THE ACTION OF DRUGS ON THE OUTFLOW OF AQUEOUS HUMOUR IN THE RABBIT

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SUMMARY

Although adrenaline has been reported to lower intraocular pressure (IOP) in both the conscious and anaesthetised rabbit the mechanism and adrenoceptor(s) involved in this hypotensive response are unclear.

In the conscious rabbit topically applied adrenaline produced a biphasic pressure response, an initial hypertensive response followed by the expected but longer lasting hypotensive response. Adrenaline also produced mydriasis, an alpha adrenoceptor mediated response.

The mechanism whereby adrenaline lowers IOP was investigated by measuring the facility of outflow of aqueous humour from the anterior chamber of the anaesthetised rabbit using a constant pressure anterior chamber perfusion technique. Topically applied adrenaline raised facility of outflow and lowered IOP. Adrenaline produced its expected mydriasis.

To date the majority of evidence suggest that adrenaline lowers IOP by acting via a beta adrenoceptor. Timolol was used as a pharmacological tool in the present experiments to investigate the involvement of the beta adrenoceptor in the outflow response to adrenaline. Timolol largely reversed the outflow response to adrenaline.

All of the afore mentioned results imply that adrenaline lowers IOP by raising facility of outflow, an effect mediated via a beta adrenoceptor since it was reversed by timolol.

Indomethacin, an inhibitor of prostaglandin (PG), biosynthesis can antagonise the ocular hypotensive response to chronic adrenaline treatment in the conscious rabbit. In the present experiments topical indomethacin pretreatment markedly antagonised the rise in facility of outflow and resultant fall

in IOP caused by acute topical adrenaline treatment in the anaesthetised and conscious rabbit. This result suggested that following the interaction with beta adrenoceptors, PGs were involved at some stage in the outflow and pressure responses to adrenaline.

Since indomethacin has known calcium antagonistic properties, the effects of aspirin and piroxicam, whose subsidiary properties differ from those of indomethacin, on the outflow and pressure responses to adrenaline were investigated.

Systemic aspirin pretreatment had no affect on the ocular responses to adrenaline or their antagonism by indomethacin in the anaesthetised rabbit. The lack of an inhibitory effect by aspirin, be it through inhibition of PG. biosynthesis, or calcium antagonism, was perhaps explained by the comparitively low plasma levels of salicylic acid. Alternatively, the ability of aspirin to inhibit ocular biosynthesis may differ from that in other organs.

Piroxicam is a potent inhibitor of PG biosynthesis at concentrations which have no effect on calcium fluxes. Topical piroxicam pretreatment antagonised the adrenaline-induced rise in facility of outflow and fall in IOP in the anaesthetised rabbit. This result lends weight to the theory that indomethacin antagonises the outflow response to adrenaline by blocking PG synthesis and not by inhibiting calcium movements. Nonetheless the effects of verapamil, a calcium channel blocker, on the pressure responses to adrenaline were investigated. Although topical verapamil pretreatment partially inhibited the hypertensive response (which may be the result of extraocular muscle contraction) to adrenaline the hypotensive response was unaltered. Again this result strengthens the hypothesis that adrenaline interacts on the beta adrenoceptor which at some

stage results in the production of PGs which in turn could be responsible for the rise in facilty of outflow and consequent fall in IOP. This is also consistent with other workers' demonstration that low concentrations of PGs can both raise facility of outflow and lower IOP.

Measuring PG concentrations in the aqueous humour was a more direct way of investigating the possible involevement of PGs in the ocular response to adrenaline. In the present experiments PG concentrations in the aqueous humour were measured using radioimmunoussay. Unfortunately this assay could neither accurately measure the very low concentrations of PGs present in aqueous humour samples nor distinguish differences in PG concentrations before and after drug treatment.

Beta adrenoceptor stimulation usually results in the production of cAMP. cAMP can both raise facility of outflow and lower IOP in the rabbit and many workers believe that it is this cAMP which is responsible for the ocular responses to adrenaline. Since indomethacin was reported to antagonise the outflow response to intracamerally injected cAMP one might postulate that cAMP triggers PG synthesis. Therefore in these experiments the effect of piroxicam and indomethacin pretreatment on the ocular reponses to intracamerally injected cAMP were investigated. Control experiments were also carried out. However the technical problems associated with the prolonged presence of 2 perfusion needles and/or the injection of substances into the anterior chamber precluded any conclusions being drawn from these experiments.

The relevance of this work to the human eye has recently been confirmed by a report showing that systemic indomethacin reduces the hypotensive response to chronic adrenaline treatment in glaucoma patients.

INTRODUCTION

The Anatomy Of The Formation And Drainage Of Aqueous Humour.

Aqueous humour (AH) is a colourless fluid present in both the posterior and anterior chambers of the eye. The main functions of AH are two fold, firstly for the supply of nutrients to and the removal of waste products from the avascular portions of the eye, namely the lens and the cornea. Secondly, it serves a hydromechanical function providing stability to ocular dimensions and the internal structures associated with performance of visual function.

AH is formed continuously in the posterior chamber by the ciliary processes. The newly formed AH flows from the posterior chamber into the anterior chamber via the pupil. It then flows from the anterior chamber into the outflow system. In rabbits the outflow of AH occurs primarily at the iridocorneal angle where it flows through the trabecular meshwork into the angular aqueous plexus and finally into the venous system.

The formation of aqueous humour.

AH is formed by the ciliary processes in the anterior chamber. A partial filtrate of plasma forms in the stroma of the ciliary processes from the capillaries which supply it. This constitutes the fluid from which the ciliary processes form AH by two main processes, secretion and ultrafiltration, of which secretion is the most important .

Secretion.

The ciliary processes have an epithelium which consists of a double layer of cells, a pigmented and a non-pigmented epithelium. The inner layer, the non-pigmented epithelium (NPE), lies next to the AH while the outer heavily pigmented epithelium (PE) lies closer to the stroma.

Cole (1977) has postulated that the sodium ion is absorbed from the ciliary stroma by the NPE and then transported into the intracellular clefts of the ciliary epithelium. This influx of sodium into the anterior chamber is inhibited by a variety of compounds including ouabain and dinitrophenol, indicating the existence of a transport system which actively transports sodium across the ciliary epithelium.

The inhibition of sodium transport by the cardiac glycosides is related to the inhibition of a sodium-potassium ATPase. This enzyme has been localised mainly at the cell surface and the multiple infoldings which bound the intercellular channels of the NPE.

The active transport of solute into the lateral intercellular spaces between epithelial cells makes the channel fluid hypertonic. Water therefore enters the intracellular spaces and in the steady state a standing osmotic gradient is created. Since the intracellular clefts of the ciliary epithelium are closed at the stromal side by tight junctions, but are open at the posterior chamber side, fluid therefore tends to flow towards the posterior chamber.

The active transport of sodium into the intracellular clefts creates an electrochemical gradient which favours the movement of anions, in this case chloride ions, in the same direction as the sodium ions.

Since treatment with cardiac glycosides or dinitrophenol reduces sodium influx by only 50% this implies that sodium is transported across the ciliary epithelium by some other means. Some movement of the sodium ions could be linked with the active transport of anions such as chloride or bicarbonate ions.

Holland and Gibson (1970) have shown that part of the net flux of chloride ions into the intracellular clefts can be attributed to the active transport of this ion. The active transport of the chloride ion could explain why the ciliary epithelial surface is electronegative relative to the stromal surface. The in vitro active chloride transport in cat ciliary processes also correlated well with the chemically demonstrated excess of chloride in the AH (Holland and Stockwell, 1967; Holland and Gibson, 1970). Sodium ions may therefore move passively down an electrochemical gradient as a result of active chloride transport.

In 1973, Maren showed that acetazolamide lowered sodium and bicarbonate concentrations in the posterior chamber of the dog. Acetazolamide also lowers intraocular pressure and reduces the rate of production of AH (Becker and Constant, 1955; Garg and Oppel, 1970). In view of acetazolamide's well known inhibition of carbonic anhydrase, it is reasonable to suppose that bicarbonate is actively transported from the ciliary epithelium and partly coupled to this is the influx of sodium into the posterior chamber.

Ultrafiltration

Ultrafiltration is a pressure-dependent process which relies on the presence of "leaky" junctions, i.e. one which has a relatively high passive permeability to ions. Values for resistance transport potential and osmolarity ratio of the ciliary processes place the junctions between the apices of the PE and NPE within the catergory of leaky junctions (Cole, 1966, Pederson and Green, 1973).

The balance of hydrostatic, oncotic and osmotic pressures across the apical complex of the NPE determine the relative proportion of ultrafiltration and secretion.

Some workers believe that ultrafiltration plays a major role in the formation of AH (Green and Pederson 1972, Weinbaum, 1972; Pederson and Green 1973); on the other hand Bill (1975) has shown that hydrostatic pressure and oncotic pressure differences across the ciliary processes do not support the likelihood of ultrafiltration normally occurring.

The chemical composition of AH

The composition of the AH has been investigated by Kinsey and Reddy (1964) and Bito (1977). In general its composition resembles that of extracellular fluid of peripheral tissues, but with differences between freshly secreted AH and AH in the anterior chamber.

Junctions between the NPE cells are tight enough to prevent the passage of macromolecular markers. Peroxidase can enter the ciliary stroma following intravenous injection and penetrate between the PE cells, but it then accumulates at the apices of the NPE layer (Cole, 1977). These cells therefore constitute the "blood aqueous barrier" which is responsible for greatly limiting the amount of plasma protein which can accompany fluid secreted into the posterior chamber. Therefore, the greatest difference between AH and blood plasma lies in the protein concentration which are 0.02 - 0.06% and 6-7% respectively.

The sodium and potassium contents of the anterior chamber AH are essentially the same as those of the plasma. The chloride ion content of the posterior chamber AH is lower than that of plasma but rises somewhat as the fluid passes through the anterior chamber. This chloride deficiency is balanced by an excess of bicarbonate ions present in the anterior and posterior AH as compared to levels of bicarbonate in the plasma.

The ascorbic acid concentration of both anterior and posterior chamber AH is many times higher than plasma levels. In contrast, as a result of glucose utilization by the avascular cornea and lens, glucose levels are 10 to 20% lower than in plasma levels.

Unlike most species, rabbit AH has higher concentrations of amino acids compared to plasma.

The pH of both anterior and posterior AH is at least 0.2 units greater than that of plasma.

The drainage of AH

In primates and rabbits AH drains from the eye primarily through the "conventional" passageway although some may leave the eye through other less important pathways (refer to Figs A and B throughout this section).

Conventional Outflow route

In rabbits the conventional drainage pathway consists of the trabecular meshwork, the angular aqueous plexus and the intra- and episcleral plexus of collecting channels, including the aqueous veins. Although the rabbit differs from primates in certain aspects of the anatomy and morphology of the anterior chamber angle, namely the angular aqueous plexus (as opposed to its equivalent in primates, the Canal of Schlemm), an insubstantial ciliary muscle and the absence of a scleral spur, the physiological similarities have often been stressed.

The poorly developed ciliary muscle in the rabbit results in a posterior extension of the anterior chamber, as well as the major bulk of the ciliary body being loose connective tissue which consists of several layers of adventitial cells, occasional smooth muscle cells, melanocytes, scattered collagen fibrils, clumps of elastin-like material and other extracellular components. In the rabbit the patency of the ciliary cleft and drainage angle cannot be maintained by the ciliary muscle, but is

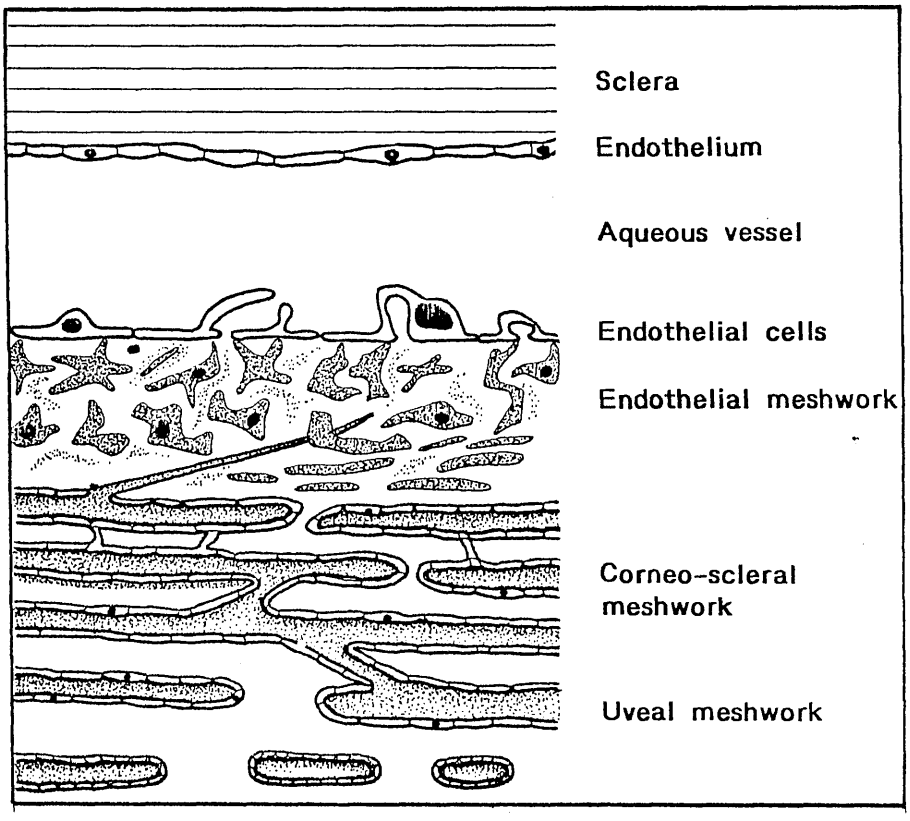

Fig. A Diagrammatic section through the trabecular meshwork of the rabbit (modified from Bill, 1975)

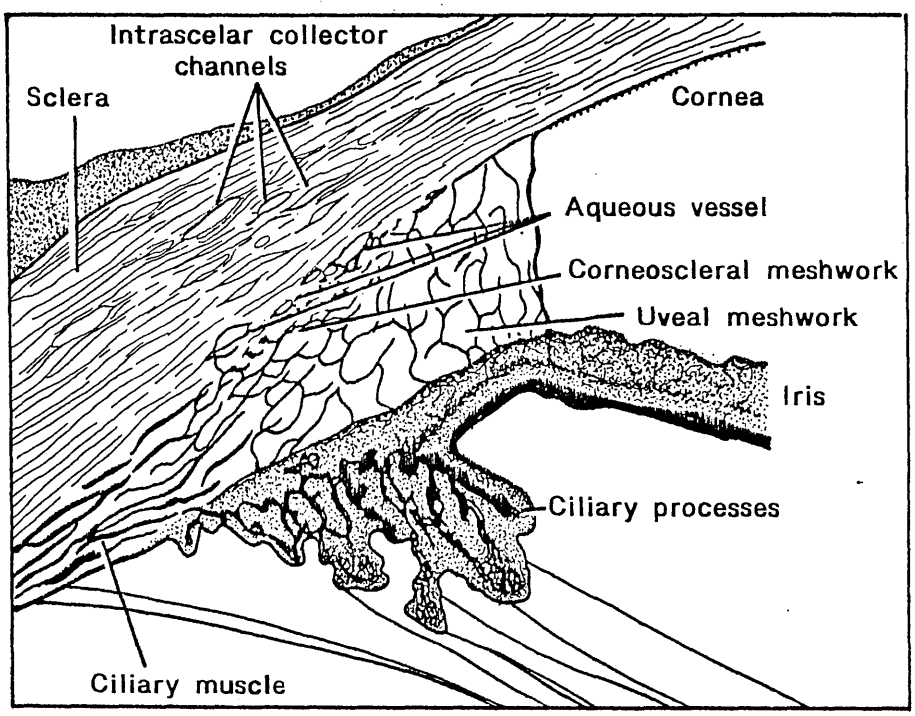

Fig. B Diagram of the aqueous pathway in the rabbit

instead supported by several connective tissue strands, now commonly referred to as the iris processes. These processes originate from the iris and proceed anteriorly towards the cornea where they merge with the corneal endothelium and its membrane (Descemet's membrane).

The spaces delineated by these processes are called the spaces of Fontana (Tripathi, 1971, 1974) and serve as passage ways for the flow of AH into the depth of the ciliary cleft.

Having entered the ciliary cleft the AH then flows through the trabecular meshwork. In general the trabecular meshwork consists of a series of endothelium-covered collagenous cords and fibrocellular perforated sheets (trabeculae). The endothelial lining of the trabeculae is a continuation of the corneal endothelium and Descemet's layer. The trabeculae are rarely fully encased by this endothelial layer (Grierson, 1976).

In rabbits, the cilary cleft is shallower but more highly developed than in primates (Prince, 1964). Although these differences do occur, the rabbit has layers of trabeculae with connections corresponding directly with a uveal and corneoscleral connection in addition to an endothelial meshwork close to the angular aqueous plexus.

Uveal meshwork.

This lies closest to the chamber angle and has a distinctive outer and inner layer.

The outer trabeculae are orientated circumferentially, parallel to the surface of the cornea at the limbus and are flattened perforated sheets.

The inner layers are cord like and are orientated radially in a net-like fashion enclosing large oval spaces. This layer therefore offers little resistance to the outflow of AH.

Corneoscleral meshwork.

This adjoins the uveal meshwork and is morphologically very similar to the outer uveal trabeculae, but has narrower inter - trabecular spaces. These are also orientated circumferentially. In rabbits there are fewer layers of corneoscleral trabeculae than in primates. (Tripathi, 1974).

Endothelial meshwork.

Close to each aqueous vessel of the angular aqueous plexus is a narrow zone where endothelial cells form a network. This is termed the endothelial meshwork. It lacks trabecular cores and the endothelial cells are surrounded by amorphous and fibrillar material which is probably dispersed in a mucopolysaccharide matrix.

The trabecular meshwork as a whole, therefore forms a labyrinth of intercommunicating intra- and inter - trabecular spaces of variable sizes and shapes and this may

be likened to a coarse filter whose porosity is probably influenced by biochemical agents acting directly on the meshwork or indirectly through the ciliary muscle (and, therefore, to a lesser extent in rabbits).

Prior to its entry into the angular aqueous plexus, insoluble material may be removed from the AH not only by the sieve-like action of the meshwork but also by its endothelial cells which can phagocytose and degrade debris which enters (Tripathi 1971, and Tripathi, 1972 and Tripathi Tripathi, 1977).

The majority of resistance to flow of AH is believed to reside in the inner trabecular meshwork and/or the inner walls of the angular aqueous plexus.

This resistance is believed to be a result of a mucinous coating, probably present on the surface of the endothelial cells of trabeculae and angular aqueous plexus (Tripathi, 1971, Tripathi, and Tripathi, 1972 and Tripathi, 1977) and perhaps the basement membrane material of the trabecular meshwork. (Armaly and Wang, 1975; Grierson and Lee, 1975a; Tripathi, 1974). This mucin coating probably consists of glycosaminoglycans (Mizokami, 1977, Schachtschabel, 1977). The origin of this coating is unclear, but it may be produced by the endothelial cells (Berggren and Vrabec, 1957). The heaviest concentrations of mucopolysaccharides are found in the extracellular spaces of the endothelial meshwork in the rabbit (Grierson, 1976).

Due to its physical presence or to its charge, such a coating may reduce the effective open area for aqueous humour percolation, thereby offering resistance to flow.

In rabbits AH drains from the trabecular meshwork into a series of drainage channels, called the angular aqueous

plexus (AAP) (Tripathi, 1971,1974). These are equivalent to the canal of Schlemm in primates.

The vessels of the AAP have a continuous endothelial lining whose ultrastructural characteristics are similar to those in the inner wall endothelium lining of the canal of Schlemm. The channels themselves have a morphology similar to Schlemm's canal.

A few reports have described the presence of smooth muscle cells in the structural elements associated with the AAP in rabbits. (Knepper et al., 1975, Sakimoto, 1979). They were most frequently observed in the corneoscleral tissue neighbouring trabecular meshwork and less frequently in the limbal region of the cornea and in the sclera external to the ciliary muscle cells. The cells were usually located on one side of the channel, but occasionally they completely surrounded the channel. Sakimoto believes that contraction of these smooth muscle cells may regulate AH outflow in the rabbit eye.

The means by which AH enters the AAP from the trabecular meshwork is a subject of great discussion. The view held by many workers is that transcellular channel formation is the main process involved (Tripathi, 1971b; Inomata, Bill and Smelser, 1972, Grierson, 1976). In this process indentations of the basal aspect of Schlemm's canal from the trabecular meshwork side results in the formation of a vacuole. This vacuole gradually enlarges and eventually opens on the apical aspect of the cell to form a transcellular channel. After a certain time interval the basal infolding is occluded by cell cytoplasm and the cell returns to its non-vacuolated stage. The initial

indentation of the cell may result from the existence of a pressure gradient across the AAP. Using morphological analysis the number of trancellular channels at any given time is low when compared with the number of cells with basal or apical openings alone.

This illustrates the temporary nature of these transcellular channnels. Tripathi (1971 and 1974) believes that the formation of a transcellular channel is a "cyclical event that constitutes a dynamic system of pores." The flow of AH through these channels is easily accounted for even if it is assumed that a transcellular channel exists for even 1/50th of its total life cycle (Cole and Tripathi, 1971). Since at any given time only a small number of channels may exist this offers another explanation for the resistance to flow in this area of the outflow system. These channels often take tortuous courses through a cell and AH flows down a pressure gradient through these channels into the lumen of the AAP. There is probably no retrograde flow of AH through these channels since the apical openings are far smaller than and not directly opposite the basal openings and because of a possible contractile property as indicated by the presence of cellular cytoplasmic filaments.

Other possible drainage mechanisms include intercellular drainage and micropinocytosis.

Intercellular drainage

The Intercellular drainage involves the passage of AH across the poorly developed tight and/or gap junctions which exist in cellular linings of the aqueous plexus. These

junctional complexes present a barrier to the flow of molecules greater than 10nm.

Although some workers believe that this is the major outflow route for aqueous humour (Shabo et al., 1973) considering the number and size of these intercellular pathways this route would appear to account for only a very small percentage of the total drainage of aqueous humour (Bill, 1975).

Micropinocytosis

Micropinocytosis is an oxygen - dependent energy - requiring process. It involves the uptake of AH into small vesicles which are transported across the cells of the inner wall endothelium. Again, views on the importance of this process in AH outflow are contradictory although the concensus of opinion is that it does not play a major role in the bulk outflow of AH at normal intraocular pressures (Fine, 1964;Feeney and Wissag, 1976; Grierson and Lee, 1975)

Non-conventional drainage routes.

Uveoscleral pathway.

In this pathway AH flows from the chamber angle into the spaces between the bundles of cilary muscle, which in turn open into the suprachoroidal and supraciliary space. From here, fluid can pass across the sclera or along the perivascular spaces of the large blood vessels penetrating the sclera. This pathway appears to be independent of IOP changes.

According to Bill (1966), the proportion of AH leaving via the uveoscleral route in rabbits is less than 2.8% compared with 35% in monkey. On morphological grounds this is expected due to the poorly developed ciliary muscle in the rabbit. However, Tripathi and Cole (1976), using blue dextran and fluorescein - dextran to trace the drainage routes, suggest that as much as 25% of the drainage of AH in the rabbit is via the uveoscleral route.

Accessory drainage routes of lesser significance include diffusion across iris vessels, posterior drainage,and trans-corneal flux (Tripathi, 1977).

Exit channels

AH is normally drained from the lumen of the AAP by the scleral (deep and intracleral), episcleral and sub - conjunctival venous plexuses.

From the AAP, AH drains into several collecting channels which are in turn connected to the deep scleral venousplexus (DSVP) which drains into the anterior ciliary veins. The DSVP is also connected to the intrascleral venous plexus (IVP). The IVP drains into the episcleral and subconjunctival venous plexuses.

In 1942, Ascher demonstrated colourless superficial vessels of the globe which contained AH, which were termed "aqueous veins".

In both rabbits and primates a variable proportion of aqueous passes forward to reach the episcleral and subconjunctival veins directly through the aqueous veins (Gazala et al., 1965). In rabbits these aqueous veins sometimes contain blood mixed with aqueous.

Innervation of the tissues in the iridocorneal angle.

Many studies have been undertaken to demonstrate the structure and function of nerve in the angular region of vertebrates. Both sensory and autonomic nerve endings have been demonstrated with species variations. The rabbit has a sparse innervation of the irido-corneal angle (Ehinger 1966).

Ciliary body and ciliary processes.

In the rabbit eye, the ciliary processes have a delicate but rich innervation of both adrenergic and cholinergic fibres. The bulk of the ciliary body receives a sparse innervation of adrenergic fibres, although a band of adrenergic fibres is present above the ciliary epithelium. In contrast, both the ciliary stroma and ciliary muscle are crowded with a dense innervation of fine cholinergic fibres. Evidence for the presence of both adrenergic and cholinergic fibres in the trabecular meshwork is contradictory.

Using the histofluorometric technique of Falck (1962) to identify adrenergic nerve terminals, and the localization of acetylcholinesterase to identify cholinergic innervation, Laties and Jacobowitz (1964), showed that the filtration area in rabbits received a moderate, but definite supply of adrenergic and cholinergic fibres. Autoradiographic studies of the uptake of catecholamines have shown a predominance of sympathetic nerve terminals in the irido-corneal and scleral-trabecular areas of rabbits (Bhattacherjee, 1972).

In rabbits, adrenergic fibres are seen scattered in the loose tissue of the trabecular region around the spaces of Fontana (Staflova, 1969).

In monkey, adrenergic and cholinergic nerve terminals in the trabecular meshwork are located mainly in the posterior part, just anterior to the insertion of the longitudinal ciliary muscle. About one third of these were adrenergic (Nomura and Smelser, 1974). Sensory nerve, endings as loops or free terminals have also been demonstrated in the trabecular meshwork.

In contrast Holland et al., (1957) concluded that many of the trabecular axons were in fact parasympathetic in nature and only a small number were derived from the sensory trigeminal and sympathetic systems. Gwin, Gelatt and Chiou (1979) found little evidence for cholinergic axons in the meshwork.

Sensory nerves, ending as loops or free terminals have also been demonstrated in the trabecular meshwork (Laties and Jacobowitz, 1964).

In opthalmology the medical treatment of glaucoma is a subject of principle concern. Glaucoma is a disorder of the eye in which intraocular pressure (IOP) is abnormally high and if this increased IOP remains unchecked it leads to loss of visual acuity and eventual blindness. Although the therapeutic value of many anti-glaucomic drugs has been established the mechanisms whereby these drugs are effective in controlling IOP still remain uncertain. Adrenergic agonists and antagonists are effective in lowering IOP and are used to treat glaucoma.

Adrenaline is used widely in the treatment of glaucoma. The effects of adrenaline on aqueous humour dynamics and IOP have been studied mainly in rabbits. Although the rabbit differs from primates in certain aspects of the anatomy and morphology of the anterior chamber angle the physiological similarities have often been stressed. (Tripathi, 1971; Grierson, 1976). The current state of knowledge on the mechanism of adrenaline on the rabbit eye with reference mainly to primates will therefore be discussed.

It is now well established that the adrenergic responses are mediated by two major types of receptors, the so called alpha and beta receptors (Alqvist, 1948). The subdivision of the beta receptors into the beta-1 and beta-2 subtypes was proposed by Lands et al in 1967. The beta-1 subtype is involved in stimulation of the heart, whereas vasodilation and bronchodilation are mediated by beta-2 receptors.

In the past decade alpha receptors have been differentiated into alpha-1 and alpha-2 receptors on the basis of the selectivity of various agonists and antagonists for these receptors. Alpha-1 receptors usually have a presynaptic location whereas alpha-2 receptors have a postsynaptic location (Langer, 1974). In general, alpha receptors respond to catecholamines in the ascending potency order of phenylephrine, noradrenaline (NA) and isoprenaline, whereas beta receptors respond in the reverse order. NA is released from postganglionic sympathetic nerves whereas adrenaline is secreted from the adrenal medulla. Although the iris-ciliary body and the angular aqueous plexus receive a sparse innervation of adrenergic fibres, studies using mainly radioligand binding techniques have shown that these structures contain adrenergic receptors. In rabbit, the ciliary processes contains mainly beta receptors (Neufeld and Page, 1977; Neufeld et al., 1978; Page and Neufeld, 1978; Lahav et al., 1978; Dafna et al., 1979; Bromberg et al., 1980; Tsukahara et al., 1980), whereas the ciliary muscle contains mainly alpha receptors (van Alphen et al., 1965; Kern, 1970). By interacting with these receptors NA released from nerve terminals, circulating NA and adrenaline and exogenously applied NA and adrenaline have the opportunity to alter aqueous humour dynamics by effects on aqueous humour inflow and/or outflow.

The mechanisms underlying the hypotensive effects of adrenergic agonists and antagonists have been the interest of many investigators.

Many experiments have been conducted in rabbits, some in monkeys, a few in cats and other species.

Changes in IOP are the final result of many tissues interacting in a complex manner. The diversity of the adrenoceptors found in ocular tissues (Holland et al., 1957; Laties and Jacobowitz, 1964; Ehinger, 1966; Stafl~w, 1969 and Nomura and Smelser, 1974), the lack of specificity of the various adrenergic agonists and antagonists which lower IOP (Rowland and Potter 1980) and the differences in the ocular structures (Tripathi, 1971, 1974) and the responses to these drugs in different species make it difficult to describe precisely how adrenaline may bring about changes in IOP.

The Effect Of Adrenaline On IOP In The Rabbit.

Early evidence demonstrated that electrical stimulation of the cervical sympathetic nerves could produce ocular hypotension (Wesley, 1908). Subsequently, it was shown that acute or chronic administration of adrenaline to the rabbit eye lowered IOP. This response to adrenaline may or may not be preceded by a short-lived rise in IOP. The hypertensive phase occurs half-an-hour to one hour after the application of adrenaline and may last at the most for forty five minutes. The hypotensive response is not apparent usually until at least two hours following adrenaline application and is maximal after four to five hours.

Adrenaline stimulates both alpha and beta receptors. In an attempt to elucidate the receptors involved in the IOP responses to adrenaline, many studies have examined the ocular responses to selective alpha and beta agonists, as well as the IOP responses to adrenaline in the presence of selective alpha and beta receptor antagonists.

The effects of adrenaline on AH production, its drainage from the eye and on episcleral venous pressure have been the basis of experimental studies which have attempted to identify the cause of the pressure responses to adrenaline in several species. Changes in at least one of these parameters are likely to be responsible for the IOP responses to adrenaline.

The hypertensive response to adrenaline.

Depending on the dose, frequency of application, and the route of administration, adrenaline may produce a transient rise in IOP. Such a response has been reported in rabbit (Norton and Vierstein, 1972; Langham and Krieglestein, 1976; Lamble, 1977; Rowland and Potter, 1981), monkey (Bill, 1969) and in man (Lea, 1958; Kronfield 1971; Wilkie, 1974).

This rise in IOP appears to be a dose-related phenomenon. Low doses of adrenaline (0.5 to 2%) decrease IOP whereas higher doses (4 to 10%) increase and then decrease IOP. (Norton and Vierstein, 1979). In contrast, concentrations as small as 0.1 to 1% can cause a minor transient rise in IOP (Innemee and van Zwieten,1982).

The hypertensive phase can be observed with initial application (acute) (Lamble, 1977; Rowland and Potter, 1981; Potter et al., 1982, 83) or it can be manifested only on repeated exposure on consecutive days (chronic) (Unger, 1979; Langham and Krieglestein, 1981).

Norton and Vierstein (1979) reported that this hypertensive phase was a function of the administration

site. After two hours, topically applied adrenaline produced a transient elevation of IOP in 75% of the rabbits treated. No such rise was found in animals treated with adrenaline via the intravitreal or retrobulbar route.

Both alpha and beta receptors appear to be involved in the hypertensive reponse to adrenaline. When applied topically to the rabbit eye, chronic administration of low doses of adrenaline produced a hypertensive response which was partially blocked by the beta antagonists propranolol and timolol (Langham and Krieglestein, 1976) and the alpha antagonist phenoxybenzamine (Langham and Krieglestein, 1976). The hypertensive response to single doses of high concentrations of adrenaline (4,8, and 10% solutions) were similarly blocked by propranolol (Norton, 1972; Lamble, 1974). However, Langham and Krieglestein, (1976) have shown that intravenous or topical application of propranolol did not alter the hypertensive response to chronic adrenaline treatment. Concurrent topical administration of the alpha antagonists phenoxybenzamine and thymoxamine with adrenaline on the third day of treatment abolished the hypertensive response but not the hypotensive response. Pupil diameter responses remained unaltered. The effect of intravenous phenoxybenzamine on the hypertensive response to adrenaline was difficult to interpret since phenoxybenzamine lowered IOP on its own. Rowland and Potter, (1978) demonstrated a positive association between alpha receptor stimulation and the manifestation of a transient rise in IOP following single dose applications of adrenergic drugs.

Selective alpha and beta receptor agonists also produce an initial rise in IOP. Phenylephrine and St. 587 (a

selective alpha agonist), when topically administered to the rabbit eye, caused an increase in IOP with a maximal effect within one hour (Inneme et al., 1982). In contrast BHT 920, a selective alpha - 2 agonist, produced an immediate fall in IOP. These results indicate that the alpha - 1 subtype is involved in ocular hypertension.

Chronic treatment with reproterol, a relatively selective beta - 2 agonist, increased IOP, an effect antagonised by timolol pretreatment (Potter et al., 1982, 1983).

There is a temporal difference in the hypertensive response to acute as compared to chronic administration of adrenergic agonists. The ocular hypertensive response to a single dose of alpha adrenergic stimulants, for example, dopamine, NA, adrenaline or phenylephrine, peaked more rapidly (within half-an-hour after administration) and was short-lived. In contrast, the response to repeated consecutive doses of beta adrenergic agonists, for example l-adrenaline, isoprenaline and reproterol, tended to peak more slowly (one to two hours after administration), were longer lasting and were usually much greater in the magnitude of the change. In functional terms the early ocular hypertensive phase caused by adrenaline may be attributed to:

1) an increase in intra-and episcleral venous pressure,
2) an increase in aqueous humour production,
3) enhancement of extraocular muscle tone,
4) the generation of prostaglandins.

The effect of adrenaline on episcleral venous pressure.

AH leaves the anterior chamber mainly by bulk flow through the trabecular meshwork and eventually flows via the intrascleral plexus into the episceral veins. The intrascleral plexus has vessels containing blood and/or aqueous humour. Convergence of these vessels at the episcleral veins, therefore, provides several loci where vasomotor responses could conceivably facilitate or restrain flow. Langham and Krieglstein (1976) have suggested that interaction with alpha receptors on these veins could result in vasoconstriction, which would increase the resistance to flow and therefore increase IOP. Although in rabbits there is no reported evidence of a rise in episcleral venous pressure soon after the application of adrenaline, the application of alpha agonists induces intense blanching of both the conjuntival and episcleral vessels. Under these conditions the rate of blood flow is seen to decrease (Langham and Krieglstein, 1976). In the rabbit eye, a small hypertensive response to repeated daily application of NA was associated with an increase in outflow resistance (Langham and Palewicz, 1977). In man, episcleral venous pressure has been measured following adrenaline treatment (Wilke, 1974). Such measurements were made on recipient veins i.e. an episcleral vein which had just received a large aqueous vein. In these experiments topical application of a 1% solution of adrenaline produced a transient rise in IOP which was maximal within 15 minutes. Adrenaline produced an obvious vasoconstriction of conjuntival blood vessels and a rise in episcleral venous

pressure almost immediately after its administration. These workers therefore suggested that the rise in IOP may have been secondary to a rise in episcleral venous pressure.

Similarly in the monkey adrenaline and isoprenaline tended to increase recipient venous pressure, although there was also a rise in total facility of outflow (Bill, 1970). Other investigations in the human eye found no changes in episceral venous pressure following the topical application of adrenaline or isoprenaline (Kupfer et al., 1971; Gaasterland et al., 1973).

The effect of adrenaline on AH production.

Since beta-2 receptors are present in the ciliary processes and are proposed to be involved in AH formation (Nathanson, 1980) it is possible that adrenaline may well increase AH production by stimulation of these receptors (Potter et al., 1981). Several workers have reported decreases in AH formation following adrenaline treatment (Masuda, 1971; Green and Padget, 1979; Miichi and Nagataki, 1982). Although this adrenaline-induced decrease in AH inflow would tend to decrease IOP, these measurements were taken several hours after adrenaline treatment which coincides with the hypo- and not the hypertensive response to adrenaline. Therefore, to determine the role of AH production in the hypertensive response to adrenaline, AH formation should be measured soon after adrenaline administration. Lamble (1977) has investigated the acute effects of adrenaline on AH formation. Using tritiated water as a model of a rapidly diffusing molecular species,

the effects of adrenaline on its transfer from the blood to the aqueous was investigated. Following intravenous injection of triated water, topically applied adrenaline caused a concentration-related decrease in tritium penetration into the anterior chamber at thirty and sixty minutes after its administration. No effect was seen two to three hours later. The clearance of radiolabelled inulin which had been injected into the anterior chamber was also investigated. One hour after adrenaline treatment there was a significantly higher level of radioactivity in the AH, indicating decreased clearance. Again these results suggest that adrenaline decreases AH formation. It therefore seems that adrenaline's hypertensive response is not a result of increased AH production. However, it is interesting to note that approximately one hour following adrenaline treatment, Lamble (1977) reported a rise in facility of outflow but no fall in IOP and suggested that this was a result of an adrenaline-induced rise in inflow. In support of this theory Cole and Nagasubramanian (1973) have shown, in vitro, that NA and isoprenaline increased the ciliary transepithelial short circuit current.

