

PREVENTION OF EXPERIMENTAL OCULAR HYPERTENSION WITH POLYPHLORETIN PHOSPHATE*

BY

D. F. COLE

M.R.C. Ophthalmological Research Unit, Institute of Ophthalmology, University of London

It is well established that certain chemical or mechanical injuries to the blood-aqueous barrier are frequently followed by the formation of a protein-rich aqueous humour and an increase in intra-ocular pressure (I.O.P.). It seems likely that in both cases the rate of aqueous formation is increased and that the capillaries of the anterior uvea and the ciliary epithelium become more permeable to proteins. In particular, damage of this kind is produced by rapid reduction of the I.O.P., as in paracentesis (Seidel, 1918; Franceschetti and Wieland, 1928; Poos, 1931; Rohen, 1953), or by the local administration of an irritant such as mustine (Davson and Quilliam, 1947; Davson and Matchett, 1953; Davson, 1956).

More recently it has been shown that the permeability of serous membranes and of capillaries may be reduced by administering polyphloretin phosphate (PPP) (Fries, 1956, 1960), a synthetic anti-hyaluronidase of high molecular weight (M.W. = 15,000) (Diczfalusy, Ferno, Fex, Högberg, Linderot, and Rosenberg, 1953). This substance also diminishes the formation of serous exudates (Fries, 1956). It seemed probable that PPP would exert a similar action in eyes in which the blood-aqueous barrier had been rendered more permeable by injury and thus prevent or reduce the rise in I.O.P. which would otherwise occur.

In the present series of experiments, both paracentesis and mustine hydrochloride were used to induce damage of the blood-aqueous barrier in order that the effectiveness of PPP might be assessed.

Experiments using the Schiötz Tonometer

Pairs of rabbits were anaesthetized with urethane, 1.75 g./kg. body weight, administered intravenously. One animal from each pair was then given 100 mg. PPP intravenously in 10 ml. isotonic saline, whilst the other received a control injection of 10 ml. isotonic saline. Some 10 to 20 minutes later one eye of each rabbit was treated with mustine hydrochloride (0.2 per cent.

* Received for publication September 1, 1960.

in isotonic saline) instilled into the conjunctival sac, the contralateral eye in each case serving as a control. Readings with the tonometer (5.5 g. wt.) were made on both eyes at fairly frequent intervals from 30 minutes before until 30 minutes after instilling the mustine HCl. At the end of the experiment both animals were killed and the aqueous removed for examination. Each eye was then connected to a saline reservoir and manometer by means of polythene tubing and a No. 15 hypodermic needle inserted into the anterior chamber along the track of the needle used to remove the aqueous. By altering the height of the reservoir above the eye, the Schiötz tonometer was calibrated for known values of intra-ocular pressure, a stopcock between the eye and the reservoir being closed whilst tonometer readings were taken. The aqueous was tested for protein with 5 per cent. trichloro-acetic acid.

In other animals mustine HCl was instilled into the conjunctival sac some 20 minutes before administering the PPP in order to determine whether this treatment would depress an already increased I.O.P.

Direct Recording of I.O.P.

Animals were anaesthetized with urethane as before and a polythene cannula for recording the arterial blood pressure tied in the femoral artery. A fine cannula, through which PPP solution could be infused, was inserted into one lingual artery (Cole, 1959). The rabbit was turned back uppermost and its head supported on a chin rest some 2 inches above the level of the table. The I.O.P. in both eyes was measured by means of two pressure transducers (Model 267B Sanborn Co., Inc., Waltham, Mass., U.S.A.) connected with the aqueous in the anterior chamber by fine polythene tubing containing heparinized saline (1,000 I.U. per 100 ml.) and terminating in No. 27 hypodermic needles. Before insertion through the cornea each needle was carefully positioned so as to ensure the least possible traction upon the eye. A pressure reservoir, also containing heparinized saline, connected to both the transducer and the intra-ocular needle was raised to 18–20 mm. Hg and the saline was allowed to flow freely out through the needle. With the superior rectus muscle held firmly in fixation forceps the needle was then inserted through the margin of the cornea in the 12 o'clock position so as to lie in the anterior chamber with its bevel facing outwards. A tap connecting the intra-ocular needle with the pressure reservoir was closed and the I.O.P. recording commenced. A precisely similar operation was carried out upon the other eye and the blood-pressure cannula was connected to a third transducer by means of polythene tubing.

