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**THRESHOLD SHIFTS AND COCHLEAR INJURY IN CHINCHILLAS
EXPOSED TO OCTAVE BANDS OF NOISE CENTERED AT 63
AND 1000 HERTZ FOR NINE DAYS**

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20. ABSTRACT:

The use of A-weighted sound pressure levels in hearing conservation criteria is based on scant data from low frequency exposures. This study contributes additional data from exposure to low frequency noise and attempts to relate it to damage risk criteria. Audiograms were obtained on 16 binaural chinchillas using a shuttle box avoidance procedure. The chinchillas were then exposed in groups to one of the following noise conditions for 9 days. Eight animals were exposed to an octave band noise centered at 1000 Hz: four were exposed at an intensity level of 85 dB SPL (85 dBA) and four at an intensity level of 95 dB SPL (95 dBA). The 85-dB exposure produced a temporary threshold shift of 46 dB at 1.4 kHz with no permanent threshold shift. The 95-dB exposure produced a compound threshold shift of 60 dB at 1.4 kHz and a peak permanent threshold shift of 28 dB at 2.0 kHz. The eight remaining animals were exposed to an octave band noise centered at 63 Hz: four were exposed to an intensity level of 110 dB SPL (84 dBA) and four at an intensity level of 120 dB SPL (94 dBA). The 110-dB exposure produced a compound threshold shift of 23 dB at 2.0 kHz with a peak permanent threshold shift of 7 dB at 2.0 kHz. The 120-dB exposure produced a compound threshold shift of 45 dB at 2.0 kHz and a peak permanent threshold shift of 19 dB at 2.0 kHz. The results of surface preparation histology produced mixed findings with cases of no permanent threshold shift and no hair cell loss, hair cell loss and no permanent threshold shift, and permanent threshold shift with hair cell loss that did not tonotopically correspond to the lesions. The findings are inconsistent with earlier data but consistent with A-weighted level providing an adequate predictor of noise induced hearing loss.

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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INTRODUCTION

Current damage-risk criteria (DRC) for exposure to noise are expressed in terms of A-weighted levels rather than unweighted octave-band levels. The assumption implicit in the use of A-weighted levels is that low-frequency noise is not as hazardous to hearing as is high-frequency noise. A previous study (Burdick et al., 1978) showed that low-frequency noise produced high-frequency hearing losses in chinchillas. In that study, two groups of chinchillas were exposed for 3 days to octave bands of noise with center frequencies of either 63 Hz or 1000 Hz. The exposure levels of the 63-Hz octave-band noise were 100 dB SPL (74 dBA), 110 dB SPL (84 dBA), and 120 dB SPL (94 dBA). The exposure levels of the 1000-Hz octave-band noise were 75 dB SPL (75 dBA), 85 dB SPL (85 dBA), and 95 dB SPL (95 dBA). Permanent threshold shifts after exposure were measured at 1400 and 2000 Hz with both bands of noise at the highest intensity levels. The permanent threshold shift for the 63-Hz octave-band noise at 120 dB SPL was greater than that found for the 1000-Hz octave-band noise at 95 dB SPL, even though the intensities of the two bands of noise were matched within 1 dBA. These results have serious implications both for damage-risk criteria and for understanding the mechanisms of noise-induced hearing loss. In addition, two other results were of particular interest: (1) threshold shift at the half-octave frequency of 90 Hz, for the 63-Hz octave-band of noise at 120 dB SPL, was still increasing at the end of the 72-h exposure, i.e., threshold shift did not reach an asymptote, and (2) the amount of recovery from the asymptotic threshold shift at 1.4 and 2.0 kHz incurred for the high-level, low-frequency exposure was less than that found for the high-frequency exposure.

The present study extends these earlier findings and attempts to relate these results to the damage-risk criteria and to mechanisms of hearing loss.

METHOD

SUBJECTS

The subjects were 16 male, binaural chinchillas which ranged in age from 12 to 29 months at the start of the study.

APPARATUS

The audiometric and exposure instrumentation have been previously described in detail (Burdick et al., 1978). Briefly, the chinchillas were tested in a double-grille cage within a 1200 Series Industrial Acoustics Company (IAC) sound room. Mounted on the cage were a row of photocells to detect the animal's location and an electronic buzzer which was used as a

secondary reinforcer. A synthesized signal generator (Fluke Model 6010A),* an attenuator, and an amplifier were used to generate and adjust the signal level. The pure-tone signals were delivered through an Altec 418B coaxial loudspeaker. The control, duration, and sequencing of events, as well as response recording were done with a Prolog PLS 401 microprocessor and the animals were monitored on closed circuit television.

The instrumentation used for the noise exposures consisted of a random noise generator, octave-band filter set, amplifier, and eight 12-in loudspeakers. The levels of the noise bands were constantly monitored by a level recorder and the spectra of the noise bands were determined twice daily using either a Time Series Analyzer (Time Data Model 1923A) or a Nicolet Scientific Corporation Mini-Ubiquitous Spectrum Analyzer (Model 440A).

TRAINING AND TESTING PROCEDURE

Threshold Testing

The procedures for training and testing the animals were similar to those described previously (Burdick et al., 1979). Briefly, the animals were conditioned to avoid an AC electric shock (1.0 mA nominal level) by crossing from one compartment to the other of the double-grille cage during a 3.84-s trial interval during which a pulsed, puretone signal was presented. Each trial interval consisted of three tone pulses with 720-ms on-times separated by 560-ms off-times. The tone pulse had an exponential rise and decay function with a first time constant of 14 ms. When the avoidance response was made, the signal was immediately terminated and lights at the barrier of the cage were illuminated for 1.28 s. If the subject failed to cross from one compartment to the other during the trial interval, shock and buzzer were simultaneously presented until the crossing response was made. This resulted in the termination of the shock, buzzer, and signal, and the 1.28-s illumination of the lights at the barrier.

During the first two sessions of training, trials were presented on an average of every 60 s. Trials were then presented for one session on an average of 45, 30, and then 20 s. At that point all subsequent training and testing trials were presented on an average of every 20 s. The intensities of the tones varied over a 40 dB range (50-90 dB SPL re: 20 μ Pa) in all training sessions.

Each group of subjects received training sessions until all subjects scored 90% correct for three successive sessions. During the training sessions the animals were given two trials with each of the following 11 frequencies for a total of 22 trials per session. The 11 frequencies were: 63, 90, 125, 250, 500, 1000, 1400, 2000, 4000, 5700, and 8000 Hz. Once the training criterion was obtained, threshold determinations were begun.

*Appendix contains list of equipment manufacturers.

A modified method of limits (Miller, 1970; Burdick et al., 1978) was used to estimate thresholds. On the first trial of a threshold measurement, the signal was presented at an intensity level of 60-70 dB SPL. For each frequency the initial level was randomly varied over a 10 dB range. A correct response at that level resulted in a 20 dB reduction in level for the next trial and so on until the animal failed to respond. On the trial following a miss, the level of the signal was increased 20 dB and the threshold was taken as the level halfway between the lowest level that was correctly responded to and the highest level missed. After threshold values began to stabilize, which required 8 to 10 audiograms, a threshold value was discarded if it differed from those values by 15 dB and a second threshold measurement was taken. The threshold obtained on the second determination was always accepted. A sham trial always followed the last trial of each threshold determination. This was done to obtain an estimate of the rate of "spontaneous responding". These trials were identical to the regular trials except that the oscillator was unplugged from the circuit and the shock and buzzer were turned off. There was no consequence to the animal for spontaneous responding. Shock was presented on approximately 5% of the trials at or below threshold. Generally, shock was turned off and only the buzzer was used as a secondary reinforcer when the signal level was 10 dB above the animal's threshold.

Exposure Conditions

The 16 chinchillas were randomly assigned to one of four groups of four subjects each. Two of the groups were exposed to an octave band of noise with a center frequency of 63 Hz. One group was exposed at a level of 110 dB SPL and the other at a level of 120 dB SPL. The two other groups were exposed to an octave band of noise with a center frequency of 1000 Hz. One group was exposed at 85 dB SPL and the other at 95 dB SPL. The spectral characteristics of the noise bands as measured in the sound field are shown in Figure 1. The highest harmonic of the 63-Hz band occurred at 200 Hz and was 40 dB down from the peak. All other harmonics were greater than 75 dB below the peak. All exposure durations were 9 days and all of the subjects of each group were exposed at the same time.

The growth of threshold shift during all exposures was monitored at selected time intervals of 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, and 216 h into the exposure. For the subjects exposed to the 63-Hz octave band of noise, the growth of threshold shift was monitored at the frequencies of 90 and 2000 Hz. The subjects were removed from the noise at each test interval for 11 minutes. During the first 2 minutes the animal sat in the test cage. Two threshold measurements were made at 90 Hz during minutes 2 to 6 and the two threshold values were averaged to derive a TS_4 measure. Two threshold measurements were made at 2000 Hz during minutes 6 to 10 and the two threshold values were averaged to derive a TS_8 measure. The animal was returned to the noise field during minute 11 and remained in the noise for 4 more hours before the next test. The test schedule was adjusted by 11 minutes each time during exposure so that the animals had

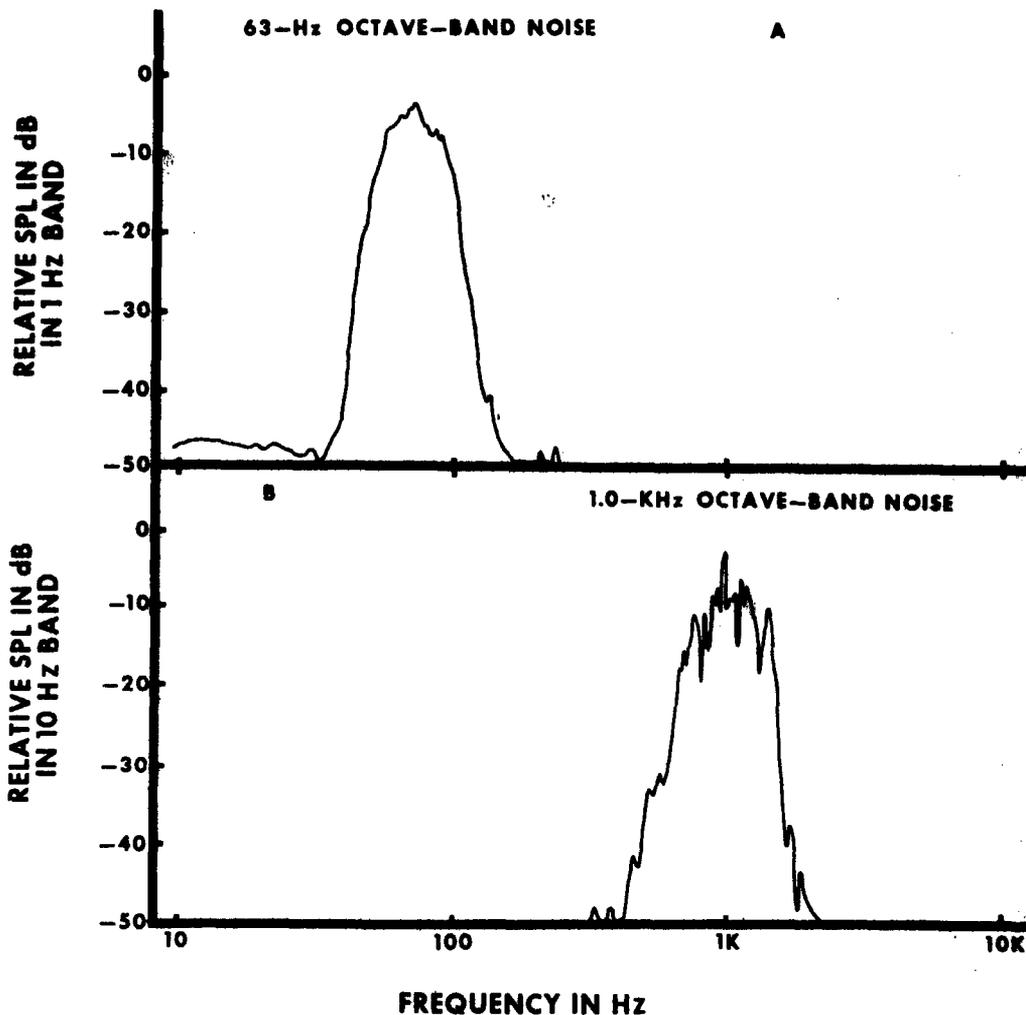


FIGURE 1. Power spectrum characteristics of the octave bands of noise used for exposure as measured in the sound field. Panel A depicts the octave band with a center frequency of 63 Hz and Panel B depicts the octave band with a center frequency of 1000 Hz.

received an actual exposure of the stated amount of time. For the subjects exposed to the 1000-Hz octave band of noise, the growth of threshold shift was monitored at the one-half octave frequency of 1400 Hz. These subjects were removed from the noise for a total testing time of 7 minutes. Again, two threshold measurements were made during minutes 2 and 6 and were averaged to derive TS_4 . The test schedule was adjusted by 7 minutes each time so that the animals received the actual exposure of the stated amount of time.

Recovery Conditions

After 9 days (216 h) the animals were removed from the noise and tested. During recovery, a complete audiogram at all 11 frequencies was

obtained at each recovery interval. Audiograms were obtained at regular intervals either until there was complete recovery of hearing or for 30 days post-exposure.

