Effect of Cervical Sympathectomy on the Ocular Response to Systemic Endotoxin[1] (36249)

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Systemic bacterial endotoxin produces a marked increase in ocular vascular permeability in the rabbit (1, 2). This effect can be quantitated by measuring the accumulation in the eye of intravenously injected human serum albumin labeled with radioactive iodine (2). The change is evident at 1 hr following systemic endotoxin, persists and reaches a maximum by 4 hr, and returns toward normal by 24 hr after endotoxin (2). The location of this prolonged increase in vascular permeability is primarily in the iridial portion of the ciliary process. The initial change is edema; but, by 4 hr, hemorrhages and thrombi are a prominent part (3).

Sympathetic denervation (4, 5) and α-adrenergic blocking agents (6) are known to suppress or prevent other reactions to endotoxin, *i.e.*, both the local and generalized Shwartzman reactions. Adrenalectomy between preparation and provocation prevents the generalized Shwartzman reaction (7); and norepinephrine is required for the synthesized Shwartzman reaction employing ellagic acid and ϵ-aminocaproic acid as well (8).

The following experiment was undertaken to determine the role of the sympathetic nervous system in the ocular response to a single systemic injection of endotoxin. It was found that superior cervical ganglionectomy suppressed the ocular protein leak and attenuated the response at the maximum stage of this reaction.

Materials and Methods. Albino rabbits of both sexes (weighing 1700–2300 g) were used. *Escherichia coli* bacterial endotoxin 055:B5 (Difco Laboratories, Detroit, MI) was dissolved in 2.0 ml of saline and injected in 200 μg quantities into the marginal ear vein.

Experimental design. All operations were done on the right side and the findings in the right eye were compared with the eye on the unoperated left side. At least 6 animals were used in each group. Superior cevical ganglionectomies were performed at three time intervals prior to injecting endotoxin: 4–6 days (Series A), 17–21 days (Series B), and 22–24 hr (Series C). Preganglionic neurectomies (Series D) and sham operations were performed 4–6 days before endotoxin. Generally, endotoxin was injected 4 hr prior to sacrifice. In one group of Series A animals, endotoxin was injected 1.5 hr before sacrifice. Ocular albumin space measurements and routine histologic sections were made for each eye. One group of Series A animals was used for morphologic study only. A microscopic quantitative estimate of the severity of damage was made on the eyes of these rabbits.

Sympathectomy. All operations were performed aseptically using sodium pentobarbital (Diabutal, Diamond Laboratories, Des Moines, IA) at half strength, 30 mg/kg of body weight. The technique of dissection was basically that of Sears and Barany (9). The superior cervical ganglion was found medial to the internal carotid artery and was often in two parts (9). The ganglion was isolated in the sham operations and isolated and removed in the ganglionectomies. Routine histologic sections were made to estimate the completeness of ganglionectomy. Preganglion-

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TABLE I. Effect of Cervical Sympathectomy on the Ocular Response to Systemic Endotoxin. Measurement of ocular albumin space.

Expt.	No. of animals	Av ocular albumin space (μl/g of eye tissue with SD) Operated side (right eye)	Unoperated side (left eye)
A 4–6 days postcervical ganglionectomy			
No endotoxin	5	6.12 ± 0.97	5.28 ± 1.22
1.5 hr after endotoxin	7	20.61 ± 7.94	21.88 ± 9.73
4 hr after endotoxin	6	15.00 ± 5.48[a]	24.96 ± 6.06[a]
B 17–21 days postcervical ganglionectomy			
4 hr after endotoxin	9	23.63 ± 11.30	27.47 ± 12.41
C 24 hr postcervical ganglionectomy			
4 hr after endotoxin	6	22.57 ± 5.60	22.40 ± 4.06
D 4–6 days postcervical ganglionectomy			
4 hr after endotoxin	6	27.66 ± 12.54	26.32 ± 11.10

[a] $p < .001$; other values: difference not significant.

ic neurectomies were made immediately proximal to the ganglion and 0.5 cm of nerve was removed.

Ocular albumin space. Measurements were made by a slight modification of the technique of Fish and co-workers (10) and used in previous studies to measure protein leak from vessels of the iridial process in the ocular response to systemic endotoxin (2, 3). Briefly, this consisted of the intravenous injection of human serum albumin (HSA) labeled with Iodine 125 (^{125}I) (in quantities of 10–15 μl) 15 min prior to killing the animal. Heparinized cardiac blood was obtained from the lightly anesthetized animal just prior to sacrifice, and the animal was killed by an intracardiac injection of pentobarbital. The eyes were enucleated with the animal in an upright position, and the globes were trimmed of excess tissue. Radioactivity was measured in 1.0 ml of cardiac plasma and in each intact eye. The "ocular albumin space" or "total albumin space" was calculated for each eye from the following formula: ocular albumin space =

$$(^{125}\text{I} - \text{HSA/g of eye tissue})/(^{125}\text{I} - \text{HSA/ml of cardiac plasma}).$$

The results were expressed as microliters per gram of eye tissue (see Table I).

