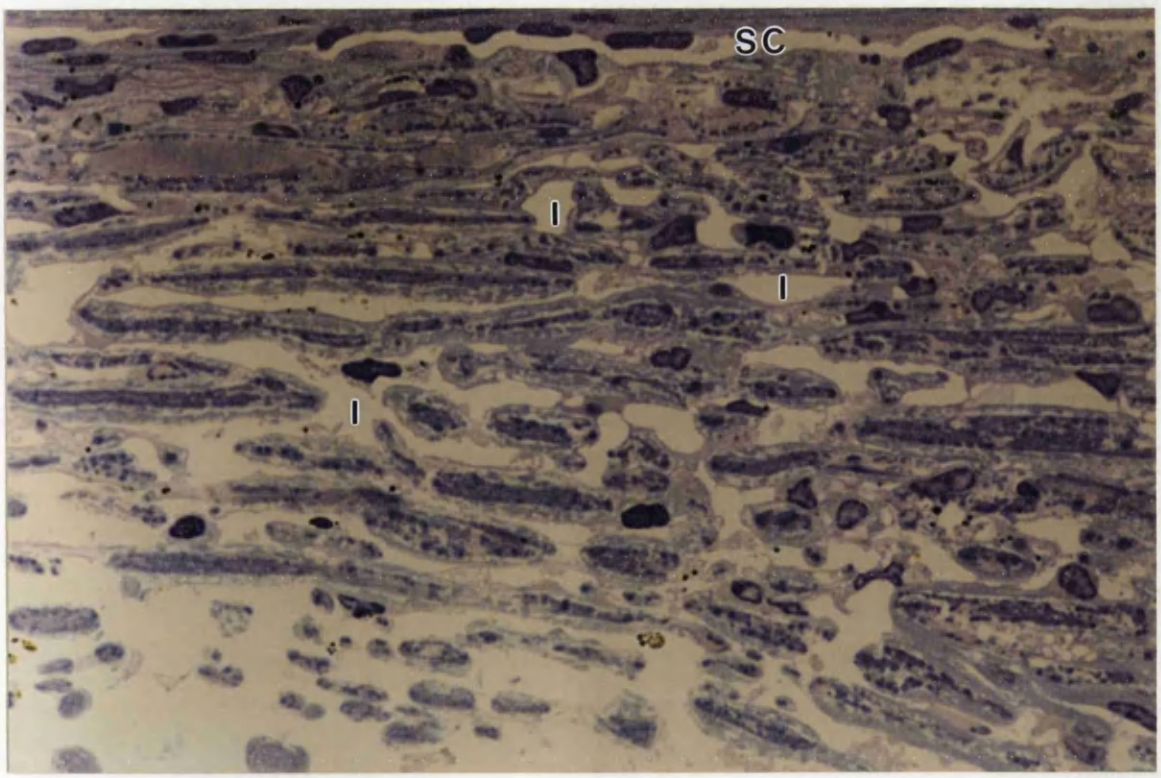

Figure 15B Light micrograph of a human eye perfused with Healon. Semithin section of the trabecular meshwork from the fellow eye of the same donor as in Figure 15A perfused with Healon. There is considerable compression of the corneoscleral layer, indicated by decrease in size of the intertrabecular spaces (I), Schlemm's canal (SC) is much narrower than the control eye and giant vacuoles are absent. Toluidine blue, x630.

TABLE 12: THE EFFECT OF DIFFERENT DILUTIONS OF HEALON ON OUTFLOW FACILITY IN THE HUMAN EYE IN VITRO

% Healon	n	C_0	C_H	C_H/C_0
100	3	0.20±0.05	0.07±0.01	0.45±0.15[c]
50	12	0.21±0.03	0.13±0.02	0.70±0.11[a]
10	5	0.22±0.04	0.17±0.04	0.88±0.25[b]

Anterior segments of human eyes were perfused with culture medium at a pressure of 15mmHg at 37°C in a 5% CO_2 environment as described in Materials and Methods. After establishment of a baseline outflow facility (C_0), the perfusion chamber was emptied and refilled with 300µl of the indicated dilutions of Healon either through an injection with a 27G needle at the limbus or through the outflow port of the perfusion apparatus, and outflow facility measurements resumed. C_H represents the maximum amount of obstruction obtained after Healon injection over a period of up to 24h. For each eye, the ratios (C_H/C_0) were calculated first and then the means. Data shown are means ± SEM for the indicated number (n) of eyes. Outflow facility is expressed as $\mu l.min^{-1}.mmHg^{-1}$. C_H/C_0 is significantly different from 1.0 by the two- tailed paired t-test ($^a p < 0.001$, $^b p < 0.05$, $^c p < 0.1$).

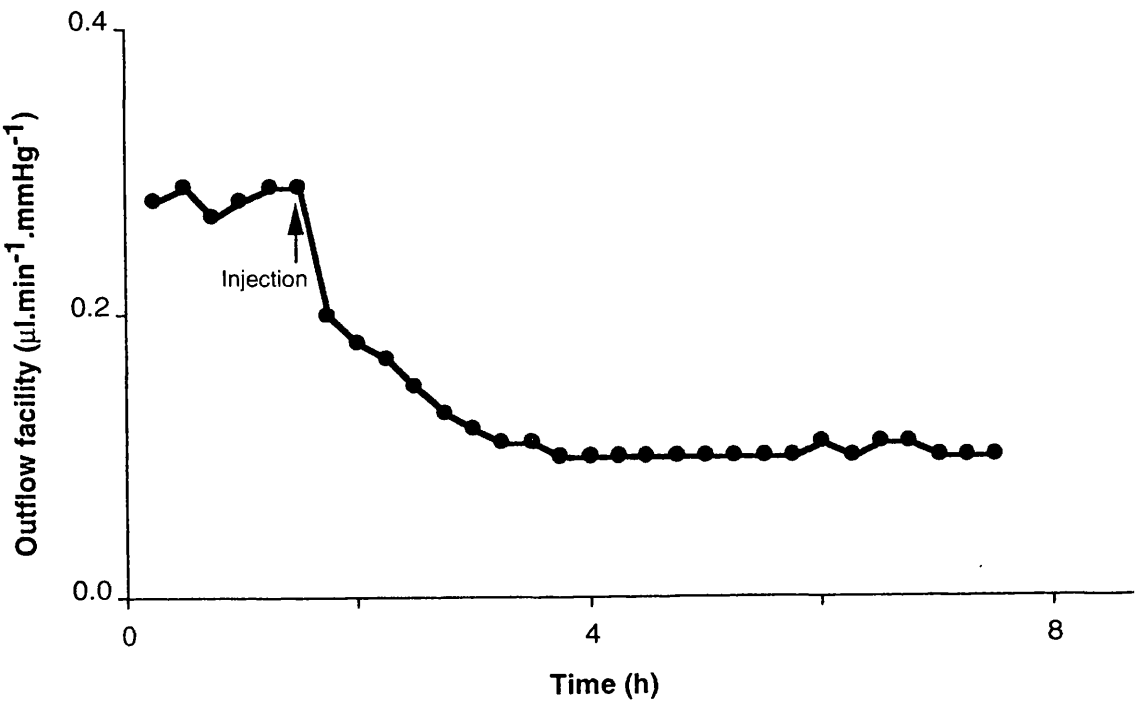

Figure 16. The effect of Healon (50% dilution) on outflow facility in the human eye in vitro.

An anterior segment from a human eye (81-year old female) was perfused with culture medium at 15mmHg at 37°C in a 5% CO_2 environment as described in Materials and Methods. After ninety minutes of perfusion (arrow) the perfusion chamber was emptied and refilled with 300µl of Healon (50% dilution) via the outflow port of the apparatus. Data show that Healon (50% dilutuion) substantially reduces outflow facility for at least 6h post-injection.

