Sympathetic Denervation Effects on Avian Eye Development and Aqueous Fluid Dynamics[1] (36457)

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Sympathetic denervation of the eye in experimental mammals, either by surgical means (1, 2), or pharmacologically (3), has been reported to cause a decrease in intraocular pressure (IOP), an increase in outflow facility (*C*) (1, 2), no change (4) or a decrease (5) in aqueous secretion, no change in episcleral venous pressure (4, mydriasis (6) or miosis (7), supersensitivity to alpha-adrenergic drugs (7), decreased vascular tone and increased blood flow to the anterior segment of the eye (4, 8). Many of these effects are transient, the peak response occurring about 1 day after anterior cervical ganglionectomy. This "24-hr ganglionectomy effect" has been attributed to the release of stored catecholamines, primarily norepinephrine, from degenerating postganglionic nerve terminals (1, 9). This hypothesis was strengthened by Eakins and Eakins (10), and by Sears *et al.* (11), who showed that catecholamine stores in the uveal tissues were depleted over a time period which coincided with postganglionectomy changes in IOP and C.

There are conflicting reports regarding the long-term effects of ganglionectomy on the eye: a return to normal may occur within 3 days (2), especially after unilateral rather than bilateral ganglionectomy. However, Sears *et al.* (11) reported that, following a transient rise, outflow facility was greatly reduced for almost 4 weeks after unilateral ganglionectomy, and *C* did not return to normal until 12 weeks after bilateral sympathetic denervation. Langham (7) found that supersensitivity to norepinephrine was still evident 8 days after unilateral ganglionectomy, and in the experiments of Holland and Mimms (3), miosis during the first 48 hr was followed by mydriasis lasting as long as 5 weeks in chemically sympathectomized monkeys. From the reduced IOP and increased *C*, these authors postulated that postganglionectomy effects were accompanied by reduced intraocular pressure and miosis (3).

It appears that some of the reported effects of sympathetic denervation are alpha-adrenergic effects, occurring only during the period of release of norepinephine from degenerating nerve endings. After this period, long-term effects may be masked by supersensitivity to adrenergic amines reaching the denervated eye via a humoral route, or via crossed innervation from an intact contralateral eye.

Thus, although a number of nonvisual ocular structures and functions are known to be under automatic control, much less is known about the operation of these under chronic sympathetic deprivation, or about the effects of such deprivation on the orderly growth and development of the eye.

We here outline a procedure for removal of the superior cervical sympathetic ganglion, unilaterally or bilaterally, in the newly hatched domestic chick. Prior work on the removal of the superior cervical sympathetic ganglion in birds has dealt mostly with the effects of sympathetic denervation on the pineal body and/or gonads (12–15). To our knowledge, none of the published papers has included a description of the method of ganglionectomy. This study was concerned with the effects of ganglionectomy on subsequent growth and development of the avian eye, and on aqueous fluid dynamics in the sympathetically denervated eye.

The avian eye is an especially favorable

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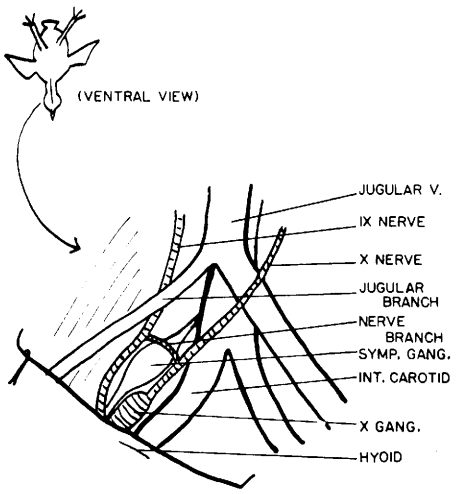

FIG. 1. Surgical approach for location and extirpation of the left superior cervical sympathetic ganglion of the newly hatched chick.

system for study of ocular physiology because of its large size relative to body size, and its apparent functional similarity to the better known mammalian eye.

Materials and Methods. The White Rock cockerels used in these experiments were reared in a diurnal (14L/10D) photoperiod. They were supplied with food, water and brooder heat *ad libitum*, according to standard rearing practice. Chicks were subjected to unilateral or bilateral superior cervical ganglionectomy during the first few days after hatching. Hatchmates of the bilaterally operated chicks served as sham-operated controls, the ganglia being exposed but left intact. Either the right or the left ganglion was removed in unilaterally ganglionectomized birds, while the contralateral ganglion was exposed but left intact.

