

Chronic perilymphatic fistula: Experimental model in the guinea pig

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Chronic perilymphatic fistulas were created in guinea pig cochleas using silicone rubber tubing placed into the scala tympani through the round window. Fistula patency was determined by fluorescein perfusion into cerebral spinal fluid. Fistulas were found to be patent in 6 of 6 animals at 7 days and 8 of 13 animals at 28 days. Analysis of ABRs revealed threshold increases of 10 to 15 dB across all frequencies at 1 hour and 7 days. However, thresholds returned to pre-fistula levels by 28 days. Animals with acute fistulas (simple laceration of the round window) had similar threshold increases at 1 hour; however, recovery to baseline levels occurred by day 7. Control animals with intact round windows did not have threshold shifts. Scanning electron microscopy revealed hair cell loss localized to the apical and basal turns of the cochlea. The morphologic changes observed occurred acutely (within 7 days) and were not progressive, despite the presence of a fistula. Hair cell loss or degeneration did not correlate with hearing loss. (OTOLARYNGOL HEAD NECK SURG 1988;99:380.)

Clinical reports of perilymphatic fistulas are abundant; however, histopathologic and physiologic correlates associated with perilymphatic fistulas are poorly understood. This is due in part to the difficulties encountered reproducing the human condition in the laboratory, as well as the difficulty in monitoring the changes in electrophysiologic parameters. Review of the current literature reveals several problems inherent to experimental evaluation of perilymphatic fistulas. First, experimental round window lesions heal spontaneously within 3 days; however, there are numerous case reports of patients with surgically proven fistulas and symptoms present for years before surgical diagnosis.¹⁻⁶ Second, documentation of fistula patency in animals, as in human beings, has been limited to visual inspection of the fenestra, leaving some doubt to the accuracy of the diagnosis. Third, the cochlear micro-

phonic (CM) is an indirect measure of auditory threshold, specifically measuring electrical activity associated with outer hair cell function without reflecting inner hair cell status. In addition, the CM is biased to favor basal turn potentials and is insensitive to lesions in the mid to apical region.⁷ It has also been speculated that electrical shunting by perilymph and granulation tissue may alter the CM response.⁸ Furthermore, serial recordings are difficult to obtain over long periods of time requiring chronic electrode implantation. Lastly, histologic analysis of fistulas has been based on light microscopy with EM studies limited to documentation of round window repair. Few studies have attempted to correlate histologic and physiologic changes.

The purpose of this study was to develop an animal model for chronic perilymphatic fistulas that would allow assessment of long-term effects on cochlear structure and function. To eliminate problems associated with cochlear microphonics, auditory brainstem response thresholds were determined and correlated with morphologic changes as seen by scanning electron microscopy.

LITERATURE REVIEW

Simmons et al.⁸ were the first to perform physiologic studies following round window laceration. They demonstrated a 35 dB loss in CM threshold in cats during the initial 24 hours, followed by gradual return to baseline over 1 to 4 weeks. They also noted that removal of perilymph from around the electrode resulted in re-

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covery of near pre-laceration levels and speculated that perilymph caused shunting. Histologic examination was not performed. Hallen et al.^{9,10} studied acute and long-term morphologic and electrophysiologic effects of lesions in the guinea pig cochlea—specifically fistulas of the scala media, vestibuli, and tympani. Using CMs and light microscopy, they found no consistent relationship between histologic and electrophysiologic findings. Changes in CM thresholds were more severe in acute studies and sensorineuro-epithelial damage occurred at the site of injury only. These comparisons were to normal control groups and individual animals were not followed longitudinally. Axelsson et al.¹¹ specifically addressed round window lacerations in guinea pigs and attempted to correlate alteration in CM and cochlear histology using light microscopy. Organ of Corti degeneration was found in only three of thirteen animals, with survival ranging from 9 to 95 days. CM sensitivity appeared to correlate with organ of Corti degeneration within the basal turn in two of three animals. The third animal exhibited apical degeneration with minimal change in CM sensitivity. CM changes were again compared to normal control animals and longitudinal data was not obtained. Weisskopf et al.¹² also removed the round window in guinea pigs and followed CM over 9 weeks. They observed an immediate postoperative hearing loss that averaged 6 dB, with recovery to near-normal levels by 6 weeks. Histology was not performed, but they speculated that the severe sensorineural hearing loss seen clinically results from some other process associated with rupture of the round window (e.g., intracochlear damage induced by pressure waves, labyrinthitis).

