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DEGENERATION AND DISTRIBUTION OF EFFERENT NERVE FIBERS IN THE GUINEA PIG ORGAN OF CORTI. A LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY

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SUMMARY

After brain stem lesions interrupting the efferent innervation to the inner ear in guinea pigs, the degeneration and the distribution of efferent nerve fibers in the organ of Corti were studied in surface preparations stained by the zinc iodide–osmic acid (ZIO) technique. Light microscopy, transmission electron, and scanning electron microscopy were used to examine tissue from animals with postoperative survival times of 1–41 days.

Following section of the crossed efferent bundle alone, degenerative changes were first evident in the outer hair cell (OHC) innervation as early as 1 day after placement of the brain stem lesion. Degeneration in the inner spiral (ISB) and spiral tunnel (STB) bundles was seen after about 3 days. After transection of both components of the efferent nerve supply all neural elements stained by the ZIO method degenerated and disappeared. This finding indicates a high specificity of the stain for the efferent nerve fibers.

It was found that both radially and spirally oriented efferent fibers innervate the OHC. The spiral fibers follow long, complex courses directed toward either the base or the apex of Corti's organ. These fibers predominate in the upper cochlear turns where they sometimes meander into the region of Hensen's cells. Radial fibers comprise the major efferent supply to the OHC in the basal portion of the cochlea. Fibers coursing toward both the base and apex were seen in the inner spiral and spiral tunnel bundles. A close structural relationship was noted between these two bundles, suggesting that the STB may, in part, serve in an accessory capacity to the ISB.

Single nerve fibers were often seen contacting the supranuclear portions of both inner and outer hair cells. These climbing efferent fibers were particularly numerous in the upper turns of the organ of Corti.

INTRODUCTION

Most investigators now agree that the cochlea receives an extensive efferent nerve supply which originates in the pontine brain stem and terminates within the organ of Corti. This concept is supported by ultrastructural studies demonstrating degeneration of the highly vesiculated nerve terminals and fibers supplying the cochlear hair cells following experimental lesions along the course of the efferent pathway[9,11,22,25]. In addition to elucidating the behavior of the cochlear efferents during degeneration, these studies have contributed much to our understanding of the organization and fine structure of these nerve fibers.

Although electron microscopy is of great value in the investigation of cochlear innervation, it is by practical necessity a sampling technique limited to study of minute fragments of inner ear tissues. In the present study, the distribution of degenerating nerve fibers in the guinea pig cochlea has been examined by the surface preparation or whole-mount method[5]. Surface preparations provide the investigator with a comprehensive view of Corti's organ in which the sensory cells, supporting structures, and neural elements can be studied together and in normal relation to one another in a single tissue specimen. The combination of the zinc iodide–osmic acid (ZIO) nerve stain of Maillet[15], which has been shown by Engström *et al.*[4] to be highly specific for the efferent innervation, with the surface preparation technique thus allowed a survey of the nerve supply from a perspective not possible by other methods. This approach permitted assessment of degeneration in all turns of the cochlea from animals surviving for various time periods after interruption of the efferent nerve supply. Moreover, the surface preparation method proved advantageous for study of the efferent system 'wiring diagram' of the organ of Corti. In cochleas displaying advanced degeneration, many nerve fibers disappeared entirely, providing a simplified view of the normally dense innervation pattern. In such preparations individual fibers (which would otherwise be lost in the tightly packed nerve bundles) could often be traced over considerable distances.

Finally, the surface preparations obtained in this investigation provided the basis for a productive combination of light microscopy with additional observations using the scanning electron microscope (SEM). Segments of the organ of Corti were first examined and photographed using the light microscope and then reprocessed for study in the SEM with its capabilities of high magnification and resolution, and great depth of field.

METHODS

Thirty-six young guinea pigs 150–300 g in weight provided experimental data for the present study. The crossed efferent fibers alone were sectioned in 22 of these, while both the crossed and uncrossed portions of the efferent tract were interrupted on one side in the remaining 14 animals.

Surgical anesthesia was induced by intraperitoneal injection of Ketamine hydrochloride, 130 mg/kg; this was supplemented when necessary by local anesthesia.

The operative approach was identical for both types of lesions. The dorsal part of the occipital bone was exposed and a small portion of the bone surrounding the foramen magnum was removed, after which the exposed dura was incised and the cerebellum gently freed from the surface of the posterior brain stem. The lesions were made by small knives inserted beneath the cerebellum into the fourth ventricle for predetermined distances rostral to the obex. The crossed efferent fibers were sectioned in the midline, between the facial colliculi. When both components of the efferent system were to be interrupted, the knife was displaced 2–3 mm lateral to the midline and an incision 1.5–2.0 mm deep was made at the same rostro-caudal level as for the midline lesions. The placement of all lesions was checked histologically by examination of brain stem sections prepared after the method of Fox and Eichman[7].

Table I shows the survival times permitted the animals with the two types of lesions.

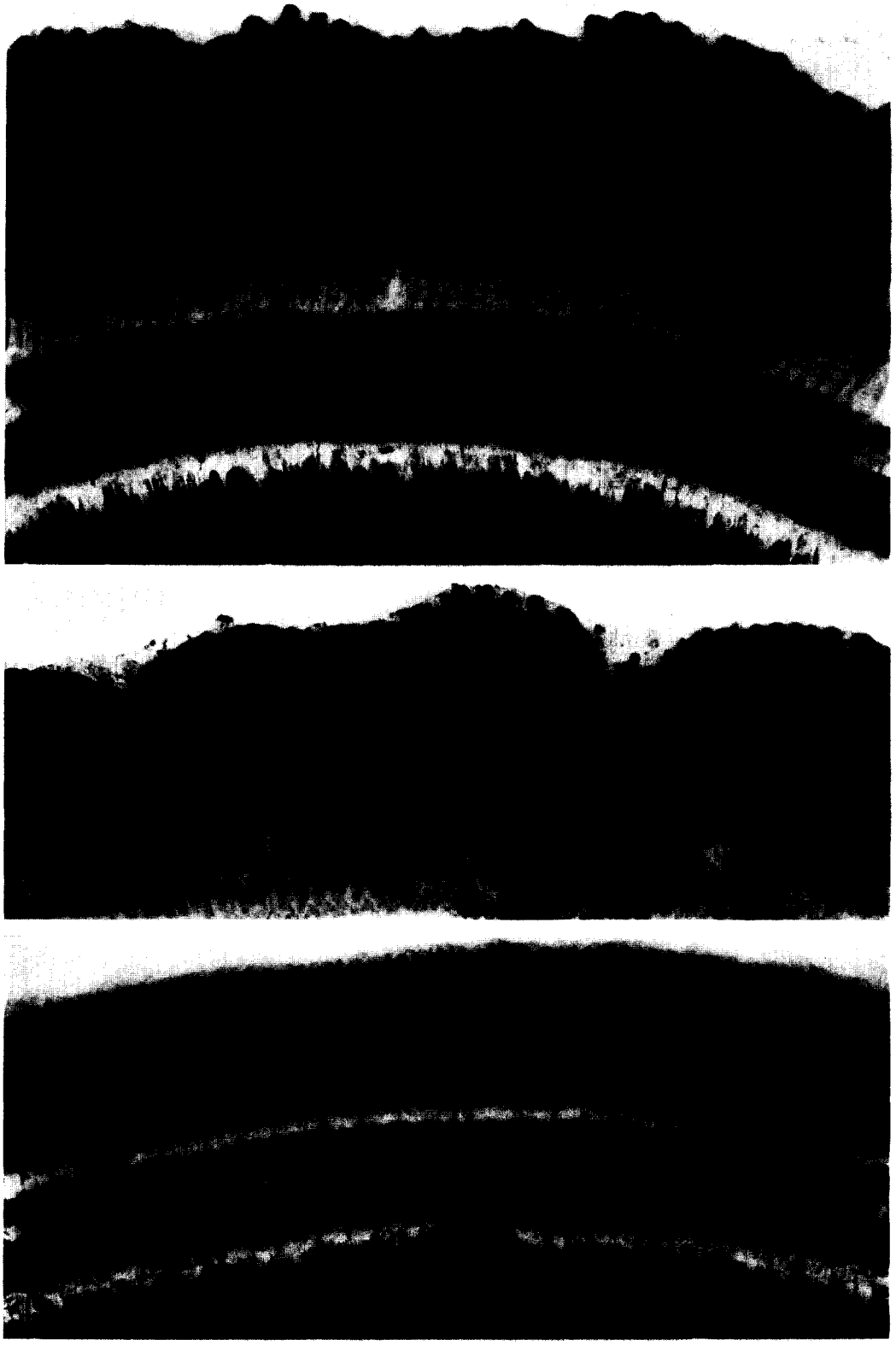
Immediately after decapitation, the otic capsules were exposed *in situ* and fixed by perilymphatic perfusion with the ZIO staining solution. The temporal bones were then removed and the inner ears perfused again before being placed in the staining fluid for 20–22 h. The preparation of the nerve stain and the general procedure used have been described by Engström *et al.*[4]. The glycerol-mounted preparations were studied and photographed using bright-field light microscopy.