The two I.O.P. transducers were connected with two Sanborn pre-amplifiers (Model 150–1100), and the blood-pressure transducer with a Sanborn Model 150–3000 pre-amplifier, and thence to three of the four channels of a Model 154–100B Sanborn Recorder.

Once steady records for blood and intra-ocular pressure had been obtained, PPP was infused at 0.4 mg/min. through the lingual artery cannula by means of a motor-driven syringe. After some 10 to 15 minutes of this infusion a few drops of 200 mg. per cent. mustine HCl in saline were instilled into each eye and the recording was continued for a further 15 to 20 minutes. In some other animals mustine HCl was instilled before commencing the intra-carotid infusion and the administration of PPP was not started until the I.O.P. had begun to rise.

Paracentesis was simulated by an abrupt fall in I.O.P. which was brought about by lowering the reservoir on each side of zero pressure. The intra-ocular needle was then connected to this low pressure for some 2 minutes, after which the reservoir was disconnected and the I.O.P. was allowed to rise. It was thus possible to observe the recovery phase in both eyes, one of which was receiving a high concentration of PPP from the infusion through the lingual artery.

Results

The Table shows the changes in I.O.P., as measured with the Schiötz tonometer, in animals treated with mustine HCl and with PPP separately and with mustine HCl after PPP. The protein content of the aqueous is recorded semi-quantitatively over a range of - to + + +. It may be seen that, whilst PPP alone has no significant action, it is effective in preventing the ocular hypertension caused by mustine HCl. The rapid increase in I.O.P. produced by mustine HCl in animals not receiving PPP is shown in Fig. 1 (opposite).

TABLE
CHANGES IN INTRA-OCULAR PRESSURE AFTER

Time from Instillation of Mustine HCl (min.)		- 30	- 20	- 10	- 5
Administration of Mustine-HCl and PPP	Mustine HCl Only	-0.1 ±0.17	+0.1 ±0.10	+0.2 ±0.05	+0.3 ±0.17
	PPP Only	-0.2 +0.11	-0.2 ±0.09	+0.2 ±0.17	+0.2 ±0.14
	Untreated	-0.3 ±0.13	-0.3 ±0.12	+0.3 ±0.10	+0.4 ±0.11
	Mustine HCl after PPP	-0.3 ±0.11	+0.2 ±0.11	+0.2 ±0.10	-0.2 ±0.09