McClure and Lycett¹³ were the first to follow serial ABRs acutely (over 24 hours) after round window removal. In cats, threshold shifts of 25 dB occurred immediately, with gradual increase to 60 dB over 4 hours and lasting 24 hours. With a new twist, H. Lamm et al.¹⁴ demonstrated preservation of CM and the auditory nerve action potential (AP) with a temporary reduction of brainstem response following round window perforation in guinea pigs with the middle ear space filled with Ringer's lactate. They inferred that air in the scala tympani was the cause of sudden SNHL. K. Lamm et al.¹⁵ also found alterations in the latency of ABR waves I and V after round window perforation. These latency shifts were not affected by immediate closure with adhesive fibrin and recovery occurred within 7 to 14 days. Scanning and transmission EM studies (unpublished) revealed *intact* cochlear structures. In addition, their study corroborated earlier data showing rapid healing of the round window lesion, by Choo¹ and Paparella et al.²

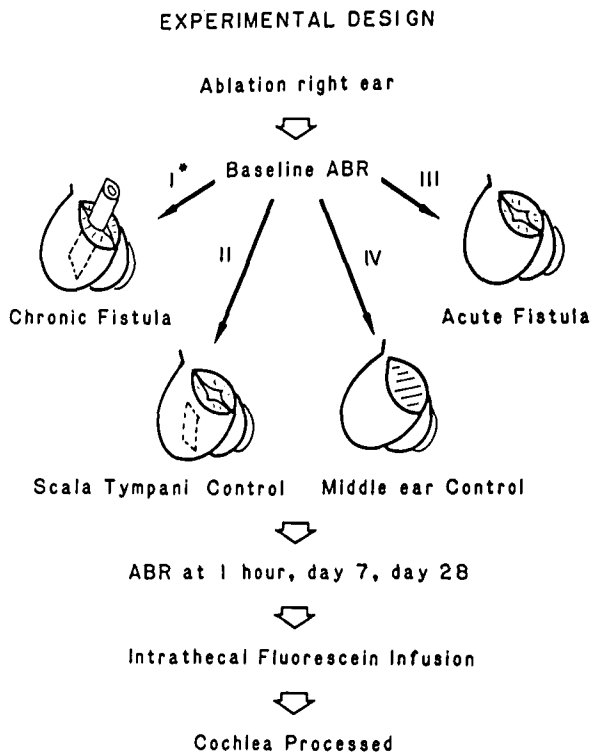


Fig. 1. Flow diagram for experimental and control groups I through IV. * Indicates group.

METHODS

Thirty-six adult male guinea pigs (300 to 350 grams each) were used in the present study. The effects of chronic round window fistulas were examined by serial ABR measurements and with SEM of the organ of Corti. All animals were anesthetized with ketamine and xylazine. Prior to manipulation of the round window, unilateral cochlear ablation was performed via a posterior approach to the mastoid bulla, eliminating the need for contralateral masking (Fig. 1).

EXPERIMENTAL DESIGN

Group I. Chronic fistulas were created in 20 guinea pigs through a posterior approach to the mastoid bulla. A 5-mm length of silicone rubber tubing (0.020 in ID × 0.037 in OD) was placed 2 to 3 mm into the scala tympani after laceration of the round window. ABR thresholds were obtained before the fistula was made, 60 minutes after fistulization, and then repeated at 7 and 28 days. Six animals were killed on day 7; the remainder were killed on day 28, at which time the cochleas were harvested and prepared for scanning electron microscopy.

Group II. Six guinea pigs underwent round window laceration and placement of a 3-mm × 1-mm silicone

POSITIVE FLUORESCEIN INFUSION





		Day 7	Day 28
I	 Chronic Fistula	6/6	8/13
II	 S. Tympani Control		1/6
III	 Acute Fistula		0/5
IV	 Middle Ear Control		0/4

Fig. 2. Number of animals in each group with fluorescein flow into the middle ear space after intrathecal fluorescein infusion. The one animal in group II with a fistula also had otitis media and a persistent fistula, presumably secondary to infection.

rubber strip into the scala tympani. The round window was then immediately closed with a fat tissue plug. ABR thresholds were obtained before the operation, then again at 1 hour, day 7, and day 28. All animals were killed at day 28 and cochleas were harvested for SEM studies.