Histology

At 88-90 days post-exposure, the animals were anesthetized with halothane and killed by decapitation. Prior to decapitation, 3-8 ml of blood were removed via a cardiac puncture. The blood samples were used to obtain normative data for another study. Four non-noise-exposed control animals were killed in the same manner. In addition, one control animal was killed after being anesthetized with halothane without having a blood sample removed and another control animal was killed after being anesthetized with an injection of sodium pentobarbital without having a blood sample removed. Following decapitation, the two auditory bullae were removed and opened widely. The cochleae were opened in the base of scala tympani and at the apex of scala vestibuli. A solution of 2.5% gluteraldehyde in 0.1 M PO_4 buffer was then perfused through the cochleae for about 2 minutes. The stapes were removed and perfusion continued for approximately 2 minutes. After a variable length of fixation (> 24 h) cochleae were post-fixed in 1% osmium tetroxide in 0.1 M PO_4 , washed, and dehydrated to 70% ETOH. The surface preparation dissections were then done and the tissue sections mounted in glycerine. Specimens were studied at 400X and 1000X using an interference contrast light microscope with nomarski optics and cochleagrams were constructed.

RESULTS

PRE-EXPOSURE AUDIOGRAMS

The pre-exposure audiograms of the four groups of animals are shown in Panel A of Figure 2. Each audiogram is the average of six or seven estimates at each frequency. These audiograms closely resemble one another. The average audiogram for all 16 animals combined is shown in Panel B. The vertical bars indicate one standard deviation above and below the mean. The thresholds of monaural chinchillas reported by Miller (1970) are also shown in Panel B. It is evident that the chinchillas had pre-exposure thresholds within accepted normal limits. The rate of false-alarm responding during the baseline audiogram determination was 0.11, with the range across the four groups equalling 0.10 to 0.14.

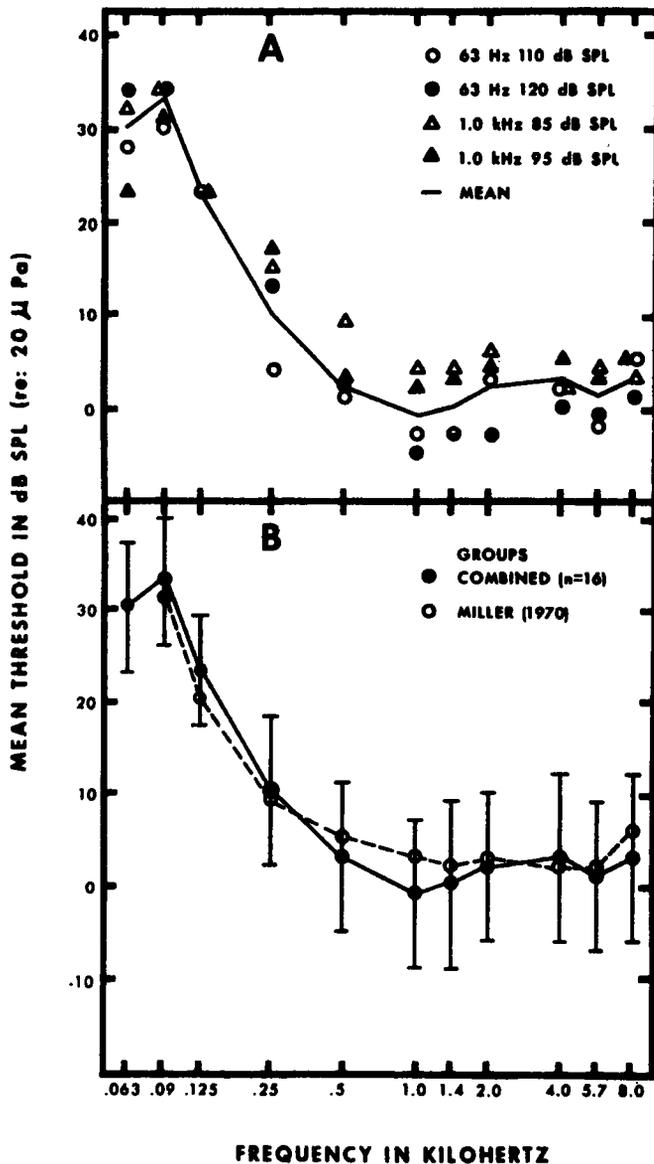


FIGURE 2. Pre-exposure and audiograms for binaural chinchillas. Panel A shows the pre-exposure audiograms for each of the four groups as well as the mean audiogram for all of the subjects. Panel B shows the mean audiogram for the 16 subjects. The vertical bars indicate one standard deviation above and below these values. Also shown are the thresholds of monaural chinchillas reported by Miller (1970).

EXPOSURE TO 63-HZ OCTAVE-BAND NOISE

Growth of Threshold Shift

The growth of TS_4 at 90 Hz and TS_8 at 2000 Hz is depicted in Figure 3 for those subjects exposed at 110 dB SPL (84 dBA). The levels of threshold shift were calculated throughout this study by averaging over exposure days 4-9. The 110-dB exposure resulted in threshold shifts of 5 dB at 90 Hz and 23 dB at 2000 Hz. While it is clear that TS_4 at 90 Hz attained an asymptotic level of threshold shift, TS_8 at 2000 Hz does not appear to have stabilized. There is an indication that it was still increasing at the end of the exposure.

The first time constant (67% of full value) for the growth of threshold shift was less than 4 h at 90 Hz and 12 h at 2000 Hz (see Table 1).

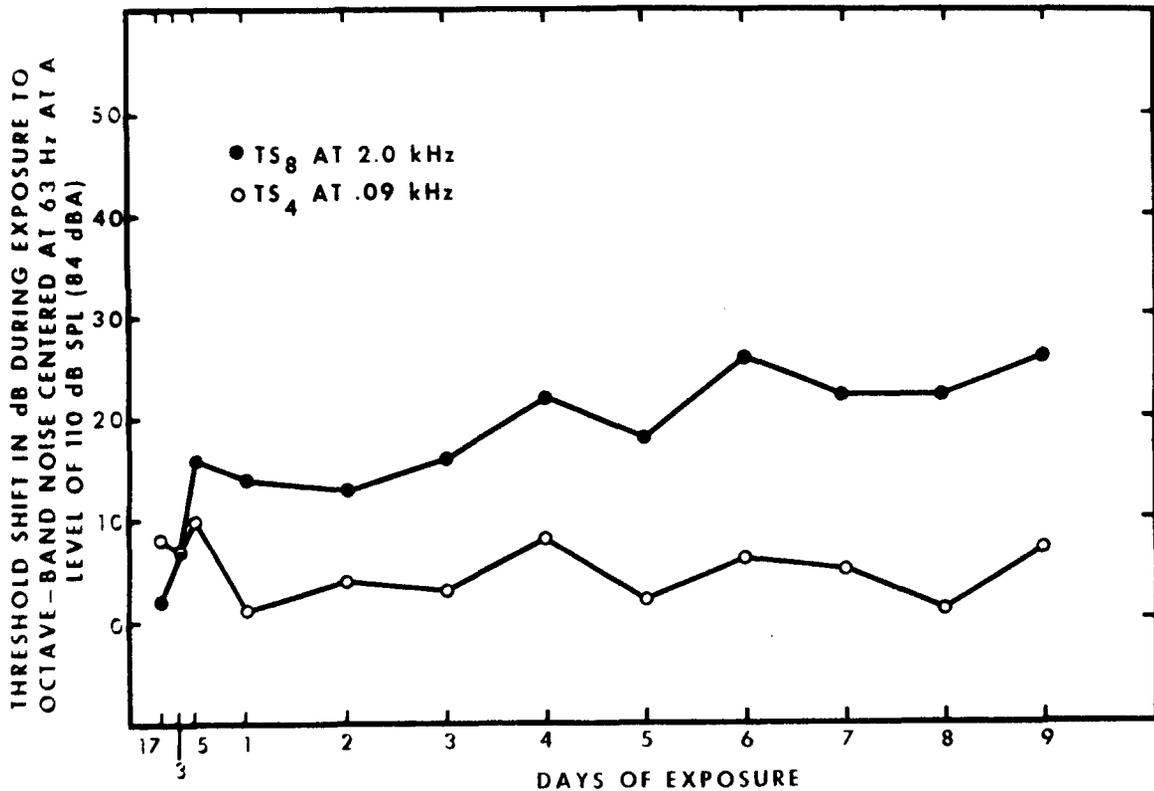


FIGURE 3. Threshold shifts in dB at 90 and 2000 Hz during exposure for 9 days to octave-band noise centered at 63 Hz at an intensity of 110 dB SPL (84 dBA).

Threshold shifts at 90 and 2000 Hz are shown in Figure 4 for those subjects exposed at 120 dB SPL (94 dBA). This condition produced asymptotic threshold shifts of 29 and 45 dB at 90 and 2000 Hz, respectively. The first time constant for growth of TS₈ at 2000 Hz was 12 h (see Table 1) as it was in the 110-dB exposure. The level of TS₈ at 2000 Hz was stable during the last one-half of the exposure. The level of TS₄ at 90 Hz developed slowly, as reflected by its time constant of 48 h (see Table 1), and never stabilized, as reflected by its apparent continued growth even at 9 days.

TABLE 1
THE FIRST TIME CONSTANTS FOR GROWTH AND RECOVERY OF THRESHOLD SHIFTS

Exposure Band							
		63 Hz			1000 Hz		
	Test Frequency	Exposure Level	First Time Constant	Test Frequency	Exposure Level	First Time Constant	
	(kHz)	(dB SPL (dBA))	(h)	(kHz)	(dB SPL (dBA))	(h)	
<u>Growth</u>	0.09	110 (84)	<4	1.4	85 (85)	<4	
	2.0	110 (84)	12				
	0.09	120 (94)	48	1.4	95 (95)	<4	
	2.0	120 (94)	12				
<u>Recovery</u>	0.09	110 (84)	48	1.4	85 (85)	8	
	2.0	110 (84)	48				
	0.09	120 (94)	48	1.4	95 (95)	48	
	2.0	120 (94)	48				

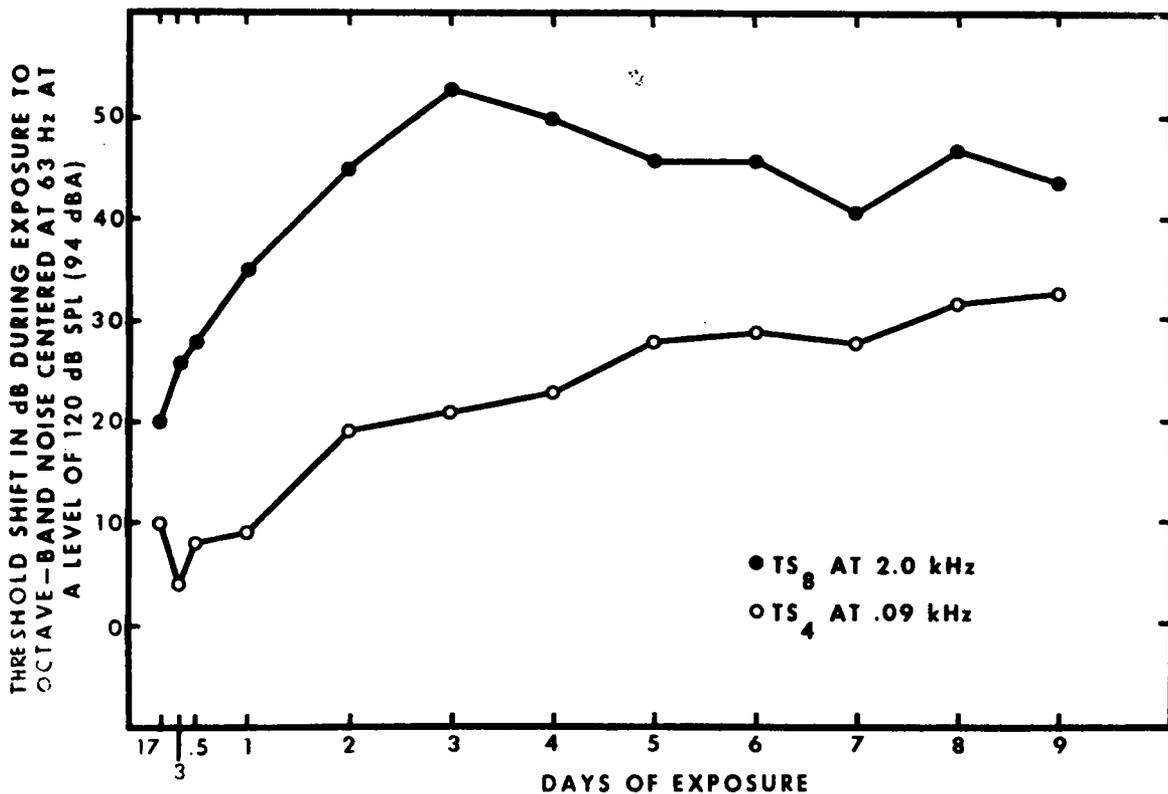


FIGURE 4. Threshold shifts in dB at 90 and 2000 Hz during exposure for 9 days to octave-band noise centered at 63 Hz at an intensity of 120 dB SPL (94 dBA).

