THE FINE STRUCTURE OF THE ASTROGLIA IN THE HUMAN OPTIC NERVE AND OPTIC NERVE HEAD*

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TO PROVIDE A BASIS FOR FUTURE STUDIES of naturally occurring and experimentally produced lesions of the optic nerve, we have been using the electron microscope to examine in detail the normal optic nerves of humans and cynomolgus monkeys. Various aspects of the findings will be reported separately. This report, dealing with the several kinds of astrocytes, is based on the examination of three human eyes. The findings are essentially the same as in our preliminary examinations of monkey optic nerves.

MATERIALS AND METHODS

The specimens were obtained from three eyes that were surgically removed because of malignant melanoma of the anterior choroid. The patients' ages were 51, 54, and 58 years. Immediately after enucleation, the eyes were immersed in a 3 to 5 per cent glutaraldehyde fixative buffered to pH 7.4 with cacodylate, phosphate, or *s*-collidine. The eyes were opened without delay. The anterior portion containing the melanoma was separated from the posterior portion with the attached optic nerve. To provide for rapid access of the fixative to the tissues, the vitreous was teased away from the retinal surface and a spatula was used to separate the choroid from the sclera except in the region adjacent to the nerve head. For the same purpose, one or two longitudinal slits were usually made in the dural sheath covering the orbital portion of the nerve.

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After 2 to 24 hours in fixative, the specimens were dissected further with a razor blade. Several thin blocks were made from each of the areas to be examined—various regions of the optic disk, the lamina cribrosa, the myelinated portion of the optic nerve, the central retinal vessels at several levels, the tissues bordering the scleral canal, and the vaginal sheaths. The blocks were rinsed in buffer and placed in buffered 1 per cent osmium fixative for two hours. They were then dehydrated in a graded series of alcohol, cleared in propylene oxide, infiltrated in a mixture of propylene oxide and Epon or Araldite, and flat-embedded in Epon or Araldite. Thick sections were mounted on a glass slide and stained with methylene blue-azure II.[1] Thin sections were cut with a Porter-Blum MT-2 or an LKB Ultratome and picked up on prepunched copper grids, some of which had been coated with Formvar. Sections were stained with aqueous or alcohol solutions of uranyl acetate for 1 to 24 hours. Most of the sections were also stained with lead citrate[2] for 1 hour.

FINDINGS

ORBITAL PORTION OF THE OPTIC NERVE

The fibrous astrocyte in this region is characterized by the many feet that arise from its cell body. Several of the processes may be included fortuitously in a plane of section, serving to identify the cell type (Figure 1). The nucleus is usually oval but can be somewhat irregular in shape. It has a pale appearance, owing to the sparseness of chromatin clumping.

The cytoplasm surrounding the nucleus (perikaryon) is paler and more empty-looking than oligodendroglial cytoplasm (Figures 2 and 3). With present techniques, fixation of the astrocytes is often poor and this exaggerates their pallor. Small channels of endoplasmic reticulum are scattered randomly throughout the perikaryon. Depending apparently on the fixative used, they may appear as flattened channels or they may be dilated and appear as empty vacuoles (Figures 2 and 3). Some of the channels of endoplasmic reticulum are of the rough variety with attached ribosomes. Free ribosomes are distributed throughout the cytoplasm but usually they are not conspicuous. In the perikaryon the mitochondria are oval or somewhat irregular but within the processes they may be in the shape of long cylinders. Here and there are vacuoles that contain smaller vesicles (Figure 4); in other cell types, these are variously called "compound vacuoles" or "multivesicular bodies." Dense granules (250–350 mμ) and tiny vesicles also occupy

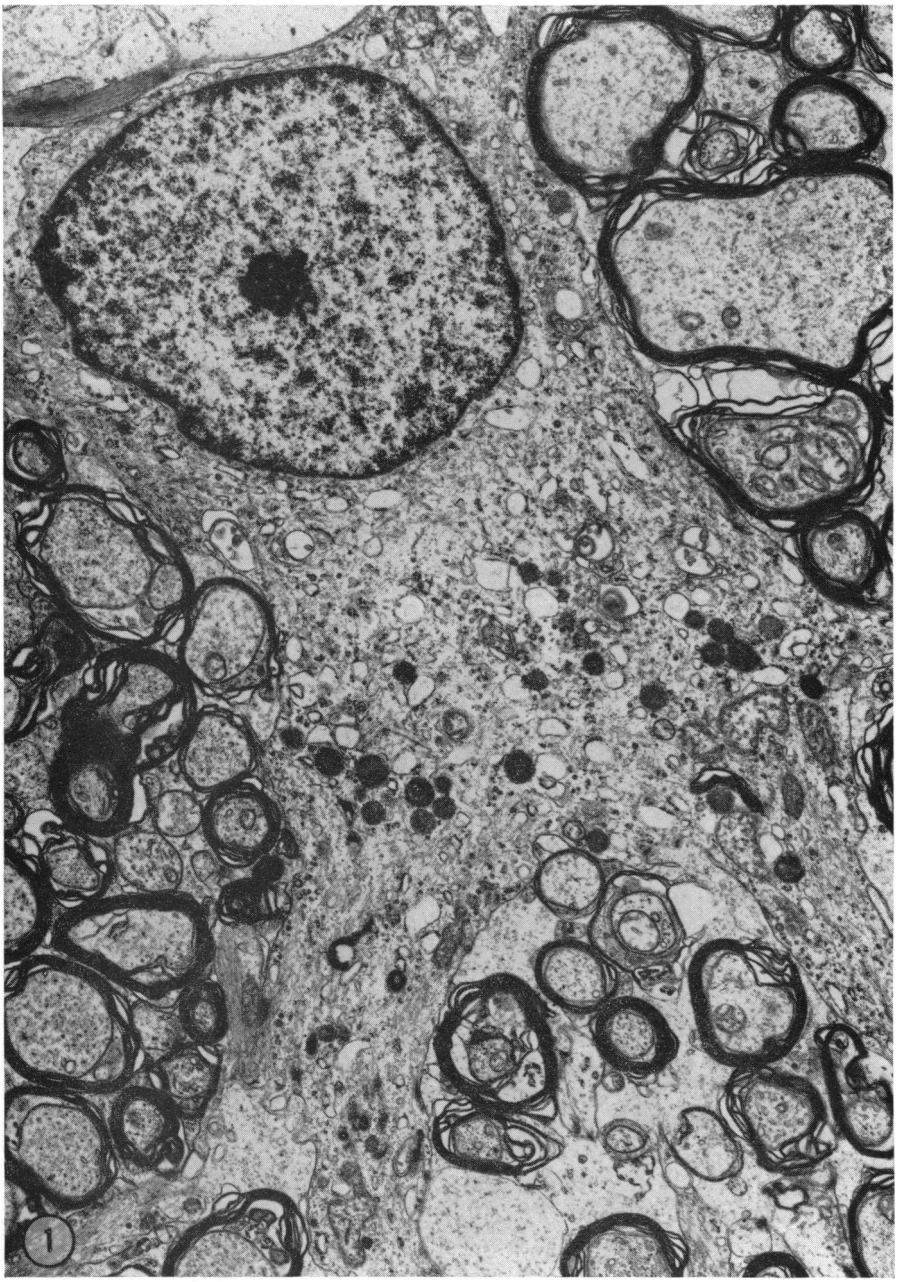


FIGURE 1
Astrocyte in the orbital portion of optic nerve. (× 12,000)

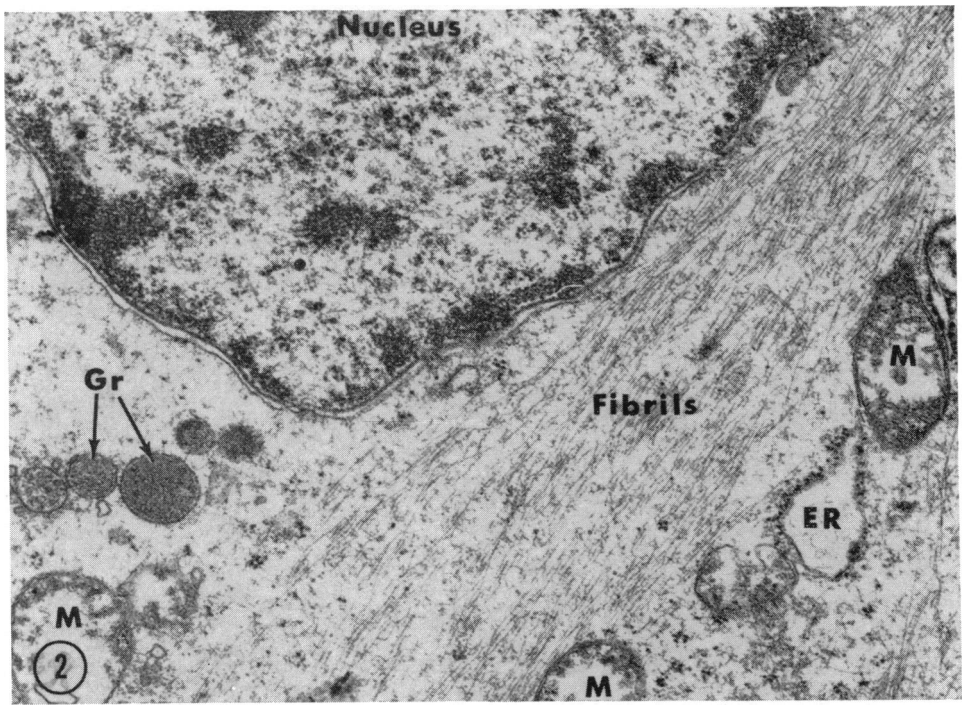


FIGURE 2
Portion of a fibrous astrocyte in the orbital portion of optic nerve. Many fibrils course near the nucleus. Dense granules (Gr.), mitochondria (M), and endoplasmic reticulum (ER) are also present. (× 33,000)