Group III. Five guinea pigs underwent simple laceration of the round window without closure. ABR thresholds were obtained prior to the laceration, and again at 1 hour, day 7, and day 28 after surgery. Animals were killed at day 28 and the cochleas were processed for SEM.

Group IV. Five guinea pigs had the bulla opened and a silicone rubber strip inserted into the middle ear space. The round window was left intact. ABR and SEM studies were obtained as in groups II and III.

ELECTROPHYSIOLOGIC EVALUATION

Baseline ABR thresholds were obtained from stimulation of the test ear at 5 dB increments with unfiltered clicks and with filtered clicks. Click stimuli for ABRs were generated using a Systron-Donner model 100A pulse generator (Systron-Donner Corp., Concord, Mass.) and presented at 20 clicks/sec. A Krohn-Hite model 3550 filter (Krohn-Hite Corp., Avon, Mass.) was used to shape filtered clicks at 4 kHz, 2 kHz, and 0.5 kHz. The signal was then attenuated using an HP 350C

attenuator (Hewlett-Packard, San Diego, Calif.). A Dynaco stereo 70 power amplifier (Dyna Corp., Philadelphia, Pa.) was used to drive the signal through a second "tail-end" attenuator, set at 30 dB attenuation, to a TDH 49 transducer (Telephonics Corp., Huntington, N.Y.) in an earbar cannula system.

The EEG recording from vertex, nuchal, and thoracic electrodes was fed to a Grass Model P511J preamplifier (Grass Instrument Co., Quincy, Mass.) (bandpass filtered 100 to 3000 Hz) and then to a signal averager (Nicolet Model 1170, Nicolet Instrument Corp., Madison, Wisc.) set to average 512 sweeps. Waves I through IV were recorded; however, wave IV was found to be most sensitive for determining thresholds.

FLUORESCEIN INFUSION

Before the animals were killed, the bulla on the side of the fistula was opened, allowing visualization of the silicone rubber tubing and round window. The incision was extended posteriorly, allowing exposure of the cysterna magna, which was cannulated with silicone rubber tubing (0.37 in OD) and infused with 0.2 cc of 0.25% sodium fluorescein using a microperfusion pump. Visualization of fluorescein flowing into the middle ear space verified patency of the fistula. Perfusion fluorometry was performed during this procedure using a Diversatronics perfusion fluorometer (Diversatronics Inc., Broomall, Pa.) to detect systemic distribution of fluorescein. Fluorescein infusion was performed on all animals studied.

SCANNING ELECTRON MICROSCOPY

All cochleas were immediately harvested at the time the animals were killed and fixed by cochlear perfusion. The apex was opened using a 26-gauge needle and 2.5% phosphate-buffered glutaraldehyde was infused via the oval window. Specimens were left in fixative for 4 hours followed by a 12-hour rinse in phosphate buffer. The tissue was post-fixed in 1% osmium tetroxide (OSO₄).

Microdissection of each cochlea was performed by thinning the bone, overlying all turns with a diamond bur, and removing all bone piecemeal with microforceps to permit inspection of the organ of Corti. The specimens were processed using the osmium-thiocarbonylhydrazide (OTO) method, dehydrated, and critical point-dried with CO₂. After mounting with silver paste on aluminum stubs, the spiral ligament and Reissner's membrane were removed. The specimens were then coated with gold approximately 150 Å thick on a rotating stage in a vacuum evaporator. Examination was performed with a JEOL 35C scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 15 kv.