The growth of threshold shifts during both exposures at 90 and 2000 Hz is shown in Figures 5 and 6, respectively. The 10 dB increase in exposure level from 110 to 120 dB produced nearly equal increases in asymptotic threshold shifts at both frequencies, i.e., a 24 dB increase at 90 Hz and a 22 dB increase at 2000 Hz. The level of false alarm responding was 0.10 for the 120-dB group and 0.06 for the 110-dB group, indicating behavior consistent with the pre-exposure phase of the study.

Recovery from Threshold Shift

The recovery from threshold shift at 90 and 2000 Hz is shown in Figures 7 and 8 for the subjects exposed at the 110 dB and 120 dB exposure levels,

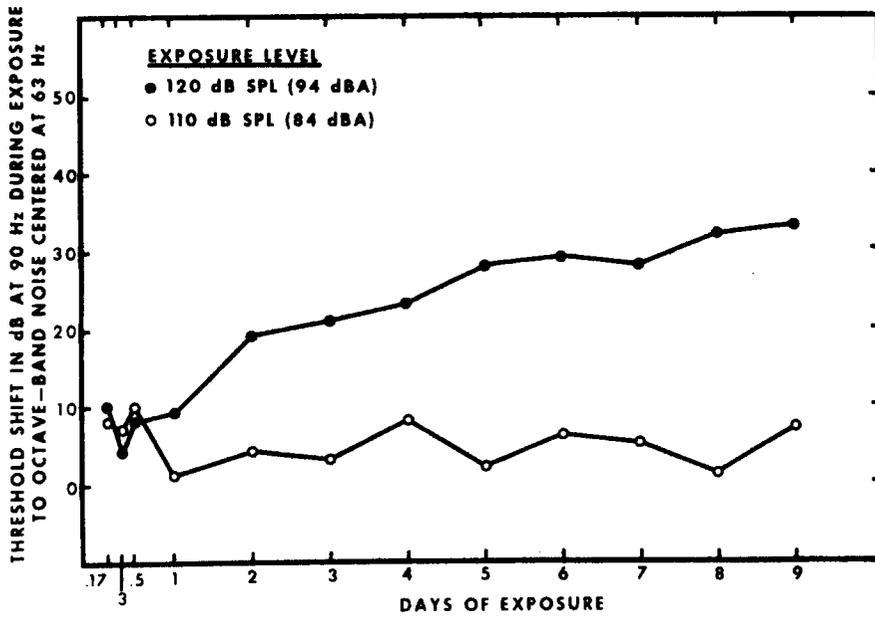


FIGURE 5. Threshold shifts in dB at 90 Hz produced by exposure to octave-band noise centered at 63 Hz at intensity levels of 110 and 120 dB SPL for 9 days.

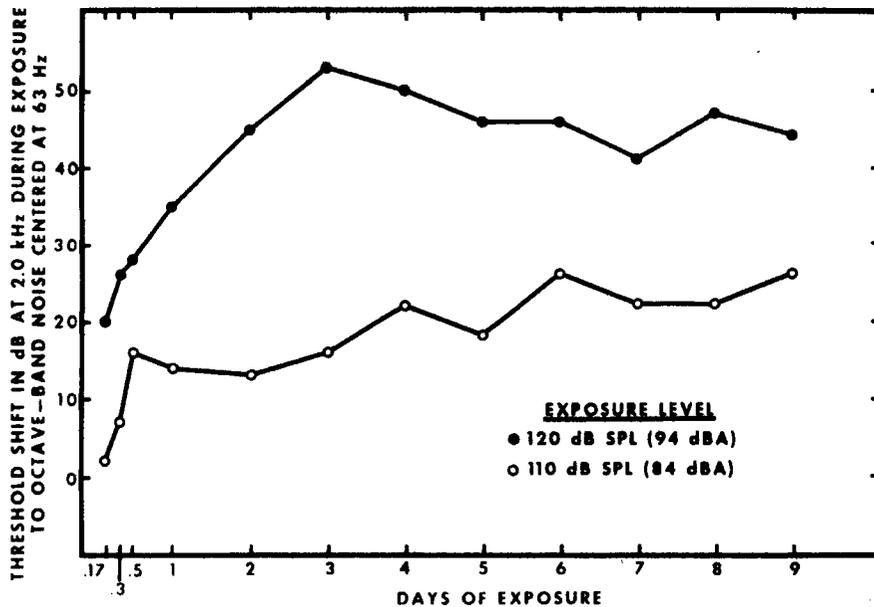


Figure 6. Threshold shifts in dB at 2.0 kHz produced by exposure to octave-band noise centered at 63 Hz at intensity levels of 110 and 120 dB SPL for 9 days.

THRESHOLD SHIFT IN dB AFTER EXPOSURE FOR
9 DAYS TO OCTAVE-BAND NOISE CENTERED
AT 63 Hz AT A LEVEL OF 110 dB SPL (84 dBA)

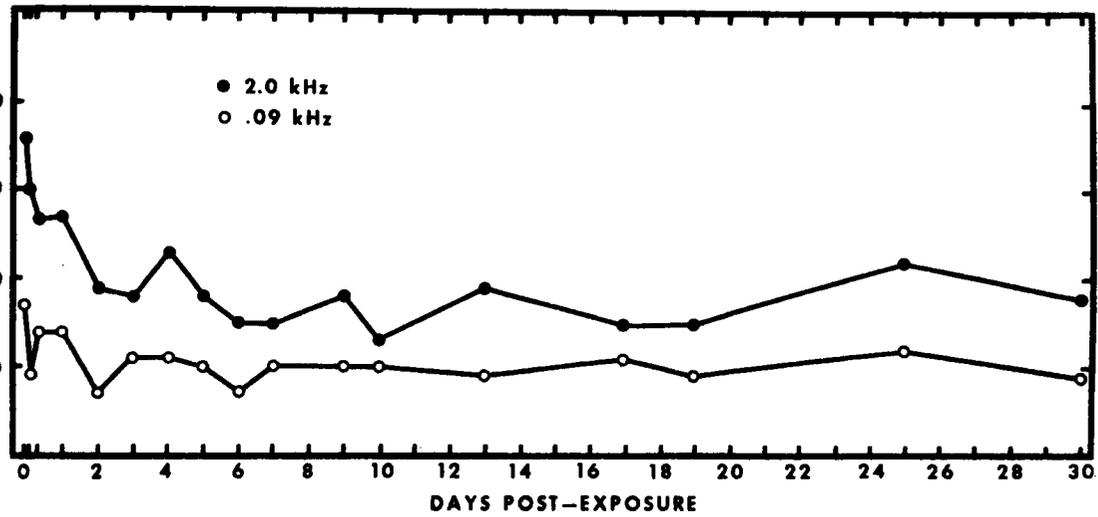


FIGURE 7. Recovery of threshold shifts at 90 and 2000 Hz over the 30-day period following 9 days of exposure to octave-band noise centered at 63 Hz at an intensity of 110 dB SPL.

THRESHOLD SHIFT IN dB AFTER EXPOSURE FOR
9 DAYS TO OCTAVE-BAND NOISE CENTERED
AT 63 Hz AT A LEVEL OF 120 dB SPL (94 dBA)

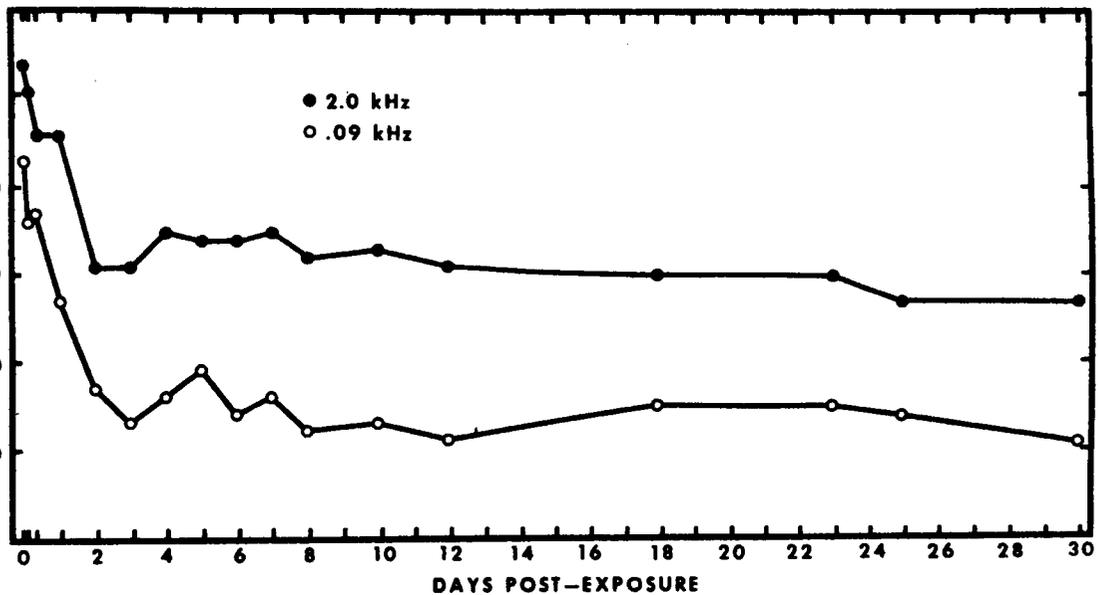


FIGURE 8. Recovery of threshold shifts at 90 and 2000 Hz over the 30-day period following 9 days of exposure to octave-band noise centered at 63 Hz at an intensity of 120 dB SPL.

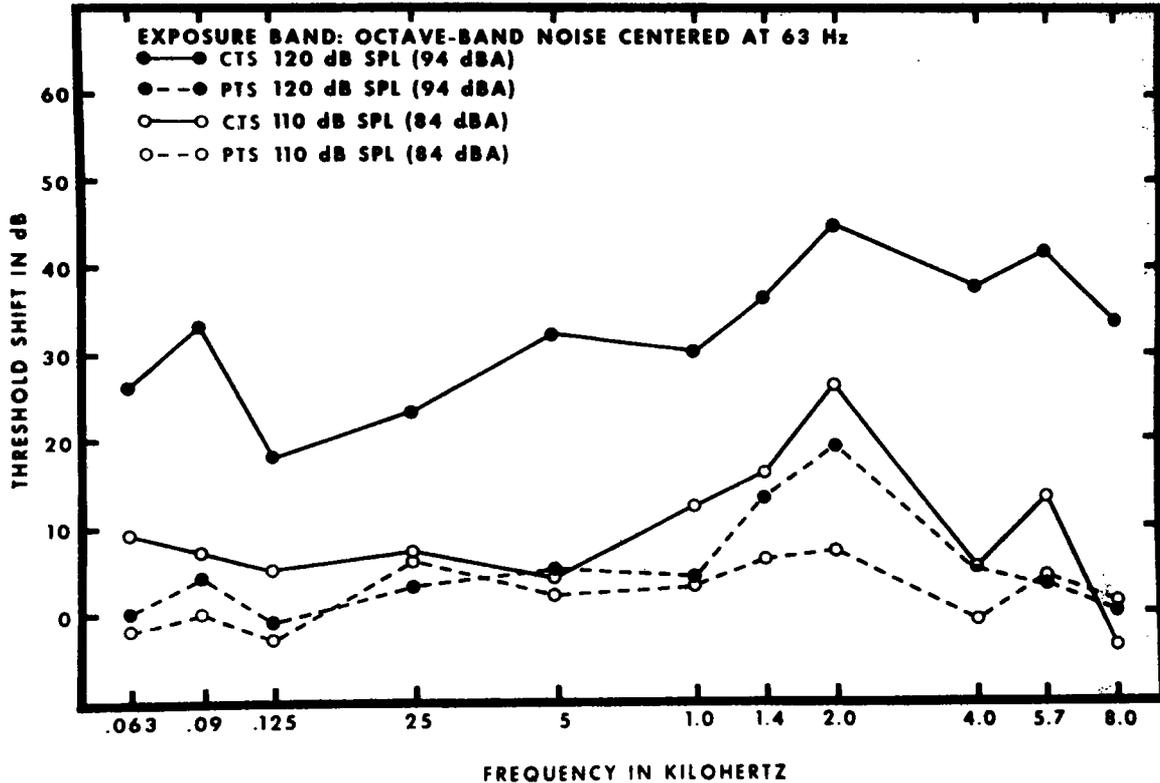


FIGURE 9. Threshold shift as a function of frequency - 63 Hz at intensities of 110 and 120 dB SPL. The solid lines depict compound threshold shifts for frequencies from 63 to 8000 Hz following 9 days of exposure to octave-band noise centered at 63 Hz at intensities of 110 and 120 dB SPL. Broken lines depict the resultant permanent threshold shifts at the same frequencies.

respectively. The thresholds of all subjects were monitored at intervals up to 30 days post-exposure. The resultant permanent threshold shifts were calculated by averaging the threshold values obtained at the last four intervals tested. The permanent threshold shift at 90 Hz was 0 dB and at 2000 Hz was 7 dB for the 110-dB exposure. The permanent threshold shift at 90 Hz was 4 dB and at 2000 Hz was 19 dB for the 120-dB exposure. Figures 7 and 8 show that both groups reached their maximum recovery levels within 10-12 days at which time they remained relatively stable for the balance of the 30-day recovery period. The time constants for recovery at both 90 and 2000 Hz were 48 h for both exposure levels (see Table 1).

