Ultrastructural aspects of the human optic nerve

Adolph I. Cohen

The ultrastructure of the human optic nerve was studied by electron microscopy. All neuro-ectodermal components are separated from the mesodermal components by a pial-glial interface. No nonmyelinated axons were seen, save for isolated single axons which probably represent myelinated axons cut within nodes of Ranvier. Myelination appears consistent with a hypothesis of spiral wrapping. Redundant myelin sheathing of one axon may superficially laminate onto another. The principal glial types are fibrous with a ribosome poor cytoplasm, and a ribosome rich variety with few cytoplasmic fibrils. The latter seems to best meet oligodendroglial characters; and the former, those of fibrous astrocytes. However, intermediate types are also seen. Nonmyelinated fibers are seen ending in the connective tissue around the central retinal artery, and their synaptic vesicles often possess dense granules.

Studies on the optic nerve present unique opportunities for students of the nervous system and for the ophthalmologist. As a relatively accessible tract of the central nervous system, it conveys the largest single class of sensory information to brain centers remote from the retina. As a stalk-like extension of the brain complete with dural, arachnoid, and pial investments, and with an associated vasculature of significance (both for the supply of the tract itself and for the retina), it provides (at the papilla) a visible index of the balanced forces of intraocular and vascular pressures, of possible edema-tous and inflammatory states of central nervous tissue, and of the survival of the ganglion cells of the retina, since the axons of these cells form the bulk of the nerve.

In addition to describing the disposition and appearance of cellular and extracellular elements, electron microscopic studies of the human optic nerve can uniquely contribute to answering questions regarding the presence of nonmyelinated fibers and the mechanism of axonal myelination, and can more precisely characterize the glial elements in order to permit the identification of the various glial types. Electron microscopy can also be useful in characterizing the mesodermal compartment, whose tortuous branching tunnels in the nerve serve as conduits for blood vessels, or contain connective tissue elements, such as collagenous bundles, which may subserve strengthening functions.

In general studies on the ultrastructure of optic nerves, the ideal means of preservation would be by vascular perfusion of the fixative fluid; but this method is...
obviously inapplicable to the human nerve, which must be fixed by immersion. In previous studies, the poor penetration powers of osmium fixatives combined with the need to fix large enough portions in order to preserve relationships have given poor results. Instead, Peters7 and Yamamoto13 have resorted to the use of potassium permanganate as an immersion fixative for mammalian optic nerves. However, this fixative, while preserving cell membranes, is sufficiently deleterious to the preservation of cell organelles as to severely handicap one's ability to distinguish glial processes from nonmyelinated neurites, varieties of glia, etc. Indeed, based on this fixative, Yamamoto11 has claimed that the human optic nerve possesses bundles of nonmyelinated axons, and that some axons branch prior to their myelination. All these questions were treated in this investigation, in which methods of fixation yielding better cytological preservation were employed.