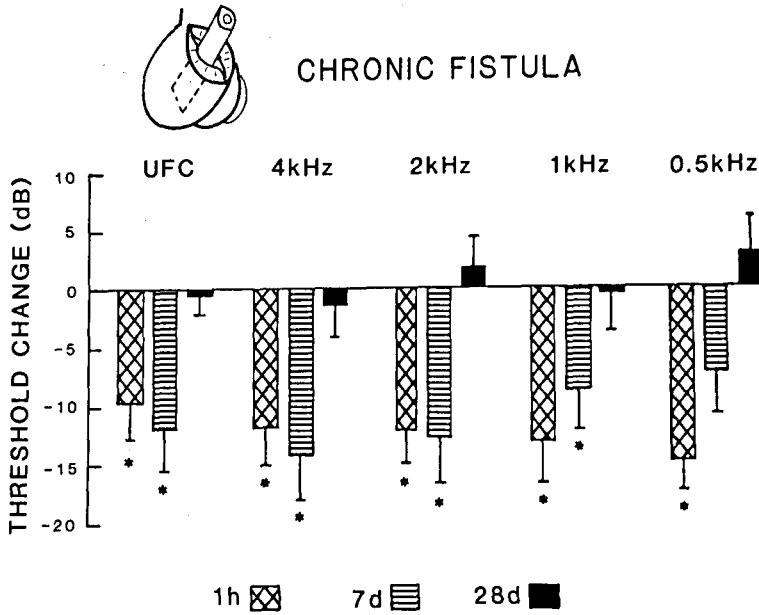


Fig. 3. Mean auditory brainstem response thresholds for group I, obtained at 1 hour, day 7, and day 28, after placement of silicone rubber tubing through the round window. Negative deflection indicates increase in threshold (n = 7). Standard error indicated by vertical bar; * = p 0.05.

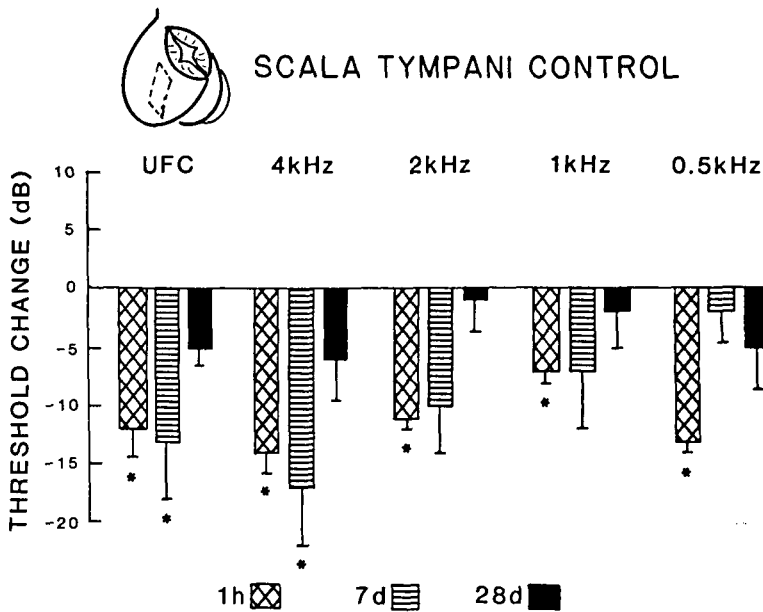


Fig. 4. Mean auditory brainstem response thresholds for group II, obtained at 1 hour, day 7, and day 28, after placement of a 3 × 1 mm silicone rubber strip through the round window into the scala tympani, followed by closure of the defect (n = 5).

RESULTS

General observations. Despite unilateral labyrinthectomy, none of the animals demonstrated significant vestibular dysfunction as manifested by head tilt or

failure to eat, with the exception of control group IV. All five animals in this group demonstrated persistent head tilting and one animal died on day 6. One animal in group I died on day 14 of unknown cause.

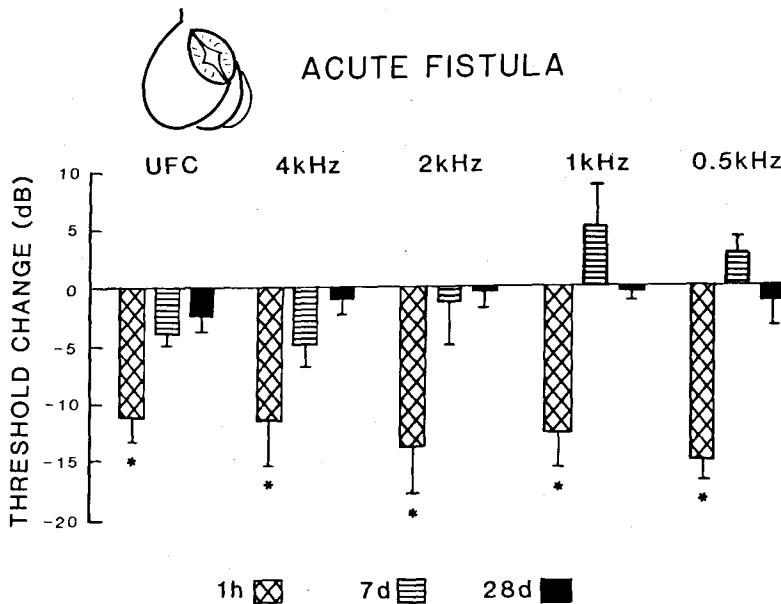


Fig. 5. Mean auditory brainstem response thresholds for group III, obtained at 1 hour, day 7, and day 28, after simple laceration of the round window ($n = 5$).