Since it is not known that frequencies other than 90 and 2000 Hz did in fact show asymptotic threshold shift, we have chosen to refer to the

threshold shifts across frequencies that were found at the conclusion of the exposure as compound threshold shifts rather than asymptotic threshold shifts. The compound threshold shifts across frequencies for both groups at the end of the 9-day exposure are shown in Figure 9. A clear peak in compound threshold shift occurred at 2.0 kHz in the 110-dB exposure condition, with the frequencies of 1.0, 1.4, and 5.7 kHz showing the next larger effects. The frequencies below 1.0 kHz all showed less than 10 dB compound threshold shift. The 120-dB exposure group displayed shifts in excess of 40 dB at 2.0 and 5.7 kHz with the peak at 2.0 kHz. Shifts in excess of 30 dB were found at 90, 500, 1000, 1400, 4000, and 8000 Hz. The minimal effect (19 dB threshold shift) occurred at 125 Hz. This clearly shows that the octave band of noise at 63 Hz produces its greatest effects at frequencies several octaves higher.

The permanent threshold shift incurred by both groups is also shown in Figure 9. The permanent effects of the 110-dB exposure were small, with the permanent threshold shifts at all frequencies tested being 7 dB or less. Exposure at the 120-dB level resulted in a clearly defined "notch" at 1.4 and 2.0 kHz with permanent threshold shifts of 13 dB and 19 dB respectively. Permanent threshold shifts at the other frequencies were 5 dB or less. The false-alarm rates during the recovery phase were 0.07 and 0.05 for the 110-dB and 120-dB group, respectively.

Histology

Both cochleae of each subject were dissected and examined. There was remarkable similarity between the two cochleae of each animal. As a result, only one cochleagram from each subject is presented to illustrate the damage found. The percentage of outer hair cells and inner hair cells missing has been plotted as a function of percent total distance from the apex. The amount of permanent threshold shift found at each frequency is also presented along a cochlear map of frequency proposed by Miller, Eldredge, Bohne, and Clark (1977).

The cochleae of both groups of subjects exposed to the 63-Hz octave band of noise were quite similar histologically. The cochleae from those exposed at 120 dB looked reasonably normal except for some scattered losses of outer hair cells in the apical one-half of the cochleae. The loss was invariably greatest at the extreme apex where it averaged about 10-15% for outer hair cells. This can be seen by examining the cochleagrams presented in Figures 10 and 12. This loss appears normal for the chinchilla, being found in control animals that were not exposed to noise. This loss probably represents a developmental anomaly in the chinchilla. The single exception for this group of subjects was found in chinchilla B-115 whose left-ear cochleagram is shown in Figure 10. This animal incurred a peak permanent threshold shift at 2.0 kHz of 14 dB. Each cochlea had a small focal lesion in the basal half. As shown in Figure 10 the lesion occurred in the left cochlea at the 70% point while it occurred at the 90% point in the right cochlea. In both ears the lesion amounted to a loss of outer and inner hair cells, collapse and/or loss of supporting elements,

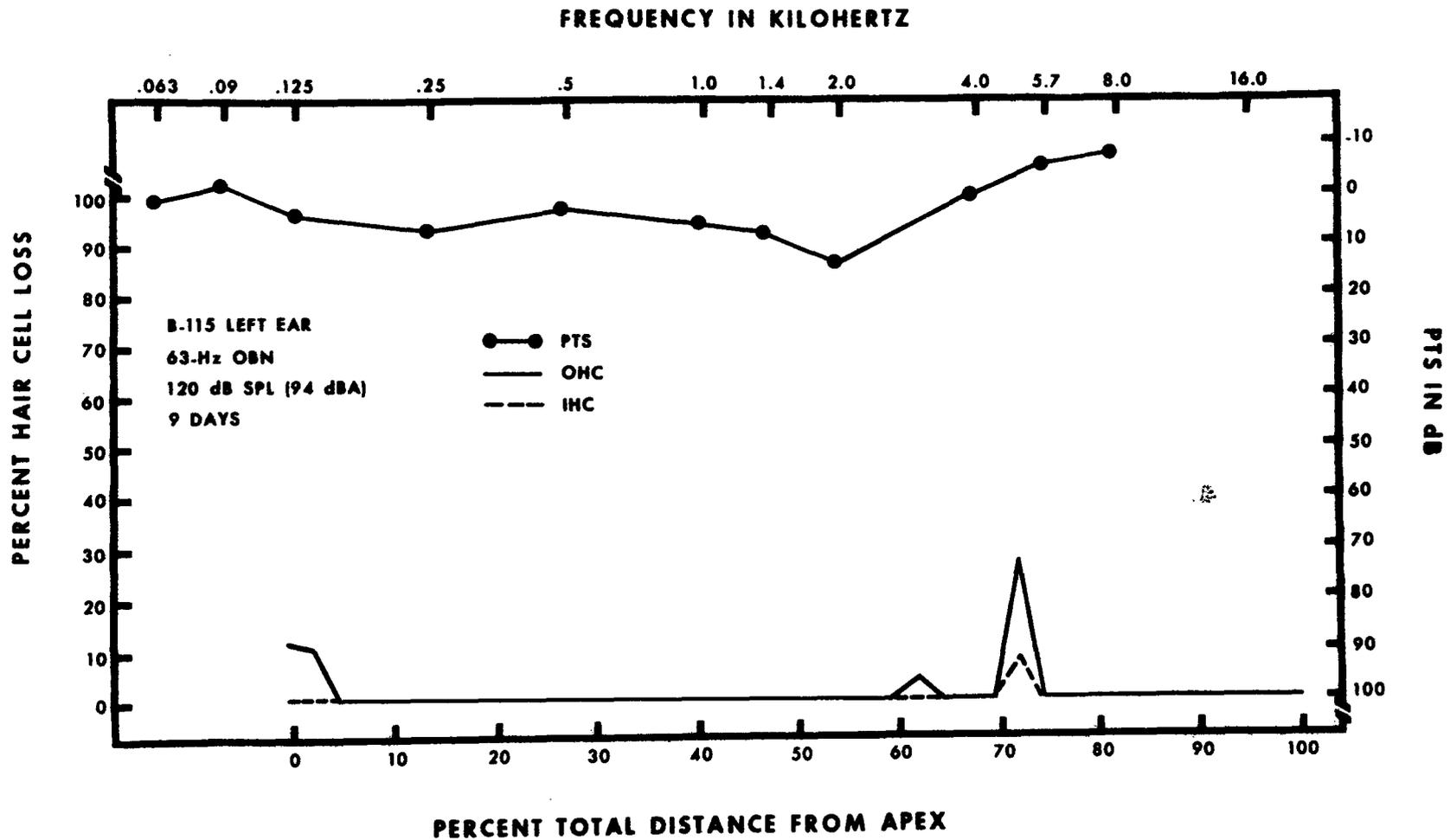


FIGURE 10. Cochleagram of the left ear of subject B-115 exposed to 63-Hz octave-band noise at 120 dB SPL for 9 days. The amount of permanent threshold shift at 11 frequencies is also shown:

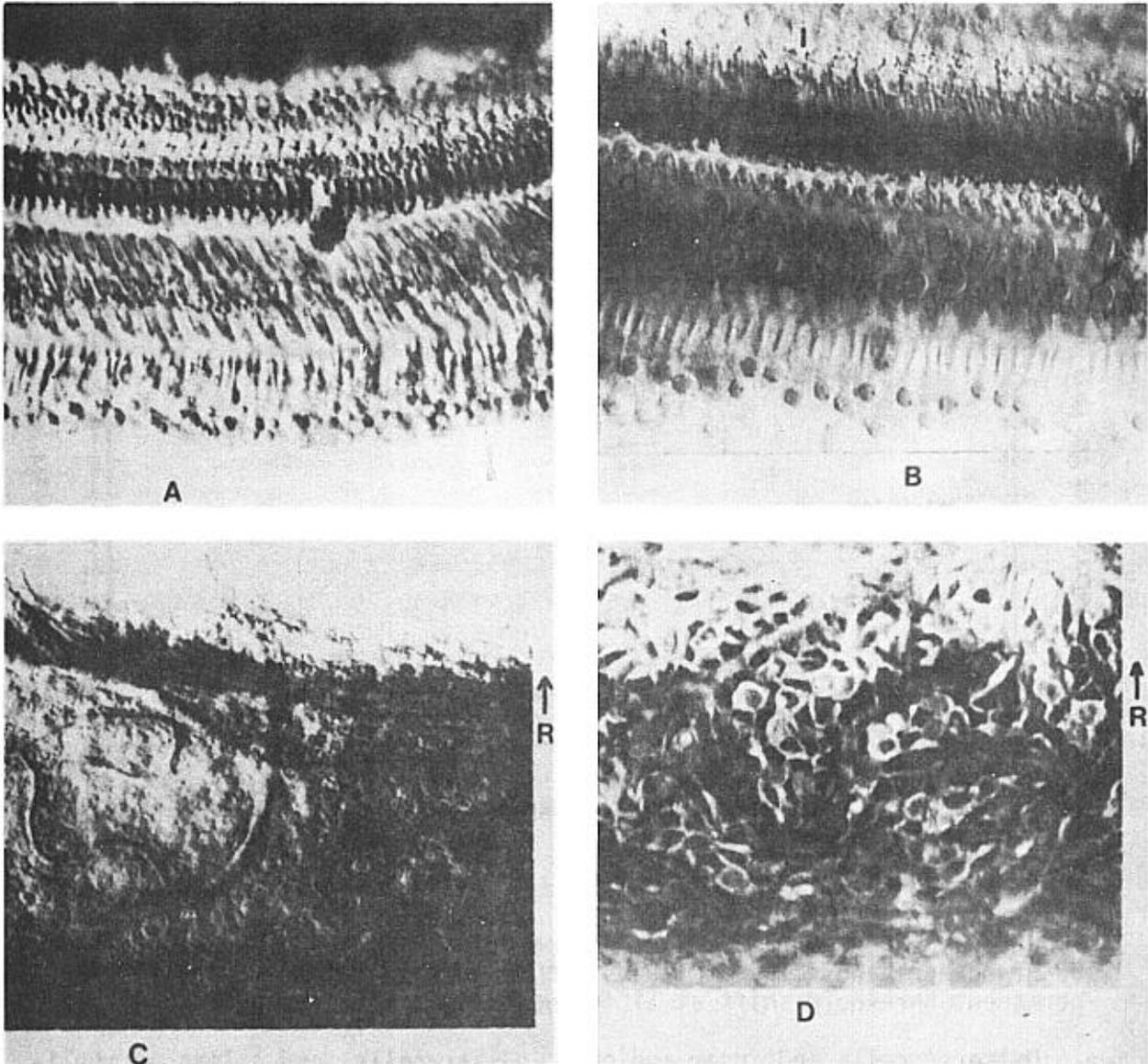


FIGURE 11. Photomicrographs of surface preparations from chinchilla cochleae. The animals were exposed to 63-Hz octave-band noise at either 110 or 120 dB SPL. (A) Typical surface view of the Organ of Corti showing scattered outer hair cell losses (X) and outer pillar cell defects (*). (B) Surface preparation view of the Organ of Corti illustrating the generally swollen outer hair cell bodies, extensive Hensen body accumulations (→), and pillar cell defects (*). (C) Endolymphatic surface of the stria vascularis illustrating the thinning of the stria tissue (patchy osmophilic character). (D) Endolymphatic surface of the stria vascularis illustrating the unusual appearance of the stria cells. I=inner hair cell; R↑=toward the attachment of Reissner's membrane; P=pillar cells; O=outer hair cell.

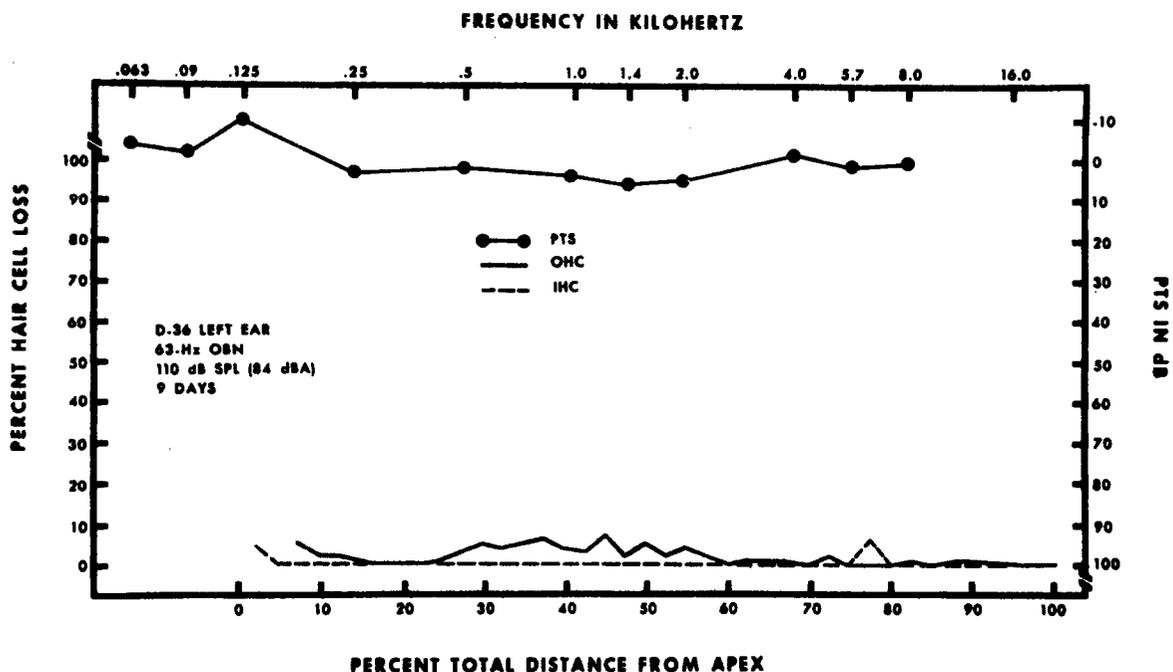


FIGURE 12. Cochleagram of the left ear of subject D-36 exposed to 63-Hz octave-band noise at 110 dB SPL for 9 days. The amount of permanent threshold shift at 11 frequencies is also shown.

i.e., Deiters' cells and inner and outer pillar cells, and a loss of myelinated nerve fibers in the vicinity of the habenula. For purposes of illustration, this lesion was identical to that shown in Figure 20C (page 30). Other presumed noise-induced defects, often seen in the apical one-half of the cochlea included: loss of an inner pillar cell (see Figures 11A and B); changes in the density of the material composing the head of the outer pillar; pillar defects with outer hair cell losses; outer hair cells with an unusually large number of cytoplasmic inclusions, presumably Hensen bodies, as seen in the micrograph in Figure 11B. Finally, in a number of cochleae, the stria vascularis appeared abnormal. In some cochleae the strial capillaries appeared in relief due to a thinning of the cell layers comprising the stria (see Figure 11C). In one animal the cell populations of the stria took on a greatly vacuolized or condensed appearance. A typical section of a surface preparation is seen in Figure 11D. Therefore, even though the cochleae had most of the inner and outer hair cells intact, the cochleae did not appear normal.