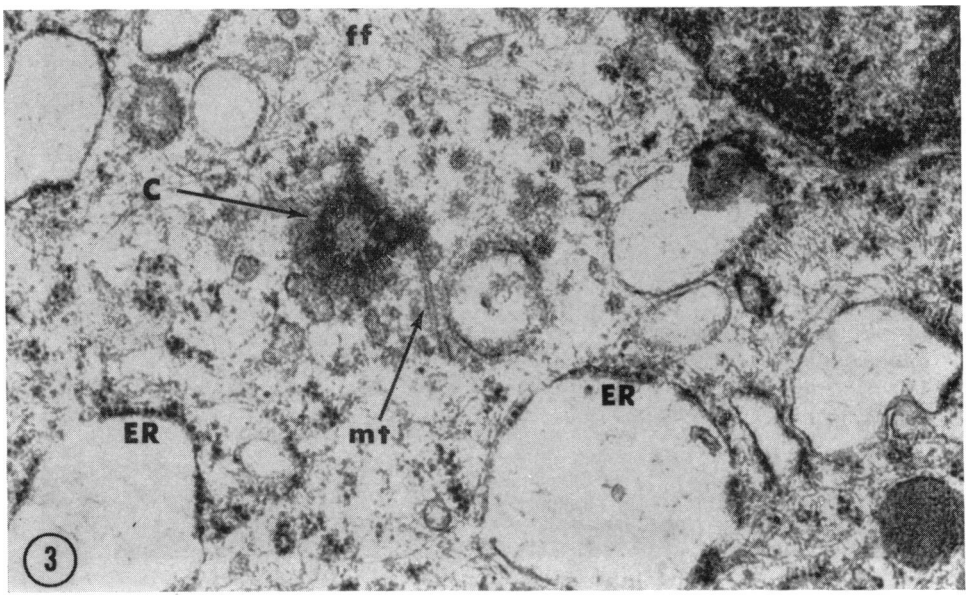
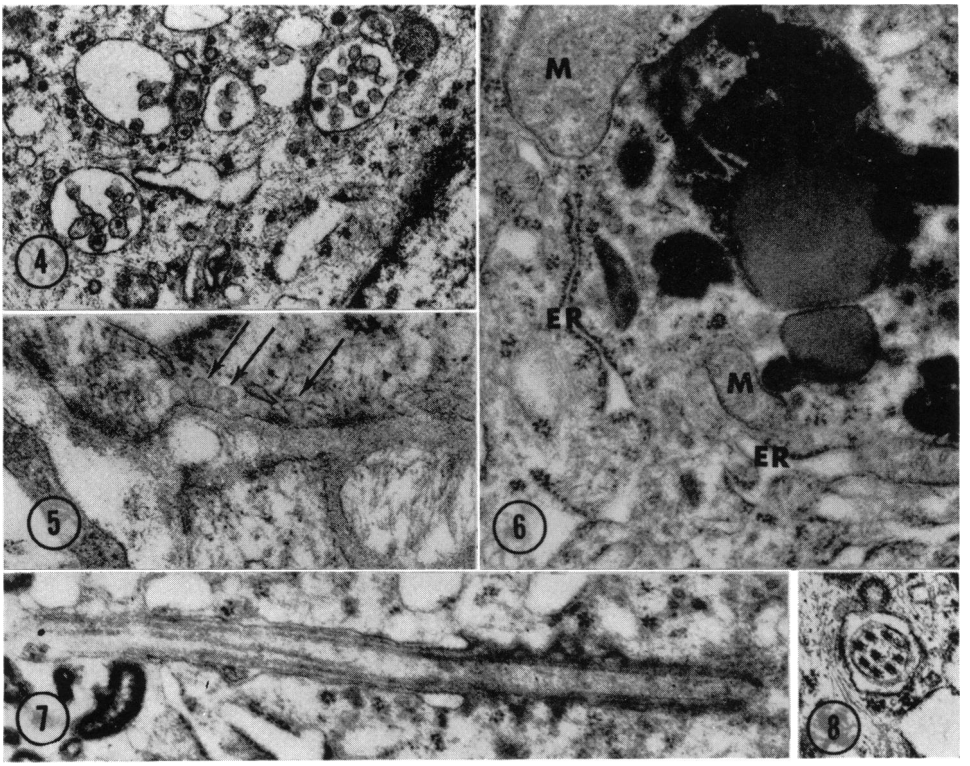


FIGURE 3
Cytoplasm of a fibrous astrocyte, including a centriole (C) and a microtubule (mt). (× 61,000)



FIGURES 4–8

4, Multivesicular bodies in cytoplasm of a fibrous astrocyte (× 25,000). 5, Pinocytotic vacuoles (arrow) at the surface of an astrocyte in the orbital portion of optic nerve (× 42,000). 6, Pigment inclusion in cytoplasm of fibrous astrocyte in orbital portion of optic nerve; mitochondria (M) and endoplasmic reticulum (ER) are also in the field (× 39,000). 7, Longitudinal section through intracytoplasmic cilium in the cytoplasm of an astrocyte in the orbital portion of the optic nerve (× 31,500). 8, Cross-section through a similar intracytoplasmic cilium; note that the cilium is in a membrane-bound space (× 31,500).

the perikaryon and extend for a very short distance into the processes (Figures 1 and 2). Many fine fibrils (60–75 Å in diameter) run longitudinally throughout the length of the astrocytic processes. These fibrils disperse in the perikaryon (Figure 2) where they are seen in small bundles or in a loose random tangle.

Astrocytes contain glycogen, but if there is much delay before the specimen is placed in fixative, the glycogen may be metabolized.[3] When present, glycogen appears as dense angulated granules distributed throughout the cytoplasm, from the perikaryon to the end

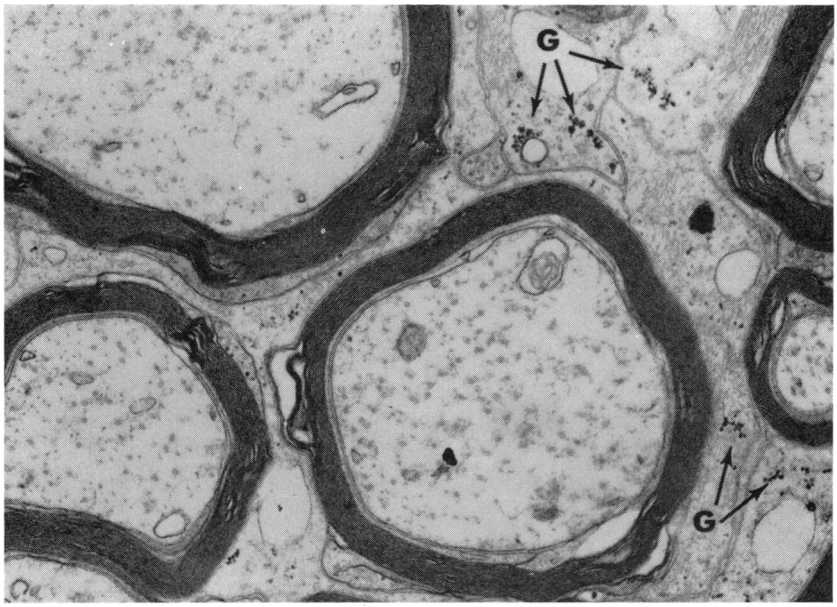


FIGURE 9
Orbital portion of optic nerve. Processes of astrocyte cytoplasm fill the space between myelinated nerve fibers. Glycogen (G) is present as dense grains in the astrocyte cytoplasm. (× 39,000)

feet. Sometimes a collection of dense material (Figure 6) is encountered within the perikaryon. It may be in the form of crystals or spikes, a course network, or fine fibrils. In some instances, a round globule of gray material is seen in association with the inclusion. The inclusion usually seems to be bounded by a membrane. A few microtubules can be found, most often clearly associated with the centriole (Figure 3). Intracytoplasmic cilia are rare (Figures 7 and 8); each cilium projects into a membrane-bound space that forms an envelope around it.

The astrocyte processes are distributed among the myelinated axons and bundles of axons. Some processes are irregular, tortuous, cytoplasmic strands, molded by the nerve fibers among which they insinuate themselves (Figure 9). Others are straight cylindrical bands that apparently do not yield to the structures surrounding them (Figure 10). In either case, the processes contain many longitudinal fibrils with only occasional mitochondria or pieces of endoplasmic reticulum.

There is an uninterrupted glial layer that separates nerve fibers from the connective tissue septa. This layer is made up mainly of footplates

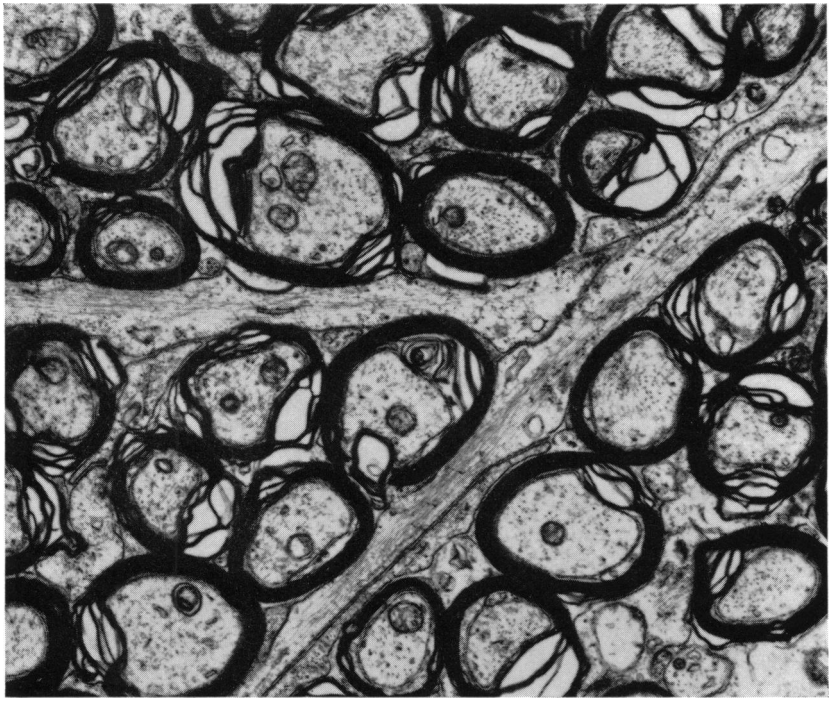


FIGURE 10
Straight astrocyte processes among the myelinated nerve fibers in the orbital portion of the optic nerve. (× 18,000)

derived from distant astrocytes, but astrocyte cell bodies that lie directly on the connective tissue are also encountered regularly (Figure 11). In a few places the foot processes seem randomly arranged, but usually all the processes are cut either in longitudinal section (Figure 12) or cross-section (Figure 13), giving the impression that there is a directional arrangement of the astrocytic feet. When a process is sectioned longitudinally, it can sometimes be seen to be in contact with the connective tissue septum over a considerable distance (Figure 12).

The surface of the astrocyte's cell body or footplate that abuts upon the connective tissue is flattened. Thus, the surfaces of glia and connective tissue are smooth where they are in contact, separated only by a basement membrane of even thickness. Where the astrocyte footplate or astrocyte cell body is in contact with basement membrane, there is often a thin dense layer just inside the plasma membrane (Figures

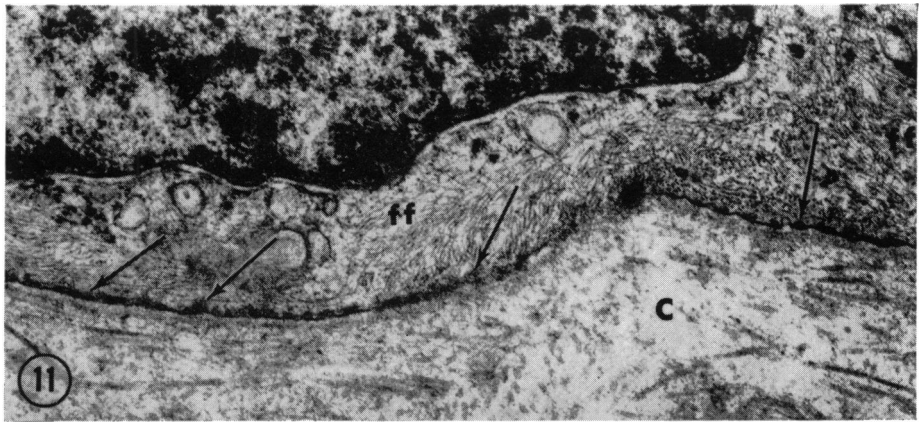


FIGURE 11

Astrocyte cell body lying adjacent to a connective tissue septum (C). There is an irregular dense zone (arrows) inside the plasma membrane, and many fibrils (ff) are present in the cytoplasm near the cell surface. (× 31,500)