Fragments of the organ of Corti to be examined by transmission electron microscopy were dehydrated and then embedded in Araldite M mixture according to the schedule of Luft[14]. Thin sections were stained with uranyl acetate and lead citrate. To examine glycerol-mounted segments in the SEM, we soaked the slides in 70% ethanol, rehydrated the tissue, and froze it in nitrogen-cooled Freon at —160 °C. Drying was accomplished on a cooled copper block in a vacuum of 5×10^{-2} torr. With the block cooled to liquid nitrogen temperature, sublimation occurred in 4–5 h, and the block and specimens were left at reduced pressure overnight or until they had returned to room temperature. After mounting tissue fragments on supports with conductive paint, a layer of gold (10–20 nm) was applied in a glow-discharge specimen coater.

Normal tissue specimens were obtained from healthy, unoperated guinea pigs and processed as outlined above. In addition to a number of normal cochleas that

TABLE I

Postoperative survival time (days)	*Number of animals*		*Postoperative survival time (days)*	*Number of animals*	
	Midline lesions	*Lateral lesions*		*Midline lesions*	*Lateral lesions*
1	2	2	8	—	1
2	2	2	15	2	1
3	1	2	18	1	—
4	3	3	21	—	1
5	2	2	28	2	—
6	3	—	30	1	—
7	1	—	40, 41	1 ea.	—



were examined throughout the course of the study, a control animal was run in parallel with each of 5 selected experimental animals. The controls were killed simultaneously with the operated animals and both were processed using the same staining solutions and identical staining times. The normal and experimental animals were of the same age and were obtained from the same supplier.

RESULTS

The innervation of the normal guinea pig organ of Corti stained by the ZIO method has been described and beautifully illustrated by Kohonen[12] and by Engström *et al.*[4]. The present report will, therefore, not include an extensive description of the normal innervation pattern as displayed in ZIO-stained preparations but will be concerned primarily with material obtained from lesioned animals.

Briefly, the highly vesiculated nerve endings and fibers associated with the outer hair cells, the upper tunnel-crossing fibers, the spiral tunnel bundle (STB), and the inner spiral bundle (ISB) are all stained intensely and clearly seen in surface preparations impregnated by the ZIO nerve stain. Examples of such preparations from normal animals are shown in Figs. 1 and 5.

The degenerative sequence

Midline lesions. In animals with lesions of the crossed efferent bundle alone, evidence of degenerative changes first appeared in the outer hair cell innervation and tunnel-crossing fibers. These changes were observed less than 24 h after placement of the brain stem lesion. Fig. 2 is an electron micrograph demonstrating vacuolization and clumping of vesicles in a large nerve terminal on a first row outer hair cell from the second cochlear turn of an animal killed 18 h postoperatively. Similar abnormalities were noted in nerve endings on other hair cells from this animal. As early as 1 day after placement of the lesion, densely stained, swollen nerve fibers were found beneath the outer hair cells by light microscopy. Figs. 6 and 13 illustrate surface preparations in which such fibers can be seen. The appearance of swollen nerve fibers was followed by disintegration of the fiber bundles and large nerve endings on the sensory cells, as illustrated in Fig. 3. The disintegrating neural structures often appeared to coalesce, forming large, irregular droplets of osmiophilic material. Finally, there was complete disappearance of a positive staining reaction over large areas of the outer spiral bundle region in all turns of the organ of Corti. Particularly in the initial stages of the process,

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Fig. 1. Surface preparations of normal guinea pig organ of Corti stained by the ZIO method. A shows the various bundles of ZIO-positive nerve fibers as they appear in the third turn. ISB, inner spiral bundle; STB, spiral tunnel bundle; arrows, bundles of tunnel-crossing fibers; OSB, spiral bundles of fibers beneath first row of outer hair cells on which large clusters of nerve endings can be seen; HC, Hensen's cells containing osmiophilic lipid droplets; MN, myelinated nerve fibers in osseous spiral lamina. In B the many spiral fibers that constitute the outer spiral bundles in the apical turn are displayed. C demonstrates the innervation pattern in the second turn. 1,2,3, nerve endings on first, second, and third rows of outer hair cells. (Endings on third row appear as a dark band slightly out of focus.)

Fig. 2. Electron micrograph of first row outer hair cell (OHC) with nerve endings clustered about its basal end. The arrow at right indicates an abnormal efferent terminal showing vacuolization and clumping of synaptic vesicles 18 h after interruption of the crossed efferent bundle.

the degeneration pattern tended to assume an irregular, patchy appearance. Patches of degeneration were found alternating with areas where the nerve fibers and endings appeared quite normal. This pattern of degeneration is illustrated in Fig. 4. In every case the neural elements beneath all 3 rows of outer hair cells seemed to degenerate simultaneously.

Fig. 3. Degenerating spironeuron (arrows) in lower third cochlear turn. Note fragmentation of the fiber and swollen terminal at extreme right. Brain stem lesion at midline; 10-day survival time. P, outer pillar cells; 1, 2, 3, nerve bundles beneath first, second, and third row outer hair cells.

Degeneration involving the inner spiral bundle was usually characterized by a more or less uniform thinning of the bundle. However, a patchy loss of nerve fibers like that seen beneath the outer hair cells was sometimes evident in animals with short survival times. ISB degeneration in an animal killed 4 days after a midline lesion is shown in Fig. 5.

Degeneration of the spiral tunnel bundle always paralleled that of the inner spiral bundle in both time of onset and extent. In preparations displaying extensive degeneration of the outer hair cell innervation with little or no inner spiral fiber loss, the tunnel bundle appeared normal. However, whenever a widespread degeneration of inner spiral fibers was apparent there was a corresponding loss of fibers within the tunnel bundle.

Lateral lesions. The sequence of degenerative changes in animals subjected to lateral brain stem lesions, which interrupted both the crossed and uncrossed components of the efferent system, was similar to that following section of the crossed bundle alone. Thus, degenerative changes in nerve fibers supplying the outer hair cells were evident 1 day after placement of the lesion. Also, as in the midline lesion group, a patchy degeneration pattern was seen in the initial phase of the degeneration sequence. Unlike those in animals with midline lesions, however, all nerve fibers normally impregnated by the ZIO stain eventually disappeared in animals having lateral lesions. After postoperative survival times of 5 or more days there was little or no positive staining reaction in the organ of Corti.

The characteristics of the ZIO nerve stain did not permit us to make a reliable quantitative assessment of nerve fiber loss in either of the two groups of experimental animals at various postoperative survival times. A survey of preparations from control animals showed that the stain sometimes fails to impregnate all nerve fibers uniformly.

Time of onset and localization of changes. In both groups of experimental animals degenerative changes were first apparent in the outer hair cell innervation and the

