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# SCANNING ELECTRON MICROSCOPIC STUDY OF THE POSTNATAL DEVELOPMENT OF THE RABBIT COCHLEA, WITH AN EMPHASIS ON INNERVATION

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## Abstract

The development of nerve fiber arrangements of the organ of Corti was studied in rabbits 1, 3, 5, 7 and 12-days-old using thick sections from celloidin-embedded cochleas which were examined under a scanning electron microscope. The arrangements of nerve fibers varied with developmental age. The tunnel spiral bundle was thick and loosely collected in the immature cochlea. The outer spiral fibers were recognized even in the narrow space of Nuel in the one-day-old cochlea. As Nuel's space is extending, the fibers course along the medial side of Deiters' cells. The arrangement of the outer spiral fibers was irregular and sparse in the five-day-old cochlea, in contrast to the regular parallel pattern of the adult cochlea. Adult-like parallel arrangement of the outer spiral fibers was seen in the twelve-day-old cochlea. In the three-day-old cochlea, irregularly running nerve fibers were seen along the outer spiral fibers. They may be efferent axons which develop afterwards. Club-like immature nerve endings were recognized at the base of the outer hair cells in the seven-day-old cochlea. Some fibers climbed high up along the medial wall of the outer hair cells. A nearly mature pattern was seen in the twelve-day-old cochlea. This study confirms previous reports on the development of cochlear innervation.

**Key Words:** Scanning electron microscopy, rabbit, organ of Corti, nerve fiber, development.

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## Introduction

The development of the afferent and efferent nerve system in the mammalian cochlea has been extensively studied. The peripheral innervation pattern has been studied in neonatal rats, mice, hamster and other species by transmission electron microscopy (Pujol *et al.*, 1978; Lenoir *et al.*, 1980; Emmerling *et al.*, 1990; Lavigne-Rebillard and Pujol, 1990), Golgi stain (Perkins and Morest, 1975; Ginzberg and Morest, 1983), horseradish peroxidase (HRP) technique (Simmons *et al.*, 1990, 1991), carbocyanine dye technique (Cole and Robertson, 1992) and immunohistochemical methods (Whitlon and Sobkowicz, 1988, 1989; Sobkowicz and Emmerling, 1989; Merchan-Perez *et al.*, 1993). The combined results of these studies is that the maturation of the inner hair cells (IHCs) and their afferent and efferent innervation occurs first and the outer hair cells (OHCs) develop more slowly. In addition, the innervation of OHCs is dynamic and plastic at early stages of development.

Although scanning electron microscopy (SEM) is suitable for investigating the three-dimensional appearance of nerve fibers in the organ of Corti, few authors have attempted to study the innervation in the developing cochlea by SEM (Hoshino and Nakamura, 1985; Lim and Anniko, 1985; Hoshino, 1990) because of the technical difficulties involved. Recently, we reported an SEM method to study the cochlea in thick sections prepared from celloidin-embedded temporal bones (Mizuta *et al.*, 1990; Morita *et al.*, 1992). This method is suitable for studying the nerve fiber arrangement inside the fluid spaces of the organ of Corti and it is easy to perform, even in the cases of small immature cochleas.

In 1965, Änggard demonstrated that the fluid spaces of Corti's organ in the rabbit cochlea begin to open on the 5th day after birth and get mature appearance toward the 11th day, in his light microscopic study. He reported that the onset of cochlear function occurs on the 5th day. In the present study, we report our SEM observations of cochleas from newborn rabbits and discuss developmental changes of the nerve fiber arrangements inside the organ of Corti.

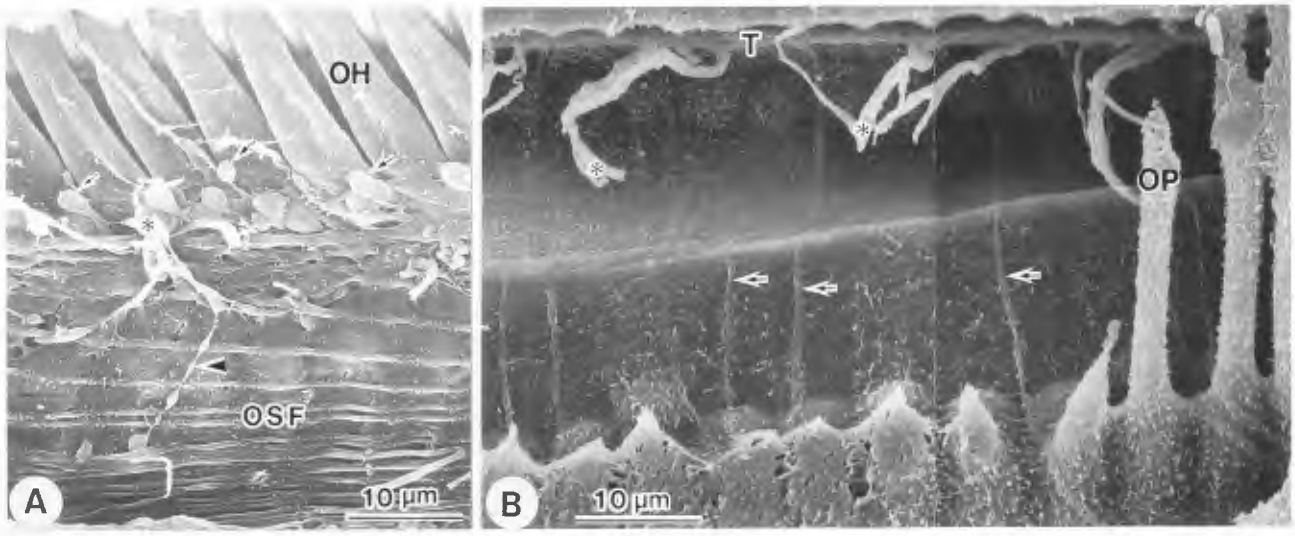



**Figure 1.** Inside views of the organ of Corti in the adult rabbit cochlea (lower basal turn). **(A)** The lateral wall of Nuel's space. The cochlear base is toward the left. The outer spiral fibers (OSF) run parallel and gradually ascend on the medial wall of Deiters' cells. Several large nerve endings (arrows) are seen at the base of the first outer hair cell (OH). A vertically running fiber with varicosities can be seen (arrowhead). The upper tunnel radial fibers have been cut (*). **(B)** Survey view of the floor of Corti's tunnel. The modiolar side is at top. The cochlear base is toward the right. The tunnel spiral bundle (T) runs at the inner corner of the tunnel. The upper tunnel radial fibers have been cut during the preparation (*). The tunnel basilar fibers run radially (arrows). OP: outer pillar cell.