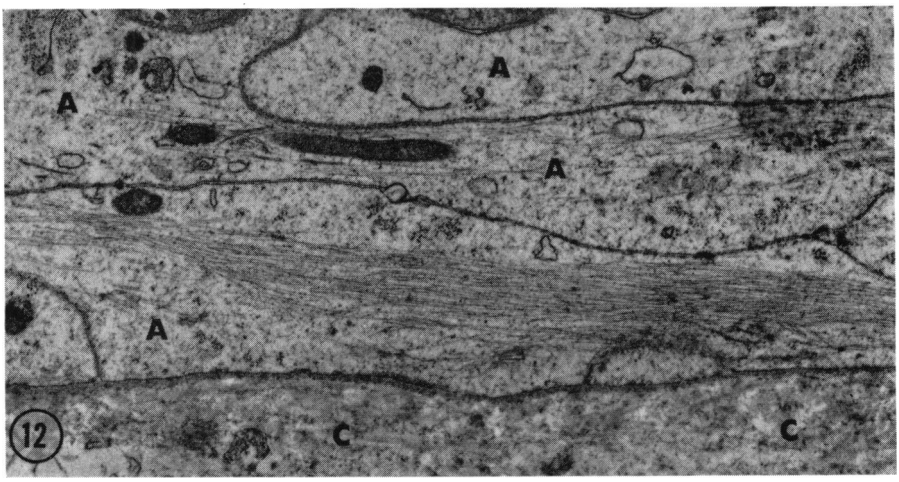
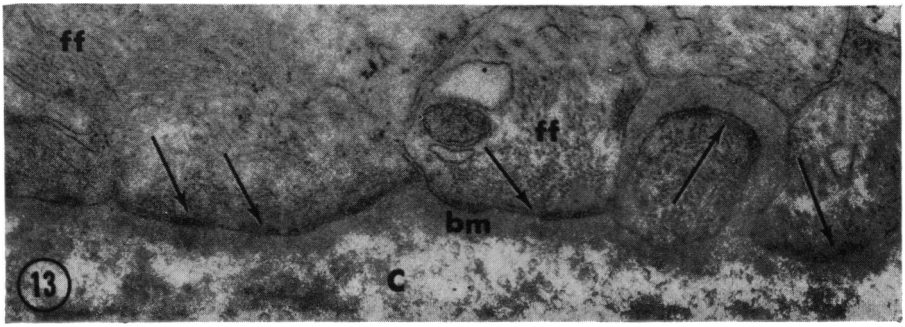


FIGURE 12

Astrocyte processes (A) cut in longitudinal section as they abut on a connective tissue septum (C). (× 18,000)



11–14). The dense layer may be interrupted and appear as clumps, or it may be absent altogether. The cytoplasmic fibrils seem to insert into this dense layer, and this arrangement somewhat resembles half a desmosome. Pinocytotic vacuoles may be seen forming on the surfaces of astrocytes or astrocyte processes (Figure 5) but they are not present everywhere.

In many places the astrocyte footplates are separated by a 120- to 150-mμ gap filled by basement membrane (Figure 13). Similar spaces filled with basement membrane material also occur within footplates (Figure 14). The significance of this unusual structural arrangement is not apparent. Occasionally in such an area, the myelin of a nerve fiber makes contact with the invaginated basement membrane (Figure 15). Except in this borderline circumstance, glial tissue always effects a complete separation between nerve fibers and basement membrane, although in places the single strand of glial cytoplasm interposed between them may be quite thin.

Beneath the connective tissue of the pia mater, there is an astroglial layer called the "peripheral glial mantle of Fuchs"[4] (or "of Greeff"[5]). The thickness of this layer varies, and in some places nerve fibers come quite near the surface (Figure 15), separated only by a thin layer of astrocyte feet similar to the layer that lines the connective tissue septa of the nerve. In most places, however, there is a more extensive tangle of fibrous astrocytic processes (Figure 16), and among them a variable number of astrocyte cell bodies (Figure 17). These cells seem better preserved than the astrocytes among the nerve fibers: their chromatin is less clumped, making the nucleus more evenly granular, and their numerous fibrils are evenly distributed through the cytoplasm, giving it a less watery appearance. The fibrils predominate in the cytoplasm, but small vesicles, endoplasmic reticulum, mitochondria, and a few free ribosomes are also distributed throughout the cytoplasm around the nucleus and in the processes.

The large central connective tissue core, containing the central retinal vessels, is surrounded by a similar glial layer of variable thickness. The ultrastructure of this glial layer is the same as that of the glial mantle of Fuchs.

FIGURE 13

Astrocyte footplates against a connective tissue septum. Fibrils (ff) of astrocyte cytoplasm approach the dense layer (arrow) within the plasma membrane. A basement membrane (bm) separates astrocyte feet from connective tissue (C). ($\times$ 46,000)

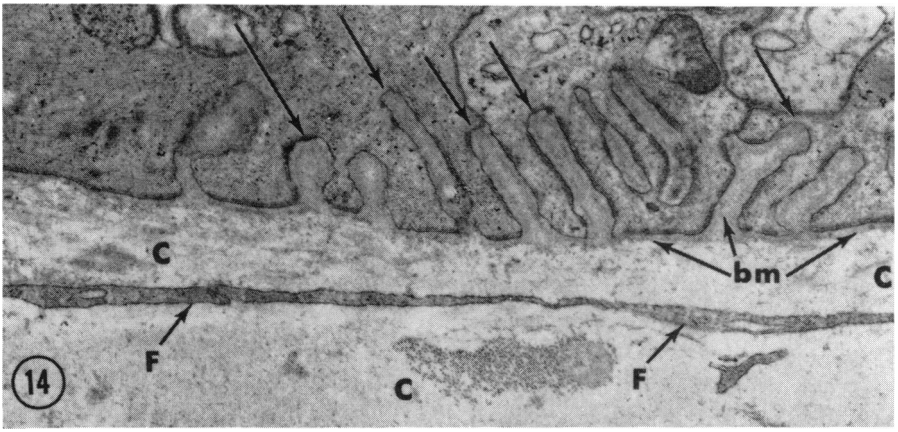


FIGURE 14

Orbital portion of optic nerve. Astrocyte processes abut upon the connective tissue (C) of the septum below. Basement membrane (bm) of the glial feet extends into invaginated spaces (arrows) between the feet and within them. A strand of fibroblast cytoplasm (F) is in the field. ($\times$ 22,500)

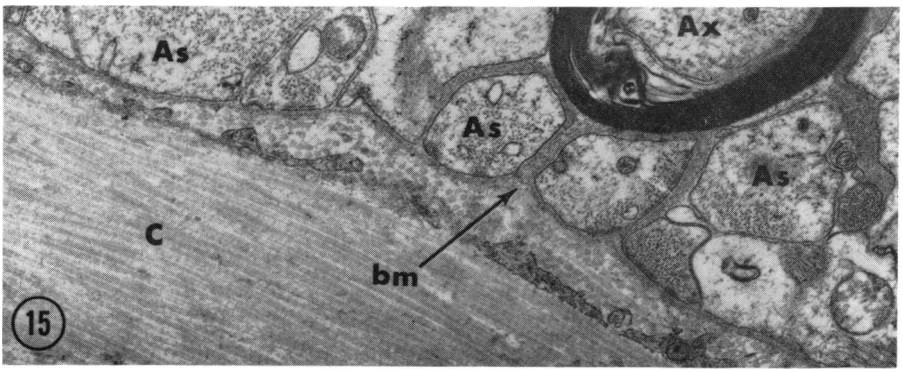
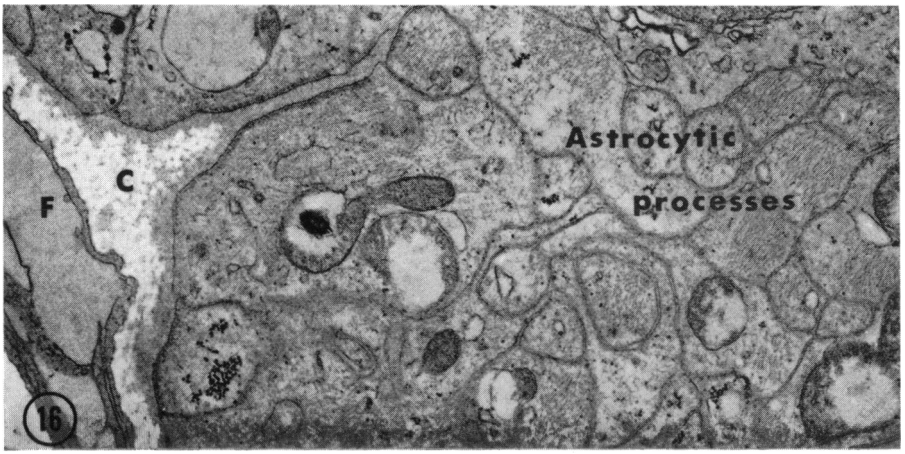


FIGURE 15

Junction of the connective tissue of pia mater (C) and its glial lining. Between some of the astrocyte processes (As) there is a gap filled with basement membrane (bm) material which comes in contact with a myelinated axon (Ax). ($\times$ 25,000)



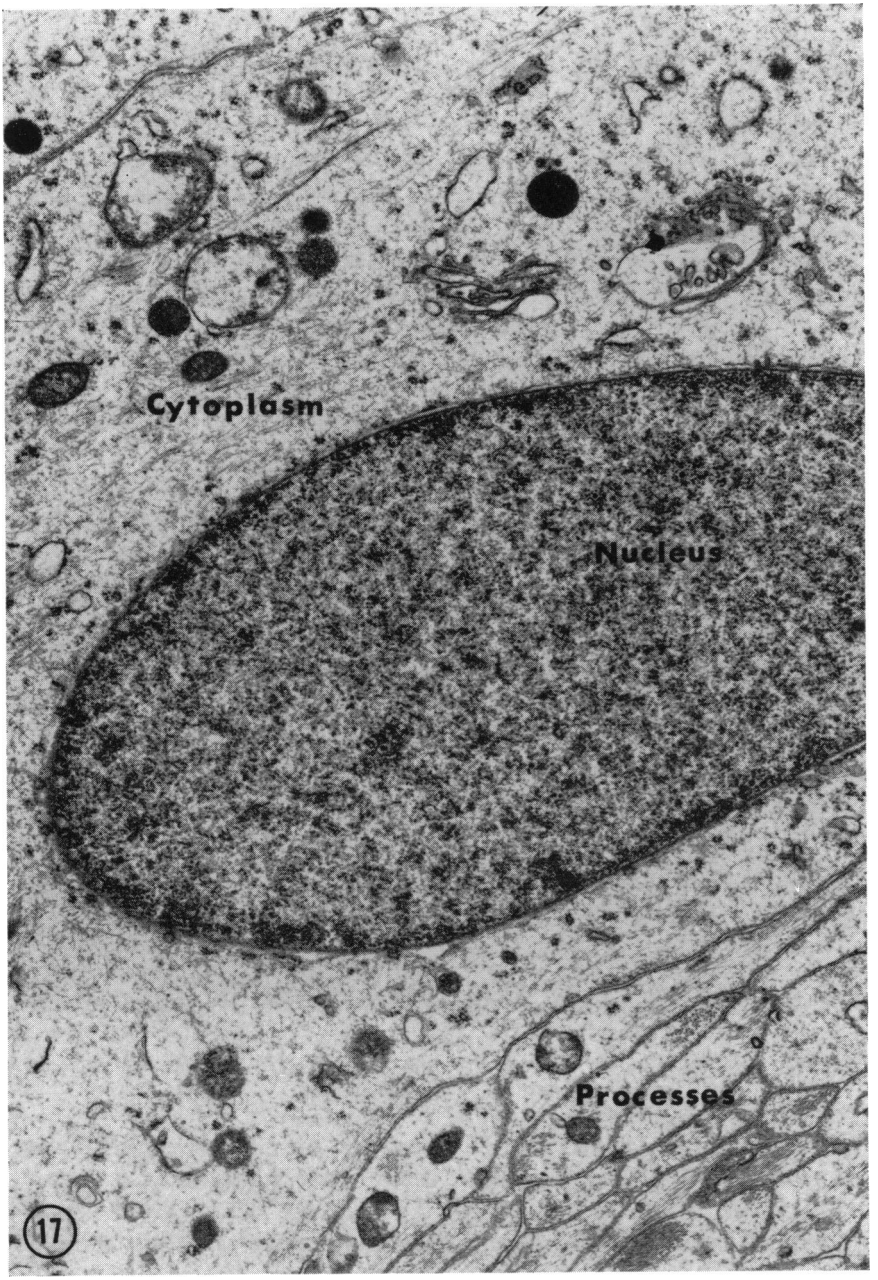


FIGURE 17
Astrocyte cell body in the tangle of astrocyte processes that lies beneath the connective tissue of the pia mater. (× 18,000)

