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The effects of prostaglandins on the intraocular pressure of the rabbit

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1. The effects of intracameral injections of prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, and A_1 were studied on the intraocular pressure (IOP) of rabbits anaesthetized with urethane.

2. With the exception of prostaglandin $F_{1\alpha}$, all the prostaglandins studied were found to be capable of producing a large, sustained rise in IOP, accompanied in many cases by miosis.

3. A marked decrease in response to repeated injections was found with all the prostaglandins studied; this effect was more pronounced following a large initial response to the prostaglandin.

4. The descending order of potency in their ability to raise IOP was as follows: prostaglandin $E_1 \approx E_2 > F_{2\alpha} > A_1 > F_{1\alpha}$.

5. Intracameral injections of prostaglandins E_1 and E_2 resulted in an increase in the protein content of the aqueous humour, which was related to the magnitude of the sustained increase in IOP.

6. Stabilization of the blood-aqueous barrier with polyphloretin phosphate markedly reduced both the IOP response and the effect of prostaglandin E_2 on the protein content of the aqueous humour.

7. It is concluded that the production of local vasodilatation and increased permeability of the blood-aqueous barrier play an important part in the effect of prostaglandins on the IOP. The involvement of prostaglandins in the response of the rabbit eye to irritation is discussed.

Injections of prostaglandins into rabbit eyes have been shown by Waitzman & King (1967) to produce a sustained rise in intraocular pressure (IOP). This effect on the IOP was thought to result from an action of prostaglandins on the metabolic processes involved in aqueous humour production, rather than from vascular or permeability changes (Waitzman, 1968), although it was well known that many prostaglandins are powerful vasodilators. Moreover, it had been shown earlier that the response of the rabbit eye to irritation is associated with the release into the aqueous humour of irin, an ether-soluble spasmogen with vasodilator activity consisting of unsaturated hydroxy fatty acid(s) (Ambache, Kavanagh & Whiting, 1965); subsequently, two prostaglandins, E_2 and $F_{2\alpha}$, were identified as components of rabbit irin (Ambache, Brummer, Rose & Whiting, 1966; Ambache & Brummer,

1968). Thus, there was good evidence that prostaglandins are involved in the response of the rabbit eye to irritation. It was also well known that the rabbit eye responds to chemical or mechanical irritation with a prolonged miosis, vasodilatation, increased capillary permeability and a sustained rise in IOP (Duke-Elder & Duke-Elder, 1931 ; Davson & Quilliam, 1947 ; Perkins, 1957) ; these effects cannot be prevented by the previous administration of atropine and mepyramine (Ambache *et al.*, 1965), and the prolonged miosis is mimicked both *in vivo* and *in vitro* by the administration of irin (Ambache, 1956, 1957). This strongly suggests that both vascular effects and alterations in the permeability of the blood-aqueous barrier are in fact involved in the IOP response of rabbits to prostaglandins.

In the experiments described in this paper we have re-examined the effects of prostaglandins on the rabbit IOP. In order to study the actions of prostaglandins on ocular permeability, we have observed the effects of prostaglandins on the protein content of the aqueous humour and their interaction with polyphloretin phosphate, a compound known to antagonize the breakdown of the blood-aqueous barrier.

Methods

New Zealand white rabbits weighing 2·5–3·5 kg were anaesthetized with 1–2 g/kg urethane injected into a marginal ear vein as a 25% solution in 0·9% NaCl solution. The femoral artery and vein were cannulated and mean arterial blood pressure was measured with a Statham P23Db transducer. All recordings were made by a Beckman R Dynograph. Each animal received heparin, 250–500 i.u./kg intravenously before cannulation of the eyes.