## Materials and Methods

Postnatal rabbits (New Zealand White), ranging in age from 1 to 12 days after birth, and adult rabbits aged 4 weeks were used. The day of birth was defined as day 0. We examined two animals at one, three and twelve days of age, and four animals five to seven days of age and four adult rabbits. None of them showed inflammatory changes in the middle ear. After anesthesia by intraperitoneal injection of pentobarbital, the temporal bones were dissected and 1% phosphate-buffered glutaraldehyde (pH 7.4) was perfused via the round and oval windows. The temporal bones were decalcified in ethylenediaminetetraacetic acid (EDTA), dehydrated in graded ethanol and embedded in celloidin. After hardening of the celloidin, the specimens were cut in 80-100 $\mu$m sections with a sliding microtome so that the cutting plane was parallel to the basilar membrane. After removing the celloidin in an ether-alcohol (1:1) solution, the sections were freeze-dried using t-butyl alcohol (Inoue and Osatake, 1988). The sections were then mounted on metal stubs with carbon paste, coated with gold using a sputter coater (JEOL JFC-1100), and observed under a Hitachi S-800 SEM.

## Results

### Adult rabbit cochlea (Fig. 1)

**Nuel's space:** The outer spiral fibers ran parallel and gradually ascended toward the cochlear base along the medial walls of Deiters' cells. In the basal and lower middle cochlear turns, large nerve endings were seen attached to the base of the outer hair cells (OHCs) (Fig. 1A), but were absent in the upper turn; only spirally running nerve fibers covered the basal portions of the OHCs. The upper tunnel radial fibers reached the medial side of Deiters' cells near the base of the OHCs.

**The tunnel of Corti:** Inside the tunnel of Corti, the tunnel spiral bundle ran attached to the base of inner pillar cells (Fig. 1B). The upper tunnel radial fibers entered the tunnel just below the tunnel spiral bundle or through the bundle, then ran apart from the tunnel floor. The upper tunnel radial fibers had varicosities.

The tunnel basilar fibers, parallel to each other, crossed the tunnel floor (Fig. 1B). In the upper portion of the cochlea, the basilar fibers were well-exposed to the tunnel fluid, while in the basal turn they were scarcely seen through the thin covering of the cytoplasm of outer pillar feet.

### One-day-old cochlea (Fig. 2)

As in the adult cochlea, two and a half turns were distinguished in the one-day-old cochlea. In the apical turn, there was no fluid space in the organ of Corti. In the middle and the basal turn, the tunnel of Corti was still closed while Nuel's space was recognized as a small opening (Figs. 2A and 2B). There was a spiral nerve

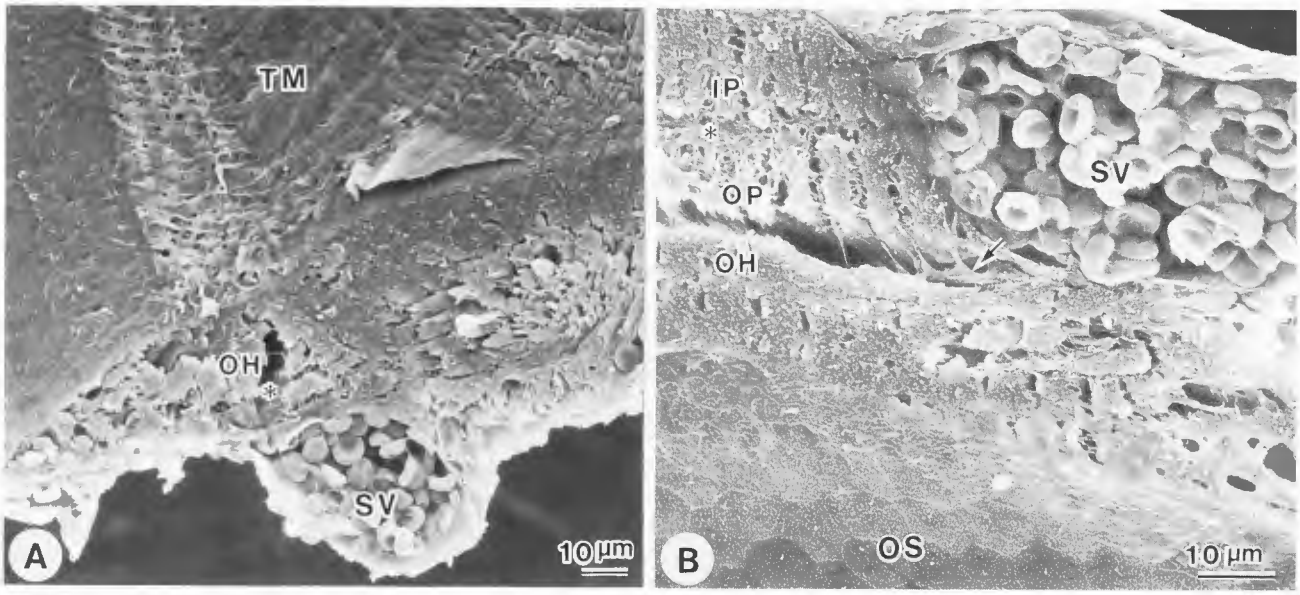



**Figure 2.** One-day-old rabbit cochlea. **(A)** A cross-section through the organ of Corti in the apical turn. The tunnel of Corti is not open, while Nuel's space (*) is recognized as a small opening. The tectorial membrane (TM) adheres to the organ of Corti. **(B)** Survey view of a horizontal section of the organ of Corti in the lower basal turn. The modiolar side is at top. Spirally running nerve bundle can be seen in Nuel's space (arrow). The area between the outer pillar cells (OP) and the inner pillar cells (IP) is filled with nerve fibers (*). SV: spiral vessel, OS: outer sulcus.

bundle in Nuel's space (Fig. 2B). The area between the outer and inner pillar cells (the incipient Corti's tunnel) was filled with nerve fibers (Fig. 2B). The underside of the tectorial membrane appeared adhered to the organ of Corti and its outer margin was tightly attached to the outermost row of Deiters' cells (Fig. 2 A).

**Three-day-old cochlea (Fig. 3)**

The fluid spaces in the organ of Corti were open in every turn in the three-day-old cochlea. The fluid spaces were larger in the basal portion of the cochlea than in the apical portion.

**The tunnel of Corti**: In the apical turn, the tunnel of Corti was narrow and filled with spirally running fibers. These fibers are probably part of the tunnel spiral bundle. In the middle turn, the medial part of the tunnel space was occupied by loosely collected fibers of the tunnel spiral bundle (Fig. 3A). The nerve fibers in the tunnel spiral bundle seemed more tightly collected in the basal turn than in the apical portion of the cochlea. In the basal turn, the upper tunnel radial fibers ran apart from the tunnel floor (Fig. 3B), while in the middle turn they ran near the floor, in the narrow space of the tunnel. In the apical turn, we could not distinguish the upper tunnel radial fibers because of the tunnel's narrowness. In the three-day-old cochlea, the tunnel basilar fibers could not be distinguished in any turn because the floor was thickly covered with microvilli of the outer pillar cells.

**Nuel's space**: Nuel's space seemed to be larger than the space of Corti's tunnel in every turn in the three-day-old rabbit cochlea. In every turn, spirally running nerve fibers were observed below the OHCs (Fig. 3A). They seemed to be young outer spiral fibers. In addition, there were irregularly running fibers along these spiral fibers. These irregularly running fibers were of various calibers with irregular swellings. They were numerous in the basal turn where they formed a complicated network (Fig. 3B). They seemed to connect to the upper tunnel radial fibers. Some fibers were seen attached to the basal portion of the OHCs (Fig. 3B).

