

Kanamycin and bumetanide ototoxicity: Anatomical, physiological and behavioral correlates

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Severe hair-cell degeneration and cochlear dysfunction was observed in chinchillas examined at 60 days (or longer) after administration of a single injection of 150 mg/kg kanamycin, followed 2 h later by a single injection of 20 mg/kg bumetanide. Outer hair cells in the cochlear base were most severely affected. While inner and outer hair-cell loss was common, some animals showed large regions along the basilar membrane where almost all inner hair cells were present and almost all outer hair cells were absent. Wherever areas of complete degeneration of the organ of Corti occurred, a small, diffuse population of nerve fibers within the spiral lamina was always present. Single-unit tuning curves correlated best with anatomical observations, compared with the other functional measures of auditory sensitivity that were obtained (behavioral audiogram and compound action potential thresholds). Results indicated that behavioral detection of auditory stimuli is relatively independent of innervation density as long as a few inner hair cells are present. Thus, the cross-fiber threshold envelope of the single-unit tuning curves appeared very similar to the behavioral audiogram.

Key words: AP thresholds; behavioral audiogram; bumetanide; cytochrome; kanamycin; potentiation; single-unit tuning curves.

Introduction

Severe permanent hearing losses have been reported in patients receiving the combined administration of the loop-inhibiting diuretic ethacrynic acid and the aminoglycoside antibiotic kanamycin [16,24]. Cochlear dysfunction and hair-cell loss have also been reported in guinea pigs after a single combined dose of kanamycin and either of two loop-inhibiting diuretics, ethacrynic acid or furosemide. No significant cochlear damage was evident when the drugs were given separately at the same doses.

The present study has two purposes. The first is to determine if an ototoxic reaction occurs after a single combined dose of kanamycin and bumetanide. Bumetanide is a relatively new loop-inhibiting diuretic, with clinical importance, since it appears to be more potent and less ototoxic than either ethacrynic acid or furosemide [3]. The second purpose of this study was to examine two hypotheses that rely on neural data to relate the behavioral audiogram to structural damage. The first hypothesis, previously proposed by Schuknecht and Woellner [34], states

that behavioral detection of auditory stimuli is relatively independent of innervation density, so that the responses from only a few hair cells can account for behavioral sensitivity thresholds. The second hypothesis, alluded to by Kiang and coworkers [19,23], is that the cross-fiber threshold envelope of single-unit tuning curves closely approximates the behavioral audiogram, provided that tuning curves are obtained from an adequate sample of nerve fibers.

Methods

Twenty normal chinchillas, approximately one year old, were randomly divided into two control and one experimental groups. One control group (BUM) contained five animals, each of which received a single intravenous injection of bumetanide (20 mg/kg; Hoffmann-La Roche). The other control group (KAN) contained five animals, each of which received a single subcutaneous injection of kanamycin (150 mg/kg; Kantrim, Bristol). The experimental group (KAN-BUM) contained ten animals, each of which received an injection of kanamycin (150 mg/kg) followed 2 h later by an injection of bumetanide (20 mg/kg). To prepare the bumetanide solution, the powder was dissolved in 0.2 ml/kg NaOH (1 N) and added to 0.7 ml of saline. Bumetanide was injected over a period of 15 s into the right jugular vein.

Four of the ten KAN-BUM animals had been previously monauralized by surgical destruction of a cochlea and were subsequently used for behavioral testing. Preinjection behavioral thresholds were based upon the average of the thresholds obtained during the last 12 test sessions preceding the drug treatment. After the injections, the animals were tested 3 times per week until thresholds had reached stable values for at least 10 test sessions. Final thresholds were based upon the average of the thresholds obtained during the last 10 sessions.

An automated conditioned avoidance behavioral testing procedure was employed to test sensitivity at octave frequencies. This procedure used an adaptive threshold testing paradigm. Briefly, the technique required a chinchilla to cross the barrier in a shuttle-box apparatus in response to a series of test tones. A buzzer served as a conditioned negative reinforcer, and on a percentage of the 'miss' trials a mild electric shock was delivered to the animal's feet to maintain response behavior. The buzzer and shock presentations, as well as the test tones, were under control of an automated testing program. Test tones were 500 ms in duration and were switched with 25 ms rise-decay times. A complete description of the behavioral testing procedure is described by Nelson et al. [27]. After behavioral testing (65–165 days following the drug treatment) the four KAN-BUM animals were physiologically tested.

Animals were initially deeply anesthetized with an intraperitoneal injection of sodium pentobarbital. Supplementary doses were administered regularly after surgery to maintain relatively light anesthesia. A tracheal cannula was inserted but artificial respiration was not used. A rectal probe was used in a feedback system that employed a d.c.-current heating pad to maintain a body temperature at 37.8°C. The left pinna and the lateral bony wall of the external meatus were removed, to allow a

short and direct sound path in the closed acoustic system. The bulla was opened widely and the tensor tympani ligament was cut. The auditory nerve was approached from the posterior cranial fossa by aspiration of the overlying cerebellum. Micro-pipettes (30–120 M Ω impedance) were positioned over the acoustic nerve and remotely advanced into the internal auditory meatus. Compound action potentials were monitored by means of a silver wire positioned on the round window membrane.

The sound delivery system consisted of a metal-encapsulated Beyer DT-48 earphone and a plastic speculum whose tip, inserted in close proximity to the eardrum, contained a metal probe tube connected to a miniature (electret) microphone. At the beginning of each experiment, the calibrated probe-tube microphone was used to create tables of sound levels and phases for fixed-voltage input to the earphone at 240 frequencies between 100 Hz and 24 kHz. These tables, stored on a disk, were used by a PDP11 computer to automatically adjust the input to the earphone in order to generate tonal acoustic stimuli with known levels and phases. In addition, the stimulus signal path included a 1/3-octave band equalizer, which was reset in each experiment to yield a relatively flat acoustic frequency response. All stimuli were generated by digital computation and presented to the earphones via a waveform buffer equipped with 16k 16-bit words and 2 digital-to-analog converters. Compound action potential (AP) thresholds were determined for each stimulus frequency from averages of 256 responses using a criterion of 0.3 μ V peak N₁-to-baseline magnitude. Single-unit tuning curves were obtained with an automated procedure identical to that used by Kiang et al. [19] and Liberman [22], except that the level steps used were 1 and 2 dB, instead of 2/3 and 4/3 dB.

