CENTRIFUGAL NERVE FIBERS IN THE ADULT HUMAN OPTIC NERVE: 16 DAYS AFTER ENUCLEATION*

BY *J. Reimer Wolter*, MD

THE EXISTENCE OF CENTRIFUGAL (ANTIDROMIC, EFFERENT) nerve fibers in the human retina and optic nerve is still not totally accepted. Brindley[1] in his recent book on *Physiology of the Retina and Visual Pathway,* for example, states in his discussion of the anatomical evidence for centrifugal fibers in the human optic nerve and retina that: "none of these constitutes compelling evidence in favour of centrifugal fibres, and there is some evidence against." Talking about the function of centrifugal fibers to the human retina Brindley says: "If I take a more skeptical attitude towards the question of centrifugal fibres — it is in part because of the difficulty of finding any convincing suggestions of how the centrifugal control of retinal activity postulated could be of sufficient use — to justify the occupation of so much valuable space in the overcrowded optic nerve . . ." Statements like this are surprising to all those who have seen the anatomic and physiologic evidence for the existence of these centrifugal fibers.

It should be recalled that Polyak[2] wrote in 1957: "Whether all axis cylinders of the optic nerve, in man and other primates belong to the category of afferent nerves — that is those conveying impulses generated in the retina to the brain — or whether there are efferent axons, or those originating in the brain and terminating in the retina, by means of which central influences may reach the photoreceptors, is a problem still to be solved. From the investigations of Ramon y Cajal, Bouin, Dogiel, Weber and others, the presence of such fibers is fairly certain in birds and axolotl, but they have not yet been definitely shown to exist in mammals." In 1956 numerous surviving axons were found in two human optic nerve stumps 12 and 16 years after enucleation and this observation was considered the first definite evidence for the existence of cen-

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trifugal nerves in the human optic nerve.[2] Terminal branches of centrifugal nerves were discovered in the normal human optic nerve and retina[4-5] and it was concluded that at least some of these fibers supply blood vessels. The observation of interrupted neurites with terminal swellings pointing away from the disk in the nerve fiber layer of the diseased human retina again was taken as additional evidence for the existence of centrifugal fibers.[6] Attempts at regeneration of interrupted centrifugal nerve fibers were found in a child's optic nerve stump 11 days after enucleation of the corresponding eye[7] and similar fibers seen 4 days after enucleation in the optic nerve stump of another child showed terminal swellings, but they were without any signs of regeneration.[8] In 1966 the stumps of centrifugal nerve fibers were observed in the optic nerve head of an eye that had suffered occlusion of the central retinal artery.[9] Survival of numerous nerve fibers in the optic nerves and chiasm 50 years after bilateral enucleation was recorded in 1965 as further morphologic evidence for the existence of centrifugal nerve fibers in man.[10] Photocoagulation burns in the human retina were used to histologically show reactive terminal swellings of interrupted centrifugal fibers at the proximal aspects of these burns.[11-12] Sacks and Lindenberg[13] in 1969 found centrifugal nerve fibers in the optic nerve of a patient with a bilateral congenital ocular anomaly precluding the formation of retinas and estimated the number of these fibers at about 9-10% of the normal optic nerve complement. They suspected that these centrifugal fibers came from the anterior hypothalamus. The exciting studies of Honrubia and Elliott[14-15] have added much to the understanding of the course and the distribution of centrifugal nerves in the retina of man and monkey. These authors observed the centrifugal fibers in flat mounts of the retina to emerge from the optic disk and to form many bifurcations towards the retinal periphery.

Along with the morphologic findings supporting the existence of centrifugal fibers in the anterior human visual system there were numerous reports of electrophysiologic studies indicating the presence of centrifugal elements.[1,16] These are best summarized in volume one of *Clinical Neuro-Ophthalmology* by Walsh and Hoyt.[17]

The present paper has three aims: to give additional evidence for the presence of centrifugal nerves in the human optic nerve 16 days after removal of the corresponding eye, to present the first electron micrographs of these centrifugal fibers with their terminal swellings, and to show that the centrifugal fibers in the optic nerve of this 56-year-old man are without any evidence of attempted regeneration.

CASE REPORT

This 56-year-old white male had noted a shadow coming over his left visual field early in May 1977. His local ophthalmologist made a clinical diagnosis of malignant melanoma in the left eye. This eye was enucleated on June 15, 1977 and the eye was sent to me for histopathological study.

