Anterior segment chemical sympathectomy by 6-hydroxy-dopamine

I. Effect on intraocular pressure and facility of outflow

Monte G. Holland and James L. Mims, III

Histofluorometric techniques have confirmed that topical ocular application of 6-hydroxy-dopamine, a norepinephrine congener, causes a selective and reversible destruction of sympathetic nerve terminals in the anterior segment. An investigation of the effects of “chemical sympathectomy” on the pupil, intraocular pressure, and facility of outflow showed: the pupil underwent a sequence of changes characteristic of surgical sympathetic denervation, but with a different time course; the intraocular pressure was significantly lowered, transiently in rabbits and of longer duration in monkeys; the facility of outflow was transiently increased in monkeys and probably in rabbits; the episcleral venous pressure was unchanged in both species. It was concluded that the lowered intraocular pressure and lowered outflow pressure were the result of a reduction of aqueous inflow. There was no unequivocal experimental demonstration of supersensitization to topical norepinephrine or isoproterenol following chemical sympathetic denervation; however, the experiments were not conclusive on this important point. It was concluded that chemical sympathectomy with 6-hydroxy-dopamine reproduces many of the ocular phenomena of surgical sympathectomy. 6-Hydroxy-dopamine is a useful drug for experimental ophthalmology, and may be useful clinically.

Key words: chemical sympathectomy, surgical sympathectomy, 6-hydroxy-dopamine, sympathetic denervation effects, denervation supersensitization, intraocular pressure, facility of outflow, episcleral venous pressure, norepinephrine.

Several investigators have reported that the norepinephrine congener 6-hydroxy-dopamine, produces sympathectomy by selective destruction of adrenergic nerve endings, leaving parasympathetic and other nerve terminals unaffected.

The possibility of producing ocular sympathectomy by chemical means is interesting and potentially useful. Since Horner’s early observation of lowered intraocular pressure as a part of his well-known syndrome, there have been attempts to lower intraocular pressure in glaucoma patients.
by superior cervical ganglionectomy or cervical sympathectomy. However, because of the unpredictable results from this surgical intervention and its attendant risks, the procedure has been abandoned.

More recently there has been a renaissance of interest in the effects of ocular sympathetic denervation and the mechanisms of action of sympathetic drugs on aqueous secretion and outflow. Because 6-hydroxy-dopamine has the unique effect of producing a reversible degeneration of sympathetic nerve terminals, an investigation of the effects of its topical administration on the intraocular pressure, facility of outflow, and episcleral venous pressure of owl monkeys and New Zealand rabbits was carried out.

Methods

Experimental animals. Both sexes of adult owl monkeys (Aotus trivirgatus), weighing 0.6 to 0.9 kilograms, and adult New Zealand strain rabbits, weighing 2.4 to 3.6 kilograms, were used. Three of the eight rabbits were pigmented.

Anesthesia. Owl monkeys were anesthetized with 15 to 20 mg. per kilogram of sodium pentobarbital given intramuscularly and supplemented as necessary by intravenous or intramuscular administration. Other agents were tried and found to be less satisfactory.

Rabbits were anesthetized with sodium pentobarbital by marginal ear vein.

Drugs. 6-Hydroxy-dopamine* was prepared as a ten per cent aqueous solution, buffered to pH 7 with sodium carbonate. Sodium bisulfite was added as a reducing agent in equimolar concentration to 6-hydroxy-dopamine in the final solution.

DL-Isoproterenol hydrochloride and norepinephrine (1-Arterenol bitartrate†) were prepared as two per cent aqueous solutions.

All drugs were dissolved just prior to their topical administration.

Techniques of topical administration. As 6-hydroxy-dopamine is a highly polar compound, it would be anticipated that its lipid solubility and corneal penetration would be low. This was verified experimentally utilizing the pupillary responses to the drug as criteria. To enhance corneal penetration, 0.5 per cent topical proparacaine (one to two drops) (Ophthetic, Allergan Pharmaceuticals, Irvine, Calif.), was given prior to tonography in monkeys. Ten per cent 6-hydroxy-dopamine (0.2 ml.) was dropped onto the cornea after tonography, making use of the slight epithelial damage to enhance penetration.

In rabbit experiments, where tonography was not done, 6-hydroxy-dopamine (0.2 ml.) was applied after six applications of proparacaine spaced over 5 minute intervals.

It should be noted that the control eyes in all experiments were treated identically to the treated eyes, except that the 6-hydroxy-dopamine was omitted from the solution.

Experimental techniques

Measurement of intraocular pressure. The intraocular pressure was measured by three methods: electronic Schiötz tonometry (Crescent Instrument Co., El Monte, Calif.); applanation tonometry (Draeger hand applanation tonometer, Edward Weck & Company, Long Island City, N. Y.); and by cannulation with the use of pressure transducers (Statham Instrument Co., Oxnard, Calif.). Appropriate technical details are given below.

Electronic Schiötz tonometry. The Crescent electric tonometer was utilized to make intraocular pressure measurements in anesthetized monkeys just prior to tonography. In a separate set of experiments, an open manometric calibration of this Schiötz tonometer was done in the cannulated eyes of anesthetized monkeys. The P, values of the owl monkey eye were sufficiently close to the 1955 Schiötz calibration tables for human eyes, so that these tables could be utilized for tonometric and tonographic calculations.

Tonometric measurements were taken with 5.5, 7.5, and 10 gram weights in each experimental animal on several occasions; the mean value of ocular rigidity in each animal was used to correct intraocular pressure estimates and facility of outflow. Average ocular rigidity in the owl monkey (16 eyes) was 0.0173 (± 0.0015).

Applanation tonometry. The Draeger hand applanation tonometer was modified so that the doubling prism was set for a 4 mm. diameter of applanation, as recommended by Schmidt. A series of standardization measurements in cannulated monkey eyes yielded a regression line for a 4 mm. diameter of applanation, which related tonometer readings to manometrically determined intraocular pressure.

In a series of measurements in 16 eyes of anesthetized monkeys, it was found that the Schiötz pressures averaged 0.77 (±0.47) mm. Hg higher than the applanation pressures.

