AN ELECTRON MICROSCOPIC STUDY OF NORMAL OPTIC NERVE AND OF AN OPTIC-NERVE GLIOMA*

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The optic-nerve glioma, although a rare tumor, has continued to be of interest because of the enigma concerning both its cell of origin and its relationship with generalized neurofibromatosis. The first report of an optic-nerve glioma was that of von Graefe. In 1912 Hudson analyzed 182 intra-orbital neural tumors which he considered as a manifestation of abnormal glial hyperplasia rather than of neoplasia. The astrocytic glia were implicated in the formation of gliomas of the optic nerve by Verhoeff, who reported a series of 11 intraneural optic tumors studied by the Mallory neuroglial stain (phosphotungstic acid hematoxylin). Davis also agreed that a majority of the cells were astroglial and, although he considered it a neoplasm, tended to agree with both Hudson and Verhoeff that the early optic atrophy suggested a degenerative process as the primary lesion followed by a secondary neoplastic gliosis. Dissenting views concerning these tumors are those of Oberling and Nordmann who considered the optic-nerve tumor as derived from cells of the leptomeninges, and Lundberg who considered them as oligodendrogliomas.

The cytology of the normal optic nerve has been studied in detail by Cone and MacMillan. This nerve, actually a tract of the central nervous system, is unlike the brain in that it is separated into fascicles of myelinated axons by the inward extensions of the pia mater which form the vascularized connective-tissue septa. Myelinated axons within the fascicles of the optic nerve are surrounded by glial cells, whereas Schwann cells, characteristic of peripheral nerves, are absent.

By means of electron microscopy cytological structures now may be studied in greater detail than has been possible previously. Furthermore, the various types of cells, both neural and glial, may be distinguished in a single preparation rather than by use of various fixatives and staining procedures. It is the purpose of this paper to describe the fine structure of an optic-nerve glioma and to compare its glial components with the glia of normal optic nerve.

MATERIALS AND METHODS

Normal Tissue. Optic nerves from normal adult rabbits and mice, and an adult rhesus monkey were removed immediately after decapitation. Small pieces, less than 1 mm. in greatest diameter, were fixed for 1 hour in 1 per cent osmium tetroxide in potassium dichromate at pH 7.4 as recommended by Dalton. They were dehydrated rapidly in 10 per cent increments of ethanol (10 per cent to 100 per cent). After completion of dehydration, the tissue was transferred to a mixture of absolute ethanol and toluene for a few minutes and then to toluene for 20 minutes. It was transferred from the toluene to a half-and-half mixture of toluene and methacrylate. Infiltration in methacrylate was for 90 minutes in 3 changes of the plastic. A catalyst, 0.2 per cent benzoyl peroxide, was added to the last change of the 8:1 butyl-methyl methacrylate. The tissue was embedded in partially prepolymerized methacrylate plus catalyst and polymerization was completed at 60°C overnight.

Optic-Nerve Glioma. A biopsy of a typical optic-nerve glioma was obtained at the time of intracranial exploration by Dr. Henry Schwartz. Immediately following excision of the biopsy specimen it was placed in Dalton's fixative and treated

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in the same way as that described for normal optic nerves.

The patient from whom the specimen was obtained was a boy 7 years of age with a 2-year history of gradually diminishing vision in the left eye. Vision in the left eye was limited to perception of motion. Vision in the right eye was 20/40. Both pupils reacted to light. Numerous café au lait spots were present on the skin, and on one arm there was a soft subcutaneous nodule. Roentgenograms of the skull disclosed widening of the optic foramen on the left and a lesser enlargement on the right. Pneumoencephalograms showed a mass of soft tissue, 2.5 cm. in size, that filled the chiasmatic cistern and bulged posteriorly into the interpeduncular cistern, indenting the anterior portion of the third ventricle and obliterating the optic recess. Both lateral ventricles were enlarged. At surgical exploration, the enlarged left optic nerve was biopsied.

OBSERVATIONS

Normal Optic Nerve. The optic nerve (Fig. 1) is surrounded by leptomeninges, of which the innermost, the pia mater, extends into the nerve to outline the connective-tissue septa separating the axons into fascicles. The septa contain blood vessels, fibroblasts, and collagen. The surface of the optic nerve adjacent to the connective-tissue septa is bounded by the pia mater which by electron microscopy proves to be principally a basement membrane (Fig. 2). Astrocytic fibrils insert into the inner surface of this pial basement membrane, thus forming the pia-glial limiting membrane. Collagen with its repetitive 640 Å pattern is evident external to the basement membrane. The axons of the optic nerve vary in size but are all myelinated. Surrounding the myelinated axons are processes of the interspersed glial cells (Fig. 5). Astrocytes and oligodendrocytes are present as well as the scattered small dense microglial cells. The interstitial space in the optic-nerve fascicles is scant, just as in brain. The septa of the normal nerve, however, have an abundant extracellular space separating the nerve into fascicles (Figs. 3 and 6).

Optic-Nerve Glioma. Light microscopy. Paraffin sections revealed the typical pattern of an optic-nerve glioma (Fig. 4) in which connective-tissue septa partially divided the tumor into giant fascicles reminiscent in configuration of a greatly expanded normal optic nerve. The dense fibrous connective-tissue partitions contained the neoplastic blood vessels. The tumor was composed, for the most part, of sparse fibrous astrocytes with ovoid nuclei and indistinct cytoplasmic outlines. Mitotic figures were not present.

Electron microscopy. Connective-tissue septa deeply indented the tumor and partially separated it into funiculi. A rather delicate homogeneous amorphous basement membrane, a part of the pia mater, separated the tumor from connective tissue. Firmly attached to this basement membrane were the expanded fibrous glial "feet", the two together forming the pia-glial membrane (Fig. 11). Within the basement membrane-lined clefts there were variable numbers of collagen fibers, fibroblasts, inflammatory cells, and blood vessels (Fig. 9). Deep within the fascicles of the tumor there were compressed folds of basement membrane extending out from the perivascular septa, but appearing to be isolated from any connection with the septa (Figs. 7 and 8).

A majority of the cellular elements of this tumor were fibrous astrocytes of the type often referred to as piloid since most of their cytoplasmic processes were crowded with dense fine fibrils (Figs. 11 and 12). Nuclei of these cells were often elongated with many of the cells oriented in a similar plane. Some of the nuclei were irregular, but no bizarre or giant forms were evident. Occasionally a single nucleolus was present in the plane of section. Extending from the two poles of the nucleus there often were a small amount of cytoplasm with a fine dense stippling, occasional vesicles, and rare small mitochondria. The astrocytic cytoplasm, except for that immediately around the nucleus, was subdivided into intertwined processes packed with fibrils.

In many areas all available space was occupied by the closely interlaced cytoplasmic expansions of distant cells. Most of these processes were extensions of fibrous astrocytes (Fig. 11) but interspersed amongst them were rare myelinated axons (Fig. 14), and occasional pale processes without fibrils.