The eye was of normal size. There was a dark brown tumor on the outside of the sclera in the area of the inferior nasal vortex vein. Transillumination showed the shadow of a large intraocular tumor involving the inferior nasal portion of the choroid. After opening the eye in the plane of the extraocular tumor extension, the intraocular tumor was also found to be of brown color and it measured 15 × 15 × 10 mm. Histologic sections showed the anterior segment of this eye to be normal except for some diffuse mononuclear infiltration in iris and ciliary body. A serous transudate was seen under the retina on and next to the tumor. The outer retinal layers and the pigment epithelium exhibited degenerative changes in the region of the tumor. Bruch's membrane had remained intact, however, and many drusen were observed on its surface in the area of the choroidal tumor. The choroidal tumor was composed of rather irregular cells most of which were unpigmented. Some of the cells were spindle shaped whereas others were of an epithelioid cell type. Most of these cells had nucleolated nuclei and occasional tumor giant cells were recognized. On its scleral side the tumor was seen to extend through the sclera along the pathway of the vortex vein. On the outside of the sclera the tumor formed a relatively well defined nodule that appeared to have been removed as a whole. The optic nerve was not involved by the choroidal neoplasm and appeared normal. A diagnosis of a large malignant choroidal melanoma of a mixed spindle B and epithelioid type with direct extraocular extension was made. Additional surgical removal of orbital contents was recommended.

The patient was admitted to this University Hospital on June 30, 1977. A partial exenteration of the left orbit was done under general anesthesia on July 1, 1977 without difficulties. Along with the inner portions of both lids, the conjunctival sac and the structures of the muscle cone of the left orbit containing a mesh-covered plastic implant as well as the optic nerve stump measuring 8 mm in length were obtained. Histological study of these tissues did not show additional malignant melanoma.

HISTOPATHOLOGY

The optic nerve stump was isolated after proper fixation in formalin and cut length-wise on the freezing microtome. The pyridine silver carbonate stain of Hicks[18] was used. Next to the distal end of the optic nerve stump there was a sharply limited zone of total destruction of all nerve fibers and most of the glia (Figure 1). Active microglia was accumulated in this zone and filled the space of the absent neuroectodermal elements. The number of axons in this optic nerve stump was found greatly reduced as compared to the number of axons seen in the normal human optic nerve. However, numerous axons were present and their number was estimated to be about one tenth of that found in a normal optic nerve (Figure 2).

Next to the zone of destruction at the end of the optic nerve stump there were numerous axons pointing with terminal swellings (end bulbs) towards the distal cut end of the nerve stump. These terminal swellings were mostly simple club-like formations with more complex structures in a few instances. No evidence of attempted regeneration was recognized (Figure 3).

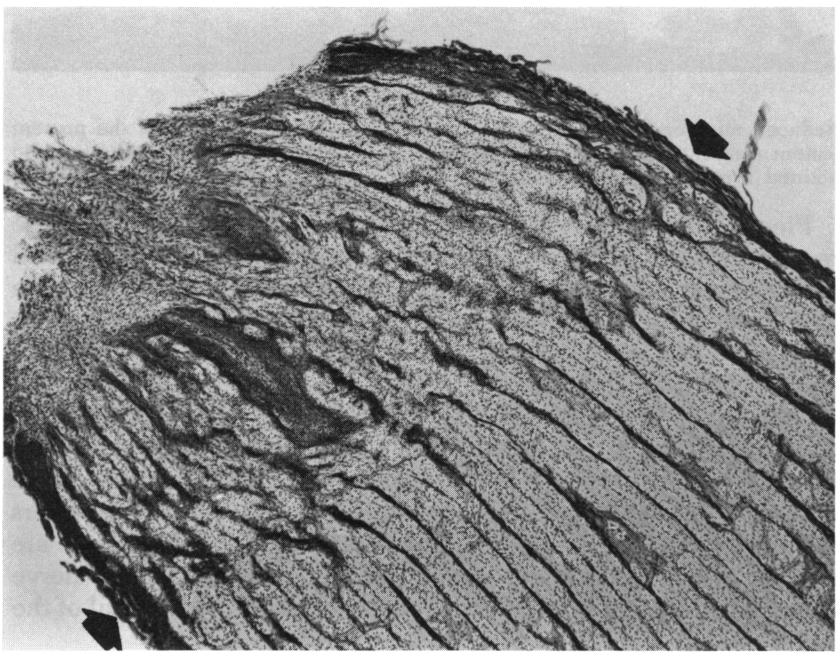

FIGURE 1

Longitudinal section of the optic nerve stump in the present case with its distal end pointing to the left and up. The zone of total destruction caused by the surgical cut 16 days before the removal of this nerve is indicated by two arrows. (Formalin fixation, frozen section, pyridine silver stain, photomicrograph × 100).