Two hypodermic needles were then introduced through the cornea into the anterior chamber of each eye. One needle (23 gauge) was connected by polyethylene tubing to a Sanborn pressure transducer (model 267b) and the IOP measured as described previously (Eakins, 1963), with the modification that in the present experiments no Perspex block was used, all connections being made directly to the transducer by means of Hamilton three-way stopcocks (Hamilton Company, Whittier, California, U.S.A.). The other needle (27 gauge) was connected by polyethylene tubing to a calibrated Agla (Burroughs Wellcome) micrometer syringe for the intracameral injections. All intracameral injections were made after at least 5 min recordings of steady state IOP.

Stock solutions of prostaglandins (10 mg/ml.) were stored at $-10°$ C in 95% ethyl alcohol and diluted to the desired concentration in 0·9% NaCl immediately before use. A constant dose-volume of 10 μl. was used for the intracameral injections in all these experiments. Each injection of prostaglandin into the test eye was accompanied by a simultaneous injection of an equal volume of saline into the contralateral control eye. Periodic checks were made of the activity of prostaglandin in stock solutions by injecting a standard dose with a known effect on IOP. Stock solutions were discarded when the increase in IOP was significantly lower than the established value. Under these conditions stock solutions were usually used for no longer than 4 weeks.

Close-arterial infusions of polyphloretin phosphate

In one series of experiments, simultaneous intracameral injections of 1 μg prostaglandin E_2 were made into both eyes of each rabbit. Polyphloretin phosphate (10

mg/ml.) was infused into the peripheral stump of the lingual artery on one side at a rate of 0·5 mg/min. The infusion was started 15 min before the prostaglandin injection and continued throughout the response period. The polyphloretin phosphate was obtained from A. B. Leo, Hälsengborg, Sweden.

Determination of protein concentration in aqueous humour

Samples of aqueous humour were removed from the anterior chambers of both eyes 30 min after the intracameral injection of prostaglandin into the test eye. A semi-quantitative estimation of protein was made by the addition of an equal volume of 8% trichloroacetic acid. Samples of normal aqueous humour from cannulated eyes yield a barely perceptible to slight turbidity (0 to +) under these conditions. Moderate turbidity has been classified + + and actual flocculation + + +. Quantitative, colorimetric determinations of the protein in aqueous humour were made as described by Lowry, Rosenbrough, Farr & Randall (1951).

Results

Control experiments

No change in either IOP or the protein content of the aqueous humour was observed following the intracameral injection of 10 µl. 1% ethanol. This was equivalent to the highest concentration of alcohol in any prostaglandin test solution.

Effect of prostaglandins on IOP

A typical response to the intracameral injection of prostaglandin (PG) is shown in Fig. 1. In this rabbit, the left eye received 0·5 µg PGE_1 at time 0, while the right eye was used as a control and received an equal volume of 0·9% NaCl solution. Following the injection artefact, the IOP of the control eye returned to normal within 5–10 min. The test eye, on the other hand, showed a rise in IOP after the injec-