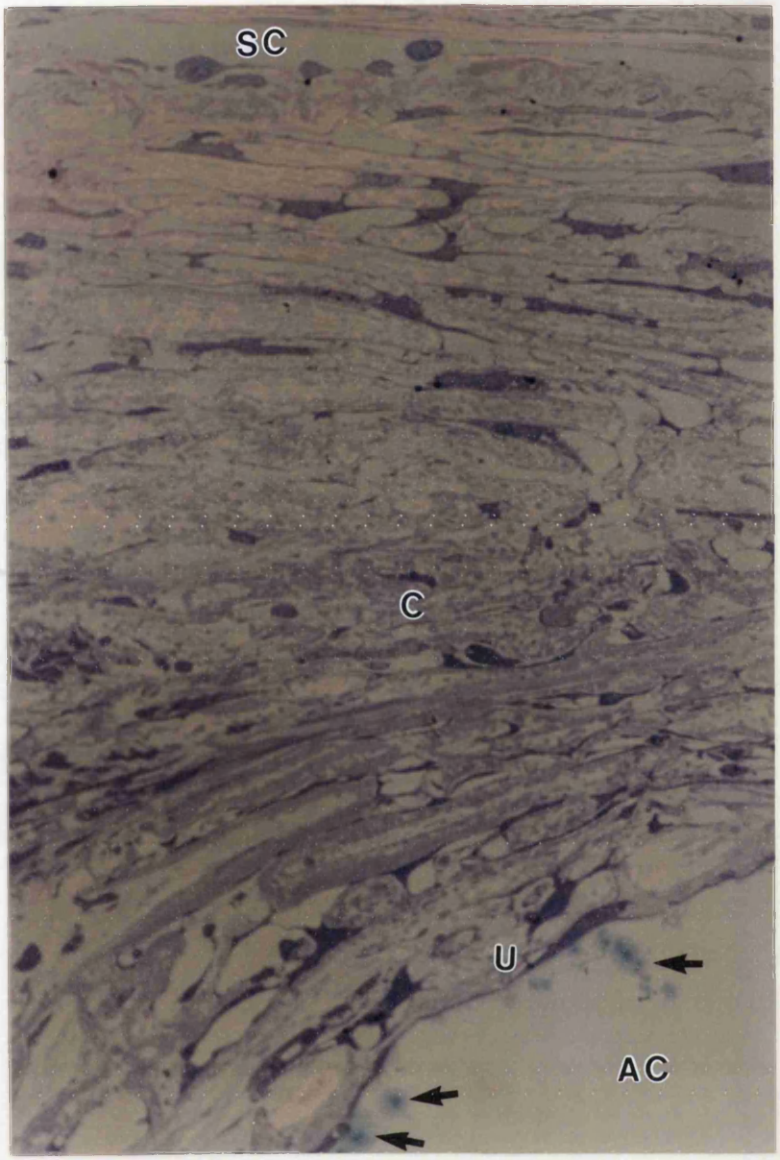

Figure 17
Light micrograph of a human eye perfused with Healon (50% dilution).

Semithin section of the trabecular meshwork of an eye (81-year old female) after a 6h perfusion with Healon (50% dilution). Fixation with Karnovsky's fixative followed by immersion in alcian blue was carried out as described in Materials and Methods. The Healon (arrowed) is located at the junction between the uveal layer (U) and the anterior chamber (AC). Note the absence of Healon from the corneoscleral layer (C), the cribriform layer and Schlemm's canal (SC). Both the uveal and corneoscleral layers are compressed and Schlemm's canal is collapsed. Toluidine blue, x630.

4.3 THE EFFECT OF SURGERY AND HEALON ON POSTOPERATIVE ASCORBATE CONCENTRATION IN THE RABBIT AQUEOUS HUMOUR (Specific aim B.1)

The effect of surgery (corneal incision alone) on aqueous ascorbate levels was studied in the rabbit eye in vivo in two series of experiments. In the first set of experiments, aqueous samples were taken at 6, 12, 24, and 48h after an initial corneal incision was made at time zero in 7 rabbits. These data show that there is a substantial drop in aqueous ascorbate levels during the first 24h postoperatively with a slight recovery at 48h (Figure 18, top). In a second series of experiments in 7 rabbits at slightly different timepoints (2, 5, 8, 24, 48h) the results were similar (Figure 18, bottom). In these experiments, at 24h the ascorbate levels dropped to an average of 45% of the zero-time value with a recovery to about 54% at 48h (Table 13). Comparison of fellow control eye at 48h with the experimental eyes at time zero shows that the ascorbate levels were approximately 30% higher in the control eyes than the baseline levels in the experimental eyes (Table 13). Therefore, at 48h, there was a net decrease in ascorbate levels of 57% when comparing the experimental eye after multiple paracenteses with the fellow control eye (Table 13).

A similar effect on ascorbate levels was observed at 48h when a corneal incision alone was made at time zero where a decrease of 54% was observed, relative to the contralateral control eye (Table 14A). Lens removal did not appear to further compromise the ascorbate levels at 48h where a 56% decrease in ascorbate levels relative to the control eye was observed (Table 14A). Collectively, these data indicate that a considerable decrease in aqueous humour ascorbate concentration occurs in the early postoperative period and

that additional lens removal does not further compromise the levels of ascorbate.

Figure 19 shows that plasma ascorbate levels following oral administration of 50mg/Kg body weight of ascorbic acid peaks within 2-3h and remains high for about 9-10h.

The results after pretreating rabbits with systemic ascorbate (a single oral dose of 50mg/kg body weight, 3h preoperatively), show that, although initial levels of aqueous humour ascorbate were significantly higher at time zero as compared with unloaded animals, pretreatment did not appear to enhance aqueous ascorbate at 6h and 48h (Tables 14A and 14B).

The intraoperative use of Healon did appear to influence ascorbate levels. There was a small (8%) (but not statistically significant) attenuation of the loss of ascorbate when intraoperative Healon was used in eyes which received corneal incision alone without lens removal (Table 14A). However, the use of Healon intraoperatively with lens removal resulted in dramatic reduction in the recovery of ascorbate levels. In this instance, ascorbate levels were only 26% of contralateral control at 48h, ie, there was an 18% increase in the loss of ascorbate as compared with eyes which did not receive Healon (Table 14A). The significance of the above differences in ascorbate levels is presented in Table 15.

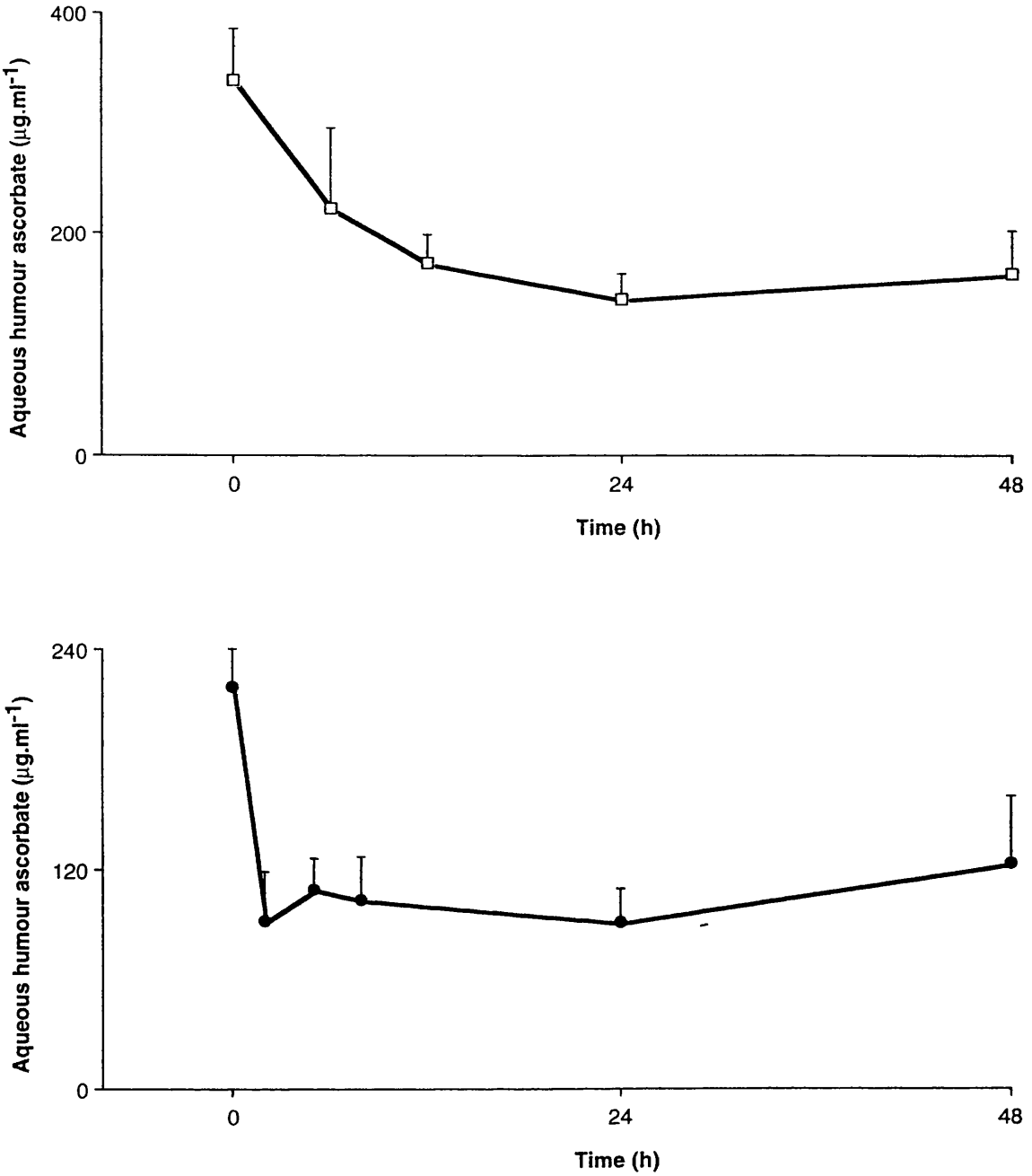

Figure 18 **Timecourse of the effect of surgery on rabbit aqueous humour ascorbate concentration.**

Aqueous humour ascorbic acid levels were measured in the rabbit eye in vivo in two series of experiments during the early postoperative period. In the first set of experiments (top) aqueous humour samples were taken at the indicated timepoints after an initial corneal incision at time zero in 7 rabbits. In the second set of experiments (bottom) aqueous humour samples were taken at slightly different timepoints as indicated. Data are the mean ascorbate concentrations in $\mu g.ml^{-1}$ and vertical bars show the SEM.