Fluorescein infusion. Six animals in group I were killed at day 7 and all six had fluorescein flow into the middle ear space through the fistula. Of the remaining thirteen animals in group I, eight had fluorescein flow through the fistula on day 28. The five animals with closed fistula were separated into group IA. One animal in group II was found to have a positive infusion on day 28, and none of the animals in group III or IV were found to have a patent fistula (Fig. 2). None of the animals studied were found to have systemic absorption of fluorescein as determined by dermofluorometry.

ABR ANALYSIS

Figs. 3 through 5 show changes in ABR thresholds as a function of time after surgery for groups I through III. These results are summarized later. The statistical significance of threshold changes for each group were determined by the *t*-test for related samples using *PC Statistician, The Statistical Report Program* (Human Systems Dynamics, Northridge, California, 1983). Probability values <0.05 (two-tailed) were accepted as statistically reliable.

Group I. Significant increases in ABR thresholds that averaged 10 to 15 dB for all frequencies tested occurred at 1 and 7 days (Fig. 3). Recovery to baseline levels (± 5 dB) occurred somewhere between 7 and 28 days. None of the animals killed at day 7 had evidence of otitis media (OM). One animal in group I, killed at 28 days, had cloudy debris in the middle ear space

consistent with OM and a persistent 5 to 10 dB threshold increase across all frequencies. Of the five animals with closed fistula (group IA, not shown), two recovered to pre-fistula ABR threshold levels by day 28—one without evidence of OM had a persistent 10 to 20 dB increase in all thresholds—and two animals with OM had severe losses in all frequencies (>60 dB) at 28 days.

Group II. ABR analysis revealed significant increases in thresholds across all frequencies at 1 hour, which persisted in the higher frequencies (≥ 2 kHz) at 7 days (Fig. 4). Recovery was complete at 28 days, with the exception of two animals; one had OM and 10 to 20 dB threshold shift at 2 to 4 kHz, and the other had a normal appearing middle ear space and a 10 to 15 dB threshold shift at 0.5 to 1 kHz.

Group III. All animals demonstrated a similar pattern of hearing loss observed in groups I and II at 1 hour. However, all recovered to within normal range of thresholds by day 7 (Fig. 5). One animal with purulent OM was found to have persistent threshold shifts at day 28.

Group IV. With the exception of two animals with a low frequency (0.5 kHz) loss of 10 to 15 dB at 1 hour, all animals had normal hearing at 1 hour and 7 days. Two animals demonstrated late onset hearing loss at day 28, with findings of purulent OM at the time they were killed (10 to 20 dB loss at 1 to 4 kHz).