The authors are aware of the problem of relating change in cochlear histology to changes in the audiogram when animals are binaural and field tested. However, the agreement between the left and right ear cochleagrams was so good in most animals that it made comparisons between cochlear pathology and hearing loss worthwhile. When such comparisons are made, the number of missing hair cells is actually less than one would predict from the hearing loss.

The cochleae of the 110-dB exposure group had minor losses or were normal. The most severely affected subject of this group was D-36 and the cochleagram of his left ear is shown in Figure 12. This subject incurred little, if any, permanent threshold shift. As with the 120-dB exposure group, the hair cell loss amounted to scattered outer hair cell losses, generally in the apical one-half of the cochlea. This ear had a small inner hair cell lesion between the 70% and 80% points. The Organ of Corti was otherwise similar to the 120-dB group with numerous cytoplasmic densities in the outer hair cells. Large numbers of outer hair cells in both groups could also be found whose stereocillia were clearly clumped or beaded in appearance. The cell bodies on each side of the lesion were often distorted. In general, there was good agreement between the cochleagrams of this group and their audiograms, i.e., there was little or no permanent threshold shift, and the cochleae were normal or had minor losses.

EXPOSURE TO 1000-HZ OCTAVE-BAND NOISE

Growth of Threshold Shift

The growth of TS_4 at 1.4 kHz for the groups of chinchillas exposed to the 1.0-kHz octave band of noise at intensities of 85 and 95 dB SPL (dBA) is shown in Figure 13. Threshold shifts for both groups developed very rapidly to their asymptotic levels of 46 dB at 85 dB SPL and 60 dB at 95 dB SPL. This rapid growth is reflected in the time constants for growth of less than 4 hours (see Table 1). The 95-dB exposure level resulted in very stable threshold shifts with the asymptotic level of 60 dB reached in 12 h. The apparent decrease at days 8 and 9 of exposure at the 95-dB level is believed to be a reflection of test variability rather than a decrease in threshold shift. The 10 dB increase in exposure level from 85-95 dB SPL produced a difference in asymptotic threshold shift of 14 dB. The false alarm rates of the two groups were 0.14 for the 85-dB group and 0.15 for the 95-dB group.

Recovery from Threshold Shift

The recovery functions for the two groups exposed to the 1.0-kHz octave band of noise are shown in Figure 14. The subjects exposed at the 85-dB level rapidly and completely recovered within 72 h. After 4 days of stable

thresholds, testing was terminated. The time constant for recovery for this group was 8 h (see Table 1). The subjects exposed at 95 dB incurred 17 dB of permanent threshold shift at 1.4 kHz. This was calculated by averaging threshold shifts over days 18, 20, 25 and 30. The recovery of this group was considerably slower than that of the 85-dB group, as reflected by the time constant for recovery of 48 h (see Table 1). The false alarm rates during recovery were 0.10 for the 85-dB group and 0.13 for the 95-dB group.

The levels of compound threshold shift and permanent threshold shift across 11 frequencies are shown in Figure 15 (page 26). The levels of compound threshold shift were those present at the termination of the 9-day exposure. Both levels of exposure produced maximum effects at the one-half octave frequency of 1.4 kHz. In addition, the 1.0-kHz octave band of noise at 95 dB produced a relatively large compound threshold shift of 40 dB at 63 Hz, while the 85-dB exposure produced 9 dB of threshold shift at 63 Hz.

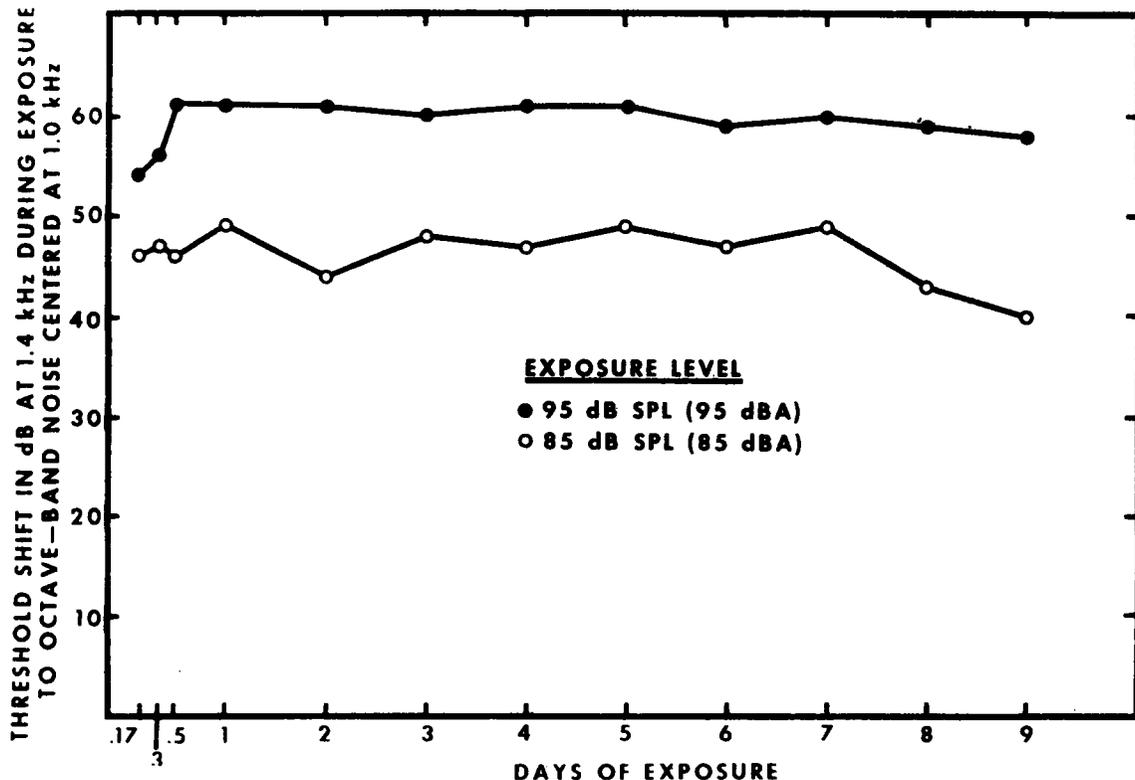


FIGURE 13. Threshold shifts in dB at 1.4 kHz produced by exposure to octave-band noise centered at 1.0 kHz at intensity levels of 85 and 95 dB SPL for 9 days.

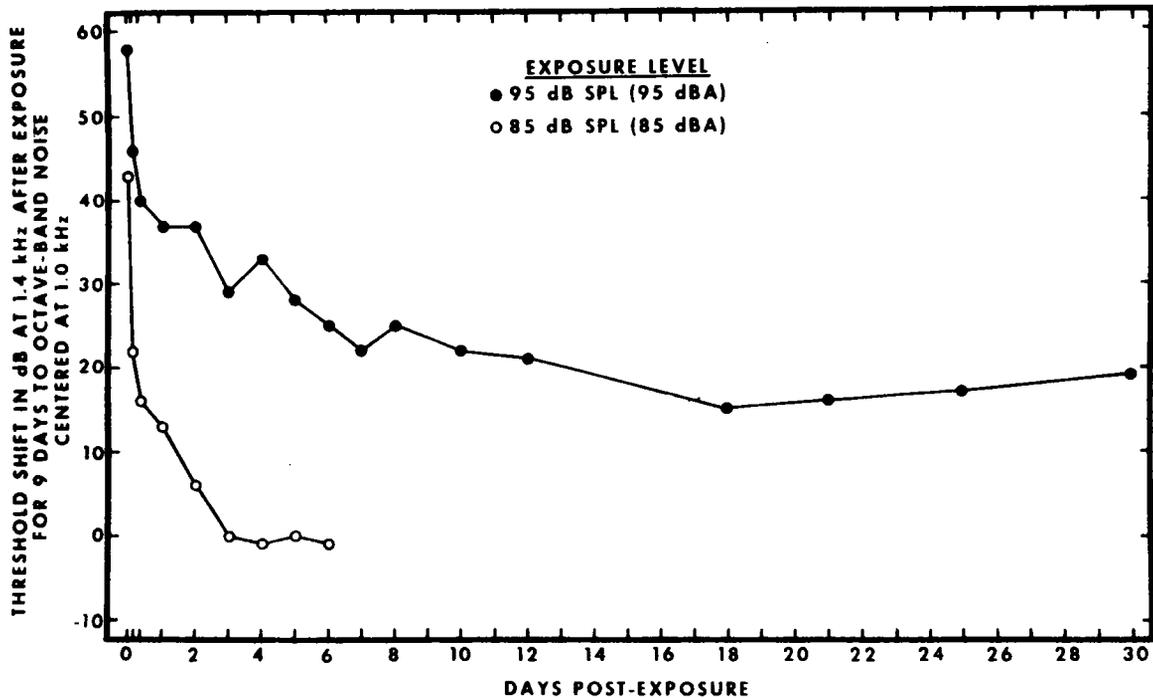


FIGURE 14. Recovery of threshold shifts at 2.4 kHz over the 30-day period following 9 days of exposure to octave-band noise centered at 1.0 kHz at intensity levels of 85 and 95 dB SPL.

The group exposed at the 85-dB level incurred essentially no permanent hearing loss. However, the group exposed at the 95-dB level incurred its maximum hearing loss at 2.0 kHz. As seen in Figure 15, peaks in permanent threshold shift occurred at 63, 500, and 5700 Hz as well.

Histology

The animals exposed at 85-dB had a variety of cochlear lesions. However, the most typical lesion was confined to the apical one-half of the cochlea. One pattern of hair cell loss found in this group is shown in Figure 16. This subject had a significant outer hair cell lesion between the 27% and 50% points along the cochlea. As was typical for this group, no permanent hearing loss was found for this animal. It would be expected that at least a small permanent threshold shift would be found in light of

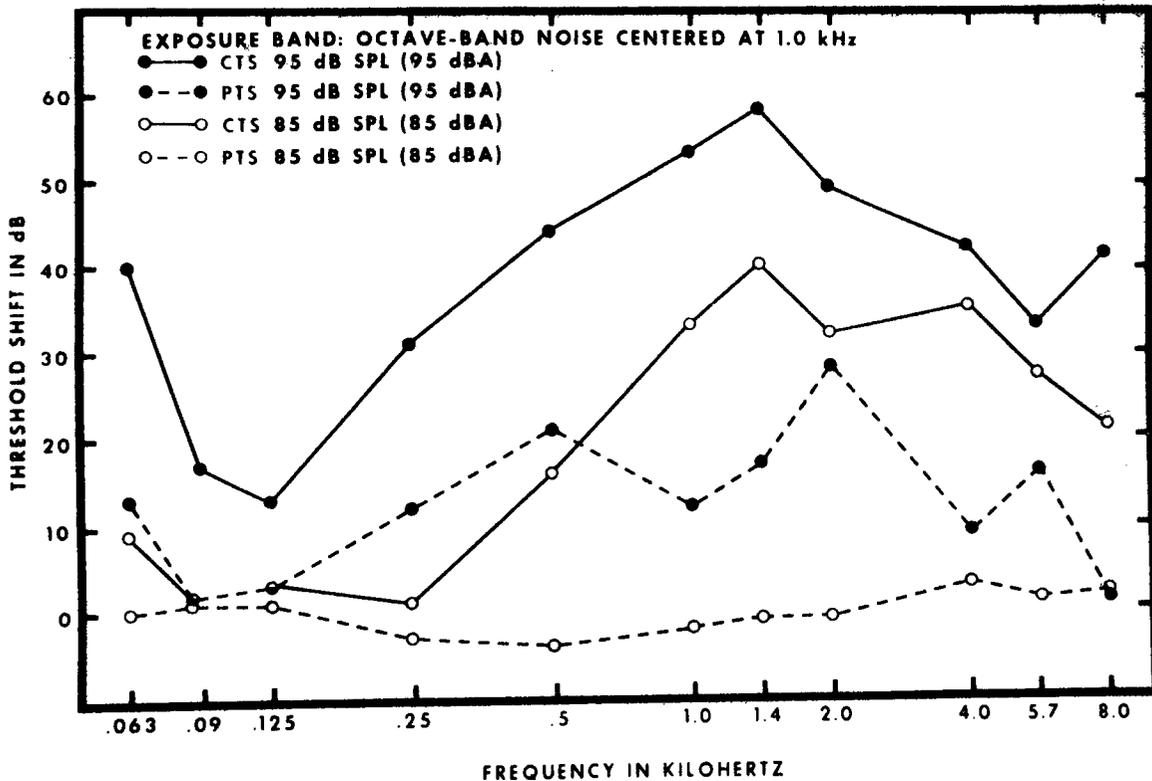


FIGURE 15. Threshold shift as a function of frequency - 1000 Hz at intensities of 85 and 95 dB SPL. The solid lines depict compound threshold shifts for frequencies from 63 to 8000 Hz following 9 days of exposure to octave-band noise centered at 1000 Hz at intensities of 85 and 95 dB SPL. The broken lines depict the resultant permanent threshold at the same frequencies.