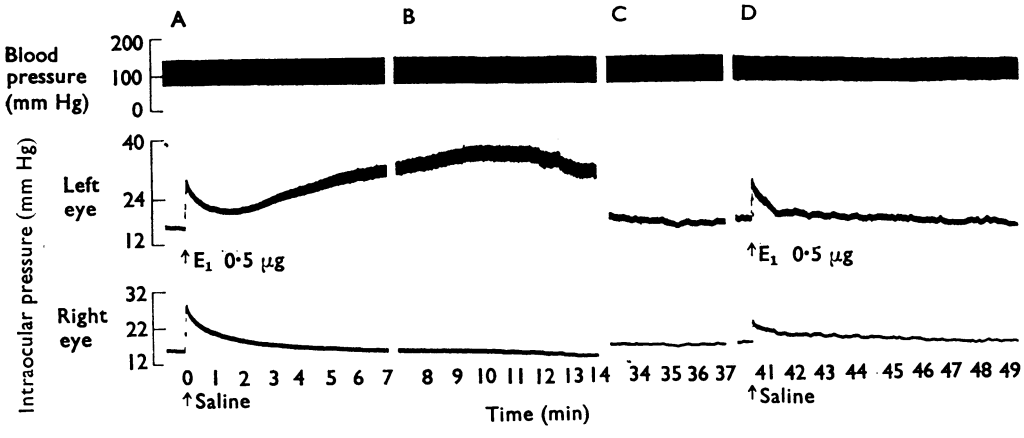

FIG. 1. Rabbit, urethane anaesthesia. Effect of PGE_1 on IOP. A, Intracameral injection of 0·5 µg PGE_1 in 10 µl. saline (0·9% NaCl) solution into the left eye, 10 µl. saline solution injected into the control right eye. B, Peak response in test eye 9–12 min after the injection. C, Return of IOP in the test eye to normal. D, Intracameral injections repeated as in A. Note the failure of the second injection of PGE_1 to produce a sustained rise in IOP.

tion artefact, associated with an increased amplitude of pulsation. In general, the IOP in the test eyes reached a maximum by 10–15 min, remained at this level for a variable length of time, then slowly returned to the initial value within 40–120 min, depending on the particular prostaglandin used and the dose injected. In general, the rise in IOP following intracameral injections of prostaglandin was associated with a congested appearance of the blood vessels in the iris.

Following the initial response, a second injection of the same dose of the prostaglandin usually had no effect on the IOP (Fig. 1D). This lack of response to repeated injections was found with all the compounds studied, and was more pronounced following a large pressure response to the initial injection of prostaglandin.

In a number of experiments, injection of prostaglandin into one eye resulted in a delayed rise in IOP in the contralateral eye, as seen in Fig. 2. In this experiment the left eye received an injection of 0·5 μg PGE_2; it showed a characteristic increase in IOP, reaching a maximum after 15 min. The IOP in the contralateral eye returned to normal following the injection artefact and remained there during the height of the response of the test eye. A gradual increase in IOP of the control eye then took place some 20–60 min later as the IOP of the test eye declined. There was a considerable variation in this contralateral response among individual animals receiving an identical dose of the same prostaglandin. Out of thirty-two animals receiving either prostaglandin A_1, E_2 or $F_{2\alpha}$, 13 exhibited this contralateral response; the frequency of this response appeared to be lower in animals receiving PGE_1.

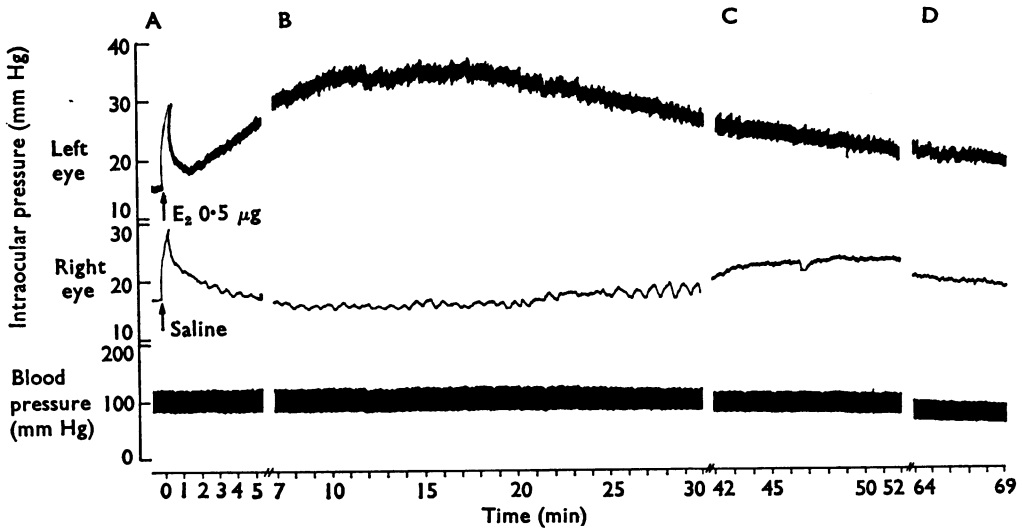

FIG. 2. Rabbit, urethane anaesthesia. Recordings of IOP and blood pressure, showing the delayed rise in IOP sometimes observed contralaterally in the control eye. A, Intracameral injection of 0·5 μg PGE_2 into the test left eye, 10 μl. saline (0·9% NaCl) solution injected into the control right eye. B, Peak response in test left eye, start of contralateral response in the right eye. C, Return of IOP to normal in left eye, increase in pressure in the right eye. D, IOP response still present in both eyes 1 hr after the injections.