FIGURE 16
Tangle of glial processes adjacent to the connective tissue (C) of the pia mater. Part of a fibroblast (F) is included in the micrograph (× 25,000)

LAMINA CRIBROSA

Most of the astrocytes in the optic nerve head are included in the structure of the lamina cribrosa and are its main component. The anterior (or "choroidal") portion of the lamina cribrosa is made up of a large number of specialized astrocytes whose arms lie more or less in a single plane perpendicular to the course of the nerve fibers. These cells, which are aligned to form tube-like channels, support the nerve fibers as they undergo their ninety-degree turn into the optic nerve. In longitudinal section, the cells are seen as columns of nuclei which follow the course of the nerve fibers (Figure 18). In electron micro-

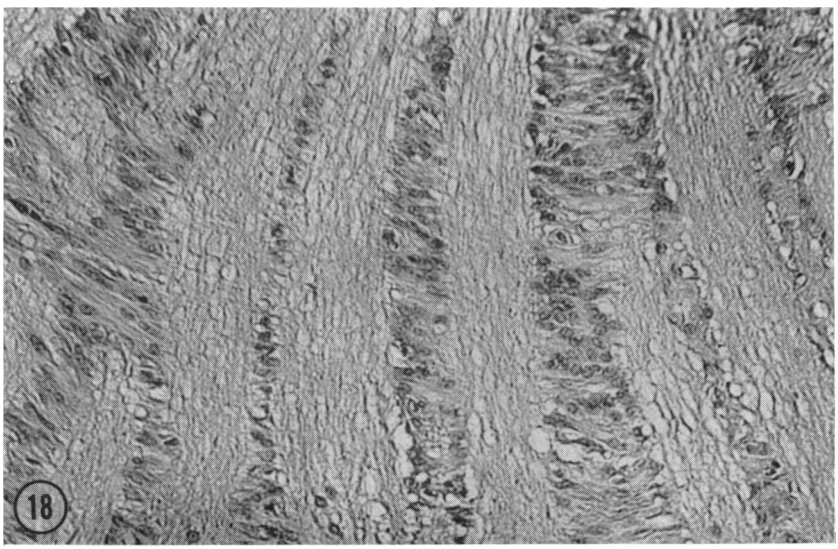


FIGURE 18
Anterior portion of lamina cribrosa showing columns of astroglia nuclei between bundles of axons. (Masson trichrome, × 250)

graphs, the arms of the flattened astrocytes are often oriented in the same direction: in one place all the cells are cut in longitudinal section (Figure 19) while in another all the cells are cut in cross-section (Figure 20). The nuclear chromatin is more condensed than it is in astrocytes of the orbital portion of the optic nerve. The circumference of the nucleus is surrounded by a very thin layer of cytoplasm which contains ribosomes and fibrils. The Golgi zone, several mitochondria, and scattered bits of endoplasmic reticulum are concentrated at the nuclear poles. The long arms of these astrocytes are made up almost

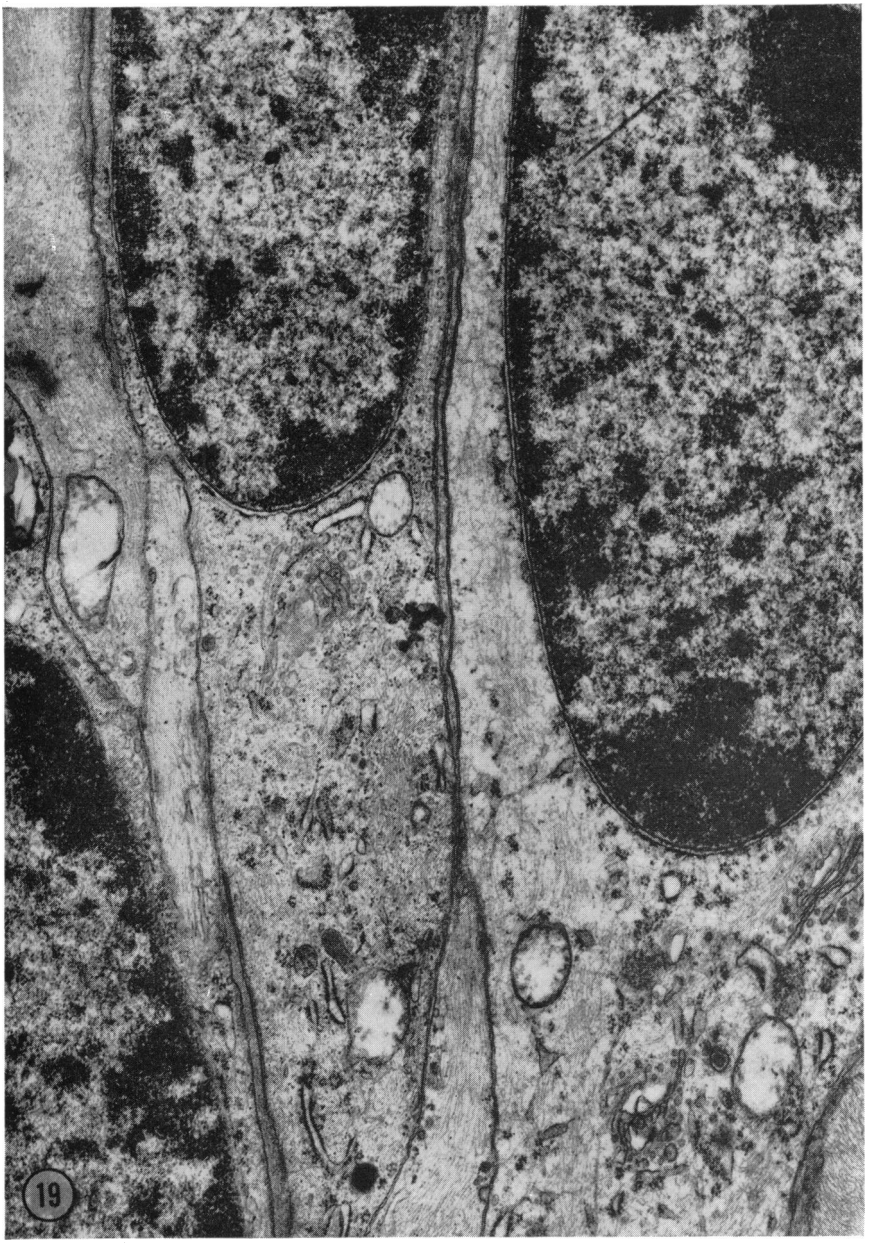


FIGURE 19
Astrocytes of the anterior portion of the lamina cribrosa. The section passes through the long axis of these cells. (× 22,000)

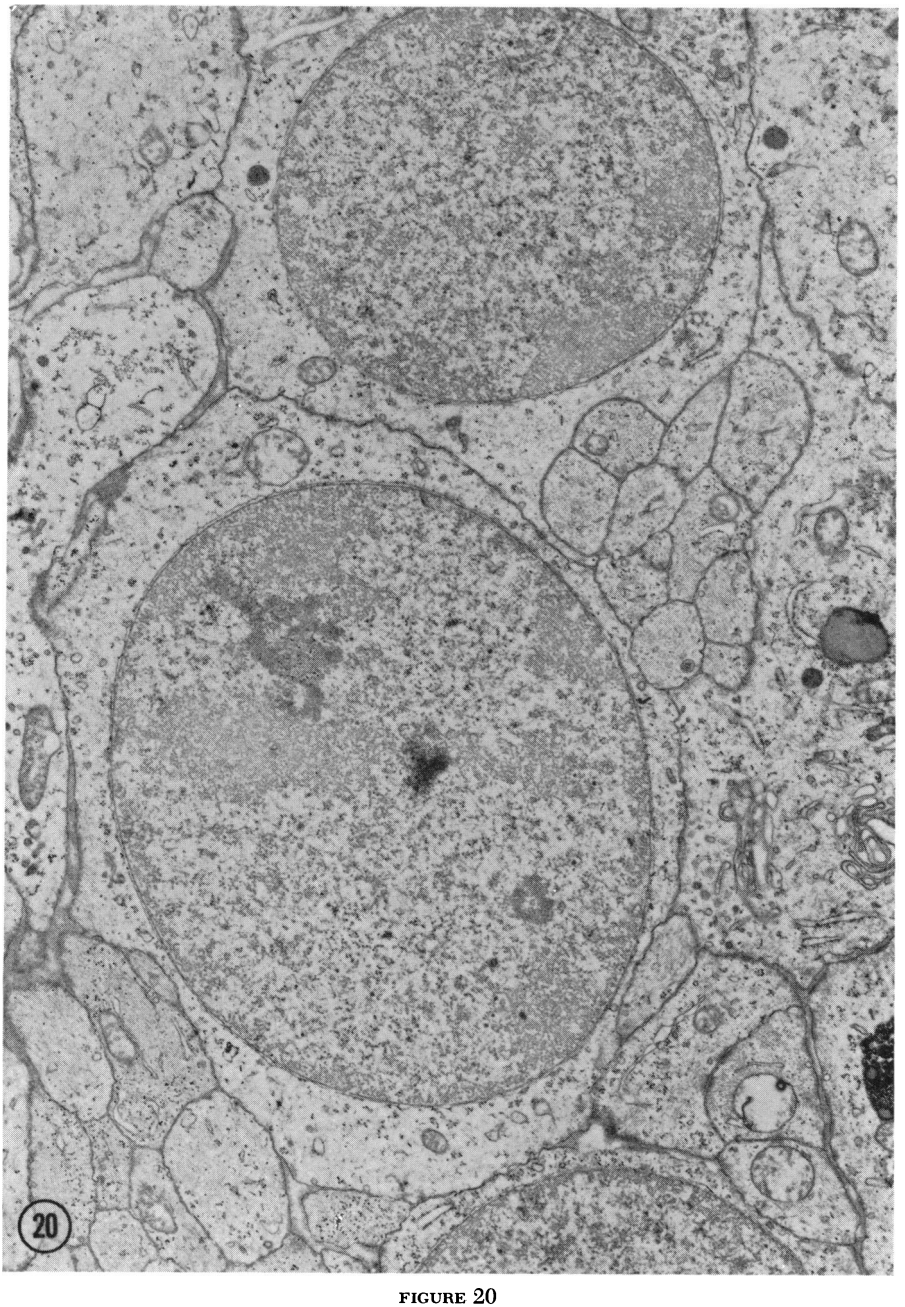


FIGURE 20
Astrocytes of anterior portion of lamina cribrosa. The section cuts across the long axis of astrocyte cell bodies and processes. (× 16,000)