Morphological study. Following radioactivity determinations the eyes were fixed in 4% buffered formalin. The eyes were bisected at the equator, the lens was removed, and the anterior segment was divided into quadrants. The iridial portion of the ciliary processes was the primary site of the morphologic changes in a previous study (3). A section across these processes at right angles to an iris meridional plane was taken from each quadrant. To quantitate and compare the severity of morphologic change in the group most affected by sympathectomy, a more elaborated fixation technique was used. Immediately following sacrifice, the eyes of these 6 animals were enucleated, opened in the usual fashion, and the lens was removed and immersed in 1.75% glutaraldehyde in 0.050 *M* cacodylate buffer. The anterior segment was subdivided, the lens capsule was reflected and carefully removed. Fixation was at 4° overnight, the tissues were washed briefly in buffer, and sections were made of the iridial processes throughout the circumference of both eyes. These sections were then fixed in 2% osmium tetroxide, dehydrated through alcohol, and embedded in Araldite. Sections of 1–2 μ in thickness were stained with methylene blue and azure II and examined by light microscopy. The number of processes showing hemorrhage and/or thrombi were expressed as a percentage of total processes counted (see Table II).

TABLE II. Effect of Cervical Sympathectomy on the Ocular Response to Systemic Endotoxin.[a] Percentage of iridial processes showing hemorrhage and/or thromboses.

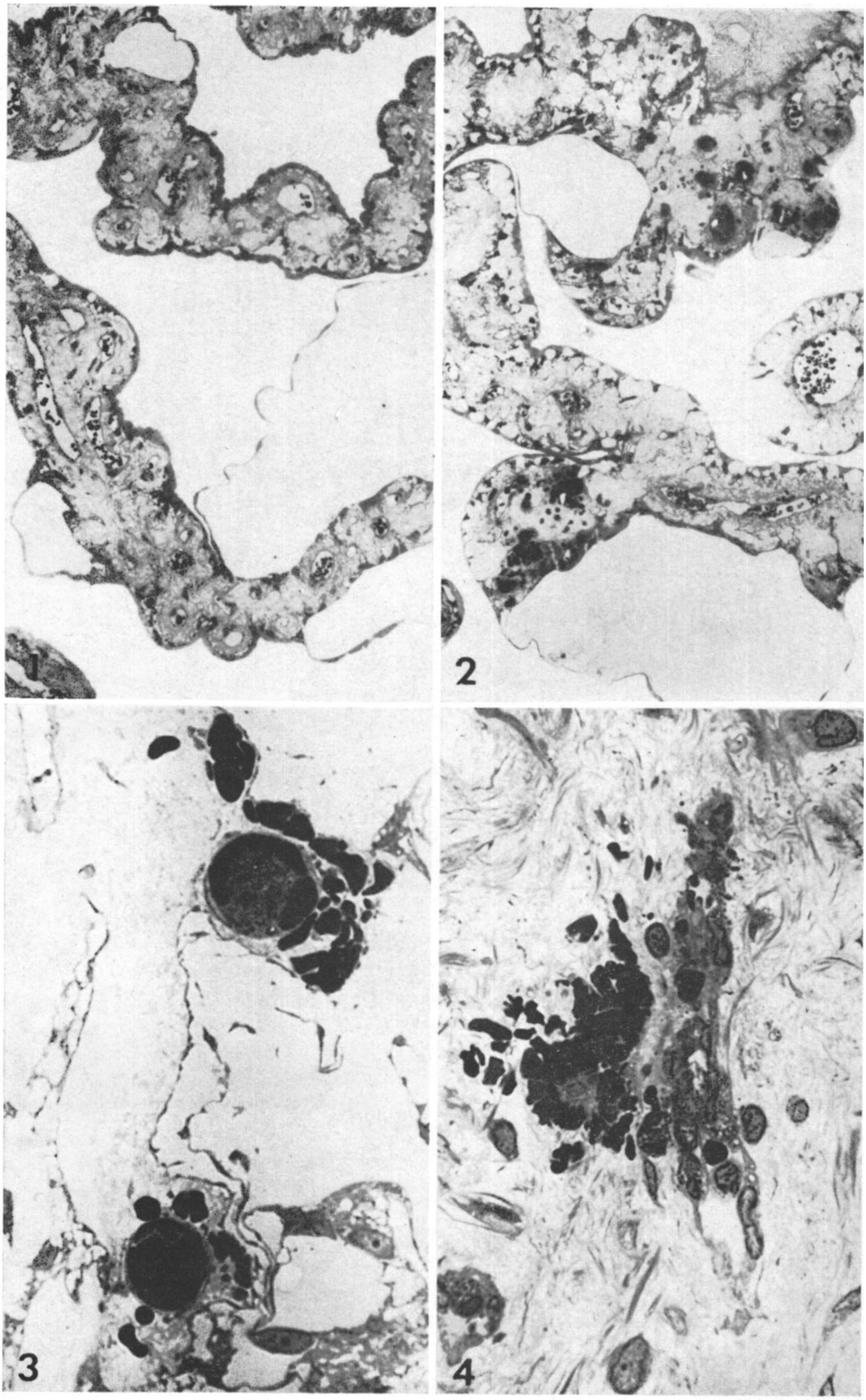
Expt. animals						
Rabbit no.	Eye	No. of processes	Hemorrhage and thrombi	Hemorrhage	Thrombi	% of iridial processes with hemorrhage and/or thrombi
55	Right	39	4	6	3	33
55	Left	42	13	15	1	69
56	Right	40	13	11	0	60
56	Left	40	27	10	0	93
57	Right	39	17	18	0	90
57	Left	38	18	13	0	82
58	Right	39	0	3	2	13
58	Left	37	1	13	0	38
75[b]	Right	36	20	8	1	81
75[b]	Left	35	30	5	0	100
76	Right	38	4	20	0	63
76	Left	38	16	20	0	95