the outer hair cell lesion that was present. Another pattern of lesions was found in subject C-100 whose cochleagram is shown in Figure 17. This subject, unlike the other three, had outer hair cell lesions of 20-30% scattered throughout the basal-half of the cochlea. Again, the audiogram of this animal was essentially normal. Photomicrographs showing the various features of the pattern of hair cell loss in chinchilla C-102 are shown in Figure 18. In addition to the scattered losses, the remainder of the outer hair cells present were often distorted (see Figure 18) and had swollen cell bodies (Figure 18C) with numerous cytoplasmic densities evident. The stria vascularis in two of the four cochleae were abnormal. There was a total loss of the stria through most of the apical one-half of the cochlea. The

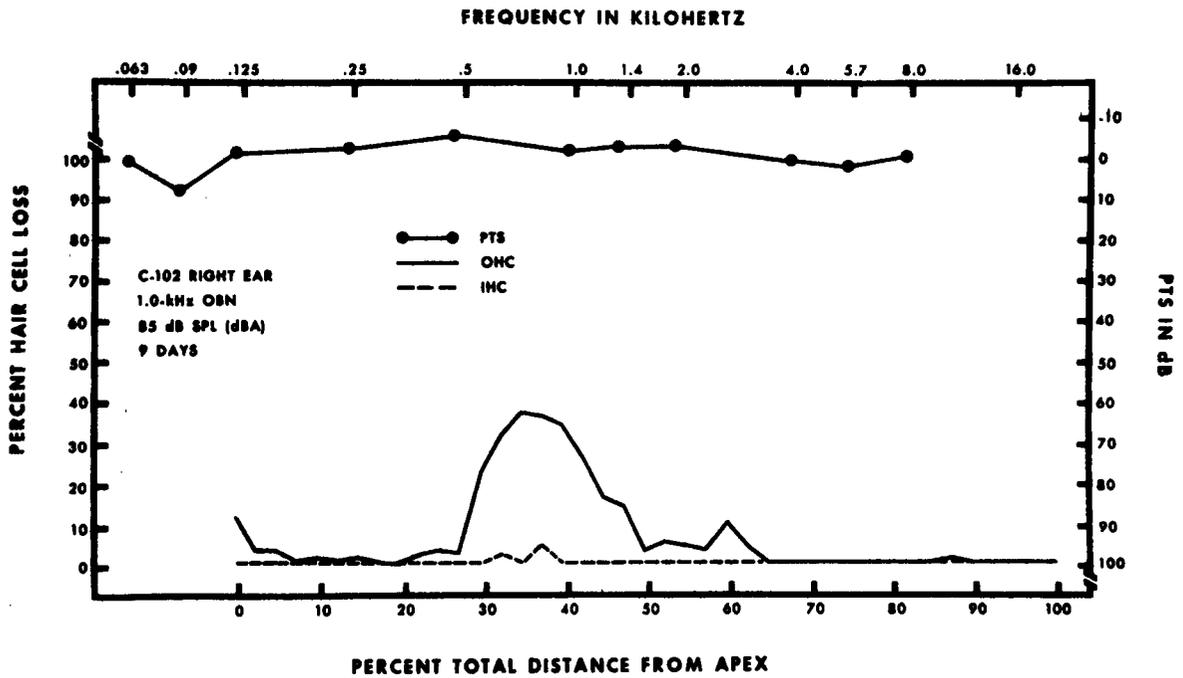


FIGURE 16. Cochleagram of the right ear of subject C-102 exposed to 1.0-kHz octave-band noise at 85 dB SPL for 9 days. The amount of permanent threshold shift at 11 frequencies is also shown.

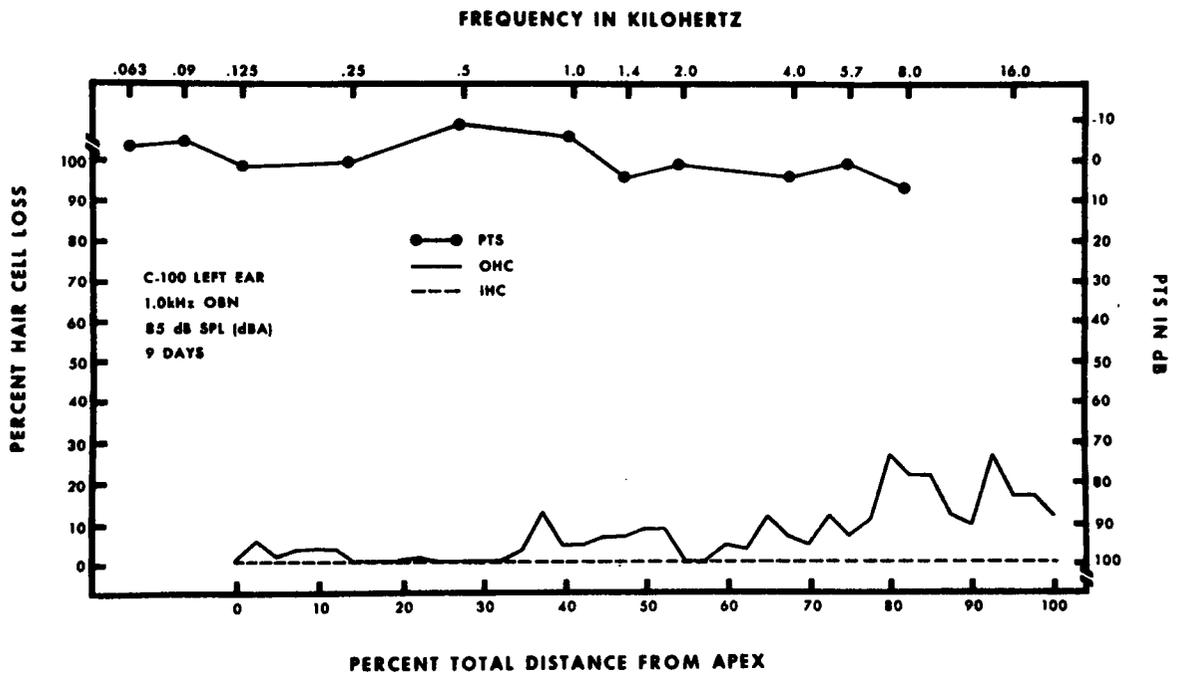


FIGURE 17. Cochleagram of the left ear of subject C-100 exposed to 1.0-kHz octave-band noise at 85 dB SPL for 9 days. The amount of permanent threshold shift at 11 frequencies is also shown.

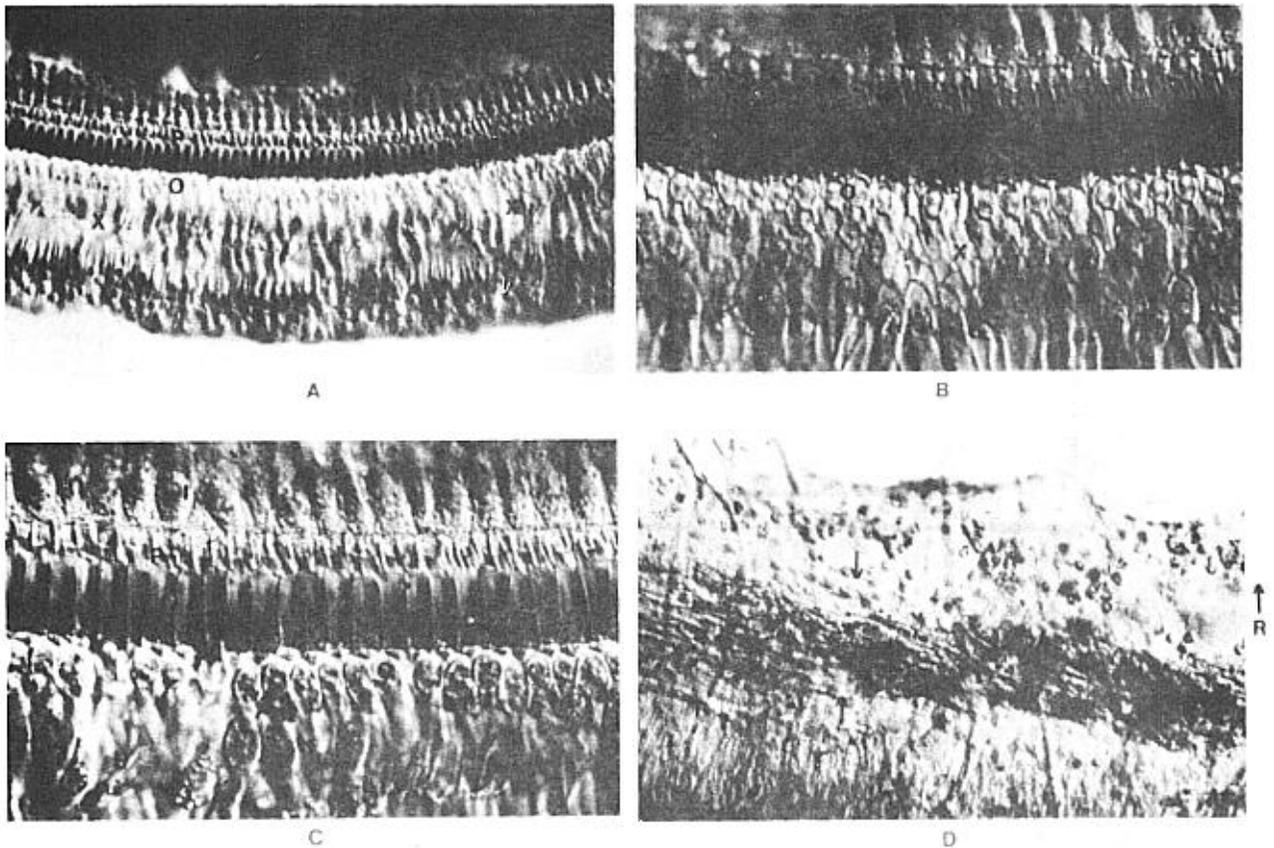


FIGURE 18. Photomicrographs of surface preparation from the cochlea of chinchilla C-102 exposed to 1.0-kHz octave-band noise at 85 dB SPL. (A) Surface of the Organ of Corti showing considerable scattered losses of outer hair cells (X). (B) Surface view of the Organ of Corti at the level of the reticular lamina showing the typical scar configuration in noise-damaged cochleae. (C) Surface view of the Organ of Corti illustrating swollen outer hair cells and the extensive accumulation of Hensen bodies (T). (D) Surface view of the lateral wall of the cochlea showing complete loss of the stria vascularis; only remnants of the strial tissues remain (X). I=inner hair cell; R↑= toward the attachment of Reissner's membrane; P=pillar cells; O=outer hair cell.