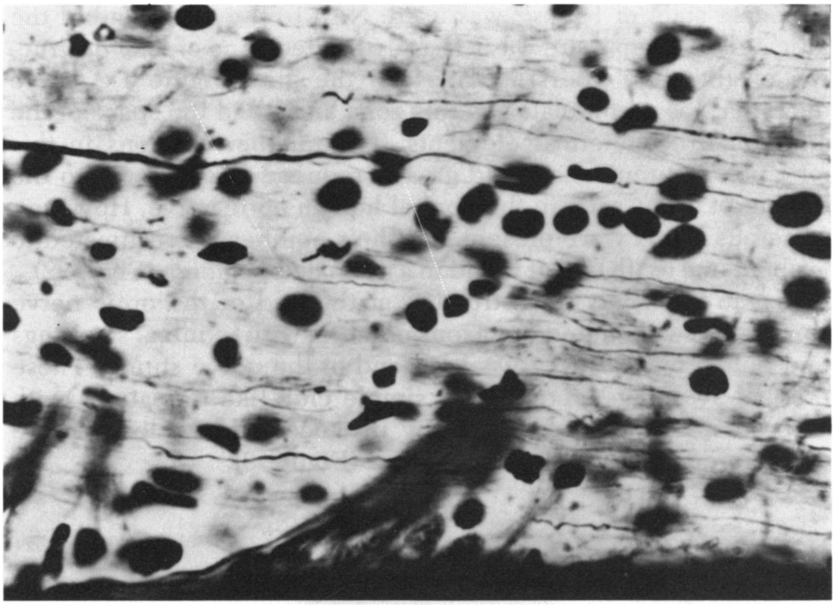

FIGURE 2

Reduced number of nerve fibers found in the optic nerve stump of the present patient running from left to right in the picture. The nuclei of neuroglia are also stained (Longitudinal frozen section, pyridine silver stain, photomicrograph × 800).

Pieces of optic nerve tissue from the zone where most of the terminal swellings had been observed by silver stain were isolated for electron microscopy. Electron micrographs clearly show the surviving nerve fibers to be surrounded by dark staining myelin (Figures 4 and 5). In many instances the electron micrographs also show portions of the swollen distal ends of the nerve stumps (Figures 6 and 7). These terminal swellings are also almost completely covered by the extended myelin sheath, but defects are seen through which the swollen substance of the axon appears to be exposed. Active glia is demonstrated between the nerve fibers (Figures 4 to 7). Very few irregular pieces of myelin (Figure 6) are all that is left of the great number of afferent (centripetal) nerve fibers which have undergone degeneration after the removal of the eye.

DISCUSSION

The presence of nerve fibers in this optic nerve stump 16 days after removal of the corresponding eye is in itself believed to be new

