Optic nerve transection in cats.

II. Effect on vessels of optic nerve head and lamina cribrosa

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Key words: optic nerve transection, optic disc vessels, feline eye, transection.

In a prior communication we demonstrated that transection of the optic nerve of the cat with consequent retrograde neuronal degeneration did not lead to alteration of the ipsilateral retinal vasculature. We interpreted this to mean that retinal neuronal degeneration does not, of itself, cause an associated vascular degeneration. In the present study the vasculature of the optic disc region of the cat was examined after optic nerve transection.

Materials and methods

Unilateral optic nerve transection was performed on eight domestic cats as previously described. In four of these cats, isolation of the opposite optic nerve without transection was performed as a control procedure. Patency of the retinal circulation was ascertained for all cats by indirect ophthalmoscopy and in three cats by fluorescein angiography pre- and postoperatively. Cats were sacrificed at 24 hours and at 3, 7, 14, 30, 60, and 90 days postoperatively, and the ocular vessels were flushed with 0.2 percent sodium nitrite and filled with India ink by infusion through the left ventricle. The eyes were then removed and fixed in 10 percent neutral buffered formalin. After fixation, the eyes were hemisected at the ora serrata and the posterior segment was placed into a clearing solution (Spalteholz technique), examined with a dissecting microscope, and photographed. The optic nerve and peripapillary tissue were then dissected en bloc and further prepared for routine paraffin sections which were cut serially at 20 µ parallel to the lamina cribrosa. Sections were stained with hematoxylin and eosin (H & E), periodic acid–Schiff (PAS), Luxol fast blue (myelin), and Feigin-Naoumenko (axon) and examined by light microscopy.

Results

In all animals optic nerve transection was accomplished without disturbing the retinal or optic nerve head circulations. No difference in myelin staining, axon staining, or angioarchitecture was definable at 24 hours or 3 days (Fig. 1). By 7 days myelin sheaths were noted to be irregularly broken down and alterations in axon stain were seen on the side of optic nerve transection. There was no vascular alteration at the level of the lamina cribrosa or anterior optic nerve head (Fig. 2). The specimens at 14, 30, 60, and 90 days showed increasing evidence of degenerative
changes of myelin and axons, but no evident change in the microcirculatory pattern on the side of the transected nerve (Fig. 3). Glial proliferation was very noticeable by 60 days. In no instance could India ink be found leaking from the disc or lamina vessels on either the control or transected side (Table I).

Discussion
The present work expands our prior observations concerning the relationship of
Fig. 2. Photomicrograph of nontransected (A) and transected (B) optic nerves 7 days postoperatively. Note that B is slightly more anterior than A and demonstrates well the thicker tissue of the lamina cribrosa. The vascular pattern is normal for each side. (H & E; ×25.)

neuronal atrophy to surrounding microvasculature. In essence, the nutrient blood vessels of the optic nerve head and lamina seem to be unaffected by the loss of adjacent neural tissue. While the capillaries persist both in patency and pathway we cannot, of course, be certain of their physiologic function. Our prior work on retinal vessels showed no fluorescein angiographic differences of flow or permeability between the control and transected sides. Studies have not yet been carried out to determine
whether ultrastructural differences exist between the vessels of the normal and severed side.

This study may have pertinence for increasing our understanding of phenomena such as optic pallor, optic atrophy, and cupping. For example, it has been assumed that optic pallor reflects dropout of the capillaries of the nerve head: "Pallor (atrophy) of the optic disc occurs due to closure of blood vessels no longer needed after death of axons of ganglion cells."
Table I. Summary of results