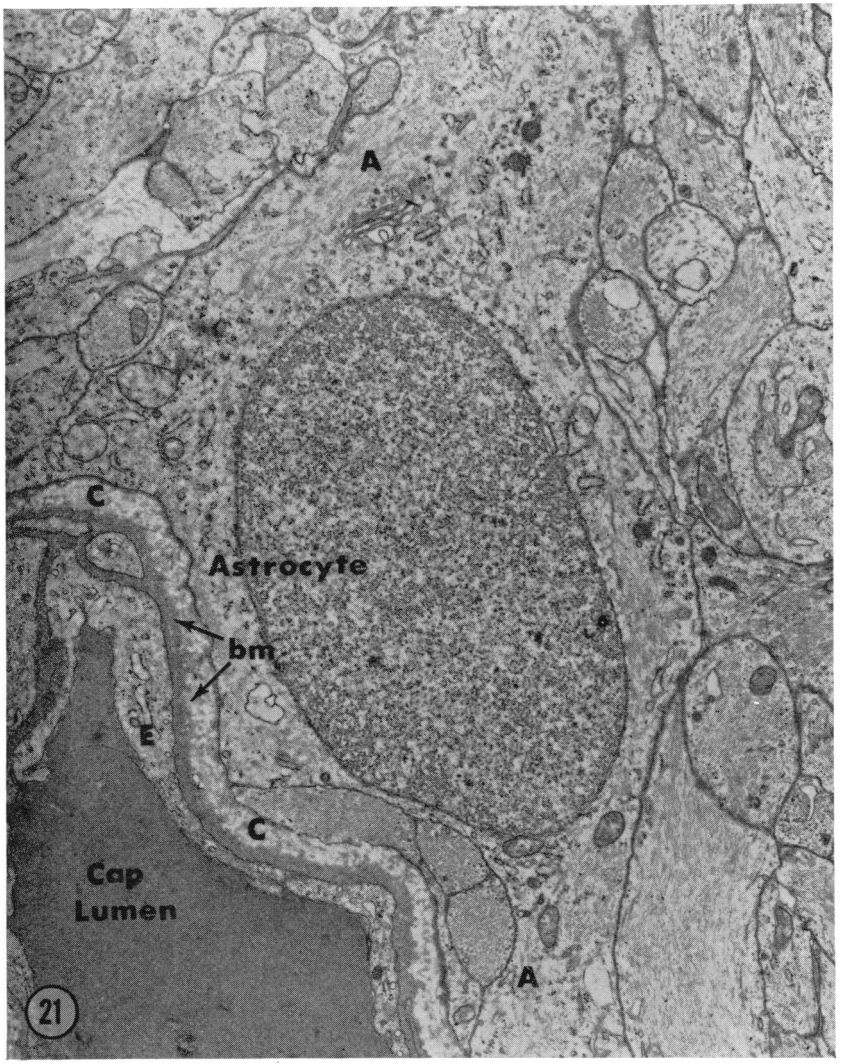


FIGURE 21
Capillary in anterior portion of lamina cribrosa. The lumen is enclosed by endothelial cells (E) with their basement membrane (bm). A small amount of collagen (C) accompanies the capillary. A perivascular astroglial cell (A) sends processes around the capillary. ($\times$ 13,500)

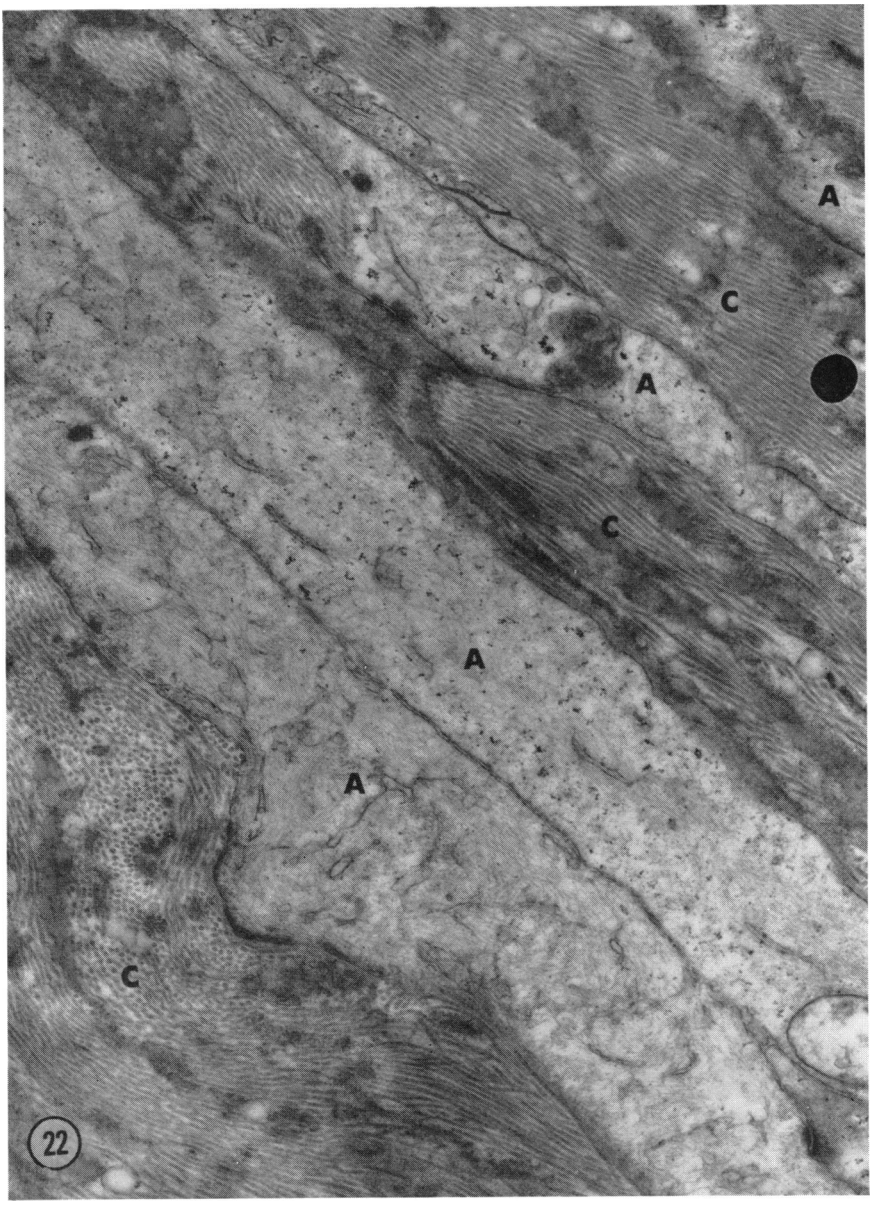


FIGURE 22
Alternating layers of astroglia (A) and collagenous connective tissue (C) in the posterior part of the lamina cribrosa. ($\times$ 25,000)

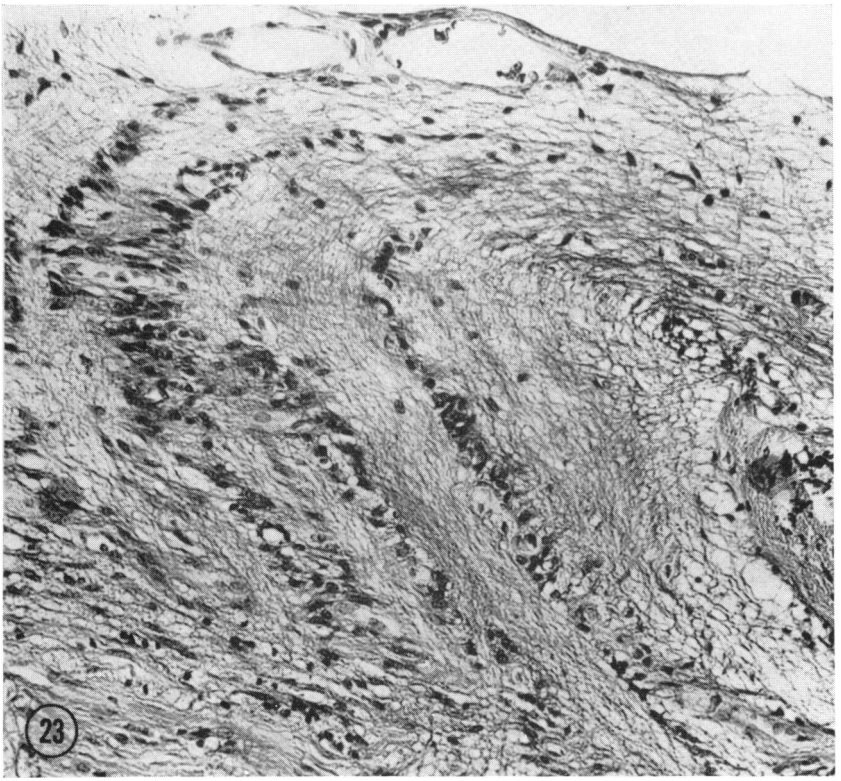


FIGURE 23
Optic nerve head, showing the column of astroglia nuclei making a ninety-degree turn toward the retina. (Masson trichrome, × 200)

entirely of fibrils. In some places the astrocytes are joined by desmosomes.

Capillaries mingle in the glial tissue of the lamina cribrosa (Figure 21) and are accompanied by a small amount of connective tissue. Often collagen is not present, and the glial basement membrane fuses with the capillary basement membrane, as it does in the retina and most of the central nervous system.

The posterior (or "scleral") portion of the lamina cribrosa is made up of several sheets of connective tissue separated from each other by sheets of glial tissue. Each of the alternating sheets of connective tissue and glia is perforated, and the congruent perforations of all the layers are aligned to form a passage through which the nerve fibers pass. By electron microscopy, astrocytes of the posterior portion of the lamina