In order to estimate best frequencies (BFs) for neurons whose tuning curves were relatively flat, a simple algorithm was devised which relates the estimated BF to the high frequency cutoff at a fixed SPL (80 or 90 dB) of the tuning curve. The algorithm, calibrated on the basis of several hundred normal tuning curves, always yields correct BFs for normal tuning curves. Robertson et al. [29], who recorded from spiral ganglion neurons innervating damaged regions of the cochlea, indicate that the most sensitive portions of tuning curves probably consistently underestimate the 'true' (pre-damage) BF. On this basis, it is almost certain that our BF estimates for abnormal neurons are biased toward low frequencies. The magnitude of the bias is uncertain, but the data of Robertson et al. [29] suggest that it should not be much greater than 1/4 octave, equivalent to a distance of 0.6 mm on the basilar membrane. With this *caveat*, our estimates are useful in that they provide an objective, consistent and automated means to assign predamage BFs to neurons whose tuning curves may vary from normal to extremely pathological. After physiological testing the four KAN-BUM animals were killed along with all the remaining animals in the three groups for anatomical examination.

All animals were anesthetized and killed by decapitation. Their left cochleae (and right cochleae in the four functionally tested animals) were fixed by perilymphatic perfusion of 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Fixation by immersion continued for 24 h. Cochleae were subsequently postfixated for 2 h with 0.5% osmium tetroxide in phosphate buffer containing 0.4% potassium dichromate.

Whole-mount, 'soft surface' preparations [1] of the organ of Corti were produced by decalcification in 0.1 M EDTA containing 2% glutaraldehyde [2]. Hair-cell stereocilia were stained with alcian blue and cell nuclei were stained with ruthenium red [33]. Segments of tissue containing the organ of Corti were mounted on slides in Aqua Mount (Sigma) for light microscope examination.

To produce cytochleograms from organ of Corti surface preparations, the condition of each hair cell was recorded with the aid of a semiautomatic device. This device consisted of a microcomputer with a digitizing tablet, two foot pedals and a microscope equipped with a drawing tube. Hair cells were serially assessed along the entire length of the basilar membrane and each hair cell was classified as present or absent. A hair cell was counted present based upon the presence of stereocilia (including distorted stereocilia) and the presence of a cell nucleus. A hair cell was counted absent if it had been replaced by a phalangeal scar, if it lacked a nucleus, or if all of the stereocilia were missing. In areas of complete cellular degeneration of the organ of Corti, the length of the defect was measured along the region of the pillar cells and the number of degenerated hair cells was estimated from normal hair-cell density measurements. Cytochleograms were produced with the aid of a computer. All photographs in this paper were taken using Hoffman modulation contrast illumination.

Results

Anatomical observations

Greater hair-cell loss occurred in those animals that received the combined administration of kanamycin and bumetanide (KAN-BUM, Fig. 2) than in those animals that received either kanamycin (KAN) or bumetanide (BUM) alone (Fig. 1). The BUM group showed slightly more hair-cell loss than the KAN group (Fig. 1).

In the KAN-BUM group there was a broad range (from 13 to 99%) of hair-cell loss among animals (Fig. 2). Hair-cell loss was most frequent in the cochlear base. Median values for outer hair cell (OHC) loss (78.8%) exceeded inner hair cell (IHC) loss (34.4%). Across the three rows, loss of OHCs was greatest in row one and least in row three. In addition, some animals showed large regions along the length of the basilar membrane where only IHCs were present with no corresponding OHCs. In animal 2045 (Fig. 2) a region approximately 11 mm in length (over 60% of the basilar membrane) contained an almost full complement of IHCs without any corresponding OHCs.

Where IHCs were present with complete loss of OHCs, most of the supporting cells of the organ of Corti were also present (Fig. 3). However, when damage consisted of complete loss of IHCs and OHCs, some or all of the supporting elements of the organ of Corti degenerated and were replaced by a low cuboidal epithelium (Fig. 4). When complete loss of both sensory and supporting cells of the organ of Corti occurred, a sparse population of nerve fibers was always present within the corresponding segment of the osseous spiral lamina. IHC loss was always accompanied by a substantial decrease in nerve fiber density. Without IHC loss,