Cannulation and pressure transducers. In seven monkeys the intraocular pressure was measured in the control and treated eyes by cannulating the anterior chambers with 25 gauge needles.
connected to Statham, Model P23BB, pressure transducers. Schiötz pressures averaged 1.6 (-1.6) mm. Hg higher than the transducer pressure, whereas the applanation pressure averaged 1.1 (±2.3) mm. Hg below transducer pressures. Applanation tonometry in conscious topically anesthetized rabbits was attempted. However, in a large series of measurements the standard errors were large and the technique gave no reliable information about the effects of 6-hydroxydopamine on the intraocular pressure. Applanation measurements on conscious rabbits after intra-peritoneal water loading had smaller standard errors and were usable.

Applanation tonometry in the anesthetized rabbit was highly satisfactory and was the method used in rabbits to determine intraocular pressure. Facility of outflow. The total facility of outflow was measured by tonography and by perfusion, utilizing the two-pressure perfusion method of Barány. Tonographic facility. Tonography was carried out on the anesthetized monkey by placing the animal in a specially constructed box for stabilization. The tonometer was also stabilized upon the animal's eye by a mechanical support; the latter made it possible to acquire technically superb tonograms. The average P't was calculated by averaging the P's of the midpoints of each minute of the 4 minute tonography. Scleral rigidity was measured in each animal, and the C values appropriately corrected.

The average radius of corneal curvature was determined for the experimental group of monkey eyes and was found to be 7.32 mm. (±0.09). Appropriate corrections in C were introduced in each facility calculation by using this average value.

Tonography in anesthetized rabbits was not technically satisfactory in our hands and was not used in these experiments.

Facility of outflow by perfusion. The two-level constant pressure perfusion technique described by Barány was used to measure total facility of outflow in ten monkey eyes. The femoral artery and vein were cannulated to monitor blood pressure and administer anesthesia. A type SC-II Beckman dynagraph simultaneously recorded intraocular pressures, systemic blood pressure, and the weights from hanging reservoirs supplying fluid to each eye (Barány's polyelectrolyte fluid). An integrating amplifier for each hanging reservoir recorded the change in the fluid weight over the 5 minute intervals of perfusion. Each eye was perfused six times, alternating between 5 and 10 mm. Hg above P, (steady-state or baseline intraocular pressure). After each pair of perfusions, the eyes were allowed to come to a new equilibrium P, for 5 minutes. This new P, was used for the next pair of perfusion measurements.

The facility of outflow was calculated from the perfusion pressure and change in weight of the reservoirs for each minute. The average of three different perfusion pairs at 5 and 10 mm. above P, were used to calculate the facility.

Perfusion facility estimates were also done in a small series of rabbit eyes. The technical quality of the experiments was poor and are not included in this report.

Episcleral venous pressure. The episcleral venous pressure was measured in a series of three treated and three control monkey eyes and in nine treated and nine control rabbit eyes to determine whether chemical sympathectomy altered the episcleral venous pressure. The method was a modification of that described by Brubaker. A pelotte pressure chamber was connected to a calibrated Statham pressure transducer and a 500 ml micrometer syringe. Adjustment of the micrometer made it possible to change the pressure in the saline-filled system from 0 to 40 mm. Hg. The chamber pressure was continuously recorded. Frog pericardial membranes were used initially, but it was found that a thin, highly elastic, transparent polyurethane membrane was superior to the frog pericardial membranes. The characteristics of this system and experimental techniques are described in a separate report.

The episcleral vessel selected in the rabbits was approximately 2 to 3 mm. from the nasal limbus. In the owl monkey, because of the anatomical features of the episcleral vessels, a point of measurement 1 to 1.5 mm. from the temporal limbus was selected.

Intraperitoneal water loading. McDonald and associates reported that water loading, by gavage, in the anesthesized rabbit was a sensitive test for measuring the pressure lowering effects of drugs. However, intraperitoneal water loading (75 ml. per kilogram) in the conscious rabbit, proved to be a safer, more reproducible and more human procedure than the gavage method.

Standard intravenous equipment was used to give the water intraperitoneally within a period of five to seven minutes. Five applanation pressure measurements were taken on each eye every ten minutes for the following hour. The pressures were then plotted as a function of time, and the difference in intraocular pressures (con-
control minus the treated) was integrated over the entire hour and expressed as average intraocular pressure difference per minute. This technique was found to be very sensitive and reliable in the conscious animals.

**Pupils.** Due to the varying excitation of the animals during handling, it was found that quantitatively precise pupillary measurements were not possible with the equipment available; however, a consistent pattern of pupillary changes could be seen after repeated handling and observation of the animals.

**Experimental protocols and results**

The effects of topical application of 6-hydroxy-dopamine on the pupils, intraocular pressure, and facility varied with the time elapsed following treatment. Accordingly, experimental protocols were designed to take into consideration early and late effects.

**Pupillary changes.** The pupillary responses could be grouped into three different types which are illustrated in Fig. 1.

Phase 1 pupils were observed 30 minutes to 3 hours after the first treatment. The pupil of the treated eye was widely dilated and did not appear to react to light. Our interpretations of this Phase 1 pupil were that it probably corresponded to "denervation contraction" from release of endogenous norepinephrine resulting from chemical sympathectomy. It could not be elicited on repeated treatments of the animal, unless the animal had been allowed to recover for at least two full weeks after the last treatment.

In Phase 2 (3 to 48 hours after treatment), the pupil of the treated side remained miotic at all times. When the animal was excited or angry, the control pupil would dilate as indicated in the figure, whereas the treated pupil would remain miotic.

Phase 3 pupils occurred as early as 24 hours and lasted as long as five weeks. The pupil of the treated eye would dilate slowly and remain dilated when the animal was excited. Under conditions of rest, however, the pupil appeared to be approximately the same as the control pupil (whether or not it was slightly miotic could not be ascertained under the conditions of these observations).

The Phase 2 pupil might be interpreted as a phase of Horner’s miosis and the Phase 3 pupils, in excitation, might represent a denervation supersensitivity to circulating endogenous catecholamines.

Phase 1 and 2 pupils could be seen in rabbits, but Phase 3 pupils were seen only in monkeys. These changes were characteristic and appeared in every monkey after sufficient treatment. They were used as a guideline as to whether sufficient amounts of the drug had been transferred through the cornea.