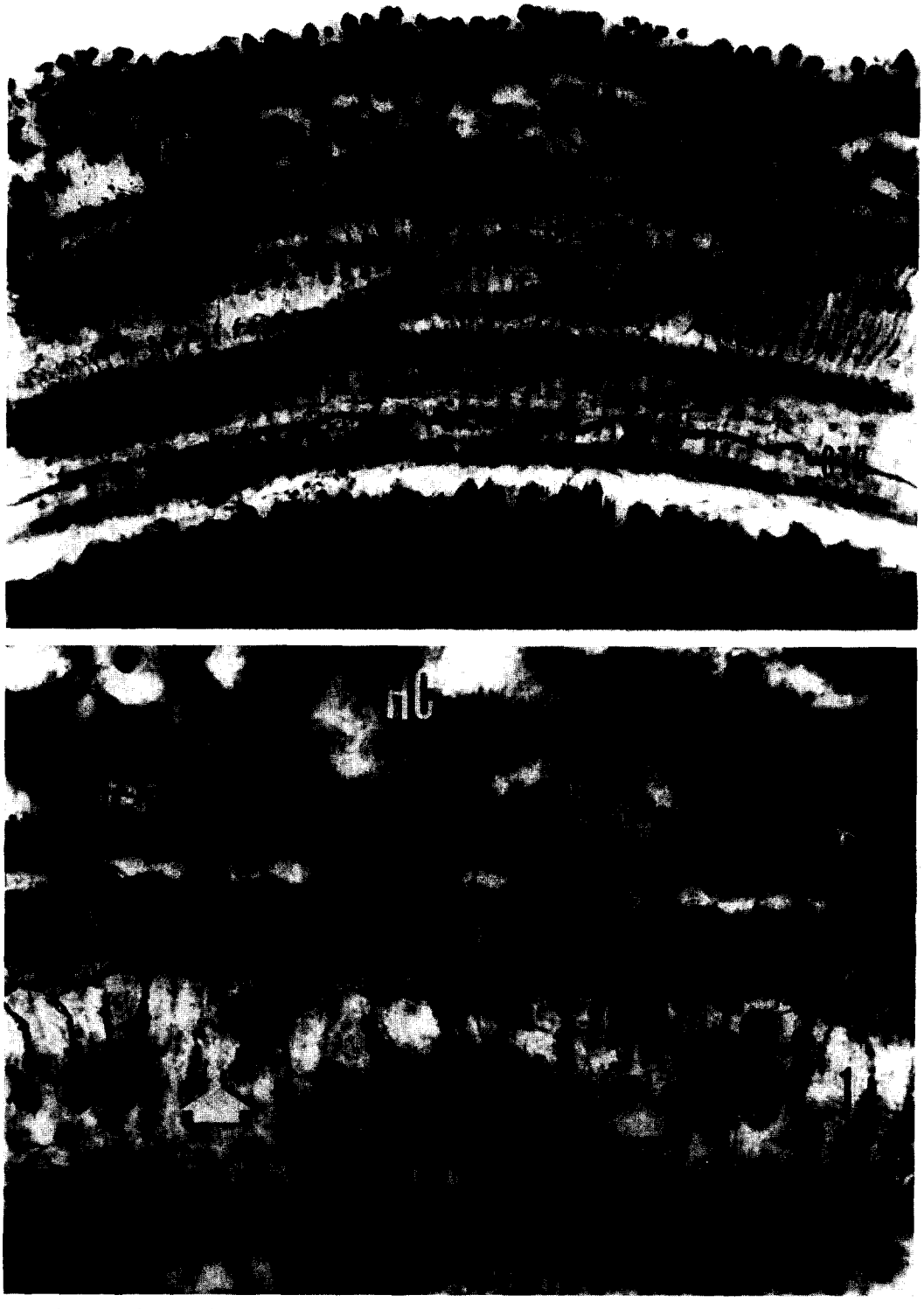

Fig. 4. Patchy neural degeneration beneath outer hair cells of the upper third turn. The central area in A is shown at higher magnification in B. Large, upward-pointing arrows indicate areas of degeneration of neural elements; small, downward-pointing arrows show darkly stained clumps of degenerating neural matter. Brain stem lesion at midline; 6-day survival time. STB, spiral tunnel bundle; 1,2,3, neural elements under first, second, and third row outer hair cells; HC, Hensen's cells containing many osmiophilic lipid droplets.

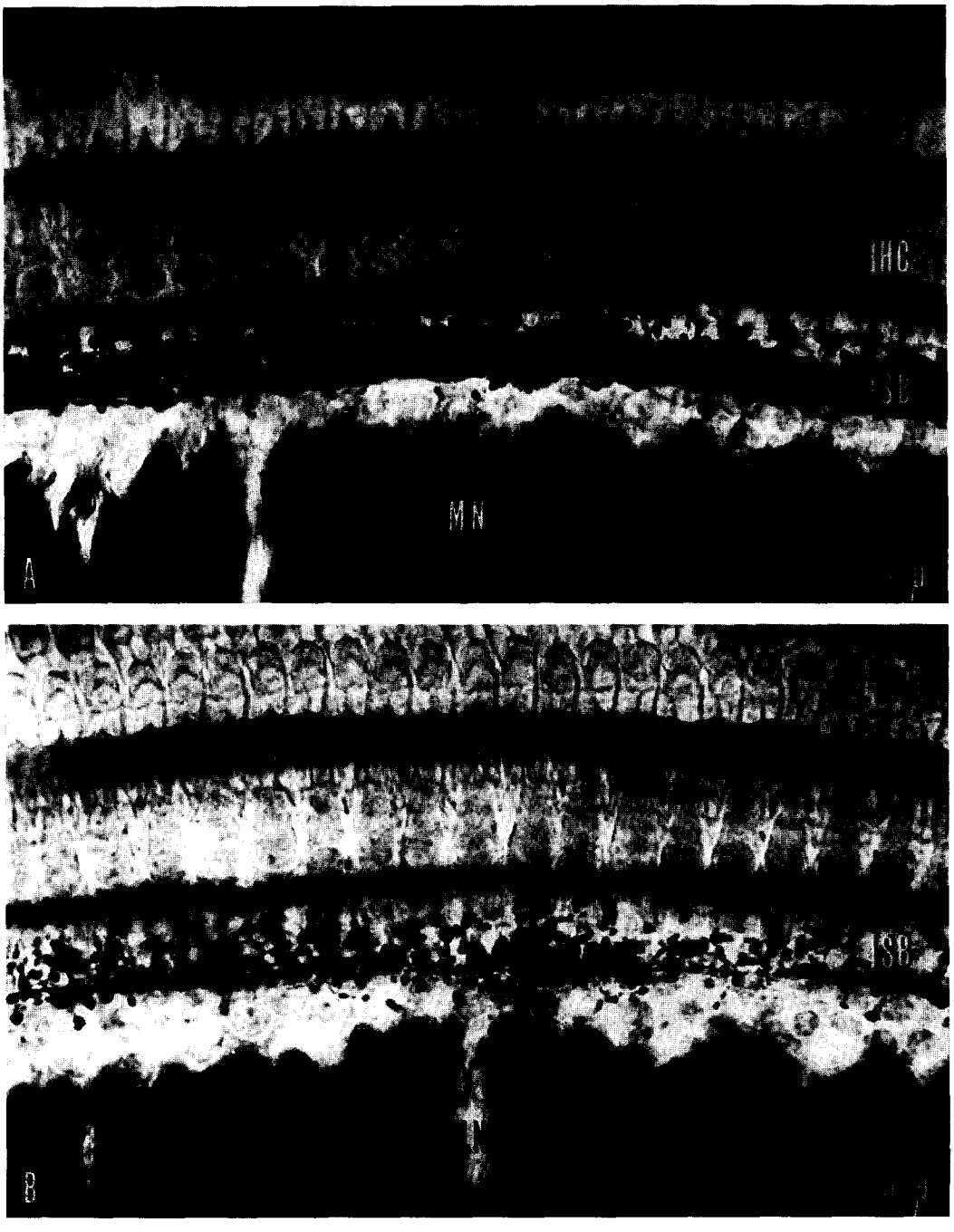

Fig. 5. A: normal inner spiral bundle (ISB) in third turn of a ZIO-stained preparation. B: ISB from third turn showing loss of neural elements 4 days after placement of a brain stem lesion in the midline. MN, myelinated nerve fibers in osseous lamina; IHC, inner hair cells.

tunnel-crossing fibers. However, we noted considerable variation from animal to animal in time of onset and rate of development of the degenerative process. Thus, in two animals killed 24 h postoperatively, neural degeneration was obvious in all turns of Corti's organ, while in other animals killed at survival times of 3 and 4 days only a few swollen nerve fibers (which might appear in any turn) could be found in an otherwise normal innervation pattern. We did, however, see extensive disintegration of nerve fibers and endings in all animals with postoperative survival times of 5 or more days.

After both midline and lateral brain stem lesions, the time of onset of degeneration in the inner spiral and tunnel bundles tended to lag behind that in the outer hair cell innervation. Some evidence of degeneration was apparent in most cases in the outer spiral bundle area 1 or 2 days postoperatively. The inner spiral and tunnel bundles did not consistently display degenerative changes until 2 or 3 days later.

In an effort to determine which portion of Corti's organ is first affected by neural degeneration, a number of cochleas from animals with short postoperative survival times were surveyed to locate the area of maximum degeneration. This was done upon the assumption that the greatest loss of nerve fibers would be found in the area where the degenerative process had begun. An examination of 12 ears from animals surviving 1–4 days revealed maximum degeneration of the outer hair cell innervation in the middle portion of the cochlea (turns 2 and 3) in 7 cases. Of the remaining 5 ears, 2 showed maximum degeneration in the base and 3 in the apex. A survey of 8 ears from animals with midline lesions having survival times of 4 and 5 days show maximum degeneration of the ISB in the apical turn in 5 cases and about equal degeneration throughout all turns in the remaining 3. Quite similar patterns were found in short survival time animals with lateral brain stem lesions. In most of these, degeneration tended to be of about equal intensity in all turns, with no well-defined focus of maximum nerve fiber loss in any one turn.

It should also be noted that no correlation could be found between the time of onset or extent of neural degeneration and the small variations in the position and depth of brain stem lesions.

Organization and distribution of nerve fibers

Outer hair cell innervation. An advantage of the method used in this study is the relative ease with which the distribution of nerve fibers within the organ of Corti can be studied. In surface preparations where most, but not all of the stained fibers had degenerated, it was often possible to make fairly detailed observations on the course taken by single nerve fibers. An example of one such fiber running beneath the outer hair cells is shown in Fig. 6. In this instance a tunnel-crossing fiber located in the third turn enters the outer spiral bundles, turns toward the apex, and finally terminates on 2 or 3 first row outer hair cells in the apical turn after meandering from row to row under some 295 outer hair cells. In the basal two-thirds of its trajectory, this fiber gives off only 4 identifiable branches, while in the apical one-third a total of 12 branches are seen, most of which terminate on first and second row hair cells.