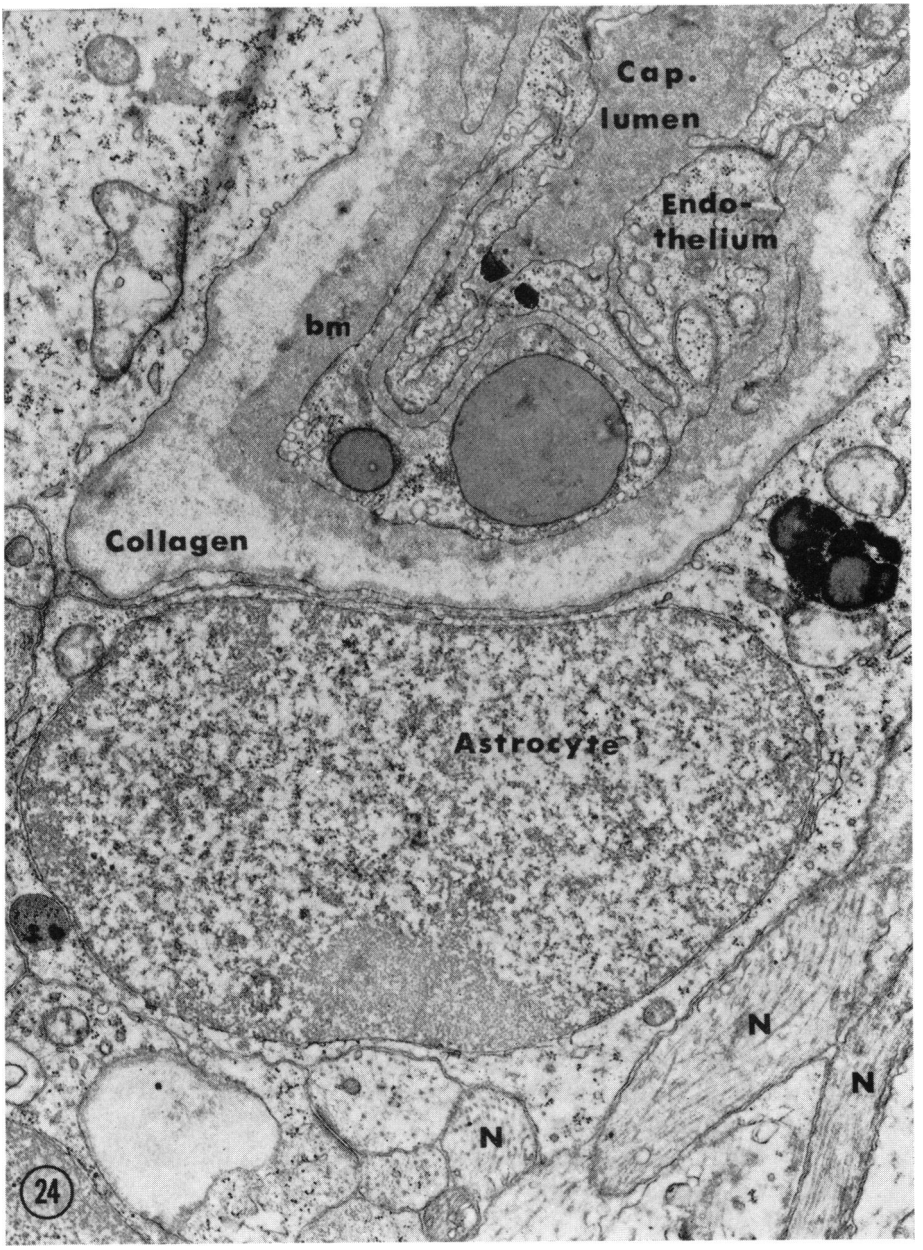


FIGURE 24

Capillary surrounded by a perivascular astrocyte in the region where retina and optic nerve head meet. Nerve fibers (N) are unmyelinated. ($\times$ 19,000)

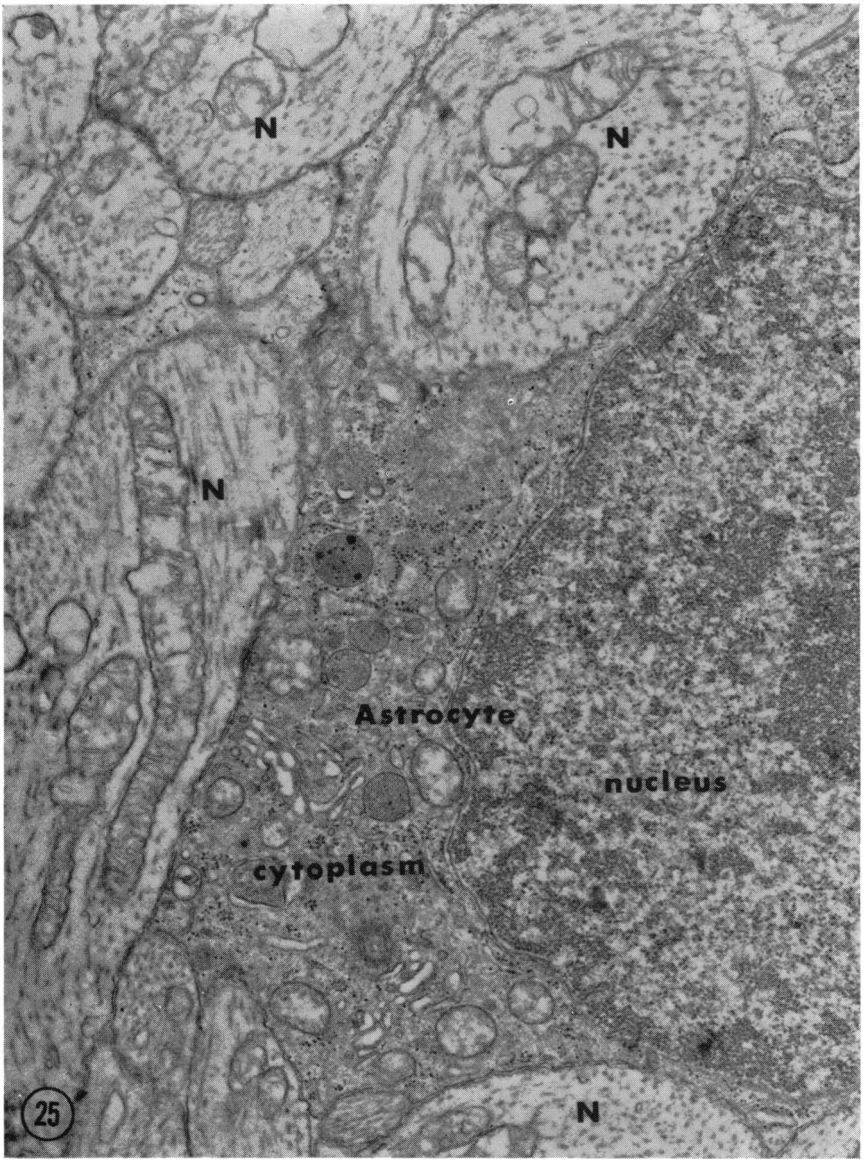


FIGURE 25
Astrocyte with dense cytoplasm among unmyelinated nerve fibers (N). ($\times$ 25,000)

cribrosa are similar to those of the anterior portion, but they are sandwiched between the layers of connective tissue (Figure 22). As elsewhere, the glia have a basement membrane separating them from collagenous tissue. Where the anterior and posterior portions of the lamina cribrosa merge, the interdigitations of glia and connective tissue are intricate; eventually the layer of connective tissue between glia is so thin that it consists of little more than basement membrane material. Posteriorly the connective tissue becomes more and more prominent. Finally it merges into the septa of the orbital portion of the nerve where there is a transition of the astrocytes to the type with multidirectional arms.

OTHER ASTROCYTES OF THE OPTIC PAPILLA

As the nerve fiber bundles are followed from the region of the lamina cribrosa onto the retina, the glial columns between them taper and finally become discontinuous (Figure 23). Gradually the glial processes are no longer at right angles to the nerve fibers and the glia are reduced to less conspicuous cells that lie among the numerous axons, with the long axes of their nuclei parallel to the nerve fibers. Two types of glial cells are encountered in this zone of transition between retina and optic disk. One type is a pale cell, similar to the astrocytes of the lamina cribrosa but smaller, less fibrous, and often running parallel to the course of the axons. Some of these pale cells are seen to surround capillaries (Figure 24) and may be equivalent to the spirocytes that surround capillaries in the retina.[6,7] Without serial sections it is not possible to be sure that seemingly isolated pale cells have no connection with a capillary. The second type of cell in this region is more condensed (Figure 25); its dense nucleus is surrounded by a thin tag of dense cytoplasm in which ribosomes are conspicuous. These dense cells have not been seen associated with capillaries. It is not certain at present whether this dark cell is a distinct type of astrocyte, or whether it merely reflects a different degree of fixation that depends on the location of the cell near the retinal surface or on some other contingency.

The arrangement of glia at the scleral margin of the nerve head varies. Sometimes there are several connective tissue projections from the collagenous tissue that bounds the scleral canal. These projections interdigitate with glial processes that extend into the surrounding collagenous tissue. This arrangement is presumed to mark the place where the posterior lamina cribrosa takes its origin from the sclera. Elsewhere there is a smooth boundary between the collagenous border tissue and the astroglial layer that lines it (Figure 26). This glial

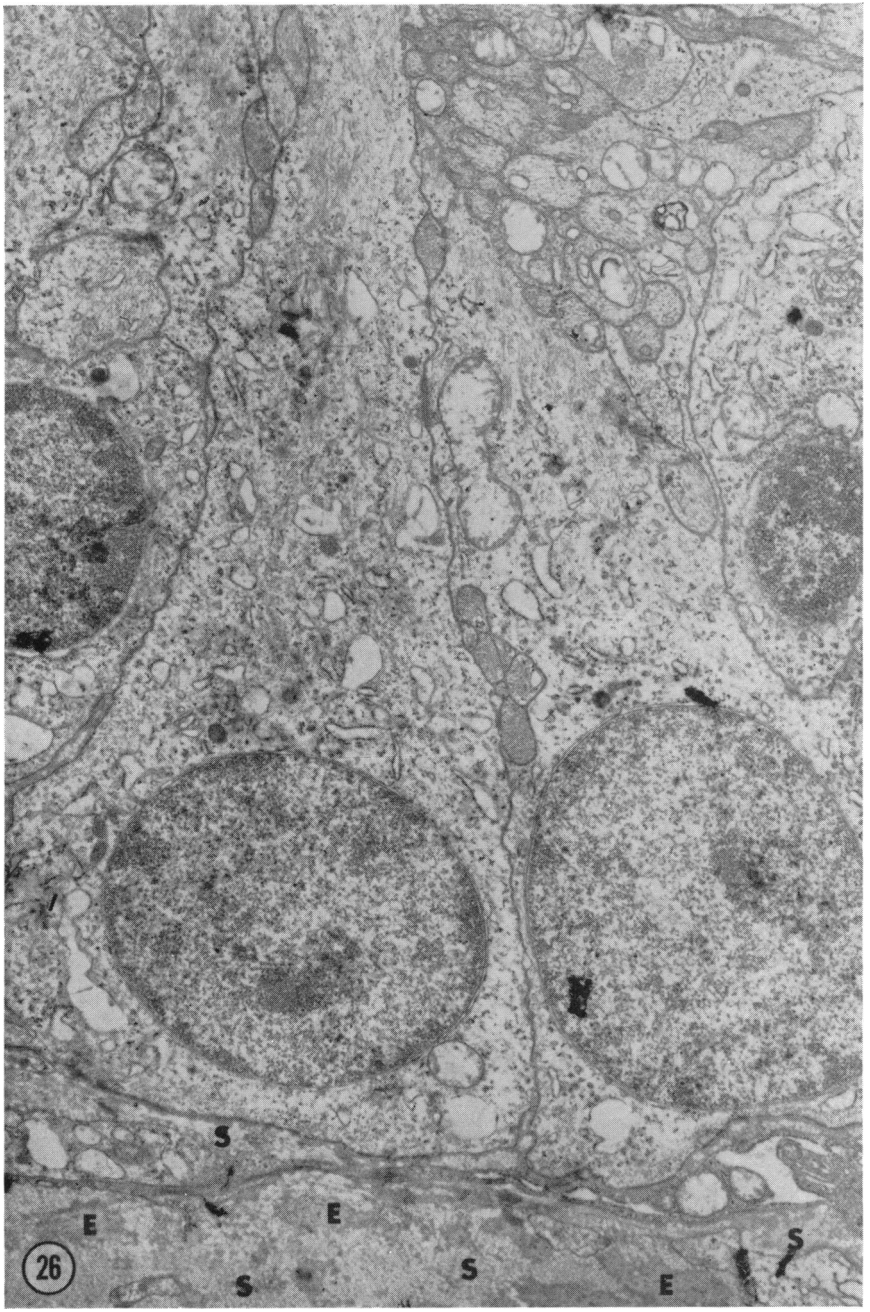


FIGURE 26
Astroglia bordering on scleral connective tissue (S) containing elastic fibers (E). (× 12,000)