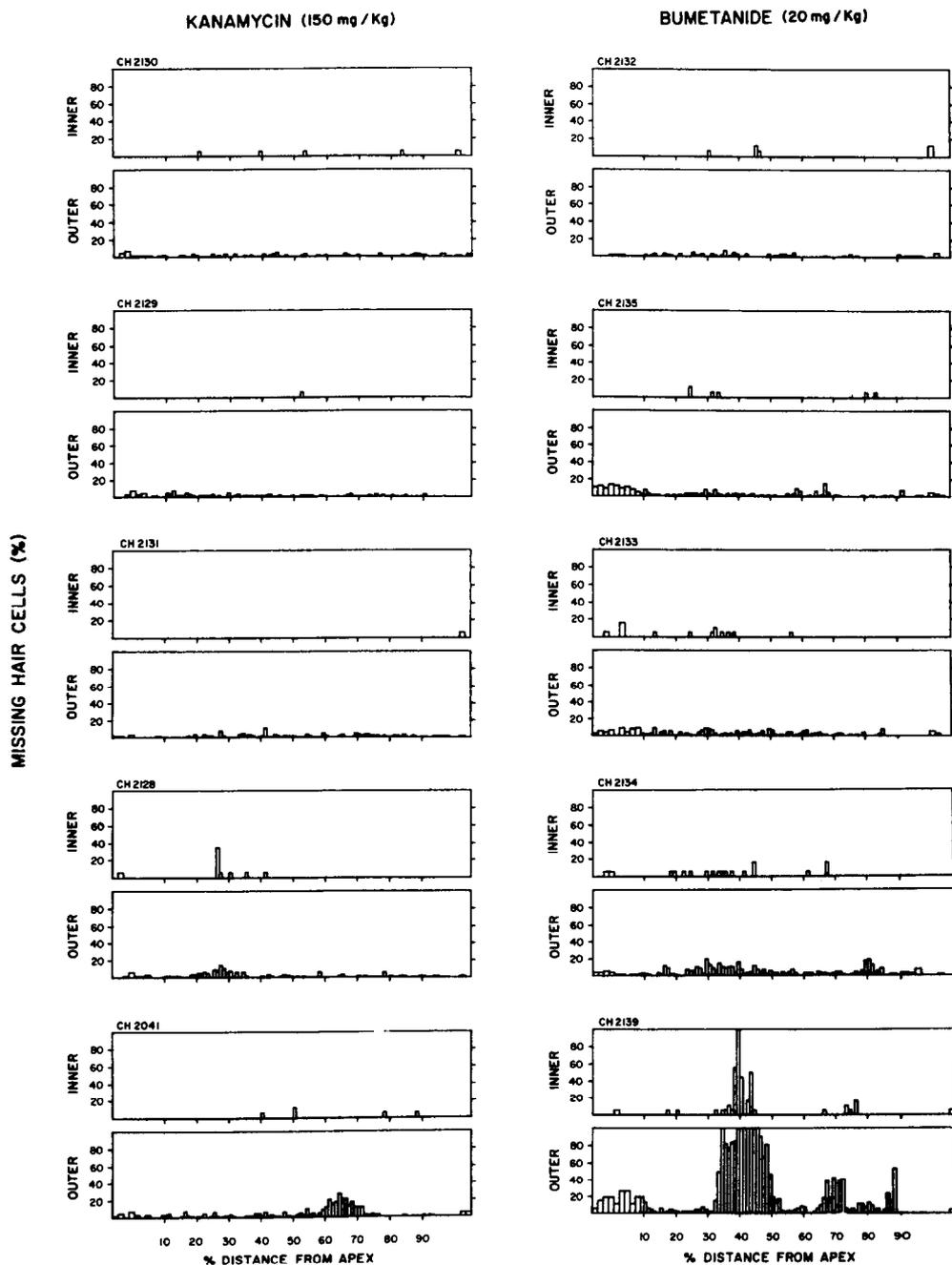


Fig. 1. Ten cytocholeograms from chinchillas that were treated with either a single subcutaneous injection of 150 mg/kg kanamycin or a single intravenous injection of 20 mg/kg bumetanide. All animals were allowed to recover for at least 60 days following the drug treatment. A cytocholeogram from each animal consists of a pair of panels. The upper panel represents the IHCs while the lower panel represents the combined three rows of OHCs. The ordinate is percent IHCs or OHCs missing, while the abscissa is percent distance from the cochlear apex. The cytocholeograms are ordered from least to greatest total hair-cell loss.

KANAMYCIN (150 mg/Kg) and BUMETANIDE (20 mg/Kg)

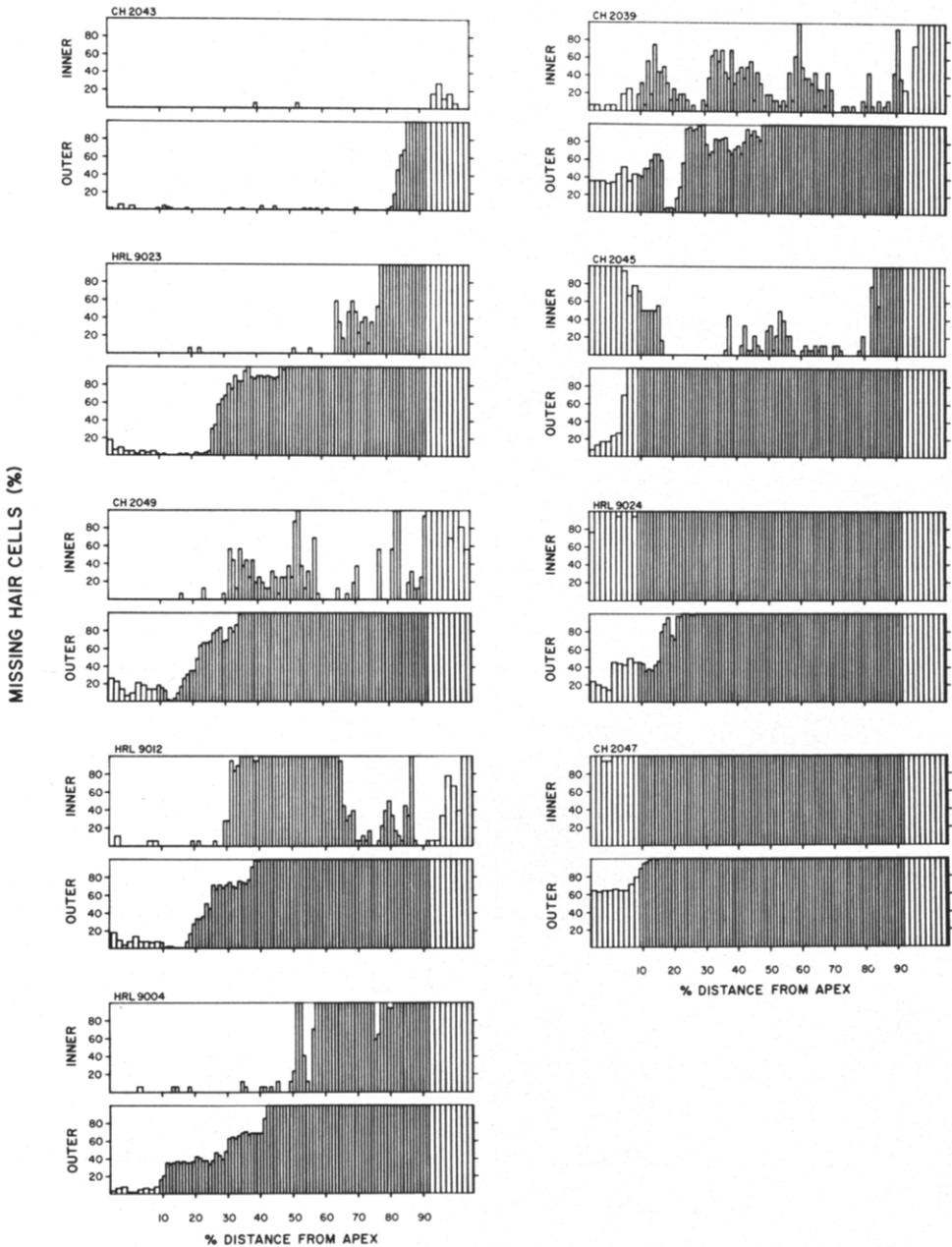


Fig. 2. Cytochromeochleograms from the nine surviving animals that received the combined administration of kanamycin and bumetanide. There were large differences in individual susceptibility to the drug combination; however, all animals showed significant hair-cell loss. The cytochromeochleograms are ordered from least to greatest total hair-cell loss. In general, hair-cell loss was greatest in the cochlear base and a greater percentage of OHCs were missing than IHCs. In addition, some animals (e.g., 2045, 9023) showed large regions of complete OHC loss with correspondingly small IHC loss.

