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THE CONTROL OF THE INTRA-OCULAR PRESSURE IN THE RABBIT

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Since the demonstration by Henderson & Starling in 1904 that, in the cat, stimulation of the cervical sympathetic caused a transitory rise, followed by a fall, in the intra-ocular pressure, comparatively little work has been done on the general problem of the nervous factors in the control of this important function. In rabbits, Adler, Landis & Jackson (1924) have shown that cervical sympathectomy exaggerates the increase in intra-ocular pressure following occlusion of the abdominal aorta; a fact suggesting that the sympathetic acts by reducing the intra-ocular pressure. Yata (1930) reported no effect of sympathectomy *per se* on the normal intra-ocular pressure, whilst stimulation lowered it, at the same time lowering the general blood pressure. More recently, Jaffe (1948) has stated that cervical sympathectomy induces a temporary fall in the intra-ocular pressure of cats; and Schmerl & Steinberg (1949) that stimulation of the superior cervical ganglion lowers the intra-ocular pressure whilst stimulation of the ciliary ganglion raises it. Finally, clinical studies in humans suggest a chronically lowered intra-ocular pressure following stlectomy.

The investigations to be reported here grew out of studies of the effect of *N*-mustard, the hydrochloride of methyl-*bis*(β -chloroethyl)-amine, on the eye; this substance, when instilled into the conjunctival sac, was found by Davson & Quilliam (1947) to cause a marked breakdown of the barrier between blood and aqueous humour, so that Evans Blue, which normally does not penetrate from the blood into the intra-ocular fluid, appears therein in significant quantities. It was observed that this effect was not confined to the treated eye, in the sense that the dose required to produce a lesion in one eye was smaller when the other eye was treated simultaneously. It was observed later (Davson & Huber, 1950*a*), that *N*-mustard produced an acute rise in intra-ocular pressure of the treated eye, but as only one manometer was available at the time, the intra-

ocular pressure of the other eye could not be determined. In the present work the pressures in both eyes have been recorded simultaneously. Besides the effects of *N*-mustard, those of cervical sympathectomy, stimulation of the peripheral end of the cut sympathetic, and unilateral carotid occlusion, were studied in the normal eye and in the eye treated with *N*-mustard. The observation that carotid occlusion caused a rapid fall in intra-ocular pressure on the same side, prompted a reinvestigation of the effects of this procedure on the rate of penetration of sodium into the aqueous humour (Bárány, 1947*a*), and this led to a measurement of the effects of carotid occlusion on the rate of drainage of aqueous humour from the eye.

METHODS

Intra-ocular pressure was measured simultaneously in both eyes by two separate membrane-type manometers, recently described by Davson & Purvis (1950). Movements of the membrane were magnified by a mirror which reflected a spot of light on to a scale on the wall; changes of 1 mm. Hg could be measured with ease. Hypodermic needles were connected to the body of the manometer by a length of polythene tubing. Nembutal was used as a general anaesthetic and pantocaine was applied locally to the eye. When nerve stimulation was involved, the cervical sympathetic nerve was isolated with a ligature, and the animal placed in the prone position over a hole on the operating table; section and stimulation of the nerve could then be carried out through this hole when the manometer needles were in position. This procedure was adopted to avoid the exophthalmos which occurs with the rabbit in the supine position; moreover, the needles are most conveniently inserted from above the eye; with the animal upright this permits steadying of the eye by holding the easily accessible superior rectus with forceps. The nerve was stimulated for 15–30 sec. with tetanic shocks from a Palmer induction coil.

The relative rates of penetration of sodium into the two eyes were determined by giving a single intravenous injection of 2–4 ml. of isotonic $^{24}\text{NaCl}$ and counting, after dilution, the samples of aqueous humour, withdrawn half an hour later, in the chamber described by Maurice (1948). Counts were accurate to within 1%. The rate of drainage of aqueous humour was determined by measuring the rate of removal of *p*-amino-hippuric acid, by the technique described by Bárány & Kinsey (1949). The principle of this method will be indicated in the Results Section; *p*-amino-hippuric acid was determined chemically by the method of Bratton & Marshall (1939). Arterial pressure was measured by the classical manometric technique.