The effect of adrenaline on extraocular muscles.

In 1976, Allen and Langham found an initial rise and then fall in IOP after the local administration of clonidine (an alpha agonist). Subsequently it was found that this rise could be blocked by phenoxybenzamine or muscle relaxants. Innemee and van Zwieten (1978), therefore proposed that this rise in IOP involved alpha receptors and was a result of the contraction of extraocular muscles.

Earlier experiments showed that systemic adrenaline increased the tension in extraocular muscles in the cat eye (Eakins and Katz, 1968). In an attempt to determine whether or not the hypertensive phase to adrenaline involved extraocular muscles, Rowland and Potter (1980) surgically transected the three major extraocular muscles in the rabbit eye. In control animals a single dose of 2% adrenaline applied topically evoked a biphasic response which was significantly hypertensive after half to one hour. In contrast, the hypertensive responses to chronically administered reproterol and adrenaline were not abolished in rabbits with transected extraocular muscles (Potter, Nicholson and Rowland 1982, 1983). However, in conjunction with these experiments a separate series of control experiments was carried out in which adrenaline failed to raise IOP. This suggests that the hypertensive response to acute adrenaline may be inconsistent as was implied by the observation by Boas et al., (1981) that only 10 to 15% of rabbits tested elicited an initial hypertensive response to 2% adrenaline.

The involvement of prostaglandins in the hypertensive response to adrenaline.

Destruction, either surgically or chemically of the cervical sympathetic nerves in rabbits was found to enhance the ocular hypertensive response to topically applied NA or phenylephrine (Unger, 1979; Waitzman et al., 1979).

This enhanced response was blocked by indomethacin or phentolamine pretreatment. These results suggest that prostaglandins released under such circumstances are closely linked to alpha receptor stimulation. However, the ocular hypertensive response to chronic administration of NA, phenylephrine and isoprenaline remained unaltered following pretreatment with inhibitors of prostaglandin biosynthesis. (Unger, 1979; Potter et al., 1982, 1983) suggesting that the generation of prostaglandins was probably not involved in raising IOP.

The Hypotensive Response To Adrenaline

In the rabbit eye, adrenaline lowers IOP in a dose-dependent fashion (Norton and Vierstein, 1972). This hypotensive response is independent of the administration site since topical application, intravitreal, retrobulbar or intravenous injection of adrenaline lowers IOP (Norton and Vierstein 1972; Langham et al., 1973). Either acute or chronic administration of adrenaline lowers IOP (Langham and Krieglstein, 1976), although there is conflicting evidence that this response may gradually decline during continuous use (Langham and Diggs, 1972; Bhattacherjee and Hammond, 1977).

Following the topical application of low doses of adrenaline (0.1 to 1%), the change in IOP follows a characteristic timecourse. In the absence of a hypertensive response there is no significant alteration for at least one hour, then IOP begins to fall and reaches a maximal response four to six hours after the administration of adrenaline (Lamble, 1974; Langham and Krieglestein, 1976; Lamble, 1977; Inneme and van Zwieten, 1982). This slow process is in marked contrast to the characteristic rapid fall in IOP produced by beta agonists. Selective beta agonists, for example, salbutamol, metaproterenol, BHT-920 and terbutaline, produced a significant pressure decrease within one hour with a maximal response attained within two to three hours (Langham and Diggs, 1974; Innemee et al., 1982).

Several experiments have suggested that the delay in the onset of the hypotensive response to adrenaline is due to the hypertensive response which temporarily negates the

hypotensive response (Lamble, 1977; Rowland and Potter, 1978). However, although the hypertensive response to adrenaline is blocked by propranolol, propranolol did not alter the time of onset of the hypotensive response (Lamble, 1974). Thymoxamine, an alpha antagonist, reduced the delay of the onset of this response, which suggests a role of alpha receptors in mediating this delay (Lamble, 1977). The ocular responses to adrenaline applied topically to primate and human eyes are qualitatively very similar to those in rabbits (Kitazawa and Langham, 1978; Langham et al.,1971).

Adrenoceptors Involved In The Hypotensive Response To Adrenaline.

Adrenaline stimulates both alpha and beta adrenoceptors. To date the majority of evidence suggests that the hypotensive response to adrenaline in the rabbit eye involves mainly beta adrenoceptors although an alpha adrenoceptor-mediated effect on inflow may also be involved. However, the relative importance of each of these subclasses remains uncertain. The availability of highly selective beta-1 and beta-2 receptor antagonists and relatively selective alpha receptor antagonists may allow a more profound analysis of this ocular hypotensive response.

The effects of selective and non- selective beta antagonists on the ocular responses to dipivalyladrenaline (DPA), a prodrug for adrenaline, were investigated by Innemee and van Zwieten (1982). The selective beta-1 antagonists, atenolol and metoprolol did not lower IOP and had no effect on the hypotensive response to DPA.

Although low concentrations of the non-selective beta antagonists (timolol) and selective beta-2 antagonists (IPS 339,ICI 118-551 and 32-468 hmo) lowered IOP they also antagonised the hypotensive response to DPA.

Although intravenous injection of propranolol (5mg kg^{-1}) did not alter IOP, it did reduce the hypotensive response to 0.5% adrenaline (Langham et al., 1973). Similarly, in the rabbit and monkey eye, pretreatment with propranolol antagonised the rapid fall in IOP induced by topically applied isoprenaline and salbutamol (Langham and Diggs, 1974). However, topical pretreatment with timolol

had no effect on the hypotensive response to adrenaline (Boas et al., 1982). In these experiments 2% adrenaline produced a fall in IOP as late as six hours following its administration with a maximal response between eight and twelve hours.

In 1968, Potter and Rowland concluded that relatively selective alpha receptor agonists, for example phenylephrine and NA were minimally effective in lowering IOP whereas drugs with more beta adrenoceptor stimulating activity, for example, adrenaline and isoprenaline were very active in lowering IOP. These authors, therefore, suggested that selective beta adrenoceptor stimulation is an effective mechanism for lowering IOP.

In earlier papers, Langham et al., (1972, 1973, 1976, 1977) concluded that alpha receptors were primarily involved in the ocular hypotensive response to adrenaline.
However, examination of these papers shows that such a conclusion cannot be drawn from the results presented. Topical, IV, or intravitreal administration of phenoxybenzamine, propranolol and thymoxamine lowered IOP and, therefore, the ability of these drugs to antagonise the acute hypotensive response to adrenaline could not be properly investigated. Similarly, intravenous injection of phenoxybenzamine failed to block the hypotensive response to NA (Langham and Paliewicz, 1977). In contrast, IV pretreatment with phenoxybenzamine one day prior to adrenaline treatment blocked the outflow response to intracamerally injected NA (Sears and Sherk, 1974).

Despite some conflict in the literature most observations are compatible with the theory that adrenaline lowers IOP by stimulating beta adrenoceptors.

The effect of adrenaline on AH formation.

It has been suggested that adrenaline lowers IOP at least in part by decreasing AH formation. A fall in AH production which precedes the adrenaline-induced fall in IOP has been reported (Lamble, 1977). Adrenaline may decrease inflow by acting on blood vessels supplying the ciliary processes.

Although there are no nerves within the ciliary epithelial layers, alpha receptors are probably present in the blood vessels supplying the stroma. Vasoconstriction of these vessels would decrease blood flow thereby limiting the plasma filtrate entering the stroma and AH production may therefore be decreased.

Sympathetic cervical stimulation causes intense uveal vasoconstriction, an increase in vascular resistance and a fall in uveal blood flow. These vessels also respond to injected NA but not to isoprenaline suggesting the presence of alpha but a lack of beta receptors. (See review by Sears, 1967).

In rabbit and monkey, stimulation of the sympathetic cervical chain, which releases NA from nerve terminals, produces a decrease in AH formation and a fall in IOP (Langham and Rosenthal, 1966; Bill, 1970).

The rate of AH formation can be determined experimentally using a variety of tracer dilution techniques. By perfusing the anterior chamber of the rabbit eye with fluorescein-dextran, and measuring its concentration in samples of AH taken from the anterior chamber, Miichi and Nagataki (1982) investigated the effects of several adrenergic agonists and antagonists on AH formation in the rabbit eye. Two hours after the topical application of adrenaline and NA into a conjunctival reservoir both AH formation and IOP were lowered. Topical adrenaline and NA also reduced AH formation as determined by volumetry after cannulation of the anterior chamber (Masuda, 1971). Green and Padgett (1979) determined the rate of AH formation by means of radioactive inulin dilution and similarly showed that two hours after the topical application of adrenaline and NA, AH formation was decreased although pseudofacility was increased. It should be noted that in all of these procedures experimental manipulation may well result in trauma of the eye which could ultimately alter the parameters measured. In 1977, Lamble also reported that adrenaline decreased inflow in the rabbit eye. However, by the time the hypotensive response to adrenaline was apparent, the observed decrease in inflow was already waning.

In the rabbit eye, neither salbutamol, a selective beta-2 agonist, nor timolol, a non-selective beta antagonist altered AH formation (Miichi and Nagataki, 1982).

The effects of relatively selective alpha and beta agonists on AH production have been investigated in the primate eye.

In the monkey eye, adrenaline had no effect on the rate of AH formation (Bill, 1969). This may be due to the antagonistic effects of adrenaline on AH formation, i.e., a direct stimulatory effect on the secretory mechanism could be masked by a reduction in AH formation resulting from a vasoconstriction of blood vessels supplying the stroma.

Bill (1970) determined the rate of AH formation in the vervet monkey and found that intracamerally perfused isoprenaline (1 $g.ml^{-1}$) increased the rate of AH formation. This response was antagonised by propranolol pretreatment. In these experiments NA had only a slight effect on inflow.

Miichi and Nagataki (1983) studied the effects of salbutamol and timolol on the rate of AH formation in the cynomolgus monkey. Although these drugs were applied into a conjunctival reservoir, anterior chamber concentrations were comparable to the concentrations infused by Bill (1970) into the vervet monkey eye. Salbutamol increased, whereas timolol decreased the inflow rate. Thus both these studies agree that beta adrenergic agonists tend to increase and beta adrenergic antagonists tend to decrease the rate of AH formation in the monkey eye.

In man, AH formation is usually determined by a fluorophotometric technique. Development of this technique has allowed a more direct measurement of AH inflow which had previously been calculated from manometrically determined facility of outflow values. Nevertheless, results from studies using this technique have been in disagreement. Adrenaline was reported to decrease AH inflow, one, ten and twenty four hours following its instillation (Weekers, 1954; Gaasterland et al, 1973; Nagataki, 1977). In contrast,

Townsend and Brubaker (1980) observed that a single dose of adrenaline increased AH production two to three hours following its administration. Similarly, Schenker et al., 1981) showed that adrenaline, when given for a week, produced an increase in AH production two to five hours after the last dose.

Following a detailed analysis of the reponses of ocular hypertensive patients to adrenaline, Kronfeld (1971) concluded that adrenaline failed to show consistent effects on AH inflow, either initially or during chronic treatment.

In the human eye, NA increases inflow and decreases IOP, whereas phenylephrine has no effect on either inflow or IOP (Gaasterland et al., 1973; Lee and Brubaker, 1982, 1983). The concensus of opinion suggests that alpha agonists have no appreciable effects on inflow. Isoprenaline, a non-selective beta agonist, and timolol, a non-selective beta antagonist decrease both inflow and IOP in man (Gaasterland et al., 1973; Zimmerman, 1977; Sontag et al., 1978; Boger, 1978; Coakes, 1978; Yablonski et al., 1978; Higgins et al., 1980; Thomas et al., 1981).

The effect of adrenaline on the facility of outflow of AH.

Adrenergic agents have pronounced effects on the facility of outflow of AH. Topical or intracameral injection of adrenaline increases the facility of outflow of AH from the rabbit eye (Eakins, 1963; Bill, 1969; Lamble, 1974; Lamble, 1977). Using pressure decay curves to calculate the facility of outflow, Eakins (1963) showed that in the rabbit eye intravitreal injections of adrenaline, NA,

but not isoprenaline increased the facility of outflow after their administration.

Lamble (1974) measured facility of outflow using a constant pressure perfusion technique. This measures total facility of outflow which is a combination of both true and pseudofacility. In these experiments, topically applied adrenaline increased the facility of outflow of AH one to six hours following its administration. A conspicuous feature of this study was the lack of a relationship between adrenaline's effects on facility and on IOP. Large facility effects were not accompanied by significant differences in tension. However, in these and subsequent experiments (Lamble, 1977) the effect of adrenaline on ocular tension was apparently reduced or reversed by the procedures of anaesthesia and cannulation performed to allow facility determination and may, therefore, explain this apparent disparity. Thus it was reasonable to conclude that the fall in IOP caused by adrenaline was related to the facility effect.

The resistance to the outflow of AH associated with the hypotensive response to NA has been analysed manometrically by a two-level constant pressure perfusion technique in the anaesthetised rabbit (Langham and Palewicz, 1977). Five hours following a single dose of NA, a significant decrease in IOP and outflow resistance was observed. In a separate series of experiments, facility was determined by measuring the steady-state increase in IOP caused by infusion of saline (Sears, 1960; Sears and Scherk,1964). Although NA

increased facility of outflow in both the innervated and denervated eye, the response was larger following unilateral superior cervical ganglionectomy. In these experiments, NA injected intracamerally was more effective in raising facility than NA injected subconjunctivally.

Using a manometric technique to measure facility of outflow in the monkey eye, topically applied adrenaline increased the gross facility of outflow one hour after its application (Bill, 1969). Since pseudofacility was unaffected by adrenaline, this rise in gross facility of outflow was attributed to the observed rise in pressure-dependent and independent outflow, that is through the canal of Schlemm and uveoscleral routes respectively. The lack of an effect on pseudofacility was probably due to the high arterial blood pressure found in these experiments.

Topical or intracameral injection of adrenaline produced a rise in true facility of outflow thirty to sixty minutes after its administration (Barany, 1968; Kaufman and Barany, 1981). Associated with this early rise in facility was an increase in pseudofacility (Barany, 1968).

Adrenergic agents which have a greater selectivity for either alpha or beta receptors can also alter facility of outflow.

Bill (1970) examined the effects of intracamerally injected isoprenaline and adrenaline on AH dynamics. Isoprenaline increased the gross facility of outflow, enhanced uveoscleral flow and increased AH inflow. These effects were blocked by pretreatment with propranolol. Although isoprenaline increased recipient venous pressure and therefore tended to increase IOP, these changes were small and statistically insignificant.

Stimulation of the cervical sympathetic nerves in monkey, which releases NA into the uvea, increased AH formation and decreased IOP but had no effect on the facility of outflow (Bill, 1970). However, exogenous NA had no effect on any of the afore-mentioned parameters. In contrast, Kaufman and Barany (1981) reported that NA injected into the anterior chamber of the monkey eye increased the gross facility of outflow.

In man the facility of outflow of AH is measured indirectly using tonography. Unlike constant pressure perfusion, tonography measures only pressure-dependent outflow while pressure-independent outflow through the uveoscleral route has to be calculated.

In humans, Sears (1966) proposed that topical adrenaline lowered IOP in three ways: (1) an early decrease in IOP related to a decrease in AH inflow, (2) an early rise in facility of outflow overlapping with the fall in inflow and (3) a progressive increase in outflow facility with chronic medication.

In 1971, Kronfeld further investigated the early effects of single and repeated doses of adrenaline in patients with ocular hypertension and primary open angle glaucoma. In these patients single doses of adrenaline did indeed produce a fall in IOP, approximately one hour after its administration, which was associated with a rise in facility of outflow. However, no consistent effect on outflow could be observed. These effects were most pronounced on the day of treatment, were still present on the post-treatment day and were returning to control values on the second post-treatment day. Chronic administration of adrenaline appeared to produce a steady state, where the responses were on the whole somewhat greater than those to single doses although no significant upward or downward trend was apparent.

Although in normal or hypertensive human eyes, acute or chronic administration of adrenaline increases tonographic facility of outflow this rise alone cannot explain the fall in IOP. In addition to this rise, adrenaline has been reported to increase uveoscleral outflow, and together these increases can account for the fall in IOP (Townsend and Brubaker, 1980; Schenker et al., 1981).

Salbutamol also increases both tonographic facility of outflow and uveoscleral flow in the normal human eye, although pressure-dependent flow was reduced (Coakes and Siah, 1984). Contrasting results from Gaasterland et al., (1973) demonstrated an adrenaline-induced fall in facility although this could be attributed to a reduction in pseudofaclity.

Structures Involved In the Adrenaline-Induced Rise In Facility Of Outflow.

The primary target structures responsible for the adrenaline-induced rise in facility are unknown. Candidates proposed include:

1) the anterior portion of the ciliary muscle
2) the iris
3) the trabecular meshwork (and/or the canal of Schlemm in primates)

Iris and ciliary muscle involvement

In primates the ciliary muscle inserts at the scleral spur and trabecular meshwork, so that ciliary muscle tone may markedly influence outflow facility. Adrenergic terminals found in primates, are for the most part, located just anterior to the insertion of the ciliary muscle. the adrenoceptors of the ciliary muscle of several monkey species are purely beta (Van Alphen *et al.*, 1965). Activation of these receptors tends to relax the ciliary muscle and to decrease outflow facility.

In rabbits, the possible involvement of ciliary muscle tone in modulating AH outflow is more tentative. In the rabbit the ciliary muscle is poorly developed and due to the absence of the scleral spur and Schlemm's canal, its insertion at the anterior chamber angle differs from that in monkeys. Nonetheless there is a forward prolongation of

ciliary muscle cells into the filtration angle (Sakimoto, 1979). Adrenergic innervation of this muscle is very difficult to find, although Staflova (1969) and Bhattacherjee (1972) have reported the presence of adrenergic fibres in the loose tissue of the trabecular region and the spaces of Fontana. Rabbit ciliary muscle contains mainly alpha receptors (Van Alphen et al., 1965). Contraction of the ciliary muscle, as a result of interaction with these receptors would tend to increase outflow faclity.

In rabbit, stimulation of the cervical sympathetic nerves contracts the ciliary muscle (Van Alphen et al., 1965) but does not alter outflow facility (Langham and Rosenthal, 1966; Paterson, 1966). The lack of any rise in facility may be a result of the sparsity of the ciliary muscle. Eakins and Eakins (1964) found that in rabbits pretreated with cocaine, stimulation of the sympathetic nerves raises the concentration of NA in the AH, but without cocaine no rise was observed. Paterson (1966) found that in cocaine-pretreated rabbits there is a marked rise in outflow facility during sympathetic stimulation.

Stimulation of the cervical sympathetic nerves in the monkey relaxes the ciliary muscle (Tornqvist, 1966) which, as expected decreases outflow facility (Casey, 1966). However, both adrenaline and NA increase facility of outflow (Barany, 1968; Bill, 1969; Bill, 1970). This can be explained by an adrenaline-induced rise in pseudofacility (Barany, 1968) although this is not enough to account totally for the rise in outflow facility. Alternatively, a rise in uveoscleral flow could be responsible.

In the monkey, uveoscleral flow accounts for at least 30% of the total aqueous flow (Bill, 1971). The uveoscleral flow increases after topical adrenaline and also anterior chamber perfusion with isoprenaline. This is accounted for by ciliary muscle relaxation which widens the spaces in the intermuscular connective tissue. (Bill, 1969; Bill, 1970).

The influence of ciliary muscle tone on outflow facility can be eliminated by disinserting and retrodisplacing the muscle from the spur. Kaufman and Barany (1981) demonstrated that the percentage facility increases caused by adrenaline and NA were similar in disinserted and nondisinserted eyes. Drug-induced alterations in ciliary muscle tone, therefore, could not have been responsible for the adrenaline-induced rise in facility of outflow.

The idea has been advanced that dilation of the pupil by adrenaline might transmit mechanical pull on the trabeculae and therefore increase outflow facility. This idea is not favoured for several reasons. Kaufman and Barany (1981) have shown that iridectomy has no effect on the outflow responses to adrenaline or NA. The increase in outflow produced by cAMP, the second messenger supposedly responsible for adrenaline's activity at beta adrenergic receptors, occurs without mydriasis (Neufeld et al., 1975). Beta receptor agonists, which have no effect on pupil diameter, lower IOP. (Langham and Diggs, 1974; Innemee, 1982).

Trabecular meshwork/canal of Schlemm

In the monkey eye adrenaline and NA increase facility of outflow before and after ciliary muscle disinsertion and iridectomy. Kaufman and Barany (1981) therefore concluded that adrenaline increased outflow facility by acting directly on the trabecular meshwork and/or the endothelial lining of Schlemm's canal. However, the precise mechanism of action of adrenaline at these sites remains obscure. It has been considered most likely that adrenaline activates beta adrenoceptors on the endothelial cells lining the outflow pathway.

In rabbit and monkey there is an increase in the level of cAMP in the AH in response to adrenergic agonists. In an attempt to identify the tissue origin of this cAMP Neufeld and Sears (1974) investigated the ability of ocular tissues from rabbit, monkey and man, to generate cAMP following the administration of adrenergic agonists. Because of the lack of cAMP in the posterior chamber, it appeared that the cAMP in the AH must originate from some tissue lining the anterior chamber. Although adrenaline, NA and isoprenaline raised the levels of cAMP in a *in vitro* preparation of the iris-ciliary body and the cornea, of particular importance was the rise and subsequent release of cAMP from rabbit scleral trabecular rings. This increase in cAMP content of the scleral trabecular ring tissue, which contains the site of decreased outflow resistance, may represent the initial biochemical response of the trabecular meshwork and/or endothelial cells to adrenergic agonists.

Similarly adrenaline increased the cAMP content of both human trabecular explants and cultured trabecular endothelial cells 15 minutes following its administration (Tripathi and Tripathi, 1984).

Although in most species the endothelial cells of the meshwork and of the canal of Schlemm are only occasionally innervated, nevertheless the cells lining the canal have mitochondria, indented nuclei and cytoplasmic rod-like structures, all characteristic of cells with contractile properties. Muscle activity of these cells triggered by cAMP, would by analogy with smooth muscle elsewhere, most likely consist of relaxation. Relaxation of cells within the meshwork would increase facility of outflow, if, when contracted they tended to draw together the trabecular bars. Recent studies by Tripathi and Tripathi (1984), using continuous phase-contrast, time-lapse micrography, have revealed striking changes in the dynamic cellular movement of human cultured trabecular endothelial cells following adrenaline treatment. Cell contraction was noted within fifteen minutes of exposure of the cells to adrenaline. Although cell contraction resulting from the interaction with a beta receptor is unusual,it would still be compatible with increased facility of outflow if contraction pulled the meshwork open in the same way as the ciliary muscle does in primates. An alternative hypothesis is that cAMP may well act as an intercellular as opposed to an intracellular messenger; cAMP, released from cells lining the anterior chamber, may act directly on "receptors" within the trabecular meshwork or the canal of Schlemm.

It is known that the meshwork is coated with a mucinous material. In rabbit, it is coated with the hyaluronidase - sensitive hyaluronic acid. Such a coating provides resistance to outflow and may be influenced directly by adrenergic agents, or indirectly through cAMP. Experiments done in the aorta of rabbit indicate that adrenaline may decrease the production of mucoid substances (Sears, 1975). Similar effects in the trabecular meshwork, eliminating resistance produced by a mucoid coat, could in time decrease the resistance to outflow of AH.

The Biochemical Mechanism of Action of Adrenaline In Lowering IOP.

A majority of results from studies which have investigated the effects of selective adrenergic agonists and antagonists on AH dynamics suggests that adrenaline increases outflow facility and consequently lowers IOP via a beta receptor. Similarly, biochemical evidence suggesting a beta adrenergic mechanism has also been compiled.

In many tissues adrenergic agents activate adenylate cyclase to produce adenosine-3',5'-cyclic monophosphate (cAMP) and it is this second messenger which appears to mediate many of the physiological events that the catecholamines initiate. The action of cAMP is clear, for example, in the effects of adrenaline on glycogenolysis in the liver (Exton, Robison et al., 1971) and skeletal muscle (Walsh et al., 1970) and on lipolysis in fat cells, (Butcher et al., 1968). cAMP may also play a role in ion and fluid transport (Orloff and Handler, 1977).

Evidence from several sources suggests that cAMP may play a central role in mediating several ocular responses to catecholamines.

In the rabbit eye, topically applied adrenaline, NA and isoprenaline increased cAMP levels in the AH (Neufeld, Jampol and Sears, 1972) with a concomitant rise in outflow facility and fall in IOP. This rise in cAMP was directly related to the adrenaline concentration (Neufeld et al., 1973). The cAMP in the AH increased rapidly (within half an hour), preceded the fall in IOP by about thirty minutes and peaked about the same time as the minimum IOP was reached.

This increased level of cAMP was sustained for several hours.

To test the relevance of this rise in cAMP to the ocular effects of catecholamines, the effects of exogenous cAMP on various ocular parameters were investigated in the rabbit (Neufeld, Jampol and Sears, 1972; Neufeld et al., 1975). Intracameral injections of cAMP increased outflow facility and decreased IOP. Pupil diameter was unaltered. These responses followed a time-course similar to that of adrenaline. Although in the monkey eye cAMP failed, 8-methylthio-cAMP, a potent analoque of cAMP, increased outflow facility. (Neufeld and Sears, 1975).

Factors which are known to influence the IOP responses to adrenaline also alter the levels of cAMP in the AH (Neufeld, Chavis and Sears, 1973). Repeated daily topical treatment with adrenaline leads to a progressive decrease in the magnitude of the IOP response to the drug, while cAMP levels in the AH showed less increase than in acutely treated controls. Superior cervical ganglionectomy produces an increased sensitivity of IOP to adrenaline and was accompanied by enhanced rises in AH cAMP concentrations.

There are, however, findings which discount a causal relationship between elevated cAMP levels and a fall in IOP following the activation of a beta receptor. The relative potency of catecholamines in increasing aqueous cAMP was reported to be in the order adrenaline, NA and isoprenaline (Neufeld, Jampol and Sears, 1972). This may not represent the order of potency, since the actual drug concentration in the biophase of the target tissue is unknown.

Roland and Potter (1979) found that the potencies of various catecholamines to produce a hypotensive response and to increase cAMP concentrations in the AH were not necessarily in direct relation. Systemic propranolol failed to block the pressure decrease and the elevation of cAMP in responses to NA or adrenaline treatment, whereas phenoxybenzamine partially inhibited them both (Neufeld, Jampol and Sears, 1972). Topical timolol effectively blocked the rise in cAMP production following treatment with isoprenaline in the rabbit eye, but it did not alter the IOP responses to isoprenaline (Bartels et al, 1980). Similarly, topical pretreatment with timolol blocked the rise in cAMP but not the fall in IOP following adrenaline treatment (Boas et al., 1981). In these experiments cAMP levels peaked as expected between sixty and ninety minutes followed by a second peak at 240 minutes, and had returned to control values by 360 minutes, However, there was no change in IOP until six hours after adrenaline treatment, an unusually long delay.

The binding of beta adrenergic agonists and antagonists with the receptor shows stereospecificity; the L-isomer shows approximately 100 times greater affinity for the receptor than the D-isomer. In accordance with this, topical D-isoprenaline did not increase the cAMP concentration in the AH but it induced a hypotensive response (Kass et al., 1976; Seidehamel, 1975) which was dose-dependent and was suppressed by the beta receptor antagonist sotalol. (Seidehamel et al., 1975).

Timolol and Aqueous Humour Formation.

Timolol is a non-selective beta-adreneceptor antagonist which is relatively devoid of sympathomimetic and local anaesthetic properties. Timolol has been shown to lower IOP in rabbit, monkey and man.

In rabbits the ocular hypotensive response to timolol is controversial. Topical application of a 1% solution of timolol failed to lower IOP in both normo- and hypertensive rabbit eyes. (Boas et al 1981, Palkama, 1985). Similarly, Vareilles et al., (1977) reported that timolol produced only a slight but inconsistent lowering of IOP in the normotensive rabbit eye. In contrast, both timolol, and the highly selective beta-2 antagonist, IPS 339, lowered IOP in the normotensive rabbit (Nathanson et al., 1981). Timolol also reduced IOP which had been raised by alpha chymotrypsin and water loading (Vareilles et al., 1977).

In man, timolol produces a dose-dependent lowering of IOP in the normotensive eye (Katz et al., 1978) and is particularly effective in the hypertensive eye. (Boger et al., 1978; Ritch et al., 1978; Williams et al., 1978). Zimmerman and Kaufman (1977) reported that timolol lowered IOP in man with no significant effect on facility of outflow. Based on these results the authors suggested that timolol lowered IOP by decreasing AH formation. Using fluorophotometry several workers have found that timolol decreases AH formation in the human normotensive and hypertensive human eye while outflow remains unaltered (Coakes and Brubaker, 1978; Yablonski et al., 1978; Korey et al., 1982).

The effect of timolol on AH formation in the rabbit eye was investigated using fluorescein-dextran where the dye was infused into the anterior chamber (Miichi and Nagataki, 1982). In these experiments, timolol had no significant effect on either AH formation or IOP. However, using the same technique in monkey eye, Miichi and Nagataki (1983) have shown that topically applied timolol lowered both IOP and AH formation.

The Involvement of Prostaglandins In The Ocular Hypotensive Response To Adrenaline.

It has been reported that the ocular hypotensive response to adrenaline declines during continuous treatment (Langham and Diggs, 1974) and it has been suggested that this tachyphyllaxis to adrenaline was due to negative feedback mediated by PGs. To test this theory, Bhattacherjee and Hammond (1977) investigated the effect of indomethacin on the hypotensive response of the rabbit eye to topically administered adrenaline. Indomethacin is a cyclo-oxygenase inhibitor and therefore inhibits PG synthesis. Although a 2% solution of adrenaline administered twice daily for twenty one days lowered IOP, no tachyphyllaxis was observed. However, pretreatment with indomethacin (0.125% or 0.25%) inhibited the ocular hypotensive response to adrenaline. Although this antagonism appeared to be fully effective after two days of pretreatment, there was considerable variation. If adrenaline does release PGs which antagonise the hypotensive response to adrenaline then inhibition of PG production by indomethacin, should potentiate the hypotensive response to adrenaline. Since indomethacin antagonised the hypotensive response to adrenaline, it appears that any PGs released by adrenaline in fact lowered IOP directly or facilitated the effect of adrenaline. This possibility is perhaps not so surprising considering that PGs can lower IOP (Starr, 1971; Camras *et al.*, 1977; Weizmann *et al.*, 1982 and Hoyng, 1984).

The ocular responses to PGs and the possible mechanisms involved will now be described in greater detail.

In addition to their well-known ocular hypertensive effect, PGs can also produce a significant and prolonged reduction in IOP, which can be preceded by a rise in IOP. (Starr, 1971; Camras et al., 1977; Weizmann et al., 1982; Hoyng, 1984). In man, monkey and rabbit this response is a dose-related phenomenon.

Topical or intravitreal injections of PGE_2 (10 to 25ng) or $PGF_{2\alpha}$a (50 to 200ng) produced a biphasic IOP response in the rabbit eye; a relatively short initial hypertensive phase followed by a prolonged hypotensive phase (Camras et al., 1977). However, low doses of $PGF_{2\alpha}$a (1 to 5ng) produced prolonged hypotension without any prior rise in IOP. With such low doses, the hypotensive response was maximal within five hours and over within fifteen hours, whereas with higher doses the hypotensive response was not apparent until five hours after PG administration, was maximal within ten hours and was still apparent after 24 hours.

In a majority of experiments investigating the ocular effects of PGs, IOP was not monitored for such a lengthy time and this may explain why this hypotensive response is poorly documented. In a separate series of experiments, low concentrations of PGE, (0.2 to 0.8ng) injected intravenously or intracamerally, produced the expected rise in IOP. However, in 25% of the animals studied IOP continued to fall below control levels and remained depressed for at least three hours (Starr et al., 1971).

It has been suggested that these opposing hypertensive and hypotensive responses may run concurrently and that the depressor phase only becomes evident after the strong pressor phase has worn off.

The Ocular Responses To Prostaglandins.

The effects of prostaglandins (PGs) on the eye have been the subject of extensive studies in recent years.

In the rabbit eye, PGs of the E and F type can increase and decrease IOP, whereas PGA_2 has very little effect on IOP.

Topical or intravitreal injection of several PGs (0.1 to 10ng), including PGE_2, $PGF_{2\alpha}$, 6-keto $PGF_{1\alpha}$ and prostacyclin (PGI_2) produced a dose-dependent increase in IOP (Camras et al., 1977; Waitzman et al., 1979; Golubovic and Radmanovic, 1982; Kulkarni and Srinivasan, 1982). This rise was apparent within ten to thirty minutes and lasted for approximately two hours. Similarly, the stable PG endoperoxide, U46619, also increased IOP. The PG antagonist NO164 had no effect on IOP but prevented the rise in IOP produced by PGE_2 and U46619 (Kulkarni and Srinivasan, 1982). Arachidonic acid, the precursor of the PG endoperoxides, produced a rise in IOP following its infusion into the arterially-perfused enucleated cat eye. Indomethacin pretreatment did not alter this response (van Alphen 1981, 1982).

However, indomethacin did antagonise the hypertensive response to both topical and intravitreal injections of PGE_2 (Camras et al., 1977). Polyphloretin phosphate, a PG antagonist, blocked the rise in IOP following treatment with PGE_1 (Starr, 1971).

These results suggested that following the application of exogenous PGs, either these, or endogenous PGs synthesised de-novo, act directly at a site within the eye to increase IOP.

Using a combination of suction cup and constant pressure perfusion to calculate AH inflow and pseudofacility and constant pressure perfusion to measure outflow facility, Masuda and Mishima (1973) examined the effects of injected PGs on AH dynamics in the rabbit eye. Both $PGE_{1\alpha}$ and PGE_2 produced a dose-dependent rise in AH formation which lasted for approximately three hours; while pseudofacility was increased true outflow facility was unaltered.

Similarly, using the constant pressure perfusion technique, Green and Kim (1985) showed that although IOP increased following topical application or the infusion of PGE_1, or PGE_2, total outflow was unchanged. By infusing the anterior chamber with fluorescein-dextran, Miichi and Nagataki (1982) also showed a rise in pseudofacility one and a half hours after the administration of high doses of PGF_2 a It has, therefore, been suggested that the rise in total outflow facility observed during PG-induced ocular hypertension was in turn due to a breakdown of the blood-aqueous barrier. This rise in pseudofacility could be caused by the vasodilatory effect of PGs.

Topically applied PGs cause marked vasodilation concurrent with an increased vascular permeability (Whitelocke and Eakins, 1973). Vasodilation would cause a change primarily in capillary pressure and hence vascular permeability, due to an increase in vessel diameter.

This increase in capillary pressure would force more fluid out of the capillaries, thereby increasing ultrafiltration. A breakdown of the blood aqueous barrier is reflected in the increased protein levels found in the AH following PG administration (Kass et al., 1972; Camras et al., 1977; Golubovic and Radmanovic, 1982; Kulkarni and Srinivasan, 1982). Alternatively or in addition, PGs may stimulate active transport across the ciliary body, therefore increasing aqueous production. Cole and Nagasubramanian (1972) found that PGE_1 increases transepithelial short-circuit current in isolated ciliary body preparations. This is thought to indicate increased active transport.

Although PGI_2 is a major product of AA metabolism it is an unstable compound (with a half-life of 10 minutes) which readily breaks down into the stable compound 6-keto $PGF_{1\alpha}$. Recently, it has been shown that both the iris-ciliary body and conjunctiva of the rabbit eye possess the ability to synthesise PGI_2 (Bhattacherjee et al., 1979).

Intravenous infusion of PGI_2 lowered IOP in a dose-dependent fashion (Weizmann et al., 1982). Low doses of PGI_2 (20 to 70ng.min^{-1}) rapidly lowered IOP, whereas higher doses (100ng min^{-1}) raised IOP.