**Five-day-old cochlea (Figs. 4 and 5)**

In the five-day-old cochlea, Nuel's space seemed smaller than the space of Corti's tunnel in every turn. The basal turn apparently showed far more mature features than the apical turn (Figs. 4 and 5).

**The tunnel of Corti**: The tunnel spiral bundle ran along the inner wall of the tunnel in each turn. In the apical turn, the tunnel spiral bundle was loosely collected and wider than that of a mature cochlea (Fig. 4B). The bundle became compact in the basal half of the cochlea, and within the basal turn it showed almost an adult-like appearance.

The tunnel basilar fibers crossed the tunnel radially in each turn as in the adult cochlea. In the five-day-old cochlea, the basilar fibers were well-exposed (Figs. 4B and 5B), while in the mature cochlea they were almost

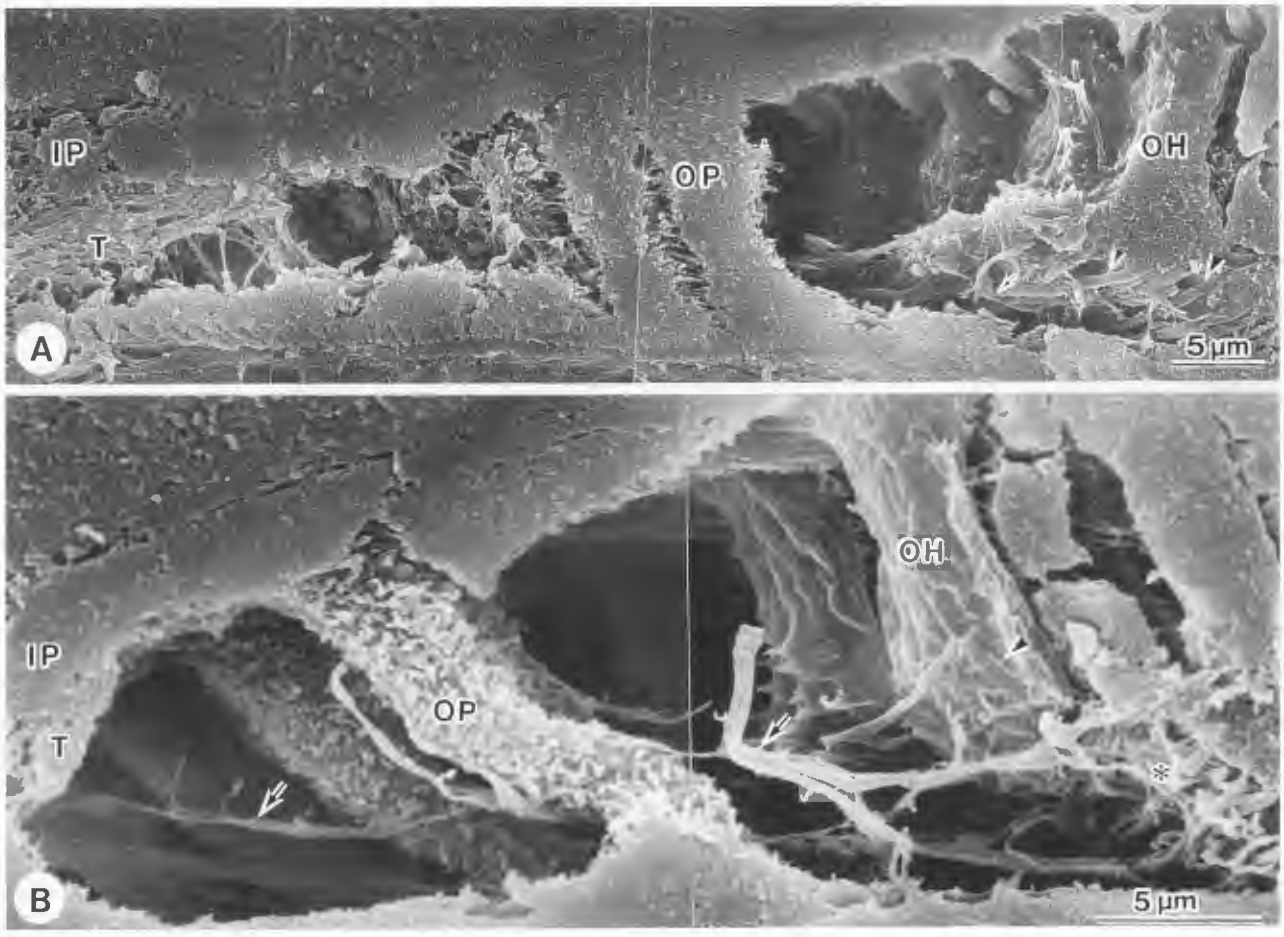



**Figure 3.** Three-day-old rabbit cochlea. (A) A cross-section through the organ of Corti in the lower middle turn. The fluid spaces are still narrow. A loosely collected, thick tunnel spiral bundle (T) is seen in the medial portion of the tunnel of Corti. The microvilli of the outer pillar cells (OP) are evident. Below the first outer hair cells (OH), spirally running fibers (arrowheads) and irregularly running fibers (arrow) are seen. (B) A cross-section through the organ of Corti in the lower basal turn. The tunnel spiral bundle (T) is compact as compared to that in A. The upper tunnel radial fibers (arrows) run across the tunnel and Nuel's space. The irregularly running fibers are evident (*) below the first outer hair cell (OH). Some fibers are attached to the base of the first OHC at their lower pole (arrowhead). IP: inner pillar cell.

covered by the cytoplasm of the outer pillar cells, especially in the basal turn.

**Nuel's space:** Spiral running fibers along the medial side of the first OHCs were prominent, especially in the apical turn (Fig. 4A). Some irregular running fibers were seen along the spiral fibers. Another feature of the nerve fiber arrangement in the apical turn was lack of parallel fibers gradually ascending the medial walls of Deiters' cells. In contrast, there were many nerve fibers assembled at the bottom of Nuel's space (Fig. 4A). Some fibers ascended diagonally toward the cochlear base, but they were few in number and their ascending angle was sharper as compared to that of the mature cochlea (Fig. 4A).

In the upper basal turn, the spiral fibers along the medial side of the OHCs were less evident than were those in the apical turn (Fig. 5A). They covered only the basal portions of the OHCs. The outer spiral fibers ascended Deiters' cells gradually, but this arrangement was irregular (Fig. 5A).

In the lower basal turn, the outer spiral fibers showed an adult-like parallel pattern along the surface of Deiters' cells. The arrangement of the second and third bundles of the outer spiral fibers was partly seen at the cut-edge and appeared to be similar to that of the first bundle (Fig. 5C).