Methods

Human eyes were obtained following enucleation procedures for reasons not involving the eye. With one exception (that of a child) these eyes were from elderly humans; but, in the final studies made and reported here, no nerves were used if routine fundal observations by trained ophthalmologists gave evidence of retinal disease, or if subsequent observations of the retina revealed the presence of disease which would affect the nerve. On the other hand, all the globes had been exposed to some degree of x-irradiation directed at the maxilla or orbit. After obtaining the eye by surgery, it was totally immersed in ice-cold 2.5 per cent glutaraldehyde in Earle’s saline containing 450 mg. of glucose and 1 to 2 ml. of M/15 phosphate buffer (pH 7.4) per 100 ml. This is a moderately hypertonic fixative (555 milliosmols). About 5 minutes later, on arrival at the laboratory, the nerve stump was cut off close to the globe, and placed in a shallow, ice-surrounded dish containing the same fixative. In this condition, the dura was removed under microscopic observation by means of judicious teasing with fine-tipped scissors. The naked nerve was now allowed to remain in the cold fixative for 5 minutes, during which time its surface hardened and became somewhat yellow in color. After this time, it was gently grasped with blunt forceps and sliced into thin discs with a sharp razor blade. The discs were individually stored in the same cold fixative for one hour, and records were kept as to the order of their proximity to the globe. Following the one hour fixation of the discs in tubes surrounded by ice, a second hour of fixation was permitted at room temperature. The glutaraldehyde fixative after this period was replaced, without washing, by a 2 per cent osmium tetroxide fixative prepared in the same buffered saline. This second fixative turned black during the first hour or two, and was replaced with fresh fixative. The second fixation was allowed to proceed overnight; but, in any event, for not less than twelve hours. The discs were then dehydrated with alcohol and embedded in epoxy resin (Araldite or Durcupan) in a flat bottomed polyethylene container. Using a glass knife, sections of about 1 to 2 microns thick were obtained from each disc, and stained with Azure II and methylene blue dyes in a borax solution.9 The stained sections were studied by light microscopy, and a simple sketch was made of the nerve outline and the disposition of main trabeculae and blood vessels. The plastic-embedded disc was then cut into nine sectors of similar area, which were cemented with epoxy glue to wood pegs for microtomy. Again, 1 to 2 micron sections were obtained for light microscope reference, and thin sections were obtained for electron microscopy. For viewing, these sections were mounted on copper discs containing a single 1 by 2 mm. hole, which was covered with a formvar film, and stained with uranium and lead salts. As the entirety of a section lay within the hole, no area of a
cross section of the optic nerve escaped viewing by the electron microscope. To obtain sections in another plane, the blocks were cut free from the pegs, turned appropriately, and reaffixed.

Results

In the optic nerves studied, there was perfect isolation of the neuroectodermal and mesodermal compartments by a pial-glial barrier. The mesodermal compartment, consisting of blood vessels and connective tissue cells and elements, infilrates (Fig. 1) the neuroectodermal mass of axons and glial elements within a tortuous arrangement of septae and tunnels which often branch. The tunnel walls consist of glial cells covered by a basement membrane (Figs. 5, 15, 16). This pial membrane is topologically continuous with the similar pia mater of the nerve surface, and also with the basement membrane on the retinal face. The latter union is achieved via the surface of the glia-lined tunnel containing the major blood vessels. Except for a long central space containing the central retinal artery and one or more veins (Fig. 3), the tunnels generally contain a single blood vessel (Fig. 1). In the cribriform plate area, collagenous bundles occupy tunnels of a similar nature; however, a detailed description of the nerve head will be deferred for another report.

Nonmyelinated nerve fibers may be seen in the connective tissue space (Fig. 4) near the central retinal artery. However, in the five specimens examined, they have never been seen in front of the cribriform area. Considering the known variability of blood vessels throughout the body, one may expect some variation in where these nerves terminate in the papilla, but it is highly improbable that they ever extend into the retina, since no nerves accompanying blood vessels have been seen in numerous human retinas which have been studied by electron microscopy in the course of other investigations. Laites has reported the absence of adrenergic innervation of retinal blood vessels based on fluorescence techniques. The endings of the nonmyelinated nervous elements often contain vesicles with dense centers, a feature often associated by electron microscopists with a content of catechol-amine derivatives.9

When the tubular cytoplasmic element was used as a necessary component of any axons (Figs. 7-12, 16), no nonmyelinated axons were seen in the neuroectodermal compartment of the myelinated region of the optic nerve. Since this technique avoided the use of grid bars, the entire cross section of the nerve was examined. Seen in cross section, the nerve appears to consist of isolated or semi-isolated fascicles containing myelinated axons (Fig. 1). However, none of these fascicles is truly isolated, since they are united with one another at different levels. The incomplete fasciculation is due to the incomplete mesodermal septation and tunneling. As seen in cross section, the superficial, sub-pial portion of the nerve often exhibits extensive fillets of neuroectodermal tissue where few or no myelinated axons are evident (Figs. 1, 2, 5). Careful examination of deeper lying fascicles also reveals a tendency for the neuroectodermal tissue at the fascicle surface to be relatively free of myelinated axons (Fig. 3). In the sub-pial area, the extensiveness of this fillet has suggested to some that this is a site of nonmyelinated axons. With glutaraldehyde fixation, however, all axons, myelinated (as in the optic nerve) or unmyelinated (as in the retina or in front of the cribriform area) contain tubular elements, although fibrous materials may also be present. Such tubular elements have never been seen in the cytoplasm of the cells occupying the subpial fillets, or on the surface of fascicles. Instead, the cells in these regions (Figs. 5, 6) possess a cytoplasm which is dominated by irregularly distributed masses of fibrous elements. This suggests that these cells are fibrous astrocytes. These presumptive astrocytes are the dominant glial species in the nerve, to judge by the high percentage of the cross-sec-
Fig. 1. A light micrograph from an Azure-stained plastic section of optic nerve. This illustrates the surface of a nerve from which the dura had been removed, and shows several fascicles of myelinated axons and glia, as well as dividing septae of connective tissue. Note that the pale subsurface fillets are continuous with the fascicles. ($\times 270.$)