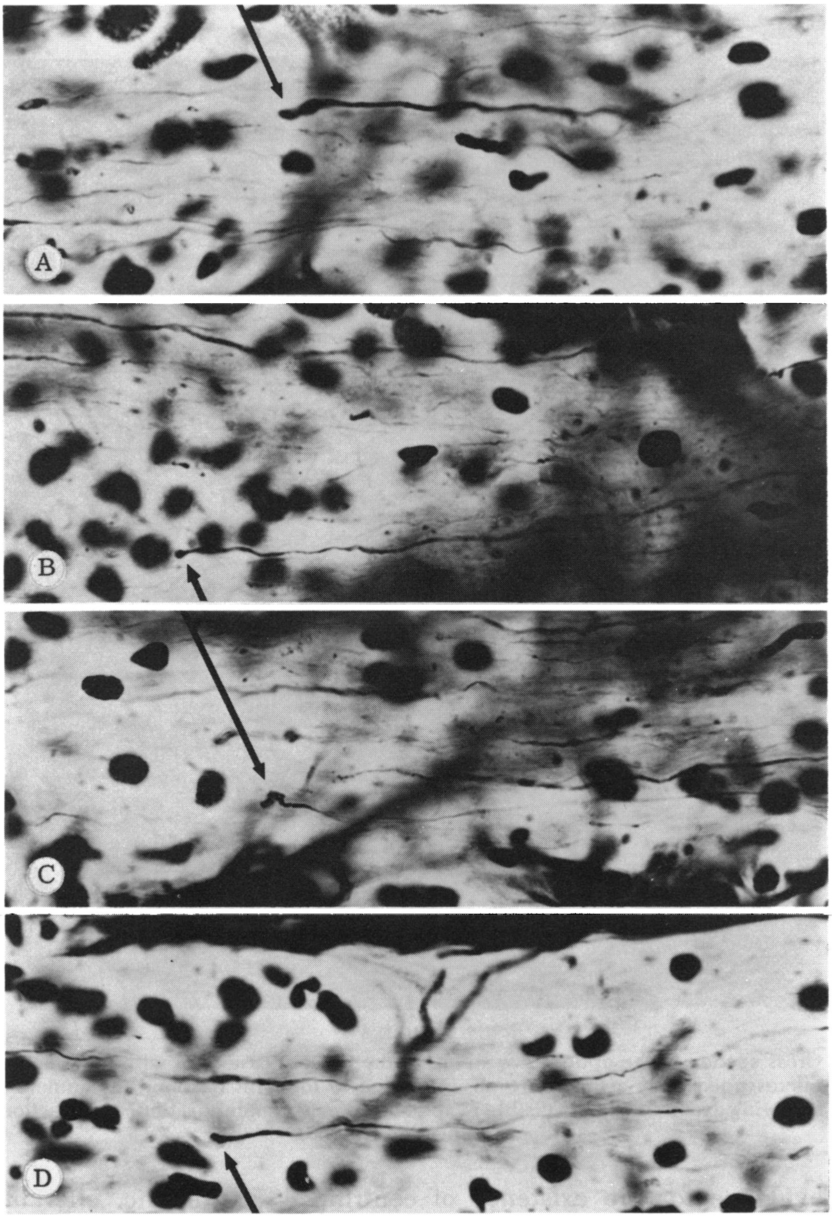

FIGURE 3

Four photographs (a,b,c and d) showing club-like terminal swellings of centrifugal nerves (arrows) pointing towards the distal end of the optic nerve stump in the present case (Longitudinal frozen sections, pyridine silver stains and photomicrographs × 800).

FIGURE 4
Cross section of the optic nerve stump in the present case seen with the electron microscope. Many artifacts are observed due to formalin fixation, but numerous surviving axons are clearly visible in spite of these (Formalin fixation, cross section, magnification × 6000).

evidence for the existence of centrifugal (antidromic, efferent) nerve fibers in the anterior human visual system. All afferent nerve fibers are expected to have degenerated and disappeared within a few days after the removal of the eye. All afferent nerves are axonal processes of ganglion cells located in the retinal nerve fiber layer of this eye, of course.