**Seven-day-old cochlea (Figs. 6 and 7)**

The arrangement of outer spiral fibers was still

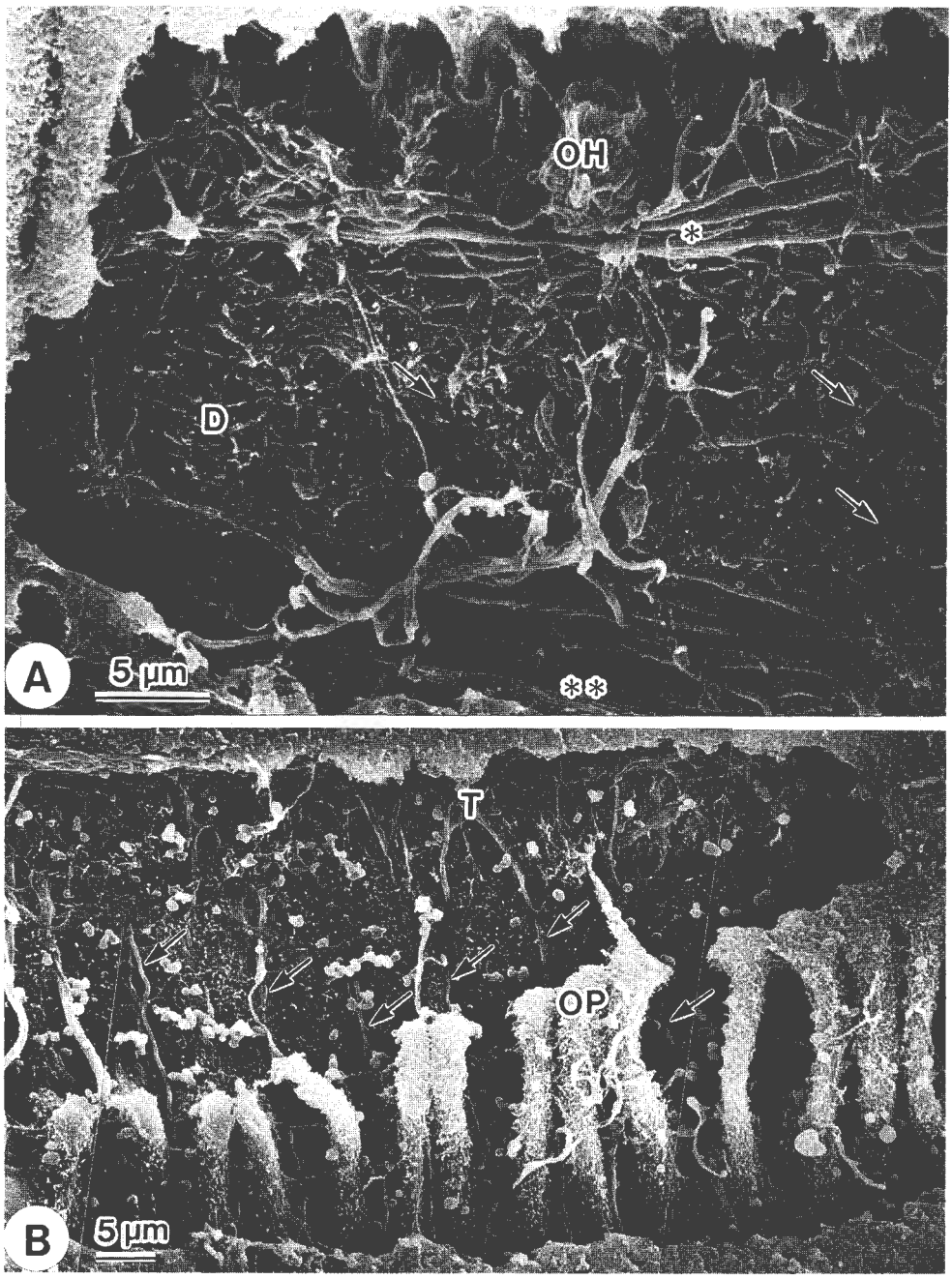



**Figure 4.** Five-day-old rabbit cochlea (apical turn). (A) The lateral wall of Nuel's space. The cochlear base is toward the right. Spirally running fibers on the medial side of the outer hair cells (OH) are evident (*). The ascending fibers on the Deiters' cells (arrows) are seen, but they are not parallel and the ascending angle is sharp. There are many spiral fibers at the bottom of Nuel's space (**). (B) Survey view of the tunnel of Corti. The modiolar side is at top. The tunnel spiral bundle (T) is loosely collected. Radially running basilar tunnel fibers (arrows) can be seen.

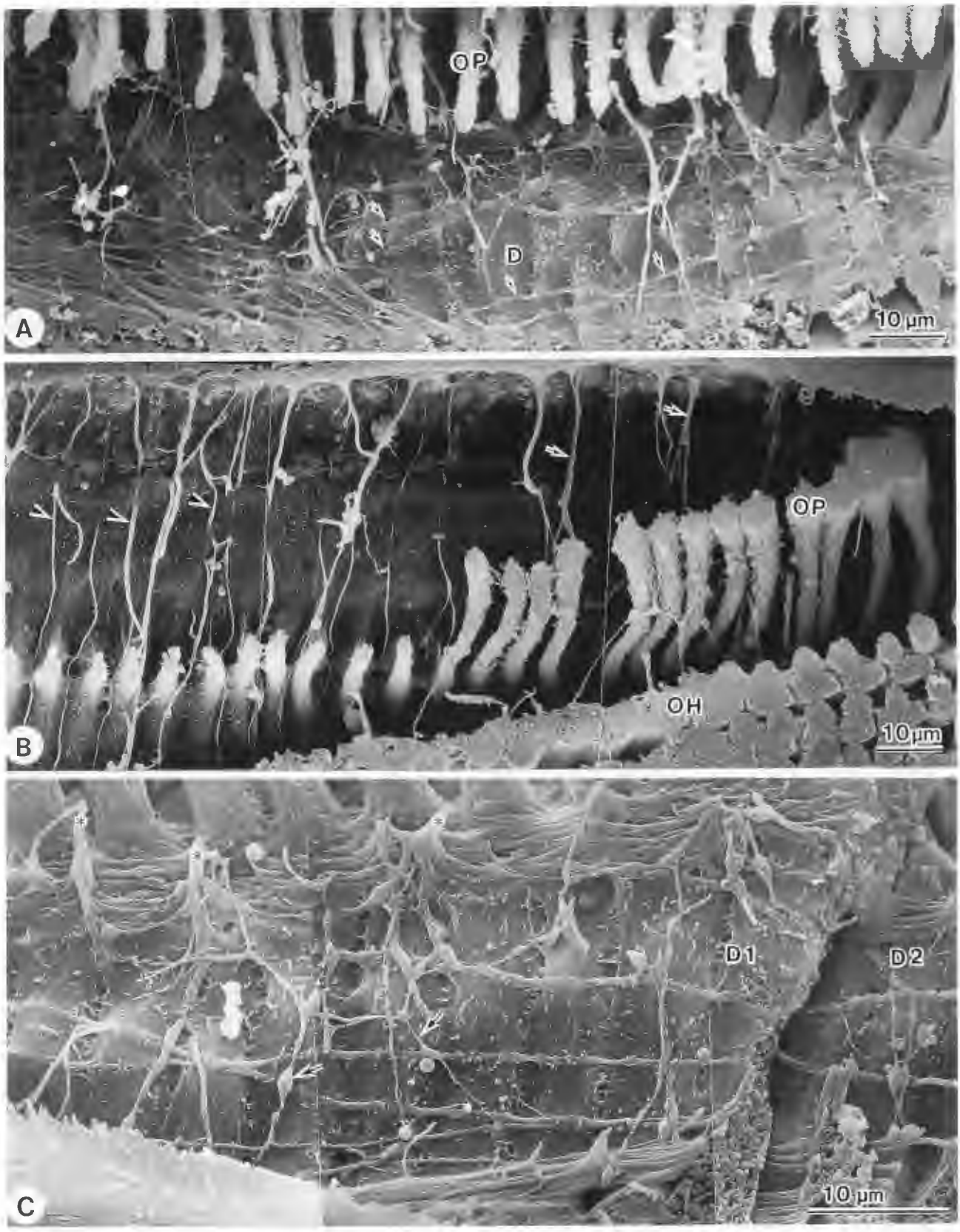
irregular in the apical and middle turns, while in the basal turn they ran regularly and parallel (Fig. 6A).