**Intraocular pressure following topical application of 6-hydroxy-dopamine.**

Schiötz tonometry in monkey eyes following topical 6-hydroxy-dopamine. Seven owl monkeys were subjected to Schiötz tonometry and tonography three times to establish control values for each eye. Before each animal awoke after the tonogram, the eye which had the higher Schiötz pressure in the control measurements was selected for topical treatment. Five ani-
mals were treated with 10 per cent 6-hydroxy-dopamine, one was treated with 5 per cent, and one with 2½ per cent. Because of limitations imposed by repetitive anesthesia, the intraocular pressure was measured at weekly intervals; therefore, the time of the earliest occurrence of an effect on the intraocular pressure is not known precisely.

The intraocular pressure of all control measurements averaged 14.8 (± 0.5) mm Hg.

One week following treatment, the intraocular pressure measured by Schiötz tonometry averaged 3.8 mm Hg or 29.5 per cent lower in the treated eyes. This is indicated in Fig. 2 at days six through nine. The statistical significance of this difference, based upon a paired sample t test, is shown below the bar graph. The effect on intraocular pressure appeared to be somewhat greater the following week when the intraocular pressure difference was 5.0 mm Hg or 35.2 per cent lower in the treated eyes. Two weeks after the last treatment, the effect was diminished, although still significant (2.9 mm Hg or 19.2 per cent lower in the treated eyes). The intraocular pressure returned to control levels three weeks after the last treatment and remained at normal levels.

An attempt was made to determine if the response of the pupil and intraocular pressure were dose dependent. It was determined quickly that, because of the variable penetrance of the drug, the response to 2.5 per cent 6-hydroxy-dopamine was at least as good as the 10 per cent dose in several cases, depending upon the penetration of the drug and its degree of enhancement by proparacaine and/or tonography.

Intraocular pressure measurements by applanation tonometry in monkey eyes following 6-hydroxy-dopamine treatment. The intraocular pressure in this same group of animals was measured with the applanation tonometer. Five measurements were made on the treated and control eyes of each animal immediately preceding tonography.

Fig. 3 illustrates applanation intraocular pressure differences taken at the same time as the Schiötz measurements. The per cent differences are somewhat less, but the statistical significance is the same for the pressure-lowering effect by either method.
The mean intraocular pressure in the control eyes by applanation tonometry was 11.6 (± 0.7) mm Hg.

The average pressure differences measured by applanation were 1.4, 1.6, and 1.2 mm Hg lower in the treated eyes which correspond to 9.4, 15.2, and 11.6 per cent lowering of intraocular pressure. The difference between the applanation and Schiøtz pressures were greater than an earlier series; the higher Schiøtz pressures were probably the result of errors in estimating ocular rigidity.

Intraocular pressure measurements by cannulation in eyes treated topically with 6-hydroxy-dopamine. Five additional monkeys were treated topically in one eye with 6-hydroxy-dopamine after tonography. Four to six days after the first treatment, a measurement of intraocular pressure by cannulation was done in the control and treated eye of each animal. Initial pressure after equilibrium (about 1 minute after...
Another group of five monkeys was treated three times at weekly intervals after tonography. The intraocular pressure was measured by cannulation 20 to 22 days after the initial treatment.

In this separately treated series of monkey eyes, the pattern of intraocular pressure change was the same as observed in the group measured by Schiöttz and application methods.

The intraocular pressure measured by cannulation in the control eyes averaged 11.8 (± 1.1) mm Hg. The treated eyes averaged 2.5 (± 0.5) mm Hg or 26.2 per cent lower than controls on approximately the fifth day (Fig. 4). One week following the last treatment (three weeks after the first treatment), the intraocular pressure difference was less (i.e., 19.1 per cent ± 4.3 lower) in the treated eyes. On the twenty-first day after the first treatment, the intraocular pressure averaged 2.8 (± 0.9) mm Hg lower in the treated eyes. These results were of high statistical significance.

COMMENT. Using three different techniques of measuring intraocular pressure, it was demonstrated that approximately one week following an initial treatment with 6-hydroxy-dopamine, the mean intraocular pressure in the treated eyes was about 25 to 30 per cent lower than the control eyes. If the treatment were continued weekly, by the second week the effects seemed to be greater, and by the third week the effects on the intraocular pressure seemed to be definitely diminishing. The long-term duration of the lowered intraocular pressure following continued weekly applications has not been explored. One might
Fig. 6. Measurement of facility by perfusion showed an increase in the treated eyes one week after treatment; because of the small number of animals and large variance, the change was not significant statistically.

expect that it would resemble surgical sympathectomy in its duration.

Facility of outflow following topical 6-hydroxy-dopamine

Tonographic facility. In the group of seven owl monkeys used for Schiötz and applanation P_o measurements, three control tonograms were done in each eye prior to treatment. This control series showed there was an average difference in facility between the two eyes (randomly occurring between right and left) of about 33 per cent. The eye with the higher facility was usually associated with a higher P_o. Because of these differences in control measurements, the eye with higher P_o was selected for treatment and its opposite for control. A tonogram was always done prior to treatment. Measurements continued for two weeks following the last treatment.

Fig. 5 summarizes this series of experiments. The number of animals in each group is included in parenthesis below the bar graph in the figure. The average control facility was 1.12 (± 0.27) μl per minute per millimeter of Hg (hereafter called facility units). A week after a single treatment, the mean difference in facility between treated and control eyes was 66.7 per cent (an increase in facility of treated eyes by approximately 33 per cent, 0.01 < p < 0.05). One week after the second
Fig. 7. Intraperitoneal water loading of rabbits following topical 6-hydroxy-dopamine showed a significantly lower pressure rise per minute in the treated eyes, probably reflecting a transiently increased facility of outflow (ganglionectomy effect). This change was not demonstrable one and two weeks later.

treatment, the facility difference was approximately the same as the control difference prior to treatment. Subsequent weekly measurements without treatment showed a variation about the control difference in facility.

COMMENT. Thus, it can be seen that tonographic facility of outflow was increased significantly following topical 6-hydroxy-dopamine, but the effect did not persist through the second week, even though the intraocular pressure was lowered significantly at this time.