[a] 4–6 days postcervical ganglionectomy, 4 hr after endotoxin: right eye, sympathectomized side; left eye, unoperated side.

[b] Although not strikingly different in percentage of processes involved, the left eye had 15 major hemorrhages involving full width of the process, while the right had only 3 such hemorrhages.

Results. Four to six days following the removal of the right superior cervical ganglion, and 1.5 hr after endotoxin, an ocular albumin content 3–4 times greater than the noninjected operated controls was noted. The magnitude of change was about the same in both eyes (see Table I). In this same operative group 4 hr after the injection of endotoxin, a significant decrease in protein leak had occurred, an average of 15.00 μl on the operated side compared to 24.96 on the unoperated side ($p < .001$). This decrease was not found in animals who were sham operated; it could not be detected in those animals operated on 24 hr before, and it could not be demonstrated in those animals with preganglionic neurectomy 4–6 days before (see Table I). Two and one-half to 3 weeks following ganglionectomy and 4 hr after endotoxin injection, the average ocular albumin space was less than in the eye on the unoperated side, but there was not a significant decrease in averages. Eight of the 9 animals had less of a protein leak on the sympathectomized side, the differences ranging from 1.0 to 8.2 μl with great overlap in the ocular albumin space for the same eye among different animals. One of the 9 animals had an ocular albumin space 3.4 μl greater on the right.

Histologic study of the iridial process showed changes described previously: marked stromal edema, epithelial vacuolization and cyst formation, scattered stromal hemorrhages of variable size, and numerous thrombi composed of platelets and/or fibrin with red blood cells admixed (3). These findings were more pronounced on the unoperated side. To define these differences more exactly, 1–2 μ sections of the iridial processes were examined for the entire circumference of each eye of a group of Series A animals. The number of iridial processes showing hemorrhage and/or thrombi were expressed as a percentage of the total number of processes counted. These results are shown in Table II. Clear-cut differences were evident in all but one of the animals (No. 57). The typical histologic findings are shown in Figs. 1 and 2. In Fig. 1, stromal edema and cyst formation are seen. In Fig. 2, both hemorrhages and thrombi are seen in addition. The vascular obstruction was primarily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by platelet thrombi with some fibrin and varying quantities of red cells (Fig. 3). In one animal (No. 56), there was a more striking component of fibrin in the thrombi. In the animal showing only a slight response to endotoxin (No. 58), thrombi were rare and microscopic hemorrhages the rule. An additional finding was diffuse microscopic iris hemorrhages in 3 animals on the unoperated side (Fig. 4) and only focal hemorrhage in one eye on the right.

Discussion. The most effective form of sympathectomy to suppress the ocular response to endotoxin was superior cervical ganglionectomy done 4–6 days prior to giving endotoxin. It has been shown that norepinephrine content of the iris and ciliary processes of the rabbit is no longer measurable 3 days following such a ganglionectomy (11), and that, 4–10 days after ganglionectomy, adrenergic nerve endings with rare exception are no longer detectable morphologically by fluorescence microscopy (12). Thus, it would appear that the local depletion of adrenergic substances is what is important for the attenuation of the response. It is also concluded that central sympathetic stimulation by endotoxin does not play a role in this reaction for preganglionic neurectomy failed to alter the response.