Fig. 2. A light micrograph view of a pale subsurface fillet at higher magnification. ($\times 700.$)
Fig. 3. A light micrograph of a central tunnel in the optic nerve showing a major artery and vein as well as minor vessels. Note the pale character of the surface of the adjacent nerve fascicles (arrows). (×280.)

Fig. 4. An electron micrograph showing unmyelinated nerve fibers in the connective tissue near the central artery. A completely naked axon (n) contains synaptic vesicles, some of which (arrow) contain dense cores. A Schwann cell (s) with other axons is also seen nearby as is collagen (c). (×55,000.)
Fig. 7. An electron micrograph of a small myelinated axon (a) which shows an internal mesaxon (inside arrow) and an external mesaxon (outside arrow). (×91,000.)

Fig. 8. An electron micrograph of a myelinated axon (a) cut close to a node of Ranvier. Here the compacted myelin-forming membranes have expanded to reveal a dense cytoplasm. (×45,000.)
Fig. 9. An electron micrograph of a myelinated axon (a) cut through the node of Ranvier. (×62,500.)

Fig. 10. An electron micrograph of a longitudinal section of a myelinated axon (a) entering a node of Ranvier which illustrates the total cessation of sheathing at the node (arrow). Note the neural tubules coursing longitudinally in the axon cytoplasm. (×35,000.)
Fig. 11. An electron micrograph which shows extra myelin surrounding the sheathing of one axon (a) and superficially laminating onto the myelin system of another (a'). Also note cytoplasm enclosed within the expanding myelin and containing tubular elements (arrow). (~21,700.)

Fig. 12. An electron micrograph showing the close relationship of a dense, ribosome rich glial cell to myelinated axons. (~30,000.)
tional area that is occupied by fibrous non-neural processes.

Among the myelinated axons, one occasionally finds a solitary nonmyelinated axon (Fig. 9). However, as longitudinal sections of myelinated fibers demonstrate that, at nodes of Ranvier, myelinated axons become quite devoid of any investment by the myelinating glial cells (Fig. 10), it seems highly probable (in view of their low frequency and isolated occurrence) that these single nonmyelinated axons represent sections through nodes of Ranvier between regions of myelination.

According to Polyak, the primate retina is free of oligodendroglia. This is also true in the cat. Since oligodendroglia are said to be responsible for myelination in the central nervous system, comparison of the precribriform area with the postcribriform area (where the myelination of the retinal ganglion cells begins), might well reveal a uniquely postcribriform glial species, and thus provide a critical test for identification of glial cells in the central nervous system. There has been some disagreement among electron microscopists as to the correspondence of glial types seen in the electron microscope with those seen in silver preparations.

In carrying out the above comparison, it is first evident that, in the precribriform area, the axons are without organized glial separation (Fig. 16) and often are contiguous with one another, sometimes for 200 μ at least. The principal glial cytoplasm seen, as in the postcribriform area, is rich in fibrous elements. However, in the zone just behind where myelination is beginning, one often notes lines of cell somas (Fig. 13). This is said to be a characteristic of oligodendroglia. These cells possess a cytoplasm that is relatively devoid of fibrous material, but rich in ribosomes and mitochondria. Well into the optic nerve, among numerous low density fibrous processes, one encounters isolated cells with fewer processes and a denser cytoplasm rich in ribosomes (Figs. 6, 12), and one often notes that these cells have very large centrioles (Figs. 14, 17). These glial cells also occasionally exhibit small myelin figures (Fig. 18), but these might result from the x-irradiation of these cells.

