fluid cannot explain their presence in ocular fluids. If vascular leakage was the sole source of these immune reactants, then immunoglobulin binding and/or complement consumption must play a role in the pathogenesis of some of these diseases. The present studies provide suggestive evidence that complement and immunoglobulin(s) may be active in the pathogenesis of ocular tissue damage and demonstrate that currently available microassay techniques are applicable for further studies in this area.

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The arterial pressure dependency of the increased aqueous humor formation induced by Ach+eserine.

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Abstract. The magnitude of the increased rate of formation of aqueous humor produced by Ach+eserine is dependent and proportional to the arterial perfusate head pressure. Because of this, it may be assumed that the mechanism of the Ach+eserine response is due to an increase in ultrafiltration. It is suggested that the increase in ultrafiltration is brought about by a constriction of efferent ciliary process blood vessels.

It has recently been reported that the administration of acetylcholine plus eserine (Ach+eserine) to enucleated, arterially perfused cat eyes produces a marked increase in the rate of aqueous humor formation.[1] The present study was undertaken to determine the vascular pressure dependency of this increased aqueous humor formation rate.

Methods. Cat eyes were enucleated and arterially perfused according to methods already reported.[1] The perfusate pressure head, which was regulated by a pressure bulb, was always placed at the highest levels at the beginning of the experiments. Two pressure decrements were evaluated in each experiment. The perfusate pressures were measured through one port of a differential pressure flowmeter[2] utilized to monitor the arterial perfusate flow rate. Intraocular pressures (IOP) were measured manometrically and maintained continuously at 15 mm. Hg by cannulation of the anterior chamber. The rate of aqueous humor formation was determined by the inulin-^{14}C dilution technique as previously reported.[3] The anterior chamber fluid was continuously mixed using a stirrer especially designed for this purpose.[4]

Results. Prior to the administration of Ach+eserine, the rate of aqueous humor formation in seven experiments was found to be 6.82 ± 0.71 (S.E.) μl per minute, at an average perfusate pressure head of 115 ± 0.71 (S.E.) mm. Hg. Within 30 minutes after the intra-arterial (IA) infusion of Ach (0.01 ng. per milliliter) and eserine (10.0 μg per milliliter), the mean rate of aqueous humor formation increased to 42.17 ± 7.22 (S.E.) μl per minute at the same mean (115 mm. Hg) head pressure. Decreases of perfusate head pressure, to levels approaching 70 mm. Hg, produced

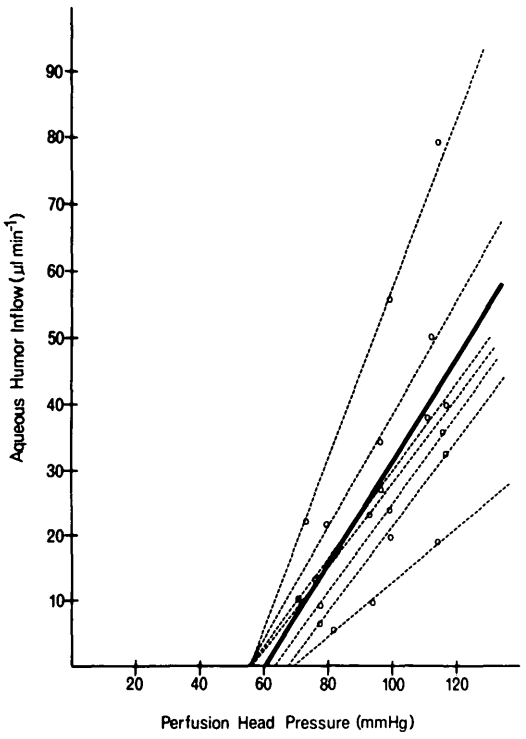

Fig. 1. Effect of lowering arterial perfusate pressure head on aqueous humor formation. Dashed lines are results of individual experiments. Solid line is the mean slope of the seven individual experiments.

in each case a straight-line arithmetic decrease in the rate of aqueous humor production. The mean of the individual slopes was -0.76 μl per minute per mm. Hg with an intercept on the perfusate pressure axis at 60.61 mm. Hg. A plot of the inflow rate versus the perfusate pressure, at the three pressure intervals studied, is shown in Fig. 1. Raising the perfusate head pressure to beginning levels caused an increase in the rate of aqueous humor formation to anticipated levels in five of the seven experiments. The average arterial perfusate flow rate at the initial perfusate head pressure was 568 μl per minute ± 26 (S.E.). After the administration of Ach+eserine, a decline in perfusate rate was obtained in all cases. The average fall was 34 μl per minute ± 10 (S.E.) which is equivalent to a 6.0 per cent decrease in perfusate flow rate.

Discussion. The eye pressure was maintained constant at 15 mm. Hg, in seven enucleated, arterially perfused cat eyes. The IA administration of Ach+eserine produced a mean increase in the rate of aqueous humor formation from 6.82 μl per minute to 42.17 μl per minute, and a 6.0 per cent decrease in arterial flow. These results are in close agreement with previously reported data.[1] Altering the arterial perfusate pressure head between the limits of 115 and 77 mm. Hg produced a linear change in the aqueous humor formation rate which was equivalent to -0.76 μl per minute per mm. Hg. The intercept of the curve on the arterial pressure axis was at 61 mm. Hg. Since the IOP was being maintained at 15 mm. Hg, the transmural pressure at which no aqueous would be assumed to be formed must be equal to or less than 46 mm. Hg (61 minus 15 mm. Hg).