Values indicate mean changes (\pm S.E.M.) from the average

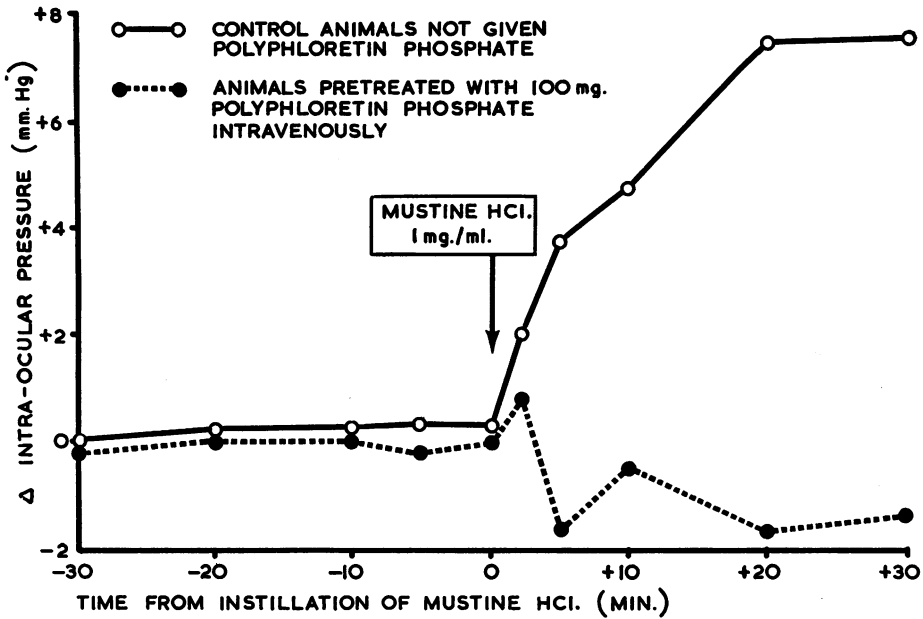


FIG. 1.—Mean changes in intra-ocular pressure measured with the Schiötz tonometer after the instillation of mustine hydrochloride at Time=0, in control animals and in animals pre-treated with 100 mg. polyphlorethin phosphate intravenously. In all cases tonometer readings were calibrated against the actual intra-ocular pressure at the end of the experiment. Each point represents the mean from not less than six eyes.

Two typical recordings of the I.O.P. are shown in Figs 2 and 3 (overleaf).

In Fig. 2 the I.O.P. in both eyes was reduced to zero for 2 minutes in simulation of paracentesis and was then allowed to increase, the left eye being treated with PPP administered *via* the left lingual artery throughout

ADMINISTRATION OF MUSTINE HCl AND PPP (mm. Hg)

0	+	+5	+10	+20	+30	Protein Content of Aqueous Fluid
+0.3 ±0.11	+2.0 ±0.15	+3.1 ±0.23	+5.2 ±0.36	+7.7 ±0.41	+7.4 ±0.42	++ to +++
-0.1 ±0.15	-1.1 ±0.47	-0.7 ±0.32	+0.3 ±0.21	+0.3 ±0.21	+1.5 ±0.89	- to ±
+0.3 ±0.15	-0.5 ±0.25	-0.3 ±0.31	+0.4 ±0.41	+1.2 ±0.67	+2.3 ±0.98	- to ±
+0.1 ±0.12	+0.9 ±0.43	-1.5 ±0.74	-0.7 ±0.42	-1.5 ±0.77	-1.4 ±0.31	± to +

I.O.P. taken between -30 min. and 0 min.

the experiment. In this experiment the final pressure in the right (untreated) eye was 30 mm. Hg, as compared with 21 mm. Hg in the treated eye.