TABLE 13: ASCORBATE LEVELS IN RABBIT AQUEOUS HUMOUR FOLLOWING CORNEAL INCISION AND SUBSEQUENT REPEATED PARACENTESES

		Ascorbate ($\mu g.ml^{-1}$)				
Time(h)	**n**	**Experimental**	**n**	**Control**	**n**	**E/C**
0	13	283±32				
2	7	92±27				
5	7	109±17				
6	7	23±72				
8	6	105±23				
12	7	172±27				
24	13	117±16				
48	11	148±28	14	333	11	0.43±0.05***
2/0	6	0.44±0.12*				
5/0	6	0.58±0.09**				
6/0	7	0.60±0.15**				
8/0	5	0.52±0.13*				
12/0	7	0.56±0.09**				
24/0	12	0.45±0.05***				
48/0	11	0.54±0.09***				
Control/0			13	1.30±0.15***		

Ascorbate levels (mean ± SEM) in aqueous humour samples from rabbit eyes were measured after an initial corneal incision (0) and subsequent repeated paracenteses at the hours indicated. Ascorbate levels were measured in the fellow control eye at 48h intervals with no prior surgery. Eye numbers in each group are indicated (n).

Data for 0,24 and 48h are combined means from both series of experiments. Ratios are shown for ascorbate levels at each time interval relative to the zero-time value (2/0, 5/0, 6/0, 12/0, 24/0, 48/0), as well as in the experimental eye relative to control eye at 48h (E/C) and control eye relative to zero-time value in the experimental eye (Control/0). These ratios are significantly different from 1.0 by the two-tailed paired t-test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

TABLE 14A: ASCORBATE LEVELS IN RABBIT AQUEOUS HUMOUR AFTER CORNEAL INCISION WITH AND WITHOUT LENS REMOVAL (WITH AND WITHOUT INTRAOPERATIVE USE OF HEALON)

		Ascorbate ($\mu g.ml^{-1}$)					
Group	**n**	**0h**	**6h**	**48h**	**control**	**6h/Oh**	**E/C**
Corneal incision alone/no Healon	5	248±38		189±36	433±96		0.46±0.04*
Corneal incision alone/Healon	5	219±19		110±15	206±24		0.54±0.05*
Corneal incision + lens removal /no Healon	6	237±26		91±13	204±22		0.44±0.02*
Corneal incision + lens removal /Healon	6	396±85		123±30	458±76		0.26±0.03*
Corneal incision + lens removal / Healon + ascorbate pretreatment	5	613±95	148±22	78±19	218±73	0.25±0.03*	
Corneal incision + lens removal /Healon	4	436±29	138±51	84±38	379±33	0.29±0.09*	

Ascorbate levels (mean ± SEM) were measured in the aqueous humour of rabbit eyes with or without pretreatment with ascorbate (50mg/kg body weight, 3h preoperatively) after an initial corneal incision (0h) with or without lens removal and subsequent paracentesis at the time(s) shown. Eye numbers in each group are indicated (n). In some groups, Healon was injected during the surgical procedure. Ascorbate levels were measured in the fellow control eye at 48h. Ratios of ascorbate levels in experimental eyes compared to control eyes at 48h (E/C) and in experimental eyes compared to zero time (6h/Oh) are significantly different from 1.0 by the two-tailed paired t-test (*$p < 0.001$).

TABLE 14B: THE EFFECT OF ASCORBATE PRETREATMENT ON THE CONCENTRATION OF ASCORBATE IN RABBIT AQUEOUS HUMOUR

	n	Oh
Ascorbate pretreatment	5	613±95*
No ascorbate pretreatment	26	297±26

This Table shows combined data from Table 14A. Ascorbate levels (mean ± SEM) were measured in the aqueous humour of rabbit eyes with and without pretreatment with ascorbate (50mg/kg body weight, 3h preoperatively) after an initial corneal incision (Oh). Eye numbers in each group are indicted (n). The difference in aqueous humour ascorbate levels between pretreated and non-pretreated rabbit eyes are significantly different from zero by the two-tailed unpaired t-test (*$p<0.05$).

TABLE 15: SIGNIFICANCE OF DIFFERENCES IN ASCORBATE LEVELS IN RABBIT AQUEOUS HUMOUR AFTER CORNEAL INCISION WITH AND WITHOUT LENS REMOVAL (WITH AND WITHOUT INTRAOPERATIVE USE OF HEALON)

Groups Compared	p
Corneal incision only	
No Healon vs Healon	ns
Corneal incision + lens removal	
No Healon vs Healon	<0.01
Corneal incision + lens removal vs corneal incision	
Healon	<0.01
No Healon	ns

The difference in ascorbate levels between operated (corneal incision only or corneal incision and lens removal) and control eyes 48h postoperatively was compared among groups of rabbit eyes receiving Healon or no Healon intraoperatively. Statistical significance (p) was determined by the two-tailed unpaired t-test.

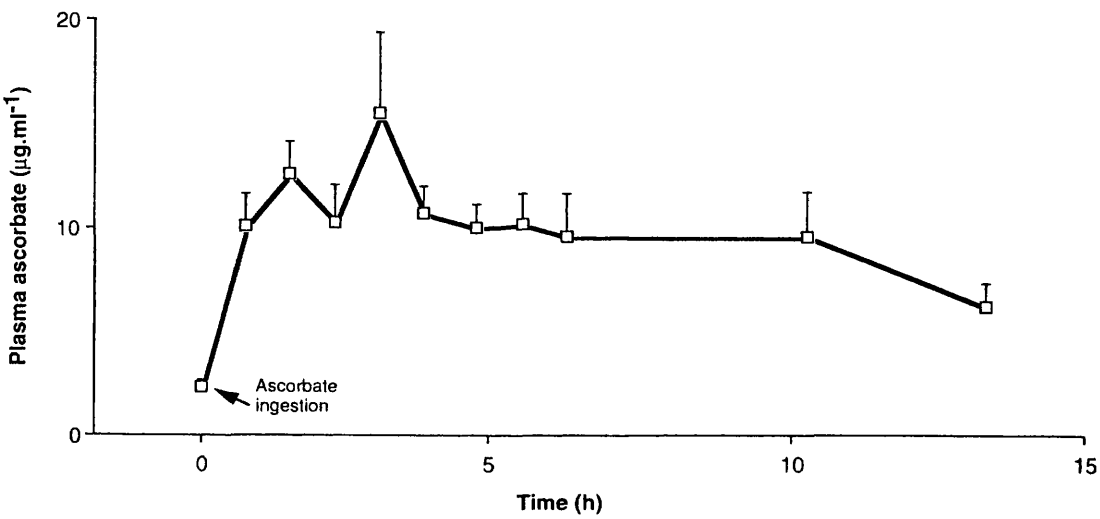

Figure 19 Timecourse of rabbit plasma ascorbate levels following a single oral dose (50mg/kg body weight) of ascorbate.

Seven rabbits received orally 50mg/kg body weight of ascorbate at time zero (arrow) and blood samples were obtained for ascorbate analysis at the indicated timepoints. Data are the mean ascorbate concentrations in $\mu g.ml^{-1}$ and vertical bars show the SEM.

4.4 THE EFFECT OF SYSTEMIC ASCORBATE PRETREATMENT ON AQUEOUS HUMOUR ASCORBATE LEVELS IN THE HUMAN EYE (Specific aim B.2)

The results of a pilot study designed to determine the timecourse of plasma ascorbate levels following ingestion of a single 1g dose of ascorbate are presented in Figure 20. This Figure shows that plasma ascorbate levels peak within 2-4h, remain high for 3-5h and fall to the preloaded concentration about 10h after the ingestion.