RESULTS

The manometric method of determining intra-ocular pressure is not entirely satisfactory in that the trauma associated with the insertion of the needle produces an upset that is not readily compensated; thus it was rare to find identical pressures in the two eyes. A series of control runs, however, showed that the intra-ocular pressure changed only very slowly, and that frequently the two pressures approached each other. The slightest damage to the iris, or even the lens, caused by an inexpertly inserted needle, produced an immediate rise in the intra-ocular pressure. The curve in Fig. 1 illustrates the effect of an accidental touching of the lens by the needle. Associated with the acute rise in tension following this type of damage, the pupil constricts, so that any rise due to this adventitious cause can be recognized at once by the associated

meiosis. It is particularly important that this source of error be recognized in all determinations of intra-ocular pressure in which contralateral effects of some agency are being studied.

Fig. 2 shows the effects of a subconjunctival dose of 0.06 ml. of 1% *N*-mustard, in isotonic saline, on the pressures in the two eyes. Curves I represent the more usual result, the effect on the untreated eye being negligible, whilst curves II

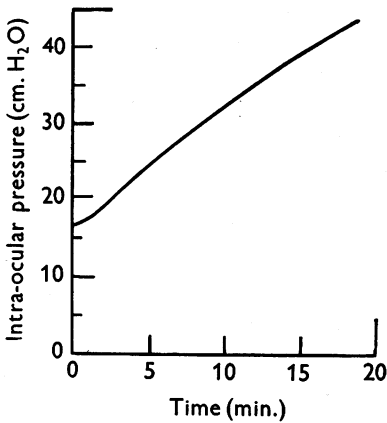


Fig. 1.

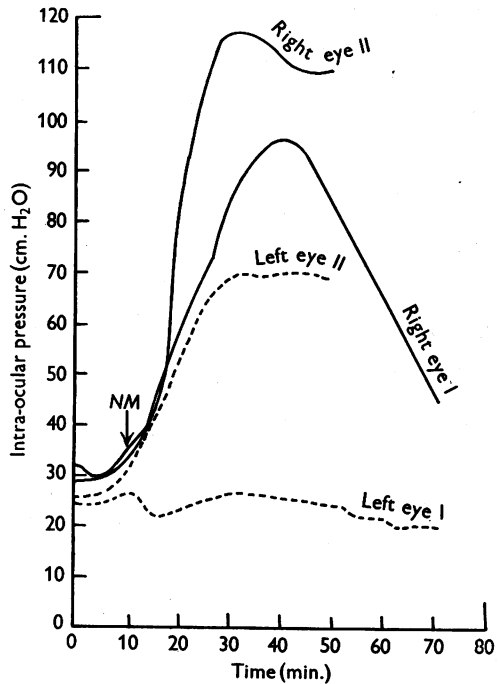


Fig. 2.

Fig. 1. Effect of accidental injury to the lens, during insertion of the manometer needle, on the intra-ocular pressure.

Fig. 2. Effect of a subconjunctival injection of *N*-mustard in the right eye on the intra-ocular pressures of both eyes. *NM* indicates moment of application of *N*-mustard. Curves I represent the more common result.

show a much less frequent type of response, the pressures in both eyes rising. This rise in pressure in the contralateral eye is always associated with a normal pupil in contrast to the extreme meiosis in the treated eye. Among twenty-one experiments carried out to determine the frequency of occurrence of the contralateral effect, a rise of more than 20 cm. H₂O was obtained in four cases, and a rise of less than 20 but greater than 10 cm. H₂O in six cases. The contralateral effect may be a reflex rise in pressure following on the rise in the treated eye, or it may be due to the presence of *N*-mustard circulating in the blood. The latter hypothesis is very unlikely in view of the small amount

injected (0.6 mg.); nevertheless, a number of control experiments, in which *N*-mustard was injected subcutaneously, were carried out. These experiments are difficult to execute as a subcutaneous injection makes an animal, although anaesthetized, very restive. In four animals doses of 0.1 ml. had no effect, and in one animal a dose of 0.5 ml., i.e. eight times the dose applied to the eye, administered with procaine to reduce the local irritation, was also ineffective, so that we may exclude this explanation of the contralateral effect.