Especially in the apical and third turns many long spiral fibers were present,

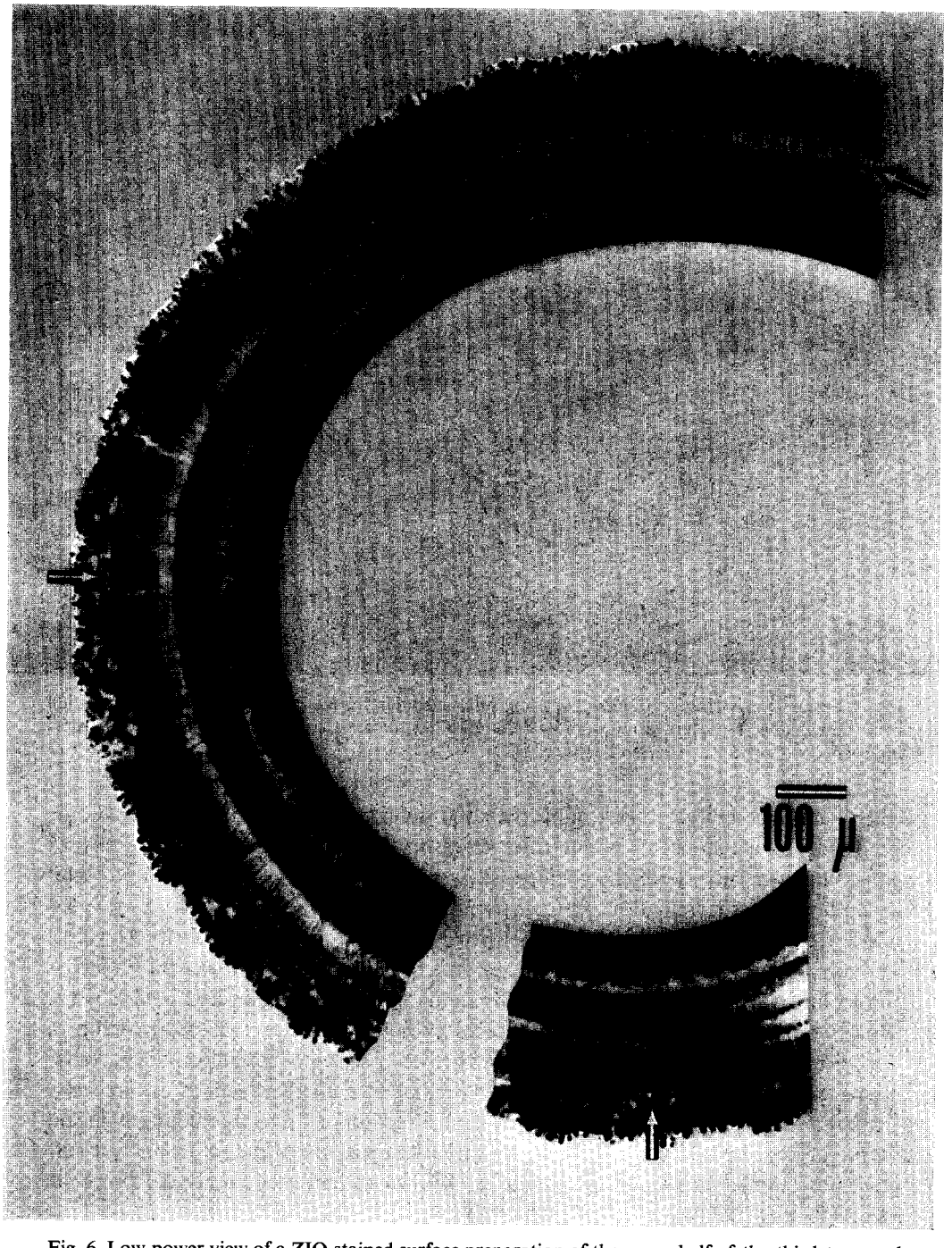

Fig. 6. Low-power view of a ZIO-stained surface preparation of the upper half of the third turn and basal portion of the apical turn from a midline-lesioned animal with a 4-day survival time. The break in the lower part of the photograph represents the scissor cut made to divide the third from the apical turn in preparation of the specimen. The arrows indicate the course of a large, swollen spironeuron which crosses the tunnel in the third turn (upper arrow) and courses toward the apex, finally terminating in the area indicated by the lower arrow.

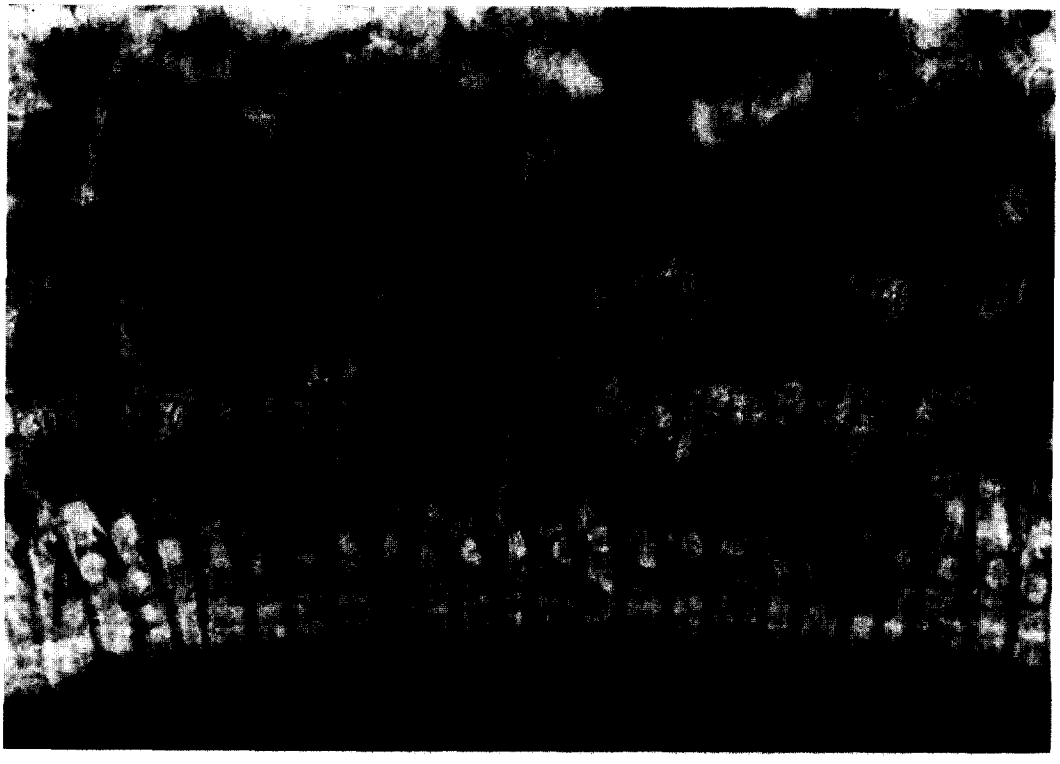

Fig. 7. Terminal arborization of a degenerating spironeuron in the lower third turn. Note the swollen nerve ending at extreme left. Brain stem lesion at midline; 10-day survival time. P, pillar cells bordering tunnel of Corti; HC, Hensen's cells.

often with courses subtending more than 100 outer hair cells. Typically these large, beaded fibers wandered from one row of sensory cells to another, traveling toward either the apex or base of the cochlea. They gave off few branches or endings until near the point of termination. In their terminal portions, these fibers usually divided into several branches supplying anywhere from 3 or 4 to about a dozen outer hair cells. The terminal arborization of a typical spironeuron located in the lower third turn is shown in Fig. 7. A single fiber sometimes supplied endings to hair cells in all 3 rows. Moreover, we saw single hair cells receive endings from nerve fibers traveling in opposite directions and lying under different rows of sensory cells. Thus, fibers coursing beneath the third row of hair cells often provided terminals to cells of the first row. Occasionally, these spiral fibers reversed their direction of travel by making 'hairpin' loops. For example, a fiber traveling toward the apex under the first hair cell row might turn out to the second row, make a 180° turn and proceed basalward along the second row of hair cells.

In all cases in which it was possible to trace a spironeuron back to the tunnel-crosser of which it was a continuation, the tunnel-crossing fiber was found either to run directly across the spiral tunnel bundle without entering it at all, or to pursue only a very short spiral course there. In a few instances, we were able to trace such fibers

Fig. 8. Terminal arborization of a radial nerve fiber supplying outer hair cells (OHC) of the second turn. Note that the fiber has endings on hair cells in all 3 rows. Brain stem lesion at midline; 10-day survival time. P, pillar cell stalks; MN, myelinated nerve fibers in osseous lamina.

back to the foramina nervosa of the habenula perforata; they always ran straight through the inner spiral bundle or, at most, coursed with it under 4 or 5 inner hair cells before turning outward to cross the tunnel.