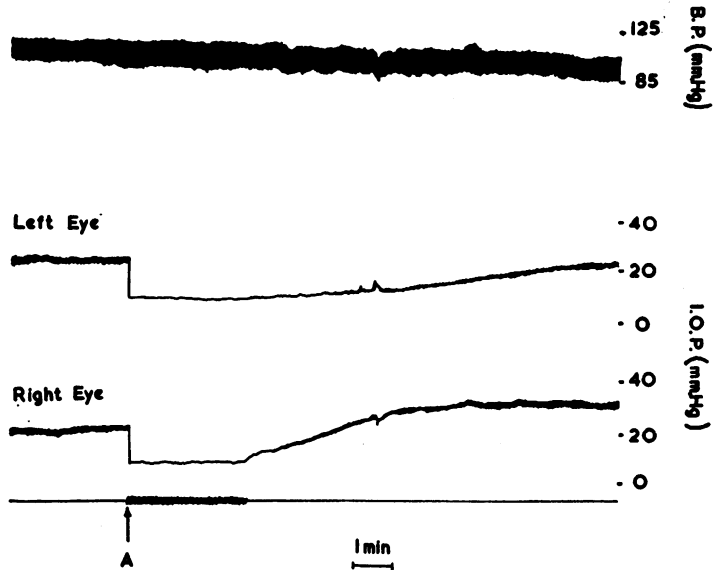


FIG. 2.—Recording of arterial blood pressure and intra-ocular pressure from both eyes, using the Sanborn Recorder. 1 per cent. polyphloretin phosphate was administered continuously throughout the experiment *via* the left lingual artery. At (A) both eyes were connected with a low pressure reservoir, abruptly reducing the intra-ocular pressure to 7 mm. Hg for 3 minutes. The formation of "secondary aqueous" was indicated by the rise in intra-ocular pressure in both eyes, and was more rapid on the untreated right side than on the left. The final pressure in the untreated right eye was 30 mm. Hg, as compared with 21 mm. Hg in the treated left eye.

Fig. 3 (opposite) shows the action of PPP, given *via* the left lingual artery as indicated at B, in limiting the rise in I.O.P. caused by the previous instillation of mustine HCl to both eyes at A. In this case the final pressure in the untreated eye was 36 mm. Hg, as compared with 28 mm. Hg in the treated eye. In Figs 2 and 3 the upper tracing represents the arterial blood pressure.

When the I.O.P. has been raised by treatment with mustine HCl, treatment with PPP reduces it to within the normal range over a period of 30 to 40 minutes (Fig. 4, opposite).

Discussion

PPP caused no significant alteration in the blood-pressure recordings. Hence, when attempting to explain the action of PPP upon the I.O.P., the occurrence of changes in systemic B.P. may be discounted both in these experiments and in those in which the Schiötz tonometer was used. This

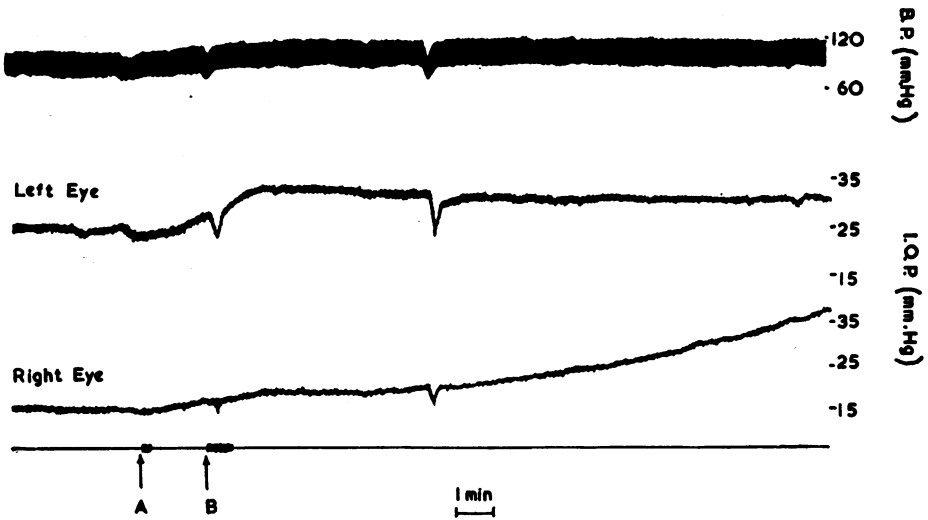


FIG. 3.—Recording of arterial blood pressure and intra-ocular pressure from both eyes, using the Sanborn Recorder. At (A) mustine hydrochloride, 1 mg./ml., was instilled into each eye, and, after the intra-ocular pressure had begun to rise, a 1 per cent. polyphloretin phosphate infusion was commenced *via* the left lingual artery (B). There was a gradual decline in the pressure in the left eye after starting the infusion of PPP, but the pressure in the right eye continued to rise. The final pressure in the untreated right eye was 36 mm. Hg as compared with 28 mm. Hg in the treated left eye.