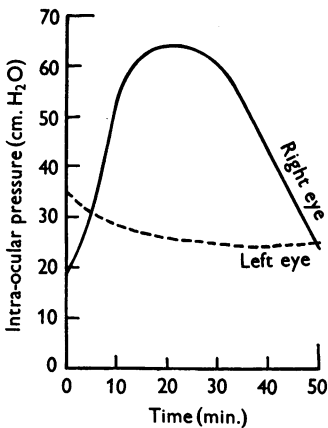


Fig. 3.

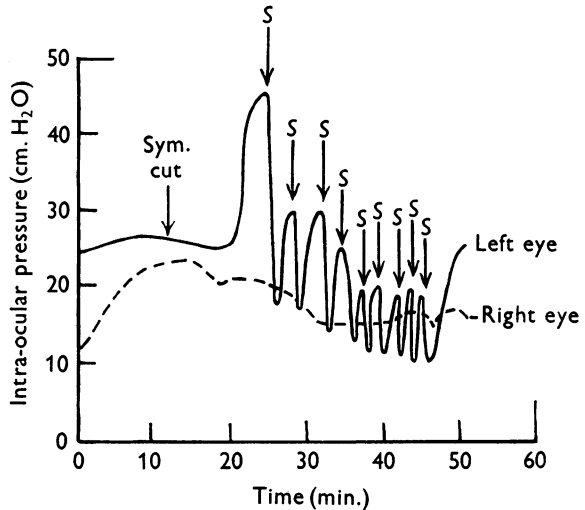


Fig. 4.

Fig. 3. Effect of right cervical sympathectomy on the intra-ocular pressure. The right sympathetic was cut some 5–10 min. before recording began.

Fig. 4. Effect of left cervical sympathectomy on the intra-ocular pressure. *S* indicates 30 sec. period of faradic stimulation of the peripheral end of the cut sympathetic.

Whilst studying the effects of sympathectomy on the responses to *N*-mustard the intra-ocular pressure was frequently found to be high in the eye on the sympathectomized side. Fig. 3 illustrates a typical finding, the cervical sympathetic having been cut some 5–10 min. before insertion of the manometer needle. The rise was large, was accompanied by a normal pupil, and subsided within some 50 min. after insertion of the needle. That the effect was definitely due to section of the cervical sympathetic is shown by the experiment depicted in Fig. 4, in which the pressures were recorded for some minutes before section of the nerve; the rise occurred rapidly, and was cancelled by stimulation of the peripheral end of the nerve. This rise in intra-ocular pressure was by no means an invariable consequence of cervical sympathectomy; more frequently, section of the nerve produced no effect. Of forty-nine experiments performed during this work, a rise of more than 20 cm. H_2O occurred in fifteen experiments, a rise of less than 10 cm. H_2O in eleven experiments and no rise at all in twenty-

three experiments. However, in all cases examined, stimulation of the peripheral end of the nerve produced a fall in intra-ocular pressure; by repeated stimulation this low pressure could be maintained apparently indefinitely, so that the effects of sympathetic stimulation are not simply due to a decreased volume of blood in the eye, since this factor can be compensated within a few minutes by the formation of more aqueous humour; presumably the rate of formation, or ease of drainage, of fluid is also significantly affected.

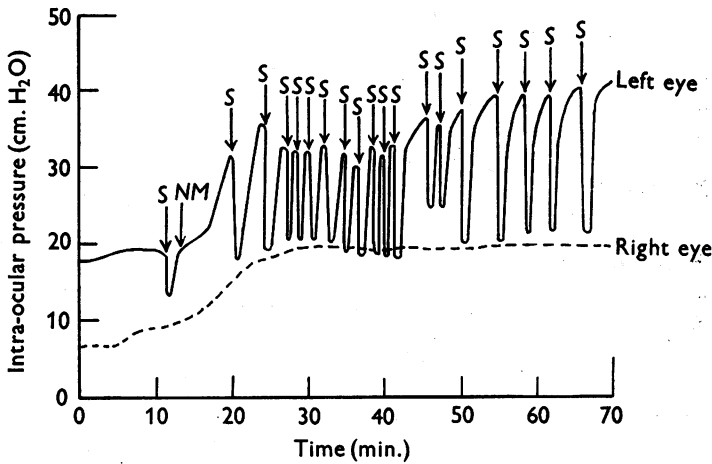


Fig. 5. Influence of left sympathetic stimulation on the effects of *N*-mustard applied to the left eye. *S* indicates 30 sec. period of faradic stimulation of the peripheral end of the cut sympathetic. *NM* indicates moment of application of *N*-mustard.