Figures 21A and 21B show the aqueous humour and plasma ascorbate levels respectively in cataract patients receiving no ascorbate or a total dose of 1, 2, 3 or 5g oral ascorbate in two equal doses, administered the evening before surgery and the morning of surgery. Aqueous humour ascorbate levels ($\mu g.ml^{-1}$) measured in this study ranged from 80 to 518 (266±14), 110 to 509 (268±41), 194 to 1066 (643±73), 207 to 1956 (655±96) and 497 to 798 (649±86; mean±SEM) for the 0, 1, 2, 3 and 5g group, respectively.

Plasma ascorbate levels ($\mu g.ml^{-1}$) ranged from 1.2 to 11.1 (5.1±1.1), 5.2 to 15.9 (8.7±0.9), 6.8 to 22.9 (13.6±1.8), 15.7 to 45.9 (27.7±4.2) and 42.6 to 103.3 (72±12.4; mean ± SEM) for the 0, 1, 2, 3 and 5g group, respectively.

These results show that plasma ascorbate increased with each incremental dose, the 1g dose of ascorbate producing approximately double the normal level. Aqueous ascorbate increased to a much lower extent than did plasma, with no significant further increase on administration of the 3 or 5g total dose. These data show that the maximal aqueous humour levels are attained after a total dose of 2g of ascorbate, representing an approximate two-fold increase over non-pretreated patients. Further regression analysis did not reveal any correlation between aqueous humour ascorbate levels and age or sex of the patients in any of the groups.

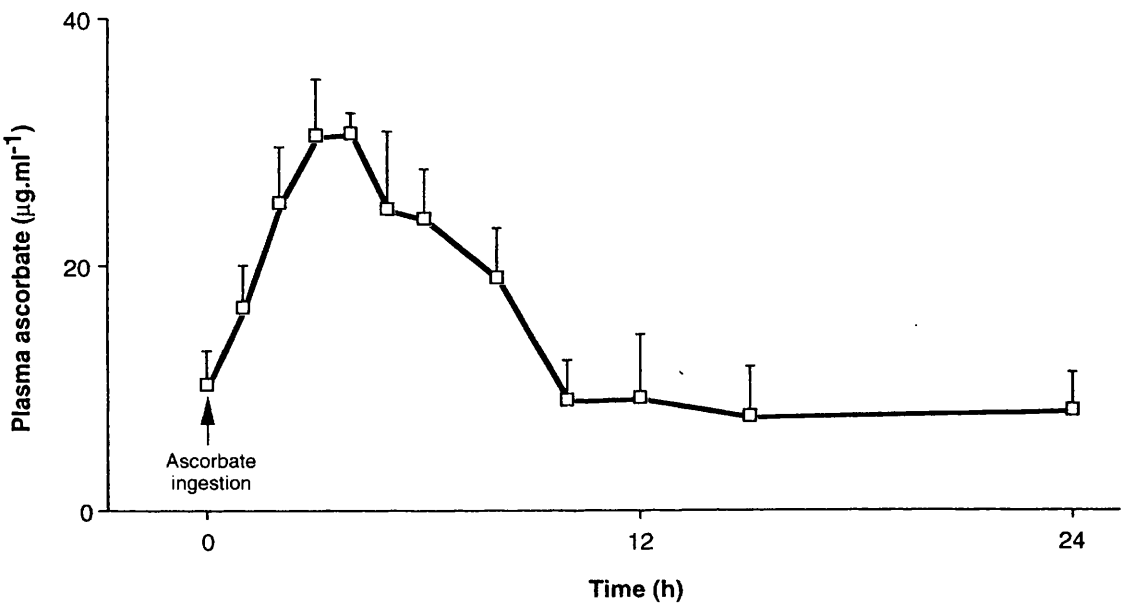

Figure 20 Timecourse of human plasma ascorbate levels following oral administration of a single 1g dose of ascorbate.

Five adult volunteers received 1g of ascorbate orally at time zero (arrow) and blood samples were obtained for ascorbate analysis at the indicated timepoints. Data are the mean ascorbate concentrations in $\mu g.ml^{-1}$ and vertical bars show the SEM.

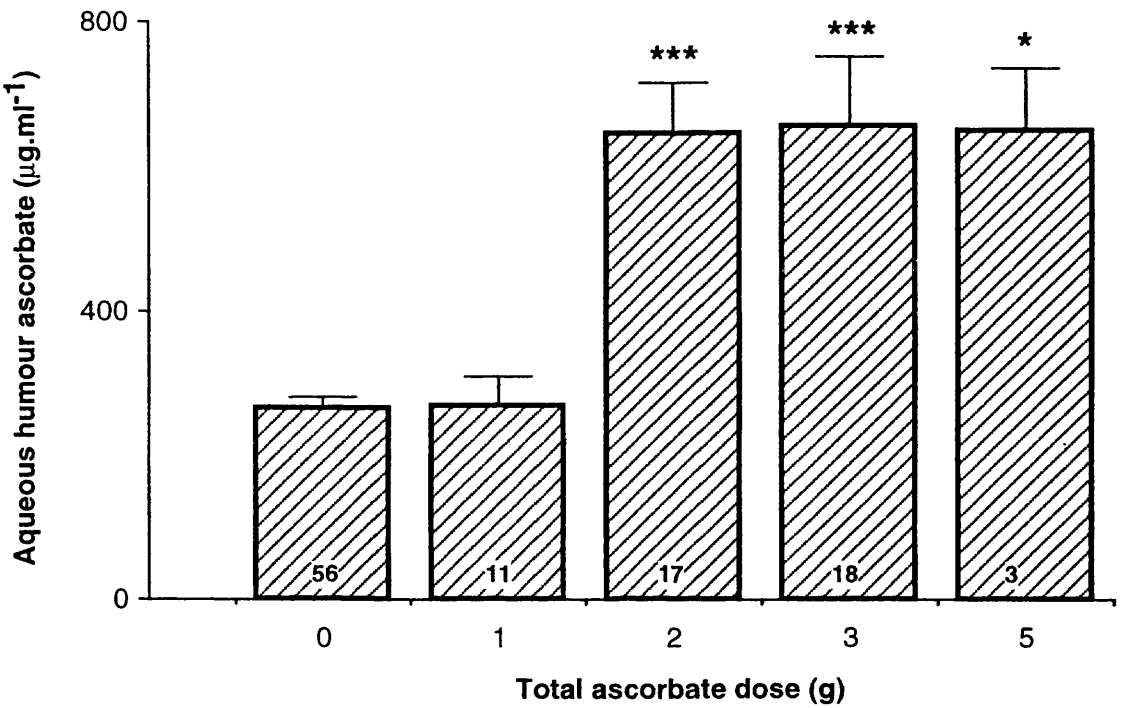

Figure 21A Human aqueous humour ascorbate levels following systemic ascorbate pretreatment.

Aqueous humour ascorbate levels were measured in cataract patients receiving no ascorbate or a total dose of 1, 2, 3 or 5g oral ascorbate preoperatively as described in Materials and Methods. Patient numbers in each group are indicated within the blocks. Data are the mean ascorbate concentrations in $\mu g.ml^{-1}$ and vertical bars show the SEM. The difference in ascorbate level between patients pretreated with 2, 3 or 5g total dose and those who did not receive ascorbate is significantly different from zero by the two-tailed unpaired t-test: (*** $p<0.001$; * $p<0.05$).

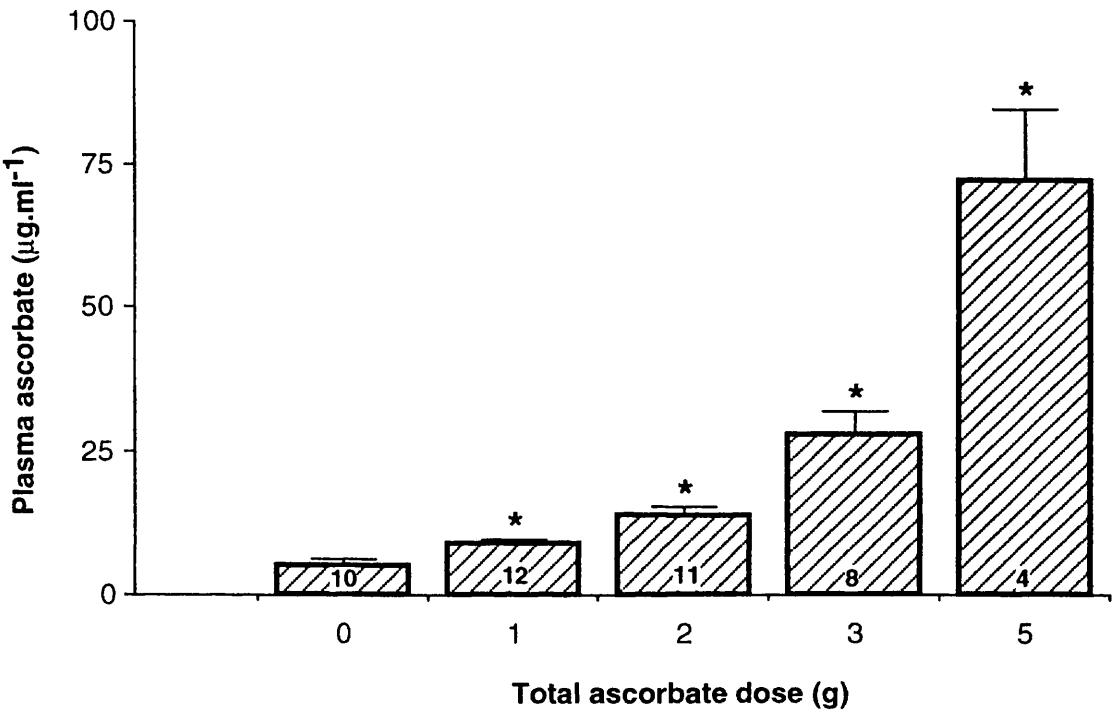

Figure 21B Human plasma ascorbate levels following systemic ascorbate pretreatment.