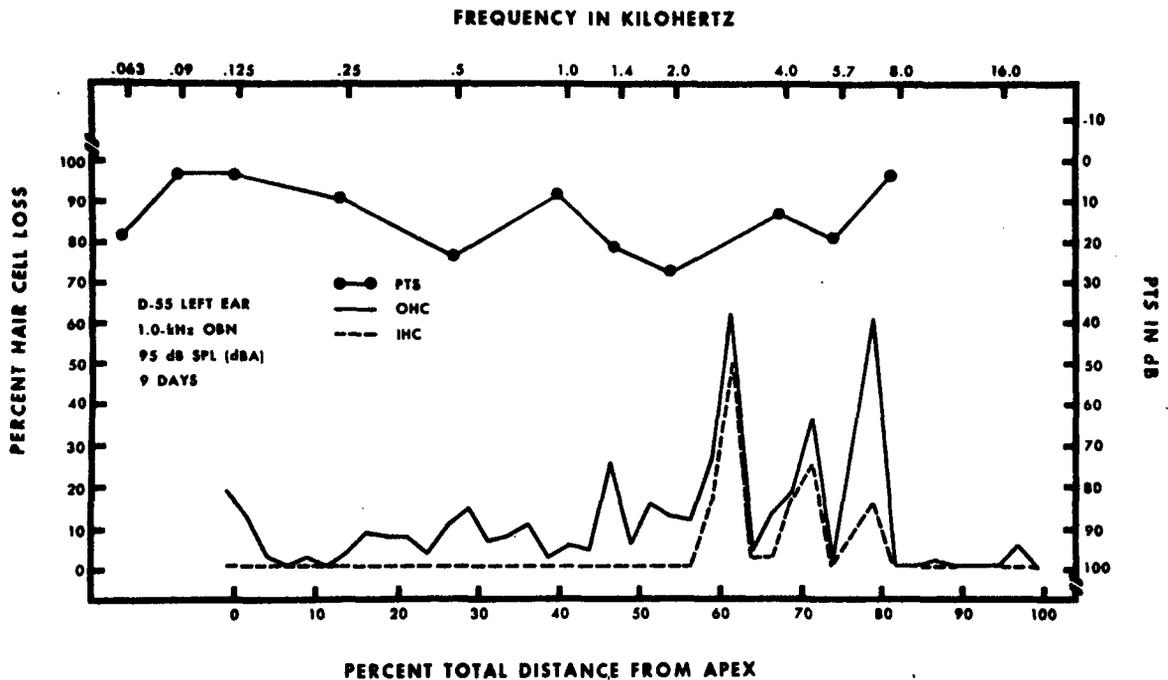


FIGURE 19. Cochleagram of the left ear of subject D-55 exposed to 1.0-kHz octave-band noise at 95 dB SPL for 9 days. Also shown is the amount of permanent threshold shift at 11 frequencies.

appearance of the lateral wall of the cochleae in these animals is shown in Figure 18D. Another animal (C-74) showed strial atrophy in discrete locations of the apical one-half of the cochlea along the attachment of Reissner's membrane. This type of loss was similar to that shown in Figure 20B. The remainder of the stria appeared thin, or otherwise did not stain uniformly.

The relation between the cochleagrams of the 85-dB, 1000-Hz octave-band noise group and the audiogram is essentially the converse of the 120-dB, 63-Hz octave-band noise group. In the 85-dB, 1.0-kHz group, all of the subjects had significant outer cell loss, but none showed any significant hearing loss.

The eight cochleae of the animals of the 95-dB exposure group were

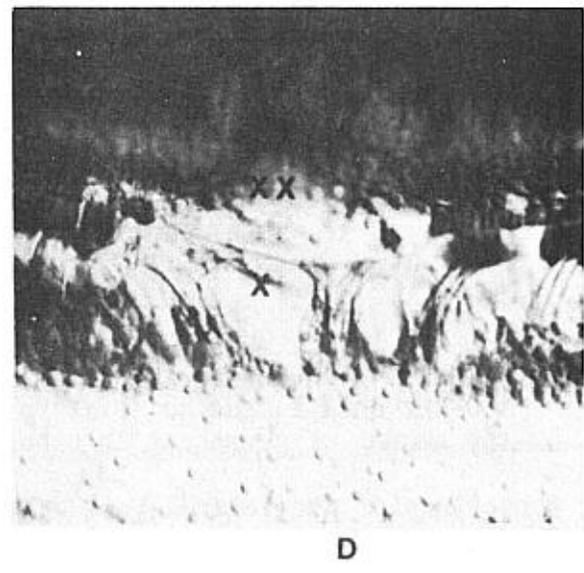
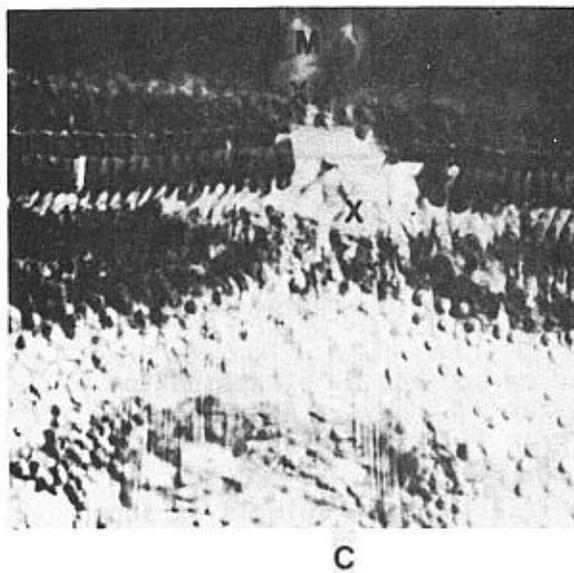
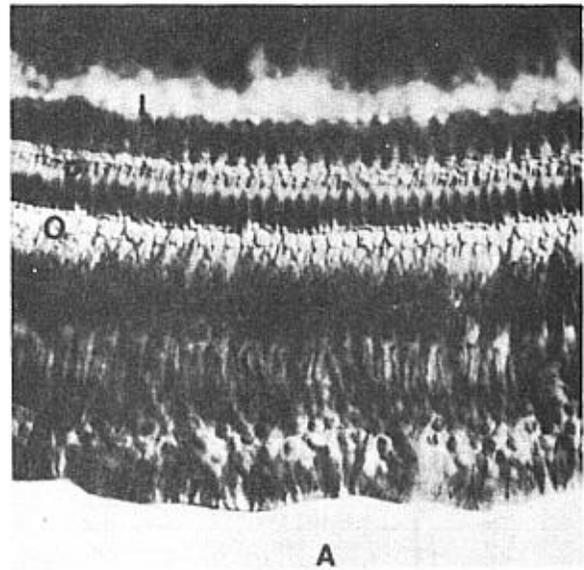
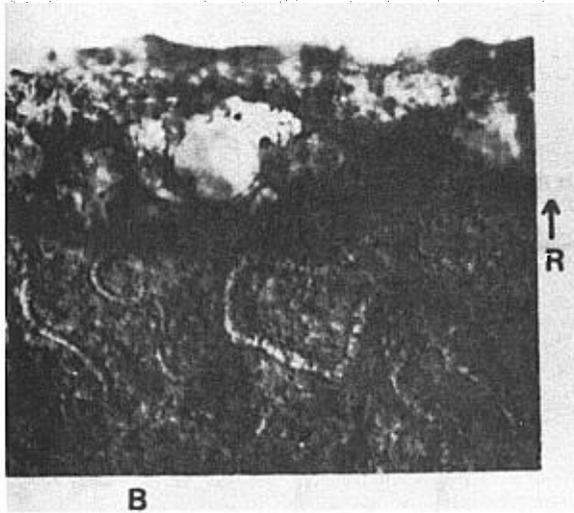


FIGURE 20. Photomicrographs of surface preparations from the cochleae of chinchillas exposed to 1.0-kHz octave-band noise at 95 dB SPL for 9 days. (A) Surface of the Organ of Corti showing scattered losses of outer hair cells (X). (B) Surface view of a section of the stria vascularis showing a well defined lesion in the stria vascularis at the attachment of Reissner's membrane. (C) and (D) Surface of the Organ of Corti showing two different focal lesions having losses of outer (X) and inner (XX) hair cells, pillar cells, Dieter cells, and a loss of radiating, myelinated nerve fibers (Mm). I=inner hair cells; R↑=toward the attachment of Reissner's membrane; P=pillar cells; O=outer hair cells.

dissected and evaluated. Four cochleae, those of subjects D-55 and D-35, had scattered outer hair cell losses throughout most of their entire length. The most severe losses were found in chinchilla D-55 whose left-ear cochleagram is shown in Figure 19. Several severe outer and inner hair cell lesions were found in the basal half of the cochleae of this animal. This animal displayed permanent threshold shift in excess of 19 dB at 63, 500, 1000, 1400, 2000, and 5700 Hz. The correspondence between the location of the lesions and the frequencies of hearing loss as defined by the tonotopic map are, in general, not good. Photomicrographs of particularly severe localized lesions are shown in Figures 20C and 20D. Damage extended to all of the supporting elements of the Organ of Corti, the inner and outer hair cells, and the myelinated nerve fibers. The remaining two animals had losses that were comparable to the animals of the 85-dB exposure group. While the pattern of loss often extended to within 20% of the base of the cochlea, most of the losses occurred in the middle and through the apical one-half of the cochlea. This pattern is illustrated by Figure 21, which shows the left-ear cochleagram of subject D-79. This subject incurred a 37-dB permanent threshold shift at 2.0 kHz and the severity of the hair cell lesions again does not reflect a hearing loss of this magnitude. Stria aberrations were similar to those previously described and varied from areas of total loss in the apex, as pictured in Figure 18D in chinchilla D-79, to localized areas of strial atrophy along the line of attachment of Reissner's membrane, and occasionally just above the spiral prominence. The appearance of the stria in these areas was much the same as shown in Figure 20B. While all animals of this group have both hearing losses and losses of hair cells, the relationship between the two measures of noise trauma is not clear. Admittedly, there are ambiguities about the tonotopic map of the cochlea, but discrepancies between the position of the hair cell loss and the pattern of hearing loss are greater than one can attribute to inaccuracies in the cochlear map.

COMPARISON OF THE TWO BANDS OF NOISE WITH REGARD TO A-WEIGHTING

The intensities of the two bands of noise were matched within 1 dBA. The compound threshold shifts and permanent threshold shifts produced by the two bands of noise at the low-level exposure are shown in Figure 22. The 1000-Hz octave band of noise at 85 dB SPL (85 dBA) produced somewhat more threshold shift than did the 63-Hz octave band of noise at 110 dB SPL (84 dBA). The frequency region showing the greatest effect in both conditions was generally the same. Neither exposure produced significant amounts of permanent threshold shift. Histologically, however, there was a difference between the two groups. The subjects of the 63-Hz exposure had little, if any, hair cell loss while the subjects of the 1000-Hz exposure had significant outer hair cell losses.

The compound threshold shifts and permanent threshold shifts of the subjects exposed at the higher intensity levels are shown in Figure 23. Although the 1000-Hz band produced more compound threshold shift than did the 63-Hz band at 250 to 4000 Hz, both exposure bands affected the same frequencies. The major exception to the similarity between the effects of

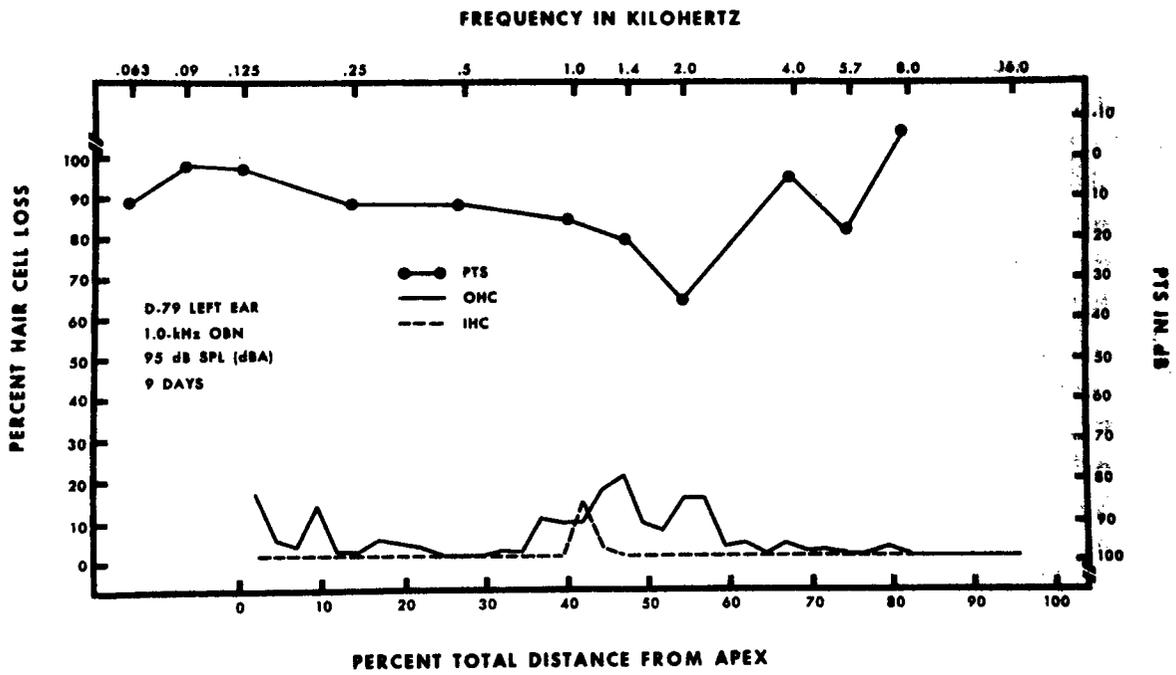


FIGURE 21. Cochleagram of the left ear of subject D-79 exposed to 1.0-kHz octave-band noise at 95 dB SPL for 9 days. The amount of permanent threshold shift at 11 frequencies is also shown.