Perfusion facility. Fig. 6 summarizes the results of facility measurements by Bárány's technique in ten owl monkeys treated with 6-hydroxy-dopamine. Five animals were perfused approximately five days after the first treatment. The average per cent difference in facility (control minus treated eye) was 28.9 per cent \( (\pm 23.6) \) higher in the treated eyes. One week after the last treatment, the difference in facility was 7.2 per cent. The average total facility of all controls was 0.55 \( (\pm 0.07) \) facility units. The average increase in facility in the treated eyes on the fifth day was 0.29 facility units. Because of the large standard errors of these measurements and the small sample size, none of the differences in perfusion facility were statistically significant. However, the differences in \( P_o \) in these same animals measured by cannulation at the same time were statistically significant.

COMMENT. The pattern of facility change after treatment was similar to the group measured tonographically in which the facility change one week after treatment was statistically significant.

It should be noted that the "C" values obtained by tonography in control eyes averaged 0.57 facility units higher than those obtained by perfusion. This difference is probably explainable by the combination of errors in using human calibra-
tion tables, as well as possibly greater pseudofacility effects during tonography.

Results of studies of 6-hydroxy-dopamine on the rabbit eye

Water provocative testing. Eight rabbits were treated topically twice a day for seven days with 6-hydroxy-dopamine in one eye, the opposite eye receiving buffer solution. On the third and seventh days after treatment, and also seven days after discontinuing treatment, intraperitoneal water loading was done in the conscious animal. The mean of five applanation readings was taken every five minutes in both eyes as the pressure rose from intraperitoneal water absorption.

Fig. 7 illustrates the results. Three days after the institution of daily treatment, the difference in pressure rise per minute (control minus treated eye) was 11.2 (± 3.8) mm. Hg lower in the treated eyes. This difference was statistically significant (p < 0.01). On the seventh day of continued treatment, the average difference had dropped to 4.8 mm. Hg per minute, and one week after discontinuing treatment it was 2.2 mm. Hg per minute higher in the treated eye. The latter differences were not statistically significant.

Comment. Topical treatment with 6-hydroxy-dopamine produced a lower intraocular pressure rise after intraperitoneal water loading, which was significant three days after daily treatment, but not thereafter. The lower pressure rise at this time is suggestive of the ganglionectomy effect on facility in rabbits as measured by constant-rate perfusion techniques, where equilibrium pressures are much lower on the side of the sympathectomy.16

Applanation pressures. Under pentobarbital anesthesia, applanation tonometry was done on a separate group of nine rabbits. The mean intraocular pressure in all control eyes was 21.9 (± 1.3) mm. Hg. These eyes averaged 1.1 mm. Hg higher than those eyes which were selected for treatment (Fig. 8). Following three days of daily treatment, the intraocular pressure was 3.3 (± 1.3) mm. Hg lower in the treated eyes, a total change of 4.4 mm. Hg (0.01 < p < 0.05). Seven days after initiating daily treatment, the pressure difference between the control and treated eyes was not statistically significant.

Episcleral venous pressure in the rabbit treated with 6-hydroxy-dopamine. Immediately after applanation tonometry in the nine rabbits as described above, episcleral venous pressure measurements were made. The results are given in Fig. 9. The average episcleral venous pressure in the control eyes was 9.4 (± 0.3) mm. Hg, which is almost identical to that reported by Linner17 in the rabbit. The treated eyes averaged 0.7 (± 0.4) mm. Hg lower episcleral venous pressure three days after a single treatment, while the intraocular pressure averaged 3.3 mm. Hg lower in the treated eyes. This change in episcleral venous pressure is slight and not statistically significant. Seven days following a single treatment with 6-hydroxy-dopamine, the mean difference in episcleral venous pressure was 0.5 (± 0.3) mm. Hg lower in treated eyes, which also was not statistically significant.

Comment. It is evident that the episcleral venous pressure is not significantly affected by chemical sympathectomy by 6-hydroxy-dopamine, even at a time when the effects on intraocular pressure are easily demonstrated. This is in agreement with the measurements of episcleral venous pressure in rabbits by Linner17 following surgical sympathectomy.

The effect of topical norepinephrine and isoproterenol on the intraocular pressure of monkeys pretreated with topical 6-hydroxy-dopamine

Norepinephrine—effects on intraocular pressure. Five monkeys were used in this experiment (Fig. 10). One week after a single treatment with 6-hydroxy-dopamine the intraocular pressures averaged 23.1 per cent (± 4.9) lower in the treated eyes (p << 0.01). The eyes were treated each week for three weeks. One week after the last treatment, three applications of 2 per cent norepinephrine (0.1 ml.) were given
Fig. 8. Applanation pressure differences showed a significantly lowered IOP in treated eyes of rabbits approximately three days after a single treatment. Seven days later no significant difference could be detected.

to both eyes after proparacaine. Ninety minutes later, the intraocular pressure and facility were measured. After norepinephrine, the eyes previously treated with 6-hydroxy-dopamine showed an average of only 0.1 (± 0.2) mm Hg lower pressure than the control eyes, a result which was not statistically significant.

COMMENT. The possibility of sensitization to topical norepinephrine after chemical sympathectomy was not demonstrable under these experimental conditions, even though the 6-hydroxy-dopamine had exerted an intraocular pressure-lowering effect the preceding week. It is interesting to note, however, that the intraocular pressure of control eyes averaged 20.7 (± 1.1) mm Hg one week prior. This increase in pressure was statistically significant. It would be easily explainable by an increase in blood pressure. However, similar experiments done in anesthetized monkeys, in which the intraocular and blood pressures were monitored by cannulas in the anterior chamber and femoral artery, showed that there was no increase in blood pressure following topical application of norepinephrine.

Norepinephrine—effects on facility of outflow. Control measurements of facility on these five monkeys averaged 0.88 (± 0.09) facility units (Fig. 11). One week following the first treatment with 6-hydroxy-dopamine, the per cent difference in facility of outflow (control minus treated
eye) averaged 64 per cent (± 21) higher on the treated side (0.01 < p < 0.05). This reconfirmed the previous measurements. One week after the second treatment, the facility was slightly lower in the treated eyes by 1.6 per cent. These eyes were given a third treatment with 6-hydroxy-dopamine and treated a week later with topical norepinephrine three times as described above. The facility of outflow after norepinephrine was 13 per cent higher in the treated eyes, but the difference between the control and treated eyes was not statistically significant.