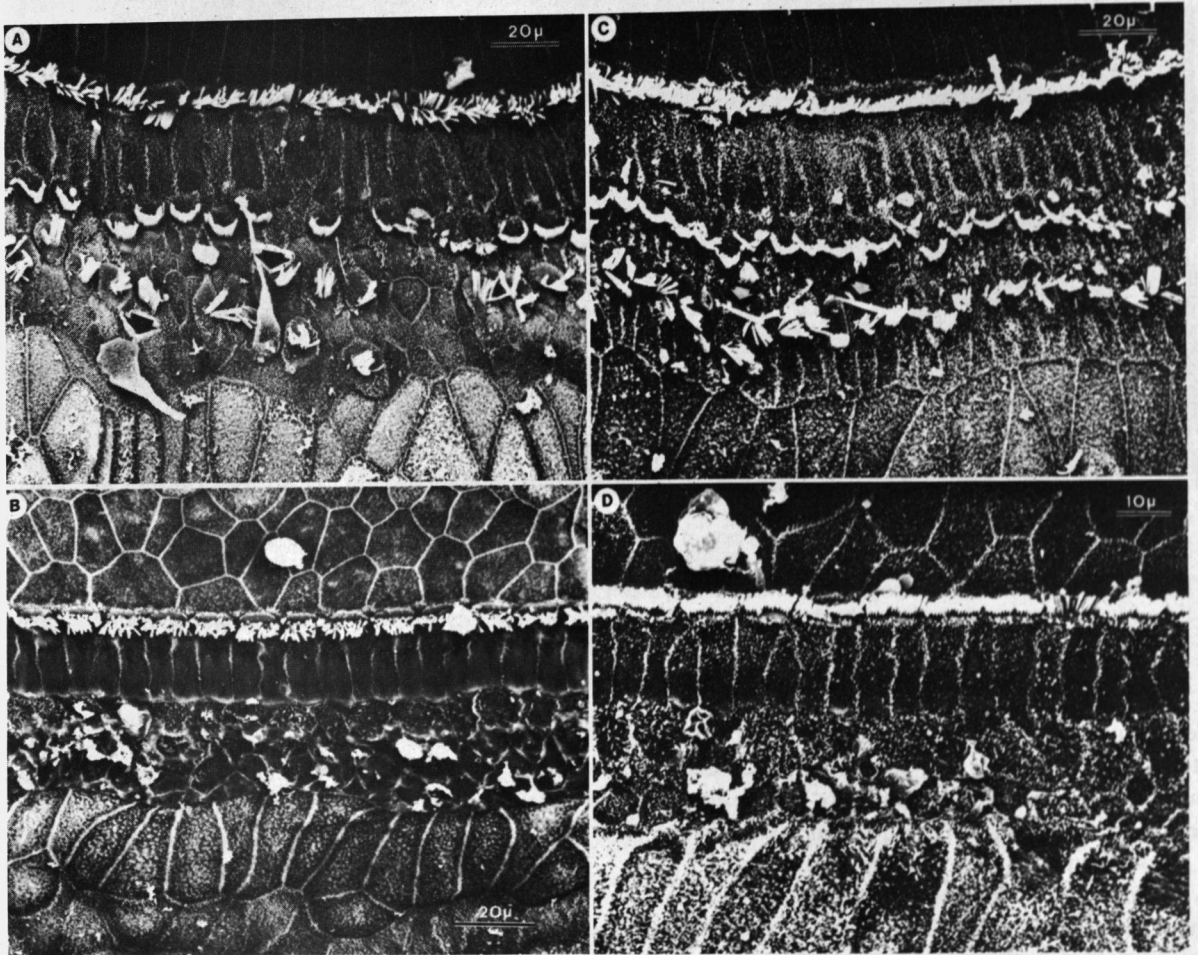


Fig. 6. **A**, Scanning electron micrograph of apical turn of a guinea pig cochlea with a chronic fistula (group I) killed at day 7, demonstrating sensory cell damage. Arrows identify outer hair cells with advanced stereocilia degeneration. The inner hair cells are intact (Magnification $\times 700$.) **B**, Photomicrograph of basal turn of the same cochlea, showing total loss of outer hair cells and preservation of inner hair cells. Integrity of the reticular lamina is preserved by the phalangeal processes of the Deiters' cells (Magnification $\times 750$.) **C**, Photomicrograph of the apical turn from guinea pig cochlea in group I killed at 28 days. Degenerative changes are limited to the outer hair cells. The sensory cell population is reduced to two-thirds of normal. Surviving outer hair cells demonstrate distorted, fused, and giant stereocilia (arrows). (Magnification $\times 800$.) **D**, Photomicrograph of basal turn of cochlea from same animal killed at 28 days. Outer hair cell degeneration is complete. (Magnification $\times 1100$.)

MORPHOLOGY

The cochleas of animals from each group were examined using the scanning electron microscope. For technical reasons, examination of the most apical half-turn and basal hook region were not possible.

Group I. In all but two animals killed on day 7, damage was restricted to the apex and basal turn 2 mm from the hook region. In the apex, two-thirds of the outer hair cells were either missing or displayed degenerative changes, including fused or absent stereocilia (Fig. 6, A). Cuticular plates were extruded and other cellular debris was scattered across the reticular

lamina. Five animals were found to have 50% to 100% loss of outer hair cells in rows 2 and 3 ($\text{OHC}_{2,3}$) with less than 10% loss in outer row 1 (OHC_1). Absent hair cells were replaced by the phalangeal processes of Deiters' cells, maintaining the endolymphatic surface of the organ of Corti.