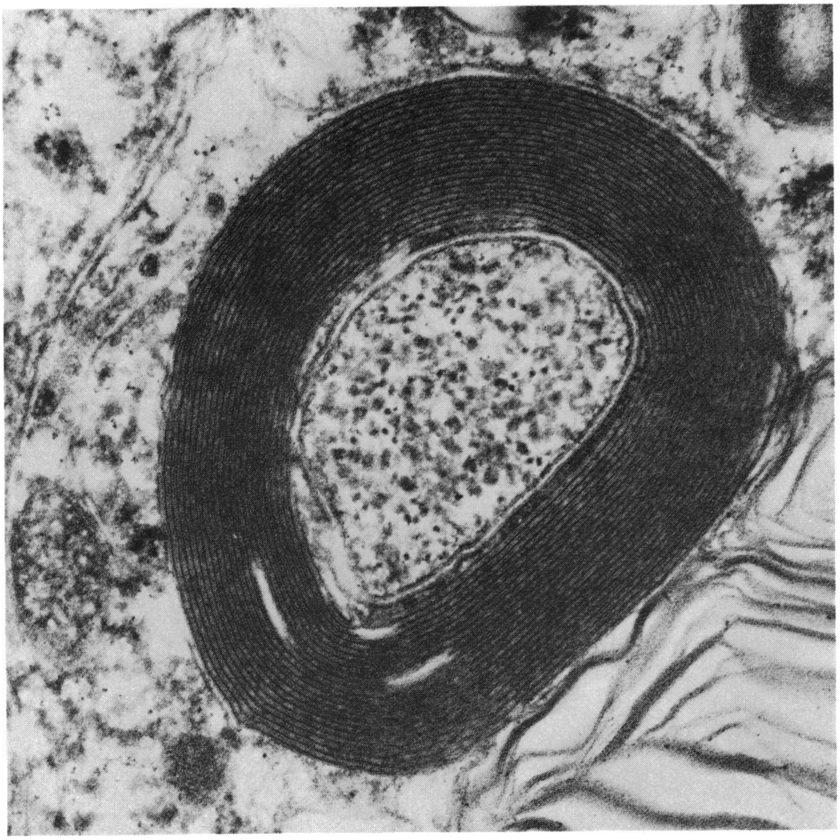

FIGURE 5
High power view of one well preserved axon with its distinct myelin sheath seen in a cross section of the present optic nerve stump (Formalin fixation, electron micrograph × 100,000).

Interrupted centrifugal nerve fibers in the optic nerve can be recognized by the direction of their terminal swellings. These point in a distal direction and most of them are found on the distal end of the optic nerve stump next to the separating surgical cut. These terminal swellings indicate axoplasmic flow towards the distal end of the optic nerve stump.

Early attempts at regeneration starting out from the terminal swellings of interrupted centrifugal nerves were observed in earlier studies[7] in the optic nerve stump of a child 11 days after enucleation of the corresponding eye, but this change was not seen

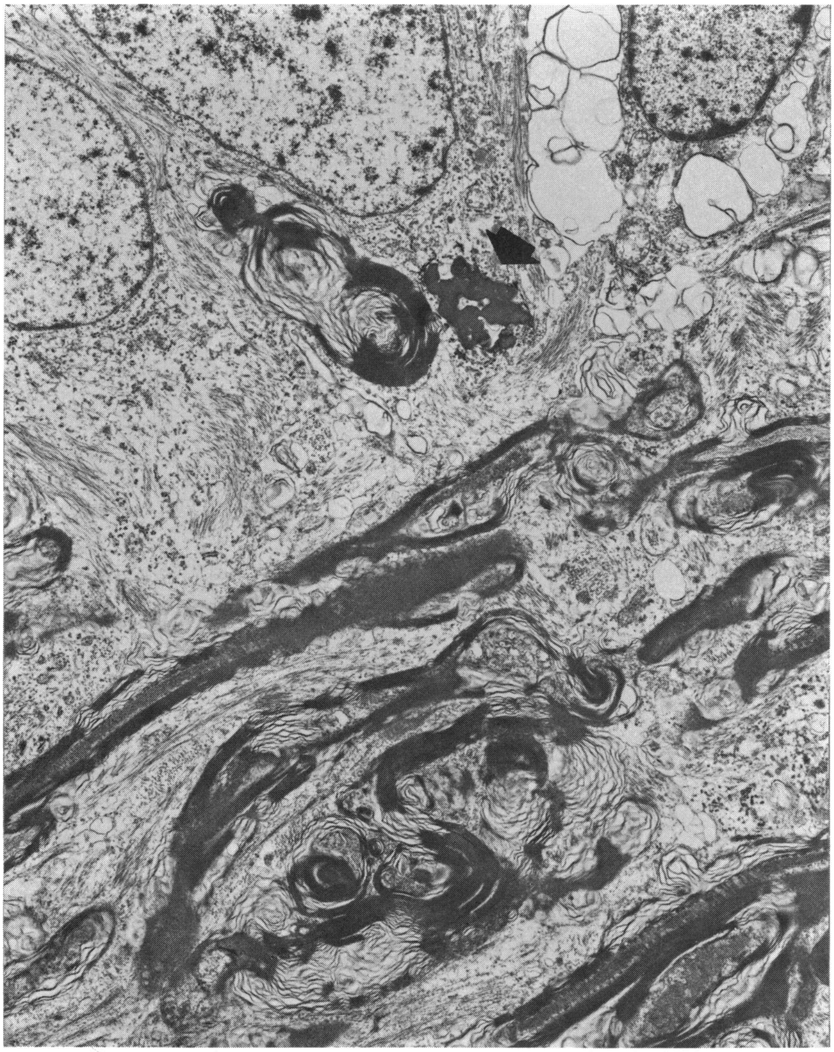

FIGURE 6

Electron micrograph of longitudinal section from an area near the distal end of the present optic nerve stump showing several terminal swellings in upper half of picture, a piece of myelin (arrow) and nuclei of active glia in lower half of picture (Formalin fixation, electron micrograph × 20,000).