Since PGI_2 tends to be rapidly inactivated in air, the IOP responses to topically applied PGI_2 are unpredictable. Nonetheless, Hoyng (1984) has investigated the effect of topically applied PGI_2 on IOP. Low doses of PGI_2 (5ng) lowered IOP after one hour, with a rapid return to control values within six hours. Three consecutive doses of 5ng of PGI_2 reduced IOP for at least four hours. In both cases no hypertensive phase was apparent. Topically applied 6-keto

$PGF_{1\alpha}a$ (250ng) produced only a transient rise in IOP whereas 250ng of PGI_2 produced an initial rise followed by a fall in IOP. Although these results suggest that the PGI_2-induced hypotension is produced by PGI_2 itself and not by its stable end-product, it would be of interest to study the effect of low doses of 6-keto $PGF_{1\alpha}$ on IOP.

In the rabbit eye there is a very narrow margin between the doses of PGs which produce only ocular hypotension and those which cause both hypotension and hypertension. This appears not to be the case in cat and monkey eyes. Topical application of very high doses of $PGF_{2\alpha}a$ and PGE_2 caused a long lasting fall in IOP with negligible breakdown of the blood-aqueous barrier (Camras and Bito, 1981; Stern and Bito, 1982). In the cat eye, topical application of up to 500ng of PGE_2 or $PGF_{2\alpha}a$ lowered IOP with a maximum reduction occurring between one and eight hours after PG administration. Indomethacin pretreatment had no effect on these hypotensive responses. This hypotension was not preceded by an initial hypertensive phase. Similarly in rhesus monkey eyes, topical application of 100 or 500ng of PGE_2 or $PGF_{2\alpha}$ respectively, produced a fall in IOP within two hours which was preceded by an insignificant rise in IOP.

The sequence of events leading to this PG-induced hypotony remains obscure.

Unlike the hypertensive responses to PGs, this hypotensive response cannot be accounted for by a maintained breakdown of the blood-aqueous barrier. Following the rise in IOP and AH protein levels, AH protein levels returned to normal during the fall in IOP. (Camras et al., 1977).

The fact that a significant increase in IOP resulted when a second dose of PGE_2 was applied to the eye during the hypotensive phase also suggests that the blood-aqueous barrier was functional (Beitch and Eakins, 1969). During the hypertensive response PGs produce vasodilation of ocular blood vessels (Whitelocke, 1973; Wizemann et al., 1982); however, following intravenous infusion of $20ng.min^{-1}$ of PGI_2, which lowered IOP, the luminal diameters of vessels of the posterior uvea were unaltered (Wizemann et al., 1982).

Associated with the fall in IOP produced by topically applied or intravitreally injected PGE_2 or $PGF_{2\alpha}$ was a reduction in outflow resistance in either the anaesthetised or freshly killed rabbit (Camras et al., 1977). This effect could not be attributed to pseudofacility nor alterations in secretory mechanisms since the secretory mechanism returned to normal levels during maximal hypotony.

Since small amounts of PG activity could be detected in the AH by either immunoassay or bioassay six to eighteen hours after topical PG application, it is possible that the hypotensive phase may result from the continual presence of PGs in the eye.

It is also possible that the prolonged hypotonic effects of PGs is due to the accumulation of a second messenger. PGs are known to exert their effects in many biological systems by altering intracellular levels of cAMP

PGs have been shown to increase the cAMP content of several ocular tissues in vitro including the sclero-trabecular ring, iris-ciliary body and cornea (Neufeld et al., 1972; Neufeld and Sears, 1974) and intracameral injections of cAMP have been shown to increase

outflow facility in the rabbit eye (Neufeld et al., 1975; Neufeld and Sears, 1975). Therefore, following exogenous PG administration, the prolonged residual levels of PGs may increase the cAMP content of intraocular tissues and fluids which in turn may account for the long-term increase in outflow facility.

The possibility that PG-induced hypotension depends on the de-novo synthesis of PGs or other cyclo-oxygenase products has also been ruled out, because the hypotensive phase was not abolished by systemic pretreatment with indomethacin (Camras et al., 1977).

It is also unlikely that the IOP-lowering effect of PGs is related to sympathetic activity or to the release of endogenous catecholamines, since the time-course of the IOP response was very similar in both normally innervated and sympathetically denervated eyes. In fact, the only difference was a faster transition from the hypertensive phase to the hypotensive phase in the denervated eye (Camras et al., 1977).

Other Properties Of Indomethacin.

Indomethacin as a calcium antagonist.

Indomethacin, while a prototype inhibitor of cyclo-oxygenase, is known to have other pharmacological effects, one of which is inhibition of calcium movements (Northover, 1972,1977,1982).

Calcium has been implicated in alpha and beta receptor-mediated contraction, relaxation and metabolic effects on

vascular and other smooth muscle. (Fitzpatrick et al., 1972; Wauah, 1972; Anderson, 1973).

The relaxing and metabolic effects in smooth muscle mediated by beta-receptors are related to an increased cAMP level, which in turn, stimulates an energy-dependent, calcium-binding mechanism, which probably decreases myoplasmic calcium. A reduction in cAMP levels due to alpha receptor stimulation results in the release of bound calcium (Anderson, 1972). In ocular tissues, topical NA, adrenaline and isoprenaline increase the cAMP content of the AH and ocular tissues (Neufeld et al., 1972; Neufeld and Sears, 1974). It is this cAMP which is believed to be responsible for the hypotensive action of adrenaline. Since calcium is involved in cAMP-mediated responses, in this case ocular hypotension, then release or binding of intracellular calcium is likely to affect the hypotensive response to adrenaline. Indomethacin has been reported to inhibit the uptake of calcium by cell membranes of various tissues and to inhibit the depolarization of vascular endothelial cells (Northover, 1977). Similar inhibition by indomethacin of calcium binding in ocular tissues may have affected the ocular responses to adrenaline. Recently, a new class of non-steroidal anti-inflammatory drug, the oxicams, has been developed. Piroxicam, a member of this class , appears to be an ideal drug to study the effects of PG inhibition on the ocular responses to adrenaline. In contrast with indomethacin, not only is piroxicam a more potent inhibitor of PG synthesis but at concentrations vastly greater than those which inhibit PG synthesis, it has no effect on calcium fluxes (Burche et al., 1983).

Inhibition Of Phosphodiestase By Indomethacin.

Indomethacin is also an inhibitor of phosphodiesterase (Flores and Sharp, 1972; Hall and O'Regan, 1975). If there were an inhibition of phosphodiesterase activity, then the increased levels of CAMP would, if anything, have enhanced the effect of adrenaline in lowering IOP.

AIM OF RESEARCH

In the rabbit eye adrenaline can produce either ocular hypertension and/or hypotension depending upon the dose, frequency and route of administration of the drug. Broadly, this research set out to study the mechanism of the hypotensive response to adrenaline with particular reference to its inhibition by indomethacin.

Initial experiments in the conscious rabbit attempted to confirm the type, magnitude and duration of the pressure responses to topically applied adrenaline and to establish a control pattern against which inhibition by other drugs was to be detected.

Since this hypotensive response to adrenaline is thought to result from an increased facility of outflow of aqueous humour, this parameter would be measured in anaesthetised rabbits and the time course of the effect determined.

Although indomethacin has been shown by others to block the hypotensive response to chronic adrenaline treatment in conscious rabbits, it would be necessary first to show that such inhibition also occurred following acute dosage with adrenaline. Pretreatment of such animals with indomethacin would then indicate whether this drug's blockade of the adrenaline induced hypotension was exerted at the level of facility of outflow.

Further definition of the mechanism whereby indomethacin inhibits the responses to adrenaline might then be acquired by substituting indomethacin with other cyclo-oxygenase inhibitors, such as piroxicam or aspirin, whose subsidiary properties differ from those of indomethacin. Since these other properties of indomethacin include calcium channel blockade, testing verapamil might indicate whether such activity could explain indomethacin's ability to block the adrenaline effect.

The use of timolol should confirm whether the effect of adrenaline on facility of outflow is mediated via beta adrenoceptors.

If the experimental evidence suggests that the responses to adrenaline are inhibited by indomethacin because of cyclo oxygenase blockade, then more definitive information might be available by measuring PG concentrations in the aqueous humour following adrenaline treatment with or without indometh - acin pretreatment.

Since topical adrenaline raises the levels of cAMP in the aqueous humour, the present experiments will attempt to confirm whether indomethacin can indeed block the cAMP-induced rise in facility of outflow in the rabbit eye. Such experiments should perhaps help to trace any link which exists between cAMP and PGs in the responses of the outflow tissues.

MATERIALS AND METHODS

Measurement of Intraocular Pressure

IOP can be measured directly by manometric techniques or indirectly by tonometric techniques.

Manometric measurement of IOP involves the insertion of a needle into the anterior chamber of the eye. During this procedure the rabbit is under general anaesthesia. A major drawback of this technique is that cannulation of the eye and the prolonged presence of the needle within the chamber can damage the eye. The indirect method involves measurement of pressure by indentation or applanation of the cornea. In these studies applanation tonometry was used. This method measures the force required to flatten an area of the cornea as an index of pressure in the eye. This procedure requires only local anaesthesia. The applanation tonometer requires careful calibration and it should be remembered that there are dissimilarities in the elasticity of the cornea between different species.

Tonometric Measurement of Intraocular Pressure

Before being used in any experiment Dutch rabbits (1.5 to 3.5 kg) of either sex were acclimated to laboratory conditions for several weeks.

On the morning of each experiment rabbits were restrained by placing them in close-fitting canvas bags. The animals were then left to settle for at least 15 min.

Following topical anaesthesia with 50µl of a 0.5% solution of benoxinate hydrochloride, IOP measurements in the conscious rabbit were taken with a Digilab Pneumatonometer calibrated on the cannulated rabbit eye.

Five applanations of the cornea with the pneumatic floating tip were made for each measurement and an average taken. To avoid any unnecessary pressure to the eye, great care was taken to gently pull back the eyelid when reading IOP.

Usually two control measurements of IOP, 15 min apart, were taken prior to drug application. All drugs were slowly dropped over the surface of the cornea from a micropipette. A constant volume of 50µl of each drug was administered. Following drug administration, IOP was measured at hourly intervals for at least 4h.

Since repeated application of local anaesthetic to the eye appeared to cause corneal drying, each population of rabbits was left for 2 to 3 days before being used in another experiment.

Calibration of the Digilab pneumatonometer

The Digilab pneumatonometer is a device normally used to measure IOP in the human eye. Since the curvature and elasticity of the human cornea differs from that of the rabbit, the pneumatonometer has to be recalibrated in order to compensate for this.

Dutch rabbits were killed by an overdose of sodium pentobarbitone (approx. 500mg) injected into the marginal ear vein. The anterior chamber of the eye was then cannulated with the aid of a needle gun. The cannula consists of a 30G needle attached to a length of nylon tubing (Portex: 0.25mm bore, wall thickness 0.25mm). The cannula was connected to a manometer via a 3-way stopcock. To ensure that no air bubbles were introduced into the system during cannulation, mock aqueous humour was allowed to flow through the needle from the reservoir whilst cannulating the eye.

The pressure in the system was raised to 30mmHg by raising the reservoir 408mm above the level of the needle in the anterior chamber. The pressure was allowed to equilibrate for 5 min and 5 readings of IOP were made using the pneumatonometer. A further 3 min were allowed to pass and a second set of five readings were taken. This procedure was repeated until there was no substantial difference between the two sets of readings, i.e. no greater than 0.4mmHg between consecutive sets of readings. The manometer pressure was lowered by 5mmHg increments and the procedure repeated until a pressure of 5mmHg was reached. This procedure was carried out simultaneously in the contralateral eye.

Having plotted manometric pressure against the tonometer readings, regresion analysis was used to calculate the slope(S) and the intercept (I) of the line. The relationship between tonometer reading(T) and IOP may be expressed as follows.

$$\text{IOP (mm Hg)} = \frac{T - I}{S}$$

Following each measurement of IOP, pupil diameters (PD) were measured visually under uniform artificial illumination with a transparent millimeter ruler, held horizontally.

In Vivo Measurement of Facility of Outflow of Aqueous Humour in the Perfused Rabbit Eye.

In these experiments the main aim was to measure the facility of outflow of aqueous humour i.e. the ease with which aqueous humour can leave the anterior chamber. Secondary to outflow measurements, IOP was continuously recorded throughout the course of the experiment.

Dutch rabbits (1.5 to 3.5 kg) of either sex were anaesthetised with a 25% solution (w/v, in water) of urethane (ethylcarbamate). Urethane solution (approx. 6ml kg^{-1} body weight) was slowly infused, via a polythene catheter (O/D 0.75mm Portex), into the marginal ear vein until the animal showed no corneal reflex. A tracheal cannula was then inserted to ensure that respiration was not impaired by nasopharyngeal mucus, which is usually secreted under urethane anaesthesia. In some animals the femoral artery was cannulated with a polythene catheter (O/D 0.75mm Portex). By attaching this catheter to a Gould pressure transducer (model P23ID) which was then attached to a polygraph, arterial blood pressure was recorded. The animals were then laid prone in a restrainer box designed to hold the head steady.

A diagrammatic representation of the apparatus used to determine facility of outflow is illustrated in Fig.(1).

Using a needle gun (Edwards and Yerlett,1972) a single perfusion needle (30G hubless 0.5" blade: NI medical) was inserted into the anterior chamber of each eye. The needle was inserted tangentially to ensure a firm seal and to avoid touching the iris or lens during or after insertion. Attached to the needle was silicon-rubber tubing (bore 0.5mm, wall thickness 0.25mm: Esco Rubber Ltd.) and then polythene tubing

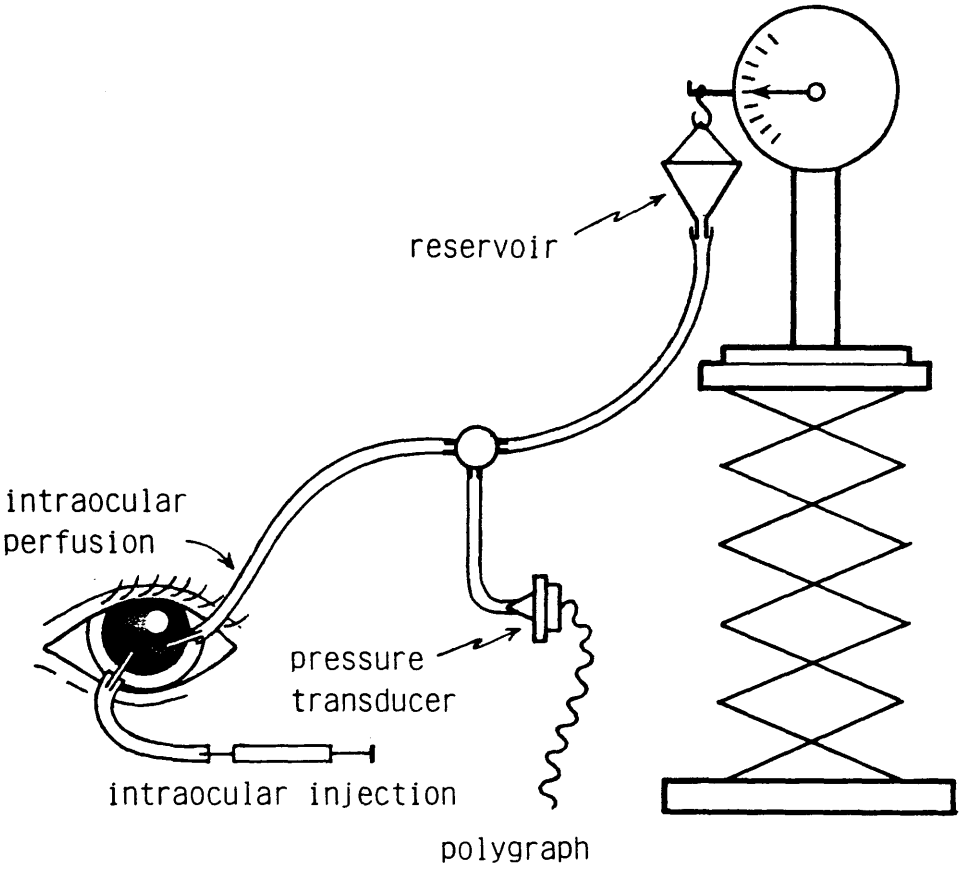

Fig. 1 Diagram of the apparatus used to measure the apparent facility of outflow and IOP in the anaesthetised rabbit.

(Portex Ltd) which connected it to a 3-way stopcock (Henleys Medical Supplies).

The stopcock was in turn connected to a polythene reservoir and a Gould pressure transducer (model P23ID). The reservoir was hung on the arm of a Gallenkamp torsion balance (fullscale deflection 1000mg) which stood upon a labjack of adjustable height.

The reservoir, tubing and pressure transducer were filled with an aqueous humour substitute (AHS) of the following composition (Barany, 1964).

NaCl	110mM
KCl	3.0mM
$CaCl_2$	1.4mM
$MgCl_2.6H_2O$	0.5mM
K_2HPO_4	0.9mM
$NaHCO_3$	30mM
Glucose	6.0mM
Ascorbic Acid	1.0mM
Heparin	20 IU ml^{-1}

The labjack allowed the reservoir to be adjusted to a height which exerted a known pressure greater than resting IOP. By connecting the pressure transducer to a Grass polygraph recorder (model 7) a continuous record of IOP was obtained.

The resistance of this system was checked visually by ensuring that the flow of AHS from the needle was far greater than the rate at which AHS could possibly be required to perfuse the eye.

If the stopcock is opened from the reservoir to the eye and a known pressure applied, then fluid runs from the reservoir

into the eye and forces an equal volume of aqueous humour to drain through the meshwork (assuming there is no compliance by the eye). Knowing this volume of fluid and the applied pressure required to force it through the meshwork, the facility of outflow (C) of aqueous humour can be calculated as follows.

$$C\ (\mu l.min.^{-1}mm^{-1}Hg) = \frac{\text{ouflow}\ (\mu l.min^{-1})}{P_{app}\ (mm^{-1}\ Hg)}$$

Where P_{app} is equal to the additional pressure applied by the reservoir. (For a worked example and analysis of precision, see Appendix IV.)

The outflow or volume of fluid lost from the reservoir is measured on the balance as its weight in mg, assuming a density of $1g.ml^{-1}$.

The applied pressure was set 4mmHg above ambient IOP by raising the height of the reservoir while monitoring IOP changes on the polygraph. A typical polygraph trace of the pressure within the anterior chamber is shown in Fig. (2).

When the stopcock is opened to the eye, perfusion begins. During the first minute of perfusion, IOP rapidly rises to the applied pressure (usually within 10-15s) and some compliance of the eye may occur. After one min, perfusion has reached steady state during which it can be assumed that each µl leaving the reservoir causes 1µl to drain from the anterior chamber. An average of 3 one-min readings of the balance during steady state perfusion were used to calculate C. Such determinations of facility of overflow were carried out at 30 min intervals for a period of 2.5h.

Before each determination of facility of outflow, PDs were measured horizontally under standard illumination using calipers with a vernier scale

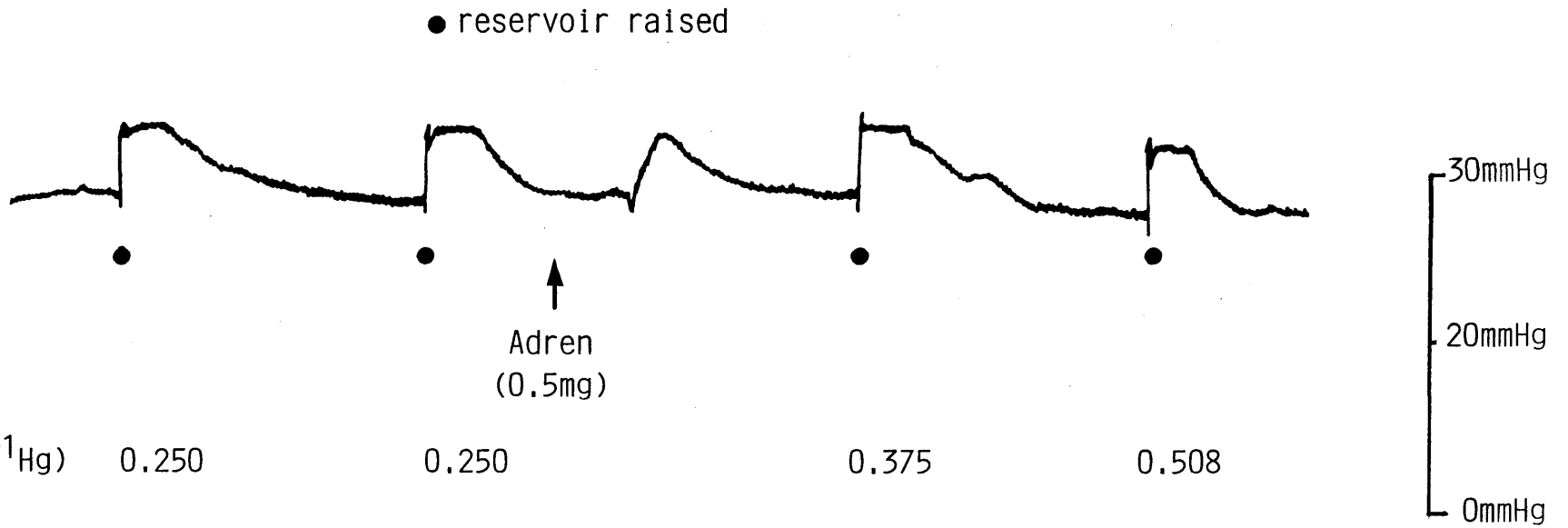

Fig. 2 Polygraph record of the pressure within the anterior chamber of the anaesthetised rabbit and values for the apparent facility of outflow of aqueous humour. Effect of adrenaline on pressure and the apparent facility of outflow.

Measurement of the Prostaglandin Concentration in the Aqueous Humour Using Radioimmunoassay.

The sensitivity and specificity of radioimmunoassay (RIA), allows us to accurately measure very small concentrations (10^{-6}-10^{-9}g) of PGs in the aqueous humour.

RIA is based on an antigen-antibody reaction. This reaction depends on the formation of weak bonds that can be readily dissociated, so one antigen molecule can be displaced by another.

If a radiolabelled antigen is used to form an antigen-antibody complex and then a further unknown amount of the same, but unlabelled antigen is added, some of the radiolabelled antigen will be displaced. Once equilibrium has been obtained the percentage of total label which is bound is inversely proportional to the concentration of unlabelled antigen (i.e. the substance to be measured).

The concentration of antibody to be used in the RIA, in this case antibodies against PGE_2 and 6 keto $PGF_{1\alpha}$ raised in horses is calculated using an antibody titer. A working definition of titer is "that dilution of antibody which binds a specified percentage of a standard amount of radiolabelled antigen", in this case (^3H) PGE_2 or (^3H) 6 keto $PGF_{1\alpha}$, under set conditions. However, the percentage binding required is restricted to a certain range by two factors, the precision and sensitivity required of the assay. Precision is a term which explains the amount of variation in the estimation of unlabelled ligand whereas sensitivity refers to the theoretically smallest quantity detectable.

As the percentage bound decreases, sensitivity increases whereas precision decreases; the opposite is true as the percentage bound increases. Therefore the best balance between sensitivity and precision occurs when 30 to 50% of the antigen is bound. The appropriate antibody titers for PGE_2 and 6 keto $PGF_{1\alpha}$ were estimated by incubating various dilutions of the antibody (1/500 to 1/10,000) with a fixed amount of radiolabelled PG (45nCi) under set conditions. The total amount of PG bound by the antibody was calculated by including a sample containing no exogenous PG ie 100µl of antibody plus up to 200µl of RIA buffer.

Radioimmunoassay of PGE_2 and 6-keto $PGF_{1\alpha}$

This assay is a modified version method of Sors et al., 1978.

Volumes up to 200µl of AH were placed in plastic Eppendorf tubes (1.5ml) and 100µl of the appropriate antibody dilution was added followed by volumes up to 200µl (45nCi,0.25 fmol) of (^3H) PG. These samples were mixed and incubated for 24h at 4^oC. Following incubation, 100µl of ice-cold dextran-coated charcoal was added. This porous charcoal readily takes up small molecules thereby separating unbound radiolabelled PG from the larger protein-bound complex. The reaction was stopped by placing the samples on ice. These samples were spun at 3,500xg for 5 min at 5^oC in a Damon/IEC centrifuge. 200µl of the resultant supernatant was transferred to plastic vials containing 10ml of scintillant (Packard).

Radioactivity was then counted in a Tricarb Liquid Scintillation Spectrometer for 5 min. Activity of non-specifically bound radiolabelled PGs was calculated by including samples which contained no antibody, ie 200µl of aqueous humour and 100µl of RIA buffer. For the composition of both the RIA buffer and dextran-coated charcoal solution see Appendix I.

A series of standards containing known amounts of PGs (0.1pg to 3ng) dissolved in RIA buffer, were used to construct a standard curve from which the concentration of PG in test samples could be calculated.

Extraction and Concentration of PGs from the AH.

It proved impossible to improve the sensitivity of the assay enough to measure endogenous PGs in the AH by adjusting the antibody titer or (^{3}H) PG concentration. Therefore the amount of PG to be assayed was increased by pooling and then extracting and concentrating AH from several rabbits.

Approximately 150µl of AH was withdrawn from each eye (control or drug-treated) using a syringe fitted with a 26G needle. These samples were pooled and the pH adjusted to 3 using 0.1M citric acid. 1ml aliquotes were taken through the following extraction procedure. 3ml of a cyclohexane-ethylacetate mixture (1:1 v/v) was added to each sample which was mixed for 1min and spun in a Damon/IEC centrifuge at 300xg at 0°C for 5min. The organic solvent layer was carefully removed using a Pasteur pipette. This procedure was carried out 5 times in total. Pooled solvent samples in glass tubes were placed in a heated metal block at 40°C and concentrated by blowing down the sample under a stream of N_2 (O_2 free).

The dried sample was reconstituted in a volume of 200µl, consisting of 160µl of RIA and 40µl of 0.28mM Na_2CO_3 solution. This 200µl sample was then assayed for 6 keto-$PGF_{1\alpha}$.

The efficiency of this extraction procedure was estimated by adding 100µl of (^{3}H) 6 keto-$PGF_{1\alpha}$ (containing 45nCi) to a 900µl sample of either 0.9% saline or AH and counting the radioactivity in samples of the solvent following extraction.

The percentage recovery of PG after both the extraction and concentration procedure was estimated by adding a known amount of 6 keto-$PGF_{1\alpha}$ (100µl) to a 900ul sample of either 0.9% saline or AH.

In some instances in this series of experiments a white flocculent precipitate formed when the cyclohexane-ethylacetate mixture was added to samples of AH. After spinning, this precipitate formed a layer between the aqueous and solvent phases. Care was taken not to remove any of this layer when removing the solvent phase.

Protein Determination

The assay used for protein determination was based upon the method of Lowry, Rosebrough, Farr and Randall (1951), as modified by McKean (1982).

Aliquots of aqueous humour were made up to a 100µl volume with N-sodium hydroxide solution. A standard curve was prepared for each batch of samples by assaying a series of bovine serum albumin standards (0 to 50µg) made up in distilled water and diluted to 100µl with N-sodium hydroxide. 1ml of solution C (Appendix I) was added to each test or standard sample at staggered time-intervals and mixed.

After 15 min, 100µl of N Folin-Ciocalteu reagent was added to each sample. The solutions were mixed immediately and allowed to stand at room temperature for 30 min before the measurement of their extinction at 750nm on either a Cecil grating spectrophotometer (model CE303) or a Shimadzu u.v. visible recording spectrophotometer (model u.v.240). Distilled water was used as the reference.

Estimation of Salicylic Acid

Aspirin is rapidly metabolised to salicylate. The time course of aspirin absorption and distribution was followed by assaying plasma salicylate concentration at 30 min intervals. The colour formed by the reaction of salicylate with acidified ferric nitrate was used as a measure of plasma salicylate concentration.

Following each measurement of outflow facility, approx. 1.5ml of blood was taken from the femoral artery. The blood was placed in plastic Eppendorf tubes (1.5ml) and spun at 10,000xg for 3 min in a Quickfit microcentrifuge. 1ml of the resultant plasma was mixed with 0.5ml of trichloracetic acid (20%,w/v) and spun for 5 min in the microcentrifuge. The supernatant was removed using a Pasteur pipette. 0.5ml of protein-free plasma was mixed with 3ml of a ferric nitrate solution (see Appendix I).

The optical density of these solutions was read at 525nm on a Cecil grating spectrophotometer. Distilled water was used as the reference.

Salicylic acid samples (0 to 200µg) made up in distilled water were assayed and a standard curve constructed.

Drug Preparation and Route of Administration

Topical application

The eyelid was gently pulled back and the drug was slowly applied to the surface of the cornea from either a "dropper" or a Hamilton Microsyringe. A volume of 50µl of each solution was usually applied.

Injection

Following the insertion of the perfusion needle, a second needle (30 G, hubless, 0.5" blade:- NI medical) was inserted into the anterior chamber of the eye. This needle was attached to the Hamilton microsyringe via silicone rubber tubing (bore 0.5mm, wall thickness 0.25mm, Esco Rubber Ltd). Both the syringe and tubing were filled with the appropriate drug solution. Before injection of the drug solution, the stopcock was opened to the reservoir, whose height was adjusted such that the pressure it applied equalled ambient IOP. This precluded any rise in pressure which would otherwise have occurred during intraocular injection. The drug, in a volume not greater than 10µl was then slowly (approx. $1\mu l.min^{-1}$) injected into the chamber.

Rectal administration

Aspirin suppositories were inserted rectally by hand.

Drug Preparation

Adrenaline

The adrenaline solution applied topically to the eye was Eppy (Smith and Nephew), a 1% (w/v) solution in an isotonic buffered ophthalmic solution. Since this vehicle or its formulation were unavailable, the control solution used was 0.9% (w/v) saline.

Aspirin

Aspirin suppositories were administered rectally (approx. 200mg.kg^{-1}) 1h prior to the insertion of the perfusion needle.

Cyclic nucleotide analoques

8 bromoadenosine 3',5' cyclic monophosphate (8 bromo-cAMP) and N^6, 0^2, dibutyryladenosine 3',5' cyclic monophosphate (d-cAMP) were obtained as the sodium salt. A 10mM solution was prepared by dissolving the appropriate weight of each drug in distilled water. This solution (5µl) was injected into the anterior chamber. Distilled water was used as the vehicle control.

Indomethacin

A 0.25% (w/v) solution of indomethacin was prepared in 1.9mM Na_2CO_3. The pH of this solution was then adjusted to between 7 and 8 by slowly adding small volumes of 0.1N-NaOH, while monitoring the pH on a Metrohm (model E520) pH meter. This solution was continuously stirred. 1.9mM solution of Na_2CO_3 was used as the vehicle control.

Piroxicam

A 0.5% (w/v) solution of piroxicam was prepared as previously described for the indomethacin solution. Since piroxicam was prepared in 1.9mM Na_2CO_3 this was used as the vehicle control.

Timolol

A 0.25% (w/v) ophathalmic solution of timolol maleate ("Timoptol", M.S.D.) was topically applied to the eye. An ophthalmic solution of the timolol vehicle was supplied by Merck Sharp and Dohme.

The Effect Of Topical Application Of Adrenaline On IOP, PD And The Apparent Facility Of Outflow Of Aqueous Humour From The Anterior Chamber.

The reproducibility of IOP measurements in the concious rabbit taken with the tonometer is shown in Fig.3.

The initial IOP measurement is usually higher since it is taken when the animals are adjusting to experimental conditions. Over a period of 5h there was no significant variation in IOP within or between eyes. The latter is particularly important since in subsequent experiments only one eye was usually treated with the drug.

The effect of topically applied adrenaline on IOP in the conscious rabbit is shown in Fig. 4. Unlike most other experiments the drug treatment (0.25mg adrenaline) was identical in both left and right eyes of 10 rabbits. Adrenaline has a biphasic effect on IOP, an initial hypertensive phase, followed by a longer lasting hypotensive phase. Compared with the immediate pre-adrenaline reading at -15min, the initial rise in IOP (5.2 ± 0.2mmHg, $P \geqslant 0.001$) was maximal within 1h, while the fall in IOP (4.7 ± 0.4mmHg, $P \geqslant 0.001$) was maximal within 2h and had almost returned to control values 4h following adrenaline administration.

The effect of adrenaline on pupil diameter (PD) is also shown in Fig.4. As expected adrenaline produced mydriasis. This increase in PD of 2.2 ± 0.2mm ($P \geqslant 0.001$) was maximal after 1h and remained increased throughout the experiment.

The effects of adrenaline on PD, IOP and facility of outflow in the anaesthetised rabbit are shown in Figs 5 and 6 .

Adrenaline (0.5mg) topically applied to both eyes (Fig 5) produced the expected fall in IOP and rise in PD.

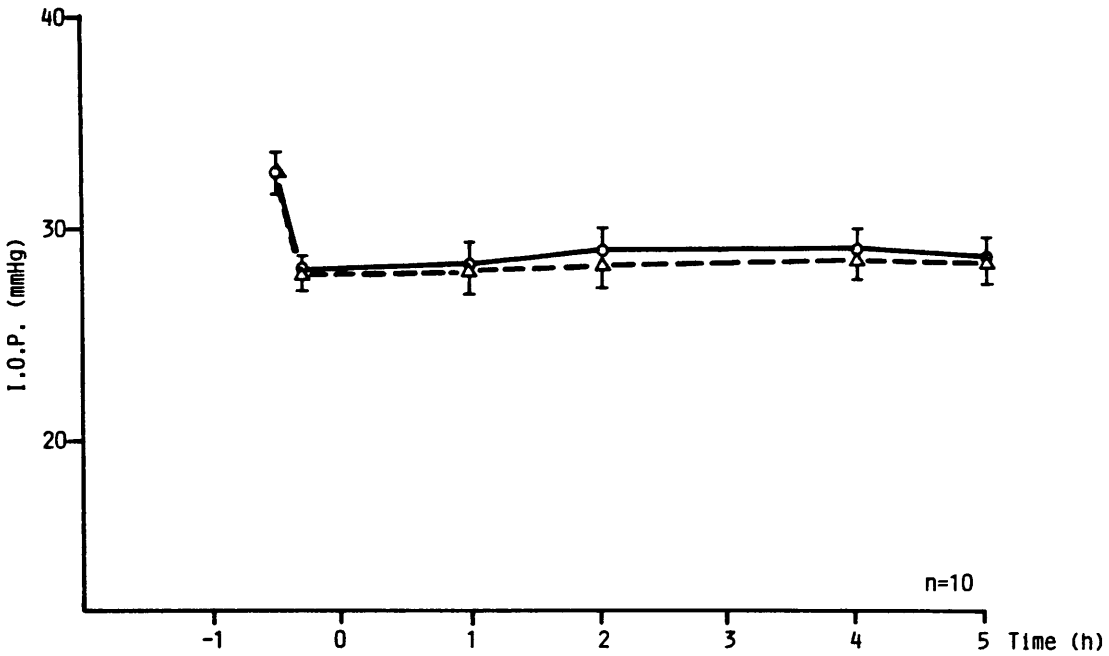

Fig. 3 Diagram illustrating the reproducibility of IOP measurements taken in the conscious rabbit. Each point is a mean ± SEM of values obtained in 10 eyes.

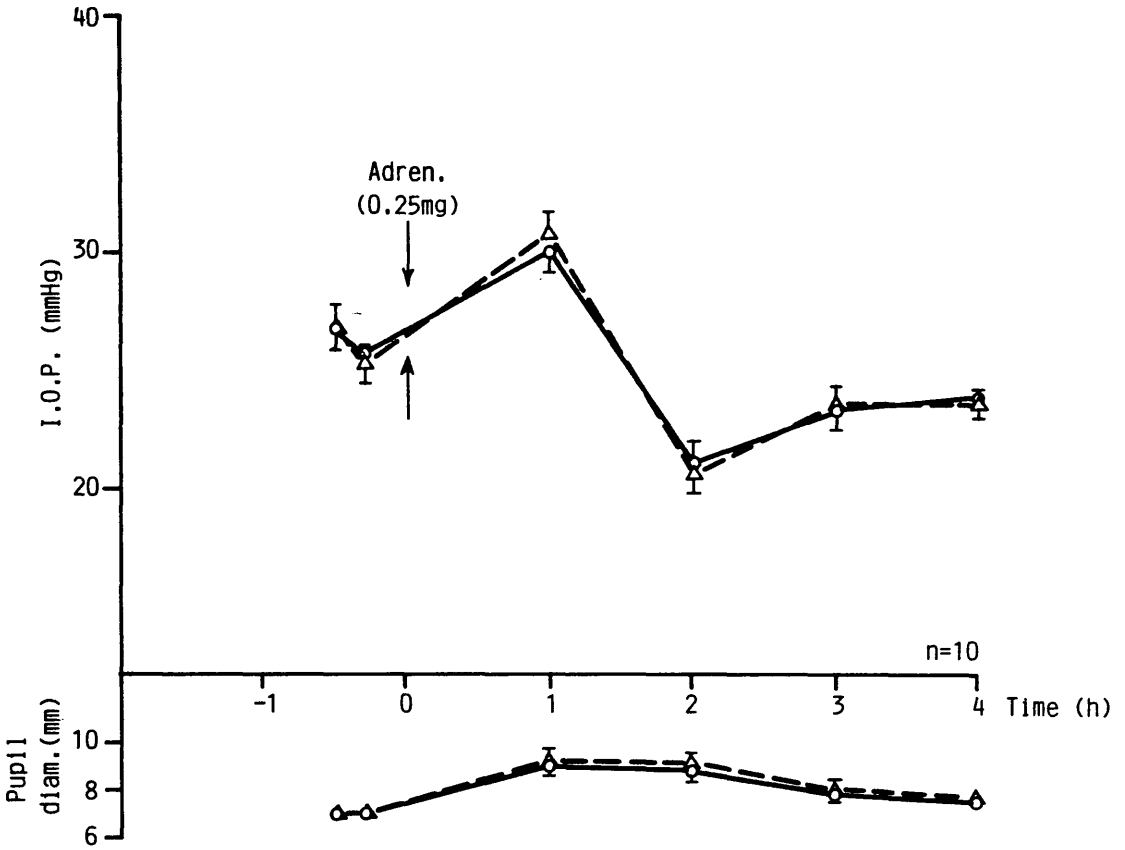

Fig. 4 The IOP and PD responses to adrenaline (0.25mg) which was topically applied as an aqueous buffered solution to both eyes of the conscious rabbit. Each point is a mean ± SEM of values obtained in 10 eves.