Within 2 mm of the hook portion of the basal turn the outer hair cells were uniformly absent (Fig. 6, B). The reticular lamina was preserved by the phalangeal cell processes of Deiters' cells. A transition to normal sensory epithelium occurred within a 1-mm zone, 2 to 3 mm from the hook, within which there were varying

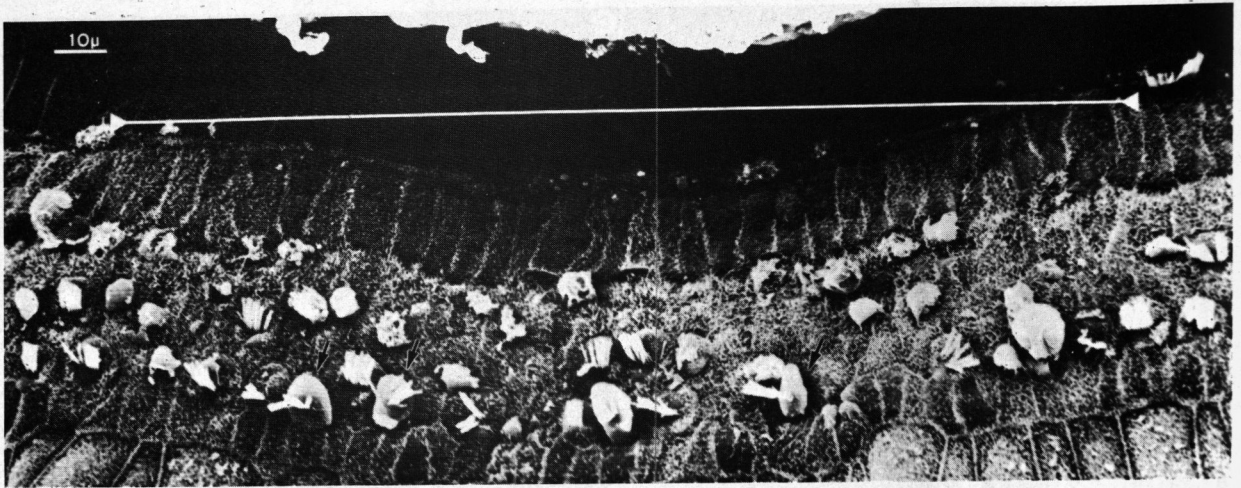


Fig. 7. Scanning electron micrograph of the apical turn in a guinea pig cochlea killed 28 days after simple laceration of the round window (group III). Both inner and outer hair cells demonstrate degenerative changes. Inner hair cell loss is almost complete and outer hair cells are either absent or are in the process of degenerating. Cuticular plates are displaced and appear turned "up-on-end" (arrows). Debris is scattered across the intact reticular lamina. (Magnification $\times 975$.)

degrees of outer hair cell degeneration (fused and absent stereocilia). The inner hair cells were intact, except in two animals in which approximately 10% of inner hair cells were absent or displayed degenerative changes.

Group I animals killed on day 28 with patent fistulas were found to have morphologic changes similar to those examined after 1 week (Fig. 6, C and D). Damage was restricted to the apical turn and basal turn within 2 to 3 mm of the hook portion of the cochlea. Two animals were found to have approximately 50% of the apical hair cells missing and the remaining apical sensory cells possessed disorganized or fused stereocilia. The outer two rows of hair cells were, again, more severely affected ($OHC_3 > OHC_2$). Only one of these two animals had concomitant basal turn damage. A third guinea pig had isolated apical degenerative changes (disarray and fusion of stereocilia).

Within the basal turn 2 to 3 mm from the hook, five animals were found to have more than 75% outer hair cell loss involving all three rows; and, only one of these animals showed occasional (<10%) inner hair cell loss in this region.

Group IA animals were found to have similar morphologic changes as described earlier. Two animals with OM had diffuse hair cell loss (75% to 100% loss) involving all turns of the cochlea.

Group II. Consistent findings included normal-to-moderate hair cell loss in the apex involving the outer hair cells, and outer hair cell damage in the region of the basal turn adjacent to the hook. Two animals were found to have isolated apical hair cell damage—one

with 75% outer hair cell loss and approximately 10% loss of inner hair cells. One animal had both apical and basal-turn hair cell loss (approximately 25%) affecting the outer two rows only. In one specimen without apical damage, inner and outer hair cell degeneration occurred in the basal turn 1 to 2 mm from the hook, with 50% to 75% depletion of hair cells.