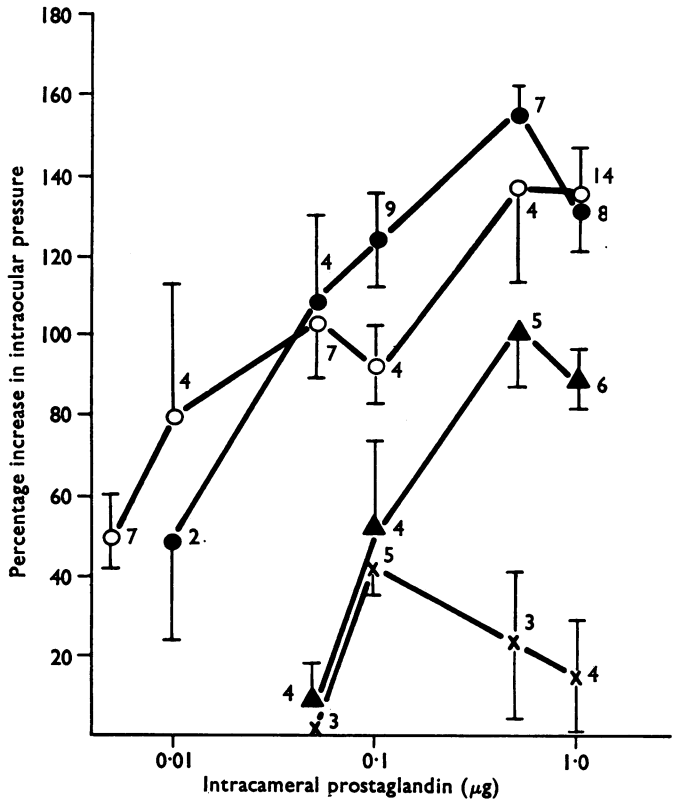

FIG. 3. Dose-response curves for the effect of various prostaglandins on IOP. ○——○, E_1; ●——●, E_2; ▲——▲, $F_{2\alpha}$; ×——×, $F_{1\alpha}$. The number of experiments at each point is indicated on the graph. Vertical bars represent the standard error of the mean.

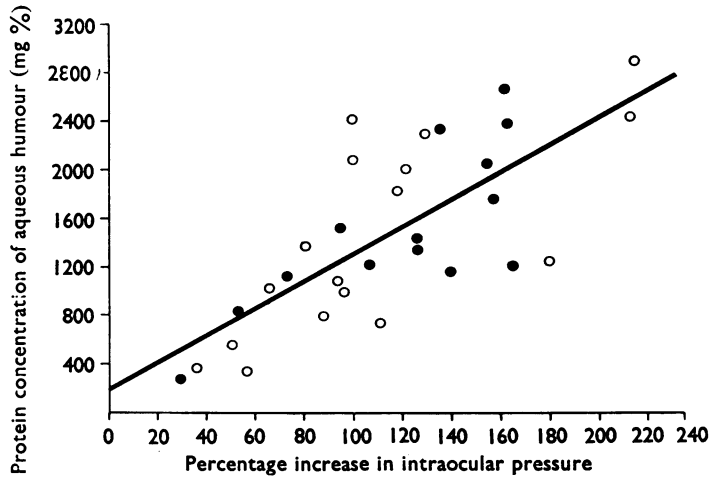

FIG. 4. Relation between the increase in IOP and the protein content of aqueous humour following the intracameral injections of PGE_1 and PGE_2. ●, E_1; ○, E_2. Regression line calculated from the combined values $y=11{\cdot}2x+195$ ($r=0{\cdot}75$; $P<0{\cdot}001$).