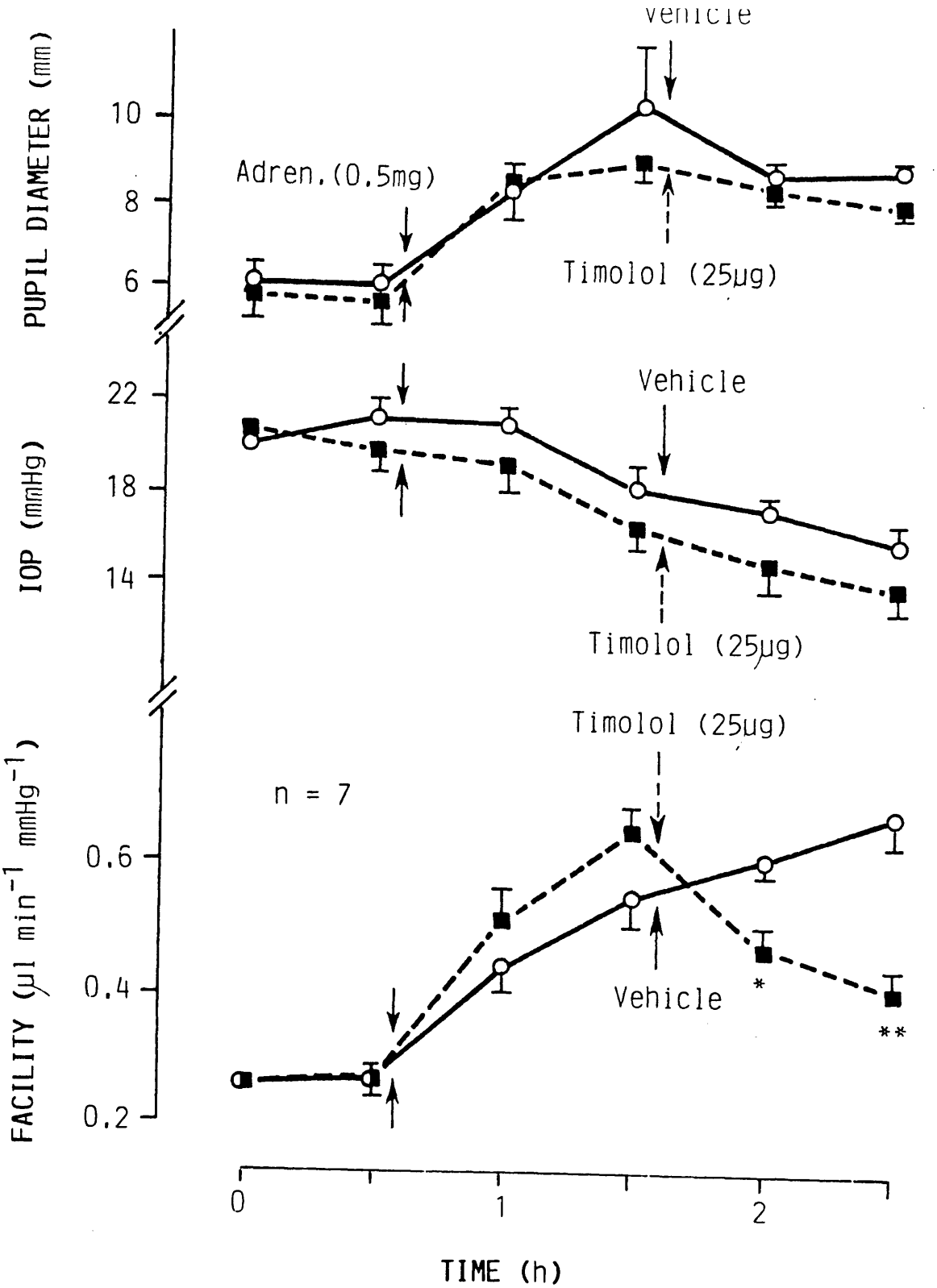

Fig. 5 The effect of topical timolol (--▲--) on the facility of outflow. IOP and PD responses to topical adrenaline (-○-) in the urethane-anaesthetised rabbit. Each point is a mean ± SEM of results obtained in 7 eyes. Significant differences between timolol treated and vehicle control eyes are shown as *$0.05 \geq P \geq 0.01$; **$0.01 \geq P \geq 0.001$.

The adrenaline-induced rise in IOP seen in the conscious rabbit was also seen in the anaesthetised rabbit. However, although IOP measurement was continuous throughout the experiment, the values used to construct the IOP curve are those taken immediately prior to each measurement of C. The speed of this hypertensive response meant that it was over before the first post adrenaline measurement of IOP was taken. The response was rapid in onset, maximal after apppox. 10min, and complete within 20min. A typical hypertensive response to adrenaline is shown in Fig.2. After 1.5h, adrenaline had lowered IOP in both eyes (Fig.5). IOP had fallen from 21.4 $\pm$ 0.7 to 16.5 $\pm$ 0.9mmHg ($0.01 \geq P \geq 0.001$), in the adrenaline-timolol treated eye. Adrenaline also appeared to lower IOP in the adrenaline-vehicle-treated eye from 19.9 $\pm$ 0.7 to 18.5 $\pm$ 0.6. However, this fall in IOP was not significant ($0.1 \geq P \geq 0.05$)

Adrenaline maximally increased PD in both eyes after 1.5h. PD had risen from 6.2 $\pm$ 0.4 to 10.5 $\pm$ 1.5mm ($0.05 \geq P \geq 0.02$) in the adrenaline and timolol-treated eye and from 5.8 $\pm$ 0.5 to 9.1 $\pm$ 0.4mm ($0.01 \geq P \geq 0.001$) in the adrenaline+vehicle-treated eye.

Of particular importance is the adrenaline-induced rise in facility of outflow (Fig.5). After 1.5h facility of outflow had increased from 0.27 $\pm$ 0.01 to 0.52 $\pm$ 0.01 $\mu l.min^{-1}$ mm^{-1} Hg ($P > 0.001$) in the adrenaline+vehicle-treated eye and from 0.27 $\pm$ 0.01 to 0.67 $\pm$ 0.03 μl min^{-1} mm^{-1} Hg ($P > 0.001$) in the adrenaline + timolol-treated eye. The maximal rise of outflow facility appears to precede the major fall in IOP following adrenaline treatment.

In a separate series of experiments, the effects of perfusion alone on IOP and facility of outflow, as well as the ocular responses to adrenaline were investigated (Fig.6).

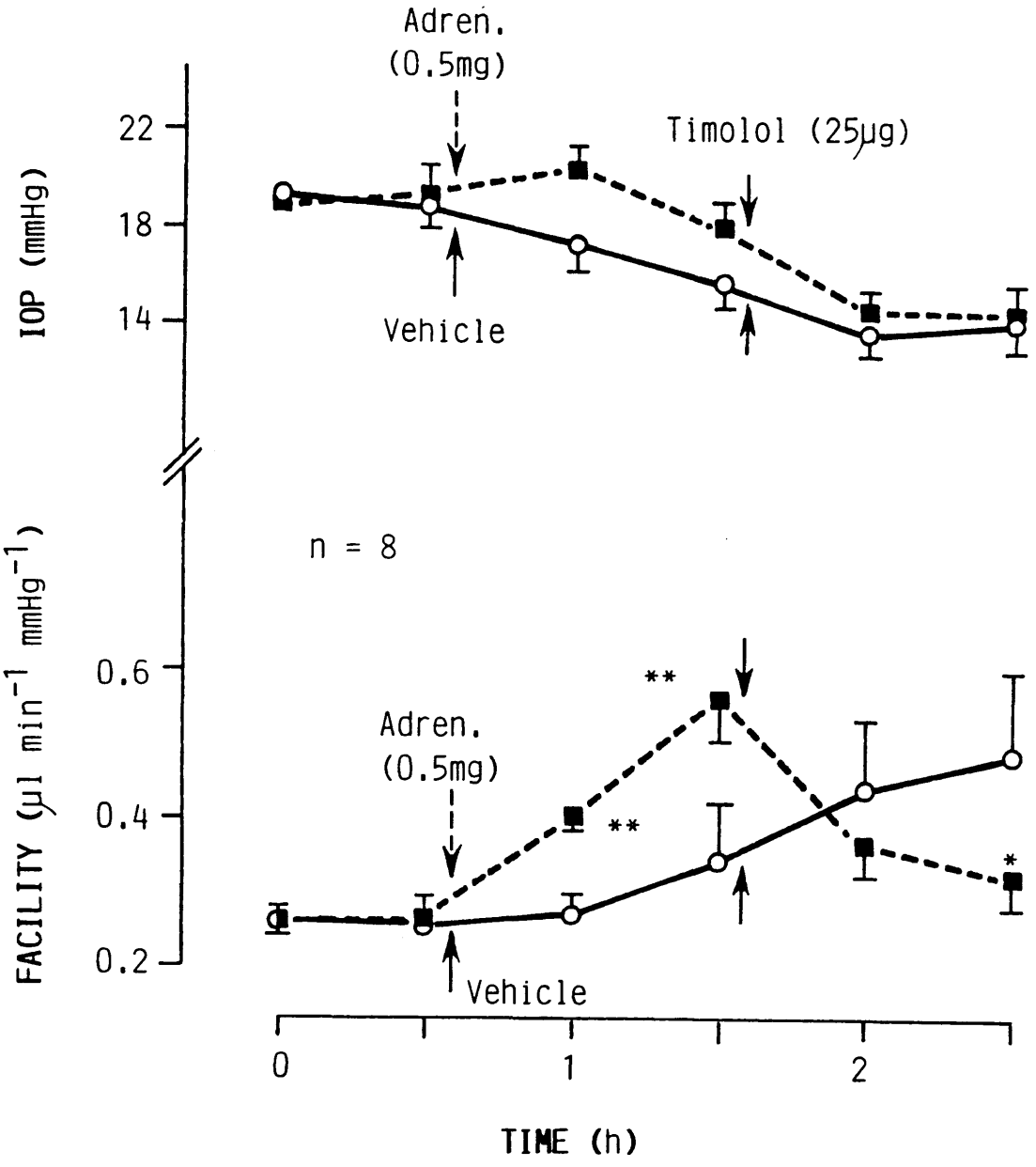

Fig. 6 The effect of topical timolol (--■--) on the ocular responses to topical adrenaline (-○-) in the anaesthetised rabbit. Each point is a mean ± SEM of results in 8 eyes. Significant differences between timolol-treated and vehicle control eyes are shown as *$0.05 \geq P \geq 0.01$; **$0.01 \geq P > 0.001$.

In these experiments adrenaline (0.5mg) was topically applied to one eye only, while the contralateral eye received its vehicle.

Again, after 1.5h adrenaline had increased facility of outflow from 0.27 $\pm$ 0.01 to 0.56 $\pm$ 0.06 μl min^{-1} mm^{-1} Hg ($\underline{P}$> 0.001). After 1.5 h IOP had not significantly changed. However, after 2.5 h IOP had fallen from 19.5 $\pm$ 1.1 to 14.9 $\pm$ 0.8mmHg (0.01> $\underline{P}$ $\geqslant$ 0.001).

Topical application of vehicle to the contralateral eye (Fig.6) significantly increased facility of outflow and lowered IOP. Facility of outflow had increased from 0.26 $\pm$ 0.01 at t=0.5h to 0.49 $\pm$ 0.12 μl min^{-1} mm^{-1} Hg at t=2.5 h. However, after 2.5h, facility of outflow was significantly lower (0.05$\geqslant\underline{P}\geqslant$ 0.01) than facility of outflow seen in the adrenaline-treated eye. IOP had fallen from 18.9 $\pm$ 0.9 at t=0.5 h, to 15.8 $\pm$ 1mm Hg at t=2.5h (0.05$\geqslant\underline{P}\geqslant$0.02). These values were not significantly different from those in the adrenaline-treated eye.

The Effect Of Topical Timolol Treatment On The Ocular Responses To Adrenaline In The Anaesthetised Rabbit.

The ocular responses to adrenaline in the presence of timolol are shown in Figs 5 and 6 . 1.5 h after the application of adrenaline (0.5mg) to both eyes, a solution of timolol (25ug) was topically applied to one eye only (Fig 5).

Timolol had no significant effect on the PD and IOP responses to adrenaline.

Although 1h after the application of timolol, IOP was still significantly lower than the starting IOP, ie at t=0.5h ($\underline{P} > 0.001$), it did not differ significantly from measurements taken 1h after the administration of adrenaline.

Similarly, following timolol treatment, PD remained unaltered but was still significantly greater than the original PD, ie measurements taken at t=0.5 h ($0.01 \geqslant \underline{P} \geqslant 0.001$).

After 0.5h and 1h, timolol had partially reversed the adrenaline-induced rise in facility of outflow (Table 1). These values were significantly lower (see table) than those 1h after the application of adrenaline. They are also significantly lower than facility of outflow values measured simultaneously in the contralateral eye which received vehicle only (Fig.6).

Fig.6 again shows the reversal of the adrenaline-induced rise in facility of outflow by timolol. Timolol lowers the facility of outflow from 0.56 $\pm$ 0.06 at t=1.5 h to 0.33 $\pm$ 0.04μl min^{-1} mm^{-1} Hg at t=2.5h ($0.01 \geqslant \underline{P} \geqslant 0.001$).

Of similar importance was the failure of timolol to reverse the gradual rise in facility of outflow seen during the perfusion experiment. 1h after timolol treatment, facility of

	C measurement ($\mu l\ min^{-1} mm^{-1} Hg$) following timolol treatment		
Drug treatment	0h	1/2 h	1h
Adrenaline - timolol	0.66 ± 0.04	0.48 ± 0.04**	0.42 ± 0.03***
Adrenaline - vehicle	0.56 ± 0.05	0.61 ± 0.03 N.S.	0.70 ± 0.04 N.S.

Table 1 The apparent facility of outflow response to topical adrenaline (0.5mg in both eyes): C values were measured in both the timolol-treated and control eyes at the time of timolol administration and 0.5 and 1h later. Each value represents the mean ± SEM of results from 7 eyes. Significances shown are those obtained by comparing outflow measurements taken at 0.5 and 1h with those taken at 0h: **$0.01 \geq P \geq 0.001$; ***$0.001 \geq P$.

outflow had further increased from 0.34 $\pm$ 0.03 at t=1.5h to 0.49 $\pm$ 0.12μl min^{-1} mm^{-1} Hg at t=2.5h ($0.05 > P \geq 0.02$)

Although IOP continued to fall in both eyes following timolol treatment it was probably not induced by saline or adrenaline.

The Effect Of Indomethacin Pretreatment On The Ocular Responses To Adrenaline In The Conscious Or The Anaesthetised Rabbit.

In the conscious rabbit topical pretreatment with indomethacin (0.125mg) for 30min reduced the hypertensive response to adrenaline (0.25mg), Fig 7. In the indomethacin-treated eye pressure increased from 26.2 $\pm$ 0.7 at t= -0.5 h to 30.3 $\pm$ 0.6mmHg at t= 1h. Although after 1h IOP was significantly higher than IOP at t= -0.5 h and -1.5 h ($0.01 \geqslant P \geqslant 0.001$ in both cases), it was significantly lower than IOP in the contralateral eye which received adrenaline only ($0.01 \geqslant P \geqslant 0.001$).

Of greater importance was the lack of a hypotensive response to adrenaline following topical indomethacin pretreatment (Fig 7). In the indomethacin + adrenaline-treated eye, IOP after 2 and 3h was not significantly different from IOP at t= -0.5h and t= -1.5h ($0.05 \geqslant P > 0.01$ in both cases). However, in the absence of indomethacin, adrenaline produced the expected fall in IOP. In the vehicle-treated eye, IOP had fallen from 26.4 $\pm$ 0.7 at t= -0.5 h to 24.1 $\pm$ 0.7mm Hg ($0.01 > P \geqslant 0.001$) after 2h and to 24.7 $\pm$ 0.5 ($0.01 \geqslant P > 0.001$) after 3 h.

The PD repsonses to adrenaline were unchanged following indomethacin pretreatment. After 1h PD was maximally increased in both eyes. In the indomethacin + adrenaline- treated eye, PD had increased from 6.9 $\pm$ 0.1 at t= -0.5h to 9.6 + 0.4mm ($0.01 \geqslant P \geqslant 0.001$) in the vehicle-treated eye. After 2,3 and 4 h, PD was still significantly higher than control values, ie t= -0.5h ($0.01 \geqslant P \geqslant 0.001$ at 2 and 3 h and $0.05 \geqslant P \geqslant 0.01$ at 4h).

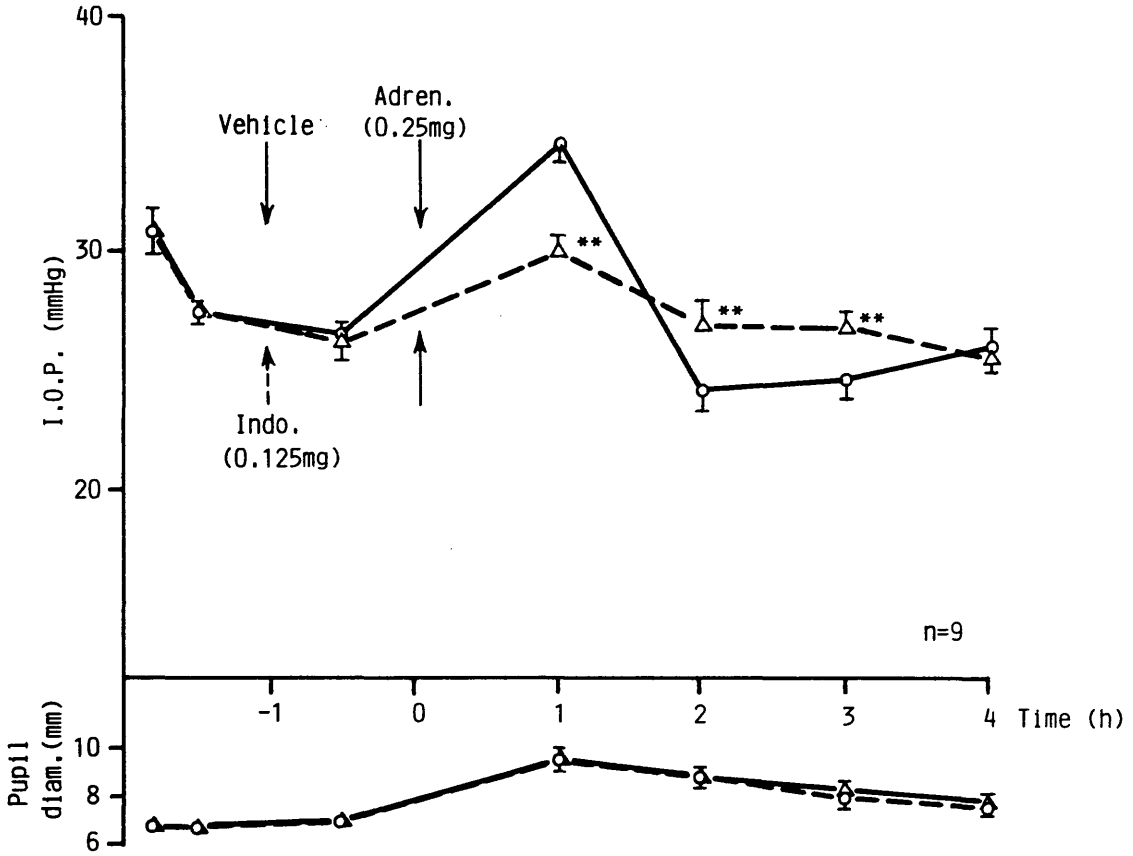

Fig. 7 The effect of pretreatment (at -1h) with indomethacin (--Δ--) or vehicle (-O-) on the ocular responses to topical adrenaline in the conscious rabbit. Each point is a mean ± SEM of values obtained in 9 eyes. Significant differences between indomethacin pretreatment and vehicle control are shown as **$0.01 \geq P \geq 0.001$.

The effect of topical indomethacin treatment on the ocular responses to adrenaline in the anaesthetised rabbit are shown in Fig.8

Following indomethacin pretreatment (0.125mg), adrenaline (0.5mg) failed to lower IOP. After 2h adrenaline had lowered IOP from 20.6 $\pm$ 1.9 (t=0.5 h) to 13.9 $\pm$ 0.9mmHg ($0.02 \geqslant P \geqslant 0.01$), whereas in the adrenaline + indomethacin-treated eye, IOP was unaltered (22.1 + 1.9mmHg at t= 0.5h and 19.9 $\pm$ 0.8mm Hg at t=2h). At t= 2h, IOP in the indomethacin-treated eye was significantly higher ($0.01 \geqslant P \geqslant 0.001$) than IOP in the contralateral eye which received vehicle only.

Of particular importance was the antagonism of the adrenaline-induced rise in facility of outflow by indomethacin. In the absence of indomethacin, adrenaline increases facility of outflow from 0.28 $\pm$ 0.01 at t= 0.5h to 0.67 $\pm$ 0.08μl min^{-1} mm^{-1} Hg at t= 2h ($P > 0.001$) but following indomethacin pretreatment adrenaline failed to increase facility of outflow which was 0.23 $\pm$ 0.02μl. min^{-1} mm^{-1} Hg at t= 0.5h and 0.32 $\pm$ 0.04μl min^{-1} mm^{-1} Hg at t= 2h. These values were not significantlly different.

In the presence or absence of indomethacin, adrenaline produced its expected mydriasis in both eyes. Adrenaline increased PD from 7.3 $\pm$ 0.7mm at t= 0.5h to 10.2 $\pm$ 0.5mm at t= 2 h. ($P > 0.001$) in the adrenaline vehicle-treated eye and from 7.7 $\pm$ 0.8mm at t= 0.5h to 11.5 $\pm$ 0.3mm at t= 2h ($P > 0.001$). Again PD remained increased for the duration of the experiment.

The effect of indomethacin alone on IOP, PD and the facility of outflow is shown in Fig.9.

Following the application of indomethacin (0.125mg) IOP

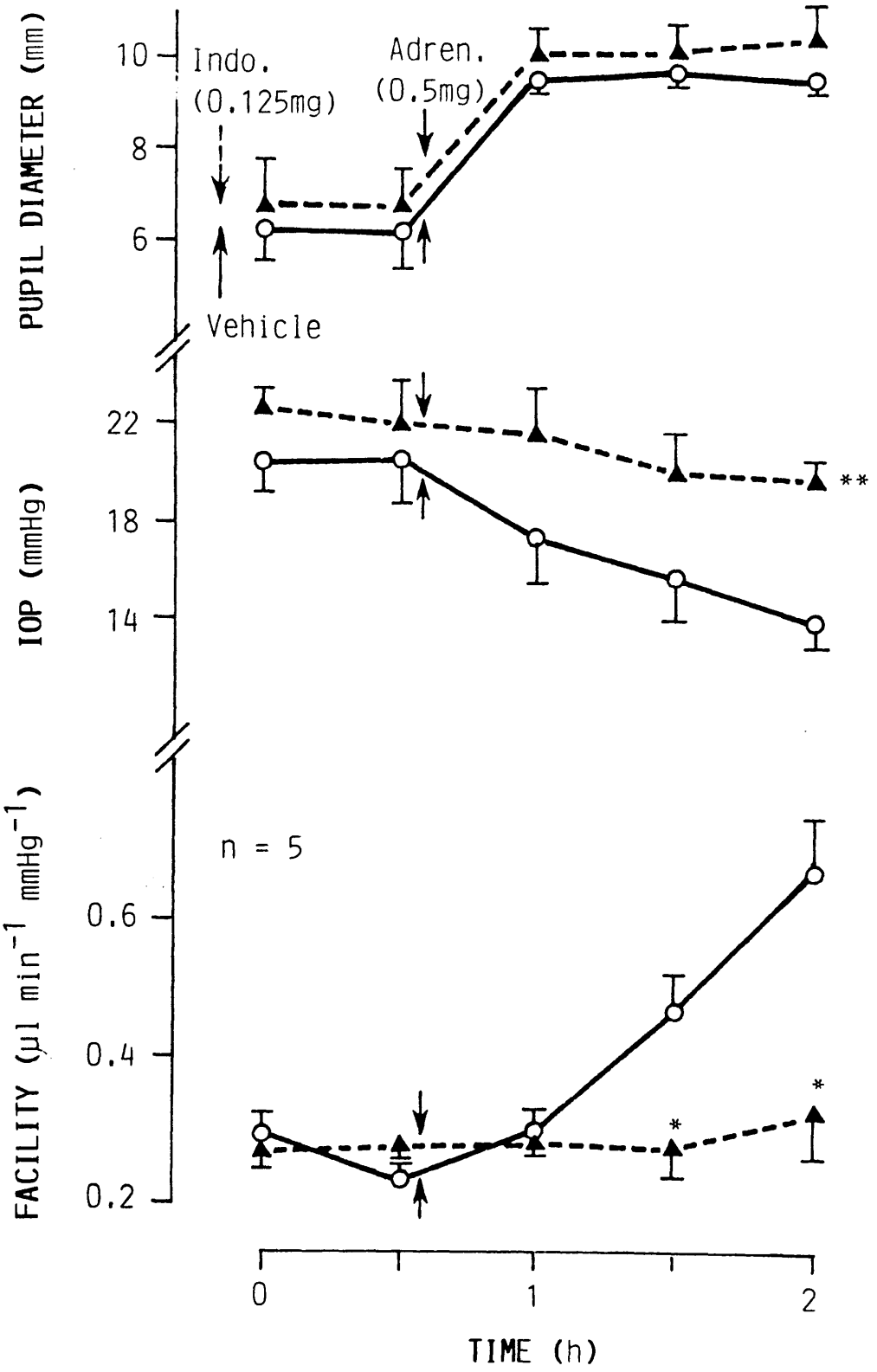

Fig. 8 The effect of pretreatment (at -30 min) with indomethacin (--▲--) or its vehicle (-○-) on the ocular response to topical adrenaline in the anaesthetised rabbit. Each point is a mean ± SEM of values obtained in 5 eyes. Significant differences between indomethacin-pretreatment and vehicle control are shown as *$0.05 \geq P \geq 0.01$; **$0.01 \geq P \geq 0.001$.

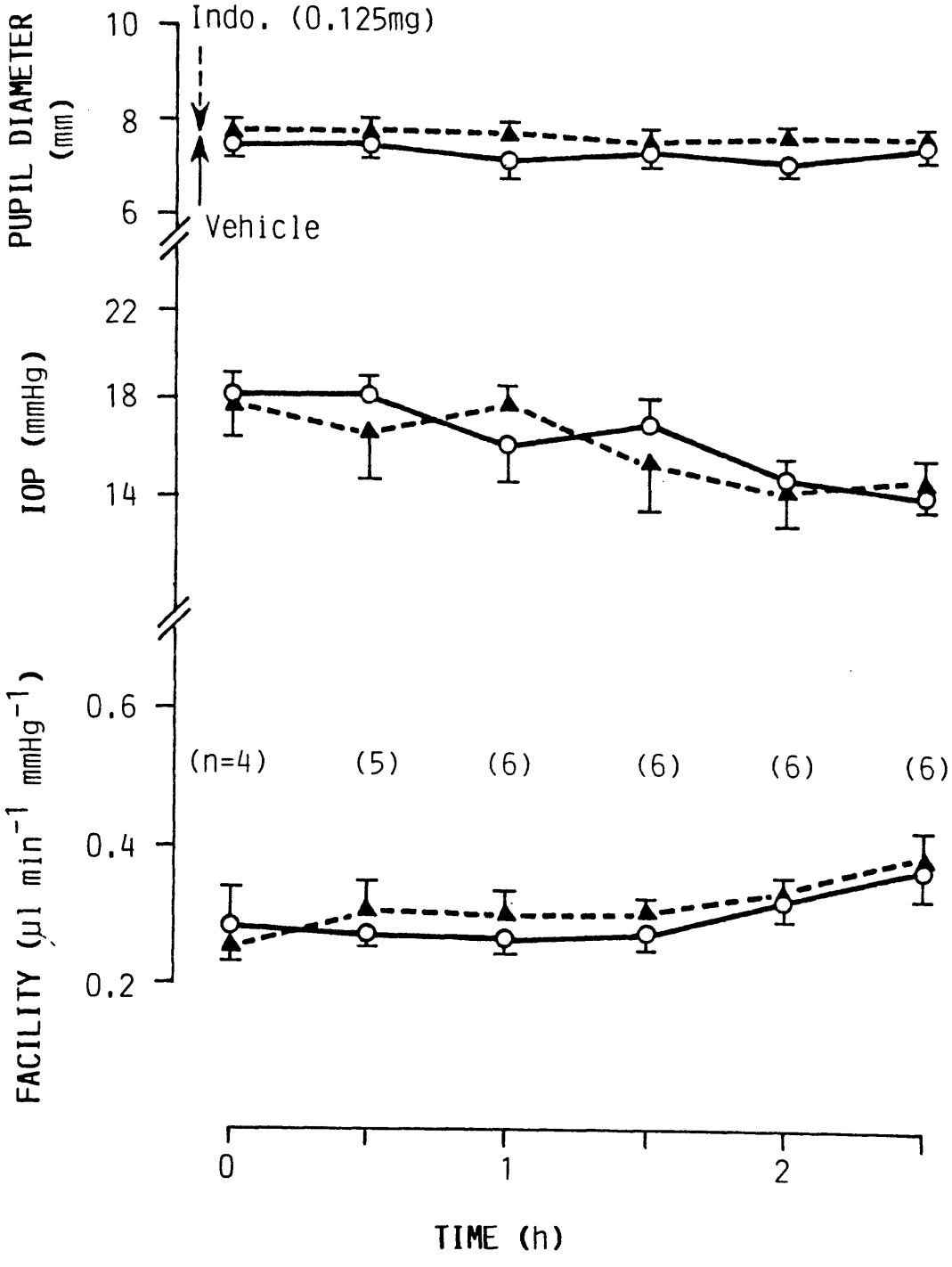

Fig. 9 Time course of the ocular effects of topical indomethacin in the anaesthetised rabbit. At -30 min drug solution was applied to one eye (--▲--) and vehicle to the contralateral eye (-○-). Each value is a mean ± SEM of values obtained in the number of eyes shown.

appears to fall from 17.7 ± 1.3 mmHg at t= 0.5h to 14.8 ± 1 mmHg at t= 2.5h. This fall in pressure was not significant. Similarly, following the application of the vehicle, IOP fell from 18.2 ± 0.8mmHg at t= 0.5h to 14.2 ± 0.7mmHg at t= 2.5h ($0.05 \geqslant P \geqslant 0.02$).

Topically applied indomethacin (0.125mg) had no effect on the slow but steady rise in facility of outflow which occurs spontaneously during the course of a perfusion experiment; facility of outflow increases from 0.25 ± 0.02 at t= 0 h to 0.39 ± 0.04µl min^{-1} mm^{-1} Hg at t= 2.5 h ($0.05 \geqslant P \geqslant 0.02$). A similar rise in facility of outflow from 0.28 ± 0.05µl min^{-1} mm^{-1} Hg at t= 2.5 h occurred following the application of the vehicle. This rise in outflow was not significant. Throughout the experiment there was no significant difference between outflow values in the indomethacin and vehicle-treated eyes.

After the application of either indomethacin or its vehicle to separate eyes, PD remained unaltered during the experiment.

The Effect Of Verapamil On The IOP And PD Responses To Adrenaline In The Conscious Rabbit

Topical application of verapamil (0.1mg), or its vehicle to the rabbit eye had no significant effect on either IOP or PD over a period of 4h (Fig.10). When the concentration of verapamil was increased to 0.25mg, verapamil appeared to lower IOP within 1h and after 2h IOP had fallen from 24.9 $\pm$ 0.5 to 19.5 $\pm$ 3.1mmHg (Fig 11). Although verapamil appeared to lower IOP in the treated and vehicle-treated eye, neither of these reductions in IOP were statistically significant. However, large doses of verapamil (0.5 to 1mg) produced dose-dependent, statistically significant, reductions in IOP in both the treated and contralateral untreated eye (data not shown).

The application of verapamil (0.25mg) or its vehicle did not alter PD through the course of the experiment.

Since 0.1mg of verapamil had no effect on IOP or PD, this dose and a very small dose of verapamil (0.05mg) were chosen to investigate the ocular responses to adrenaline in the presence of verapamil.

Topical application 0.05mg of verapamil had no significant effect on IOP or PD, nor did it alter either the pressure responses to adrenaline nor the adrenaline-induced mydriasis (data not shown). The effect of 0.1mg of verapamil on the IOP and PD responses to adrenaline are shown in Fig. 12

Although following topical pretreatment with verapamil adrenaline increased IOP from 25 $\pm$ 0.3 to 28.3 $\pm$ 0.8mm Hg ($P > 0.01$) after 1h, IOP was still significantly lower ($0.01 \geqslant P \geqslant 0.001$) than IOP in the contalateral eye which received adrenaline only. IOP had increased from 25.6 $\pm$ 0.5 at -0.5 to 33.7 $\pm$ 0.6mm Hg at t=1h ($0.01 \geqslant P \geqslant 0.001$).

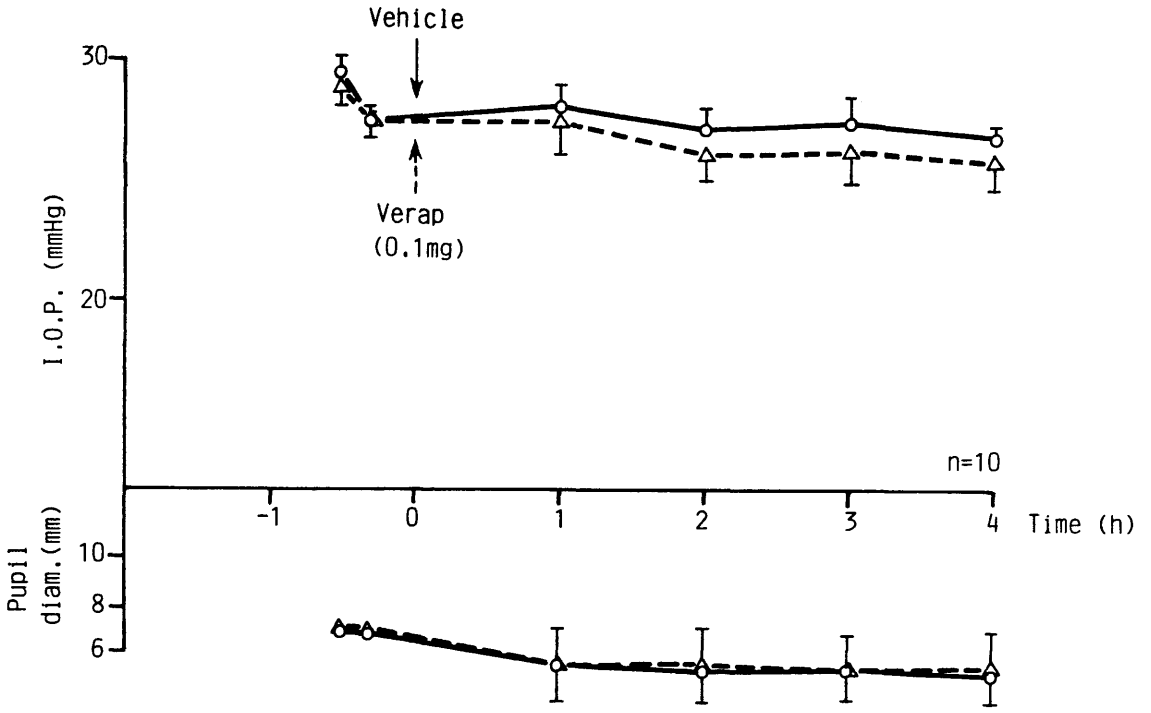

Fig. 10 The IOP and PD responses to topical verapamil (--Δ--) or its vehicle (-○-) in the conscious rabbit. Each point is a mean ± SEM of results in 10 eyes.