Surgical procedure. Chicks 1 to 5 days of age were anesthetized by intramuscular Combuthal (Nembutal–Pentothal) (16). The chick was held supine on a Styrofoam platform by passing a straight pin through the tip of the beak and through the webbing of each foot. A ventral midline incision was made, extending from the hyoid cartilage to a point about halfway down the neck. The skin was freed and retracted with skin hooks. Minor bleeding was controlled by direct pressure, using cotton pledgets soaked in a 1:50,000 adrenaline solution.

Under the dissecting microscope, the superficial fascia was cut parallel to the hyoid cartilage and displaced laterally. This procedure exposed a branch of the jugular vein lying lateral to the common carotid artery. Coursing beside the jugular vein are cranial nerve IX, located laterally, and cranial nerve X, medial and somewhat inferior to the jugular. The angle formed by the exposed branch of the jugular vein and the hyoid cartilage was used as a landmark for location of the superior cervical sympathetic ganglion. The hyoid was then dislocated and displaced in an anterolateral direction. By careful blunt dissection, a communicating branch between nerves IX and X was exposed. Rostral to the junction of this communicating branch with the tenth nerve, the latter enlarges to form the superior ganglion of the vagus.

From the communicating branch, cranial nerve IX was followed rostrally to the foramen through which it leaves the skull, several millimeters dorsolateral to the foramen of cranial nerve X. The superior cervical sympathetic ganglion lies in the triangle formed by the communicating branch as a base, cranial nerves IX and X as respective sides, and the two foramina as the apex (Fig. 1). This ganglion lies immediately lateral to the carotid, where that artery plunges dorsally to gain entry to the skull.

The investing fascia of the ganglion is continuous with the carotid sheath, and extreme care was required to avoid rupture of the artery. The exposed sympathetic ganglion was pinched off at either end with serrated forceps, and lifted away. Carotid pulse sometimes became weak after the second ganglion was removed; as a precautionary measure, adrenaline (0.05 ml of 1:50,000) was kept ready for intramuscular injection if needed. Success of the operation was judged by ipsilateral ptosis, evident immediately after the chick recovered from anesthesia and remaining as a permanent feature.

Aqueous fluid dynamics. The rate of aqueous humor inflow was measured simultaneously in both eyes after intravenous sodium fluorescein injection, according to a method previously described (17, 18). This mea-

TABLE I. Effects of Sympathetic Denervation on Dimensional Parameters and Ocular Fluid Dynamics in the Avian Eye.[a]

Age (weeks)	Eye wt (g)	Aqueous vol (mm^3)	IOP (mm Hg)	C (μl/min/mm Hg)	F (μl/min)
25 (unilateral gang X)	3.95 ± 0.22 (6)	105.8 ± 6.5 (6)	14.5 ± 1.1 (6)	1.06 ± 0.15[b] (6)	13.1 ± 1.4 (6)
25 control	3.77 ± 0.23 (6)	110.7 ± 4.5 (6)	12.2 ± 0.9 (6)	2.28 ± 0.39 (5)	16.7 ± 2.0 (6)
42 (bilateral gang X)	3.51 ± 0.16 (6)	70.7 ± 4.1[b] (5)	13.6 ± 0.4 (12)	1.72 ± 0.25[b] (6)	—
42 control	3.45 ± 0.21 (5)	111.1 ± 6.5 (6)	12.5 ± 0.5 (10)	2.89 ± 0.33 (5)	—

[a] Values shown are the arithmetic mean ± SEM. The number of experimental subjects is shown in parentheses below each value.

[b] The experimental value is significantly different from the control ($p < .05$).

surement was made first (before IOP and C measurements), in birds undisturbed except for Nembutal anesthesia and retraction of the eyelids, to minimize disturbance of homeostatic control mechanisms for aqueous flow, which may occur after cannulation. Intraocular pressure (IOP) was measured manometrically after cannulation of the anterior chamber, and outflow facility (C) was determined by constant rate perfusion, as previously described (19). In unilaterally ganglionectomized birds, the contralateral eye served as a control: saline infusion to determine C was initiated in one eye at a time, the second eye being infused only after the IOP in the first had returned to normal, and stabilized.