For a quantitative evaluation of individual prostaglandins in raising IOP, dose-response curves were obtained for prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$. The maximum increase in IOP was expressed as a percentage of the pre-injection pressure level; the results are shown in Fig. 3. The relative potencies can be expressed as follows: prostaglandin $E_1 \approx E_2 > F_{2\alpha} > F_{1\alpha}$. With $PGF_{1\alpha}$ a small increase in IOP was seen following the injection of 0·1 μg; however, the IOP response was less following the injection of 1 μg $PGF_{1\alpha}$. In six animals, intracameral injections of PGA_1 were also found to raise IOP. However, PGA_1 was less effective than prostaglandins E_1, E_2 and $F_{2\alpha}$ in that larger doses were required to produce a given elevation in IOP. In four eyes receiving 1 μg PGA_1, the mean increase in IOP was 88%, and in two eyes, injection of 2·5 μg PGA_1 yielded increases of 122 and 138%.

The rise in IOP resulting from the prostaglandin was frequently accompanied by constriction of the pupil, as found with irin (Ambache, 1956). The most effective miotic was PGE_1. It was of interest that miosis was never observed in control eyes even when a delayed rise in IOP was evident.

TABLE 1. *Effect of intracameral injection of prostaglandin E_2 on protein content of aqueous humour in rabbits*

Prostaglandin E_2 (μg)	Aqueous humour protein (mg% ± S.E.)
0	200±21 (29)
0·005	683±145 (4)
0·01	892±158 (4)
0·05	991±136 (10)
0·1	1190±271 (4)
1·0	2000±243 (12)

Protein determined by the method of Lowry *et al.* (1951). The number of experiments is shown in brackets.

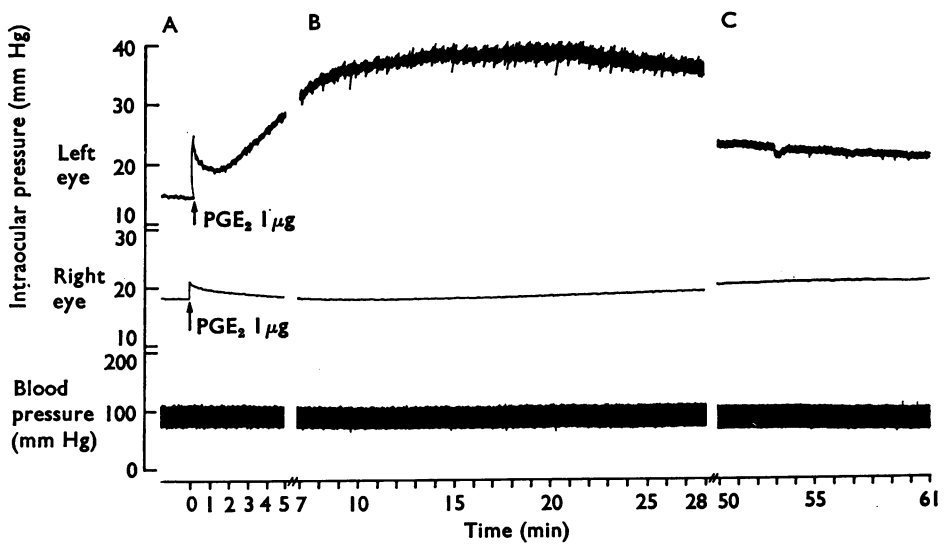

FIG. 5. Rabbit, urethane anaesthesia. Effect of polyphloretin phosphate on the response of the IOP to PGE_2. Polyphloretin phosphate (0·5 mg/min) was infused into the right lingual artery for 15 min before the intracameral injection of 1 μg PGE_2 into both eyes. Note the lack of response of the IOP in the right eye to the PGE_2.