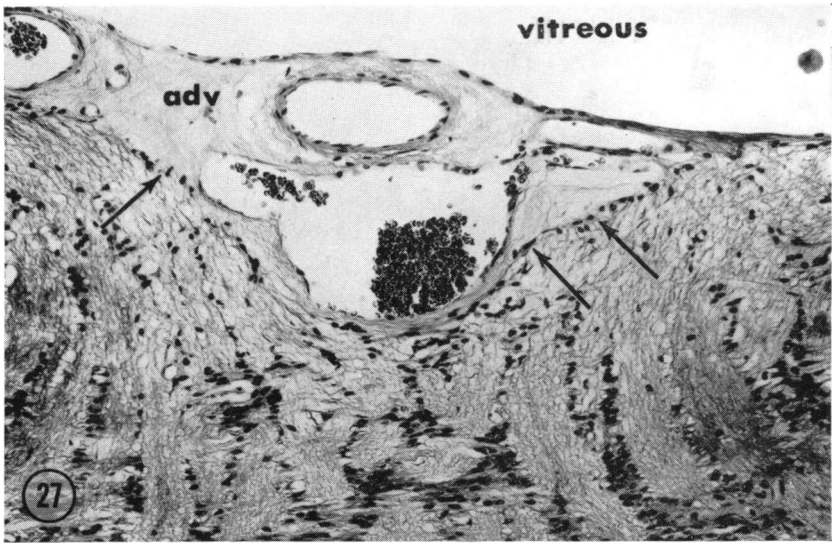


FIGURE 27
Optic nerve head, showing the connective tissue adventitia (adv) of the central retinal vessels. Note the glia deep to the vessels (arrows) and covering of the disk. (Elastic, Van Gieson, × 130)

lining, the "border tissue of Jacoby,"[8] varies in thickness and consists of glial processes and cell bodies. (When this layer extends anteriorly beyond the level of the choroid so that it lies between the optic disk and the outer retinal layers, it is called the "intermediary tissue of Kuhnt."[9]) Sometimes, as in Figure 26, the cell bodies are in a column, sending processes into the nerve head. In these areas, the glial layer appears to be nothing more than the most peripheral of the glial columns that make up the anterior lamina cribrosa.

The astroglial investment of the central retinal vessels (see Figure 27) varies from a single strand of astroglial cytoplasm to a thicker tangle of astrocyte processes and cell bodies (Figure 28). These glial cells have the same fibrous intracellular ultrastructure as the other astrocytes observed in this study.

GLIAL COVERING OF THE DISK

The optic disk surface is covered by an astroglial layer called "the limiting membrane of Elschnig."[10] Over the adventitia of the major vessels, as well as over other parts of the disk, there may be several layers of densely fibrillar astrocytic processes intermingled with cell

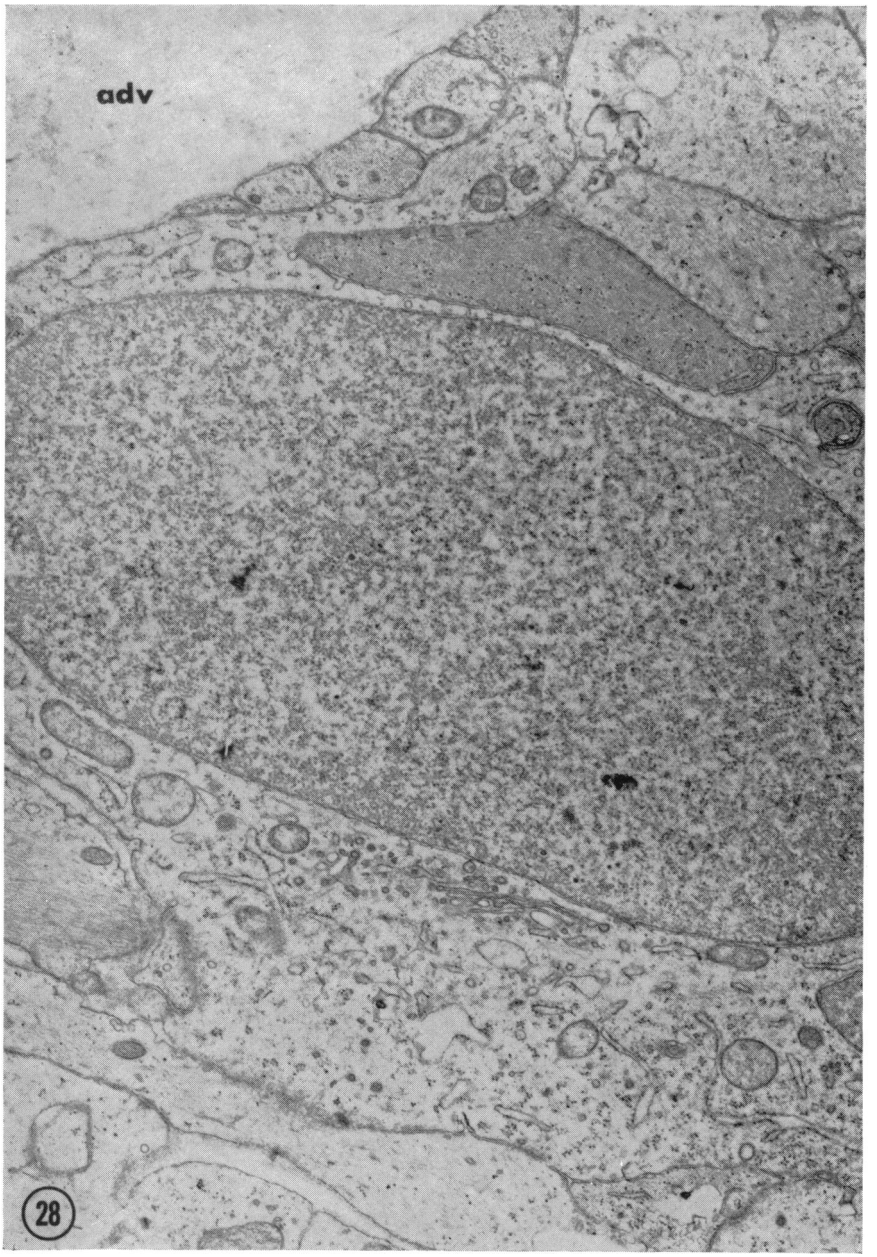


FIGURE 28
Astrocyte cell body and processes lining the connective tissue adventitia (adv) around the central retinal vessels in the optic disk. (× 18,000)

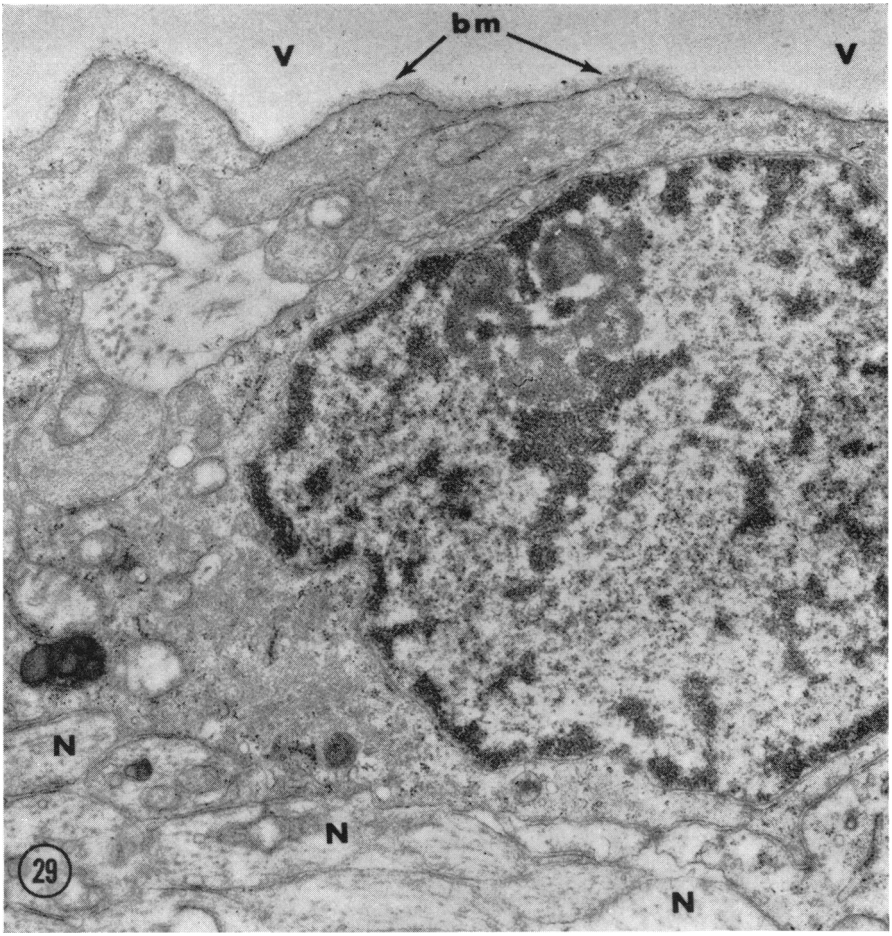


FIGURE 29
Astrocyte at the vitreous (V) surface of the optic disk. A thin basement membrane (bm) is part of the limiting tissue. Unmyelinated nerve fibers (N) are in the field. (× 22,000)

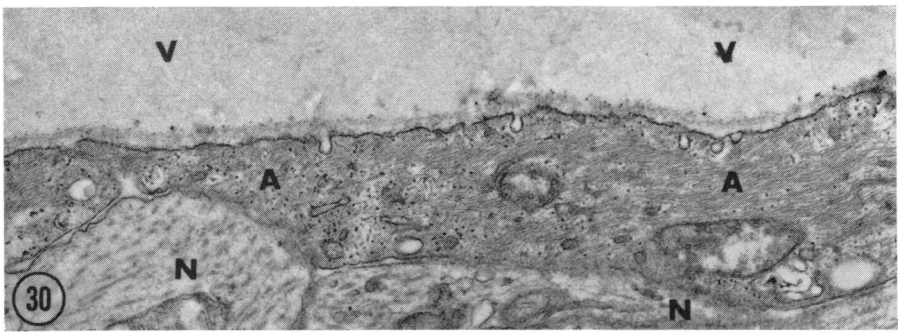


FIGURE 30
A thin strand of astroglial cytoplasm (A) separates nerve fibers (N) from the vitreous space (V). (× 25,000)

bodies (Figures 27 and 29). In other regions, the glial layer is reduced to a single thin strand of glial cytoplasm (Figure 30). On occasion there may be a small space between two glial processes so that only a basement membrane separates the vitreous from a superficial nerve fiber. Since this kind of gap was observed only in areas where there was evidence of disruption of the underlying tissue, the apparent gap is probably an artifact produced when vitreous was removed to allow fixative to reach the nerve head more rapidly.

The basement membrane over the disk is considerably thinner than that over the retina. It is of even thickness, following the irregularities in the glial surface; this is a contrast to the thick basement membrane over the retina which fills in the irregularities in the glial surface and presents a smooth face toward the vitreous.