Although we found some long spiral fibers coursing beneath as many as 220 outer hair cells in the basal and second turns, the predominant type of innervation in the lower turns of the organ of Corti appeared to be radial. That is, the majority of fibers ran directly outward, giving off short branches to a few hair cells in each of the 3 rows. Altogether, 6–12 outer hair cells were usually supplied by such arborizations, and there was extensive overlapping of areas innervated by neighboring radial fibers. An example of this type of fiber distribution is shown in Fig. 8. Like the long spiro-neurons, many of the radial nerve fibers were continuations of tunnel-crossers that ran across the spiral tunnel bundle without any significant spiral course there. Radially oriented nerve fibers were also present in the upper turns of the cochlea but they were difficult to identify due to the presence of large numbers of spiral fibers.

Inner spiral bundle. The smaller diameter fibers of the closely packed inner spiral bundle were more difficult to follow over long distances, but fibers coursing along 60–70 inner hair cells were found in all turns. The longest inner spiral fiber that could be traced ran beneath 78 hair cells in the second turn. Fibers coming from the habenula perforata were observed turning both apicalward and basalward into the inner spiral bundle. In a few cases fibers emerging from the habenula appeared to divide in the form of the letter 'T' as they entered the bundle, sending a branch in either direction.

In one preparation, a fiber came through the habenula, entered the inner spiral bundle, and ran toward the apex along 3 inner hair cells before turning outward across

the tunnel. In its short course within the ISB, this fiber gave off several small branches each of which ended in a cluster of bud-like enlargements that appeared to be nerve terminals establishing contact with other fibers of the bundle. After crossing the tunnel, this fiber also supplied endings to a number of first and second row outer hair cells.

In many specimens, the inner spiral bundle appeared to consist of a compact, central core of spironeurons surrounded by a dense tangle of profusely beaded fibers somewhat resembling a coil of barbed wire wrapped haphazardly around a telephone transmission cable. Even in preparations where many fibers had degenerated, it was very difficult to identify branches and nerve endings belonging to a particular ISB fiber.

Spiral tunnel bundle. The inner spiral and tunnel bundles are interconnected by closely spaced, radial fascicles of fibers that run between the inner pillar cells (see Fig. 1). In our specimens with simplified innervation patterns we found fibers entering the tunnel bundle via these radiating fascicles which turned to course either apicalward or basalward; occasionally they appeared to split, sending a process in each direction. Spiral fibers were followed in the tunnel bundle over as many as 50 inner hair cells, and fibers running along 30–40 inner hair cells were found in all turns. However, it was usually impossible to discover the point of entry or destination of these fibers. Few of the darkly stained spiral fibers in the tunnel bundle seemed to turn outward and cross the tunnel to the outer hair cells. As indicated above, the majority of tunnel-

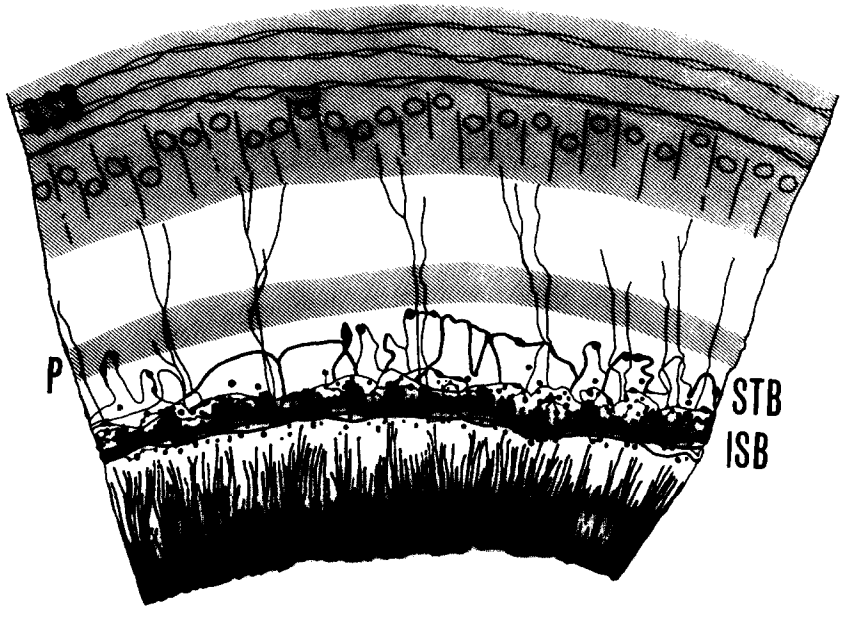

Fig. 9. Camera lucida drawing illustrating the spiral tunnel bundle (STB) breaking up into a series of arcades that originate from the inner spiral bundle (ISB) near the apex of Corti's organ. The STB and ISB were drawn from an actual specimen. The myelinated nerve fibers (MN), pillar cell area (P), tunnel-crossing fibers, and outer spiral nerve fibers (OSB) beneath the outer hair cells are shown schematically for orientation purposes.

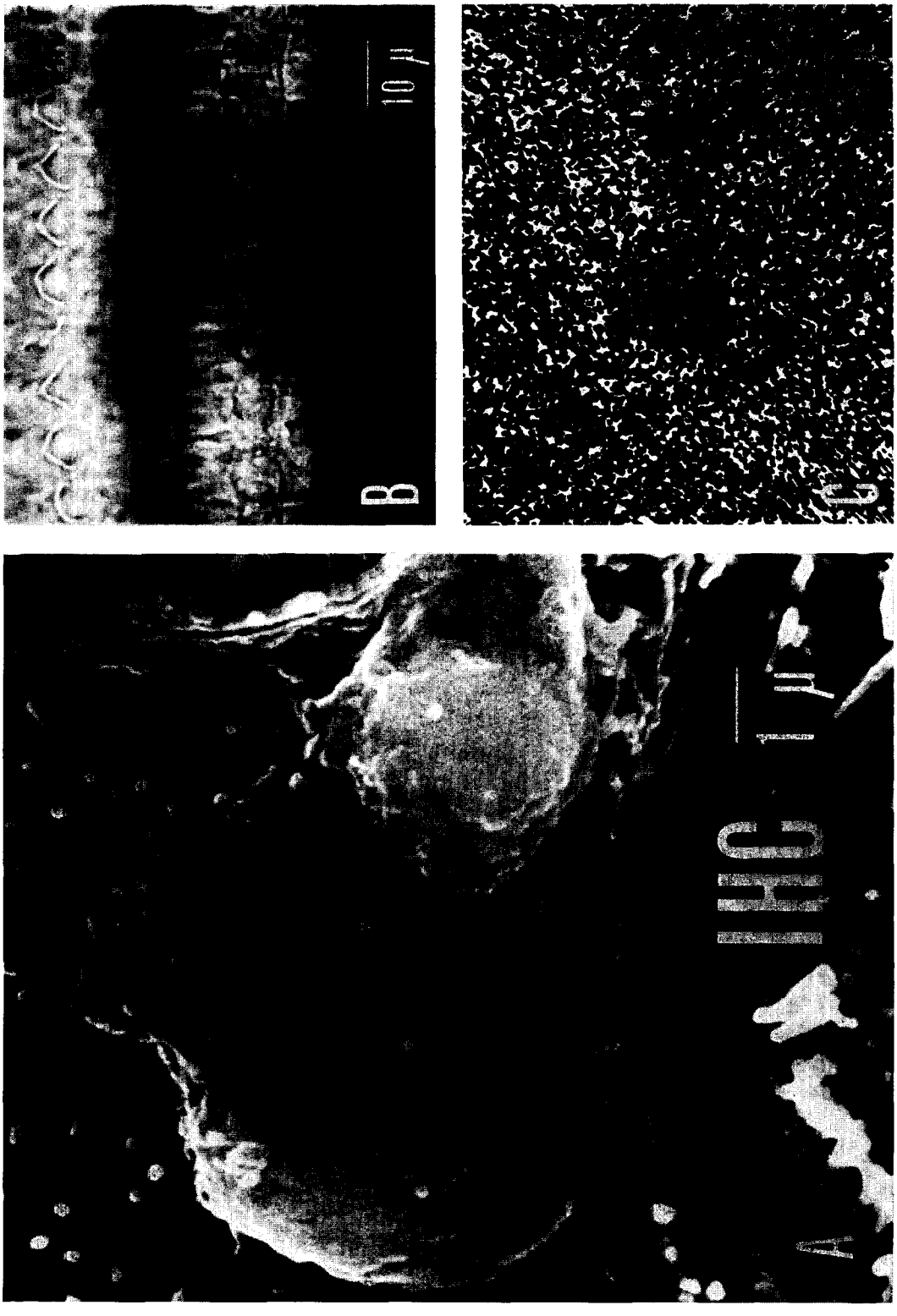

Fig. 10. Swellings on a degenerating nerve fiber near the hair-bearing end of an inner hair cell (IHC). A: SEM picture, secondary electron mode. B: bright-field light microscope survey view of the same area showing the 2 ZIO-stained swellings. C: SEM picture, X-ray mode (solid-state detector; pulse-height analyzer) showing distribution of Zn and Os in the region of the swellings. Brain stem lesion at midline; 6-day survival time.