The inside view of the tunnel of Corti showed an almost mature appearance, though kinocilia of hair cells and the marginal attachment of the tectorial membrane persisted (Fig. 6B). The tunnel basilar fibers were partly covered.

In the lower middle and basal turns, many immature nerve endings were observed along the medial sides of the OHCs (Figs. 7A, 7B, 7C and 7D). They were small in size compared to those in the adult cochlea. They seemed to be terminal branches of spiral fibers running at the level of the OHC bases (Fig. 7B and 7D). Though rarely seen in this preparation, several small nerve endings were attached to the lateral side of the base of an OHC (Fig. 7E). Some fibers climbed high up along the medial side of the first OHCs to the level of the reticular lamina (Fig. 7A).

### Twelve-day-old cochlea (Fig. 8)

Though there still were some traces of the marginal attachment of the tectorial membrane on the reticular lamina, the kinocilia in the OHCs had disappeared (Fig. 8A). The appearance of structures inside the fluid space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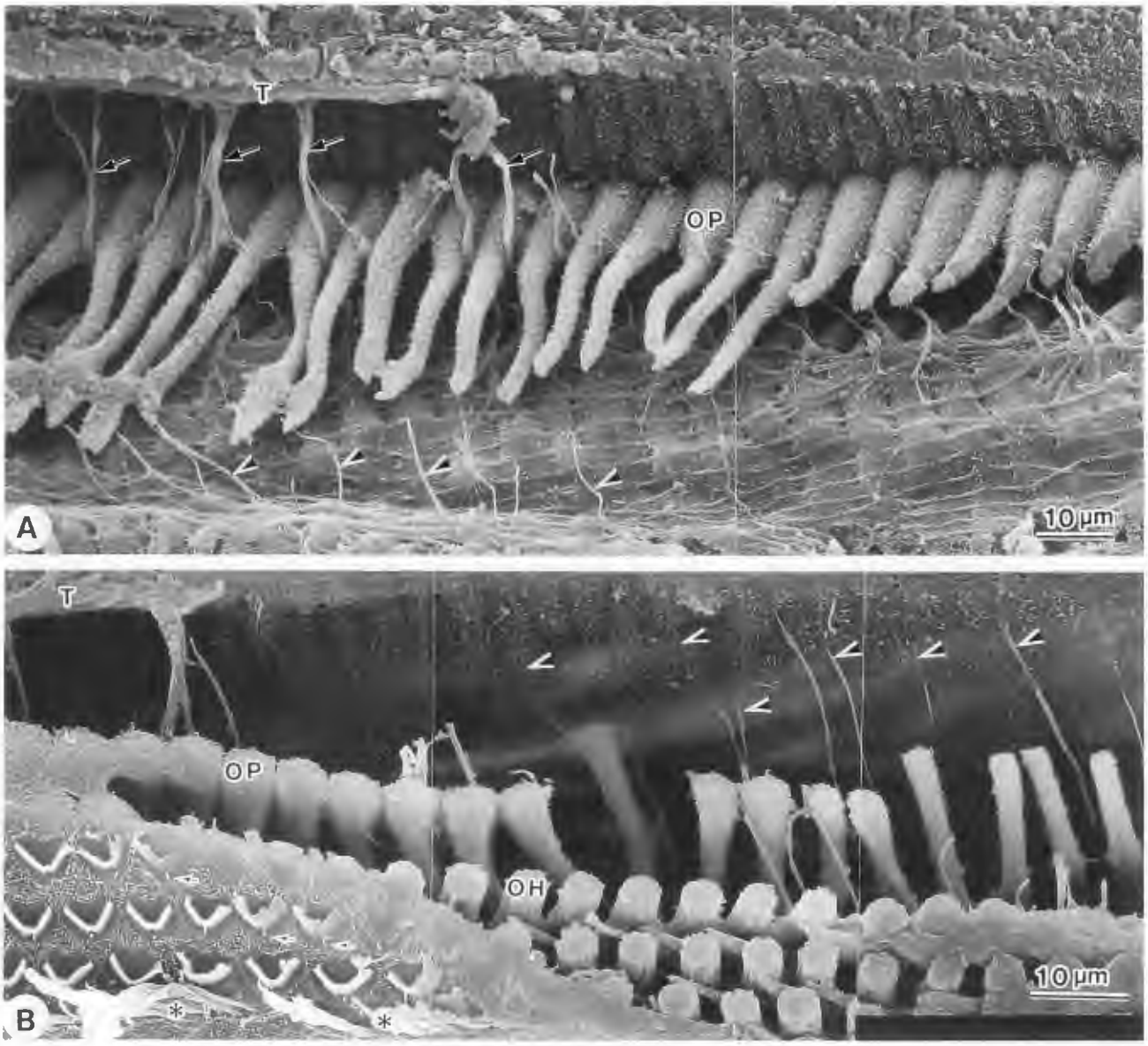


**Figure 6 (above).** Seven-day-old rabbit cochlea (upper basal turn). **(A)** The lateral wall of Nuel's space. The cochlear base is toward the right. Note that the outer spiral fibers run parallel as in the mature cochlea. The upper tunnel radial fibers (arrows) reach the upper portion of Deiters' cells, and tunnel basilar fibers reach the medial wall of Deiters' cells near the bottom of Nuel's space (arrowheads). The tunnel spiral bundle (T) has been cut. The microvilli of the outer pillar cells (OP) are shorter than those in the three- or five-day-old cochlea. **(B)** Survey view of the tunnel of Corti. The modiolar side is at top. The aspect of structures inside the tunnel is almost adult-like, though kinocilia (arrows) and the marginal attachment (*) of the tectorial membrane persist. The tunnel basilar fibers (arrowheads) are partly covered as compared with those in the five-day-old cochlea. P: phalangeal process of Deiters' cell.