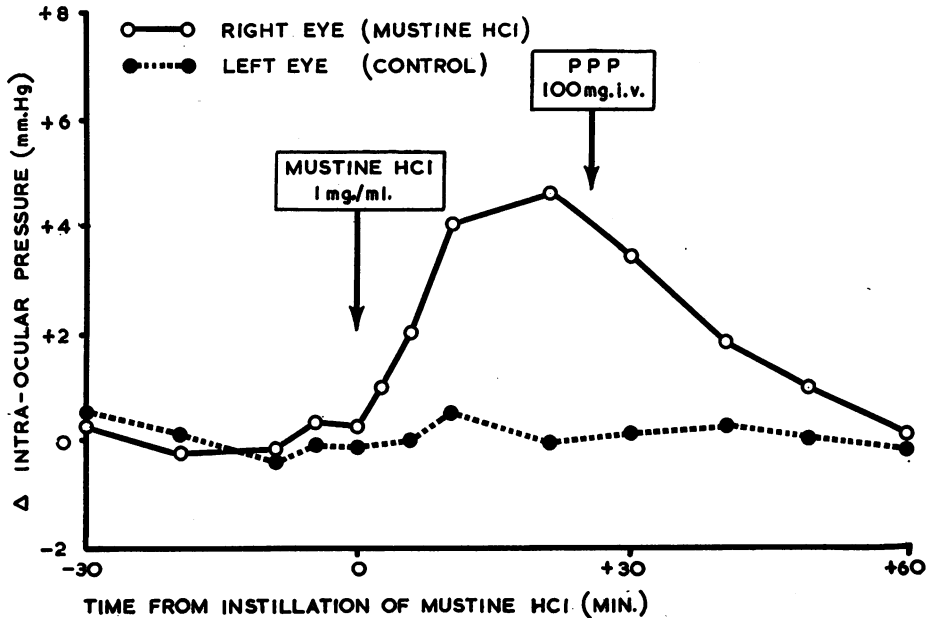


FIG. 4.—Changes in intra-ocular pressure, measured with the Schiötz tonometer after the instillation of mustine hydrochloride to the right eye at Time=0. 25 minutes later 100 mg. polyphloretin phosphate was given intravenously, resulting in a marked decrease in intra-ocular pressure in the right eye. The left eye was untreated throughout the experiment.

lack of effect on blood pressure is in keeping with other reports of the low toxicity of PPP (Fries, 1956; 1960).

It is known that, after the blood-aqueous barrier has been damaged by paracentesis, the anterior chamber becomes filled with fluid of high protein content, formed by exudation from the capillaries of the anterior uvea, notably those of the ciliary body (Seidel, 1918; Franceschetti and Wieland, 1928; Poos, 1931; Rohen, 1953). This increased capillary permeability facilitates the more rapid entry of fluid into the eye and also leads to the inflow channels becoming obstructed by clot formation in the secondary aqueous humour (Zwiauwer, Bornschein, and Deutsch, 1951). The doses of PPP used in the experiments described above are comparable with those which have been shown to prevent increased capillary permeability in response to trauma in other regions (Fries, 1960) and it seems highly probable that its present action was similar. In these experiments if the PPP treatment prevented exudation into the eye, obstruction of the drainage channels by fibrin would be precluded.

The hypertensive action of mustine HCl is likewise dependent upon increased permeability of the blood-aqueous barrier (Davson and Huber, 1950; Davson and Matchett, 1953) which the present results show to be effectively prevented by PPP (Fig. 1; Table). This again must be due to prevention of the increase of the permeability of the barrier, since aqueous from eyes treated with mustine HCl and PPP contained very much less protein than that from eyes treated with mustine HCl only (Table). When PPP was administered after the barrier had been damaged by mustine HCl and the increase in I.O.P. had begun, this rise was arrested or reversed (Figs 3 and 4), a finding which indicates that the aqueous drainage remained adequate. PPP given alone did not lower the I.O.P. in normal (untreated) eyes and there is, therefore, no reason to suppose that it increases facility of outflow or diminishes inflow unless the barrier is abnormally permeable.