Plasma ascorbate levels were measured in cataract patients receiving no ascorbate or a total dose of 1, 2, 3 or 5g oral ascorbate preoperatively as described in Materials and Methods. Patient numbers are indicated within the blocks. Data are the mean ascorbate concentrations in $\mu g.ml^{-1}$ and vertical bars show the SEM. The difference in ascorbate levels between patients pretreated with 1,2, 3 or 5g total dose and those who did not receive ascorbate is significantly different from zero by the two-tailed unpaired t-test: (*$p<0.001$).

4.5 THE EFFECT OF ASCORBATE ON THE HEALON-INDUCED REDUCTION IN OUTFLOW FACILITY IN THE HUMAN EYE IN VITRO (Specific aim B.3).

Table 16 shows the results of a study which assessed the effect of a high constant concentration of ascorbate on the Healon-induced reduction in outflow facility in human eyes in vitro.

Although this was a small study,these data show that the Healon-induced reduction in outflow facility did not improve in the presence of a high ($1000\mu g.ml^{-1}$) constant concentration of ascorbic acid over a period of 6h. In fact, a paired comparison shows a higher degree of maximum obstruction observed in the presence of ascorbate as compared with contralateral control (pairs 1 and 2). In pair 3, although the outflow facility in the presence of ascorbate was modestly improved as compared with control, a substantial amount of obstruction still remained (25%) in this eye.

Histological examination did not reveal any difference between ascorbate and non-ascorbate treated eyes and the findings were similar to those presented in Figure 17.

TABLE 16: THE EFFECT OF ASCORBATE ON THE HEALON-INDUCED REDUCTION IN OUTFLOW FACILITY

	C_e	C_c	C_{H+A}	C_H	C_{H+A}/C_e	C_H/C_c	$\frac{C_{H+A}/C_e}{C_H/C_c}$
Pair 1	0.13	-	0.04	-	0.31	-	0.39
	-	0.28	-	0.22	-	O.78	
Pair 2	0.23	-	0.07	-	0.30	-	0.79
	-	0.29	-	0.11	-	0.38	
Pair 3	0.24	-	0.18	-	0.75	-	1.5
	-	0.30	-	0.15	-	0.5	

Anterior segments of 3 pairs of human eyes were perfused at a pressure of 15mmHg with culture medium at 37°C in a 5% CO_2 environment as described in Materials and Methods. After establishment of a baseline outflow facility (C_e for the experimental eye and C_c for the control eye), the perfusion chambers were emptied and Healon (50% dilution in culture medium) was injected in both eyes through the outflow port of the perfusion apparatus. In the experimental eye, the perfusion continued with added ascorbate (1000 $\mu g.ml^{-1}$ in culture medium) while the contralateral control eye was perfused with culture medium with no added ascorbate, and subsequent outflow facility determinations were made. C_{H+A} and C_H represent the maximum amount of obstruction obtained after Healon injection in the experimental and control eyes respectively (expressed as $\mu l.min^{-1}.mmHg^{-1}$), over a period of 6h. Data in columns 6 and 7 show comparisons of post-Healon outflow facility over baseline (C_{H+A}/C_e and C_H/C_c) in the experimental and control eyes, respectively. Paired comparisons of these $\frac{C_{H+A}/C_e}{C_H/C_c}$ are presented in column 8.

4.6 THE EFFECT OF SYSTEMIC ASCORBATE PRETREATMENT ON POSTOPERATIVE IOP FOLLOWING CATARACT SURGERY WITH INTRAOPERATIVE USE OF HEALON

Specific aim B.4)

A comparison of the IOP measurements among groups of cataract patients receiving Healon with and without pretreatment with a total dose of 2g of oral ascorbate shows that ascorbate administration did not influence the early (6h and 24h) postoperative rise in IOP (Table 17).

Further, regression analysis did not reveal any correlation between aqueous humour ascorbate levels at the time of surgery and postoperative rise in IOP in any of the Healon groups.

Further, two-way analysis of variance did not indicate any effect of ascorbate pretreatment on postoperative IOP rise in any of the Healon groups.

TABLE 17: POSTOPERATIVE IOP IN HUMAN EYES WITH OR WITHOUT INTRAOPERATIVE USE OF HEALON AFTER ASCORBATE OR PLACEBO PRETREATMENT

		n	Baseline(O)	Baseline(C)	6h(O)	6h(C)	6h(O-C)	24h(O-C)
No Healon	Ascorbate	10	13.3±0.8	13.6±0.8	21.7±1.8	11.9±0.9	9.8±1.9	0.7±1.4
	No Ascorbate	10	13.7±1.2	13.0±0.9	17.1±1.7	12.2±0.9	4.9±1.7	-2.1±1.6
Healon	Ascorbate	28	13.8±0.5	13.0±0.5	24.5±2.0	12.7±0.6	11.8±2.1	0.1±1.1
	No Ascorbate	28	13.5±0.7	14.3±0.6	22.7±2.0	12.9±0.5	9.8±2.2	1.8±1.1
Vol. of Healon (ml)								
<0.2	Ascorbate	5	15.2±1.3	15.6±1.7	18.4±2.1	15.8±2.2	2.6±3.7	-4.4±2.6
	No Ascorbate	5	13.0±0.9	13.8±1.0	12.0±2.5	13.8±0.7	1.8±3.2	-1.2±1.5
0.2-0.35	Ascorbate	9	13.2±0.8	11.9±0.7	10.3±1.9	11.1±0.9	7.2±2.7	-1.3±1.4
	No Ascorbate	10	12.5±1.2	13.6±1.4	21.0±2.8	12.0±1.3	9.0±3.5	1.1±1.1
>0.35	Ascorbate	14	13.7±0.6	14.5±0.6	31.9±2.5	12.7±0.5	19.2±2.3	2.7±1.7
	No Ascorbate	13	14.5±1.1	14.6±0.9	27.3±3.0	13.3±0.6	14.0±3.1	3.5±1.9

The top half of the Table shows data gathered from all patients in the study, comparing those who received oral ascorbate (2g) pretreatment with those who did not. Data are segregated into eyes treated with Healon and those which did not. The bottom half of the table shows the same data subdividing the Healon-treated eyes according to the volume of Healon used intraoperatively. Patient numbers in each group are indicated (n). IOP (mmHg) measurements (mean ± SEM) were taken in operated (O) and contralateral control (C) eyes prior to surgery (baseline) and 6h and 24h postoperatively. The difference in IOP between operated and control eyes (O-C) in patients receiving ascorbate vs placebo were not statistically significant in all the groups using two-way analysis of variance.

CHAPTER 5

DISCUSSION

5.1 DISCUSSION OF THE STUDY

The original reports on the use of Healon during cataract surgery did not mention any postoperative IOP elevation. In several studies no statistically significant differences in IOP were observed after use of Healon compared with controls (Pape and Balazs, 1980; Miller and Stegmann, 1981; Stegmann and Miller, 1982; Holmberg and Philipson, 1984a, 1984b). Other studies showed significant elevations in postoperative IOP following the use of Healon and advocated irrigation and aspiration of Healon at the end of surgery (Pape, 1980; Lazenby and Broocker, 1981; Olivius and Thorburn, 1985). Subsequent studies described that despite irrigating/aspirating Healon at the end of surgery, significant postoperative IOP rises occurred (McGuigan et al, 1986; Ruiz et al, 1987). However, a too vigorous attempt to irrigate/aspirate the Healon at the end of surgery should be avoided as a potential damage to the corneal endothelium may occur (Olivius and Thorburn, 1985).