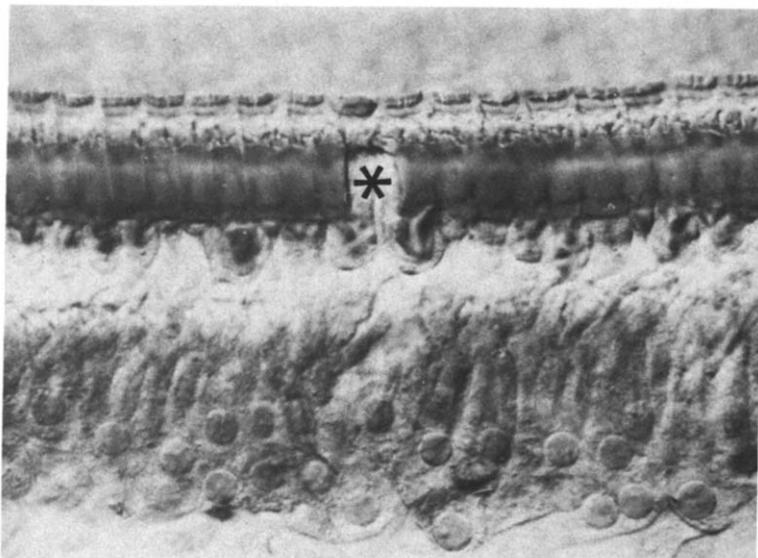


Fig. 3. A segment of the organ of Corti in the first turn of animal 2045. No OHCs are present whereas all of the IHCs are present. Most supporting cells are present with the exception of some pillar cell degeneration (*). Magnification, $\times 400$.

even complete OHC loss did not appear to significantly reduce nerve fiber density. In several KAN-BUM animals a few small bundles of nerve fibers appeared in regions where the organ of Corti had completely degenerated. These nerve fibers were myelinated and some traveled in a radial course for considerable distances along the basilar membrane (Fig. 4), while other nerve fibers made a U-turn at the habenula perforata and traveled back into the spiral lamina.

In all the KAN-BUM animals, the stria vascularis contained greater amounts of granular material (that resembled lipofuscin) than was observed in the stria vascularis of KAN or BUM animals. Strial atrophy occurred in only two animals (both in the KAN-BUM group) and it was located in the cochlear hook.

Functional measures

Anatomical, physiological and behavioral results from animal 9023 are shown together in Fig. 5. The lower graph displays the percentage of missing hair cells as a function of relative distance (from the apex) along the basilar membrane. The counts from the three rows of OHCs are pooled across rows into a single average measure of OHC loss in the lowest panel; counts for the IHCs are in the next lower panel. This animal shows a typical kanamycin-like cochlear lesion. There was a total loss of hair cells in the basal one-third of the cochlea. The middle third of the cochlea showed almost complete loss of OHCs with almost no loss of IHCs. The apical third of the cochlea showed almost no hair cell loss.

The upper graph of Fig. 5 shows the functional measures from this animal.



Fig. 4. Two myelinated nerve fibers (*) that are traveling beneath the external sulcus cells and low cuboidal epithelium that has replaced the sensory and supporting cells of the organ of Corti. Magnification, $\times 2000$.