COMMENT. When one considers control eyes alone, it is interesting to observe that prior to any treatment the facility averaged 0.88 (± 0.09) units, and three weeks later with norepinephrine the facility averaged 1.47 (± 0.31) units which is statistically significant (0.01 < p < 0.05).

Also, it is interesting to note that the average difference between the facility of control eyes on approximately day 14 before norepinephrine and the facility of control eyes on approximately day 21 after norepinephrine (0.43 ± 0.36 units) is almost a statistically significant difference (p < 0.1; t = 2.0) when compared to the average difference between the facility of 6-hydroxy-dopamine-treated eyes on day 14 and the facility of these same treated eyes on day 21 after norepinephrine (0.71 ± 0.34). This difference suggests, but does not demonstrate, the possibility that 6-hydroxy-dopamine may produce supersensitivity to the facility-increasing effect of norepinephrine.
Perfusion measurements of facility of outflow following 6-hydroxy-dopamine and norepinephrine. Four monkeys were treated topically with 2 per cent norepinephrine (0.1 ml.) at the end of three pairs of control perfusions (at 5 and 10 mm. Hg above $P_o$). The norepinephrine treatment was repeated four times at 5 minute intervals. One hour after the first treatment, three additional pairs of perfusion were done.

The facility of outflow in control eyes prior to norepinephrine treatment averaged 0.603 (± 0.14) units and after norepinephrine the facility had increased to 0.873 (± 0.13) units. This difference was not significant ($t = 1.68$).

The facility of eyes treated with 6-hydroxy-dopamine and norepinephrine averaged 3.6 per cent higher than the control eyes also treated with norepinephrine. These eyes had a mean facility of 0.213 (± 0.32) units higher than control eyes, but this difference was not statistically significant.

**COMMENT.** A statistically significant increase in facility after topical treatment with norepinephrine was not seen in the 6-hydroxy-dopamine-treated eyes. However, in comparing facility of control eyes before and after topical norepinephrine,
it appeared that there was a facility increase, although further control measurements are needed.

**Perfusion facility following 6-hydroxydopamine and isoproterenol.** A pilot experiment was done in three monkeys in which control perfusion facilities were measured prior to topical isoproterenol application. The mean facility in control eyes was 0.503 (± 0.056) units. Two per cent isoproterenol was applied topically to both eyes in the same manner described for norepinephrine. Sixty minutes after isoproterenol, the facility in the control eyes averaged 1.09 (± 0.22) units. The blood pressure was 146 (± 16) mm. Hg before and 97 (± 18) mm. Hg after topical isoproterenol.

In the eyes treated with 6-hydroxydopamine, the facility of outflow following isoproterenol averaged 5.2 per cent (± 9) lower. These differences are not statistically significant.

**Episcleral venous pressure measurements in the monkey eye.** As treatment with 6-hydroxy-dopamine lowered the intraocular pressure, it was important to determine whether a lower episcleral venous pressure could account for the lowering of intraocular pressure.
In three monkeys treated in one eye with 6-hydroxy-dopamine, episcleral venous pressure and applanation pressures were measured the second, third, and fourth weeks of treatment.

One week after the first 6-hydroxy-dopamine treatment, the applanation intraocular pressure in the treated eyes averaged 11.0 (± 0.5) and 12.7 (± 0.5) mm. Hg in the control eyes. The episcleral venous pressure \( (P_e) \) in these treated eyes averaged 11.1 (± 0.4) and in the control eyes 11.4 (± 1.1) mm. Hg. The end point used for these episcleral venous pressure measurements (complete collapse of the blood column) produced higher \( P_e \) values than the end point utilized in subsequent measurements (a 50 per cent reduction in blood column\(^{10}\)). These \( P_e \) values are estimated to be 0.5 to 2 mm. Hg higher.\(^ {18}\) However, the end points selected were the same for the treated and control eyes and there was no difference in episcleral venous pressure in spite of a 1.7 mm. Hg lower intraocular pressure in treated eyes.

One week after the second treatment with 6-hydroxy-dopamine, the applanation pressure in treated eyes averaged 16.2 (± 4.4) mm. Hg and 18.1 (± 3.7) mm. Hg in control eyes. The episcleral venous pressure in these treated eyes averaged 9.9 (± 1.1) mm. Hg while control eyes averaged 10.0 (± 0.6) mm. Hg (using 50 per cent collapse of the blood column as the end point\(^ {10}\)).

Thus, there was no significant difference in \( P_e \) between the 6-hydroxy-dopamine-treated and control eyes, even though the intraocular pressure effects were quite significant.

Ninety minutes prior to the fourth measurement, 2 per cent norepinephrine (0.1 ml.) was given topically to both eyes after pretreating with proparacaine. Two additional 0.1 ml. doses of norepinephrine were given at 5 minute intervals.

Measurements of \( P_e \) in control eyes treated with norepinephrine, and in eyes treated with both 6-hydroxy-dopamine and norepinephrine, similarly showed no significant difference (12.3 and 13.2 mm. Hg, respectively).

Discussion

Our knowledge of the effects of ocular sympathectomy on aqueous inflow-outflow dynamics is woefully incomplete in the primate eye. This is probably a result of the relative expense of studying the primate, the technical difficulty in accomplishing superior cervical ganglionectomy in the monkey,\(^ {19}\) and the small number of patients available for study with postganglionic Horner's syndrome. Indeed, part of the motivation for the present investigation was to explore the possibility of the production of chemical sympathectomy by topical applications of 6-hydroxy-dopamine, which would obviate the surgical difficulties of sympathectomy, as well as possibly open up new therapeutic avenues in the treatment of human disease.

Several points to be raised in this discussion are: (1) a review of the present knowledge of the effects of superior cervical ganglionectomy on aqueous inflow-outflow dynamics of the rabbit eye and a comparison with the primate eye; (2) a comparison of the effects of chemical sympathectomy with surgical sympathectomy; (3) consideration of the question of the presence of supersensitization to adrenergic amines following chemical sympathectomy; (4) and finally, the pharmacologic mechanism of action of 6-hydroxy-dopamine, its side effects, toxicology, and its relationship to other sympatholytic agents will be discussed.