The effects of *N*-mustard on the eye are apparently due to a very strong dilatation of the small blood vessels; the rise in intra-ocular pressure is associated with the rapid exudation of a protein-containing fluid. If the primary mode of action of the sympathetic is the constriction of the small vessels of the eye, the effects of *N*-mustard should be, at least partially, cancelled by sympathetic stimulation. That this is true is shown by Fig. 5, which depicts the effects of a subconjunctival injection of *N*-mustard into one eye, followed by repeated stimulation, for periods of 30 sec., of the sympathetic nerve of the same side as soon as the pressure rose above 30 cm. H₂O; it will be seen that the large rise in intra-ocular pressure that usually occurred was annulled during the stimulation.

Effects of carotid occlusion

The extra-cerebral circulation to the head of the rabbit is apparently functionally separate on the two sides (vide, for example, Lubsen, 1940), so that tying one common carotid should reduce the pressure in the homolateral ophthalmic artery only. The effects on the normal intra-ocular pressure of such a procedure have been studied by Bárány (1947*b*), who, using a tonometric technique, found an average fall of only 3 mm. Hg on the occluded side. In the

present work the effects of this procedure on the action of *N*-mustard were studied, in order to confirm the vascular nature of the effects of this drug. Fig. 6 shows the effect on the normal intra-ocular pressure; the fall is immediate and usually greater than 4 cm. H₂O (on an average it was 10 cm. H₂O, but it depended in some measure on the initial intra-ocular pressure being greater, the greater the latter); release of the clamp caused a rise above normal. The effects of this procedure on the action of *N*-mustard were invariably to reduce the rise

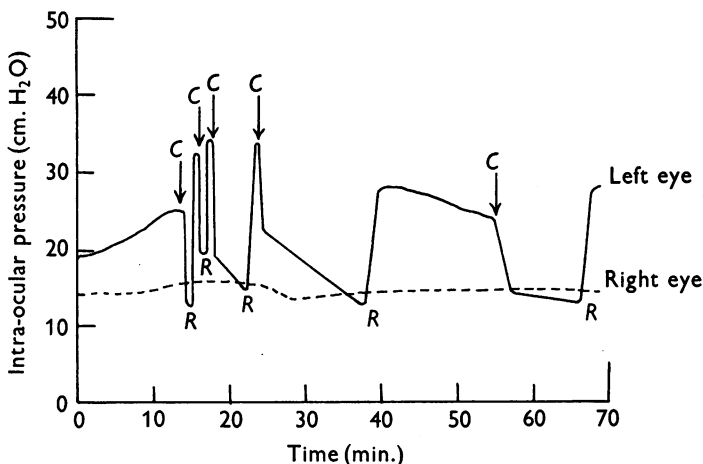


Fig. 6. Effect of left carotid occlusion on the intra-ocular pressure. *C* indicates moment of occlusion; *R* indicates moment of release.

in intra-ocular pressure that would have occurred in the normal animal; the results of eight experiments are shown in Table 1 where, besides the rises in intra-ocular pressure, the pressures in the head-end of the occluded carotid are

TABLE 1. Influence of carotid occlusion on the effect of *N*-mustard

Pressure in head end of occluded common carotid (mm. Hg)	Rise in intra-ocular pressure due to <i>N</i> -mustard (cm. H ₂ O)
35	4
63	2
70	0
76	5
80	12
90	25
92	35
96	0

also stated. With the exception of the last experiment, there is a correlation between the magnitude of the effect of *N*-mustard and the height of the arterial pressure, when this is greater than 70 mm. Hg.

Formation and drainage of aqueous humour

One may expect that the unilateral fall in the intra-ocular pressure, following carotid occlusion, will be reflected in a change in the rate of formation of the aqueous humour; there is some evidence (Kinsey & Bárány, 1949) that the rate of penetration of ^{24}Na from the blood into the aqueous humour is largely determined by the rate of renewal of this fluid, so that carotid occlusion may well influence this rate. Bárány (1947*a*) reported measurements on six rabbits, but on account of the variability in his results, the small difference in rates which he found was not significant. The results of eleven experiments carried out in this work were more consistent, in all cases the rate being less on the occluded side; the average difference was $7\% \pm 1.5$ (s.e.) with extremes of 0.7 and 18.4%. This result suggests that the rate of formation of aqueous humour is decreased by carotid occlusion.