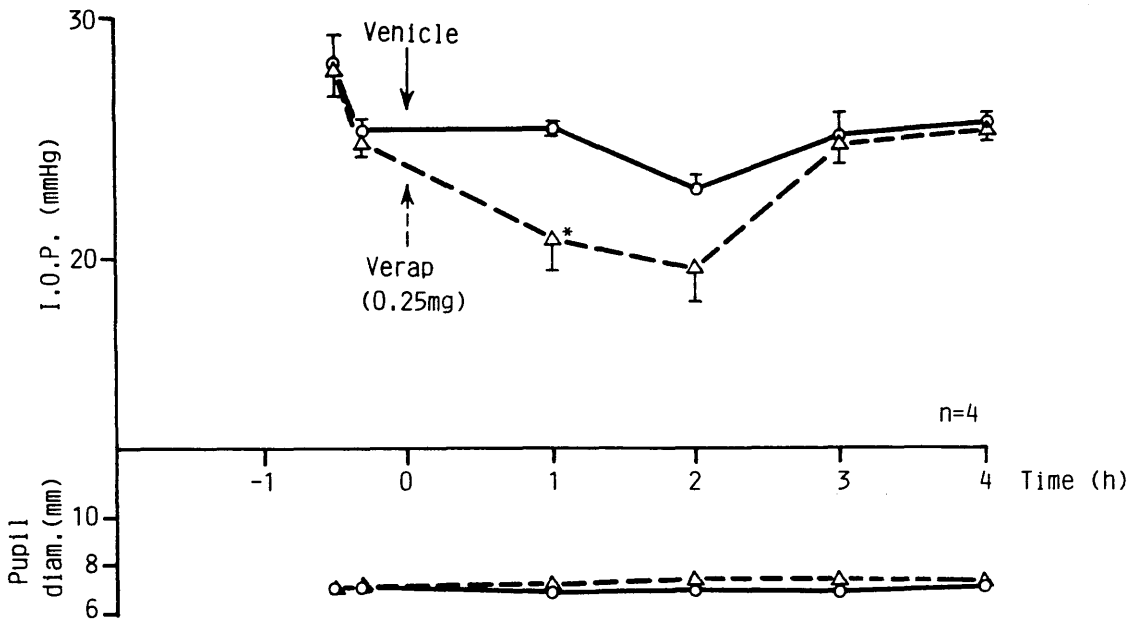

Fig. 11 The effect of topical verapamil (--Δ--) or its vehicle (-○-) on IOP and PD in the conscious rabbit. Each value is a mean ± SEM of results obtained in 4 eyes.

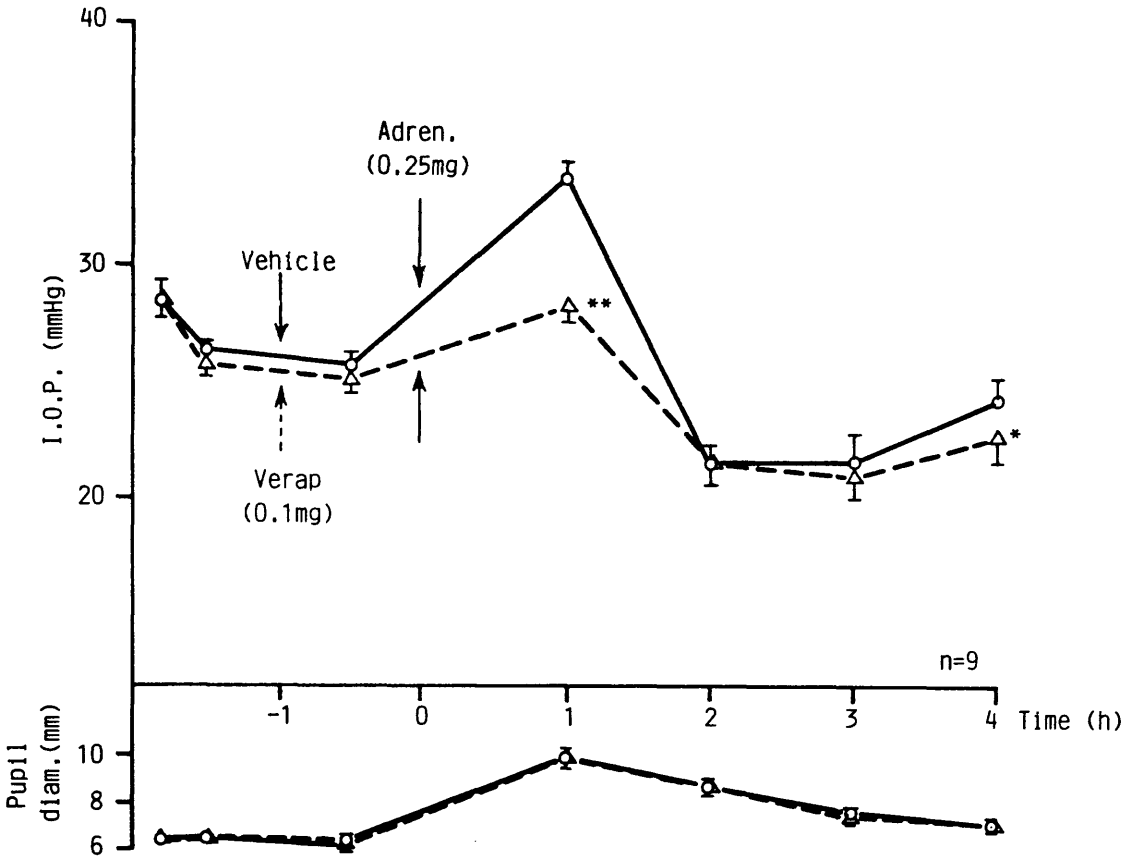

Fig. 12 The effect of pretreatment (at - 1h) with verapamil (--△--) or vehicle (-○-) on the ocular responses to topical adrenaline in the conscious rabbit. Each point is a mean ± SEM of values obtained in 9 eyes. Significant differences between verapamil - and vehicle - pretreated eyes are shown as *$0.05 \geq P \geq 0.01$; **$0.01 \geq P \geq 0.001$.

Of greater interest was the failure of verapamil to alter the hypotensive response to adrenaline. After 2 and 3h adrenaline significantly lowered IOP ($0.01 \geqslant \underline{P} \geqslant 0.001$ in both cases) the verapamil-and vehicle-pretreated eyes.

The adrenaline-induced mydriasis remained unaltered following verapamil pretreatment.

The Effect Of Systemic Aspirin Pretreatment On The Ocular Responses To Adrenaline In The Anaesthetised Rabbit.

Following the systemic administration of aspirin (approx. 200mg. kg^{-1}) the effect of indomethacin (0.125mg) on the ocular responses to adrenaline (0.5mg) were investigated. The response to these drugs in one population of animals are shown in Fig 13.

In the presence of aspirin, but in the absence of indomethacin, adrenaline lowered IOP from 17.1 $\pm$ 0.7 at t= 0h to 13.3 $\pm$ 0.5mmHg at t= 2h ($P > 0.001$). Following aspirin-pretreatment in IOP measurements taken in the indomethacin + adrenaline-treated eye and the vehicle + adrenaline-treated eye, the apparent drop in IOP from 16.4 $\pm$ 0.9mmHg at t= 0h to 14.8 $\pm$ 0.3mmHg at t= 2h, in the indomethacin + adrenaline-treated eye was not stastically significant.

Adrenaline raised facility of outflow from 0.28 $\pm$ 0.03µl min^{-1} mm^{-1} Hg at t= 0.5h to 0.60 $\pm$ 0.07µl min^{-1} mm^{-1} Hg ($P \geqslant 0.001$) at t= 2h in the absence of indomethacin. Following indomethacin pretreatment, facility of outflow was significantly lower at t= 1.5h and 2h ($0.05 \geqslant P \geqslant 0.01$ in both cases) in the indomethacin + adrenaline-treated eye compared to the vehicle + adrenaline - treated eye. However, the rise in facility of outflow from 0.28 $\pm$ 0.01 at t= 0.5h to 0.40 $\pm$ 0.03µl min^{-1} mm^{-1} Hg at t= 2h in the indomethacin + adrenaline - treated eye was significant ($0.01 \geqslant P \geqslant 0.001$).

After aspirin administration, adrenaline significantly increased PD in both the indomethacin-and-vehicle-treated eyes ($P > 0.001$ in both instances).

The above results were obtained in 9 out of 13 animals

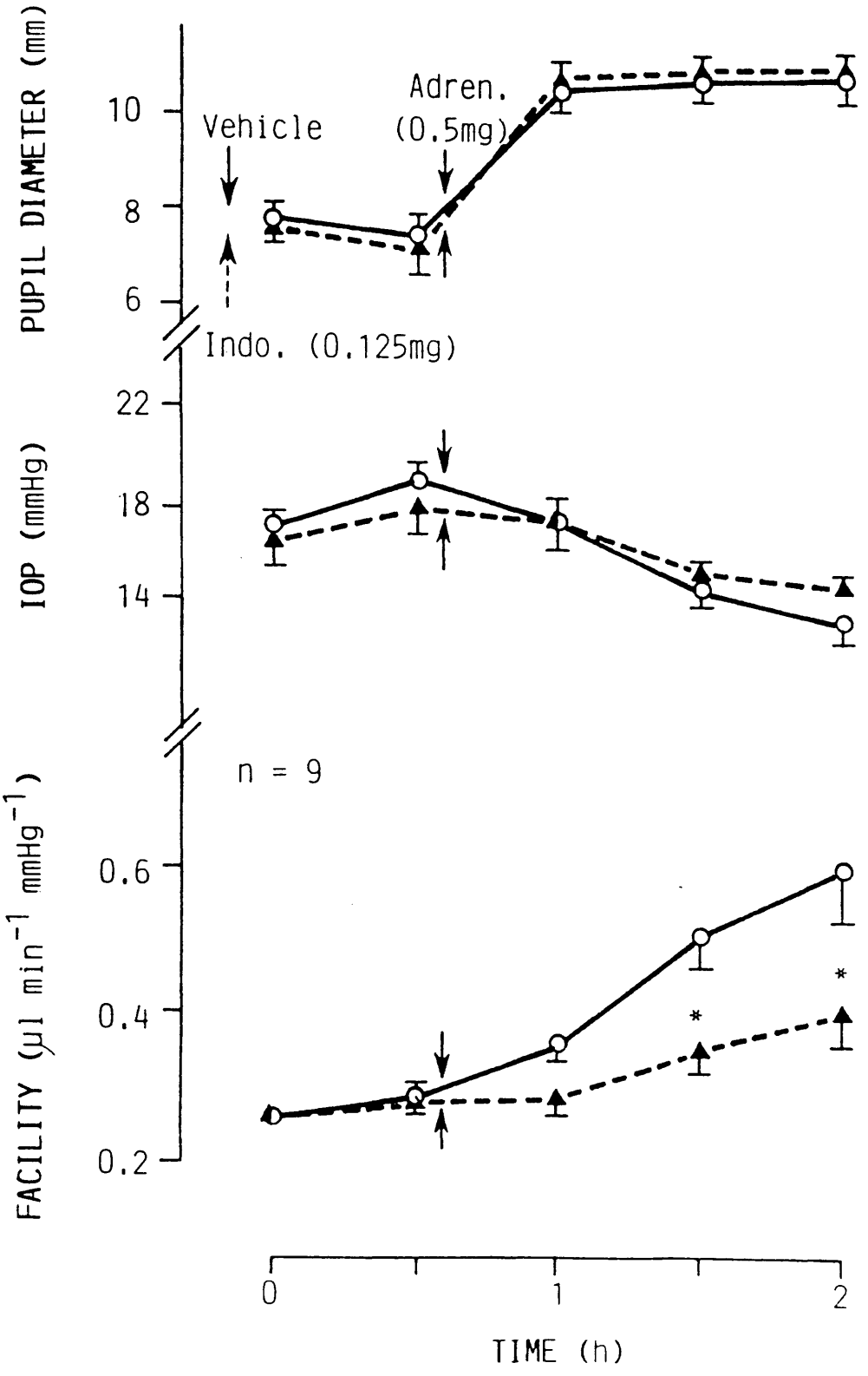

Fig. 13 The effects of systemic aspirin and indomethacin pretreatment on the ocular responses to adrenaline in the anaesthetised rabbit. Following the systemic administration of aspirin (approx. 200 mg kg^{-1}), indomethacin (--▲--), or its vehicle (—O—) were topically applied to the eye at -30 min. Adrenaline was then topically applied to both eyes as shown. Each point represents a mean ± SEM of values obtained in 9 eyes. Significant differences between indomethacin and vehicle pretreatment are shown as *0.05≥P≥0.01; **0.01≥P≥0.001.

tested. Because 4 animals showed no response to adrenaline in either eye, these were grouped together for the purpose of analysing the results (Fig 14). Adrenaline increased PD in both the indomethacin and vehicle-treated eye.

In these animals adrenaline failed to raise facility of outflow and lower IOP both in the presence and absence of indomethacin.

In both eyes the starting facility of outflow appeared to be abnormally high, but stayed constant throughout the experiment. In the vehicle + adrenaline-treated eye, IOP fell significantly during the course of the experiment ($0.05 \geqslant \underline{P} \geqslant 0.02$), whereas in the indomethacin + adrenaline-treated eye the fall in IOP was not significant.

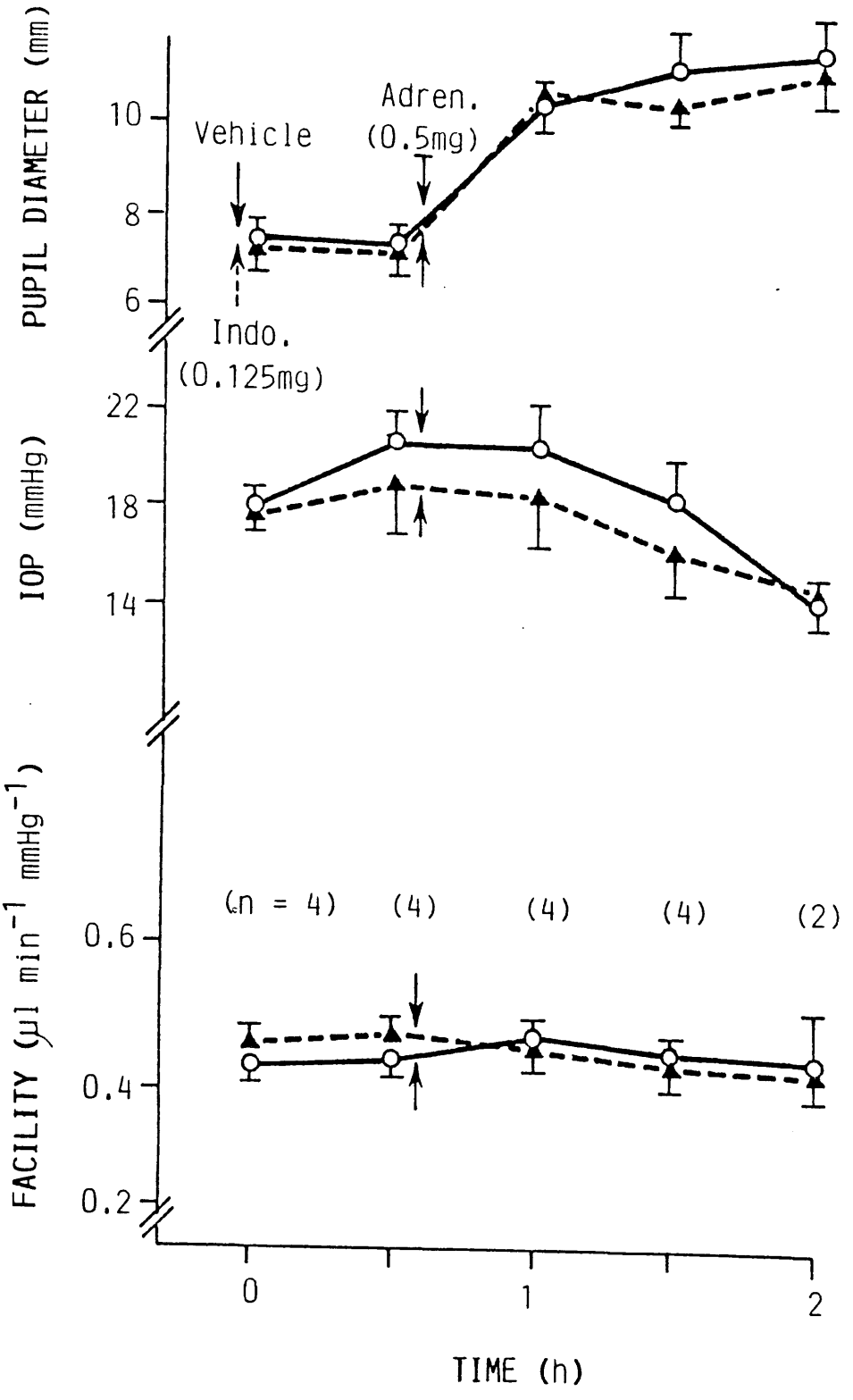

Fig. 14 Ocular responses in anaesthetised rabbits to topical adrenaline following systemic aspirin pretreatment (approx. 200mg kg^{-1}) and topical pretreatment with indomethacin (--△--) or its vehicle (-○-). Each point represents a mean ± SEM of values obtained in the number of eyes shown.

The Effect Of Piroxicam Pretreatment On The Ocular Responses To Adrenaline In The Conscious And/Anaesthetised Rabbit.

In the conscious rabbit, topical application of 0.05 mg of piroxicam (data not shown) or 0.25 mg of piroxicam (Fig.14) to the eye had no significant effect on either the IOP or PD during the course of the experiment. Increasing the concentration of piroxicam to either 2.5mg (data not shown) or 5mg (Fig.15) produced a dose-dependent fall in IOP in both the treated and untreated eyes. Although after 1h the fall in pressure from 26.4 $\pm$ 0.5 to 22 $\pm$ 0.6 mm Hg in the piroxicam treated eye was statistically significant ($0.05 \geqslant P \geqslant 0.002$) the apparent fall in pressure in the vehicle-treated eye was not. IOP had returned to pre-drug values (t= -0.5h) after 2h.

Several different doses of piroxicam (0.05mg to 5mg) had no effect on PD.

One hour after the application of piroxicam (0.25mg) to one eye only, adrenaline (0.25mg) was then applied to both eyes. Fig.16 shows that piroxicam has no effect on the biphasic pressure response to adrenaline. In both eyes adrenaline increases IOP after 1h ($0.05 \geqslant P \geqslant 0.01$) and by 2h IOP had returned to pre-adrenaline values ie t= -0.5h. After 3 and 4h adrenaline had significantly lowered IOP. Pressure had fallen from 25.4 $\pm$ 0.5 at t= -0.5h to 19.5 $\pm$ 0.4mmHg at t= 3h ($0.05 \geqslant P \geqslant 0.01$) in the piroxicam + adrenaline-treated eye and from 25.1 $\pm$ 0.5mmHg at t= -0.5h to 19.4 $\pm$ 0.4mmHg at t= 3h ($0.05 \geqslant P \geqslant 0.01$) in the vehicle + adrenaline-treated eye.

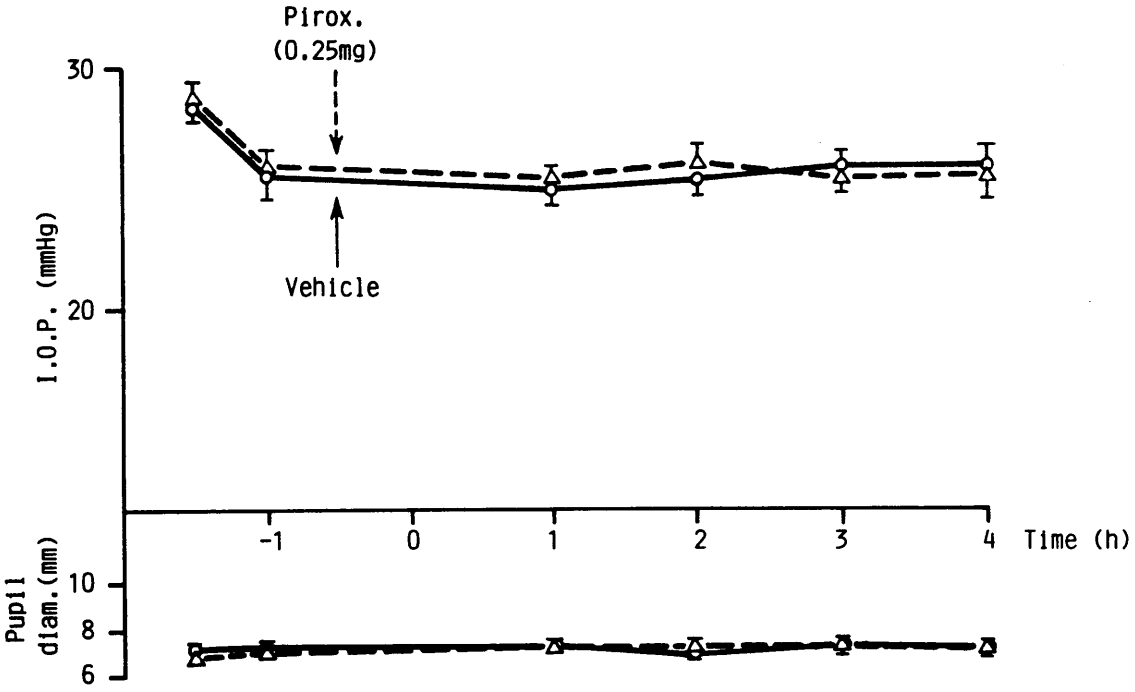

Fig. 15 Ocular response to topical piroxicam in the conscious rabbit. At -30 min drug solution was applied to one eye (--Δ--) and vehicle to the contralateral eye (-○-). Each value is a mean ± SEM of values found in 3 eyes.

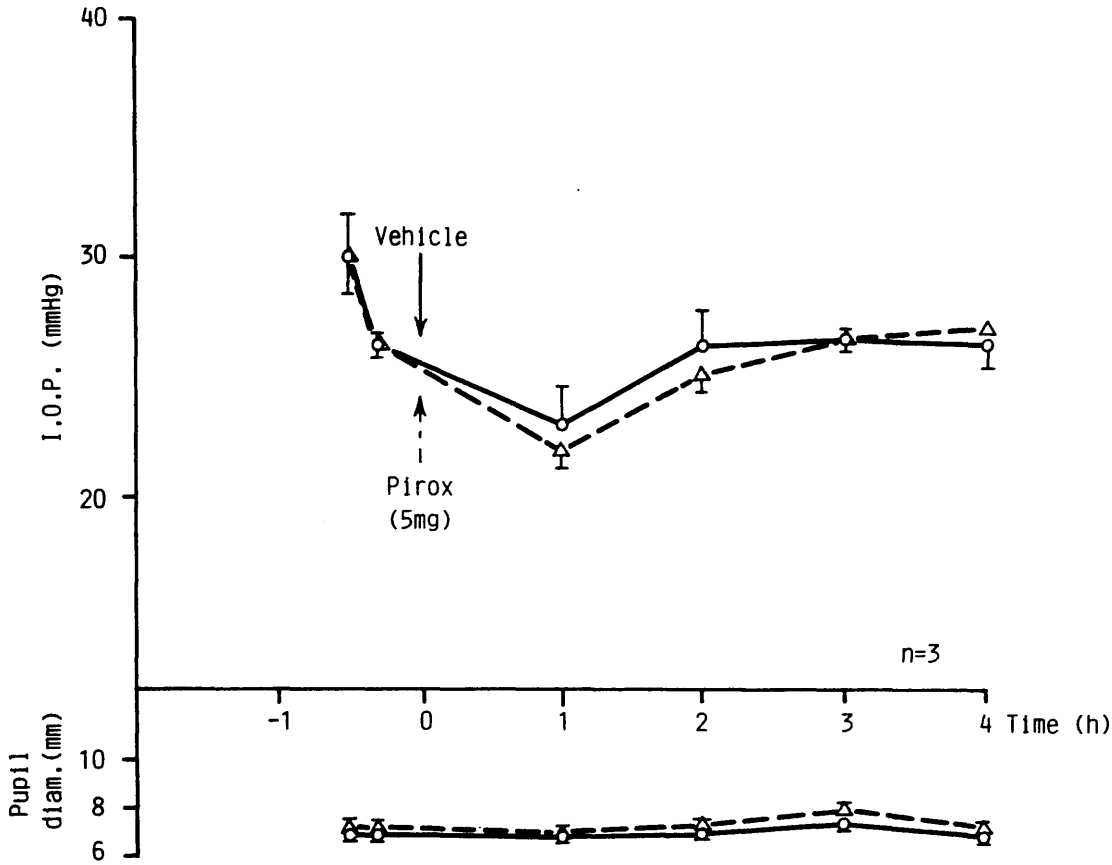

Fig. 16 IOP and PD responses to piroxicam in the conscious rabbit. At -30 min drug solution was applied to one eye only (--△--) and its vehicle to the contralateral eye (-○-). Each value is a mean ± SEM of values found in 9 eyes.

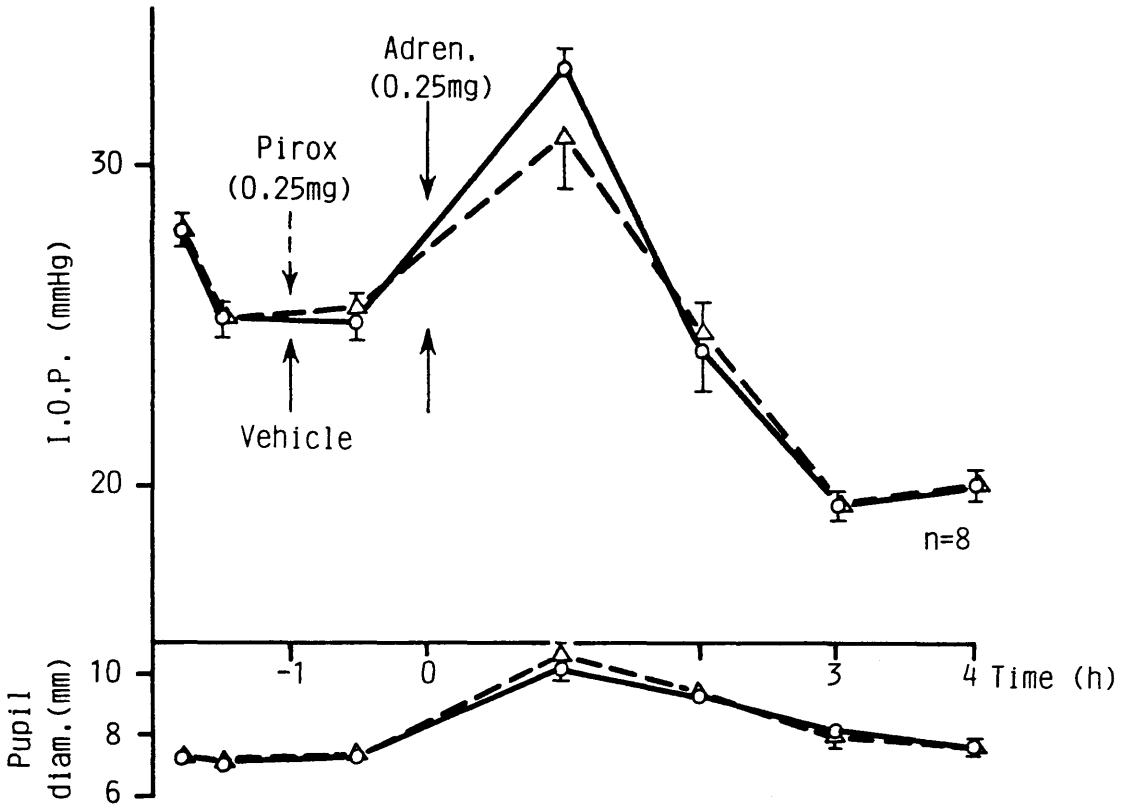

Fig. 17 The effect of pretreatment (at - 1h) with piroxicam (--Δ--) or its vehicle (-○-) on the ocular responses to topical adrenaline in the conscious rabbit. Each point is a mean ± SEM of values obtained in 8 eyes.

Again the adrenaline-induced mydriasis remained unaltered in the presence of piroxicam.

Since piroxicam (0.25mg) failed to inhibit the pressure responses to adrenaline, these results indicated that a higher dose of piroxicam should be tested. Unfortunately at this stage, the tonometric apparatus was no longer available to check such a response in conscious rabbits. A higher dose of piroxicam was therefore chosen to study the effect of piroxicam on the ocular responses, in particular the outflow responses, to adrenaline in the anaesthetised rabbit.

The ocular responses to piroxicam (0.75mg) and its vehicle in the anaesthetised rabbit are shown in Fig.18.

During the experiment a gradual rise in facility of outflow, from 0.24 $\pm$ 0.02 at t= 0.5h ($0.01 \geq P \geq 0.001$) to 0.33 $\pm$ 0.03μl.min.$^{-1}$ mm^{-1} Hg at t= 2.5h ($0.01 > P > 0.001$) was seen in the vehicle-treated eye. Such a rise in facility of outflow was unaltered by piroxicam since a similar rise from 0.24 $\pm$ 0.02 at t= 0.5h to 0.36 $\pm$ 0.03μl min^{-1} mm^{-1} at t= 0.5h ($0.01 \geq P \geq 0.001$), was seen in the piroxicam-treated eye (Fig.17). There was no significant difference between facility of outflow in both eyes throughout the experiment.

Topical pretreatment with piroxicam (0.75mg) for 30min, antagonised both the pressure and outflow responses but not the pupil responses to adrenaline (0.5mg) (Fig.19). In the vehicle-treated eye, adrenaline raised facility of outflow from 0.26 $\pm$ 0.01 at t= 0.5h to 0.52 $\pm$ 0.16μl min^{-1} mm^{-1} Hg at t= 2.5h ($0.01 > P > 0.001$), whereas in the presence of piroxicam, adrenaline failed to raise facility of outflow which was 0.28 $\pm$ 0.02μl.min.$^{-1}$ mm^{-1} Hg at t= 0.5 h and 0.29 $\pm$ 0.03μl min^{-1} mm^{-1} Hg at t= 2.5h. Compared to the vehicle + adrenaline-treated eye, facility of outflow was significantly lower in the piroxicam.

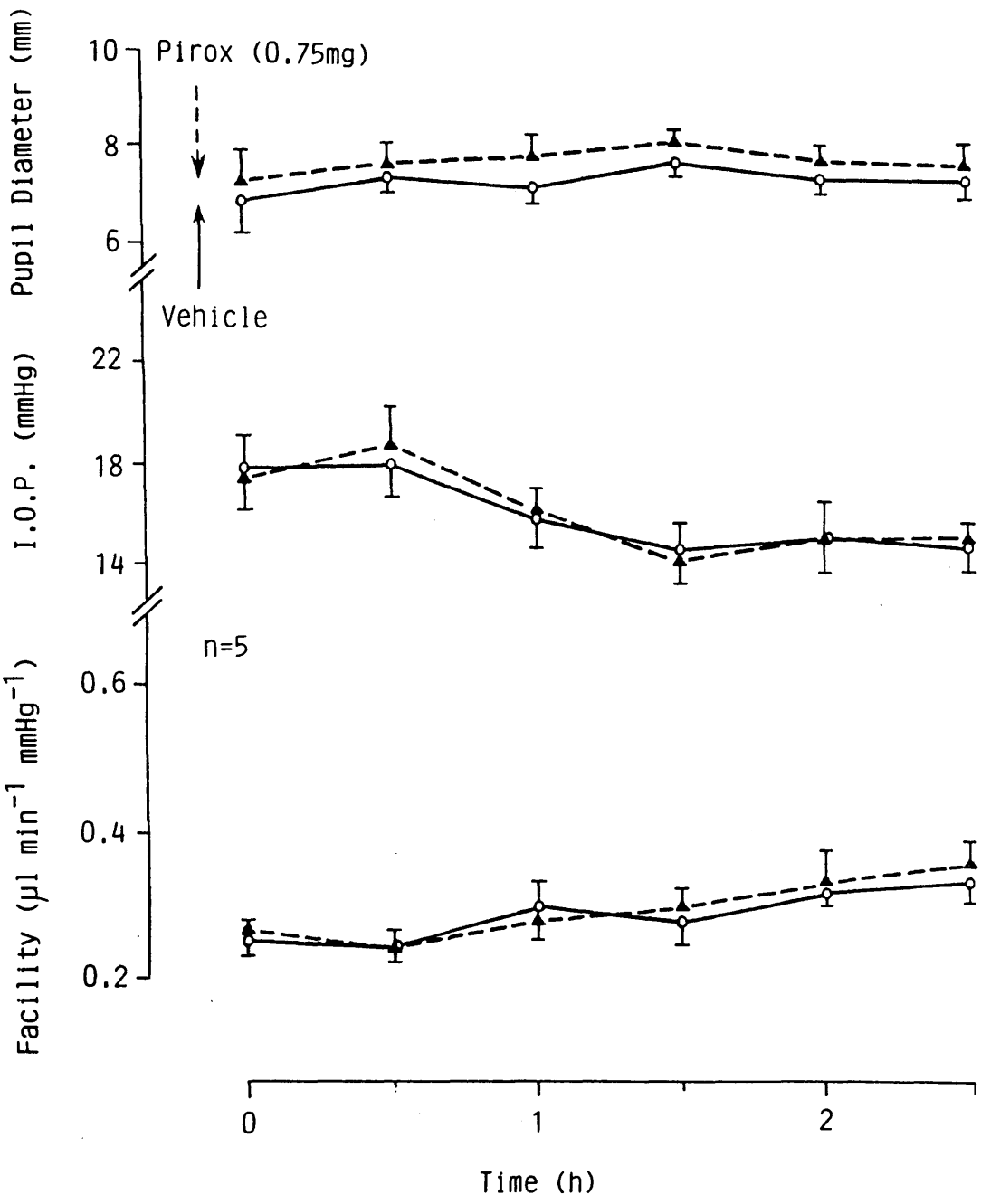

Fig. 18 Time course of the ocular responses to topical piroxicam in the anaesthetised rabbit. At -30 min drug solution was applied to one eye (--▲--) and vehicle to the contralateral eye (-○-). Each point is a mean ± SEM of values obtained in 5 eyes.

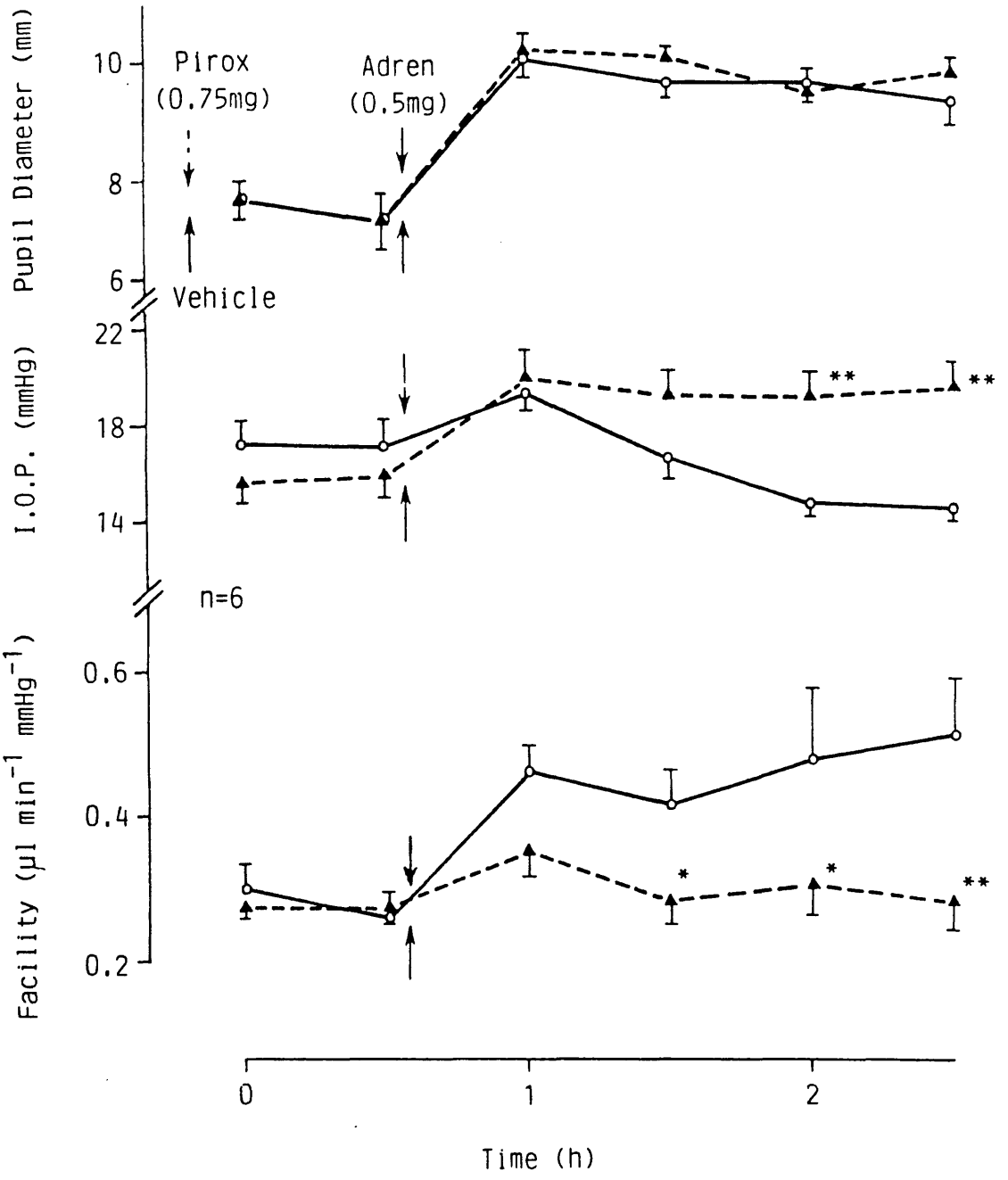

Fig. 19 The effect of pretreatment with piroxicam (--▲--) or vehicle (-O-) on the ocular responses to topical adrenaline in the anaesthetised rabbit. Each point is a mean ± SEM values obtained in 6 eyes. Significant differences between piroxicam-pretreatment and vehicle control are shown as *0.05>P>0.01; **0.01≥P>0.001.

adrenaline-treated eye at t= 1.5, 2 and 2.5h.