DISCUSSION

IDENTIFICATION OF ASTROCYTES

By light microscopy, astrocytes and oligodendroglia are defined and differentiated from one another mainly on the basis of their metallic staining reactions and three-dimensional architecture. Since it is difficult to judge three-dimensional architecture in ultrathin sections, the identification of glial cells in electron micrographs has been in debate. In studying the orbital portion of the optic nerve, we found two types of glia and had to decide which was the astrocyte. We concluded that the paler cell with numerous fibrils was an astrocyte because of its more abundant perinuclear cytoplasm, its more numerous processes, and its relationship to vessels and connective tissue septa. The other cell type had a thinner rim of denser cytoplasm, lacked fibrils, and contained more numerous microtubules. Processes of this second type of cell were less often included within the plane of section, and the cell was identified as an oligodendroglial cell. Moreover, the pale cells with numerous fibrils had the same basic fine structure as the cells in the optic nerve head where the nerve fibers are unmyelinated and there are no oligodendroglia. Our identification agrees with that of other authors who have examined glial cells in other parts of the central nervous system.[3,11-16]

REGIONAL MODIFICATIONS OF ASTROCYTES

In the region of the optic nerve head, the modifications that astrocytes undergo to suit special circumstances are well illustrated. Although they retain the basic fine structure of astrocytes, their arms may be multidirectional (orbital portion), confined to a plane perpen-

dicular to the nerve fibers (lamina cribrosa), or parallel to the nerve fibers (in the peripapillary retina). The cells may be loosely dispersed throughout the nerve fibers (orbital portion, retina) or highly organized into columns (lamina cribrosa). In several regions, aggregates of glia apparently serve only to fill in the space where the longitudinal bundles of nerve fibers fail to match the contour of the surrounding collagenous tissues. Thus, depending on local variations of architecture, there is a variable amount of glial tissue beneath the pial collagen, around the central vessels, along the scleral canal, and within the physiologic cup. Cone and MacMillan[17] suggested that these aggregates are produced during fetal development when the visual axons grow through the previously formed glial framework, and in some areas do not completely fill the framework.

Presumably, astrocyte function is reflected in its morphology. Thus, the close relationship of astrocytes to capillaries reflects their importance to the nutrition of neural tissue. Nutrients are envisioned as passing from the capillaries into the glial cytoplasm, through which they diffuse to axons remote from the capillaries (see deRobertis and Gerschenfeld[12] for discussion). The supportive function of astrocytes is well demonstrated in the anterior portion of the lamina cribrosa. Here, this function is reflected not only in the arrangement of the cells, but also in the intracellular morphology. The fibrils are prominent, whereas the metabolic organelles (mitochondria, endoplasmic reticulum) are sparse. In addition, the chromatin of the nucleus is relatively condensed, a feature of specialized cells with limited metabolic needs.[18] The presence of desmosomes, which serve to hold the astrocytes in a stable relation to one another, also reflects the supportive function of astrocytes in this region.

DISK-VITREOUS AND RETINA-VITREOUS RELATIONSHIPS

Vitreous—a connective tissue of mesodermal origin—is separated from the nerve fibers by a layer of glial tissue. In the retina the footplate of Müller cells forms the glial layer. These Müller cell footplates and their thick basement membrane together constitute the internal limiting membrane of the retina. Only occasional astrocytes are found among the Müller cell footplates.[19] At the disk margin, the character of the superficial glial covering changes. The covering over the optic disk (the limiting membrane of Elschnig) is derived entirely from astrocytes, and the basement membrane is considerably thinner. The rather abrupt thinning of the basement membrane probably accounts for the impression gained from light microscopy that the inner limiting membrane of the retina ends at the disk margin. Since we removed

vitreous, hoping to improve fixation, the relation of vitreous fibrils to the disk surface could not be studied adequately for comparison with vitreoretinal relationships.

CYTOPLASMIC PIGMENT OF ASTROCYTES

The dense inclusions in astrocytes in each region of the optic nerve head (Figures 6 and 24) have been found in other astrocytes. They have been called "heterogeneous dense bodies,"[11] "lipochrome,"[12] and "lysosomes."[3] These must be the same as the pigment accumulations that are seen in astrocytes by light microscopy and known to increase with age.[20] We feel they are lipofuscin ("age pigment") because they resemble published micrographs of lipofuscin in other locations.[18,21–23] Lipofuscin is a brown pigment, usually stained by lipid stains. It is composed of long-chain lipids that accumulate with age in the cytoplasm of certain cells which do not proliferate or regenerate in the course of a lifetime. Barring disease, astrocytes would not undergo mitosis in the course of a lifetime and would therefore seem eligible to develop lipofuscin inclusions. The most familiar sites of lipofuscin formation are neurons[21,22] and heart muscle.[24] Biochemical and ultrastructural studies suggest that lipofuscin is derived from lysosomes.[21,25] Perhaps lipofuscin represents collected material that cannot be broken down by lysosomal enzymes and thus cannot be removed from the cell.

SUMMARY

In contrast to oligodendroglia, the astrocytes in the orbital portion of the optic nerve are pale and contain bundles of 60 to 75 Å filaments. The perinuclear cytoplasm is more abundant, and some of the astrocytes' multidirectional processes are often included in the plane of section. In the lamina cribrosa, the astroglia are modified to provide support for the nerve fibers as they make a ninety-degree turn. These cells are flattened, their arms extending perpendicularly to the nerve fibers. Filaments predominate in the cytoplasm, and desmosomes hold the astroglia in a stable relationship to one another.

As is true throughout the central nervous system, astroglia closely invest the nutrient capillaries of the retina and optic nerve head. In the orbital portion of the optic nerve, the capillaries are found within connective tissue septa, which are invested by an astroglial lining. Not only capillaries, but all tissues derived from mesoderm (pia mater, central retinal vessels, vitreous, etc.) are separated from contact with nerve fibers by an uninterrupted astroglial layer. This astroglial lining is of varying thickness, in some places expanding into aggregates of

astroglia which occupy spaces where the contour of the nerve tissue does not exactly fit the contour of the adjacent connective tissues.

The various kinds of astrocytes in the optic nerve and optic nerve head have the same basic fine structure with some variations, and they are similar to astrocytes in other parts of the central nervous system. The pigment accumulations in astrocytes would seem to be the same as lipofuscin, a pigment that accumulates with age in certain cells that do not divide or regenerate throughout life.

ACKNOWLEDGMENT

We gratefully acknowledge that Miss Kristina Eriksen skillfully performed all the technical work of this project.

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DISCUSSION

DR. J. REIMER WOLTER. Careful studies of all the details of the normal ocular structures with all the available techniques are the basis for an understanding of the normal functions as well as the pathology of the eye. The present paper gives many new and important details of the structure and arrangement of the astroglia in the optic nerve and disk as observed with the electron microscope. The fact that astroglia actually is one of the simpler cell types in the nervous portions of the eye indicates the gigantic task that lies ahead for the morphologists who will have to carry detailed studies of this kind on to the more complex neuronal systems of the eye.

One important point of this contribution is that it again shows that in the normal eye astroglia always separates the fibrovascular (mesodermal) from the neuronal (neuroectodermal) tissues. The observation that a continuous basement membrane formed by processes of astroglia (spider cells) of the disk separates the tissues of the optic nerve head from the vitreous is also extremely important.

Several questions have to be asked: Would the continuous basement membrane on the disk permit macrophages (microglia) to migrate between vitreous and optic nerve? Would this membrane be a barrier to a flow of fluids from the inner eye into the optic nerve head? Were migrating macrophages seen to cross this border in any of your preparations? Was inactive microglia recognized to be present in the disks or optic nerves of your cases? Were there no astroglial cells with several nuclei?

DR. ROBERT N. SHAFFER. I want to point out the clinical possibility of this explaining some of the glaucoma findings. We have always been puzzled, especially in younger patients, by the way that disk cup can form without obvious functional change in the field of vision. It would seem quite possible that pressure acting upon these astrocytes might result in a loss of actual tissue from the disk without at first interfering with neuron function. This would then explain how cupping and then nutritional loss to the neuron could result in chopping off one segment of the neurons. Perhaps this could be the origin of the arcuate scotoma.

I am told Dr. Elschnig also mentioned this possibility many years ago. It fits also with the fact that in children with increased intracranial pressure there is a great loss of their astrocytes in the brain tissue, without an initial loss in the ganglion cells.

DR. ALEXANDER IRVINE. I think Dr. Anderson has shown us that, with the electron microscope, we are going to have to take a new look at a number of old concepts. He mentioned that there is normally no extracellular space in the nerve or the retina, and hence we need to take a new look at papilledema and intraretinal edema.

I would like to ask him a simple question. Now that he has looked at the vitreous with the electron microscope and tells us that it looks like a mesodermal structure, are we going to have to change our concept as to where this vitreous comes from? Will we have to throw out the old concept that the vitreous is produced by the Müller cells of the retina?

DR. ANDERSON. I would like to thank Dr. Wolter for his complimentary remarks, and answer some of his questions.

Concerning the continuous membrane which covers the surface of the disk and its interference either with invasion by macrophages or diffusion of nutrient materials, I would imagine it would be quite possible for nutrient material and wandering cells to go across the glia and basement membrane without any difficulty, although this is speculation since we have not actually observed cells doing so. However, elsewhere in the body, basement membranes—such as those around capillaries—are no barrier to the passage of nutrients or cells. Many investigators have observed leukocytes and macrophages crossing capillary walls, for example.

I cannot say we have not seen microglia. We do not know exactly what they look like. If I interpret correctly the current ultrastructural studies having to do with the central nervous system in general, there is some feeling that perhaps mesodermal elements, or microglia, are not normally present at all. Rather, what we have been calling microglia on the basis of light microscopic studies are really a kind of oligodendroglial cells, and the real macrophages of mesodermal origin which are present during a disease state invade from blood vessels at the time when the disease arises. I think this is all speculation at the present time and the electron microscope is being used to help re-evaluate the status of microglia.

In the optic nerve we have seen some cells which look somewhat like oligodendroglial cells except that they are much denser. We thought these might be microglial cells, but it is very difficult to say for sure whether these will ultimately turn out to be microglia or a variety of oligodendroglia. One confusing thing about the cell is that it has no basement membrane around it, and one might expect a mesodermally derived cell present in an ectodermal structure to have such a basement membrane. This is another reason for thinking it might be truly an oligodendroglial rather than a microglial cell.

Concerning the presence of several nuclei, I think it is a previously established fact that astroglia can have more than one nucleus. In the ultrathin sections used for electron microscopy, however, you can never be quite sure when you encounter a cell which seems to have two nuclei that you have not sectioned through an irregularly shaped nucleus twice. Therefore, the electron microscope is not a really good tool for deciding whether there is more than one nucleus or not. We have seen a few cells which appeared to have two nuclei, but they were usually very, very close together, and we had the impression that we probably were sectioning through a single nucleus twice.

I appreciate the comments of the other discussers, Dr. Stocker and Dr. Shaffer. There are no questions that need to be answered concerning their discussions, however.

Dr. Irvine has asked a very difficult question of me. The usual concept is that vitreous or at least a portion of it is derived from the glia of the retina which is an ectodermal structure. Yet it has many characteristics of a connective tissue and contains collagen which would imply that it is a mesodermally derived structure. It would be more pleasing to me if the vitreous came from the mesoderm, because I am not sure I like the idea of collagen being produced by an ectodermal structure. Probably the question of the origin of vitreous cells needs to be considered separately from the question of the origin of the vast collection of extracellular material. When the truth is finally known, it will probably be that both mesoderm and ectoderm contribute chemical constituents to extracellular vitreous material.

May I comment finally that it is not really accurate to say there is *no* extracellular space in the central nervous system. There is some space, however small, between the cells, and there is evidence that at least some materials diffuse through this extracellular space between cells rather than through astrocyte cytoplasm. Furthermore, this space is able to expand during disease to accommodate not only fluids which accumulate extracellularly but also inflammatory cells.