The intravenous injection of 1 g. of *p*-amino-hippuric acid into a rabbit causes a rapid rise in its concentration in the blood, which falls during the course of 60–90 min. to a negligibly small value. During the period of high concentration this substance penetrates into the eye in measurable quantities; at the end of 60–90 min., however, the concentration in the blood is so low that inward diffusion has ceased, the gradient being reversed. During the subsequent hour or so the rate of disappearance of *p*-amino-hippuric acid from the aqueous humour, measured by withdrawing the aqueous humour from the separate eyes at an interval of 60 min., is far greater than can be accounted for by simple diffusion, and is due to the bulk flow of fluid out of the eye through a non-selective channel, i.e. a channel opening directly into the blood, with no selective membrane separating it from this fluid. The studies of Bárány & Kinsey (1949) indicate that the amount of *p*-amino-hippuric acid leaving the eye by diffusion is a small factor, owing to the low permeability of the barrier to this substance; so that the rate of drainage can be approximately computed from the change in the concentration of aqueous humour observed in the fluids withdrawn from the two eyes at intervals of say 75 and 135 min. after the intravenous injection.

In nine control experiments the rate (uncorrected for the effects of simple diffusion) of flow of fluid was $0.97 (\pm 0.15)\%$ of the total volume per min., whilst in the same number of animals with one carotid tied immediately after withdrawal of the contralateral aqueous humour, the rate was $0.99 (\pm 0.1)\%$ /min. The variability was such that only an observed difference of greater than 30% in the average drainage rates would have been significant. Since both eyes of the same animal are required to assess the rate of drainage, it is not possible to compute quantitatively, in the same animal, the rates in a normal eye and in the opposite eye with the carotid occluded; however, evidence for a *difference* in rate of drainage can be provided by the simultaneous concentrations of *p*-amino-hippuric acid in the two eyes some 2 hr. after the intravenous

injection, since at this time the concentration will be mainly determined by the rates of drainage during the previous hour. Consequently, in five rabbits, the right carotid was occluded 75 min. after the injection, and, 60 min. later, both fluids were withdrawn. In a further, control, group of five animals the same procedure was carried out, but the carotid artery, although isolated, was not tied. The results are shown in Table 2. It will be seen that the concentration of *p*-amino-hippuric acid was, on the average, 7% lower in the eye with carotid ligatured; but as the standard error was rather greater than this figure, and since, moreover, the difference between control eyes was of the same order, the result cannot be considered significant. We must conclude, therefore, that the clearance of *p*-amino-hippuric acid from the aqueous humour is not a sufficiently refined test for the present purpose.

TABLE 2. Effect of carotid occlusion on clearance of *p*-amino-hippuric acid from aqueous humour

	Concentration of <i>p</i> -amino-hippuric acid in aqueous humour (mg./100 ml.)		
	Left eye	Right eye	Mean difference (algebraic) (right eye - left eye)
Controls (mean of five experiments)	4.0	4.6	+9% ± 9 (S.E.)
Right carotid tied (mean of five experiments)	4.0	3.4	-7% ± 9 (S.E.)

Because in this work the intra-ocular pressure was measured in two eyes simultaneously, it is only of secondary interest to determine the effects of sympathetic stimulation on the general blood pressure, since any influence of this factor should affect both eyes equally; nevertheless, because of the functional separation of the circulation in the two halves of the head in the rabbit, it is of interest to determine what local changes in the pressure in the ophthalmic artery of the stimulated side are likely to take place. To this end, a technique employed by Hürthle in 1889 was used, a cannula being inserted into the clamped carotid pointing distally to the clamp; the pressure so recorded was low, as we should expect, since it depended on anastomoses with the vertebral circulation. Stimulation of the sympathetic of the same side caused a rapid rise in blood pressure of about 30 mm. Hg, the rise being sustained for as long as the stimulation; stimulation of both the sympathetic and depressor caused a marked rise which, however, subsided as the depressor effect became manifest. No significant effect of stimulating the contralateral sympathetic was observed. These results confirm the generally accepted view that stimulation of the sympathetic reduces blood flow in the head generally; they cannot be taken to indicate that, under normal conditions of blood flow, the pressure in the external carotid rises to any great extent; but one can conclude that the fall in intra-ocular pressure following stimulation of the cervical sympathetic is