**Figure 5 (on the facing page 170).** Five-day-old rabbit cochlea (**A** and **B**: upper basal turn; **C**: lower basal turn). **(A)** The lateral wall of Nuel's space. The cochlear base is toward the right. Several spiral fibers (arrows) gradually ascend on the medial side of Deiters' cells (D). They are not spaced parallel. **(B)** Survey view of the tunnel of Corti. The modiolar side is at top. The upper tunnel radial fibers (arrows) and the tunnel basilar fibers (arrowheads) can be seen simultaneously. **(C)** The lateral wall of Nuel's space in the lower basal turn. The cochlear base is toward the left. An adult-like parallel arrangement of the outer spiral fibers is observed along the medial walls of the first (D1) and second (D2) row of Deiters' cells. Some thin fibers with varicosities are seen running irregularly (arrows). The upper tunnel radial fibers have been cut during the preparation (*). OP: Outer pillar cells; OH: outer hair cells.

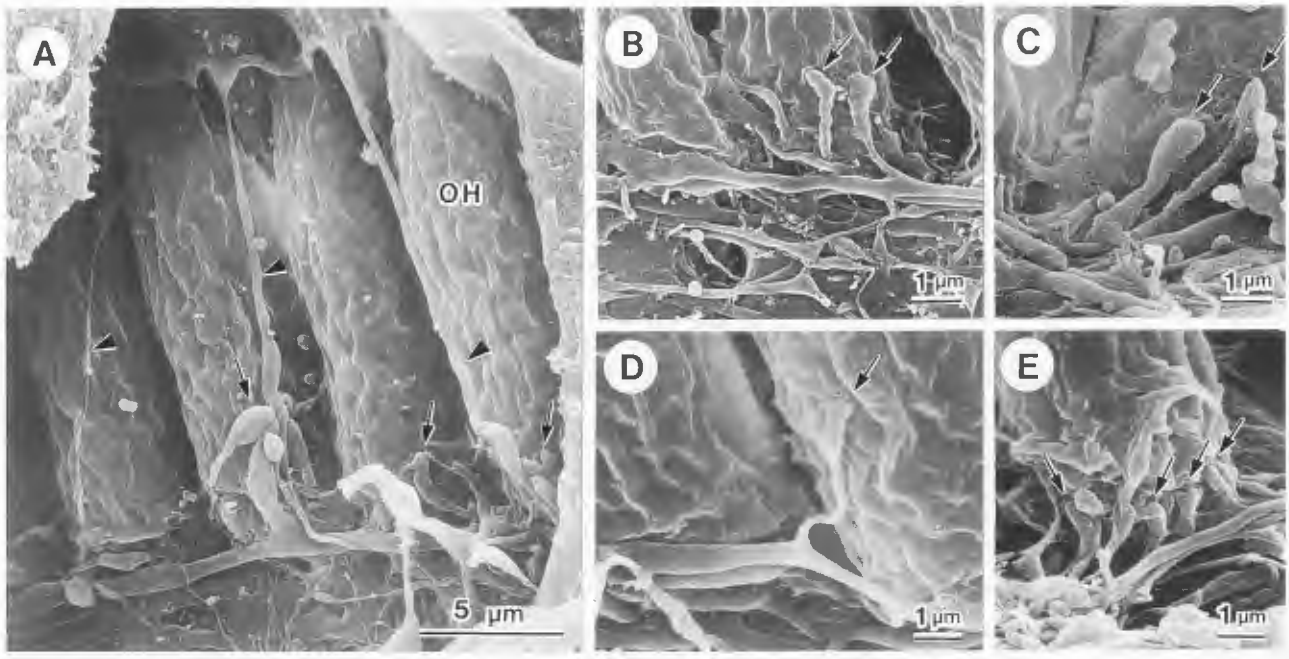


**Figure 7.** Immature nerve endings in the seven-day-old rabbit cochlea (lower middle turn). **(A)** The lateral wall of Nuel's space. Immature nerve endings (arrows) are seen at the basal portions of the first outer hair cells (OH). Some nerve fibers can be seen climbing along the side of the outer hair cells (arrowheads) until they reach the level of the reticular lamina (*). **(B, C, and D)** Immature nerve endings along the medial sides of the bases of the first row outer hair cells (arrows). **(E)** Nerve endings along the lateral sides of the bases of the second row outer hair cells (arrows). The wrinkled surfaces of the hair cells appear to be artifactual.

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had become very close to that of a mature cochlea. The tunnel spiral bundle and the upper tunnel radial fibers showed almost adult-like appearance. The nerve endings along the medial side of the OHC bases were large and more adult-like (Fig. 8B). On the floor of the tunnel of Corti, in the basal turn, as in the mature cochlea, the tunnel basilar fibers were covered by the cytoplasm of outer pillar cells (Fig. 8C).

## Discussion

Thus far, there have been not many reports involving SEM observation of the interior view of the developing organ of Corti (Linderman and Ades, 1971; Hoshino and Nakamura, 1985; Lim and Anniko, 1985; Hoshino, 1990), probably because of the technical difficulties involved in the preparation of small immature cochlea for microscopic examination. The method we used in this study allowed us to expose the fluid spaces with few artifacts (Mizuta *et al.*, 1990; Morita *et al.*, 1992).

This is the first SEM report on innervation in the developing rabbit cochlea. Änggard (1965), in a light microscopic study of the development of the rabbit cochlea, demonstrated that the tunnel of Corti begins to open on the 5th day and the organ of Corti attains a mature appearance on the 11th day. His electrophysiological study revealed that the onset of cochlear function occurs on the 5th day and that functional maturation is attained at the age of 15 to 20 days. The result of our SEM study is in agreement with his report, as the tunnel of Corti was open in every turn after the age of 5 days and the inside view of the organ of Corti had a mature appearance in the twelve-day-old cochlea.

Recent studies revealed the general gradients of cochlear maturation; from base to apex, from inner hair cells (IHCs) to outer hair cells (OHCs), from afferent nerve system to efferent system (Lenoir *et al.*, 1980; Pujol, 1985). The present study confirmed these findings with respect to the developmental changes of the OHC innervation, since we could not obtain good exposure of the IHC area due to the lack of space around the IHCs.

In the mature rabbit cochlea, spirally running fibers in Nuel's space, or the outer spiral bundle, gradually ascended along the medial side of Deiters' cells, showing a regular and parallel arrangement, as Bredberg (1981) previously reported. Even in the one-day-old cochlea there were spirally running nerve fibers in the narrow opening of Nuel's space, and in the three-day-old cochlea the fibers were distinctly seen along the medial side