Similarly, adrenaline lowered IOP from 17.2 $\pm$ 1 at t= 0.5h to 14.7 $\pm$ 0.5mmHg at t= 2.5h ($0.05 \geqslant P \geqslant 0.02$) in the vehicle-treated eye. It appears that adrenaline has increased IOP in both eyes after 1h. Although adrenaline appears to raise IOP in the vehicle-treated eye ie from 17.2 $\pm$ 1 at t= 0.5h, to 19.4 $\pm$ 0.7mm Hg at t=1h, this was only verging on significance ie $0.1 \geqslant P \geqslant 0.05$. In the piroxicam-treated eye adrenaline significantly raises IOP from 16 $\pm$ 0.9 at t= 0.5h, to 19.9 $\pm$ 1.2mmHg at t= 1h ($0.05 \geqslant P \geqslant 0.02$).

However, in the same eye adrenaline had failed to lower IOP after 2.5h, at which time pressure was still significantly higher ($0.05 \geqslant P \geqslant 0.02$) than pre-adrenaline values, ie t= 0.5h.

The Effect Of Indomethacin And Piroxicam On The Ocular Responses To Analogues Of cAMP In The Anaesthetised Rabbit.

The results from this series of experiments are presented in a chronological order.

In 1982 Wilson and Campbell.A. reported that indomethacin antagonised the outflow responses to dcAMP. Using the previously described protocol for measuring facility of outflow initial experiments attempted to confirm this report.

The effect of indomethacin pretreatment on the ocular responses to dcAMP are shown in Fig 20. In the absence of indomethacin, injection of dcAMP (25.5µg) into the anterior chamber significantly increased facility of outflow ($0.02 \geqslant P > 0.01$) after 0.5h ie t= 1h. However, the maximal response to dcAMP occurred after 1h,ie t= 1.5h, at which time facility of outflow had increased from 0.27 ± 0.04 at t= 0.5h, to 0.60 ± 0.06µl min^{-1} mm^{-1}Hg ($P > 0.001$).

Following pretreatment with indomethacin (0.125mg) injection of dcAMP continued to increase facility of outflow ($0.05 \geqslant P \geqslant 0.02$) after 0.5h,ie t= 1h, but again the maximal response was apparent after 1h,ie t= 1.5h, at which time facility had risen from 0.25 ± 0.05 at t= 0.5h, to 0.35 ± 0.05µl min^{-1} mm Hg ($0.01 \geqslant P \geqslant 0.001$). Although facility increased following the injection of dcAMP, values in the indomethacin ± dcAMP-treated eye were significantly lower than those in the vehicle ± dcAMP-treated eye at t= 1, 1.5, 2 and 2.5h.

Although in the vehicle + dcAMP-treated eye, facility of outflow increased following the injection of dcAMP, IOP was not significantly altered during the experiment.

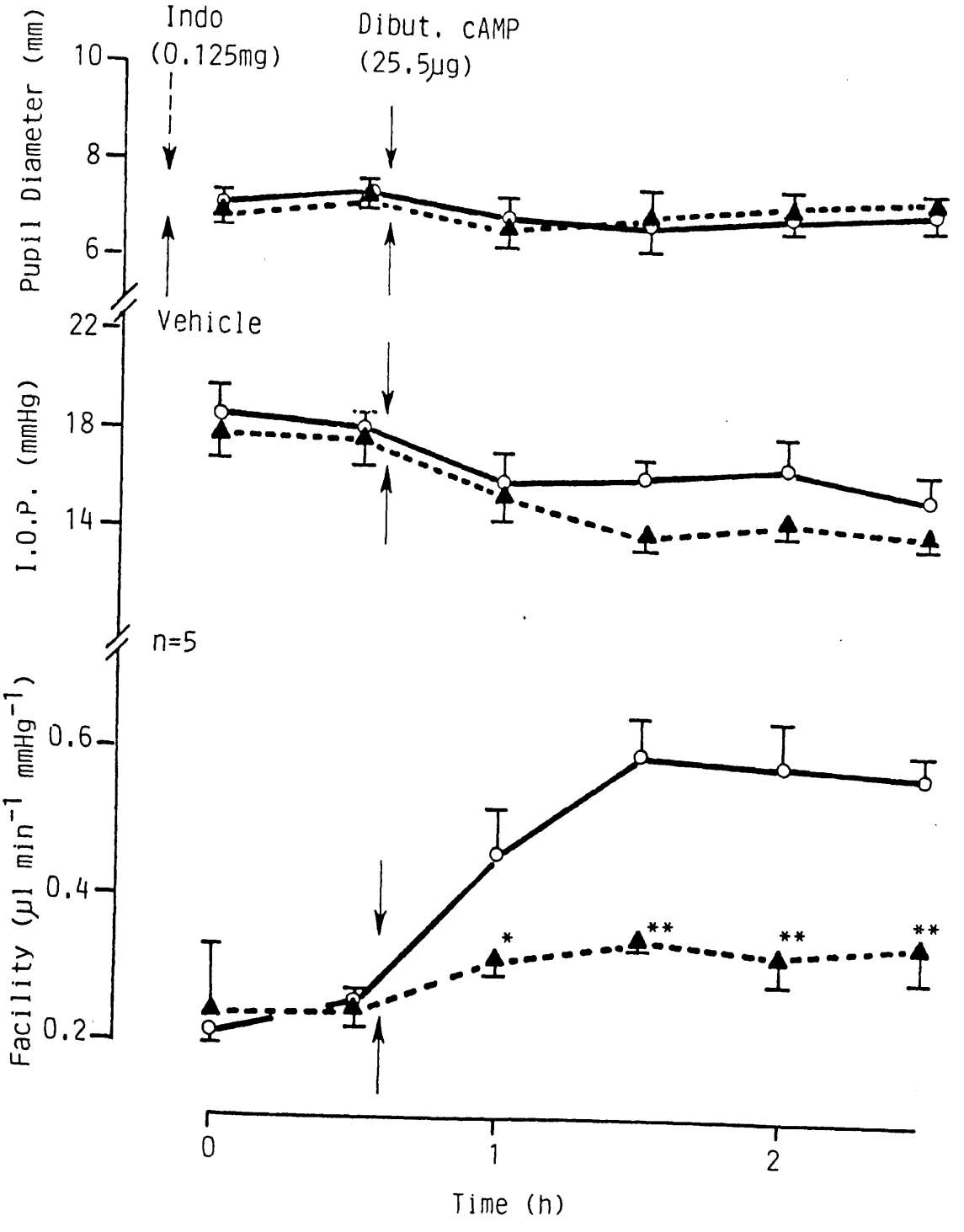

Fig. 20 The effect of topical pretreatment (at -30 min) with indomethacin (--▲--) or its vehicle (-○-) on the ocular responses to intracamerally injected dcAMP in the anaesthetised rabbit. Each point represents the mean ± SEM of results in 5 eyes. Significant differences between indomethacin - and vehicle - pretreated eyes are shown as *$0.05 \geq P > 0.01$; **$0.01 \geq P \geq 0.001$.

Even in view of the comparatively small rise in facility of outflow by dcAMP in the indomethacin + dcAMP-treated eye, IOP was significantly lower ($0.05 \geqslant P \geqslant 0.02$) after 1h and by 2h pressure had fallen from 17.9 $\pm$ 0.8mmHg at t= 0.5h, to 14.4 $\pm$ 0.5mmHg. At no time were there any significant differences between IOP in left and right eyes.

PD was unaffected by either dcAMP or indomethacin.

The responses to dcAMP (25.5 µg) following piroxicam (0.75mg)-pretreatment are shown in Fig.21.

In both the vehicle and piroxicam-treated-eyes facility of outflow was significantly higher ($0.05 \geqslant P \geqslant 0.02$ and $0.02 \quad P$ 0.01, respectively) 0.5h following the injection of dcAMP and remained higher for the remainder of the experiment. The rise was maximal after 1.5h, facility of outflow had increased from 0.27 $\pm$ 0.04 at t= 2.5h, to 0.63 $\pm$ 0.9µl.min.$^{-1}$ mm^{-1}Hg at t= 0.5h, ($P > 0.001$) to 0.58 $\pm$ 0.08µl.min.$^{-1}$mm^{-1}Hg in the piroxacam-treated eye. ($P > 0.001$).

IOP was significantly lowered in both eyes 1h after the injection of dcAMP. The peak fall in pressure occurred after 1.5h, IOP fell from 18.8 $\pm$ 2 at t= 0.5h, to 14.6 $\pm$ 2.6mm Hg ($P > 0.001$) at t= 2h in the vehicle-treated eye and from 16.2 $\pm$ 1.4mm Hg at t= 0.5h to 12.6 $\pm$ 1mm Hg ($P > 0.001$) at t= 2h in the piroxicam-treated eye.

Neither dcAMP nor piroxicam altered PD

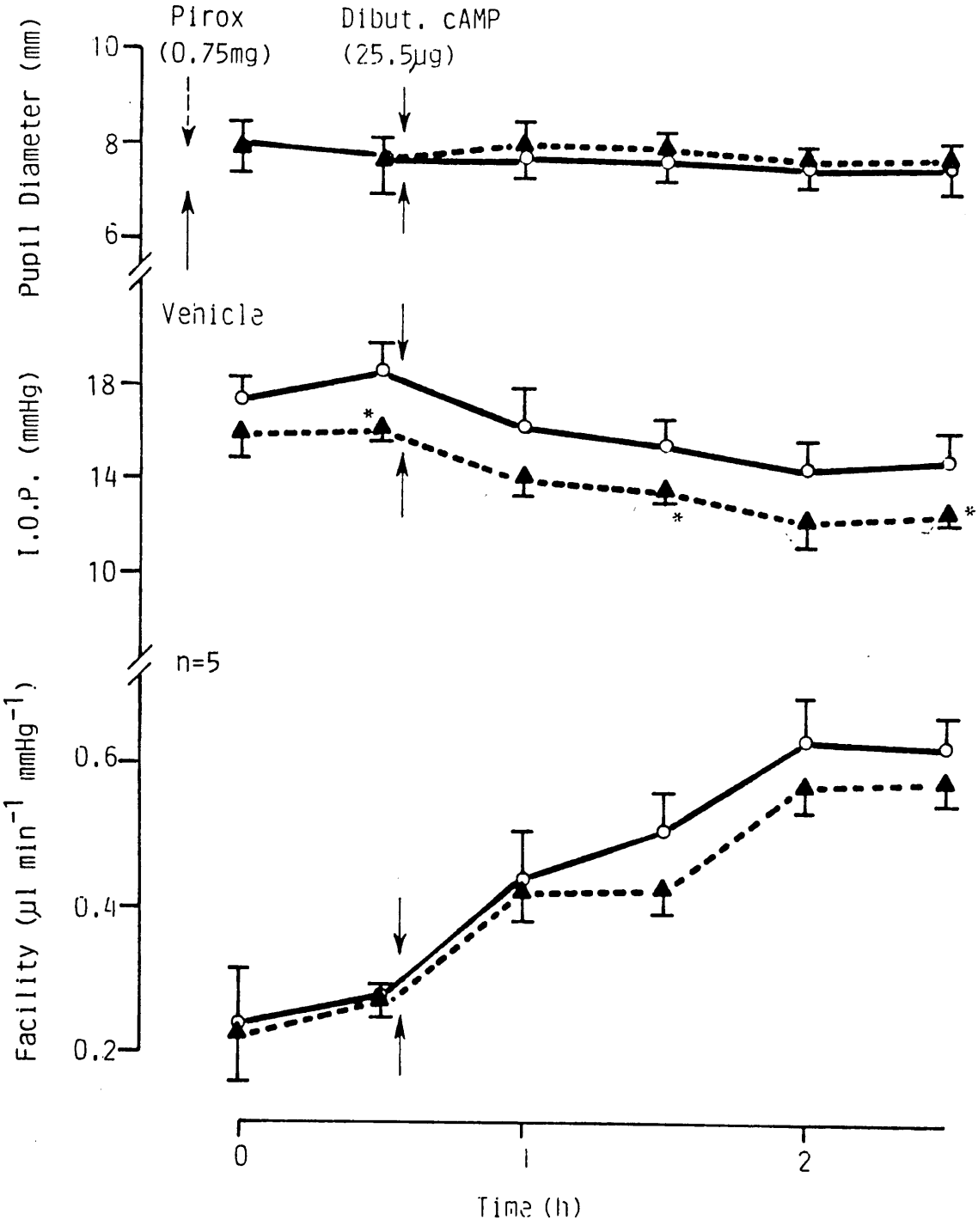

Fig. 21 The effect of topical pretreatment (at -30 min) with piroxicam (--▲--) or its vehicle (-○-) on the ocular responses to intracamerally injected dcAMP in the anaesthetised rabbit. Each point represents the mean ± SEM of results from 5 eyes. Significant differences between piroxicam and vehicle-pretreated eyes are shown as *$0.05 \geq P \geq 0.01$; **$0.01 \geq P \geq 0.001$.

The ocular responses to injected 8-bromo cAMP in the anaesthetised rabbit following topical pretreatment with piroxicam or its vehicle are shown in Fig.22.

In the vehicle-treated eye 8-bromo cAMP rapidly increases facility of outflow. The peak outflow response occurred only 0.5h after the injection of 8-bromo cAMP, at which time it had increased from 0.25 $\pm$ 0.04 at t= 0.5h, to 0.68 $\pm$ 0.13μl.min^{-1}. mm^{-1}Hg ($\underline{P} > 0.001$). Facility of outflow remained significantly higher than pre-8-bromo cAMP values throughout the experiment. Piroxicam pretreatment had no effect on this 8-bromo cAMP-induced rise in facility of outflow. Again 8-bromo cAMP rapidly raised facility of outflow with a maximal response 1h later; outflow had risen from 0.26 $\pm$ 0.03 at t= 0.5h, to 0.63 $\pm$ 0.10μl min^{-1}.mm^{-1} Hg ($\underline{P} > 0.001$).

In comparison with the outflow response to dcAMP, the rise in facility of outflow produced by 8-bromo cAMP appears to be more rapid in its onset.

Accompanying this rise in outflow was a fall in IOP in both eyes. In the vehicle-treated eye, 8-bromo cAMP produced a maximal fall in pressure after 1h, ie from 17.5 $\pm$ 1.5 at t= 0.5h, to 13.3 $\pm$ 1.6mm Hg at t= 1.5h. ($\underline{P} > 0.001$). Likewise in the piroxicam + dAMP-treated eye, pressure was at its lowest after 1.5h. IOP had fallen from 15.3 $\pm$ 2.4 at t= 0.5h, to 11.7 $\pm$ 0.8mm Hg at t= 1.5h ($\underline{P} > 0.001$). Although the pressure drop in both eyes followed a similar pattern, compared to the vehicle-treated eye,IOP was significantly lower at t= 0, 1 and 2h, in the piroxicam-treated eye.

Topical application of piroxicam or its vehicle, or the injection of 8-bromo cAMP,had no effect on PD.

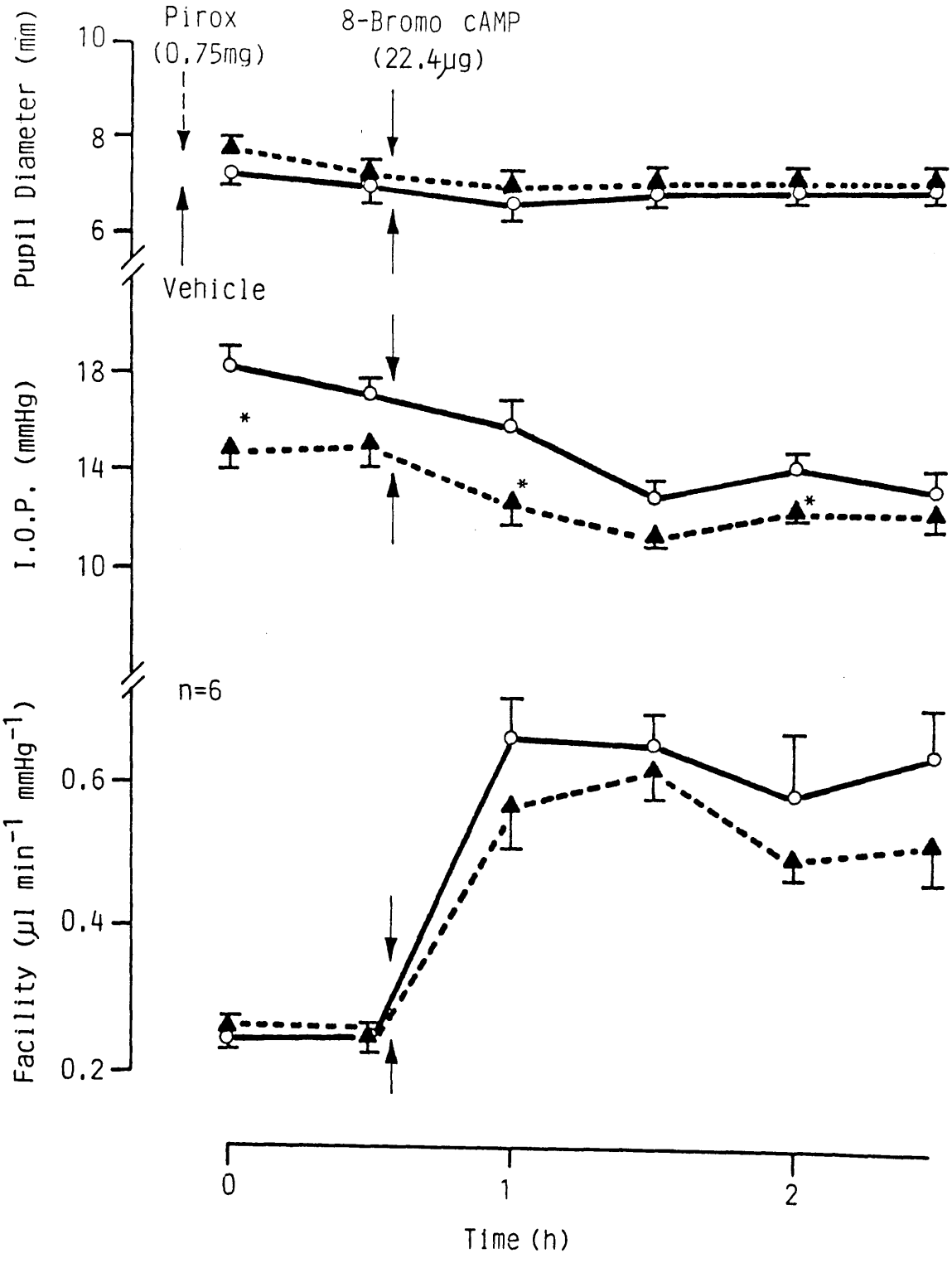

Fig. 22 The effect of piroxicam on the ocular responses to intracamerally injected 8-bromo-cAMP in the anaesthetised rabbit. 8-bromo-cAMP was injected into the anterior chamber of each eye following topical pretreatment (at -30 min) with piroxicam (--▲--) or its vehicle (-○-). Each point represents the mean ± SEM of values obtained in 6 eyes. Significant differences between piroxicam-and-vehicle-pretreated eyes are shown as *$0.05 \geq P > 0.01$; **$0.01 \geq P \geq 0.001$.

The Effect Of The Intraocular Injection Of dcAMP On PD, IOP And The Apparent Facility Of Outflow In The Anaesthetised Rabbit.

In this experiment dcAMP (25.5µg) was injected into the anterior chamber of the eye while its vehicle was injected into the contralateral eye. (Fig 23) Although injection of saline appeared to increase facility of outflow this rise was only statistically significant 1.5 and 2h after the injection. ($0.05 \geqslant P \geqslant 0.02$ in both cases). After 2h outflow had increased from 0.29 ± 0.04 at t= 0.5h, to 0.68 ± 0.27 µl min^{-1} mm^{-1} Hg. However, the outflow responses to injected vehicle were variable as indicated by the large standard errors.

Injection of dcAMP rapidly raised facility of outflow. This rise was statistically significant after 1, 1.5 and 2h. ($P > 0.001$ in all cases) This rise was maximal after 1h; outflow had increased from 0.29 ± 0.05 at t= 0.5h, to 0.81 ± 0.15µl min^{-1} mm^{-1} Hg. As a result of the large standard errors in the data from the vehicle - treated eye there is no apparent difference between outflow values in the vehicle and dcAMP treated eye.

Following the injection of saline, IOP was unaltered in all but one instance. 1.5h after the injection of saline IOP had fallen significantly from 15.8 ± 2 at t= 0.5h to 11.7 ± 1.9mm Hg at t= 2h ($0.05 \geqslant P \geqslant 0.02$) However, in the dcAMP-treated eye IOP was significantly lower after 1.5, 2 and 2.5h. After 2.5h IOP had fallen from 16.5 ± 0.9 at t= 0.5h, to 12.4 ± 1mm Hg ($0.05 \geqslant P \geqslant 0.02$) There were no significant differences between IOP values in the vehicle- and dcAMP-treated eyes.

As expected injection of dcAMP or its vehicle had no effect on PD.

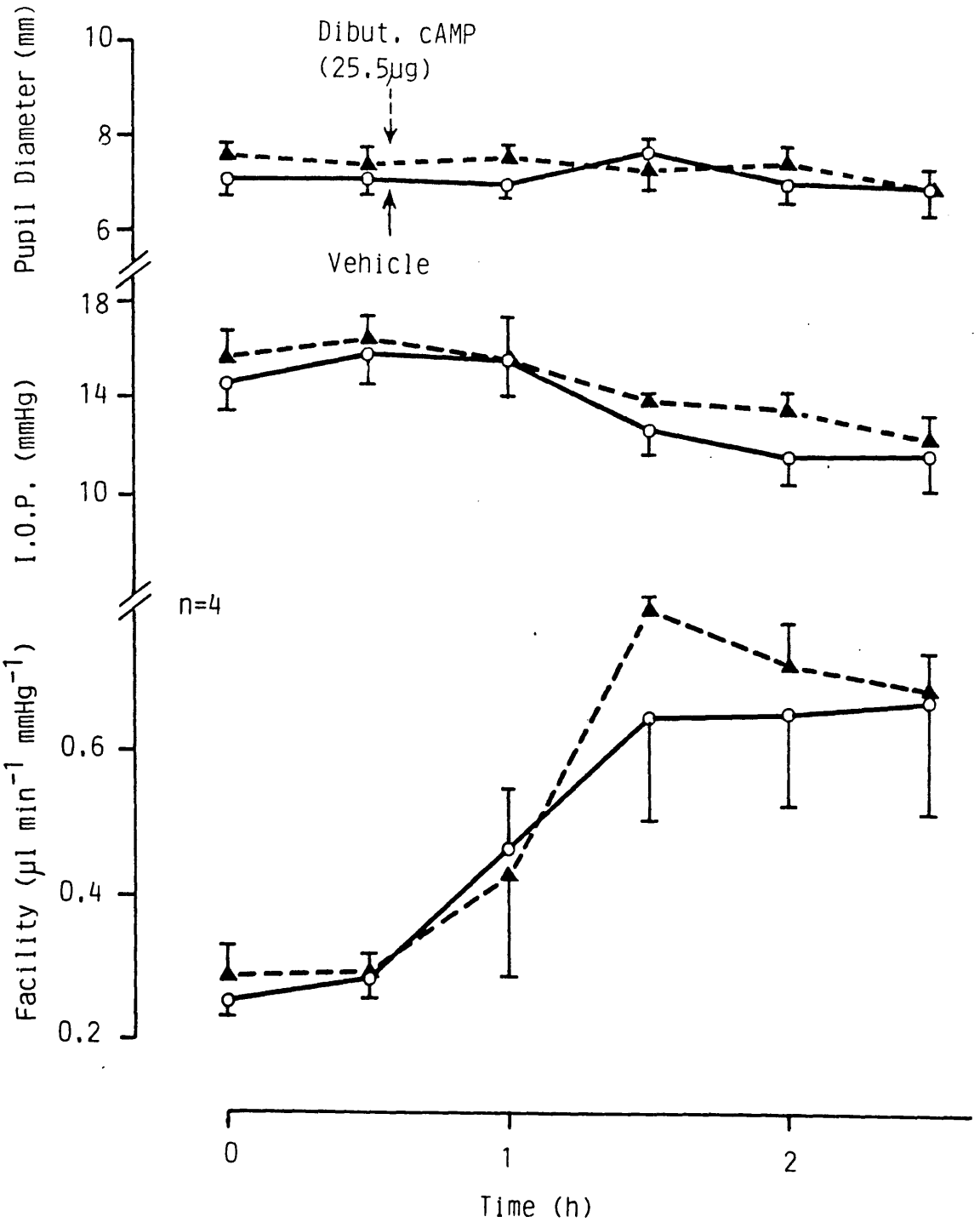

Fig. 23 The ocular responses to the injection of dcAMP (--▲--) or its vehicle (-O-) into the anterior chamber of the anaesthetised rabbit. Each value is a mean ± SEM of results obtained in 4 eyes.

The Effect Of The Intraocular Injection Of Saline On IOP, PD And The Apparent Facility Of Outflow In The Anaesthetised Rabbit.

From the aforementioned experiments it appeared that the injection of saline into the anterior chamber increased facility of outflow. Alternatively, the injection of dcAMP into one eye in some way may have produced or altered the response to saline in the contralateral eye. Therefore, in the next series of experiments facility of outflow was measured before and after the injection of 5 µl saline (0.9% w/v) into the anterior chamber. Although 2 needles were also inserted into the anterior chamber of the contralateral eye saline was not injected. Fig.25 shows the results of these experiments.

Although saline was injected into one eye only, facility of outflow appeared to rise rapidly and steadily in both eyes during the experiment. In the saline-treated and untreated eye experimental variation was marked, which is reflected in the large standard errors. Therefore, in the saline-treated eye, facility of outflow was not significantly higher until 2h after saline injection, at which time outflow had risen from 0.27 $\pm$ 0.02 at t= 0.5h, to 0.72 $\pm$ 0.13 µl min^{-1} mm^{-1} Hg ($0.02 \geqslant \underline{P} \geqslant 0.01$). Similarly, in the untreated eye the increase in facility above starting values became significant at t= 2.5h. At t= 2.5h facility had increased from 0.40 $\pm$ 0.08 to 0.78 $\pm$ 0.16 µl min^{-1} mm^{-1} Hg ($0.02 \geqslant \underline{P} \geqslant 0.01$).

In the untreated eye IOP had not fallen significantly during the experiment. However, 1h after the injection of saline IOP had decreased ($0.01 \geqslant \underline{P} \geqslant 0.001$). The fall in IOP was maximal at t= 2.5h, having fallen from 17 $\pm$ 0.5 at t= 0.5h to 11.4 $\pm$ 0.7 mm Hg ($\underline{P} > 0.001$).

PD was not altered after the injection of saline.

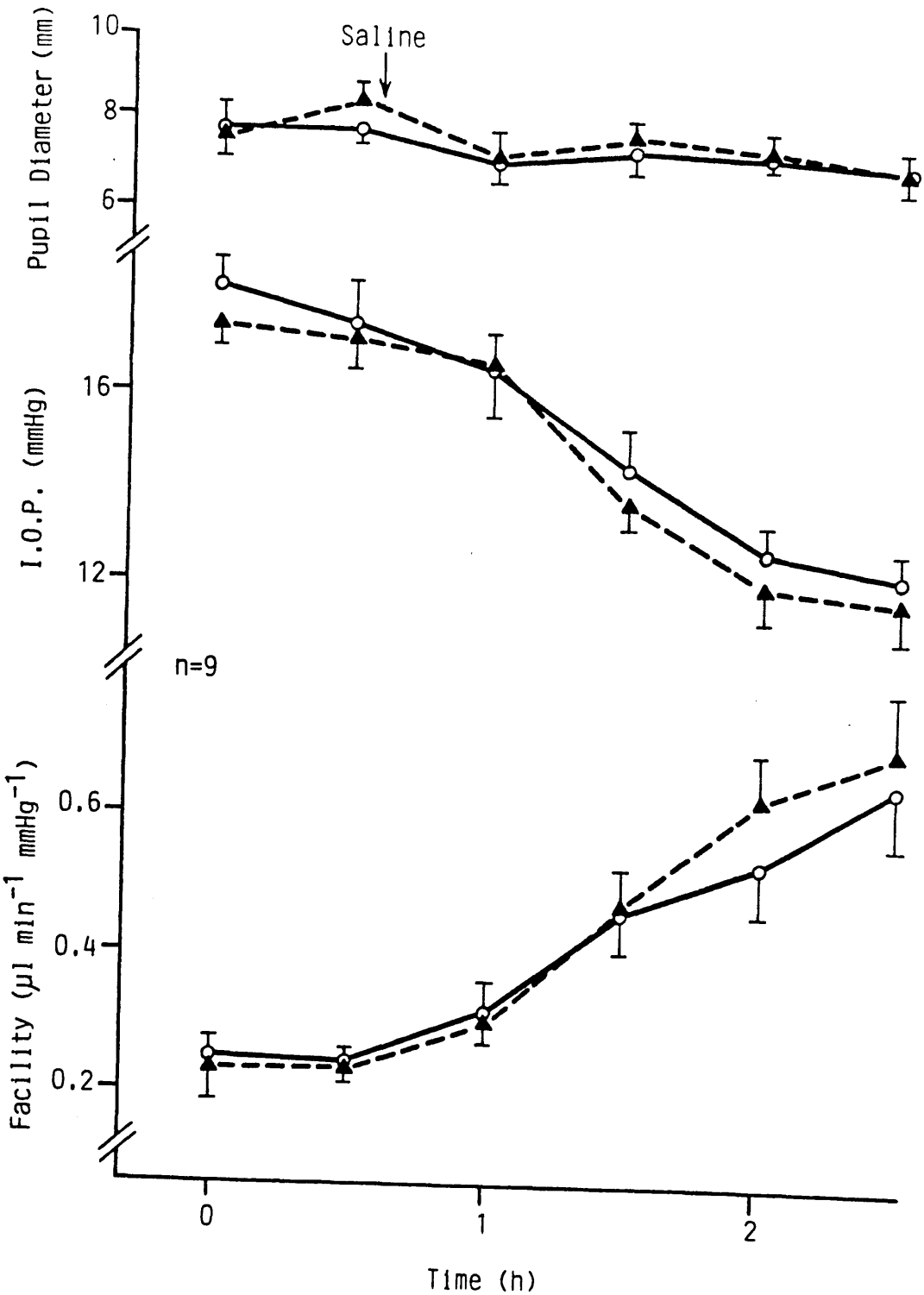

Fig. 24 The ocular responses to intracamerally injected saline in the anaesthetised rabbit. Saline was injected into the anterior chamber (--▲--) via a separate needle which was inserted into each eye at the beginning of the experiment. The contralateral eye was untreated (-○-). Each point represents the mean ± SEM of values obtained in 9 eyes.

However, 1h after the injection of saline IOP had decreased ($0.01 \geqslant P \geqslant 0.001$). The fall in IOP was maximal at t= 2.5h, having IOP had fallen from 17 $\pm$ 0.5 at t= 0.5h to 11.4 $\pm$ 0.7 mm Hg ($P > 0.001$).

PD was not altered after the injection of saline.

The Effect Of Saline Injection On IOP, PD And The Apparent Facility Of Outflow In The Anaesthetised Rabbit Following The Insertion Of Only A Perfusion Needle Into The Anterior Chamber.

Since trauma to the eye, induced by the insertion of 2 needles and/or the presence of these needles within the chamber, may have been responsible for the varied and apparently pathological response of the eye to saline injection, the following modifications were made.

Prior to needle insertion, a small metal "T" piece was attached to the perfusion needle by silicone rubber tubing. One branch of this "T" piece was attached, again using silicone rubber tubing, to a Hamilton microsyringe. The second branch was connected to the perfusion apparatus as previously described. This arrangement enabled both injection into and perfusion of the chamber to be carried out using only 1 needle. Fig 24 illustrates the ocular responses to injected saline using this apparatus. Following the injection of 5 µl of saline (0.9%, w/v) into one eye only, facility of outflow increased and IOP fell in both eyes. PD was unaltered.

In the saline-treated and untreated eye facility of outflow was significantly greater at t= 1.5h and later, than the starting value at t= 0.5h. In both cases the rise was apparently maximal after 2.5h. In the saline-treated eye facility of outflow had increased from 0.25 $\pm$ 0.01 at t= 0.5h, to 0.65 $\pm$ 0.08 µl min^{-1} mm^{-1} Hg at t= 2.5h ($P > 0.001$) and in the untreated eye from 0.25 $\pm$ 0.02 at t= 0.5h to 0.70 $\pm$ 0.1µlmin^{-1} mm^{-1} Hg at t= 2.5h ($P > 0.001$).

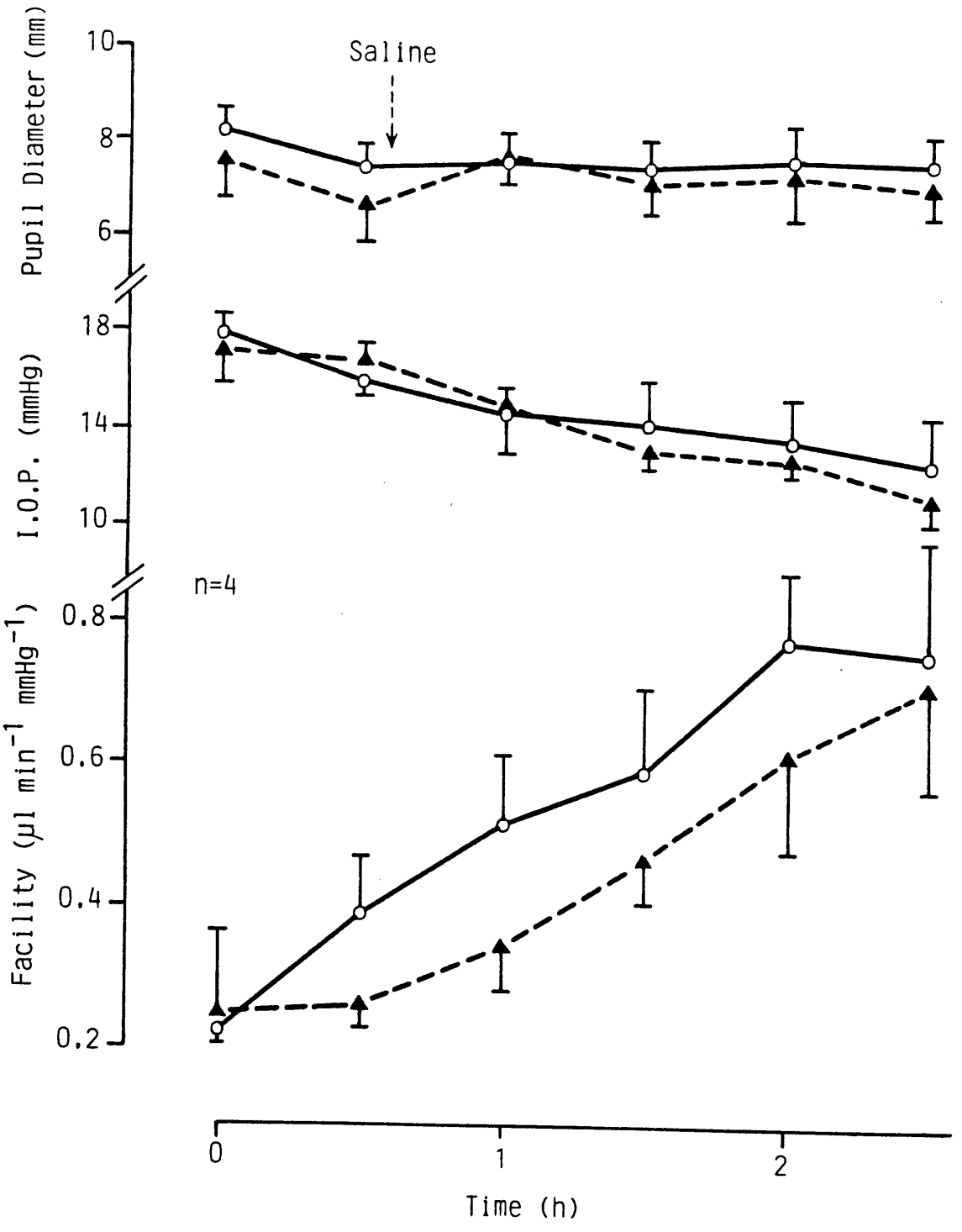

Fig. 25 The ocular responses to intracamerally injected saline in the anaesthetised rabbit. Saline was injected via a perfusion needle into the anterior chamber of one eye only (--▲--) while the contralateral eye (-O-) remained untreated. Each point is a mean ± SEM of values obtained in 4 eyes.