not due to a fall in pressure of the blood supply to the eye, any influence of sympathetic stimulation on the extra-ocular arterial pressure being such as to raise this, the decreased blood flow in the extra-cranial (and probably the cranial vessels too (Lubsen, 1940)) ensuring a greater than normal pressure-head at the branching of the ophthalmic artery from the external carotid. In the introduction it was mentioned that Yata found a fall in the general blood pressure on stimulation of the peripheral end of the cut sympathetic of the rabbit. In this animal we find, in agreement with Lubsen (1940), among others, that stimulation of the peripheral end of the cervical sympathetic is without significant effect on the general blood pressure; it would seem that Yata had included depressor fibres in his preparation. In cats this is apparently unavoidable in the left sympathetic (Koopmans, 1938); in the rabbit the depressor nerve frequently runs with the sympathetic trunk, but can always be separated by following the latter up to the superior cervical ganglion. In the majority of the experiments recorded here this precaution was not adopted since the pressures in both eyes were measured simultaneously, so that any effect due to a general lowering of the blood pressure would be reflected in a fall in the intra-ocular pressure on the unstimulated side. Such an effect was indeed sometimes observed, although the fall in intra-ocular pressure was insignificant compared with that in the stimulated side, being rarely more than 2.5 cm. H_2O . In the experiments actually shown here, Figs. 4 and 5, and a number of others, the sympathetic was carefully separated from the depressor, and its freedom from this nerve proved by observing the effect of stimulation on the pressure in the femoral artery at the end of the experiment.

DISCUSSION

The results of sympathectomy indicate that the intra-ocular pressure is normally held in check by this division of the autonomic system; whether there are two antagonistic nervous mechanisms, the one acting to raise the intra-ocular pressure and the other lowering it, has not been settled by these experiments, although the immediate deduction from the large rise in pressure which frequently follows sympathectomy would be that, under these conditions, the intra-ocular pressure is under the unopposed influence of a pressure-raising mechanism. We are not inclined to make this inference, however, without further proof, since neither atropine nor eserine has any significant influence on the intra-ocular pressure, a finding that would appear to exclude the parasympathetic from this function. We may note, however, that Schmerl & Steinberg (1949) found a small increase in intra-ocular pressure on stimulating the ciliary ganglion.

Because of the well-known action of the sympathetic in causing a constriction of the arterioles, it may well be asked whether the effect of sympathetic stimulation on the intra-ocular pressure can be accounted for by the reduced

volume of blood, combined with reduced capillary and venous pressures, in the eye. The reduced volume of blood *per se* would lower the intra-ocular pressure merely by decreasing the volume of the intra-ocular contents, but in the absence of any other influence this should be compensated by the formation of new fluid; since it is possible, by repeated stimulation, to maintain the intra-ocular pressure at a low level for long periods, we must conclude that, not only is the volume of the intra-ocular contents reduced, but also that the rate of formation of the aqueous humour, or the resistance to drainage, is decreased. Both these possibilities are reasonable; a decreased capillary pressure would decrease the rate of filtration from the capillaries and so decrease the amount of fluid available for formation of aqueous humour; moreover, in man at least, it would seem that the resistance to outflow of aqueous humour is determined by the height of the venous pressure; consequently the reduced venous pressure following arteriolar constriction may tend to lower the intra-ocular pressure. Thus it is highly probable that the sympathetic controls the intra-ocular pressure by way of vascular reflexes. Experimental support for this is given by the effects of carotid occlusion; here the capillary pressure is most probably reduced for as long as occlusion is maintained, and the intra-ocular pressure falls, apparently permanently; it is interesting, moreover, that the rate of penetration of ^{24}Na , which must be influenced, if not determined, by the rate of flow of the aqueous humour, is reduced by this procedure. The effects of *N*-mustard can be described as an acute inflammatory oedema; the capillaries of the iris are strongly dilated and a protein-containing filtrate exudes into the anterior chamber. In order that such a capillary dilatation may cause the high intra-ocular pressure observed, the arterioles must be strongly dilated too, the combined effect of capillary and arteriolar dilatation being to cause an exudation into the eye so rapid that the outflow mechanism is overloaded and the pressure rises. Stimulation of the sympathetic, by constricting the arterioles, may be expected to check the exudation and keep the intra-ocular pressure within normal limits; as we have seen, this is indeed the case, so that once again a purely vascular interpretation of the results of sympathetic stimulation is possible. The contralateral effect of *N*-mustard, occasionally observed, is presumably the reflex response of the irritative effects of this substance; whether or not the homolateral response is also reflex in origin has not been definitely proved, but it is interesting that the homolateral effect may be delayed and diminished by a retrobulbar injection of procaine (Davson & Huber, 1950*b*).