Group III. Twenty-eight days after simple laceration of the round window, morphologic changes included degeneration and hair cell loss in the apical and basal turns, similar to groups I and II. Along the apical turn, outer hair cell counts were reduced from 10% to 75% of normal in all specimens examined. Outer hair cells were either absent or identified by "toppled" cuticular plates, which in some areas resembled tombstones (Fig. 7, arrows). Inner hair cells were intact apically, except for a single specimen with only scattered cells surviving (Fig. 7, bracket). Hair cell loss within the basal turn of this specimen occurred in both inner (50% depletion) and outer (100% loss) hair cells within 3 mm of the hook. The remaining specimens were found to have severe (75% to 100%) outer hair cell loss in the basal turn with intact inner hair cells.

Group IV. No morphologic changes were identified in these specimens in which the round window was not opened. Inner and outer hair cell morphology was preserved throughout.

DISCUSSION

Understanding the histopathology and physiology associated with perilymphatic fistulas is limited by the

difficulty of reproducing the human condition in laboratory animals. In such models, documentation of fistula patency is essential. The communication between cerebrospinal fluid (CSF) and perilymph has been studied extensively in the guinea pig, cat, and rabbit.^{16,17} Studies by Jako et al.¹⁶ demonstrated CSF to perilymph diffusion of fluorescein within 60 seconds, whereas intravenous fluorescein infusion resulted in cochlear staining after 6 to 90 minutes. In our guinea pigs fluorescein could be seen staining the basal turn of the cochlea (and in the presence of a fistula flowing into the middle ear space) within 30 to 60 seconds. To control for systemic diffusion we monitored perfusion fluorometry and could not detect the presence of fluorescein within cutaneous capillary vessels during the two-minute middle ear observation period. Our study demonstrated that fluorescein is a reliable marker for fistula patency, at least in animals in which the cochlear aqueduct allows communication between CSF and perilymph.

ABR threshold shifts following round window fistulization have been documented prior to this study; however, the return to normal threshold levels with a patent fistula has not been previously reported. Statistical analyses of our data demonstrated significant increase in thresholds for groups I and III after fistulization, though long-term effects (at 28 days) were not seen. The differences in group size (n) did not adversely affect the analysis of our ABR data. The magnitude of differences and variances obtained indicated that average threshold changes greater than 5 dB would be statistically significant.

Unexpected results were found when correlating physiologic and histologic data. Changes in hair cell morphology were indistinguishable among the various groups, with the exception of group IV, in which no changes were observed. In addition, the observed changes within hair cell populations (all groups) did not correlate with alterations in ABR thresholds. Group I animals killed at 7 days were found to have apical and basal lesions associated with significant threshold shifts throughout all frequencies. However, in animals with patent fistulas, that were killed on day 28, the hearing loss reverted to normal, whereas morphologic changes persisted. The transient threshold increase observed is difficult to explain, especially since diffuse stereocilia changes were not seen in the animals killed on day 7.

Previous studies using light microscopy have not demonstrated consistent hair cell loss localized to the apical and basal turns, although sporadic reports have appeared in the literature.^{11,18} Schuknecht and Gao,¹⁸ for example, reported basal and apical hair cell loss

occurring after labyrinthectomy via the oval window in cats. Explanations for the observed morphologic changes are only speculative, since there is no strong evidence supporting a mechanical, chemical, or metabolic event. Furthermore, the cellular degeneration and hair cell loss observed in our animals are nonspecific, having been shown to result from a variety of insults, including acoustic trauma, electrical stimulation, and ischemia.^{19,20} It may be reasonable to assume that alterations in perilymph or perilymph flow may be disruptive to the normal metabolic events maintaining the functional and anatomic integrity of the organ of Corti.

The lack of correlation between hair cell loss and threshold changes may be explained on the basis of frequency localization along the basilar membrane, and/or the apparent responsiveness of the basal turn to a wide spectrum of frequencies. The 4 kHz filtered click used in this study would correspond to the region on the basilar membrane 8 to 10 mm from the hook, well beyond the region of injury observed; whereas the lowest frequency (0.5 kHz) tested *does* correspond to the region with apical hair cell loss. The responsiveness of the basal turn to stimuli at this frequency could explain why permanent threshold shifts were not observed in the face of hair cell loss. Alternatively, the presence of intact inner hair cells may be sufficient to maintain normal threshold responses.

SUMMARY

A chronic fistula model in the guinea pig was developed using a silicone rubber tube introduced through the round window. Patency of the fistula was documented by CSF fluorescein infusion. Hearing loss was found to be reversible despite the presence of a fistula. Morphologic changes (hair cell loss) occurred acutely, were localized to the apical and basal region, and were not progressive. The morphologic changes did not correlate with hearing loss.

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