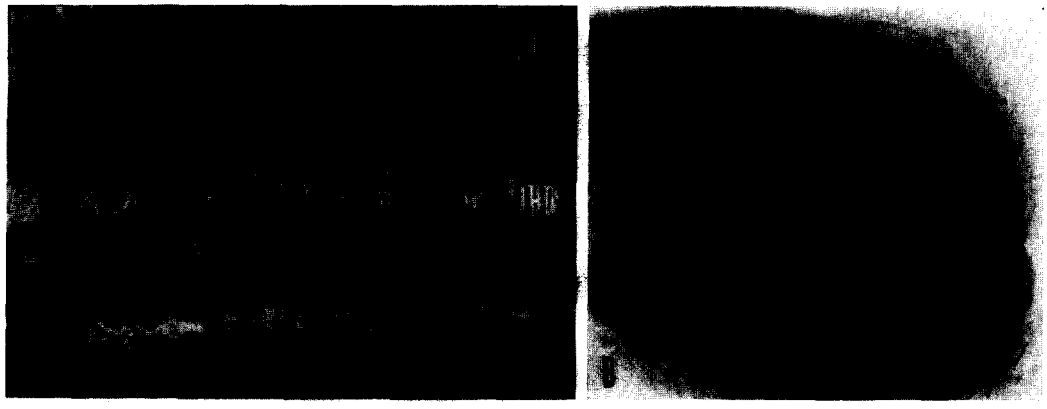

Fig. 11. Climbing nerve fibers related to apical portions of inner (A) and outer (B) hair cells. The arrows in A indicate fibers forming arcades near the hair-bearing ends of inner hair cells (IHC) of the third turn. The arrow in B shows a fiber climbing up the body of a third row outer hair cell (OHC) of the second turn. ISB, inner spiral bundle; DC, Deiters' cells; HC, Hensen's cells; P, headplates of inner pillar cells.

crossers either ran directly across the spiral tunnel bundle or entered it for a very short spiral course before turning outward across the tunnel of Corti.

Many of the fibers linking the inner spiral and tunnel bundles were found to form arcades or loops from the inner spiral bundle. That is, fibers radiating out to the tunnel bundle sometimes appeared to take a short spiral course there (usually along one or two inner pillar cells) then return to the inner spiral bundle. Although these looping fibers were most easily identified in the apical turn, they were present throughout the organ of Corti. At the extreme apex, the inner spiral bundle tended to break up into a series of discrete clumps of nerve fibers and endings, each of which was evidently associated with a single inner hair cell. In this region the spiral tunnel bundle was quite thin and often broke into a discontinuous series of arcades obviously looping outward from the ISB. An example of such a series of arcades is illustrated in Fig. 9. Sometimes the tunnel bundle was observed to approach the inner spiral bundle and actually merge with it near the apical end of the organ of Corti.

Nerve fibers related to the supranuclear portions of hair cells. From both the inner and outer spiral bundles single, beaded nerve fibers were frequently seen climbing up the sides of hair cells, sometimes ending very near the hair-bearing end of the sensory cell just beneath the surface of the reticular lamina. Such nerve fibers were most abundant in the apical and third turns where they appeared to be distributed at random to as many as 25% of the hair cells; however, they were also found scattered here and there throughout the organ of Corti. Among the inner hair cells, these climbing nerve fibers were found to form elaborate arcades looping up from the inner spiral bundle. Such a fiber occasionally separated from the ISB, ran straight up the side of an inner hair cell to a point just below the cuticular plate, then turned to take a spiral course along the tops of several adjacent hair cells before diving down again to the inner spiral bundle. During the process of degeneration these fibers sometimes devel-

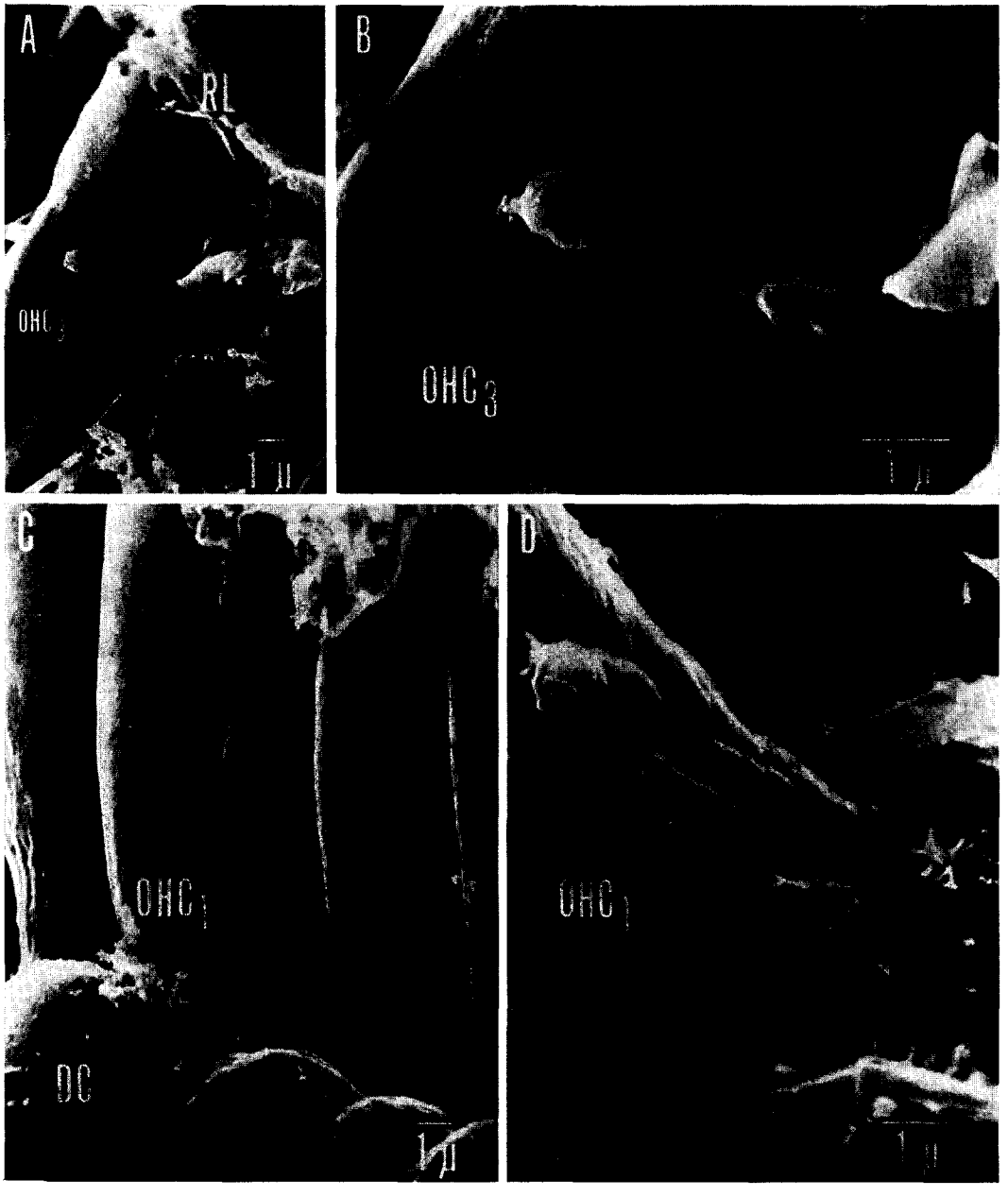

Fig. 12. Scanning electron micrographs of normal nerve fibers terminating on outer hair cells. A and B: lower and higher magnification views of a beaded fiber ending on an outer hair cell of the third row (OHC_3). C shows a climbing fiber ending near the hair-bearing end of a first row hair cell (OHC_1). In D a beaded fiber terminating near the base of a first row outer hair cell (OHC_1) is seen. A, B, and D, apical turn; C, lower third turn; RL, reticular lamina; DC, cup-like portion of Deiters' cell.