Ocular dimension measurements. After removal of the cannulas, the bird was allowed to recover from anesthesia and reform the anterior chamber during the ensuing 24 hr. The animal was then sacrificed by cervical dislocation, each eye was enucleated and trimmed of extraocular tissue, then weighed and photographed in front and side view. Each enlarged photograph included a millimeter scale against which measurements could be made of corneal diameter and radius of curvature, corneal height, and pupil diameter. The volume of the aqueous space, needed for calculation of aqueous inflow, was determined geometrically as previously described (18).

Results. The presence of ptosis of the upper eyelid immediately after the operation, and persisting for as long as 42 weeks, was accepted as evidence of the success of the ganglionectomy. Erection of the head feathers, noted by Langley (20) in ganglionectomized pigeons, was sometimes noted in our chicks, but this was transient and was only seen several days to a week after the operation. No consistent change in pupil size or response was noted.

Aqueous flow. In birds unilaterally ganglionectomized soon after hatching, and tested at 25 weeks of age, outflow facility was reduced from a normal value of 2.28 ± 0.39 μl/min/mm Hg to 1.06 ± 0.15 ($p < .05$) in the ipsilateral eye. Bilaterally ganglionectomized birds examined at 42 weeks had a mean C value of 1.72 ± 0.25 μl/min, compared with a control value of 2.89 ± 0.33 ($p < .05$) (Table I). Although outflow facility was greatly impaired, sympathetic denervation had much less dramatic effects on mean aqueous inflow and mean IOP.

Ocular dimensions. The volume of the aqueous space was reduced by one third, from a control value of 111.1 ± 6. 5 mm^3 to 70.7 ± 4.1 ($p < .05$) in bilaterally ganglionectomized birds examined at 42 weeks. However, the aqueous space volume on the ipsilateral side of unilaterally ganglionectomized birds was not significantly different from that of the contralateral side, in birds tested at 25 weeks (Table I). Corneal radius

TABLE II. Ocular Parameters 25 Weeks After Unilateral Sympathetic Denervation.[a]

	Eye wt (g)	Aqueous vol (mm^3)	IOP (mm Hg)	C (μl/min/mm Hg)	F (μl/min)
Ipsilateral (gang X)	3.89 ± 0.14	111.2 ± 4.9	14.5 ± 1.1	1.00 ± 0.15	14.3 ± 1.2
No. of pairs	(11)	(11)	(6)	(5)	(9)
Control eye	3.74 ± 0.15	114.7 ± 5.7	12.2 ± 0.9	2.28 ± 0.39	17.6 ± 1.5
Significant difference gang X: control eyes in same subject	$p < .01$	NS	$p < .05$	$p < .025$	$p < .05$

[a] Values given are the arithmetic mean $\pm$ SEM. The experimental (gang X) eye and control eye of each subject were compared by paired t test to yield the levels of confidence shown in each case.

of curvature was not affected by ganglionectomy.

Pupil diameter on the operated side was within normal range at 25 weeks after unilateral sympathetic denervation. In bilaterally ganglionectomized birds, the pupil was slightly but not significantly smaller than normal (5.5 ± 0.1 vs 6.1 ± 0.3) at 42 weeks. Comparison of mean eye weights showed that sympathetically denervated eyes were slightly but not significantly enlarged at both 25 and 42 weeks.

In Table II are summarized the mean values for several ocular parameters in unilaterally ganglionectomized birds. Although biological variation appears to mask any apparent differences between values, some of this variability may be eliminated by comparing the ipsilateral with the contralateral eye in the same unilaterally ganglionectomized bird. When values were thus compared, the ratio of operated/sham operated was greater than 1 for IOP and eye weight, and less than 1 for aqueous inflow and outflow facility. A paired t test showed that the effects of unilateral sympathetic denervation included significantly greater eye weight, elevated IOP, reduced aqueous inflow, and impaired outflow facility at 25 weeks but no differences in pupil diameter, aqueous space volume, or corneal radius of curvature.