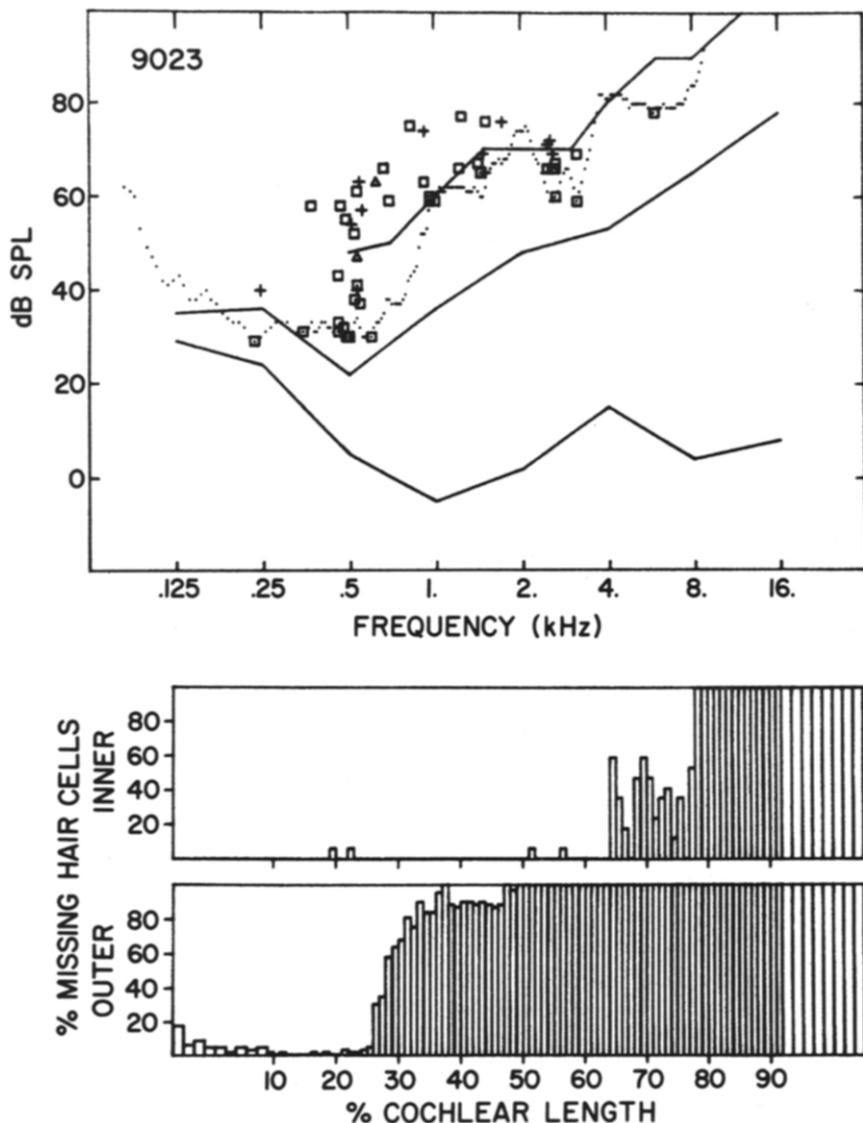


Fig. 5. This figure consists of three panels. The lower two represent the cytocochleogram as shown before (Figs. 1 and 2). The upper panel shows the functional measures. The lower of the 3 solid curves represents pretreatment behavioral thresholds, the middle curve represents posttreatment behavioral thresholds and the upper curve represents compound action potential (AP) thresholds. The symbols represent the estimated best-frequency thresholds (BFs) from single-unit tuning curves. The square symbols represent high-spontaneous-activity neurons (>10 spikes/s), the plus symbols represent medium spontaneous activity neurons (1-10 spikes/s) while the triangles represent low spontaneous activity neurons (<1 spike/s). The curve represented by the dotted line is the cross-fiber threshold envelope. This curve represents, at any given frequency, the lowest level at which any fiber responds. This animal (9023) shows a typical kanamycin-like lesion with basal loss of IHCs and OHCs and midcochlear loss of only OHCs. The post-treatment behavioral audiogram shows a sloping high frequency hearing loss that parallels the AP thresholds and the cross-fiber threshold envelope. Note also the correspondence between the presence and absence of OHCs and the apparent spreading of thresholds of a number of neurons at the 500 Hz BF region.

Frequency-distance mapping of the cochlea was similar to the map of Siegel [35], which was based on data of Eldredge et al. [11,12]. The lowest solid curve is the pre-treatment behavioral threshold. The threshold values for this animal were similar to those measured in normal chinchillas [25,27]. The post-treatment behavioral audiogram (middle solid curve) revealed elevated thresholds (10–70 dB) for all frequencies, characterized as a sloping high-frequency hearing loss. As mentioned earlier, these behavioral thresholds were based on averages of a number of test sessions. Standard deviations about the mean threshold values averaged 7.9 dB in all animals for both pre- and post-treatment tests; these error terms are comparable to those obtained previously from normal-hearing chinchillas [25,27]. During the period of behavioral testing starting from day 6 to day 60 after drug treatment there was no progressive increase in threshold shift.

Post-treatment (AP) thresholds for animal 9023 (top solid curve, Fig. 5), determined from the compound action potential (AP) recordings, were parallel to and elevated from the behavioral thresholds. These parallel differences between AP thresholds and behavioral thresholds are consistent with Henderson's et al. [14] concept of temporal integration and differences in test signal duration. No single-unit tuning curves (symbols represent BF thresholds) were recorded from neurons with BFs above 8 kHz, corresponding to the region of complete hair-cell loss. In the 500 Hz frequency region, there was a clustering of single units with thresholds scattered over a 30 dB range. This frequency region corresponds anatomically to that region where there is a sharp transition between the presence and absence of OHCs. In regions where there was complete OHC loss with the presence of at least some IHCs (corresponding to 2 and 4 kHz), the behavioral thresholds were elevated above normal values by about 40 dB. The cross-fiber threshold envelope of the single-unit tuning curve is plotted as a dotted line. This curve represents, at any given frequency, the lowest level at which any fiber responds. A plot of the cross-fiber threshold envelope parallels both the post-treatment behavioral sensitivity and AP threshold curves in this animal.

Fig. 6 shows data from the second functionally-tested animal, 9012. The pattern of OHC loss was almost identical to that seen in the previous animal (9023), while the pattern of IHC loss was strikingly different. Specifically, there was a substantial population of present IHCs in the basal third of the cochlea, and a gap of no hair cells was found in the middle one-third of the cochlea. Single unit recordings generally agree with the anatomical findings, in that only two units were recorded with estimated BFs corresponding to the 0.8–4 kHz frequency region. (These two units are presumed to have innervated a region slightly basal to the gap.) Both behavioral thresholds and AP thresholds failed to reveal this area of the cochlea that is devoid of nearly all hair cells: the 1–4 kHz thresholds were elevated only to the same degree as the higher frequencies. Threshold AP latencies at 1 and 2 kHz were not what one would expect for 1–2 kHz tone bursts, suggesting that the origin of the AP response was from cochlear regions outside of the gap*. As in the previous animal, the cross-fiber threshold envelope reflected the general shape of the behavioral and AP threshold curves.

* See Note added in proof, p. 279.