Direct opportunities to trace cell membranes from regions where cell somas permit characterization of the cytoplasm into the myelin sheathing have not materialized. However, cytoplasm associated with the sheathing is more evident close to nodes of Ranvier, and here it appears to be non-fibrous and ribosome rich (Fig. 8).

While the above findings at first suggested that, with the fixation employed, the probable oligodendroglial cell possessed a cytoplasm that was dense and rich in ribosomes, and was in contradistinction to a fibrous astrocyte as the other principal glial type of the human optic nerve, other findings suggested that cells with a spectrum of cytoplasmic characters extend between these extremes. With more searching, cells with somas that fit the above typical description for oligodendroglial cells were seen to possess bundles of fibrillar material and cytoplasm of intermediate density (Fig. 14). It is possible that irradiation might have modified the appearance of some glial cells.

Another problem besetting students of the nervous system has been the means of myelination of central nervous axons, particularly whether this followed the spiral wrapping evident for peripheral myelinated axons. Cross sections of small myelinated axons obtained in this investigation fit the concept of myelination by means of the spiral wrapping of axons by glial membranes, inasmuch as internal and external mesaxons were observed (Fig. 7). Thus, myelination in the optic nerve would resemble that in peripheral nerves. On the other hand, it was difficult to analyze the mode of myelination of the larger axons because the units of wrapping could not be resolved around the entire circumference. Tracing sheathing depends very much on the orientation of the section plane and the sheathing plane. When this relation deviates from the perpendicular, the mem-
Fig. 13. An electron micrograph which illustrates the tendency for rows of glial cells to occur in the area where myelin begins in the cribriform region. These usually have dense cytoplasm. (×7,650.)

Fig. 14. An electron micrograph of the cytoplasm of a glial cell with intermediate characteristics. The cytoplasm is of moderate density and contains fibrils (f). Ribosomes (r) and an oblique section of a centriole (c) are also evident. (×31,400.)
branes become too hazy to trace with precision. Not rarely, sheathing myelin layers were seen to surround the myelin wrappings of one axon and superficially laminate onto the myelin system of another (Fig. 11). When such gross asymmetries occur, it is difficult to explain what has happened, since cytoplasm with tubular elements resembling axon cytoplasm may be involved in the myelin sheeting (Fig. 11). But these presumptive abnormalities, which probably have no functional significance, can complicate attempts to analyze the myelination process if the source of origin of the extra sheathing is not evident in a section. Similar and numerous examples of the same myelin phenomenon have been observed in the cerebellum of the toad.12

Discussion

These findings show that all surfaces of the neuro-ectodermal compartment of the optic nerve, both externally and internally (on the faces of septae or tunnels), are characterized by a basement membrane overlying fibrous glial cells. In a subsequent report on the papilla, it will be shown that this arrangement persists in the structure of the nerve head. Where, in embryo, the hyaloid artery emerged from the embryonic papilla, the basement membrane on the retinal face is continuous with the basement membrane of the tunnels through which course the central artery and veins. Thus, there is a potential passage from the vitreal chamber to the subdural space of the nerve, through the connective tissue-filled space between the vasculature and the pial-glial surface of the walls of the optic nerve tunnels and septae. The collagenous bundles of the dural sheath of the nerve continue as the scleral and corneal stroma, encompass the globe, but also pass through pial-glial tunnels across the nerve as the cribiform plate.

Nowhere in the optic nerve have I encountered any surfaces facing the mesodermal compartment that did not consist of fibrous glia covered by a basement membrane, although the glial covering of neurons in the prescribriform region can be quite thin (Fig. 16). The same situation applies to the retina and nerve head, and suggests that there may be some functional significance to the absence of an immediate apposition of neurites and the mesodermal space.