Discussion. Method of ganglionectomy. The procedure given here for removal of the superior cervical sympathetic ganglion of the chick appears to be the first description in the literature of a practical operative procedure for birds, although several reports have been published concerning the effects of the operation (12–15, 20). In our experience, the operation was easily performed on newly hatched chicks, but became more difficult with each succeeding day after hatching. Already by 1 week of age, the ganglion is deeply embedded in the neck, and the problems of finding and exposing it are increased, as is the risk of trauma to nearby vital structures.

Aqueous fluid dynamics. Outflow facility remained low in birds tested at 25 weeks after unilateral and 42 weeks after bilateral ganglionectomy. This is in contrast with the finding that, in the rabbit, outflow facility was reduced but had returned to normal 4 weeks after unilateral and 12 weeks after bilateral ganglionectomy, respectively (11).

The possibility that bilateral crossed sympathetic innervation to the eye exists in the rabbit was suggested by Sears and Bárány (21) and by Sears *et al.* (11), in view of the fact that Holland, Von Sallman and Collins (cited in Ref. 21) had reported that ipsilateral trabecular sympathetic control appeared to be reestablished 6 weeks after unilateral ganglionectomy. Crossed sympathetic innervation presumably facilitated recovery and control of outflow facility in the unilaterally denervated eye, according to the experiments of Murray and Thompson (22). By extrapolation, it appears from IOP, F and C values that sympathetic control was still not completely reestablished in the chicken eye 25 weeks afer unilateral ganglionectomy or 42

weeks after bilateral sympathetic denervation.

Eye dimensions. Reduced aqueous space volume in bilaterally denervated avian eyes is a new finding, not previously reported for mammalian eyes after ganglionectomy. If this reduction in aqueous volume results from deficient sympathetic innervation during development, it is somewhat surprising that no significant reduction was noted after unilateral ganglionectomy. It may be that such crossed sympathetic innervation as does exist in the chicken was sufficient to support normal development of the anterior segment of the denervated eye on the unilaterally operated side, although perhaps insufficient to provide for full recovery of physiological parameters after ganglionectomy.

Mean eye weight was not significantly altered by sympathetic denervation, although there was a trend toward eye enlargement, in spite of the reduced aqueous volume. In the more exacting test based on comparison of the two eyes of a unilaterally ganglionectomized animal, which should reduce errors due to biological variation, the ipsilateral eye was always heavier and this difference was significant at the 1% level.

There is a paucity of information on the effects of sympathetic deprivation on normal tissue, especially during development. No known syndrome is associated with adrenal medullary insufficiency or extirpation of those glands. Sympathectomy has been practiced clinically since the beginning of the century (23). Beyond the beneficial effects on the afflicted tissue, such ancillary effects as have been reported appear to the explainable by hyperemia, increase in temperature and decrease in activity of sweat and other glands in the denervated area (24). In expermental animals, total sympathetic deprivation has had no serious consequences as long as the animals were maintained in a protected environment. However, such animals were unusually sensitive to stressful conditions such as cold or forced exercise (25).

With the discovery of nerve growth factor by Levi-Montalcini and Booker (26) and preparation of its antiserum by Cohen (27), it has become possible to selectively destroy all sympathetic ganglia without surgical intervention. Newborn mice so treated did not suffer any adverse effects on visceral functions, and in fact were later capable of delivering normal litters.

The changes in aqueous outflow facility and in ocular dimensions, which occurred in these experiments after sympathetic denervation, are reminiscent of glaucomatous changes we have previously reported in the eyes of birds reared under continuous light (18, 19, 28–30). The lesions of light-induced avian glaucoma include ptosis, eye enlargement, elevated intraocular pressure, reduced aqueous inflow, impaired outflow facility, reduced volume of the aqueous space, and eventual blindness. The primary lesion in this condition has so far been elusive: we have searched for but found no evidence of hyperplasia that would explain the enlargement of the globe, nor do histological or physiological investigations support blockage of the aqueous drainage angle as a mechanism which might explain the changes in aqueous space volume. While the changes in light-induced avian glaucoma are more severe than the effects reported here following ganglionectomy, and we have not yet completed similar histological studies on ganglionectomized eyes, the similarities are striking. It is tempting to speculate that the two experimental conditions, rearing under continuous light and sympathetic denervation, may operate through the same mechanism(s) in setting a pathological course for the eye during development.

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