In conclusion, the bearing of these results on the pathology of the human eye may be briefly indicated; the effects of *N*-mustard have their analogue in the condition of hypertensive iritis with the difference that in the human the signs may persist for a long time. The rise in intra-ocular pressure following sympathectomy has much in common with the hypertension of chronic glaucoma;

there is no obvious inflammatory reaction and the pupil remains dilated. A central nervous origin has frequently been assigned to this type of hypertension, and it is therefore of great interest that the mere section of a nerve may induce an increase in tension comparable with that found in this disease.

SUMMARY

1. Injury to the lens or iris causes a rapid rise in intra-ocular pressure associated with a constriction of the pupil.

2. Subconjunctival injection of *N*-mustard raises the intra-ocular pressure to very high levels, of the order of 80 cm. H₂O; the effect is not always confined to the treated eye.

3. Section of the cervical sympathetic is sometimes followed by a large rise in intra-ocular pressure (10–40 cm. H₂O) which subsides within 30–60 min. Stimulation of the peripheral end of the cut nerve always causes a fall in intra-ocular pressure.

4. The effects of *N*-mustard may be prevented by either sympathetic stimulation or occlusion of the carotid on the same side as the treated eye.

5. In the normal eyes unilateral carotid occlusion causes an immediate fall in intra-ocular pressure (average 7.5 mm. Hg); there is a significant fall in the rate of penetration of ²⁴Na into the aqueous humour of the occluded side.

6. It is concluded that the effects of both sympathectomy and *N*-mustard on the intra-ocular pressure are predominantly mediated by vascular changes.

We would like to express our indebtedness to Mr David Maurice of this Institute for carrying out the determinations of radioactive sodium described here.

REFERENCES

- Adler, F. H., Landis, E. M. & Jackson, C. L. (1924). *Arch. Ophthalm., N.Y.*, **53**, 239.
 Bárány, E. (1947*a*). *Acta physiol. Scand.* **13**, 55.
 Bárány, E. (1947*b*). *Acta Ophthalm., Kbh.*, **25**, 81.
 Bárány, E. & Kinsey, V. E. (1949). *Amer. J. Ophthalm.* **32**, 177.
 Bratton, A. C. & Marshall, R. O. Jr. (1939). *J. biol. Chem.* **128**, 537.
 Davson, H. & Huber, A. (1950*a*). *Brit. med. J.* **1**, 939.
 Davson, H. & Huber, A. (1950*b*). *Ophthalmologica*, **120**, 118.
 Davson, H. & Purvis, C. (1950). *Brit. J. Ophthalm.* **34**, 351.
 Davson, H. & Quilliam, J. P. (1947). *Brit. J. Ophthalm.* **31**, 717.
 Henderson, E. E. & Starling, E. H. (1904). *J. Physiol.* **31**, 305.
 Hürthle, K. (1889). *Pflug. Arch. ges. Physiol.* **44**, 561.
 Jaffe, N. S. (1948). *Amer. J. Ophthalm.* **31**, 1597.
 Kinsey, V. E. & Bárány, E. (1949). *Amer. J. Ophthalm.* **32**, 189.
 Koopmans, S. (1938). *Arch. néerl. Physiol.* **23**, 256.
 Lubsen, N. (1940). *Acta brev. neerl. Physiol.* **10**, 183.
 Maurice, D. M. (1948). *J. Physiol.* **107**, 26 P.
 Schmerl, E. & Steinberg, B. (1949). *Amer. J. Ophthalm.* **32**, 947.
 Yata, S. (1930). *Acta Soc. ophthalm. jap.* **34**, 550.