Twenty-four hours after ganglionectomy, no difference in the permeability alteration between the two eyes was noted. Interesting effects in the rabbit eye have been described 24 hr following ganglionectomy. These are in large part attributed to a local release of adrenergic substances from degenerating sympathetic nerve endings (9, 13). These changes include a relative decrease in intraocular pressure resulting from an increased outflow of aqueous fluid from the anterior chamber of the eye on the operated side (9, 13). Other adrenergic effects 24 hr following ganglionectomy have been described in the rabbit ear and iris (14). Although norepinephrine depletion is marked in the uveal tract at 24 hr, it is not complete (11); and sufficient released adrenergic substances must be present locally to maintain the effect equally in both eyes.

Partial recovery from the effect of ganglionectomy was evident 2–3 weeks following operation. A measurable quantity of norepinephrine has been found in the iris and ciliary processes 2 weeks after ganglionectomy (11). A second explanation of this effect may be related to an increased sensitivity in the eye to exogenous adrenergic substances. It has been estimated that there is an increase in sensitivity to exogenous norepinephrine of approximately 10-fold 7 days after ganglionectomy and a 100-fold increase in sensitivity by 2 weeks (15). This supersensitivity results in an increased outflow facility as a response to minute quantities of norepinephrine, which, by itself, produces some aqueous protein (16). Systemic endotoxin promotes an outpouring of catecholamines from the adrenal gland in the rabbit (17), and thus increased sensitivity, perhaps related to outflow facility, may play a role in the apparent recovery from the earlier ganglionectomy effect.

One and one-half hours after systemic endotoxin in the 4–6 days postganglionectomy rabbits, no difference was observed in the protein leak in the two eyes and yet the extent of this enhanced vascular permeability was nearly that at 4 hr. From morphologic observations (3), it has been suggested that different mechanisms are operative at 1.5 and 4 hr after endotoxin. The initial permeability change has not as yet been studied, but histamine or histamine-like substances may

FIG. 1. Portions of two iridial processes: There is some edema of the stroma and epithelial cyst formation can be seen, ×150.

FIG. 2. Portions of two iridial processes: Edema is more marked. Multiple hemorrhages and vascular occlusions are evident in this more severe reaction; ×140.

FIG. 3. Two vessels are obstructed and there is surrounding hemorrhage: One of the vessels is filled with red blood cells (below) and the other contains a platelet thrombus; ×950.

FIG. 4. A small hemorrhage can be seen in the iris stroma adjacent to a small blood vessel, a finding seen primarily in the eyes of the nonsympathectomized side; ×740.

play a role (3). It is only after a prolonged permeability change that adrenergic substances take part in the reaction. In one study, 3 hr after endotoxin, a suppression of histidine decarboxylase activity was demonstrated in the kidneys of two-thirds of the rabbits suggesting that a decreased local histamine activity might enhance responses to adrenergic substances (18). It is possible that this suppression is actually a depletion of activity and that a similar change may be occurring in the rabbit eye.

The effect of sympathetic denervation has been studied in other types of endotoxin reactions, in the local (5) and generalized Shwartzman reactions (4, 5). In these studies, sympathectomy produced a significant depression of the reactions. On the basis of a microradiographic study with contrast media in the local Shwartzman, Lambert *et al.* (5) have suggested that sympathectomy alters vasomotor tone, stressing the arteriolar component in the reaction. The intense arteriolar over-dilatation in response to endotoxin was modified by sympathectomy (5). Thomas (19) and Zweifach *et al.* (20) have demonstrated a markedly altered vascular response to endotoxin in the presence of locally applied or injected epinephrine or norepinephrine. Depending on whether small or large quantities of endotoxin were used, a venous constriction or a hyporeactivity and over-dilatation of arteries and veins could be produced. Zweifach *et al.* (20) suggested that some tissues may be sufficiently supplied with epinephrine and norepinephrine to be placed in jeopardy by the action of endotoxin. Although the exact changes in the microcirculation of the ciliary processes remain to be elucidated, the rabbit eye, because of its high local concentration of adrenergic substances, seems peculiarly susceptible to the action of endotoxin and this susceptibility plays a role in the prolongation and severity of the reaction.

Summary. Postganglionic sympathectomy decreases the severity of the ocular response to systemic endotoxin. This depressant effect is not present at 1.5 hr, but is present 4 hr after endotoxin. There is a decrease in the altered vascular permeability as measured by ^{125}I-human serum albumin and a decrease in stromal hemorrhages and small vessel thrombi of the iridial portion of the ciliary process in the eye on the sympathectomized side. It is concluded that a high local concentration of adrenergic substances is partly responsible for making the eye unusually sensitive to the effects of circulating endotoxin.

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