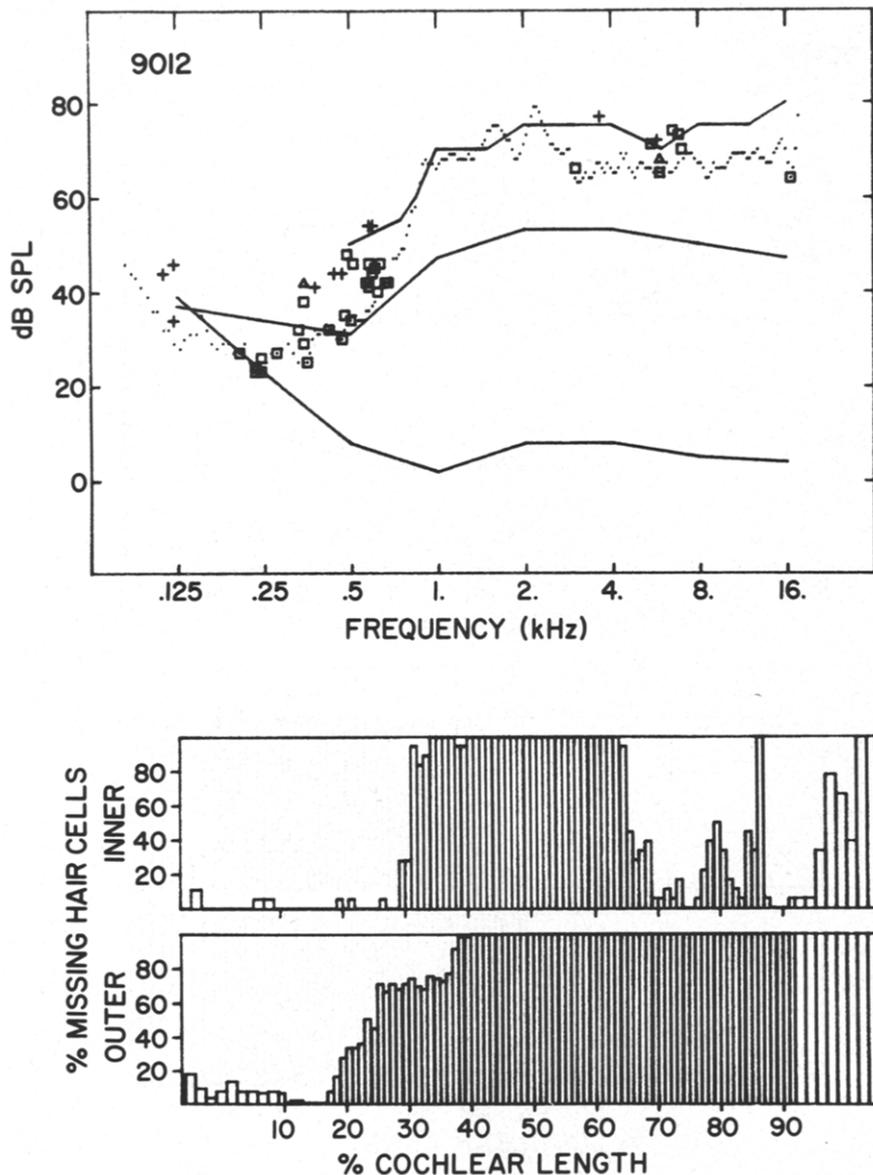


Fig. 6. Animal 9012 shows a gap of completely missing IHCs and OHCs in the middle of the cochlea. This gap of missing hair cells was not reflected in either the behavioral or AP thresholds. The best functional correlate of the anatomical lesion is provided by the recorded single-unit BFs thresholds.

The third animal, 9004 (Fig. 7), showed a pattern of OHC damage similar to the two previous animals. Most IHCs were missing in the basal half of the cochlea. There was a small area of the organ of Corti that contains 17 IHCs at approximately the 7 kHz frequency region. Anatomical observations revealed a substantial popu-

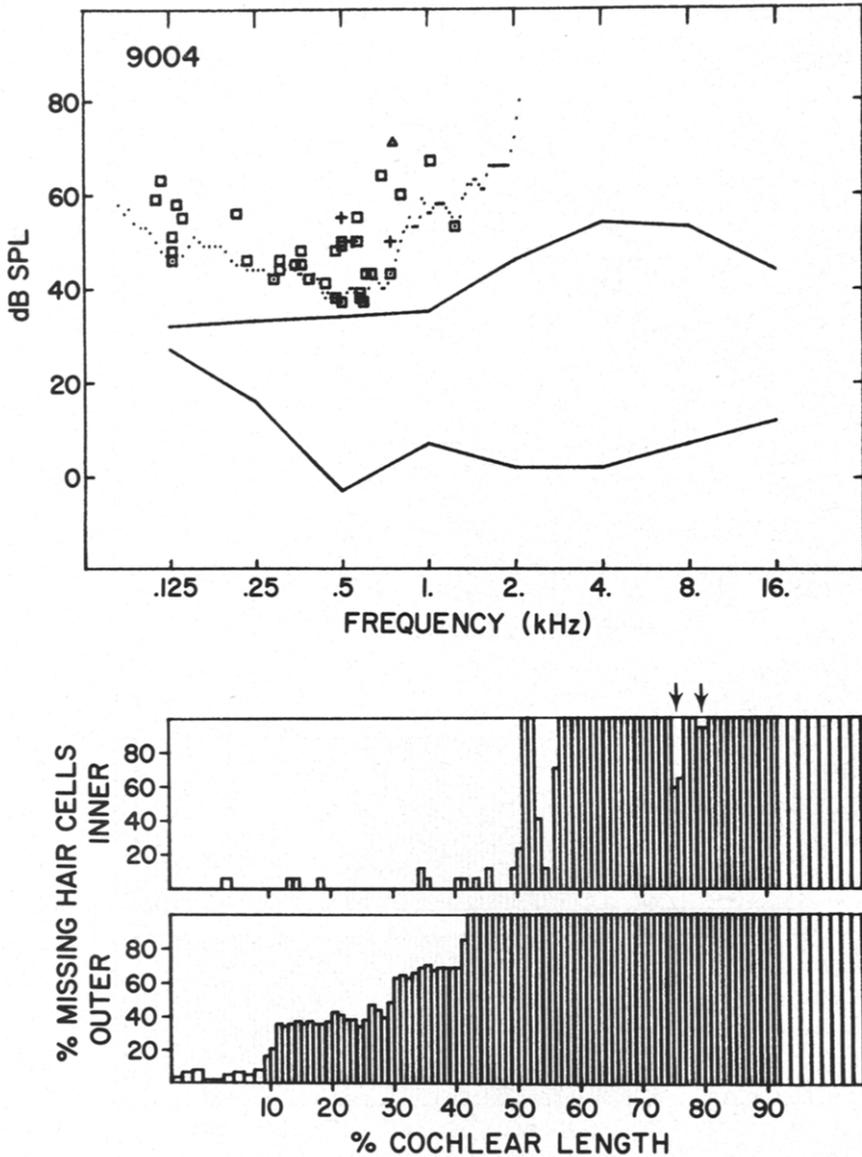


Fig. 7. Animal 9004 shows almost complete hair-cell loss in the basal half of the cochlea. However, 17 IHCs are present at the 7 kHz region (indicated by small arrows). The behavioral sensitivity thresholds are elevated above 1 kHz, but it appears that they are not dependent upon the density of IHCs as long as some of them are present.

lation of nerve fibers innervating this region of the cochlea. However, no single units were recorded with BFs in this region. This was not surprising, since the chance of recording from these particular neurons is extremely small. Reliable behavioral