Similarly, IOP was significantly lower than starting values (ie t= 0.5h) at t= 1.5, 2 and 2.5h in both eyes. Although one eye was untreated. IOP fell from 17.6 $\pm$ 0.9 at t= 1.5h, to 12.3 $\pm$ 0.7 mmHg at t= 2.5h ($\underline{P} > 0.001$). Following the injection of saline, IOP fell from 17.3 $\pm$ 0.6 to 11.8 $\pm$ 0.7 mmHg at t= 2.5h ($\underline{P} > 0.001$).

In these experiments the experimental variation appeared to be smaller than that in experiments where 2 needles had been inserted into the eye.

In the non-perfused non-drug-treated (NPND) eye protein concentration of the AH was 0.53 $\pm$ 0.03 mg ml^{-1} (n=15). The effect of perfusion as well as drug treatment on AH protein concentrations are shown in Table 2 and in all instances, protein levels following drug treatment are compared to levels found in the NPND eyes, as well as to concentrations occurring in the aqueous of the contralateral eye within the same experiment. The effect of adrenaline, indomethacin and piroxicam on AH protein concentrations, coupled with perfusion, were also investigated.

The effect of perfusion alone may be seen by comparing the vehicle-pretreated eye in the indomethacin control experiment (Table 2, line 1) or the piroxicam control experiment (Table 2 line 5), with the NPND eyes: no significant difference is seen. If one can assume that pretreatment with Na_2CO_3 solution does not prevent aqueous protein from increasing, then perfusion alone does not induce protein entry into the AH. Topically applied adrenaline (0.5mg) significantly raised the concentration of protein in the AH of all the rabbits tested (levels of significance shown in table 2), whether pretreated with an anti-inflammatory drug or not.

The one exception to this generalisation was that among the 4 aspirin pretreated animals which failed to show a response to

Systemic aspirin pretreatment	Drug pretreatment	Drug treatment	No. of eyes	Protein concentration in A.H ($mg.ml^{-1}$)	
				Vehicle-pretreated eye	Drug-pretreated eye
-	Indomethacin	-	5	0.60 ± 0.18	0.60 ± 0.17
-	Indomethacin	Adrenaline	4	1.48 ± 0.40	1.25 ± 0.32
+	† Indomethacin	Adrenaline	7	1.80 ± 0.30	1.80 ± 0.28
+	‡ Indomethacin	Adrenaline	4	2.44 ± 0.83	1.15 ± 0.24
-	Piroxicam	-	5	0.62 ± 0.19	0.66 ± 0.20
-	Piroxicam	Adrenaline	6	1.93 ± 0.71	1.32 ± 0.48

Table 2 The effect of topically applied adrenaline (0.5mg), indomethacin (0.125mg) and piroxicam (0.75mg) on AH protein concentration at the end of each experiment. 1h prior to the cannulation of the anterior chamber several animals received systemic aspirin pretreatment (approx. 200mg kg^{-1})

†: one population of animals elicited an outflow response to adrenaline.

‡: another population did not show an outflow response to adrenaline.

Each value is a mean ± SEM of results obtained in the number of eyes shown.

adrenaline, protein concentration in the indomethacin-pretreated eye was not significantly greater than indomethacin- pretreated eyes where no systemic aspirin had been given.

The Effect Of The Injection Of Cyclic Nucleotide Analogues On AH Protein Concentration Following Pretreatment With Indomethacin Or Piroxicam

Injection of solutions into the anterior chamber was carried out through a second needle which was inserted into the chamber. The effects of these procedures on AH protein concentrations are shown in Table 3.

Compared to the contralateral untreated eye (whose anterior chamber had also been cannulated) intracamerally injected saline did not raise AH protein concentrations. Similarly, protein concentrations in the AH following the injection of dcAMP (25.5µg) into the anterior chamber were not significantly higher than AH protein concentrations following the injection of saline. However, in one series of experiments (Table 3 line 3, control) injected dcAMP had significantly raised ($0.05 \geqslant P \geqslant 0.02$) the concentration of the AH when compared with values found in the AH following the injection of saline. (Table 3 line 2, control). Topical pretreatment with indomethacin (0.125 mg) appeared to suppress this rise in AH protein concentration ($0.05 \geqslant P \geqslant 0.02$). However, the high proteins recorded in the control eyes (Table 3 line 3) were subject to high variability and were not corroborated in other similarly treated eyes (Table line 2, test and line 4, control); hence, perhaps it would be unwise to conclude that dcAMP significantly raises aqueous protein or that indomethacin can suppress such an effect. Topical pretreatment with piroxicam (0.75 mg) had no effect on protein concentrations following the injection of dcAMP or 8-bromo cAMP (22.4µg)

Drug pretreatment	No. of eyes	Control eye		Test eye	
		i.c. injection	Protein concentration in A.H. (mg ml^{-1})	i.c. injection	Protein concentration in A.H. (mg ml^{-1})
-	4	None	1.0 ± 0.22	Saline	0.87 ± 0.10
-	4	Saline	0.82 ± 0.12	dcAMP	1.05 ± 0.14
Indomethacin	5	dcAMP	3.09 ± 0.79*	dcAMP	0.98 ± 0.15
Piroxicam	5	dcAMP	0.71 ± 0.13	dcAMP	0.68 ± 0.08
Piroxicam	5	8-bromo cAMP	1.51 ± 0.42	8-bromo cAMP	1.65 ± 0.34

Table 3 AH protein concentrations at the end of each perfusion experiment in anaesthetised rabbits following topical pretreatment with indomethacin (0.125mg) or piroxicam (0.75mg) and/or intracamerally injected saline, dibutyryl cAMP (25.5mg) or 8-bromo-cAMP (22.4mg). Each value is mean ± SEM of results from the number of eyes shown.

Antibody Titer.

It was found that antibody titers of 1/5,000 for PGE_2, bound approx. 26% and 1/6,000, for 6-keto $PGF_{1\alpha}$ bound 22% of the total amount of PG present. These antibody dilutions were used in subsequent experiments, but were later altered to 1/2,000 and 1/4,000, respectively, in an attempt to improve the sensitivity of the assay, but without success.

Standard Curve.

Standard curves for the RIA of known amounts of each PG are shown in Figs 26 and 27. These results show that amounts of PG greater than 10pg displaced radiolabelled PG from the appropriate anitbody in a concentration-dependent fashion but concentrations of PGs less than 10pg did not.

Assays Of Endogenous AH Prostaglandins.

Assays of PGs present in samples of rabbit AH up to 200μl indicated that the concentrations of PGs present were below the sensitivity of the method.

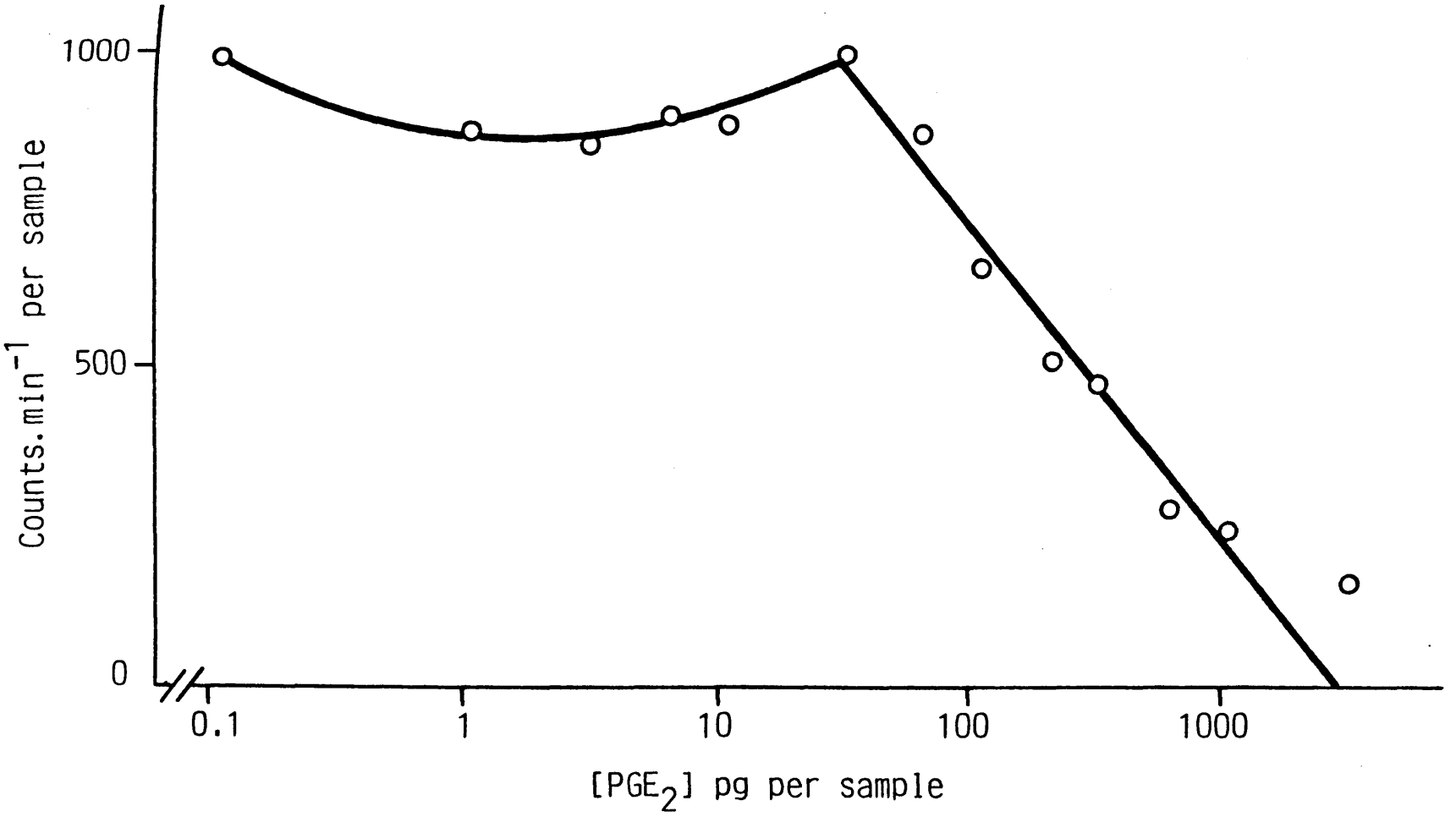

Fig. 26 Standard curve for PGE_2

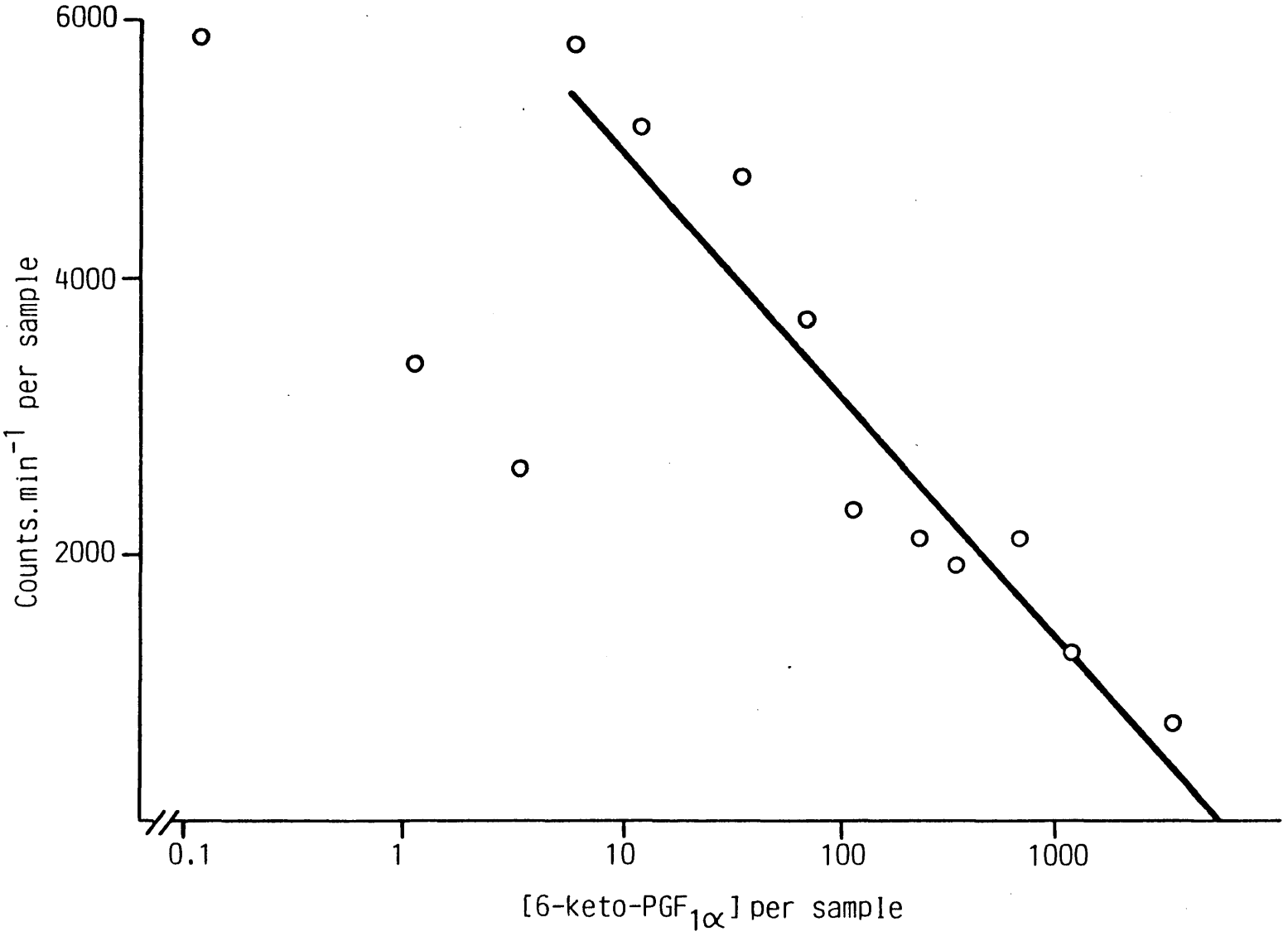

Fig. 27 Standard curve for PGF_1

The recovery of (^{3}H) 6-keto $PGF_{1\alpha}$ from standards extracted in cyclohexane-ethylacetate was found to be 63.5% (n=2) on extraction of saline and 67.5% (n=2) on extraction of AH.

The percentage recovery after extraction and concentration was estimated by adding a known amount of 6-keto $PGF_{1\alpha}$ (100µl) to a 900 ul sample of either saline or AH (an internal standard). Following the extraction and drying procedures the PG concentration of the reconstituted sample was estimated using RIA. In addition, a series of 200µl samples which had not been extracted or dried and which contained a known amount of PG (0 to 1000pg) were also assayed. The sample containing no exogenous PG represented the maximal concentration of radiolabelled PG which the antibody could bind. Results obtained from the RIA showed that no PG had been recovered from the samples which had been extracted and concentrated, in fact in the presence of these samples the antibody appeared to bind more labelled PG than the "total-bound" sample which contained neither extract nor exogenous PG. Since the dried extracts were reconsituted in a solution which contained Na_2CO_3 the effect of $Na_2\ CO_3$ on binding of PG to the antibody was investigated.

In these experiments, samples containing 10, to 1000pg of 6-keto $PGF_{1\alpha}$ were extracted, dried and then reconstituted in either of 200µl RIA buffer or 160 µl of RIA + 40 µl Na_2CO_3.

Fig. 28 shows that there was no difference in the amount of PG bound by the antibody when either of the afore mentioned solutions were used. This shows that the prescence of $Na_2\ Co_3$ in the reconstituted samples was not responsible for the ability of the antibody to bind greater amounts of PG when a

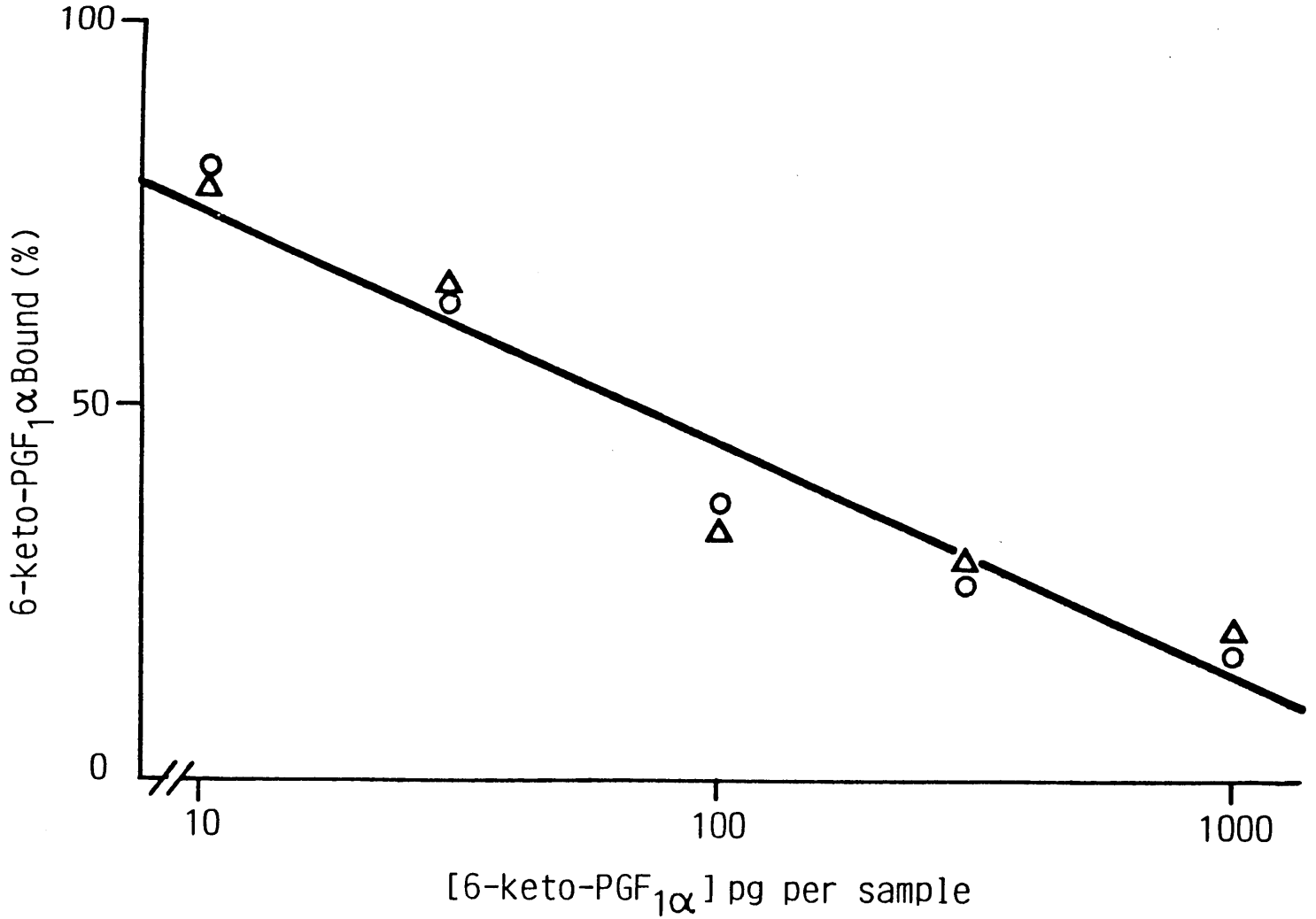

Fig. 28 The influence of Na_2CO_3 on the extraction of 6-keto-$PGF_{1\alpha}$ from a dried extract of AH to which was added 6μg Na_2CO_3 in RIA buffer (—△—) or RIA buffer alone (—○—).

reconstituted dried extract of AH was assayed using RIA. It therfore seemed more likely that the extraction procedure may be responsible for this phenomenon. Changing the solvent used to extract the PG from the AH from a cyclohexane-ethylacetate mixture to ether did not eliminate this problem. Therefore, in subsequent experiments, samples of saline containing known amounts of PG (10 to 1000pg) were extracted, dried and the reconstituted samples assayed using RIA. Results from these experiments indicated that following the aforementioned procedures, the ability of the RIA to distinguish between relatively high (1000pg) and low concentrations (10pg) was so poor that it would be extremely difficult to measure accurately any differences in the concentration of PGs in different samples of AH.

Plasma Salicylic Acid Concentrations Following Systemic Aspirin Pretreatment.

The concentration of SA in samples of protein-free plasma over a period of 3.5h are shown in Fig 29.
At zero time aspirin was administered rectally. After 1h the anterior chamber was cannulated and after a further 1h adrenaline (0.5mg) was topically applied to the eye.

0.5h after aspirin administration, plasma SA levels had risen to 8.17 $\pm$ 2mg.100ml^{-1}. Although SA levels increased to 13.97 $\pm$ 1.5mg.100ml^{-1} at t= 1h, they were not significantly higher than SA levels after 0.5h. However, after 1.5h SA levels had risen to 15.45 $\pm$ 0.96mg.100ml^{-1} which were significantly higher ($0.01 \geqslant P \geqslant 0.001$) than levels found after 0.5h. Peak plasma SA levels were found after 2.5h and values remained similar for the remainder of the experiment.

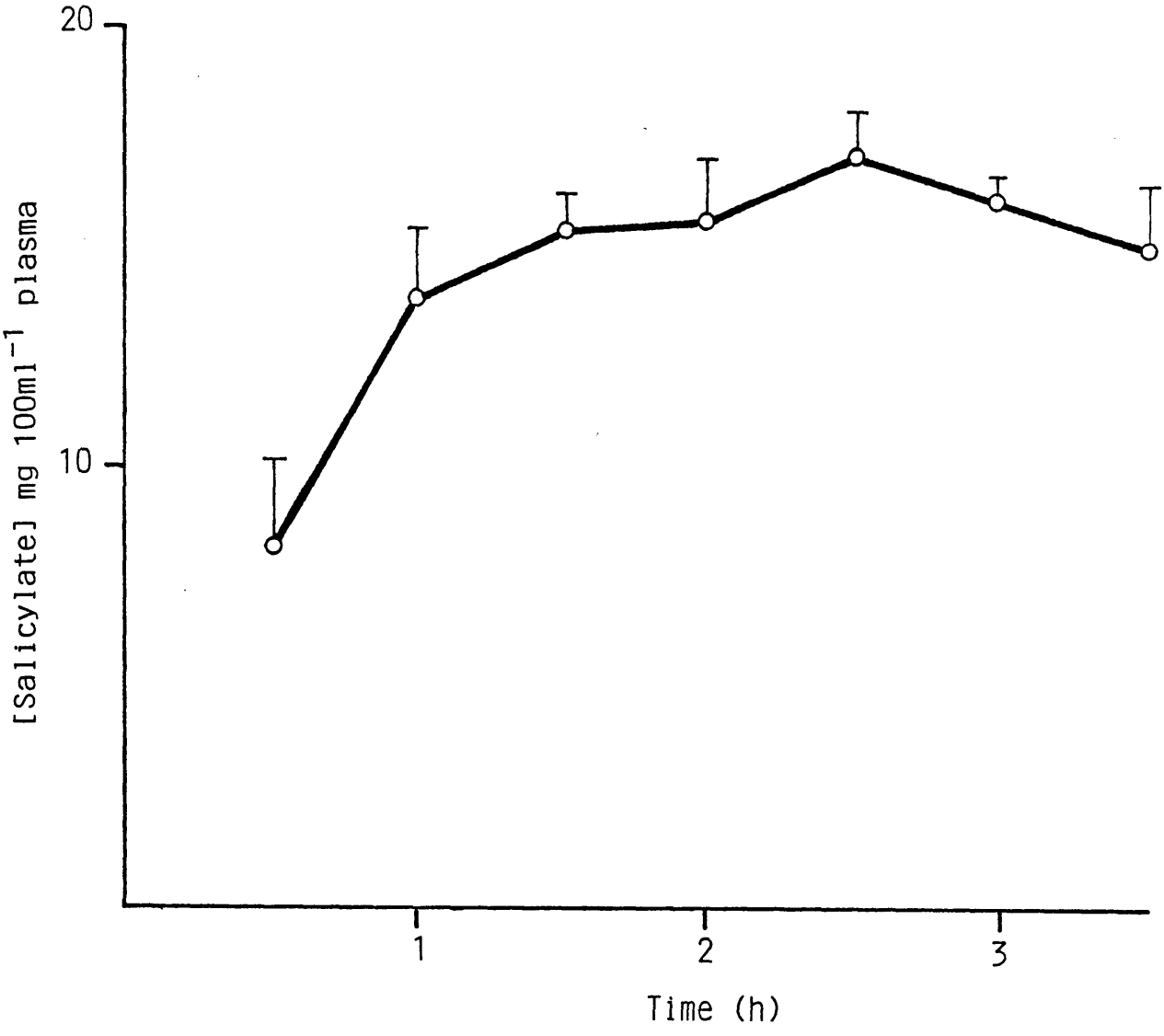

Fig. 29 Plasma salicylate concentrations following systemic aspirin pretreatment (approx 200 mg kg^{-1}) at t = 0. Each point is the mean ± SEM of values found in 5 rabbits.

DISCUSSION

In the present experiments a single dose of 0.25mg of adrenaline applied topically to the rabbit eye produced an initial rise followed by a fall in IOP. Using similar experimental procedures, Innemee and Van Zwieten, (1982) reported that a dose of adrenaline as low as 0.05mg applied topically raised IOP by approximately 2mmHg within half an hour, although IOP remained elevated after 1 hour. In contrast, other workers have reported that although topical application of 0.25 or 0.5mg of adrenaline lowered IOP, it was not preceded by a rise in IOP (Lamble, 1974; Langham and Krieglstein 1976). However, in general, although a hypertensive response of similar duration has been reported following acute topical administration of adrenaline (Norton and Viernstein, 1972; Lamble, 1976; Rowland and Potter, 1980) the doses required to elicit such a response were comparatively much higher (2 to 10mg).

It should be noted that under similar experimental conditions the pressure responses to adrenaline may differ within and between populations of rabbits. Norton and Viernstein (1972) reported that only 75% of the rabbit eyes tested showed a transitory elevation of IOP following the topical application of 4 or 8mg of adrenaline. Similarly, the hypertensive response to topically applied adrenaline (2mg) was not reproducible between different rabbit populations (Rowland and Potter,1980).

Of greater interest in these experiments was the adrenaline induced fall in IOP. The ability of adrenaline to produce a

dose-dependent fall in IOP has been observed on many occasions, not only in rabbit but also in primates and in man (See Introduction).

Although both the magnitude and the duration of the adrenaline-induced hypotensive response in present and previous studies are in general very similar a direct comparison cannot be made since previous studies used several different sub-maximal doses of adrenaline. A direct comparison can be made between present results and those obtained by Langham and Krieglstein, (1976) who also investigated the IOP and PD responses to a single dose of adrenaline (0.25mg) applied topically to the eye of the conscious rabbit. Both sets of data are in good agreement; in the present experiments, although adrenaline produced a maximum fall in IOP of approximately 5mmHg, IOP remained significantly lower for the remainder of the experiment, ie up until 4 hours, Langham and Krieglstein (1976) showed that athough adrenaline significantly lowered IOP after 2 hours the response was maximal after 4 to 5 hours and again remained lower for several hours. The maximum fall in IOP was approximately 6mmHg. The hypotensive response induced by the topical application of 0.05mg of adrenaline (Innemee and Van Zwieten, 1982) was extremely similar to the present results in both the magnitude and the duration of the hypotensive response. Adrenaline lowered IOP by approximately 4mmHg after 2 hours. The hypotensive response was maximal between 2 and 5 hours.

Before considering the effect of adrenaline on facility of outflow it is important to examine the tendency of facility of outflow to rise throughout the course of an experiment. In the present experiments facility of outflow rose steadily over a period of 2 to 3 hours in vehicle-treated eyes. It is therefore particularly important to discuss the factors involved in this phenomenon and their relative importance.

When facility of outflow is measured there appear to be three main factors which can quantitatively alter facility of outflow; a) the "washout" effect b) pseudofacility and c) trauma.

a) The "washout" effect

Perfusion of the anterior chamber can lead to a slow increase in the apparent facility of outflow, the so-called "washout effect". This washout effect is generally recognised as representing the removal of some coating substance from the trabecular fibres of the filtration angle.

In the present study the effect of perfusion on facility of outflow is seen in experiments where only the vehicle was applied to the eye. In these experiments mean facility of outflow values at the start of the experiment ranged between 0.24 and 0.28μl min^{-1} mm^{-1}Hg and rose to values between 0.36 and 0.50μl min^{-1} mm^{-1}Hg after two and a half hours. A washout effect of similar magnitude occurred in the dead rabbit eye perfused in situ, where facility of outflow increased from 0.20 to 0.4μl min^{-1} mm^{-1}Hg following a five hour perfusion (Ruben et al, 1985).

Early experiments by Barany and co-workers (1954; 1955) implicated the importance of hyaluronic acid, a glycosaminoglycan, as an element of resistance in the outflow pathway. Removal of this substance by perfusion could be responsible for the washout effect. In these experiments the perfusion of testicular hyaluronidase (TH) into enucleated cattle and rabbit eyes decreased outflow resistance by 50%. Therefore, although these results imply that HA offers some resistance to outflow, some other barrier which is insensitive to TH also exists. The existence of another barrier was also not excluded in experiments by Knepper et al, (1984). In these experiments the glycosaminoglycan profile of the trabecular meshwork was examined before and after chamber perfusion of the rabbit eye in vitro. Although facililty of outflow increased by 40% in sixty minutes, when compared to the non-perfused control eyes, no effect on the glycosaminogylcan profile was seen.

Although in the present experiments the "washout" effect appears to be time-related it can be demonstrated in a comparatively short period of time. In 1967, Pandolfi determined outflow resistance by measuring the time taken for a known volume of fluid to flow from the reservoir into the chamber under an applied pressure of 20mmHg. When the volume of fluid perfused over several minutes was varied from 50 to 500µl the apparent facility of outflow subsequently increased from 0.24 to 0.36µl min^{-1} mm^{-1}Hg. This result implies that it is the total volume of fluid passing through the meshwork, which determines the extent of the washout effect.

It has been suggested that perfusate washout of some endogenous fibrinolytic inhibitor is responsible, at least in part, for the washout effect. E - aminocaproic acid (EACA), an established inhibitor of plasminogen activation and/or plasmin activity, exerted a powerful stabilizing effect during prolonged perfusion experiments (Ruben et al, 1985). However perfusion of the enucleated rabbit eye with plasmin did not alter outflow resistence (Pandolfi, 1967). In addition little plasminogen activator activity, plasminogen or inhibitors of plasminogen activator activity have been demonstrated in the region of the collector channels or in the normal aqueous humour of the rabbit eye (Pandolfi et al, 1964, Saiduzzafar, 1970).

In conclusion although perfusate washout of HA or fibrinolytic inhibitors may be responsible for the "washout" effect, the precise mechanism(s) remains to be elucidated.

2) Pseudofacility

In the rabbit eye a majority of AH is formed by a pressure-independent secretory process, although a smaller proportion is formed by ultrafiltration, the pressure sensitive part of AH formation. As a result of a pressure difference between the capillaries which supply the ciliary processes and the ciliary stroma (Bill, 1975. Fig 2.) an ultrafiltrate is formed in the ciliary storma. Although the leaky junctions present at the apices of the pigmented and non-pigmented ciliary epithelium usually oppose the movement of materials from the stroma to the posterior chamber, they do allow the possibility of a pressure-dependent flow of salts and water (but not proteins) from the stroma to the posterior chamber. Since the pressure difference between the ciliary stroma and the posterior chamber is very small, the amount of filtrate entering the posterior chamber is comparatively small. Nevertheless, if the following assumptions are made.

a) that the driving force for the formation of the ultrafiltrate is the difference between hydrostatic pressures in the ciliary stroma and the posterior chamber,
b) capillary permeability and pressure remain constant during the experiment,
c) the integrity of the ciliary epithelium (the blood-aqueous barrier) remains constant,

then any change in IOP may alter the amount of ultrafiltration. Certain changes in blood pressure could also influence ultrafiltration.

Determinations of facility of outflow by perfusion under constant pressure measure gross facility of outflow, ie $C_{Total} = C_{True} + C_{Pseudo}$.

Pseudofacility is the suppressibility of the pressure-dependent portion of AH formation. In the present experiments facility of outflow is measured when IOP is artificially raised by 4mmHg. This rise in pressure can alter the pressure gradient across the ciliary epithelium such that the driving force for ultrafiltration can decrease. In fact, there would be a tendency for fluid to flow back across the ciliary epithelium into the ciliary stroma. Therefore, during facility determinations fluid entering the eye replaces AH forced to leave the eye by the applied pressure and by decreased or reversed ultrafiltration ie true and pseudofacility, respectively.

During the course of the present experiments, IOP gradually decreased, tending to increase the pressure gradient across the ciliary epithelium which in turn would increase the amount of AH formed by ultrafiltration. Although a certain amount of pseudofacility probably exists throughout the experiment, it need not necessarily rise as IOP falls since the applied pressure during facility determination is constant throughout the experiment. In the perfused eye of the urethane anaesthetised rabbit pseudofacility was estimated at 0.056ul min^{-1} mm^{-1}_{Hg} (Green and Padget, 1979). Unfortunately the contribution of pseudofacility to the gross facility measurements in these experiments is unknown.

The effects of various drugs on pseudofacility will be discussed in the appropriate sections.

3) Trauma

In an attempt to minimize the extent of trauma induced by the experimental procedures necessary to measure facility of outflow, pilot experiments investigated the optimal conditions under which these experiments were to be conducted. These procedures are detailed in the Materials and Methods Section. Nevertheless since the rabbit eye is a particularly sensitive stucture, needle insertion, perfusion of an artificial solution, and/or the prolonged presence of a needle within the anterior chamber, may be enough to induce trauma. Possible mediators of ocular trauma responses include PGs. (for a more detailed discussion of the ocular responses to PGs see Introduction). When applied to the eye larger doses of PGs produce responses similar to those induced by trauma, ie an increase in IOP, a breakdown of the blood-aqueous barrier, aqueous flare and pupil constriction. Closely correlated with such trauma responses is the appearance of PGs in the AH. If PGs are released in the present experiment in large enough concentrations then perhaps some of the ocular responses to PGs may have developed during the present experiments. In control eyes there was neither visible aqueous flare, pupil constriction, nor a rise in IOP. As an indication of the integrity of the blood aqueous barrier, which perhaps reflects the degree of trauma manifested in the eye during the experiment, AH protein concentrations were measured. When compared to non perfused - non drug treated eyes, perfusion of control or vehicle treated eyes did not significantly alter AH protein concentrations. This suggests that only very small concentrations of PGs, if in fact any, are released in these experiments. In support of this contention was the failure of indomethacin and piroxicam pretreatment to suppress the gradual rise in facility of outflow observed in control perfusion

experiments in the anaesthetised rabbit.