The exacerbating effect of Healon use on the immediate postoperative IOP elevation after cataract surgery has been described by a number of authors in more recent studies. The maximal IOP increase has been reported to occur within 6h (Cherfan et al, 1983; Barron et al, 1985; Naeser et al, 1986; Anmarkrud et al, 1992), 12h (Ruusuvaara et al, 1990) or 16h (Passo et al, 1985) postoperatively and returns to baseline levels within 24- 48h. The results of the present study are consistent with these previous observations.

In the present study (where the Healon-treated patients were also subdivided in three different groups according to the volume of Healon used intraoperatively), patients' IOP was significantly higher in eyes which received Healon in amounts larger than 0.35 ml when compared with operated eyes which did not receive Healon. The pressure elevations were evident by 6h postoperatively and with the exception of eyes which received more than 0.35 ml Healon, IOP returned to preoperative levels by 24h (Table 7). These IOP elevations occurred despite an attempt to remove by aspiration or irrigation the remaining Healon at the end of the procedure which has been recommended by many authors (Pape, 1980; Lazenby and Broocker, 1981; Miller and Stegmann, 1981, 1982; Hoffer, 1982; Stegmann and Miller, 1982; Cherfan et al, 1983; Glasser et al, 1986; Calissendorff and Hamberg-Nyström, 1993) in order to prevent or attenuate postoperative increases in IOP.

The dose-dependence of the postoperative Healon-induced IOP elevations has not been systematically addressed in previous studies. Polack et al (1981) and Polack (1982, 1986) have, however, shown that in keratoplasty with or without IOL implantation, IOP elevation occurs only when more than 0.4 ml Healon is injected into the anterior chamber. These authors concluded that IOP elevation may be dose-dependent. The results of the present study suggest that there is a Healon dose-dependence of both the magnitude and duration of the postoperative IOP elevation.

The clinical trial showing this effect as described in this thesis was constrained by a number of limitations:

a) it was not possible to mask the use and the injected volume of Healon for every case.

b) it was neither practicable nor ethical to randomize the volume of Healon used in each operation.

c) it was thus impractical to match patients in different treatment groups with respect to age, sex and pathology (eg, the various type and grade-maturity of cataract).

d) there was no uniformity of surgical cataract extraction procedure (although all participated surgeons were experienced and ECCE with p/c IOL was performed in all cases in a similar fashion) nor of IOL type.

e) there was no uniformity in the procedure employed to wash Healon out of the eye (volume and direction of Hartmann's solution).

f) there was variation in the anaesthetic procedures employed, although similar percentages of each group underwent general and local anaesthesia

g) there was variation in diet, general pathology and medication among the patients.

However, despite these caveats, highly significant variations in IOP were observed with respect to the volumes of Healon used during the routine cataract surgery performed by five ophthalmic surgeons. The results have thus revealed a major potentially avoidable hazard.

The results of this trial suggest that eyes which received less than 0.2ml do not show an elevation in postoperative IOP at 6h, those which receive 0.2-0.35 and greater than 0.35ml of Healon do, and in the latter case, higher IOP elevations are expected. Further, while IOP returns to preoperative levels at 24h in all eyes receiving less than 0.35ml Healon, the pressure would still be elevated at 24h in eyes which receive greater than 0.35ml Healon.

The present study also suggest that potentially harmful high IOP spikes at 6h postoperatively occur when more than 0.35ml of Healon is used intraoperatively (Tables 10 and 11).

The absence of an IOP increase in the eyes receiving less than 0.2ml Healon may have important implications for surgical procedures using Healon. While the results of this part of the study are based on a small population (ie, 10 subjects) they suggest that the use of a small amount of Healon during cataract surgery may be adequate to ensure a good surgical outcome without raising IOP. It should be noted that the range of IOPs at 6h postoperatively in this group was from 10mmHg less than to 12mmHg greater than the fellow eye. Three out of the ten eyes in this group were hypotonic (ie. the IOP deficit from the fellow eye was 5mmHg or greater). However, there were no complications or remarkable clinical sequelae to the surgical procedures. Further, in this particular group, Healon was used only to deepen the anterior chamber and not for the reformation and expansion of the capsular bag.

The residual amount of Healon left in the eye varies with different extracapsular cataract extraction techniques. One study reported that the use of Healon in the "envelope-like" technique of cataract removal (as performed in the present study) clearly caused significantly greater postoperative elevations in IOP than the "can-opener" technique, as some Healon remains unavoidably

in the capsular bag and "may obstruct mechanically the outflow channels or may shallow the anterior chamber" (Ruusuvaara et al, 1990). Another clinical study in a larger population is necessary to confirm conclusions based on these results.

More recent studies (Öhrström et al, 1989; Smith and Burt, 1991; Assia et al, 1992) using sodium hyaluronate tinted with sodium fluorescein (Healon Yellow, Kabi Pharmacia, Uppsala, Sweden) during cataract surgery described that a considerable amount of Healon is inadvertently left in the eye, especially behind the IOL, despite an attempt to dilute and aspirate this highly viscous solution at the end of surgery. However, in these studies details of the type of extracapsular technique used are not included. Healon Yellow is not available in the UK yet, but it seems promising in order to study the amounts of Healon trapped behind the IOL and also to assess the dynamics of Healon removal at the end of intraocular surgery.

Although it is postulated that in most patients the transiently elevated IOP may have no adverse effect, there may be serious consequences of highly elevated IOP postoperatively, even in normal eyes without a compromised outflow system. Sustained elevations in IOP may result in optic neuropathy (glaucomatous or ischaemic), inhibition of wound healing, retinal vascular occlusion, severe pain or corneal oedema (Kolker, 1977; Hayreh, 1980; Bartov et al, 1984).

In the glaucomatous eye, modest and even transiently elevated IOP may be disastrous for the remaining visual field as additional and irreversible damage of the optic nerves may ensue (Kolker, 1977; Hayreh, 1980). IOP rises following cataract extraction have been reported to occur more frequently and to higher levels in eyes with preexisting glaucoma or exfoliation syndrome

(Savage et al, 1985; McGuigan et al, 1986; Krupin et al, 1989; Ruusuvaara et al, 1990; Callissendorff and Hamberg- Nyström, 1993).

The results of the present study suggest that if Healon has to be used intraoperatively, a volume less than 0.2ml should be used in compromised eyes in order to prevent post operative IOP elevations. Further, the use of Healon for reforming the capsular bag before IOL insertion (which usually requires considerably more than 0.2ml Healon) is not recommended in high risk eyes (ie, glaucoma, exfoliation, ischaemic optic neuropathy).

The observation that IOP increases with Healon use during surgery even when attempts are made to remove Healon at the end of the procedure has led investigators to explore methods of attenuating the Healon-induced IOP elevation. Typically, beta blockers or other IOP lowering medications are administered in an attempt to block the Healon-induced IOP elevation (Haimann and Phelps, 1981; Miller and Stegmann, 1981, 1982; Percival, 1981, 1982; Lewen and Insler, 1985; Wood, 1988; Linn et al, 1989; Anmarkrud et al, 1992). However, the pre- or postoperative use of anti-glaucoma drugs is often ineffective or slow to take effect (Passo et al, 1985; Naeser et al, 1986; Ruiz et al, 1987). Therefore, a method of depolymerizing Healon in situ and thereby allowing its passage through the outflow pathways might attenuate post operative IOP rises.

Ascorbic acid at levels known to be present physiologically in the aqueous humour is known to depolymerize hyaluronic acid in vitro (Niedermeir et al, 1967; Wong et al, 1981; Motohashi et Mori, 1985; Fink and Lengfelder, 1987). This depolymerization appears to be concentration-dependent (Wong et al, 1981; Motohashi and Mori, 1985) at least for ascorbate levels up to 1,000μM (176μg.ml^{-1}). These observations suggest that raising

aqueous humour levels of ascorbic acid or trying to maintain these levels high (as a fall is expected due to the damaging effect of surgery on the BAB) may aid in depolymerizing Healon and thus, preventing or attenuating Healon-induced increases in postoperative IOP.

Based on these in vitro observations, the feasibility of preloading the aqueous humour with ascorbate was explored as a method to depolymerize Healon and thereby to attenuate postoperative IOP elevations. In initial clinical studies, cataract patients were pretreated with oral ascorbate prior to surgery and aqueous humour ascorbate levels at the time of surgery were measured. These initial studies suggested that aqueous ascorbate levels could be increased to a maximum of about two-fold (Figure 21A) following a 2g total dose over levels obtained in patients who were not pretreated.