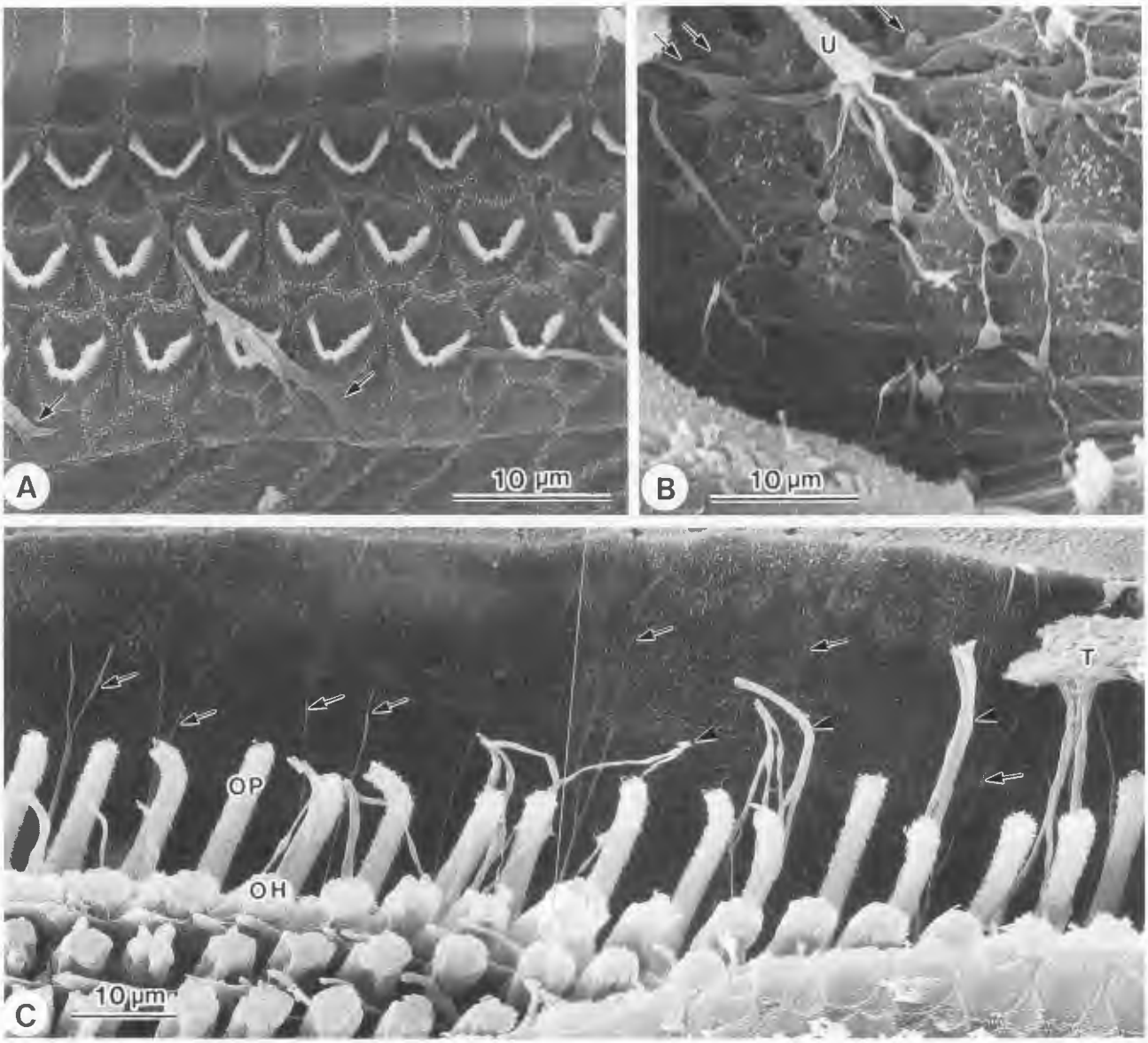


**Figure 8.** Twelve-day-old rabbit cochlea. **(A)** Stereocilia of the outer hair cells in the lower basal turn. The kinocilia in each outer hair cells disappeared. There are still a few traces of the marginal attachment of the tectorial membrane (arrows). **(B)** A cross-section through Nuel's space in the lower basal turn. The cochlear base is toward the left. The nerve endings (arrows) at the bases of the first row outer hair cells are larger than are those seen in the seven-day-old cochlea. **(C)** Survey view of the floor of Corti's tunnel in the upper basal turn. The modiolar side is at the top. The tunnel basilar fibers (arrows) are almost covered by the cytoplasm of the outer pillar cells. The tunnel spiral bundle (T), the upper tunnel radial fibers (arrowheads) and the stereocilia of the outer hair cells has been cut during preparation. U: upper tunnel radial fiber; OP: Outer pillar cells; OH: outer hair cells.

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of Deiters' cells in the first row.

There was another type of fiber with varicosities which ran irregularly over the spiral fibers. They seemed to be branches of the upper tunnel radial fibers and were evident in the three-day-old cochlea. In the hamster cochlea and using HRP method, Simmons *et al.* (1990, 1991) showed that the afferent outer spiral fibers acquire almost mature features by the age of 6 to 8 days. In the mouse cochlea, fine efferent fibers were observed at the IHC region shortly after birth, whereas in the OHC region they were seen for the first time 7 to 10 days after birth (Kikuchi and Hilding, 1965). From these reports, we suppose that the spiral fibers we observed below the OHCs are the afferent outer spiral

fibers and that the irregular fibers are the efferent fibers which develop afterwards.

In the five-day-old cochlea, spiral nerve fibers running along the medial side of the OHCs were prominent, especially in the apical portion of the cochlea. They were less evident in the twelve-day-old cochlea because they were limited to the base of the OHCs and were partly covered by Deiters' cells. Though we could not determine the nature of these fibers, we suppose that the spiral fibers running at the level of the base of the OHCs are a mixture of afferent and efferent fibers, because some of them were connected to the upper tunnel radial fibers while others were connected to the outer spiral fibers.

Another prominent group of spiral fibers was observed at the bottom of Nuel's space in the five-day-old cochlea. We think these fibers are afferent outer spiral fibers because of their connection to the tunnel basilar fibers. The spiral fibers at the floor of Nuel's space expanded gradually as they matured to run parallel and regularly along the medial sides of Deiters' cells. The fibers appeared mature in every turn by 12 days after birth. Thus, the final arrangement of the outer spiral fibers took place relatively late compared to the early development of the OHCs afferent system as reported previously (Pujol, 1985; Simmons *et al.*, 1991).

In the present SEM study, it was difficult to differentiate afferent from efferent nerve endings at the OHC base, except for the large efferent endings because the basal portion of the OHC was almost covered by Deiters' cells and nerve fibers. Thus, we could not confirm the synaptic competition of OHC afferent and efferent endings reported by Pujol (1985).

In the three-day-old cochlea, we observed nerve fibers attached to OHCs by thin end collaterals that may be precursors of the endings. In the five-day-old cochlea, we first recognized club-like nerve endings in contact with the OHCs of the first row. The nerve endings became more evident by the 7th day. We suppose that these immature nerve endings are efferent because they were located at the medial side of the OHCs as in mature efferent endings (Bredberg, 1981; Takasaka *et al.*, 1983). Some of these endings were connected to the upper tunnel radial fibers, and toward the 12th day, they appeared enlarged, similar to those in the mature cochlea. Mizuta *et al.* (1990) also reported the presence of immature nerve endings at the medial side of OHCs in kittens. Though small in size, these endings indicate the synaptogenesis of the efferent nerves. In developing mice, the greatest increase of acetylcholinesterase activity occurred between the 4th and 10th day, which suggests the differentiation of the efferent synaptogenesis (Sobkowicz and Emmerling, 1989; Emmerling *et al.*, 1990). In this study, we recognized the presence of immature nerve endings as early as in the five-day-old cochlea, which coincides with the reported date of onset of cochlear function in the rabbit (Änggard, 1965).