4 days after enucleation in another child.[8] The absence of any attempts at regeneration in our 56-year-old man studied 16 days after enucleation is of interest. It may indicate that the regeneration

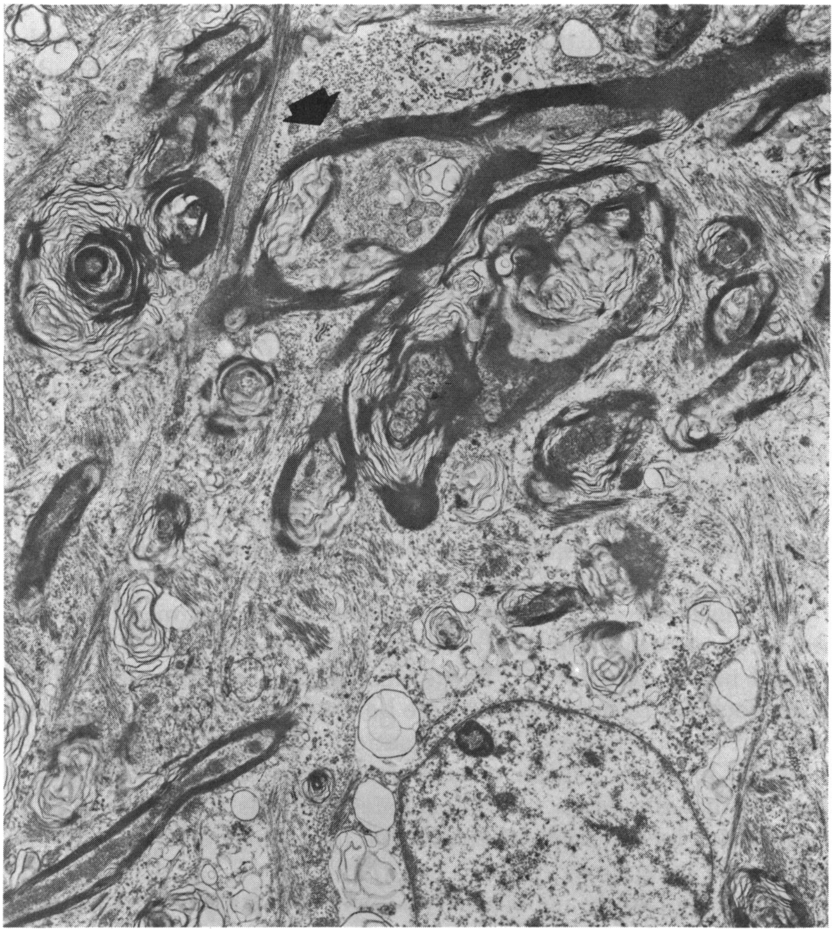

FIGURE 7
Terminal swelling of a centrifugal nerve (arrow) seen in longitudinal section. Nucleus of a glial cell and a well preserved axon seen in the lower part of the picture (Formalin fixation, electron micrograph × 20,000).

potential of the centrifugal fibers may decrease with advancing age. This fits the basic concept that repair, regrowth and regeneration in general are most exuberant early in life and decrease with age.

The number of surviving nerve fibers in the present optic nerve stump was again estimated at about 10% of the number of fibers seen in a normal human optic nerve. This observation is in agreement with our own earlier estimates[10] and with that of Sacks and Lindenberg.[13]

The present paper offers the first electron-microscopic view of centrifugal fibers of the human optic nerve. It is obvious that the numerous well preserved axons with their myelin sheaths found in the optic nerve stump cannot be afferent nerves originating in the retina of the corresponding eye that was removed 16 days before. These axons were alive and connected to their ganglion cells somewhere in the brain at the time of the removal of the nerve stump. The electron-microscopic demonstration of these nerves is new evidence for the presence of centrifugal (efferent) fibers in the human optic nerve. Electron microscopy was used not only to confirm the presence of nerve fibers in this optic nerve stump, but it was also used to get a view of the terminal swellings. It was no surprise that the centrifugal nerve fibers are myelinated and resemble in the electron micrograph by size and shape all other nerve fibers seen in the human optic nerve under normal conditions.[17,19] It is of some interest that the myelin sheath is seen to extend in an obvious attempt to surround the terminal swellings. However, there appear to be defects in this covering where the swollen substance of the axonal core is exposed.