Fig. 13. Outer spiral nerve fibers (upward-pointing arrows) looping outward toward Hensen's cells (HC) in lower third turn of Corti's organ. Downward-pointing arrows indicate swollen nerve fibers apparently in the initial phase of degeneration. Brain stem lesion at midline; 24-h survival time. MN, myelinated nerve fibers in osseous lamina; 1,2,3, neural elements beneath first, second, and third row outer hair cells; HC, Hensen's cells containing some osmiophilic lipid droplets.

oped large swellings which appeared to lie at the surface of the reticular lamina when studied by light microscopy. These swellings were visible in scanning electron micrographs as bulges or protuberances on the surfaces of the inner pillar cells. They were located on the laminar processes of the pillars immediately adjacent to the inner hair cells. Fig. 10 illustrates two such swellings as seen in both scanning electron and light microscopy. Examples of nerve fibers reaching the supranuclear and infracuticular portions of both inner and outer hair cells are shown in Fig. 11. Fig. 12 shows efferent endings on various outer hair cells as seen in scanning electron microscopy.

Relation of nerve fibers to non-sensory cells. Engström *et al.*[4] have described nerve fibers looping out into the outer tunnel area in the guinea pig. Such fibers were also present in our specimens. Highly varicose nerve fibers were seen leaving the outer spiral bundles to enter the outer tunnel and closely approach Hensen's cells, often appearing to run among Hensen's cells for varying distances before returning to the outer spiral bundles. In the third and apical turns these fibers were numerous. They formed complex arcades subtending 50–100 outer hair cells and having many interconnections with the outer spiral bundles. Fibers of this type are illustrated in Fig. 13. These arcading nerve fibers were found only in the apical portion of the cochlea; they

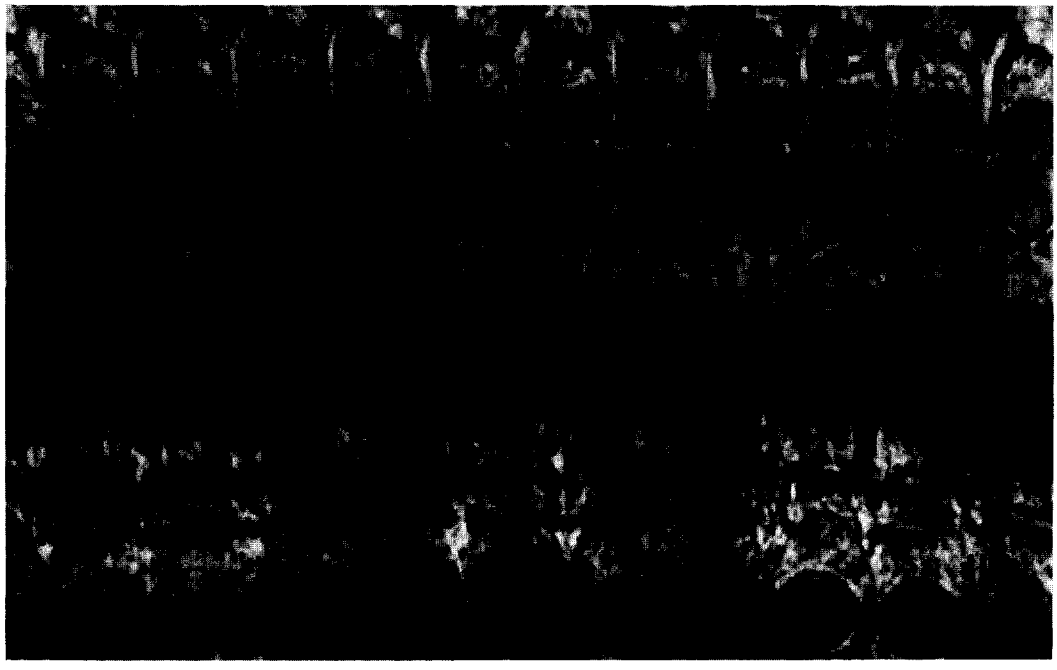

Fig. 14. ZIO-positive nerve fiber terminating on pillar cell (P) headplates in the third turn. IHC, inner hair cells; OHC, outer hair cells.

disappeared at approximately the same level as did the osmiophilic lipid droplets in the Hensen's cells.

As Engström *et al.*[4] have also reported, tunnel-crossing fibers were found, in rare instances, to send branches up to end in bulbous enlargements near the head plates of pillar cells as shown in Fig. 14.

DISCUSSION

The present study, in agreement with a number of previous investigations, demonstrates degeneration of a portion of the cochlear nerve supply after interruption of fiber tracts within the pontine brain stem. Obviously, this finding may be interpreted in favor of the existence of an efferent innervation to the inner ear which originates in the central nervous system and terminates upon hair cells within the organ of Corti. However, it must be remembered that not all workers are in agreement on this point and alternative interpretations have been offered. For example, the occurrence of degeneration after lesions like those made in this study might also be viewed as support for the findings of Ross[21] who has suggested that such degeneration may in fact be due to a disruption of fluid balance in Corti's organ rather than to transection of nerve fibers ending on hair cells.

Evidence of degenerative changes in nerve fibers supplying the outer hair cells

was found at rather short postoperative survival times in the present material. This finding was confirmed by electron microscopy as early as 18 h after placement of a midline lesion. Although Kimura and Wersäll[11] found degeneration of efferent terminals in the guinea pig no sooner than 2 days postoperatively, other workers have noted changes at shorter survival times. Thus, Terayama and Yamamoto[26] reported degeneration beginning in both myelinated and unmyelinated nerve fibers near the internal auditory meatus in the guinea pig 20 h after a brain stem lesion. Iurato[9] found advanced degeneration of efferent endings in the rat at a survival time of 16 h. Considerable variation in the time of onset of degeneration was found from one animal to another in the present study; this fact might represent an important consideration when comparing the results obtained by various workers.

Engström *et al.*[4] have demonstrated a selective affinity of the efferent innervation for the ZIO stain. At the ultrastructural level they showed that ZIO treatment produces heavy impregnation of the efferent neural elements but not of the afferent nerve endings and fibers. Spoendlin[24] has also reported that the stain shows a high specificity for efferent nerve fibers in the inner ear. These findings were confirmed in the present study. All positive nerve staining in the organ of Corti disappeared a few days after both components of the efferent nerve supply were interrupted. The ZIO staining method therefore proved to be an effective tool for study of the distribution of efferent nerve fibers within the organ of Corti. The disadvantage encountered with the technique was its occasional failure to impregnate all efferent fibers in preparations from normal animals, thus conveying a false impression of missing fibers. For this reason we were unable to make reliable quantitative assessments of degeneration in experimental animals, either for the purpose of comparing amounts of degeneration present at various survival times or for accurately evaluating the relative contributions of the crossed and uncrossed efferent bundles to the innervation of a given area of Corti's organ.

Although it is known that the ZIO stain does impregnate certain elements of both cholinergic and adrenergic terminals, as well as catecholamine-containing granules of chromaffin cells, the biochemical basis for the specific impregnation of cochlear efferent fibers is unknown[1,10,16–19].

Curiously enough, apart from neural elements, the ZIO stain is taken up by certain cells in the outer sulcus, the spiral limbus, the stria vascularis, and occasionally even by the inner hair cells.

The efferent fibers within the cochlea are known to vary widely in diameter[23]. It is generally agreed that nerve fibers of differing size undergo degeneration at different rates. Thus, variations in degeneration rate between individual fibers supplying a given area of Corti's organ may account for the irregular, patchy degeneration we observed in animals with short postoperative survival times. This principle might also explain the finding that degenerative changes in the outer hair cell innervation consistently preceded degeneration of fibers beneath the inner hair cells by 2–3 days. The inner spiral bundle has been shown to contain a higher proportion of small diameter fibers than the outer spiral bundles[6,13,23]. Many investigators believe that smaller diameter nerve fibers degenerate more slowly than larger ones. (See the review by van

Crevel and Verhaart[2].) Therefore, the later time of onset of degeneration in the inner hair cell innervation could be accounted for on this basis.

A survey of cochleas from animals having short postoperative survival times failed to provide evidence that nerve fiber degeneration consistently begins in any one turn of the cochlear spiral. There did, however, appear to be some tendency for outer spiral bundle degeneration to develop first in the middle portion of the cochlea while degeneration of the inner spiral bundle was often first evident in the apical turn. In a number of cases, though, we found scattered, patchy neural degeneration throughout all turns with no obvious area of maximum involvement in any single turn; this was true even in animals that were allowed to survive only 2 or 3 days.