Several PGs have been reported to increase gross facility of outflow, an effect attributed to pseudofacility. Although it appears that the concentrations of PGs released in the present experiments did not alter the integrity of the blood aqueous barrier such that proteins entered the AH, enough may have been released to alter pseudofacility. Doses of exogenous PGs as small as 10 to 250ng have been shown to increase AH formation and pseudofacility (See Introduction - The Ocular Responses To Prostaglandins). Although the time-course of these responses are not identical to those of present experiments, nevertheless the data do show that PGs have the ability to increase AH formation and pseudofacility. PGs may increase AH formation by increasing ultrafiltration or active secretion across the ciliary epithelium. PGE_1 increases the passive permeability of the isolated rabbit ciliary processes (Green and Padgett, 1977). PGE, increased the pressure index of the capillaries supplying the ciliary processes. This in turn could increase the amount of ultrafiltrate entering the ciliary stroma from the capillaries. This alone may not increase the amount of ultrafiltrate entering the posterior chamber, but coupled with the increased passive permeability of the ciliary epithelium it would tend to increase the amount of AH formed by ultrafiltration. Therefore in the present experiments trauma-induced production of PGs may increase pseudofacility, which in turn would be a part of the gross facility measured in the present experiments. However neither indomethacin or piroxican pretreatment prevented this time-related rise in facility of outflow. Experiments where gross facility of outflow showed signs of rising spontaneously by greater than 20% in thirty minutes were discontinued in an attempt to minimize the contribution of trauma in an experiment.

The Effect of Adrenaline On Facility of Outflow In The Anaesthetised Rabbit

In the present experiments topically applied adrenaline (0.5mg) raised facility of outflow. This outflow response was observed half an hour following the application of adrenaline and was sustained for the remainder of the experiment. Such an early outflow response to adrenaline in the rabbit eye has not been reported elsewhere. Similar results were obtained in earlier perfusion studies by Lamble (1974;1977). However in these experiments adrenaline (0.5mg) was applied to the eye prior to the experimental procedures necessary to measure facility of outflow. This meant that the earliest possible measurement of outflow was 50 minutes following the application of adrenaline. Although adrenaline raised the facility of outflow, this rise, relative to the vehicle treated eye, was only half as great as observed in the present work. Whereas in these experiments responses to adrenaline did not significantly increase beyond 50 minutes, in the present work the adrenaline-induced rise in facility continued at least until 90 minutes after administration. In view of the similar starting values for facility of outflow and methods used to measure outflow facility, other than biological variation, there is no apparent reason for such marked differences in the outflow response to adrenaline. However an outflow response of similar magnitude and duration has been reported in the monkey eye (Kaufman and Barany, 1981). Approximately 15 to 35 minutes following the intracameral injection of adrenaline (10ug) or NA (10µg) facility of outflow had increased by 0.185 and 0.201µl min^{-1} $mm^{-1}Hg$ respectively.

Similarly, 45 minutes following the topical administration of a 2% solution of adrenaline, facility of outflow was raised by 0.35µl min^{-1} mm^{-1}Hg (Bill,1969).

Although it has been assumed that the rise in facility of outflow following adrenaline treatment was due to adrenaline, other factors may contribute to this rise.

During a perfusion experiment there is a gradual rise in facility of outflow in both control or vehicle-treated eyes. Since this gradual rise will also exist in the adrenaline - treated eyes, it is therefore particularly important to note that the rise in outflow facility seen in the adrenaline - treated eye was always significantly higher than the rise seen in the contralateral vehicle-treated eye, implying that adrenaline had further increased facility of outflow. Kaufman and Barany (1981) have also shown that the percentage increase in facility of outflow caused by adrenaline or NA was significantly greater than that caused by perfusion alone.

Although pseudofacility exists in the present experiments we believe that in the control situation it is constant throughout the experiment. However, it is possible that a drug may alter pseudofacility. An adrenaline induced rise in pseudofacility has been reported in both the rabbit and monkey eye (Barany, 1968; Bill, 1969; Green and Padgett, 1979).

In experiments where the hydraulic conductivity of the isolated ciliary body-iris preparation was measured, adrenaline (10^{-8} to $^{-4}$M) increased the passive water permeability of the ciliary epithelium by 15 to 86 percent (Green and Griffen, 1978) This increase in passive permeability would suggest that pseudofacility should be increased.

Following the infusion (^{3}H) inulin into the anterior chamber of the urethane-anaesthetised rabbit, Green and Padgett (1979) investigated the pressure-dependency of AH formation and the effect of adrenaline on pseudofacility. Pseudofacility was calculated from values obtained for facility of outflow during a period of artificially elevated IOP according to the method of Macri (1967). The collection of fluid effluent from the eye approximately four hours following the topical application of a comparitively high dose of adrenaline (2mg), showed that adrenaline had raised pseudofacility from 0.056μl min^{-1} mm^{-1}Hg in the control eye to 0.086μl min^{-1} mm^{-1}Hg. Comparison of this data with results obtained in the present experiments illustrates two points. Firstly, in the control experiments pseudofacility can account for as much as 22% of the gross facility of outflow. Secondly and perhaps more importantly, the adrenaline-induced rise in pseudofacility can account for only a small proportion of the rapid rise in facility of outflow following adrenaline treatment.

In an attempt to establish the effect of adrenaline on true and pseudofacility, facility of outflow in the vervet monkey eye was studied using both a conventional and a new anterior chamber perfusion technique (Barany, 1968). In the conventional two level constant pressure perfusion technique, gross facility of outflow was measured by periodically raising and lowering IOP while extraocular venous pressure remained unaltered. With the newer technique the pressure head required to force AH through the outflow channels was obtained by lowering and raising venous pressure while IOP was unaltered. Venous pressure was altered by changing the positive pressure against which the animal was breathing. Since IOP was unaltered during

this technique AH formed by ultrafiltration was minimally affected. Therefore in these experiments facility measured approached true facility. Pseudofacility was estimated as the difference between the results of these two techniques in the same eye. Approximately one hour following the topical application of two drops of a two percent solution of adrenaline, pseudofacility had increased from 0.092 in control eyes to 0.144$\mu l min^{-1}$ $mm^{-1}Hg$ in the adrenaline-treated eyes. In 1969 Bill calculated that one drop of a two percent solution of adrenaline raised pseudofacility from 0.025 to 0.036$\mu l min^{-1}$ $mm^{-1}Hg$. Although these values are lower than those reported by Barany (1968), they may be explained by the higher arterial blood pressure measured in these experiments which would tend to decrease pseudofacility (Bill, 1970).

As expected, topically applied adrenaline significantly lowered IOP. Unexpected was the significant fall in pressure in the contralateral vehicle-treated eye which meant that there was no significant difference between IOP in the adrenaline and vehicle-treated eyes. This phenomenon cannot be explained by a consensual action of adrenaline since the same concentration of adrenaline which lowered IOP in the drug-treated eye, failed to lower IOP in the contralateral vehicle-treated eye of the conscious rabbit. However, it has previously been reported (Lamble, 1974,1977) that the effect of adrenaline on ocular tension was apparently reduced or reversed by the procedures of anaesthesia and cannulation performed to enable facility determinations. Thus when IOP was measured manometrically there was often no significant difference between values observed in adrenaline and control eyes, whereas when IOP was measured using tonometry IOP was significantly lower in the adrenaline-treated eye. In both these and present experiments it was frequently observed that the administration of urethane subsequent to initial doses of anaesthetic and cannulation of the anterior chamber were often accompanied by a irreversible lowering of IOP. This and the gradual fall in IOP seen during the experiments could be the result of a urethane-induced reduction in aqueous humour formation which has been reported in the rabbit eye (Barany, 1950). One must therefore conclude that the fall in IOP normally induced by adrenaline may often be obscured in the urethane anaesthetised rabbit.

The PD Responses To Adrenaline In The Conscious And Anaesthetised Rabbit

Adrenaline (0.25 and 0.5mg) applied topically to the rabbit eye produced mydriasis. The duration of the PD response in these experiments agrees with previously reported data, ie a rapid mydriasis which is maximal within half to one hour (Langham and Krieglstein, 1973; Langham *et al*, 1976; Lamble, 1977). However the magnitude of this response (2mm in the conscious and approximately 3.6mm in the aneasthetised rabbit) was smaller than the peak increase of approximately 5mm reported elsewhere (Langham and Krieglstein, 1973). This is probably due to the substantially higher starting PD values encountered in the present work.

Intravenous pretreatment with either phenoxybenzamine or phentolamine (5mg kg^{-1} in each case) completely blocked the PD response to 0.5 and 1.5mg of adrenaline (Langham *et al*, 1973; Langham and Krieglstein, 1976). These results suggest that adrenaline induced mydriasis is predominantly an alpha adrenoceptor mediated response. Therefore it is not surprising that in the present experiments in the anaesthetised rabbit topical timolol failed to reverse the pupillary responses to adrenaline (0.5mg). However, topical pretreatment with thymoxamine (2.5mg) inhibited the mydriatic responses to topically applied adrenaline (0.5mg) by approximately 75% (Lamble, 1977). These findings suggest that although alpha induced contraction of the dilator pupillae is primarily responsible for the adrenaline induced mydriasis, there may be another smaller component consisting of beta receptor mediated relaxation of the iris sphincter.

The Effect Of Timolol Treatment On The Ocular Responses To Adrenaline In The Anaesthetised Rabbit.

Timolol was used as a pharmacological tool in the present experiments to investigate the involvement of the beta - adrenoceptor in the outflow response to adrenaline. However, the mechanism of action of timolol is complicated since in addition to being a beta receptor antagonist, timolol is also a potent hypotensive agent in the normo- and hypertensive human eye (Katz et al, 1977; Zimmerman and Kaufman, 1977). Although timolol did not alter the progressive rise in outflow facility seen during the course of control experiment it did largely reverse the outflow response to adrenaline, confirming that this was infact a true drug response mediated via a beta adrenoceptor. Although the exact location of this receptor is unknown the concentration of timolol present in the eye is believed to be in excess of that required to block beta receptors. Following its topical administration timolol rapidly appears in the aqueous humour; for example, the topical application of 25µg results in 5.7µM timolol in the AH 30 minutes later. (Varielles et al, 1977;). Nathanson et al,(1980) have shown that as little as 2.5uM timolol was needed to block the stimulation of ciliary process adenylate cyclase by isoprenaline. If it is true that adrenaline lowers IOP by raising outflow facility then the failure of timolol to reverse the pressure response, while reversing the outflow response to adrenaline is perhaps unexpected.

In an extensive study by Vareilles et al, (1977) the hypotensive action of timolol (1 to 100µg) in the normo tensive rabbit eye was shown to be inconsistent. In contrast Innemee and van

Zwieten (1982) have shown that timolol applied to the normal rabbit eye significantly reduced IOP 1 to 2 hours later. In direct conflict, Boas et al, (1981), have shown that a dose of timolol identical to that used in the present experiments, failed to lower IOP in the normotensive rabbit eye and also failed to block adrenaline (100µg) induced ocular hypotension.

The pressure responses to timolol in the hypertensive rabbit eye are more consistant than those in the normotensive rabbit eye. Timolol (1 to 100ug) reduced IOP elevated by either an oral water load or by pretreating intracamerally with alpha chymotrypsin (Vareilles et al, 1977 and Schmitt, et al, 1981). In these experiments concentrations of timolol as low as 0.1µg blocked the hypotensive activity of isoprenaline (10ug) in the water-load model of ocular hypertension.

In the present experiments timolol reversed the adrenaline induced rise in outflow. The apparent failure of timolol to significantly alter the hypotensive response to adrenaline could be explained by the direct effect of timolol in lowering IOP in the rabbit eye (Innemee and van Zwieten, 1982), or by the slow reduction in IOP observed during any long experiments in the anaesthetised rabbit. Alternatively since the volume changes involved are small perhaps one would expect reversal of the pressure response to adrenaline to occur beyond the time restraints of the present experiments. It has also been proposed that the reduction of IOP by catecholamines in the presence of timolol was due to alpha adrenoceptor stimulation which is not blocked by the beta-adrenoceptor antagonist.

The Effect of Indomethacin Pretreatment on the IOP Responses to Adrenaline in the Conscious Rabbit.

In 1977, Bhattacherjee and Hammond showed that topical pretreatment with indomethacin (0.125mg) abolished the hypotensive response to chronic adrenaline (1mg) treatment. Although the present results confirmed these early findings, more importantly, they showed that acute indomethacin pretreatment could infact antagonise the acute hypotensive response to adrenaline. A similar finding has recently been reported in the human eye (Camras et al, 1985). In a randomized double-masked study, a 2% solution of adrenaline applied topically, twice daily for 2 weeks, to the eyes of patients with glaucoma or ocular hypertension caused a 29% reduction in IOP of placebo-treated patients, but only a 9% reduction in IOP in patients treated orally with indomethacin (25mg, 4 times daily).

Results obtained in both rabbit and human eye, therefore, suggest that at some stage following the interaction of adrenaline with its receptor, PGs are produced which may be responsible, at least in part, for adrenaline-induced hypertension.

In the present experiments adrenaline also produced initial ocular hypertension, a response which appeared to be partially blocked by indomethacin pretreatment. This is perhaps unexpected in view of previously published results. Potter et al, (1982,1983) have shown that pretreatment with a competitive inhibitor of PG endoperoxide synthetase, flurbiprofen, for 3 days, did not inhibit the development of isoprenaline-induced

ocular hypotension. Similarly Unger, (1979) has shown that when topical adrenaline (50mg) is given for 4 days and on the fifth day is preceeded by topical indomethacin pretreatment (25mg topically and 10mg kg^{-1} intraperitoneally) subsequent adrenaline treatment continued to initially raise IOP. However, Langham and Palewicz(1977) have proposed that this initial hypertensive response which is independent of PG biosynthesis, is the result of the interaction with alpha-adrenoceptors on intra- and episcleral blood vessels as well as aqueous veins causing vasoconstriction which offers resistance to the flow of AH and subsequently causes ocular hypertension. Alternatively, if adrenaline-induced hypertension in the rabbit eye is the result of extraocular muscle contraction (Rowland and Potter,1980), then it is conceivable that indomethacin could block such contraction by virtue of its inhibition of calcium movements (Northover, 1977,1980,1982).

The Effect Of Indomethacin Pretreatment On The Pressure And Outflow Responses To Adrenaline In The Anaesthetised Rabbit.

Since adrenaline appeared to lower IOP by raising facility of outflow the effect of indomethacin pretreatment on this more fundamental response to adrenaline was particularly interesting.

In the present experiments there was a tendency for facility of outflow to increase during the course of an experiment, a trauma response to adrenaline probably induced by the procedures required to measure outflow facility and which may involve PGs. It was therefore important that indomethacin pretreatment failed to alter this response. Nonetheless, indomethacin pretreatment inhibited the adrenaline-induced rise in outflow facility and subsequent fall in IOP. Although these results strengthen the idea that PGs are involved at some stage in the adrenaline-induced rise in facility of outflow and fall in IOP, one must also consider the other properties of indomethacin.

Indomethacin possesses several actions which are antagonistic to those of calcium in several biological situations. The calcium content of smooth muscle following electrical stimulation is reduced by indomethacin (Northover, 1971,1972: Burche et al, 1983). By reducing the availability of of calcium within the cell, indomethacin disrupts excitation-contraction coupling and therefore it is not surprising that indomethacin can inhibit the contraction of most types of vascular smooth muscle (Northover,1967). Similarly, the release of lysosomal enzymes from polymorphonuclear lymphocytes, a process which also requires calcium, is inhibited by indomethacin (Ignarro and George, 1974; Rossi et al , 1976).

By investigating the effect of other PG synthesis inhibitors and calcium antagonists on the ocular responses to adrenaline in both the conscious and anaesthetised rabbit it was possible to determine that indomethacin was not acting as a calcium antagonist.

The Effect Of Verapamil Pretreatment On The Pressure Responses To Adrenaline In The Conscious Rabbit.

Verapamil is a calcium channel blocker which prevents the influx of extracellular calcium into cells. If indomethacin were acting mainly as a calcium antagonist in the present study then verapamil might have been expected to have similar effects on the pressure responses to the adrenaline.

Present results show that verapamil produces a dose-dependent lowering of IOP, with consensual effects at higher concentrations.

In direct conflict, Beatty et al,(1984), have shown the topical administration of similar doses of three different calcium channel blockers (verapamil, diltiazem or nifedipine) produced a transient rise in IOP. Although facility of outflow and episcleral venous pressure were unchanged, AH inflow appeared to have increased when estimated by the Goldman equation or by changes in anterior chamber fluorescein-labelled dextran concentration. However, AH ascorbate and turnover of radioactive iodide did not differ from that in the untreated eye. It is believed that the ocular hypertensive effect of verapamil is related to ocular vasodilation. Since adrenaline (1%) and phenylephrine(10%) prevented the elevation of IOP by verapamil, it is possible that the vasconstricive properties of these agents may be preventing the verapamil-induced vasodilation.

The cellular mechanism involved in the ocular hypo-and hypertensive responses to verapamil are unclear. Intraperitoneal indomethacin pretreatment (10 to 50mg kg -1) left the hypertensive response to verapamil unaltered (Beatty et al,1984). AH protein concentrations were also unaltered. Thus the effect of calcium channel blockers on IOP does not seem to be PG mediated.

If the hypertensive response to adrenaline is the result of extraocular muscle contraction then the partial anatagonism of this response by the verapamil in the present experiments is not surprising. Of greater importance was the failure of verapamil to alter the hypotensive response to adrenaline which suggests that in previous experiments the ability of indomethacin to antagonise the outflow and pressure response to adrenaline was probably not related to calcium antagonism.

The Effect Of Systemic Aspirin Pretreatment On The Pressure and Outflow Responses To Adrenaline In The Anaesthetised Rabbit.

Systemic pretreatment with aspirin (200 mg kg^{-1} rectally) had no effect on baseline outflow or IOP measurements in the anaesthetised rabbit. Similarly in the conscious rabbit iv injection of aspirin (2.5 to 17.5 mg kg^{-1}) had no influence on IOP (Wizemann et al ,1982).

In view of the present observation that indomethacin blocks both the outflow and pressure responses to adrenaline, perhaps it is surprising that aspirin pretreatment altered neither of these responses nor their inhibition by indomethacin. It is possible that this concentration of aspirin is well below that required to inhibit PG biosynthesis or the reason is aspirin's far lower potency as an inhibitor of cyclooxygenase.

Although Miller et al ,(1973) have shown that PG biosynthesis by microsomal fractions of the anterior uvea are suppressed an hour after aspirin pretreatment (200mg kg-1,rectally), compared with the present experiments plasma salicylate levels were considerably higher, ie 18 and 30 mg percent respectively.

In 1974, Bhattacherjee and Eakins. investigated the inhibitory activity of several non-stereoidal anti-inflammatory drugs on ocular PG - like activity by microsomal fractions of the anterior uvea and ID-50 values calculated. Indomethacin had an ID-50 of 18.5μl ml^{-1} whereas with aspirin no inhibition could be detected even with concentrations as high as 200 to 300 μg ml^{-1}.

Depending upon their response to adrenaline, the animals in this series of experiments appeared to fall into two categories, those which showed an outflow and pressure response to adrenaline and those which did not. The former have already been discussed.

Since adrenaline produced mydriasis in both populations of animals it seems that drug penetration cannot explain the lack of an outflow and/or pressure response to adrenaline. It is possible that the absence of these responses are a result of high plasma salicylate levels . Unfortunately plasma salicylate levels were measured in only 2 of these rabbits and in both instances the levels were similar to those of the rabbits whose IOP did respond to adrenaline. Although it appears that for no apparent reason some rabbits fail to show a pressure response to adrenaline this is a phenomenon commonly observed in both the conscious and anaesthetisted rabbit (personal communications, D.Gilbert, S.Bartels).

The Effect Of Piroxicam Pretreatment On The Ocular Responses To Adrenaline In The Conscious And Anaesthetised Rabbit

In 1983 Burche et al , assessed simultaneosly the effects of indomethacin and piroxicam on both the uptake of $^{45}Ca^{2+}$ into hamster liver mitochondria and microsomes and PG synthesis in these organelles. Indomethacin inhibited calcium movements into these subcellular fractions at concentrations only twenty times those required to block PG synthesis (ID_{50} of 90 and 5μM, respectively). In contrast piroxicam did not alter calcium movements at concentrations up to 300 μM., while the ID_{50} for the inhibition of PG synthesis was 0.35 μM. The mechanism for inhibition of $^{45}Ca^{2+}$ transport by indomethacin is unclear but does not appear to be related to cyclo-oxygenase inhibition since it was not reversed by the addition of exogenous PGs. The use of piroxicam in the present study distinguished between direct effects on calcium movements versus actions mediated through cyclo-oxygenase inhibition.

In the conscious rabbit low concentrations of piroxicam had no effect on IOP while higher concentrations lowered IOP suggesting that endogenous PGs may be involved in maintaining IOP. Using direct or tracer dilution techniques to measure AH formation, PGs have been reported to raise AH formation in the rabbit eye (Masuda and Mishima, 1973; Green and Padgett, 1979). If endogenous PGs have similar effects on AH formation, and are involved in the maintenance of IOP then inhibition of PG production could result in a lowering of IOP. However, in the

anaesthetised monkey indomethacin (3mg kg^{-1} injected iv) had no clear effect on AH formation (Sperber and Bill 1984). Nonetheless in - vivo and in - vitro studies have shown that indomethacin enhances spontaneous fluid absorbtion in the rabbit small intestine and reduces the amount of fluid that accumulates in response to several secretory stimuli (Wald et al, 1977; Smith et al, 1981).

Following piroxicam pretreatment in the conscious rabbit, both the hyper - and hypotensive response to adrenaline were unaltered. The failure of piroxicam to block either of these responses could not be attributed to the hypotensive action of piroxicam since at this concentration it had no effect on IOP. Although piroxicam is a relatively more potent inhibitor of PG biosynthesis than indomethacin in the liver, this does not necessarily mean that a similar situation exists in the eye. The pharmacological profile of activity of a viriety of non-steroical anti-inflammatory compounds including indomethacin and asprin, was found to differ in ocular tissues from that in other tissues such as the spleen and seminal vesicles (Bhattacherjee and Eakins, 1974). It is also possible that piroxicam cannot penetrate the cornea as easily as indomethacin.

Subsequent experiments in the anaesthetised rabbit using a higher dose of piroxicam, yielded particulary important results. While indomethacin, a known calcium antagonist, partially inhibited the hypertensive response to adrenaline, piroxicam, a compound relatively devoid of calcium antagonistic properties had no effect on this transient rise in pressure. These results therefore suggest that calcium may be involved, possibly through inhibition of extraocular muscle contraction, in the hypersensive response to adrenaline. However of greater interest was the

ability of both indomethacin and piroxicam to largely inhibit the adrenaline - induced rise in facility of outflow and subsequent fall in IOP, strongly suggesting that PGs are involved at some stage in these responses to adrenaline.

The Involvement of The Beta Adrenoceptor and PGs In The Outflow and Pressure Responses To Adrenaline.

The reversal of the adrenaline induced rise in facility of outflow by timolol is in keeping with the theory that a beta-adrenergic mechanism is involved in the hypotensive response to adrenaline (See Introduction:- The Biochemical Mechanism of Action of Adrenaline In Lowering IOP). However the ability of indomethacin and piroxicam to antagonise the outflow and pressure response to adrenaline suggests that following the interaction with the beta adrenoceptor PGs are produced which may also be responsible for the afore mentioned responses to adrenaline. The more widely accepted view is that beta adrenoceptor stimulation results in the production of cAMP and many workers (Neufeld et al, 1972; Neufeld and Sears, 1974 and Neufeld et al, 1975) believe that it is this cAMP which is responsible for the ocular responses to adrenaline. In the light of the ability of indomethacin to antagonise the cAMP-induced rise in facility of outflow one may be tempted to postulate that cAMP triggers PG synthesis. Although there is little evidence in the literature to support such a hypothesis the present attempt to raise facility with cAMP and block the rise with piroxicam might have lent weight to this idea had the technical problems been overcome.

The formation and release of PGs in response to adrenergic stimuli has been demonstrated in the eye. Bhattacherjee et al, (1979) demonstrated an adrenaline-induced rise in PG synthesis by rabbit ocular tissues.

Similarly, Engstrom and Dunham (1982) examined the effect of phenylephrine on the synthesis and release of PGs from isolated rabbit iris-ciliary body (ICB) slices. Phenylephrine (10μM) enhanced the synthesis of PGs E_2 and $F_{2\alpha}$ from superfused ICB slices, a response abolished by indomethacin (5g ml^{-1}). Therefore, from these and present results one could well imagine adrenaline stimulating PG production. However the ability of phenoxybenzamine to antagonise the phenylephrine-induced rise in PG generation by ICB slices (Engstrom and Dunham, 1982) suggests that adrenergic induced production of PGs may occur through an alpha adrenoceptor. In general, relatively selective alpha adrenoceptor agonists, including phenylephrine and St-587, are minimally effective in lowering IOP in both rabbits and humans (Potter and Rowland, 1978; Innemee et al, 1982; Lee and Brubaker, 1982/83) and do not seem to have a profound effect, if any, on AH formation or outflow (Green and Griffen, 1978; Green and Padgett, 1979 and Lee and Brubaker, 1982/83). Since adrenaline raises facility of outflow and subsequently lowers IOP, and timolol reverses these responses, it is difficult to envisage an alpha adrenoceptor being involved in these responses to adrenaline.

The Effect Of Drug Treatment On AH Protein Concentrations.

Since the rabbit eye is a particularly sensitive structure it is possible that the present experimental procedures may traumatise the eye. Trauma responses could include miosis, hyperaemia, a rise in IOP and/or a breakdown of the blood aqueous barrier. Therefore in the present experiments AH protein concentrations, measured at the end of each perfusion experiment were used as one indication of the health of the eye. Pupil size, IOP and flare were also watched.

Although Miller et al (1973) reported that the protein content of primary AH was 1.2mg ml^{-1}, levels found in the present experiments were comparatively smaller, ie 0.5mg ml^{-1}. Since AH protein levels were unaltered following perfusion of the eye it appears that only minimal if any, breakdown of the blood aqueous barrier occurred during the present experiments.

Topically applied adrenaline raised AH protein concentrations. Similar concentrations (approximately 2mg ml^{-1}) were found in the AH following topical treatment with either 0.1% NA or 10% phenylephrine (Unger, 1979).

In 1971, Takase reported a NA-induced fall and an iso prenaline-induced rise in AH protein concentrations. While pretreatment with propranolol suppressed the isoprenaline induced rise, phentolamine inhibited both this rise and the NA induced fall in protein concentrations. Consequently it was thought that alpha adrenoceptor stimulation reduced and beta stimulation increased the permeability of the blood aqueous barrier. Since adrenaline can stimulate both alpha and beta receptors it is possible that a similar situation exists in the present work and

that a moderate increase in protein is the net effect of adrenaline.

Although indomethacin can antagonise the paracentesis-induced rise in AH protein concetration, (van Haeringen et al, 1982), neither indomethacin nor piroxicam altered the adrenaline-induced rise in AH protein concentrations. This suggests that this was a true drug effect and not a trauma response.

Again in the present experiments, the injection of saline into the AC raised facility of outflow, an apparent trauma response. Nonetheless AH protein concentrations were unaltered. It therefore appears that certain trauma responses can occur without any significant breakdown of the blood aqueous barrier.

Measurement of AH PG Concentrations Using RIA

Measuring PG concentrations in the AH was a more direct way of investigating the possible involvement of PGs in the ocular responses to adrenaline. Therefore in the present experiments AH was obtained by paracentesis from the eyes of conscious rabbits following adrenaline treatment and PG levels measured using RIA.

Initial experiments showed that the assay was not sensitive enough to detect the apparently low concentrations of PGs present in both vehicle-and adrealine-treated eyes. In an attempt to concentrate the levels of PGs to be measured, pooled AH samples were extracted, dried, reconstituted and then assayed. However, when samples treated in this fashion were assayed, the antibody appeared to bind more labelled PG than the "total bound" sample which contained neither extract or exogenous PG. This implied that these procedures in some way altered the binding properties of the antibody. Alternatively, some component of the AH may be responsible. Although the effect of extraction alone could not be directly determined it did not appear to be involved since changing the solvent system did not eliminate the afore mentioned phenomenon.

In view of the low concentrations of PGs detectable it was clearly necessary to attempt to concentrate the samples. Hence the use of internal standards of PG in the pooled AH samples, so that any loses of material or changes in binding due to any component of the AH would affect the standard PG to the same extent as the endogenous PG. Although following these

procedures the samples containing AH no longer bound more exogenous PG than the "total bound" sample, which was used to construct a standard curve, the sensitivity of the assay was so poor that it could not differentiate between relatively high and low concentrations of standard PG. It therefore seemed that the RIA used in the present experiments could be used neither to accurately measure low concentrations of PG nor differences in PG levels before and after drug treatment. Nontheless it might be estimated that AH from rabbits treated with either adrenaline or its vehicle contained less than 10pg of $PGF_{2\alpha}$ or PGE2.

PG levels in AH have been measured by other workers using bioassay. When estimated by the inhibition of ADP-induced platelet aggregation, basal levels of AH prostacyclin were estimated at $3ng.ml^{-1}$ (Hoyng et al, 1982), whereas AH assayed on the isolated rat fundus strip had PG - like activity of $0.7ng.ml^{-1}$ which was reduced to $0.2ng.ml^{-1}$ following aspirin pretreatment (Miller et al,1978). By comparison with the present study, it appears that prostacyclin concentrations exceed PGE_2 and $PGF_{2\alpha}$ concentrations. Since these values are greater than the present study it appears that either these bioassays are measuring more than just the compounds claimed or else AH prostacyclin concentrations exceed PGE_2 and $PGF_{2\alpha}$ concentrations. Alternatively there is a serious unexplained loss occurring in our extraction.

The Effect Of Indomethacin and Piroxicam Pretreatment On The Ocular Responses To Analogues of cAMP In The Anaesthetised Rabbit.

It is widely accepted that stimulation of beta adrenoceptors by adrenaline raises intracellular levels of cAMP and this has been demonstrated in the AH. (See Introduction - The Biochemical Mechanism of Action of Adrenaline in Lowering IOP). Many workers believe that it is this cAMP which is responsible for the outflow and pressure responses to adrenaline. However, present results imply that PGs are produced which may also mediate these responses to adrenaline. In 1982, W.S.Wilson and Alisdair Campbell, reported that indomethacin pretreatment inhibited the outflow response to intracamerally injected dibutyryl cAMP, implying that cAMP stimulates PG production which in turn raises facility of outflow. Although similar results were obtained in the present study, the failure of piroxicam to antagonise the outflow response to either 8 bromo or dibutyryl cAMP suggested that this effect of indomethacin was not a result of inhibition of PGbiosynthesis. In the previous study (Wilson and Campbell, 1982) control observations of the effect of intracamerally injected vehicle had not been carried out and were therefore investigated in the present study. Since both dibutyryl cAMP and its vehicle raised facility of outflow. It appeared that neither dibutyryl cAMP was having a consensual effect or that the injection procedure alone and not the drug was responsible for this rise. Although in subsequent experiments saline was injected into one

eye only, facility of outflow was raised in both eyes. It therefore appeared that the dibutyryl cAMP -induced rise in outflow facility may have been an injection artifact.

In all of these experiments 2 needles had been inserted. Drugs were injected through one needle and the eye perfused through the other. However, since the eye is a particulary sensitive structure it is possible that the presence of these 2 needles traumatised the eye which in turn caused a rise in facility of outflow. By inserting a "T" piece into the tubing attached to the perfusion needle, it is possible to both perfuse and inject into the anterior chamber using only one needle. Nonetheless the injection of only small quantities of saline into the anterior chamber continued to raise facility outflow in both eyes. When compared to the NPND eye, perfusion of the anterior chamber alone did not raise AH protein concentrations. However, perfusion of the chamber in the presence of a second did raise protein levels (approximately 50% increase). If AH protein concentrations are indicative of the extent of trauma in the eye, then this result suggests that this was traumatic to the eye. Unfortunately these technical problems precluded any conclusions being drawn from these experiments. Therfore it has also been impossible to confirm a link between cAMP and the adrenaline induced rise in facility.

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APPENDIX

Appendix 1

Assay Solutions

(1) Radioimmunoassay buffer

KH_2PO_4	1.79g
NaCl	0.78g
Distilled water	to 250ml

The pH of the solution was adjusted to 7.4 and then 0.5g of bovine gamma globulins were added.

(2) Dextran-coated charcoal solution

Dextran-coated charcoal	0.5g
KH_2PO_4 Buffer	to 100ml

(3) KH_2PO_4 Buffer (10mM)

KH_2PO_4	0.136g
Distilled water	to 100ml

(4) Solution C for protein determination

Solution C — 98 parts of solution A
2 parts of solution B

These are mixed on the day of use.

Solution A — Sodium carbonate 2g
Sodium hydroxide (0.1N) add 100ml

Solution B — Cupric Sulphate 0.5g
Sodium Potassium Tartrate 1.0g
Distilled Water add 100ml

(5) Ferric nitrate solution for salicylate assay

Ferric nitrate	40g
Mercuric chloride	40g
HCl (0.1N)	to 1 litre

APPENDIX (11)

Chemicals and drugs used

Chemical	Manufacturer/Supplier
Adrenaline B.P. (Eppy)	Smith and Nephew (Gift)
L-ascorbic acid	Sigma
Aspirin B.P. (Suppositories)	Macarthys Ltd.
Bovine Gamma Globulins	Sigma
Calcium Chloride	Hopkin and Williams Ltd
Dextran-Coated Charcoal	Sigma
Ferric Nitrate	May and Baker Ltd
Folin Ciocalteu Reagent	Sigma
D-Glucose	Hopkin and Williams Ltd
Halothane	May and Baker Ltd
Heparin Sodium(Mucous) (Pularin)	Duncan Folckhart Ltd
Hydrochloric acid	Hopkin and Williams Ltd
Indomethacin	Sigma
Liquifilm Tears	Allergan
Magnesium Chloride	Hopkin and Williams Ltd
Mercuric Chloride	Hopkin and Williams Ltd
Nitrogen (oxygen free)	British Oxygen Company
Nitrous Oxide	British Oxygen Company
Oxygen	British Oxygen Company
Piroxicam	Pfitzer Ltd (Gift)
Potassium Hydrogen Phosphate	Hopkins and Williams Ltd
PGE_2 Anti-sera	Pharmacology Dept G.U. (Gift)
6 Keto PGF_1 Anti-sera	Pharmacology Dept G.U. (Gift)
Scintillant	Packard
Sodium Carbonate	Hopkin and Williams Ltd
Sodium Chloride	Koch Light Ltd

Sodium Chloride (Minims)	Smith and Nephew (Gift)
Sodium Hydroxide	Hopkin and Williams Ltd
Timolol Maleate (Timoptol)	Merk Sharp and Dohme (Gift)
Vehicle for Timolol	Merk Sharp and Dohme (Gift)
Verapamil	Sigma

All chemicals were of Analar quality.
All radioactively labelled compounds were obtained from the Radiochemical Centre, Amersham.

APPENDIX (111)

Statistics

(a) Statistical comparisons in the conscious rabbit.

Before IOP was measured using the tonometer the rabbits were accustomed to both the tonometer and laboratory conditions. Nonetheless the animals were frightened by sudden movements or noises. As a result inter and intra rabbit measurements of IOP were variable. To minimize this variability 't' tests were carried out on the mean differences between values at each time point within or between rabbits.

(b) Statistical comparisons in the anaesthetised rabbit.

Comparisons of responses within or between eyes were carried out using a 't' test which compared the means of absolute values.

Appendix IV

(a) Precision

An example of the method for determining total facility of outflow (C) is given below.

	Time				
	0 min	1 min	2 min	3 min	4 min
Weight of reservoir	635mg	631mg	630mg	629.5mg	628.5mg
Fluid loss from reservoir		4.0mg	1.0mg	0.5mg	1.0mg

Assuming a density of 1.00g ml^{-1} 4µl of perfusion fluid left the reservoir during the first minute which probably represents compliance. (see Methods). C is calculated from the quantity of fluid leaving the reservoir over the next 3 minutes, ie. 2.5µl in 3 minutes. If during this period the record of IOP shows that the pressure applied by the reservoir was 3.8mmHg above ambient IOP, then

$$C = \frac{2.5}{3 \times 3.8} = 0.22 \mu l min^{-1}. \ mm^{-1}Hg$$

Analysing the precision fo this method, the most reliable component of this result is the value for time, determined correct to ± 1sec. Precision is therefore to within approx. 0.5%. The applied pressure is read from the recorder chart within 0.2mm giving a precision of approx. 5%

The least reliable component is the value for weight of fluid, determined on the balance scale whose smallest division is 1mg. Precision here is certainly to within 0.5mg, ie 17%, of the normal control outflow (values under drug treatment always being higher than this).

(b) The relationship between IOP, total facility of outflow (c), episcleral venous pressure (EVP) and the rate of formation of aqueous humour (F) are given by the following equation

$$IOP = \frac{F}{C} + EVP$$

$$F = (IOP-EVP)C$$

Most workers assume that EVP is relatively constant and has a value in rabbit of approx. $9mm^{Hg}$. Assuming this to be true in the present experiments, values for F can be deduced for any of the data shown in the Results section, e.g. in Fig 9 using zero time data for the vehicle treated eye,

$$F = (18.2-9)\ 0.25$$

$$= 2.3\mu l min^{-1}$$