Effect of prostaglandins on the protein content of aqueous humour

Addition of trichloroacetic acid to samples of aqueous humour withdrawn from cannulated control eyes resulted in a barely perceptible to faint turbidity. In contrast, samples of aqueous humour withdrawn from eyes treated with either PGE_1 or PGE_2 yielded a moderate turbidity to actual flocculation when treated with trichloroacetic acid. It was frequently observed that after large loses of prostaglandin (0·5–1·0 μg), the samples of aqueous humour contained haemolysed blood. These experiments indicated that the amount of protein found in the aqueous humour was proportional to the magnitude of the elevation of IOP produced by the prostaglandin. Quantitative determinations of aqueous humour protein concentrations confirmed these observations (Table 1). In all cases, aqueous humour from the test eyes contained more protein than that withdrawn from control eyes.

A positive correlation was found between the effects of prostaglandins on IOP and aqueous humour protein concentrations (Fig. 4). The Pearson correlation coefficient (Underwood, Duncan, Taylor & Cotton, 1954) is significant at the 99·9% level of confidence when calculated from both the individual values for PGE_1 ($r=0·76$) and PGE_2 ($r=0·73$) and the combined values ($r=0·75$). The calculated regression line for the combined results is also shown in Fig. 4.

A series of experiments was conducted to ascertain whether the pH of the prostaglandin solution influenced its effect on IOP and protein concentration. The action of prostaglandin when delivered in 0·1 M sodium phosphate buffer (pH 7·8) did not differ from that of prostaglandin in 0·9% NaCl.

Effect of polyphloretin phosphate on the response of the IOP to prostaglandin E_2

Close-arterial infusion of polyphloretin phosphate (a synthetic phosphorylated, polyanionic phloridzin derivative of high molecular weight, with anti-hyaluronidase activity) was found to inhibit or markedly antagonize the rise in IOP normally seen after the intracameral injection of 1 μg PGE_2. A typical result of such an experiment can be seen in Fig. 5. While the IOP in the left eye increased by 162% after PGE_2, an IOP response was not evident in the right eye, which received the close-arterial infusion of polyphoretin phosphate. In five such experiments, polyphloretin phosphate markedly reduced both the IOP response and the effect of PGE_2 on the protein content of the aqueous humour (Table 2).

TABLE 2. *Effect of close-arterial infusions of polyphloretin phosphate (PPP) on the rise in IOP produced by intracameral injections of prostaglandin E_2 in the rabbit*

	Left eye (1 μg prostaglandin E_2)		Right eye (1 μg prostaglandin E_2 + PPP)	
	% increase IOP	Protein	% increase IOP	Protein
	286	+++	36	++
	124	+++	41	++
	162	+++	14	0
	64	++	2	0
	196	+++	35	+
Mean	166		26	
± S.E.	37		8	

Polyphloretin phosphate (10 mg/ml.) was infused into the right lingual artery at a rate of 0·5 mg/min. Protein content of the aqueous humour was estimated by the use of 8% trichloroacetic acid; slight turbidity 0 to +, moderate turbidity ++, flocculation +++.

Discussion

In contrast to the findings of Waitzman & King (1967), we have observed that intracameral injections of as little as 10 ng PGE_1 or PGE_2 resulted in a significant increase in the protein content of aqueous humour, indicating an increase in the permeability of the blood-aqueous barrier. Furthermore, we have found a good correlation between the concentration of protein in the aqueous humour and the sustained increase in IOP produced by either prostaglandin. These findings on the protein content of aqueous humour, together with the congested appearance of blood vessels in the region of the iris, suggest that both vasodilatation and increased capillary permeability play a significant role in the response of the IOP to prostaglandins in the rabbit. Prostaglandins are known to be vasodilators, particularly the ketonic PGEs. Local vasodilatation resulting in an increase in ocular blood volume will produce a rise in IOP ; however, such an effect would be transient in nature, and although it could be responsible for the initial rapid rise in IOP, it could not be the sole cause of the sustained rise in IOP seen after the intracameral injection of prostaglandin. A sustained rise in IOP could occur, however, after a breakdown of the blood-aqueous barrier, associated with local vasodilatation, by exudation of fluid into the aqueous compartment of the eye from intraocular blood vessels.