In vitro experiments have shown that the outflow resistance may be decreased by adding hyaluronic acid to the fluid perfusing the anterior chamber (Bárány and Scotchbrook, 1954; Bárány and Woodin, 1955; François, Rabaey, and Neetens, 1956), and in the present experiments one would have anticipated, by virtue of its anti-hyaluronidase activity, that PPP, if it had exerted any action at all on the outflow system, might have favoured a rise in I.O.P. There was no evidence of such an effect in either the damaged or the control eyes, possibly because the high molecular weight of PPP limits its rate of penetration from blood to aqueous.

There is probably no simple relationship between the condition produced by paracentesis or mustine HCl and the protein exudation of anterior uveitis, and the findings of Böhringer (1957) indicate that the ability of the blood-aqueous barrier to secrete ascorbate was diminished by uveitis but not by paracentesis although massive exudation occurred in both conditions. Nevertheless, PPP may have some clinical application in limiting ocular

hypertension in so far as this is due to increased permeability of the blood-aqueous barrier.

Summary

The action of polyphloretin phosphate (PPP) upon the ocular hypertension caused by paracentesis and by mustine hydrochloride instilled into the conjunctival sac has been studied in rabbits:

(1) PPP prevents or limits the rise in I.O.P. which normally follows these procedures.

(2) PPP reduces the raised I.O.P. caused by previous administration of mustine.

(3) PPP prevents the rise in the protein concentration of the aqueous which is caused by mustine HCl.

It appears that these effects are due to a reduction of the capillary permeability in the anterior uveal tract of the damaged eye.

The author's thanks are due to Sir Stewart Duke-Elder for advice and encouragement, to the National Council to Combat Blindness, Inc., New York City, for defraying the expense of the Sanborn pressure recording apparatus, and to A. B. Leo, Halsingborg, Sweden, for a generous gift of polyphloretin phosphate.

REFERENCES

- BÁRÁNY, E. H., and SCOTCHBROOK, S. (1954). *Acta physiol. scand.*, **30**, 240.
 ——— and WOODIN, A. M. (1955). *Ibid.*, **33**, 257.
 BÖHRINGER, H. R. (1957). *Docum. Ophthalm.*, **11**, 281.
 COLE, D. F. (1959). *Brit. J. Ophthalm.*, **43**, 268.
 DAVSON, H. (1956). "Physiology of the Ocular and Cerebrospinal Fluids". Churchill, London.
 ——— and HUBER, A. (1950). *Ophthalmologica (Basel)*, **120**, 118.
 ——— and MATCHETT, P. A. (1953). *J. Physiol. (Lond.)*, **122**, 11.
 ——— and QUILLIAM, J. P. (1947). *Brit. J. Ophthalm.*, **31**, 717.
 DICZFALUSY, E., FERNO, O., FEX, H., HÖGBERG, B., LINDEROT, T., and ROSENBERG, T. (1953). *Acta chem. scand.*, **7**, 913.
 FRANCESCHETTI, A., and WIELAND, H. (1928). *Arch. Augenheilk.*, **99**, 1.
 FRANÇOIS, J., RABAËY, M., and NEETENS, A. (1956). *A.M.A. Arch. Ophthalm.*, **55**, 193.
 FRIES, B. (1956). *Acta chir. scand.*, Suppl. 217.
 ——— (1960). *Ibid.*, **119**, 1.
 POOS, F. (1931). *v. Graefes Arch. Ophthalm.*, **127**, 489.
 ROHEN, J. (1953). *Ber. dtsh. ophthal. Ges.*, **58**, 65.
 SEIDEL, E. (1918). *v. Graefes Arch. Ophthalm.*, **95**, 1.
 ZWIAUER, A., BORNSCHEIN, H., and DEUTSCH, E. (1951). *Amer. J. Ophthalm.*, **34**, 1403.