Summary of the effect of superior cervical ganglionectomy on the inflow-outflow dynamics of aqueous humor in the rabbit eye. Our understanding of the mechanisms of lowering of intraocular pressure following superior ganglionectomy in the rabbit ("ganglionectomy effects") was initiated by Linner and Prijot,\(^ {17}\) and developed in an elegant series of studies by a number of investigators, but particularly by Bárány\(^ {16, 19, 20-24}\); Sears\(^ {16, 25-30}\); Langham\(^ {31-39}\); Eakins\(^ {40-43}\); and their co-workers. Excel-
lent summaries of this work can be found in the papers of Sears and Rosser, Kramer and Potts, and Treister and Bárány. Approximately 14 hours after ganglionectomy, neurotransmitter begins to leak from storage sites in degenerating sympathetic nerve terminals stimulating the adjacent iris dilator muscle and producing a degeneration mydriasis. The duration and course of the pupillary changes are a function of the rate of release of norepinephrine and the rate of development of supersensitization of the dilator muscle. Norepinephrine accumulates in the aqueous humor, reaching 25 per cent of its maximum concentration four hours after the onset of degeneration mydriasis. At this time, the intraocular pressure begins to fall (reaching a maximum five hours later), a result of an increased facility of outflow produced by norepinephrine acting on sensitized alpha-adrenergic receptors in the outflow pathway. The exact site of the latter is unknown; however, opinion favors a vascular structure located close to the chamber angle.

Denervation supersensitization to norepinephrine gradually occurs during the phase of degeneration mydriasis, and correlates temporally with the loss of norepinephrine and the failure of normal uptake inactivation. Several investigators have demonstrated lower intraocular pressure and facility augmentation from supersensitization to exogenously applied norepinephrine.

The intraocular pressure returns to normal levels in two to three days, but, curiously, in the absence of nonadrenergic tone the resistance to outflow becomes higher than normal.

Review of the effects of ocular sympathetic denervation on the aqueous inflow-outflow dynamics of the primate eye. There is, of course, no priori reason to expect the same result from sympathetic denervation of the primate eye because of well-known anatomical and innervational differences. Bárány observed that adrenergic effects on the facility in the rabbit are easy to demonstrate, but clear-cut similar effects are difficult to establish in the monkey.

Langham found an alpha-receptor response in the monkey 7 to 14 days after sympathetic denervation. The intraocular pressure dropped after topical adrenalin, but there was no facility change. Langham (with David Honey) has also repeatedly made reference to unpublished rhesus monkey experiments in which a prolonged intraocular pressure-lowering effect has been seen following superior cervical ganglionectomy; a sustained fall in intraocular pressure associated with an increase in outflow facility was also mentioned. Sensitization to topical norepinephrine (intraocular pressure and pupil) was said to be increased a thousandfold.

Hoffman found in the Cynomolgus monkey that the intraocular pressure was not significantly lower in the side of sympathetic denervation. One per cent norepinephrine applied topically produced a pressure rise in control eyes, but not denervated eyes, and it was without statistical significance. Injection of norepinephrine intracamerally caused a fall in intraocular pressure in the denervated and control eyes. At high perfusion pressures (but not at low), norepinephrine caused a significant increase in facility of outflow. Hoffman concluded that superior cervical ganglionectomy in the monkey caused only a slight drop in intraocular pressure from the ganglionectomy; facility was slightly higher in the denervated eye, and with high perfusion pressure, facility was increased and secretion was stimulated by intracameral norepinephrine.

Resume of observations in human subjects. Sears and Sherk mentioned that a greater facility of outflow occurred on the homolateral side of patients with Horner's syndrome and that the resistance to outflow further decreased after topical norepinephrine. Sears also reported supersensitization to adrenalin in these patients; the magnitude of these ef-
 Infects was reported to be inversely proportional to the duration of the sympathetic denervation in Horner’s syndrome patients.

Swegmark\textsuperscript{48} reported a case of postganglionic Horner’s syndrome in which the intraocular pressure was 35 per cent lower on the affected side; the difference in intraocular pressure was presumed due to diminished secretion as measured by suction cup techniques. There was no difference in facility of outflow by tonography with several measurements. Topical treatment of the normal eye with guanethidine reproduced the same changes seen in the eye with Horner’s syndrome. Sensitization of the denervated pupil to adrenaline 1/1,000 was demonstrated.

Langham and Weinstein\textsuperscript{35} reported three patients with Horner’s syndrome (one preganglionic and two postganglionic). Measurements of pressure, facility, and flow, before and after topical norepinephrine, were incomplete. The authors concluded that the outflow facilities measured by Schiötz tonography were not significantly different in the three patients. They assumed episcleral venous pressures were unchanged and inferred that aqueous inflow was diminished by sympathetic denervation. In one patient, topical epinephrine produced a lower intraocular pressure in both eyes and a greater aqueous humor formation in the denervated eye, along with an increase in outflow facility.

Weinstein and Langham\textsuperscript{36} reported a case of Horner’s syndrome, in association with bilateral glaucoma, in which the eye with Horner’s syndrome had a relatively lower intraocular pressure and less visual field loss. Supersensitivity to topical norepinephrine was claimed for the pupil and the pressure on the denervated side. There was no change in facility of outflow following norepinephrine in this patient.

Drance in the Gilston Glaucoma Symposium\textsuperscript{34} reported that Christansen’s follow-up studies of six patients with cervical sympathectomy demonstrated a marked fall in intraocular pressure which was of short duration and gradually became normal.

Bron\textsuperscript{50} studied a patient with postganglionic Horner’s syndrome and a normal patient treated with topical guanethidine. Intraocular pressure was lower in the sympathetically denervated eye due to a lower aqueous humor production. Guanethidine did not significantly lower the intraocular pressure, but did lower aqueous humor formation. Topical norepinephrine restored flow to normal levels in both eyes. Pupillary supersensitization to norepinephrine was demonstrated following guanethidine application. There was no intraocular pressure-lowering effect produced by topical norepinephrine in either the Horner’s patient or the guanethidine-treated eye, an observation which is similar to that reported by Drance (see above).