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Transected</th>
<th>Duration of transection</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-234</td>
<td>OD</td>
<td>OS</td>
<td>24 hr.</td>
<td>The vessels appear patent and filled with ink at both the area of the lamina and anterior optic nerve head</td>
</tr>
<tr>
<td>F-240</td>
<td>OD</td>
<td>OS</td>
<td>24 hr.</td>
<td>Same as F-234</td>
</tr>
<tr>
<td>F-235</td>
<td>OD</td>
<td>OS</td>
<td>3 days</td>
<td>The vessels appear normal on each side and are filled at both the lamina and optic nerve head</td>
</tr>
<tr>
<td>F-236</td>
<td>OS</td>
<td>OD</td>
<td>7 days</td>
<td>The vessels of the lamina and optic nerve head appear patent and normal. A beginning breakdown of myelin and loss of axon staining is observed</td>
</tr>
<tr>
<td>F-239</td>
<td>OD</td>
<td>OS</td>
<td>14 days</td>
<td>The vessels look normal. The filling appears equal. The myelin and axon stains show evidence of optic nerve degeneration</td>
</tr>
<tr>
<td>F-6</td>
<td>OS</td>
<td>OD</td>
<td>30 days</td>
<td>The vessels appear normal and the filling is equal. The myelin and axon stains show evidence of optic nerve degeneration</td>
</tr>
<tr>
<td>F-134</td>
<td>OD</td>
<td>OS</td>
<td>60 days</td>
<td>The vessels appear normal and the filling is equal. The myelin and axon stains show degeneration and there is marked glial proliferation</td>
</tr>
<tr>
<td>F-237</td>
<td>OD</td>
<td>OS</td>
<td>90 days</td>
<td>There is no change in the vascular pattern both at the area of the lamina and at the optic nerve head. There is marked evidence of neuronal degeneration</td>
</tr>
</tbody>
</table>

Axons may be lost either as a result of direct damage or of retinal disease. Is this indeed true? Certainly in a situation of primary vascular embarrassment such as seen in ischemic optic neuropathy one might expect loss of vessels. On the other hand, the situations of neuronal degeneration secondary to trauma to the optic nerve or chiasmal tumors, etc. are followed by pallor of the disc, but this pallor is not due to vascular dropout. More likely it is caused by secondary glial overgrowth. Furthermore, we could not find evidence in a most recent neuropathology textbook that neuronal dropout in the central nervous system is followed by capillary loss, but the converse is said to be true.

Glaucomatous cupping has remained somewhat of a mystery concerning its pathogenesis. Authors have postulated primary neuronal damage, primary glial damage, and a few have suggested primary vascular damage. If the damage were primarily to the neurons, and since the neurons of the central nervous system are the most sensitive elements of this system, then one might expect replacement of neurons by glial elements, and no loss of vessels. This is not the case, for glia does not fill in the cup, and the vessels of the optic nerve head are indeed absent.

As regards the possibility that glial alteration in glaucoma, this would be contrary to observations in the central nervous system: "Astrocytes are less vulnerable than nerve cells and fibers, but they are more easily damaged than connective tissue. They react to all noxae which damage neurons." This would seem to leave as an important possibility that vascular damage may be the primary event leading to glaucomatous cupping. This hypothesis needs more evidence before it can be considered to be proven. However, should it prove to be correct, then one major consideration concerning the problem of visual field loss would depend intimately on assuring the healthiness of the vascular bed of the optic nerve and retina.

REFERENCES
Permeability and patency of retinal blood vessels in experimental diabetes

Ingolf H. L. Wallow and R. L. Engerman

Increased permeability of retinal blood vessels in human diabetic retinopathy is well known clinically. Its morphologic equivalent is unknown. In dogs with 5 years of poorly controlled alloxan diabetes and nonproliferative diabetic retinopathy comparable to that of man, permeability and patency of retinal blood vessels were tested with the protein tracer horseradish peroxidase and evaluated by electron microscopy. A breakdown of the blood-retinal barrier was found associated with extensive tracer leakage around retinal blood vessels. Tracer had seemingly permeated endothelial junctions, and was not transported through the endothelial cytoplasm. Blood vessels which had lost their endothelial cells and were partially occluded by glial cells retained some patency to tracer. These findings suggest the following. (1) Endothelial tight junctions are not a static cell specialization but one that can open due to chronic metabolic or osmotic factors prevailing in diabetes. Opened tight junctions may account for plasma leakage seen clinically in human diabetic retinopathy. (2) In the absence of endothelial cells perfusion does not necessarily end abruptly. The tracer method and electron microscopy may show details of vascular obstruction that are not readily demonstrated clinically.

Key words: retinal blood vessels, experimental alloxan diabetes, intercellular junctions, permeability, patency, horseradish peroxidase.

Clinical evidence indicates that alterations of vascular permeability are among the very early changes of human diabetic microangiopathy. Even the normally tight blood-retinal barrier appears to be affected early: extravascular leakage of fluorescein is well known clinically, has been observed reportedly without recognizable anatomic changes, and may even precede any clinically visible lesions such as capillary closure and microaneurysms. The morphologic basis of these permeability changes...