Because of the marked sensitivity of the aqueous humor inflow rate to changes in the arterial perfusate pressure, it is not unreasonable to assume that the Ach+eserine effect is due to ultrafiltration brought about by either an increase in capillary permeability, or an increase in capillary pressure, or more likely, both. The decrease in perfusate blood flow induced by Ach+eserine in these eyes, which were maintained at a constant IOP, strongly indicates a vascular constriction as do data previously reported for the isolated iris-ciliary body.[5] Enucleated eyes have no centrally mediated neurogenic tonus and, possibly, because of this, vasodilation has not been observed.[6] If this were the case, utilizing the enucleated eye for these studies had a beneficial, unmasking effect in that it allowed the vasoconstrictor component of the Ach+eserine action to become apparent.

The receptor mechanism for the Ach+eserine response in producing both vasoconstriction and increasing aqueous humor formation has been demonstrated to be neurogenic, i.e., a stimulation of E-2 sites on sympathetic, ganglion-like receptors.[1, 5] As a working hypothesis we would like to suggest that drugs such as Ach+eserine which stimulate E-2 sites of these ganglion-like receptors cause the neuronal release of norepinephrine to produce vasoconstriction of the distal or efferent blood vessels of the ciliary processes. An increase in back pressure would be produced to increase filtration pressure and concomitantly a possible passive dilation of these blood vessels could be produced to increase capillary permeability. The status of increased capillary permeability, under the these conditions, is currently being studied.

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The regeneration of rhodopsin following the removal of detergent.

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The visual pigment, rhodopsin, is a membrane-bound glycoprotein.[1, 2] Detergent-solubilized rhodopsin retains lipid from rod outer segments (ROS) during extraction,[3, 4] much of which can be removed, but only at the expense of the chemical and physical properties of rhodopsin.[1, 4] Certain detergents can also modify the properties of rhodopsin. For example, photobleached rhodopsin cannot be regenerated with exogenous 11-*cis*-retinal, its prosthetic group, after solubilization with cetyltrimethylammonium bromide (CTAB),[5] Triton X-100,[4] or Emulphogene BC-720.[6] Two laboratories have reported that the removal of detergent restores certain properties to rhodopsin. Zorn and Futterman[4] described the inability to regenerate rhodopsin after the removal of Triton X-100 unless supplemented by phospholipid. Chabre and co-workers[7] report the formation of lamellar structures which undergo light-induced changes after the removal of Triton X-100 from detergent-solubilized preparations.

This report describes the ability of Triton X-100 and CTAB-solubilized rhodopsin preparations to regenerate in the presence of 11-cis-retinal after the partial removal of detergent.

Detergent extraction. ROS were prepared from frozen bovine retinae (G. A. Hormel & Co.) according to Heller,[1] and stored as a pellet in liquid nitrogen. Thawed ROS pellets were suspended in phosphate buffer and washed with 1/15 M sodium phosphate buffer, pH 7.0. Following centrifugation, the ROS pellets were suspended in 1 per cent Triton X-100 or 40 mM CTAB in phosphate buffer and mixed. Triton X-100 extracts were centrifuged immediately (40,000 × g, 20 minutes, 5° C.), while CTAB extracts were maintained at 4° C. for 4 hours prior to centrifugation.[1]

Table I. Triton X-100-solubilized rhodopsin was incubated with Bio-Beads SM-2 as described in methods. Aliquots were removed at the times indicated and rhodopsin content determined from the decrease in $A_{500\ nm.}$ after photobleaching. Excess 11-*cis*-retinal was added to the photobleached sample and the degree of regeneration was calculated from the increase of $A_{500\ nm.}$. The amount of photopigment formed by regeneration was calculated by measuring the decrease in $A_{500\ nm.}$ after the regenerated sample was rebleached

Incubation time (min.)	*Recovered*	*Rhodopsin regenerated (%)*	*Re-bleached*
0	100	21.5	2.2
30	96.5	94.0	60.9
60	94.0	104.0	59.3
90	77.9	84.1	56.1
120	76.4	96.5	49.4

Detergent removal. Bio-Beads SM-2 (Bio-Rad Laboratories) were washed and detergent removed by the batch procedure of Holloway.[8] A 2 ml. aliquot of detergent-solubilized rhodopsin solution was added to 0.6 Gm. of moist Bio-Beads in a capped vial and mixed at room temperature by inversion. Aliquots were removed periodically for assay.

Rhodopsin content was determined spectrophotometrically on a Gilford Model 2400S from the difference in $A_{500\ nm.}$ before and after photobleaching assuming a molar extinction coefficient of 40,600 M^{-1} $cm.^{-1}$.[9]

Rhodopsin regeneration was performed by adding an excess of 11-*cis*-retinal (Hoffman-LaRoche) dissolved in acetone, to photobleached preparations of rhodopsin. Some samples were regenerated in the absence of detergent but assayed after extraction with 1 per cent Triton X-100 in order to reduce light-scattering effects. Regeneration was determined from the increase in $A_{500\ nm.}$. As a final control, all regenerated samples were photobleached again and the loss of $A_{500\ nm.}$ was determined to assure measurement of a light-sensitive species.

The purity of ROS prepartions can be characterized by the spectral ratio, $A_{400\ nm.}/A_{500\ nm.}$, of the detergent-solubilized rhodopsin in the preparation. The lowest spectral ratio (i.e., highest purity) obtained directly from ROS was reported by DeGrip, Daeman, and Bonting,[10] as 0.20. The ROS preparations employed in this study are characterized by spectral ratios ($A_{400\ nm.}/A_{500\ nm.}$) of 0.40 in Triton X-100. The lack of great purity in this preparation should not interfere with these experiments or their interpretation. The ability of the rhodopsin in this preparation to regenerate