Comment. The quality and quantity of data on the primate eye concerning the effects of ocular sympathectomy are not sufficient to form a complete and convincing picture. Trends which have emerged from the existing studies are: (1) a lower intraocular pressure on the side of sympathetic denervation which may persist indefinitely or disappear (depending on duration); (2) no change in facility of outflow in chronically denervated eyes; (3) lower aqueous secretion (accounting for the lower intraocular pressure if it is assumed episcleral venous pressure is unchanged); (4) denervation supersensitization to topical catecholamines sometimes occurs, but it is not a regularly observed event in all investigations. Supersensitization of pupils is a frequent observation. Intraocular pressure may remain unchanged following topical adrenergic amines because of mutually opposing influences of increased facility of outflow in sensitized outflow pathways and increased aqueous humor secretion. The intraocular pressure may fall following topical application of adrenergic amines when a marked increase in the outflow facility occurs.

It seems likely that the variable results
of these investigations of supersensitivity phenomena may be due to different thresholds of the different ocular tissues, as well as differences in catecholamine concentrations reaching the target sites after topical applications.

**Comparison of the effects of ocular chemical sympathectomy by 6-hydroxydopamine with surgical sympathectomy.** There are many similarities between these methods of producing ocular sympathectomy, but, also, there are some outstanding differences.

**Pupil.** Degeneration contraction (mydriasis) is seen one half to three hours following topical application of 6-hydroxydopamine, but occurs approximately 14 hours after superior cervical ganglionectomy. This difference is probably related to the great rapidity of uptake of 6-hydroxydopamine by sympathetic nerve terminals compared to the relatively large time interval required for anatomic degeneration and release of endogenous norepinephrine.

**Intraocular pressure.** Two effects can be observed in the 6-hydroxy-dopamine-treated eyes which depend on the species.

**RABBIT.** In the rabbit, the intraocular pressure is significantly lower on the third day after 6-hydroxy-dopamine treatment. At this time, the pressure rise in the treated eyes on water loading is much lower, probably because of an increased facility of outflow. After surgical sympathectomy, the intraocular pressure and facility effects occur between 19 and 36 hours following ganglionectomy. Since our experiments were done every two days, because of anesthesia limitations, the earliest occurrence of the ganglionectomy effect from 6-hydroxy-dopamine is unknown.

One week after treatment, the ganglionectomy effect of 6-hydroxy-dopamine-treated rabbits is not demonstrable. This is reminiscent of the transient (lasting two to three days) effects on intraocular pressure and facility produced by surgical ganglionectomy.

**MONKEY.** In monkeys, the intraocular pressure-lowering effect of 6-hydroxydopamine persists at least two to three weeks following topical treatment. This agrees with the available data on surgical sympathectomy. One outstanding difference of the effects of surgical sympathectomy on rabbits and monkeys is the longer duration of effects on the latter species. The exact duration of the 6-hydroxy-dopamine effect on intraocular pressure has not been investigated.

**Tonographic facility.** The tonographic facility is increased transiently by 6-hydroxy-dopamine approximately one week following treatment. This increase in facility, however, disappears by the second week. The time of earliest occurrence of this increased facility has not been investigated. The transient increase in facility seen in the monkey is certainly reminiscent of the similar effect observed in rabbits following surgical sympathectomy.

**Epicleral venous pressure.** The epicleral venous pressure is unchanged by chemical sympathectomy in both monkeys and rabbits, agreeing well with the single measurement reported in the literature by Linner and Prijot in the rabbit. This permits the inference to be made that aqueous secretion is reduced following chemical sympathectomy by 6-hydroxy-dopamine, agreeing well with the reported effects of surgical sympathectomy.

Haeusler, Haefely, and Thoenen have compared the effects of chemical sympathectomy in the cat produced by 6-hydroxy-dopamine to the effect of surgical sympathetic denervation. The norepinephrine content of smooth muscles and the nictitating membranes denervated surgically was reduced to almost zero, whereas chemically sympathectomized membranes average 8 per cent of the control norepinephrine levels. Similar results were obtained in the iris in which corresponding values for norepinephrine were zero and 3 per cent, respectively. The adrenergic sensitization of the surgically denervated membranes was increased by a factor of approximately 96 compared to the con-
Fig. 12. Metabolic pathway involved in synthesis of norepinephrine and epinephrine. The rate-limiting enzyme, tyrosine hydroxylase, is presumed to be inhibited by 6-hydroxy-dopamine. The similarity in structure between 6-hydroxy-dopamine and norepinephrine is apparent.

trol values, whereas chemically sympathectomized membranes were increased in sensitivity by a factor of approximately 71.

Supersensitization phenomena. Our experiments failed to demonstrate an increased pressure-lowering response to topically applied adrenergic amines in chemically sympathectomized eyes. There was no statistically significant change in facility in the few experiments which were designed to explore this. There was an increase both in facility and intraocular pressure in the control eyes and a smaller increase in the 6-hydroxy-dopamine-treated eyes, similar to the observations of Bron and Hoffman. However, there was no increased effect due to chemical sympathectomy as might have been anticipated. We wish to emphasize that these experiments are preliminary and of a pilot nature, and we conjecture that under other experimental conditions, supersensitization effects of topically applied adrenergic amines may be demonstrable. Possibly, the dose delivered to the target sites may be the critical issue. Nonetheless, our present experiments do not demonstrate a facility-increasing or additional pressure-lowering effect of topically applied adrenergic amines in the 6-hydroxy-dopamine chemically sympathectomized eye.

Pharmacologic mechanisms of action of 6-hydroxy-dopamine. 6-Hydroxy-dopamine (2,4,5-trihydroxyphenylethylamine) is a congener of norepinephrine (Fig. 12). It has the same formula weight and similar structure, except that the –OH group attached to the beta-carbon of norepinephrine is transposed to the 2 position of the benzyl ring of 6-hydroxy-dopamine. 6-Hydroxy-dopamine is very similar to norepinephrine in its chemical properties, including its propensity for rapid oxidation and rapid uptake into sympathetic nerve terminals.
A single injection of 6-hydroxy-dopamine markedly reduces the norepinephrine content of sympathetically innervated organs of several species for days or weeks, and restoration of norepinephrine may not be complete for as long as 80 to 90 days.1, 3, 51-55 Thoenen and Tranzer5 developed a treatment regimen which led to a generalized and almost complete destruction of adrenergic nerve terminals. Adrenergic nerve cell bodies, stem axons, and other nerve endings (e.g., cholinergic) are unaffected by 6-hydroxy-dopamine. These authors proposed the term "chemical sympathectomy" to describe these unique effects.