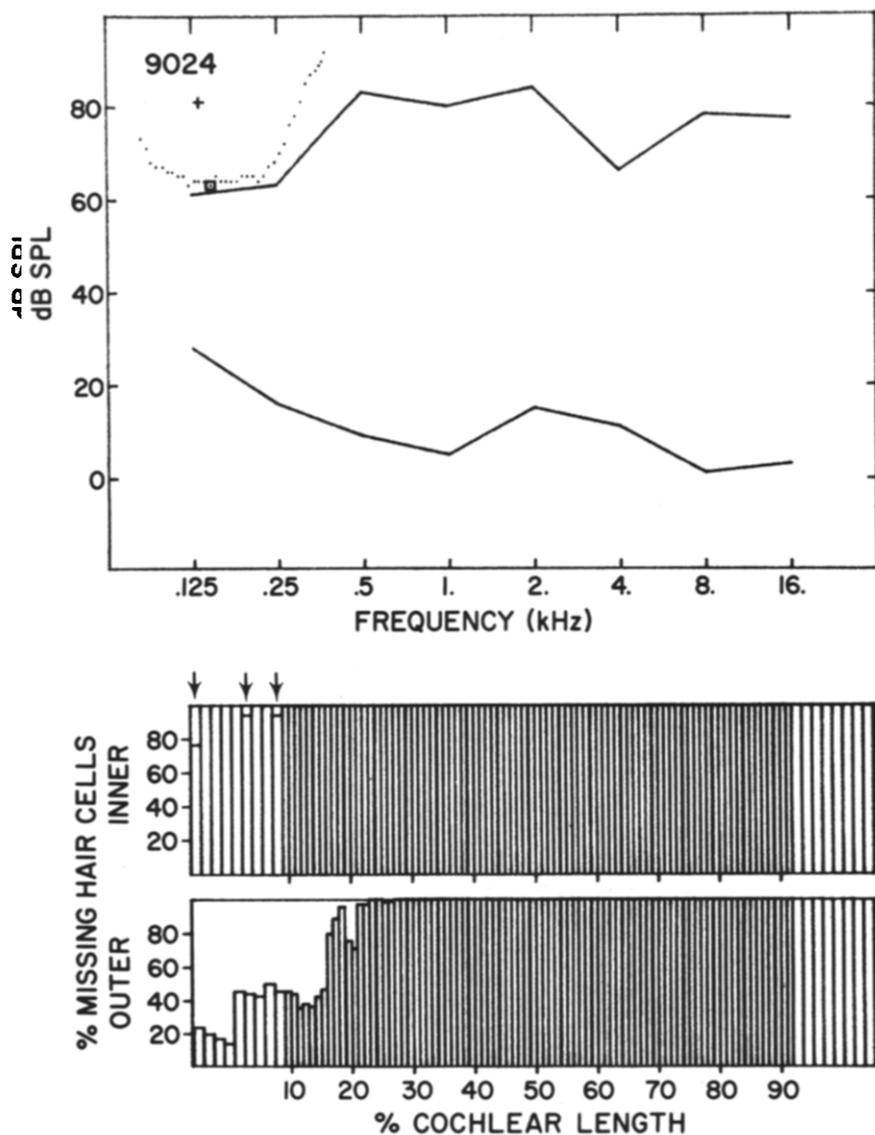


Fig. 8. Animal number 9024 shows a severely damaged cochlea with only 6 IHCs (indicated by arrows) and some 800 corresponding OHC in the apical end of the cochlea. Only two tuning curves were obtained from this animal. Behavioral sensitivity above 500 Hz was evident but we are unable to explain how it was mediated.

thresholds at about 50 dB SPL were measured across the 1–16 kHz frequency region in this animal. The behavioral audiogram of this animal was similar to the audiogram of the previous animal (9012), in spite of the different populations of

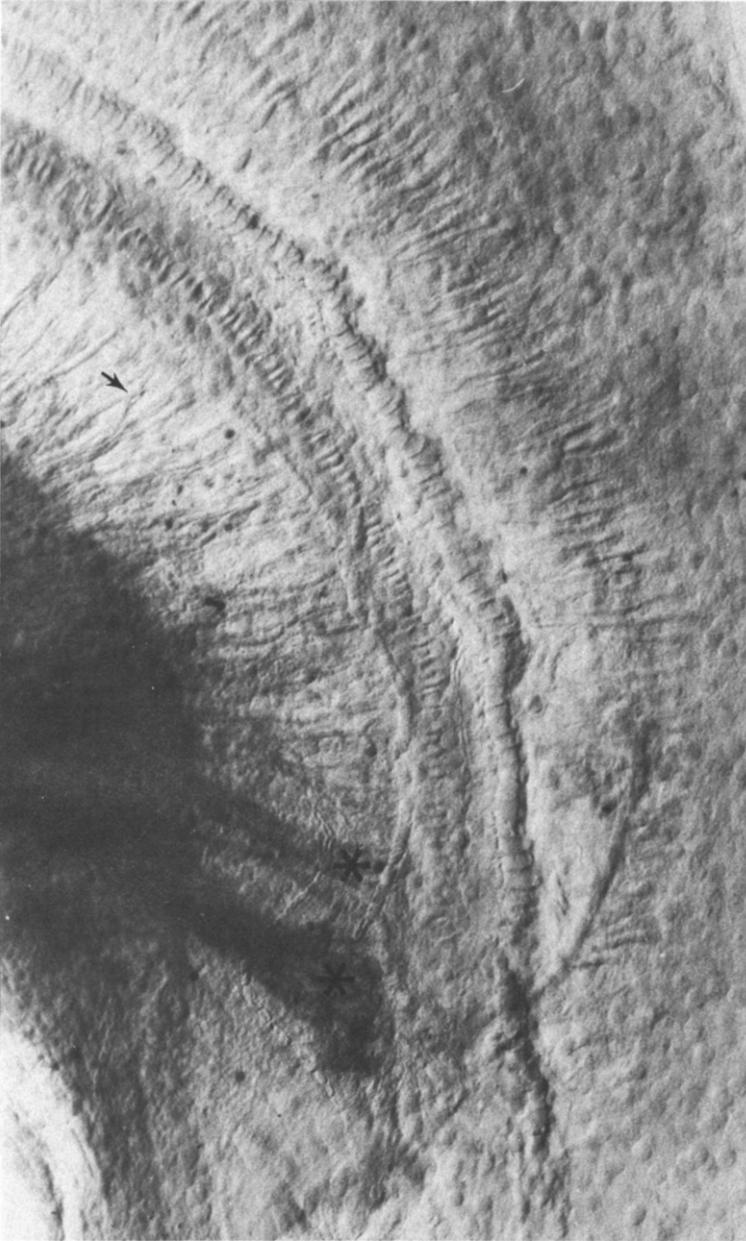


Fig. 9. A photomicrograph of the apical end of the basilar membrane in animal 9024. Note the bundles (*) of nerve fibers corresponding in location to the remaining 6 IHCs and a sparse population of nerve fibers (arrow) in more basal regions of the cochlea. Magnification, $\times 200$.

remaining IHCs at the base of these cochleae. There was agreement between the cross-fiber threshold envelope and the behavioral thresholds below 2 kHz.