The observation of efferent nerve fibers in the human inner ear are an interesting parallel to the findings in the optic nerve discussed in this paper.[20,21,22] These nerves are sometimes called the olivo-cochlear bundle and they are believed to control the auditory input at the periphery. Their function has been compared to a feedback loop and modern neurophysiologists and neuroanatomists believe that the brain in all instances controls the sources of its information by sending out centrifugal (efferent) nerve fibers to the peripheral receptors.

The present study again is only a very small step towards a better understanding of the efferent component of the anterior visual pathway in man. However, it is hoped that it will stimulate the interest of those investigators who have the knowledge and the facilities to reveal the origin and function of this efferent nerve system.

SUMMARY

The optic nerve stump of a 56-year-old patient was removed 16 days after enucleation of the corresponding eyeball. The stumps of numerous centrifugal (efferent) nerve fibers are demonstrated histologically in this optic nerve central to the 16-day-old surgical cut.

Electron-microscopic views of the centrifugal nerve fibers are offered for the first time. These findings are further evidence for the existence of centrifugal fibers in the human optic nerve. The nerve fiber stumps exhibit reactive terminal swellings pointing towards the surgical cut indicating axoplasmic flow in that direction. It is of special interest that the centrifugal nerve fibers of this 56-year-old patient lack any evidence of attempted regeneration that has been observed under similar conditions in the optic nerve stump of a child.

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DISCUSSION

DR JOHN WOODWORTH HENDERSON. This important paper is a further confirmation of the thesis which Doctor Wolter presented for membership in the American Ophthalmological Society in 1965. He demonstrated surviving centrifugal fibers in an optic nerve examined ten days after occlusion of the central retinal artery, and showed also surviving centrifugal nerve fibers in both the chiasm and optic nerves in a brain specimen 50 years after bilateral enucleation. The present paper adds important electron-microscopic evidence to support the existence of such nerve fibers.

Despite this evidence and that of many other workers in such species as fish, dog, cat and monkey, as well as the work in human retinal flat mounts by Honrubia and Elliott, there still remains a reluctance to accept the existence of centrifugal fibers by other investigators.

This attitude is probably due to the fact that a nucleus of origin for these fibers has not yet been demonstrated in man. As recently as 1976, Pearlman and Hughes in their work on efferent fibers in the control of retinal ganglion cell receptive fields in the pigeon, stated that a great deal of controversy surrounds the question of the existence of efferent fibers in species other than birds, noting that only in the bird has a nucleus of origin been clearly demonstrated.

These authors note that efferent fibers to the retina in the bird arise in the isthmo-optic nucleus, a distinct cell mass in the caudal midbrain and form the final link in a closed nerve loop. In the bird, each point on the retina projects to a restricted area of the tectum and there is a similar retinotopic projection from the tectum to the isthmo-optic nucleus which is also arranged in a retinotopic manner.

The projection back to the retina from the isthmo-optic nucleus reaches the same retinal quadrant from which the original input arises.

The centrifugal fibers have been shown in birds to terminate in retinal amacrine cells along the inner aspect of the inner nuclear layer.

Hoyt and Walsh point out that some and probably the majority of the centrifugal fibers synapse with amacrine cells in other species as well and that the multiple synapses of the amacrine cells with ganglion cells would permit relatively few efferent fibers to influence a large number of ganglion cells. This correlates with Doctor Wolter's observation that only about

10% of the optic nerve fibers are efferent — a figure also presented by Sacks and Lindenberg.

Hoyt and Walsh also note that Ogden and Brown's electrophysiologic investigations of these fiber systems in monkeys suggest that they function in an inhibitory retinal feed-back system which modifies the output of receptors and the input of ganglion cells.

As far as man is concerned, we still are not certain of the origin of the fibers or their specific sites of termination within the eye. Wolter and Liss concluded in earlier papers that at least some of the fibers supply blood vessels. In his earlier thesis, Doctor Wolter found some thin unmyelinated centrifugal nerve fibers coming into the chiasm from the direction of the pituitary stalk. Sacks and Lindenberg suspected that they arose in the anterior hypothalamus in their reported case.

It is probable that the efferent fibers in man reach retinal amacrine cells as a part of a feed-back loop — a mechanism by which the brain controls the sources of its information by sending out efferent nerve fibers to the peripheral sensory receptors.

The total feedback system in man must be more complex than that of the pigeon where visual reception is a midbrain rather than a cortical function.

The author is to be congratulated on a further important addition to his long study on centrifugal optic nerve fibers. His skill with silver staining techniques has now been supplemented by the electron microscope.