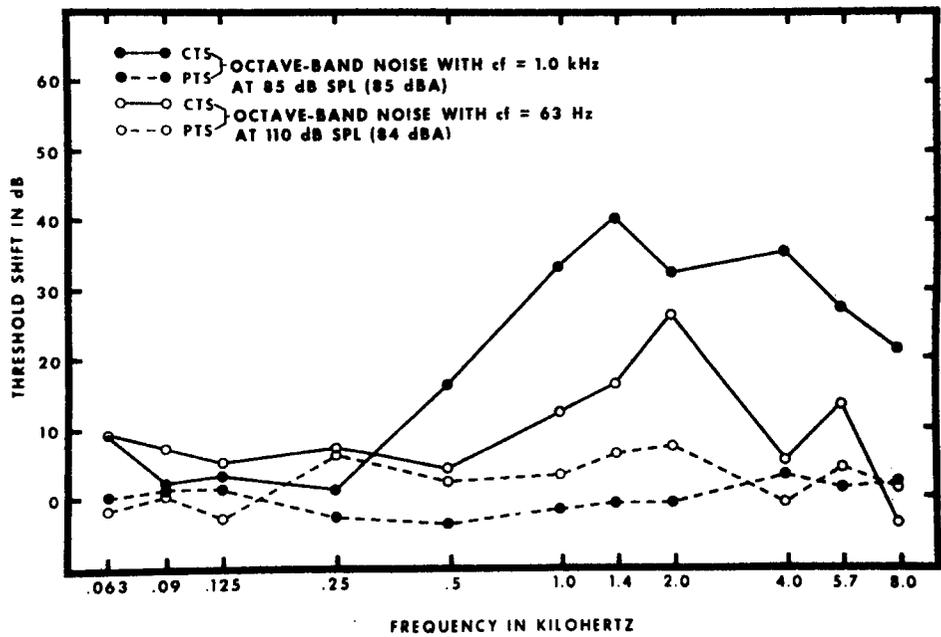


FIGURE 22. Compound threshold shifts and permanent threshold shifts of the subject exposed to 63-Hz octave-band noise at 110 dB SPL (84 dBA) and the subjects exposed to 1000-Hz octave-band noise at 85 dB SPL (85 dBA) for 9 days.

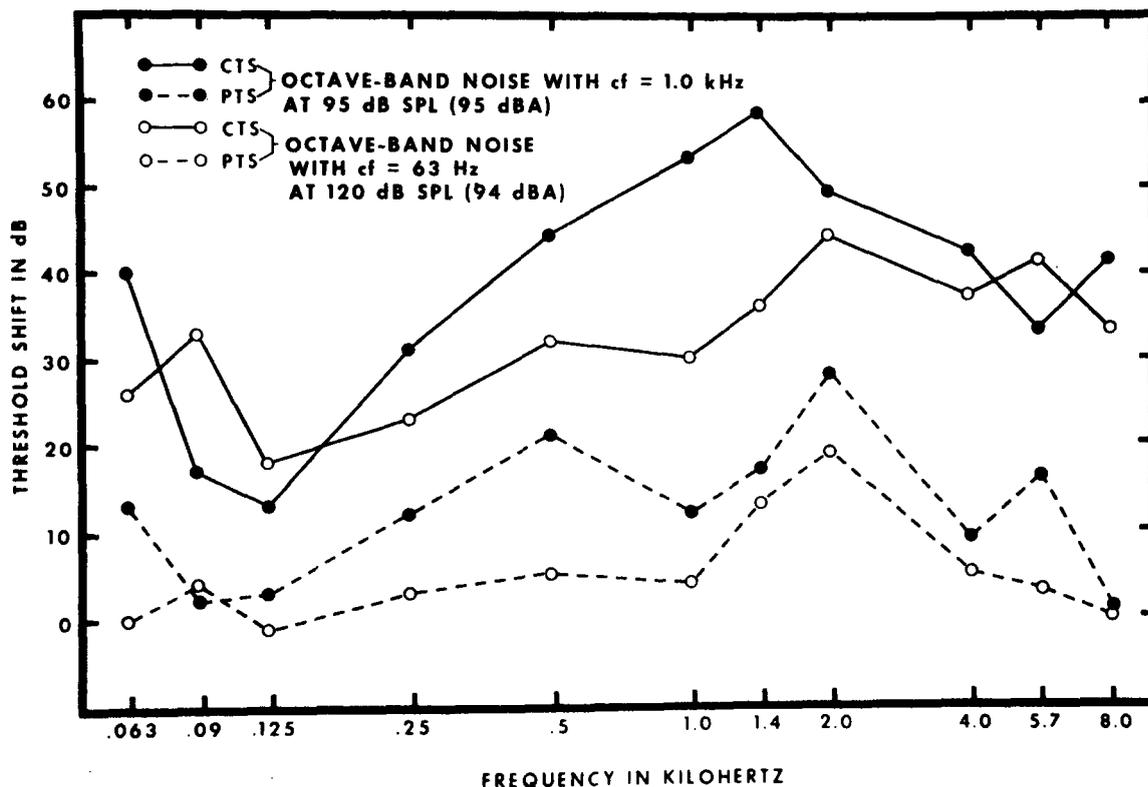


FIGURE 23. Compound threshold shifts and permanent threshold shifts of the subjects exposed to 63-Hz octave-band noise at 120 dB SPL (94 dBA) and the subjects exposed to 1000-Hz octave-band noise at 95 dB SPL (95 dBA).

the two bands of noise was the threshold shift produced at 63 Hz by exposure to the 1000-Hz octave-band noise. Somewhat paradoxically the 1000-Hz band of noise produced a larger threshold shift than did the 63-Hz band of noise and there was a resultant permanent threshold shift of 13 dB at 63 Hz produced by the 1000-Hz noise band while no permanent threshold shift was produced by the 63-Hz noise band. This is shown in Table 2, which gives the values of the permanent threshold shifts at each frequency for the two noise bands at their highest intensity level. In terms of overall permanent threshold shifts, the 1000-Hz octave band of noise produced more permanent threshold shift than did the 63-Hz octave band of noise. Both bands produced similar levels of permanent threshold shift at 1.4 and 2.0 kHz. The 1000-Hz octave bands produced more permanent threshold shift at the lower

TABLE 2

PERMANENT THRESHOLD SHIFTS IN dB FOLLOWING 9-DAYS OF EXPOSURE TO OCTAVE-BAND NOISE CENTERED AT 63 HERTZ AT 120 dB SPL (94 dBA) AND TO OCTAVE-BAND NOISE CENTERED AT 1000 HERTZ AT 95 dB SPL (95 dBA)

Frequency (Hz)	63	90	125	250	500	1000	1400	2000	4000	5700	8000
63-Hz											
Exposure Band	0	4	-1	3	5	4	13	19	5	3	0
1000-Hz											
Exposure Band	13	2	3	12	21	12	17	28	9	16	1

NOTE: Each value is the average shift found over the last four days tested: days 18, 23, 25, and 30 post-exposure for the 63-Hz exposure and days 18, 21, 25, and 30 post-exposure for the 1000-Hz exposure.

frequencies of 63, 250, 500, and 1000 Hz than did the 63-Hz octave band of noise. The 63-Hz octave band of noise had a narrowly defined effect with the major permanent threshold shifts occurring at 1400 and 2000 Hz.

DISCUSSION

As in the previous study (Burdick et al., 1978) in which a 3-day exposure was used, the 63-Hz octave band of noise had its major permanent effects at 1400 and 2000 Hz. One difference between the 3-day and the 9-day exposures was that in the 9-day exposure, the 1.0-kHz octave-band noise produced permanent threshold shifts at a larger number of frequencies than it did in the 3-day exposure. Following the 3-day exposure, the only permanent threshold shifts produced by the 1.0-kHz band were found at 1.4 and 2.0 kHz. This difference in the number of frequencies affected was most likely the result of the longer duration exposure. Another difference between the 3- and 9-day exposure was that in the 9-day exposure the 1000-Hz noise band produced slightly more hearing loss at 1400 and 2000 Hz than did the 63-Hz noise band, while in the 3-day exposure these results were reversed. Again, this may be the result of the longer exposure. An interesting effect found in the present study was the production of both compound threshold and permanent threshold shift at 63 Hz by the 1000-Hz exposure band. This same effect was found previously but it was less pronounced than in the present investigation. The mechanism of this effect is unclear. Since, to our knowledge, no one has previously tested for threshold shift at frequencies as low as 63 Hz, it would be useful if other investigators would consider such tests when exposing subjects to bands of noise to determine if the low frequencies are affected by other higher frequencies.

It was recently pointed out by Mills et al. (1979) that a number of studies (e.g., Miller, Watson, and Covell, 1963; Patterson, Burdick, Mozo, and Camp, (1977)) have found a second, smaller peak or maximum in threshold shift at frequencies above the major peak at the one-half octave frequency. The 63-Hz octave-band noise exposures produce just the opposite effect. That is, the major peak in threshold shift occurred at frequencies higher than the one-half octave frequency.

Histologically, none of the ears exposed to the low-frequency noise evidenced any significant hair cell lesions in the local regions that correspond to the permanent threshold shifts found at the high frequencies. Such lesions have been found, however, in chinchillas exposed to the frequency of 500 Hz (Fried, Dudek, and Bohne, 1976; Salvi, Henderson, and Hamernik, 1977; Clark and Bohne, 1978). The high-frequency hearing loss and the absence of hair cell lesions in the basal turn indicate that the mechanics of the cochlea involved in noise-induced hearing loss, at least at 63 Hz, may be different for frequencies below 500 Hz.

The effects of exposure to the low-frequency noise can be characterized as slow to develop and slow to recover. The 10-dB change in exposure level did not affect the rate of recovery. The 1.0-kHz exposures produced much more rapidly developing effects than did the 63-Hz exposures. The 1.0-kHz, 85-dB exposure resulted in rapid recovery and the 10 dB increase in exposure level lengthened the time constant for recovery from 8 to 48 h, indicating that the rate of recovery from high-frequency noise exposure may be more dependent upon exposure level than the rate of recovery from low-frequency noise exposure.

The debate concerning the role of the inner and outer hair cells in hearing has clearly not been resolved by the results presented in this paper. In fact, the issue may be more confused. Following the 120-dB, 63-Hz exposure, the cochleae had almost normal numbers of hair cells but the audiograms showed an average hearing loss of 19 dB. Conversely, after the 85-dB, 1.0-kHz exposure, the cochleae had significant lesions without any permanent hearing losses. Finally, after the 95-dB, 1-kHz exposure, the cochleae had significant hair cell losses, but not in the locations that are consistent with the pattern of hearing loss. The issue is even more confused when one recognizes that hair cells may be present but have a myriad of intracellular changes or the supporting elements may be altered. One of the pervasive changes seen in these cochleae was a general swelling of the hair cells. Swelling such as that seen in the micrographs of Figures 11B and 18C was not seen in the cochleae of control animals. One possible reason for the swelling is that the membrane characteristics of the hair cells may have been permanently altered because of the noise trauma, leaving the cells especially vulnerable to the fixation process.

The relationship between hair cell changes and hearing loss may also be "masked" by the extensive changes seen in the stria vascularis of the 1.0-kHz groups. The strial changes appear to be the consequence of this noise exposure and not a fixation artifact because the strias of control animals appear normal. The pattern of strial damage ranges from a thinning of the strial complex to a complete obliteration of the stria for several millimeters. Moreover, the changes in stria do not appear to be specifically localized to the site of the hair cell lesion. The strial atrophy at the attachment of Reissner's membrane (see Figure 20B) may be a reflection of minor tears of Reissner's membrane with local poisoning due to the mixing of endolymph and perilymph. Regardless of the origins of the strial changes, they are extensive and are likely contributors to the hearing loss, but there is no obvious way of separating strial from Organ of Corti components of the hearing loss.

One major question that arises from the results with the low-frequency noise concerns the mechanisms of the high-frequency hearing loss. At this time, there seem to be three possible ways for this to occur. First, the outer and middle ear transfer functions may "modify" the 63-Hz band of noise to an effective exposure frequency of around 1000 Hz. This could be argued in light of the nearly identical pattern of high-frequency hearing loss incurred by subjects exposed to both the 63-Hz and 1000-Hz octave bands of noise. This would not seem probable because of the rather large difference

in the magnitudes of the compound threshold shifts found between the two groups. Second, the high-frequency effect could result from harmonics generated within the cochlea itself. The resolution of this possibility must await data derived from electrophysiological investigations which are currently in progress. Third, the effect may result from a "weakness" in the basilar membrane associated with the frequency region of 2.0 kHz that results from possible physical changes of the membrane such as decreasing width and/or thickness. If this proposition were true, then any low-frequency that generates a traveling wave that stimulates the 2.0 kHz region along the basilar membrane would have an effect on 2.0 kHz. This argument does not seem particularly attractive since 500 Hz does not appear to affect the 2000 Hz region of hearing. In addition, there is no histological evidence in our study to support such an idea.

CONCLUSION

With regard to the issue of the measurement of noise for damage-risk criteria in terms of A-weighted levels, the present results are contrary to previous results (Burdick, et al., 1979). The present findings indicate that both exposure bands are either nearly equal in effect or that the 1000-Hz band was actually more hazardous than the 63-Hz band. Based on the earlier results with the 3-day exposure it would seem that the 26 dB difference in octave-band level between these two noise bands would have resulted in even larger differences in hearing loss during this 9-day exposure with the low-frequency effects becoming even greater. This clearly did not occur. The results are equivocal and we believe it is best at this time to await further data, particularly since so little is known about the mechanisms involved in exposure to low-frequency noise.

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APPENDIX A

LIST OF EQUIPMENT MANUFACTURERS

Altec Lansing Corporation
1515 S. Manchester Avenue
Anaheim, California 92803

Industrial Acoustics Company, Inc.
380 Southern Boulevard
Bronx, New York 10454

John Fluke Manufacturing Company, Inc.
P. O. Box 43210
Mountlake Terrace, Washington 98043

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