The practicalities of theatre list arrangements prevented absolute accuracy in establishing a set interval between ascorbate ingestion and the start of cataract surgery. However, the present data on the timecourse of plasma ascorbate in plasma following oral administration of a single 1g dose show that absorption is rather slow and the ascorbate concentration in plasma peaks within 2-4h, remains high for 3-5h and falls to the preloaded level about 10h after ingestion (Figure 20). These results are in good agreement with earlier determinations of peak plasma concentrations of ascorbate 3h after oral administration of a single 1g dose (Yung et al, 1981; Melethil et al, 1986). These findings indicate that the interval (1 to 6h) between dosing on the morning of surgery and sampling of aqueous humour from cataract patients at the commencement of surgery was approximately correct. This was important in order to achieve a high and persistent level of plasma ascorbate, so that the ciliary body active transport system could best attain a high aqueous humour

ascorbate level. It also suggests that the ingestion of a dose of ascorbate the night before surgery may be of relatively little additional value in boosting aqueous ascorbate concentration. Nevertheless, the night-and-morning regimen was adopted for the main clinical study, on the basis of the claim by Ringvold et al (1985) that this regimen is preferable to a single dose.

Plasma ascorbate is itself subject to considerable variation. Levels measured in the present work in untreated individuals ranged from 1.2 to 11.1 $\mu g.ml^{-1}$ (5.1±1.1; mean ± SEM) which are similar to the value of 7±5.2 (mean ± SD) determined by HPLC (Taylor et al, 1991).

Aqueous humour ascorbate levels were also variable in the present study, but corresponded closely to published values measured by HPLC (Ringvold et al, 1985; Taylor et al, 1991).

The results of the initial clinical study confirmed that increasing doses of ascorbate would increase the plasma ascorbate concentration almost linearly (Figure 21B) and showed that aqueous humour ascorbate levels can be increased by oral dosing with ascorbic acid. Oral dosing with more than 2g (total dose) did not lead to a further increase in the aqueous ascorbate levels (Figure 21A). The probable explanation of this is that the ciliary body active transport system for ascorbate is approaching saturation and is therefore unable to utilise any further increase in plasma ascorbate concentration. The results of the present study are in good agreement with previously published human and animal studies (Kinsey, 1947; Linner, 1954; Becker, 1967; Reim et al, 1978; Giblin et al, 1984; Ringvold et al, 1985; Taylor et al, 1991) and confirm that the ascorbate transport system into the aqueous humour is saturable. However, the increased aqueous humour ascorbate levels did not influence postoperative IOP. While there was a direct correlation between the injected value of Healon

and the postoperative IOP at 6 and 24h (Tables 7 and 9 and Figures 12 and 13), there was absolutely no correlation between aqueous humour ascorbate concentration at the time of surgery and postoperative IOP levels in any of the Healon groups (Table 17). Therefore, despite the strong evidence that hyaluronic acid can be depolymerized by ascorbic acid in vitro, this did not occur in vivo, or if it did, the depolymerization was not sufficient to reduce the Healon molecule to a size compatible with free clearance from the anterior chamber.

In order to better understand the failure of ascorbic acid pretreatment to prevent Healon-induced postoperative IOP elevation, measured 6 and 24h after cataract extraction,studies were conducted in the rabbit eye to determine whether the trauma of surgery may have been responsible for the absence of sufficient levels of ascorbic acid in the aqueous humour postoperatively. In these studies the effects of surgery (corneal incision alone and corneal incision plus lens removal) on aqueous humour ascorbate levels were determined. Some of the eyes also received intraoperative Healon.

Breakdown of the blood-aqueous barrier is a well known sequel of intraocular surgery (Sanders et al, 1982, 1983; Liesegang et al, 1984; Miyake et al, 1984a; Ferguson and Spalton, 1991). Clinical and rabbit studies also showed that residual Healon can contribute to and/or enhance the breakdown of the blood-aqueous barrier following intraocular surgery (Miyake and Mizuno, 1986; Machi et al, 1989; Tsurimaki and Shimizu, 1991a, 1991b).

The results of the present studies showed that in the first 2-6 hours postoperatively aqueous humour ascorbic acid levels were decreased to about half of the baseline levels (Table 13 and Figure 18) which persisted at 48h. This was true of eyes which received a corneal incision alone with or without

added Healon (Table 14A), and also of eyes which underwent extracapsular lens extraction without added Healon. Interestingly, eyes which underwent extracapsular lens extraction with intraoperative Healon showed an additional statistically significant 18% drop in aqueous ascorbate relative to the contralateral control eyes at 48h (Tables 14A and 15). This result is presumably due to the damaging effect of Healon on the blood-aqueous barrier.

It is possible that during the intraoperative use of Healon residual lens debris is pushed out of the posterior chamber and may adhere to intraocular structures. In such a situation, where the presence of debris is prolonged, reactions may occur at the ciliary body, leading to release of mediators such as prostaglandins, which are well known to cause blood-aqueous barrier breakdown. (Unger et al, 1975; Eakins, 1977). Perhaps the intraoperative use of Healon is safer in cases of secondary IOL implantation in aphakic eyes where no lens material is present during the surgical procedure.

It is interesting to note that in the present work the ascorbate concentration in the contralateral eye was consistently greater by about 30%, 48h after surgery, than in the experimental eye at the time of surgery (Table 13).

Kottow and Seligman (1978) found that trauma induced by performing a limbal paracentesis consistently results in fluorescein leakage (indicating blood-aqueous barrier breakdown) in both the operated and contralateral eye. In a clinical study, Miyake et al (1984b) reported a consensual reaction of the blood-aqueous barrier in the contralateral eye following intraocular surgery.

The results of the present study suggest that in contradiction to the results of the aforementioned workers, the blood-aqueous barrier and associated membrane pumps were fully operational in the contralateral eye at this time.

Alternatively, it is possible that if the blood-aqueous barrier did break down in the contralateral eye at an earlier timepoint, a compensatory mechanism was in place increasing the rate of transport of ascorbate into the anterior chamber. Further, recovery of the blood-aqueous barrier immediately after cataract surgery is perhaps more rapid in the primate, since Jampel et al (1992) found that ascorbic acid levels were unchanged in the monkey eye 24h after paracentesis.

After these initial experiments, an attempt was made to increase aqueous ascorbate levels by pretreating rabbits with oral ascorbate before surgery. Ascorbate pretreatment resulted in a moderate increase (about two-fold) in aqueous humour ascorbate levels at the time of surgery, which was statistically different from ascorbate levels after no pretreatment (Table 14B). Subsequent measurements 6h and 48h after surgery showed that pretreatment with ascorbate 3h before surgery did not attenuate the surgically-induced drop in aqueous ascorbate levels (Table 14A), although the plasma ascorbate concentration reached a maximum level within 2- 3h and remained elevated for about 10h before it fell to the preloaded concentration (Figure 19). Therefore, it seemed possible that the lack of an effect of ascorbate on Healon-induced IOP elevations postoperatively in the clinical situation may have been due to the failure to maintain elevated ascorbic acid levels in the anterior chamber, due to surgically-induced and Healon-induced damage to the blood-aqueous barrier.

Since the results of these rabbit studies suggested that ascorbic acid levels may decrease postoperatively, even with ascorbate pretreatment, experiments were conducted to determine whether the obstructive effect of Healon could be eliminated under conditions where a constant high

concentration of ascorbate could be maintained.

The mechanism of IOP elevation is thought to involve a blockage of the outflow pathways and a consequent decrease in outflow facility (Berson et al, 1983). Therefore, experiments were conducted in an in vitro human eye model designed to assess the effect of surgical and pharmacological manipulations on outflow facility. Initial studies were conducted to confirm that Healon decreases outflow facility. These studies, performed over a period of up to 24h, showed that there was a concentration-dependent decrease in outflow facility due to Healon administration with a maximal decrease of 55% (Table 12) occurring when the perfusion chamber was filled with the undiluted commercial preparation of Healon. The facility decrease was maximal within 6h of administration, lasted for at least 1h and in some preparations persisted for 6h or more. The morphological correlate to these findings was the collection of Healon at the uveal surface of the TM which apparently blocked the egress of perfusion medium in much the same manner as the mechanical obstruction by cells, material or debris in some of the secondary forms of open angle glaucoma (eg, haemolytic, phacolytic, melanomalytic glaucoma, pigmentary glaucoma, exfoliative glaucoma, glaucoma due to hyphaema).

In some instances of the aforementioned studies in perfused anterior segments, the reduction in outflow facility was apparent immediately after the instillation of Healon into the perfusion chamber. In other cases, there was a delay of up to 6h before the maximum obstruction became apparent.

It is possible that on these occasions, when perfusion of culture medium was recommenced immediately after introduction of Healon into the chamber, small residues of medium remained trapped on the chamber walls from the pre-Healon perfusion, allowing formation of outflow channels of low-viscosity

fluid. These would then presumably carry medium around the Healon to reach the trabecular meshwork with relatively little mixing with the highly viscous Healon. This might explain the observation that on two occasions (two eyes out of twelve in the 50% Healon group) the outflow facility did not rise until approximately 6h after the introduction of Healon.