Yamamoto,13 in reporting that the human optic nerve contained nonmyelinated fibers, was aware of the problem of confusing these with axons cut in nodes of Ranvier and with glial processes. He based his positive conclusion on the persistence of circular cross sections in serial sections. However, even 100 serial sections of 600 A (Yamamoto does not indicate the number of consecutive sections) cover but 6 μ of optic nerve. Glial processes may easily extend for such lengths, and are often oriented in the length of the nerve. The use of glutaraldehyde (with its preservation of neural tubules) provides far more reliable identification of axons, and this weighs against the presence of nonmyelinated fibers in the orbital region of the human optic nerve. Sparse tubular elements occasionally have been noted by others in many cell types (including certain glial cells in particular locations), and also are often seen in dendrites. The point here is that they are an invariant feature of axons of retinal ganglion cells when fixed with glutaraldehyde-osmium. Any nonmyelinated optic nerve axon should reasonably be expected to possess cellular elements characteristic of these axons, even if the tubules are not unique features of axonal cytoplasm. The absence of nonmyelinated fibers in the primate supports the findings of Bruesch and Arey2 who used light microscopy in a vertebrate survey.

It is more difficult to decide on the existence of branching of the axons. Yamamoto reports that in the human optic nerve, as in other myelinated regions of the central nervous system, such branches occur at nodes of Ranvier. In his report,13 Fig. 8
Figs. 15-18. For legends see opposite page.
shows an axon protuberance which might well be a beginning branch at a node. His interpretation of Figs. 10 and 11, from serial sections, is less convincing. His Fig. 10 shows an axon completely surrounded by myelin, and the outermost myelin sheaths also envelop a second axon. In his Fig. 11, the same two axons are now separately myelinated. The hypothesis here is that the myelinating mechanism, after a branch, now encompasses two axons. If myelination by spiral wrapping of oligodendroglial membrane begins next to an axon, it is not readily evident how two axons could be separately wrapped by the same glial cell. His Fig. 10 arrangement is highly similar to that in Fig. 11 of this report, which I interpret as an abnormal situation. I am not aware of any evidence in the classical literature to indicate anything other than separate myelin sheathing of axonal branches and main axons, but Yamamoto’s interpretation is not inconceivable—if one glial cell can wrap two axons. Thus, while I have as yet not encountered evidence of branching of axons in my material, such negative evidence does not permit one to rule out this possibility. Fig. 7 of this report, Yamamoto’s report,’ and the work of Peters on the developing optic nerve of the rat leave little doubt that spirally wrapped myelin sheaths are encountered in the mammalian optic nerve.

The problem of correlating glial characteristics in the electron microscope with those seen in conventional preparations is a difficult one. The weight of the evidence in this report seems to indicate that the bulk of the glial processes in the nerve are fibrous, and derived from fibrous astrocytes. Since it is possible that x-irradiation may have influenced the glial fibrosity, it is worth noting that similarly prepared optic nerves of normal rhesus monkeys and mice present an identical appearance with respect to the dominant glial species being fibrous.” In addition, as one nerve examined was that of a child, and as this was identical with respect to the reported glial characteristics, age would not seem to be a factor. However, fibrous materials have also been seen in oligodendroglia. Fibrous, but ribosome poor glial processes are found both in front of and behind the area of myelination. At, and after the zone where myelination begins, one finds the less numerous ribosome rich cells and processes. These occur near the axons, and sometimes in strings of cells. Thus, the weight of evidence compels one to call these oligodendroglia. Yet, the occurrence of some of these presumptive oligodendroglia, with processes containing considerable fibrous material, suggests that the distinction between glial cell types may be more a discernment of extremes of a spectrum, than a hard and fast distinction encompassing separate and irreversible lines of differentiation. Again, the uncertainties here are in the possible effects on the glial characters of the irradiation given to these eyes. However, preliminary examination of optic nerves of normal ma-
caques suggest that these are minimal, since they are highly similar in appearance.

An electron microscopic view of non-myelinated nerve fibers in the connective tissue about the human central retinal artery has been previously reported upon, and is only briefly illustrated in this report—in contradistinction to the axons of the optic nerve proper, and to illustrate the dense granules seen in some synaptic vesicles.

No evidence bearing on the existence of centrifugal fibers in the optic nerve proper was sought or obtained. If such exist, they must be myelinated.

REFERENCES

Erratum
In the April issue of the Journal the legend for the cover illustration should read "Vesicle-bounded vacuole in nonpigmented epithelium of rabbit ciliary body" instead of "Anterior region, air embolism (×47,500.)."