Further support for the hypothesis that permeability changes play a major part in the effect of prostaglandins on IOP comes from the observation that polyphloretin phosphate either prevented or markedly antagonized both the rise in IOP and the increase in protein content of aqueous humour seen after intracameral injection of PGE_2. Polyphloretin phosphate is an anti-hyaluronidase and has been shown to prevent increased capillary permeability in response to trauma in the rat paw and guinea-pig lung (Fries, 1960). Moreover, Cole (1961) has found that close-arterial infusions of polyphloretin phosphate either totally abolished or markedly antagonized the rise in IOP seen in the rabbit eye following mechanical or chemical irritation, and prevented the rise in the protein concentration of aqueous humour which normally followed chemical irritation with mustine hydrochloride. Thus, when breakdown of the blood-aqueous barrier was prevented, PGE_2 was without effect on the IOP in our experiments.

We can offer no satisfactory explanation for the differences between the present results and those of Waitzman and King (1967) on the effect of prostaglandins on the protein content of aqueous humour. However, there is good evidence that prostaglandins are involved in the response of the rabbit eye to irritation. The characteristic changes known to occur when the rabbit eye responds to irritation, namely miosis, vasodilatation, an increase in capillary permeability and a sustained rise in IOP, are now thought to result from the release of irin (i.e. PGE_2 and $PGF_{2\alpha}$) into the aqueous humour (Ambache *et al.*, 1965). Furthermore, intradermal injections of irin in man (Ambache, 1961) produced erythema and of PGE_1 in the guinea-pig (Horton, 1963) increased capillary permeability ; and prostaglandins are known to be vasodilator in the rabbit (compare Bergström, Carlson & Weeks, 1968). At the present time, we cannot rule out the possibility that other factors, such as an increase in the rate of secretion of aqueous humour or a decrease in the rate of outflow of aqueous humour from the anterior chamber of the eye, may contribute to the response of the IOP to prostaglandins in the rabbit, although Waitzman &

King (1967) have reported that in their experiments prostaglandins did not affect outflow facility.

The tachyphylaxis observed in the response of the IOP to prostaglandins has not been reported before, although tachyphylactic responses to prostaglandins have been observed in a variety of biological systems (Eliasson, 1959; Änggård & Bergström, 1963; Avanzino, Bradley & Wolstencroft, 1966). The present observations may be related to the increased protein content of the aqueous humour in the eyes which received prostaglandins, since Duke-Elder & Duke-Elder (1931) observed that the IOP in the rabbit became refractory to repeated mechanical stimulation of the iris, and Ambache (1959) showed that protein may interfere with the action of irin on the hamster colon. Thus, the presence of plasma proteins in the aqueous humour may curtail the action of intracamerally administered prostaglandin.

The delayed rise in IOP sometimes observed in the control eye following injection of prostaglandin into the test eye resembles the consensual changes in IOP observed after either chemical (Davson & Matchett, 1951) or mechanical (Perkins, 1957) irritation of one eye. There is evidence that the consensual response is reflex in origin (Davson & Huber, 1950; Perkins, 1957) and may involve the release of prostaglandins in the contralateral eye, since Ambache *et al.* (1965) were able to demonstrate the presence of irin in samples of aqueous humour removed from one eye 30 min after paracentesis of the other eye.

We conclude from these results that local vasodilatation and increased permeability of the blood-aqueous barrier play an important part in the ability of prostaglandins to produce a sustained rise in intraocular pressure in the rabbit eye. There is evidence from the results presented here and the work of others that prostaglandins are involved in the response of the rabbit eye to irritation. Whether or not prostaglandins have a similar function in the eyes of other species, including man, requires further investigation.

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