Animal 9024 showed a severely damaged cochlea in which IHC loss exceeded OHC loss (Fig. 8). Only 6 IHCs in the extreme apical portion of the cochlea and some corresponding 800 OHCs were present. As in the other functionally tested animals, the contralateral cochlea was examined and monauralization was complete (i.e., no hair cells were present). Figure 9 shows the nerve fibers innervating the 6 IHCs and some 800 of the OHCs remaining in this cochlea. As seen in the other animals, there was a substantial population of nerve fibers in regions where IHCs were present; in regions where no hair cells existed or only OHCs were present, there was only a sparse population of nerve fibers within the spiral lamina. Only two tuning curves from single units were obtained from this animal, both with BFs corresponding to the extreme apical region. Behavioral thresholds were reliably measured at all frequencies for this animal, indicating a sensitivity loss of 30–40 dB at 125 and 250 Hz, and greater losses on the order of 60–80 dB for frequencies of 500 Hz and above. Analysis of the sound field used for behavioral testing ruled out the possibility that low-frequency distortion components were responsible for the higher-frequency threshold responses, as distortion products were well below this animal's behavioral thresholds across frequencies. Therefore, the origin of the behavioral responses for frequencies of 500 Hz and above is not obvious. There was agreement between the cross fiber threshold envelope and the behavioral thresholds below 500 Hz.

Discussion

The pattern of hair-cell loss after treatment with a single combined dose of kanamycin and bumetanide was similar to that previously reported after treatment with kanamycin and either ethacrynic acid or furosemide [5,6,30,40]. That is, there was severe, basal cochlear damage with loss of outer and inner hair cells. However, our results differ from those reported by Ohtani [28]. They reported extensive OHC loss, but found little IHC loss after kanamycin and bumetanide treatment. However, there are a number of important methodological differences between their study and our own, which may account for the different pattern of damage. In their study, rabbits were used, a low dose of bumetanide (< 1 mg/kg) was administered for 10 days and the animals were allowed to survive for only 10 days after the last drug treatment.

An ototoxic potentiation has been described between kanamycin and three different types of loop-inhibiting diuretics; however, no ototoxic potentiation has been observed between kanamycin and four other classes (thiazides, mercurials, carbonic anhydrase inhibitors or osmotics) of diuretics [4]. The site(s) and mechanism(s) for the ototoxic potentiation produced by the combined administration of kanamycin with loop-inhibiting diuretics remains unclear [30].

The pattern of nerve-fiber degeneration within the spiral lamina followed IHC loss. This presumably reflects the greater innervation density of IHCs [26,36].

Contrary to a report by Johnsson [17] and our observations on other pathological cochleae, there did not appear to be a correlation between the presence of the supporting elements of the organ of Corti and the density of nerve fibers within the spiral lamina. However, we believe that this is due to the relatively short recovery period (68–165 days) after kanamycin and bumetanide treatment. In other chinchillas that were exposed to intense sound in our laboratory, there were regions of complete degeneration of the organ of Corti and regions where only supporting cells (mainly pillar cells) were present. In these animals, which survived for over two years before killing, spiral-lamina nerve-fiber density was greater in areas containing supporting cells than in areas without supporting cells. One can speculate that this same pattern of nerve-fiber density might have emerged in the KAN-BUM treated animals if longer survival times had been allowed. Similarly, the presence of myelinated nerve fibers on the basilar membrane, or the turning back of nerve fibers into the spiral lamina that was observed in some of these KAN-BUM treated animals, are probably temporary phenomena. These phenomena were not observed in acoustically traumatized animals that had a long recovery period (> 2 years) after exposure. The presence of myelinated nerve fibers onto the basilar membrane has also been observed in the guinea pig 64 days after perilymphatic perfusion of streptomycin [18].

When only IHCs were present in the organ of Corti, both single-unit and behavioral thresholds were elevated about 40–50 dB from normal as seen in animals 9004, 9012 and 9023. This finding is in agreement with those of Dallos [9] and Stebbins et al. [37], and it also supports the notion that OHCs and their associated structures are responsible for the sensitive tips of neural tuning curves [10].

When no inner or outer hair cells were present in the basal 75% of the cochlea, as in animal 9024, consistent (70–80 dB SPL) behavioral thresholds were nevertheless measured at frequencies above 500 Hz. There are several possible mechanisms by which this may occur. These include, (1) stimulation of the vestibular system, (2) stimulation of the somatosensory system, such as via the whiskers, (3) direct stimulation of remaining auditory nerve fibers. None of these possibilities can be ruled out. Other investigators have reported similar findings, indicating that cochlear hair-cell loss alone will not eliminate behavioral responses to intense acoustic stimuli. Hunter-Duvar et al. [15] reported a human with a hearing threshold of 80–90 dB HL who had no cochlear sensory hair cells. Cazals et al. [7] recorded AP-like responses and brainstem evoked potentials in response to acoustic stimuli between 80–90 dB SPL from guinea pigs whose cochlea were essentially lacking hair cells. Most recently, Cazals et al. [8] have studied this phenomenon of ‘hearing without hair cells’ in more detail. Their results suggest that these responses originate from the saccule.

On the other hand, Schuknecht and Woellner [34] found no behavioral responses in cats when both hair cells and nerve fibers were destroyed following surgical lesions. In addition, Ryan and McGee [31] and Stebbins et al. [37], reported animals in which no high-frequency behavioral responses were obtained after complete basal hair-cell degeneration. Unfortunately, no mention was made in the latter two studies about remaining nerve fibers. At this point in time, growing evidence suggests that

extreme caution should be exercised when interpreting behavioral thresholds over 70–80 dB SPL as reflecting the state of the cochlea.

Even at lower intensities, the behavioral sensitivity audiogram may inaccurately reflect the state of the damaged cochlea. Animal 9012 (Fig. 6) provides an illustration of this point. In this animal, neither the behavioral nor the AP sensitivity audiograms reflect the complete loss of both inner and outer hair cells that occurred in the middle of the cochlea. In this case, the tails of the tuning curves from fibers originating in the basal third of the cochlea would appear to be responsible for both the AP and the behavioral sensitivity thresholds measured at the mid-frequencies [20]. Therefore, a simple sensitivity-threshold detection measurement appears to be inadequate for revealing a gap of sensory cells in the cochlea, provided that some IHCs and their accompanying nerve fibers are present at a more basal region. On the other hand, the plot of single-unit BF thresholds tended to reflect the state of the cochlea quite well. Usually no neurons could be recorded with BFs in frequency regions where both inner and outer hair cells were missing. Although a simple sensitivity-threshold measurement, either behavioral or AP, may not accurately reflect cochlear pathology, it may be that masked AP tuning curves [13] or psychophysical tuning curves [38] would reveal such a cochlear lesion.

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Note added in proof (see footnote on p. 270)

Threshold AP latencies at frequencies 1.5 kHz and below were substantially elevated, indicating that the origin of the AP responses was from the apical region of the cochlea. At 2 and 3 kHz the threshold latencies were somewhat shorter than normal, suggesting that these AP responses resulted from stimulation of basally located neurons.