I would like to ask whether he has any further theories on the site of origin and possible function of these fibers. Are there any which are possibly inter-retinal in origin?

DR MICHEL MATHIEU. Mr President, Mr Secretary, Gentlemen. I was most interested in Doctor Wolter's presentation because we did some work on the subject some years ago. In fact, it was Doctor Wolter's initial research on centrifugal nerve fibres of the retina, published in the German Archives, in 1956 and 1957 that prompted our studies. We used flat-mounted retinas with silver staining and were able to identify such fibres in man, dog, rabbit, and cat.

I would like to show you two figures taken from a paper we published in the Archives of Ophthalmology in 1960. The first is a sketch showing the way these fibres seem to lie in the dog retina. They come out of the disc and proceed quite directly to the periphery where they branch and curl somewhat. The second slide is a microphotograph of these exogenous fibres in man. As you can see and as Doctor Wolter said, they appear at the disc and branch as they go towards the periphery. Therefore these fibres originate outside the eye.

For sometime we tried to find the origin of these fibres. We made sections at different levels of the optical pathways in rabbits. The eyes were enucleated two months later. Retrograde degeneration of the exogenous fibres indicated that the section had been made between the eye and the cell bodies of these fibres. We were only able to find that these cells were quite far back. We were not able to localize them in a more precise way.

These exogenous fibres have certainly a role to play in the physiology of the eye and much more has to be learned about them. I would like to compliment Doctor Wolter for this new and excellent contribution on the subject and ask him if he believes that the time when we will be able to find the origin of these fibres is in the near future.

DR JOEL SACKS. Doctor McPherson, Doctor Hollenhorst, members, and guests: Doctor Wolter is to be congratulated for pushing back the frontiers of knowledge by more of his meticulous work in the laboratory. He is gracious enough, as was Doctor Henderson, to refer to some related work that Doctor Lindenberg and I reported (Sacks JG, Lindenberg R: Efferent nerve fibers in the anterior visual pathways in bilateral congenital cystic eyeballs. *Am J Ophthalmol* 68:691-695, 1969). We showed that the efferent fibers did go from the brain forward through the optic nerve. We traced the origin of the fibers to the hypothalamus. This is an area which regulates salt and water metabolism and probably contains osmoreceptors. As I recall, a few years ago the glaucoma group at Washington University in St. Louis published a paper in which they suggested that these fibers had something to do with the control of intraocular pressure. They showed that microinjections of hypertonic solutions in that area of the brain rapidly affected intraocular pressure. Perhaps Doctor Kolker can comment about this.

While we do not yet have firm answers as to the function of these fibers, Doctor Wolter, we cannot resist speculating about them. I would be grateful to know what your inner thoughts are and if there is any possibility in your mind that these fibers do control intraocular pressure.

DR J. REIMER WOLTER. In the first place I would like to thank Doctor Henderson not only for the thorough discussion of my paper, but also for the generous support and friendship that he has given me for my more than 25 years in Ann Arbor. For the last ten years Doctor Henderson has been to me both, a friend and a chairman — a rare and precious combination, as you well know.

I would also like to thank the Doctors Mathieu and Sacks for their comments and questions. The origin of the centrifugal fibers in the optic nerve would be of the greatest interest, of course. My earlier attempts at tracing these fibers back to their origin in the brain of a patient who had a bilateral enucleation fifty years before his death have failed due to lack of time, proper training, and knowledge. The distribution of the centrifugal nerves in the retina is now also known, but we don't know what these fibers do. One of the next steps would be to find clinical conditions where the centrifugal nerves have become atrophic while the afferent (centripetal) fibers persist. Study of these latter cases would very likely give clues to the functions of centrifugal nerves.

A question of the greatest importance is whether or not some of these fibers could find their way to the ciliary body. Years ago I have described tremendous reactive nerve changes in the ciliary body of cases with early open angle glaucoma *(Arch Ophthalmol* 62:99, 1959). In earlier studies I looked very carefully for nerves that cross the ora serrata to go from the retina to the ciliary body, but I did not find any. I did find whole bundles of nerves bridging Elschnig's marginal tissue between optic disk and choroid. With these bundles, nerves certainly could get from the optic nerve to the ciliary body and, thus, explain a change in intraocular pressure regulation after cutting of the optic nerve.

It is my hope that a team of workers better qualified than I will go on and discover the origin and the functions of the centrifugal nerve component in the human visual system.