Thoenen (personal communication) points out that the sympathetic nerve terminals accumulate the amine very efficiently. Oxidation products of 6-hydroxy-dopamine undergo covalent bonding to nucleophilic groups of macromolecules which leads to destruction of the nerve terminals. Miller, Thoenen, and Axelrod50 postulate that the rate-limiting enzyme in norepinephrine synthesis, tyrosine hydroxylase (Fig. 12), is destroyed by 6-hydroxy-dopamine since the enzyme's disappearance from the heart is coincident with the destruction of cardiac sympathetic nerve endings.

Electron microscopic studies of nerve terminals following 6-hydroxy-dopamine treatment show sympathetic nerve terminal degeneration.4, 5, 55 Using the histofluorometric technique of Falck, Malmfors and Sachs53 and Knyihar and associates5 have shown a depletion of catecholamines from sympathetic axonal terminals with relative preservation of catecholamines in nerve cell bodies and stem axons. This depletion of endogenous norepinephrine was due to a destruction of nerve terminals by 6-hydroxy-dopamine or some metabolite, making the terminals unable to retain endogenous norepinephrine. Inhibition of norepinephrine uptake occurred simultaneously with the disappearance of the fluorescence of sympathetic nerve terminals.

Studies in our laboratory with the use of the Falck histofluorometric technique (in collaboration with Dr. Jeffrey Ellison) have demonstrated a loss of sympathetic nerve terminals in the anterior segment following topical 6-hydroxy-dopamine (Fig. 13). These observations have been correlated with electron microscopic observation of the effects of topical administration of 6-hydroxy-dopamine in a report now in preparation.

The concept of "chemical sympathectomy" is not a new one in ophthalmology. Depletion of endogenous norepinephrine has been accomplished experimentally by reserpine,20 cocaine,57 tyramine analogs,50 and guanethidine. It is the latter that 6-hydroxy-dopamine most closely resembles.

It may be informative to compare the effects of guanethidine with those of 6-hydroxy-dopamine.

In rabbits, guanethidine has been reported to lower the intraocular pressure and to increase the facility of outflow following intracameral injection.43 In humans, guanethidine has been reported to lower the intraocular pressure following intravenous58 and topical application.48, 50, 59-60 Topical application in normal eyes lowers the pressure 1 to 3 mm. Hg. Topical or intravenous administration in glaucomatous eyes lowers the pressure about 8 mm. Hg.

Four to 12 hours after the first administration of topical 10 per cent guanethidine, a significant increase in facility of outflow has been reported.60 This increase in normal eyes is about 0.045 μl per millimeter of mercury per minute. Beyond 12 hours after the first administration, there is no effect on facility but the intraocular pressure is still significantly lowered. Anselmi, Bron, and Maurice59 have demonstrated directly, with a fluorophotometric technique, that eyes treated twice a day with 10 per cent guanethidine for 3 to 36 days ("chronic pharmacological sympathectomy") have a significantly decreased rate of aqueous flow and increased vascular permeability. The effect of guan-
Fig. 13. (Right) Sections of control albino rabbit iris treated by Falck histofluorometric method showing fluorescence of catecholamine containing axons. (Left) Three days following a single treatment with 6-hydroxy-dopamine, there is a marked diminution of terminal and preterminal sympathetic axons, demonstrating "sympathectomy" by chemical means. (Slides courtesy of Dr. Jeffrey Ellison.)

Ethidium on episcleral venous pressure has not been reported.

Like guanethidine, 6-hydroxy-dopamine produces a transient increase in outflow facility followed (with continued treatment) by a decrease in intraocular pressure due to diminished aqueous inflow. The transient increase in facility caused by 6-hydroxy-dopamine lasts much longer (at least six to seven days) than that caused by guanethidine. Also, the duration of the effect is considerably longer than that of guanethidine.

Other significant differences exist between 6-hydroxy-dopamine and guanethidine. For example, there is a slightly greater potency of 6-hydroxy-dopamine in depleting cardiac norepinephrine. Reserpine, guanethidine, and tyramine reversibly release norepinephrine from the tissues, whereas 6-hydroxy-dopamine destroys or irreversibly alters the norepinephrine binding sites. The slow recovery of norepinephrine after 6-hydroxy-dopamine is a consequence of the rate required for regeneration of binding sites.

Histofluorometric experiments by Csilik showed that guanethidine caused a reduction of axonal fluorescence, but did not produce a complete depletion of catecholamines, which is in marked contrast to the complete loss of axonal terminals produced by 6-hydroxy-dopamine.

Toxicology of topically applied 6-hydroxy-dopamine. There were no animal deaths in any experiments performed in this investigation which could be attributed to the use of 6-hydroxy-dopamine.

In early experiments, adrenochrome-like staining of the deep corneal lamellae occurred when an ascorbate was used as an antioxidant at pH 5.2. However, when bisulfite was substituted at a pH of 7, no corneal staining or oxidation products were observed on repeated slit lamp examinations.

Iris vasodilation was a regular occurrence in the 6-hydroxy-dopamine-sympa-
thectomy and control eyes. Slit lamp examinations of eyes showed no increased aqueous flare compared to control eyes. The semiquantitative trichloroacetic acid test of Langham and Taylor showed no significant elevation of protein in the aqueous humor of control and treated eyes in four monkeys used for perfusion studies.

Histology of these eyes has shown no abnormalities in rabbits and a slight vacuolization of the pigment epithelium of the iris in monkeys.

It is our impression that this drug is reasonably safe for clinical investigation. Limited clinical investigations are in progress and will be reported on in the near future.

The authors wish to acknowledge the assistance of Mrs. Parveen Walia and Mr. Fred Clark and the collaborative assistance and efforts of Dr. Jeffrey Ellison.

REFERENCES


51. Hausler, G., Haeceley, W., and Thoenen, H.: Chemical sympathectomy of the cat