In four preparations (two at the 10% and two at the 50% Healon) no obstruction was noted and actually the outflow facility slightly increased. The cause of the disparate results is not immediately apparent. There were no indications that cause of death or sex of the donor played a role. Although there was no apparent leakage from the injection point at the limbus through the course of these four experiments, technical difficulties cannot be ruled out. Alternatively, it is possible, like the clinical situation where Healon does not cause a postoperative IOP elevation in all patients, it does not cause obstruction in the outflow pathways of all eyes.

The significant decrease in outflow facility occurring with concentrations of Healon much lower (ie, 10% and 50%) than the commercial preparation, suggests that despite dilution of Healon at the end of cataract surgery, high postoperative IOP rises may be expected.

Similar to the Healon-induced rises in IOP observed in the clinical studies, Healon-induced reduction in outflow facility did not appear to improve in the presence of a constant high concentration of ascorbic acid ($1000\mu g.ml^{-1}$) over a period of 6h (Table 16). Further study is necessary to confirm these preliminary findings.

Overall, the results of the <u>in vitro</u> studies confirm that the mechanism of the postoperative IOP elevation induced by Healon is a physical blockage of the aqueous humour outflow pathways. There is also clear evidence that this

phenomenon is dose-related, which is consistent with the Healon dose-dependent rise in IOP observed in the clinical studies. Further, the failure of ascorbate to attenuate the Healon-induced IOP elevation in vivo was probably not due to insufficient levels of ascorbate in the aqueous humour, since the concentration used ($1000\mu g.ml^{-1}$) in the perfused human ocular anterior segment preparation was at the top end of the range which can occur in vivo when oral ascorbate supplementation is used (Figure 21A), and this level was not adequate to attenuate the Healon-induced reduction in outflow facility.

The mechanism by which ascorbate depolymerizes hyaluronate is thought to depend on trace quantities of Fe^{2+} amd Cu^{+} which are the actual catalysts of depolymerization. Ascorbate, presumably regenerates these ions to the appropriate reduced state, Fe^{2+} or Cu^{+} (Harris et al, 1972; Wong et al, 1981). Although hyaluronate can be depolymerized by ascorbate in vitro, the difference in origin and in the content of proteins and especially of heavy metal ions (eg, iron and copper) from the commercial preparation of Healon could be considerable and might influence the depolymerization reaction in either direction. Due to commercial confidentiality, Kabi Pharmacia is currently unwilling to divulge analytical data of this sort.

It is probable that either Healon is not depolymerized by ascorbate in vivo, or if it is, that depolymerization is not sufficient to break the hyaluronic acid molecules down to a size small enough to allow easy egress through the outflow pathways.

5.2 SUGGESTIONS FOR FUTURE WORK

It is clear that although attempts have been made to attenuate Healon-induced IOP rises, either by anti- glaucoma drugs or by irrigating/aspirating Healon at the end of surgery, their success has been, at best, limited.

The present study showed that ascorbic acid pretreatment failed to prevent or to attenuate the immediate postoperative IOP elevation following the use of Healon during cataract surgery. Further study should be made on the involvement of heavy metal ions in the depolymerization reaction and on the levels of these ions occurring in normal and postoperative aqueous humour.

The finding that IOP rises were not as extreme when lower volumes of Healon were used intraoperatively has potentially important implications for the techniques employed in cataract surgery. Further clinical studies are recommended to verify the adequacy of using a low volume (<0.2ml) of Healon during cataract surgery.

A novel approach involving the use of hyaluronidase to depolymerize Healon has been suggested (Berson et al, 1983; Hein et al, 1986; Calder and Smith, 1986). Hyaluronidase has been used experimentally in cadaver human eyes. In enucleated eyes, anterior chamber irrigation with hyaluronidase showed a dramatic restoration toward normal of the reduced outflow facility following hyaluronate instillation (Berson et al, 1983). In an animal model, hyaluronate decreased outflow facility, but with a mixture of hyaluronate and hyaluronidase no significant change in outflow facility was noted (Hein et al, 1986). Calder et al (1986) found that the instillation of hyaluronidase into the anterior chamber in patients undergoing ICCE with IOL implantation resulted

in a statistically significant lowering of postoperative IOP on the first and second postoperative days.

Although further research is needed to determine the smallest effective dose and to verify the safety of its use, the enzyme hyaluronidase shows promise as an effective treatment and it is suggested as a possible means of eliminating the potentially harmful postoperative IOP elevation following the use of Healon in cataract surgery.

5.3 ADDENDUM TO DISCUSSION

5.3.1 Variation in the clinical trial

Several sources of variations may have contributed towards the variability of the data in the clinical ascorbate-Healon trial.

Many factors affect the amount of Healon remaining in the eye at the end of surgery. These are related:

a) to the volume, direction and speed of injection, and also to the angle and position of the cannula during the injection of Healon, and

b) to all these same factors during the irrigation/aspiration procedure by which the surgeon attempts to wash Healon out of the eye with Hartmann's solution.

All these factors combine to make it very difficult to estimate both the amount of Healon actually injected into the eye and the residual Healon volume. In a future study, the use of Healon Yellow would offer the opportunity to quantify the remaining amount of Healon, and the dynamics of its removal at the end of intraocular surgery. The technique of fluorophotometry potentially provides a method of quantification

The lack of uniformity of the type of the incision during cataract surgery (limbal or corneal) may influence the postoperative IOP elevation (Rich et al,1974; Cherfan et al,1983). A limbal section is more likely to lead to synthesis and release of prostaglandins, since the incision is performed through a vascular tissue and this may exacerbate the breakdown of the blood-aqueous barrier during the immediate postoperative period, resulting in higher IOP elevation. This is another source of variation which could be eliminated in a future study by ensuring that the same incision technique is used throughout.

However, in the present study similar percentages of each group underwent limbal and corneal incision. Further, regression analysis did not reveal any correlation between the type of the incision used and postoperative rise in IOP in any of the Healon groups.

The use of adrenaline (1 in 10^{-6}) in Hartmann's solution is intended to prevent miosis during intraocular surgery. Since adrenaline from the irrigation solution may increase the facility of aqueous humour outflow, this could affect the postoperative IOP rise following cataract surgery. The influence of this factor upon the results obtained may, however, be negligible, since the concentration used is low and the same reagent was used uniformly throughout the present study. The volume of irrigating

solution passing through the eye was not, however, measured in the present study and thus the total amount received by each operated eye cannot be estimated.

Further studies would be necessary to evaluate the role and the importance of the above factors.

5.3.2 The failure of ascorbic acid to prevent Healon-induced intraocular pressure rise

A number of hypotheses may be invoked in order to explain the failure of ascorbic acid to prevent or attenuate postoperative IOP elevation following intraoperative use of Healon in cataract patients and to explain the failure of high constant concentration of ascorbic acid to reduce the Healon-induced decrease in aqueous humour outflow facility in the human eye model in vitro. These hypotheses are now presented and possible strategies to investigate them are described.

The mechanism of ascorbate-induced depolymerization of hyaluronate is unclear. There are several studies suggesting that hyaluronic acid of different origins (e.g. rooster comb, umbilical cord, synovial fluid) can be depolymerized by ascorbic acid in vitro and heavy metal ions may enhance this reaction (Niedermeier et al, 1967; Harris et al, 1972; Wong et al, 1981; Miyauchi and Iwata, 1984; Motohashi and Mori, 1985; Fink and Lengfelder, 1987). However, the depolymerization of the commercial preparation of sodium hyaluronate 1% (Healon) by ascorbic acid has not

from the anterior chamber at the end of cataract surgery giving the appearance of "plastic anterior uveitis" (Cherfan et al, 1983; Larson et al, 1989). This may be important in preventing ascorbic acid from penetrating a globule of unmixed, highly viscous Healon, thereby greatly reducing the rate of any depolymerization reaction. This hypothesis is supported by the fact that all previous in vitro experiments on the ascorbate-induced depolymerization of hyaluronate have been carried out following thorough mixing of the reagents.

In order to investigate this hypothesis, a study should be made on the effect of different concentrations of ascorbic acid solution on the reduction of aqueous outflow facility induced by low volumes of Healon, with and without thorough mixing with culture medium, in the perfused human eye model. The role and importance of heavy metal ions should also be studied in this preparation by adding different concentrations of these ions to the perfusion medium.

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APPENDIX

STATISTICAL ANALYSIS

The statistical analyses of all studies were carried out with Minitab statistical software for IBM PC.

Statistical analysis included: the two-tailed paired t- test, the two-tailed unpaired t-test, the chi-squared test, the Mann-Whitney U test, a simple regression analysis and a two-way analysis of variance.

P-value <0.05 was regarded as statistically significant.