We observed thin, climbing fibers on the side of OHCs in the seven-day-old cochlea. We have recognized similar climbing fibers in the adult cochlea of the rabbit, guinea pig and dog. Some of the climbing fibers were connected to the nerve endings at a high level on the OHCs. Simmons *et al.* (1990) reported that some of the tunnel-crossing efferent fibers stained by HRP climbed up high along the side of the first OHCs in the 10-day-old hamster. Whitlon and Sobkowicz (1989) also demonstrated similar fibers in developing mice by the gamma aminobutyric acid (GABA) immunoreactive method. In the latter study, the upper tunnel radial fibers innervated the OHCs at the age of 12 days, and some of the fibers climbed upward to just beneath the reticular lamina. The GABA immunostained fibers climbing along OHCs were also shown in guinea pig (Eybalin *et al.*, 1988) and rat (Merchan-Perez *et al.*, 1990). The course of those fibers was quite similar to that of the climbing fibers that we found. Thus, we suppose that the climbing fibers are efferent in nature and that some efferent fibers may take an irregular course in the organ of Corti of various mammals. We found the climbing fibers in the middle turn, however, the previous studies show that the GABA immunostained climbing fibers are seen mainly in the apical turn. We could not clarify this discrepancy in this study.

The tunnel of Corti, which opened shortly after Nuel's space, was still narrower than Nuel's space in the three-day-old cochlea. Recent studies had shown that the OHC efferent fibers cross the tunnel after the 6th day in hamsters (Simmons *et al.*, 1991) and after the 4th day in mice (Sobkowicz and Emmerling, 1989, Emmerling *et al.*, 1990). In the present study, the upper tunnel radial fibers were recognized in the basal turn in the three-day-old cochlea. It was difficult, however, to find them in the apical portion of the cochlea because of the narrowness of the tunnel.

As to the afferent nervous system, though we assumed that the tunnel basilar fibers had already crossed the tunnel in the three-day-old cochlea, we could not observe them because of the microvilli of the outer pillar cells covering the floor of the tunnel. The method used in the present study is not useful when the fluid spaces are small or filled with microvilli, and thus, may not be suitable for studying the development of nerve fibers that cross the incipient tunnel.

In the one-day-old cochlea, we could observe spirally running fibers which filled the area between the inner and outer pillar cells, even though the tunnel of Corti was not open. We think these fibers belong to the tunnel spiral bundle. This finding supports the early matu-

ration of IHC efferent innervation compared to that of OHCs (Pujol, 1985). The tunnel spiral bundle was loosely collected just after the opening of the Corti's tunnel in every turn. It becomes compact in the basal and the middle turn of the five-day-old cochlea and in the apical turn of the seven-day-old cochlea. The tunnel spiral bundle was located at the inner side of the tunnel in each turn, as in the mature cochlea. In kittens, however, the tunnel spiral bundle runs at the center of a barely opened tunnel floor and is compact (Mizuta, unpublished data). There may be some species differences in the developmental patterns of nerve fiber arrangements.

So far, there have been no reports on the development of the rabbit cochlea using HRP or immunohistochemical methods. Further investigations are necessary to determine the exact nature of the fibers found in this SEM study.

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### Discussion with Reviewers

**R.V. Harrison**: How does the rabbit compare to human cochlear development post-natally?
**Authors**: The cochlea of the neonate rabbit is less developed compared with that of the human neonate. The onset of the cochlear function in the human is reported to be five months before birth, while in the rabbit it is five-day after birth (Pujol, 1985).

**Reviewer II**: Is the celloidin technique responsible for blurry or pasty appearance of some of the pictures?
**Authors**: There was no difference in appearance between tissue prepared by the celloidin technique and by other methods (Hoshino, 1991).

**B.A. Bohne**: What about the time and initial pattern of entrance of the nerve fibers into the developing organ of Corti? How about the innervation of the IHCs?
**Authors**: In this SEM study, it was not possible to observe the interior view of the fluid spaces before they open, so SEM appears to be inadequate for detecting the initial pattern of the innervation to the organ of Corti. Also, we could not observe the innervation of the IHCs in this SEM study because of the lack of space around them.

**B.A. Bohne and Reviewer II**: What was the difference between the afferent and efferent nerve endings on the OHC bases?
**Authors**: In this SEM study, it was not possible to determine the nature of the nerve endings. The large nerve endings at the medial side of the OHCs and the high nerve endings are reported to be efferent endings in TEM studies. The other types of nerve endings were usually hidden by the cytoplasm of Deiters' cells.

**Reviewer V**: Why did not the authors consider to use mid-modiolar sections of the cochlea?
**Authors**: We chose the horizontally cut plane of the cochlea so as to observe the floor of the tunnel, the lateral wall of the Nuel's space and the course of the nerve fibers in the fluid spaces. Mid-modiolar sections are not adequate for getting such interior views.

**Reviewer V**: In Figure 2 of a one-day-old cochlea, how do you know that the tectorial membrane was "tightly" adhered to Deiters' cells?
**Authors**: In this preparation method, the tectorial membrane was usually lifted up during preparation in the mature cochlea. In the immature cochlea, however, the tectorial membrane was not lifted up due to its marginal attachment to Deiters' cells.

**Reviewer V**: In Figure 3 of three-day-old cochlea, why is the space between outer pillar cell and the outer hair cell so large? Is this typical at this age? What are some specific differences between middle and basal turns?
**Authors**: In the rabbit cochlea, Nuel's space was larger than the space of Corti's tunnel at the age just after the opening of the spaces. Also in the immature hamster cochlea, at the age of seven days, Nuel's space was larger than the space of Corti's tunnel. The remarkable difference between middle and basal turns was the size of fluid spaces and the number of the irregular running fibers. The fluid spaces were larger in the basal turn than in the middle turn, as the cochlea develops from the basal portion towards the apex.

**Reviewer V**: What was the frequency of the irregularly running fibers in the three-day-old cochlea? How much did they spiral?
**Authors**: The irregularly running nerve fibers were recognized in every turn. We could not measure the spiral course of nerve fibers in this study.

**Reviewer V**: In Figures 4 and 5 of the five-day-cochlea, did the upper tunnel crossing fibers differ between base and apex, and in what way? What about the nerve endings on hair cells, base to apex?
**Authors**: There seemed to be no significant differences in the appearance of the upper tunnel radial fibers between base and apex in the five-day-old cochlea. At this age, the immature nerve endings on OHCs were observed in the basal and lower middle turn, while they were not recognized in the upper cochlear portion because the basal portion of the OHCs was covered by the spirally running nerve fibers.

**Reviewer V**: In Figures 6 and 7 of the seven-day-old cochlea, what makes a nerve ending immature?
**Authors**: The nerve endings found in the seven-day-old cochlea were small in size compared to those in the adult cochlea at the same cochlear location, so we suppose that the nerve endings are immature at seven days.

**Reviewer V**: In the twelve-day-old cochlea, might different regions have different size endings? What is the interpretation of swellings seen on nerve fibers?
**Authors**: In the basal turn, large nerve endings were recognized at the bases of OHCs, while in the apical and middle turn nerve endings were not so large and they were almost covered by Deiters' cells except for the high nerve endings. We do not know what the swellings of the nerve fibers in Figure 8B represent, or whether they have synaptic contacts to outer spiral fibers.