Our observations suggest a close relationship between the spiral tunnel bundle and the inner spiral bundle. In all preparations, degeneration of the STB and ISB was found to occur simultaneously. However, the spiral tunnel bundle appeared to degenerate independently of both the tunnel-crossing fibers and the nerve fibers underlying the outer hair cells. In material from animals with midline lesions, it was not uncommon to find almost total degeneration of the outer hair cell innervation and tunnel-crossing fibers combined with normal appearing inner spiral and tunnel bundles. Although some spiral tunnel bundle fibers were traced for distances up to about 400 μm, very few long spironeurons were found to turn out of the tunnel bundle and cross over to the outer hair cells. In agreement with the observations of Engström *et al.*[4], we found that the majority of tunnel-crossers ran directly across the tunnel bundle. On the other hand, a portion of the STB appeared to be composed of fibers looping up from the inner spiral bundle to run short distances within the tunnel bundle before returning to the ISB. This finding calls to mind the classical description of Held[8], who reported that some fibers supplying the inner hair cells course first in the spiral tunnel bundle. In the apical turn particularly, the tunnel bundle was found to have a close structural relationship to the inner spiral bundle, since a sizable portion of the STB in this area is apparently composed of an elaborate series of arcades from the inner spiral bundle. Finally, it is of interest to note that Engström and Engström[3] reported the existence of a system of fibers in the ISB which contains dense-cored vesicles. According to these workers, the only other place in Corti's organ where nerve fibers containing cored vesicles are numerous is the spiral tunnel bundle. These findings strongly suggest that at least a portion of the spiral tunnel bundle is closely related to the inner hair cell innervation and may indeed function as a kind of accessory inner spiral bundle which lies on the peripheral side of the inner pillar cells.

According to Retzius[20] and Fernández[6] fibers of the inner spiral bundle course in the direction of the round window as well as toward the apex of the cochlea. In preparations from young mice Retzius also described fibers entering the ISB which split and sent branches in either direction. Nerve fibers taking similar courses were found in the present material stained by the ZIO method. In addition, spiral fibers could be traced for distances up to 500–600 μm within the bundle.

The long spironeurons with few branches that we observed innervating the outer hair cells in this study evidently correspond to the spiral fibers described by Engström *et al.*[4] in preparations also stained by the ZIO method. They reported that

these fibers travel for distances of 2000–3000 μm in the outer spiral bundles. The longest such fiber that we were able to trace ran beneath 295 outer hair cells, a distance equal to approximately 2400 μm. These fibers had very complex courses, wandering from one row of sensory cells to another and occasionally looping outward toward the cells of Hensen. In agreement with Held[8] who noted the presence of 'ruckläufige Innervationsfibrillen' under the outer hair cells, we found that in passing from one row of hair cells to another, the outer spiral fibers sometimes reversed their direction of travel.

It was sometimes possible to trace the spironeurons back to the tunnel-crossing fibers from which they originated. Some of these tunnel-crossers turned into the outer spiral bundles to run toward the base, but a number were found as well that bent towards the apex. Such a fiber is shown in Fig. 6.

As illustrated in Fig. 1, ZIO-positive spironeurons to the outer hair cells are numerous in the upper turns of the guinea pig cochlea. In animals with brain stem lesions they were often found in the process of actual disintegration. (An example of such a fiber appears in Fig. 3.) After lesions involving both components of the cochlear efferent nerve supply, they disappeared completely. We therefore conclude that the ZIO-positive spiral fibers described in this study are efferent in nature.

The presence of spiral as well as radial efferent nerve fibers to the outer hair cells is somewhat surprising in view of Spoendlin's[23] finding that in the cat the efferents have a predominantly radial orientation. It must be remembered, however, that we found a predominance of spiral fibers only in the third and fourth turns of the guinea pig cochlea which comprise less than one-half the total length of Corti's organ. In these upper turns the organization is less regular and more variable than in the lower portions of the cochlea. The morphology of the upper turns may well reflect a species-related functional specialization. In the lower portion of the guinea pig cochlea we did find that the majority of efferent fibers distribute radially to the outer hair cells in a way similar to that described in the cat.

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REFERENCES

1 AKERT, K., AND SANDRI, C., An electron-microsopic study of zinc iodide–osmium impregnation of neurons. I. Staining of synaptic vesicles at cholinergic junctions, *Brain Research*, 7 (1968) 286–295.
2 CREVEL, H. VAN, AND VERHAART, W. J. C., The rate of secondary degeneration in the central nervous system. I. The pyramidal tract of the cat, *J. Anat. (Lond.)*, 97 (1963) 429–449.
3 ENGSTRÖM, B., AND ENGSTRÖM, H., Structural and physiological features of the organ of Corti, *Audiology*, 11 (1972) 6–28.
4 ENGSTRÖM, H., ADES, H. W., AND ANDERSSON, A., *Structural Pattern of the Organ of Corti*, Almqvist and Wiksell, Stockholm, 1966, 172 pp.
5 ENGSTRÖM, H., ADES, H. W., AND HAWKINS, J. E., JR., Cytoarchitecture of the Organ of Corti, *Acta oto-laryng. (Stockh.)*, Suppl. 188 (1964) 92–99.
6 FERNÁNDEZ, C., The innervation of the cochlea (guinea pig), *Laryngoscope (St. Louis)*, 61 (1951) 1152–1172.
7 FOX, C. A., AND EICHMAN, J., A rapid method for locating intracerebral electrode tracks, *Stain Technol.*, 34 (1959) 39–42.
8 HELD, H., Die Cochlea der Säuger und der Vögel, ihre Entwicklung und ihr Bau. In A. BETHE, G. v. BERGMANN, G. EMBDEN AND A. ELLINGER (Eds.), *Handbuch der Normalen und Pathologischen Physiologie, Band XI*, Springer, Berlin, 1926, pp. 467–534.
9 IURATO, S., Efferent fibers to the sensory cells of Corti's organ, *Exp. Cell Res.*, 27 (1962) 162–164.
10 KAWANA, E., AKERT, K., AND SANDRI, C., Zinc iodide–osmium tetroxide impregnation of nerve terminals in the spinal cord, *Brain Research*, 16 (1969) 325–331.
11 KIMURA, R., AND WERSÄLL, J., Termination of the olivo-cochlear bundle in relation to the outer hair cells of the organ of Corti in guinea pig, *Acta oto-laryng. (Stockh.)*, 55 (1962) 11–32.
12 KOHONEN, A., Effect of some ototoxic drugs upon the pattern and innervation of cochlear sensory cells in the guinea pig, *Acta oto-laryng. (Stockh.)*, Suppl. 208, 70 pp.
13 LORENTE DE NÓ, R., The sensory endings in the cochlea, *Laryngoscope (St. Louis)*, 47 (1937) 373–377.
14 LUFT, I. H., Improvements in epoxy resin embedding methods, *J. biophys. biochem. Cytol.*, 9 (1961) 409–414.
15 MAILLET, M., Le réactif au tétraoxyde d'osmium–iodure de zinc, *Rev. méd. Tours*, 4 (1963) 247–268.
16 MATUS, A., Ultrastructure of the superior cervical ganglion fixed with zinc iodide and osmium tetroxide, *Brain Research*, 17 (1970) 195–203.
17 NIKLOWITZ, W. J., The interaction of hippocampal tissue of the rabbit with OsO_4–zinc iodide stain after treatment with 3-acetylpyridine, reserpine, and iproniazid, *Acta anat. (Basel)*, 81 (1972) 570–585.
18 PÁRDUCZ, A., HALÁSZ, N., AND JOÓ, F., Lack of correlation between the zinc-iodide–osmium positivity of cholinergic terminals and the cholinergic transmission in the sympathetic ganglia of the cat, *J. Neurochem.*, 18 (1971) 97–100.
19 PELLEGRINO DE IRALDI, A., AND GUEUDET, R., Action of reserpine on the osmium tetroxide zinc iodide reactive site of synaptic vesicles in the pineal nerves of the rat, *Z. Zellforsch.*, 91 (1968) 178–185.
20 RETZIUS, G., Die Endigungsweise des Gehörnerven, *Biol. Unters. N.F.*, 3 (1892) 29–36.
21 ROSS, M. D., The general visceral efferent component of the eighth cranial nerve, *J. comp. Neurol.*, 135 (1969) 453–478.
22 SMITH, C. A., AND RASMUSSEN, G. L., Recent observations on the olivo-cochlear bundle, *Ann. Otol. (St. Louis)*, 72 (1963) 489–506.
23 SPOENDLIN, H., *The Organization of the Cochlear Receptor*, Karger, Basel, 1966, 227 pp.
24 SPOENDLIN, H., Ultrastructure and peripheral innervation pattern of the receptor in relation to the first coding of the acoustic message. In A. V. S. DE REUCK AND J. KNIGHT (Eds.), *Hearing Mechanisms in Vertebrates*, Churchill, London, 1968, pp. 89–119.
25 SPOENDLIN, H., AND GACEK, R. R., Electronmicroscopic study of the efferent and afferent innervation of the organ of Corti in the cat, *Ann. Otol. (St. Louis)*, 72 (1963) 660–686.
26 TERAYAMA, Y., AND YAMAMOTO, K., Olivo-cochlear bundle in the guinea pig cochlea after central transection of the crossed bundle, *Acta oto-laryng. (Stockh.)*, 72 (1971) 385–396.